Joslyn, A.

AN ANALYSIS OF INCREASED TEMPERATURE AND ULTRAVIOLET RADIATION AS CAUSES OF CORAL BLEACHING

by

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A CRITICAL ESSAY

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CHAPTER I

INTRODUCTION

Symbiosis, the cohabitation of two or more different organisms, is represented across species as well as phyla. A unique symbiosis has developed in the marine environment between the phylum Cnidaria and members of the Division Dinophyta ("zooxanthellae"). The relationship is one of mutualism, as both organisms benefit from the relationship. Although cnidarians, such as sea anemones, hard and soft corals, scyphozoans, and hydrocorals, comprise the majority of the hosts that take part in this symbiosis, some nudibranchs and sponges also contain endosymbiotic dinoflagellates. Alternatively, some cnidarians may contain chlorophyte endosymbionts. The symbionts are usually held within vacuoles inside the host endoderm cells (Glider et al., 1980; Trench, 1987), but they have also been found within the epidermis and mesoglea of some cnidarians.

Zooxanthellae can be acquired by the host in one of two ways: a) through the egg from parent to offspring or b) from the external environment. The first mechanism, known only in the Cnidaria (see Table 1), requires the parent's investment of symbiotic dinoflagellates into the egg or egg plasm, and the eventual uptake of the dinoflagellates into the endodermal cells of the developing embryo. The second mechanism requires the larval or adult stage to be

TABLE 1. Two methods of symbiont acquisition by several invertebrate hosts. (From Trench, 1987)

	References
(A) Direct transmission via the egg or larvae (closed systems)	
Hydrozoa	
Millepora sp.	Magnan 1909
Myrionema amboinense	Fraser 1931; Trench 1981
Velella velella	Kuskop 1921; Brinkmann 1964
Aglaophenia pluma	Faure 1960
Alcyonaria	
Eunicella stricta stricta	Theodor 1969
Actiniaria	
Anthopleura sp.	Atoda 1954
Scleractinia	
Six species	Durden 1902
Pocillopora damicornis	Atoda 1947a
*Stylophora pistillata**	Atoda 1947b
Porites spp. (4 species)	Kojis 1982
(B) From the ambient environment, by post-larval stages (open systems)	
Scyphozoa	
Mastigias papua	Sugiura 1963, 1964
Cassiopeia andromeda	Ludwig 1969
C. xamachana	Bigelow 1900; Trench *et al.* 1981a
Alcyonaria	
Eunicella stricta aphyta	Theodor 1969
Briarium asbestinum	Kinzie 1974
Pseudopterogorgia elizabethae	Kinzie 1974
P. bipinnata	Kinzie 1974
Actiniaria	
Anthopleura elegantissima	Siebert 1974
A. xanthogrammica	Siebert 1974
Aiptasia tagetes	R. D. Steele, pers. comm.
A. pulchella	G. Muller-Parker, pers. comm.
Scleractinia	
Favia doreyensis (= *F. pallida*)	Marshall & Stephenson 1933
Acropora bruggemanni	Atoda 1951
Pocillopora meandrina	J. Stimpson, pers. comm.
Fungia scutaria	Krupp 1981
Goniastrea australensis	Kojis & Quinn 1981
Astrangia danae	Szmant-Froelich *et al.* 1980
Turbinaria mesenterina	B. Willis, pers. comm.
Acropora formosa	J. Oliver, pers. comm.
Platyhelminthes	
Amphiscolops langerhansi	Taylor 1971b
Mollusca	
Corculum cardissa	Kawaguti 1950
Tridacna squamosa	LaBarbera 1975; Fitt & Trench 1981
T. derasa	Beckvar 1981
T. maxima	LaBarbera 1975; Jameson 1976
T. gigas	Beckvar 1981
T. crocea	Jameson 1976
Hippopus hippopus	Jameson 1976; Fitt *et al.* 1984

* Rinkevich & Loya (1979) report no algae in eggs of *S. pistillata*, but algae were present in the brooded planulae.

reinfected by endosymbionts directly from the water column. Zooxanthellae may develop motile forms or be dispersed by water currents, and through chemotactic behavior may gain access to the host (Trench, 1987). This mechanism occurs in members of the Cnidaria, Platyhelminthes, and Mollusca (see Table 1).

The dinoflagellate produces organic compounds via photosynthesis which are translocated to its host as glycerol, glucose, or alanine (Brusca and Brusca, 1990). Muscatine et al. (1981) estimated the daily contribution of carbon from zooxanthellae to coral (Pocillopora damicornis) by calculating the amount of carbon fixed by the algae, the percentage translocated to the host, and the carbon required daily by the host. They concluded that 63-70% of the respiratory carbon of the host is provided by the symbiont. The remainder of the carbon needed by the host is acquired through intake of particulate organic carbon or absorption of dissolved organic carbon.

In addition, the scleractinian corals, which form hard calcium carbonate skeletons, benefit further from the association because the dinoflagellates aid in increasing the rate of calcium carbonate deposition (Fig. 1). $Calcium^{+2}$ is actively transported into the cells of the coral polyp. Within the coral calicoblastic epidermis, carbonic acid is concentrated as a by-product of calcium carbonate deposition thereby decreasing the pH within the

Possible pathways of calcium and carbonate during calcification in a hermatypic coral. In this diagrammatic cross section of the calicoblastic body wall at the base of a polyp, the parts are not drawn to scale. The coelenteron and the flagellated gastrodermis containing a zooxanthella are shown at the top of the figure, the calicoblastic epidermis is in the middle, and the organic membrane with crystals of calcareous matter is at the bottom. The direction of growth is upward (i.e., calcium deposition is in a downward direction). C.A., carbonic anhydrase. (After Goreau 1959.)

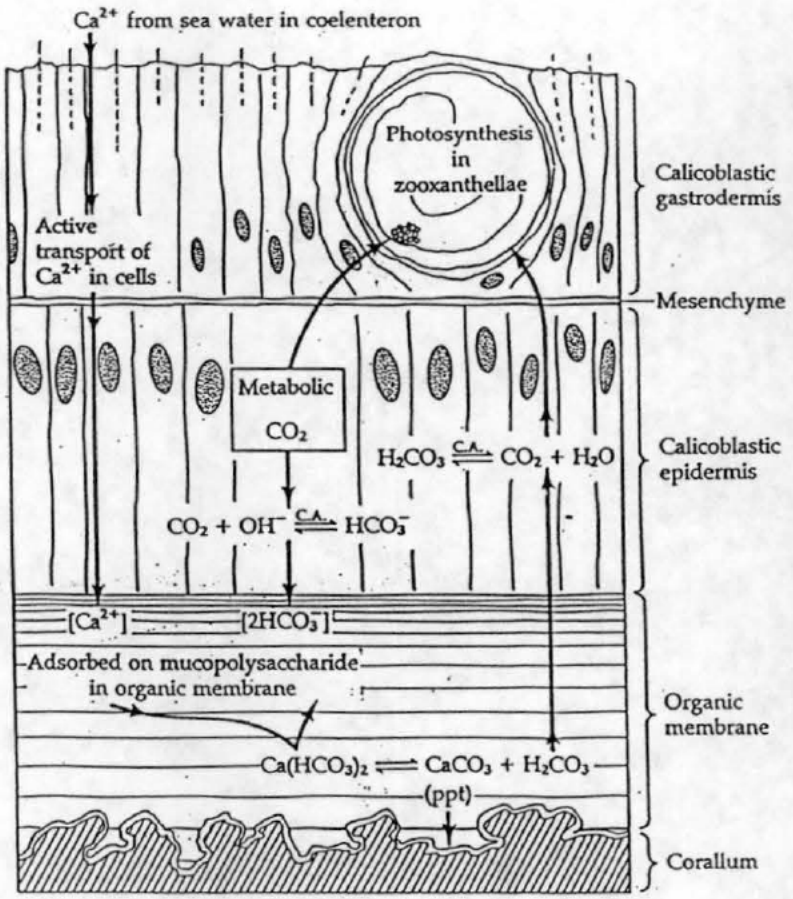


Figure 1. Possible pathways of calcium and carbonate during calcification in a hermatypic coral

(From Brusca and Brusca, 1990)

coral tissue. Carbonic acid itself may slow down deposition or dissolve calcium carbonate. However, the zooxanthellae utilize carbon dioxide and water, the constituents of carbonic acid, during photosynthesis. By removing carbonic acid and thus increasing the pH within the coral tissue, the symbionts increase the rate of calcium carbonate deposition. If the zooxanthellae were not present, calcium carbonate deposition rates would be reduced. The zooxanthellae allow the equilibrium to be shifted in favor of calcium carbonate deposition by removing carbonic acid and therefore increasing the pH within the coral tissue.

Benefits to the dinoflagellates include the availability of the host's excretory products, which contain nitrogen and phosphorus and can be utilized by the zooxanthellae as nutrient sources. Since most dinoflagellates are auxotrophic, they may require specific external organic compounds (usually 3 vitamins: B_{12}, thiamine, and biotin) in small amounts presumably as catalysts (Gaines and Elbrächter, 1987). Although Symbiodinium microadraticum, a common cnidarian endosymbiont, does not require B_{12}, thiamine, or biotin (Hastings and Thomas, 1977; Loeblich, 1966), it may require another organic compound such as a vitamin from the animal host. In addition, the host's carbon dioxide, a by-product of respiration, can be fixed by the dinoflagellates during photosynthesis. Jokiel (1984) has also suggested that the

animal host may produce effective UV-absorbing ectodermal pigments that may protect the symbiotic dinoflagellates from harmful UV radiation. Therefore, by participating in the symbiosis, the alga benefits from protection against UV radiation it would otherwise not receive in its free-living state. Under normal conditions, the association between host and zooxanthellae is quite stable and beneficial to both organisms.

In 1987, coral reef communities world-wide suffered a drastic change. Corals that normally appeared brown or green acquired white patches. Loss of coral pigmentation or "coral bleaching" was attributed to the dissociation of dinoflagellate and animal host. Since coral tissue without endosymbionts is clear, a white calcium carbonate skeleton was seen through the animal body. Localized, small-scale bleaching events are not unusual. However, data collected on global coral bleaching demonstrated that the 1987 event was the most severe and widespread ever observed (Bunkley-Williams and Williams, 1990). Bleaching was evident on the Great Barrier Reef in Australia, northern Caribbean, the Bahamas, southern Florida, and subsequently in the southern Caribbean. In addition, data also showed that widespread bleaching had occurred two other times: 1979-1980 and 1982-1983. Subsequent research focused on the possible causes of large-scale bleaching.

In this essay, I will begin by describing the

endosymbionts and further defining coral bleaching. Then, I will concentrate on two possible causes of bleaching that have been the subject of numerous studies: increased temperature and increased ultraviolet radiation as it relates to oxygen toxicity. Lastly, I will discuss my thoughts on these two issues in relation to global warming, ozone layer depletion, and El Niño Southern Oscillation and possible directions for future research.

CHAPTER II

DESCRIPTION OF ENDOSYMBIONTS

In 1962, Freudenthal described the zooxanthellae isolated from the scyphozoan, Cassiopea sp., and named it Symbiodinium microadriaticum. It was thought that endosymbiotic zooxanthellae of most host species were members of this single species. In 1979, Kinzie and Chee alluded to the idea that the zooxanthellae were in fact different "strains" of the same species. They isolated symbiotic dinoflagellates from a scyphozoan, two molluscs, and one nudibranch, and then experimentally reinfected anemones of the genus Aiptasia with dinoflagellates from different original hosts. They then compared the growth rates of the anemones using change in number of tentacles as an indicator of growth. After allowing the experiment to proceed 200 days, they discovered that the anemones grew

differentially depending on the "strain" of dinoflagellate they were infected with. They concluded that not only do strains of endosymbiotic dinoflagellates exist, but also that they differ in their ability to enhance the growth of the host.

More recently, Rowan and Powers (1991) utilized restriction length fragment polymorphisms in nuclear genes that encode small ribosomal subunit RNA to compare dinoflagellates isolated from 131 individuals from 22 host taxa. Speculations on zooxanthellae taxonomy included two hypotheses: 1) a single, pandemic zooxanthella species, Symbiodinium microadraticum or 2) one zooxanthella taxon for every host taxon (Rowan and Powers, 1991). Rowan and Powers (1991) discovered ten alga genotypes distributed among 22 host taxa, suggesting an intermediate hypothesis concerning algal-host specificity. They concluded that considerable variability exists within the genus Symbiodinium. In addition, corals in the same family may have dissimilar zooxanthellae and similar alga can be isolated from corals in different families. The possibility that the hosts are capable of acquiring a combination of algal symbionts and may be reinfected when rendered asymbiotic is interesting given the recent coral bleaching events.

Buddemeier and Fautin (1993) addressed this possibility with a hypothesis that coral bleaching has the following attributes: 1) physiological responses of the symbiotic unit

(host and dinoflagellate) are primarily characteristic of the combination, rather than of the host or symbiotic algal partner alone; 2) multiple types of both zooxanthellae and host species are commonly available on reefs; 3) bleaching provides an opportunity for the host to be repopulated with a different type of partner; and 4) altered environmental conditions favor establishment of combinations of symbionts that were less adaptive under previous conditions.

Buddemeier and Fautin (1993) referred to evidence provided by Rowan and Powers (1991) that animal hosts may be infected by more than one type of algal host and by Kinzie and Chee (1979) that hosts may grow differentially when infected by different algal symbionts. Moreover, they suggested that the more severe the bleaching, the more likely the corals are to be repopulated by algae from the external environment, resulting in a higher probability of change.

In conclusion, although bleaching may represent instability in the short term, it promotes long-term stability by enhancing survival chances of both zooxanthellae and hosts under conditions that are not those of the prestress environment (Buddemeier and Fautin, 1993).

CHAPTER III

CORAL BLEACHING

Coral bleaching can result from a decrease in the amount of chlorophyll *a* and accessory pigments per zooxanthellae cell, a reduction in the population density of zooxanthellae, or both (Gates et al., 1992). Kleppel et al. (1989) quantitatively studied the changes in pigmentation in bleached and normal stony coral, Montastrea annularis. They estimated that chlorophyll *c*, peridinin, and diadinoxanthin levels were 35, 17, and 20 times higher, respectively, in normal corals than in bleached ones. In addition, loss of zooxanthellar pigment from remaining endosymbiotic cells accounted for 72% of the decrease in chlorophyll *c* (Kleppel et al., 1989). They concluded that not only do bleached corals contain less zooxanthellae, but also the zooxanthellae within bleached tissues contain less pigment.

In response to environmental stresses, endosymbiotic zooxanthellae may be expelled which may lead to animal host death. As seen in Figure 2, zooxanthellae could be released in one of five ways: a) exocytosis of the algal cell, b) programmed host cell death, c) release of algal cell and host cell contents, d) pinching off vacuole containing algal cell and plasma membrane, and e) detachment of entire endodermal cell with endosymbiotic algal cell (Gates et al., 1992). Gates et al. (1992) utilized Aiptasia pulchella, a

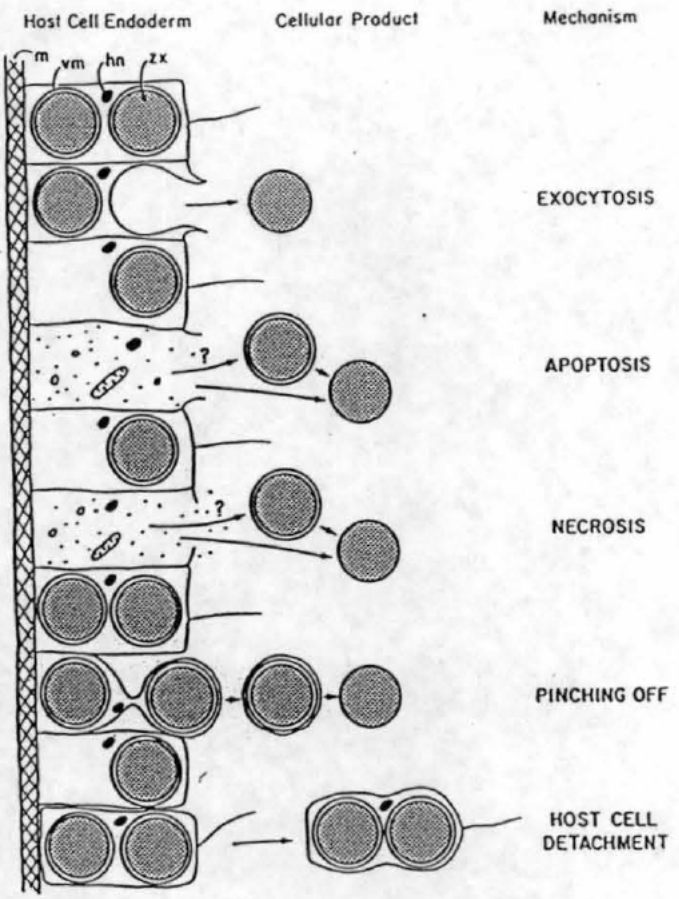


Figure 2. Five potential ways by which zooxanthellae could be released from the endoderm of cnidarians

(From Gates et al., 1992)

tropical sea anemone, and Pocillopora damicornis, a reef coral, to discern what actually happens to the zooxanthellae during a bleaching event. As described more fully in the next section, they thermally stressed these two organisms and discovered that intact host cells were released rather than portions of cells.

Isolated instances of coral bleaching have been observed and attributed to high and low temperature, subaerial exposure, calm sea conditions, freshwater dilution, high and low turbidity, sedimentation, storm shock, high and low light levels, UV radiation, parasite infections, and pollutants (Bunkley-Williams and Williams, 1990). For example, RÜtzler et al. (1983) investigated the black band disease of several Western Atlantic shallow reef corals. They discovered that the cyanophyte, Phormidium corallyticum, had invaded susceptible corals wherever lesions or stress had weakened the ectoderm. The cyanophyte progressed along the coral head in a characteristic black band leaving behind a patch of white coral skeleton. Unlike coral bleaching which also leaves behind white areas of coral skeleton seen through clear coral tissue, this disease actually destroys coral tissue. A microbial mat may develop under the cyanophyte creating an anoxic, sulfide-rich environment. Sulfide poisoning may be the cause of coral polyp mortality (Castenholz, R., 1994, personal communication).

Sedimentation can also cause coral death in a number of ways including smothering and increasing the likelihood of bacterial infection. Corals may suffer from decreased light availability, reduced ability to capture food, abrasion, and decreased calcification rates, which can be attributed to increased sedimentation rates (Stafford-Smith, 1993). However, the ability of a coral to withstand the effects of sedimentation depends on each coral species' ability to actively remove and reject sediments, coral morphology, and sediment size. Different species of corals have been shown to react differently to the same sedimentation environment. Lastly, sedimentation events can be very localized and can have differing effects on different coral species ranging from mild bleaching to mortality.

The expulsion of zooxanthellae may also lead to a reduction in growth rates in certain scleractinian corals (Goreau and MacFarlane, 1990). Goreau and MacFarlane (1990) conducted a study on the growth rate of Montastrea annularis following a 1987-8 coral bleaching event in Discovery Bay, Jamaica. They measured growth over three months by implanting stainless steel nails in the uppermost surface of the coral heads. Their results indicated that unbleached colonies grew at a mean rate of 0.0149 mm/day and bleached corals grew at a significantly different mean rate of 0.0057 mm/day. Although these numbers seem small, the mean rate of unbleached corals translates to a rate close to 6

mm/annum which is normal for the area (Goreau, 1977).

Since most bleached corals were reinfected by normally colored zooxanthellae by May, 1988, but ceased to accrete skeleton, Goreau and MacFarlane (1990) suggested that the bleaching of M. annularis inhibited calcium deposition more than photosynthetic translocation. Without zooxanthellae, calcium deposition was diminished, leading to reduced growth rates. Since almost all the bleached corals in this area eventually recovered, the lack of nutrition, otherwise received from the endosymbiotic zooxanthellae, was not enough to lead to this species' mortality. However, Goreau and MacFarlane (1990) did not measure the rate of photosynthesis of the dinoflagellates, so it is difficult to conclude that differential growth rates of bleached versus unbleached corals was due to a decline in calcium deposition rather than the absence or low levels of photosynthates received by the host from the symbiotic dinoflagellate. A reduction in the amount of fixed carbon translocated to the host would also eventually reduce the growth rate of the host. In addition, calcium carbonate deposition and photosynthetic translocation are correlated. The decline in zooxanthellar pigments or numbers of endosymbiotic zooxanthellae will decrease photosynthetic translocation as well as hinder the removal of carbonic acid which slows down calcium carbonate deposition. Thus, it is difficult to separate the two processes and justify a single process as

the culprit of slow growth.

Thermal Stress

In recent years coral bleaching events have often been attributed to increases in seawater temperature. Thermal stress can affect the host as well as the endosymbiotic dinoflagellate. Iglesias-Prieto et al. (1992) conducted a study on the effects of thermal stress on Symbiodinium microadriaticum cultured in isolation from any host species. They monitored the photosynthetic response of the dinoflagellate to elevated temperatures. Cells were grown at 26°C and then exposed to an increase in temperature from 20 to 35°C (intervals of 5°C) over a total period of 45 minutes. Oxygen evolution and whole cell fluorescence data were used as measures of photosynthesis.

Their results indicated that photosynthesis is impaired at 30°C and stops at approximately 34°C. Beyond 34°C, the cells continued to respire but did not photosynthesize. They hypothesized that photosynthesis may have been hindered by changes in the lipid characteristics of the thylakoid membranes caused by the increased temperatures. They further suggested that coral bleaching may be the result of a decrease in the flow of nutrients to the host and consequent breakdown of the association. Since the cell culture was exposed to such a wide range of temperatures in

such a short period of time it is difficult to say that the dinoflagellates would react in the same manner under field conditions in which the temperature only increases slightly over a much longer period of time. In addition, conditions experienced by dinoflagellates in culture are much different than conditions experienced in host tissues. Regardless, the results suggest that the level of translocation of photosynthates to the host may be reduced under abnormally high seawater temperatures due to hindered photosynthesis.

Gates et al. (1992) conducted another study on coral bleaching as a result of thermal stress. In this instance, they thermally stressed Aiptasia pulchella, a sea anemone, and Pocillopora damicornis, a soft coral, and observed changes in dinoflagellate concentrations and host bleaching. They exposed A. pulchella and P. damicornis to cold temperature (12°C), 13°C below normal seawater temperatures for these organisms, for 2.5 and 4 hours, respectively. In addition, they monitored the effect of warm temperatures by exposing the cnidarians to 32°C seawater, 7°C above their ambient temperature, for 16 hours. Water surrounding the organisms was sampled and checked microscopically every hour. Their research shows that the hosts release their own endodermal cells containing dinoflagellates when thermally stressed with either sub- or super-ambient temperatures. Once released, the host cells degrade rapidly. Gates et al. (1992) did not address the fate of the dinoflagellates but

indicated that all that is left in the seawater are isolated algal cells. The transmission electron micrographs also indicated that the algal cells are intact once expelled.

Host cell adhesion dysfunction may have been the cause of zooxanthellae and endodermal host cell expulsion. Gates et al. (1992) speculated that temperature stress may cause denaturation of proteins responsible for cell adhesion. To confirm this statement, Gates et al. (1992) cited works by Suzuki and Choi (1990) and Watson and Morris (1987). The former dealt with the repair of cryogenic injury and the latter with cold shock injury in animal cells. In general, these situations would probably not pertain to bleaching since bleached corals usually experience increased seawater temperatures. Thus, the temperatures experienced by corals during bleaching events would probably not cause denaturation of proteins. However, temperature-induced bleaching may be due to changes in membranes and their related proteins rather than protein denaturation. They concluded that other environmental variables such as sedimentation, salinity changes, and ultraviolet radiation also cause bleaching but the mechanisms behind these may be very different. They further suggest that coral bleaching needs to be redefined according to cause and mechanism.

It is difficult to make a jump from these observations to natural episodes of coral bleaching. The cnidarians were exposed to very low and very high temperature changes for

short periods of time. In the field, seawater temperatures have been known to rise over periods of weeks and when doing so only increase 1-3°C above expected seasonal temperatures. These results do not demonstrate conditions that would be apparent in the field. In addition, coral bleaching need not be the result of one discrete event. A set of mechanisms may be the cause of coral bleaching due to a combination of environmental changes. Therefore, it is hard to pinpoint a single cause, such as temperature changes, to coral bleaching. In this case, the cnidarians were shocked into releasing their endosymbionts so the mechanisms of expulsion under laboratory conditions will not necessarily correlate to what happens in the field. However, Gates et al. (1992) has been one of the few research projects that has actually suggested a mechanism for the expulsion of algal cells. The instability of cell membranes and their related proteins at increased temperatures leading to cell adhesion dysfunction may be a possible way of explaining the sloughing off of endodermal cells.

In contrast Glynn and D'Croz (1990) conducted a thorough study over a period of ten weeks on the effects of thermal stress on the coral, Pocillopora damicornis. Coral individuals were collected at two sites off the Pacific coast of Panama, the Uva Island in the Gulf of Chiriqui and the Uraba Island in the Gulf of Panama. Four temperature treatments were set up per site and the actual mean

experimental temperatures were: Uraba corals (ambient, 27.8; 28°C, 28.4°C; 30°C, 29.6°C; and 32°C, 31.6°C) and Uva corals (ambient, 26.2°C; 26°C, 26.4°C; 28°C, 27.8°C; and 30°C, 30.3°C). The corals were maintained in laboratory tanks and heated seawater was delivered to each tank according to treatment. Their results showed a significantly lower number of zooxanthellae per coral area in the corals exposed to the higher temperatures (30°C and 32°C). In addition, they recorded significantly lower chlorophyll *a* per zooxanthellae cell in the corals exposed to higher temperatures (Fig. 3). Observations included severe bleaching in the 32°C treatment tank with mortality of all corals occurring by four weeks. The 30°C treatment group also exhibited bleaching with mortality occurring by nine weeks.

In comparison to the work of Gates et al. (1992), Glynn and D'Croz (1990) used more naturally occurring conditions. The experiment was conducted over a longer period of time, ten weeks, and individual corals were maintained at four different temperatures over a narrow temperature range from ambient to 30°C or 32°C as would be seen in the field during a warm temperature cycle. Comparisons can be drawn from all temperature treatments and also from site to site. All other factors were kept constant between treatments, and the control group (see ambient temperature regression lines in Fig. 3) showed no detrimental signs of being in the

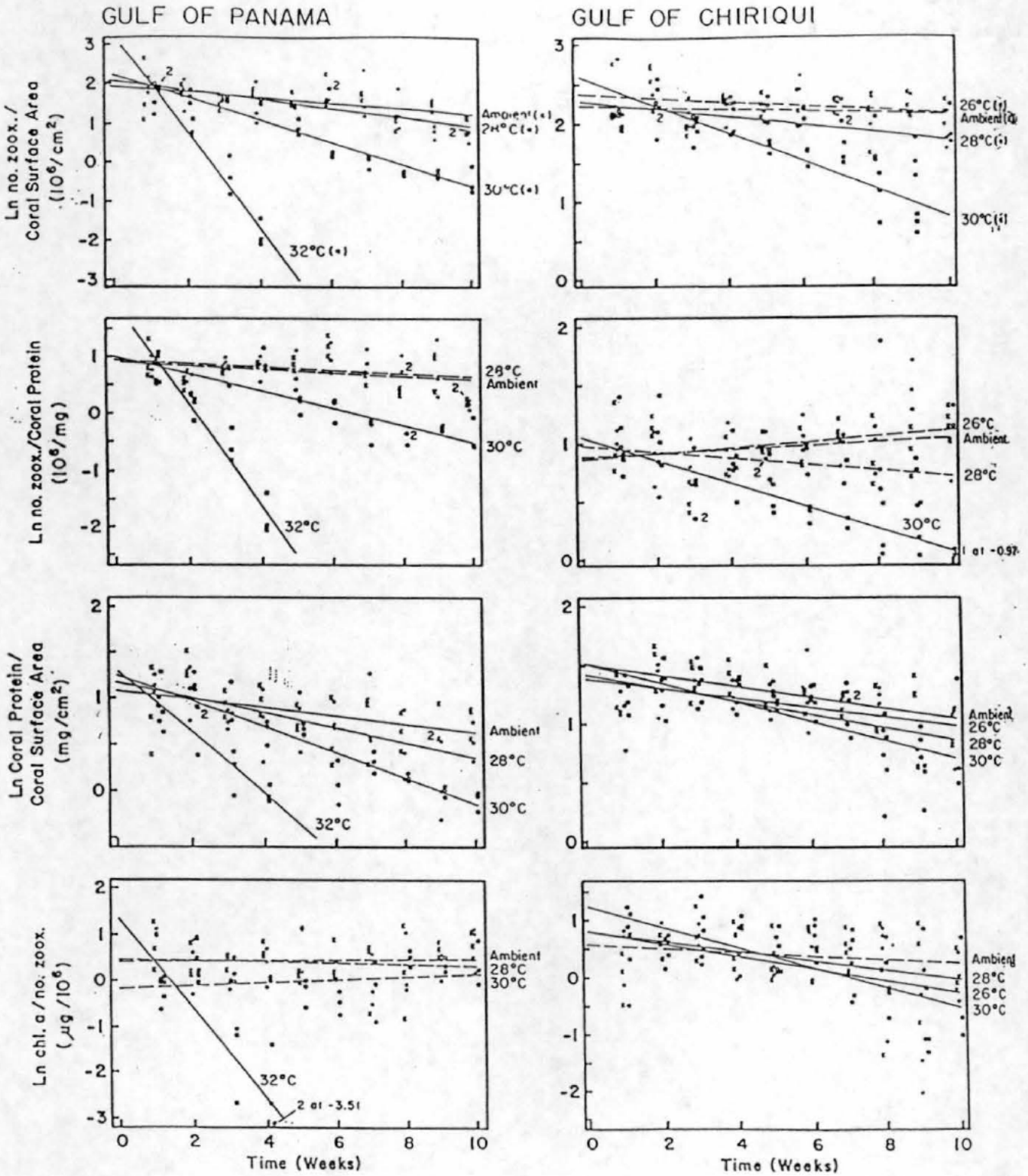


Figure 3. Zooxanthellae densities, coral tissue protein, and chlorophyll *a* concentrations in experimental coral colonies (at four temperatures) from the Gulf of Panama and the Gulf of Chiriqui.

(From Glynn and D'Croz, 1990)

laboratory tanks, except perhaps with regard to coral protein / surface area. Glynn and D'Croz (1990) did not address the decline in coral protein / surface area but this may be due to lack of food and thus unfavorable laboratory conditions for the corals. Unlike the work of Gates et al. (1992), Glynn and D'Croz (1990) did not suggest a mechanism for coral bleaching but only prove that bleaching and death occurs at elevated temperatures. However, Glynn and D'Croz (1990) observed the following lesions in the coral tissues in both experimental groups with increasing time and temperature: deterioration of epidermal mucous secretory cells, erosion of epidermal and gastrodermal cell layers, atrophy of longitudinal retractor muscles, loss of mesogleal pleat structure, and the appearance of necrotic nuclei in calicoblastic layer and epidermal and gastrodermal cells. It appears that the expulsion of zooxanthellae may have been due to host cell necrosis and/or the loss of host cells, which may indirectly demonstrate cell adhesion dysfunction.

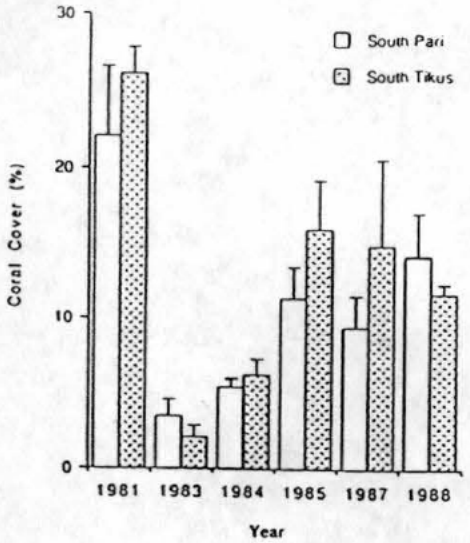
A long-term study on coral community changes was carried out at an Indo-Pacific location in the Thousand Islands, Indonesia, from 1979 through 1988, during which an El NiÑo event took place. From March to August, 1983, mean monthly sea surface temperatures were 2-3°C higher than those recorded in 1984, 1985, and 1986 (Brown and Suharsono, 1990). Coral bleaching was first observed in April, 1983, followed by the deaths of species of Acropora and

Pocillopora. It was estimated that 40-50% of the existing reef flat corals were bleached, of which 80-90% died by May, 1983. Corals affected by bleaching included scleractinians, hydrocorals, and soft corals; in total 70 species from 33 genera (Suharsono and Kiswara, 1984). Coral cover and number of living coral colonies were estimated and the difference between 1981 and 1983 was drastic (Fig 4).

Brown and Suharsono (1990) suggested that increased seawater temperatures were the major cause of bleaching on shallow water reef tops in the Thousand Islands in 1983. This project is one of the few research projects in which the period of study actually brackets an El NiÑo event. Seawater temperatures increased four to six weeks before bleaching of corals was observed. More long-term studies are needed to attribute major coral bleaching events to specific causes such as increased seawater temperatures. Moreover, controlled experiments, combined with the monitoring of other environmental variables are needed before other causes of coral bleaching can be ruled out.

Increased Ultraviolet Radiation and Oxygen Toxicity

Dykens and Shick (1982) investigated the effect of increased oxygen production of endosymbiotic dinoflagellates on the host's superoxide dismutase (SOD) levels. SOD breaks down harmful oxygen radicals ($\cdot O_2^-$) within host tissues.



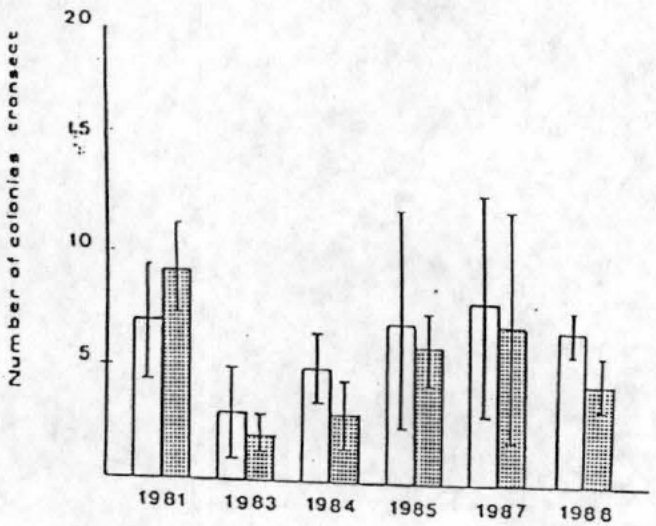


Figure 4. Average coral cover and coral colonies per transect over the period 1981-1988 at South Pari and South Tikus

(From Brown and Suharsono, 1990)

Animals do not normally encounter increased O_2 pressures unless they are inhabited by algal endosymbionts (Dykens and Shick, 1982). When photosynthesizing, the algal species produces more oxygen than the host can use. Therefore, hosts must presumably increase the levels of enzymes such as SOD to inactivate harmful oxygen radicals. Oxygen radicals may induce lipid peroxidation, depolymerize hyaluronate, inactivate ribonuclease, and destroy phagocytized bacteria (Dykens and Shick, 1982).

Dykens and Shick (1982) discovered that SOD activity is positively correlated with chlorophyll content of symbiotic anemones and that host SOD activities are altered in response to the levels of oxygen generated by endosymbiotic dinoflagellates. These findings suggest that this symbiosis may sometimes have deleterious effects. This is an interesting concept in terms of coral bleaching: The host may respond to increased levels of oxygen by losing its dinoflagellates.

Otto Siebeck (1988) conducted a laboratory study on the effects of ultraviolet radiation (280 - 400 nm) on scleractinian corals of various genera. He collected corals from two depths, 0-1.5 meters and 18-20 meters, and exposed them to a variety of light conditions. He measured the effect of different light regimes by calculating the value LD_{50}, the UV dose at which 50% of the corals irradiated died. A mortality of 20% was observed in corals exposed to UV

radiation and subsequent irradiance with 450 nm visible light, instead of 100% mortality in corals exposed to UV radiation and subsequent darkness. His experiments showed that corals at 0-1.5 m depths are more UV tolerant than corals at 18-20 m depths, and that UV-induced damage (280 - 400 nm) can be reversed by subjecting the coral to a wavelength of 450 nm. Siebeck (1988) observed the extrusion of mesenterial filaments as a result of UV exposure, but did not explain coral bleaching in terms of dinoflagellate expulsion or loss of pigment. In this case, he might have implied that coral death with the extrusion of mesenterial filaments indicated a form of coral bleaching. He also observed an increase in dissolved organic carbon (DOC) in the water a few hours after UV exposure ceased. This may have indicated expulsion of symbionts, unattached host endodermis, and/or chlorophyll *a* expulsion, but Siebeck did not pursue this matter or attempt to identify its source. Siebeck concludes that depth dependent differences in LD_{50} may reflect differences in the concentration of UV-absorbing compounds.

Siebeck's experiment did not try to mimic naturally occurring conditions. The corals were first exposed to UV radiation alone, and then to either darkness or artificial daylight for 48 hours. Neither of these light regimes corresponds to spectra reaching these corals in nature. It can be concluded that UV radiation alone does harm corals

and that UV harms corals normally found at depth (18-20 m) more than corals found in shallower waters (0-1.5 m). Because Siebeck (1988) did not discriminate between UV-A and UV-B, no conclusions can be drawn about what type of UV radiation causes coral bleaching. Another aspect of the study indicated that when the corals were exposed to short wavelength visible light (450 nm) after UV radiation exposure, the corals somehow repaired the damage caused by UV radiation through photoreactivation. Therefore, under natural circumstances where UV radiation and visible light, both components of natural sunlight, irradiate the coral concurrently, the corals might not show the detrimental effects of UV radiation because they might be repaired simultaneously by short wavelength visible light. Lastly, Siebeck (1988) could have measured or observed characteristics of corals exposed to non-fatal doses of UV radiation. This information may have shed some light on the detrimental effects of UV radiation on surviving corals. The results of this study were rather confusing, but it may be concluded that UV radiation alone does have detrimental effects on corals.

Lesser and Shick (1989) conducted a study on photoadaptation and defenses against oxygen toxicity in dinoflagellates removed from cnidarian hosts. As mentioned earlier, reactive oxygen itself can oxidize membrane lipids and cause proteins and nucleic acids to denature (Fridovich,

1986). Dinoflagellates and other algae have developed ways to protect their photosynthetic components from oxygen radicals which are more harmful than oxygen alone. The enzymes SOD, catalase (CAT), and ascorbate peroxidase (ASPX) in host and zooxanthellar "tissues" function together to inactivate oxygen and hydrogen peroxide thereby preventing the formation of HO· and subsequent cellular damage (Lesser and Shick, 1989). Lesser and Shick (1989) stated that the production of defensive enzymes might represent an unwarranted energetic cost to the host. However, if the host produced UV-absorbing ectodermal pigments that shaded the zooxanthellae from harmful UV radiation and prevented photoinhibition (loss of photosynthetic capability), the host would continue to benefit from the association regardless of the costs.

Lesser and Shick (1989) measured chlorophyll *a* and c_2 levels in dinoflagellates isolated from various coral species and found that these pigment contents were inversely related to the levels of irradiance (UV radiation and visible light: 280-700 nm) at the site of collection. The enzymes, SOD, CAT, and ASPX, also showed increased activity with an increase in irradiation. In addition, Lesser and Shick (1989) conducted transplant experiments in which they exposed Aiptasia pallida individuals usually found in dim light to bright light and vice versa. Then, freshly isolated zooxanthellae were obtained by homogenizing

specimens. They found that these isolated zooxanthellae from transplanted A. pallida individuals exhibited phenotypic plasticity. That is, the levels of SOD, CAT, and ASPX increased with increasing light and decreased with decreasing light.

This study gives a good overview of oxygen toxicity within dinoflagellate tissues and the plasticity exhibited by dinoflagellates in response to changes in irradiance. However, it is still not clear what happens to host tissues under increased levels of oxygen radicals. Trench (1987) has suggested 3 mechanisms, which may indirectly change oxygen radical levels within the host, relating to the regulation of algal numbers: 1) the host cell divides in 'synchrony' with the algal population, 2) the host eliminates excess algae by exocytosis or digestion, and 3) the host cell 'controls' increases in algal population by withholding critical nutrients. The first mechanism has been refuted and the question of digestion of algae remains controversial (Trench, 1987). However, the host may change its "chlorophyll content" by expelling its own symbionts (Trench, 1987) or by withholding inorganic nitrogen which seemed to limit algal growth (Falkowski et al., 1993). It is possible that bleaching events are the host's response to increased oxygen toxicity of host tissues due to changing environmental conditions such as increasing irradiance which includes ultraviolet radiation.

Lesser et al. (1990) followed up the above study on dinoflagellates with a study on bleaching in coral reef anthozoans in response to ultraviolet radiation (280 - 400 nm), irradiance (photosynthetically active radiation = PAR; 400-700 nm), and temperature. Increases in temperature, or highly energetic UV radiation, can increase the production of oxygen radicals, particularly in photosynthetic tissues. Then oxygen toxicity could potentially initiate a bleaching event (Lesser et al., 1990). In addition, for symbiotic cnidarians exposed to high irradiances (PAR) and ultraviolet radiation, the additional exposure to sublethal temperatures could result in photoinhibition and irreversible damage to the photosynthetic machinery of the symbiont. Photoinhibition may occur at high temperatures due to a decrease in photosynthetic electron transport. This, combined with the continued absorption of excitation energy in the presence of molecular oxygen, can cause the production of active oxygen radicals which will harm the photosynthetic apparatus (Asada and Takahashi, 1987).

Lesser et al. (1990) exposed the zoanthid Palythoa caribaeorum and its endosymbionts to four different treatments (HUV: high visible irradiance with UV; DUV: low visible irradiance with UV; HWO: high visible irradiance without UV; and, DWO: low visible irradiance without UV) at low (26°C) and high (31°C) temperatures as seen in Figure 5. They then isolated zooxanthellae, assayed enzymes

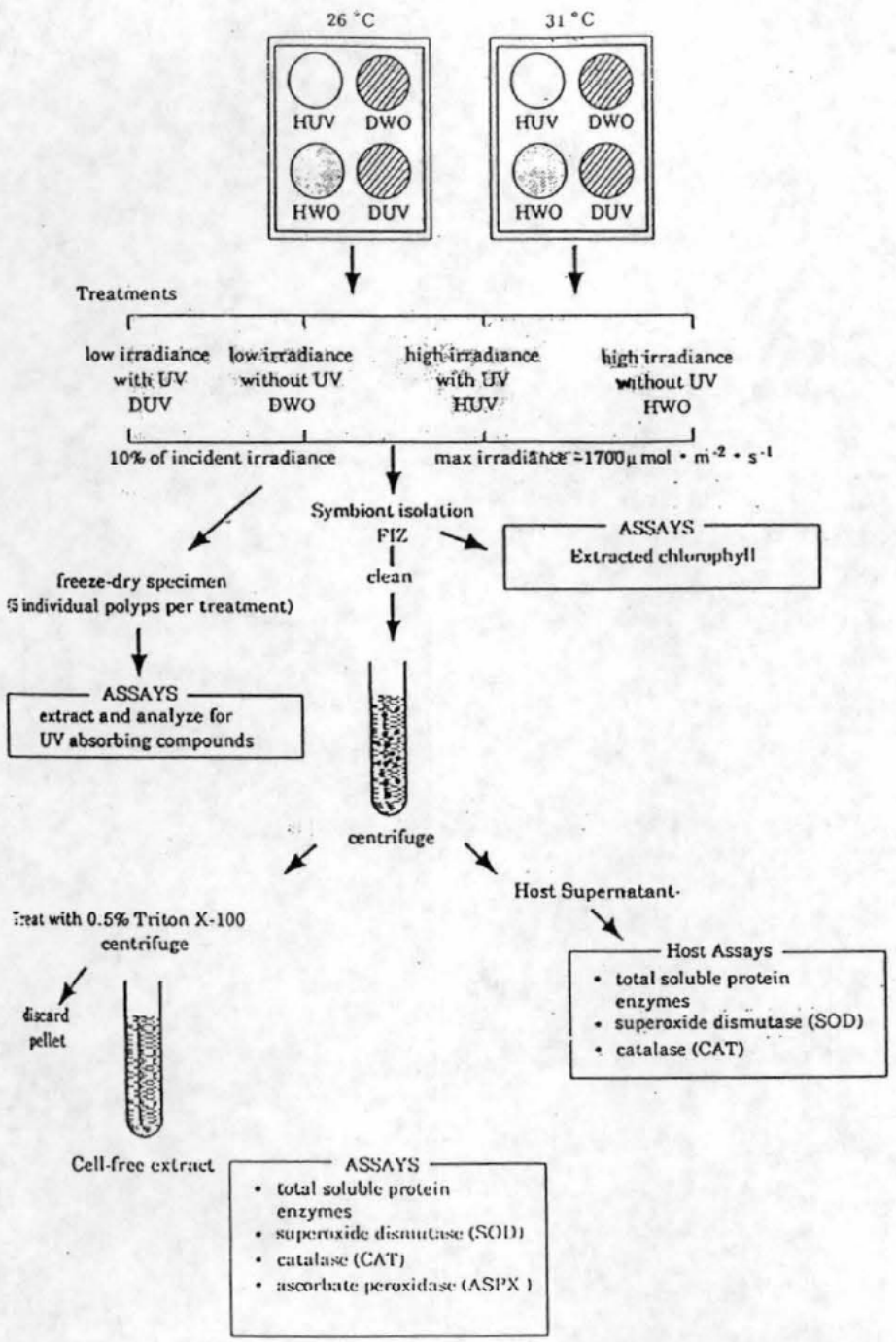


Figure 5. Flow chart of experimental design and analysis for the host and freshly isolated zooxanthellae of Palythoa caribaeorum

(From Lesser et al., 1990)

responsible for protection against oxygen toxicity in host and isolated zooxanthellae, and measured the levels of ultraviolet absorbing compounds in the host. Their results indicate that the number of zooxanthellae per polyp decreased with increased temperature and ultraviolet radiation exposure, tested separately, but not with increasing irradiance (PAR). In addition, all chlorophyll contents showed a significant decrease with increased temperature, and no statistically significant change in chlorophyll contents with or without ultraviolet radiation. Correspondingly, there was a significant interaction between irradiance (PAR) and ultraviolet radiation. SOD activity within the host increased only at high visible irradiance including ultraviolet radiation. In the host, CAT activities showed a significant increase with increased temperature but no effects from ultraviolet or visible irradiance, tested separately.

Lesser et al. (1990) did not measure the production of active forms of oxygen and argued that the relationship between the production of active forms of oxygen and the induction of specific antioxidant enzymes is well established (Fridovich 1981; Asada and Takahashi 1987). One of the major findings was that within the host, the highest activities of SOD as well as the lowest numbers of zooxanthellae were observed in the high visible irradiance/ ultraviolet radiation treatment regardless of high or low

temperature conditions. This may indicate that high oxygen production by the symbiont has occurred as a result of ultraviolet radiation exposure and high visible irradiance. Although ultraviolet radiation alone may cause bleaching, ultraviolet radiation in conjunction with high visible irradiance causes bleaching while increasing the production of SOD which scavenges $\cdot O_2^-$. Neale (1987) demonstrated that including a significant UV component in the photoinhibitory treatment magnifies the effects (photooxidation of the reaction centers as well as oxidation of electron transport intermediates) seen with a similar intensity of only visible light. In addition, photoihibition may be exacerbated by other stresses including high temperatures (Ludlow, 1987). Interestingly, many species of algae lose sensitivity to visible light photoinhibition when adapted to high light, but such adaptation cannot increase tolerance to high UV radiation (Jokiel, 1984). That is, some algal species may adapt to changes in visible light levels, but most algae cannot tolerate changes in UV radiation levels.

However, when looking at the high and low temperature treatments not exposed to ultraviolet radiation, there were significantly less zooxanthellae per polyp in the high treatment as opposed to the low temperature treatment. This indicates that temperature also plays a role in zooxanthellae expulsion. These results were probably not

related to oxygen toxicity, but rather may have been a physical response to increased temperatures. High temperatures may damage host tissue, as seen in the previous section, allowing zooxanthellae to be sloughed off. Increased temperatures may have also damaged the photosynthetic apparatus thereby decreasing photosynthate translocation.

The specific signal that induces this expulsion is presently unknown (Lesser et al., 1990). High PAR or high temperature may damage the thylakoid membrane and increase the permeability of hydrogen peroxide and $\cdot O_2^-$ which may be exported to host tissues. Another signal proposed by Lesser et al. (1990) is the decrease in photosynthates delivered to the host by weakened zooxanthellae.

If Siebeck (1988) and Lesser et al. (1990) are compared, their results may seem contradictory at first glance. Siebeck (1988) demonstrated a 20% mortality rate of corals exposed to UV radiation and subsequent artificial daylight for 48 hours. Lesser et al. (1990) observed that most bleaching occurred in zoanthids exposed for 10 days to UV radiation and visible light concurrently. In addition to differences in methodology, there are others differences in the two experiments: 1) zoanthids were collected from the low intertidal, less than one meter depth (Lesser et al., 1990); 2) scleractinian corals were collected from 0-1.5 m and 18-20 m (Siebeck, 1988); 3) Siebeck (1988) did not

collect data on coral bleaching; and 4) Lesser et al. (1990) did not observe mortality of zoanthids. Since the zoanthids in Lesser et al. (1990) did not die, they may have been exhibiting some form of photoreactivation by being exposed to visible light and UV radiation simultaneously. In addition, the zoanthids, which were collected from the low intertidal, may have been more adapted to changes in visible irradiance and UV radiation than the corals which were taken from 0-1.5 m and 18-20 m depths. Thus, without observations of bleaching in Siebeck (1988), it is impossible to demonstrate whether these two experiments contradict each other.

With the exception of not measuring oxygen levels within host tissues, Lesser et al. (1990) have produced a well organized study that encompasses temperature, ultraviolet radiation, and irradiance (PAR) changes with enough treatment groups to be able to compare their effects on zoanthid and symbiont. Effects of ultraviolet radiation and temperature can be looked at alone or together. Ultraviolet alone may induce bleaching as well as high temperatures alone, regardless of light levels. However, SOD activities will only increase if ultraviolet radiation is coupled with high visible irradiance.

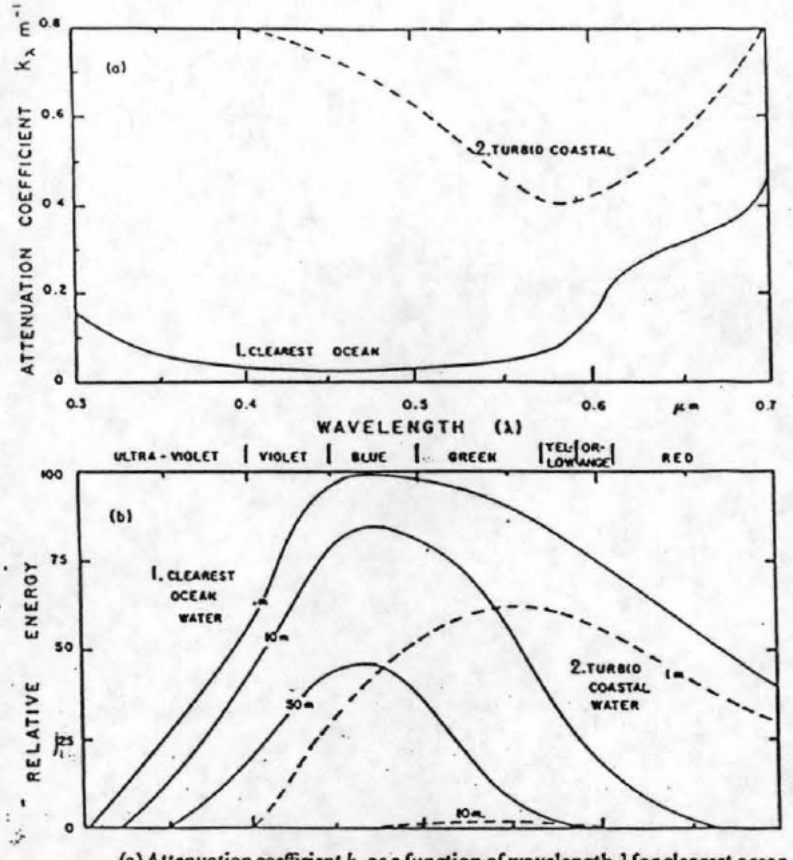
Gleason and Wellington (1993) conducted a field experiment on San Salvador Island in the Bahamas, on the stony coral Montastrea annularis. They experimentally

transplanted boulders from 24 meters depth to 12, 18, and 24 meters depth. At each depth, coral colonies were exposed to ambient ultraviolet radiation or protected from natural ultraviolet radiation by an acrylic cover which blocked only ultraviolet radiation and transmitted visible light. Ultraviolet radiation levels found at 12 m occur at other times of the year at 24 m when waters are calmer and clearer. Therefore, this experiment mimicked conditions that actually occur naturally. Water temperatures were also monitored during the study and were found to be normal for the area for that time of year.

Their results showed that coral colonies transplanted to 12 m began to show bleaching signs within seven days and corals moved to 18 m and 24 m did not bleach during the course of the 21 days of the study. The number of zooxanthellae per cm^2 for colonies at 12 m was significantly lower than for any other treatment group. Zooxanthellae densities and chlorophyll contents were not significantly different between UV-excluded treatments at 12 m or 24 m. As a result they concluded that bleaching was a result of increased ultraviolet radiation and not increased visible irradiance.

In water, light is attenuated by absorption and scattering. Absorption of longer wavelengths (red visible light) is greatest in water, and shorter wavelengths (blue light) are the least absorbed in water (Lee, 1989). In open

oceanic waters the percentage of transmitted irradiance per meter is higher for blue light than for any other visible light. In coastal waters, however, green-yellow visible light has the highest percent transmitted irradiance per meter (Lee, 1989). In general, the percentage of surface irradiance of a given amount reaches deeper in oceanic waters than in coastal waters. As a result, since corals inhabit tropical waters which are clear and calm, the corals will be most affected by blue light which will penetrate the deepest. Ultraviolet radiation (UV-A and -B), on the other hand, does not penetrate turbid coastal waters but can penetrate clear ocean waters to a great extent with UV-A (320-400 nm) radiation penetrating to almost the same extent as blue light (see Fig. 6), and thus can affect coral communities. In addition, Jerlov (1950) concluded that clear ocean water is very transparent to short wave-length solar radiation and that solar UV radiation must be significant biologically in the upper photic zone of oceanic regions. Gleason and Wellington (1993) tabulated the UV-B (300-320 nm) and UV-A (320-400 nm) radiation intensities measured in W/m^2 for 12, 18, and 24 meter depths as seen in Table 2.



(a) Attenuation coefficient k_λ as a function of wavelength λ for clearest ocean water (full line) and turbid coastal water (dashed line). (b) Relative energy reaching 1, 10 and 50 m depth for clearest ocean water and reaching 1 and 10 m for turbid coastal water.

Figure 6. Attenuation coefficients and relative energy in relation to wavelength in turbid coastal and clearest ocean waters

(From Pickard and Emery, 1990)

Table 2. UV-B and UV-A light intensities (W/m^2) for 12, 18, and 24 meter depths

Date	Depth(m)	UV-B	UV-A
Sept.	12	0.32	15.70
Sept.	18	0.12	10.06
Sept.	24	0.05	7.59
July	24	0.19	18.17

Synthesis and accumulation of UV-absorbing compounds known as mycosporine-like amino acids (MAA) are means by which corals may possibly neutralize damaging UV-B (280 - 320 nm) radiation (Gleason and Wellington, 1993). However, since there has been no evidence for the protective effect of MAAs, this study only suggested a correlation between high MAA content and exposure to ultraviolet radiation. This study indicated that MAA concentrations are lower in coral colonies at depth than ones in shallower waters. In addition, Gleason and Wellington (1993) suggested that Montastrea annularis was unable to counter rapid increases in ultraviolet radiation intensities through rapid production of MAAs.

Gleason and Wellington (1993) concluded that an increase in UV radiation intensity due to anthropogenic ozone depletion will be minimal at low latitudes and should not be a factor in bleaching, but an increase in the frequency or duration of exceptionally calm (doldrums) and clear water conditions, which accompany El Niño Southern

Oscillation events, could lead to a greater penetration of UV radiation and UV-induced bleaching. In addition, increased ultraviolet radiation causes the expulsion of zooxanthellae, where as warm seawater temperatures may cause both decreased pigments within symbionts and the expulsion of the endosymbiont.

Gleason (1993) conducted a 104 day study on the distributional pattern of green and brown morphs of Porites asteroides Lamarck. Green coral colonies were significantly more abundant than brown ones on the shallow forereef (≤ 2 m deep) where UV intensities were high (Gleason (1992) as cited in Gleason (1993)). He hypothesized that green and brown morphs may differ in the types and quantities of UV protective pigments present within tissues. Gleason (1993) transplanted green and brown colonies from 6 m to 1 m and exposed each to three treatment groups: 1) exposure to ambient UV radiation present at 1 m (no cover); 2) UV-blocked (polycarbonate cover that blocks all UV-A and UV-B but transmits 92% of visible light); and 3) cover control (acrylic cover transparent to 70-84% UV-B, 85-91% UV-A and provided control for any shading and other effects associated with the cover).

Gleason (1993) demonstrated that by transplanting coral colonies to 1 m depth, the corals were exposed to an increase of 305% UV-A intensity and 640% UV-B intensity. His results indicated that, although not significantly

different, brown P. asteroides exposed to ambient UV radiation at 1 m exhibited lower numbers of zooxanthellae; and, that green morphs showed similar zooxanthellae densities in all treatments. Using a linear skeleton extension method to measure growth, Gleason (1993) demonstrated that brown morphs protected from UV radiation grew significantly more than brown colonies exposed to UV radiation; and, that green P. asteroides in all three treatment groups grew the same amount. Exposure to UV radiation also caused significant photoinhibition of the algal mitotic index in brown colonies. Green morph mitotic indices were not altered by irradiance with UV (Gleason, 1993).

In addition, Gleason (1993) measured levels of four mycosporine-like amino acids (MAAs): mycosporine-glycine (310 nm), playthine (320 nm), asterina-330 (330 nm), and shinorine (334 nm). His results indicated that brown individuals taken from 6 m had significantly more mycosporine-glycine on average and that green morphs harbored more asterina-330. After transplantation, brown and green colonies changed similarly. Levels of asterina-330 and shinorine showed significantly higher concentrations in both morphs. Gleason (1993) indicated that this effect was primarily due to enhanced UV radiation. When analyzing the overall quantities of MAAs in transplanted corals, brown morphs had significantly greater amounts of mysosporine-

glycine and green morphs had significantly greater amounts of asterina-330.

Gleason (1993) concluded that UV radiation may contribute to the pattern of zonation observed for the two morphs of P. asteroides. In addition, the wavelengths responsible for photoinhibition in brown colonies appeared to be concentrated near the boundary between UV-A and UV-B (310-350 nm) because it is the light range where extracts from the green morphs exhibited higher light-blocking efficiency. Gleason (1993) also clarified that the changes in MAA concentrations may have been in the endosymbiont, the host, or both, since coral core samples assayed contained both organisms.

Gleason and Wellington (1993) and Gleason (1993) are good examples of experimental conditions that manage to mimic natural conditions. They distinguished between high irradiance (PAR) and increased ultraviolet radiation and come to sound conclusions about increased UV radiation. They did not however distinguish between the effects of UV-A and UV-B. In the work by Gleason and Wellington (1993), increased UV radiation caused expulsion of zooxanthellae but increased visible irradiance alone did not. In the study by Gleason (1993), increased UV radiation caused decreased growth rates and an altered zooxanthellar mitotic index only in the brown morph of P. asteroides. Additionally, Gleason (1993) managed to correlate the UV radiation effects to the

levels and types of MAAs found within the brown and green morphs of P. asteroides. Gleason and Wellington (1993), Gleason (1993), and Lesser et al. (1990) gave a good overview of the causes of coral bleaching in terms of varying physical conditions that may be experienced in nature.

CHAPTER IV

CAUSES OF INCREASED TEMPERATURES AND ULTRAVIOLET RADIATION: GLOBAL WARMING, OZONE LAYER DEPLETION, AND EL NIÑO

Since 1983 large scale coral bleaching has been observed. Areas affected during two events (1982-83 and 1986-88) included the Caribbean, eastern Pacific, Hawaiian, Central American, and Australian coral reefs (Fig. 7). These events have received much attention, and have led to hypotheses about global warming and the depletion of the ozone layer. These two phenomena may cause increased seawater temperatures and increased levels of UV-B radiation, respectively.

Six "greenhouse" gases including carbon dioxide, water vapor, methane, ozone, nitrous oxide, and human-produced chlorofluorocarbons (CFCs), delay heat from leaving the earth by trapping it in the lower atmosphere. The amount of heat trapped varies with the concentration of the gases in the atmosphere. Recent human activities including burning wood, coal, oil, and gas; deforestation; and the use of

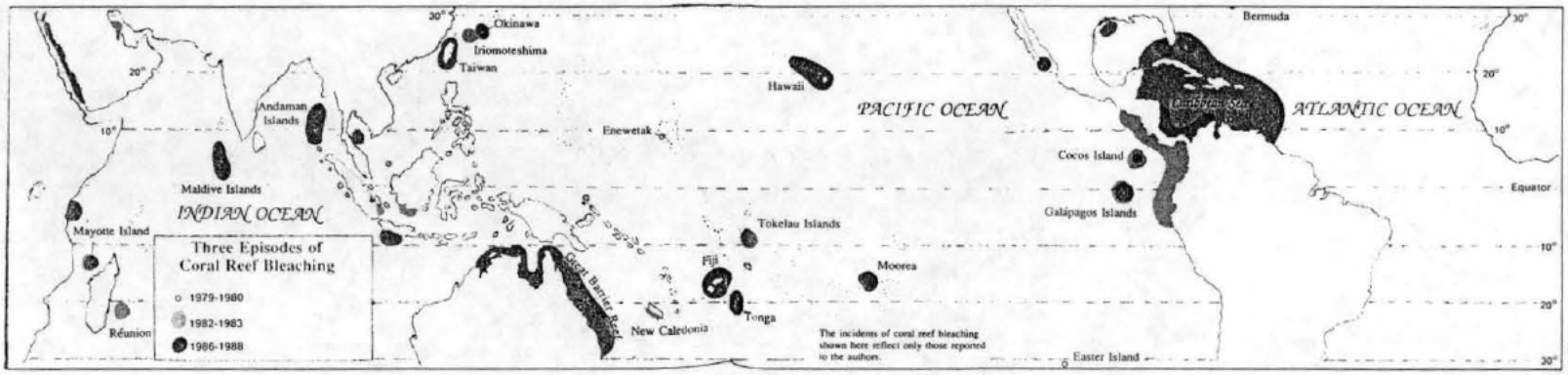


Figure 7. Areas affected by large scale coral bleaching

(From Bunkley-Williams and Williams, 1990)

CFCs, have increased the concentration of these gases in the atmosphere. Over the past 100 years, the temperature of the earth's surface as a whole has increased by ≈0.5°C (Jones et al., 1988; Hansen and Lebedeff, 1988, as cited in Mann and Lazier, 1991). With an increase in surface temperatures, the concentration of water vapor in the atmosphere increases. This, in turn, increases the earth's surface temperature and creates a positive feedback loop. Global surface warming could increase seawater temperatures. However, there is no present evidence that global warming is causing seasurface temperature anomalies like the El NiÑo Southern Oscillation, which will be discussed later (Mann and Lazier, 1991).

Another global pattern in the atmosphere is the depletion of the ozone layer. Ozone forms when UV-C (190-280 nm) radiation from the sun strikes oxygen molecules in the atmosphere. The ozone layer absorbs about 99% of the UV-B (280-320 nm) radiation that would otherwise strike the planet's surface (Postlewait and Hopson, 1992). Recently, atmospheric ozone concentrations have been declining and the amount of UV-B radiation reaching the earth has been increasing. Scientists have detected a huge hole in the ozone layer over Antarctica. They have also discovered a 3-5% thinning in the ozone layer worldwide (Postlewait and Hopson, 1992). Human use of CFCs to propel substances from spray cans, to create plastic-foam containers and cushions,

and as coolants in refrigerators and air conditioners, has increased the concentration of CFCs in the atmosphere. CFCs convert ozone back to oxygen thus destroying the ozone layer. The depletion of the ozone layer may result in an increase in UV-B radiation reaching the earth's surface. Therefore, a greater amount of UV-B radiation may penetrate the earth's oceans.

An alternate hypothesis that has gained support is the occurrence of repeated and long-lasting El NiÑo events. The El NiÑo event begins with an oscillation in the atmospheric pressures over the equatorial Pacific called the Southern Oscillation. During the cool phase, high atmospheric pressure exists over the southeastern Pacific and low atmospheric pressure over Indonesia. Trade winds along the equator blow westward due to the presence of the low pressure zone over the western Pacific Ocean. The easterlies cause ocean waters to be pushed towards the west and offshore from the coast of South America. The end result is coastal upwelling of cold, nutrient-rich waters. In the warm phase of the El NiÑo Southern Oscillation (ENSO) cycle, a decrease in the trade winds as well as a decrease in the east to west pressure gradient is experienced. This causes an increase in thermal stratification of the surface waters and a deepening of the thermocline. Waters in the eastern Pacific are still upwelled but they come from warmer sources above the thermocline and are therefore relatively

nutrient-poor and clear allowing penetration of UV radiation. This event causes temperature anomalies which may persist from one to six months (Fig. 8). In the severe El NiÑo of 1982-3, the maximum temperature anomaly was about 6°C (Mann and Lazier, 1991).

An increase in the frequency and severity of El NiÑo events, resulting in large scale increases in temperature and clarity of surface waters could explain the large-scale bleaching of corals observed during the past decade. As already discussed, both of these conditions (increased temperatures and increased UV transmission) have been identified in lab and field experiments as causes of coral bleaching. In the Southeastern Pacific, ENSO events may have caused warmer seawater temperatures over long time periods. In the Caribbean, ENSO events may have caused doldrums which created calmer and clearer conditions, thereby increasing penetration of ultraviolet radiation (UV-A (320-400 nm) and UV-B). As a result, the oceanographic conditions occurring during ENSO events create environmental situations conducive for the appearance of coral bleaching.

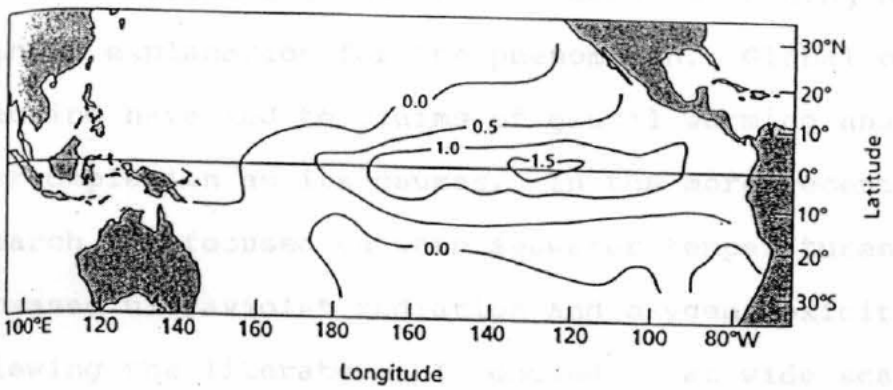


Figure 8. Temperature changes from the equatorial Pacific Ocean, averaged from six El Niño events between 1951 and 1973. Isopleths indicate change in temperature.

(From Mann and Lazier, 1991)

CHAPTER V

DISCUSSION

Most scientific research on coral bleaching has sought a single explanation for the phenomenon. Global patterns of bleaching have led to claims of global warming and ozone layer depletion as its causes. In the more recent past, research has focused on warm seawater temperatures and increased ultraviolet radiation and oxygen toxicity. After reviewing the literature, I conclude that wide scale bleaching is likely the result of environmental changes working in concert.

Gates et al. (1992) showed decisively that warm temperatures cause the detachment of host endodermal cells with endosymbionts. They hypothesized that cell adhesion dysfunction is the mechanical effect of increasing temperatures. However, their laboratory experiments were restricted to short time scales (hours) and drastic temperature changes that cannot be compared to natural conditions. Glynn and D'Croz (1990) achieved coral bleaching over a longer time scale (weeks) and lower temperature ranges, thus more closely mimicking conditions in the field. Their results showed that both the numbers of zooxanthellae and the pigment concentration per algal cell declined with increasing temperature. Lastly, Brown and Suharsono (1990) observed and described bleaching in the

field over a much longer (decadal) time scale and implicated warm seawater as the cause but their data do not allow other possible causes to be ruled out.

It is well documented that warmer seawater temperatures can lead to coral bleaching. However, more research needs to focus on the way in which zooxanthellae are expelled, such as the question of whether it is a physical mechanism like cell adhesion dysfunction. More research could be devoted to the chemical signals received by the host that commence bleaching. Several hypotheses have been postulated including a reduction in photosynthate received by the host and damage to host tissue by oxygen radicals. These could be tested by measuring the change in concentration of photosynthate translocated to the host or by measuring the amount of oxygen radicals formed inside the host during a bleaching event. Based on present evidence, three large-scale bleaching events have been correlated to different El NiÑo events. However, oceanographic processes like the ENSO event, which may lead to warmer seawater temperatures and increased transmission of ultraviolet radiation, should be monitored in conjunction with future bleaching events to better document the correlation between the two. Other parameters such as high irradiance, sedimentation, and the presence of pollutants, need to be monitored as well so that these parameters can be excluded or included as causes of bleaching.

The research on increased ultraviolet radiation and oxygen toxicity as causes of coral bleaching seems more compelling. From the works of Siebeck (1988), Gleason and Wellington (1993), and Gleason (1993), it can be concluded that coral bleaching and subsequent mortality can be caused by increased ultraviolet radiation, and that corals that grow at depth are more prone to its deleterious effects. Lesser et al. (1990) provide the best data in terms of increased temperatures, increased ultraviolet radiation, and changes in visible irradiance. In addition to demonstrating the effects of all these different parameters acting alone and in concert, they have suggested a biochemical mechanism underlying coral bleaching. Lesser et al. (1990) allude to the translocation of hydrogen peroxide or $\cdot O_2^-$ or the reduction of photosynthate to host tissues as signals for initiating a bleaching response in the coral. High temperatures and high ultraviolet levels can produce highly active forms of oxygen. In addition, photoinhibition may result from high visible and UV irradiance, accompanied by sublethal temperature increases. This also may cause the production of oxygen radicals. As a result, it is becoming more and more evident that despite its benefits, coral/dinoflagellate symbioses may also have deleterious effects under extreme changes in the environment.

Research has indicated that some responses to UV radiation, like increases in MAA levels, are slow to none in

the short term (Gleason and Wellington, 1993), but in the long-run MAA levels do increase (Gleason, 1993). Responses to increases in visible irradiance, such as the increase in SOD levels, can be very pronounced (Lesser et al., 1990). In their 21 day study, Gleason and Wellington (1993) showed that MAAs were not accumulated rapidly enough to counter rapid changes in UV radiation levels. Gleason (1993) showed that MAA levels increased in 2 morphs of Porites asteroides. Green morphs, which were found in shallower waters, did not suffer from increased UV radiation. But, brown morphs, which were usually found at greater depths, demonstrated detrimental effects as a result of higher UV radiation intensities. In terms of the overall amounts of MAAs, green morphs had higher quantities of asterina-330 and brown morphs had higher quantities of mycosporine-glycine. It is possible that the type of MAA accumulated is an adaptation to increased UV radiation levels. However, as stated earlier, there is no direct evidence that MAAs are capable of providing UV protection.

In conjunction with higher seawater temperature, the concentrations of MAAs may decrease, suggesting some thermal lability, decrease in production, or some other process by which the rate of production cannot keep up with the rate of degradation (Lesser et al., 1990). SOD, the enzyme responsible for deactivating oxygen radicals, increases in the host in the presence of high visible irradiance with

ultraviolet radiation, which may indirectly indicate the presence of oxygen radicals. Production of active oxygen in illuminated chloroplasts is inevitable but it is normally countered by SOD. Thus, under normal conditions, active oxygen is scavenged to protect the symbiont and host from damage. However, under stressful conditions, the concentration of active oxygen is likely to increase due to an increase in production or a reduction in the ability to scavenge oxygen radicals. This excess can attack target molecules in the photosynthetic system, inducing photoinhibition (Asada and Takahashi, 1987). With the breakdown of photosynthesis, there follows a decrease in photosynthates to the host. In addition oxygen radicals may leak into the host tissue causing cellular damage. With the symbiosis now disrupted, one or both of these situations could be the stimulus inducing the bleaching response.

Even though most coral bleaching research tends to focus on a single environmental variable, it is likely that more than one factor causes the dissociation of the host/symbiont system. These include increases in water temperature and/or increases in irradiance including visible light and UV radiation. Other questions that remain to be addressed include the fate of expelled symbionts and of live bleached corals and the cellular mechanism that causes the expulsion of zooxanthellae once the signals for bleaching have been received. Research in the field should span a

longer time and various parameters should be measured simultaneously during a single project including temperature, irradiance, ultraviolet radiation levels, zooxanthellae per polyp, pigment per zooxanthella, etc., as Lesser et al. (1990) have done with their laboratory experiment.

CHAPTER VI

CONCLUSIONS

Coral reefs are among the most productive marine ecosystems. They form and maintain the physical foundations of many islands, serve as barriers to wave action, and sustain fishing industries in many Caribbean and Pacific countries (Brown and Ogden, 1993). Along with the death of many coral species due to bleaching comes the recruitment of other more rapidly colonizing organisms. Benthic algae and sea urchins have colonized large coral areas that have become bleached (Brown and Ogden, 1993). Along with coral death, the crown-of-thorns sea star, Acanthaster planci, has been able to invade a new niche in the eastern Pacific, successfully prohibiting new coral settlement. Massive numbers of invertebrates and coral reef fishes have been displaced due to mass coral bleaching (Brown and Ogden, 1993).

The research projects discussed in this essay have

demonstrated that two changes in the environment, increased seawater temperatures and ultraviolet radiation, may lead to large-scale coral bleaching events. Cell adhesion dysfunction, decreased photosynthate translocation, and oxygen toxicity have been suggested as some physical and biochemical mechanisms that initiate the bleaching response.

A study on coral bleaching should include experimentation on the causes and mechanisms as well as observations of atmospheric and oceanographic processes. For instance, a coral initiative, including a long-term study involving a large number of scientists each on a site in the Caribbean, Eastern Pacific, Australia, and Central America, should be undertaken. In addition, corals in each area should be found at a range of depths before bleaching occurs. Seawater temperatures should be monitored as well as visible light and UV radiation intensities at a range of depths. Along with these two variables, a set of other parameters, such as the presence or absence of the warm phase of an ENSO event, should be measured to exclude or include other environmental changes. Laboratory experiments on the corals from each study site should include measurements of $\cdot O_2^-$ concentrations and photosynthate translocation, as well as observations of physical characteristics of bleached and unbleached corals before, during, and after a bleaching event. A study like this may indicate the causes and mechanisms of bleaching over a

large-scale as seen in the recent past.

There is no present evidence that global warming is the cause of large scale coral bleaching. Ozone layer depletion caused by human activities may be correlated to coral bleaching events. Gleason and Wellington (1993) have suggested that equatorial latitudes where corals are prevalent do not show a diminished ozone layer. However, according to Postlewait and Hopson (1992) the ozone layer has thinned 3-5% worldwide. If coral reefs are sensitive to a 3-5% thinning of the ozone layer and to UV-B radiation increases, then bleaching may occur because of ozone layer depletion. One of the main questions that remains to be addressed is whether UV-A, UV-B, or both, cause coral bleaching. Since the ozone only traps UV-B, a depletion of the ozone layer only increases UV-B radiation. As a result, if coral bleaching is caused only by increases in UV-A radiation, then ozone layer depletion can be ruled out as a large-scale cause of bleaching.

Oceanographic processes such as the warm phase of the ENSO have mediated 3 large-scale coral bleaching events by temporarily increasing temperatures and ultraviolet radiation levels, which have both been shown to induce bleaching in the field and in the laboratory. Doldrums, an effect of ENSO events, may very well cause calmer, clearer waters and increase ultraviolet light transparency over many coral communities. Clear and calm waters, which

exist in tropical latitudes, may also be prime for coral bleaching.

In conclusion, major environmental changes that are detrimental to coral reef communities may be attributed to anthropogenic causes such as ozone layer depletion, but not global warming. Coral bleaching may be a natural response to changing environmental and oceanographic conditions, like the warm phase of El NiÑo Southern Oscillation, acting over a wide range of spatial scales.

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