SARAH KATHRYN LEWIS
An Examination of the Effects of Environmental Stresses on Stony Corals
(Under the direction of JAMES W PORTER)

Understanding threats to a resource may allow development of management techniques to limit resource degradation. I chose to examine the impact of four threats on stony corals; elevated ultraviolet radiation, elevated visible irradiance, elevated salinity, and elevated temperature.

Colonies of the Pacific stony coral *Montipora verrucosa* were exposed to elevated ultraviolet radiation and elevated visible irradiance by transplanting them from 10m depth to an in-situ respirometer at 0.5m depth. The corals were exposed to full sun or 30% sun, with and without elevated ultraviolet radiation.

Corals exposed to elevated ultraviolet radiation exhibited decreased photosynthesis and chlorophyll levels, but no change in respiration, suggesting that ultraviolet radiation may be more damaging to photosynthetic algae than coral tissue. No change was seen in the UV absorbing compounds, mycosporine-like amino acids, suggesting that longer exposure times to elevated ultraviolet radiation are necessary before changes occur.

Stony corals that were photoadapted to low visible light levels exhibited decreased maximum photosynthesis rates, respiration rates, and photosynthetic efficiency following acute exposure to dramatically increased visible irradiance. These results suggest that increased visible irradiance was detrimental to both the photosynthetic algae and to the coral tissue.

Colonies of the Atlantic stony coral *Montastrea annularis* were monitored during exposure to elevated salinities of 40‰, 45‰, and 60‰. As salinity levels increased, algal photosynthesis decreased but coral respiration increased. Chlorophyll concentration was also reduced by exposure to elevated salinities. These effects were more pronounced the higher the salinity and the longer the exposure. Furthermore, this study revealed a threshold lethal salinity, since

corals exposed to 40‰ or lower survived, while corals exposed to 45‰ or higher all eventually died.

*Montastrea annularis* was also exposed to elevated temperatures of 33$^{o}$C and 36$^{o}$C while metabolic measurements were collected. These corals showed decreased photosynthesis and photosynthesis to respiration ratios. Chlorophyll levels were also lower in corals exposed to elevated temperatures. These effects were more pronounced the higher the temperature and the longer the exposure.

These results indicate that corals respond dramatically to short-term exposures to these environmental stressors, suggesting that these conditions do not need to persist for long before damage occurs.

INDEX WORDS: Chlorophyll, Coral reef, *Montastrea annularis*, *Montipora verrucosa*, Mycosporine-like amino acid, Photosynthesis, Respiration, Salinity, Stony coral, Stressor, Temperature, Ultraviolet radiation, Visible irradiance

# AN EXAMINATION OF THE EFFECTS OF ENVIRONMENTAL STRESSES ON STONY CORALS

by

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# INTRODUCTION AND LITERATURE REVIEW

Conservation biology involves protection and management of biodiversity (Meffe and Carroll 1994). This integrated discipline requires both an understanding of an ecosystem and its associated threats, as well as an understanding of the management of the system as a resource. In order to develop management techniques that can limit resource degradation, we need a clear understanding of the role of threats to the resources. The focus of this dissertation is assessing threats to resources, using coral reefs as a model system.

For the purposes of this dissertation, I have defined a threat as any external factor that may induce a substantial reduction in diversity and/or productivity. I chose to examine the impact of threats on stony corals, since the existence of reefs depends upon stony corals to develop and maintain the reef structure. Death of corals typically results in death or migration of other reef organisms (Johannes 1975). Furthermore, stony corals can act as bioindicators for other reef organisms (Jokiel and Coles 1990).

Coral reefs are calcium carbonate structures found in the tropical oceans, typically in a band around the equator from $30^{o}$N to $30^{o}$S (Wells 1988). Coral reefs thrive in shallow, warm, well-oxygenated, well-illuminated, oligotrophic, clear waters (Kinsman 1964, Glynn 1973). Although reef corals can be found as deep as 100m (Wells 1957), the richest reef coral development occurs to depths with 30-40% or more of subsurface irradiance (Achituv and Dubinsky 1990), typically within the first 20m (Kinsman 1964, Falkowski et al. 1990). As depth increases, light decreases, resulting in decreased diversity and growth of reef corals (Achituv and Dubinsky 1990). Coral reefs are found in areas with

salinities of approximately 35‰ (Kinsman 1964, Coles and Jokiel 1992) and annual mean temperatures between 23$^{o}$ and 25$^{o}$C (Wells 1957, Nybakken 1988). No reefs are found where annual minimum temperatures are less than 18$^{o}$C (Vaughan 1919). Development is limited in certain regions, such as west Africa and the eastern Pacific because of cool upwellings or currents (Milliman 1973, Dana 1975, Glynn and Wellington 1983). Additionally, corals grow in well-oxygenated water, with dissolved oxygen levels that are 90-125% of saturation (Jaap and Wheaton 1975).

Coral reefs are one of the most productive ecosystems with gross productivity between 5 and 20 $gCm^{-2}day^{-1}$ (Odum and Odum 1955, Gordon and Kelley 1962, Smith 1973, Marsh 1974, Lewis 1981). Interestingly, the bulk of the energy of the system is kept within the organisms, and thus, the surrounding water is clear and very low in nutrients (Crossland 1983, Kinsey 1985). Biological productivity per square meter is 50-100 times that in the surrounding oligotrophic waters (Sorokin 1990). Odum (1971) described a Pacific reef atoll as “an oasis in a desert ocean.” High gross primary productivity is maintained in nutrient poor waters by very efficient recycling of nutrients (Erez 1990).

Not only are coral reefs incredibly productive, they are among the most diverse ecosystems in nature (Odum 1971). Indeed, Meffe and Carroll (1994) note that coral reefs are often richer in species per unit area than tropical forests. 7500 species of corals have been identified, though 5000 species are now extinct (Hyman 1940). Eighty genera and 500 species of hermatypic corals are found in the Indo-Pacific and 20 genera and 65 species of hermatypic corals in the Atlantic (Newell 1971). Many organisms unique to reefs would be lost if these areas are not protected.

In addition to the intrinsic value of reefs, they also provide many benefits to humans. They are the foundation of thousands of islands, many of which are

inhabited (Craik et al. 1990). Reefs also act as breakwaters, protecting coastal areas of larger islands and continents from storms, exemplified by the Great Barrier Reef off the northeast coast of Australia (Craik et al. 1990). Reefs allow the development of mangroves, essential nursery areas for commercial fishes (Nybakken 1988, Craik et al. 1990). Furthermore, reefs are economically important for tourism and recreation, fishing, and the aquarium trade (Endean 1976, Gomez et al. 1981, Rogers 1985, Craik et al. 1990).

Reefs are calcium carbonate structures built by mollusks, polychaetes, and sponges, however, scleractinian corals are by far the most important contributors to reef formation (Achituv and Dubinsky 1990). Stony corals, the building blocks of reefs, are comprised of colonies of genetically identical polyps that grow over a calcium carbonate skeleton. Coral polyps are both heterotrophic and autotrophic. They gain energy by capturing organisms with the ring of tentacles around the mouth, as well as by photosynthesizing. These reef-building animals harbor endosymbiotic dinoflagellate algae within their tissues that allow them to harness the energy of the sun (Trench and Blank 1987, Rowan and Powers 1991). This symbiotic relationship allows the host coral animal to provide a nutrient-rich microenvironment for the algae and the algae in turn produce reduced organic carbon, through photosynthesis. This carbon can then be translocated to the host animal.

It is not clear how much energy stony corals garner from autotrophy versus heterotrophy. While corals in shallow water may attain up to 100% of their energy from photosynthesis (Porter 1976, Porter 1985), corals in deeper water may not receive enough light to sustain a gross photosynthesis to respiration ratio greater than two (Porter 1985) and therefore require supplementation from zooplankton feeding. Porter (1974) found zooplankton feeding provided 0.2-10% of the energy requirement for *Montastrea cavernosa*,

while Johannes and Tepley (1974) recorded 10% of the energy for *Porites lobata* came from feeding. Although the energy provided from zooplankton feeding may be low, zooplankton may be an important source of nutrients, such as phosphorus and nitrogen (Odum and Odum 1955, Johannes et al. 1970).

Traditionally, coral reefs were thought to be stable ecosystems (Endean 1976). Anthropogenic disturbances were thought to be more likely to result in permanent changes to a community than natural disturbances (Endean 1976, Johannes 1975). However, Grassle (1973) has described reefs as "temporal mosaics" in space: reefs are comprised of a collection of communities in various stages of recovery from various types of disturbances. It is now more commonly believed that reefs are unstable and that change is more typical than constancy (Grigg and Dollar 1990). Connell (1973) developed the Intermediate Disturbance Hypothesis using the Great Barrier Reef as a model and discovered that an intermediate level of disturbance keeps diversity high. However, high levels of disturbance can be very damaging and prevent reef recovery (Connell 1973). If disturbance to an area is so severe, few fragments of reef-building corals may remain and reef recovery would depend upon sexual reproduction (Highsmith 1982). Typically, reef recovery is faster through asexual reproduction and regeneration (Highsmith 1982).

Coral reefs are exposed to a wide array of natural and anthropogenic threats. Man-made threats to reefs include direct disturbances, such as removal of resources, and indirect disturbances, such as pollutants (Craik et al. 1990). Natural threats include storm damage (Stoddart 1963, Goreau 1964) and El Nino Southern Oscillation events (Glynn 1984, Glynn 1988, Glynn et al. 1988). Additionally, reefs are threatened by diseases, such as Black Band Disease seen in *Montastrea annularis* (Rutzler et al. 1983, Taylor 1983) and White Band Disease seen in *Acropora palmata* (Gladfelter 1982). Coral reefs can also be

severely impacted by population pulses in coral predators, such as the Crown of Thorns seastars (*Acanthaster planci*), that devoured much of the stony coral in Australia during the 1970's and 1980's (Endean and Stablum 1973, Endean 1976, Birkeland and Lucas 1990, Endean and Cameron 1990).

Anthropogenic threats to coral reefs can occur on a global scale, including ozone depletion leading to increased penetration of ultraviolet radiation (Fleischmann 1989, Blumthaler and Ambach 1990). Additionally, reefs can be impacted by climate change (Glynn 1991) including elevated water temperatures (Glynn et al. 1988, Atwood et al. 1992), elevated sea levels (Neumann and Macintyre 1985, Buddemeier and Smith 1988, Hopley and Kinsey 1988), and changes in salinity (Mitchell et al. 1990). Localized anthropogenic threats include diminished water quality from various sources, including wastewater run-off at water treatment plants (Smith et al. 1981, Pastorok and Bilyard 1985), pesticides (Glynn et al. 1989), oil (Rinkevich and Loya 1977, Loya and Rinkevich 1980, Brown and Howard 1985), and metals (Brown and Howard 1985, Glynn et al. 1989). Corals can also be highly sensitive to sedimentation from canal dredging and deforestation leading to soil run-off (Bak 1978, Dollar and Grigg 1981, Rogers 1990). Resource exploitation including boating damage (Dustan 1977), overfishing (Rogers 1985), dynamite fishing (Endean 1976, Gomez et al. 1981), and shell-collecting (Endean 1976), and fish-collecting (Jaap and Wheaton 1975, Endean 1976, Gomez et al. 1981) can also seriously threaten coral reefs.

Since coral reefs are exposed to a variety of stressors, it is important to assess the impact of these threats. Traditional monitoring techniques, including surveys, quadrats and transects, provide information on diversity and abundance, recruitment and growth (Stoddart 1972, Done 1977, Bak and Engel 1979). Unfortunately, these techniques typically only detect gross changes, such

as organism death, and little or no information regarding the mechanism of those changes.

More complex monitoring techniques include sampling tissues for toxins, determining weight and pigment changes, and *in-situ* respirometry. These kinds of monitoring approaches may require innovative field techniques, such as *in-situ* respirometry to measure organism metabolism (Porter 1980). These approaches may provide the ability to detect finer scale change and therefore, may allow pre-emptive intervention before damage is really severe.

A further advance involves experimentally assessing the effects of a potential threat by exposing organisms to threats under controlled conditions and measuring their responses. This approach ties experimental studies to conservation concerns by exploring the potential impact of future threats. I used this approach and exposed stony corals to a range of experimentally manipulated stressors: elevated ultraviolet radiation and elevated visible irradiance (Chapter 1), elevated salinity (Chapter 2), and elevated temperature (Chapter 3).

The continuing destruction of stratospheric ozone by chlorofluorocarbons is resulting in increased ultraviolet radiation penetration on a global scale (Baker et al. 1980, Smith and Buddemeier 1992). Furthermore, more UV radiation passes through the tropical atmosphere because the ozone layer is thinner over the tropics than over temperate latitudes (Baker et al. 1980, Madronich 1993). Additionally, the smaller solar zenith angle in the tropics results in greater penetration of ultraviolet radiation than in temperate regions (Madronich 1993).

Ultraviolet radiation effects on coral reefs have not been extensively studied because it was believed that ultraviolet radiation was attenuated rapidly by ocean water (Smith and Baker 1979). However, it is now well known that ultraviolet radiation penetrates well through clear tropical water (Fleischmann

1989). Ultraviolet radiation is known to be harmful to organisms in both terrestrial and aquatic systems (Harm 1980, Wood 1987, Cullen and Neale 1993), including coral reef epifauna (Jokiel 1980). Ultraviolet radiation levels in shallow water have reduced coral photosynthesis (Sisson 1986, Lesser and Shick 1989) and lowered zooxanthellae growth rates (Jokiel and York 1982, Jokiel and York 1984) and are also thought to detrimentally impact reproduction in reef organisms (Smith and Buddemeier 1992). The unknown effects of increasing ultraviolet radiation on reefs, coupled with the increasing ultraviolet radiation levels over tropical regions suggest that this stressor deserves additional study. Therefore, I examined the effects of elevated ultraviolet radiation on stony corals in chapter 1.

Climate change models do not predict increases in the peak clear-day visible irradiance (Glynn 1977). However, climate related changes in cloud cover, sea state, or turbidity may result in increases in the seasonal or annual irradiance incident on a reef (Glynn 1977). Higher visible irradiance levels will reach the ocean surface in areas with reduced cloud cover, and will penetrate through calm sea surfaces and clear water (Gleason and Wellington 1993).

Stony corals depend on visible light, or photosynthetically active radiation (PAR), for photosynthesis (Kinsman 1964). Their symbiotic dinoflagellates, zooxanthellae, have a broad peak of absorption between 400-550 nm and a second, narrower peak from 650-700 nm (Dustan 1979). PAR is absorbed by the zooxanthellae and energy is stored in organic compounds produced during photosynthesis (Falkowski et al. 1990). While several factors play a role, light is primarily responsible for maximum biodiversity occurring to depths above 30m and maximum reef accretion occurring between 5-15m (Falkowski et al. 1990). Corals depend upon visible irradiance for survival, however too much light can be detrimental. High visible irradiances can enhance coral sensitivity to other

stressors (Jokiel and Coles 1990) and has played a role in coral bleaching events (Brown et al. 1994). I examine the effects of elevated visible irradiance on stony corals in chapter 1.

It is unlikely that the future will bring significant shifts in salinity on a global scale (Smith and Buddemeier 1992). The global change models do not predict large-scale shifts in the magnitude of salinity levels that will affect reefs, however, they do predict alterations in local salinity regimes. In particular, they predict a change in the frequency, magnitude, or geographic distribution of major tropical storms resulting in increased frequency of local salinity deviations (Mitchell et al. 1990). Additionally, shifts in salinity may well occur as a result of changes in run-off patterns caused by changing land uses, such as urbanization and deforestation (Smith and Buddemeier 1992). Indeed, changing land uses have affected salinity patterns in southern Florida.

Historically, the estuarine nature of Florida Bay was maintained by freshwater inputs from the Everglades resulting in average salinities of 18‰ within the Bay (Smith et al. 1989). However, following the construction of canals through southern Florida, almost all of the freshwater was diverted from the Everglades eastward to populated areas of south Florida, eventually emptying into the Atlantic Ocean (Smith et al. 1989, Tilmant 1989, McIvor et al. 1994). This diversion of freshwater from the Everglades resulted in a severe reduction of freshwater into Florida Bay (Tilmant 1989, McIvor et al. 1994), producing average salinities of 40-45‰, with seasonal highs over 70‰ (Fourqurean and Zieman 1992, Fourqurean et al. 1993). There is a net flow of water southward from Florida Bay, between the islands of the Keys and out over the coral reefs (Smith et al. 1989, Smith 1994). Higher salinity water similar to Florida Bay, 38.5‰, has been reported over the reefs of the Florida Keys (Porter et al. 1999)

and this higher salinity water may detrimentally impact the offshore reefs. Therefore, in chapter 2, I examine the effects of elevated salinity on stony corals.

Unlike salinity, there are expected changes to temperature on a global scale in the future. Global climate change models predict a warming of seawater temperatures and/or an increased frequency of more extreme temperature excursions (Smith and Buddemeier 1992). Increased temperatures pose a serious threat to coral reefs, since corals live near their thermal maximum (Moore 1972, Vernberg and Vernberg 1972, Jokiel and Coles 1990).

The effects of seawater warming from global climate change will depend on the time scales of change. It takes generations for selection to shift the thermal tolerance of local coral populations (Jokiel and Coles 1990) to allow them to tolerate higher temperatures (Jokiel and Coles 1990). However, the rate of warming from the greenhouse effect is expected to occur too fast for genetic selection to occur (Buddemeier and Smith 1988, Glynn 1993).

While there are numerous reported cases in the literature of changes in regional temperature levels, my interest in temperature effects on coral reefs was triggered by observations in south Florida. The low average water depth of the Florida Bay (<1m, Tilmant 1989) allows for significant warming of the water by the intense tropical sunlight (Tilmant 1989, Fourqurean et al. 1993). Since water moves from the bay, between the islands of the Keys and onto the reefs (Smith et al. 1989, Smith 1994), this warmer water may be detrimentally impacting the coral reefs. Therefore, in chapter 3, I examine the effects of elevated temperature on stony corals.

These experiments examine how stony corals react to a range of various environmental stressors. These results may be used in conservation efforts by providing information on the mechanisms by which coral reefs may be affected by, and acclimate to future stresses.

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# CHAPTER 1

## RESPONSE OF A PACIFIC STONY CORAL TO SHORT-TERM EXPOSURE OF ULTRAVIOLET AND VISIBLE LIGHT[1]

[1]Lewis, S (1995). *In*: Gulko, D., P. L. Jokiel (eds.); Ultraviolet radiation and coral reefs. HIMB Tech. Report #41. UNIHI-Sea Grant-CR-95-03. p. 89-106. Reprinted here with permission of publisher.

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

## INTRODUCTION

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

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

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

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

UV radiation has been implicated in damaging organisms both in terrestrial and aquatic systems (Harm, 1980; Wood, 1987; Cullen & Neale, 1993). Worrest *et al*. (1981a; b) correlated altered species compositions in standing crops of algae with increased long-term UV dosage. Lesser and Shick (1989) reported 30% lower growth rates in zooxanthellae from *Aiptasia pallida* acclimated under high visible light conditions with UV radiation than those acclimated under high visible light conditions without UV or acclimated in low light conditions. Jokiel and York (1982; 1984) also found reduced growth rates in a number of algal species, including zooxanthellae, when exposed to visible light with UVA + UVB radiation. There is also evidence of UV induced photosynthetic inhibition (Sisson, 1986; Lesser & Shick, 1989). Studies of this photoinhibition suggest that UV damages or destroys chlorophyll and/or chloroplasts (Gessner & Diehl, 1951; Smith *et al.*, 1980; Hader & Worrest, 1991). Lesser and Shick (1989) found reduced levels of chlorophyll in *Aiptasia pallida* in the presence of ultraviolet radiation. It has been suggested that increased UV radiation has been instrumental in causing widespread bleaching observed in tropical oceans (Fisk

& Done, 1985; Harriot, 1985; Oliver; 1985; Goenaga *et al*., 1988). However, the evidence is strictly correlational and is confounded by increases in visible irradiance.

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

There is some debate regarding the relative contribution of visible irradiance and UV radiation in damaging reef organisms. Brown *et al*. (1994) speculate that the bleaching patterns observed in corals in Thailand result from longer wavelength, photosynthetically active radiation (PAR) and that UV radiation played a nominal role, if any at all. They contend that there is increasing evidence that high levels of PAR negatively affect algal photosynthetic systems (review in Powles, 1984). Contrastingly, Jokiel and York (1984) consider that the role of PAR in photoinhibition has been overestimated and that long-term photoinhibition effects are primarily caused by UV radiation. They discovered that algae in their study could rapidly photoadapt to increased PAR (92% surface irradiance), but the addition of UV resulted in growth photoinhibition. Their study agreed with previous studies (Steemann-Nielsen, 1962; Steemann-Nielsen *et al*., 1962; Prezelin & Matlick, 1980) that showed that

some microalgae can rapidly photoadapt to high levels of visible light (<< 24 hrs). This debate can only be settled by further, non-correlational research on the relative impacts of these two light components. In addition to the impact of UV radiation or visible irradiance on reef organisms, there may be an interaction between these two effects. It is possible that the combination of these two factors produces greater detrimental effects than either of the two acting alone. It is equally possible that one factor may ameliorate the effects of the other.

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

## MATERIALS AND METHODS

### Collection Site and Study Organism

This study was conducted at the Hawaii Institute of Marine Biology (HIMB), Coconut Island, Kaneohe, Oahu during the summer of 1994. All coral pieces were collected off the Coconut Island's lighthouse dock from large colonies of plating *Montipora verrucosa* at a depth of 10m. A pair of coral pieces was taken from the same location on each colony; one piece of the pair was used in the experimental treatments while the other piece of the pair was used immediately for lab analysis to obtain initial estimates for chlorophyll *a* levels,

and MAA levels. A total of 36 pieces from 18 colonies was used for this experiment.

Experimental Design

The collected pieces of *M. verrucosa* were transported approximately 300 m in shaded fresh seawater to the site of the *in-situ* respirometer in the evening before each experimental run. The respirometer was located on a suspended platform at a constant depth of 0.5 m. Six pieces of *M. verrucosa* were collected at a time. One piece from each pair was randomly placed in each of the three chambers of the respirometer, while the other pair-member was taken to the lab for immediate processing.

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

Metabolic Measurements

Photosynthetic measurements were made using the suspended respirometer. The experimental corals were placed inside sealed 2.3 liter plexiglass chambers for the duration of the experiment. The chamber lids were quartz and therefore transparent to UVA and UVB radiation. Each chamber was

connected to a submersed impeller pump that fully exchanged all the water in the chambers every hour. Temperature readings taken periodically inside the chambers showed that this flushing rate prevented significant heating inside the chambers (< 0.5 $^{o}$C higher than surrounding water). Uniformity of oxygen levels throughout the chamber was achieved by rotating stir bars below a perforated pedestal that held the coral. Oxygen production (photosynthesis) and consumption (respiration) were measured by YSI oxygen probes and recorded every 4 minutes by an Omnidata datalogger. Light was recorded every 4 minutes through use of a LiCor light meter and 4pi steradian spherical sensor attached to the respirometer.

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

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

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

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

Surface Area Determination

After removal from the chambers, each coral was taken to the lab in seawater and surface area was determined. Each coral was videotaped and projected surface areas were then calculated. Because *M. verrucosa* has tissue on both the top and underside of the plate, the total surface area of the coral involved in photosynthesis and respiration was obtained by doubling the measured surface area.

Photosynthetic Pigment Determination

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

Chlorophyll levels were determined photometrically. A small plug was taken from the middle of each piece using a 1 cm diameter cork borer and then ground in 90% acetone. The ground coral and solvent were placed in a dark refrigerator to extract overnight. The tubes were then spun in a refrigerated centrifuge at 3500 x g and the absorbance of the supernatant was then measured on a scanning spectrophotometer at 750nm, 663nm, and 630nm. Chlorophyll *a* values were determined using the equations of Jeffrey and Humphrey (1975).

MAA Assays

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

Methods of Statistical Analysis

The response of an individual coral piece to a treatment effect may be influenced by pretreatment factors. Hence, a covariate analysis with these factors (ANCOVA) might provide a more powerful test than a simple non-covariate parametric test (ANOVA). Covariates were only used when the covariate model was significantly different than the reduced, non-covariate model. The choice between different significant covariate models follows the method outlined by Mallows (1973). Independence of the covariates was tested by a linear correlation procedure.

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

## RESULTS

Covariate Analysis

The set of potential covariates were: MAA, chlorophyll, respiration normalized by surface area, respiration normalized by chlorophyll, and

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

Chlorophyll and MAA were evaluated as covariates for all metabolic response variables. The respiration rates were only used for the metabolic response variables with the same normalization. Table 1.1 shows the correlation analysis for the covariates. The only covariates that showed a significant correlation were R by wet weight and R by surface area (correlation coefficient = 0.73, p=0.001). Since these two covariates were always used separately in any analysis, all covariate models tested used a set of independent covariates. Tables 1.2, 1.3, and 1.4 show the covariates used in the analyses. Of all the covariates tested, only two were used: Chl-before and MAA-before. An ANCOVA procedure was used to analyze the following response variables: R normalized by Chl using Chl-before as covariate, net $P_{max}$ normalized by Chl using MAA-before as covariate, and net $P_{max}$ normalized by surface area using MAA-before and Chl-before as covariates. All other response variables were analyzed using an ANOVA procedure.

Assay Analysis - Chl and MAA

*Within 10m light corals - UV effects:*

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

Table 1.1. Pearson correlation coefficients for analysis for covariates (* indicates significantly correlated, $p < 0.05$).

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

Table 1.2. Metabolic data - comparison of UV and visible light treatments for first day of exposure. Significance levels from ANOVAs for UV and visible light treatment effects for normalization independent variables (* indicates $p < 0.05$).

| Response Var. | Covariates | UV Effect | PAR Effect | UVxPAR |
|---|---|---|---|---|
| $\mathbf{I_c}$ | none | 0.59 | 0.57 | 0.06 |
| $\mathbf{I_k}$ | none | 0.39 | 0.12 | 0.99 |
| **P:R** | none | 0.52 | 0.46 | 0.62 |

Table 1.3. Metabolic data - comparison of UV and visible light treatments for first day of exposure. Significance levels from ANOVAs or ANCOVAs for UV and visible light treatment effects for variables normalized to surface area (SA), wet weight (Wgt), and chlorophyll (Chl) (* indicates $p < 0.05$).

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

Table 1.4. Metabolic data - comparison of UV and day treatments for full light corals. Significance levels from ANOVAs for metabolic response variables for UV treatment effect and day effect (* indicates $p < 0.05$).

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

*Within full light corals - UV effects:*

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

The covariates used and the significance levels of the treatment effects are summarized in Table 1.5, and the means and standard errors are provided in Table 1.6.

Metabolic Analysis Within Day 1 - UV and PAR Effects

There was no observed interaction between the UV treatment effects and the PAR treatment effects for any of the metabolic response variables. Significance levels for both unnormalized and normalized response variables are provided in Tables 1.2 and 1.3, respectively. The means and standard errors are provided in Table 1.7.

*UV Effects:*

Compensation irradiance ($I_c$), saturation irradiance ($I_k$), and net P:R ratio did not show a significant UV effect (p=0.59, 0.39, 0.52, respectively). There were no significant differences between the means for each of these response variables among the different UV treatments (Table 1.7).

Of the metabolic response variables normalized by surface area, wet weight, and chlorophyll, the only significant UV effect occurred with net $P_{max}$ normalized to surface area (p=0.02, Table 1.3). The corals that were shielded from UV had a higher net $P_{max}$ than those exposed

Table 1.5. Significance levels from ANOVAs and ANCOVAs for UV treatment effects for chlorophyll *a* (µg $cm^{-2}$) and total MAA (nmol $mg^{-1}$ protein) for 10m light corals after 1 day exposure and for full light corals after 2 days exposure.

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

Table 1.6. Mean and standard errors for chlorophyll *a* (µg $cm^{-2}$) content and total mycosporine-like amino acids (nmol/mg protein) for 10m light corals (after 1 day of exposure) and full light corals (after 2 days of exposure).

| | **10m Light Corals - 1 day exposure** | | | | | **Full Light Corals - 2 day exposure** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Chl *a*** | | | **Total MAA** | | **Chl *a*** | | | **Total MAA** | |
| Treatment | N | Mean | SE | Mean | SE | N | Mean | SE | Mean | SE |
| PAR only | 3 | 12.3 | 0.92 | 9.47 | 3.44 | 3 | 14.7 | 1.51 | 702 | 645.80 |
| PAR+UVA | 3 | 7.53 | 0.92 | 49.5 | 14.32 | 3 | 13.1 | 1.65 | 176 | 120.00 |
| PAR+UVA+UVB | 3 | 7.91 | 1.85 | 35.28 | 16.53 | 3 | 13 | 0.04 | 191 | 152.50 |

Table 1.7. Metabolic data - comparison of UV and visible light treatments for first day of exposure. Mean values for metabolic response variables, sample sizes, and standard errors (letters represent t-test groupings. Values in different groups are significantly different at $p < 0.05$).

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

| **Respiration** | **normalized by SA** | | | **normalized by Wgt** | | **normalized by Chl** | |
|---|---|---|---|---|---|---|---|
| Treatment | N | Mean | SE | Mean | SE | Mean | SE |
| PAR - only | 6 | -4.79 | 0.77 | -10.20 | 1.58 | -0.387 | 0.06 |
| PAR + UVA | 6 | -4.78 | 0.40 | -10.99 | 1.54 | -0.529 | 0.06 |
| PAR + UVA + UVB | 6 | -4.52 | 0.75 | -10.50 | 1.90 | -0.563 | 0.06 |
| 10 m Light | 9 | -5.86 **a** | 0.36 | -13.25 **a** | 1.13 | -0.628 **a** | 0.05 |
| Full Light | 9 | -3.53 **b** | 0.30 | -7.86 **b** | 0.69 | -0.358 **b** | 0.05 |

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

| **Alpha** | **normalized by SA** | | | **normalized by Wgt** | | **normalized by Chl** | |
|---|---|---|---|---|---|---|---|
| Treatment | N | Mean | SE | Mean | SE | Mean | SE |
| PAR - only | 6 | 0.065 | 0.01 | 0.140 | 0.018 | 0.006 | 0.0004 |
| PAR + UVA | 6 | 0.061 | 0.010 | 0.141 | 0.029 | 0.006 | 0.0012 |
| PAR + UVA + UVB | 6 | 0.061 | 0.01 | 0.144 | 0.035 | 0.007 | 0.0016 |
| 10 m Light | 9 | 0.081 **a** | 0.01 | 0.187 **a** | 0.022 | 0.008 **a** | 0.0010 |
| Full Light | 9 | 0.044 **b** | 0 | 0.097 **b** | 0.057 | 0.004 **b** | 0.0007 |

to UVA (Table 1.7, Figure 1.1). However, this design could not detect a significant difference between those exposed to UVA+UVB from those shielded from UV or those receiving UVA-only (means and t-groupings - Table 1.7).

The light saturation curves shown in Figure 1.2 provide an overall view of the UV treatment effects on the metabolism of *M. verrucosa*. The higher net $P_{max}$ for corals receiving only visible light is evident.

*PAR Effects:*

There was no significant PAR effect on $I_c$, $I_k$, and P:R ($p=0.57$, 0.12, 0.46, respectively, Table 1.2; means - Table 1.7). There was a highly significant PAR effect for each of the metabolic response variables for each of the three normalizations. The 10m light corals showed significantly higher net $P_{max}$, R and alpha values (all $p < 0.05$ - Table 1.3; means and t-groupings - Table 1.7). Figure 1.1 shows mean values and 95% confidence intervals of net $P_{max}$ normalized by surface area. The same trend was evident for the other two metabolic response variables normalized by surface area, as well as for all metabolic response variables normalized by wet weight and chlorophyll.

Figure 1.3 shows the light saturation curves for the two PAR treatments, irrespective of UV treatment. They clearly indicate the effects of increased visible irradiance on the photosynthetic ability of *M. verrucosa*.

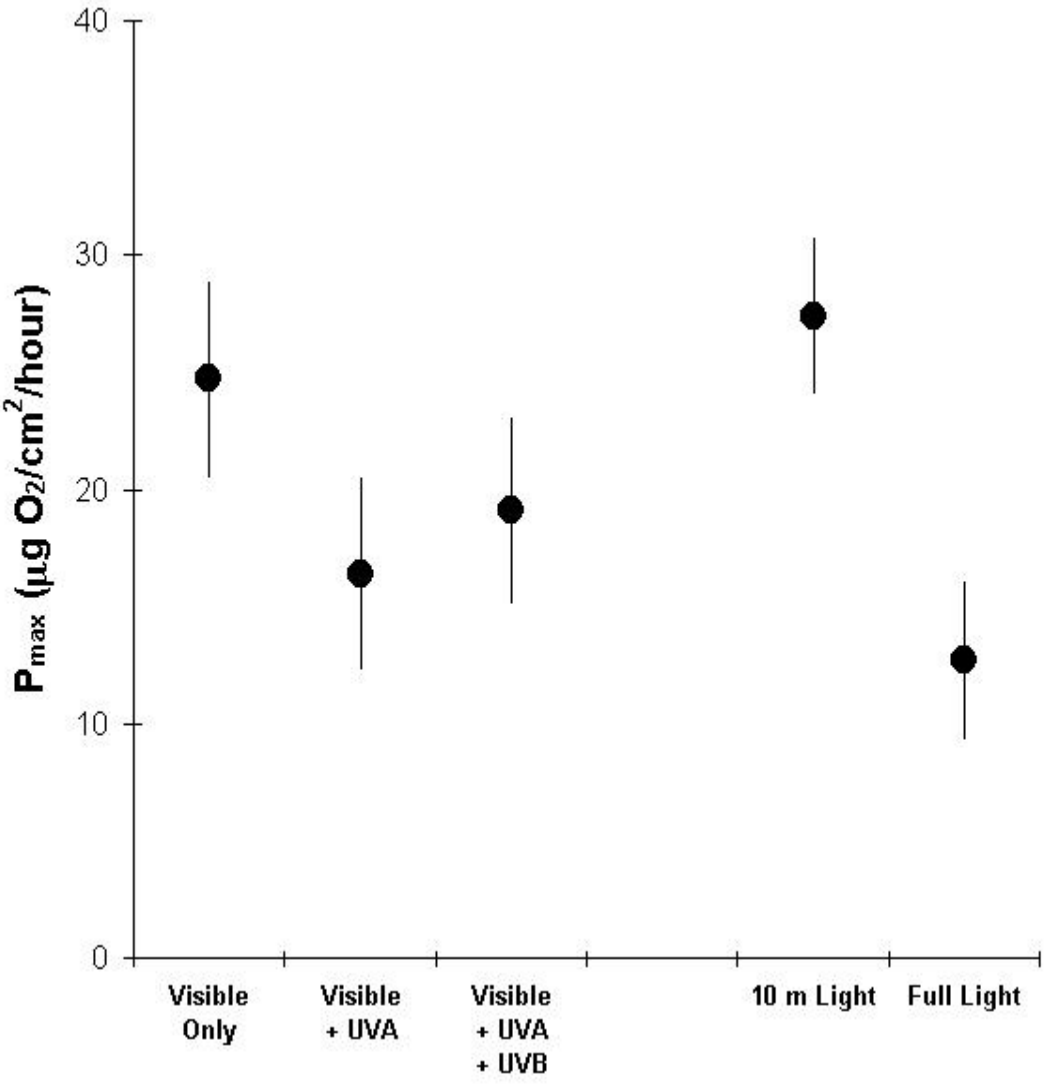


Figure 1.1. Significant UV and PAR treatment effects after 1 day of exposure for maximum net photosynthesis normalized by surface area. Means and 95% confidence intervals.

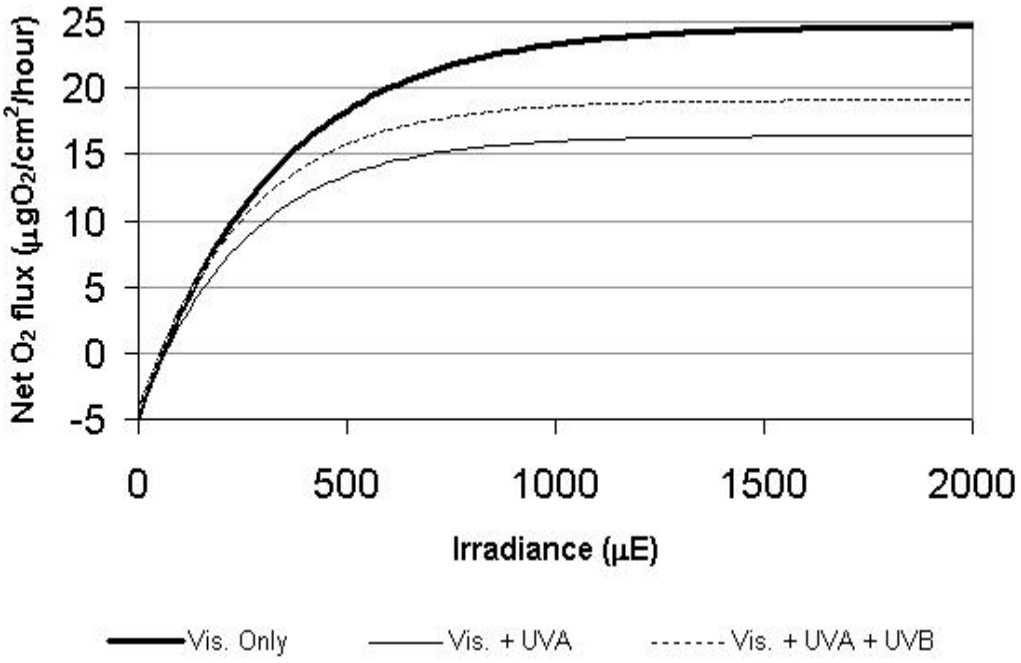


Figure 1.2. Light saturation curves for all corals for first day of exposure to the three UV treatments.

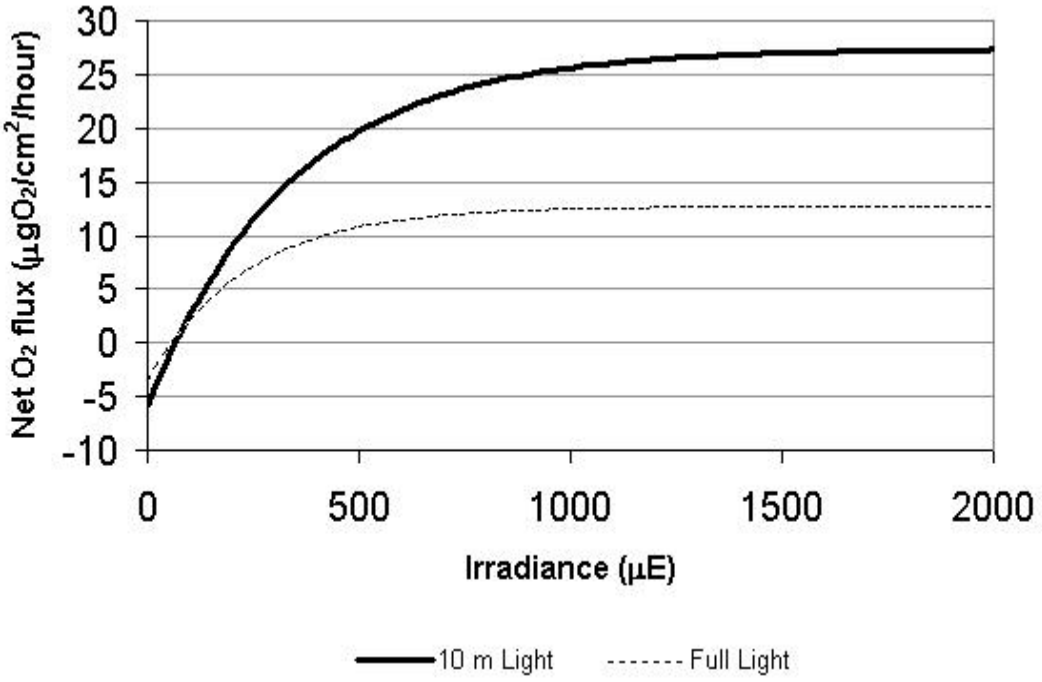


Figure 1.3. Light saturation curves for first day of exposure to the two different PAR treatments.

Metabolic Analysis Within Full Light Treatment - UV and Day Effects

There was no interaction observed between the UV treatments and the day of exposure for any of the metabolic response variables (Table 1.4).

*UV Effects:*

When full light corals from the three UV treatments were compared for the first and second day of exposure, saturation irradiance ($I_k$) and net P:R ratio were not different among the UV treatments (p=0.72, 0.33, respectively, Table 1.4). However, there was a significant UV effect for compensation irradiance (p=0.01, Table 1.4). Corals shielded from UV had significantly lower compensation points than those exposed to UVA (means and t-groupings, Table 1.8). However, it was not possible to distinguish the compensation point of corals exposed to UVA+UVB from that of corals exposed to UVA or shielded from UV (Table 1.8). Figure 1.4 shows the lower compensation irradiance for corals shielded from UV. Maximum net photosynthesis, respiration, and photosynthetic efficiency normalized to surface area, wet weight, and chlorophyll did not show significant UV effects (p values -Table 1.4; means and standard errors - Table 1.8). Figure 1.5 shows the light saturation curves for the full light corals for both days of exposure to the different UV treatments. Although corals shielded from UV appear to have a higher photosynthesis, this was not significant.

*Day Effects:*

There was no significant difference for saturation irradiance or net P:R ratio between the first day and second day of exposure for the full light corals (p=0.14 and 0.70, respectively, Table 1.4). However, the

Table 1.8. Metabolic data - comparison of UV and day treatments for full light corals. Mean values, sample sizes, and standard errors for metabolic response variables (letters represent t-test groupings. Values in different groups are significantly different at $p < 0.05$).

| | Variable: Ic | | | Variable: Ik | | | Variable: P:R | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | N | Mean | SE | N | Mean | SE | N | Mean | SE |
| PAR only | 6 | 69.32 **x** | 5.25 | 6 | 442.00 | 66.54 | 6 | 4.97 | 0.98 |
| PAR+UVA | 6 | 97.98 **y** | 8.14 | 6 | 390.33 | 53.07 | 6 | 2.57 | 0.55 |
| PAR+UVA+UVB | 6 | 81.75 **xy** | 9.89 | 6 | 373.17 | 62.80 | 6 | 3.68 | 1.30 |
| Day 1 | 9 | 69.48 **a** | 5.44 | 9 | 344.78 | 38.98 | 9 | 3.99 | 1.00 |
| Day 2 | 9 | 95.56 **b** | 6.24 | 9 | 458.89 | 50.42 | 9 | 3.49 | 0.67 |

| **Respiration** | | normalized by **SA** | | normalized by **Wgt** | | normalized by **Chl** | |
|---|---|---|---|---|---|---|---|
| Treatment | N | Mean | SE | Mean | SE | Mean | SE |
| PAR only | 6 | -3.76 | 0.33 | -8.23 | 0.93 | -0.409 | 0.056 |
| PAR+UVA | 6 | -4.45 | 0.24 | -10.21 | 1.20 | -0.475 | 0.072 |
| PAR+UVA+UVB | 6 | -3.62 | 0.35 | -8.22 | 0.69 | -0.390 | 0.080 |
| Day 1 | 9 | -3.53 **a** | 0.30 | -7.88 | 0.69 | -0.395 | 0.071 |
| Day 2 | 9 | -4.36 **b** | 0.14 | -9.89 | 0.80 | -0.454 | 0.065 |

| **Pmax** | | normalized by **SA** | | normalized by **Wgt** | | normalized by **Chl** | |
|---|---|---|---|---|---|---|---|
| Treatment | N | Mean | SE | Mean | SE | Mean | SE |
| PAR only | 6 | 17.42 | 2.33 | 38.92 | 6.43 | 1.93 | 0.30 |
| PAR+UVA | 6 | 11.29 | 2.40 | 25.16 | 5.17 | 1.14 | 0.23 |
| PAR+UVA+UVB | 6 | 11.85 | 3.46 | 27.10 | 7.51 | 1.26 | 0.50 |
| Day 1 | 9 | 11.93 | 1.74 | 26.79 | 4.43 | 1.24 | 0.21 |
| Day 2 | 9 | 15.11 | 2.81 | 33.99 | 6.19 | 1.64 | 0.37 |

| **Alpha** | | normalized by **SA** | | normalized by **Wgt** | | normalized by **Chl** | |
|---|---|---|---|---|---|---|---|
| Treatment | N | Mean | SE | Mean | SE | Mean | SE |
| PAR only | 6 | 0.048 | 0.0024 | 0.105 | 0.0053 | 0.005 | 0.0004 |
| PAR+UVA | 6 | 0.038 | 0.0029 | 0.086 | 0.0086 | 0.004 | 0.0004 |
| PAR+UVA+UVB | 6 | 0.037 | 0.0037 | 0.086 | 0.0086 | 0.004 | 0.0008 |
| Day 1 | 9 | 0.043 | 0.003 | 0.097 | 0.0053 | 0.004 | 0.0004 |
| Day 2 | 9 | 0.039 | 0.003 | 0.088 | 0.0080 | 0.004 | 0.0008 |

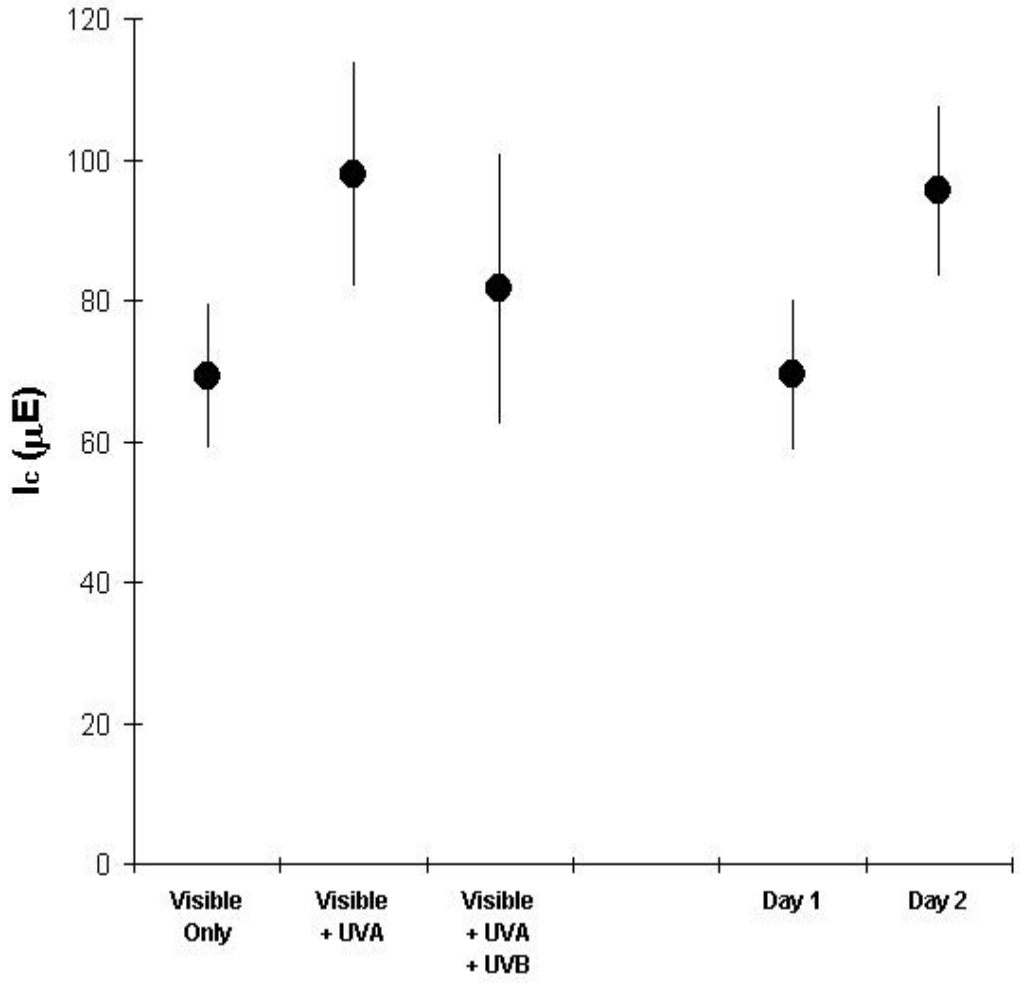


Figure 1.4. Significant UV treatment effects after 2 days of exposure to the 3 UV treatments and significant day treatment effects for compensation irradiance ($I_c$) for full light corals. Means and 95% confidence intervals.

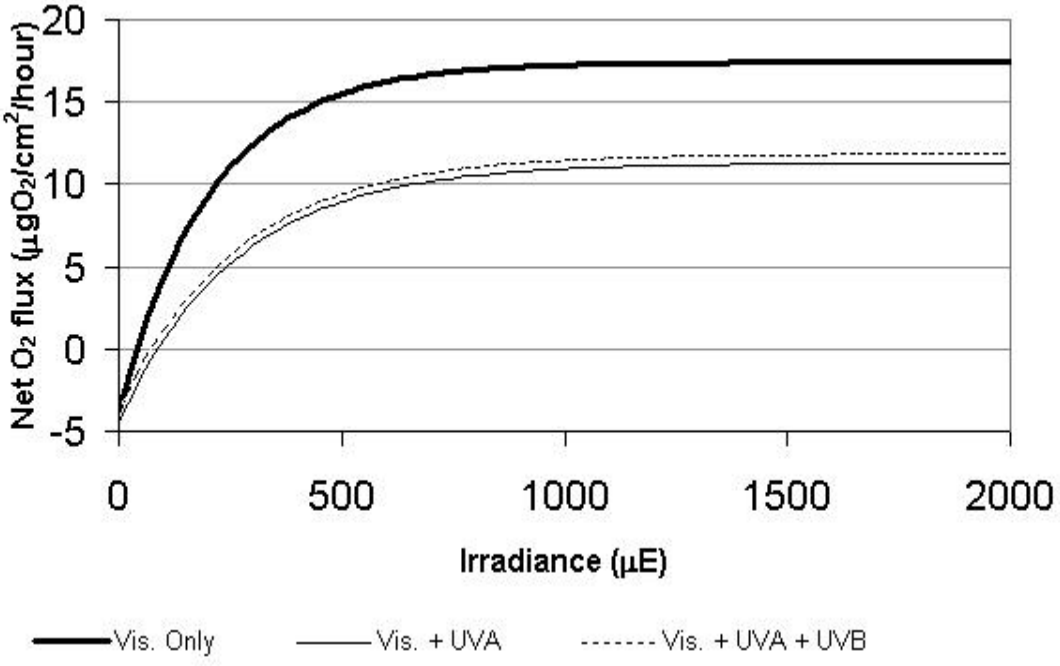


Figure 1.5. Light saturation curves for full light corals for both days of exposure to the three UV treatments.

compensation irradiance was significantly lower during the first day of exposure and increased during the second day of exposure (p=0.002, Table 1.4; means and t-groupings - Table 1.8, Figure 1.4).

Of the three variables normalized to surface area, wet weight, and chlorophyll, only respiration normalized to surface area showed a significant day effect (p=0.02, Table 1.4, means and t-groupings, Table 1.8). Figure 1.6 shows that respiration rates were significantly higher on the second day of exposure.

## DISCUSSION

### Assay Analysis - Chl and MAA

*UV Effects:*

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

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

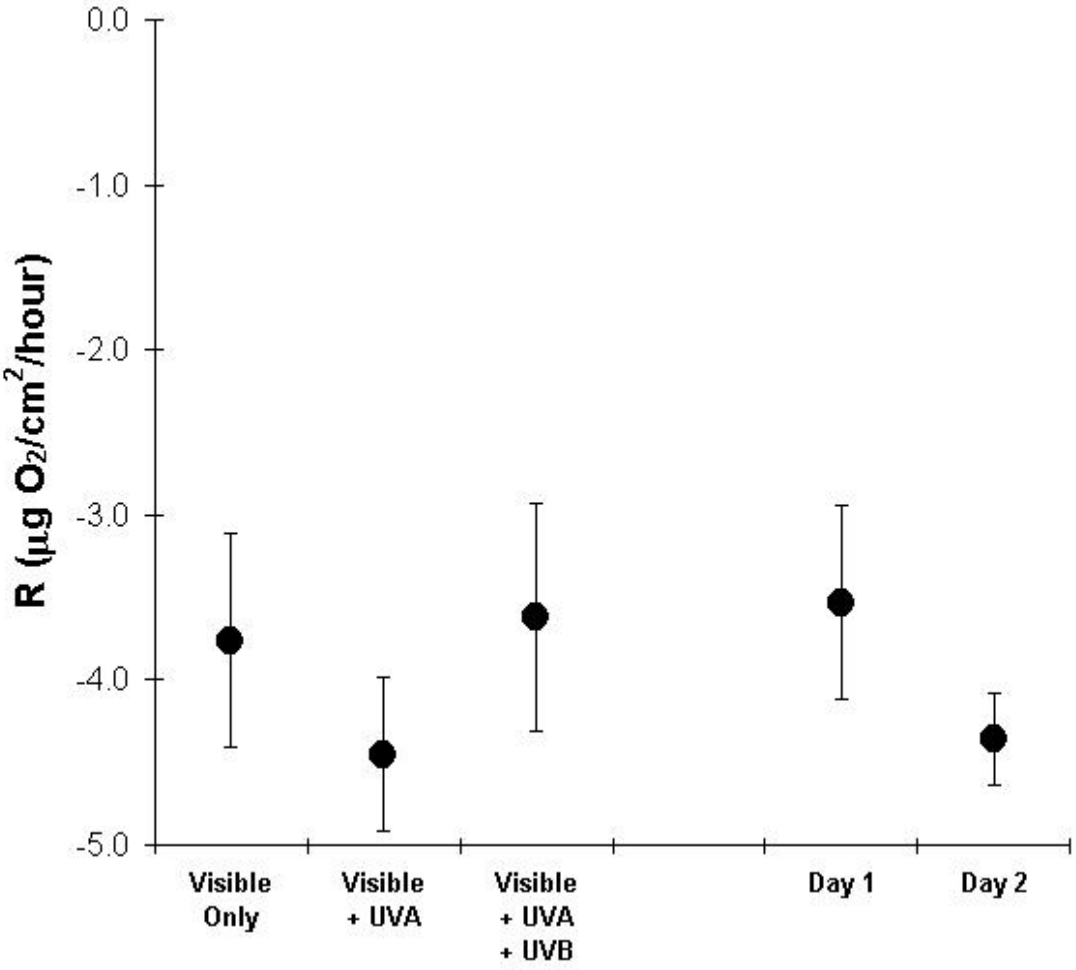


Figure 1.6. Significant day effects for respiration normalized by surface area for full light corals. Means and 95% confidence intervals.

Metabolic Analysis Within Day 1 - UV and PAR Effects

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

*UV Effects:*

In this experiment, only one of the metabolic response variables, the maximum net photosynthesis rate, showed a significant UV effect after one day of exposure. The observation that net $P_{max}$ was highest in those corals shielded from UV suggests that UV radiation may be damaging the photosynthetic components of zooxanthellae. These results are consistent with previous studies. For example, Kinzie (1993) found enhanced photosynthetic ability in full sun by *Montipora verrucosa* acclimated to PAR+UV compared to corals acclimated to PAR only. Lesser and Shick (1989) found UV exposure decreased net $P_{max}$ in freshly isolated zooxanthellae but not cultured zooxanthellae from *Aiptasia pallida*.

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

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

*PAR Effects:*

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

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

Metabolic Analysis Within Full Light Treatment - UV and Day Effects

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

*UV Effects:*

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

*Day Effects:*

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

## CONCLUSION

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

other differences in metabolic response variables were observed. Significantly, the treatment effects observed in this experiment occurred following exposure to natural levels for only one to two days.

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# CHAPTER 2

# PHYSIOLOGICAL RESPONSES OF A STONY CORAL TO ELEVATED SALINITIES: A CASE STUDY WITH *MONTASTREA ANNULARIS*[1]

[1]Lewis, S. K. To be submitted to *Marine Biology*

**Abstract.** Stony corals cannot osmoregulate, therefore, changes in salinity can lead to physiological stress. The consequences of changing salinity levels on reef organisms have not been extensively studied because of the belief that ocean salinity is a stable 35‰. However, changes in salinity levels over coral reefs do occur, with hyposalinities down to below 10‰ and hypersalinities up to 50‰. Therefore, studying effects of salinity changes on corals is warranted. High salinity water from Florida Bay (38.5‰) has been recorded over the offshore reefs of the Florida Keys Reef Tract and may have contributed to the marked decline in coral cover noted since the mid 1980's.

In this study, physiological responses of colonies of one of the dominant reef building stony corals in the Florida Keys Reef Tract, *Montastrea annularis*, were monitored in the laboratory during exposure to elevated salinities of 40‰, 45‰, 60‰ (or 5‰, 10‰, and 25‰ above ambient). Even brief exposures (6 hours) to elevated salinity levels consistent with water conditions in Florida Bay were damaging to stony corals. In addition, the longer the exposure to elevated salinity, the more detrimental the effect, suggesting that an inability to osmoregulate prevented the corals from acclimating to these conditions.

As salinity levels increased, the autotrophic potential of the corals decreased, as indicated by a drastically reduced photosynthesis to respiration ratio. Under these stress conditions, symbiotic algal photosynthesis decreased, while coral respiration increased. Additionally, chlorophyll concentration of the corals was strikingly reduced by exposure to elevated salinity.

This study revealed a threshold lethal salinity, since corals exposed to 40‰ or lower survived for the duration of the experiment, whereas corals exposed to 45‰ or higher all eventually died. The results from this experiment indicate that salinity levels consistent with Florida Bay water can be extremely

damaging to one of the main reef-building stony corals. A mild elevation of salinity for even a brief time can produce significant physiological changes.

**Introduction**

The effects of elevated salinity on stony corals have been rarely studied because of the long-held belief that tropical ocean salinity is a generally stable 35‰ (Kinsman 1964). However, variations in salinity do occur, including both stable hyposalinity (<35‰) and stable hypersalinity (>35‰), as well as short-lived episodic hyposalinity or hypersalinity. These variations are more likely to occur in shallow areas, since shallow water salinity is affected by freshwater influxes or by evaporation (Goodbody 1961; Goreau 1964). Coral reefs thrive in shallow water, with most vigorous growth above 20m (Kinsman 1964), so they can be influenced by salinity variations.

Stable hyposalinity in tropical oceans can occur near river mouths, where a steady supply of freshwater enters the surrounding seawater, producing a gradient from freshwater eventually increasing to 35‰. This consistently lowered salinity typically causes breaks in the nearby fringing reefs because very few coral species can tolerate the lowered salinity conditions (Crossland 1928; Squires 1962; Stoddart 1969; Achituv and Dubinsky 1990). Stable hyposaline conditions also exist over reefs in Southeast Asian waters, where a few coral species survive in salinities of 25‰ (Coles and Jokiel 1992).

Stable hypersalinity can be found around the world. Coral reefs at atolls can experience high salinities when lagoon water becomes isolated from the open ocean, such as 35-39‰ at Canton Island, in the Indo-Pacific (Jokiel and Maragos 1978; Smith and Jokiel 1978) and Laysan Atoll, in the Hawaiian chain, has waters typically near 50‰ (Caspers 1981). Extensive hypersaline conditions (above 40‰) also occur regularly in the Red Sea and the Arabian Gulf (Kinsman

1964; Downing 1985; Coles 1988). While stony corals can survive in areas of stable hypersalinity, relatively few species comprise the total coral cover. As salinity increases, diversity of coral species decreases (Kinsman 1964; Jokiel and Maragos 1978; Smith and Jokiel 1978; Coles 1988). Sheppard (1988) found 8 coral species growing in 48‰ in Bahrain, but only 3 species in 50‰. Kinsman (1964) documented 11 genera of coral in the Persian Gulf, where salinities typically reach 42-48‰ compared with 80 genera of coral in the Indo-Pacific, where 35‰ is more the norm. The recorded world maximum for coral survival under stable hypersaline conditions is 51-52‰ at Christmas Island saline lake, in the Indo-Pacific, which is naturally hypersaline due to consistently high evaporation and low precipitation (Coles and Jokiel 1992). However, only a single stony coral species, *Acropora grandis*, survives under these conditions.

While these salinity extremes reduce species diversity and coral cover, the stability of the conditions allow some euryhaline corals to survive. Some coral species can tolerate elevated salinities; *Porites compressa* is found in Hawaii growing in 33-35‰, but is also found growing in the Arabian Gulf at 40-41‰. When corals from both of these populations were exposed to an experimental range of salinities from 19-53‰ for 20 days (Marcus and Thorhaug 1981), the corals found growing in the higher salinity environment had a maximum salinity tolerance about 5‰ higher than their Hawaiian counterparts. Conversely, the corals found growing in the lower salinity environment had a minimum salinity tolerance approximately 5‰ lower than their Arabian Gulf counterparts (Marcus and Thorhaug 1981). Muthiga and Szmant (1987) also found that stony corals could adjust to hypersaline conditions when given time to acclimate. The authors slowly raised the salinity by 12-14‰ over 30 days and *Siderastrea siderea* was able to acclimate and showed no adverse effects. Conversely, rapid, episodic salinity extremes do not allow reefs to acclimate.

Departures from regional salinity norms tend to be short-lived and limited geographically. Hyposalinity may result from rain and/or freshwater run-off following severe storms (Goreau 1964; Jokiel et al. 1993). Studies have indicated that mild or short-lived hyposalinity may not show significant effects on corals. When Muthiga and Szmant (1987) experimentally reduced salinity by 5‰, *Siderastrea siderea,* did not show a significant change in any metabolic variable. Hoegh-Guldberg and Smith (1989) also found that 30‰ had no effect on the biomass and physiology of *Stylophora pistillata* and *Seriatopora hystrix*. Marcus and Thorhaug (1981) saw no obvious stress in *Porites compressa,* in either the Atlantic or the Pacific when they lowered salinity by 10‰, to 25‰, for 20 days.

Moderate levels or longer-lived hyposalinity can induce physiological changes in corals. When Muthiga and Szmant (1987) lowered salinity by 14‰, both photosynthesis and respiration rates of *Siderastrea siderea* were reduced. Similarly, Moberg et al. (1997) found lower photosynthesis and photosynthesis to respiration ratios in *Porites lutea* and *Pocillopora damicornis* following sudden exposure to decreased salinities of 20‰ and 10‰. Hyposalinity decreases the net productivity of the coral-algal complex, which decreases the vitality of the coral (Coles and Jokiel 1992). *Porites* spp. has been reported to release mucus when exposed to salinities of 27‰ (Coffroth 1985) and 20‰ (Marcus and Thorhaug 1981). Marcus and Thorhaug (1981) also reported a loss of the photosynthetic pigment chlorophyll in *Porites* spp. exposed to 20‰.

Severe levels or prolonged exposure to hyposalinity can induce dramatic responses in corals. In 1963, Hurricane Flora lowered the salinity of the Port Royal, Jamaica area from its usual 35‰ to 3‰ and the salinity stayed below 30‰ for five weeks (Goreau 1964). This resulted in widespread bleaching of organisms, including corals, to a depth of 3m (Goreau 1964). Bleaching in corals

leads to reduced autotrophic capacity (Hoegh-Guldberg and Smith 1989; Porter et al. 1989) and eventually reduced tissue biomass (Glynn et al. 1985; Porter et al. 1989; Szmant and Gassman 1990). Extensive freshwater influxes can also lead to widespread reef mortality (Goodbody 1961; Glynn 1973; Jokiel et al. 1993). High rainfall in May 1965, led to surface salinities of 8‰ and subsurface salinities of 20-25‰ over the reefs in Kaneohe Bay, Hawaii, which led to rapid bleaching and high levels of mortality (Maragos et al. 1985). In early 1988, a severe storm produced dramatically lowered salinities (to 15‰) for many days on the fringing reefs of Kaneohe Bay, Hawaii (Jokiel et al. 1993). This led to massive mortality of many reef organisms, including corals to a depth of 2m. If reduced salinities occur for an extended time (months to years), or are severe enough, there can be changes in abundance and diversity of coral species. Following the massive mortality in Kaneohe Bay, Hawaii from the freshwater input (salinities lowered to 15‰) of a 1988 storm, coral cover and diversity were dramatically reduced (Jokiel et al. 1993). In many cases, it takes several decades for the coral populations to begin to recover to pre-disturbance levels. Maragos et al. (1985) noted that coral populations decimated by high rainfall in a 1965 storm began showing signs of recovery in 1983.

While it is clear from the above discussion that there are numerous reported cases of episodic hyposalinity, there are very few cases of episodic hypersalinity. Mild or short-lived hypersalinity may not trigger significant effects in corals. When *Montipora verrucosa* was exposed to a 5‰ higher salinity (40‰), it did not show any visible signs of damage after a few days (Coles and Jokiel 1978). Similarly, when *Porites spp.* was exposed to a 2‰ higher salinity (37‰), it also showed no visible signs of stress after short exposure times (Marcus and Thorhaug, 1981).

Moderate levels of hypersalinity or longer exposure times can induce more pronounced effects. When Muthiga and Szmant (1987) experimentally raised salinity of *Siderastrea siderea* by 10‰ (to 45‰) they did not observe a change in respiration, but there was a significant reduction in photosynthesis and photosynthesis to respiration ratios, suggesting a diminishing of the autotrophic capacity of these organisms. A 10‰ increase in salinity was also found to bleach corals (Marcus and Thorhaug 1981; Glynn and D'Croz 1990). Similarly, a longer exposure of mild hypersalinity, 20 days at 40‰, caused *Porites porites* to release mucus and resulted in 30-40% mortality (Marcus and Thorhaug, 1981).

Severe levels or prolonged exposure to elevated salinities can induce dramatic effects in stony corals. Salinities above 43‰ for even relatively short times (<12 hours) resulted in high mortality in *Montastrea annularis* (Wells 1932). Longer exposures (many days) to hypersalinity (45‰) can lead to sloughing of tissue by corals (Vaughan 1916; Marcus and Thorhaug 1981; Hoegh-Guldberg and Smith 1989). As previously discussed, prolonged hypersalinity produces the reduced diversity patterns observed in areas such as the Red Sea and the Arabian Gulf (Kinsman 1964; Downing 1985; Coles 1988), as well as numerous coral atolls (Jokiel and Maragos 1978; Smith and Jokiel 1978; Caspers 1981). I chose to examine the effects of hypersalinity in this experiment because they are less understood and less studied than the effects of hyposalinity. Furthermore, there is some evidence to suggest that stony corals are more sensitive to elevated salinities than lowered salinities. Edmondson (1928) found that *Montipora verrucosa* was more sensitive to experimentally raised salinities of 4‰ to 39‰ than to lowered salinities of 10‰ to 25‰.

The majority of salinity studies have been observational field studies. Patterns of changes in reef organisms have been observed and then a hypothesized correlation to the environmental factors has been established.

Local responses in coral reefs have been correlated with local environmental changes, but there are no direct causal mechanisms offered. Experimental studies can be used to explore causal relationships under controlled conditions, thereby attempting to discover underlying mechanisms of changes or responses. I elected to examine the effects of hypersalinity under controlled experimental conditions because the results are more easily interpreted and the causative factors are more easily determined.

Early studies of the effect of salinity on corals utilized very crude measurement techniques, such as mortality (Vaughan 1916; Edmondson 1928). Later studies measured additional responses to salinity changes, such as reduced reproduction and reduced growth (Jokiel and Coles 1977; Coles and Jokiel 1978), as well as bleaching and release of mucus (Marcus and Thorhaug 1981; Glynn and D'Croz 1990). The disadvantage of relying on visually observable, gross changes in an organism's health is that subtle changes cannot be detected. By measuring an organism's metabolic response to a treatment, early, subtle effects can be seen before a physical consequence is visible. Three studies have measured coral metabolic responses to salinity changes, however, two only examined the effects of reduced salinities. Moberg et al. (1997) measured the effects of two reduced salinities on *Porites lutea* and *Pocillopora damicornis*. Hoegh-Guldberg and Smith (1989) measured the metabolism of *Stylophora pistillata* and *Seriatopora hystrix*, after, not during, exposure to only one reduced salinity treatment. Muthiga and Szmant (1987) examined metabolism of *Siderastrea siderea* exposed to reduced and elevated salinities. I chose to use metabolic response variables in this study to allow me to examine subtle changes in coral health.

Few studies have looked at the effect of exposure time by taking repeated measurements over time. The few experimental studies have generally exposed

corals to an altered salinity level and then measured the effects only once. For example, many studies have simply determined whether a coral has lived or died after treatment exposure (Vaughan 1916; Edmondson 1928; Kinsman 1964). I was particularly interested in the effects of exposure time to see if the level of damage may be directly affected by the length of time that an organism is exposed to the altered environmental conditions. Additionally, the responses of corals to hypersalinity may change over time, for example a coral may not respond until after a threshold exposure time has been reached. By utilizing non-destructive metabolic measurements, I repeatedly measured responses over time to explore the effects of exposure time.

I chose to examine the effects of hypersalinity on corals in the Florida Keys Reef Tract because of the potential influence of Florida Bay water on the reefs. High evaporation and low freshwater input yielded average salinities of 52‰ and highs of 70‰ in Florida Bay (Robblee et al. 1989; Smith et al. 1989; Fourqurean and Zieman 1992). While the extreme salinity conditions of Florida Bay limit coral growth to just a few hardy species, with none growing in areas of most severe salinity fluctuations (Hudson et al. 1989), it is possible that water from the bay may impact the offshore reefs. Florida Bay water moves southward, between the islands of the Keys and out over the coral reefs (Smith et al. 1989; Smith 1994). There have been few studies of salinity conditions over reefs in the Florida Keys. However, one study notes higher salinity water (38.5‰) was recorded on the reefs of the Florida Keys (Porter et al. 1999) and anecdotal evidence suggests that similar conditions have repeatedly occurred (J. Porter, pers. comm.). However, it is not clear how this water affects the corals. A marked decline in coral cover and diversity has been recorded in Florida since the mid 1980's, with decline noted in five of six monitoring stations and as much as 46% reduction in coral cover at one station (Porter and Meier 1992).

However, the reasons for this decline are not clearly understood. I chose to conduct this experiment using *Montastrea annularis* as my study organism because it is one of the dominant reef-building corals in the Florida Keys. According to Jaap (1984), *Montastrea annularis* is one of the two stony corals most responsible for reef building in southern Florida. In order to understand the effects of hypersaline water on the Florida Keys Reef Tract, it is important to know how these conditions would affect a dominant coral species. The existence of reefs depends upon the survival of stony corals, since death of corals typically results in death or migration of other reef organisms (Johannes 1975).

**Materials and methods**

*Collection and maintenance of corals*

All coral samples were collected from Admiral Patch Reef off Key Largo, Florida ($25^{o}00.323$ N, $80^{o}23.066$ W). *Montastrea annularis* was the dominant stony coral and most closely resembled the sibling species *Montastrea annularis* cf. *faveolata* (Knowlton et al. 1992). All samples consisted of healthy tissue without scars or bleached areas and were clean of algae and boring organisms. A total of 24 corals were used in this experiment, with a sample size of 6 corals in each treatment. Samples were placed in racks secured to the sea-floor at the patch reef for a minimum of 2 weeks to allow for acclimation and recovery from collection stress prior to use in the experiment.

Upon return from the patch reef, the corals were placed in scrubbed aquaria filled with newly collected seawater. Each aquarium was equipped with 500 W halogen lights providing an irradiance of $600\mu Em^{-2}s^{-1}$ and the corals received 12 hours of light each day. Air bubblers and water pumps provided aeration and continuous water movement. The temperature was maintained at

local ambient reef conditions of 30$^{o}$C and was monitored with thermographs and thermometers. Salinity regulation was achieved by adding Instant Ocean™ or purified freshwater and was monitored with a refractometer every hour.

*Response variables*

This experiment utilized several response variables: survivorship, chlorophyll concentration, and three metabolic response variables, net photosynthesis rate, respiration rate, and gross photosynthesis to respiration ratio.

Corals were defined as dead when they began sloughing tissue. After each coral was removed from the experimental treatments, the chlorophyll density was measured. A stream of filtered high-pressure seawater was used to remove the tissue from each coral and the coral skeleton was then sun-dried. The tissue blastate was measured and homogenized and samples were spun down in centrifuge tubes and the supernatant poured off. The remaining pellet was ground with cooled acetone in a tissue grinder and then placed in the freezer overnight. Spectrophotometer readings were taken the next day at 750 nm, 663 nm and 630 nm. Chlorophyll content of each coral was calculated using the equations of Jeffrey and Humphrey (1975).

Net photosynthesis rate, $P_{net}$, was the measured rate of oxygen production under light conditions and respiration rate, R, was the measured rate of oxygen consumption under dark conditions for the intact coral. Gross photosynthesis to respiration ratio, $P_g$:R, utilizes gross photosynthesis which can be estimated by summing net photosynthesis and respiration measurements, assuming equivalent light and dark respiration. By examining the amount of oxygen produced by the algal symbionts and the amount of oxygen consumed by the coral-algae symbiosis, this dimensionless ratio gives an indication of the

autotrophic potential of the coral. However, it does not indicate how much photosynthate is translocated to the host.

*Oxygen flux experiments*

Rates of net photosynthesis and respiration were measured every six hours by placing each coral piece inside an airtight and watertight glass chamber and monitoring the changes in dissolved oxygen with YSI oxygen electrodes and meters. The chambers were flushed every half to one hour with fresh seawater in order to maintain oxygen concentrations within 25% of ambient. Rotating stir bars inside each chamber maintained uniformity of dissolved oxygen levels throughout the chambers. Oxygen production (net photosynthesis) was measured under saturating light conditions and oxygen consumption (respiration) was measured under dark conditions. Oxygen probe consumption rates were determined on a daily basis by monitoring the oxygen levels in empty chambers.

Dissolved oxygen readings were automatically logged every 2 minutes, and oxygen production and consumption rates were determined by linear regression and subsequently normalized by coral surface area. Pre-treatment photosynthesis and respiration measurements were taken for each coral under control conditions (35‰) representing ambient summer conditions at the collection site. No measurements were taken at midnight and no photosynthesis measurements were taken during nighttime hours. One respiration rate and one photosynthesis rate were determined for each six-hour period following the initial exposure to the treatment conditions. Measurements were terminated when the subject began sloughing tissue or the exposure time exceeded 48 hours.

*Experimental design*

Four treatment groups were utilized in the experiment with a sample size of 6 in each group. One treatment group was used as a control (35‰) and three groups were assigned to elevated salinity treatments: 40‰, 45‰, and 60‰. The levels chosen are representative of salinity levels measured in Florida Bay (Robblee et al. 1989; Smith et al. 1989; Smith 1994). The corals were randomly assigned to the treatment groups and initially placed in tanks with water at control conditions (35‰) for 36 hours. Salinity was then raised slowly over the next 6 hours until the desired treatment level was reached. As a test of the experimental set-up, another set of corals was maintained at control conditions and showed no detrimental changes after 144 hours (6 days). Validation of the experimental procedures is indicated by the physiological consistency of this set of corals.

*Statistical analysis of covariates*

The response of corals to a given treatment may be influenced by pre-treatment factors, such as individual coral differences and past coral health. Therefore, these pre-treatment differences among treatment groups may mask differences in post-treatment responses. For example, a treatment group with significantly higher respiration rates prior to treatment exposure than the other groups may tend to have significantly higher respiration rates after treatment exposure independent of responding to the treatment. The degree to which pre-exposure differences influence post-exposure differences can be estimated through Analysis of Covariance. Hence, an analysis of covariance including these factors might provide a more powerful test than the more typical analysis of variance. Typically, the significance of pre-treatment measurements of the response variables is tested in a linear fashion. If these pre-exposure

differences do not significantly explain the post-exposure differences, one may appropriately ignore these differences in the analysis of variance of the post-exposure response variables.

The analysis of covariance procedure was used to test for significance ($\alpha$ = 0.05) of two different pre-exposure measurements for each response variable for all treatments and for all time periods. These two pre-exposure measurements were of the same type as the response variables, so that pre-treatment respiration was compared with post-treatment respiration, and so on. The two measurements utilized were a measurement immediately prior to exposure to the stress and the average of four prior measurements. Covariate analysis indicated that pre-treatment exposure differences had no influence on the metabolic responses at any post-exposure time period. Therefore, analysis of variance was used for examination of treatment and time effects.

Pre-exposure measurements of chlorophyll density, although desirable for analysis of covariance, are difficult to obtain. Typically, measurements of chlorophyll require destruction of the organism, thus rendering the subject unavailable for further stress experiments. Therefore, no pre-treatment levels of chlorophyll were obtained in this experiment.

*Hypothesis testing*

Significance levels for all hypothesis tests were 0.05, and all statistical analyses were carried out using a PC SAS package. The analysis of variance of the salinity factor was performed in two ways: (1) analysis of variance (2-way ANOVA) within each time period to test for differences among the treatments; (2) analysis of variance (2-way ANOVA) within each treatment to test for temporal changes in the responses.

## Results

*Survivorship*

Elevated salinity had a dramatic negative effect on the survival times of the corals (Fig. 2.1). Control corals exposed to ambient salinity (35‰) showed no detrimental changes for the duration of the experiment and corals exposed to 40‰ also survived. However, there appears to be a threshold between 40‰ and 45‰, since corals exposed to 45‰ died between 36 and 42 hours. Further deviation beyond the threshold shows an even more pronounced decline, since beyond 45‰ there is a very steep drop-off in survivorship with 60‰ corals surviving between 18 and 24 hours.

Before corals began sloughing tissue and died, they released mucus. This occurred after 30 hours for corals exposed to 45‰ and after 12 hours for corals exposed to 60‰. Neither the control corals or those in the 40‰ treatment group released any mucus.

*Net photosynthesis*

As salinity level increased, net photosynthesis ($P_{net}$) decreased, and this response was more dramatic as exposure time increased (Fig. 2.2). Additionally, corals responded rapidly to elevated salinities. Corals exposed to 60‰ and 45‰ showed a rapid and dramatic decline in $P_{net}$, whereas corals exposed to 40‰ show little change over the course of the experiment, with a slightly lower $P_{net}$ than controls only after extended exposure. The differences among treatments are more clearly seen when the percent change in $P_{net}$ is examined over time (Fig. 2.3). Clearly, corals have a dramatically lower $P_{net}$ following exposure to high salinities than before treatment exposure.

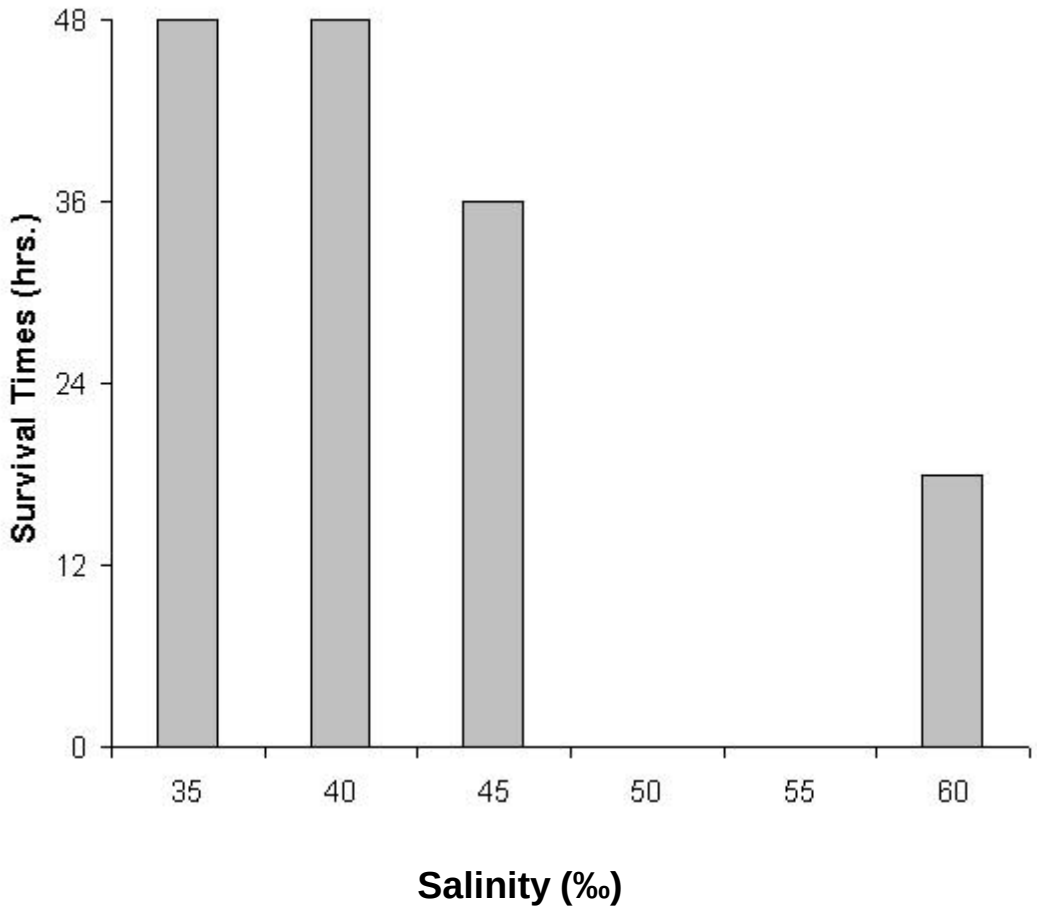


Figure 2.1. Coral survival times (n=6) for *Montastrea annularis* exposed to a range of salinities. Note corals exposed to 35‰ and 40‰ survived beyond the termination of the experiment.

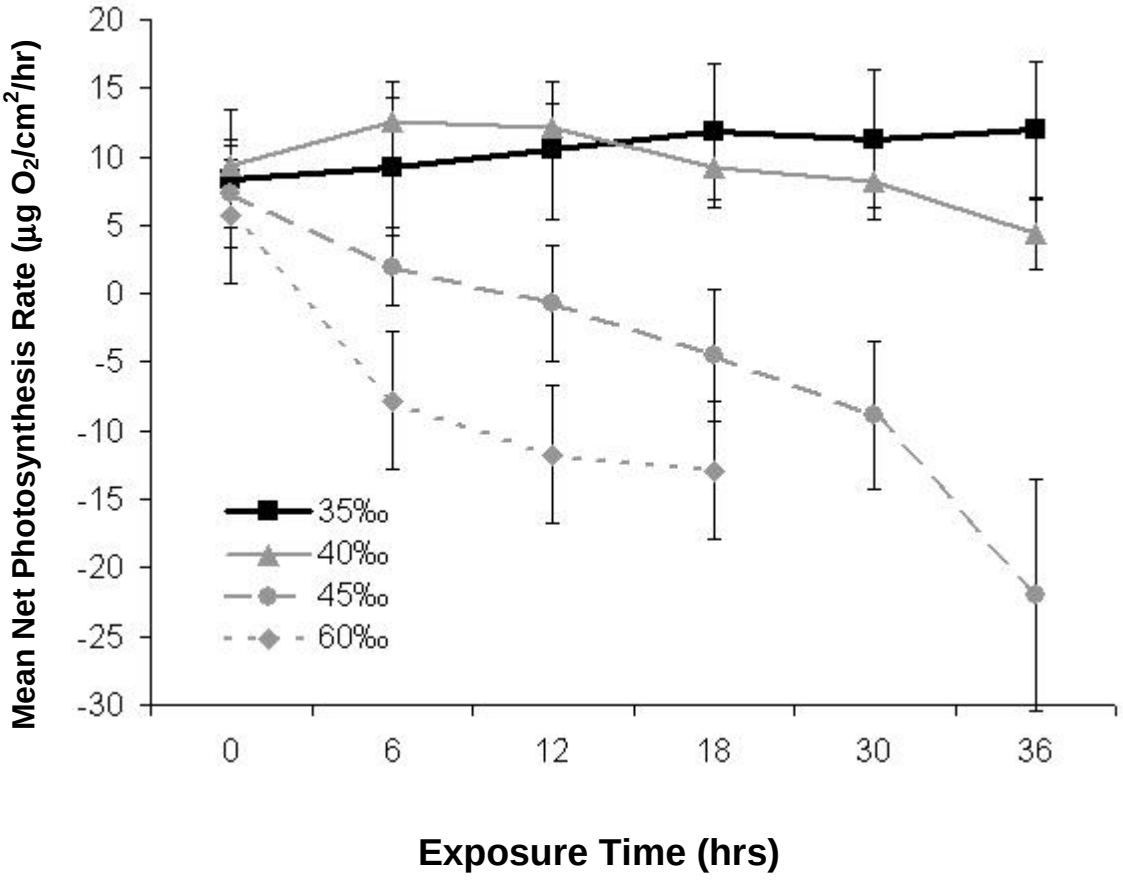


Figure 2.2. Mean net photosynthesis rates (± 1 S.D., n=6) for *Montastrea annularis* during increasing exposure times to different levels of salinity. Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

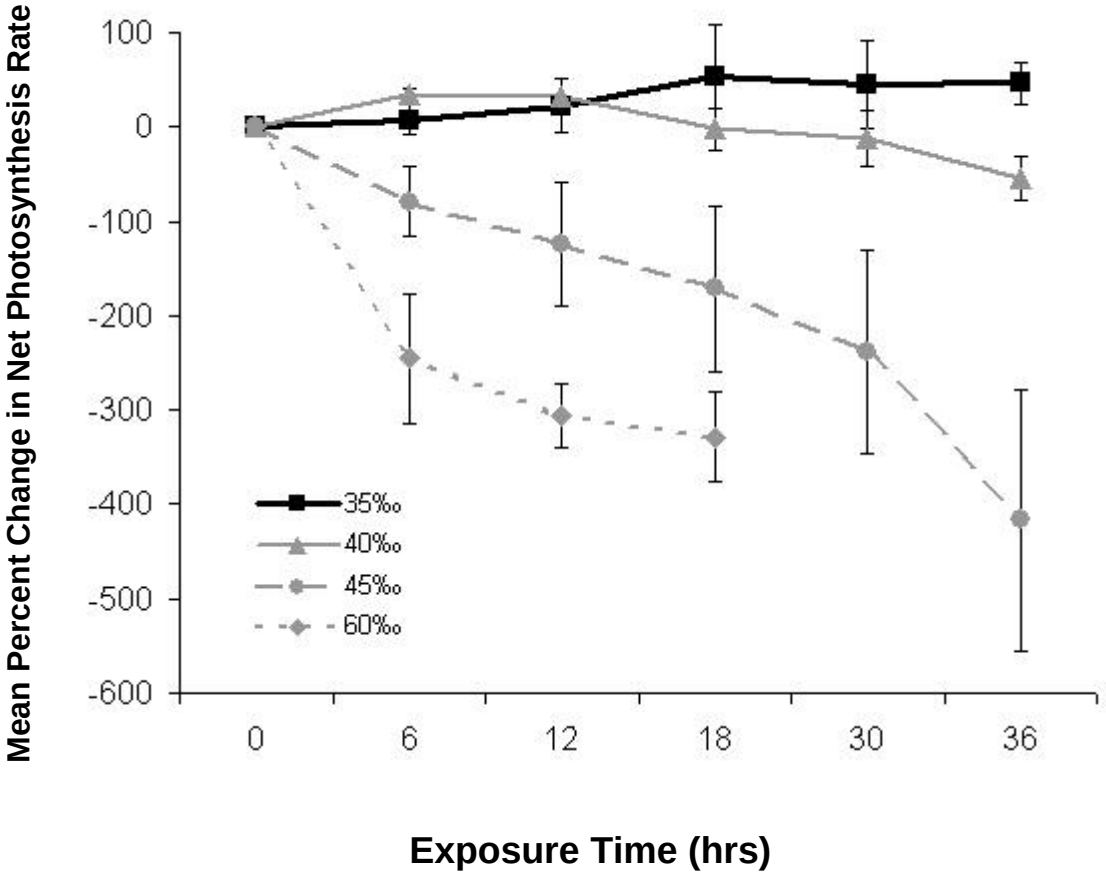


Figure 2.3. Mean percent change in net photosynthesis rates (± 1 S.D., n=6) for *Montastrea annularis* during increasing exposure times to different levels of salinity. Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

To investigate the differences among treatments in greater detail, I examined the responses for each salinity treatment by each time period (Fig. 2.4). $P_{net}$ is significantly lower the higher the salinity for all time periods ($p<0.001$, Table 2.1). The means and Tukey groupings indicate that after only 6 hours of exposure, corals in 60‰ water have the lowest $P_{net}$ (Table 2.1, Fig. 2.4), 250% lower than before treatment exposure (Table 2.2, Fig. 2.5). Corals exposed to 6 hours of 45‰ also show a significantly lower $P_{net}$ than control or 40‰ corals (Table 2.1, Fig. 2.4) and this response is 80% lower than before treatment (Table 2.2, Fig. 2.5). This trend is more pronounced as exposure time increased, with corals in 60‰ consistently maintaining the lowest $P_{net}$ (Fig. 2.4) and the greatest percent change in $P_{net}$ (Fig. 2.5). While corals exposed to 45‰ also show a more dramatic decline in net photosynthesis rates over time (over 400% lower after 36 hours), corals exposed to 40‰ are not significantly different from control corals until 36 hours of exposure when their $P_{net}$ has dropped 50% (Table 2.2, Fig. 2.5).

When the standard deviations of each sample of corals are examined, corals in 45‰ show the highest variation and this variation within the sample increases over time (Fig. 2.2). By examining the response of each individual coral, it is evident that the response to 45‰ is often dominated by one or two particularly sensitive corals (Fig. 2.6). Contrastingly, all corals within each of the 40‰ and 60‰ treatment groups respond similarly when exposed.

*Respiration*

Although respiration, R, was the least sensitive and consistent response variable, a trend was still clear: as salinity level increased, respiration rate increased and grew more pronounced as exposure time increased (Fig. 2.7). Extended exposure was required before the differences among the treatments became clear. However, when the percent change in respiration rate is

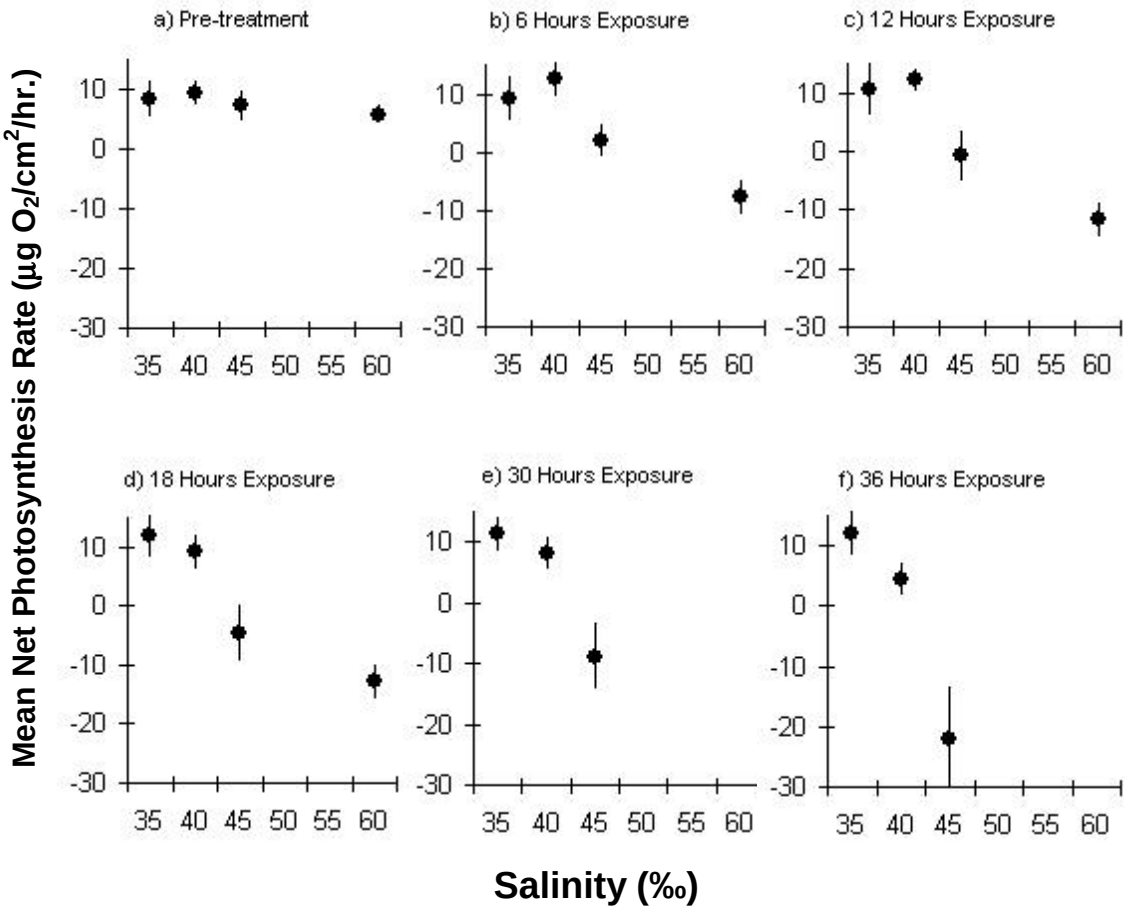


Figure 2.4. Mean net photosynthesis rates (± 1 S.D., n=6) for *Montastrea annularis* exposed to a range of salinities: before treatment exposure (a); during increasing exposure times (b-f). Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

Table 2.1. Effect of increased salinity on *Montastrea annularis* (n=6) for different exposure times. a) Means of net photosynthesis rates - statistical significance was assessed using ANOVA and a significance level of 0.05 (WA indicates that Welch's ANOVA was utilized due to unequal variances). Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of net photosynthesis rates - values with different labels indicate standard deviations significantly different with the Brown-Forsythe test for unequal variances.

**a**

**Means of Net Photosynthesis Rates (μg $O_2$/$cm^2$/hr)**

| Exposure Time | 35‰ | | 40‰ | | 45‰ | | 60‰ | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| Pre-Treatment | 8.4 | | 9.3 | | 7.3 | | 5.8 | | .076 |
| 6 hrs | 9.3 | a | 12.5 | a | 1.9 | b | -7.8 | c | <.001* |
| 12 hrs | 10.5 | a | 12.1 | a | -0.7 | b | -11.7 | c | <.001* |
| 18 hrs | 11.8 | a | 9.1 | a | -4.5 | b | -12.9 | c | <.001* |
| 24 hrs | Night | | | | | | | | |
| 30 hrs | 11.3 | a | 8.1 | a | -8.9 | b | | | <.001* |
| 36 hrs | 12.0 | a | 4.4 | b | -22.0 | c | | | <.001* (WA) |

**b**

**Standard Deviations of Net Photosynthesis Rates (μg $O_2$/$cm^2$/hr)**

| Exposure Time | 35‰ | 40‰ | 45‰ | 60‰ | |
|---|---|---|---|---|---|
| Pre-Treatment | 3.0 | 1.9 | 2.5 | 1.5 | .0535 |
| 6 hrs | 3.8 | 2.9 | 2.8 | 2.8 | .846 |
| 12 hrs | 4.5 | 1.8 | 4.3 | 2.8 | .137 |
| 18 hrs | 3.5 | 2.8 | 4.8 | 2.9 | .925 |
| 24 hrs | Night | | | | |
| 30 hrs | 2.8 | 2.7 | 5.4 | | .693 |
| 36 hrs | 3.7 a | 2.5 a | 8.4 b | | <.001* |

Table 2.2. Effect of increased salinity on *Montastrea annularis* (n=6) for different exposure times. a) Means of percent changes in net photosynthesis rates - statistical significance was assessed using ANOVA and a significance level of 0.05 (WA indicates that Welch's ANOVA was utilized due to unequal variances). Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of percent changes in net photosynthesis rates - values with different labels indicate standard deviations significantly different with the Brown-Forsythe test for unequal variances.

**a**

**Means of Percent Changes in Net Photosynthesis Rates**

| Exposure Time | 35‰ | 40‰ | 45‰ | 60‰ | p-value |
|---|---|---|---|---|---|
| 6 hrs | 7.9 a | 33.6 a | -79.0 b | -245.3 c | <.001* (WA) |
| 12 hrs | 22.6 a | 32.7 a | -123.5 b | -306.2 c | <.001* (WA) |
| 18 hrs | 52.6 a | -2.5 a | -171.6 b | -328.3 c | <.001* |
| 24 hrs | Night | | | | |
| 30 hrs | 44.9 a | -11.9 a | -238.4 b | | <.001* |
| 36 hrs | 46.8 a | -55.2 b | -416.8 c | | <.001* |

**b**

**Standard Deviations of Percent Changes in Net Photosynthesis Rates**

| Exposure Time | 35‰ | 40‰ | 45‰ | 60‰ | |
|---|---|---|---|---|---|
| 6 hrs | 15.9 a | 8.1 b | 37.7 a | 69.2 a | .023* |
| 12 hrs | 28.2 a | 18.5 b | 65.3 b | 34.5 b | .011* |
| 18 hrs | 56.0 | 21.4 | 88.4 | 47.1 | .542 |
| 24 hrs | Night | | | | |
| 30 hrs | 45.8 | 29.6 | 107.5 | | .292 |
| 36 hrs | 22.0 | 23.1 | 139.1 | | .131 |

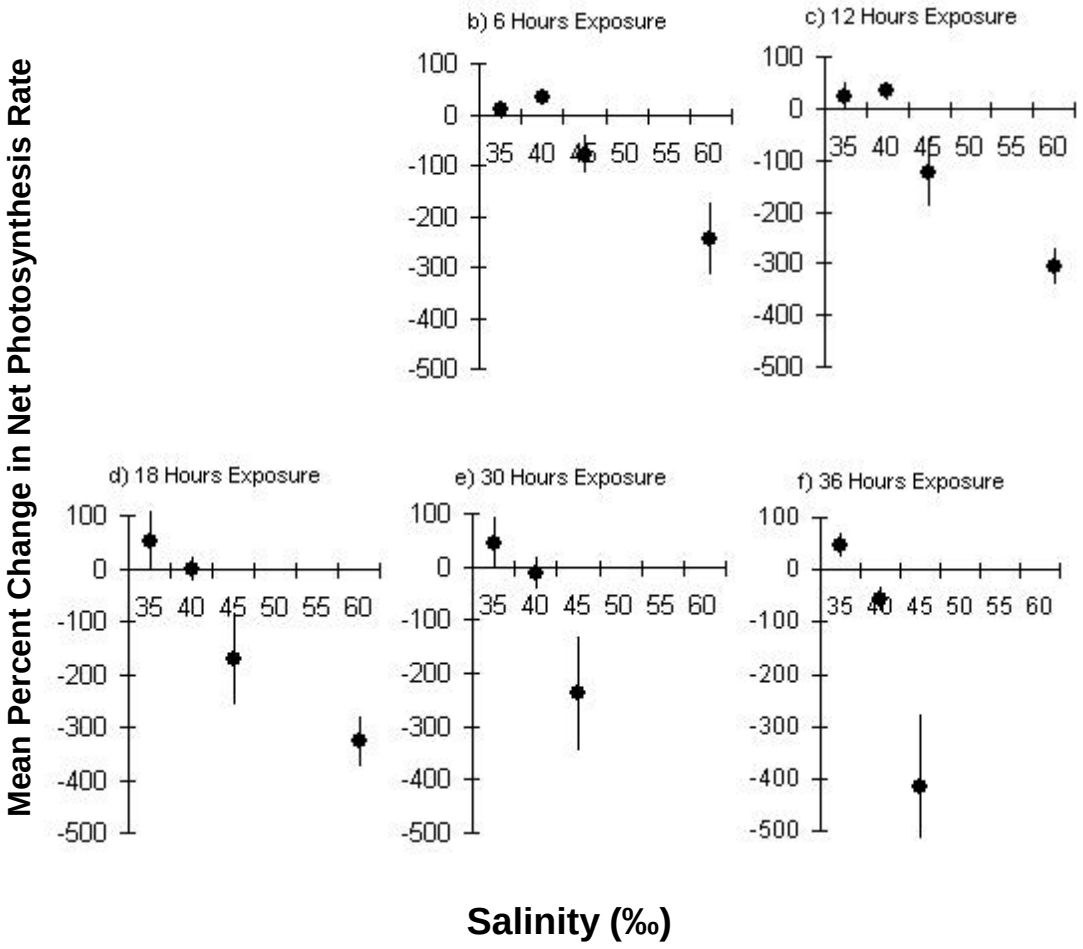


Figure 2.5. Mean percent change in net photosynthesis rates (± 1 S.D., n=6) for *Montastrea annularis* exposed to a range of salinities: before treatment exposure (a); during increasing exposure times (b-f). Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

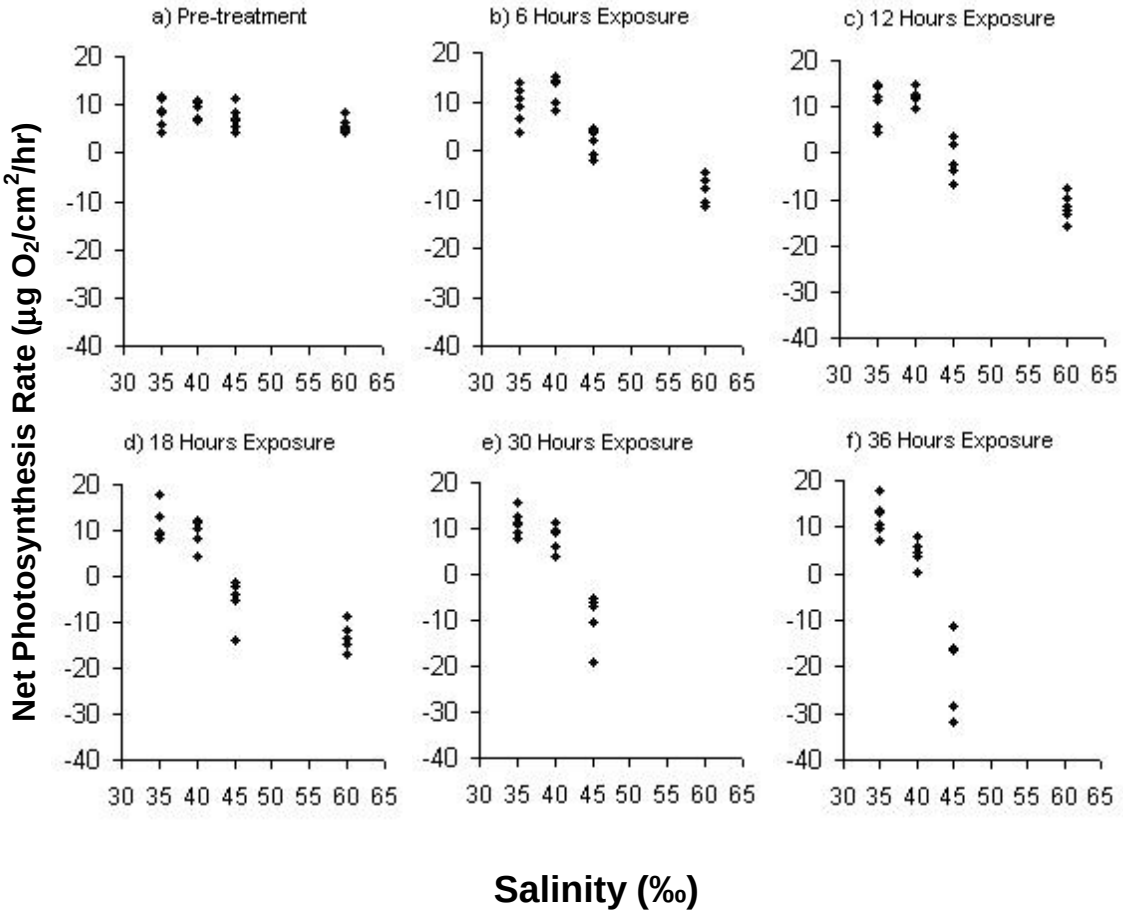


Figure 2.6. Net photosynthesis rates for *Montastrea annularis* exposed to a range of salinities: before treatment exposure (a); during increasing exposure times (b-f). Each plotted point represents a photosynthesis rate of an individual coral (n=6 corals per salinity treatment group). Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

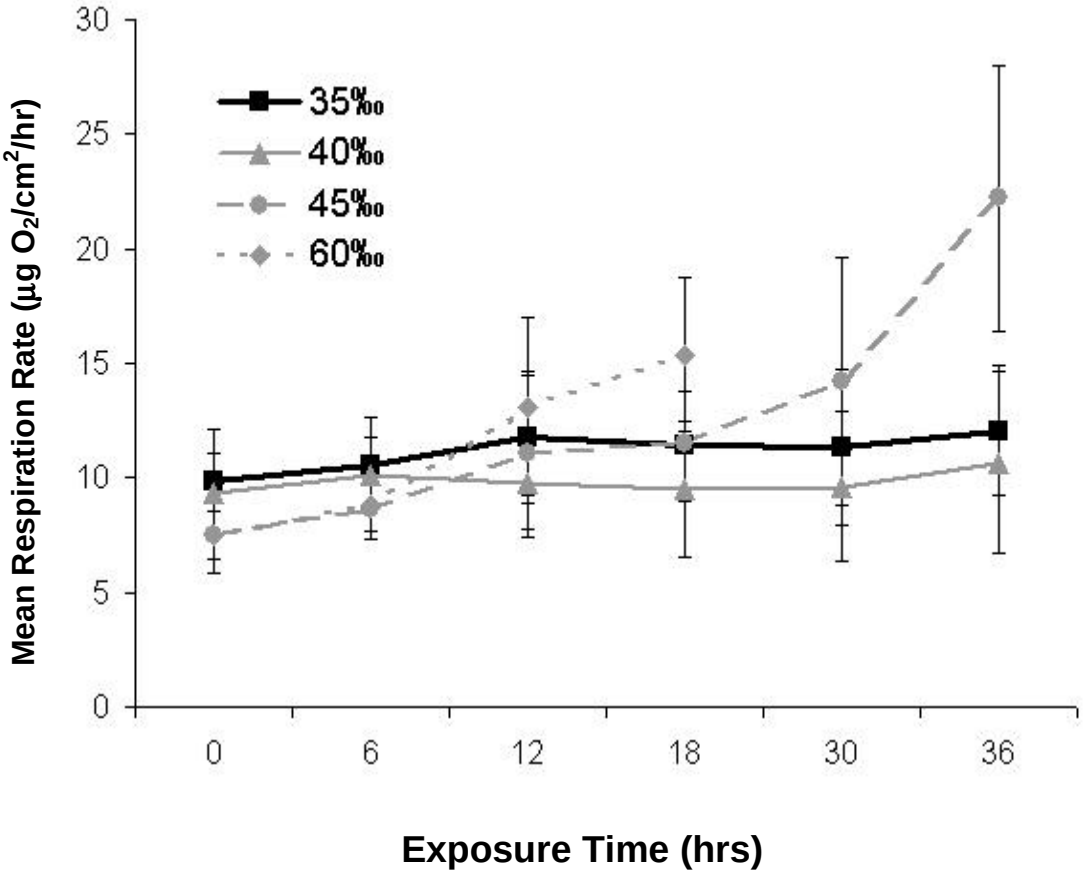


Figure 2.7. Mean respiration rates (± 1 S.D., n=6) for *Montastrea annularis* during increasing exposure times to different levels of salinity. Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

examined, the differences among the treatments are readily apparent (Fig. 2.8). While control and 40‰ corals show no change in R over the course of the experiment, corals in both the higher salinity treatment groups show a striking increase in R. Corals exposed to the highest salinity level, 60‰, showed the highest R after the shortest exposure time. Corals exposed to 45‰ also show an increase in R, though the increase is not as rapid.

I examined the responses to salinity within each time period to further investigate the differences among treatments (Fig. 2.9). There is no significant difference among treatments until 18 hours of exposure, when corals in the 60‰ treatment show significantly higher R (Table 2.3). Corals exposed to 45‰ show a significantly higher R than either the control or 40‰ corals after 36 hours of exposure. However, when the percent change in respiration rate is examined, significant differences are detected after 12 hours and the differences become more pronounced as exposure time increases (Table 2.4, Fig. 2.10). After 18 hours, corals exposed to 60‰ show a 100% increase in their respiration rate, while corals exposed to 45‰ show a 200% increase after 36 hours (Table 2.4). However, corals in the 40‰ treatment group never differ from the control corals.

Similar to the results for net photosynthesis rates, corals exposed to 45‰ again show the greatest variation in respiration rates, however there is no significant difference until 36 hours of exposure (Table 2.3, Fig. 2.7). By examining the response of each individual coral, it is clear that some corals are reacting more rapidly to the increased salinity than others in the treatment group (Fig. 2.11). By contrast, corals exposed to low and high salinities (40‰ and 60‰) appear to react similarly (Fig. 2.11), as indicated by the lower standard deviations for these groups (Table 2.4).

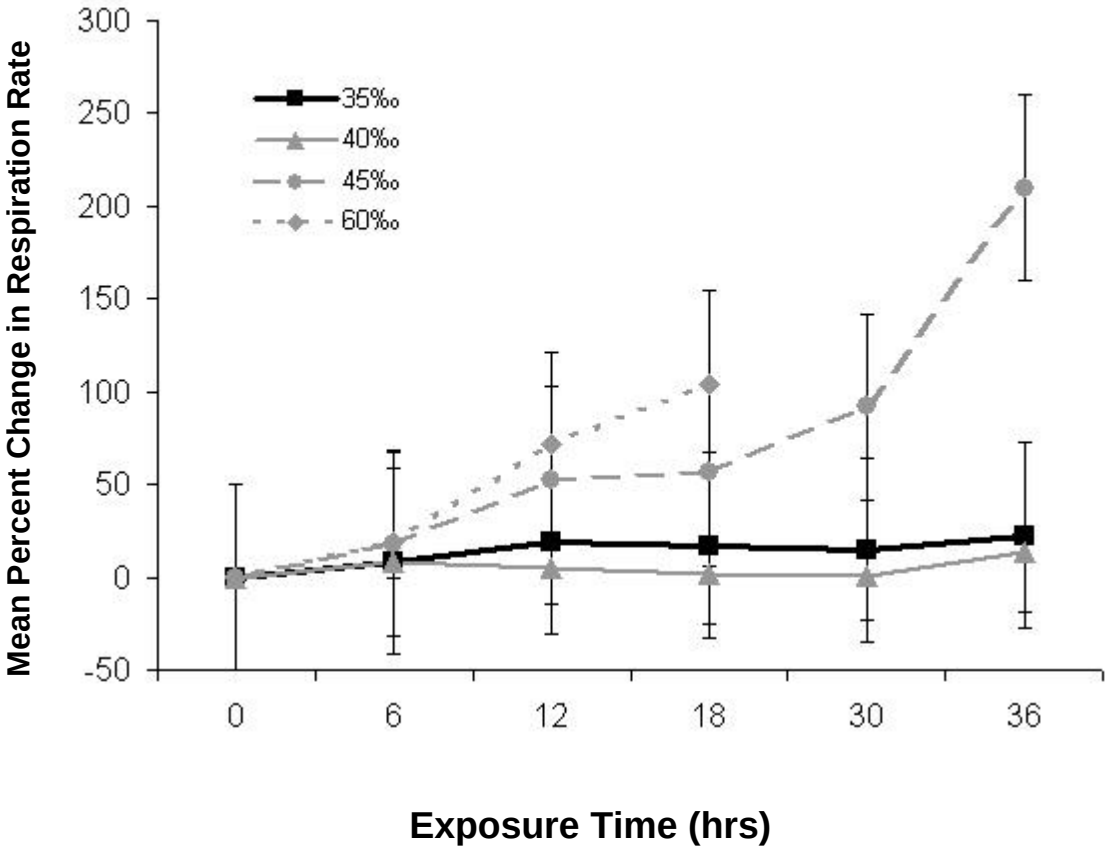


Figure 2.8. Mean percent change in respiration rates (± 1 S.D., n=6) for *Montastrea annularis* during increasing exposure times to different levels of salinity. Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

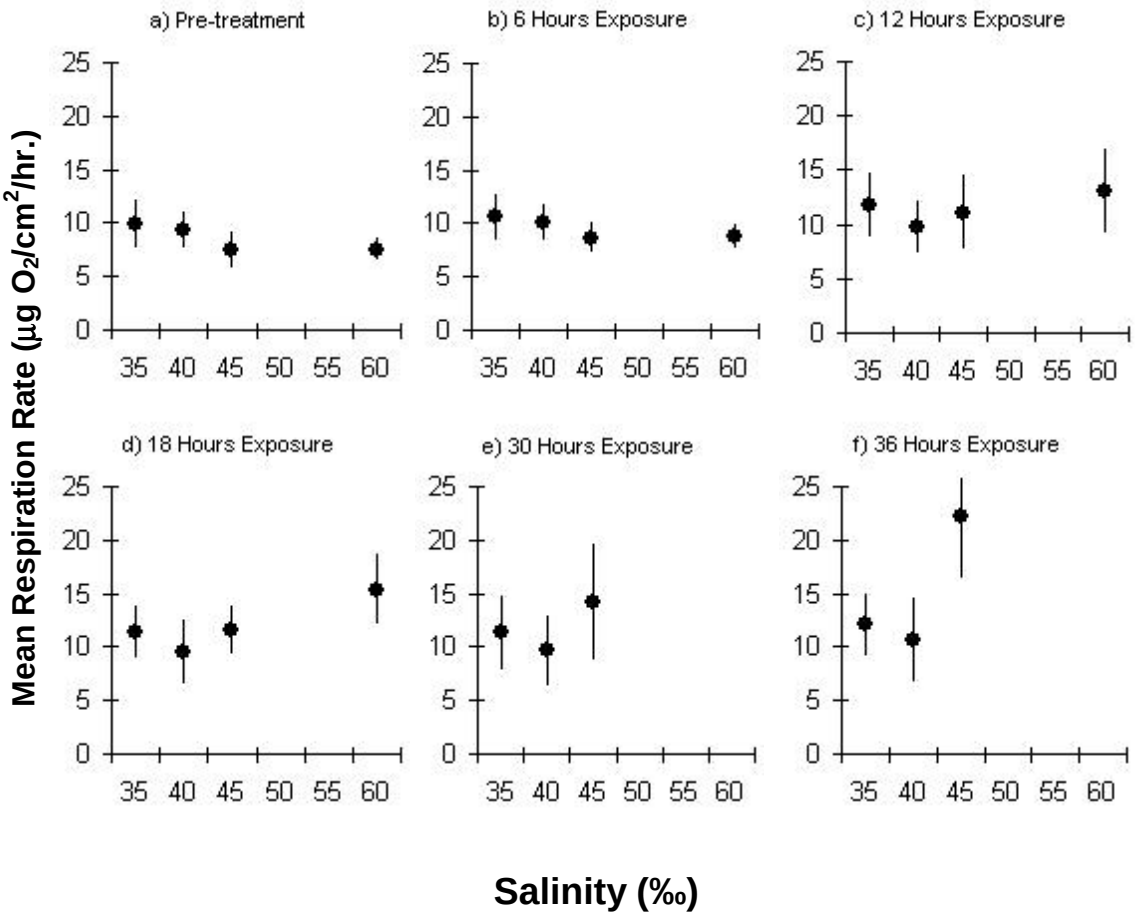


Figure 2.9. Mean respiration rates (± 1 S.D., n=6) for *Montastrea annularis* exposed to a range of salinities: before treatment exposure (a); during increasing exposure times (b-f). Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

Table 2.3. Effect of increased salinity on *Montastrea annularis* (n=6) for different exposure times. a) Means of respiration rates - statistical significance was assessed using ANOVA and a significance level of 0.05 (WA indicates that Welch's ANOVA was utilized due to unequal variances). Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of respiration rates - values with different labels indicate standard deviations significantly different with the Brown-Forsythe test for unequal variances.

**a**

**Means of Respiration Rates (µg $O_2$/$cm^2$/hr)**

| Exposure Time | 35‰ | 40‰ | 45‰ | 60‰ | p-value |
|---|---|---|---|---|---|
| Pre-Treatment | 9.8 | 9.4 | 7.5 | 7.5 | .051 |
| 6 hrs | 10.6 | 10.1 | 8.7 | 8.8 | .130 |
| 12 hrs | 11.8 | 9.8 | 11.1 | 13.1 | .361 |
| 18 hrs | 11.4 a | 9.5 a | 11.5 a | 15.4 b | .011* |
| 24 hrs | Night | | | | |
| 30 hrs | 11.3 | 9.6 | 14.2 | | .185 |
| 36 hrs | 12.1 a | 10.7 a | 22.2 b | | .008* (WA) |

**b**

**Standard Deviations of Respiration Rates (µg $O_2$/$cm^2$/hr)**

| Exposure Time | 35‰ | 40‰ | 45‰ | 60‰ | p-value |
|---|---|---|---|---|---|
| Pre-Treatment | 2.3 | 1.7 | 1.6 | 1.0 | .308 |
| 6 hrs | 2.1 | 1.7 | 1.3 | 1.1 | .672 |
| 12 hrs | 2.9 | 2.4 | 3.3 | 3.9 | .848 |
| 18 hrs | 2.4 | 3.0 | 2.2 | 3.3 | .646 |
| 24 hrs | Night | | | | |
| 30 hrs | 3.4 | 3.3 | 5.4 | | .766 |
| 36 hrs | 2.8 a | 4.0 a | 5.8 b | | .038* |

Table 2.4. Effect of increased salinity on *Montastrea annularis* (n=6) for different exposure times. a) Means of percent changes in respiration rates - statistical significance was assessed using ANOVA and a significance level of 0.05 (WA indicates that Welch's ANOVA was utilized due to unequal variances). Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of percent changes in respiration rates - values with different labels indicate standard deviations significantly different with the Brown-Forsythe test for unequal variances.

**a**

**Means of Percent Changes in Respiration Rates**

| Exposure Time | 35‰ | | 40‰ | | 45‰ | | 60‰ | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| 6 hrs | 8.5 | | 8.3 | | 17.9 | | 18.5 | | .391 (WA) |
| 12 hrs | 19.3 | ab | 4.8 | a | 52.7 | bc | 71.8 | c | .006* (WA) |
| 18 hrs | 17.1 | a | 1.5 | a | 56.5 | b | 104.1 | c | <.001* |
| 24 hrs | Night | | | | | | | | |
| 30 hrs | 14.7 | a | 1.0 | a | 91.9 | b | | | .002* |
| 36 hrs | 22.7 | a | 13.1 | a | 209.8 | b | | | <.001* |

**b**

**Standard Deviations of Percent Changes in Respiration Rates**

| Exposure Time | 35‰ | | 40‰ | | 45‰ | | 60‰ | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| 6 hrs | 8.9 | a | 8.8 | a | 12.2 | a | 21.3 | b | .013* |
| 12 hrs | 6.3 | a | 19.2 | a | 44.7 | b | 27.8 | a | .019* |
| 18 hrs | 12.2 | | 26.9 | | 20.2 | | 30.1 | | .282 |
| 24 hrs | Night | | | | | | | | |
| 30 hrs | 13.8 | | 24.2 | | 61.4 | | | | .119 |
| 36 hrs | 9.4 | | 32.1 | | 112.6 | | | | .052 |

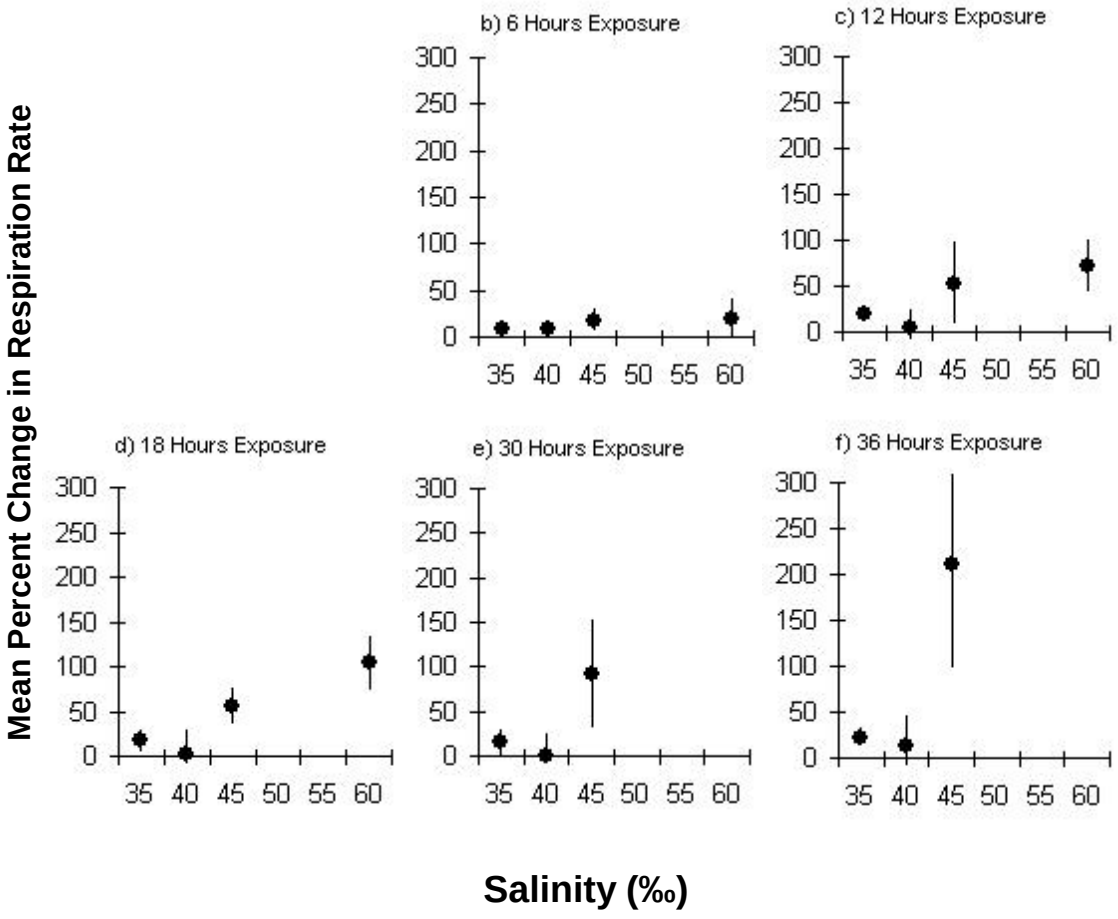


Figure 2.10. Mean percent change in respiration rates (± 1 S.D., n=6) for *Montastrea annularis* exposed to a range of salinities: before treatment exposure (a); during increasing exposure times (b-f). Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

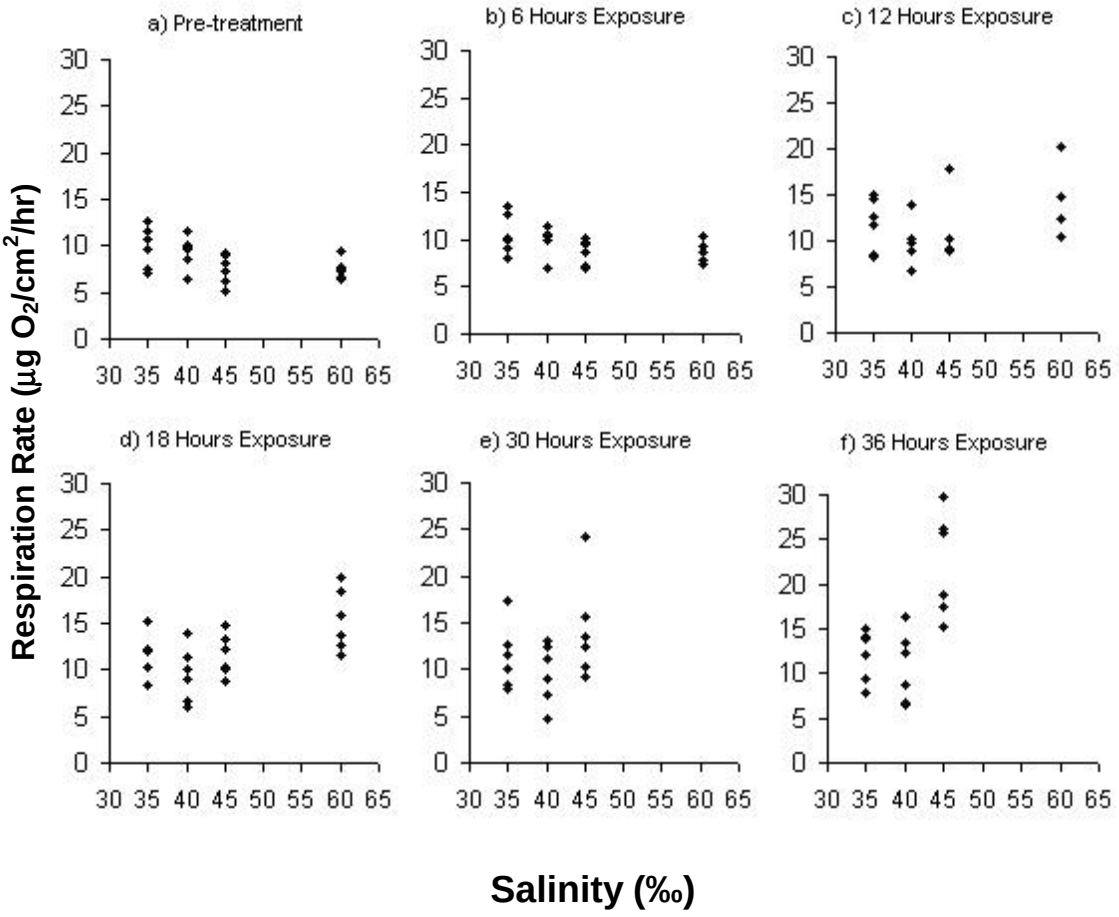


Figure 2.11. Respiration rates for *Montastrea annularis* exposed to a range of salinities: before treatment exposure (a); during increasing exposure times (b-f). Each plotted point represents a respiration rate of an individual coral (n=6 corals per salinity treatment group). Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

*Photosynthesis to respiration ratio*

As salinity level increased, gross photosynthesis to respiration ratio, $P_g$:R, decreased and this response was more pronounced as exposure time increased (Fig. 2.12). This decline is clearly apparent after only 6 hours of treatment exposure. The most rapid and severe decline in $P_g$:R occurs in corals in the 60‰ treatment group, with corals in the 45‰ treatment group also declining rapidly (Fig. 2.12). There is little difference between corals in 40‰ and control corals, however, after extended exposure corals in 40‰ do show a slightly lower $P_g$:R. The differences among treatments are even more clearly distinguishable when the percent change in $P_g$:R is examined over time (Fig. 2.13).

$P_g$:R for 60‰ corals is significantly lower than any other treatment group after only 6 hours of exposure, as well as during all subsequent measurement periods ($p<0.001$, Table 2.5, Fig. 2.14). Corals exposed to 60‰ have a $P_g$:R reaching near 0 after only 6 hours, over 90% lower than before treatment application (Table 2.6, Fig. 2.15). The same effect is evident for 45‰ corals for each time period ($p<0.001$, Table 2.5, Fig. 2.14). Corals in this treatment group have a $P_g$:R near 1 after 6 hours, 40% lower than before treatment exposure, and eventually reaching 0, almost 100% lower, after 36 hours of exposure (Table 2.6, Fig. 2.15). By contrast, both corals in 40‰ and control corals maintain $P_g$:R of 2 until after 36 hours, when the $P_g$:R for 40‰ had dropped by 30% (Table 2.5, Table 2.6, Fig. 2.14). Unlike the other response variables, there are no differences among standard deviations of the different treatment groups for $P_g$:R (Table 2.5), except that the standard deviations for percent change in $P_g$:R for 60‰ are significantly higher than the other treatments (Table 2.6).

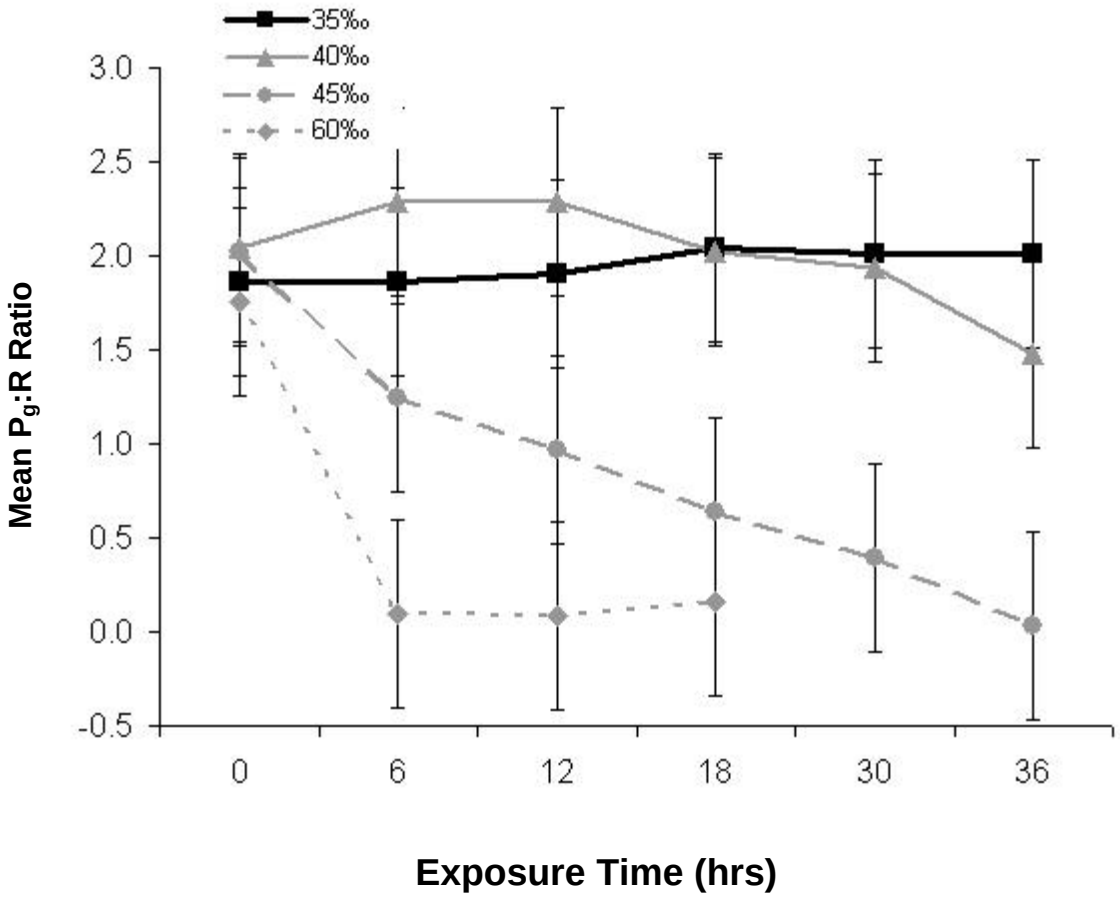


Figure 2.12. Mean $P_g$:R Ratios (± 1 S.D., n=6) for *Montastrea annularis* during increasing exposure times to different levels of salinity. Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

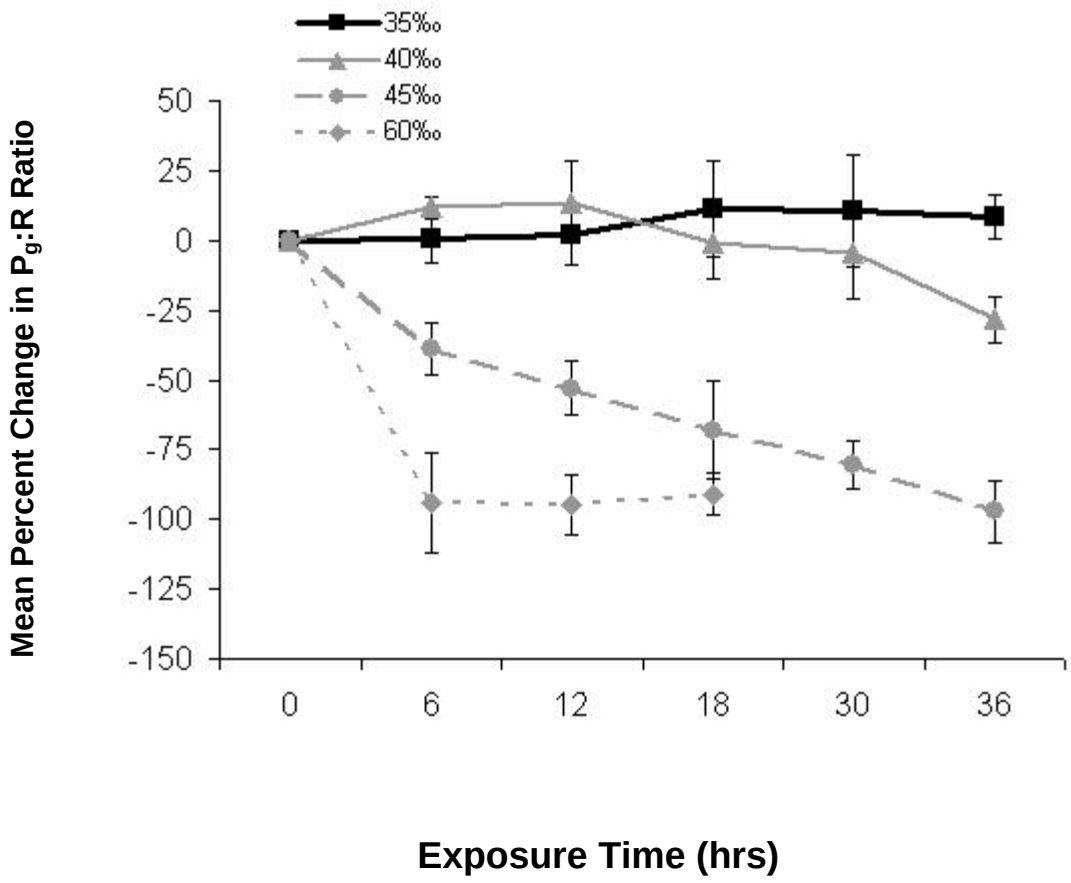


Figure 2.13. Mean percent change in $P_g$:R Ratios (± 1 S.D., n=6) for *Montastrea annularis* during increasing exposure times to different levels of salinity. Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

Table 2.5. Effect of increased salinity on *Montastrea annularis* (n=6) for different exposure times. a) Means of $P_g$:R Ratios - statistical significance was assessed using ANOVA and a significance level of 0.05 (WA indicates that Welch's ANOVA was utilized due to unequal variances). Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of $P_g$:R ratios - values with different labels indicate standard deviations significantly different with the Brown-Forsythe test for unequal variances.

**a**

**Means of $P_g$:R Ratios**

| Exposure Time | 35‰ | | 40‰ | | 45‰ | | 60‰ | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| Pre-Treatment | 1.9 | | 2.0 | | 2.0 | | 1.8 | | .353 |
| 6 hrs | 1.9 | a | 2.3 | a | 1.2 | b | 0.1 | c | <.001* |
| 12 hrs | 1.9 | a | 2.3 | a | 1.0 | b | 0.1 | c | <.001* |
| 18 hrs | 2.0 | a | 2.0 | a | 0.6 | b | 0.2 | c | <.001* |
| 24 hrs | Night | | | | | | | | |
| 30 hrs | 2.0 | a | 1.9 | a | 0.4 | b | | | <.001* |
| 36 hrs | 2.0 | a | 1.5 | b | 0.0 | c | | | <.001* |

**b**

**Standard Deviations of $P_g$:R Ratios**

| Exposure Time | 35‰ | 40‰ | 45‰ | 60‰ | |
|---|---|---|---|---|---|
| Pre-Treatment | 0.3 | 0.4 | 0.4 | 0.1 | .074 |
| 6 hrs | 0.3 | 0.4 | 0.3 | 0.3 | .880 |
| 12 hrs | 0.4 | 0.3 | 0.4 | 0.2 | .124 |
| 18 hrs | 0.2 | 0.4 | 0.3 | 0.1 | .334 |
| 24 hrs | Night | | | | |
| 30 hrs | 0.1 | 0.4 | 0.2 | | .083 |
| 36 hrs | 0.3 | 0.4 | 0.2 | | .485 |

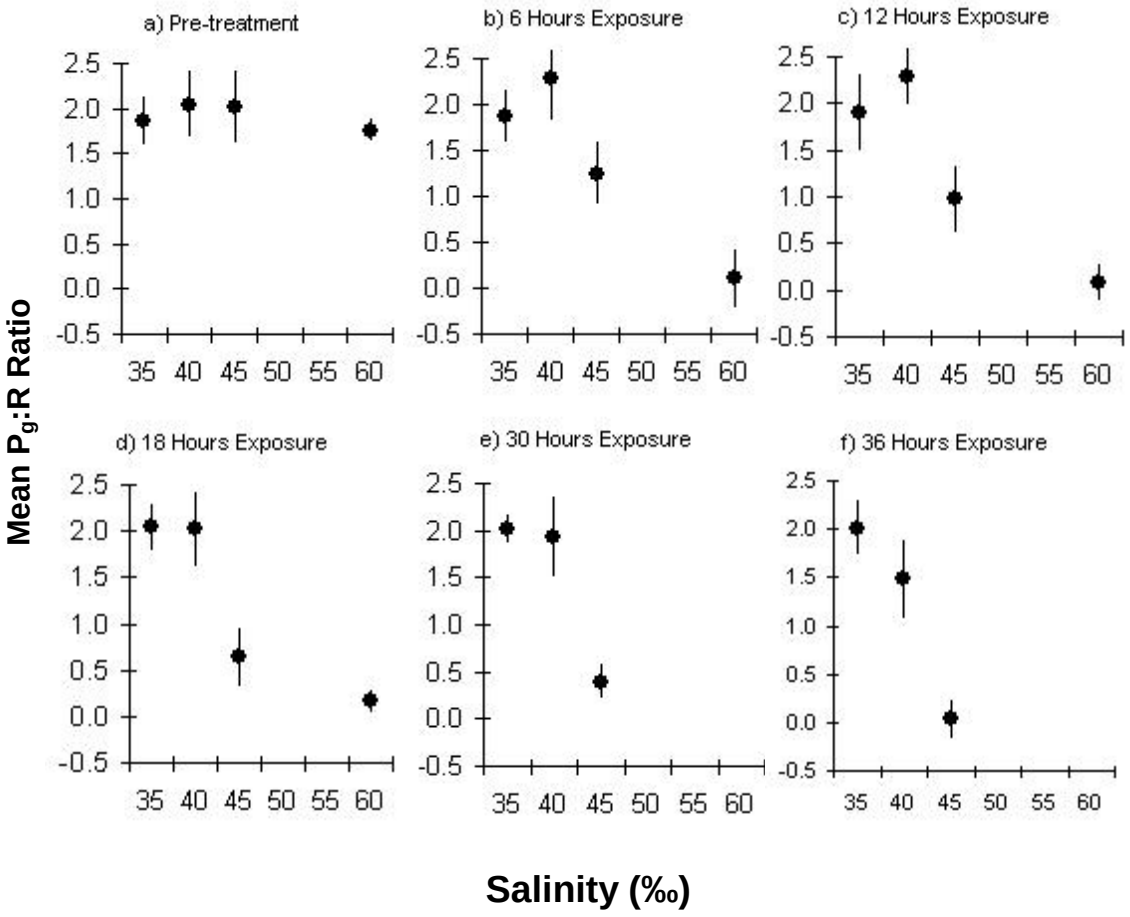


Figure 2.14. Mean $P_g$:R Ratios (± 1 S.D., n=6) for *Montastrea annularis* exposed to a range of salinities: before treatment exposure (a); during increasing exposure times (b-f). Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

Table 2.6. Effect of increased salinity on *Montastrea annularis* (n=6) for different exposure times. a) Means of percent changes in $P_g$:R Ratios - statistical significance was assessed using ANOVA and a significance level of 0.05 (WA indicates that Welch's ANOVA was utilized due to unequal variances). Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of percent changes in $P_g$:R ratios - values with different labels indicate standard deviations significantly different with the Brown-Forsythe test for unequal variances.

**a**

**Means of Percent Changes in $P_g$:R Ratios**

| Exposure Time | 35‰ | 40‰ | 45‰ | 60‰ | p-value |
|---|---|---|---|---|---|
| 6 hrs | 0.3 a | 11.7 a | -38.8 b | -94.3 c | <.001* (WA) |
| 12 hrs | 2.1 a | 13.5 a | -52.9 b | -95.0 c | <.001* |
| 18 hrs | 11.2 a | -0.6 a | -68.0 b | -90.9 b | <.001* |
| 24 hrs | Night | | | | |
| 30 hrs | 10.4 a | -4.8 a | -80.5 b | | <.001* |
| 36 hrs | -8.5 a | -28.3 b | -97.3 c | | <.001* |

**b**

**Standard Deviations of Percent Changes in $P_g$:R Ratios**

| Exposure Time | 35‰ | 40‰ | 45‰ | 60‰ | |
|---|---|---|---|---|---|
| 6 hrs | 8.2 a | 4.0 a | 9.5 a | 18.0 b | .018* |
| 12 hrs | 11.0 | 14.7 | 9.5 | 10.9 | .814 |
| 18 hrs | 17.3 | 13.2 | 17.6 | 7.6 | .305 |
| 24 hrs | Night | | | | |
| 30 hrs | 20.0 | 16.2 | 8.3 | | .570 |
| 36 hrs | 8.0 | 8.1 | 11.2 | | .948 |

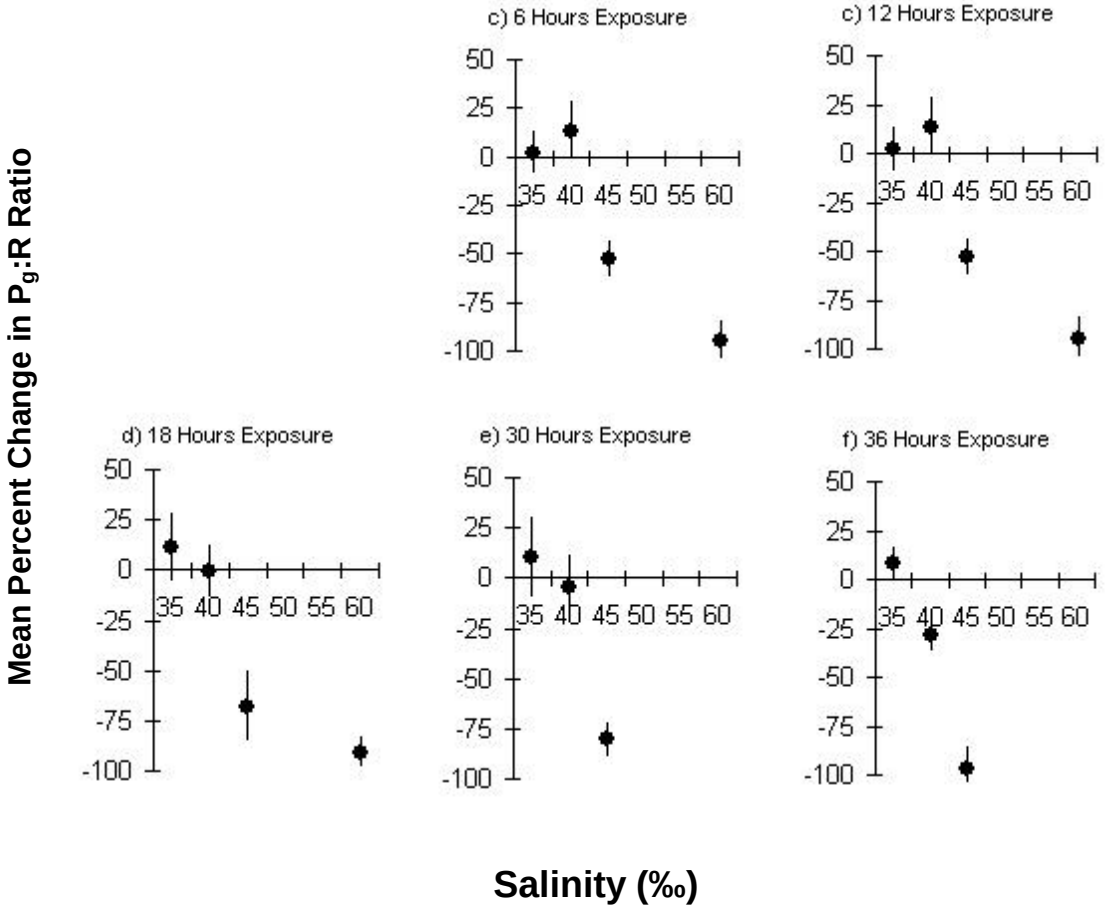


Figure 2.15. Mean percent change in $P_g$:R Ratio (± 1 S.D., n=6) for *Montastrea annularis* exposed to a range of salinities: before treatment exposure (a); during increasing exposure times (b-f). Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

*Chlorophyll density*

Chlorophyll density decreases markedly as salinity increases (Fig. 2.16). Chlorophyll content is significantly higher in the control corals than any of the higher salinities (Table 2.7, $p<0.001$). Corals exposed to 40‰ for the same length of time as the control corals (48 hours) had approximately one-half the chlorophyll density. The chlorophyll densities of corals exposed to 45‰ for 36 hours and corals exposed to 60‰ for 18 hours were only fractions of the density of the control corals.

Caution should be used when interpreting the chlorophyll density measurements, since exposure times were different for different treatments and chlorophyll was measured at the end of the run for each coral. Corals in control and 40‰ conditions were kept in the experimental set-up for 48 hours when these experiments were terminated and chlorophyll *a* content was determined. However, corals in higher salinities were exposed for shorter times since these corals died before the 48 hour truncation point of the experiment. Therefore, corals in 45‰ were only exposed for 36 hours and corals in 60‰ were only exposed for 18 hours. However, evidence from this experiment showed that higher salinities were more stressful than lower salinities, suggesting that the longer the corals were exposed to these high salinities the more stressful it was and therefore, the lower their chlorophyll content. Consequently, if all treatment groups were exposed for the same length of time, it is reasonable to suggest that the higher salinity treatment corals would actually show a more significant reduction in chlorophyll when compared with the controls.

*Correlations among response variables*

When photosynthesis is plotted against chlorophyll concentration, there is a strongly positive correlation between chlorophyll concentration and net

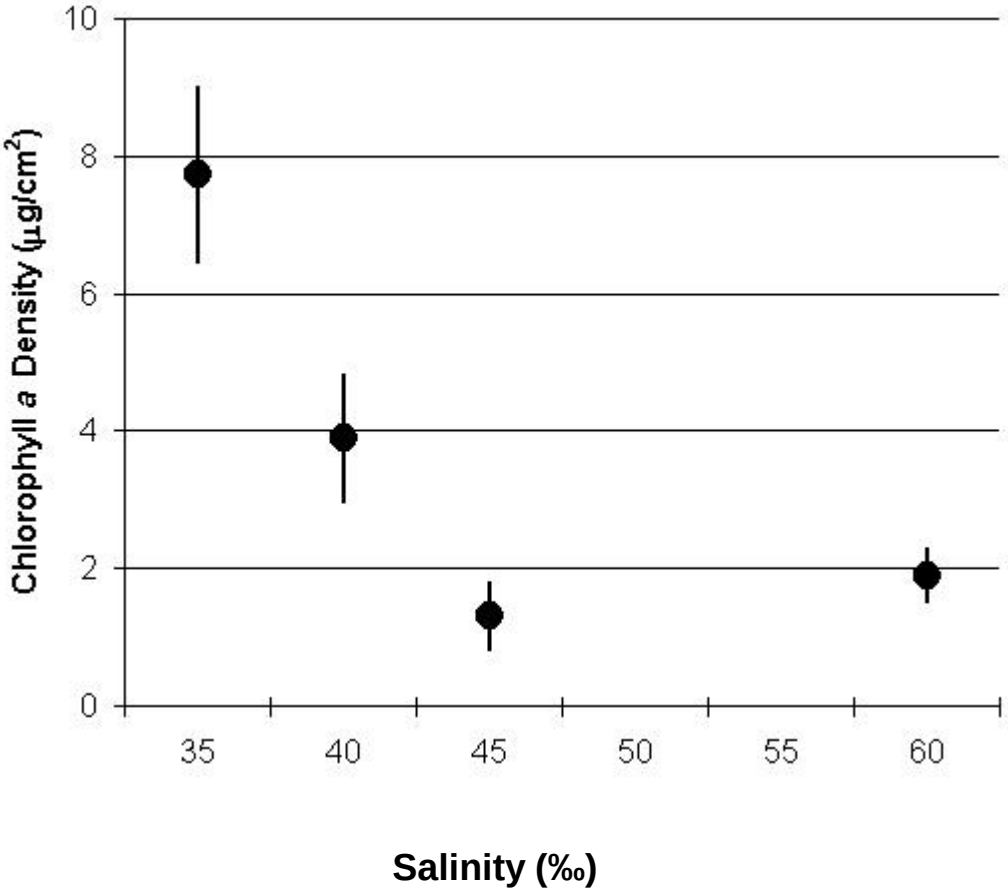


Figure 2.16. Mean Chlorophyll *a* densities (±1 S.D., n=6) for *Montastrea annularis* exposed to a range of salinities. Note corals exposed to 35‰ and 40‰ survived beyond the termination of the experiment.

Table 2.7. Effect of increased salinity on *Montastrea annularis* (n=6). a) Means of chlorophyll *a* density - statistical significance was assessed using ANOVA and a significance level of 0.05. Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of chlorophyll *a* densities.

**a**

**Means of Chlorophyll *a* Densities (µg/cm$^2$)**

| 35‰ and 48 hour exposure | 40‰ and 48 hour exposure | 45‰ and 36-42 hour exposure | 60‰ and 18-24 hour exposure | p-value |
|---|---|---|---|---|
| 7.74 a | 3.9 b | 1.31 c | 1.9 c | <.001* |

**b**

**Standard Deviations of Chlorophyll *a* Densities (µg/cm$^2$)**

| 35‰ and 48 hour exposure | 40‰ and 48 hour exposure | 45‰ and 36-42 hour exposure | 60‰ and 18-24 hour exposure | p-value |
|---|---|---|---|---|
| 1.27 | 0.93 | 0.50 | 0.40 | .153 |

photosynthesis (Fig. 2.17). There is a non-linear, saturation curve relationship between the two. The same relationship is seen between chlorophyll and photosynthesis to respiration ratio (Fig. 2.18). Conversely, there is a negative non-linear relationship between chlorophyll concentration and respiration (Fig. 2.19).

## Discussion

### *Survivorship*

This study clearly indicates that even short-lived pulses of high salinity water can trigger massive mortality among corals. The higher the salinity, the shorter the exposure time necessary to cause major damage to a reef. There are several areas around the world where reef organisms have adapted to survive in hypersaline conditions (Kinsman 1964; Jokiel and Maragos 1978; Smith and Jokiel 1978; Caspers 1981; Downing 1985; Coles 1988). However, this adaptation has occurred through prolonged exposure of many years where natural selection has resulted in the survival of only the few most euryhaline, tolerant species. Florida's reefs cannot adapt or acclimate to the sudden, short-lived (several hours to many days) pulses of high salinity water from Florida Bay.

The results from this experiment are consistent with early studies of salinity stress. Edmondson (1928) noted that the higher the salinity, the faster the mortality response. Seven of 12 Hawaiian coral species died within 2 weeks after a 6‰ salinity increase, while 5 of 12 species died within 2 days after a 12‰ increase and only 2 species survived 24 hours at 52‰ (Edmondson 1928). Wells (1932) also found a very rapid mortality with extreme hypersalinity. When he exposed Floridian coral species to 70‰, he observed 100% mortality within 12 hours.

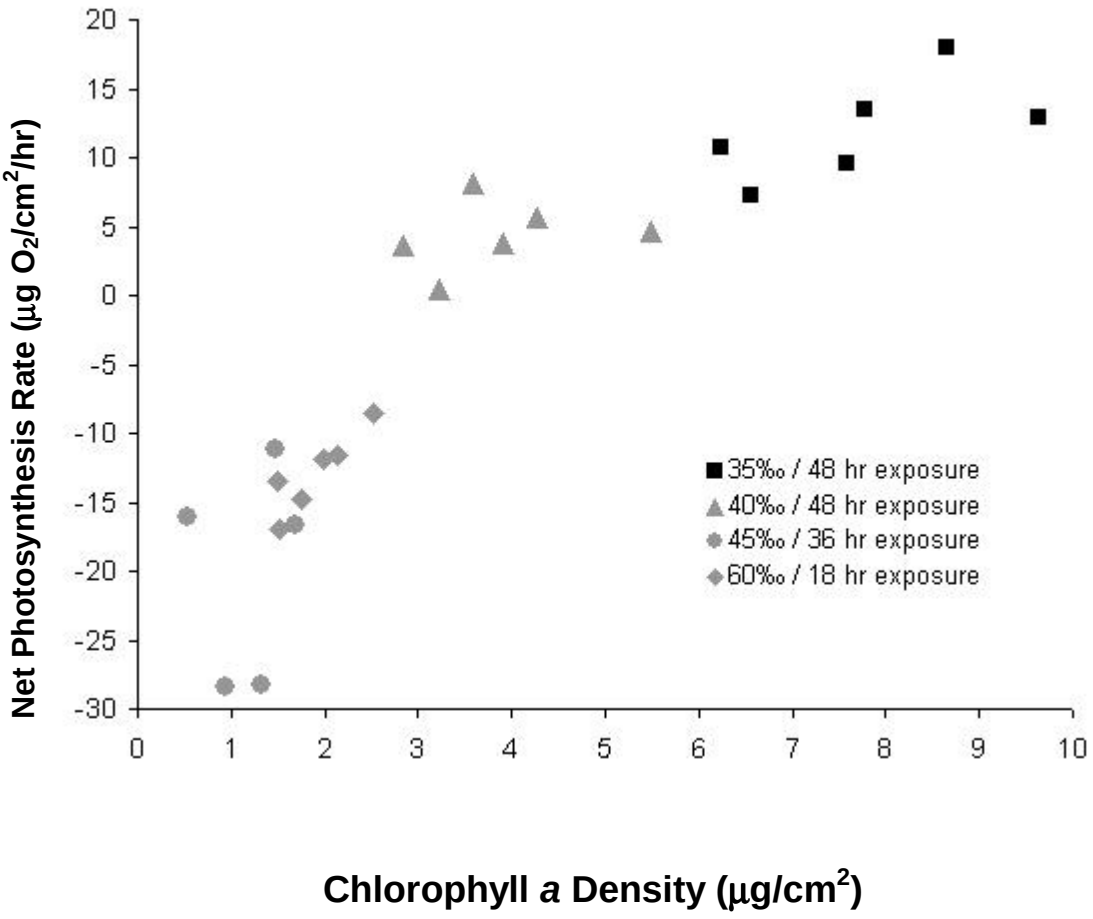


Figure 2.17. Relationship between net photosynthesis rate and chlorophyll *a* for *Montastrea annularis* (n=6 per treatment group) exposed to a range of salinities and exposure times. Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

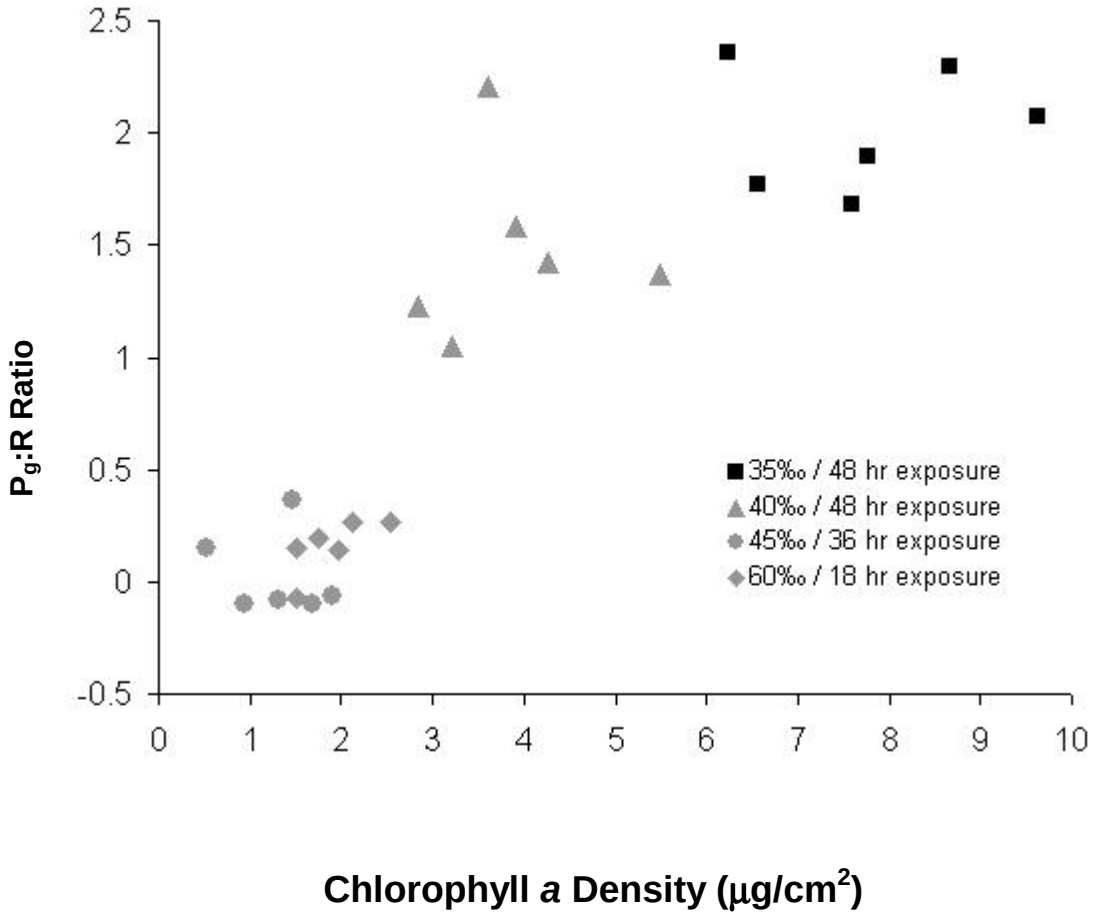


Figure 2.18. Relationship between $P_g$:R ratio and chlorophyll *a* for *Montastrea annularis* (n=6 per treatment group) exposed to a range of salinities and exposure times. Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

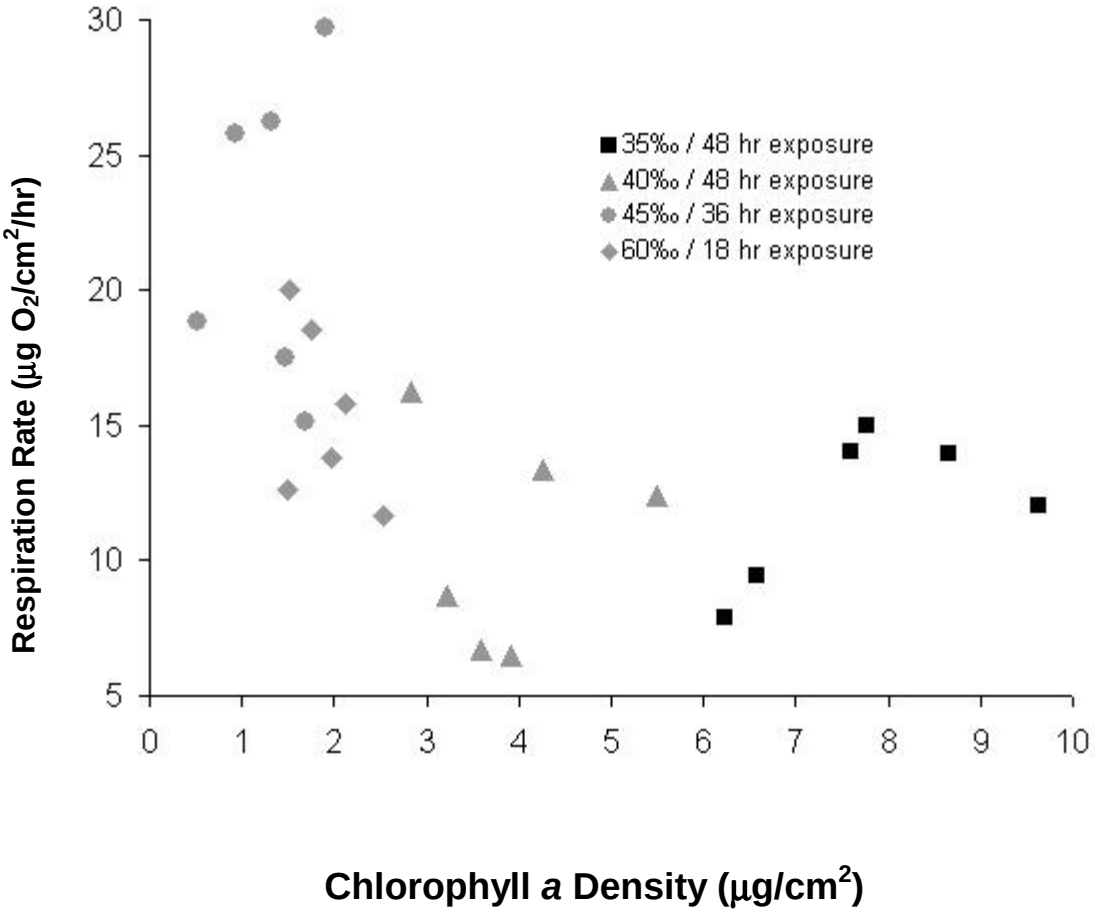


Figure 2.19. Relationship between respiration rate and chlorophyll *a* for *Montastrea annularis* (n=6 per treatment group) exposed to a range of salinities and exposure times. Notes: Corals exposed to 45‰ died between 36 and 42 hours; Corals exposed to 60‰ died between 18 and 24 hours.

My results also suggest that some corals are less stenohaline than originally proposed by Wells (1957), since *Montastrea annularis* survived extended exposure to a mild increase in salinity (40‰) with little detrimental effect. However, there is also evidence of a threshold salinity, between 40‰ and 45‰, above which all corals eventually died. Other studies have also suggested a survival threshold between 40‰ and 45‰. Hypersalinity of 40‰ was not lethal to several Hawaiian coral species (Coles and Jokiel 1978) and *Porites* spp. in Florida and Hawaii (Marcus and Thorhaug 1981), whereas corals exposed to 45‰ died within 3 days (Marcus and Thorhaug 1981). These results suggest that corals can osmoconform to mild salinity increases (up to 40‰) but not more severe salinity increases (greater than 40‰). Once the threshold has been reached, hyperosmotic stress producing cell shrinkage from water loss leads to disruption of cell function and structure and eventually death (Muthiga and Szmant 1987).

*Metabolic responses*

The photosynthetic ability of corals is dramatically reduced as salinity levels increase and the response is proportional to exposure time. Furthermore, this reduction occurs very rapidly, suggesting that coral reefs need not be exposed to hypersalinity for long before their autotrophic capacities are compromised. Within 6 hours of exposure, net photosynthesis for corals in the 60‰ treatment became negative, indicating that not enough oxygen was being produced to off-set oxygen consumption. Similarly, net photosynthesis for corals in the 45‰ treatment was only slightly above 0 after 6 hours, suggesting some net production of oxygen, but not enough for sustaining healthy coral growth. After 12 hours of exposure to 45‰ water, corals showed no net production of

oxygen and following even longer exposure, they used significantly more oxygen than they produced.

There was evidence of a threshold between 40‰ and 45‰, since $P_{net}$ for corals exposed to 40‰ was the same as control corals for the duration of the experiment and only showed a slightly lower $P_{net}$ after prolonged exposure. However, $P_{net}$ for corals exposed to 45‰ showed a rapid and dramatic decline. Muthiga and Szmant (1987) also found that mild increases in salinity did not change photosynthesis rates, while raising salinities by 10‰ resulted in a significant decrease in photosynthesis.

Since most cnidarians are osmoconformers (Wells 1957; Ranklin and Davenport 1981), the increased salinity causes the osmotic pressure of the extracellular fluid to fall, resulting in cell shrinkage. With increasing salinity, cell shrinking becomes more pronounced, disrupting cell functions and hence interfering with photosynthesis. Consequently, decreased net energy is available to corals exposed to elevated salinities, which can lead to reduced growth and coral vitality.

Experiments presented here indicate that there was a striking difference in sensitivity of metabolic response variables. The most responsive and consistent variable was net photosynthesis, while respiration was the least sensitive and least consistent metabolic variable. A striking and clear reduction in $P_{net}$ occurred after only 6 hours at 45‰, however, respiration did not show any consistent change until 18 hours of exposure to 60‰ or 36 hours of exposure to 45‰. Muthiga and Szmant (1987), similarly observed a significant decrease in photosynthesis after raising salinities by 10‰, but they did not see any change in respiration. Furthermore, changes in salinity triggered a response in photosynthesis, but little response in respiration from *Porites lutea* and *Pocillopora damicornis* (Moberg et al. 1997). These results suggest that the

endosymbiotic algae and/or the coral-algal complex are less resilient and more easily damaged than the coral animal.

Although changes in respiration were not as dramatic as changes in photosynthesis, increasing salinity did result in elevated respiratory rates. There was again evidence of a threshold between 40‰ and 45‰, since exposure to 40‰ did not change respiration rates, however higher salinities caused significant respiration changes.

Salinity stress can result in decreased respiration, increased respiration, or no change in respiration (Vernberg and Vernberg 1972); the current study found the latter two effects. Other studies have found that respiration decreased after the application of stresses (Muthiga and Szmant, 1987; Porter et al. 1989; Moberg et al. 1997), while others have shown that respiration increased (Jokiel and Coles 1974; Coles and Jokiel 1977; Hoegh-Guldberg and Smith 1989). Muthiga and Szmant (1987) found that hypersalinity (and hyposalinity) decreased coral respiration and they explained these observations by suggesting that hypersalinity shuts down the activities of the coral. The current experiment contrasted these results, since corals exposed to the highest salinities showed the highest respiration rates. Studies using other stressors have found elevated respiration rates following treatment exposure. Hoegh-Guldberg and Smith (1989) found increased respiration with increased temperature beyond ambient levels. Coles and Jokiel (1977) also found that high temperature stress resulted in increased respiration rates in corals. They also found that corals with the highest respiration rates were most susceptible to bleaching and mortality (Jokiel and Coles 1974; Coles and Jokiel 1977).

Hoegh-Guldberg and Smith (1989) hypothesize that differences in observed results for respiration rates among these different studies could be due to differences in experimental methodologies. Respiration is known to go up

after photosynthesis because of greater availability of respiratory substrates (McCloskey and Muscatine 1984; Edmunds and Davies 1988). Previous studies have measured photosynthesis during the day and then respiration at night, meaning that for most of the night, respiratory substrates were being depleted. Muthiga and Szmant (1989) measured respiration before photosynthesis and subsequently they observed a decrease in respiration. However, Hoegh-Guldberg and Smith (1989) measured photosynthesis first and then respiration and saw an increase in respiration. In the current study, respiration was measured immediately following photosynthesis for each time period. However, given that the photosynthetic mechanism was completely breaking down for corals in the high salinity treatments, it could not be providing respiratory substrates to the corals and thus this cannot explain why respiration increased for these stressed corals.

In studies showing increasing respiration following stress, it is also possible that respiration increased due to increased bacterial colonization following weakening of the corals. However, I measured oxygen probe consumption daily by placing the probes inside the sealed containers without a coral. While there was an increase in respiration rate when the coral was in the chamber, there was no increase in respiration when the coral was removed, suggesting that bacterial colonization was not responsible for the observed increase.

One of the best ways to estimate the effects of environmental change on a photosynthetic organism's metabolism is to compare their photosynthesis to respiration ratio with the photosynthesis to respiration ratio for control corals. While this ratio is an estimate of the autotrophic potential of the coral, it does not indicate how much photosynthate is translocated to the host coral. Thus the benefit to the host of photosynthesis is unclear.

Gross photosynthesis to respiration ratio, $P_g$:R, was a sensitive and consistent response variable; differences between treatments were detected early and were consistent. Corals exposed to the highest salinity, 60‰, rapidly passed the point where they were only able to produce as much oxygen as they consumed, meaning there was no net production of oxygen ($P_g$:R=1). For the duration of the experiment, these corals exhibited $P_g$:R ratios below 1 indicating that more oxygen was consumed than produced. Indeed, the ratios hover near 0, suggesting no oxygen was produced at all, though respiration continued. This was demonstrated by the oxygen flux readings within the 60‰ chambers producing the same readings both with and without irradiance. Corals exposed to 45‰ also showed an immediate drop in their $P_g$:R, and produced no net amount of oxygen after 12 hrs. of exposure ($P_g$:R=1). These corals also eventually reached a $P_g$:R of 0 after 36 hrs., again indicating no production of oxygen.

There was again further evidence for a threshold between 40‰ and 45‰, since there was little difference between the $P_g$:R for control and 40‰ corals. Although there was a trend of decreasing $P_g$:R for corals exposed to 40‰ over time, the values always remain above 1, suggesting that corals exposed to this treatment continue to produce a net amount of oxygen.

Although both the increase in respiration and the decrease in photosynthesis contributed to the observed decrease in $P_g$:R, photosynthesis has a larger magnitude of change and had the greatest influence on the photosynthesis to respiration ratio. Consequently, the decline in $P_g$:R is primarily due to a breakdown in the photosynthetic process. It is clear that hypersalinity can dramatically reduce the autotrophic ability of corals. To maintain a healthy autotrophic relationship, assuming a 12 hour light-dark cycle, stony corals should

maintain a $P_g$:R above 2 (Coles and Jokiel 1992). The corals exposed to 45‰ and higher salinities fall below this level very rapidly.

In this experiment, corals exposed to moderate salinity (45‰) show the highest variation for each metabolic response variable. This suggests that some corals in the sample were more detrimentally impacted by moderately elevated salinity than others. Therefore, when corals are exposed to moderate stresses, some may fair better than others and these survivors would then allow the reef to survive and recover. Contrastingly, all corals within the highest salinity treatment (60‰) respond similarly to the stress, suggesting that reefs exposed to extreme hypersalinity may not have any coral survivors to replenish the reef.

*Chlorophyll*

Chlorophyll density decreased with increasing salinity. This study revealed a positive correlation between chlorophyll level and photosynthesis rates. Interestingly, the results from this study suggest that once a saturation level of chlorophyll is reached, photosynthesis rates do not increase further. Therefore, the observed dramatic decline in chlorophyll density for the higher salinities is most likely responsible for the observed decline in photosynthesis. Indeed, the treatment with lowest chlorophyll had lowest photosynthesis. This signals the breakdown of the algal-host symbiotic process, leading to decreased nutritional input from the zooxanthellae. These results are consistent with other reports; salinity elevations produced lower chlorophyll density in *Siderastrea siderea* (Muthiga and Szmant 1987) and bleaching was observed in *Porites* spp. subjected to hypersaline conditions (Marcus and Thorhaug 1981). This loss of pigmentation is considered a common sign of sublethal stress (Coles and Jokiel 1992).

Szmant and Gassman (1990) studied bleached colonies of *Montastrea annularis* and found that they showed reduced growth and concluded that prolonged exposure to suboptimal physical conditions causes sterility in *Montastrea annularis* (Szmant and Gassman 90). Harriott (1983) also found that prolonged exposure to suboptimal conditions lowered larval production for *Pocillopora damicornis*.

This experiment has showed that salinity elevations can have a strong negative impact on *Montastrea annularis* even after short exposure times. Future research could focus on the effects of multiple factors under highly controlled conditions.

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# CHAPTER 3

## EFFECTS OF ELEVATED TEMPERATURES ON A FLORIDIAN HERMATYPIC CORAL

[1]Lewis, S. K. To be submitted to *Marine Ecology Progress Series*

**ABSTRACT:** Colonies of one of the dominant reef building stony corals in the Florida Keys Reef Tract, *Montastrea annularis*, were monitored during exposure to elevated temperatures and selected physiological responses were recorded. As temperature increased, net photosynthesis of corals markedly decreased, respiration increased, and gross photosynthesis to respiration ratio dramatically decreased. The higher the temperature, the quicker these responses occurred. Furthermore, each of these responses was stronger as treatment exposure time increased. Additionally, chlorophyll levels of the corals were strikingly reduced by exposure to elevated temperatures. This experiment indicated that even brief exposures (6 hours) to elevated temperature levels consistent with Florida Bay conditions could be very damaging to the stony corals. Consequently, if high temperature water from Florida Bay reaches the reefs, it does not need to stay long to inflict damage.

## INTRODUCTION

Temperature patterns affect reef distribution and diversity. Coral reefs are generally centered around the tropical regions of the world, and, while both light and temperature vary latitudinally (Clark & Jensen 1982), there is some evidence to suggest that temperature is the dominant factor (Glynn 1973). Reefs occur in relatively high latitudes in the proximity of warm currents, as seen in Bermuda (Glynn 1973, Veron 1974), and are conspicuously absent or much reduced in growth near upwellings of cold, deep ocean water even within the tropics (Glynn & Stewart 1973). Cool currents and local upwellings limit reef development in the eastern Pacific (Yonge 1940, Dana 1975, Glynn & Wellington 1983).

Reef-building corals are considered stenothermic (Wells 1957, Johannes 1975, Endean 1976), tolerating a narrow range of temperatures and generally flourishing in areas with annual mean temperatures between 23$^{o}$C and 25$^{o}$C (Wells 1957, Nybakken 1988). Although corals can tolerate 16$^{o}$-18$^{o}$C for a few days (Wells 1957, Kinsman 1964), no significant reef development occurs in areas with prolonged low temperatures because corals are outcompeted for space by other benthic organisms (Yonge 1940, Birkeland 1977, Porter et al. 1982). Calcification and growth increase as temperature increases, reaching a peak between 25$^{o}$-29$^{o}$C (Kinsman 1964, Clausen & Roth 1975, Jokiel & Coles 1977). However, these temperatures are very near the upper lethal temperature limits of corals (Mayer 1914, Moore 1972, Vernberg & Vernberg 1972, Johannes 1975, Coles et al. 1976, Jokiel & Coles 1990).

In addition to growing within a narrow annual temperature range, corals cannot tolerate wide daily temperature fluctuations (Wells 1957). For example, Mayer (1918) found no corals in areas of the Torres Straits where there was a diurnal temperature change of 12.3$^{o}$C, but the seaward edge of these areas had less than 3$^{o}$C daily change in temperature and had abundant coral growth.

Similarly, Wells (1952) found few or no corals on inshore reefs in the Marshall Islands where the diurnal temperature flux was 7.5$^{o}$C, but the outer reefs had a change of only 1$^{o}$C and, again, had abundant coral growth.

While coral reefs typically develop within the temperature range described above, stable temperature extremes exist and some corals are able to adjust to these stable environments (Coles et al.1976). For example, corals on offshore reefs in the Arabian Gulf tolerate summer temperatures greater than 34$^{o}$C for weeks or months with no visible change (Coles 1988), while the hardiest corals can survive prolonged exposure to 36$^{o}$-38$^{o}$C (Kinsman 1964, Shinn 1976, Coles 1988). Similarly, corals in tropical Enewetak can tolerate 2$^{o}$C higher summer maximum temperatures than the same species in subtropical Hawaii (Coles et al. 1976, Coles & Jokiel 1977). When exposed to elevated temperatures, corals from Enewetak displayed an upper lethal limit 2$^{o}$C higher than corals from Hawaii (Coles et al. 1976, Coles & Jokiel 1977). Marcus and Thorhaug (1981) also found a higher thermal tolerance in *Porites* from Florida than *Porites* from Hawaii. They attributed this difference to adaptation to the higher than average summer temperatures in Florida.

The normal tolerable temperature range for reef corals has a dramatic impact on the organism's physiology. Since Cnidaria are not capable of homoiothermy (Muthiga & Szmant 1987, Hoegh-Guldberg & Smith 1989), the temperature of stony corals fluctuates with the environment. Enzymatic rates are temperature dependent (Schmidt-Nielsen 1990), thus, as temperature increases within the normal range, metabolism increases (Johannes & Betzer 1975, Coles & Jokiel 1977). Muthiga and Szmant (1987) examined the effects of salinity changes on *Siderastrea siderea* using temperatures between 22$^{o}$-26$^{o}$C. They collected corals in winter at 16$^{o}$C and then raised them to 22$^{o}$C over 72 hours. This increase in temperature increased photosynthesis by 39% and increased

respiration by 30%. Feeding activity and reproductive capability also increase as temperature increases (Achituv & Dubinsky 1990). While calcification and growth increase as temperature increases, the optimum temperature is near the upper lethal temperature limit of corals (Houck et al. 1977, Jokiel & Coles 1977, Coles & Jokiel 1978, Berkelmans & Willis 1991).

When temperatures extend beyond the tolerable range, changes in metabolism are among the first signs of stress in reef corals. Hoegh-Guldberg and Smith (1989) experimentally exposed *Stylophora pistillata* and *Seriatopora hystrix* to elevated temperatures and found that as temperature increased, net photosynthesis decreased, respiration increased, and photosynthesis to respiration ratio decreased. Warner et al. (1996) found the photosynthetic efficiency of zooxanthellae in *Montastrea annularis* and *Agaricia lamarki* to be markedly reduced following exposure to elevated temperatures. Similarly, isolated zooxanthellae exposed to temperatures beyond their ambient range exhibit lower net photosynthesis rates (Iglesias-Prieto et al. 1992) and lower photosynthesis to respiration ratios (Fitt & Warner 1995). Indeed, temperatures above ambient result in decreased photosynthesis to respiration ratios for a wide range of photosynthetic systems (Coles & Jokiel 1977), thus as temperature increases outside the normal range, autotrophic ability is diminished at the community level (Jokiel & Coles 1990).

Temperatures above ambient also reduce the reproductive capabilities of reef corals (Jokiel & Coles 1990). Increasing temperature may stimulate planula release (Edmondson 1946, Harrigan 1972), but the planulae may be immature and less likely to survive. At the Great Barrier Reef, temperatures above the thermal optimum lowered planula production in *Pocillopora damicornis* (Harriott 1983) and Jokiel and Guinther (1978) noted an order of magnitude fewer number of settled corals under temperature conditions outside the optimal range.

Periods of unusually high temperatures can lead to reduced calcification and reduced growth in reef corals (Jokiel & Coles 1977, Coles & Jokiel 1978, Hudson 1981, Goreau & Macfarlane 1990, Glynn 1993). Reduced growth in stony corals makes them more susceptible to being overgrown by other benthic reef organisms (Glynn 1993). Several investigators have reported that weakened and dying corals are rapidly outcompeted by other organisms in Panama and the Galapagos (Glynn 1990), and Indonesia (Brown & Suharsono 1990).

Elevated temperatures are also thought to trigger bleaching, the loss of the symbiotic algae and/or the loss of algal photosynthetic pigments. Large-scale bleaching events around the world have been correlated with higher than normal temperatures (Cook et al. 1990, Gates 1990, Glynn & D'Croz 1990). Porter et al. (1989) point out that areas affected by the 1987 Caribbean bleaching event experienced $0.5^{o}$-$1.0^{o}$C higher than normal seawater temperatures, while Bermuda experienced normal temperatures and showed no bleaching. However, one year later, Bermuda recorded the highest seawater temperatures in 30 years and corals showed signs of bleaching (Porter et al. 1989). Additionally, direct experimental evidence has demonstrated that corals exposed to elevated temperatures bleach (Jokiel & Coles 1974, Jokiel & Coles 1977, Coles & Jokiel 1978, Hoegh-Guldberg & Smith 1989).

Severe temperature elevations or prolonged exposures trigger mortality in reef corals. During the 1982-83 El Nino Southern Oscillation Event, reefs were exposed to temperatures 0.5-$1.5^{o}$C above normal summer maxima throughout the Pacific for several weeks (Fisk & Done 1985, Harriott 1985, Oliver 1985, Glynn & D'Croz 1990). Glynn (1990) reported 50-99% coral mortality in the eastern Pacific over the bathymetric range of all reef-building species, with 95-99% coral mortality in shallow water (0-8m) in the Galapagos. Brown and

Suharsono (1990) reported 80-90% mortality on shallow reefs in Indonesia. Experimentally increased temperatures of 5-6$^{o}$C above ambient was lethal to corals in both Enewetak and Hawaii (Coles et al. 1976).

In this experiment, I chose to investigate the effects of temperature because temperature is a critical environmental factor for all coral reefs. Additionally, I chose to examine elevated temperature, since corals live very near their maximum thermal tolerance during summer months (Mayer 1914, Moore 1972, Vernberg & Vernberg 1972, Johannes 1975, Coles et al. 1976, Jokiel & Coles 1990), thus, even small increases in temperature may have a detrimental impact.

Many studies of temperature extremes have been observational field studies. Patterns of changes in reef organisms have been observed and then a hypothesized correlation to the environmental factors has been established. However, the inferences from these studies may be unclear. For example, several researchers attributed the observed coral bleaching in the Caribbean in 1987 to elevated seawater temperatures (Williams et al. 1987, Porter et al. 1989). Conversely, other researchers attributed the observed bleaching to unusually calm seas which caused water column clearing and resulted in increased exposure to high levels of photosynthetically active radiation and ultraviolet radiation (Gleason & Wellington 1993). Experimental studies attempt to explore causal relationships under controlled conditions, thereby attempting to discover underlying mechanisms of changes or responses. Therefore, I decided to explore this stress under controlled, experimental conditions.

I conducted this study in Florida because of the possibility that higher temperature water from Florida Bay may detrimentally impact the reefs of the Florida Keys, and contribute to the decline in coral cover and diversity noted since the 1980's (Porter & Meier 1992). The shallow nature of Florida Bay (avg.

<1m depth) creates high summer water temperatures (34$^{o}$C), due to solar heating (Tilmant 1989). Since water moves from Florida Bay, between the islands of the Keys and over the reefs (Smith 1994), high temperature water may be damaging the corals. An oceanographic survey recorded pulses of high temperature water (31.5 $^{o}$C) moving out from Florida Bay and over the reefs (Porter et al. 1999). I chose *Montastrea annularis* as the study organism because it is one of the main reef-building corals in the Florida Keys and is a crucial species to the existence of the reef.

## MATERIALS AND METHODS

**Collection and maintenance of corals.** Pieces of the reef-building coral, *Montastrea annularis* were collected from Admiral Patch Reef off Key Largo, Florida (25$^{o}$00.323 N, 80$^{o}$23.066 W). Collected specimens most closely resembled the sibling species *Montastrea annularis* cf. *faveolata* (Knowlton et al. 1992). All collected samples were placed in racks at the patch reef for 2 weeks to allow for acclimation and recovery from collection stress prior to use in the experiment. A total of 18 coral pieces were used in this experiment.

The corals were placed in scrubbed aquaria filled with freshly collected seawater. Each aquarium was equipped with 500 W halogen lights providing a saturating irradiance of 600μE $m^{-2}$ $s^{-1}$ for 12 hours of light each day. Air bubblers and water pumps provided aeration and continuous water movement. The temperature was increased with submersible heaters and monitored with thermographs and thermometers. Salinity was maintained at ambient reef conditions of 35‰.

**Oxygen flux experiments.** Metabolic measurements were made by placing each coral piece inside a sealed glass chamber and monitoring the level of

dissolved oxygen with YSI oxygen electrodes and meters. The chambers were periodically flushed with fresh seawater to maintain oxygen concentrations within 25% of ambient. Uniformity of dissolved oxygen levels throughout the chambers was achieved by rotating stir bars within the chambers. Oxygen production (net photosynthesis, $P_{net}$) was the measured rate of oxygen production under light conditions and oxygen consumption (respiration, R) was the measured rate of oxygen consumption under dark conditions. Gross photosynthesis (estimated by summing net photosynthesis and respiration measurements) to respiration ratio, $P_g$:R, is a dimensionless ratio that gives an indication of the autotrophic potential of the corals. It describes the relationship between the amount of oxygen produced by the algal symbionts and the amount of oxygen consumed by the coral-algal symbiosis. It does not, however, indicate how much photosynthate is actually translocated to the host. Oxygen probe consumption rates were determined on a daily basis by monitoring the oxygen levels in empty chambers.

Dissolved oxygen readings were automatically logged every 4 minutes, and oxygen production and consumption rates were determined by linear regression and subsequently normalized by coral surface area. Pre-treatment photosynthesis and respiration measurements were taken for each coral under control temperatures ($30^oC$), representing ambient summer conditions at the collection site. Corals were kept in the control conditions during an initial 36 hour acclimation period and then temperatures were slowly raised over a 6 hour period until the desired treatment conditions were achieved. Measurements were terminated when the subject began sloughing tissue or the exposure time exceeded 48 hours. No measurements were taken at midnight and no photosynthesis measurements were taken during nighttime hours.

**Photosynthetic pigment analysis.** At the end of the experimental runs, all tissue was removed from each coral using a stream of focused, high pressure filtered seawater, and the coral skeleton was sun-dried. The volume of the tissue blastate was measured and homogenized and samples of the tissue blastate were spun down in centrifuge tubes and the supernatant was poured off. The remaining pellet was ground with cooled acetone in a tissue grinder and then placed in the freezer overnight. Spectrophotometer readings were taken the next day at 750 nm, 663 nm and 630 nm. Chlorophyll *a* content of each coral was calculated using the equations of Jeffrey and Humphrey (1975).

**Experimental design.** There was a sample size of six in each of three treatment groups; the control group, 30$^{o}$C, and two elevated temperature treatments, 33$^{o}$C and 36$^{o}$C. The intermediate temperature level is consistent with temperatures recorded within Florida Bay and the high temperature level was chosen to bound the effects of this stressor.

**Covariate analysis.** Differences among individual corals and past coral health may mask differences in post-treatment responses. For example, a treatment group with significantly higher respiration rates prior to treatment exposure may tend to have significantly higher respiration rates after treatment exposure independent of responding to the treatment. The degree to which pre-exposure differences influence post-exposure differences can be estimated through analysis of covariance. If these pre-exposure differences do not significantly explain the post-exposure differences, one may appropriately ignore these differences in the analysis of variance of the post-exposure response variables.

The analysis of covariance procedure was used to test for significance ($\alpha$ = 0.05) of two different pre-exposure measurements for each response variable

for all treatments and for all time periods. These two pre-exposure measurements were of the same type as the response variables, so that pre-treatment respiration was compared with post-treatment respiration, and so on. The two measurements utilized were: (1) a measurement immediately prior to exposure to the stressor, and (2) the average of four prior measurements. Covariate analysis indicated that pre-treatment exposure differences had no influence on the metabolic responses at any post-exposure time period. Therefore, analysis of variance was used for examination of treatment and time effects.

Pre-exposure measurements of chlorophyll density, although desirable for analysis of covariance, are difficult to obtain. Typically, measurements of chlorophyll require destruction of the organism, thus rendering the subject unavailable for further stress experiments. Therefore, no pre-treatment levels of chlorophyll were obtained in this experiment.

**Hypothesis testing.** All statistical analyses were carried out using a PC SAS package and significance levels for all hypothesis tests were 5%. An analysis of variance was performed within each time period to test for differences among treatments. Additionally, an analysis of variance was performed within each treatment to test for temporal changes in the responses.

## RESULTS

### Survivorship

Elevated temperature had a profound effect on survival times of corals; as temperature increased, survival rapidly decreased (Fig. 3.1). Corals exposed to the highest temperature treatment, 36$^{o}$C, died between 24 and 30 hours, while

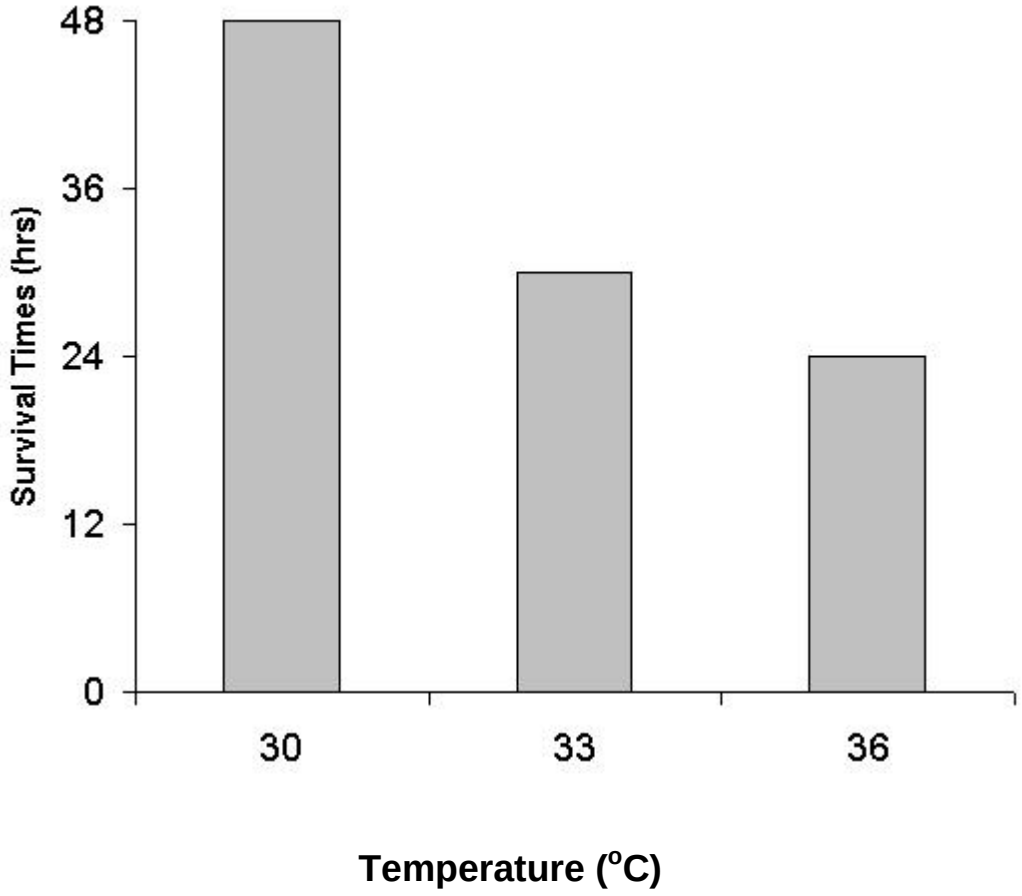


Figure 3.1. Coral survival times for *Montastrea annularis* exposed to a range of temperatures (n=6). Note corals exposed to 30°C survived beyond the termination of the experiment.

corals exposed to 33$^{o}$C died between 30 and 36 hours of exposure. However, the control corals survived for the duration of the experiment and were healthy at the termination of the experiment (48 hours of exposure). Another set of corals kept at control conditions survived well beyond 144 hours.

**Net photosynthesis**

Net photosynthesis ($P_{net}$) decreased as temperature increased and the response was more pronounced as exposure time increased (Fig. 3.2). Corals exposed to the highest temperature, 36$^{o}$C, showed the most rapid and most striking decline in net photosynthesis rates. Corals exposed to 33$^{o}$C did not respond as quickly, but also showed a striking drop in $P_{net}$. Control corals showed little change in their net photosynthesis rates for the duration of the experiment (Fig. 3.2).

Corals exposed to elevated temperatures show significantly lower net photosynthesis rates than control corals after only 6 hours ($p=0.008$, Table 3.1), and the trend is more clear the longer the exposure time (Fig. 3.3). $P_{net}$ is significantly lower the higher the salinity after 12, 18, and 30 hours of exposure ($p<.001$, Table 3.1, Fig. 3.3). $P_{net}$ rates for corals in the 36$^{o}$C and 33$^{o}$C treatments were approximately 40% lower after 6 hours of exposure, while control corals remain unchanged ($p<.001$, Table 3.2, Fig. 3.4). After only 12 hours of exposure, corals exposed to 36$^{o}$C reach their minimum $P_{net}$, 400% lower than before treatment exposure and remain at that level until dying after 18 hours of exposure (Table 3.2, Fig. 3.4). After 12 hours of exposure, $P_{net}$ for corals exposed to 33$^{o}$C are over 100% lower, reaching 270% lower after 30 hours of exposure (Table 3.2, Fig. 3.4). By contrast, there is no significant change in $P_{net}$ for control corals, suggesting that the experimental set-up successfully maintained healthy corals.

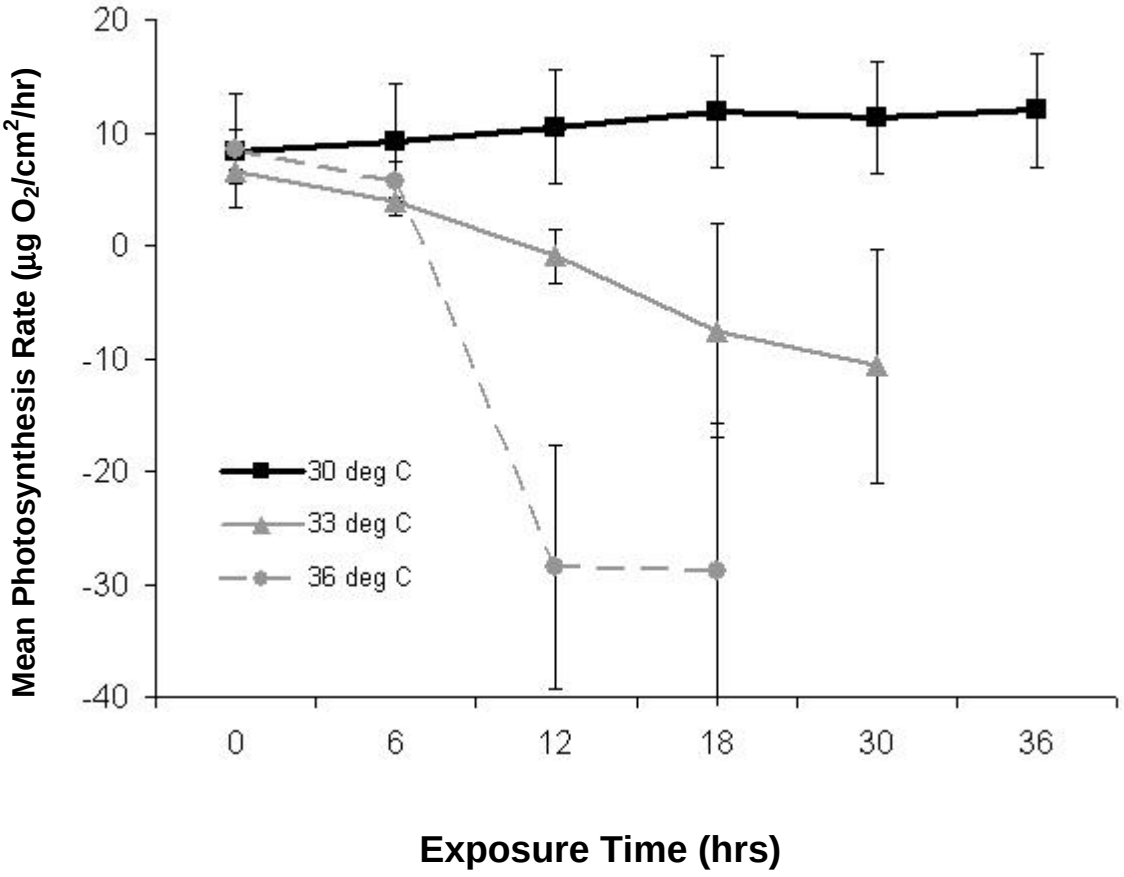


Figure 3.2. Mean net photosynthesis rates (± 1 S.D., n=6) for *Montastrea annularis* during increasing exposure times to different levels of temperature. Notes: Corals exposed to 33°C died between 30 and 36 hours; Corals exposed to 36°C died between 18 and 24 hours.

Table 3.1. Effect of increased temperature on *Montastrea annularis* (n=6) for different exposure times. a) Means of net photosynthesis rates - statistical significance was assessed using ANOVA and a significance level of 0.05 (WA indicates that Welch's ANOVA was utilized due to unequal variances). Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of net photosynthesis rates - values with different labels indicate standard deviations significantly different with the Brown-Forsythe test for unequal variances.

**a**

**Means of Net Photosynthesis Rates (μg $O_2$/$cm^2$/hr)**

| Exposure Time | 30°C | | 33°C | | 36°C | | p-value |
|---|---|---|---|---|---|---|---|
| Pre-Treatment | 8.4 | | 6.6 | | 8.5 | | .229 |
| 6 hrs | 9.3 | a | 4.0 | b | 5.7 | ab | .008* |
| 12 hrs | 10.5 | a | -1.0 | b | -28.5 | c | <.001* (WA) |
| 18 hrs | 11.8 | a | -7.5 | b | -28.9 | c | <.001* |
| 24 hrs | Night | | | | | | |
| 30 hrs | 11.3 | a | -10.6 | b | | | .001* |

**b**

**Standard Deviations of Net Photosynthesis Rates (μg $O_2$/$cm^2$/hr)**

| Exposure Time | 30°C | | 33°C | | 36°C | | |
|---|---|---|---|---|---|---|---|
| Pre-Treatment | 3.0 | | 1.1 | | 1.7 | | .179 |
| 6 hrs | 3.8 | | 1.3 | | 1.8 | | .059 |
| 12 hrs | 4.5 | a | 2.4 | a | 10.7 | b | .001* |
| 18 hrs | 3.5 | | 9.5 | | 13.2 | | .291 |
| 24 hrs | Night | | | | | | |
| 30 hrs | 2.8 | | 10.4 | | | | .084 |

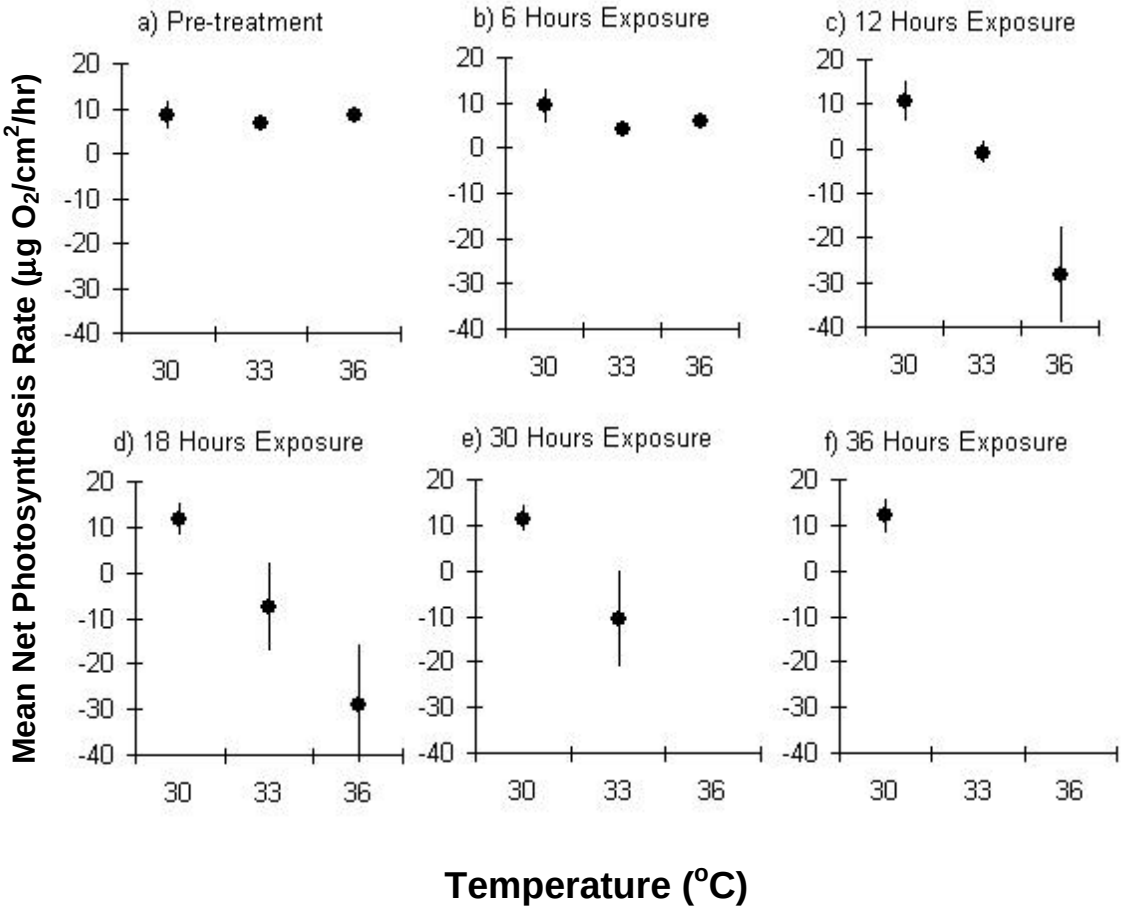


Figure 3.3. Mean net photosynthesis rates (± 1 S.D., n=6) for *Montastrea annularis* exposed to a range of temperatures: before treatment exposure (a); during increasing exposure times (b-f). Notes: Corals exposed to 33°C died between 30 and 36 hours; Corals exposed to 36°C died between 18 and 24 hours.

Table 3.2. Effect of increased temperature on *Montastrea annularis* (n=6) for different exposure times. a) Means of percent changes in net photosynthesis rates - statistical significance was assessed using ANOVA and a significance level of 0.05 (WA indicates that Welch's ANOVA was utilized due to unequal variances). Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of percent changes in net photosynthesis rates - values with different labels indicate standard deviations significantly different with the Brown-Forsythe test for unequal variances.

**a**

**Means of Percent Changes in Net Photosynthesis Rates**

| Exposure Time | 30°C | | 33°C | | 36°C | | p-value |
|---|---|---|---|---|---|---|---|
| 6 hrs | 7.9 | a | -40.8 | b | -32.4 | b | <.001* |
| 12 hrs | 22.6 | a | -115.1 | b | -426.3 | c | <.001* (WA) |
| 18 hrs | 52.6 | a | -219.8 | b | -431.7 | c | <.001* |
| 24 hrs | Night | | | | | | |
| 30 hrs | 44.9 | a | -270.8 | b | | | .002* |

**b**

**Standard Deviations of Percent Changes in Net Photosynthesis Rates**

| Exposure Time | 30°C | | 33°C | | 36°C | | |
|---|---|---|---|---|---|---|---|
| 6 hrs | 15.9 | | 11.1 | | 15.6 | | .733 |
| 12 hrs | 28.2 | a | 39.0 | a | 69.5 | b | .033* |
| 18 hrs | 56.0 | | 156.2 | | 105.6 | | .459 |
| 24 hrs | Night | | | | | | |
| 30 hrs | 45.8 | | 182.4 | | | | .149 |

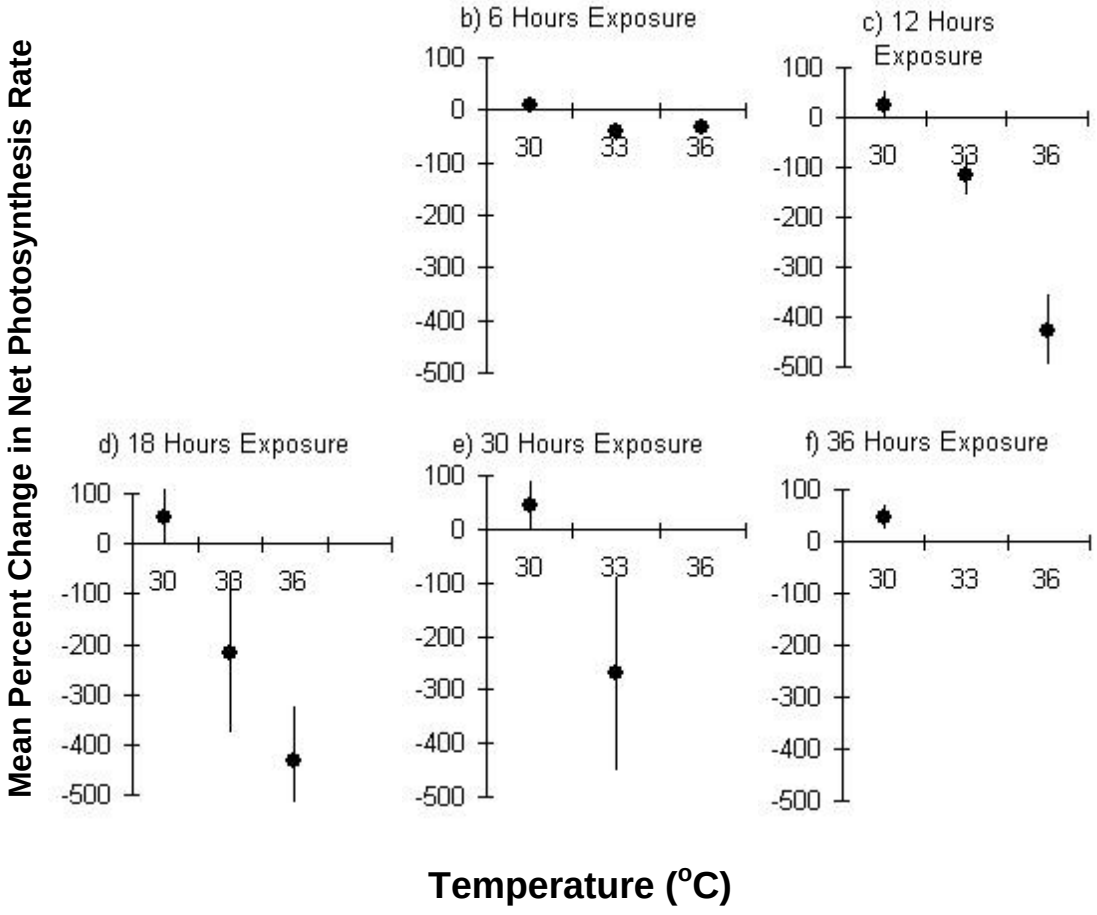


Figure 3.4. Mean percent change in net photosynthesis rates (± 1 S.D., n=6) for *Montastrea annularis* exposed to a range of temperatures: before treatment exposure (a); during increasing exposure times (b-f). Notes: Corals exposed to 33°C died between 30 and 36 hours; Corals exposed to 36°C died between 18 and 24 hours.

When the standard deviations of each sample of corals are examined, the greatest variation exists among corals exposed to 36$^{o}$C, although the differences among treatments are only significant at 12 hours of exposure (Fig. 3.5, Table 3.1).

## Respiration

There is a trend of increasing respiration rates, R, as temperature increases and this trend is more obvious the longer the exposure time (Fig. 3.6). Corals exposed to the highest temperature show the highest R after the shortest exposure time. The differences among the treatments are more pronounced when percent change in respiration following treatment application is examined (Fig. 3.7).

Although there is a significant difference among the treatments after 6 hours of exposure ($p=.006$, Table 3.3), a consistent trend is not apparent until after longer exposures (Fig. 3.8). Corals exposed to 36$^{o}$C have significantly higher R after 12 and 18 hours ($p<.001$, Table 3.3), over 400% higher for both time periods Table 3.4, Fig. 3.9). Corals exposed to 33$^{o}$C show a slightly elevated R after prolonged exposure, about 80% higher than before treatment exposure after 18 and 30 hours (Table 3.4, Fig. 3.9), but the values are not significantly different than R for control corals (Table 3.3, Fig. 3.8). There is no significant change in R values for control corals throughout the experiment.

There is greatest variation among corals within the highest temperature treatment (Table 3.3, Fig. 3.10). However, the variation is only significantly higher for corals in 36$^{o}$C after 12 hours (Table 3.3, Fig. 3.10).

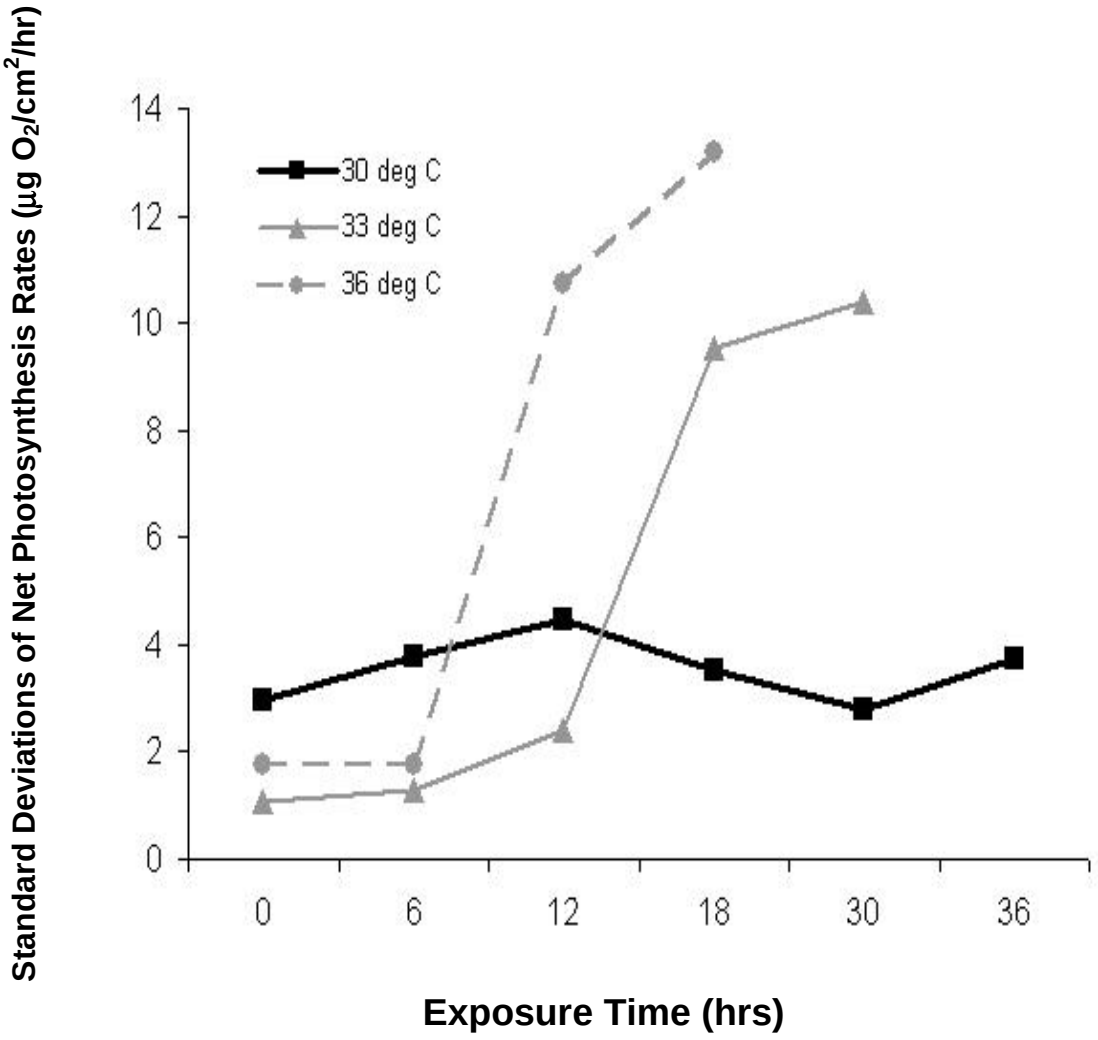


Figure 3.5. Standard deviations of net photosynthesis rates (n=6) for *Montastrea annularis* during increasing exposure times to different levels of temperature. Notes: Corals exposed to 33$^{o}$C died between 30 and 36 hours; Corals exposed to 36$^{o}$C died between 18 and 24 hours.

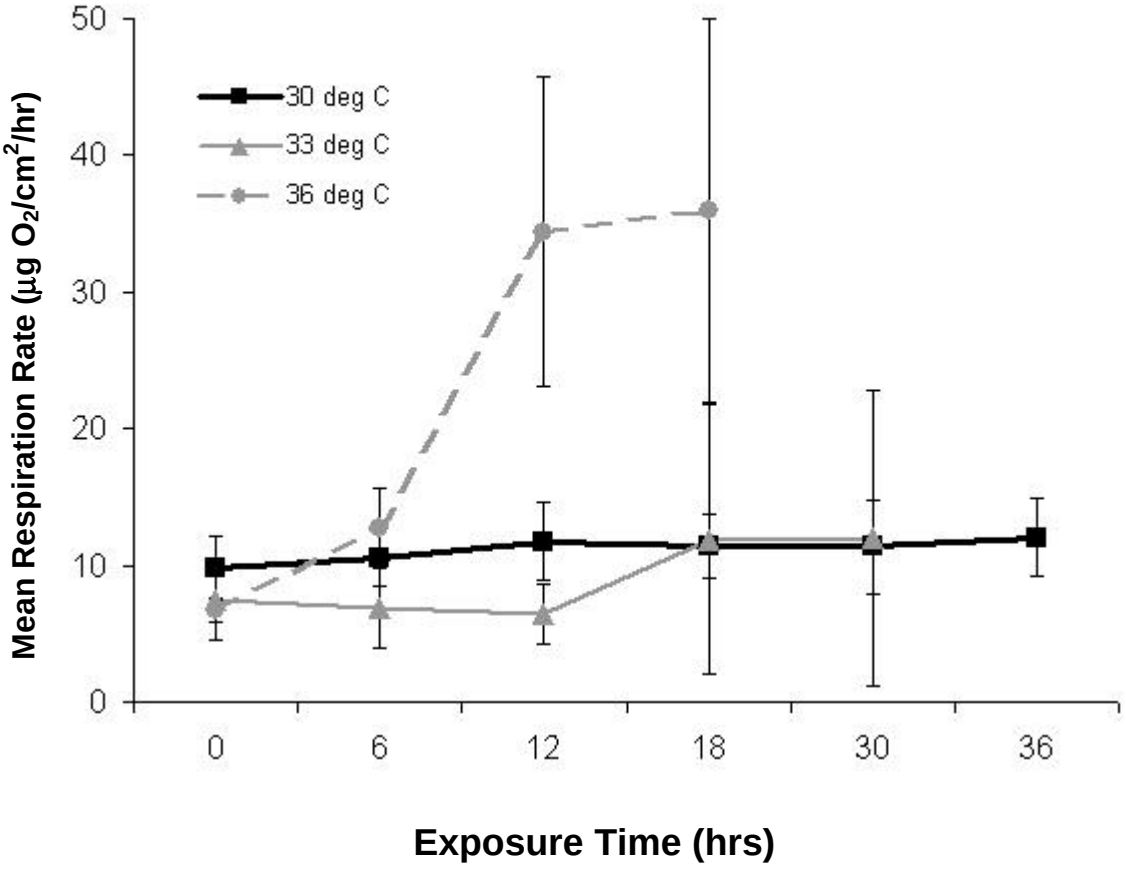


Figure 3.6. Mean respiration rates (± 1 S.D., n=6) for *Montastrea annularis* during increasing exposure times to different levels of temperature. Notes: Corals exposed to 33°C died between 30 and 36 hours; Corals exposed to 36°C died between 18 and 24 hours.

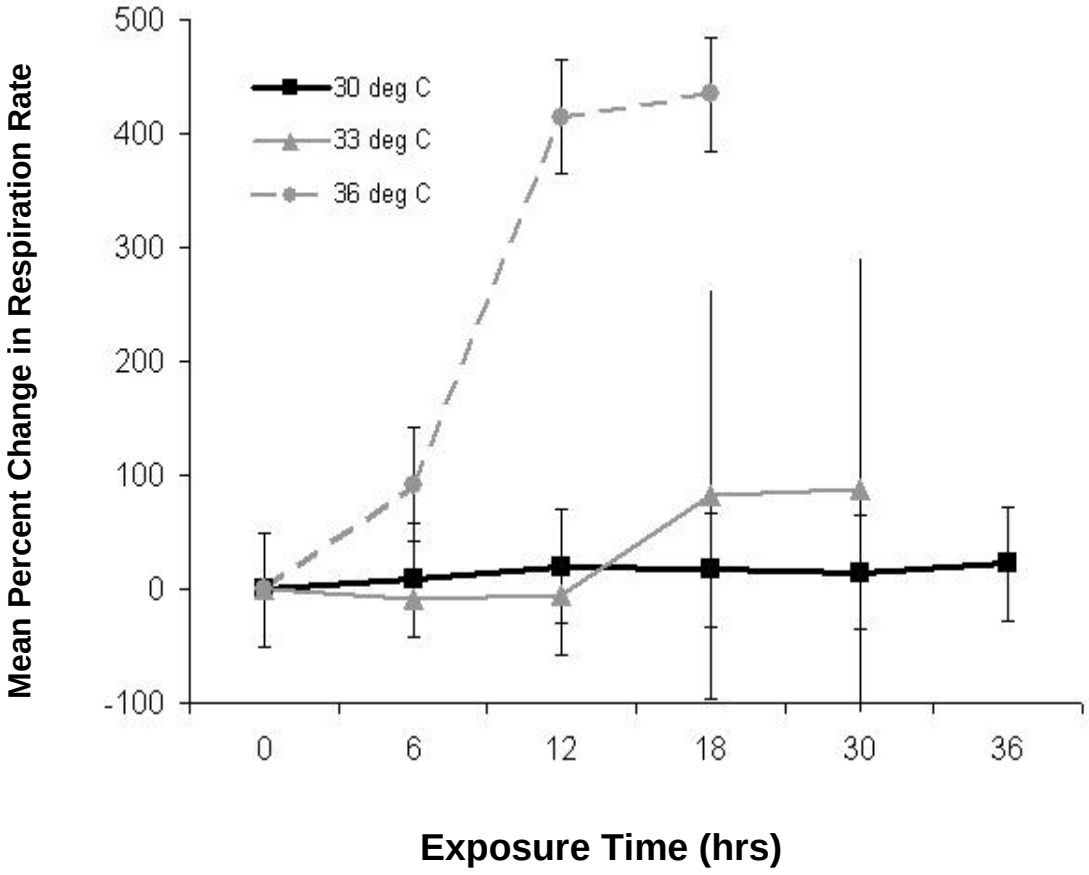


Figure 3.7. Mean percent change in respiration rates (± 1 S.D., n=6) for *Montastrea annularis* during increasing exposure times to different levels of temperature. Notes: Corals exposed to 33$^{o}$C died between 30 and 36 hours; Corals exposed to 36$^{o}$C died between 18 and 24 hours.

Table 3.3. Effect of increased temperature on *Montastrea annularis* (n=6) for different exposure times. a) Means of respiration rates - statistical significance was assessed using ANOVA and a significance level of 0.05 (WA indicates that Welch's ANOVA was utilized due to unequal variances). Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of respiration rates - values with different labels indicate standard deviations significantly different with the Brown-Forsythe test for unequal variances.

**a**

**Means of Respiration Rates (μg $O_2$/$cm^2$/hr)**

| Exposure Time | 30°C | | 33°C | | 36°C | | p-value |
|---|---|---|---|---|---|---|---|
| Pre-Treatment | 9.8 | | 7.4 | | 6.7 | | .054 |
| 6 hrs | 10.6 | ab | 6.9 | a | 12.7 | b | .006* |
| 12 hrs | 11.8 | a | 6.4 | a | 34.4 | b | <.001* (WA) |
| 18 hrs | 11.4 | a | 11.9 | a | 35.9 | b | .001* |
| 24 hrs | Night | | | | | | |
| 30 hrs | 11.3 | | 12.0 | | | | 0.888 |

**b**

**Standard Deviations of Respiration Rates (μg $O_2$/$cm^2$/hr)**

| Exposure Time | 30°C | | 33°C | | 36°C | | p-value |
|---|---|---|---|---|---|---|---|
| Pre-Treatment | 2.3 | | 2.8 | | 0.8 | | .080 |
| 6 hrs | 2.1 | | 2.9 | | 3.0 | | .663 |
| 12 hrs | 2.9 | a | 2.2 | a | 11.3 | b | .022* |
| 18 hrs | 2.4 | | 9.9 | | 14.0 | | .155 |
| 24 hrs | Night | | | | | | |
| 30 hrs | 3.4 | | 10.8 | | | | .150 |

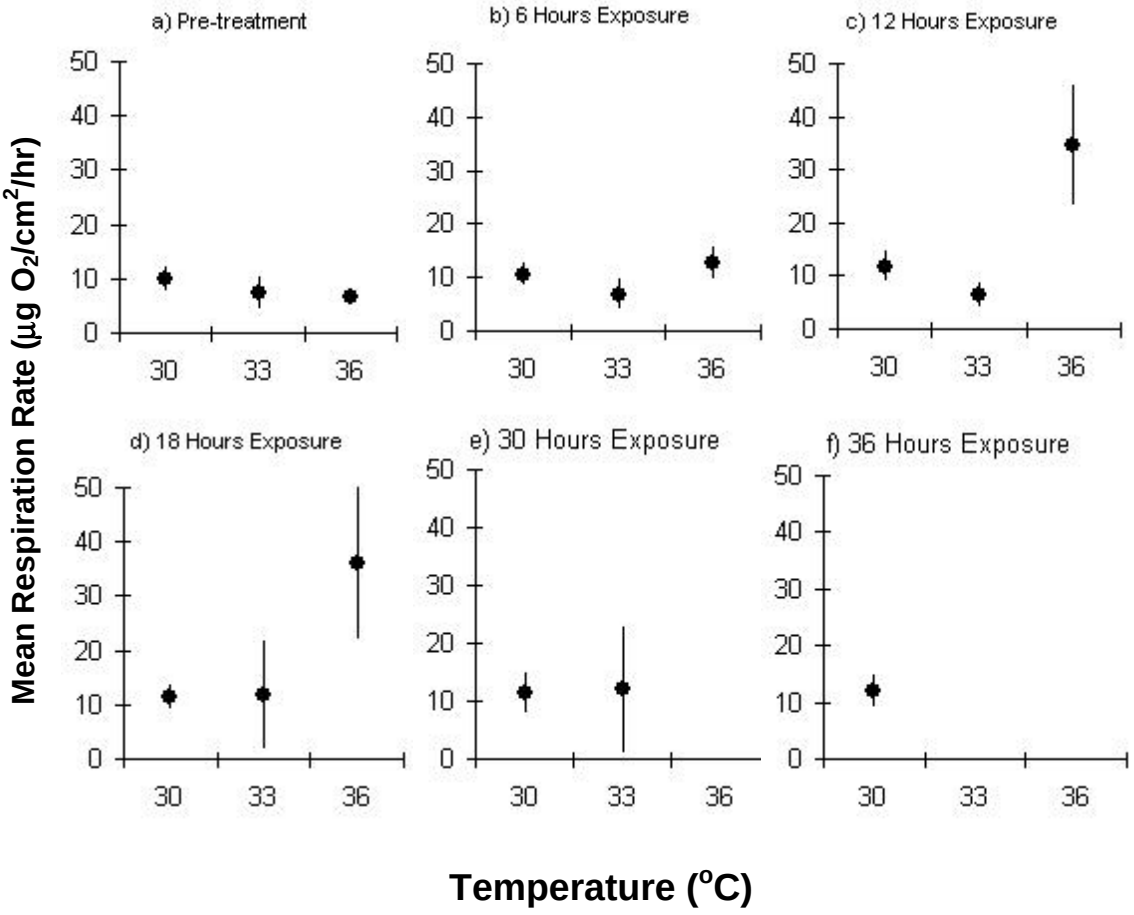


Figure 3.8. Mean respiration rates (± 1 S.D., n=6) for *Montastrea annularis* exposed to a range of temperatures: before treatment exposure (a); during increasing exposure times (b-f). Notes: Corals exposed to 33°C died between 30 and 36 hours; Corals exposed to 36°C died between 18 and 24 hours.

Table 3.4. Effect of increased temperature on *Montastrea annularis* (n=6) for different exposure times. a) Means of percent changes in respiration rates - statistical significance was assessed using ANOVA and a significance level of 0.05 (WA indicates that Welch's ANOVA was utilized due to unequal variances). Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of percent changes in respiration rates - values with different labels indicate standard deviations significantly different with the Brown-Forsythe test for unequal variances.

**a**

**Means of Percent Changes in Respiration Rates**

| Exposure Time | 30°C | | 33°C | | 36°C | | p-value |
|---|---|---|---|---|---|---|---|
| 6 hrs | 8.5 | a | -8.4 | a | 91.3 | b | <.001* (WA) |
| 12 hrs | 19.3 | a | -4.9 | a | 414.4 | b | <.001* (WA) |
| 18 hrs | 17.1 | a | 82.7 | a | 435.0 | b | .001* |
| 24 hrs | Night | | | | | | |
| 30 hrs | 14.7 | | 87.2 | | | | .407 |

**b**

**Standard Deviations of Percent Changes in Respiration Rates**

| Exposure Time | 30°C | | 33°C | | 36°C | | p-value |
|---|---|---|---|---|---|---|---|
| 6 hrs | 8.9 | a | 8.0 | a | 44.0 | b | <.001* |
| 12 hrs | 6.3 | a | 53.6 | b | 159.9 | c | .002* |
| 18 hrs | 12.2 | | 179.9 | | 199.5 | | .182 |
| 24 hrs | Night | | | | | | |
| 30 hrs | 13.8 | | 204.9 | | | | .182 |

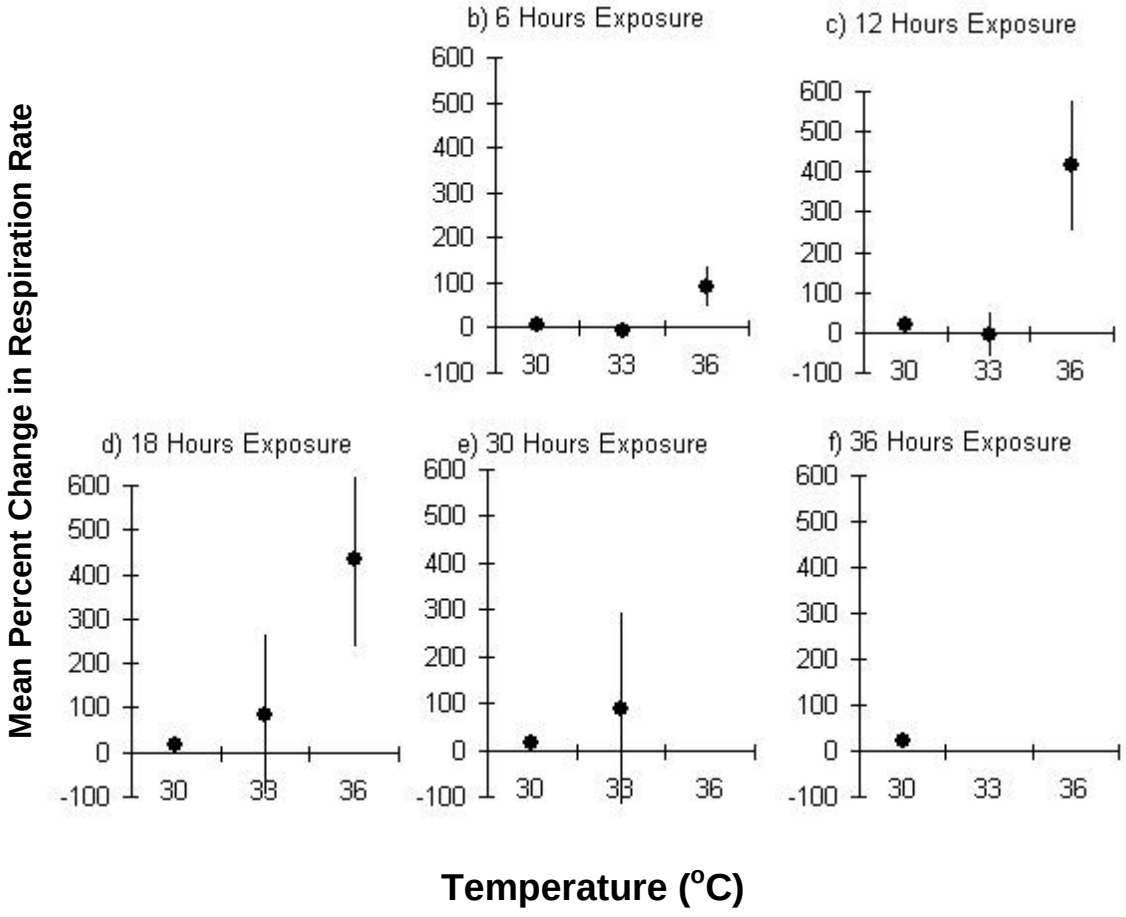


Figure 3.9. Mean percent change in respiration rates (± 1 S.D., n=6) for *Montastrea annularis* exposed to a range of temperatures: before treatment exposure (a); during increasing exposure times (b-f). Notes: Corals exposed to 33°C died between 30 and 36 hours; Corals exposed to 36°C died between 18 and 24 hours.

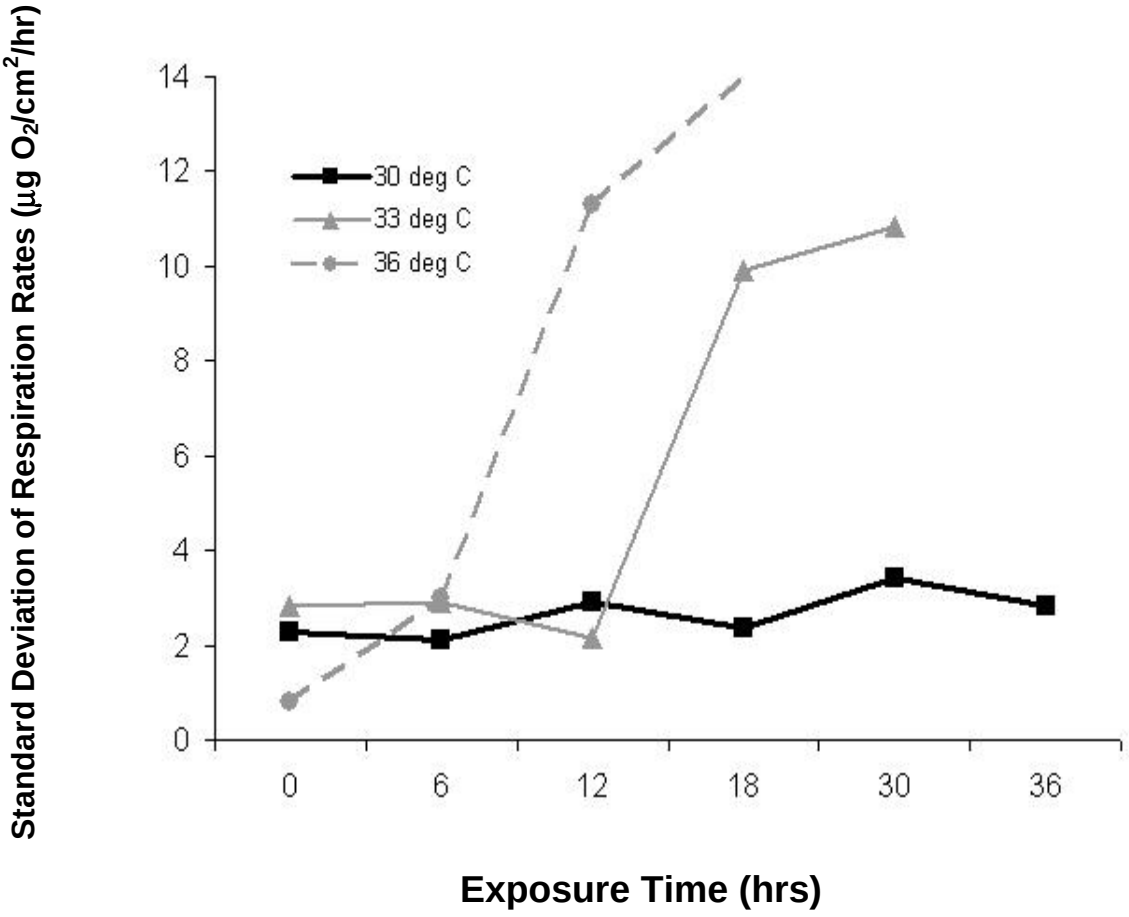


Figure 3.10. Standard deviations of respiration rates (n=6) for *Montastrea annularis* during increasing exposure times to different levels of temperature. Notes: Corals exposed to 33$^{o}$C died between 30 and 36 hours; Corals exposed to 36$^{o}$C died between 18 and 24 hours.

**Gross photosynthesis to respiration ratio**

The gross photosynthesis to respiration ratio, $P_g$:R, decreases dramatically with increasing temperature, and the differences among treatments become more pronounced as exposure time increases (Fig. 3.11). $P_g$:R was highest for the control corals and lowest for the 36$^o$C treatment corals following treatment exposure (Fig. 3.11).

Control corals maintain a $P_g$:R of 2 for the duration of the experiment, however, corals exposed to the higher temperature treatments had a significantly lower $P_g$:R than controls for each time period (Table 3.5). After 6 hours of treatment exposure, corals exposed to the highest temperature maintained a $P_g$:R above 1, however, it is significantly lower than the control corals ($p=.004$, Table 3.5, Fig. 3.12). After 12 hours, $P_g$:R for 36$^o$C corals reached a minimum near 0, significantly lower than either the 33$^o$C corals or the controls ($p<.001$, Table 3.5, Fig. 3.12). $P_g$:R for corals exposed to 33$^o$C for 12 hours was near 1, and reached a minimum near 0 after 30 hours of exposure (Table 3.5, Fig. 3.12). These same relationships are evident when the percent changes in $P_g$:R for each coral are examined. The highest temperature treatment corals have the lowest $P_g$:R for each measurement period ($p<.001$, Table 3.6, Fig. 3.13), and reach their minimum $P_g$:R the quickest, after only 12 hours of exposure. While corals exposed to 33$^o$C eventually, reached the same minimum $P_g$:R, it required 30 hours of exposure.

There is no significant difference among standard deviations for the different treatment groups for any time period (Table 3.5). However, when the percent changes are examined, corals exposed to 33$^o$C have a higher variation after 12 hours, than either control or 36$^o$C corals (Table 3.6).

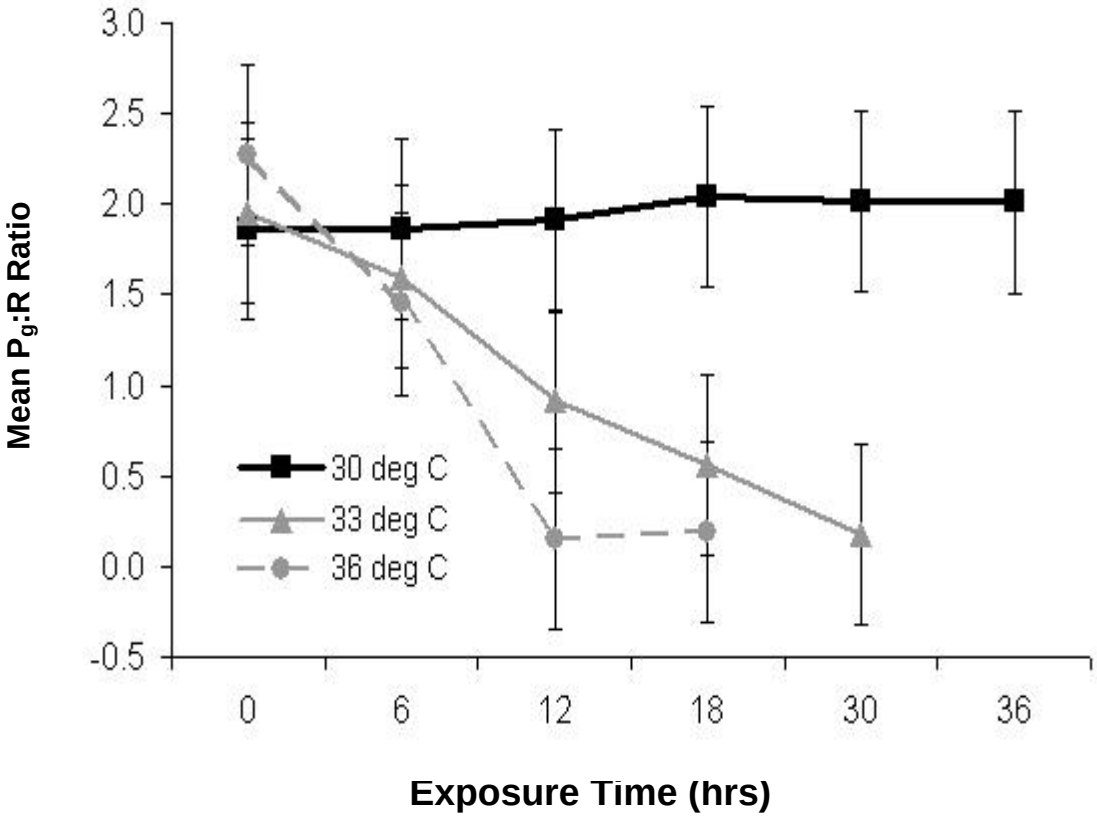


Figure 3.11. Mean $P_g$:R Ratios (± 1 S.D., n=6) for *Montastrea annularis* during increasing exposure times to different levels of temperature. Notes: Corals exposed to 33$^{o}$C died between 30 and 36 hours; Corals exposed to 36$^{o}$C died between 18 and 24 hours.

Table 3.5. Effect of increased temperature on *Montastrea annularis* (n=6) for different exposure times. a) Means of $P_g$:R Ratios - statistical significance was assessed using ANOVA and a significance level of 0.05 (WA indicates that Welch's ANOVA was utilized due to unequal variances). Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of $P_g$:R ratios - values with different labels indicate standard deviations significantly different with the Brown-Forsythe test for unequal variances.

**a**

**Means of $P_g$:R Ratios**

| Exposure Time | 30°C | | 33°C | | 36°C | | p-value |
|---|---|---|---|---|---|---|---|
| Pre-Treatment | 1.9 | b | 2.0 | ab | 2.3 | a | .021* |
| 6 hrs | 1.9 | a | 1.6 | ab | 1.4 | b | .004* |
| 12 hrs | 1.9 | a | 0.9 | b | 0.2 | c | <.001* |
| 18 hrs | 2.0 | a | 0.6 | b | 0.2 | b | <.001* |
| 24 hrs | Night | | | | | | |
| 30 hrs | 2.0 | a | 0.2 | b | | | <.001* |

**b**

**Standard Deviations of $P_g$:R Ratios**

| Exposure Time | 30°C | 33°C | 36°C | |
|---|---|---|---|---|
| Pre-Treatment | 0.3 | 0.2 | 0.2 | .792 |
| 6 hrs | 0.3 | 0.1 | 0.1 | .091 |
| 12 hrs | 0.4 | 0.3 | 0.3 | .223 |
| 18 hrs | 0.2 | 0.4 | 0.1 | .172 |
| 24 hrs | Night | | | |
| 30 hrs | 0.1 | 0.2 | | .588 |

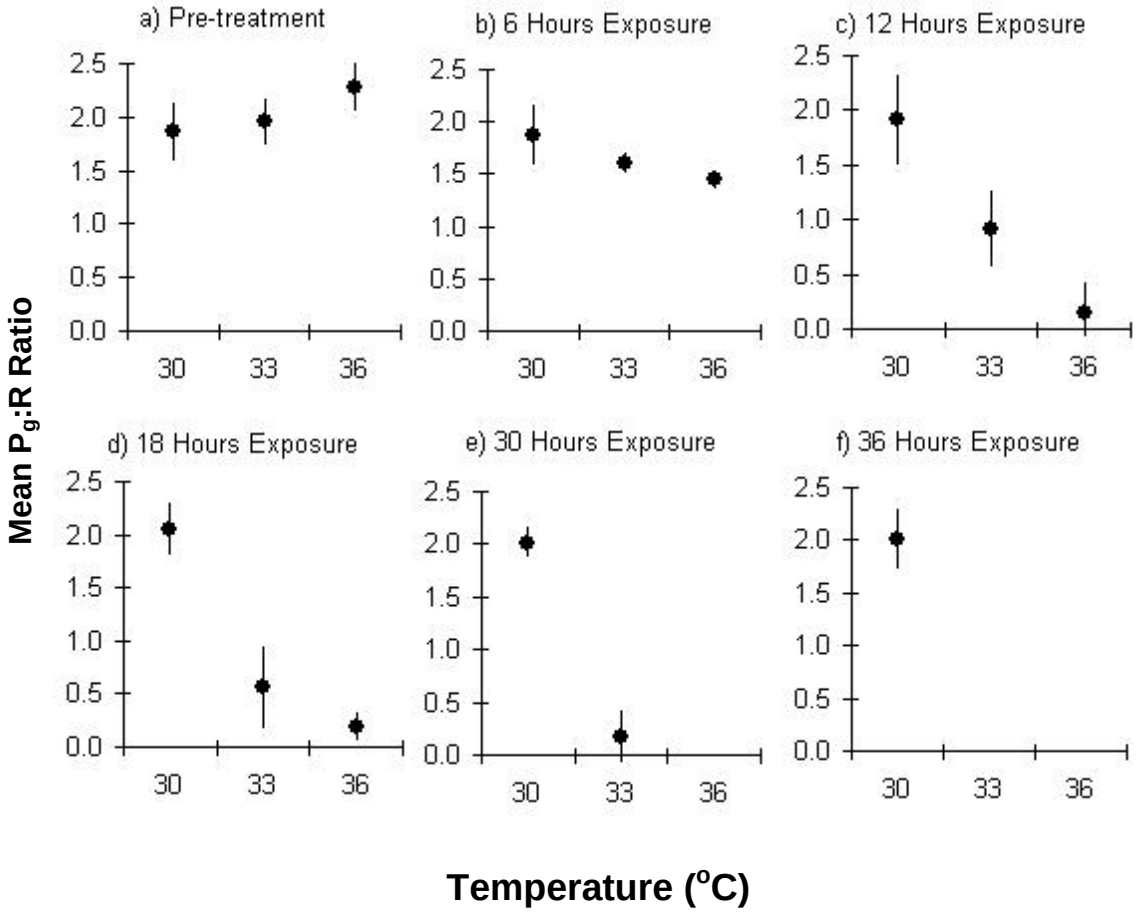


Figure 3.12. Mean $P_g$:R Ratios (± 1 S.D., n=6) for *Montastrea annularis* exposed to a range of temperatures: before treatment exposure (a); during increasing exposure times (b-f). Notes: Corals exposed to 33°C died between 30 and 36 hours; Corals exposed to 36°C died between 18 and 24 hours.

Table 3.6. Effect of increased temperature on *Montastrea annularis* (n=6) for different exposure times. a) Means of percent changes in $P_g$:R Ratios - statistical significance was assessed using ANOVA and a significance level of 0.05 (WA indicates that Welch's ANOVA was utilized due to unequal variances). Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of percent changes in $P_g$:R ratios - values with different labels indicate standard deviations significantly different with the Brown-Forsythe test for unequal variances.

**a**

**Means of Percent Changes in $P_g$:R Ratios**

| Exposure Time | 30°C | | 33°C | | 36°C | | p-value |
|---|---|---|---|---|---|---|---|
| 6 hrs | 0.3 | a | -17.5 | b | -35.7 | c | <.001* |
| 12 hrs | 2.1 | a | -53.4 | b | -92.5 | c | <.001* |
| 18 hrs | 11.2 | a | -70.7 | b | -91.3 | b | <.001* (WA) |
| 24 hrs | Night | | | | | | |
| 30 hrs | 10.4 | a | -91.3 | b | | | <.001* |

**b**

**Standard Deviations of Percent Changes in $P_g$:R Ratios**

| Exposure Time | 30°C | | 33°C | | 36°C | | |
|---|---|---|---|---|---|---|---|
| 6 hrs | 8.2 | | 6.3 | | 7.7 | | .752 |
| 12 hrs | 11.0 | | 17.0 | | 11.9 | | .37 |
| 18 hrs | 17.3 | a | 20.2 | a | 6.5 | b | .034* |
| 24 hrs | Night | | | | | | |
| 30 hrs | 20.0 | | 12.4 | | | | .521 |

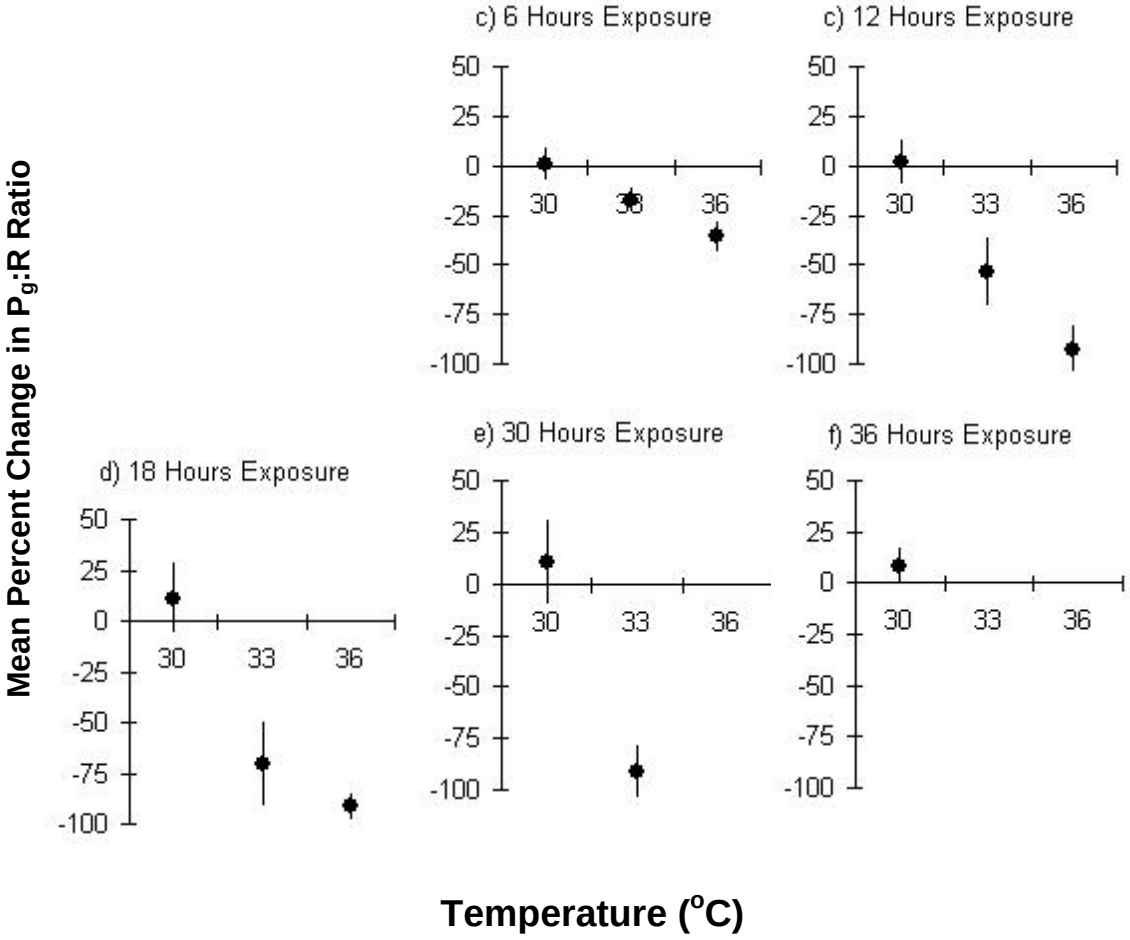


Figure 3.13. Mean percent change in $P_g$:R Ratio (± 1 S.D., n=6) for *Montastrea annularis* exposed to a range of temperatures: before treatment exposure (a); during increasing exposure times (b-f). Notes: Corals exposed to 33°C died between 30 and 36 hours; Corals exposed to 36°C died between 18 and 24 hours.

## Chlorophyll density

There is a significant decrease in chlorophyll density as temperature increases ($p<0.001$, Table 3.7). Chlorophyll per unit area was highest in control corals (Fig. 3.14). Corals exposed to 33$^{o}$C had approximately half the amount of chlorophyll as the control corals and corals exposed to 36$^{o}$C had approximately a quarter of the amount of chlorophyll as the control corals.

The chlorophyll results should be interpreted with caution, since exposure times were different for the different treatments. Corals in the highest temperature were exposed for the shortest time, since they died before the termination of the experiment. Corals in the intermediate temperature treatment also died before the termination of the experiment and, thus, were not exposed for as long as the control corals. However, evidence from this study suggests that higher temperature were more stressful than lower temperatures, suggesting that the longer the corals were exposed to these high temperatures, the more stressful it was and the lower their chlorophyll content. Therefore, if all treatment groups were exposed for the same length of time, it is reasonable to suggest that the corals in the higher temperature treatments would actually show a more significant reduction in chlorophyll when compared with the controls than was observed in this experiment.

## Correlations among response variables

There is a positive, non-linear relationship between net photosynthesis and chlorophyll concentration (Fig. 3.15). The relationship indicates that as chlorophyll concentration increases, photosynthesis increases until a saturation level is reached. There is also a positive relationship between gross photosynthesis to respiration ratio and chlorophyll concentration (Fig. 3.16), however, the relationship appears to be a step-function. A threshold chlorophyll

Table 3.7. Effect of increased temperature on *Montastrea annularis* (n=6). a) Means of chlorophyll *a* density - statistical significance was assessed using ANOVA and a significance level of 0.05. Values with different labels indicate means that are statistically different using the Tukey-Kramer method for multiple comparisons. b) Standard deviations of chlorophyll *a* densities.

**a**

**Means of Chlorophyll *a* Densities (µg/cm$^2$)**

| 30°C and 48 hour exposure | 33°C and 30-36 hour exposure | 36°C and 24-30 hour exposure | p-value |
|---|---|---|---|
| 7.74 a | 3.59 b | 1.37 c | <.001* |

**b**

**Standard Deviations of Chlorophyll *a* Densities (µg/cm$^2$)**

| 30°C and 48 hour exposure | 33°C and 30-36 hour exposure | 36°C and 24-30 hour exposure | p-value |
|---|---|---|---|
| 1.27 | 1.02 | 0.48 | .223 |

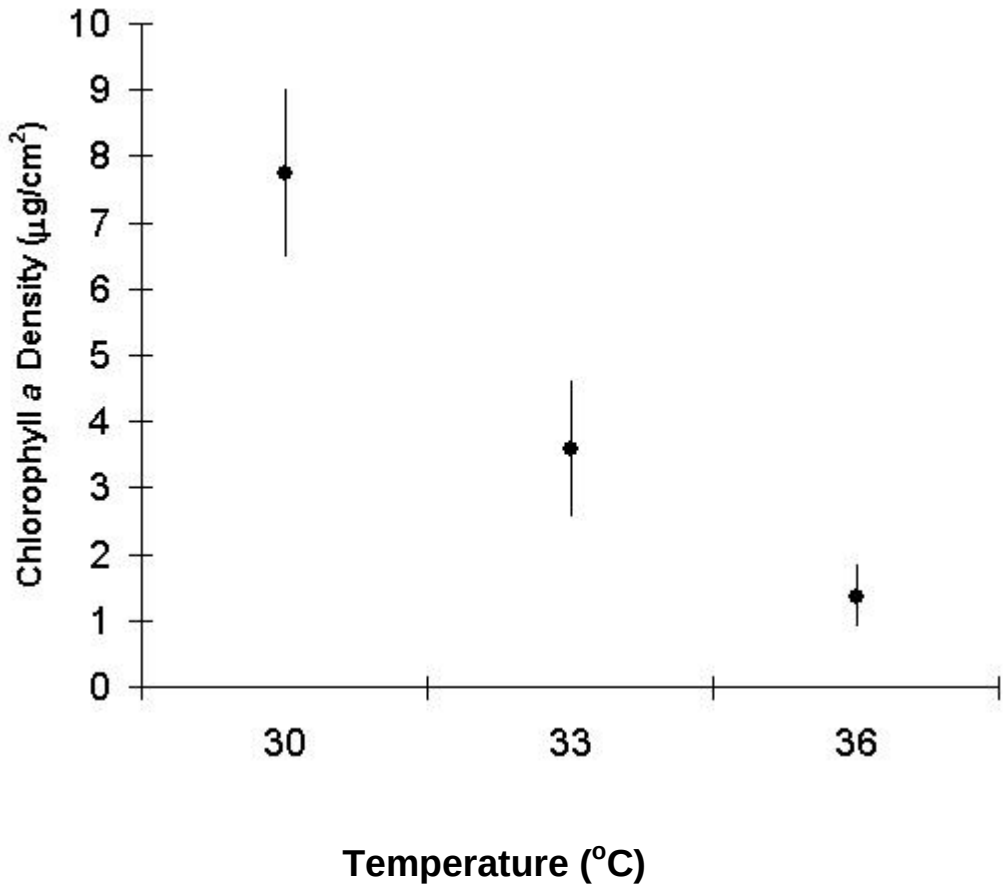


Figure 3.14. Mean Chlorophyll *a* densities (± 1 S.D., n=6) for *Montastrea annularis* exposed to a range of temperatures. Notes: Corals exposed to 33°C died between 30 and 36 hours; Corals exposed to 36°C died between 18 and 24 hours.

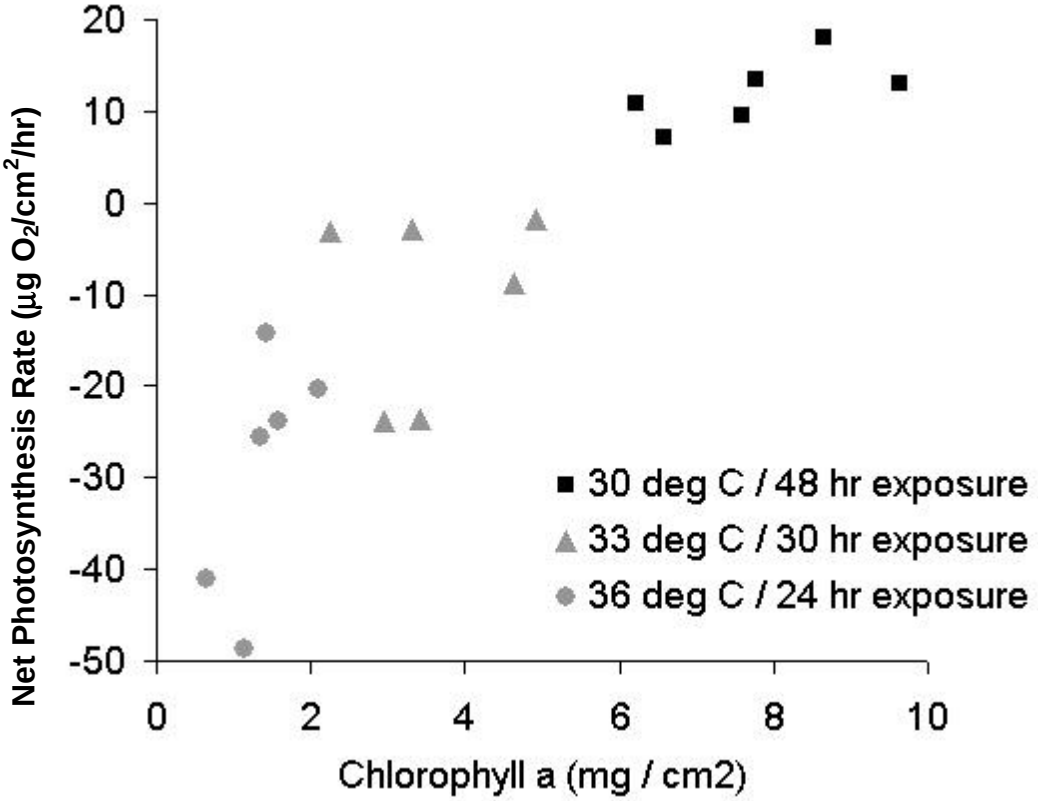


Figure 3.15. Relationship between net photosynthesis rate and chlorophyll *a* for *Montastrea annularis* (n=6 per treatment group) exposed to a range of temperatures and exposure times.

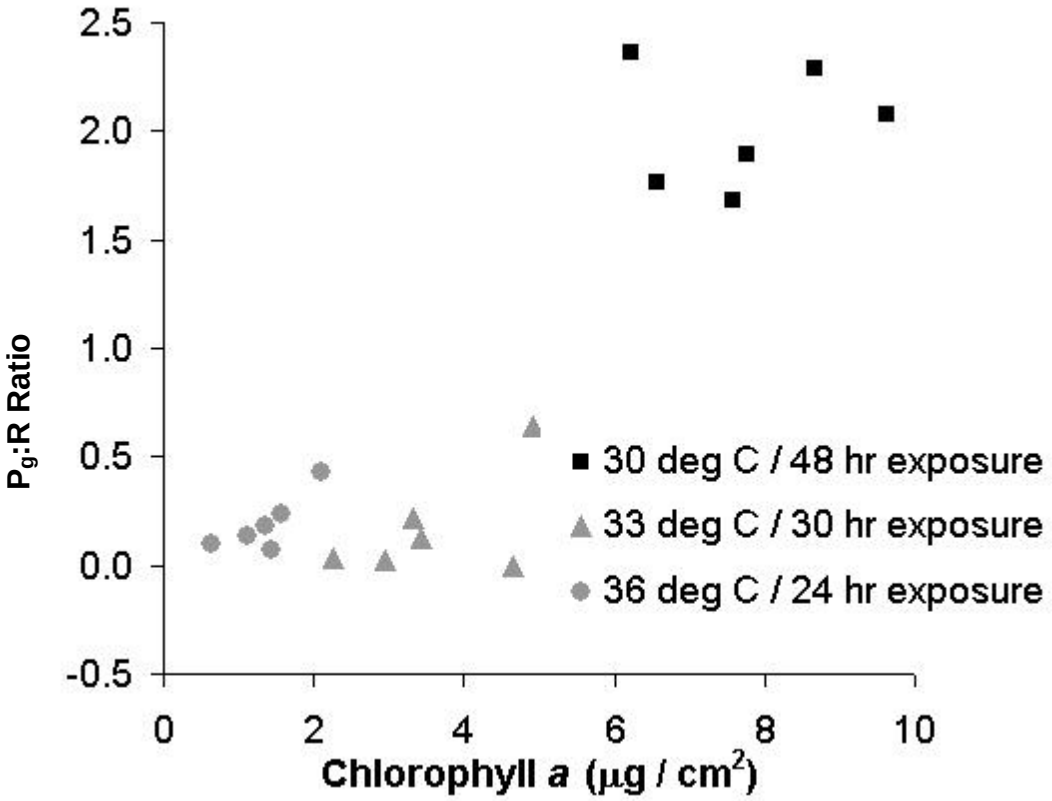


Figure 3.16. Relationship between $P_g$:R ratio and chlorophyll *a* for *Montastrea annularis* (n=6 per treatment group) exposed to a range of temperaturesand exposure times.

level exists that must be reached to see a marked increase in $P_g$:R. Conversely, there is a strong negative, non-linear relationship between respiration and chlorophyll level (Fig. 3.17).

## Discussion

### Effect of elevated temperature on survivorship

Survivorship of corals is influenced by both the length of exposure, as well as the level of temperature; the higher the temperature above ambient, the shorter the exposure time necessary to kill the corals. *Montastrea annularis* exposed to a 6$^{o}$C increase in temperature died between 24 and 30 hrs., while a longer exposure was needed to produce mortality in corals exposed to a 3$^{o}$C increase. Similarly, Jokiel and Coles (1977) found that an increase of 6$^{o}$C was lethal to *Montipora verrucosa, Pocillopora damicornis,* and *Fungia scutaria* within one day, while prolonged exposure to an increase of 3$^{o}$C was necessary to produce mortality. Hoegh-Guldberg and Smith (1989) found similar results with *Stylophora hystrix* and *Seriatopora pistillata.*

Corals exposed to smaller temperature increases have lower mortality rates than the same exposure times for large temperature increases (Jokiel & Coles 1977, Coles & Jokiel 1978). One to 2 days of exposure to temperature elevations of 4-5$^{o}$C above summer ambient resulted in high mortality in stony corals (Jokiel & Coles 1977). However, one to 2 days of exposure to 2-3$^{o}$C above ambient temperatures resulted in less than 10% mortality (Jokiel & Coles 1977, Coles & Jokiel 1978).

Corals in some areas of the world tolerate and survive stable high temperatures. For example, parts of the Arabian Gulf reach 36$^{o}$C, however, these *Porites* spp. dominated reefs have low diversity (Downing 1985). This selection occurred over generations and only those species that could

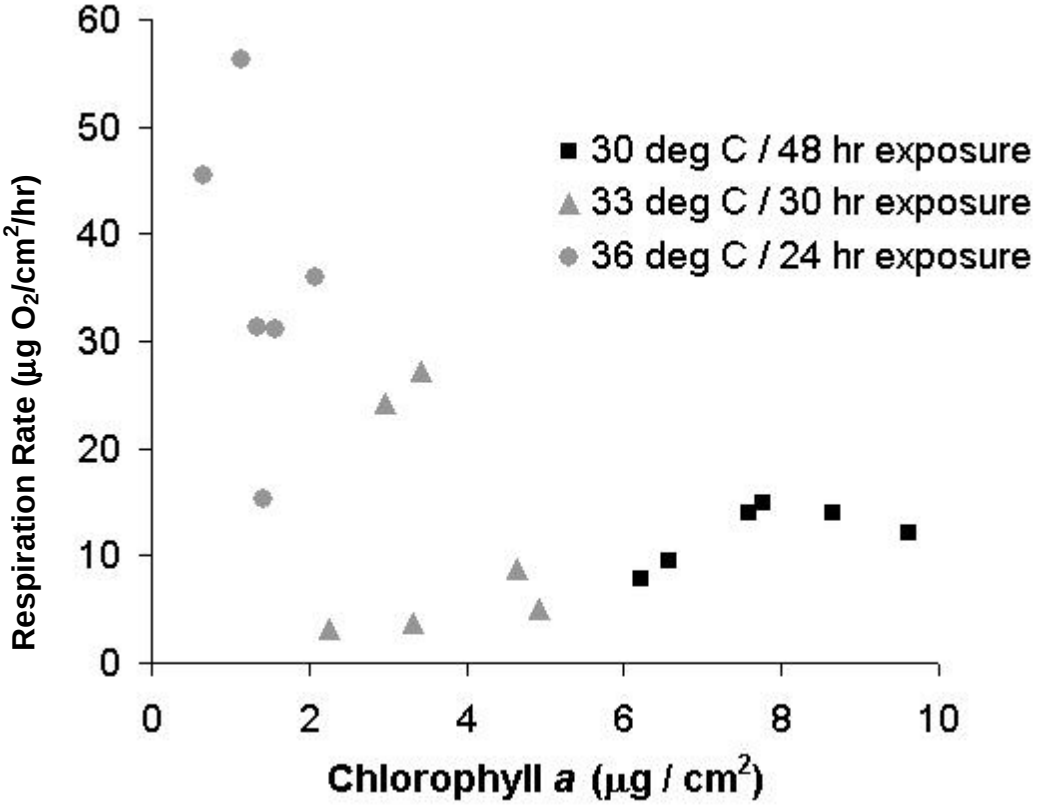


Figure 3.17. Relationship between respiration rate and chlorophyll *a* for *Montastrea annularis* (n=6 per treatment group) exposed to a range of temperatures and exposure times.

genotypically adapt to these temperature extremes are now found in these areas. Conversely, Florida's reefs cannot acclimate to the sudden, periodic pulses of high temperature water from Florida Bay.

**Physiological responses to elevated temperature**

Corals cannot thermoregulate, thus their temperature fluctuates with the environment (Muthiga & Szmant 1987, Hoegh-Guldberg & Smith 1989). There is a linear relationship between metabolic processes and temperature for poikilotherms (Coles & Jokiel 1977). Photosynthesis increases for corals as temperature increases within a tolerable range (Muthiga & Szmant 1987, Jokiel & Coles 1990, Iglesias-Prieto et al. 1992). However, exposure to temperatures beyond typical ambient conditions can be detrimental to reef organisms.

In this study, exposure to temperatures beyond ambient resulted in lower net photosynthesis proportional to the magnitude of the increase. Furthermore, net photosynthesis became relatively lower as exposure time increased for the higher temperatures. There was a rapid response of decreasing net photosynthesis with higher temperatures, suggesting that coral reefs need not be exposed to elevated temperatures for long before their autotrophic capabilities are compromised. After 12 hours of exposure to temperatures $6^{o}$C above ambient, net photosynthesis for corals became negative, indicating that not enough oxygen was being produced by the algae to compensate for oxygen consumption by the algal-coral association. However, after 12 hours of exposure to temperatures $3^{o}$C above ambient, net photosynthesis for corals was near 0, suggesting that enough oxygen was still being produced to off-set oxygen consumption. Longer exposures to an increase of $3^{o}$C were necessary to decrease photosynthesis to the point where more oxygen was being consumed than produced. Similarly, Iglesias-Prieto et al. (1992) found net photosynthesis

to be 0 or negative for isolated zooxanthellae exposed to 7$^{o}$C higher temperatures. Fitt and Warner (1995) recorded no photosynthesis for zooxanthellae after 24 hours of exposure to an increase of 8$^{o}$C. Hoegh-Guldberg and Smith (1989) and Jokiel and Coles (1977) also found lower photosynthesis in whole corals as temperature increased beyond ambient levels.

The break-down of photosynthesis by the zooxanthellae is extremely damaging for corals, since algal translocate provides up to 100% of a coral's daily energy requirements (Porter et al. 1989), as well as materials for growth and reproduction (Wethey & Porter 1976, Muscatine & Porter 1977, Muscatine et al. 1981, Muscatine et al. 1984). Reduced coral growth makes them more susceptible to competition from other benthic organisms, such as algae (Brown & Suharsono 1990, Glynn 1990, Glynn 1993).

Iglesias-Prieto et al. (1992) suggest that reduced photosynthesis at high temperatures is the result of uncoupling of energy absorption and photochemistry. This effect is most likely caused by changes in the electron transport capacity of the thylakoid membranes (Lange et al. 1981, Havaux et al. 1991). Reef corals exposed to elevated temperatures led to damage of the reaction center of Photosystem II of zooxanthellae (Warner et al. 1996). Warner et al. (1996) noted striking differences in high temperature tolerance among coral species. They concluded that zooxanthellae in tolerant coral species are more capable of dissipating excess energy than zooxanthellae in less tolerant coral species. Regardless of the mechanism, the breakdown in photosynthesis results in a reduction or cessation of algal metabolites moving to the coral host, possibly dissolving the host-symbiont association.

Photosynthesis was the most sensitive response variable examined, while respiration was the least sensitive. A larger temperature change from ambient was required to produce a significant response for respiration than for

photosynthesis. Similarly, Iglesias-Prieto et al. (1992) observed clear changes in photosynthesis of zooxanthellae in response to elevated temperatures but saw no consistent change in respiration. Photosynthesis is known to be a sensitive indicator of thermal stress in plants (Thebud & Santarius 1982) and the current study indicates that it is also an effective indicator of thermal stress in the algal-coral symbiosis.

Iglesias-Prieto et al. (1992) were unable to detect a significant change in respiration of isolated zooxanthellae (*Symbiodinium microadriaticum*) even at 35$^{o}$C. However, in the present study, there is an increase in respiration as temperature increases above ambient conditions, although only corals exposed to the highest temperature, 36$^{o}$C, resulted in a significantly higher respiration than the other treatments.

Some studies have found that respiration rates in corals decrease with increased temperature beyond the ambient range (Fitt & Warner, 1995), while other studies have shown that respiration increased (Jokiel & Coles 1974, Coles & Jokiel 1977, Hoegh-Guldberg & Smith 1989). High temperature stress was found to produce high colony respiration rate for *Stylophora pistillata* and *Seriatopora hystrix* from the Great Barrier Reef (Hoegh-Guldberg & Smith 1989). Coles and Jokiel (1977) found a linear increase in respiration with increasing temperature (up to 7$^{o}$C above ambient) for 4 coral species in Hawaii and 2 in Enewetak. In the present study, however, respiration did not increase in a regular, linear fashion, but rather increased exponentially. Previous studies have suggested that corals with the highest respiration rates were most susceptible to bleaching and mortality (Jokiel & Coles 1974, Coles & Jokiel 1977). Similarly, in the current experiment, corals exposed to 36$^{o}$C showed the highest respiration rates, as well as the lowest chlorophyll content and highest mortality.

Gross photosynthesis to respiration ratio ($P_g$:R) is a sensitive and consistent response variable. There is a steady decline in $P_g$:R with increasing temperature and the decline is most rapid in higher temperature treatments. Several other studies have also recorded a decrease in photosynthesis to respiration ratio with increasing temperature (Coles & Jokiel 1977, Hoegh-Guldberg & Smith 1989). Indeed, temperatures above ambient result in decreased photosynthesis to respiration ratios for a wide range of photosynthetic systems (Coles & Jokiel 1977), thus as temperature increases outside the normal range, autotrophic ability is diminished at the community level (Jokiel & Coles 1990).

Most reef-building corals are autotrophic in shallow water with oxygen production greater than oxygen consumption (Muscatine et al. 1989). This is evident for the control corals with $P_g$:R remaining above 2 for the duration of the study indicating a net production of oxygen, and thus the potential for transfer of algal products to the coral host (Achituv & Dubinsky 1990, Patterson et al. 1991). A $P_g$:R equal to 1, indicates that total production of oxygen equals consumption of oxygen and that there is no net production of oxygen. This point is reached shortly after 6 hours of exposure to temperatures 6$^o$C above ambient and 12 hours exposure to temperatures 3$^o$C above ambient. Continued exposure to elevated temperatures dropped $P_g$:R near 0, indicating no production of oxygen, although respiration was still occurring; there is a complete break-down of the photosynthesis process. Fitt and Warner (1995) found the photosynthesis to respiration ratio for *Montastrea annularis* reduced to the same level within 24 hours of exposure to temperatures 6$^o$C above ambient.

The increases in respiration and the decreases in photosynthesis with increased temperature both contributed to the lowered $P_g$:R. However, the

relatively greater decline in photosynthesis means that change in photosynthesis was the major contributing factor to the change seen in $P_g$:R.

**Effects of elevated temperatures on chlorophyll**

Chlorophyll density decreased with increasing temperature and this loss of pigmentation was evident as bleaching. Previous investigations of stony corals have discovered similar negative correlations of colony pigmentation with temperature both in the laboratory and in the field. Experimental investigations of elevated temperatures have produced declines in photosynthetic pigment in stony corals (Coles & Jokiel 1978, Hoegh-Guldberg & Smith 1989, Glynn & D'Croz 1990, Fitt & Warner 1995). Similarly, widespread bleaching throughout the Caribbean (Williams et al. 1987, Williams & Bunkley-Williams 1988) and Pacific (Fisk & Done 1985, Harriott 1985, Oliver 1985, Glynn & D'Croz 1990) has been attributed to unusually elevated seawater temperatures. Bleached corals have been reported to show lowered photosynthetic and lowered nutritional potential following a period of bleaching (Coles & Jokiel 1978, Hoegh-Guldberg & Smith 1989, Porter et al. 1989). This in turn leads to reduced growth and reduced reproductive capacity (Glynn & D'Croz 1990, Jokiel & Coles 1990, Szmant & Gassman 1990).

There is a positive, non-linear relationship between net photosynthesis and chlorophyll concentration. The relationship indicates that as chlorophyll concentration increases, photosynthesis increases until a saturation level is reached.

There are cases of genotypic adaptation of corals to higher than usual temperature levels, however, this adaptation has occurred over many generations and only a few species are able to adapt (Coles et al. 1976, Coles & Jokiel 1977, Downing 1985). There are also cases of phenotypic acclimation,

where corals have been able to acclimate to increased temperatures which enables them to better tolerate subsequent temperature increases than non-acclimated corals (Coles & Jokiel 1978, Marcus & Thorhaug 1981). Berkelmans and Willis (1999) also noted a seasonal acclimatization in various coral species at the Great Barrier Reef. However, reefs exposed to sudden, episodic elevated temperatures cannot acclimate to these rapid environmental changes.

This work has bounded the effects of one of the main reef building corals in Florida, *Montastrea annularis*, to the range of potential environmental temperature extremes from Florida Bay water. This experiment demonstrated that temperature elevations have a strong negative impact even after short exposure times. Since corals are living near their thermal maximum, even small increases in temperature can be very damaging. Future research could address the recovery of corals following a range of stress events.

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## CONCLUSION

The experiments outlined in this dissertation indicate that stony corals are susceptible to damage from a variety of environmental stressors. Corals exposed to elevated levels of ultraviolet radiation, visible irradiance, salinity, and temperature all showed detrimental effects following acute exposure. Moreover, the strong responses from the corals suggest that reefs need not be exposed to stressors for long for damage to occur.

Corals exposed to elevated levels of ultraviolet (UV) radiation exhibited decreased photosynthesis and chlorophyll *a* levels, although there was no change in respiration, suggesting that UV radiation may be more damaging to photosynthetic algae than the coral tissue. Solar UV radiation reaching the surface of the ocean is greater in the tropics than temperate or subpolar regions (Calkins and Thordardottir 1980). Ozone is the primary absorber of UV radiation in the upper atmosphere (Madronich 1993) and more radiation passes through the atmosphere in the tropical regions because the ozone layer is thinner over the tropics than temperate latitudes (Madronich 1993). Coral reefs are exposed to increasing levels of UV radiation as ozone depletion continues (Baker et al. 1980, Smith and Buddemeier 1992). Reef organisms may not be able to adapt quickly enough to survive the changing conditions. Furthermore, water column clearing events from unusually calm periods (Gleason and Wellington 1993) can result in greater exposure of reef organisms to UV radiation.

It is believed that many reef invertebrates employ UVAbsorbing compounds, called mycosporine-like amino acids (MAA's) to protect their tissues from the damaging effects of UV radiation (Tsujino et al. 1980, Dunlap and Chalker 1986, Karentz et al. 1991). The study described in this dissertation did

not reveal any change in MAA levels in corals exposed to elevated UV radiation. However, given that exposure was limited to just a few days, it is reasonable to conclude that longer exposure times may be needed to see changes in MAA levels. Previous studies have found changes in MAA levels following multiple weeks of exposure to elevated UV (Jokiel and York 1982, Scelfo 1985, Kinzie 1993). Further studies could determine the effects of intermediate exposure times (many days to a few weeks) on MAA levels.

Stony corals that were photoadapted to low visible light levels exhibited decreased maximum photosynthesis rates, respiration rates, and photosynthetic efficiency following acute exposure to dramatically increased visible irradiance. These results suggest that increased visible irradiance was detrimental to both the photosynthetic algae and to the coral tissue.

There is still some debate regarding the relative contribution of visible irradiance and UV radiation in damaging reef organisms. Some authors have attributed coral damage to high photosynthetically active radiation (PAR) (Brown et al. 1994), while others have found UV more damaging (Jokiel and York 1984). In the studies described in this dissertation, elevated visible irradiance had a stronger negative effect on stony corals than elevated UV radiation. However, future studies would need to address this issue in a more controlled environment, where the same relative increase in each factor could be studied.

Stony corals exposed to elevated salinities exhibited decreased autotrophic capacity, with significantly reduced photosynthesis. Chlorophyll *a* levels were also much lower in corals exposed to elevated salinities. The study described in this dissertation also demonstrates a threshold lethal salinity. Corals exposed to salinities up to 40‰ survived for the duration of the experiment. However, corals exposed to salinities above 40‰ all eventually

died. The detrimental effects of this stressor were more pronounced the longer the exposure time.

Although salinity is generally considered stable, there are many reports of salinity fluctuations. Salinity fluctuations often occur following storms (Goreau 1964, Jokiel et al. 1993), or at isolated lagoons and atolls (Jokiel and Maragos 1978, Smith and Jokiel 1978, Caspers 1981, Coles 1988). Additionally, elevated salinity levels were reported over reefs in the Florida Keys (Porter et al. 1999). This study demonstrates that even brief exposures of elevated salinity can be damaging. Stony corals cannot osmoregulate, so salinity fluctuations can lead to physiological stress.

When stony corals were subjected to elevated temperatures, they showed decreased photosynthesis, as well as decreased photosynthesis to respiration ratio. Chlorophyll *a* levels were also significantly lower in corals exposed to elevated temperatures. These changes occurred after even short exposures and were more pronounced the longer the exposure time.

Since corals are living near their thermal maximum (Moore 1972, Vernberg and Vernberg 1972, Johannes 1975, Coles et al. 1976, Jokiel and Coles 1990), even small increases in temperature can be very damaging. Temperature increases can occur from localized warming on shallow reefs (Jokiel and Coles 1990) or from large-scale El Nino Southern Oscillation Events (Glynn 1984, Harriot 1985, Brown 1987, Glynn and D'Croz 1990). It is also possible that global warming may trigger more frequent and more prolonged seawater warming events (Jokiel and Coles 1990, Glynn 1993).

This dissertation outlines the effects of acute stress from several environmental factors. One significant area for future research would be to explore the long-term effects of exposure to these stressors. Additionally, monitoring of reef organisms throughout extended exposure to stressors, as well

as after the stressor has been removed would determine how quickly and completely organisms may recover from these different factors.

Recovery of reef organisms is largely impacted by the severity of the stress and the length of exposure. Recovery depends upon removal of the stress. However, prior stress to corals has been found to increase the likelihood that an individual will contract a disease and subsequently be killed (Peters et al. 1986). Some corals affected by diseases such as White Band Disease (Gladfelter 1982), Black Band Disease (Rutzler et al. 1983, Taylor 1983), and others (Peters et al. 1986) may have previously been affected by another stressor. Life history strategies may also be important in determining reef recovery after stress, since corals with high levels of asexual reproduction may recover more quickly than those with high sexual reproduction (Brown and Howard 1985).

Finally, perhaps the most significant area for future research is investigating the effects of multiple stressors. I explored this topic, with two other authors, in a publication focusing on the combined effects of temperature and salinity (Porter et al. 1999). When elevated salinity and temperature were combined, there was a short-term mitigative effect, since the combined stressors were less damaging than the stressors acting independently (Porter et al. 1999). This effect was also seen by Coles and Jokiel (1977). Their study revealed that corals exposed to elevated temperatures experienced higher survival when also exposed to elevated salinities than those in normal salinities. However, in both cases, this mitigative effect was temporary and as exposure time increased, corals exposed to combined elevated salinity and temperature all eventually died.

Information from studies on the effects of stressors can be used to influence reef conservation management decisions. There are three areas of

relevant research for coral reef planning and management: resource analysis, analysis of use, and information management. This dissertation has focused on the first of these three areas, however, the other two areas are also necessary for creating an effective conservation management plan. The hallmarks of a successful management plan are acceptance by users of the resource, effective enforcement by managers of the resource, and the ability of the plan to ensure long-term sustainability (Craik et al. 1990). As Meffe and Carroll (1994) stated, 93% of coral reefs have already been damaged and at current depletion rates, it is predicted that up to 60% of reefs will be lost within the next 20-40 years (Achituv and Dubinsky 1990). Given the diversity, productivity, and value of coral reefs, every effort to conserve these unique environments is warranted.

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