THE PHOTOSYNTHETIC BIOLOGY OF THE REEF CORAL

*Pocillopora damicornis*

AND SYMBIOTIC ZOOXANTHELIACE

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN OCEANOGRAPHY

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ACKNOWLEDGEMENTS

A research problem of this complexity could not have been investigated without considerable interdisciplinary interaction. Many people contributed substantially to the success of this research effort. Experiments involving the isolation and in vitro culture of zooxanthellae and microalgae were carried out in collaboration with Richard H. York, Jr. The studies of response of corals to light of different spectral composition within the visible range were conducted jointly with Dr. Robert A. Kinzie III. Dr. Janice I. Morrissey assisted in the studies of the photosynthesis-irradiance relationship and canopy development. Dr. David Krupp and Tom Hunter provided a great deal of help on statistical and computer analysis of data. Studies on effect of night irradiance on coral reproduction were facilitated by the dedicated assistance of Peter M. Liu and Russell Y. Ito. I am grateful to many others at the Hawaii Institute of Marine Biology, the Department of Oceanography and the Department of Zoology who helped me in many ways. I am especially thankful to the members of my committee for the role that they played in the development of this dissertation.
ABSTRACT

The reef coral *Pocillopora damicornis* and its symbiotic algae (zooxanthellae) shows strong biological responses to subtle changes in the spectrum, intensity and modulation of the natural radiation environment in the 280 nm to 700 nm range. Corals and *in vitro* cultures of zooxanthellae were grown in full spectrum solar radiation and in filtered sunlight having the same Photosynthetically Active Radiance (PAR) but lacking ultraviolet (UV) radiation. Skeletal growth rate of the corals was decreased by approximately 50% in the treatment with full solar UV radiation. Corals grown in the treatment without solar UV radiation contained less of the "S-320" UV-absorbing substances. The UV-screening material apparently protected both the contained symbiotic algae and the host from the detrimental effects of UV radiation. *In vivo* zooxanthellae apparently were protected by the S-320 pigments of the host and were not damaged by UV radiation. In contrast, cultures of the algae grown *in vitro* in UV-transparent quartz vessels were severely inhibited by solar UV radiation. The impact of UV was far more severe in a "shade-loving" genetic strain of zooxanthellae than in a "sun-loving" strain. Growth of cultures of zooxanthellae and other unicellular algae was not inhibited even at full solar intensity if the UV portion of the spectrum was blocked with a filter.
Corals and in vitro cultures of zooxanthellae were also grown under identical levels of PAR but with different regimes of spectral composition. Corals and algae showed maximal growth rate and photosynthetic pigment concentration under conditions of "blue" PAR. Growth and pigmentation was extremely low when the organisms were grown under prolonged exposure to "red" PAR. An intermediate response was observed under conditions of "white" and "green" PAR.

All of the basic parameters that define the photosynthesis-irradiance relationships in coral are influenced by the size of the coral. Maximum photosynthetic rate, saturation constant, and the photosynthesis to respiration ratio increased with increasing size of the coral. Initial slope (alpha) of the P-I curve and oxygen consumption per unit biomass in darkness decreased as a function of size. Net primary production of the coral per unit reef area increased dramatically with increasing size.

The "lunar cycle" of coral reproduction is controlled by night irradiance. Alteration of the night pattern of "lunar" irradiance caused the coral to shift reproductive periodicity. Lack of a regular lunar signal eliminated reproductive synchronization.
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LIST OF ABBREVIATIONS AND SYMBOLS

**alpha** = the initial slope of the photosynthesis-irradiance curve.

**chl** = chlorophyll.

**d** = day.

**h** = hour.

**HIMB** = Hawaii Institute of Marine Biology, Coconut Island, Hawaii.

**I** = Irradiance. Irradiance is the radiant flux incident on a receiving surface from all directions, per unit area of surface.

**Ic** = Compensation intensity. The I value of the point where the P-I curve crosses the abcissa (P=0). This is the level of irradiance where photosynthesis compensates for respiration, resulting in no net oxygen exchange.

**Ik** = irradiance saturation constant. This is the level of irradiance where the initial slope of the photosynthesis-irradiance curve intercepts the horizontal asymptote Pm (see Talling, 1957).

**l** = liter.

**min** = minute.
ml = milliliter.

nm = nanometer.

P = Photosynthetic rate measured as rate of oxygen exchange.

PAR = Photosynthetically Active Radiation. Radiation in the 400 nm to 700 nm waveband. PAR is a general term that includes photon units and energy units.

P-I curve = Plot of photosynthetic rate (ordinate) versus irradiance (abscissa).

Pm = Maximum net photosynthetic rate. Also called photosynthetic capacity. This is the horizontal asymptote that photosynthetic rate approaches as irradiance increases.

(Pm-R) = Maximum gross photosynthetic rate.

PPFD = Photosynthetic Photon Flux Density. Photon flux density of PAR. Also called the quantum flux density. The number of photons in the 400 nm to 700 nm waveband incident per unit time on a unit surface.

ppm = parts per million

PQ = Photosynthetic Quotient. Defined as the molar ratio of oxygen produced during photosynthesis to carbon dioxide consumed during photosynthesis.
PSU = Photosynthetic Unit (see Schmid and Gaffron, 1968). The organized assemblage of pigment molecules and associated membranes that carry out the complete set of light reactions during photosynthesis.

R = Respiration rate. Defined in this study as oxygen flux during nighttime darkness. By this convention R is a negative number.

rpm = revolutions per minute.

RQ = Respiratory Quotient. Defined as molar ratio of carbon dioxide produced to oxygen consumed during respiration.

S.A.S. = Statistical Analysis Systems

S. D. = Standard Deviation.

sec = second.

S. E. = Standard Error.

S-320 = UV-blocking pigment found in the ectoderm of corals. Term coined by Shibata (1969) for an unknown water soluble substance with peak absorbance at 320 nm. These chemical substances are now known to be mycosporine-like amino acids (Dunlap and Chalker, in preparation).
UV = Ultraviolet. Radiation at wavelengths between visible and x-radiation.

UV-A = Near ultraviolet radiation. Defined in this study as solar radiation in the 320 nm to 400 nm range.

UV-B = Middle ultraviolet radiation. Defined in this study as solar radiation in the 280 nm to 320 nm region.

UV-C = Far ultraviolet radiation. Defined in this study as solar ultraviolet radiation at wavelengths shorter than 280 nm. These wavelengths do not penetrate the atmosphere. UV-C has not been ecologically important at the surface of the earth since the Pro-phanerozoic.

μE = microeinstein. The einstein is used to designate Avogadro's number of photons. It has been suggested that this quantity is more properly referred to as one mole of photons (6.20 x 10^{23}) because the mole is a SI unit (see Incoll et al., 1977).

μm = micron (micrometer).
PREFACE

Statement of dissertation problem: background, purpose, conceptual framework, scope of work and hypotheses to be tested.

Solar radiation is required for the continued existence of life on this planet. Sunlight drives photosynthetic reactions and hence is the ultimate energy source for most of the major ecosystems on earth. The importance of solar radiation in this respect is generally understood and accepted.

This dissertation is concerned with some of the more complex, subtle and poorly understood photobiological phenomena occurring on coral reefs. My interest in this topic was stimulated by frustrations encountered in previous research. Often, I could not explain observations using conventional concepts concerning solar radiation. Gradually, I became aware of the fact that many important aspects of coral reef photobiology have gone unstudied.

Originally, I chose to accept all of the simplifying assumptions that were widely used in coral reef research. I accepted the conventional idea that corals "light saturate" at low intensity. Likewise, I ignored the possible importance of altered spectral distribution
because it was difficult to measure. Normally, I did not even measure actual irradiance. It was much more convenient to calculate "relative" solar radiation intensity at a given depth using crude measurements of optical transparency obtained with a secchi disc. I followed the practice of correlating biological measurements with such calculated values. Naturally, I assumed that a simple "animal" such as a coral could not detect or respond to subtle variations in the photic environment. After all, corals have no eyes, no complex nervous system and no complex endocrine system. How could they "see" such small changes in the photic environment? How could they possibly detect low level night irradiance coming from the moon? I embraced many other simplifying assumptions, especially those used in the calculation of gross primary production. I also accepted the assumption of many workers that solar ultraviolet radiation does not penetrate sea water in quantities sufficient to be biologically important on coral reefs. Gradually, I came to the conclusion that many of these assumptions are very weak. Certainly all were in need of testing. My first studies on the ultraviolet portion of the spectrum (Jokiel, 1980) further supported this suspicion. Therefore, I selected the area of coral reef photobiology as my primary research topic.
Boundaries were placed on the proposed work to develop a coherent approach to the problem. I chose to unify the work by focusing the study on the reef coral *Pocillopora damicornis*. This coral is abundant, easy to handle in experimental situations, readily produces larvae and has been the subject of extensive research throughout the Indo-Pacific for many years. In some cases other organisms were compared to this species in order to evaluate important similarities or differences in photobiological response.

Solar radiation has numerous important dimensions and one must clearly specify the technical features being evaluated by a particular measurement or experiment. I found it conceptually useful to clearly differentiate various aspects of *spectrum, intensity and modulation* (SIAM) as suggested by Thorington (1980). Other dimensions, such as polarization, direction and degree of scattering might be important, but were not dealt with in this dissertation. The acronym SIAM provided a very useful framework for this work.

The photic environment of a "typical" reef coral such as *Pocillopora damicornis* is constantly changing. Spectrum, intensity and modulation of solar input varies throughout the day and throughout the year. These parameters also vary with location. The daily rotation of
the earth on its axis and seasonal revolution around the sun are primary causes. Others are atmospheric conditions (e.g. amount of cloud cover), latitude, depth, optical transparency of the water, bottom topography and bottom composition. Measurement of solar radiation in the sea can be an expensive and technically difficult task to accomplish properly. Many biologists are poorly trained in optical oceanography and the physics of radiation. The problems of producing different artificial experimental regimes of intensity, spectral composition and periodicity can be immense. Much of the difficulty lies in the challenge of learning the basics of photobiology, discovering new areas that are open to investigation, and being able to devise experiments that test widely accepted assumptions. Aspects of this research can be carried out with relatively inexpensive materials as shown in the following chapters.

The major features of solar radiation (S1AM) that are dealt with in this dissertation are as follows:

**Spectrum**

Downward directed radiation from the sun mixes with scattered refracted and reflected sky radiation in varying proportions depending on the changing angle of the sun, cloud cover and atmospheric composition (e.g. dust, water
vapor, ozone). Differential reflection, refraction and absorption of energy at various wavelengths at the air-sea interface modify irradiance. Within the water column the absorption of photons by dissolved material, particulate suspended material and the water itself radically alter spectral composition along with decreasing the intensity with increasing depth. This pattern changes radically among different water types. Difficulty of measurement has led to the widespread practice of avoiding consideration of this very important dimension of the photic environment. Most studies measure total irradiance with depth and assume either that spectral distribution does not vary with depth or that spectral distribution is not an important factor. Such studies inherently assume that photons have the same photobiological effect on the organism regardless of wavelength.

Irradiance

Total irradiance, unlike spectral irradiance, is often measured in coral reef studies. Increased availability of instrumentation capable of radiometric measurement has enabled coral reef biologists to abandon photometric measurements (foot-candles, lux) which are of less value in studies of primary production (Tyler, 1971). Increasingly, the quantumeter (Jerlov and Nygård, 1969) is coming into use. Studies of the photosynthesis vs.
irradiance response are becoming fashionable and the study of photoadaptation in reef corals and other photosynthetic organisms is of increasing interest to many investigators.

Modulation

Alteration of the frequency and amplitude of solar radiation follows complex patterns in nature. Predictable changes accompany rotation of the earth and produce modulation with a 24 h period. Solar radiation reflected from the moon adds a circamonthly night irradiance component. Another predictable modulation is seasonal. This results from the earth's movement around the sun along with the inclination of the earth's axis of rotation. The incoming solar radiation is modulated unpredictably by random events such as the passing of clouds or storm systems. Such "noise" is difficult to predict or interpret. The surface of the sea is seldom flat and the refractive-reflective "lens" effects of ripples and waves cause high frequency modulation or "flashing" of solar radiation (Gerard, 1984).
Statement of Purpose

The scope of this dissertation was restricted to exemplary studies on the effects of spectrum, intensity and modulation (as described above) of the photic environment of the common reef coral *Pocillopora damicornis* and its symbiotic dinoflagellate alga. Various aspects of its biology were investigated. In some cases direct comparison was made with other species to emphasize similarities or differences. The central hypothesis of this dissertation can be stated as follows:

Subtle changes in the spectrum, intensity and modulation of the natural photic environment can produce a profound effect on growth, reproduction, primary production and general metabolism of the reef corals.
CHAPTER 1

INFLUENCE OF SPECTRUM AND INTENSITY WITHIN THE ULTRAVIOLET PORTION OF THE SOLAR SPECTRUM

1.1 INTRODUCTION

Solar ultraviolet (UV) radiation is detrimental to many forms of life (e.g. Giese, 1964; Urbach, 1969; Halldal and Tande, 1972; Nachtewey and Caldwell, 1975). High levels of UV radiation reach the surface of the earth in the tropics due to thinning of the earth's protective ozone layer near the equator (Bener, 1969; Schultz and Gröfe, 1969; Green et al., 1974; Baker et al., 1980). Clear ocean water, such as found over many coral reefs, is notably transparent to solar UV radiation (Jerlov, 1950; Smith and Baker, 1979) and affords relatively little protection to shallow water reef communities. UV radiation is an important factor in the biology of shallow water tropical epifauna (Jokiel, 1980) and among the reef corals (Siebeck, 1981).

Reef corals contain symbiotic dinoflagellate algae commonly known as zooxanthellae (Symbiodinium microadriaticum = Gymnodinium microadriaticum). Such corals will die if deprived of light for long periods of time (Yonge and Nicholls, 1931). On the other hand,

1Part of the work in this chapter has been published in Jokiel and York (1982, 1984) and Jokiel (in press).
High levels of solar radiation can be detrimental. Coral calcification can be inhibited by high solar irradiance (Barnes and Taylor, 1973). In Guam, corals held in continuous flow aquaria and exposed to full sunlight died while corals that were partially shaded showed no ill effects (Jones and Randall, 1976). High levels of solar radiation can increase coral mortality, inhibit skeletal growth, decrease carbon fixation and reduce photosynthetic pigment concentration (Coles and Jokiel, 1978). Corals suffer from prolonged exposure to intense artificial ultraviolet radiation (Catala, 1959). Coral growth is inhibited by UV radiation (Roth et al., 1982). Some corals contain materials that are transparent to visible light but absorb heavily in the UV with peak absorption near 320 nm (Shibata, 1969). These "S-320" substances might act as a filter to protect the coral and its zooxanthellae. Concentration of S-320 decreases in corals growing at greater depths (Maragos, 1972), possibly in response to attenuation of UV by seawater. Less of this protective pigment would be required at greater depths. Other coral pigments reflect UV or fluoresce the energy into the visible portion of the spectrum (Kawaguti, 1944; 1969; Catala, 1959; Shibata, 1969).

The UV absorbing, reflecting and fluorescing pigments persist in corals that have lost their symbiotic algae

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(Jokiel and Coles, 1974). These substances are concentrated in the coral ectoderm (Kawaguti, 1944; 1969) where they would be most effective in shielding the symbiosis from UV damage. Although the UV-protective substances apparently are located mainly in the animal fraction of the symbiosis, they may actually be produced by the plant fraction (Kawaguti, 1944). This appears to be the case in tropical sarcoglossan molluscs. Carbon fixed by chloroplasts is incorporated in a UV-absorbing compound that presumably shields the symbiosis (Ireland and Scheuer, 1979). Photosynthesis in unicellular algae is inhibited by UV (Bell and Merinova, 1961; Lorenzen, 1979; Smith and Baker, 1980; Smith et al., 1980). Similar effects have been observed in higher plants (Caldwell, 1971; 1977). UV photoinhibition apparently results partially from chloroplast damage (Bell and Merinova, 1961; Jones and Kok, 1966; Brandle et al., 1977). Severe photooxidation has been observed at UV wavelengths below 300 nm in the action spectrum of zooxanthellae isolated from coral host tissue (Halldal, 1963).

Thus UV could be a very important environmental factor for reef corals. The notable success of corals and other coelenterates containing symbiotic algae might be attributed to their ability to tolerate UV and derive a net benefit from solar radiation (Jokiel, 1980). Areas of
coral reef shallows exposed to full sunlight are notably devoid of other epifauna. Corals that flourish in shallow areas obviously tolerate extremely high levels of solar radiation. One cannot assume, however, that UV is innocuous to these creatures. Aquatic organisms generally must expend energy in order to cope with UV irradiation in the upper euphotic zone (Calkins and Thórdardóttir, 1980; Jokiel, 1980). The purpose of this investigation was to evaluate the possible impact of UV of reef corals and their symbiotic unicellular algae. The ubiquitous Indo-Pacific coral _Pocillopora damicornis_ is a good subject for such studies because it normally reaches maximum abundance in subtidal environments (e.g. Maragos, 1972; Neudecker, 1977) and prospers under the highest levels of UV radiation encountered in the ocean.

The term ultraviolet normally is used in reference to that portion of the electromagnetic spectrum between x-radiation and visible light. Separation of the electromagnetic radiation spectrum into various divisions is arbitrary and artificial. Consequently some ambiguities are encountered. The 400 nm boundary between UV and visible light is widely cited (e.g. Klein and Klein, 1970; Parrish et al., 1978). Some authorities, however, prefer to define a 380 nm or 390 nm boundary because the human eye is slightly sensitive to radiation
in the 380-400 nm region. It is advantageous to accept the 400 nm boundary in this study of photosynthetic organisms because 400 nm is widely recognized as the lower limit of photosynthetically active radiance (PAR). PAR generally is defined as quantum flux within the 400-700 nm range. Ultraviolet between 200 nm and 280 nm is often called far-ultraviolet or UV-C. UV radiation below 286 nm does not reach the surface of the earth (Benner, 1969; Schultz and Gröfe, 1969; Green et al., 1974; Baker et al., 1980) and is not of direct concern in this study. Radiation at wavelengths between 280 and 320 nm has severe biocidal properties. Energy at these wavelengths damages nucleic acids (Smith, 1969; Setlow, 1974) and inhibits chloroplast function (Jones and Kok, 1966). Atmospheric ozone is the principal absorber of energy in this region. Atmospheric ozone concentration (and therefore flux of 280-320 nm radiation reaching the earth) is controlled by a number of variable factors (Callis and Nealy, 1978; Callis, 1979). This study utilizes a frequently cited definition of UV-B as wavelengths between 280 and 320 nm. The 320 nm value coincides approximately with the cutoff point of polyester film that often is used as a UV-B filter. Others have defined upper boundaries for the UV-B ranging from 315 nm (Caldwell, 1977) to 340 nm (Smith and Baker, 1979). The remainder of the UV spectrum (wavelengths between 320 and 400 nm) will be termed UV-A.
Jokiel (1980) proposed that photosynthetic symbiotic coelenterates dominate the shallow tropical benthos because the animals can shield their contained algae from UV radiation damage. The symbiosis can thereby derive a net benefit from life in full sunlight. But are all microalgae UV sensitive? The immense literature concerning the effects of ultraviolet radiation on algal physiology and biochemistry was reviewed by Hallidal and Taube (1972). Unfortunately, the early studies were generally conducted with germicidal lamps that produce UV radiation at 254 nm. Wavelengths shorter than 280 nm do not penetrate the earth's atmosphere so the earlier studies involving UV-C radiation are of uncertain value in predicting the impact of natural solar ultraviolet radiation on algal populations (Nachtway, 1975).

Concern about the potential ecological importance of solar UV radiation on microalgae begins with the work of Jerlov (1950). He found that clear ocean water is very transparent to short wavelength solar radiation and concluded that solar UV radiation must be a significant biological factor in the upper photic zone of oceanic regions. Gessner and Diehl (1951) demonstrated that natural levels of solar UV radiation destroyed the chlorophyll of freshwater unicellular algae while natural visible light had no effect. Furthermore, they observed
that tolerance to UV radiation varied among different species; UV resistant species occurred at shallow depths, while sensitive species were found at greater depths. Subsequent studies (Steemann Nielsen, 1964; Jitts et al., 1976) showed that incident levels of UV radiation at the surface of the sea could severely inhibit photosynthesis. Approximately 50% of short-term photoinhibition observed at surface intensity can be attributed to wavelengths shorter than 390 nm (Smith et al., 1980). However, Smith and his co-workers strongly emphasized that short-term studies employing the static bottle technique might not accurately assess the long-term ecological consequences of UV radiation. Consequently, attention focused on other types of experiments that tested long-term ecological effects. One approach was to compare the lethal limit of organisms to the levels of UV flux found in their normal environments. Micro-algae and other aquatic organisms from different habitats in both temperate and high-latitude waters show little or no reserve capacity to withstand increased UV radiation dosage (Calkins and Thördardóttir, 1980). This implies the presence of genetic limits to UV photo-adaptation among various species. Long-term species interaction studies showed that increases in UV-B radiation dosage can alter species composition and standing crop of microalgae (Norrest et al., 1981a; 1981b).
Long-term monoculture experiments using natural sunlight and combinations of UV filters and neutral density filters could be useful in determining the relative importance of present day levels of solar UV radiation and PAR in causing microalgal growth inhibition. These types of experiments last for many algal generations and could demonstrate whether or not various species can eventually adapt to full spectrum sunlight. The purpose of the present investigation was to conduct such tests. These experiments, incidentally, are similar in design to the classic studies by Gessner and Diehl (1951) and Steemann Nielsen (1964). The present investigation simply expanded this design to include more treatments, much longer incubation times, and more sophisticated culture techniques. Hallidial and Taube (1972) pointed out that both of these studies were flawed because PAR was not equal in the different UV radiation treatments. The present investigation was designed to avoid this potential problem.

1.2 MATERIALS AND METHODS

1.2.1 Corals

1.2.1.1 Coral Culture Experiment 1

The influence of UV on coral growth was measured in a long fiberglass aquarium (180 cm long x 55 cm wide x 30 cm
high) located in full sunlight and supplied with seawater from Kaneohe Bay, Oahu at a rate of 20 l min\(^{-1}\). The aquarium was vigorously aerated at both ends to insure near saturation levels of dissolved oxygen, optimal water motion (Jokiel, 1978) and thorough mixing. During the experiment the temperature in the aquarium remained within the growth optimum of 27-28\(^\circ\)C for this species (Glynn and Stewart, 1973; Clausen and Koch, 1975; Jokiel and Coles, 1977). One end of the aquarium was shielded with acrylic plastic sheet. This material was opaque to the UV portion of the spectrum but transmitted approximately 90\% of the energy at longer wavelengths (Fig. 1). The other half of the aquarium was covered with neutral density black mesh screening that was adjusted to transmit the same levels of PAR as the acrylic filter.

PAR was measured as PPFD with a Li-Cor Inc. Model LI-185 quantameter with LI-185SB quantum sensor. Measurements were taken above the experimental aquarium and within each treatment at various times of day from sunrise to late afternoon under clear and cloudy conditions. Percent of ambient quantum flux reaching the corals increased in each treatment from sunrise to local apparent noon and subsequently decreased. At sunrise only about 45\% of surface quantum flux reached the corals. This increased to a maximum of 69\% near local
Figure 1: Transmission characteristics of the UV filters

Fluorohalocarbon filter material (dash-dot line), polyester filter material (dotted line) and polycarbonate filter material (solid line) were used in the experiments. Treatments under fluorohalocarbon filters were subjected to the full solar spectrum. Treatments under polyester lacked UV-B radiation while those under polycarbonate lacked UV-A and UV-B radiation. A typical relative solar spectral irradiance curve for the surface of the earth is included for reference as a dashed line (e.g. Gast, 1965; Baker et al., 1980).
apparent noon. Reflection, refraction and shading are influenced by solar angle and changed throughout the day as the position of the sun shifted. The readings at a given time were usually identical in both treatments, but differences of a few percent were observed at certain sun angles or under diffuse light (heavy cloud cover) as opposed to direct sunlight. The filter materials were originally matched with great care to insure equal PAR transmission. Uncontrolled differences between the two treatments probably were due to the slightly different optical characteristics of the two filters, the configuration of the aquarium and optical effects at the surface of the water. These differences were barely detectable and did not appear to consistently favor either treatment. Measurements were made on 25 occasions ranging from shortly after sunrise (33 μE m⁻² sec⁻¹) to local apparent noon on a clear day (2300 μE m⁻² sec⁻¹). Measurements were not clustered at a particular time of day and were taken on various days. The combined measurements for the treatment with UV (mean=56.1; S.D.=6.19; range=45-69) compare favorably with the combined measurements made in the treatment without UV (mean=56.7; S.D.=6.02; range= 47-69). The mean difference between paired simultaneous measurements is only 0.92% (S.D.=2.87). A two tailed paired sample t-test (Zar,
indicated that sample means for the two treatments were not significantly different (p > 0.50). PAR was, therefore similar in both treatments, but UV was lacking in one treatment. Total daily solar radiation above the experimental aquarium was monitored continuously using a standard Eppley pyroheliometer with integrator.

Twenty-two colonies of *Pocillopora damicornis* (10 cm diameter) were removed from the reef (depth 1 m) and carefully transferred into the experimental aquarium. Eleven colonies were randomly assigned to each of the two treatments and acclimated for two weeks. The coral skeletons were stained with Alizarin Red-S (Lamberts, 1974). The water flow was interrupted and sufficient dye added to bring the concentration of Alizarin to 20 ppm. Staining was allowed to continue for 8 h in sunlight before flow was resumed. New white carbonate material deposited on the branch tips during the following 40 d was clearly visible at the end of the experiment. Elongation from the stained portion to the branch tips was measured to the nearest 0.1 mm with vernier calipers. Twenty randomly selected branch tips were measured from each colony. The growth treatment of individual corals was not revealed to the person making the measurements to prevent unconscious biasing of the data. Differences between the two samples were tested using the non-parametric Mann-Whitney Test (Zar, 1974, p. 109).
Water extracts of coral pigments (Shibata, 1969) were also made at the conclusion of the experiment. A tip measuring 1 cm in length was cut from the top branch of each colony at 1400 h and placed in 5 ml of demineralized water. Extraction was allowed to continue for 16 h at 4°C. Samples were cleared of suspended material by centrifugation under refrigeration at 1700 rpm for 20 min. The extracts were analyzed with a GCA/McPherson Corp. Model EU701 Spectrophotometer using a 1 cm quartz cell.

The photosynthetic pigments were analyzed in a similar manner but using 100% acetone for extraction. Determinations were made according to procedures described in Strickland and Parsons (1972) and using the equations of Jeffrey and Humphrey (1975). Pigment concentration was normalized to surface area calculated geometrically from tip length and diameter.

The population of zooxanthellae was estimated for the corals in both treatments. Branch tips measuring 1 cm in length were removed from the colonies and placed in 5 ml of 20% acetic acid at 4°C for 24 h to decalcify the skeleton. The tissue was gently broken apart in a tissue grinder. Finally, ground glass was added to each vial. The vials were thoroughly agitated for 2 min on a vortex mixer to completely dissociate the cells. The cell walls of zooxanthellae are very tough and the cells withstood
such handling without damage. Replicate counts of plant cells in the resulting suspension were made using a hemacytometer. The mean cell count was normalized to branch surface area calculated from dimensions of the tip.

Relative rate of planula larva production was estimated near the period of maximum planula release in this species (Harrigan, 1972). Colonies previously held in each experimental treatment for three months were placed in large individual containers of seawater overnight. Planulae released in the containers were retrieved by filtering the water through a 0.1 mm mesh sieve and counted.

1.2.1.2 Coral Culture Experiment 2

In experiment 1 planula release was measured at only one point on the well-documented monthly reproductive cycle of pocillopora damicornis (Marshall and Stephenson, 1933; Atoda, 1947; Stimson, 1978; Richmond and Jokiel, 1984). Absence of UV radiation might alter the timing as well as the amplitude of planula release. Therefore, planulation over several cycles was measured on a continuous basis in the presence and absence of UV radiation.
1.2.2 **Microalgae**

Algal culture experiments also were conducted. Previous work (Jokiel, 1980) suggested that photosynthetic coelenterates shield symbiotic algae from UV radiation. This hypothesis was tested by culturing zooxanthellae and various unicellular algae in full spectrum natural solar radiation, in solar radiation lacking UV-B, and in solar radiation lacking both the UV-A and the UV-B bands.

All cultures were maintained in UV-transparent quartz test tubes (12.5 cm x 1.5 cm diam.). The culture tubes were washed with 4% Haemo-sol, rinsed with tap water, rinsed with 10% HCl, rinsed again with tap water, stoppered and autoclaved. Seawater was passed through a Nucleopore 0.2 micron filter, enriched to make "f" medium (Guillard and Ryther, 1962), and autoclaved in borosilicate flasks. The flasks were inoculated with algae from laboratory cultures. Initial cell counts were made on these cultures using a hemacytometer. Aliquants of 5 ml were placed in the sterile quartz tubes which were stoppered with sterile cotton. The cotton stoppers were capped with Parafilm before the tubes were placed in their respective incubation chambers.

Incubations were carried out in three continuous-flow water baths maintained outdoors at 24±1°C under full natural solar irradiance. Culture tube racks and water
baths were aligned on an east-west axis with the culture tubes sloping toward the south at 45°. This configuration allowed the cultures to receive unshaded radiation throughout the day. One tray was fitted with a UV-stabilized polycarbonate (Rohm and Haas Tuffak brand) cover. This material was opaque to UV radiation but highly transparent to longer wavelengths. Its spectral transmission curve is shown in Fig. 1. The second water bath was fitted with a cover of polyester film (DuPont Mylar brand) that blocked out wavelengths below 320 nm but transmitted approximately 90% of the energy at longer wavelengths (Fig. 1). Polyester film eventually is photochemically altered by UV radiation, necessitating replacement every few days. The remaining water bath was covered with the same neutral density filter used in the coral growth experiments. Thus each culture received approximately the same PAR.

1.2.2.1 Algae Culture Experiment 1

Five algae were tested in a preliminary experiment: zooxanthellae originally isolated from the scyphozoan Cassiopea sp. by D. A. Schoenberg and designated CS31b clone 4; zooxanthellae originally isolated from the anemone Aiptasia tagetes by C. Fisher in 1978 and designated clone CF1; the tropical flagellate Isochrysis
sp. from Tahiti; and the temperate marine diatoms *Phaeodactylum tricornutum* Bohlin, and *Thalassiosira pseudonana* (Hust.) Hasle and Heimdal (clone JH). The last two species are popular experimental laboratory organisms. They provide a useful comparison.

Zooxanthellae derived from *Pocillopora damicornis* could not be obtained at the time of these experiments. It is difficult to isolate and successfully culture such algae from corals. Fortunately, zooxanthellae can be isolated from a wide range of other coelenterates. These algae have been successfully maintained *in vitro* for long periods of time in the laboratory and are routinely utilized for experimental studies of zooxanthellae. The clones derived originally from the scyphozoan *Cassiopea* sp. and the anemone *Aiptasia tagetes* were selected for comparison because their hosts occupy different photic environments. *Aiptasia* is restricted to low light environments and could be described as "shade-loving". In contrast, *Cassiopea* is a jellyfish that inhabits shallow environments exposed to full sunlight and could be described as "sun-loving".

One culture of each of the five algae was placed in each treatment. Every few days the cultures were taken into the laboratory. The sterile cotton was replaced with a sterile cap and the culture shaken vigorously to
resuspend the cells. Small aliquants were removed with sterile capillary tubes for counting on a hemacytometer. The cultures were again stoppered with sterile cotton and Parafilm and returned to their respective treatments.

1.2.2.2 Algae Culture Experiment 2

The technique used in the preliminary experiment proved to be satisfactory. A second experiment was conducted using the same methods but with greater emphasis on replication of cultures and cell counts. Only the two clones of zooxanthellae were used. Four replicates of each clone were included in each of the three treatments. The cultures in the first experiment were moved from the laboratory directly into the sunlight. Results of the first test might have been influenced by the acclimation process. This complication was avoided in the second experiment by holding the algae used to inoculate the cultures for two weeks in full spectrum solar irradiance prior to the start of the experiment. Cells in each culture were counted at the beginning and at the end of the incubation period.

The cell count data were analyzed directly using a two-level, mixed model, nested analysis of variance (Sokal and Rolf, 1969, p.268) for the two sets of count data separately. This analysis requires an initial square root
transformation of cell count data to stabilize variance. Three sources of variation in the data were examined: (1) variation attributable to differences in UV radiation exposure; (2) variation resulting from unexplained differences between the cultures within a treatment, and (3) variation related to counting error in estimating the cell density within a particular culture. Cell growth rate (doublings per day) was calculated for each treatment in the second in vitro experiment so the two sets of data could be compared. The two way analysis of variance was performed on the resulting data matrix.

1.2.2.3 Algae Culture Experiment 3

Another series of experiments was designed to evaluate the relative importance of portions of the UV radiation spectrum versus the visible (PAR) spectrum in producing the observed growth photoinhibition. Comparisons were made between symbiotic zooxanthellae and other microalgae. The previous experimental design was expanded to include four relative intensity treatments within each of the three UV radiation treatments. Twelve incubation chambers (shallow black plastic trays) were installed on the Point Laboratory of HIMB. Each was flushed rapidly with recirculating fresh water (T = 24\(\pm\)1\(^\circ\)C) pumped to the roof from a thermally regulated reservoir. As in the first two
experiments, the culture tube racks were designed to allow incubations to be carried out with the lower three-fourths of the culture tubes immersed in the temperature-regulated recirculating freshwater. These temperature baths and culture tube racks were aligned on an east-west axis with culture tubes sloping toward the south at an angle of 45°. This configuration allowed the cultures to receive direct solar radiation throughout the day. Spectral intensity and distribution within each of the twelve incubation chambers was altered with covers made from various types of filter material.

Twelve solar radiation treatments were produced by superimposing three UV radiation treatments over four relative intensity (neutral density filter) treatments. Intensity was reduced with different grades of woven black polypropylene mesh. The treatments were not intended to simulate natural attenuation in the water column which changes the relative distribution of energy at different wavelengths. The purpose was to maintain the same spectral distribution within the four relative intensity treatments within each UV radiation treatment. The three different UV radiation treatments were created by underlying the mesh with sheets of either Allied Chemical Corp. Aclar brand fluorohalocarbon film (highly transparent to all UV and visible wavelengths), DuPont
Corp. Mylar brand polyester film (opaque to wavelengths below approximately 320 nm) or Rohm and Haas Tuffak brand UV-stabilized polycarbonate (opaque to wavelengths below approximately 400 nm). Spectral transmission curves for all of these materials are shown in Fig. 1. Spectral distribution was unaltered in the "control" fluororhalocarbon filter treatment. The polyester filter was identical to the control filter in the region of PAR and UV-A radiation, but blocked UV-B radiation. The polycarbonate filter had the same optical characteristics as polyester and fluororhalocarbon in the PAR region but blocked both UV-A and UV-B radiation. PAR within the 12 treatments was measured with a Li-Cor Inc. Model LI-195 quantameter with LI-192SB sensor. Levels of 92%, 38%, 20%, and 6% of surface PAR flux were measured as PPFD in the four relative intensity treatments. PPFD was essentially the same in the three UV radiation treatments at each of the four intensity levels because the optical characteristics of the three UV filters are similar between 400 nm and 700 nm (Fig. 1). Only a small amount of total solar energy lies in the UV portion of the spectrum (Fig. 1, dashed line). The UV filters used in these experiments differed basically in absorbance characteristics outside of the PAR range. PPFD was similar regardless of what UV filter was employed in a given treatment.
Replicate cultures and treatment positions were randomly assigned within the matrix. Algal cells are most sensitive to UV radiation while in the log growth phase (McLeod and McLachlan, 1959), so the study was restricted to cultures undergoing rapid growth.

Six representative types of algae were studied. The symbiotic dinoflagellate originally isolated from the anemone Aiptasia tagetes was chosen as the representative zooxanthella. The temperate estuarine naked diatom Phaeodactylum tricornutum Clone TFX-1 originally isolated by H. Stanley in 1972 at Woods Hole Oceanographic Institution was selected along with the tropical Pacific silicious diatom Chaetoceros gracilis. Tetraselmis sp. Clone Platy I = Platymonas sp. 1 (UTEX 818) is a green alga isolated by R. R. Guillard from Great Pond, Falmouth, Massachusetts in 1954. The tropical flagellateIsochrysis sp. Parke isolated by J. L. Martin in Tahiti was also tested. Oscillatoria lud (UTEX 1953) was included as a representative blue-green alga. This organism grows in short filaments. Cell doubling rate was calculated for this alga from total length of filaments per ml rather than number of cells to facilitate counting. Average cell size did not vary, so doubling rate of length is numerically equal to doubling rate.
The second algae experiment allowed a rigorous statistical analysis of within-treatment and between-treatment variation for a larger number of replicate cultures grown under the three UV treatments at 92% of surface intensity. The expansion of the earlier experimental design to include four relative intensity treatments within each UV radiation treatment necessitated use of only two replicates per treatment. Estimation of cell counts for the large number of resulting cultures is time consuming and only 24 of the expensive quartz culture tubes were available. The smaller number of replicate cultures enabled gross characterization of the growth response among the 12 treatments, but precluded use of rigorous statistical tests for detecting subtle differences in growth between treatments. This was not a serious drawback because UV radiation resistance or sensitivity was quite dramatic among the various species. Acute relative response was readily and convincingly discernable. Cells that settled or aggregated during the incubation became evenly distributed in the medium after shaking the culture tubes. Therefore it was not necessary to rotate, shake, or otherwise disturb the culture tubes during the incubations.
1.3 RESULTS

1.3.1 Coral

1.3.1.1 Coral Experiment 1

The observed difference in coral growth (Table 1) is significant (p<0.01) by the Mann-Whitney nonparametric test. This is regarded as the most appropriate statistical treatment of branch elongation growth data on Pocillopora damicornis (Glynn, 1977; Jokiel, 1978). Various investigators have reported growth rates of 1.6 to 3.3 mm month⁻¹ for this species (summarized in Buddemeier and Kinzie, 1976). Slightly higher growth rates ranging from 4.5 mm month⁻¹ were subsequently reported by Glynn (1977). The median colony growth for the treatment with solar UV radiation was 5.7 mm for a 40 d growth period (4.3 mm month⁻¹). This value agrees with the most rapid growth measured for this species by Glynn (1977). Growth of colonies maintained without UV radiation was 3.3 mm (or 6.2 mm month⁻¹), a value that greatly exceeds the previously reported maximum of approximately 4.5 mm month⁻¹. Total solar radiation at the experimental site during the 40 d was 17,933 cal cm⁻² (daily mean = 448, S.D. = 87, range = 310-578).

The water extract absorption spectra measured in this experiment were very similar to those of the S-320 UV-absorbing substances described by Shibata (1969) for
**Pocillopora.** Absorption occurred between 280 nm and 380 nm with a peak near 320 nm. The extract was transparent at wavelengths between 400 nm and 800 nm (Fig. 2). The violet reflecting substances and green fluorescent substances described by Kawaguti (1944) and Shibata (1969) were not visible on the coral tips nor were they observed in the extract. The green fluorescent material was, however, observed in polyps located near the base of some of the colonies. The extinction of the 11 samples from the treatment with UV radiation (Table 1) was significantly higher \((p < 0.001\) by the two sample t-test) than the extinction of the 11 samples from the treatment without UV radiation. In contrast, the symbiont population and chlorophyll concentration were not significantly different (Table 1). Severe photoinhibition normally is followed by chlorophyll breakdown (Bell and Merinova, 1961; Jones and Kok, 1966; Coles and Jokiel, 1978). Apparently the S-320 blocked UV irradiance and protected the algae from damage. Phaeopigments were not detectable. Chlorophyll-\(b\) was absent also, indicating lack of contamination by epiphytes and absence of endolithic algae.

These results support Shibata's (1969) observation that S-320 is an extremely effective UV shield. For example, Fig. 2 compares the typical absorbance spectrum of an S-320 extract (1 cm branch tip in 5 ml demineralized...
Figure 2: UV radiation, action spectra and absorbance spectra.

Comparison between surface incident UV radiation, coral pigment absorbance, photosynthetic action spectrum of zooxanthellae and photosynthetic action spectrum.

A. Typical spectral distribution of solar irradiance at the surface of the earth (e.g. Green et al., 1974; Baker et al., 1980).

B. Coral pigment extraction comparison: UV-blocking pigment "S-320" (1 cm branch tip extracted in 5 ml of deionized water) versus photosynthetic pigment extraction (1 cm branch tip extracted in 5 ml acetone).

C. Action spectrum of zooxanthellae isolated from the coral Pavia (Halldal 1968).

D. Effectiveness of various wavelengths at producing photosynthetic inhibition in the green unicellular alga Chlorella (Bell and Merinova, 1961).
TABLE 1. Pigment concentration, symbiont cell population, growth rate and planula release measurements for *Pocillopora damicornis* in the two treatments.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Median</th>
<th>Mean±S.D.</th>
<th>Range</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-absorbing Pigment S-320 (absorption at 320 nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV Present</td>
<td>11</td>
<td>0.26</td>
<td>0.26±0.06</td>
<td>0.19-0.35</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>UV Absent</td>
<td>11</td>
<td>0.12</td>
<td>0.11±0.04</td>
<td>0.07-0.17</td>
<td></td>
</tr>
<tr>
<td>Photosynthetic Pigments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chl a (ug cm⁻²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV Present</td>
<td>10</td>
<td>6.0</td>
<td>6.0±0.94</td>
<td>4.5-7.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>UV Absent</td>
<td>10</td>
<td>4.7</td>
<td>5.2±1.62</td>
<td>3.0-9.0</td>
<td></td>
</tr>
<tr>
<td>chl c (ug cm⁻²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV Present</td>
<td>10</td>
<td>2.1</td>
<td>2.2±0.65</td>
<td>1.2-3.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>UV Absent</td>
<td>10</td>
<td>3.3</td>
<td>3.6±1.82</td>
<td>1.6-7.0</td>
<td></td>
</tr>
<tr>
<td><em>Symbiodinium microadriaticum</em> population (10³ cells cm⁻²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV Present</td>
<td>10</td>
<td>377</td>
<td>383±83</td>
<td>267-522</td>
<td>n.s.</td>
</tr>
<tr>
<td>UV Absent</td>
<td>10</td>
<td>386</td>
<td>396±66</td>
<td>311-505</td>
<td></td>
</tr>
<tr>
<td>Coral Growth (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(during 40 day experiment)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV Present</td>
<td>11</td>
<td>5.7</td>
<td>6.2±1.2</td>
<td>5.0-8.5</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>UV Absent</td>
<td>11</td>
<td>8.3</td>
<td>8.1±1.2</td>
<td>5.3-9.5</td>
<td></td>
</tr>
<tr>
<td>Planulae Release Rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(per colony per night)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV Present</td>
<td>10</td>
<td>26.5</td>
<td>55.3±75.7</td>
<td>9-251</td>
<td></td>
</tr>
<tr>
<td>UV Absent</td>
<td>10</td>
<td>4.5</td>
<td>12.9±23.0</td>
<td>2-77</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>
water) to a typical photosynthetic pigment extraction (1 cm tip in 5 ml of 100% acetone). One might not expect to see such high solubility of an organic compound such as S-320 in water, but nevertheless the amount extracted was extremely effective at blocking UV-B radiation. It is noteworthy that S-320 absorbs the detrimental UV radiation without attenuating beneficial PAR (Fig. 2).

The number of planulae released was significantly greater (p<0.01 by the Mann-Whitney Test) for the corals grown in full spectrum solar radiation (Table 1) after 3 months of treatment. All planulae released in the treatments appeared to be mature and fully pigmented.

1.3.1.2 Coral Experiment 2

Results of the long term planulation experiment in the presence and absence of UV radiation are presented in Fig. 3. The data are in good agreement with results of Exp. 1 (Table 1).

1.3.2 Microalgae

1.3.2.1 Algae Culture Experiment 1

Overcast conditions occurred throughout the preliminary in vitro algae culture experiment. Average daily solar radiation during the 10 d experiment was 347 cal cm⁻² d⁻¹ (S.D. = 123, range = 177-550). This was less than the 449
Figure 3: Production of coral larvae in the presence and absence of UV
cal cm⁻² d⁻¹ recorded in the previous coral culture experiment. Even so, growth was clearly suppressed by UV radiation (Table 2). The same pattern is apparent in all five algae. The zooxanthellae showed a lower growth rate than the other species. The effect of UV radiation on the three nonsymbiotic species is most noticeable on days 3 and 7. By the 10th day these species reached high cell densities and may have been approaching senescence.

During the first algal culture experiment it became apparent that zooxanthellae from Aiptasia were far more sensitive to UV than the zooxanthellae isolated from Cassiopea. Both clones grew at nearly the same rate when solar UV radiation was blocked, but cultures of the Aiptasia clone did poorly in the treatments with UV radiation (Table 2). The second in vitro culture experiment was designed to confirm this observation. Extensive replication of the cultures and cell counts allowed a statistical analysis of several possible sources of variation.

Results of the two level nested ANOVA on the cell count data are presented in Table 4 for both clones. The effect of UV treatment was highly significant (p<0.001) and the photoinhibitory effect is attributable to UV-A as well as UV-B radiation. Wide variations in cell count estimates occurred within some cultures (Table 3). This was not significant for the clone isolated from Cassiopea.
TABLE 2. Growth of algae in the first UV culturing experiment. Final counts of *Symbiodinium microadriaticum* ± S.D. based on four aliquants of each culture.

<table>
<thead>
<tr>
<th>Species and Treatment</th>
<th>Cell Count (10^3 cells ml⁻¹)</th>
<th>Cell Count (10^3 cells ml⁻¹)</th>
<th>Cell Count (10^3 cells ml⁻¹)</th>
<th>Cell Count (10^3 cells ml⁻¹)</th>
<th>doublings day⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symbiodinium microadriaticum</strong> (Clone from Aiptasia)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>full solar spectrum</td>
<td>130</td>
<td>143</td>
<td>147</td>
<td>109±8</td>
<td>0.000</td>
</tr>
<tr>
<td>wavelengths&gt;320 nm</td>
<td>130</td>
<td>197</td>
<td>93</td>
<td>173±21</td>
<td>0.041</td>
</tr>
<tr>
<td>wavelengths&gt;400 nm</td>
<td>130</td>
<td>107</td>
<td>209</td>
<td>439±56</td>
<td>0.175</td>
</tr>
<tr>
<td><strong>Symbiodinium microadriaticum</strong> (clone from Cassiopea)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>full solar spectrum</td>
<td>120</td>
<td>117</td>
<td>127</td>
<td>126±15</td>
<td>0.007</td>
</tr>
<tr>
<td>wavelengths&gt;320 nm</td>
<td>120</td>
<td>237</td>
<td>262</td>
<td>342±46</td>
<td>0.151</td>
</tr>
<tr>
<td>wavelengths&gt;400 nm</td>
<td>120</td>
<td>118</td>
<td>225</td>
<td>406±99</td>
<td>0.175</td>
</tr>
<tr>
<td><strong>Isochrysis sp.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>full solar spectrum</td>
<td>280</td>
<td>1470</td>
<td>1910</td>
<td>2890</td>
<td>0.337</td>
</tr>
<tr>
<td>wavelengths&gt;320 nm</td>
<td>280</td>
<td>2850</td>
<td>2320</td>
<td>3620</td>
<td>0.369</td>
</tr>
<tr>
<td>wavelengths&gt;400 nm</td>
<td>280</td>
<td>2840</td>
<td>3190</td>
<td>3000</td>
<td>0.342</td>
</tr>
<tr>
<td><strong>Phaeodactylum tricornutum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>full solar spectrum</td>
<td>170</td>
<td>790</td>
<td>4670</td>
<td>5590</td>
<td>0.504</td>
</tr>
<tr>
<td>wavelengths&gt;320 nm</td>
<td>170</td>
<td>2480</td>
<td>5500</td>
<td>8190</td>
<td>0.559</td>
</tr>
<tr>
<td>wavelengths&gt;400 nm</td>
<td>170</td>
<td>2510</td>
<td>4710</td>
<td>8390</td>
<td>0.562</td>
</tr>
<tr>
<td><strong>Thalassiosira pseudonana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>full solar spectrum</td>
<td>120</td>
<td>420</td>
<td>1860</td>
<td>3860</td>
<td>0.501</td>
</tr>
<tr>
<td>wavelengths&gt;320 nm</td>
<td>120</td>
<td>590</td>
<td>2690</td>
<td>4680</td>
<td>0.528</td>
</tr>
<tr>
<td>wavelengths&gt;400 nm</td>
<td>120</td>
<td>980</td>
<td>3270</td>
<td>5590</td>
<td>0.544</td>
</tr>
</tbody>
</table>
TABLE 3. Growth of the two clones of zooxanthellae during the second experiment.

Counts show ± S.D. based on ten aliquants of each culture.

<table>
<thead>
<tr>
<th>Cell Count (thousands of cells ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>A. Clone from Aiptasia</td>
</tr>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Full solar spectrum</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Wavelengths</td>
</tr>
<tr>
<td>&gt;320 nm (no UV-B)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>&gt;400 nm (no UV-A or UV-B)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>B. Clone from Cassiopea</td>
</tr>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Full solar spectrum</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Wavelengths</td>
</tr>
<tr>
<td>&gt;320 nm (no UV-B)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>&gt;400 nm (no UV-A or UV-B)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mean</td>
</tr>
</tbody>
</table>
A significant difference in culture replication was observed for the clone isolated from *Aiptasia*. This can be attributed partially to the lower cell densities encountered in the UV-inhibited cultures. Low cell density leads to a much larger counting error (Guillard, 1973). Also, the UV irradiated cultures were extremely stressed; replication is difficult under such conditions. In any event, this error is small compared to the extreme variation attributable to UV treatment.

1.3.2.2 Algae Culture Experiment 2

The overcast conditions of the preliminary algal culture experiment were not encountered during the second test. Incident surface radiation during the second experiment was (459 cal cm\(^{-2}\) d\(^{-1}\), S.D. = 60, range = 362-521). Also, fully acclimated algae were used as inoculum. The results, however, are quite similar to those of the preliminary experiment (Table 3).

As in the first *in vitro* culture experiment, the shade-loving clone of zoanthella originally isolated from *Aiptasia* did not grow well in full sunlight. After 13 d of growth the sun-loving clone isolated from *Cassiopea* had reached high cell density in all treatments. Counting of cells of the clone derived from *Aiptasia* was postponed for four days. This was necessary because cell densities in
the UV-irradiated cultures were extremely low. Growth rates (no. of doublings d\(^{-1}\)) was not appreciably different for the two clones grown in the absence of UV (Tables 2 and 3). Differences of several-fold, however, are evident in the cultures grown in the presence of UV radiation. Cells of the clone isolated from Aiptasia were visibly altered by the presence of full solar UV radiation. The damage was similar to that reported for UV-irradiated cells of the green alga Chlorella (Bell and Merinova, 1961). Cell contents were shrunken and the chloroplasts were discolored. In contrast, cells of the "sun-loving" clone appeared to be undamaged by this treatment. The two-way ANOVA on doubling time data (Table 4) yielded a highly significant interaction effect between clone type and UV treatment (p<<0.001, F = 12.8372, DF1 = 2, DF2 = 8). The analysis supports the subjective observation that response of the clone derived from Aiptasia was different from the response of the clone originally derived from Cassiopea. During the second experiment the cultures were observed for 30 days. The clone derived from Aiptasia remained at extremely low cell density in the treatment with full UV radiation. In contrast, the cultures of both clones grew to high cell density in the treatment lacking UV radiation and eventually appeared to reach senescence.
TABLE 4. Results of two level mixed model nested ANOVA applied to final cell counts of zoxanthellae in second culturing experiment.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Clone from <em>Aiptasia</em>)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV Treatment</td>
<td>1638</td>
<td>2</td>
<td>818.9</td>
<td>350.6</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Replicate Cultures</td>
<td>21</td>
<td>9</td>
<td>2.3</td>
<td>2.6</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Cell Count Error</td>
<td>96</td>
<td>108</td>
<td>0.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Within Culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1755</td>
<td>119</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Clone from <em>Cassiopea</em>)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV Treatment</td>
<td>1570</td>
<td>2</td>
<td>784.7</td>
<td>122.7</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Replicate Cultures</td>
<td>57</td>
<td>9</td>
<td>6.4</td>
<td>1.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cell Count Error</td>
<td>436</td>
<td>108</td>
<td>4.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Within Culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2063</td>
<td>119</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
TABLE 5. Chronology, surface radiation, incubation duration and initial cell density for the third culturing experiment.

<table>
<thead>
<tr>
<th>Species</th>
<th>Experimental culture period (1981)</th>
<th>Days</th>
<th>Surface Irradiance (cal cm$^{-2}$ d$^{-1}$)</th>
<th>Initial cell count ($10^3$ cells ml$^{-1}$)</th>
<th>Initial cell volume per unit medium volume (ml cells l$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symbiodinium microadriaticum</td>
<td>6-20 Aug</td>
<td>14</td>
<td>416 ± 86</td>
<td>9.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Chaetoceros gracilis</td>
<td>2-8 Sept</td>
<td>6</td>
<td>448 ± 38</td>
<td>6.9</td>
<td>58.1</td>
</tr>
<tr>
<td>Phaeodactyllum tricornutum</td>
<td>10-16 Sep</td>
<td>6</td>
<td>472 ± 39</td>
<td>4.8</td>
<td>49.0</td>
</tr>
<tr>
<td>Tetraselmis sp.</td>
<td>17-22 Sept</td>
<td>5</td>
<td>416 ± 70</td>
<td>1.1</td>
<td>25.6</td>
</tr>
<tr>
<td>Oscillatoria lub</td>
<td>23-29 Oct</td>
<td>6</td>
<td>305 ± 39</td>
<td>2.3</td>
<td>4.6</td>
</tr>
<tr>
<td>Isochrysis sp.</td>
<td>16-23 Nov</td>
<td>7</td>
<td>298 ± 46</td>
<td>10.4</td>
<td>67.4</td>
</tr>
</tbody>
</table>
1.3.2.3 Algae Culture Experiment 3

Pertinent data describing the chronology, duration, initial cell counts and surface incident solar irradiance during each culturing period within the third alga culturing experiment are presented in Table 5.

Calculated cell division rate (no. of doublings d⁻¹) is plotted against relative intensity in Fig. 4. Growth rates of replicate cultures were reasonably close. Replication was not good among cultures of sensitive algae damaged by UV radiation. *Symbiodinium microadriaticum* and *Oscillatoria lud* grew at a slower rate (0.4 doublings d⁻¹ maximum) than the four other species (1.0 to 1.3 doublings d⁻¹ maximum) under the described culture conditions.

1.3.2.4 Algae Experiments - General Observations

During the second experiment the cultures were moved directly from laboratory lighting (40 watt standard fluorescent lamps) into the respective solar treatments. Even at high levels of PAR (92% of surface PPFD), little or no photoinhibition of growth rate was observed in the absence of solar UV radiation. A question remains, however, as to whether or not the algae that were photoinhibited by UV radiation could eventually adapt to the new regime of spectral irradiance. The resistance of *Symbiodinium microadriaticum* to solar UV radiation was not
Figure 4: Algal growth under various regimes of solar irradiance.

Growth rate (doublings d⁻¹) for various species of algae cultured under the experimental regimes of solar irradiance. Squares joined by solid lines represent treatments lacking UV-A and UV-B radiation (polycarbonate filter), triangles and dashed lines represent treatments without UV-B radiation (polyester filter), while circles and dashed lines are for treatments with full spectrum irradiance (fluorocarbon filter).
enhanced by holding the cultures in full intensity sunlight (quartz culture tubes) for two weeks prior to testing growth rate during the second culture experiment. Also, the three UV radiation resistant species (Chaetoceros gracilis, Tetracelmis sp. and Isochrysis sp.) showed no evidence of division delay in any of the full intensity UV radiation treatments. These data show that rapid adaptation to extremely high levels of solar UV radiation and PAR is possible in many species. It is, however, also possible that the UV radiation sensitive forms might eventually recover and resume normal growth rate in full solar UV radiation.

The symbiotic dinoflagellate Symbiodinium microadriaticum proved to be extremely sensitive to near-surface levels of UV-A and UV-B radiation as demonstrated in the first two experiments. The third experiment showed that sensitivity did not carry into treatments below 20% of surface irradiance. At 92% of surface intensity Phaeodactylum tricornutum was sensitive to UV-B radiation but not UV-A radiation. High UV tolerance was observed in the silicious diatom Chaetocerus gracilis, the green alga Tetracelmis sp. and in the flagellate Isochrysis sp. The blue-green alga Oscillatoria lud was sensitive to both UV-A and UV-B radiation. This species was cultured in the experimental
series when total solar irradiance was diminished (Table 1), but strong UV growth photoinhibition was still noted. This species also seemed to show some inhibition due to PAR at the 92% level, but this was slight compared to that caused by UV-A and UV-B radiation. The polycarbonate filter used in these experiments blocked UV, but also cut off a small amount of the shortest wavelength PAR (see Fig. 1). Perhaps this explains why cultures grown under the conditions of light limitation (6% intensity treatments of *Symbiodinium microadriaticum*, *Phaeodactylum tricornutum*, and *Tetraselmis* sp.) seemed to grow slightly faster in the treatments with UV radiation. Some species of algae can utilize UV radiation photosynthetically under light-limited growth conditions. McLeod and Kanwisher (1962) tested two species of unicellular algae (*Phaeodactylum tricornutum* and *Dunaliella tertecolata*) and found them to be capable of utilizing UV in photosynthesis. The action spectrum for photosynthesis in *Symbiodinium microadriaticum* shows oxygen production in the UV-A range (Halldal, 1968). In the experimental treatments lacking UV radiation, maximum growth rate was reached between 20% and 40% of surface intensity and generally did not diminish at higher levels (Fig. 4). Growth photoinhibition, where observed, always could be attributed largely to UV radiation.
1.4 DISCUSSION

The reef coral *Pocillopora damicornis* thrives under incident doses of UV radiation that would kill or severely damage many forms of life (e.g. Hunter et al., 1979; Calkins and Thördardóttir, 1980; Jokiel, 1980). The UV-blocking pigment S-320 (Shibata, 1969) appears to be a primary UV-defense mechanism of this species that shields the symbiosis from the biocidal effects of UV radiation. Symbiotic dinoflagellate algae are known to be UV sensitive as are most unicellular algae (e.g. Bell and Merinova 1961; Lorenzen, 1979; Smith and Baker, 1980; Calkins and Thördardóttir, 1980). The host appears to block the harmful portion of the spectrum without attenuating potentially beneficial PAR wavelengths (Fig. 2) and thereby produces an ideal photic environment for the culture of its zooxanthellae. This adds one more dimension to our knowledge of benefits derived by the plants in these symbioses (most recently reviewed by Trench, 1979). The symbiotic unit as a whole can derive a net benefit from life in full solar irradiance. The plant and animal fractions might not be capable of independently offsetting the high metabolic cost of UV radiation encountered on shallow reefs, but can do so as a symbiotic unit. Therefore, corals and other photosynthetic symbioses can exploit areas that are forsaken by UV-sensitive epibionts (Jokiel, 1980).
The \textit{in vitro} algal culture experiments demonstrated that populations of zooxanthellae can be severely damaged by long-term exposure to UV-A radiation as well as by UV-B radiation. Research on the biological effects of UV has, unfortunately, often been confined to the highly biocidal UV-B and UV-C regions. Interest in the effects of longwave UV-A radiation on humans has been increasing among medical researchers (reviewed by Parrish et al., 1978), but relatively little is known about its ecological importance. Until recently there was little evidence to suggest that UV-A is a significant ecological factor among the microalgae. For example, the short-term incubation action spectrum for zooxanthellae from the coral \textit{Favia} shows no detrimental effect in the UV-A (Halldal 1968). A classic study describing ultraviolet dose and wavelength effects on the green alga \textit{Chlorella} also showed no effects in this region (Bell and Merinova, 1961). In both studies, however, the investigators recognized that UV-A could be an important agent in long-term photoinhibition because chlorophyll absorbs radiation in this region. Subsequently it was shown that UV-A does, in fact, cause chloroplast inhibition (Jones and Kok, 1980). More recent studies involving phytoplankton (Smith et al., 1980; Smith and Baker, 1980) suggested that 25\% of the photoinhibition observed at surface irradiance is caused by wavelengths
below 390 nm. The recent review of the UV-A literature by Parrish et al. (1978) clearly points to a need for more photobiological research on the subject, especially in the area of ecological effects.

Short-term incubation tests of less than one generation time might not adequately assess the ecological importance of UV radiation. Many effects might become evident only when cells attempt repair or reproduction (Smith et al., 1980; Smith and Baker, 1980). The incubations conducted in the present study extended over many algal generations and the results agree in some respects to those obtained by Smith and his co-workers. Areas of non-agreement will be dealt with later in this discussion.

Karakashian and Siegel (1965) argued that endocellular symbionts can be viewed as part of the genetic apparatus of the symbiotic unit. New phenotypes are acquired by infection with new genetic partners and the symbiotic complex acquires novel physiological capabilities and ecological potentialities. For example, _Aiptasia_ that lack zooxanthellae can be reinfected with zooxanthellae from _Cassiopea_ (Kinzie and Chee, 1979). In theory, this new combination would have higher UV tolerance than other anemones containing zooxanthellae of the "shade-loving" type. The new combination may or may not be a successful competitor in nature, but certainly it would possess new
"ecological possibilities" as predicted by Karakashian and Siegel (1965).

The probability of high genetic diversity among these symbionts was emphasized by McLaughlan and Zahl (1969). They pointed out that symbionts in surface-dwelling invertebrates are probably physiologically different than symbionts from intermediate or deep zones. Recently these concepts have become popular among researchers. For example, the possibility that genetic differences in algal symbionts can influence thermal tolerance among reef coral was raised by Jokiel and Coles (1977). Likewise, the existence of "sun" and "shade" zooxanthellae was proposed to account for the inability of different ecotypes of the same coral species to photoadapt to new environments (Dustan, 1979). Several genetic strains of zooxanthellae can be recognized on the basis of host specificity, morphology, and isoenzyme patterns (Kinzie and Chee, 1979; Schoenberg and Trench, 1980a, 1980b, 1980c; Chang et al., 1983).

The striking difference observed in the response of the two clones of zooxanthellae is not without precedence. Species of other plants contain races or strains that are adapted to specific habitats (e.g. Mooney and Billings, 1961; Milner et al., 1962). Ecotypes of higher plants from exposed and shaded areas show differences in
photosynthetic capacity that are interpreted as genetic adaptations to original habitat. These differences persist in plants that have been cultured under laboratory conditions for years (Björkman and Holmgren, 1963). Numerous examples of physiological differences among clones of the same species are known (e.g. Carpenter and Guillard, 1971; Murphy and Guillard, 1976). Natural selection might operate rapidly on the algal fraction of a symbiosis. The generation time of such unicellular algae is measured in h while the coral animal has a generation time that is measured in years. Also, the number of zooxanthellae is many orders of magnitude greater than number of hosts. Therefore, the plants should be able to produce new genetic combinations more rapidly and presumably are capable of more rapid genetic adaptation through natural selection.

This study demonstrated that Shibata's (1969) S-320 substances are produced in direct response to UV radiation. The chemical structure of these water-soluble substances has recently been determined (Dunlap and Chalker, in preparation). These workers extracted the material in tetrahydrofuran-methanol and used high-performance liquid chromatography to resolve the compounds. The "S-320" substances can be resolved into a series of mycosporine-like amino acids including
mycosporine-gly (λ max=310 nm), palythine (λ max=320 nm) and palythinol (λ max=322 nm). Mycosporine-gly and palythine are, by far, the most abundant of these compounds. Their observations document a bathymetric decrease in the materials from depths of 1 m to 20 m. If these compounds function as UV-protective mechanisms, then reef-building corals must be able to detect and biochemically respond to the presence of UV radiation at depths up to 20 m. Jerlov (1950) had previously suggested that UV must be biologically significant to depths of 20 m in the clearest ocean waters. Their UV-absorbing properties immediately suggested an aromatic structure. The inclusion of aromatic secondary metabolites such as quinones, phenols, melanins, and melanoids in the biochemistry of aquatic and terrestrial organisms has long been attributed to their value as UV-blocking agents (Jackson, 1965).

The UV-blocking substances are largely transparent to UV-A radiation. Wavelengths in the UV-A portion of the spectrum are most effective at stimulating fluorescence in the green animal pigments and presumably are reflected by the violet animal pigments (Kawaguti, 1944; Shibata, 1969). The fluorescent pigments of the animal appear to channel UV radiation into photosynthetic pathways be reemitting the energy at longer wavelengths where it can
be absorbed by the photosynthetic pigments of the algae (Kawaguti 1969). This property is potentially of great value to coelenterates living under conditions of light limitation. Also, the UV-A is that portion of the spectrum showing maximum penetration in clear oceanic seawater (Smith and Baker, 1979). The majority of coral species contain at least some individuals with the fluorescent green or violet color phase. Apparently a high metabolic cost is associated with the production of such materials (Kawaguti, 1944). The photoinhibition resulting from UV-A radiation might be tolerable in some situations when weighed against the cost of producing the protective fluorescent material. Another possible adaptive pattern is substitution of UV-tolerant symbiotic algae for the more sensitive strains. The wide range of potential pigment and symbiont clone combinations might afford a great deal of photobiological plasticity to these symbioses. A species could thereby extend its range into many photic environments. The fluorescent and reflective pigments might be optional, but it appears that the S-320 pigments probably are necessary for life in shallow water. These pigments absorb the biocidal UV-B radiation that is extremely damaging to nucleic acids. It is doubtful that corals could maintain genetic integrity in the face of such extreme exposure to damaging radiation without and effective shield such as S-320.
UV radiation appears to reduce growth while increasing reproduction in the coral *Pocillopora damicornis*. Perhaps this serves to increase reproduction at the expense of skeletal growth in corals growing in very shallow water, where there is no selective advantage to rapid vertical growth. Increased skeletal growth is advantageous in deeper water where the corals run less risk of mortality, but must compete for space.

There is wide variation in the ability of various corals as well as zooxanthellae to adapt to high solar irradiance (Siebeck, 1981), and this could be partially due to the type of zooxanthellae that they possess. *Pocillopora damicornis* is a representative of what has been termed a "sun-loving" species. In contrast, the coral *Montipora verrucosa* is intolerant of high light intensity (Coles and Jokiel, 1978) and could be described as a "shade-loving" species. Under identical conditions these two species show dissimilar responses to solar radiation (Houck et al., 1977; Houck, 1978). For example, growth of *P. damicornis* correlates directly with light intensity over the range of 100 to 500 cal cm⁻² d⁻¹ while *M. verrucosa* growth shows an inverse correlation.

This investigation was conservative in design because a sun-loving species of coral was employed. The UV-induced effects noted in *P. damicornis* should be widely
applicable. Obviously UV radiation should be even more damaging to species known to be highly photosensitive. The experiments described in this chapter were carried out with all other important physical factors (temperature, salinity, dissolved oxygen concentration, water motion, etc.), within the optimal range for P. damicornis. The detrimental effect of high solar radiation is far more severe when other environmental conditions are suboptimal (Coles and Jokiel, 1978). Also, these experiments were conducted near the northermost latitudinal extent of coral reefs. The observed effects should be far more pronounced near the equator where total annual UV dosage is much greater. Therefore, it is reasonable to conclude that such effects should be observed among other coral species and at other geographic locations.

Results of the algae culture experiments suggest the presence of genetic limits to UV photoadaptive capacity to natural sunlight conditions among some species of microalgae. Other species showed a remarkably high tolerance of solar UV radiation. The wide range of growth response patterns can be related to photic conditions encountered by each species in its normal environment. The symbiotic dinoflagellate Symbiodinium microadriaticum lives within the tissues of animal hosts that can produce effective UV-blocking ectodermal pigments. Even so, this
alga consists of sun-loving and shade-loving genetic variants isolated respectively from sun-loving and shade-loving hosts. Niche specialization is quite advanced in this respect. This alga might be at an extreme competitive disadvantage during its free-living stage in high-UV environments. In contrast, the silicious diatom Chaetoceros gracilis is very tolerant of both UV-A and UV-B radiation and is well suited for life in shallow tropical seas as a non-symbiotic alga. The naked diatom Phaeodactylum tricornutum appeared to be very sensitive to UV-B radiation, but can withstand UV-A radiation. It seems that P. tricornutum can prosper under full solar UV-A and UV-B in some situations (P. A. Laws, personal communication). This species has been cultured in outdoor ponds under unobstructed solar radiation. In the first culture experiment involving this species (Table 2) it appeared that growth of P. tricornutum was inhibited by full sunlight during the first few days, but the alga seems to have eventually adapted somewhat to full UV radiation. During this first experiment P. tricornutum experienced irradiance of only 347 cal cm\(^{-2}\) d\(^{-1}\). Irradiance was much higher during the second experiment involving this species (472 cal cm\(^{-2}\) d\(^{-1}\)). Consequently, growth photoinhibition in the full UV treatment (Fig. 4) was far more severe, probably because of higher UV
irradiance. Obviously, more information is needed on possible UV tolerance differences between different clones of this species and possible long-term photoadaptive processes.

The most surprising result in the alga experiments was that long-term growth photoinhibition (where observed) appeared to be due almost entirely to UV radiation. Extremely high levels of natural PAR (92% of surface flux) did not severely inhibit growth. Previous short-term incubations had demonstrated that about half of the $^{14}C$ fixation photoinhibition at surface irradiance could be attributed to wavelengths in the PAR portion of the spectrum. In the present study all six species of algae readily photoadapted to intense PAR at little or no metabolic expense. Three of the species also adapted readily to near-surface levels of UV radiation.

Attempts have been made to produce a generalized UV action spectrum for plants. This seemed to be appropriate because modes of action for various short-term UV photoinhibitory processes have similar spectral shapes (Caldwell, 1971). The weight of existing evidence (e.g. Gessner and Diehl, 1951; Smith et al., 1980) suggested that UV radiation damage to chl or chloroplasts is the major inhibitory effect. On the other hand, Mcleod (1953, p.243) found that different algae show differences in the
UV portion of their "delayed action light spectra" that could not be related to chlorophyll action spectra and concluded that "pigments other than chlorophyll must be sensitizing or shielding the algae in the ultraviolet region". Smith and his co-workers (Smith et al., 1980) suspected that time dependent processes (such as production of screening pigments) could modify the observed short-term response. Apparently, their warnings were justified.

Photoinhibition by radiation in the PAR portion of the spectrum can be demonstrated during short incubations. Based on the results of this study, however, it seems likely that energy in the PAR portion of the spectrum is not important in growth photoinhibition. Algae can rapidly photoadapt to high PAR flux. The time dependence of these adaptive processes were not established, but in general it seems to have occurred rapidly. This might not be true in the UV portion of the spectrum. As mentioned previously, adaptation to full UV radiation in Phaeodactylum tricornutum might take several days. Some of the microalgae must have adapted quite rapidly to full surface PPFD. For example, the three UV resistant species showed the same overall growth rate at 92% of surface PPFD as in the 40% treatment. These observations are consistent with the general notion that photoadaptation in
some microalgae can be accomplished in much less than 24 h (e.g. Steemann Nielsen, 1962; Steemann Nielsen et al., 1962; Prézelin and Matlick, 1980). Wherever UV growth photoinhibition did occur, it was very severe and apparently persisted throughout the incubation period. This was true in the full spectrum, high intensity treatments of Symbiodinium microadriaticum, Phaeodactylum tricornutum and Oscillatoria lud. Three of the six test species readily adapted to extremely high levels of UV radiation. This was totally unexpected. Two of these were isolated from shallow tropical oceanic regions, where free-living algae must be UV tolerant in order to compete. Some communities seem to contain few, if any, UV-tolerant species. For example, UV photoinhibition seems to account for the extreme paucity of algae found in the shallow waters of stratified high-altitude "blue water" lakes (Rodhe et al., 1966).

Results of these investigations support the contention of many workers that UV is an important ecological factor in aquatic and marine ecosystems (e.g. Gessner and Diehl, 1951; Calkins and Thórdardóttir, 1980; Worrest et al., 1981a, 1981b). Furthermore, UV-A as well as UV-B is deleterious to some species of microalgae (i.e. Symbiodinium microadriaticum and Oscillatoria lud). This was not previously demonstrated. The diversity of
response among various species is consistent with McLeod's (1958) observation that different algae contain substances other than chlorophyll which protect or sensitize them within the UV region of the solar spectrum. The importance of PAR in long-term natural photoinhibitory processes probably has been generally overestimated, although some exceptions (i.e. Volvox) are known (Gessner and Diehl, 1951). Ecologically significant long-term photoinhibitory effects probably are caused almost entirely by UV radiation. In any event, oceanic water is known to be highly transparent to UV radiation (Jerlov, 1950; Smith and Baker, 1979) and its role as a niche dimension should not be underestimated.

It is important to recognize that UV radiation is not clearly a "harmful" ecological factor on coral reefs. UV radiation might be utilized photosynthetically to a net advantage among some coelenterates living under conditions of light limitation (Kawaguti, 1969). UV tolerance affords a competitive advantage to many coral reef organisms that occur only in shallow water (Jokiel, 1980). Therefore, we can only say that UV radiation is an important environmental factor that influences the basic structure and function of these ecosystems. The pervasive influence of short-wavelength radiation on coral reef should not be surprising. Such radiation has a dramatic
impact on many biochemical compounds and processes. UV radiation has been present in significant amounts throughout the entire evolutionary history of these ecosystems. The end result apparently has been a wide range of UV mediated biological processes that we are now just beginning to recognize on coral reefs.
CHAPTER 2

INFLUENCE OF SPECTRUM AND INTENSITY WITHIN THE PAR REGION

2.1 INTRODUCTION

Several recent studies comparing corals from shallow versus deep environments have demonstrated "sun" and "shade" adaptation (Wethey and Porter, 1976; Falkowski and Dubinsky, 1981; Duschan, 1979, 1982). The observed physiological differences were assumed to result from differences in solar radiation intensity. This might be an oversimplification. Spectral differences can be important. For example, Chapter 1 of this dissertation deals with the effects of UV radiation. Obviously, shallow environments receive relatively high doses of solar UV radiation while deep environments receive little or none. Hence the ratio of UV to PAR can be an important dimension of the photic environment. Attenuation of PAR with depth is also accompanied by marked alteration in spectral distribution within the PAR region. Furthermore, the relative contribution of different parts of the spectrum to total PAR at any given site is controlled

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Part of the work in this chapter has been published in Kinzie, Jokiel and York (1984).
by the amount and type of particulate and dissolved material in the water (Jerlov, 1968). Blue wavelengths at approximately 480 nm generally have the greatest penetration in the clear oceanic water types characteristic of many reef areas. Solar radiation at shorter or longer wavelengths penetrates to a lesser extent. Green penetrates to a relatively greater extent than blue in waters of high phytoplankton standing crop.

Algal response to spectral quality has been demonstrated using filtered solar radiation of altered spectral quality or artificial sources of different spectral quality. Responses studied include changes in pigment composition (Hess and Tolbert, 1967; Brody and Emerson, 1959; Jones and Meyers, 1965; Faust et al., 1982), metabolism (Bird et al., 1981; Hess and Tolbert, 1967), modified chloroplast ultrastructure (Lichtenthaler et al., 1980) and changes in regulatory processes (Dunbier and Dring, 1975; Forward, 1970; Dring, 1971). The classic concept of "complementary chromatic adaptation" is based on the hypothesis that different plant pigments represent adaptations to different spectral regimes occurring at different depths. This hypothesis was intended to explain the observed depth zonation of the major groups of seaweeds in the ocean (see Rabinowitch, 1945 for a discussion). The actual situation is not as simple as was
originally postulated as shown by Dring (1981), but it is clear that the spectral changes associated with depth do, in fact, influence these plants (Ramus et al., 1976a, 1976b).

Absorption spectra and action spectra have been determined for zooxanthellae from several host species (Halldal, 1968; Scott and Jitits, 1977; Dustan, 1982), but the effect of long term depth related changes in spectral quality has not been investigated for intact symbiotic corals. As mentioned previously, experimental studies comparing response of corals from different depths have generally been interpreted only in terms of differences in intensity. Spectral quality, while likely to be a confounding influence, often has not been considered in previous experimental designs.

Thus we are led to the following question: Does spectral distribution within the PAR wavelength band influence the physiology of reef corals and their contained zooxanthellae? Long term growth experiments under carefully defined spectral regimes of equal PAR were designed to deal with this question. The basic experimental approach was to grow corals and algal cultures under "red", "green", "blue" and "white" (neutral density) filters in outdoor aquaria.
Justification for this experiment was provided by colleagues who initially offered expert opinions on the probable outcome of such experiments. Some felt that published action and absorbance spectra (Halldal, 1968) predicted that "green" PAR would not be very effective at promoting growth while "blue" and "red" PAR should be photosynthetically useful to the symbiosis. Others suggested that corals would grow well in any spectral regime within the PAR because all wavelengths are utilized with equal effectiveness under the higher intensities that would be encountered in such experiments. This argument was based on the findings of Scott and Jitts (1977). Also, it was postulated that accessory pigments would change in concentration and transfer energy into the cnid system. Some concluded that red PAR would not be effectively absorbed. Dr. John Coll (personal communication) has shown that live coral appears bright red when photographed using aerial infra-red film. Hence corals reflect radiation in the near red region. Others raised the possibility that the red treatment would prove to be the most productive because a great deal of additional energy is potentially available at wavelengths longer than the 700 nm cutoff of a standard quantumeter. Filters available for this type of work transmit these longer wavelengths, but they are not measured on a
quantameter. Therefore more photons are potentially available outside of the PAR region being measured by the instrument. In any case, it was clear that experimental data were needed.

2.2 MATERIALS AND METHODS

Two coral incubation experiments and one in vitro zooxanthella growth experiment were carried out at HIMB. The approach was to grow organisms under different "color" and neutral density filters in treatments having equal PAR of different spectral distribution.

2.2.1 Culturing Techniques

2.2.1.1 Corals (Exps. 1 and 2)

The corals used in these experiments were obtained from the Coconut Island reef adjacent to HIMB. Small heads (approx. 9 cm diam.) of Pocillopora damicornis were carefully detached from the reef and transported in seawater filled pails to continuous-flow holding aquaria located in full unobstructed solar radiation. A single large head of the branching form of Montipora verrucosa was similarly collected and brought to the laboratory where it was broken into fragments of roughly equal size yielding numerous clone-mates for the experiments. Pocillopora damicornis is restricted to shallow reef flats
in Hawaii and was selected as a representative "sun-loving" coral. *M. verrucosa* reaches maximum abundance in deeper water and might be termed a "shade-loving" species. Controlled experiments on the two species support this interpretation (Houck et al., 1977). The aquaria were covered with black polypropylene mesh (shade cloth) which reduced the solar radiation to about 25% of incident intensity. The *Montipora* fragments and the *Pocillopora* heads were allowed to acclimate in the running seawater for about two weeks. At the end of the acclimation period, one branch tip was collected from each experimental coral for pigment analysis and algal cell counts as described below. Plastic identification tags were attached to the corals, dead branches removed, and the coral skeletons were stained in Alizarin Red S at 20 ppm for 24 h (Lamberts, 1974). After the staining was completed, the corals were placed in the experimental treatments on plastic-coated wire racks. Positions within the treatment series were randomly determined as were the allocations of the spectral treatments to the aquaria. Each experimental aquaria had an identical configuration of corals, that is, position was controlled throughout the series. *Pocillopora* heads or clone mates of *Montipora verrucosa* occupied the same position within each treatment.
Figure 5: Transmission spectra for the colored filters used in this study.

"Blue-green" refers to the filter used in the preliminary coral growth experiment (Exp. 1). "Green" refers to the filter used in the second coral growth experiment (Exp. 2) and in the algal growth experiment (Exp. 3). The "red" and "blue" filters were the same in all experiments. The neutral density or "white" treatment employed an unpigmented acrylic filter that passed all wavelengths in the PAR portion of the spectrum (400-700 nm), but was opaque to UV radiation as were the other filters.
The experimental culturing aquaria (117 cm square by 46 cm deep) were located in unobstructed solar radiation and supplied with seawater from Kaneohe Bay at a rate of 20 l min⁻¹. The aquaria were continuously aerated with a uniform and vigorous supply of filtered air to insure near saturation levels of oxygen as well as optimal water motion (Jokiel, 1978). The sides and bottom of the aquaria were opaque. Spectral irradiance within the aquaria was controlled by fitting clear or colored acrylic plastic covers of Rohm and Haas "Plexiglas brand" acrylic sheet. These had quite different transmission characteristics (Fig. 5). Each cover was carefully adjusted to the same PPFD (Shibles, 1976) by lightly spraying flat black paint on the underside of the covers. This treatment is essentially a neutral density attenuation of total PPFD. Measurement of PPFD was made with a Li-Cor Inc. Model LI-185 quantum meter with a LI-185SB submersible quantum sensor. The covers were fitted with black foam rubber gaskets. The junction between the cover and the aquaria walls was also sealed with a skirt of black plastic sheeting to further guard against leakage of external radiation into the treatments. PPFD was carefully measured within each treatment at the beginning and at the end of each experiment to insure that no alteration of the optical properties had occurred over
the experimental time period. No change was detected. 
Exp. 1 was a preliminary experiment that ran from 7 
September to 4 January (3.97 mo). Average solar radiation 
at the experimental site was 283±127 cal cm\(^{-2}\) d\(^{-1}\). 
Treatments consisted of "blue-green", "blue", "red" and 
"white" (neutral density) adjusted to equal PAR. 
Transmission characteristics of the filters are shown in 
Fig. 5. The preliminary experiment demonstrated the value 
and feasibility of this approach. A second experiment was 
then designed to include more extensive pigment analysis. 
The "blue-green" filter was unsatisfactory because it 
overlapped considerably with the "blue" treatment. The 
experiment was repeated as Exp. 2 when a more suitable 
"green" filter became available (Fig. 5). Exp. 2 was run 
from 13 August to 25 October (2.1 months). Solar 
radiation at this location was higher during the second 
experiment (419±97 cal cm\(^{-2}\) d\(^{-1}\)). 

2.2.1.2 Zooxanthella isolation and in vitro culturing 
techniques.

The symbiotic dinoflagellate (Symbiodinium 
microadriaticum) Freudenthal (= Gymnodinium 
microadriaticum) was isolated from Montipora verrucosa 
host tissue in the following manner: A 1 cm long branch 
tip was cut from the coral and placed in a solution of 3 
ppm sodium hypochlorite in filtered sterile (autoclaved)
seawater for 2 h. Tissue was then removed from the skeleton with a flamed scalpel. Areas of tissue with large concentrations of algae were excised and transferred to sterile seawater, shaken vigorously and diluted to a concentration of several plant cells ml⁻¹. A sterile Pasteur pipette, drawn to an inside diameter of approximately 20 μm, was used to remove single cells. These were placed in separate 125 ml cotton-stoppered Erlenmeyer flasks containing 40 ml of 'f/2' medium (Guillard and Ryther, 1962). The cells were allowed to grow out at under irradiance emitted by 40 W "Vitalite" brand fluorescent tubes. PPFD was 40 μE m⁻² sec⁻¹ operated on a daily cycle (16 h on - 8 h off). One of the resulting algal clones was selected for use in the experimental incubations.

Experimental cultures were grown in standard laboratory test tubes (15 cm long x 1.8 cm diam.). Before use the culture tubes were washed with 4% Haemo-sol, rinsed with tap water, rinsed with 10% HCl, water, rinsed again with tap water, stoppered with cotton and autoclaved. Growth medium consisted of Nucleopore brand 0.1 μm filtered seawater enriched to make 'f' medium and autoclaved in a teflon flask. The flask was innoculated with the clone of Symbiodinium microadriaticum isolated from Montipora verrucosa. After hemacytometer determinations of the
initial algal concentrations were made, aliquants of 10 ml were withdrawn from the flask and added to the sterile test tubes. Sterile cotton stoppers were inserted and the tubes were capped with Parafilm before they were placed in the incubation chambers.

Twelve incubation chambers (shallow black plastic trays) were installed on the roof of the Point Laboratory at HIMD. Each tray contained recirculating freshwater that was pumped to the chambers from a temperature regulated (T=24±1°C) reservoir. Culture tube racks were positioned to allow incubations to be carried out with the lower three fourths of the test tubes submerged in the recirculating water. The temperature baths and the culture tube racks were aligned along an east-west axis with the tubes slanted to the south at an angle of 45°. This configuration allowed the cultures to receive direct solar radiation throughout most of the day.

Twelve different treatments were produced by superimposing the four different "color" filters over 3 neutral density filter treatments. The three intensity treatments were created using two grades of neutral density shade cloth over the "color" filters which already had been adjusted to equal PPFD transmission. The end result was to produce three similar PPFD levels within each of the 4 spectral distribution (i.e. color)
treatments. PAP within each of the 12 treatments was measured as PPFD at the beginning and end of the experiment as in Exp. 1 and Exp. 2. Levels of 12%, 10% and 6% of ambient solar PAP were obtained. Culture tubes were randomly assigned to positions within the 3 x 4 matrix. Four replicate tubes were assigned randomly to each of the 12 treatments.

2.2.2 Collection of Experimental Data

2.2.2.1 Corals and in vivo zooxanthella (Exps. 1 and 2)

At the termination of the experiment, live branch tips were removed from the corals, rinsed in distilled water to remove seawater salts, and placed in 5 ml of 100% acetone and stored in a freezer at (-16°C). This operation was carried out in dim light to avoid photodestruction of the pigments. After a 24 h extraction period, chl a, chl c and 'carotinoids' were determined for each of the samples. Absorption was measured using a Beckman Model 3600 spectrophotometer. The equations of Jeffery and Humphrey (1975) were used to convert absorption to chl concentrations. Total carotinoids were determined using the equation in Strickland and Parsons (1972). Recent work on peridinin, the major carotinoid pigment of dinoflagellates (Prézelin and Haxo, 1976; Haxo et al., 1976), has produced a quantitative extraction method for
this pigment. The simpler technique used in this study is suitable where one is concerned mainly with relative changes. In this chapter, the term 'carotenoids' will refer to the value obtained by the simple extraction technique as measured spectrophotometrically.

Surface area was estimated by measuring the length and the top and bottom diameters of each coral tip and using the formula for the surface area of a truncated cone.

Subsequent to tissue sampling, the corals were removed from the aquaria and sprayed with a strong jet of fresh water from a garden hose to remove all tissue. This exposed the skeleton and revealed the presence of the original stain band. Growth of ten tips from each coral head was measured as the length of the new white skeletal material deposited distally from the red alizarin band. The tips to be measured were selected haphazardly, five across one diameter of the head and five across another diameter normal to the first row. This insured that tips from the sides as well as the tops of the colonies were measured. Such coral growth data shows skewness introduced by tips showing little or no growth. In general such growth data are not normally distributed. Therefore, it is appropriate to use median branch tip elongation as the growth estimate (Sokal and Rolf, 1969).
2.2.2.2  *In vitro* zooxanthella culture (Exp. 3)

The incubation of the algae cultures was terminated after 15 days. Several drops of formalin were added to each tube to preserve the cells. The fixed cells tended to adhere to the test tube walls in some cases. To alleviate this problem, crushed glass was added to each tube before they were shaken vigorously in a vortex mixer. This process resuspended the cells evenly in the medium without breaking cell walls. The samples were shaken again before removing a subsample with a capillary tube for counting on a hemacytometer. This process was repeated 10 times for each of the 48 cultures. The values reported are means of these counts.

2.3  RESULTS

2.3.1  Coral Growth

Table 6 shows the average median growth of the corals in mm per month. Three statements can be made about these data. First, the "shade-loving" species *Montipora verrucosa* exhibited higher growth at the low PAR intensities of the series than does the "sun-loving" reef flat species *Pocillopora damicornis*. This occurred in both experiments and in all color treatments. Second, the red treatment resulted in inferior growth for both
TABLE 6. Growth rate of corals held under PAR of the same intensity but with different spectral composition. Growth rate in mm mo⁻¹ (±1 S.D.). Values are means (n=10 corals) of the median length of 10 branches on each coral.

<table>
<thead>
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<th>Spectral Treatment</th>
<th>White</th>
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<th>Green</th>
<th>Red</th>
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<td><strong>Experiment 1</strong></td>
<td></td>
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<tr>
<td><em>Pocillopora damicornis</em></td>
<td>0.47±0.14</td>
<td>0.30±0.17</td>
<td>0.48±0.21</td>
<td>0.00±0.00</td>
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<td><em>Montipora verrucosa</em></td>
<td>2.53±1.27</td>
<td>2.06±1.42</td>
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<td>0.87±0.44</td>
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<td><strong>Experiment 2</strong></td>
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<td></td>
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<tr>
<td><em>Pocillopora damicornis</em></td>
<td>0.97±0.78</td>
<td>1.30±0.70</td>
<td>0.24±1.02</td>
<td>0.09±0.17</td>
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<tr>
<td><em>Montipora verrucosa</em></td>
<td>3.51±2.32</td>
<td>3.55±1.71</td>
<td>2.54±1.62</td>
<td>1.52±1.11</td>
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TABLE 7. Ranking of coral growth rates in Exps. 1 and 2. (Rank 1 is fastest and Rank 4 is slowest growth). Color treatments connected by lines were not significantly different from each other (p=0.05) as determined by the STP (simultaneous test procedure) *a posteriori* method (Sokal and Rohlf, 1969).

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<td><em>P. damicornis</em></td>
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<td><em>M. verrucosa</em></td>
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<td>blue-green</td>
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<tr>
<td><em>P. damicornis</em></td>
<td>blue</td>
<td>white</td>
<td>green</td>
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<tr>
<td><em>M. verrucosa</em></td>
<td>blue</td>
<td>white</td>
<td>green</td>
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¹ Significance of effects determined using Friedman's method of randomized blocks (Sokal and Rohlf, 1969).
TABLE 8. Effect of spectral quality on plant pigments in Exp. 2. Pigment area$^{-1} =$ pigment concentration (ug 10$^{-6}$) per cm$^2$ unit area; cells area$^{-1} =$ cell density x 10$^6$ per cm$^2$ unit area; pigment cell$^{-1} =$ pigment concentration (ug 10$^{-6}$) per algal cell. Sample size 10 except for Pocillopora damicornis (Pd) red and Montipora verrucosa (Mv) white where sample size = 9. Values are means ± S.D.

<table>
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<td>Pd</td>
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<td>Mv</td>
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<tr>
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<tr>
<td><strong>Pd</strong></td>
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<td>19.92±8.25</td>
<td>18.23±5.96</td>
<td>14.63±2.69</td>
<td>13.21±5.96</td>
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<tr>
<td><strong>Mv</strong></td>
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<td>7.33±3.17</td>
<td>7.39±2.30</td>
<td>6.86±1.65</td>
<td>7.87±3.46</td>
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<tr>
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<td>4.88±0.99</td>
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<tr>
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<td>4.07±1.31</td>
<td>5.23±1.83</td>
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<td>22.94±4.16</td>
<td>22.82±8.03</td>
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<tr>
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<td>2.98±0.24</td>
<td>3.03±0.26</td>
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<td>2.13±0.46</td>
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<td>1.76±0.33</td>
<td>1.55±0.34</td>
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<td>**chl_a:**carotenoids</td>
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<tr>
<td><strong>Pd</strong></td>
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<td><strong>Mv</strong></td>
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<td>0.53±0.38</td>
<td>0.53±0.06</td>
<td>0.48±0.04</td>
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1Significance levels as determined from one-way ANOVA: ***(p<0.001), **(p<0.01), *(p<0.05), -=(0.01<p<0.05). Values connected by lines were not significantly different (p=0.05) by Scheffé's multiple range test.
TABLE 9

Growth of in vitro zooxanthella cultures during Exp. 3.

Values are means of final counts (± S. E.) on each of the culture tubes in each treatment. Colors sharing the same underlining were not significantly different from each other (P=0.05) over all relative PAR intensities as determined by Scheffé test. ANOVA was run on square-root transformed data. DF = degrees of freedom; MS = mean square.

<table>
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<th>% Transmission</th>
<th>Blue</th>
<th>White</th>
<th>Green</th>
<th>Red</th>
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<tr>
<td>10</td>
<td>52.60</td>
<td>40.68</td>
<td>19.35</td>
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<tr>
<td>±3.68</td>
<td>±2.33</td>
<td>±1.55</td>
<td>±1.01</td>
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<tr>
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<td>40.55</td>
<td>44.75</td>
<td>11.55</td>
<td>5.65</td>
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<tr>
<td>±2.94</td>
<td>±2.35</td>
<td>±1.51</td>
<td>±0.57</td>
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</table>

<table>
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<td>intensity</td>
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<td>interaction</td>
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<td>error</td>
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<td>total</td>
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</tr>
</tbody>
</table>
species. The blue-green treatment led to inconclusive results in Exp. 1, but use of proper filters in Exp. 2 leads us to the third conclusion: the blue treatment produced greater growth than the white or green treatment. This is supported by the statistical results shown in Table 7. Significance among color treatments for both species is shown for both experiments, and both species showed identical ranking of the order of color effects on growth rate.

2.3.2 *In Vivo Algal Response*

Table 8 gives the results obtained from the analysis of the zooxanthellae taken from the corals at the end of the second experiment. Note that while the initial values are listed for each parameter, "before and after" comparisons are not strictly valid. This is partially because the preadaptation intensity was 25% of full ambient solar PAR while the experimental level was 11%. The neutral density filters used to preadapt the corals transmitted the UV radiation portion of the spectrum. In addition, average daily solar radiance decreased seasonally during the course of the experiment. It is better to view the "white" treatment as the true experimental control.
2.3.3 **In Vitro Algal Growth**

Table 9 presents the mean final hemacytometer counts of algal cells after the 15 d incubation under the four color and three intensity treatments as well as cell doubling rate. Two-way analysis of variance on square root transformed count data showed significant intensity as well as color effects. As expected, the final cell counts are directly proportional to PAR intensity because growth is limited by available PAR in this range. The effect of spectral treatment (color), when considered independently of intensity, is also significant (Table 9). A Schiffl's *a posteriori* test indicates that the growth rates in the blue or white treatments are significantly greater than the growth rate in the green treatment. This, in turn, is greater than growth in the red treatment. There is a significant interaction between the "color" and "intensity" effects. This interaction probably is due to the increase in absolute amount of PAR transmitted through the "tails" of the transmission spectra (Fig. 5). This, of course, would increase the absolute number of quanta available in parts of the spectrum away from the modal transmission of the filter, thus effectively broadening the spectral transmission characteristics of each of the filters. This is generally a problem with filters that do not have sharp cut-off characteristics. One-way ANOVA's
at each PAR intensity on final cell count showed significant color treatment effects with blue and white >
green > red at all PAR intensities ($p < 0.001$).

2.3.3.1 Pigment per Unit Area

If chl a is expressed as a function of the surface area of the coral, we find a significant color effect for both species. This effect appears to be most pronounced in *M. verrucosa*. Again, the decreasing ranking of blue and white > green > red is observed as in the case for coral growth. While comparison with the initial pigment concentration is not strictly valid for the reasons given above, it is noteworthy that the final areal concentration of chl a under the blue and the white treatments is higher than the starting levels, while the opposite is true for the green and red treatments. This trend is stronger for *P. damicornis* than *M. verrucosa*.

The areal concentration of chl c for *P. damicornis* shows no differences between spectral treatments, but there is an apparent decrease from the initial pigment concentration. The algae from *M. verrucosa* show significance among group differences in chl c. As with *P. damicornis*, there is a general decline in chl c concentration in all colors relative to the starting level.
The changes in "carotenoids" show trends similar to those seen in chl a. The concentration of these accessory pigments seems to have increased in the blue and white treatments relative to the initial concentrations, while decreasing in the green and red treatments. As with chl g, carotenoids of P. damicornis show only a moderate response to spectral treatment while the changes in carotenoid concentrations in M. verrucosa are highly significant.

Thus it appears that in both species there was a greater increase in the concentration of chl a under the blue and white spectral treatments than was observed under the green or red spectral treatments. M. verrucosa seems to be capable of a greater response in terms of alteration of accessory pigments than does P. damicornis. The trends under the different spectral treatments are the same as that of chl a for both species.

The observed changes in chl a concentration could be produced in three ways: either an increase in the amount of chl a per cell, or an increase in the numbers of zooxanthellae per unit area, or some combination of the two.
2.3.3.2 Zooxanthella cells per unit area of coral surface

Pocillopora damicornis showed no differences in the number of algal cells per unit coral surface area among the four color treatments while there were strong differences in the number of zooxanthellae per unit area in Montipora verrucosa. In the latter species, colonies grown in the blue treatment had higher algal cell densities than those grown under the white or green treatments. Colonies grown in the red treatment had the lowest density of symbionts. The algal densities in P. damicornis did not appear to have changed appreciably from the initial concentration per unit coral surface area, but the algal cells in M. verrucosa grown in the blue treatment might have increased.

2.3.3.3 Concentrations of plant pigments per algal cell

The alternate means of changing areal density of plant pigments would be to change the cellular concentration of pigments within the cells of the algal symbionts. The increase in chl a per unit area in P. damicornis apparently was achieved in this manner. The among-treatment differences are only marginally significant (p = 0.066), but the significant differences in chl a seem to have resulted from this change. Again the ranking of blue > white > green > red is apparent.
There was no significant difference among the color treatments in the chl a content of zooxanthellae from _M. verrucosa_, so changes observed in areal concentration of pigment must have been achieved through alteration of algal cell numbers.

Note that the accessory pigment chl c showed significant differences among treatments in _M. verrucosa_, but not in _P. damicornis_. This was true in terms of both chl c per area and chl c per algal cell. Both species exhibited highly significant differences among treatments in chl a to chl c ratios. Neither species showed a significant treatment effect on the concentration of 'carotenoids' per algal cell, yet again the chl a to carotenoid ratio showed significant effects due to color treatment.

The use of pigment ratios in assessment of chromatic adaptation has a long history in plant research, but the statistical behavior of ratios and their biological interpretation is fraught with difficulty (see Atchley 1978 for a discussion of these problems). Results of the present investigation show a marked reduction in the variance about the means of the original pigment density measurements. The most likely statistical explanation is that there is a high correlation between accessory pigments and chl a in each sample. The assumption
implicit in most discussions of chromatic adaptation is that the concentrations of the various pigments are independent, yet it is known that fairly rigid stochiometric, spatial and physical constraints govern the composition of the photosynthetic unit (PSU). It appears that the correlations between chl a and the accessory pigments are dictated by their molecular associations as reflected in the low variability in their ratios. This in turn produces spuriously inflated values among treatment F

values.

Results of this section can be summarized as follows: the concentration per unit coral surface area of dominant photosynthetic pigment, chl a, responds to differences in spectral quality of PAR as well as to differences in intensity, but this response is achieved through different mechanisms in algae associated with different species of coral. In the perforate species, Montipora verrucosa this adaptive process appears to be brought about primarily (but not entirely) by changes in the number of algal cells per unit coral surface area. In the imperforate coral Pocillopora damicornis, the ability to radically alter symbiont number may be limited by available tissue volume. In this species, photoadaptation appears to be achieved largely by changes in pigment per cell of the contained algal population.
2.4 DISCUSSION

The ability to adapt to PAR of different intensities is important to all photosynthetic organisms. "Sun" vs. "shade" adaptations have been studied both in terrestrial and aquatic plants. A generalized response to decreased PAR is an increase in plant photosynthetic pigment content as well as modifications in chloroplast structure and photosynthetic response (Falkowski and Owens, 1980). The increase in photosynthetic plant pigments by unicellular algae such as zooxanthellae increases the photon harvesting ability, either through an increase in the number or the size of the PSU (Falkowski and Owens, 1980, Prözelin 1981). In symbiotic invertebrates such as corals, there are other possible levels of homeostatic adjustment available. Alteration of number and/or spatial arrangement of the symbiotic algae within the animal can be accomplished. Chapter 3 deals with canopy-understory effects provides yet other examples. Sun-shade adaptation has been studied in situ and under laboratory conditions. The response of in vivo zooxanthellae to low levels of PAR is the same as seen in free living algae. Cellular pigment content is increased (Falkowski and Dubinski, 1981; Dustan, 1979, 1982; Chang et al., 1983), and alterations in the photosynthetic capacity of the adapted coral/algal association are observed (Wethey and Porter, 1976; Chang et al., 1983).
The earlier studies assumed that observed physiological differences in the algae result only from difference in PAR intensity. It is known that algae show adaptations to PAR of different spectral composition (Kowalik, 1970; Wallen and Geen, 1971 a, b, c; Jones and Galloway, 1979; Hess and Tolbert, 1967; Bird et al., 1981; Faust et al., 1982) as well as intensity. Also, it is well established that different wavelengths have different extinction coefficients. Therefore, relative spectral distribution changes dramatically with depth along with decreased intensity and both factors must be considered as demonstrated by the results of the current investigation.

The original concept of complimentary chromatic adaptation was intended to explain the depth zonation of seaweeds by phylogenetically determined differences in their pigment composition (see discussion by Kabinowitch, 1945). A further development in this line of thinking was that a given alga thallus could adjust its pigments to conform to the PAR field in which it was growing (see Dring, 1981 for a discussion of these ideas). In nature, however, both intensity and relative spectral distribution vary together, leading to the recent suggestion that changes formerly attributed to spectral distribution might actually be a response to decreasing intensity with increasing depth (Dring, 1981). An alternate view has
been expressed by Ramus et al. (1976a, b). Results of the present investigation demonstrate that the spectral composition of PAR per se does in fact have a dramatic effect both on the algal symbionts grown in vitro and on the intact coral-algal association. The most obvious pattern to emerge from the data is that blue PAR supports algal growth and coral growth as well or better than white PAR, at least at the low PAR levels used in these experiments. It is equally clear that red PAR is far less effective. Green PAR is intermediate in promoting growth. Calcification rate in the corals is related to photosynthesis in the algae (Goreau, 1963; Vandermeulen et al., 1972). Perhaps this is why corals generally show decreasing rates of skeletal extension with increasing depth (Dustan 1979; Barnes and Taylor, 1973). The results in Tables 6 and 7, however, show that green and particularly red PAR did not promote skeletal growth as well as blue PAR of equal quantum flux. The ambiguous results of Exp. 1 can be attributed to the fact that the blue-green experiment used in the filter had a very wide transmission band (Fig. 5).

The results of the in vitro algal growth experiment (Table 9) are similar to the coral growth data. Long term growth experiments on algae grown under altered spectral composition are lacking. Available data from studies of
less than two weeks duration suggest that there may be no
marked differences in growth rates for plants grown in red
and blue PAR (Lüning and Dring, 1975; Hess and Tolbert,
1967). It has been established, however, that pigment
composition and allocation of fixed carbon do change in
response to changing spectral quality (Brody and Emerson,
1959; Hess and Tolbert, 1967; Wallen and Geen, 1971; Bird
et al., 1981). Results of the present investigation
suggest that spectral composition is a very important
environmental factor, but possibly differences can only be
shown in long-term experiments.

The induction of chl formation in response to spectral
quality has been studied in terrestrial plants, and it
appears that blue wavelengths favor the production of
sun-type adaptive characteristics while red wavelengths
promote the development of shade-type adaptations
(Lichtenthaler et al., 1980). These authors suggest that
since terrestrial leaves exposed to full solar radiation
(canopy leaves) receive a higher proportion of blue
photons than understory leaves, blue light serves to
trigger sun-type chloroplast development.

In aquatic systems the situation appears to be
reversed. Long wavelengths are absorbed more rapidly by
water than the shorter blue wavelengths. Therefore, a
high proportion of red PAR is characteristic of shallow
environments. The deeper shaded environments that are analogous to the forest understory have little or no red PAR, but are rich in blue PAR. Perhaps marine plants key in on blue rather than red PAR. Studies have shown that the well known inverse relationship between chlorophyll content of phytoplankton and PAR intensity are mirrored by blue PAR effects rather than by the red PAR as seen in terrestrial plants (Kamiya and Miyachi, 1980). The mechanisms controlling these adaptations are not entirely understood. The possibility that altered pigment composition might allow plants to match absorption spectra with spectral distribution of available PAR ("complementary chromatic adaptation") has long been an attractive hypothesis. It appears, however, that the actual case is not so simplistic (Dring, 1981).

Jones and Meyers (1965) postulated a response termed "inverse chromatic control" whereby the relative contributions of energy to photosystem I and photosystem II are kept in balance through the operation of "pigment control". In the Rhodophyte that they studied, red PAR (or blue PAR as shown by Brody and Emerson, 1959) should decrease the amount of chl relative to the accessory pigment phycocyanin, because the quanta absorbed by chl are channeled mainly into the PS-I while the quanta absorbed by the accessory pigments in the 550 - 660 nm
hand go to PS-II. Jones and Meyers (1965) predicted that the effect of red PAR on the production of chl should be "even more dramatic" than in the blue (but see Sargent, 1934). They also noted that PAR in the region most strongly absorbed by phycocyanin does not appear to markedly reduce the relative concentration of this accessory pigment, in contrast to the reduction of chl in the blue and red region of the spectrum.

The pigments of dinoflagellates were studied by Prézelin and Alberte (1978). They presented a model that envisions a peridinin-chlorophyll-protein complex (PCP) as the major light harvesting component, a function analogous to the phycobiliproteins of the red algae. Prézelin and Alberte suggested that changes in PCP might be expected as the major mode of intensity adaptation, a hypothesis supported by in situ studies (Falkowski and Dubinsky, 1981; Dustan, 1982). PAR color treatments in the present research were not designed to test this hypothesis. All treatments were adjusted to the same PPFD, so it was not unexpected that carotenoid content of the zooxanthellae did not differ between treatments. It is possible that measurement of cellular content of peridinin rather than use of the "total carotenoids" might show differences, but these would still be very small. Thus the response to altered spectral composition must lie in changes in chl
concentration rather than changes in the accessory pigments.

Algae in both species of coral studied showed higher levels of chl a per unit coral surface area in the blue and white PAR treatments than in the green or red PAR treatments. In the perforate coral *Montipora verrucosa* this apparently was brought about by altered densities of zooxanthellae. In the imperforate coral *Pocillopora damicornis* the change in pigment per unit coral surface area appeared to result from altered concentration of pigment per algal cell. Perhaps the imperforate coral has a limited ability to sequester more zooxanthellae in its thin tissue layer. This differential response was also noted by Houck (1978) on the same two species of coral grown at different PAR intensities. It has been recently shown that zooxanthellae isolated from different hosts show different capacities in photoadaptation to PAR intensity (Chang et al., 1983). It is possible that different strains of zooxanthellae will show different capacity to photoadapt to changes in spectral regime.
CHAPTER 3

INFLUENCE OF CANOPY DEVELOPMENT ON THE P-I RELATIONSHIP

3.1 INTRODUCTION

The most complex and metabolically active communities on earth show elaborate vertical development and chlorophyll (chl) stratification in arborescent primary producers (Odum et al., 1958). Typically, these are terrestrial or benthic communities occurring in relatively stable physical environments where abundant solar radiation and moisture are available throughout the year. Rain forests, kelp beds, and coral reefs are examples (Odum et al., 1958). Such communities often represent the climax of a long period of community succession. Vertical development appears to be the result of intense competition for available solar radiation. In these cases, natural selection appears to favor photosynthetic organisms that can overtop competitors. On one hand, increased size could lead to more efficient capture of energy and consequently higher rates of primary production. On the other hand, more material and energy might be required to produce the required inert supportive material (e.g. cellulose or calcium carbonate) and the

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Part of the work in this chapter will be published in Jokiel and Morrissey (in press).
chl light-harvesting apparatus as a photosynthetic organism increases in size. Self-shading, structural limitations and material distribution problems might impose severe metabolic burdens on large primary producers. This leads to the following simple question: how is metabolism influenced by size in photosynthetic organisms?

Botanists studying terrestrial plants have attempted to define the relationship between primary production rate, light distribution, chl and plant biomass within the canopy of a community (e.g. Monsi and Saeki, 1953; Saeki, 1960; Newton and Blackman, 1970). Such information is useful in the determination of optimal planting densities (spacing for row crops, pruning rate for trees, etc.). Other studies have demonstrated the photoadaptive characteristics of sun adapted leaves from upper canopy versus shade adapted leaves from the shaded inner portion of the canopy (e.g. Young and Smith, 1980). Some general principles concerning chl distribution, light and primary production in whole communities have been proposed by Odum et al. (1958). A qualitative analysis of the canopy size vs. production problem was presented by Kanwisher (1966) for macroalgae and by Kanwisher and Wainwright (1967) for corals. The extremely high rates of primary production measured on some coral reefs might be the result of
elaborate vertical development of reef corals and algae (Kinsey, 1979; Smith, 1981). Quantitative testing of these hypotheses would be useful.

The literature concerning coral and coral reef primary productivity is diverse, and contains a wide range of estimated values. The disparate results of coral and coral reef primary production studies have been adequately summarized by others. Previous work on primary production in corals and algae has been reviewed (e.g. Stoddart, 1969; Lewis, 1977; McCloskey et al., 1978; Raven, 1981; Larkum, 1983) along with estimates for reef primary production rates (e.g. Marsh and Smith, 1978; Kinsey, 1983). An analysis of the influence of canopy development might reconcile some of the differences found in the coral and coral reef primary production literature.

Irradiance diminishes within a canopy structure (Mitchell and Calder, 1958; Odum et al., 1958; Greene and Gerber, 1967; Nomoto, 1978; Gerard, 1984). Chloroplasts and cells deeper in the canopy are more shaded and become increasingly "shade adapted" at the lower levels of irradiance. For example, zooxanthellae cultured in vitro under low levels of irradiance photoadapt by increasing photosynthetic pigmentation content and by modifying photosynthetic response (Chang et al., 1983). The zooxanthellae of the coral are analogous to the leaves on
a tree. The outer leaves of a tree become sun adapted while the leaves within the inner portion become shade adapted (e.g. Monsi and Saeki, 1978; Young and Smith, 1980). Shade adaptation involves increase in chlorophyll, increase in initial slope of the photosynthesis-irradiance curve, decrease in saturation intensity, decrease in respiration rate and other changes (e.g. Chalker et al., 1983). The response of a complete plant or coral head is therefore the sum of responses of many "layers", each with its own relative irradiance regime and with cells in different states of photoadaptation. Chloroplasts and individual cells "light saturate" at low irradiance, while reefs, coral thickets and algal stands do not (Odum et al., 1958). Talling (1961) pointed out that the unravelling of these simultaneous variables in whole canopies is rarely attempted. Therefore, little is known of the quantitative influence of depth differentiation of "sun" and "shade" characteristics upon integrated response of whole communities or large photosynthetic organisms. Generalizations based on studies of fragments, individual cells or chloroplasts do not predict the integrated response of larger systems.

The present investigation is a preliminary study directed at the quantitative description of possible "size dependent P-I effects" in two of the most common large
arborescent primary producers found on the shallow reefs of Kaneohe Bay, Oahu, Hawaii. The tightly branched hemispherically shaped coral *Pocillopora damicornis* has often served as an experimental subject for studies of reef coral physiology and was suited to this study. It is one of the most abundant corals on the shallow reef flats in Kaneohe Bay, but is very rare in deeper water. The red alga *Acanthophora spicifera* is one of the most abundant reef flat macroalgae and provides an interesting contrast to the coral.

*Pocillopora damicornis* is a relatively slow growing coral (1 to 2 cm yr⁻¹) and requires 8 to 10 years to reach full size. It is highly phototrophic and forms a rigid, highly symmetrical corallum with evenly spaced branches. The structure of the corallum appears to produce an optimal hydrodynamic environment for the polyps along with their contained symbiotic dinoflagellate algae (Chamberlain and Graus, 1975; Jokiel, 1978). Possibly the geometry of the symmetrical branched corallum optimizes self-shading and thereby enhances harvesting of available Photosynthetically Active Radiation (PAR) for the contained zooxanthellae. Chl distribution is very stratified in the coral. The tissues of the branch tips are very transparent, but color darkens gradually to chocolate brown in tissues deep in the corallum. In
contrast, the red alga *Acanthophora spicifera* grows very rapidly and apparently invests relatively little material and energy in structural support material. Chl distribution in the macroalga is more uniform than in the coral. The macroalga is easily broken away by storms and carried off the reef into deep water or deposited on the shore. It has a flimsy tangled canopy that is poorly organized and haphazardly rearranged by water movement. Hence this species is only successful in protected areas. This ephemeral alga can be regarded as an annual plant in Kaneohe Bay (Morrissey, 1985), reaching maximum abundance in the calm summer months. Winter storms remove much of the crop from the reef flats.

The coral and the macroalga both occur throughout the reef shallows and overlap somewhat in distribution. The coral, however, is found mainly on the subtidal fore-reef and flourishes in areas of moderate water motion. The alga colonizes the lower intertidal and semi-protected areas of the back reef. At low tide the flexible alga fronds become prostrate and remain submerged under tidal conditions that would expose and kill the coral. Nevertheless, some areas occupied by the macroalga are completely exposed by occasional extreme spring low tides with resulting mass mortality among the algae and other reef biota. Although reef flats in Kaneohe Bay are
normally calm, they are disrupted by occasional winter storms. In relative terms it appears that the coral inhabits a slightly more predictable subtidal benign environment, while the macroalga is adapted to exploit the more unpredictable low intertidal zone. The two experimental species thus represent two contrasting forms of arborescent development, and studies of P-I response versus size could provide useful information concerning the questions raised previously.

3.2 MATERIALS AND METHODS

Collection of material

Corals

Six corals were collected in a graded size series on the Coconut Island reef flat adjacent to the Hawaii Institute of Marine Biology. All were taken from the same area (mean depth 0.5 m, nearly uncovered at low tide) and hence had similar recent histories. Tidal range in the collection area is less than 1 m. Any metabolic differences presumably could be attributed to size and age of the corals. Each colony selected was of highly symmetrical hemispherical shape and nearly free of commensal animals such as crustaceans, fish, and echinoderms. Corals were carefully pried from the substratum at the base of the colony. The corals were
quickly and carefully moved into continuous flow aquaria. The few commensals found on the colonies were carefully removed. In each case, the coral skeleton was completely covered with living tissue, and epiphytes were absent.

Macroalgae

Four thalli of *Acanthophora spicifera* were collected in graded sizes from the Coconut Island back-reef and quickly transported to the aquaria in pails of sea water. Epibionts were carefully removed before the plants were placed in the respirometers.

**Measurement of oxygen metabolism**

**Corals**

Oxygen production measurements were carried out over a 2 day period in early October using uncovered continuous flow glass aquaria positioned in unobstructed sunlight. The size of each container was scaled approximately to the size of the coral so that geometrical relationships, ratio of coral to water volume, and ratio of water surface to water volume were roughly comparable. Volume of the containers ranged from 0.7 l for the smallest to 57.6 l for the largest. Each container was supplied with seawater pumped from the reef at a rate sufficient to replace the volume of the container within 2-3 min. Special attention was given to design features that insured high water motion in each container during
continuous flow and static conditions. Proper level of water motion was achieved with a single magnetic stirring bar in the smallest containers. The larger containers required larger stirring bars and mechanical plungers to produce water motion comparable to that encountered on the reef flat. Water motion is a critical factor because apparently it can limit exchange of materials at the tissue-water interface (Jokiel, 1978; Wheeler, 1990). Rapid flushing of the containers with water from the Bay insured that salinity, dissolved oxygen concentration and other physical factors did not depart from the optimal range for the coral. Surface irradiance in the 400 nm to 700 nm range (PAR) was measured as Photosynthetic Photon Flux Density (PPFD) with a Li-Cor Inc. Model LI-185 quantumeter with the LI-185SB sensor located next to the experimental containers. Quantum flux reaching the corals within the chambers was 75% of surface irradiance. This is similar to the level of irradiance reduction observed in the normal reef flat environment of the coral. Oxygen measurements were made with a Yellow Springs Instrument Company Model 57 oxygen meter with self-stirring probe.

The procedure used to measure oxygen production was as follows: The inlet water flowing to the container was stopped. An automatic siphon drained the excess water in the container to a pre-determined level within a few
seconds. Initial oxygen concentration was measured and recorded. After 10 min the final oxygen concentration was measured and the flow rate was resumed. Average PPFD during the incubation period was calculated from readings taken at 1 min intervals. Staggering the incubation starting times made it possible to measure the metabolism of all six corals continually during the day. In most instances, the containers were flushed for at least 30 min between the 10 min static oxygen measurements. The corals apparently were not stressed by the incubations. The polyps remained expanded throughout the experiment and mucus was not released. Water temperature within the containers remained at 26.5±1°C during the incubations. Oxygen concentration in the chambers stayed within 20% of saturation.

Macroalgae

The procedures used in the algal experiment were essentially the same as in the coral experiment. Four algae were selected for measurement. The oxygen metabolism measurement procedures previously described for the coral were employed without modification.

Calculation of oxygen flux

Oxygen production and consumption rate in corals and macroalgae were calculated from the initial and final
oxygen concentrations, water volume and incubation time. Gas exchange across the surface of the water in the open containers was calculated using the equations in Marsh and Smith (1978). The correction was inconsequential for this experiment because incubation time was very short (10 min) and wind stress in the sheltered containers was very low. Furthermore, initial oxygen tension was very close to saturation and changed by less than 20% during the measurement period. In the worst case the error caused by surface exchange in this series of measurements was calculated to be less than 1% of the oxygen being exchanged between the coral and the water within the container. The correction for surface exchange can become very important in measurements of long duration or where the oxygen concentration in the water departs markedly from saturation.

measurement of weight, chlorophyll, size and projected area

Corals

After completion of the oxygen measurements, the linear dimensions of each colony were measured. Projected "ground area" or "substratum occupied" was calculated as 3.14 times the mean radius squared. Wet weight and buoyant weight of each coral were also determined. Dry skeletal weight was calculated from buoyant weight (Jokiel 104
et al., 1978). The corals were rinsed briefly with fresh water to remove salt before being broken and placed in acetone for extraction of photosynthetic pigments. Breaking apart the colony also demonstrated the absence of organisms in the inner parts of the colony. The smaller colonies were extracted in their entirety, but whole colony extraction was not practical for the largest colonies. These were split vertically through the center and a fraction of the colony was used in the extractions.

Total amount of chl for the entire colony was calculated based on the portion of the wet weight extracted. Chl extraction was allowed to proceed for 24 h at 4°C in darkness. The acetone containing the dissolved chl was drained and replaced with a fresh volume of acetone every 24 h. Chl a and chl c were determined spectrophotometrically using the methods and equations of Jeffrey and Humphrey (1975). The entire process was repeated daily until the amount of chl extracted was less than 1% of that recovered on the first extraction. Four successive extractions were required. After pigment extraction the entire coral heads were decalcified (Brock and Brock, 1977) to recover tissues. The tissues were rinsed in tap water and dried to constant weight. This value is somewhat less than the true dry tissue weight because some organic material is removed by the acetone
extraction. Other organics were lost while dissolving the skeleton. Tissue in this species covers the skeleton very evenly, so dry tissue weight also is related to the surface area of the skeleton.

Macroalgae

At the end of the experiment the algae were measured and weighed. Subsamples were rinsed in fresh water to remove salts, crushed in a tissue grinder and placed in measured volumes of acetone for chl extraction. Dry weight was determined for the remaining fraction. Chl concentrations were determined spectrophotometrically (Strickland and Parsons, 1972).

Mathematical representation of the P-I data

Light saturation curves were obtained by plotting the rate of net oxygen flux (P) on the ordinate versus irradiance (I) on the abscissa. Numerous investigators have shown that such photosynthesis-irradiance (P-I) plots have a similar shape for a multitude of photosynthetic organisms (Talling, 1957; Jassby and Platt, 1976; Chalker, 1981). The rate of photosynthesis initially increases linearly with increasing irradiance, but eventually levels off and approaches a horizontal asymptote which can be expressed as the maximum net photosynthetic rate (Pm) or the maximum "gross" photosynthetic rate (Pm-R). The level
of irradiance at which the initial linear portion of the curve intersects \( P_m \) is termed the saturation constant \( (I_k) \). The initial slope of this linear portion of the \( P-I \) curve normalized to chl is termed \( \alpha \). Changes in \( \alpha \) accompany photoadaptive processes, so it is an important parameter to consider. The point at which the \( P-I \) curve crosses the abcissa is the compensation intensity \( (I_c) \).

A number of different equations have been used to represent this \( P-I \) relationship, but recent comparisons between the various methods suggest that the hyperbolic tangent function usually provides the best fit to experimentally derived data (Jassby and Platt, 1976; Chalker, 1981). The hyperbolic tangent \( P-I \) relationship is expressed by the equation

\[
P = (P_m - R) \tanh\left(\frac{I}{I_k}\right) + R
\]

where \( P \) is net oxygen flux between the organism and the water, \( P_m \) is maximum net photosynthetic rate, \( I \) is irradiance, \( I_k \) is the saturation constant and \( R \) is oxygen exchange rate at night. By convention \( R \) is always a negative number. This equation was fitted to the experimental \( P-I \) data derived from the corals and algae using computer programs in the SAS computer package.
Normalization of P-I equations

The curves were fitted directly to oxygen production rate per coral colony or per plant. These data need not be normalized to chl, area, dry weight etc. for the initial curve fit. Direct analysis involves the fewest complicating assumptions, and is a meaningful way to describe total colony metabolism. For example, one might want to describe net oxygen production of a colony versus colony radius. This relationship is used to illustrate the functional advantage or disadvantage of growing to large size. Excess oxygen production over 24 h is an index of the amount of surplus fixed carbon available for biological functions such as colony spawning. Also, the colony specific equations can be used directly to calculate whole colony metabolism under given photic conditions. Such calculations are relevant to size-staged population models (Hughes, 1984) that are very appropriately applied to coral populations. Each major parameter in the above equation can be normalized to specific factors as needed. Normalization simply involves division by a constant (chl, tissue, area etc.) that was determined for each coral or macroalga (Tables 10-11).
3.3 RESULTS

Photosynthesis-irradiance relationships

The photosynthesis versus irradiance plots for each coral are shown in Fig. 6 along with the hyperbolic tangent function that was fitted to each data set. The data for the macroalgae are shown in Fig. 7. Most of the scatter in the data points appeared to result from variability in irradiance during incubation. Passing clouds frequently altered intensity dramatically. Oxygen production is not a linear function of irradiance. Therefore, rapid fluctuations in PPPD alter production in a complex manner. Nevertheless, the equations appear to be good approximations of the overall P-I response measured throughout the day and provide an objectively fitted "best" curve to the available data points. Note the complete lack of photoinhibition even at full midday irradiance (Figs. 6 and 7), which is consistent with results of studies by others (e.g. Kanwisher, 1966; Franzisket, 1969; Falkowski and Dubinsky, 1981; Muller-Parker, 1984).

Tables 10 and 11 also show the calculated values for \( P_m \), \( I_k \) and \( (-\tilde{R}) \). All three parameters increase with increasing size for both the alga and the coral.

The listed value for the chl specific initial slope (alpha) is the slope of the linear portion of the fitted
hyperbolic tangent equation and is calculated by dividing "rise" over "run" and normalizing to unit chl.

\[ \alpha = \frac{(Pm-R)(Ik^{-1})}{(chl^{-1})} \]

"Rise" or "gross" production is again expressed as \((Pm-R)\). Other selected calculated ratios of structural and functional parameters normalized to area are listed in Tables 10-11 because they demonstrate a strong relationship to size. Note that the ratio of \(Pm\) to \((-R)\) increases with size, because respiration decreases with size more rapidly than does net photosynthesis. This ratio varied from 3.2 to 7.6 in the coral and from 7.2 to 13.7 in the macroalga over the size range tested. Apparently the ratio is correspondingly much lower in the coral than the macroalga due to respiration of the animal portion of the symbiosis. This relationship has been observed by Franzisket (1969). As colony size increases, the lower branch structure becomes more shaded. Among the many consequences of growing to large size is the increase in chl per unit substrate area, and a large decrease in the chl specific initial slope alpha.
Figure 6: P-I data and Tanh function for various sizes of coral.

Plot of the Photosynthesis versus Irradiance measurements made on the six corals (No. C1-C6) during the experiment. The solid line is the computer-fitted hyperbolic tangent function. Values for each of the parameters that define this relationship are shown in Table 11.
Figure 7: P-I data and Tanh function for various sizes of macroalgae.

Plot of the Photosynthesis versus Irradiance measurements made on the four algae (No. A1-A4) during the experiment. The solid line is the computer-fitted hyperbolic tangent function. Values for each of the parameters that define this relationship are shown in Table 11.
TABLE 10. Functional and structural description of various sizes of the coral Pocillopora damicornis.

<table>
<thead>
<tr>
<th>Coral mean hemisphere radius (cm)</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate area occupied (cm²)</td>
<td>13.8</td>
<td>36.3</td>
<td>50.2</td>
<td>78.5</td>
<td>211.2</td>
<td>490.8</td>
</tr>
<tr>
<td>Wet weight (g)</td>
<td>27.7</td>
<td>86.7</td>
<td>186.8</td>
<td>390.4</td>
<td>1175.0</td>
<td>2119.0</td>
</tr>
<tr>
<td>Buoyant weight (g)</td>
<td>9.2</td>
<td>40.4</td>
<td>78.2</td>
<td>198.5</td>
<td>561.0</td>
<td>967.0</td>
</tr>
<tr>
<td>Skeletal weight (g)</td>
<td>13.9</td>
<td>62.6</td>
<td>121.5</td>
<td>309.2</td>
<td>874.7</td>
<td>1508.1</td>
</tr>
<tr>
<td>Dry tissue weight (g)</td>
<td>0.21</td>
<td>1.68</td>
<td>1.78</td>
<td>4.60</td>
<td>16.21</td>
<td>34.66</td>
</tr>
<tr>
<td>Chl a (mg)</td>
<td>0.58</td>
<td>2.53</td>
<td>6.08</td>
<td>9.02</td>
<td>32.22</td>
<td>91.53</td>
</tr>
<tr>
<td>Chl c (mg)</td>
<td>0.20</td>
<td>0.81</td>
<td>1.49</td>
<td>3.83</td>
<td>12.98</td>
<td>34.92</td>
</tr>
</tbody>
</table>

Pm (maximum net oxygen production rate in mg oxygen min⁻¹) (±S.E.)

<table>
<thead>
<tr>
<th>Pm (mg oxygen min⁻¹)</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>±S.E.</td>
<td>±0.003</td>
<td>±0.013</td>
<td>±0.029</td>
<td>±0.135</td>
<td>±0.189</td>
<td>±0.287</td>
</tr>
</tbody>
</table>

Ik (saturation constant in µE sec⁻¹ m⁻²) (±S.E.)

<table>
<thead>
<tr>
<th>Ik (µE sec⁻¹ m⁻²)</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>±S.E.</td>
<td>±79</td>
<td>±73</td>
<td>±147</td>
<td>±410</td>
<td>±191</td>
<td>±147</td>
</tr>
</tbody>
</table>

-R (night oxygen uptake rate in mg oxygen min⁻¹) (±S.E.)

<table>
<thead>
<tr>
<th>R (mg oxygen min⁻¹)</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>±S.E.</td>
<td>±0.002</td>
<td>±0.008</td>
<td>±0.014</td>
<td>±0.030</td>
<td>±0.106</td>
<td>±0.135</td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ic</strong> (compensation intensity in ( \mu )E sec(^{-1}) m(^{-2}))</td>
<td>146</td>
<td>192</td>
<td>206</td>
<td>260</td>
<td>188</td>
<td>219</td>
</tr>
<tr>
<td>Ratio of ( P_m ) to ( -R )</td>
<td>3.2</td>
<td>3.4</td>
<td>5.8</td>
<td>7.6</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td>( P_m ) per unit reef area ((mg\ oxygen\ min^{-1}\ cm^{-2} \times 10^{-3}))</td>
<td>3.0</td>
<td>6.4</td>
<td>12.1</td>
<td>15.3</td>
<td>13.1</td>
<td>12.4</td>
</tr>
<tr>
<td>Chl ( a ) per unit reef area ((mg\ chl\ cm^{-2}))</td>
<td>0.042</td>
<td>0.070</td>
<td>0.121</td>
<td>0.115</td>
<td>0.152</td>
<td>0.186</td>
</tr>
<tr>
<td>Assimilation number ((P_m-R)\ per\ unit\ chl\ ( a ) ((mg\ oxygen\ min^{-1}\ mg^{-1}\ chl\ ( a )))</td>
<td>0.094</td>
<td>0.119</td>
<td>0.117</td>
<td>0.151</td>
<td>0.100</td>
<td>0.078</td>
</tr>
<tr>
<td>( -R ) per unit chl ( a ) ((mg\ oxygen\ min^{-1}\ mg^{-1}\ chl\ ( a )))</td>
<td>0.022</td>
<td>0.027</td>
<td>0.017</td>
<td>0.017</td>
<td>0.014</td>
<td>0.021</td>
</tr>
<tr>
<td>( -R ) per unit dry tissue wt. ((mg\ oxygen\ min^{-1}\ g^{-1}))</td>
<td>0.062</td>
<td>0.040</td>
<td>0.058</td>
<td>0.034</td>
<td>0.029</td>
<td>0.030</td>
</tr>
<tr>
<td>Alpha (chl ( a ) specific slope ((mg\ oxygen\ mg^{-1}\ chl\ ( a ) (min^{-1}\ \mu E^{-1}\ m^{-2} \times 10^{-5})))</td>
<td>20.8</td>
<td>18.6</td>
<td>9.1</td>
<td>7.7</td>
<td>9.1</td>
<td>6.1</td>
</tr>
</tbody>
</table>
TABLE 11. Functional and structural description of various sizes of the alga *Acanthophora spicifera*.

<table>
<thead>
<tr>
<th></th>
<th>Plant Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>4</td>
</tr>
<tr>
<td>Substrate area occupied (cm²)</td>
<td>21</td>
</tr>
<tr>
<td>Wet weight (g)</td>
<td>2.6</td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>0.5</td>
</tr>
<tr>
<td>Chl a (mg)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

\[ \text{Pm} (\text{maximum net oxygen production rate in mg oxygen min}^{-1}) \]
\[ (\pm \text{S.E.}) \]
\[ 0.079 \pm 0.005 \quad 0.902 \pm 0.065 \quad 3.78 \pm 0.048 \quad 6.41 \pm 0.61 \]

\[ \text{Ik} (\text{saturation constant in } \mu \text{E sec}^{-1} \text{m}^{-2}) \]
\[ (\pm \text{S.E.}) \]
\[ 353 \pm 105 \quad 629 \pm 215 \quad 945 \pm 503 \quad 983 \pm 382 \]

\[ \text{R} (\text{night oxygen uptake rate in mg oxygen min}^{-1}) \]
\[ (\pm \text{S.E.}) \]
\[ 0.011 \pm 0.003 \quad 0.087 \pm 0.028 \quad 0.390 \pm 0.160 \quad 0.467 \pm 0.198 \]

(continued)
<table>
<thead>
<tr>
<th></th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ic (compensation intensity in uE sec⁻¹ m⁻²)</td>
<td>50</td>
<td>61</td>
<td>98</td>
<td>72</td>
</tr>
<tr>
<td>Ratio of Pm to -R</td>
<td>7.2</td>
<td>10.5</td>
<td>9.7</td>
<td>13.7</td>
</tr>
<tr>
<td>Pm per unit reef area (mg oxygen min⁻¹ cm⁻² x 10⁻⁵)</td>
<td>3.8</td>
<td>5.4</td>
<td>7.2</td>
<td>9.1</td>
</tr>
<tr>
<td>Chl a per unit reef area (mg chl a cm⁻²)</td>
<td>0.016</td>
<td>0.022</td>
<td>0.037</td>
<td>0.049</td>
</tr>
<tr>
<td>Assimilation number (Pm-R) per unit chl a (mg oxygen min⁻¹ mg⁻¹ chl a)</td>
<td>0.273</td>
<td>0.267</td>
<td>0.215</td>
<td>0.197</td>
</tr>
<tr>
<td>-R per unit chl a (mg oxygen min⁻¹ mg⁻¹ chl a)</td>
<td>0.033</td>
<td>0.023</td>
<td>0.020</td>
<td>0.013</td>
</tr>
<tr>
<td>-R per unit dry tissue weight (mg oxygen min⁻¹ g⁻¹)</td>
<td>0.022</td>
<td>0.013</td>
<td>0.011</td>
<td>0.011</td>
</tr>
<tr>
<td>Alpha (chl a specific slope) (mg oxygen mg⁻¹ chl a min⁻¹ uE⁻¹ x 10⁻⁵)</td>
<td>77.3</td>
<td>42.5</td>
<td>22.7</td>
<td>20.1</td>
</tr>
</tbody>
</table>
TABLE 12. Hourly oxygen exchange for the experimental corals (mg oxygen h⁻¹) and daily primary production calculated for a clear day in early October using data from Table 10.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Mean Surface Irradiance (uE m⁻² sec⁻¹)</th>
<th>Coral Number (radius in parentheses)</th>
<th>Oxygen released over 24 h period (mg oxygen d⁻¹)</th>
<th>Oxygen consumed over 24 h period (mg oxygen d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0000-0600</td>
<td>0</td>
<td>C1 (2.1 cm) 0.78 C2 (3.4 cm) 4.08 C3 (4.0 cm) 6.24 C4 (5.0 cm) 9.48 C5 (8.2 cm) 27.90 C6 (12.5 cm) 62.22</td>
<td>24.5 128.1 270.9 425.3 1318.9 2745.1</td>
<td>10.0 52.6 81.5 125.3 359.9 803.5</td>
</tr>
<tr>
<td>0600-0700</td>
<td>162</td>
<td>C1 (2.1 cm) 16.2 C2 (3.4 cm) 9.85 C3 (4.0 cm) 13.59 C4 (5.0 cm) 16.55 C5 (8.2 cm) 74.45 C6 (12.5 cm) 139.51</td>
<td>162 52.6 81.5 125.3 359.9 803.5</td>
<td>162 52.6 81.5 125.3 359.9 803.5</td>
</tr>
<tr>
<td>0700-0800</td>
<td>650</td>
<td>C1 (2.1 cm) 6.5 C2 (3.4 cm) 3.4 C3 (4.0 cm) 5.0 C4 (5.0 cm) 7.5 C5 (8.2 cm) 25.0 C6 (12.5 cm) 87.5</td>
<td>650 25.0 75.0 125.3 359.9 803.5</td>
<td>650 25.0 75.0 125.3 359.9 803.5</td>
</tr>
<tr>
<td>0800-0900</td>
<td>1235</td>
<td>C1 (2.1 cm) 12.35 C2 (3.4 cm) 7.5 C3 (4.0 cm) 10.5 C4 (5.0 cm) 13.5 C5 (8.2 cm) 35.9 C6 (12.5 cm) 87.9</td>
<td>1235 35.9 105.0 125.3 359.9 803.5</td>
<td>1235 35.9 105.0 125.3 359.9 803.5</td>
</tr>
<tr>
<td>0900-1000</td>
<td>1917</td>
<td>C1 (2.1 cm) 19.17 C2 (3.4 cm) 13.9 C3 (4.0 cm) 22.0 C4 (5.0 cm) 31.8 C5 (8.2 cm) 82.8 C6 (12.5 cm) 258.6</td>
<td>1917 82.8 258.6 125.3 359.9 803.5</td>
<td>1917 82.8 258.6 125.3 359.9 803.5</td>
</tr>
<tr>
<td>1000-1100</td>
<td>2242</td>
<td>C1 (2.1 cm) 22.42 C2 (3.4 cm) 14.01 C3 (4.0 cm) 22.0 C4 (5.0 cm) 31.8 C5 (8.2 cm) 82.8 C6 (12.5 cm) 258.6</td>
<td>2242 82.8 258.6 125.3 359.9 803.5</td>
<td>2242 82.8 258.6 125.3 359.9 803.5</td>
</tr>
<tr>
<td>1100-1200</td>
<td>2340</td>
<td>C1 (2.1 cm) 23.40 C2 (3.4 cm) 14.02 C3 (4.0 cm) 22.0 C4 (5.0 cm) 31.8 C5 (8.2 cm) 82.8 C6 (12.5 cm) 258.6</td>
<td>2340 82.8 258.6 125.3 359.9 803.5</td>
<td>2340 82.8 258.6 125.3 359.9 803.5</td>
</tr>
<tr>
<td>1200-1300</td>
<td>2307</td>
<td>C1 (2.1 cm) 23.07 C2 (3.4 cm) 14.01 C3 (4.0 cm) 22.0 C4 (5.0 cm) 31.8 C5 (8.2 cm) 82.8 C6 (12.5 cm) 258.6</td>
<td>2307 82.8 258.6 125.3 359.9 803.5</td>
<td>2307 82.8 258.6 125.3 359.9 803.5</td>
</tr>
<tr>
<td>1300-1400</td>
<td>2112</td>
<td>C1 (2.1 cm) 21.12 C2 (3.4 cm) 13.99 C3 (4.0 cm) 23.90 C4 (5.0 cm) 33.07 C5 (8.2 cm) 121.86 C6 (12.5 cm) 243.35</td>
<td>2112 33.07 121.86 243.35</td>
<td>2112 33.07 121.86 243.35</td>
</tr>
<tr>
<td>1400-1500</td>
<td>1787</td>
<td>C1 (2.1 cm) 17.87 C2 (3.4 cm) 13.90 C3 (4.0 cm) 23.90 C4 (5.0 cm) 33.07 C5 (8.2 cm) 121.86 C6 (12.5 cm) 243.35</td>
<td>1787 33.07 121.86 243.35</td>
<td>1787 33.07 121.86 243.35</td>
</tr>
<tr>
<td>1500-1600</td>
<td>1137</td>
<td>C1 (2.1 cm) 11.37 C2 (3.4 cm) 13.04 C3 (4.0 cm) 23.90 C4 (5.0 cm) 33.07 C5 (8.2 cm) 121.86 C6 (12.5 cm) 243.35</td>
<td>1137 33.07 121.86 243.35</td>
<td>1137 33.07 121.86 243.35</td>
</tr>
<tr>
<td>1600-1700</td>
<td>487</td>
<td>C1 (2.1 cm) 4.87 C2 (3.4 cm) 1.83 C3 (4.0 cm) 5.16 C4 (5.0 cm) 9.13 C5 (8.2 cm) 10.32 C6 (12.5 cm) 52.31</td>
<td>487 52.31</td>
<td>487 52.31</td>
</tr>
<tr>
<td>1700-1800</td>
<td>16</td>
<td>C1 (2.1 cm) 1.6 C2 (3.4 cm) 0.66 C3 (4.0 cm) 3.63 C4 (5.0 cm) 5.71 C5 (8.2 cm) 8.82 C6 (12.5 cm) 25.09</td>
<td>16 25.09</td>
<td>16 25.09</td>
</tr>
<tr>
<td>1800-2400</td>
<td>0</td>
<td>C1 (2.1 cm) 0.0 C2 (3.4 cm) 0.78 C3 (4.0 cm) 4.08 C4 (5.0 cm) 6.24 C5 (8.2 cm) 9.48 C6 (12.5 cm) 27.90</td>
<td>0 27.90</td>
<td>0 27.90</td>
</tr>
<tr>
<td>Coral Number (radius in parentheses)</td>
<td>C1</td>
<td>C2</td>
<td>C3</td>
<td>C4</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>(2.1 cm)</td>
<td>(3.4 cm)</td>
<td>(4.0 cm)</td>
<td>(5.0 cm)</td>
</tr>
</tbody>
</table>

Excess oxygen production (mg d\(^{-1}\))
(net daily oxygen flux per coral)
14.5  75.5  189.4  300.0  959.0  1941.6

Degree of phototrophic capacity
(Ratio of oxygen produced in daylight to oxygen consumed at night)
2.4  2.4  3.3  3.4  3.7  3.4

Daily excess oxygen production per unit area
(mg oxygen cm\(^{-2}\) d\(^{-1}\))
1.1  2.1  3.8  3.8  4.5  4.0

Net Daily Primary Production (24 h)
(g C m\(^{-2}\) d\(^{-1}\))
3.9  7.8  14.1  14.3  17.0  14.9

Gross Daily Primary Production
(g C m\(^{-2}\) d\(^{-1}\))
9.0  17.9  25.3  25.2  28.9  26.2

Photosynthetic efficiency
(Atoms of carbon fixed per photon)
0.013  0.025  0.036  0.036  0.041  0.037
TABLE 13. Hourly oxygen exchange for the experimental macroalgae (mg oxygen h\(^{-1}\)) and daily primary production calculated for a clear day in early October using data in Table 11.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Mean Surface Irradiance uE m(^{-2}) sec(^{-1})</th>
<th>Plant Number (height in parentheses)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A1 (4 cm)</td>
</tr>
<tr>
<td>0000-0600</td>
<td>0</td>
<td>-0.66</td>
</tr>
<tr>
<td>0600-0700</td>
<td>162</td>
<td>1.66</td>
</tr>
<tr>
<td>0700-0800</td>
<td>650</td>
<td>4.47</td>
</tr>
<tr>
<td>0800-0900</td>
<td>1235</td>
<td>4.73</td>
</tr>
<tr>
<td>0900-1000</td>
<td>1917</td>
<td>4.74</td>
</tr>
<tr>
<td>1000-1100</td>
<td>2242</td>
<td>4.74</td>
</tr>
<tr>
<td>1100-1200</td>
<td>2340</td>
<td>4.74</td>
</tr>
<tr>
<td>1200-1300</td>
<td>2307</td>
<td>4.74</td>
</tr>
<tr>
<td>1300-1400</td>
<td>2112</td>
<td>4.74</td>
</tr>
<tr>
<td>1400-1500</td>
<td>1787</td>
<td>4.74</td>
</tr>
<tr>
<td>1500-1600</td>
<td>1137</td>
<td>4.72</td>
</tr>
<tr>
<td>1600-1700</td>
<td>487</td>
<td>4.10</td>
</tr>
<tr>
<td>1700-1800</td>
<td>16</td>
<td>-0.41</td>
</tr>
<tr>
<td>1800-2400</td>
<td>0</td>
<td>-0.66</td>
</tr>
</tbody>
</table>

Oxygen released over 24 h period (mg oxygen d\(^{-1}\))

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48.12</td>
<td>507.45</td>
<td>1942.10</td>
<td>3280.47</td>
<td></td>
</tr>
</tbody>
</table>

Oxygen consumed over 24 h period (mg oxygen d\(^{-1}\))

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.33</td>
<td>66.36</td>
<td>299.96</td>
<td>357.54</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 13. (continued)

<table>
<thead>
<tr>
<th></th>
<th>Plant Number (height in parentheses)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1 (4 cm)</td>
</tr>
<tr>
<td>Excess oxygen production (mg d⁻¹) (net daily oxygen flux per plant)</td>
<td>39.8</td>
</tr>
<tr>
<td>Degree of phototrophic capacity (Ratio of oxygen produced in daylight to oxygen consumed at night)</td>
<td>5.8</td>
</tr>
<tr>
<td>Daily excess oxygen production per unit area (mg oxygen cm⁻²)</td>
<td>1.9</td>
</tr>
<tr>
<td>Net Daily Primary Production (24 h) (g C m⁻² day⁻¹)</td>
<td>7.1</td>
</tr>
<tr>
<td>Gross Daily Primary Production (g C m⁻² d⁻¹)</td>
<td>9.9</td>
</tr>
<tr>
<td>Photosynthetic efficiency (Atoms of carbon fixed per photon)</td>
<td>0.014</td>
</tr>
<tr>
<td>Photosynthetic System</td>
<td>Daily Primary Production</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
<td>(g C m^-2 d^-1)</td>
</tr>
<tr>
<td></td>
<td>Gross</td>
</tr>
<tr>
<td>Theoretical Maximum</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>65</td>
</tr>
<tr>
<td>Coral Communities</td>
<td></td>
</tr>
<tr>
<td>Rongelap Atoll</td>
<td>4.1</td>
</tr>
<tr>
<td>Enewetak Atoll</td>
<td>9.6</td>
</tr>
<tr>
<td>North Kapaa, Kauai</td>
<td>7.9</td>
</tr>
<tr>
<td>One Tree Island</td>
<td>3.8</td>
</tr>
<tr>
<td>Piti Reef, Guam</td>
<td>7.2</td>
</tr>
<tr>
<td>Houtman Abrolhos</td>
<td>21.0</td>
</tr>
<tr>
<td>Enewetak Atoll</td>
<td>6.0</td>
</tr>
<tr>
<td>Reef Corals</td>
<td></td>
</tr>
<tr>
<td>Various species</td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>6.5</td>
</tr>
<tr>
<td>range</td>
<td>2.7-10.2</td>
</tr>
<tr>
<td>Montastrea annularis</td>
<td>2.9</td>
</tr>
<tr>
<td>Pocillopora damicornis</td>
<td>9.0-28.9</td>
</tr>
</tbody>
</table>

(continued)
TABLE 14. (continued)

<table>
<thead>
<tr>
<th>Photosynthetic System</th>
<th>Daily Primary Production (g C m⁻² d⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gross</td>
<td>Net</td>
</tr>
<tr>
<td>Plant Communities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theoretical mean annual maximum</td>
<td>-</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Fucus vesiculosus bed</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Halimeda opuntia</td>
<td>-</td>
<td>2.3</td>
</tr>
<tr>
<td>Seagrass</td>
<td>-</td>
<td>2-4</td>
</tr>
<tr>
<td>Turf algae</td>
<td>-</td>
<td>1-7</td>
</tr>
<tr>
<td>Enewetak</td>
<td>11.6</td>
<td>5.6</td>
</tr>
<tr>
<td>(algal flat)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macroalgae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acanthophora spicifera</td>
<td>9.9-19.1</td>
<td>7.0-15.6</td>
</tr>
</tbody>
</table>
Aerial chlorophyll density

The chl density of most aquatic and terrestrial communities is on the order of 1 g m\(^{-2}\) (=0.1 mg cm\(^{-2}\)) which appears to be the case for coral reefs (e.g. Odum et al., 1958; Talling, 1961; Newbould, 1963). Chl a density per unit reef area increased with coral size from 0.04 mg cm\(^{-2}\) for the smallest coral to 0.18 mg cm\(^{-2}\) for the largest coral (Table 10). In the macroalgae the increase was from 0.016 mg cm\(^{-2}\) to 0.049 mg cm\(^{-2}\) over the size range tested (Table 11). The coral appears to be extremely successful at "packing" chl onto a given reef area.

Daily primary production

In order to examine the influence of canopy "size effects" on daily primary production one must integrate oxygen flux over 24 h under the photic conditions being evaluated. Obviously phototrophy will be influenced by seasonal factors such as temperature, day length, previous history of the organism and irradiance regime. Mean hourly PAR surface readings taken on a relatively clear day in early October (the time of the measurements) were used to calculate hourly flux and exemplify the effect of arborescent structure on primary production. The calculated simultaneous hourly oxygen flux for each coral and each alga are shown in Tables 12 and 13 respectively
for this particular day. The integrated daily oxygen flux (net daily productivity) and other important calculated parameters are also listed. Note the peculiar asymmetry of the Kaneohe Bay irradiance curve. Large vertical cloud banks are formed over the Koolau Mountains by prevailing trade winds. The mountains are located to the west of the Bay. Reflection of light from these clouds produces abnormally high values early in the day.

The above analysis avoided the use of unnecessary assumptions. Oxygen production rates were calculated directly from the tanh equations for each organism (Figs. 6 and 7). Changes in dissolved oxygen were measured, so only net oxygen exchange was considered wherever possible in the analysis. The only required assumption is that curves fitted to the tanh equation adequately represent the empirical data. The equations can then be used to model the response of the coral and the macroalgae under various conditions of irradiance.

Excess daily oxygen production per coral or per macroalga is an index of energy in excess of daily maintenance needs that is available for growth and reproduction of the genetic unit represented by the macroalga or colony (Tables 12-13). This value increased
from 14 mg d⁻¹ in the smallest coral (C1) to 1900 mg d⁻¹ in the largest coral (C6) and from 40 mg d⁻¹ in the smallest macroalga (A1) to 2900 mg d⁻¹ in the largest (A4).

Calculation of gross carbon production involves use of many untested assumptions. The calculated values for net carbon fixed per day are reported (bottom of Tables 12 and 13), but only to enable comparison with previously published values that were calculated in the same manner (Table 14). The historical calculations based on oxygen measurements employed the assumption that Q and Q approximate unity and that 32 g of net oxygen flux represents 12 g of fixed carbon. This is not a problem for results based on the carbon dioxide method (e.g. Smith, 1973; Smith and Marsh, 1973; Smith, 1981). These approaches also assume that gas diffusion limitation does not occur. Use of conversions from oxygen to fixed carbon was necessary for purposes of comparison with the previously published values and is not intended to imply endorsement of the method. Calculation of gross production is potentially flawed because one must assume that respiration is constant over the day and that night oxygen uptake (apparent respiration) represents true respiration.
Assimilation number

The assimilation number is the maximum gross production rate per unit chl per unit time (Rabinovich, 1951). The maximum value was observed in opportunistic seaweeds and is approximately 0.7 mg oxygen (mg chl a)\(^{-1}\) min\(^{-1}\) (e.g. Raven et al., 1979). Previously reported values for communities vary between 0.007 and 0.070 mg oxygen (mg chl a)\(^{-1}\) min\(^{-1}\) in whole communities (reviewed by Odum et al., 1958). Assimilation numbers calculated for the corals (Table 10) range from 0.09 to 0.15 mg oxygen (mg chl a)\(^{-1}\) min\(^{-1}\). The coral shows an apparent increase in assimilation number with size up to a canopy height of 5 cm, followed by a decline. The alga assimilation numbers (Table 11) range from 0.20 to 0.27 mg oxygen (mg chl a)\(^{-1}\) min\(^{-1}\) with an apparent slight decrease with increasing size. The coral and the macroalga assimilation numbers measured in this experiment are higher than community values. This is not surprising when we consider the optimal conditions during the measurements. The maximum photosynthetic capacity is seldom reached in natural communities due to other limitations imposed by factors such as water motion, temperature, nutrient supply, inorganic carbon supply, etc.
Efficiency of incident PPFD utilization

Division of total available photons (quanta) incident per day per unit reef area by the total gross daily production (moles of carbon) gives us an estimate of efficiency of harvesting available photons (photosynthetic efficiency). This value must be interpreted with caution. Quantum flux measured as PPFD is two dimensional (photons passing through a horizontal plane). The corals and algae are three dimensional and might capture more of the scattered photons from adjacent areas as they increase in height. The smallest corals required about 80 photons to fix one carbon atom while the largest corals required only about 25 photons to fix one atom of carbon (Table 12). The smallest macroalga required about 70 photons C\(^{-1}\) and the largest alga about 40 photons C\(^{-1}\) (Table 13). The theoretical limit is about 10 photons C\(^{-1}\) (Rabinovich, 1951) considering only photons absorbed by chlorophyll.

Efficiency of incident energy utilization

The average maximum theoretical quantum yield of approximately 0.1 molecules of carbon per quantum corresponds to 22% efficiency of solar energy utilization in the spectral region of 400-700 \(\mu m\) (reviewed by Talling, 1961). Energy in the PAR region is approximately 0.46 of total solar energy. This translates into an energy efficiency of about 3% of daily PAR (1.5% of total solar
energy) for the smallest coral (C1) to a maximum of 9% of daily PAR (4.5% of total daily solar energy) for one of the larger corals (coral C5). The smallest macroalga (no. A1) gave an efficiency of approximately 3% of total daily PAR (1.5% of total daily solar energy). The largest macroalga (A4) had an energy efficiency of approximately 6% of total daily PAR (3% of total daily solar energy). These numbers agree well with previously published values (e.g. Talling, 1961).

3.4 DISCUSSION

The results of this quantitative analysis lend support to the contention of Odum et al. (1958) that whole community photosynthetic response differs markedly from the response of single cells, leaves or small plants. They predicted that increasing stratification of photosynthetic systems (light-shade adapted layering) will lead to higher saturation level, higher photosynthetic efficiency, higher chl per unit area, and higher rate of primary production. The efficiency of PPFD utilization was highest in the larger corals and algae where a greater portion of the photosynthetic tissue was shaded. A similar phenomenon has been observed in pastures (Mitchell and Calder, 1958). Arborescent development also leads to increased photosynthesis to respiration ratio expressed as Pm/-R.

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A universal limitation on the efficiency of energy utilization in high light environments is the phenomenon of light saturation (Talling, 1961). Results of the present study demonstrate that increased canopy development can overcome this limitation. Hence we can regard increasing canopy development as a photoadaptive response to high light environments. Corals and macroalgae become more "sun adapted" by increasing the amount of shaded tissues. There is an upper limit based on the theoretical maximum photosynthetic efficiency. Data on the corals suggested a possible decline in the P:R ratio as the extreme upper size is approached and self-shading begins to interfere with efficient photon capture. The largest specimens selected for testing were close to the maximum size observed in the field, so it seems that conclusions based on these data will be relevant to the natural field situation. Extensive arborescent development might not be possible in extremely unpredictable environments or in high wave energy zones where frequent damage can suppress development.

The coral forms a rigid, complex, symmetrical, and highly stratified canopy that enables it to increase net daily primary production per unit reef area by at least four-fold over the size range tested. Apparently the corallum creates optimal hydrodynamic and photic
conditions within the canopy. Assimilation number increased with size in the corals to a maximum in the coral of 5 cm height, but then decreased (Table 11). This suggests that an optimum size was exceeded. Assimilation number decreased with size among the macroalgae (Table 11). The framework of the coral holds the symbiotic algae in a rigid position relative to incoming solar radiation. This elaboration of coral surface area greatly enhances the photosynthetic rate per unit reef area and hence the ability the corals to compete in substrate-limited benthic communities. Very high densities of chl per unit reef area are achieved. A disadvantage of this adaptive pattern might be the need to invest additional material and energy in framework construction. Growth is slow and environmental conditions must remain favorable for many years before the coral can develop a large corallum. Perhaps the coral possesses many of the characteristics of a "climax species". In contrast, the macroalga produces a loose, poorly integrated and flimsy canopy structure. This "low cost" branched framework is weak but expendable, and might be characteristic of short lived "pioneer species" that are adapted to rapidly exploit unstable environments after natural disturbances. Substantial increases (at least two fold) in net primary production per unit substrate area occur with increased macroalga
size over the range tested. Also, development of the alga canopy is advantageous in that it enables this species to overtop and smother competing organisms.

The two types of arborescent development lead to many similarities in functional response. The coral apparently has achieved a much more efficient and sophisticated structure, but possibly at greater metabolic expense. Excess daily oxygen production per plant or per colony increased by three orders of magnitude (Tables 12-13) over the size range tested. This number is an index of energy available to the genetic individual (plant or colony) for growth and reproduction. Increased available energy with increased size is a possible selective advantage, but must be viewed from the standpoint of the size-staged population model (Hughes, 1984).

Results of this study suggest that it is unwise to extrapolate physiological measurements made on branch tips, small fronds, small colonies or individual leaves to estimate primary production rates of larger organisms or large stands of primary producers. The generalization that coral photosynthetic rate approaches Pm at 10% to 20% of full midday solar irradiance (e.g. Kanwisher and Wainwright, 1967; Franzisket, 1969; Chalker et al., 1983) has been developed from data taken on coral branch tips or small colonies monitored in relatively small
respirometers. Field measurements made on coral reefs with well developed arborescent framework show that photosynthesis continues to increase up to midday values (e.g. Kohn and Helfrich, 1957; Marsh and Smith, 1979; Kinsey, 1979). These observations and the results of the present study suggest that saturation ($P_m$) is not approached in large arborescent corals or macroalgae, even at the highest levels of PAR encountered on reefs. Also, the saturation constant $I_k$ is clearly a function of colony or plant size. The results of this investigation support the conclusion of Kanwisher (1966), Kinsey (1979) and Smith (1981) that maximum gross production of coral thickets and alga beds is near 20 g C m$^{-2}$ d$^{-1}$. The variation in the rate of production that can be attributed to canopy size within a single species is extremely large and of the same magnitude as variation observed in all previous studies (Table 14). Obviously size (degree of canopy development) is an important factor to be considered in such studies.

The increases in gross primary production realized through such increased size in corals probably is partially offset by the increased respiration of the coral commensal community. A wide variety of crabs, fish, shrimps, echinoderms and molluscs take up residence within the branched framework. The number and total weight of
commensals increase greatly with increasing coral size (e.g. Abele and Patton, 1976; Coles, 1980). Some of these animals derive part or all of their nutritional requirements from the coral. The increased animal component obviously will add to the respiration of the coral head and decrease net primary production. Likewise, the increase in macroalgal gross primary production is offset by increases in resident herbivore and carnivore biomass. Coral reef communities develop extremely high rates of metabolism as they mature, but community photosynthesis to respiration ratios trend toward unity and 24 h net productivity toward 0 (Kinsey, 1983). Higher respiration rate probably results from establishment of intricate food-webs within the complex canopy.

Coral reefs obviously are more complex than the coral and macroalgal models examined in this study. Reef communities contain many trophic levels and many other primary producers besides macroalgae and corals. Specialization into "canopy species" and "understory species" is apparent in some situations. The generalizations presented here, however, should be useful when applied to primary production in whole coral reef communities. Most coral reef communities show long term photosynthesis to respiration ratios of nearly 1.0 and very low 24 h net production. Because animal biomass
(respiration) increases with increased primary production. Gross primary production, however, appears to increase with increased canopy-understory development (e.g. Smith, 1974, 1981; Marsh, 1974; Kinsey, 1979). In any event, size and degree of canopy-understory development appears to be a very important metabolic parameter in the marine environment.
CHAPTER 4

INFLUENCE OF NIGHT IRRADIANCE ON THE CORAL REPRODUCTIVE CYCLE*

4.1 INTRODUCTION

The reef coral *Pocillopora damicornis* produces large (approximately 1 mm in length) and abundant planula larvae throughout the year (Edmondson, 1946). The release rate of planulae in this species shows a strong cyclic component with a period of approximately 29 days (Marshall and Stephenson, 1933; Atoda, 1947; Harrigan, 1972; Stimson, 1978; Richmond and Jokiel, 1984). Harriott (1983) found highly seasonal reproduction in *P. damicornis* at Lizard Island, Australia. The greatest number of planulae were released at full moon in the austral spring. Relatively small numbers were released in the austral summer with an apparent shift in peak release to time of new moon. A similar pattern was described by Marshall and Stephenson (1933) at Low Isles, Australia. Such monthly oscillation inevitably has been termed "lunar periodicity", but without any direct evidence of causal relationships. Many potential forcing functions in nature show oscillations with a similar frequency, so numerous

*Part of the work in this chapter has been published in Jokiel et al. (1985).*

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hypotheses can be proposed to explain this phenomenon. Solar activity, for example, shows a clear monthly periodicity. The solar flux cycle results from localized areas of intense and persistent activity on the surface of the sun combined with the solar rotational period of approximately 27 days. The most conspicuous aspect of the monthly solar cycle is the periodic change in the number of sunspots visible from the earth. A strong relationship is known to exist between sunspot activity, solar flux, and ultraviolet radiation flux (Goldberg, 1969). Changes in the intensity of solar ultraviolet radiation can influence coral planula production rate (Jokiel and York, 1982). A comparison of the Mean Daily Zurich Sunspot Number (as reported monthly in the Quarterly Bulletin of Solar Activity) and P. damicornis planulae release previously reported (Marshall and Stephenson, 1933; Atoda, 1947; Harrigan, 1972) indicated that peak production of larvae generally coincided with periods of maximum monthly sunspot number (Jokiel, unpublished). Again, such correlations do not demonstrate cause and effect.

Tidal cycles are frequently proposed as a probable controlling factor. Inundation, mechanical agitation, water pressure and water temperature vary with the tidal cycle and can entrain biological rhythms (Bünnning, 1973; Palmer, 1974; Brady, 1982). Lunar night irradiance might
influence the coral directly through photochemical reactions or indirectly by altering nightly feeding patterns of coral commensals and predators. Abundance of planktonic food sources can vary with the lunar cycle (Alldredge and King, 1980) which could indirectly influence reproductive rate. Endogenous mechanisms coupled with production of pheromones (e.g. Müller and Seferiadis, 1977) might conceivably synchronize reproduction in a manner that is largely independent of external physical forcing functions. Even cyclic monthly variations in the geomagnetic field could be a factor, but little information exists in this area. Certainly other hypotheses could be erected. Monthly cycles of barometric pressure and cosmic radiation correlate with certain biological processes showing circa-monthly periodicity (Brown et al., 1956). Experimental studies of reproduction in polychaetes reveal numerous ways in which cyclical changes in environmental parameters can influence rates of reproduction and reproductive synchronization (Olive, 1984). Reproductive cycles seem to involve the interaction of external cycles of geophysical parameters with endogenous biorhythms of circa-annual, circa-lunar or circadian periodicity. Observations of correlations between breeding and environmental factors can lead to the formulation of hypotheses concerning possible mechanisms.
These hypotheses must be tested in controlled experiments to establish cause and effect.

Attempts to solve the riddle of lunar periodicity in the reproductive cycle of this coral began during 1930 at the Mid Pacific Research Laboratory (Enewetak Atoll, Marshall Is.) and at the Hawaii Institute of Marine Biology (Coconut Is., Hawaii). Results of this geographic comparison are presented elsewhere (Richmond and Jokiel, 1984). This previous research and a much longer (over 2 yr) time-series study of coral reproduction (Chapter 5) negated the previously mentioned solar "sunspot" hypothesis. Obviously, the 27 day solar radiation cycle will continuously move into and out of phase with the 29 day lunar cycle. The lunar correlation with reproduction held, but the solar cycle moved out of phase (Chapter 5). The work of Richmond and Jokiel (1984) indicated that differences in time of peak planula release occur within and between geographically isolated populations of Pocillopora damicornis. The taxonomic status of this species is brought into question by the apparent temporal isolation of the reproductive process. At Enewetak, the time of maximum larva release occurred consistently between new moon and first quarter. In Hawaii, two morphological variants of this coral were studied: The first (Type Y) showed peak release of larvae near the time
The two "types" are now known to represent two genetically distinct clones among a number of genotypes found within Kaneohe Bay (Stoddart, in press).

All colonies of *Pocillopora damicornis* used in this study were 10±1 cm in diameter. Collection of the planulae produced by the corals was carried out in the following manner: The corals were transferred into containers (volume = 4 l) that received a flow of approximately 1 l min⁻¹ of unfiltered seawater pumped from Kaneohe Bay. The overflow from the containers was channeled into collector cups fabricated from plastic beaker bases built up with walls of 180 μm plankton netting. Water overflowed through the mesh walls while the planulae were retained within the cups. The planulae were counted daily by washing the contents of the collector cups onto a 180 μm mesh sieve. The total number of planulae was tallied with the aid of a mechanical counter. Control containers maintained without corals collected fewer than 1 planula per month in comparison to several thousand per month typically produced in containers with corals. Extraneous planulae that might have entered with the incoming water supply did not alter the count significantly.
4.2.1 Larva Production Experiment 1
Possible Influence of Tide-Related Factors on Planula Release

The first experiment was designed to examine the possible influence of tidal height, tidal range, tidal current, water motion and/or other factors influenced by tide. Type B corals were grown in outdoor laboratory aquaria for 16 months prior to the experiment. These corals received a constant flow of water pumped from a depth of 3 m on the outer Coconut Island Reef slope. Water temperature and salinity at the intake are relatively stable compared to the reef flat where this species is most common. Consequently, the corals held in the containers did not experience the tidal fluctuations or associated environmental changes (temperature, oxygen tension, water motion etc.) experienced by the reef flat population.

Five laboratory grown corals were placed in five of the continuous flow planula collectors. Planula production in these corals was compared to that of field corals. Daily planula counts were made for the laboratory corals over the period Sept. 1980 through Feb. 1981. The five "field" corals were replaced daily, but the same 5 "laboratory" corals that had not experienced tidal changes for 16 months were monitored for the entire 6 month period. Moving the corals into the container did not seem to
disturb the reproductive pattern of the corals if they were handled carefully (Richmond and Jokiel, 1984).

4.2.2 Larva Production Experiment 2

Interspecific Comparison of the Time of Planula Release

Two other species of corals found in Kaneohe Bay are known to release abundant large planulae throughout the year, but there is some question as to how their pattern of release compares to that observed in Pocillopora damicornis. The purpose of Exp. 2 was to simultaneously measure the pattern of larva release in the corals Cyphastrea ocellina, Tubastrea coccinea and P. damicornis. Comparison of synchronization between species might be useful in establishing possible forcing functions. Planula release in these two species was monitored along with Type B P. damicornis using the same methods as in Exp. 1. Planulation was monitored in 5 corals of each species over a period of 3 months (June through August, 1981). The colonies of P. damicornis measured 10±1 cm in diameter. The colonies of C. ocellina measured 8±1 cm in diameter and the colonies of T. coccinea were 7±1 cm (with approximately 16 polyps per colony).
4.2.3 Larva Production Experiment 3

The Influence of Night Time Irradiance on Planula Release

Results of the geographic comparison (Richmond and Jokiel, 1984) and results of Exp. 1 eliminated a number of possible forcing functions. Preliminary results of field experiments suggested that reproduction in the corals was disrupted during times of prolonged continuous nighttime cloud cover. Also, reproductive periodicity was disrupted among corals held in an aquarium located near an incandescent light that was left burning every night (J. Stimson, personal communication). These observations suggested night irradiance as the next factor to examine.

Exp. 3 (August through December, 1982) tested the response of both Y and B Type corals to three artificial regimes of night irradiance. The null hypothesis was that corals in the three artificial night irradiance treatments would continue to reproduce in synchrony with the field corals as in Exp. 1. Changes in synchronization would indicate that night irradiance is an important controlling factor.

Three large continuous flow aquaria located outdoors under full solar radiation were fitted with covers that shut out all external light when closed. The covers were opened and closed by means of a simple bucket and pulley system that was actuated hydraulically. A solenoid valve actuated by an electrical timer controlled water flow into
or out of the buckets. As the bucket filled with water it became heavy and dropped a distance of 2 m while pulling the aquarium lid aside through the system of pulleys. When the bucket drained counterweights closed the lid. The timer was set to uncover the aquaria at 0500 h and cover them at 1900 h daily. Reef corals require high levels of sunlight to nourish their symbiotic algae. Therefore, corals were allowed to receive full solar radiation during the daylight hours. Artificial "lunar" night radiation in the aquaria was produced by small incandescent bulbs adjusted with a rheostat so that corals received irradiance comparable to lunar intensity at full moon (approximately 0.01 uE m\(^{-2}\) sec\(^{-1}\) as measured with a Li-Cor, Inc. Model LI-188B integrating quantumeter). Corals in the "shifted phase" treatment received no night irradiance at time of the natural full moon, but received full "lunar" intensity incandescent irradiance during a 10 day period centered on time of new moon. Corals in the "constant full moon" treatment received irradiance of full lunar intensity throughout every night of the month. Corals in the "constant new moon" treatment received absolutely no night irradiance.

The fiberglass aquaria measured 90 cm X 90 cm by 45 cm deep. Each was supplied with incoming seawater at a rate of approximately 10 l min\(^{-1}\). A standpipe maintained the
water depth at 20 cm. Water in the aquaria was vigorously aerated and 5 small (6 to 8 cm length) herbivorous fish were added to control growth of unwanted macroalgae. Corals were supported 2 cm off the bottom and about 2-5 cm under the water surface on vinyl coated wire mesh. Sediments that accumulated in the aquaria were siphoned off. A small shelf in each treatment supported two of the standard planula collection containers described previously. Space restrictions and the considerable amount of time required to count planulae each day in multiple experiments limited measurement to only one Type Y and one Type B coral at a time within each treatment. This proved to be adequate for purposes of this experiment. The coral in the planula collection apparatus was replaced every day with another from the same treatment. Thus each coral was measured on only 3 or 4 days during each month. Corals exposed to unaltered field conditions of night irradiance served as the "control". These measurements were made on Type Y and Type B corals that were collected and replaced daily.

During the experiment it was noted that some of the larvae produced within the different treatments settled on various substrata within the aquaria and started growing into new colonies. It soon became apparent that possible differences in recruitment existed between the treatments.
Therefore, at the end of the experiment, the aquaria were drained and all new coral settlements on each of ten randomly selected wall areas (8 cm wide x 20 cm high strips) were counted within each treatment. The plot count data were square-root transformed to homogenize variance and analyzed using a model 1 single classification analysis of variance (Sokal and Rolf, 1969). A posteriori differences were evaluated with the Student-Newman-Keuls Test.

The data are plotted as the log of the quantity one plus the daily mean number of larvae released. Transformation of these data is useful because of the high variability encountered in the counts (3 orders of magnitude) and presence of zero values (log 0 is undefined). The smoothed curve shown in the figures is a centered running mean (n=5) of the given day plus the two preceding days plus the two subsequent days.

4.3 RESULTS

4.3.1 Larva Production Experiment 1

Possible Influences of Tide-Related Factors on Planula Release

Fig. 8 shows daily release of planula larvae by corals held in outdoor aquaria for 16 months compared to release by Pocillopora damicornis from the field "control".

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Planula release of the laboratory-held corals was far more regular and intense than that of the field corals (Fig. 8). Apparently this is because the physical environment of the laboratory corals was more moderate than that of the natural population that inhabits the reef flat. On 14 Dec. 1980 a severe rainstorm occurred at extreme low tide. Corals that were uncovered at that time were killed. Other corals growing slightly submerged were subjected to low salinity. The Type B corals from the field responded by continually aborting developing planulae and reproduced at a low rate for several months (Fig. 8). The laboratory-held Type B corals received a continuous flow of seawater from a deeper location (3 m) in the Bay during this rainy period and were not subjected to the low salinity surface waters. The laboratory-held corals maintained high production throughout the following months. Field and laboratory corals received the same solar irradiance during this time.

Holding the corals in an artificial aquarium system away from tides, waves and currents for 16 months had no apparent effect on synchronization of spawning. The laboratory corals in the aquaria continued to reproduce synchronously with the reef-flat corals even though the corals were maintained in individual containers without other corals, fish or symbiotic crustaceans.
Figure 8: Larva production in the presence and absence of external tidal influence. Results of Exp. 1. Daily planula production for the field population of "Type B" Pocillopora damicornis (top) compared to corals that had been isolated from tidal influences for 16 months in the laboratory (bottom). Thin lines connect daily values; thick lines represent 5 day centered running mean (see text).
Figure 9: Larva production comparison for three species of corals. Comparison of larva production in *Pocillopora damicornis*, *Cyphastrea ocellina*, and *Tubastrea coccinea*. Thin lines connect daily values; thick lines represent 5 day centered running mean (see text).
Pocillopora damicornis

Cyphastrea ocellina

Tubastrea coccinea

Planula Release Rate

log (P+1)

June July Aug.
The fact that reproduction continued at a high rate even though the corals were isolated in individual containers is consistent with the suggestion that planulation in *Pocillopora damicornis* is asexual (Stoddart, 1984; Martin-Chavez, in press). Furthermore, synchronization was maintained in the absence of possible external biological cues such as monthly cycles of fish predation on coral tissues or activities of coral commensals. This experiment eliminated further consideration of biological interaction and tide-related physical effects.

### 4.3.2 Larva Production Experiment 2

**Interspecific Comparison of the Timing of Planula Release**

A strong monthly planula release cycle is not evident for *Cyphastrea ocellina* and *Tubastrea coccinea* (Fig. 9). Lack of a monthly cycle of reproduction in *C. ocellina* has been observed previously (Edmondson, 1946; Stimson, 1978; Wright, in press). Often such negative results are not reported. Hence, we might wrongly conclude that lunar periodicity in larvae release is the general rule among corals because of an extensive literature involving the few species that follow this pattern (Fadlallah, 1983; Harrison et al., 1984).
4.3.3 Larva Production Experiment 3

The Influence of Night Irradiance on Planula Release

Results of the third experiment (Figs. 10-11) clearly show that night irradiance synchronizes reproduction in Pocillopora damicornis. The corals were moved into their respective treatments at the beginning of the planula release cycles for their "Type" (full moon for Type Y, new moon for Type B). Both Type Y and Type B corals shifted reproductive periodicity quickly in response to the shifted lunar phase. Also, both reduced their reproductive output in the treatment without night irradiance and in the treatment with continuous night irradiance (Table 15). One difference in the response of the two types is the apparent ability of the Type Y corals to continue synchronized reproductive periodicity into a second cycle even in the absence of an external signal. This is consistent with the two year record of field planulation (Jokiel, in press). Reproductive periodicity in the Type B corals is very sensitive to periods of heavy night cloud cover. The corals can reduce or cease reproduction during such periods.

The numbers of new colonies on the aquaria walls within each treatment at the conclusion of Exp. 2 are shown in Table 15 along with larva production within each treatment.
Figure 10: Production of larvae in Type B corals.

*Pocillopora damicornis* grown under different regimes of night irradiance (Exp. 3). Thin lines connect daily values; thick lines represent 5 day centered running mean (see text).
Type B Corals

Natural Night Irradiance

Shifted Moon Phase

Continuous Full Moon

Continuous New Moon

Planula Release Rate

Figure 11: Production of larvae in Type Y corals.

For *Pocillopora damicornis* grown under different regimes of night irradiance (Exp. 3). Thin lines connect daily values; thick lines represent 5 day centered running mean (see text).
Type Y Corals

Natural Night Irradiance

Shifted Moon Phase

Continuous Full Moon

Continuous New Moon

Planula Release Rate

TABLE 15

Larvae production and recruitment of new colonies during Exp. 3.

Sample areas were randomly chosen strips of aquarium wall measuring 8 cm wide by 20 cm tall.

<table>
<thead>
<tr>
<th></th>
<th>&quot;Constant Full Moon&quot;</th>
<th>&quot;Shifted Phase&quot;</th>
<th>&quot;Constant New Moon&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Total larvae produced per colony</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type Y</td>
<td>8700</td>
<td>10400</td>
<td>7400</td>
</tr>
<tr>
<td>Type B</td>
<td>6700</td>
<td>12700</td>
<td>3900</td>
</tr>
<tr>
<td>B. New colonies on aquaria walls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Replicate No.)</td>
<td></td>
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<tr>
<td>1</td>
<td>14</td>
<td>89</td>
<td>11</td>
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<td>2</td>
<td>4</td>
<td>85</td>
<td>12</td>
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<td>3</td>
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<td>19</td>
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<td>8</td>
<td>3</td>
<td>22</td>
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<td>4</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>13</td>
<td>16</td>
</tr>
</tbody>
</table>

Sample mean* 7.30 52.00 35.50

*Values connected by line are not significantly different at the 0.05 level.
during the experiment. The treatment that never received night irradiance (constant new moon) had a higher number of new settlements. The treatment that always received night irradiance (constant full moon) had very few settlements even though the corals produced more larvae. The treatment that received night irradiance for 10 nights near the time of new moon (shifted lunar phase) produced the greatest numbers of new colonies. Differences between settlement in the treatments were significant (p<0.005) by the one way analysis of variance on square root transformed data (Table 15). The Student-Newman-Keuls Test revealed that the "constant moon" treatment was significantly different from the other two treatments.

4.4 DISCUSSION

The cycle of planula release in the reef coral *Pocillopora damicornis* is entrained by cyclic variation in night irradiance. Hence we observe "lunar periodicity" in larva release for this species. It is surprising that such simple animals lacking complex sensory organs, endocrine systems and neurological systems could detect and respond to such low levels of night irradiance (night PPFD was 0.01 uE m⁻² sec⁻¹ compared to mid-day values of approximately 2000 uE m⁻² sec⁻¹). Many other types of organisms show lunar cycles. For example, some species of
fish migrate on a lunar cycle (Mason, 1975; Müller, 1983), but night irradiance has not been construed to be the controlling factor. The alga Dictyota dichotomata is able to maintain an endogenous 14-15 day "lunar rhythm" of gamete production that can be "reset" by exposing the plants to low intensity (3 lux) night irradiance (Müller, 1962). The mechanisms behind the observed phenomenon in corals (as in most other species) is a mystery. Perhaps the regulatory process in corals involves the photochemical control of a substance that influences early gametogenesis. Another hypothesis is that extremely low rates of photosynthesis by zooxanthellae on moonlit nights might influence metabolism and hence influence reproduction in this coral. It is also possible that this species possesses neurologically linked photoreceptors that have not yet been described.

Results of these experiments appear to provide an explanation for the decrease in reproduction and the apparent weakness of reproductive synchronization during the austral summer on the Great Barrier Reef (Marshall and Stephenson, 1933; Harriott, 1983). This is a time of heavy rain and cloud cover (e.g. Orr, 1933), which might obscure the moon for long periods and produce conditions of night irradiance similar to those encountered in the "perpetual new moon" treatment of Exp. 3. In Hawaii,
reproduction of this species appears to be influenced by heavy cloud cover during the rainy season and by volcanic events that create overcast conditions (Chapter 5).

A recent review of reproduction in corals (Fadlallah, 1983) points out that little was known about gametic cycles in corals until very recently. We are observing a dramatic increase in the amount of new information on coral reproduction, and general statements will have to await further studies. Some species brood planulae, but others are broadcast spawners. Cyclicity of spawning ranges from continuous to monthly to once per year.

The adaptive significance of lunar periodicity in coral larva release is difficult to explain. The other species of Hawaiian corals that produce abundant planulae (Tubastrea coccinea and Cyphastrea ocellina) did not show a lunar component (Fig. 9), but species of Hawaiian corals that are broadcast spawners (Fungia scutaria, Montipora spp.) release eggs and sperm shortly after full moon (Krupp, 1983; Heyward, in press). Synchronous multispecific spawning by corals on the Great Barrier Reef occurs annually after time of full moon when water temperature is at or near annual maximum (Harrison et al., 1984). Such synchronization of reproductive activity in coral populations probably has several advantages. Synchronous release of extremely large numbers of
reproductive products could satiate predators and increase overall chances for larval survival. Synchronization of time of egg and sperm release in broadcast spawners increases the probability of fertilization. In the case of *P. damicornis* synchronization might allow the coral to release larvae at a time that is "optimal" for dispersal and/or settlement.

Difficulties in interpretation arise from the observation that various "types" or clones of *P. damicornis* exist in nature (Richmond and Jokiel, 1984; Stoddart, 1984). It is puzzling that Type B corals should begin to release larvae at apparent new moon while the Type Y begins to release larvae at full moon. Do they occupy different niches? Also, both Hawaiian types are out of phase with *P. damicornis* from Rnewetak. Attempts to relate this to local conditions have not yet provided a testable hypothesis. These disparate results provide us with no unifying principle, except for the suggestion that time of peak larva release can be readily modified by selective pressures or genetic drift in local populations.

One cannot avoid mention of "biological clocks" in discussions of such periodic biological rhythms. Basic hypotheses in this area fall into two categories (Brown et al., 1970). The first category is that of the "escapement type clock" that is hypothesized to exist within the cells
of an organism. This type of endogenous biological clock generates its own time signal autonomously. The second is the "nonescapement type clock" which must receive its timing information from a periodic environmental factor that is capable of penetrating the confines of so-called "experimental constant conditions". Under conditions of either continuous night irradiance or continuous night darkness the coral populations were unable to maintain synchronized lunar periodicity for very long and must fall into the latter category. The Type B corals completed one synchronous cycle, the Type Y corals completed two cycles in synchrony. The planula release cycle in corals exposed to the phase-shifted artificial lunar cycle was quickly entrained by the new signal, suggesting that night irradiance might serve as an "environmental trigger" (Olive, 1984) to initiate gametogenesis. The Type Y corals were slightly less responsive to altered irradiance regime than the Type B. At least two cycles passed before the Type Y corals moved into synchrony with the new night irradiance regime.

It is likely that night irradiance synchronizes some stage of the gametogenic cycle of this species. Martín-Chavez (in press) has already described gametogenesis in the "Type Y". Spermares began to form, but were resorbed before maturity was reached. Ovogenesis
took approximately 5 weeks for completion. There was an overlapping of two gametic cycles as evidenced by a bimodal distribution in size of developing eggs. Perhaps this is why the Type Y corals can remain synchronized for two planula cycles after the night illumination "zeitgeber" is removed. At new moon, the mature eggs developed into "embryo-like" structures even though mature sperm was not present. Larvae appeared to develop asexually from oocytes by parthenogenesis, and mature eggs grew into larvae in about two weeks. The influence of altered night irradiance on gametogenesis is of critical concern and will subsequently be investigated.

These experiments were designed only to discover the zeitgeber and examine synchronization of planula release within populations of the coral. Population synchronization is not the same as within-colony synchronization of polyps. Synchronization of all polyps within a colony, if this actually occurs in the absence of a lunar cycle, might be achieved by a different mechanism such as pheromone control. Future experiments will examine these problems. It is not known if individual polyps are capable of endogenous "free running" cycles that must be continually synchronized within the colony and the population by the lunar signal. Slight differences in the period of the individual polyp or
colony endogenous period would quickly damp the synchronized response.

Corals in the "constant full moon" treatment produced more larvae than the corals in the "constant new moon" treatment during the experiment. Perplexingly, far fewer settlements were found in the "constant full moon" treatment. The differences in number of new coral colonies that settled in the aquaria were striking. All factors within the various treatments appeared to be identical except for night irradiance. More research is needed in this area, but we can speculate on the meaning of the observed phenomenon. Metamorphosis of the larvae of many marine species is influenced by cycles of light and darkness (e.g. Thorson, 1964; Doyle, 1974). Perhaps this is the case with Pocillopora damicornis planulae. One might envision a selective advantage for planulae that attach to substratum, but delay final metamorphosis for at least one diurnal cycle. This could prevent settlement in the intertidal or might provide a mechanism to insure wider dispersal. Alternatively, increased night activity by the small grazing fish in the continuous full moon treatment might have prevented successful recruitment. Conclusions are premature at this point.

In summary, it is clear that night irradiance serves as a "trigger" (Olive, 1984) in synchronizing the observed
monthly reproductive cycle in these corals. The mechanism is unknown, but appears to influence an early stage of gametogenesis. There is no need to invoke tide-associated factors, circa-monthly variation in solar activity, geomagnetic flux or other factors in order to explain lunar periodicity in spawning of the coral *Pocillopora damicornis*. Once the cycle is triggered, pheromones could play a role in reproductive synchronization, but we have no direct evidence. Although night irradiance controls monthly synchronization, other environmental factors such as temperature can regulate numbers of larva produced during a cycle (Jokiel, in press). Some environmental factors influence circadian rhythms. For example, recent studies by Holloran and Witteman (in press) demonstrate that diurnal larva release in this species reaches a peak at time of low tide. Results of these experiments have raised many questions concerning both the physiological mechanism and the ecological significance of lunar synchronization of planulation. The rather dramatic biological response to changes in the monthly cycle of night irradiance demonstrates that subtle modulation of the natural photic environment is an extremely important environmental factor.
CHAPTER 5

IRRADIANCE AND CORAL REPRODUCTION: A FIELD STUDY

5.1 INTRODUCTION

Results of the controlled experiments described in Chapters 1-4 demonstrate the importance of spectral irradiance regime on the biology of the coral *Pocillopora damicornis* and its contained zooxanthellae. Another experimental approach favored by many investigators is that of the field investigation. This approach involves the monitoring of a biological process through time with measurement of environmental variables that might influence the biological process in question. Subsequently, correlation analysis between the variables is used to demonstrate possible relationships between factors. The purpose of the present investigation was to produce an extremely accurate time series of biological and physical data in an attempt to gain further insight into the relative importance of various environmental factors to irradiance on the coral reproductive process. Harrigan (1972) tried this approach, but was confronted with problems that appear to be related to small sample size, use of static incubations under artificial

5Part of the work in this chapter has been published in Jokiel (in press).
irradiance and artificial laboratory temperature. The present study was similar, but sampling rate was increased from 1 colony per week to 10 colonies per day. Length of the study was increased from 1 year to over 2 years. The experiment was conducted under natural outdoor irradiance in a continuous flow system that maintained realistic field temperature and water quality. Harrigan was able to demonstrate a correlation with lunar phase, but no other factor showed significant correlation. It was hoped that a more intensive experimental effort would produce greater insight into the importance of seasonal changes in irradiance on the reproductive process.

Our knowledge of coral reproductive biology is expanding at a rapid rate. It is now apparent that the majority of coral species do not brood planula larvae, as previously thought, but are broadcast spawners (e.g. Fadlallah, 1983; Krupp, 1983; Harrison et al., 1984; Heyward, in press). Time of spawning is often related to the lunar cycle. The coral *Pocillopora damicornis* broods its planulae and has been the subject of extensive study. The monthly cycle of planula release in this coral was first documented by Marshall and Stephenson (1933) and later observed by others (Atoda, 1947; Harrigan, 1972; Stimson, 1978; Harriott, 1983). Recent investigations have shown that phase differences occur within and between
geographically isolated populations of this species (Richmond and Jokiel, 1984). Subsequent studies demonstrated that night irradiance controls the synchronization of monthly spawning (Chapter 4). This chapter presents additional evidence in support of the above conclusions, but is mainly concerned with environmental factors that influence the numbers of larvae released per monthly spawning cycle in the natural field population.

5.2 MATERIALS AND METHODS

Two different "types" (Richmond and Jokiel, 1984) of the reef coral currently described under the species name Pocillopora damicornis (Linnaeus) were used in this investigation. The "Type Y" exhibits a yellow animal pigment that is quite visible at the growing branch tips. The "Type B" has thinner branch tips and no yellow pigment, so its tips appear to be white in color. Electrophoretic studies of these two types of P. damicornis have been conducted (Stoddart, 1984). They are known to represent two genetically distinct clones among a number of genotypes found within Kaneohe Bay, Oahu, Hawaii (Stoddart, in press). Morphological characteristics of colonies and phase differences in the cycles of planula release between the two types have been documented (Richmond and Jokiel, 1984).
All corals used in this study were 10±1 cm in diameter. The corals were transferred into 10 individual containers (volume = 4 l) that received a flow of approximately 1 l min⁻¹ of unfiltered seawater pumped from Kaneohe Bay. The overflow from the containers was diverted into collector cups fabricated from plastic beaker bases built up with walls of 180 um mesh plankton netting. The planulae were collected and counted daily by washing the contents of the collector cups onto a gridded 180 um mesh sieve. The total number of planulae produced by each coral in 24 h was tallied with the aid of a mechanical counter. Control containers maintained without corals produced fewer than 1 planula per month in comparison to several thousand per month that were produced in containers with corals. Thus extraneous planula that might have entered with the incoming water supply did not alter the count significantly.

Five "Type Y" and five "Type B" colonies were gently moved in pails of seawater from the windward reef of Coconut Island and placed in the individual continuous flow planula collectors described above. After 24 h the planulae produced by each coral were counted and the corals were replaced with fresh colonies from the field. Severe weather conditions or lack of time occasionally did not allow collection of fresh colonies in the field. In
In these cases, it was necessary to monitor the same corals for an additional day. Corals were never held for more than 48 h. Afterwards, the corals were tagged with vinyl coated wire and returned to the reef. The same corals were never used twice in the experiment. This process was repeated daily without interruption for over 2 years (Aug 1980 to Dec 1982). The routine did not appear to disrupt the natural planula release cycle (Richmond and Jokiel, 1984). Incident solar radiation at the site was monitored continuously at Coconut Island using a standard Pypley pyranometer with electronic integrater. Reef water temperature was recorded daily. Rainfall was monitored with a standard rain gauge at the Coconut Island weather station adjacent to the reef. The data on mean lunar monthly planula production, temperature and rainfall were subjected to correlation analysis and multiple regression analysis using the general linear model of the SAS statistical package.

5.3 RESULTS AND DISCUSSION

The planula production data are plotted as the log of the quantity one plus the daily mean number of larvae released (Fig. 12). This transformation is useful because of the high variability encountered in the counts (3 orders of magnitude) and the presence of 0 values (log 0 is undefined). Mean production of larvae per lunar month

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Figure 12: Mean daily planula production for Type Y and Type B Pocillopora damicornis.

Solid discs represent time of new moon, open circles represent time of full moon. Times of severe environmental disturbance are indicated by arrows and symbols: e = exposure by extreme low tide, r = rainfall during exposed conditions, t = abnormally high temperature, h = Hurricane Iwa, v = volcanic material in stratosphere from eruption of El Chichón reaches Hawaii. Keep in mind that 1981 was the warmest year on record in the Northern Hemisphere and that 1982 was a year of record breaking low irradiance in Hawaii (Tables 16-17). Periods of planula abortion are indicated by asterisks (*).
Figure 13: Mean sea level in Hawaii during the study.
Summary of field data.

Total number of planulae (sum of daily means) produced per colony per lunar cycle (new moon to new moon) compared to mean lunar monthly water temperature, total rainfall for the lunar month, and mean daily irradiance (cal cm\(^{-1}\) d\(^{-1}\)) for each lunar month.

<table>
<thead>
<tr>
<th>Lunar Month</th>
<th>Planula Production (no. colony(^{-1}) d(^{-1}))</th>
<th>Mean Temp. (°C)</th>
<th>Mean Irradiance (cal cm(^{-2}) d(^{-1}))</th>
<th>Mean Rain (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Oct</td>
<td>1595</td>
<td>26.4</td>
<td>338</td>
<td>3.9</td>
</tr>
<tr>
<td>7 Nov</td>
<td>1653</td>
<td>25.5</td>
<td>301</td>
<td>1.7</td>
</tr>
<tr>
<td>7 Dec</td>
<td>5678</td>
<td>25.0</td>
<td>229</td>
<td>13.4</td>
</tr>
<tr>
<td>1981</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Jan</td>
<td>2575</td>
<td>24.1</td>
<td>312</td>
<td>4.9</td>
</tr>
<tr>
<td>4 Feb</td>
<td>5740</td>
<td>24.1</td>
<td>373</td>
<td>5.1</td>
</tr>
<tr>
<td>6 Mar</td>
<td>2319</td>
<td>24.6</td>
<td>418</td>
<td>5.2</td>
</tr>
<tr>
<td>4 Apr</td>
<td>1657</td>
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<td>438</td>
<td>10.3</td>
</tr>
<tr>
<td>3 May</td>
<td>5761</td>
<td>25.6</td>
<td>454</td>
<td>12.1</td>
</tr>
<tr>
<td>2 June</td>
<td>777</td>
<td>27.0</td>
<td>473</td>
<td>1.5</td>
</tr>
<tr>
<td>1 July</td>
<td>1526</td>
<td>27.4</td>
<td>482</td>
<td>8.1</td>
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<tr>
<td>30 July</td>
<td>713</td>
<td>27.7</td>
<td>436</td>
<td>6.5</td>
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<tr>
<td>29 Aug</td>
<td>1195</td>
<td>28.0</td>
<td>432</td>
<td>3.1</td>
</tr>
<tr>
<td>27 Sept</td>
<td>1201</td>
<td>26.9</td>
<td>376</td>
<td>2.8</td>
</tr>
<tr>
<td>27 Oct</td>
<td>1692</td>
<td>25.9</td>
<td>282</td>
<td>11.6</td>
</tr>
<tr>
<td>26 Nov</td>
<td>1568</td>
<td>23.7</td>
<td>236</td>
<td>16.5</td>
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<tr>
<td>26 Dec</td>
<td>1229</td>
<td>24.6</td>
<td>224</td>
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<td>1982</td>
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</tr>
<tr>
<td>24 Jan</td>
<td>2878</td>
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<td>310</td>
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</tr>
<tr>
<td>23 Feb</td>
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<td>23.3</td>
<td>247</td>
<td>26.8</td>
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<tr>
<td>25 Mar</td>
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<td>307</td>
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<td>402</td>
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<tr>
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<td>386</td>
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</tr>
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<tr>
<td>16 Oct</td>
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<td>271</td>
<td>24.1</td>
</tr>
<tr>
<td>15 Nov</td>
<td>6757</td>
<td>24.3</td>
<td>206</td>
<td>12.7</td>
</tr>
</tbody>
</table>
TABLE 17

Long term mean environmental parameters at Coconut Island.

Mean irradiance is based on a 5 year (1976-1980) daily record and is compared to the monthly means for 1981 and 1982. Water temperature is a 12 year daily mean (U. S. Department of Commerce, 1970). Mean monthly sea level data for Hawaii was taken at Kewalo Basin, Honolulu (long term mean = 990 mm). Also, see Fig. 13.

<table>
<thead>
<tr>
<th>Month</th>
<th>Irradiance (cal cm^-2 d^-1)</th>
<th>Water Temp. (°C)</th>
<th>Sea Level (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>287</td>
<td>302</td>
<td>243</td>
</tr>
<tr>
<td>Feb</td>
<td>320</td>
<td>367</td>
<td>299</td>
</tr>
<tr>
<td>Mar</td>
<td>381</td>
<td>417</td>
<td>254</td>
</tr>
<tr>
<td>Apr</td>
<td>406</td>
<td>443</td>
<td>346</td>
</tr>
<tr>
<td>May</td>
<td>441</td>
<td>468</td>
<td>434</td>
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<tr>
<td>June</td>
<td>453</td>
<td>461</td>
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</tr>
<tr>
<td>July</td>
<td>443</td>
<td>483</td>
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<tr>
<td>Aug</td>
<td>420</td>
<td>424</td>
<td>331</td>
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<td>Sept</td>
<td>404</td>
<td>439</td>
<td>389</td>
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<tr>
<td>Oct</td>
<td>343</td>
<td>355</td>
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<tr>
<td>Nov</td>
<td>278</td>
<td>279</td>
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<td>Dec</td>
<td>234</td>
<td>232</td>
<td>199</td>
</tr>
<tr>
<td>Mean</td>
<td>368</td>
<td>389</td>
<td>311</td>
</tr>
</tbody>
</table>
(new moon to new moon) is listed in Table 16 along with environmental data for each lunar month. The Type Y corals complete their planulation cycle before new moon while the Type B corals begin to planulate after new moon. Therefore, new moon is a useful break point that allows comparison of monthly production in the two types.

5.3.1 Variability of planula production.
The monthly cycle of planula production is very irregular in the late winter and early spring, but becomes regular with the approach of summer (Fig. 12). Note that production was very irregular from Jan-April 1981 and again from Jan-June 1982. This probably reflects the pattern of environmental instability during these months. Meteorological conditions are extremely variable from Dec until late spring in Hawaii. The Type B corals always showed a much more drastic response to environmental disruption than the Type Y corals (Fig. 12). The Type Y corals appear to be capable of maintaining synchronization for a much longer time interval than the Type B when deprived of a lunar signal of night irradiance (Chapter 4). Much of the variation in planulation can be attributed to variations in the physical environment as discussed in the following sections.
5.3.2 Correlation and multiple regression analysis.
Analysis of the data in Table 16 demonstrated that a significant (p<0.05) relationship exists between temperature and mean number of planulae spawned per lunar cycle for the Type B corals (r=0.48). None of the other factors show a significant correlation. The Type Y did not show a significant response to temperature. Note that Type Y appeared to spawn more heavily as the water temperature dropped at the start of each winter (Fig. 12). This supports a suspicion that the Type B is a warm water coral similar to the genotype found at Enewetak, where water temperature is high (270-290°C) throughout the year (Richmond and Jokiel, 1984). In contrast, the Type Y seems to be better adapted to the rigorous environment of Kaneohe Bay and is more tolerant of temperature fluctuations (Fig. 12). The Type Y genotype is very successful throughout the bay (Stoddart, in press).

5.3.3 Differences observed between the two coral types.
In the course of the experiment certain consistent differences were observed between the two types. The morphological differences have already been described (Richmond and Jokiel, 1984) along with electrophoretic differences (Stoddart 1983, in press). The phase differences reported by Richmond and Jokiel (1984) were
again observed. The planulae of the Type Y were always about 25% to 50% larger than the Type B. Type Y planulae have a higher caloric content than Type B (R. Richmond, personal communication). Type Y corals always produced mucus when placed in the collectors, but the Type B did not. The Type Y planulae clump together, but this was very rare among Type B larvae. The Type Y larvae settle very quickly after release and attached to all available surfaces, while Type B larvae usually did not settle in the first 24 h. Differences in the amount of lipid in Type Y colonies versus Type B colonies have been observed also (J. Stimson, personal communication).

5.3.4 **Importance of environmental disturbances.**

Most of the variation in the planula production data was not explained by monthly temperature, irradiance and rainfall. During this experiment it became quite apparent that extreme environmental excursions of short duration had a severe impact on coral spawning. Some very unusual environmental events occurred during this study. The pattern of spawning (Fig. 12) was relatively uniform under normal conditions, but could be altered for several months by a single unusual environmental event. The corals often responded to a severe environmental disturbance by aborting some of their developing planulae. Fully
developed planulae of this species are over 1 mm in length and dark brown in color due to the presence of zooxanthellae. If the corals were stressed early in the reproductive cycle, the aborted planulae were approximately 0.1 mm in length and did not contain zooxanthellae. If stressed later in the cycle, the aborted planulae were larger. They were generally white in color with few zooxanthellae. The smaller aborted planulae can pass through the 180 μm mesh of the collectors. They could not be quantified accurately and were not included in the tally. Aborted planulae were, however, easily noticed in the containers and the collector cups. Few abortion episodes occurred in the first year of the survey, but the second year presented a series of severe environmental problems to the corals.

5.3.5 **Major environmental disturbances.**

Major disturbance events are indicated on Fig. 12. On Dec 14, 1980 a rainfall at time of extreme low tide stressed the corals. Both Y and B Types began aborting planulae for about one week. The source of the stress was lowered salinity rather than light (Chapter 4). Planulation in the Type B corals was severely disrupted for nearly two months (Fig. 12). Rainy overcast conditions occurred during the spring of 1981, and production of planulae by
the corals was irregular. Conditions improved in May 1981. A series of extremely low tides occurred during a period of low mean sea level from June 1-4, 1981 (Fig. 13) and exposed much of the reef flat during midday. Extensive mortality and blanching occurred among the corals (Krupp, 1984). Approximately 50% of the Pocillopora damicornis colonies on the reef flat were killed. Colonies growing in deeper depressions on the reef flat survived. Reef flat temperatures in pools of water trapped on the reef exceeded 30°C (ambient bay temperature was 26°C), so the surviving corals were stressed by the abnormally high temperature. The survivors aborted planulae for about a week. Conditions were ideal during July and Aug of 1981, but surface water temperatures throughout the bay became abnormally high during the lunar cycle of 29 Aug (several weeks at 29°C) which is about 2°C above normal (Table 17). Temperatures in this range are sublethal to this species (Jokiel and Coles, 1977), so it is not surprising that some reduction in planulation was observed. Conditions were again favorable in Oct and Nov of 1981. The year of 1981 was the warmest on record in the Northern Hemisphere (Rampino and Self, 1984). Note mean water temperatures for 1981 (Table 16) in comparison with the long term mean (Table 17).
Island reef. On Nov 24, 1982 Hurricane Iwa struck the Island of Kauai and caused extensive damage on Oahu. This was the first hurricane to strike the high islands of Hawaii in modern times. Kaneohe Bay was protected from the full impact of the storm by the Koolau Mountains, and direct damage to the windward Coconut Island reef was limited. Rainfall associated with the hurricane did influence the corals. The Y type corals again started to abort planulae and continued to release immature planulae until 2 Dec 1982. An unusually long total eclipse of the full moon occurred in Hawaii on 5 July 1982 from 2000 h to 0200 h without apparent effect on the spawning of the corals.

5.3.6 Environmental disturbance and mean sea level.
Large scale meteorological and oceanic processes produce changes in local mean sea level. These fluctuations are not related to the tide. Monthly mean sea level at Kewalo Basin, Hawaii is shown in Table 17 and Figure 13 (K. Wyrski, personal communication). Note that monthly mean sea level in Hawaii varied by over 25 cm during 1981-1982. The major reef kills described above always occurred during times when mean sea level was at its lowest. These corals normally are nearly uncovered at low tide, so a drop in mean sea level can be disastrous.
5.3.7 **Timing of disturbance within breeding cycle.**

A single environmental disturbance often had a different effect on the two coral types because they breed out of phase (Fig. 12). One type might be completing its cycle at time of disturbance and not be seriously affected. At the same time, the other type would be brooding very immature planulae and begin to abort. Development of a planula from an egg takes approximately 40 days of brooding in this species (Martin-Chavez, in press). The timing of the disturbance within the monthly breeding cycle appears to be very important. Extended periods of night cloud cover can influence each type in a different manner, depending on the state of larval development. Also, the Type Y is less sensitive to environmental disturbance (Fig. 12) and can maintain synchronization longer than the Type B when deprived of the lunar cue (Chapter 4). Therefore we observe noticeable differences in the reproductive response of the two types to the same stress event.

5.3.8 **Cost of reproduction.**

The greatest release of planulae measured in one of the 10 cm diam. colonies occurred in a "Type Y" over a 48 h period starting on 30 Dec 1980. A total of 3500 planulae were released having a dry weight of 0.25 g. This colony
was decalcified and yielded a tissue weight of 6.22 g, so this particular colony released 4% of its tissue weight as planulae in a 48 hr period. During the lunar month beginning on 15 Nov 1982 a mean monthly total of nearly 7000 planulae colony⁻¹ (nearly 8% of colony tissue weight) were produced by the "Type Y" corals (Table 16). The mean monthly production for each of the 27 full lunar cycles is extremely variable. This species annually releases a minimum of from 25% to 50% of its total colony biomass as planulae. During periods with highly favorable environmental conditions, an annual weight of planulae exceeding the total tissue weight of the parent colony probably will be released as larvae. The planulae contain a great deal of stored lipid reserves, so the total caloric content of the planulae released per year probably often exceeds the total caloric content of tissue in the parent colony. Richmond (1983) estimated that 2. damicornis at Enewetak annually releases planulae having a total caloric content that is from 50% to 180% of the total caloric content of the tissues covering the colony.

Results of this investigation support the observation that phase differences in planulation exist between different genotypes of Pocillopora damicornis occurring on the same reef as shown by Richmond and Jokiel (1984). Phase relationship was maintained throughout the 27 cycles
even after severe environmental disturbances. Furthermore, the results are consistent with the conclusion that night irradiance is the environmental factor responsible for synchronization of spawning in the populations (Chapter 4). Synchronization appeared to weaken during extended periods of heavy nighttime cloud cover. The planulation cycles stayed in phase with the lunar cycle but moved out of phase with the 27 day solar cycle. The possibility remains that changes in UV flux associated with the solar cycle influence coral reproduction. It is difficult at this time to isolate such an effect from the myriad of other factors that influence spawning.

Response of the two "Types" to the same environmental regime was quite different. This variation might have been attributed to environmental factors if not understood. Harrigan (1972) was unaware of the existence of these "Types" in Kaneohe Bay and probably included colonies of both (and probably others) in her study.

Synchronization of the two types apparently is controlled only by the lunar signal, but amplitude is influenced by a variety of parameters in a complex manner. Multiple regression analysis of the relationship between planulae produced per lunar cycle, mean temperature, mean
irradiance and rainfall (Table 16) showed a significant relationship only between temperature and planula production in Type F corals. This standard analytical approach to the problem failed to account for a large part of the variation for several reasons:

1. The impact of isolated short term disturbances is one of the most important factors controlling planulation (and mortality) on the reef flats. Random events such as the chance occurrence of a heavy rainfall at time of extreme low tide severely disrupted the population, and these phenomena are not adequately represented in the mean monthly values. In view of this, it appears that an extremely large sample would be required in order to statistically establish the importance of such environmental factors.

2. Analysis is complicated because the relationships probably are not linear in most cases. For example, reproduction and temperature are not linearly related in this species (Jokiel and Guinther, 1978). The optimal temperature for this species was exceeded in Aug-Sept 1982. Even short exposure to abnormally high temperatures will cause premature release of planulae (e.g. Edmondson, 1946; Harrigan, 1972).
3. The factors being evaluated (light, temperature, salinity) act synergistically on corals in a non-linear fashion (Coles and Jokiel, 1978).

4. Light, temperature and rainfall vary with each other. Months of low temperature are generally months of low irradiance and high rainfall.

5. The most challenging problem is the observed chaotic response due to random environmental disturbances. The severity of impact was apparently related partially to degree of development of the planulae at the time of disturbance. This response was different for both Types.

6. Tidal influences and sea level were not considered in the preliminary regression analysis and it is not clear which aspects of the tides should be considered (range, LLW, MLW etc.). As shown earlier, mean sea level fluctuations are very important. Also, this species releases planulae diurnally at time of low tide (Holloran and Witteman, in press).

7. Water motion influences reproduction in this species, (Jokiel, 1978) but was not measured.

8. Sampling error accounts for an unknown portion of the variation observed.
More sophisticated time series analyses of daily data are presently being carried out in collaboration with F. J. Martinelli in an attempt to develop the analytic tools that might resolve some of these issues. Correlation analysis does not establish cause and effect. Controlled laboratory experiments will be needed to establish the relative importance of the various environmental parameters.
CHAPTER 6
SUMMARY CHAPTER

6.1 INFLUENCE OF SPECTRUM AND INTENSITY WITHIN THE ULTRAVIOLET REGION

1. Growth rate was approximately 50% higher when corals were grown in sunlight without solar UV radiation as compared to sunlight with solar UV radiation. Reproduction was curtailed and concentration of the "S-320" UV-blocking pigments was lower in the treatment without UV radiation.

2. The zooxanthellae grown in vivo did not appear to be damaged by full spectrum solar irradiance. Photosynthetic pigment concentration and number of cells per unit area were similar in treatments with UV radiation and without UV radiation. In contrast, unshielded in vitro cultures of zooxanthellae were severely impaired by the presence of solar UV radiation. Apparently the S-320 UV-blocking pigments protect the algae and the host from damage by solar UV radiation.

3. The impact of UV radiation was much greater on zooxanthellae isolated from the "shade-loving" anemone Aiptasia than it was on the zooxanthellae isolated from the "sun-loving" scyphozoan Cassiopea. Genetic differences in the algae as
well as hosts seem to be involved in the
photoadaptation of symbioses to different regimes of
solar irradiance.

4. Both the 320-400 nm portion to the UV spectrum
(UV-A) and the 280-320 nm band (UV-B) produced
significant growth photoinhibition in cultures of
zooxanthellae grown in vitro.

5. PAR produced little or no growth inhibition in
zooxanthellae and other microalgae even at full
intensity surface irradiance.

6. Growth rate of zooxanthellae is reduced by solar UV
radiation, but several species in free-living
microalgae were found to be extremely resistant.

7. Growth photoinhibition became increasingly severe
above 20% of surface intensity in the zooxanthellae
and other algae that were sensitive to UV
radiation. PAR flux in excess of that required for
maximum growth was not inhibitory. The higher
levels of UV radiation associated with high
intensity PAR reduced growth rate in some species
of algae.

8. Results of the microalga growth experiments
support a previous suggestion that other molecules
besides chl sensitize and/or shield algae within
the UV radiation portion of the spectrum.
6.2 **INFLUENCE OF SPECTRUM AND INTENSITY WITHIN THE PAR REGION.**

9. Corals and zooxanthellae grown under equal PPDF, but under different regimes of spectral distribution within the PAR region, showed quite different growth and pigment response. Therefore, wavelength as well as intensity must be considered to be important in studies of long-term photoadaptation.

10. Corals, *in vivo* zooxanthellae and *in vitro* cultures of zooxanthellae all showed the same overall generalized response pattern to PAR of different spectral composition. Growth was generally highest in the "blue" treatments and lowest in the "red" treatments. The "white" and "green" treatments gave an intermediate response. Extreme reduction of pigment, pigment per cell, and number of algal cells occurred in red PAR.

11. These data suggest that long-term photoadaptation may involve photochemical processes that are controlled by intensity of irradiation in the blue portion of the PAR. The enigmatic "blue light syndrome" exerts a strong influence on the photobiology of corals and their contained algae. Lack of blue wavelengths created long-term adaptation problems for the corals and the
zooxanthellae. An alternative hypothesis is that irradiance in the red region of the PAR spectrum is inhibitory over the long term to certain photochemical processes.

6.3 CANOPY DEVELOPMENT AND THE P-I RELATIONSHIP: INTENSITY AND MODULATION EFFECTS.

12. The branched reef coral *Pocillopora damicornis* and the branched red macroalga *Acanthophora spicifera* show changes in the P-I relationship with increasing size. Maximum net rate of oxygen production (Pm), light saturation constant (Ik) and night dark oxygen uptake rate (-R because R is a negative number by convention) all increased with increasing size, but -R increased at a lower rate than Pm. Therefore the P to R ratio (Pm/-R) was increased with canopy height.

13. Large increases in understory chl accompanied increases in size. The initial slope alpha of the chl specific P-I curve as well as Pm per unit chl decreased with increasing canopy-understory development.

14. Integrated daytime oxygen production (Pn) increased more rapidly in relation to size than did nighttime oxygen consumption. Consequently, Pn/Pn along with daily net primary production (integrated net daily
oxygen flux) increased dramatically with increasing canopy development.

15. Arborescent development in these phototrophic organisms is advantageous in shallow environments that receive a high incidence of solar radiation, but may be maladaptive in deeper low-light environments.

16. Changes observed in the P-I relationship with increasing size are consistent with previous models describing sun and shade adaptive processes at other levels of organization. Previous workers dealt mainly with changes in the photosynthetic apparatus at the molecular level as well as changes in chloroplasts, individual cell organization, and changes in branch tip or leaf function with changes in photic environment. This investigation extends these generalizations of sun and shade photoadaptation to higher levels of organization in photosynthetic systems. The integrated response of an intact canopy system is such that "sun adaptiveness" increases with increasing height and development.

17. Primary production in substrate limited reef communities can be increased many fold through arborescent development.
18. The reef coral *Pocillopora damicornis* has long been known to release planular larvae on a lunar cycle, but the forcing function was previously unknown.

19. (*Cyphastrea ocellina* and *Tubastrea coccinea*) did not show lunar periodicity in larvae release rate.

20. Corals exposed to an artificial lunar cycle that was out of phase with the natural night irradiance cycle (i.e., exposed to artificial irradiance of full moon intensity during new moon and held in complete darkness during nights of full moon) shifted reproductive periodicity. Reproduction quickly moved out of phase with the natural field populations exposed to a natural lunar cycle. The "Y Type" corals that normally start releasing larvae at actual full moon with peak production at first quarter began releasing planulae at actual new moon (apparent full moon) with peak production at third quarter. The "B Type" corals that normally start releasing larvae at actual new moon with a peak production at third quarter began to release larvae at time of full moon (apparent new moon) with peak production at time of the first quarter.
21. Corals grown without night irradiance (constant new moon) did not show evidence of monthly periodicity. Corals exposed to constant night irradiance at full moon intensity during every night of the month did not show a monthly periodic component.

22. Possibly, a relatively simple photochemical process regulates planula production in this coral. Many precedents are known among terrestrial plants.

23. Recruitment of new corals onto aquarium surfaces under conditions of continuous night irradiance was very low, even though larvae production was highest in this treatment. This suggests that a period of darkness is required for metamorphosis.

6.5 **IRRADIANCE AND CORAL REPRODUCTION: A FIELD STUDY**

24. Synchronization of reproduction apparently is controlled only by night irradiance, but number of larvae released per lunar cycle is influenced by a multitude of interacting environmental factors.

25. Impact of isolated short term environmental disturbances is extremely important and adds a great deal of random variation to the planulation data.

27. The complexity of the observed response to the multitude of environmental factors demonstrated the comparative usefulness of controlled experimentation (Chapters 1-4) in establishing cause and effect.
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