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Interactive Effects of Water Flow and Light Levels with Decreasing pH on the Growth and Survival of Tropical Cnidarians

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UNIVERSITY OF MIAMI

INTERACTIVE EFFECTS OF WATER FLOW AND LIGHT LEVELS WITH DECREASING PH ON THE GROWTH AND SURVIVAL OF TROPICAL CNIDARIANS

By

Carolyn L. Margolin

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The changes in global climate, including the observed and predicted changes to ocean chemistry are expected to have significant impacts of the future of coral reefs. A series of laboratory experiments examined the interactive effects of water flow rate, light levels, and decreased pH on the growth of several species of reef cnidarians. Under current water chemistry conditions (pH 8.04), the massive coral species, *Montastraea faveolata* shows high growth under flow conditions less than 15.7 cm/s. At this flow rate, decreased pH (pH 7.88) had no significant impact on the growth of this species. Under both water chemistry conditions, colonies showed decreased growth under low light conditions. The branching species *Pocillopora damicornis,* showed significant decreases in growth, particularly a decreased ability to add complexity under low pH conditions (pH 7.87). The massive, azooxanthellate coral, *Tubastraea coccinea* showed little growth response to decreases in pH. This indicates that branching species are more likely to show negative responses and decreased growth as oceanic pH continues to fall. *Aiptasia pallida* anemones were smaller and denser in terms of protein make-up under low pH (pH 7.85) conditions than under current water chemistry (pH 8.04) conditions. The information presented here could be considered in future conservation efforts.

This dissertation is dedicated to my grandparents, Seelig and Irene Lester.

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Chapter 1: Introduction

A) Global Climate Change and Coral Reefs

Approximately 284,300 km^2 of the shallow ocean floor support coral-reef ecosystems (Spalding *et al.* 2001). Reefs provide protection for coastal environments (Johnson and Marshall 2007) are habitat and nursery grounds for a myriad of plant and animal species (reviewed in Wilkinson and Buddemeier 1994), provide income to human populations directly and indirectly (Lugo 2008), and serve as a contributing component of the cultural identity of nearby communities (Cooley *et al.*2009).

Coral reefs grow through the accumulation of calcium carbonate, provided by the skeletal remains of corals, calcifying algae, and numerous other invertebrates. The rate of reef growth depends on the overall rate of deposition exceeding the total rate of erosion. In addition, an existing reef framework that promotes the accumulation and consolidation of calcareous sediments will facilitate reef growth (Goreau 1959a). Corals are able to build massive structures. They are exceedingly sensitive to environmental conditions and changes in those conditions. Coral growth occurs only within narrow limits of temperature, irradiance, salinity, pH, and turbidity (Crabbe 2008). In many cases, modern corals are living near the limits of their possible range of survival for one or more of these conditions. Therefore, even minor changes in any one of the limiting parameters could have devastating results for a reef and the organisms that depend upon it.

The growth and survival of reefs are affected by physical, chemical, and anthropogenic factors. Environmental conditions determine whether corals can colonize

an area, their growth after colonization, and their distribution within colonized areas (Vaughan 1916). First order determinants of coral-reef distribution include temperature, salinity, nutrients, light availability, and aragonite saturation state (Kleypas *et al.*1999b). These are the major factors that will govern whether or not corals can exist in a given region of the ocean. These first order factors govern the likelihood of reef development on a global scale. Second order factors, including hydrodynamic conditions (currents, storm frequency and wave action) and biological variables, such as bioerosion, disease, and larval sources control reef development on a local scale (Kleypas *et al.*1999b). Critical changes in any of these parameters will affect the areas in which a reef can develop as well as affect its growth rate. These factors do not exist in isolation. There are countless combinations of conditions created by these factors. Each combination will influence the affected organisms in a specific way, that depends upon all of the individual factors and the ways in which they interact.

After a reef has been established, changes in both biotic and abiotic factors may greatly influence the survival of the corals present. For instance, increases in temperature beyond specific upper limits have been linked to decreased growth (Jaap 1979), bleaching (Gates *et al.*1992, Jones *et al.*1998, Fitt *et al.*2001, Douglas 2003), and in extreme cases death (Glynn *et al.*2001) for a number of species in several reef environments. Due to their sensitive nature, researchers are turning to coral reefs as indicators of potential effects of global warming and related issues, such as ocean acidification, and sea level rise as global climate change becomes an increasing concern (Smith and Buddemeier 1992, Wilkinson 1999).

Human activities have been affecting coral reefs and tropical ecosystems far longer and more intensely than has generally been recognized (Birkeland 1997, Jackson 1997, Jackson 2001, Jackson *et al.* 2001, Pandolfi *et al.*2003). The lack of direct records of these influences does not mean that they did not occur. Over the last several decades, however, declines caused by both local and global influences have been observed on reefs world-wide (Wilkinson and Buddemeier 1994, Wilkinson 2008). These influences may be natural or may be due to anthropogenic activities. Natural influences such as geologic events are beyond the scope of human activity. However, human actions are gaining influence over corals on both local and global spatial scales through direct and indirect effects, i.e. sewage and pollutants increasing disease occurrence and virulence (Kaczmarsky *et al.*2005, Sutherland *et al.* 2010).

Local anthropogenic disturbances can include dredging, pollution, overfishing (Chadwick-Furman 1996), and vessel groundings. While these "small scale" effects can be relieved by removing the localized stressor (West and Salm 2003), global warming-related stresses cannot be alleviated so easily. The effects of global warming are felt worldwide in a multitude of ways and to varying degrees by different species, areas, and ecosystems. In recent years, corals have become "the canaries in the coalmine" as indicators of the effects of global warming (Smith and Buddemeier 1992).

The survival of coral reefs is dependent upon a relatively stable physical environment within a narrow range of conditions. This property of the reefs is one reason why their responses to environmental changes can be seen as a harbinger of possible

wide-scale declines. Coral reefs face increasing stress from factors such as rising sea temperatures, increasing levels of greenhouse gases, and assorted pressures from increasing coastal development including overfishing and pollution (Pandolfi *et al.*2003, Hughes *et al.*2003). Reefs that are currently considered to be healthy, will likely become environmentally limited as climate change continues. Those reefs will become marginal habitats, losing their ability to support healthy communities (Guinotte *et al.*2003).

Coral reefs serve as crucial habitat for innumerable plant and animal species (Hoegh-Guldberg 1999) and support one of the highest concentrations of biodiversity of all global ecosystems (Reaka-Kudla 1997, Glynn 2011). Of the 34 known metazoan phyla, the hundreds of thousands of species found on reefs contain representatives from at least 31 of them, with new species being identified every year (reviewed in Glynn 2011). In addition, millions of people depend on coral-reef ecosystems as a source of protein since they can support fisheries of all levels- subsistence, recreational, and commercial (Bryant *et.* al 1998). Reef systems also provide many other services to both humans and other species (Costanza *et al.*1997). If they are to continue to serve these functions, despite the stresses being placed upon them by global and local stressors, it is necessary to preserve and protect these fragile systems now and in the future. An understanding of the ways that these factors will affect a variety of coral species is crucial. In many cases, stressors are predicted to have a negative effect on corals. Understanding the processes of coral acclimatization and the possible extent of acclimatization to future environmental conditions is of the utmost importance (Edmunds and Gates 2008). Determining if there are conditions that may mitigate these detrimental

effects and/or influence the acclimatization process is crucial. This may allow scientists and managers to better evaluate the potential of reef areas to resist or recover from the detrimental effects of climate change (West and Salm 2003).

The most well known component of global climate change is global warming, which is coupled with an associated rise in sea surface temperature. These increases have widespread abiotic and biological effects. The abiotic consequences of rising sea surface temperature include such diverse effects as deglaciation and melting sea ice, and sea level rise, changes in water density and circulation, and changes in the expected frequency and severity of hurricanes and other storm systems.

The effects of climate change on coral reefs suggest that their future survival will depend on the rate and extent of increased thermal stress, and their resilience in the face of thermal stress. The effects of global climate change, however, extend far beyond those associated with increasing air and sea temperatures. One major factor influencing global climate change is the increasing concentration of carbon dioxide in the atmosphere (IPCC 2007).

Elevated temperature, and its acceleration of photosynthesis and other chemical processes, is acknowledged as one of the primary influences on coral reef growth and survival due to its ability to influence coral metabolism, reproduction, and larval settlement (Buddemeier *et al.*2004, reviewed in Done and Jones 2006). Elevated temperature may also increase the virulence of diseases found on reefs by making the animals more susceptible to opportunistic infection (Harvell *et al.*2002, Bruno *et al.*2007, Lesser *et al.* 2007, Dalton *et al.*2010). Temperatures of 1.5-2.0˚C above the summer

maxima for 6-10 weeks can cause coral bleaching (Berkelmans 2002). According to the 2007 IPCC report, by the end of the century average temperatures are expected to rise 1.4-5.8˚C above 1990 levels, based on a number of scenarios considering a spectrum of CO_2 output scenarios. Therefore, incidences of coral bleaching and mortality are expected to increase with future rising temperatures (Hoegh-Guldberg 1999). In order for corals to survive in the future, coral communities will need to adapt to the changing environmental conditions. This may occur through changes to the hosts themselves and to the symbionts they host. Refuge populations of species with broad depth ranges may survive on mesophotic reefs. These lie in the deeper regions of the photic zone, and will not be exposed to the more extreme temperatures of surface waters.

Carbon dioxide naturally cycles rapidly between the atmosphere, oceans, and land. CO_2 levels in the atmosphere have been increasing rapidly since the industrial revolution as more carbon is removed from long term storage in the form of oil or coal and burned, transferring carbon into the atmosphere. The terrestrial and oceanic processes that would naturally remove CO_2 from the atmosphere are slow and limited compared to the rates that humans are adding CO_2 to the atmosphere. The high solubility of CO_2 allows the ocean to remove it from the atmosphere. This ability is limited by the amount of vertical mixing that can take place. Mixing removes CO_2 from surface waters and allows less saturated waters to take their place (IPCC 2007). As more carbon dioxide is forced into surface waters, the chemical balances within the ocean shift (Feeley *et al.*2004). These shifts may be subtle, but can have significant direct and indirect impacts on a multitude of species and communities.

Rather than a readily apparent visual response, like bleached coral tissue, responses to changing chemical conditions materialize as changes in zooxanthellae growth rate and photosynthetic capacity (Brading *et al.* 2011) (rather than quantity), colony growth and calcification rates, and other less conspicuous ways, including the protein content of coral tissues and $\delta^{13}C$ and $\delta^{18}O$ content of coral skeletons (Krief *et al.*2010). Decreases in growth rates, though subtle, can have substantial long term effects. On shorter time scales, differences in growth rate may play a role in altering the species that are dominant on a reef. The varying recovery rates of colonies, or reefs, from damage or disease can impact reef communities as individuals compete for space. On longer time scales, the subtle changes related to ocean acidification will become more significant. Decreasing growth rates caused by ocean acidification may contribute to reefs "drowning" if their vertical extension and/or accretion is unable to keep pace with rising sea levels (Riegl 2003, Kleypas *et al.*1999). Acidification can also lead to decreasing skeletal density, which can increase susceptibility to bioerosion (Manzello 2008) and a related loss of reef structure and habitat.

Understanding the ways that coral calcification will respond to increasing pCO_2 (and associated changes in the atmosphere and ocean), and how that response may vary depending on changes in other environmental factors, is critical to predicting how coral reefs may change in the next 50-100 years (Langdon and Atkinson 2005). Increases in atmospheric CO_2 since the beginning of the industrial revolution have caused a decrease in mean surface ocean pH of 0.1 units. This reflects the equivalent of a 30% increase in H^+ ion concentrations in the world's oceans (Orr *et al.*2009).

We must now begin to examine how changes in water chemistry interact with other physical factors that are known to influence corals. While there is some understanding of the effects of water flow and/or light levels on a number of coral species under existing water chemistry conditions (Sebens 1991, Sebens and Johnson 1991, Lesser *et al.*1992, Lesser *et al.*1994, Helmuth *et al.*1997, Sebens *et al.*1997, Bruno and Edmunds 1998, Sebens *et al.*1998, Kuffner 2001, Nakamura and van Woesik 2001, Nakamura *et al.*2003, , Sebens *et al.*2003, Nakamura *et al.*2005, Badgley *et al.*2006, Fabricius 2006, Finelli *et al.*2006, Carpenter and Patterson 2007, Jokiel *et al.*2007, Carpenter *et al.*2010, Schutter et al 2010) there has been a very limited amount of work addressing these factors under conditions of decreasing aragonite saturation and decreased pH. This study examined how light level and water flow rate influences the growth of corals exposed to decreased aragonite saturation levels (controlled by manipulating pH).

Although corals are generally seen to survive only in very narrow ranges of abiotic conditions, some species do have a limited ability to adapt to conditions outside of their optimal ranges. Thermal tolerances of individual colonies can be dependent upon the thermal conditions of their native habitat (Coles and Brown 2003). For instance, populations of the same coral species found in thermally variable locations have been found to have thermal tolerances that differ according to the location where they have developed (populations in warmer areas will have higher thermal tolerances than those in relatively cooler areas) (Coles *et al.*1976). In addition, coral communities that grow in euryhaline conditions showed smaller ecological changes (i.e. loss of species) as a result

of high thermal conditions than communities with stenohaline histories (McClanahan and Maina 2003). Corals at Enewetak have been shown to survive in temperatures of 34˚C while temperatures of 32˚C were lethal to congeners in the sub-tropical Pacific waters of Hawaii (Coles *et al.*1976). In a single area, communities may show a shift in tolerance after exposure to severe thermal stress. Corals in the backreef of Ofu, Samoa experienced high mortality after a warm water (>32˚C) event in 1994, but now show tolerance to daily temperature fluctuations of 6.3˚C, and maxima of 34.5˚C (Craig *et al.*2001).

If populations have the ability to adapt to conditions that can be thermally stressful to some individuals, it may also be possible for populations to adapt to other environmentally stressful conditions, including changing aragonite saturation states. In order for that to occur, individuals of each species must survive the initial increases in stress, whether they are thermal, chemical, or biological in nature. The initial survivors may serve as founders of a more stress tolerant population in the future, as occurred under thermal stress in Ofu (Craig *et al.*2001). Although shifts in the tolerance limits of a species may be possible,the time necessary for these shifts to take effect on an ecologically significant scale may be longer than the rate of change of environmental factors, such as temperature (Jokiel and Coles 1990, Smith and Buddemeier 1992) and aragonite saturation state (Doney *et al.* 2009). Some models indicate that reef areas that have low thermal stress may play an important role in increasing the capacity of corals to respond to climate change and therefore should be considered for protection (Baskett *et al.* 2010). Conversely, some observations have shown evidence of increased thermal

tolerance by corals in sites that experienced frequent periods of high thermal variability. Rapid directional selection within these frequently stressed sites after extreme thermal events has been seen to confer bleaching resistance when another thermal stress occurs. This directional selection may result not only in increased thermal tolerance, but in shifts to coral community composition (Thompson and van Woesik 2009)

The multitude of stressors on reefs associated with climate change is unprecedented in human history and to date, there have been few studies of synergistic effects (Veron et al 2009). Some studies have acknowledged the fact that interactive influences of light, water flow, sedimentation, hydromechanical stress, and competition can all affect morphological and growth properties of hard corals (Helmuth *et al.*1997). The scarcity of studies directly investigating interactions of stressors is surprising since the interactions of environmental variables become particularly important as corals reach the limits of their tolerance for any parameter (Jokiel and Coles 1990).

Climate change is forcing corals closer to their tolerance limits for a number of environmental parameters, including temperature and aragonite saturation state (Kleypas *et al.* 1999b, Cooley *et al.* 2009). The experiments described in this dissertation are a step towards determining the combined effects of physical factors on coral growth under existing and predicted sea water conditions. When taken together with studies on other influences on reef systems, such as temperature, nutrient levels, and biological influences, they will provide a framework of possible effects (changes in growth, reproduction, and community composition) over the next century. This will assist in the

development of conservation policies that will protect areas that may serve as important refugia.

The general decline in worldwide coral health also necessitates improvements in coral mariculture. These studies offer insight into how marine protected areas may be chosen to maximize effectiveness in the future. In addition, they will help determine the flow and light levels that are most conducive to coral growth under current environmental conditions. This information will be useful in designing or improving protocols for culturing coral for both scientific research and reef restoration purposes.

B) Influences of Environmental Factors on Coral Growth

1) Water Flow Rate

Water flow is one of the most important abiotic factors influencing the growth of sedentary marine invertebrates (Sebens 1987). Corals and other sessile organisms must rely on water flow for a number of reasons. Water flow can influence physiological processes including photosynthesis and respiration by affecting the diffusion boundary layers around the organism. This in turn affects the associated ability of dissolved gases (Dennison and Barnes 1988, Patterson *et al.*1991, Patterson 1992, Atkinson et al 1994, Lesser et al 1994, Shashar *et al.*1996, Bruno and Edmunds 1998) and inorganic nutrients like nitrate and phosphate (Stambler et al 1991, Atkinson and Bilger 1992, Thomas and Atkinson 1997) and carbon (Lesser *et al.*1994) to reach the animal. Boundary layers also influence the removal of heat from coral tissue (Jimenez *et al.*2008)

The flow rate of water over a suspension feeder will also affect its rate of prey encounter and capture (Helmuth and Sebens 1993, Johnson and Sebens 1993, Sebens 1997, Sebens *et al.*1997, Sebens et al 1998). In addition to governing the ability of a coral to acquire materials from the water column, the rate at which detrimental materials can be removed from the vicinity of a coral is controlled by flow speed (Nakamura and van Woesik 2001). Flow rate can also influence the holobiont through its effect on the symbiotic autotrophs. High water flow can lead to enhanced removal of oxygen from autotrophs, increasing the affinity of RuBisCo for CO_2, which promotes higher rates of photosynthesis (Mass *et al.*2010).

The water flow regime that a colony experiences may be representative of the general geographic area in which it is found, or it may be heavily influenced by its position on the reef and relative proximity to other structures and organisms. Previous studies have shown that the growth rates and/or morphological development of coral under differential flow regimes are species-specific (Jokiel 1978, Helmuth and Sebens 1993; Bruno and Edmunds 1997; Helmuth *et al.*1997).

Increased respiratory rates and increased nutrient uptake (Atkinson and Bilger 1992, Atkinson *et al.* 1994, Bilger and Atkinson 1995; Thomas and Atkinson 1997) may also be responsible for higher growth under increasing water flow rates (Schutter *et al.*2010, Schutter *et al.* 2011). Although high flow rates can be beneficial, when water flow increases, it can cause deformation of polyps which can result in decreases in efficiency of nutrient and gas uptake and exchange. Under conditions of very high water motion, coral skeletons may fracture and break (Sebens *et al.*1997) due to water

movement itself, as well as the impact of projectiles carried by the fluid. Corals in high flow conditions can develop denser skeletons than members of the same species in lower flow areas which minimizes breakage (Schumacher and Plewka 1981, Scoffin et al 1992, Smith and Birkeland 2007, and Schutter *et al* 2010).

The overall morphology of coral species can be markedly influenced by water flow rate. In some species water flow affects characteristics including symmetry, branch length, thickness, and spacing (Chamberlain and Graus 1975, Jokiel and Cowdin 1976, Lesser *et al.*1994, Bruno and Edmunds 1998, Mass and Genin 2008). Together, the morphology of a colony and the water flow around it influences its success in capturing particles and prey (Helmuth and Sebens 1992). In some cases, a coral's morphology can modify water flow patterns, which improves its ability to capture particles by slowing water flow within the colony (Johnson and Sebens 1993, Sebens *et al.*1997).

There are several ways that this flow decrease can be accomplished. In branching species, the branches can create a baffling effect which slows the flow as it passes through the colony, creating conditions of lower flow near the center of the colony (Helmuth *et al.*1997). Alternatively, corals with long tentacles that grow in dense thickets can slow water flow around the mouths of polyps increasing the retention of particles that may be deposited due to gravitational influences (Johnson and Sebens 1993). At low flow rates, polyps on the upstream side of a colony are often seen to have the higher particle capture rates than those on the downstream side (Sebens *et al.*1997). High flow velocities can cause the tentacles on the upstream side of a coral to shorten or deform (Johnson and Sebens 1993), which would decrease both their own abilities to

capture and retain prey particles and minimize their ability to influence flow over the rest of the colony. Flow conditions that are high enough to deform upstream tentacles can lead to higher capture rates by polyps on the distal side of branches, where the polyps can feed from eddies that form as water moves past the colony structure.

It has been suggested that while the morphological development of corals is heavily influenced by water flow regimes, a colony's behavior can also be influenced by the flow environment it experiences. An individual with a particular morphology that is placed in a new regime may have plasticity in its physiological and biochemical activities. This allows it to enhance the delivery of carbon to assimilation sites. The colony can then better exploit its new surroundings by changing its physiological and behavioral activities (Lesser *et al.*1994). For instance, studies of *Favia favus* have shown that light levels and water flow rates are major influences on the expansion behavior of this species (Levy *et al.*2001). Polyp extension and other behavioral changes related to water flow can also influence coral growth and survival through their influences on the capacity to capture prey (Abelson *et al.*1993, Helmuth and Sebens 1993, Johnson and Sebens 1993, Helmuth *et al.*1997, Sebens *et al.*1998, Piniak 2002).

In addition to behavioral and physiological changes that are controlled by the coral directly, the exchange of dissolved materials may be influenced by water motion, largely due to boundary layer effects (Patterson and Sebens 1989, Patterson *et al.*1991, Patterson 1992, Bruno and Edmunds 1997, Gardella and Edmunds 1999, Gardella and Edmunds 2001, Kuffner 2001). Other studies have found that increased water flow rates can temper the severity of the damage caused by a variety of stresses on corals, such as

increasing temperature and photoinhibition (Jones *et al.*1998; Nakamura and van Woesik 2001; Nakamura *et al.*2003; Nakamura *et al.*2005). This may be due to one or more of the previously mentioned effects of flow rate on the coral hosts, or its direct or indirect effects on symbiotic dinoflagellates.

Water flow can have numerous influences on symbiotic zooxanthellae. For instance, experiments investigating the effects of water flow, PAR, and UVR on the concentration of mycosporine-like amino acids (MAAs) revealed that flow-modulated photosynthetic rate seems to be a major factor affecting MAA concentration (Jokiel *et al.*1997; Kuffner 2001). MAAs serve important photoprotective roles, acting as "sunscreens" for corals and their zooxanthellae. *Porites compressa* colonies exhibit lower MAA concentrations after exposure to UVR and low flow rates compared to colonies exposed to UVR and high flow conditions (Kuffner 2001). A decrease in MAAs could lead to an increase in photoinhibition of the zooxanthellae as well as cause stress to both symbiont partners. Therefore, corals that experience low flow rates may be at a higher risk from damage due to increasing levels of UVR during periods of low cloud cover and other instances of increased light penetration. Under conditions of severe photoinhibition, the ability of the zooxanthellae to contribute sustenance to their host may be significantly reduced. This could cause a decrease in the host's resources affecting their ability to grow and allocate energy toward reproduction.

Flow rates may influence zooxanthellae in healthy colonies directly by controlling the rates of their metabolic processes. Under unstirred (stagnant) conditions, photosynthesis and respiration by *Acropora formosa* and its zooxanthellae decreased 25%

compared to rates seen with circulation (Dennison and Barnes 1988). *Montastraea annularis* colonies exhibit increasing rates of both photosynthesis and respiration with increasing flow conditions (Patterson *et al.*1991). In addition, water flow rates of 20cm/s have been shown to reduce the photoinhibition of zooxanthellate photosynthetic mechanisms in *Acropora digitifera* (Nakamura and van Woesik 2001).

In colonies that have been damaged, favorable water flow conditions can contribute to improving their ability to recover. Water flow rates of 20 cm/s have been seen to facilitate the recovery of partially bleached *Stylophora pistillata* more effectively than flow rates less than 3 cm/s (Nakamura *et al.* 2003). In addition, flow rates of 50-70cms^{-1} may increase survivorship of *Acropora digitifera* colonies under high temperatures compared to colonies under low flow (2-3 cm s^{-1}) conditions (Nakamura and van Woesik 2001). It has been suggested that increased flow only relieves bleaching stress for the affected corals if the stressed or damaged animals are exposed to increased flow for short periods of time. If they are exposed to high flow for extended periods, while still experiencing elevated photosynthetic activity, their systems may become overburdened. This situation is due to the need to expend energy on metabolic processes such as molecular chaperone synthesis, which is necessary in order to preserve cellular functions within the colony at increased temperatures or under other external stressful conditions (Carpenter *et al.*2010).

At lower, less stressful irradiances, water flow may have a more limited effect on photosynthesis, since the need for inorganic carbon supply and/or removal of oxygen to optimize photosynthesis is not as demanding as it is at high irradiance levels (Nakamura

*et al.*2005, Finelli et al 2006, Smith and Birkeland 2007). At high irradiance levels, water flow rates become increasingly important for maintaining coral growth (Schutter *et al.*2011). The zooxanthellae of poritid corals that experienced sub-lethal damage were seen to demonstrate a 10% decrease in quantum yield under low flow conditions (6cm/s) and a 4% increase in yield at high flow rates of 21 cm/s. It was hypothesized that the photosynthetic and calcification processes of poritid corals following sub-lethal damage can compete for the metabolites necessary for colony repair, and the outcome of the competition is affected by flow mediated mass transfer (Edmunds and Lenihan 2010).

Water flow rates can show marked variability across a reef habitat. This variation can be attributed to a number of factors, including water depth, wind speed, and local currents. The interactions of moving water with physical features of the environment can also impact the speed and direction of water movement (Hamner *et al.* 1988). As depth increases, water flow will generally decrease. For instance, mean flow speeds in the deeper forereef zones of Discovery Bay, Jamaica were less than 5cm/s, reaching maximum speeds of 10cm/s, while shallow areas (<5m) had mean flow speeds of 10-20 cm/s (Sebens *et al.* 1997). Therefore, zooxanthellae that demonstrate similar reactions to those observed by Edmunds and Lenihan (2010) would be expected to demonstrate decreases in quantum yield in deep forereef habitats and increases in yield in shallow waters.

A colony does not always experience stress evenly across its surface. Colony orientation to flow influences how the water movement will be experienced by the polyps. *Montastraea annularis* colonies develop and sustain spatially asymmetric

patterns of stress protein synthesis across their surfaces. The upstream areas (those experiencing higher flow) express higher levels of stress protein synthesis (ie. *hsp*70) and exhibit reduced photosynthetic efficiency and quantum yield (Carpenter *et al.*2010). This differential expression may have positive and negative effects on fitness because heat shock proteins can both protect colonies at low concentrations and harm them at high concentrations. While heat shock proteins can aid a colony by acting as chaperones for other proteins within cells, they can also be detrimental if their concentrations reach high levels that may interfere with ongoing cellular processes (Lindquist 1993; Feder and Hofmann 1999) or utilize an unsustainably large portion of a cell's or colony's resources (Calow 1991; Hoffman 1995; and Feder and Hofmann 1999). As with any biological process that requires the use of limited resources, *hsp* production decreases the amount of those cellular products available for vital activities such as growth and reproduction.

From the above findings, it can be seen that water flow has the ability to either enhance or diminish negative effects of stressors on corals. In addition, the effects of flow rates are not limited to the direct influences on the holobiont. Water movement over nearby organisms can impact the interactions between corals and their neighbors. For instance, high flow rates reduce the chance of disturbance of growth by competing algae or cyanobacteria that can have negative effects through overgrowth processes (Rogers 1990, Anthony and Fabricius 2000, Box and Mumby 2007). Since corals need not expend energy and resources to minimize overgrowth, they may then allocate more to other growth processes (McCook *et al.*2001, Kuffner *et al.*2006, Schutter et al 2010).

It is imperative that the potential of water flow to mitigate or exacerbate these effects be investigated. As CO_2 levels rise, their negative impacts on corals are likely to increase in severity. Particular flow regimes can play a role in alleviating some of the possible damage that holobionts may experience in changing environments. An understanding of these interactive effects will help researchers and managers determine which areas are best suited to act as refugia for corals and their associated communities. In addition, investigating the effects of flow on coral growth under current water chemistry could help determine the best conditions to employ in mariculture programs. It may also be used in site selection for more "natural" coral nurseries like those established in Biscayne National Park (Herlan and Lirman 2009, Lirman *et al.* 2010). Cultured colonies would be useful in both scientific investigations, restoration efforts, and even the aquarium trade in order to remove any collection pressure from natural reef areas.

As with many factors that can influence biological communities, balance must be found in water flow environments. Increasing water flow rate over a coral colony is only beneficial up to a point. Every coral likely has an "optimum flow range". Increases in flow rate up to that range will increase growth and fitness (in terms of survival, reproductive ability, etc.), while further increases can become detrimental (Nakamura and Yamasaki 2005). Numerous studies have shown increased fitness with increasing water flow (Nakamura and Yamasaki 2005, Nakamura and van Woesik 2001) up to intermediate rates of approximately 20cm s^{-1}. However, tissue damage can result from exposure to flows of ~100cm s^{-1}. *Pocillopora damicornis,* exhibits reductions in coral growth rates under such high flow conditions (Jokiel 1978).

Each species is likely to have its own optimal flow range based on its morphology, polyp size, feeding behaviors, and tissue thickness. This range may widen or narrow based on other environmental factors, including light, temperature, and nutrient levels. Species optimal flow ranges may play key roles in determining which portion of the reef that the species will inhabit. *Pocillopora meandrina* exhibits higher skeletal growth under higher water flows (20-40 cm/s) than under lower flow rates, and may possess an optimal flow range that is even higher (Jokiel 1978). The morphologically similar *Pocillopora eydouxi* also shows increased growth under high water motion conditions (Smith *et al.*2008). *Acropora palmata* shows a comparable preference for high flow, thermally stable forereef environments and poor adaptation to thermally variable low flow backreef areas (Lundgren and Hills-Starr 2008). These properties make them well suited to the high water motion conditions of forereef environments where they are often found (Veron 2000).

Studying multiple species over a spectrum of flow treatments could help determine possible optimal flow ranges for each of them. More detailed information on the optimal range for various species, particularly major framework builders, could be useful to both scientists and policy makers for culturing, research, and conservation purposes.

Natural reef habitats have been observed to have average flow speeds ranging from <5cm/s (Sebens *et al.* 1997) to > 37cm/s (Carleton *et al.* 2001). The majority of previous flow studies have found healthy growth rates for most species fall within the range of 5 cm s^{-1} - 30 cm s^{-1} (Edmunds and Lenihan 2010, Purser *et al.*2010, Schutter *et*

*al.*2010). Water flow rates of approximately 5, 15, and 25 cm s^{-1} were selected as treatments conditions in both the isolated flow and interactive factor studies for this research. Based on field observations, and previous manipulative studies, these choices fall within recognized definitions of low, medium, and high flow rates (Table 1.1).

Flow rates	**Area / Conditions**	**Author**	**Year**
2.22 cm/s	Great Barrier Reef Lagoon	Hamner and Hauri	1981
1-15 cm/s	Great Barrier Reef Windward Face	Hamner *et al.*	1988
2.3-7 cm/s (max 4.1-16.7 cm/s)	Mean flows in *in situ* flumes, St. Croix	Patterson *et al*.	1991
3-50 cm/s	Discovery Bay, Jamaica	Helmuth and Sebens	1992
20 cm/s	Flume		
3, 5, 10, 15, 25 cm/s	Aquarius Habitat in flume	Johnson and Sebens	1993
3 and 15 cm/s	Belize, flumes	Helmuth *et al.*	1997
4-8 cm/s	Discovery Bay, Jamaica	Sebens *et al.*	1997
10-15 cm/s			
40-50 cm/s			
12-57 cm/s, average 37 cm/s	Helix Reef, GBR	Carleton *et al.*	2001
0-15 cm/s	Conch Reef	Finelli *et al.*	2006
19.2-36 cm/s	Aquarius Habitat flume – upstream position	Carpenter and Patterson	2007
15-24 cm/s	Aquarius Habitat flume – side position		
1.2-3.6 cm/s	Aquarius Habitat flume – downstream position		
<3 cm/s and 20 cm/s	Collected in Okinawa, outdoor flume systems	Suzuki *et al.*	2008
0-5 cm/s low flow	Santa Barbara, CA	Arkema	2009
10-12cm/s intermediate ambient flow			
>20 cm/s high flow			
approx. 5-30 cm/sec	Glover's Reef, Belize	Finelli *et al.*	2009
13.2 cm/s around coral	Red Sea, Eilat 2-15 m depth	Mass *et al*.	2010
20.6 cm/s above algae, 4.5 cm/s above seagrass			
10, 20, 25 cm/s	Burgers Ocean, Arnhem, The Netherlands	Schutter *et al.*	2010
low flow 5-10 cm/s	Burgers Ocean, Arnhem, The Netherlands	Schutter *et al.*	2011
high flow 15-25 cm/s			

Table 1.1: Summary of flow conditions observed on various natural reefs and examined in previous research studies.

2) Temperature

Increases in temperature can have both direct and indirect effects on numerous plant and animal species. Higher temperatures may cause changes to growth rates, survival, and reproduction as well as other metabolic rates within marine organisms. If some organisms are thermally tolerant or able to adapt to rising temperatures, they are more likely to survive increases in sea surface temperatures. These increases are predicted to occur at a global average of approximately 0.15˚C per decade (IPCC 2007), though local increases will likely vary with habitat (Brown 1997, IPCC 2007)

Corals are widely accepted to be stenothermal species with upper and lower lethal limits that are relatively fixed (Mayer 1918) in the ~18-31˚C range (Coles and Jokiel 1977). They are already known to be living close to their upper thermal tolerance limits in most areas. The coral's responses to increased temperature conditions will depend on multiple factors including species (Edmunds 2009), prior stress exposure (Berkelmans 2009), symbiont identity and diversity (Glynn et al. 2001, Baker 2003; Baker *et al.*2004, Berkelmans and Van Oppen 2006, Jones et al. 2008, LaJeunesse et al. 2009), intensity and duration of the temperature anomaly (Gleeson and Strong 1995, Podestá and Glynn 1997, Berkelmans 2002), and local light conditions (Lesser 1996), as well as other local and physical and biotic variables (Jokiel and Brown 2004).

Species that live in shallow water or reef flat areas are likely to be more tolerant of environmental variability than species that live sub-tidally and experience less variation on a regular basis. This tolerance is exemplified by backreef assemblages in American Samoa (Ofu Island) that withstand daily temperature fluctuations of 6.5˚C

(Craig *et al.*2001). Tolerance can be influenced both by the host and the symbiont. Transplant studies showed that coral-algal symbioses often conform to thermal environments through changes in the identity of the algal symbionts, with colonies in warmer water hosting more clade D symbionts than colonies in cooler waters. (Oliver and Palumbi 2011). In addition, the genotype of coral hosts may drive limitations to the physiological responses of corals when they are exposed to new environmental conditions (Barshis *et al.* 2010).

There is a spectrum of possible symbioses between coral and algal communities, and the associations can be influenced by physical factors (Rowan and Knowlton 1995, Baker 1999, Baker 2003, Thornhill *et al.* 2006, Mieog *et* al. 2009, Lajeunesse *et al.* 2011, Oliver and Palumbi 2011). Changes in carbon dioxide levels, light, and water flow may favor symbioses other than those currently presently associated with a particular species in a given region or depth. It is important to determine if these combined physical conditions will influence the members of each symbiosis as well as the survival and growth potential of these associations. This knowledge will help predict the viability of future coral-algal symbioses under altered environmental conditions.

Although some symbioses of corals and zooxanthellae exhibit the capacity to adapt to changing conditions, the full extent of these abilities at the time scales in question are as yet unknown (Berkelmans 2002, Brown 1997, Coles and Brown 2003, Csaszar *et al.*2010).

In general, thermal tolerance limits for corals in a particular area are approximately 1-2˚C higher than the region's historical summer highs (Coles *et al.*1976).

For example, several coral species in Kaneohe Bay, Hawaii, exhibit maximal growth at 26˚C while long term exposure to 30˚C will lead to bleaching and mortality, and exposure to temperatures of 32˚C or higher will lead to mortality after an exposure of only a few days (Jokiel and Coles 1977). In addition, low latitude reefs showing higher thermal tolerance than their counterparts in higher latitudes (Berkelmans 2002).

Increases in temperature can affect corals at all life stages. Even as larvae, corals are influenced by thermal conditions. The larvae of *Porites astreoides* are large and have high population densities of *Symbiodinium* dinoflagellates at 26.4˚ C– 27.7˚C compared with larvae at 25.8˚C and 28.8˚C which are significantly smaller with lower symbiont densities (Edmunds *et al.*2005). This sensitivity of larvae can have far reaching influences for the thermally sensitive species and the reef areas that depend on the affected reefs as a source of larvae. The reproductive abilities of adult corals can also be influenced by thermal conditions, though the effects can be both species and site specific. Periods of moderately increased temperatures caused significant decreases in gametogenesis for *Pocillopora damicornis* colonies in the Gulf of Chiriqui. Members of the same species, as well as *P. elegans* in the Galápagos show significant increases in gametogenesis with moderate warming, but decreases in reproductive activity when the temperature anomalies (+2- 4˚C) persist for a prolonged period (Colley *et al.* 2006).

In adult coral colonies, the effects of thermal stress are most often related to bleaching, a generalized stress response of corals. It occurs when symbiotic corals lose their algae and/or pigments due to the breakdown of the host/symbiont partnership. Bleaching may cause corals to exhibit decreased growth or survival. The severity of the

bleaching and its overall effect depends on the severity and duration of the stressor (Fitt *et al.*2001). Ocean warming has caused bleaching events to occur on a nearly annual basis in one or more of the world's tropical or subtropical seas since the early 1980s. Bleaching events can directly cause the catastrophic loss of coral cover in an affected area, which can also impact biodiversity, susceptibility to disease, and reef structure (Baker *et al.* 2008).

The responses of corals to elevated temperatures is quite complex, however temperature data can be used to produce a number of indicators to hindcast and forecast bleaching episodes (Goreau *et al.* 1993, Gleeson and Strong 1995, Brown *et al.* 1996, , Podesta and Glynn 1997, Winter *et al.* 1998, Berkelmans *et al.* 2004, Sammarco *et al.* 2006). Estimates of cumulative thermal tolerances in an individual area can be made using the Degree Heating Weeks (DHW) bleaching index (Gleeson and Strong 1995). This index is based on the difference between average weekly temperatures in an area compared to its average climatology. Variations on this index include the Degree Day (DD) index, which can detect variations on a shorter time scale (Podesta and Glynn 1997, Berkelmans 2002). Degree Day analysis may be utilized to determine the threshold for cumulative thermal stress that a coral can withstand before bleaching occurs. Use of a single index for determining thermal stress might be misleading, for example an extended period of temperatures slightly above calculated threshold levels will have less effect than a few days of temperatures far above the threshold, despite the fact that calculations might assign them identical degree heating index values (Berkelmans 2002). As global atmospheric and sea surface temperatures continue to rise, the number of degree days of

stress to corals will also rise. More degree days of stress are expected to lead to more frequent and severe coral bleaching events.

Large scale bleaching events (widespread bleaching in multiple regions at the same time) have become frequent occurrences in the last thirty years. Beginning in the early 1980s, mass coral bleaching has been associated with the high sea surface temperatures associated with El Niño events in equatorial eastern Pacific waters (Glynn 1984, Glynn 1991, Glynn and D'Croz 1990, Glynn 1993). During the 1980's there were four coral bleaching events recorded that occurred on an unprecedented geographic scale and frequency. Each was associated with a period of elevated temperatures (Glynn 1991).

At the time of the original observations, the connection between CO_2 and global warming had not yet been completely accepted (Glynn 1991). At the time of these initial observations, there was not sufficient evidence to support a connection between rising sea surface temperatures and global warming (Atwood *et al.*1992). Now, these connections are widely accepted by the scientific community (Hoegh-Guldberg 1999, Watson *et al.*2001). Therefore, concern over increasing CO_2 levels and the related rise in air and sea temperatures translates to concern over increases in coral bleaching events.

While bleaching is the obvious outward sign of stress, the loss of zooxanthellae and their activities can have numerous far reaching consequences for coral. The loss of zooxanthellae leads to an immediate decrease in a host's nutritional intake. If a colony remains bleached, the decrease in sustenance causes a decrease in both growth and calcification (Muscatine 1990). This can decrease its ability to compete for substrate

with fast growing species like algae (Hoegh-Guldberg 1999). In addition, a lack of sustenance can decrease a colony's reproductive capacity (Szmant and Gassman 1990, Ward *et al.*1998). When adult corals have decreased fecundity, the recruitment of individuals of that species is also decreased (Hughes *et al.*1999). It follows that if thermal stress causes a decrease in adult fecundity, it is in turn likely causing decreases in recruitment to affected reefs. A decrease in recruits can lead to a decrease in later adult coral populations, which in turn affect the abilities of a reef to recover after a thermal-related bleaching event (Hoegh-Guldberg 1999).

Recent research has determined that temperature and light related bleaching is due primarily to damage to the dinoflagellates and their effect on their hosts (Warner *et al.* 1999, Warner *et al.* 2002, Suggett *et al.* 2008, Weis 2008). The ability of the host-dinoflagellate associations to adapt to local stresses is a factor that will determine the ability of a reef to continue to carry out its ecological functions (as a source of nutrients, shelter, etc.) in an area (Baker *et al.*2008). Free living *Symbiodinium* in the C, D, and G clades were all more susceptible to damage due to heat and light stress than those examined *in hospite* (Schönberg *et al.*2008). If these zooxanthellae are exposed to physical stress in the water column, they are unlikely to survive to repopulate bleached animal hosts.

The bleaching tolerance of a colony results from the combined resistance of the symbionts to thermal and light stress, and the host's ability to cope with the stress responses of the symbionts. Stressed symbionts have been observed to produce an abundance of reactive oxygen species (ROS) (Weis 2008). When ROS concentrations

increase above host specific thresholds, a cascade is triggered in the host cell. This cascade can result in apoptosis and death of the affected host cells. Hosts that have a higher ROS threshold before triggering the caspase cascade and symbionts that produce fewer ROS under thermal and light stresses are likely to become more abundant in the coming years (Tchernov *et al.* 2011). Under controlled conditions, *Acropora hyacinthus* and *Porites solida* were resistant to temperatures as high as 34˚C for 48 hours while their symbionts exhibited high levels of necrosis and apoptosis at temperatures of 30˚C (Strychar and Sammarco 2009). Therefore, the ability of corals to adapt to increasing temperatures may lie in the plasticity of gene expression in local host populations (Polato *et al.*2010) as well as the zooxanthellae populations.

The holobiont's survival depends upon, in part, the thermal tolerance of the dinoflagellates. The identity of the symbionts and the ability of the holobiont to shuffle them to acclimate to changing conditions are of paramount importance (Baker 2001, Jones and Berkelmans 2010). Numerous species of coral have experienced shuffling or switching of their symbiont communities in response to thermal stress (Baker 2001; 2003, Baker *et al.* 2004). While the ability of corals, to host multiple types of symbionts was not considered to be a universal feature of the coral-algal symbiosis (Stat *et al.* 2009), recent work by Silverstein *et al*. (2012) showed that of 39 coral species examined, at least one individual of each species had the ability to host multiple clades of zooxanthellae.

Bleaching events cause long term reductions in growth in all colonies, even those that appear unaffected during the peak period of stress. Colonies dominated by type D

symbionts show higher survival rates than those dominated by type C, but they still exhibit long term reductions in growth after a bleaching event (Jones and Berkelmans 2010). Some members of clade D (D1a) have recently been classified as *Symbiodinium trenchi* and are a rare, but host generalist symbiont. They increase in the weeks before and during bleaching events, but are displaced in the years following the stress event. Colonies hosting clade D dominated communities show increased survival during these events, but it is not yet known if these new communities indicate an increase or decrease in overall coral health (LaJeunesse *et al.*2009).

In addition to direct influences on the coral themselves, loss or decreased activity of symbionts can have a cascading affect across the entire reef community. Reef building corals contribute a substantial portion of the total productivity of a reef, and primary production by zooxanthellae is a large fraction of this productivity. Therefore, when corals bleach, the overall productivity of the reef can drop dramatically (Coles and Jokiel 1977).

The sensitivity of corals to small temperature changes becomes a pressing concern as global warming continues. Average sea surface temperatures are expected to continue to increase by at least 2˚C by 2100 (Hoegh-Guldberg *et al.*2007, IPCC 2007). In addition to these "business as usual" temperature increases, further pressure may be placed on coral systems during El Niño events, which often include particularly high temperature and light penetration (Glynn and D'Croz 1990). These events are predicted to increase in frequency, duration, and intensity as global climate change continues (Timmerman *et al.*1999). All projections of tropical sea surface temperatures indicate

that this rise will be rapid, with the highest rates in the Caribbean, Southeast Asia, and the Great Barrier Reef, and lowest rates in the Central Pacific (Hoegh-Guldberg1999).

With each acute stress event, like those that occur during El Niño events and disease outbreaks, coral fitness (i.e. survival, growth, gamete production, symbiosis) is compromised. After the event passes, affected corals require time to recover their former metabolic and growth rates. If an additional stressor (acute or chronic) impacts the area before the community has recovered, the resulting physiological damage can be greater than would be predicted if the two events occurred completely independently in time, since the capacity of the reefs to recover has been diminished (Done 1999).

As stress events increase in frequency, and the recovery time between them becomes more truncated, their combined effects would likely take an even greater toll on the health of reef communities. The impact of stresses that cause decreases in growth can be further exacerbated by increases in storm frequency and severity. If a colony is growing slowly, it will take longer to recover from any damage it suffers, and will be less capable of competing for space with other benthic organisms. If the corals do continue to grow, but exhibit decreased calcification and associated decreased skeletal density, they will be more prone to both bioerosion and breakage due to storms and wave action. This would result in decreased coral cover, reef structure, and habitat for organisms that depend on live coral and reef shelter and associated microhabitats to survive. It is predicted that by 2050, bleaching events will occur on an annual basis in many of the world's oceans due to thermal stress rather than the less frequent El Niño events (Hoegh-Guldberg 1999). More vulnerable areas, like the Caribbean and Southeast Asia are

predicted to begin experiencing annual bleaching events by 2020 (Hoegh-Guldberg 1999).

While the association of global climate change with higher than normal seasonal maximum temperatures is widely acknowledged, its connection to colder winter weather and higher levels of precipitation is often overlooked. Cold water events can be just as detrimental to coral health as warm water events (Jokiel and Coles 1977, Lirman *et al.* 2011). An extreme cold water event in the Gulf of California during the winter of 2008 caused extensive bleaching of *Pocillopora damicornis* colonies that hosted *Symbiodinium* in the C1b-c clades. However, as with warm water stresses, colonies that hosted D1 zooxanthella clades remained largely unaffected by the low thermal anomaly (LaJeunesse *et al.* 2011).

Thermal extremes can be examined as both chronic and acute stressors and will likely play a key role in future reef survival. This kind of stress was not the focus of the present study, but an understanding of its influence on coral communities and concerns about future impacts is necessary to place this research in the context of stress and climat change. In order to avoid the effect of thermal stress on experimental organisms, (during the interaction experiments), tank temperatures were kept at levels in the usual thermal range for the studied species, i.e. approximately 26-27˚C.

3) Light Intensity

Light plays a key role not only in photosynthetic activities of zooxanthellae, but also in calcification rates, and reproductive activities of their hosts (Falkowski *et al.* 1990). Efficient harvesting and use of solar energy by zooxanthellae is essential not only for the survival of corals but for the construction and maintenance of reefs (Rodriguez-Roman *et al.*2006). Light level then is a major factor controlling the presence or absence of reefs and the manner in which corals grow (Muscatine 1990). Within the reef ecosystem, light is the most important environmental gradient to which holobionts respond (Veron 2000).

In nutrient poor waters, zooxanthellae provide the bulk of the coral's dietary needs. At low light levels, hosts rely more heavily on heterotrophic feeding than upon the sustenance created by their symbionts. The host can then utilize the heterotrophically gained compounds for a number of growth processes. In low light conditions, heterotrophic feeding can result in increased rates of growth and lipid storage. In higher light conditions, the lipids and energy gained from the captured zooplankton is directed toward increasing calcification, chlorophyll content, and protein content (Treignier *et al.*2008).

Photosynthetically Active Radiation (PAR) and Ultraviolet Radiation (UVR) can affect the zooxanthellae and host in a number of ways. These include damaging DNA and DNA repair mechanisms if PAR is present at very high levels (Lesser and Farrell 2004). Studies have shown that symbiotic corals are among the most efficient collectors of light in nature as well as extraordinarily efficient in their use of the collected light

energy (Rodriguez-Roman et al 2006). Therefore, very high levels of radiation can overwhelm the light processing mechanisms of the organism, causing damage.

PAR is used in photosynthesis, but UVR may cause damage to the holobiont (reviewed in Schick *et al.*1996). There are considerable energetic costs associated with living in high light habitats. These include increased baseline metabolic rates and low oxygen quantum yield. In addition to these costs, high-light acclimated corals acquire less carbon per unit of tissue surface area. This is due to the depleted chlorophyll concentrations the organisms maintain at high irradiances to minimize light stress (Hoogenboom *et al.*2009). Therefore, reef crests and other areas frequently exposed to intense irradiance provide numerous challenges to the animals that inhabit them.

Studies of corals from a variety of habitats have revealed a wide spectrum of sensitivity and tolerance to UVR. Colonies of multiple species, taken from depth and transplanted to increased UVR conditions (natural or artificial) survived only when shaded (Vareschi & Fricke 1986; Scelfo 1986 reviewed in Schick *et al.*1996). At less intense levels of UVR, exposed colonies may exhibit decreases in growth and calcification (Jokiel and York 1982; Roth *et al.*1982). Not all studies have found UVR to significantly influence these processes (Glynn *et al.*1993 reviewed in Schick *et al.*1996). However, some have indicated that, like exposure to high temperature, bleaching can result from exposure to excess UVR (reviewed in Schick *et al.*1996). Decreases in metabolic activities caused by UVR or excess PAR can be attributed, at least in part, to damage to photosystem II mechanisms within the zooxanthellae (Hoegh-Guldberg and Jones 1999, Lesser and Farrell 2004).

The ability of the symbionts to utilize available light and tolerate high levels of irradiance plays a key role in the health of the holobiont. The differential thermal tolerances of particular clades of symbionts have been examined, and the thermotolerant properties of some clade D genotypes have been noted (Baker 2003, Baker *et al.*2004). Similarly, some *Symbiodinium* genotypes have demonstrated adaptations to particular light conditions. The breadth of the light niche that symbionts can exploit is sensitive to the size of their photosynthetic units, absorption cross-section, and rates of respiration (Hoogenboom *et al.*2009). Light availability influences the concentrations of chlorophyll, zooxanthellae, their photosynthetic properties (Rodolfo-Metalpa *et al.*2008) and the types of pigments present within a colony (D'Angelo *et al.*2008). Together these factors allow holobionts to exploit particular light environments.

The exploitation of various light environments can be achieved through the possession of particular clades of zooxanthellae by the host, or through the physiological plasticity of a single clade. Individuals of the species *Madracis pharensis* in Curaçao exemplify the fine scale specialization of sub-cladal groups of *Symbiodinium* across light environments. Colonies located in deeper reef areas with less irradiance were dominated by symbionts in the B15 type. Individuals of this clade were present in low densities and were larger in size, had high concentrations of pigment, and possessed higher peridinin : chlorophyll *a* ratios compared to the closely related B7 type. B7 individuals dominated in shallow, high light areas of the same region (Frade *et al.*2008). Even genetically identical symbiont communities can exhibit high degrees of plasticity in regards to light adaptation. At Heron Island, *Pocillopora damicornis* colonies have genetically uniform

zooxanthellar communities, yet show plasticity in their photo-physiological acclimation to various light microenvironments (Ulstrup *et al.*2006).

Irradiance levels on a reef can vary over two orders of magnitude with changes in water depth (due to light attenuation) and shading (Falkowski *et al.* 1990). These can also influence coral growth, by exerting control over both the colony morphology and the rate and manner in which the colony grows (Marubini *et al.*2001). Incident light levels influence both the branch spacing and lengths of *Acropora humilis* and *Stylophora pistillata*. Under conditions of decreased light availability, branch spacing of both species increased, allowing more light to reach the center of the colony. However, the species have contrasting approaches of branch length when exposed to decreasing light levels. *S. pistillata* in low light levels grow longer, thinner branches than in high light conditions while *A. humilis* grows shorter, stouter branches in low light levels (Kaniewska *et al.*2008).

Through its effects on photosynthesis, light can influence the calcification of corals (Marubini *et al.*2001; Langdon and Atkinson 2005). In some species, high light levels lead to increased calcification, particularly increasing the skeletal thickening processes (Juillet-Leclerc and Reynaud 2010). Influences on calcification rates will become even more important as sea level continues to rise, aragonite saturation state decreases (decreasing calcifying abilities of many corals), and erosion (both biotic and physical in nature) continues to remove carbonate from reefs.

The negative effects of increasing UVR on unshaded coral colonies and lower tolerance of UVR for colonies from deeper depths compared to those colonies from

shallow sites is well documented (Masuda *et al.*1993, Schick *et al.*1996, Kinzie 1993 reviewed in Schick *et al.*1996). Transplants of *Montipora verrucosa* from low light environments to areas where they were exposed to increased UVR experienced a loss of chlorophyll a. When individuals experienced higher PAR levels, but not increased UVR, there was no significant increase in bleaching compared to controls (Grottoli-Everett and Kuffner 1995).

Models of increasing CO_2 predict that increased SST will cause reduced upper level cloudiness (Schroeder and McGuirk 1998, Bates and Jackson 2001, Lau and Wu 2003, Lau *et al.*2005). This will increase the amount of radiation that will penetrate the atmosphere and reach marine communities. The increases in UVB radiation that are expected to occur are likely to have a number of sub-lethal effects on shallow coral communities even when these increases are not coupled with other stressors. Small increases in UVB will affect photosynthesis, respiration, calcification, growth and planula release in affected colonies. At the community level, selection for species with greater UVB tolerance could cause changes in both coral and symbiont community composition (Schick *et al.*1996).

Author	Year	Area	Light levels
Lang and Dustan (in Dustan 1979)	1979	Dancing Lady Reef 35-60m depth	250 uW /cm^2
Titlyanov	1991	Gulf of Siam	380 to 8 W/m^2, 230 to 8Wm-^{2}, 21-0.6Wm-^{2}
Iglesias-Prieto and Trench	1997	cultures of Symbiodinium	40, 80, 250 umol m-^{2} s^{-1}
Kayanne	1996	Shiraho Reef (Ishigaki Island, Japan)	March=552 umol m^{-2} s^{-2} ,August = 864 umol m^{-2} s^{-1}
Lesser	1997	Carrie Bow Key	2100-2500 lmol quanta m~2 s~1 PAR: 400-700 nm
Muller-Parker and Cortes	2001	Costa Rica	9m depth =125-334 umol photons m^{-2} s^{-1}
			9m depth = 228 umol of photons m^{-2} s^{-1}
Titlyanov et al.	2001	Okinawa	95-0.8% PAR
Marubini et al	2001	lab Porites compressa	81, 150, 698 micromoles m^{-2} s^{-1}
Titlyanov and Titlyanova	2002	Assorted areas of the Indo-Pacific	high illumination=90-70%PAR
			Shaded Habitats at medium depths=50-10% PAR
			Low light, deep depths, highly shaded grottoes = 5-0.5% PAR
Finelli et al	2006	Conch Reef	300-400 microeinsteins m^{-2} s^{-1}
Ulstrup et al	2006	Heron Island, flow through aquaria	shaded <50 micromoles photons m^{-2} s^{-1}1
Mass et al.	2007	Red Sea Eilat, reef profile 5-65m depth	light at 50 meters =13% of light at 5m
			light at 65 meters = 9% of light at 5m
			10m PAR 400-750 micromoles m^{-2} s^{-1}
			30. PAR 250-400 micromoles m^{-2} s^{-1}
			60 PAR 50-100 micromoles m^{-2} s^{-1}
Reviewed in Kaniewska et al	2008		Reef crests can see >2000 micromoles m^{-2} s^{-1}
			cave, deep, or turbid water areas =< 50 umol m^{-2} s^{-1}
Nakamura and Yamasaki	2008	Okinawa collected, flumes	80 micromoles m^{-2} s^{-1}
			500 micromoles m^{-2} s^{-1}
Kaniewska et al.	2008	Acropora humilis and Stylophora pistilata	25-500 micromoles m^{-2} s^{-1}
Rodolfo-Metalpa, Huot, and Ferrier-pages	2008	Ligurian Sea	Winter 50 micromoles m^{-2} s^{-1}
		Cladocora caespitosa	Summer 250 micromoles m^{-2} s^{-1}
Treigner et al.	2008	Red Sea collected, flume	100 micromoles m^{-2} s^{-1}
			300 micromoles m^{-2} s^{-1}
Schonberg et al	2008	Okinawa collected, flumes	0 micromoles m^{-2} s^{-1}
			40-45 micromoles m^{-2} s^{-1}
			360-420 micromoles m^{-2} s^{-1}
Edmunds	2009	tanks in sunlight	291-560 micromoles photons m^{-2} s^{-1}
Mass et al.	2010	Red Sea, Eilat 2-15 m depth	350 micromoles m^{-2} s^{-1}
Schutter et al	2011	cultured Galaxea fascicularis	600 micromoles m^{-2} s^{-1}= high irradiance
			300 micromoles m^{-2} s^{-1}= low irradiance

Table 1.2: Summary of natural light conditions observed on natural reefs and examined in previous scientific research

Bleaching can have a variety of effects. In some cases, only the bleached colony is affected, experiencing reductions in skeletal growth (Goreau & Macfarlane 1990; Leder *et al.*1991) and calcification (Abramovitch-Gottlib *et al.*2002). This bleaching may lead to eventual colony death if severe stress continues (Wilkinson 1998). In other cases, the colony may regain its pigmentation as it either acclimatizes to the new condition or the stressor is removed (Edmunds *et al.*2003). If however bleaching persists during a period of expected gametogenesis, reproduction and recruitment may be delayed for the affected reef (Szmant & Gassman 1990). Reefs that these larvae could recruit to would also be negatively impacted (reviewed in Glynn 1996).

Incidents of bleaching have been attributed to a number of ecological causes, including anomalously high or low temperatures, solar radiation, subaerial exposure, sedimentation, fresh water dilution, increases in nutrients, exposure to xenobiotics, and epizootics (reviewed in Glynn 1996). No matter what the original stressor, bleaching occurs when the host/zooxanthellae symbiosis breaks down. This has been attributed to damage to the zooxanthellae's photosynthetic process and the consequences of that damage (Weis 2008). High irradiance leads to the over-reduction of the zooxanthellae's light reaction centers. Irradiance levels play a major role in modulating the severity of the disruption to photosystem II (Hoegh-Guldberg 1999). Higher light levels increase the formation of free radicals and greater damage to the holobiont (Lesser1996, Richier *et al.*2008). It is currently unknown whether light will influence colonies stressed by other means such as changing aragonite saturation levels in similar ways.

Light attenuation, due to absorption by dissolved materials in the water column (Falkowski *et al.* 1990), may be affected by the changing conditions associated with ocean acidification. As CO_2 levels and ocean acidification increase, oceanic waters will show decreases in the ubiquitous calcium carbonate particles, such as microscopic coccoliths that are suspended throughout ocean waters. As these particles decrease in concentration, there will be a related decrease in light scattering and attenuation. This will result in deeper euphotic zones, which may have consequences for biogeochemical processes such as export production. These predictions focused on high latitude regions (Balch and Utgoff 2009), but similar processes in tropical waters may cause increases in the amount of light (PAR and UVR) reaching coral reefs in the future.

4) Aragonite Saturation State

Carbon dioxide gas in Earth's atmosphere has significant influences on many crucial regulating systems in the environment. It is a vital ingredient for photosynthesis and a product of respiration. On a large scale, CO_2 both regulates the heat balance of the planet and influences the calcium carbonate levels in the oceans (Kleypas *et al.*2006). Increases in global CO_2 and other greenhouse gases contribute to both the global warming component of climate change and ocean acidification.

Oceanic waters absorb anthropogenic CO_2 at differential rates depending on temperature and "age". Older, colder water that comes into contact with the atmosphere will take up more carbon-dioxide from the atmosphere than warm sub-tropical waters, which rapidly become saturated at atmospheric CO_2 levels (Sarmiento *et al.*1992, Orr *et al.*2001).

Experiments by Anthony and colleagues (2008) have demonstrated that ocean acidification is likely to increase the sensitivity of reef-building corals and crustose coralline algae to elevated temperatures at high irradiances. They speculated that high CO_2 levels (decreased pH) will impact the photoprotective mechanisms of the photosystems of the coral symbionts which will induce bleaching in corals. These impacts can occur by lowering rates of photorespiration and lowering their capacity for thermal dissipation. This will likely narrow the range of temperatures in which they will be able to survive in the future (Hoegh-Guldberg 2009).

Prior to the beginning of the industrial revolution in the mid 19th century the release and absorption of CO_2 by the ocean had been in near equilibrium for thousands of years (Watson and Orr 2003). The atmospheric CO_2 levels had remained between approximately 180 and 300 parts per million by volume (ppmv) for the previous 650,000 years (Petit *et al.*1999, Siegenthaler *et al* 2005, reviewed in Kleypas *et al.*2006). The rate of carbon dioxide input to the atmosphere has greatly accelerated in recent decades (Siegenthaler and Sarmiento 1993). Compared to the relatively stable level that the Earth experienced for several thousand years before the industrial era (280 +/- 10 ppm), current average rates of increase (+0.4% per year since 1980) are substantial and are attributed to anthropogenic CO_2 emissions (IPCC 2001). Today, atmospheric CO_2 levels are approximately 380 ppmv (reviewed in Kleypas *et al.*2006).

Increases in CO_2 and other greenhouse gases are predicted to cause rates of warming that are higher than those seen in the 20th century, and are without precedent during the last 10,000 years (IPCC 2007). The climate changes that have already been

observed have been attributed to anthropogenic causes (including increased CO_2 emissions and changing land-use) based on several lines of evidence. Increases in atmospheric CO_2 are expected to continue in the future as human activities persist in producing carbon dioxide in excess of the Earth's processing capabilities (IPCC 2005).

Anthropogenic activities continue the input of CO_2 into the atmosphere (IPCC 2007). As the atmospheric gases enter into a state of equilibrium with dissolved gases, there is a forced increase in the amount of aqueous CO_2 in the ocean. While the ocean is coupled to the atmosphere primarily through fluxes of heat and freshwater (mostly relating to sea surface temperatures), they are also connected through the fluxes of greenhouse gases like CO_2 (IPCC 2007).

The quantity of dissolved CO_2 and its dominant form in the oceans is controlled by a suite of physical factors including temperature, atmospheric concentration of gases, pressure, and the presence of other dissolved substances in the system (Morse and Mackenzie 1990, Guinotte *et al.*2003). Ocean and terrestrial systems have the capacity to take up 70-80% of the projected anthropogenic CO_2 that is released to the atmosphere, but the process would take centuries due to the relatively slow rates of ocean mixing. Therefore, approximately one fourth of anthropogenic emissions will remain in the atmosphere several centuries after they occur (IPCC 2007). If CO_2 inputs continue at current rates, the acidification process will severely impact tropical seas and the corals within them by approximately 2030-2050 (Cao *et al.*2007, Meehl *et al.*2007) and oceanic pH will decrease by 0.4 ($\pm$ 0.1) pH units relative to pre-industrial conditions by the end of the current century (Meehl *et al.*2007). These pH decreases are equivalent to an

approximate 150% increase in dissolved H^+ ions and a 50% decrease in CO_3^{2-} concentrations (Orr *et al.* 2005).

The ability to predict the effects of CO_2 emissions on climate change and other environmental conditions are limited due to the unpredictable nature of human activities. These include population change, economic change, technological developments, and other human behaviors (IPCC 2007). Therefore, numerous scenarios, covering a spectrum of human activity levels have been utilized to predict a range of possible conditions for the future. While both the "best" and "worst" case scenarios tested are each unlikely, they "bound" the possible outcomes that can result from actual human activities (IPCC 2007).

Biologically produced carbonates represent the biosphere's largest carbon reservoir. The calcification activities of calcareous organisms can influence the ocean and atmosphere through their effects on oceanic pH and carbon dioxide content (Cohen and McConnaughey 2003). Approximately 11% of global $CaCO_3$ production occurs in coral reef systems (reviewed in Gattuso *et al.*1998). The removal of any carbonate or bicarbonate from seawater induces an increase in the dissolved CO_2 level. This can cause carbon-dioxide to be driven from the ocean to the atmosphere (Frankignoulle and Gattuso 1993, Gattuso *et al.* 1993) The expansion of reefs and the associated increase in calcification during the last deglaciation have been credited with approximately 25% of the increase in atmospheric CO_2 that brought an end to the last ice age. Currently, byproducts of coral reef calcification contribute only 0.4-1.4% of the carbon dioxide being released into the atmosphere each year (Houghton *et al.*1996 reviewed in Gattuso

*et al.*1998). This decrease in relative contribution (11% →1.4%) is due almost entirely to the high amounts of CO_2 being released by anthropogenic activities rather than a decrease in production by reef systems.

The question that needs to be addressed is no longer, "How do coral reefs affect climate?" but, "How will anthropogenic effects on climate influence coral reefs?" The calcification response of some organisms to these changes has been examined, but researchers have barely addressed the question of how these reduced calcification rates will influence coral reef ecosystems (ISRS 2007).

The ocean holds approximately 50 times more CO_2 than the atmosphere, due in part to its high solubility in seawater, its ability to form H_2CO_3 and dissociate into ions, and interact with other constituents in seawater (IPCC 2007). Its solubility is dependent upon temperature, with a net cooling leading to an increase in CO_2 uptake and warming causing a net decrease in CO_2 uptake (IPCC 2007). Therefore, increasing temperatures associated with global climate change could have additional indirect influences on dissolved CO_2 levels in the future. The extent of the influence of CO_2 on marine systems, including coral reefs, may be influenced by changing temperatures over time. In addition the ability of the ocean to take up anthropogenic CO_2 is decreasing as its levels increase. This decreases the ocean's ability to buffer the effects on the atmosphere and other changes in natural carbon cycling (IPCC 2007). This decrease in uptake ability could cause acceleration of CO_2 related effects (like rising temperatures) in the future.

Although CO_2 in gaseous form in the atmosphere is primarily chemically stable, the same can not be said for dissolved CO_2. When atmospheric CO_2 is absorbed by seawater it enters into the following series of chemical reactions:

$$CO_{2\,(g)} \Leftrightarrow CO_{2\,(aq)} \quad \text{(eq. 1)}$$

$$CO_{2\,(aq)} + H_2O \Leftrightarrow H_2CO_3 \quad \text{(eq. 2)}$$

$$H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \quad \text{(eq. 3)}$$

$$HCO_3^- \Leftrightarrow H^+ + CO_3^{2-} \quad \text{(eq. 4)}$$

calcification----------

$$Ca^{2+} + CO_3^{2-} \Leftrightarrow CaCO_3 \quad \text{(eq. 5)}$$

$$CO_2 + H_2O + CaCO_3 \Leftrightarrow Ca^{2+} + 2HCO_3^- \quad \text{(eq. 6)}$$

(Kleypas and Langdon 2006)

The end result of these reactions is an increase in the number of dissolved free hydrogen ions (eqs. 3 and 4) which cause the previously noted decrease in oceanic pH (Caldeira and Wickett 2003).

Models of changes in oceanic pH, coupled with observed increases and projected increases of oceanic CO_2 show decreases in pH worldwide as CO_2 increases, with more intense effects projected in shallow areas compared to deeper sites (Caldeira and Wickett 2003). Increases in dissolved CO_2, and the accompanying decreases in pH lead to an increase in the dissolution of $CaCO_3$ (eq. 6) (Hallock 1997, Kleypas *et al.*1999) a building block of coral reefs and the skeletal elements of other reef organisms. The future of coral

reefs as functional ecosystems is in doubt if oceans continue to warm and acidify at current rates, which are unprecedented in the past 420,000 years (Hoegh-Guldberg *et al.*2007).

CO_2 is well mixed in the atmosphere and aragonite saturation state is highly correlated with sea surface temperature (Guinotte *et al.*2003). Decreasing pH reflects decreases in the saturation states of biological carbonates, including aragonite, calcite, and high magnesium calcite in the world's oceans (Done and Jones 2006).

The availability of carbonate (CO_3^{2-}) ions and sufficient saturation states of carbonate minerals like aragonite are necessary for the precipitation of shells and the carbonate skeletons of corals (Kleypas *et al.*2006). Shallow tropical seawater is supersaturated with respect to aragonite, ($\Omega_{aragonite} > 4$), but saturation levels have fallen over the past century (from ~4.6 to ~4). They will continue to fall as atmospheric CO_2 continues to rise (Kleypas and Langdon 2006). It is expected that by 2060-2100, atmospheric CO_2 will be double preindustrial levels (Houghton *et al.*2001) which would lead to a corresponding decrease in aragonite saturation state to approximately 3.1 (Kleypas *et al.*1999).

Prior to the last decade, it was generally believed that since seawater is supersaturated with respect to aragonite, the carbonate chemistry of seawater was not an important factor influencing the precipitation of calcium-carbonate by corals (Gattuso *et al.*1998). However, multiple studies have shown that decreased aragonite saturation yields decreases in calcification in numerous coral species, including *Stylophora pistillata, Porites compressa,* and *Galaxea fascicularis* (Gattuso *et al.*1998, Langdon *et*

*al.*2000, Broeker et al 2001, Marubini et al 2001, Leclerq *et al.*2002, Marshall and Clode 2002 – reviewed in Langdon and Atkinson 2005, Marubini et al 2002, Reynaud et al 2003, Ohde and Hossain 2004, and, Langdon and Atkinson 2005,). Most of these studies indicate that coral calcification will decrease by 30 (±18%) when CO_2 concentrations double, as they are expected to during the next century (Gattuso *et al.*1998, Langdon and Atkinson 2005, ISRS 2007). Therefore, the saturation threshold of $\Omega = 1$ is an approximation of a minimum threshold for biomineralization, but can not be considered a strict criterion for it. Many species require a much higher saturation state in order to carry out calcification. Others may be able to continue to generate or maintain calcified structures when saturation states (Ω) are <1 despite the bioenergetic costs of maintaining them (Feely *et al.*2009).

The observed calcification rates of 12 coral species under conditions of decreasing aragonite saturation state were compiled by Langdon and Atkinson (2005). Together they illustrate the general trend of decreasing calcification rate (as a percentage of the pre-industrial calcification rate of each species) that occurs as aragonite saturation state decreases. The compiled data indicate a 60% decline (range 40-83%) in calcification by the year 2065 when aragonite saturation state will be barely greater than 3 (Langdon and Atkinson 2005). Other species show a predicted decline in calcification which is less severe, only 1-18% of pre-industrial rates (Gattuso *et al.*1998, Leclerq *et al.*2000, Marubini *et al.*2001, and Reynaud *et al.*2003 reviewed in Langdon and Atkinson 2005). The degree of decrease may vary among species, but a general decline in calcification ability with decreasing saturation state is apparent.

The reduced calcification rates of corals and other calcifying organisms can have numerous impacts on these species and the ecosystems they inhabit. The calcifying organisms may exhibit reductions in the age of sexual maturity, changes in buoyancy (Tyrell *et al.*1999), and reductions in their ability to compete with non-calcifying organisms (Kuffner *et al.*2008). In addition, changes in the water itself and calcifying organisms that remain in the water column may have noteworthy impacts on light attenuation (Tyrell *et al.* 1999) and therefore on photoresponsive organisms. Each species will express individual responses to changing water chemistry. These small variations in response could become amplified over successive generations which could drive major reorganizations of both planktonic and benthic ecosystems in the future (Doney *et al.* 2009).

Some researchers have predicted that when atmospheric pCO_2 levels reach 560 ppm, and aragonite saturation falls even farther, coral reefs will cease to grow and begin to dissolve (Silverman *et al.*2009). No matter the magnitude of the species' relationship with aragonite saturation, it is apparent from these data that calcification rates for coral will decrease as aragonite saturation continues to decrease with increasing levels of CO_2. Many corals have the ability to control their internal pH, using H^+ pumps to produce and maintain internal fluids with higher aragonite saturation states than the surrounding waters (Al-Horani *et al.* 2003, Allemand *et al.* 2004). The variation in the extent of the calcification response between different types of calcifiers or particular species stem from differences in mechanisms that the organisms utilize in order to control their internal micro-environments where the calcification process takes place (Orr et al 2009).

In order to produce skeletons, corals secrete calcium carbonate at the base of the calicoblastic epithelium into the space between the polyp and its existing skeleton (Braun and Erez 2004). This secretion is possible because the corals maintain a high saturation state for aragonite at the site of calcification. The calcification process likely differs between species, life stages within species, and for some species, between stages of calcification (Kleypas and Yates 2009). At low pH, maintaining this higher saturation state is energetically costly. The corals do not readily divert energy from other functions in order to perform or increase calcification under these conditions (Cohen and Holcolmb 2009). Corals will continue to expend energy to increase the saturation state of water at the site of calcification, but if the saturation state is low enough in the surrounding waters, the saturation state of the internal calcifying fluid will reach critically low levels (Ω_{ar} ~2). If internal saturation states fell below this level, the skeleton would cease to grow properly. At these levels, calcification can not proceed in order to produce skeletons with normal crystal growth patterns (Cohen and Holcomb 2009).

The addition of anthropogenic CO_2 since the onset of the industrial revolution has caused the aragonite saturation horizon (where Ω =1) to shoal closer to the surface of all ocean basins. This horizon can range from 50-200m depending on the location (Feely et al 2004, Orr *et al.*2005). This horizon is a demarcation of the depth below which biomineralization can not occur spontaneously and the production and maintenance of biomineral structures is energetically costly. Many organisms have optimal calcium carbonate precipitation rates at the supersaturated states that were typical in the ocean before the industrial era. It is expected that decreases in carbonate saturation states will

yield a decrease in the calcification rates of a number of species (Feeley et al 2009). As the aragonite saturation horizon becomes shallower, some organisms will find themselves in waters with aragonite saturation states that are no longer favorable for their biomineralization and their persistence in those areas will be in jeopardy.

Decreased aragonite saturation states will gradually lead to decreased average carbonate accumulation, slower extension rates, weaker skeletons, and possibly a reduction in reef cementation and stabilization (Guinotte *et al.*2003). Studies of coral skeletons have shown steady decreases in the density of annual bands from the 19th to late 20th century (Wang *et al.*2010) especially within the last 20 years (Cooper *et al.*2008, Lough 2008, De'ath et al 2009). Areas that exhibit low aragonite saturation states have less abundant inorganic sediment deposits and higher rates of erosion than areas with high aragonite saturation states (Manzello *et al.*2008). It is likely that the organic growth and extension of reefs will decline as ocean acidification continues and that inorganic cementation and the related strengthening of reefs will also decrease in future. This will likely render reefs more susceptible to the effects of bioerosion.

Although it has been hypothesized that calcification will cease when oceanic waters are undersaturated with aragonite, one study has found that calcification will indeed continue under these conditions. However, the same study discovered that although calcification continues, it is not unaffected by the change in water chemistry. When aragonite saturation state is high, the bundles of aragonite needles that form are densely packed, yet when aragonite saturation state begins to fall, instead of bundles, the minerals form into disordered aggregates of highly faceted rhombs (Cohen *et al.*2009).

So although calcification may continue, the reef structure itself may become compromised and prone to erosion processes.

Changes in water chemistry affect all calcifying organisms including crustose coralline algae (CCA) (Jokiel *et al* 2008, Kuffner *et al.*2008). Changing water chemistry has been shown to negatively impact the ability of some crustose coralline algae species to colonize substrate and the subsequent settlement of coral spat (Albright 2011). Declines in pH can therefore affect corals both directly and indirectly since calcified red algae often serve as a settlement cue for numerous coral species (Price 2010).

As with many environmental factors, changing aragonite saturation state and associated water chemistry conditions interact with other factors to create a growth response in effected organisms. For instance, increasing pCO_2 can lead to nutrient limitation in the temperate coral *Astrangia poculata* (Holcomb *et al.*2010). *A. poculata* exposed to increased pCO_2 at ambient nutrient levels show decreased calcification compared with those exposed to ambient levels of both nutrients and CO_2. The nutrient limited corals were unable to utilize the higher levels of DIC present in order to calcify. When this species was exposed to increased levels of both pCO_2 and nutrients, the calcification rates did not differ from rates at current ambient levels. The increased nutrients allowed the corals to utilize more of the DIC for calcification. These results indicate that increasing CO_2 will cause these corals to become nutrient limited, possibly due to the effects of CO_2 on the calcification process and zooxanthellae (Holcomb *et al.*2010).

The effects of CO_2 variation appear to vary according to both species and general climate region. While some zooxanthellate temperate species show nutrient limited decreases in calcification with increasing pCO_2 (Holcomb *et al.*2010), some tropical zooxanthellate species do not show effects of changing pCO_2 and temperature on calcification, photosynthesis, or photosynthetic efficiency. However, the lack of sensitivity of corals like *Cladocera caespitosa* to these factors may be due to their slow growth rates which are likely more dependent upon temperature than the aragonite saturation states over the ranges predicted for the coming century (Rodolfo-Metalpa *et al.*2010a).

Determining the response of corals to changing water chemistry requires an understanding of the host's response as well as the effects on their symbionts. Although they live within gastrodermal cells, and are not directly bathed in sea water, zooxanthellae are influenced by changing water chemistry. It can cause changes in the photorespiration and productivity of zooxanthellae. Photorespiration can function as a photoprotective mechanism, by providing an alternate pathway for excess excitation energy. Excess energy would otherwise lead to the production of reactive oxygen species, photooxidative damage of proteins, lipids, and pigments and possibly to bleaching (Niyogi 1999, Ort and Baker 2002, Smith et al 2005, Takahashi & Murata 2008).

Symbiodinium within corals are limited by dissolved inorganic carbon which is limited by the host. Photosynthesis itself is dependent on bicarbonate uptake by the host, not the diffusion gradient of carbon dioxide through the host tissues (Weis *et al.*1989, Goiran *et al.*1996, Marubini & Thake 1999, Leggat *et al.*2000, Marubini et al 2008).

Changes in water chemistry and the gases that are available for use by zooxanthellae can affect their function in many ways. Chlorophyll content of zooxanthellae increases under increased CO_2 conditions (Crawley *et al.*2010). The presence of photosynthesizing zooxanthellae within coral tissues can raise the pH of the host cytosol up to levels of 7.4 (Venn *et al.* 2009).

However, decreased water pH can cause decreased maximum net rates of photosynthesis (P_{nmax}) and decreasing net oxygen production per unit of chlorophyll (Goiran *et al.*1996, Crawley *et al.*2010). Crawley and colleagues (2010) found that under the CO_2 levels predicted for the year 2100 under "business as usual" the abilities of zooxanthellae to protect themselves from bleaching using non-photochemical quenching pathways are decreased. These effects of these changes on enzyme synthesis may be responsible for the changes in chlorophyll content and activity.

In addition to environmental factors, the life stage of a coral influenced by CO_2 increases. Larval survival rates of *Acropora* were not affected significantly by changing pH, however polyp growth and algal infection rates were significantly decreased at reduced pH levels compared with control conditions. Under future acidified conditions, members of this genus may see reduced primary polyp growth and delayed establishment of symbiosis (Suwa *et al.*2010). Other species exhibit decreases in fertilization success under predicted future water chemistry conditions (Albright 2011) which will have significant impacts on the ability of reefs to reseed after damage, or simply continue their existence as conditions continue to decline.

It has been predicted by some researchers that when atmospheric CO_2 levels reach 560 ppm, not only will some reefs cease to grow, they will in fact begin to dissolve (Veron 2008, Silverman *et al.* 2009). Fossil records support the hypothesis that past incidences of wide spread coral reef destruction coincided with major perturbations to earth's CO_2 cycle. These extinction events were then followed by 'reef gaps' that could last for millions of years (Pelejero *et al.*2010). There is paleontological support for the hypothesis that corals continued to exist as "naked corals" for 14 million years following the Permian extinction before water chemistry again became favorable for skeleton formation (Stanley and Fautin 2001)

As non-calcifying reef cnidarians, the responses of anemones to changing water chemistry can provide insight into possible responses that corals may exhibit in the future. Towanda (2008) exposed anemones of the species *Anthopleura elegantissima* and their symbiotic zooxanthellae (*Symbiodinium muscatinei*) to moderately and highly increased levels of pCO_2 (pCO_2 = 450ppmv, pH = 8.1 and pCO_2 = 2340 ppmv, pH = 7.3 respectively) and measured the contribution of zooxanthellae to the animal's respiration (CZAR). *A. elegantissima* exhibited higher rates of photosynthesis and respiration under conditions of moderately increased pCO_2 compared to rates under current pCO_2 levels. In addition, under higher pCO_2 conditions, the anemones were receiving more of their respiratory carbon and oxygen from the zooxanthellae than they did under current conditions. The response of *Anthopleura elegantissima* to hypercapnic acidification reveals the adaptability of an organism that has evolved a tolerance for high pCO_2

(Towanda 2008). This provides some hope that other organisms may also possess the ability to adapt to changing conditions over time.

Although aragonite dependent species were predicted to be the first impacted by changing water chemistry, calcite dependent organisms are expected to be affected as well. Bryozoans create their skeletal structures by depositing the mineral calcite. *Myriapora truncata* bryozoans that were subjected to short exposures to extremely high CO_2 conditions (mean pH 7.43) calcified significantly less than those at normal pH (mean pH 7.66, high pH 8.1). Established colonies of *M. truncata* seemed well able to withstand the levels of ocean acidification predicted in the next 200 years. This may be due to the soft tissues protecting the skeleton from external decreases in pH (Rodolfo-Metalpa *et al.* 2010). However all of the bryozoans died when exposed to high temperatures and high CO_2 levels. Although *M. truncata* was resilient to short-term exposure to high levels of ocean acidification at normal temperatures, it showed that its ability to calcify at higher temperatures was compromised. Therefore, this species must be added to the growing list of species now potentially threatened by global warming (Rodolfo-Metalpa *et al.* 2010).

A study of the effects of acidification on benthic ecosystems at shallow coastal sites near Ischia Island, Italy, where volcanic CO_2 vents lower the pH of the water column was carried out in 2008. Along gradients of normal pH (8.1–8.2) to lowered pH (mean 7.8–7.9, minimum 7.4–7.5) the typical rocky shore communities with abundant calcareous organisms shifted to communities lacking scleractinian corals with significant reductions in sea urchin and coralline algal abundance. The species populating the vent

sites comprise a suite of organisms that are resilient to naturally high concentrations of pCO_2 and indicate that ocean acidification may benefit highly invasive non-native algal species (Hall-Spencer *et al.*2008). Recent observations of benthic communities near similar CO_2 seeps in Papua New Guinea (Fabricius *et al.* 2011) showed reductions in coral diversity, recruitment, and the abundance of framework builders with decreasing pH. When pH conditions were decreased from 8.1 to 7.8, coral cover remained constant, but there was a marked decrease in framework building corals while massive *Porites* colonies became more abundant. At pH levels below7.7 reef development ceased completely. These results provide a glimpse of how benthic communities may change as ocean acidification continues.

Benthic community shifts may result from changing water chemistry, but species themselves may show major adaptations to these changes. During the Cretaceous period, an increase in CO_2 concentrations similar to modern increases occurred. It has been proposed that some cretaceous scleractinian species lost and regained their calcium carbonate skeletons in response to these environmental changes (Fautin and Lowenstein 1992, Stanley and Fautin 2001). It is currently unknown how many modern species possess the ability to undergo similar adaptations and survive the expected changes in water chemistry (Medina *et al.*2006).

It is important with the increased stresses on coral reefs worldwide that scientists determine what conditions can help minimize the predicted detrimental effects of these factors on the corals. It is probable that many sub-tropical reefs are already living in sub-optimal conditions and that most ocean surface waters will become perilously

undersaturated with respect to aragonite as CO_2 levels continue to rise (Veron et al 2009). By the end of this century, it is possible that only a few parts of the Pacific Ocean will maintain levels of aragonite saturation that will be adequate for coral calcification (Guinotte *et al.*2003).

Even with overall decreases in pH and aragonite saturation, certain areas may have the capacity to serve as refugia for corals and other organisms that depend on them for survival. The ability of these refugia (should they remain) to successfully continue to support coral communities will likely be dependent on the interactions of a set of specific physical conditions based on flow rate, light levels, temperature range, and nutrient influences (West and Salm 2003, Riegl and Piller 2003).

The goals of this research were:

1) To determine which light conditions, flow conditions, or combinations thereof allow for the greatest growth of each studied species under current water chemistry.

2) To determine which light conditions, flow conditions, or combinations thereof allow for the greatest growth of each studied species under projected decreased pH conditions .

3) To determine how projected decreases in pH will affect the growth of the study species under a variety of light and flow combinations.

4) To derermine if there is a combination of light and flow conditions underwhich changing pH will not cause significant changes in growth to the study species.

5) To determine if species or morphological types respond differently to decreased pH.

6) To determine if calcification activities impact the way that a species will respond to changes in water chemistry.

7) To determine if the possession of zooxanthellae will influence the response of cnidarians to decreases in pH.

The information gained from this research can be utilized in current aquaculture efforts as well as future conservation efforts including MPA design.

Chapter 2: Isolating Water Flow as a Factor

Please note, portions of this chapter have previously been published:

Margolin CL (2008) Use of the Coral-sel Technique in the Study of Small Scale Water Flow Environments on Coral Growth. Proceedings of the 11th International Coral Reef Symposium 1: 486-490

A) Introductory Remarks

In a natural environment, it would be impossible to fully control the variability of all factors other than the intended target variable. In order to minimize excess sources of variability, experimentalists rely largely on controlled procedures. Laboratory studies that examine the influence of water flow upon marine organisms like coral are most often created in fully independent experimental systems (Helmuth *et al*. 1997, Sebens *et al.*1997, Bruno and Edmunds 1998, Fujimura *et al.* 2008, Edmunds and Lenihan 2010, Purser 2010, Schutter *et al.* 2010). These independent systems were successful in creating a variety of water flow conditions. Even in a laboratory environment, however, flow speed can affect a variety of parameters beyond the amount of water moving through an area and over an object. Water moving at different speeds may also experience differing rates of heating and cooling. In addition, the availability of gases, dissolved nutrients, and food particles/zooplankton to nearby organisms can also be affected. These unintended differences between treatments prevent flow from being a "fully" independent variable in studies that keep water isolated. In order to avoid these

undesirable differences between treatments, it was necessary to design an experimental method which allowed all experimental organisms to exist in a shared water system, yet still experience different water flow speeds.

This study examined the effects of flow rate on the "colony level" metrics of size and mass as well as morphological variation on the "corallite level". Three main scales exist for morphological variations in adult corals: colony scale, corallite scale, and sub-corallite (micromorphological) scale (Chen *et al.* 2011). The colony scale plasticity that numerous coral species have is easily observed in the variations in branching patterns, lateral vs. vertical extension, pigment, and skeleton robusticity. Corallite level variation is characterized by features including septa number and arrangement. Sub-corallite differences include size and shape of septal teeth and granules (Veron 2000, Budd and Stolarski 2009).

Within a species, corallite level characteristics, such as the overall shape of and relationships between corallites, are relatively constant, and can be used as identifying taxonomic characteristics. While these characteristics are consistent for all members of a species, sub-corallite morphological changes can develop based on a combination of genetic variation and phenotypic plasticity that responds to environmental conditions (Knowlton and Budd 2001, Budd and Pandolfi 2004, Fukami *et al*. 2004).

Morphological variations can include the relative dimensions of segments of the corallite septa. The three dimensional morphologies of the calical surfaces of corallites can show significant variations when they develop under different physical conditions.

These variations can serve as useful tools in determining adaptive significance to environmental conditions. (Klaus *et al.* 2007).

Previous work (Klaus *et al.* 2007) has examined the effects of light intensity, pollution, zooxanthella diversity and photosynthetic activity on the morphology of corallites of *Montastraea faveolata* found between 5m and 20m depths in Curaçao. Approximately 41% of the morphologic variation that was detected could be attributed to the effects of the quantified environmental and physiologic variables. The rest of the observed variance remained unexplained by the factors studied. Water motion was not quantified in the original Curaçao study of *M. faveolata*. The remaining variation is produced by a combination of randomness, genetic influence, and environmental influences not measured in that study. In the Curaçao study, differences were noted in the size of corallites and costae development between sites. These differences were assumed to be related to site differences in water currents and sedimentation rates. Water movement was not investigated directly in that study, but the coral-sel experiment sought to fill in that gap.

B) Experimental Apparatus

This goal was accomplished through the construction of the "coral-sel" (Figure 2.1). It consisted of two major components. The first was an underwater rotating platform that allowed the attachment of experimental colonies at set distances from the

center of rotation (Figures 2.2, 2.3, and 2.4). The second was a driving motor and gearing system that turned the submerged component at a steady rate of 3 rpm (Figure 2.5).

Figure 2.1: The motor and drive component of the coral-sel connected to the in-tank rotating component.

Figure 2.2: The in-tank component of the coral-sel, composed of the central column, mounting arms, support frame, guiding tubes, and attached bike wheel frame.

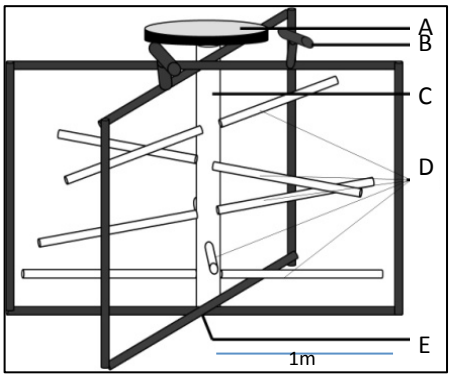

Figure 2.3: Coral-sel in-tank component schematic. A- Standard bicycle wheel frame, no tire. B- Guidance tubes. C- Central Column, 4" PVC, D- Mounting arms, E- Support Structure.

Figure 2.4: The in-tank component of the coral-sel. The flow speed experienced by each attached colony was dependent upon the colony's distance from the center of rotation.

In the coral-sel method, corals were moved through the water at particular speeds rather than moving water over the corals to create different flow treatments. The experimental speeds were determined by the distance of colonies from the coral-sel's center of rotation and the number of rotations the submerged component completed per minute. This allowed the experimental corals to experience one of the three assigned flow rates, while removing all other sources of variation associated with separate experimental systems.

The driving system consisted of a ½ horsepower electric motor secured to a PVC frame with its spindle pressed to the outside edge of a fully inflated bicycle tire whose axle was inserted into the PVC frame (Figure 2.5). The tire was positioned horizontally and spun freely. This wheel had a full set of standard bicycle gears connected to it, which spun simultaneously (Figure 2.6). The driving system was situated on a platform next to the experimental tank, with the driving wheel level with the top of the "in-tank" component. Two and a half standard bicycle chains were connected to form one large single loop which passed around the third gear of the driving system and around a second bicycle wheel frame (with no tire) secured to the top of the "in tank" rotating platform. A

platform topped by a pair of angled ceramic plates was placed under the chain to prevent the chain from interfering with the spinning wheels, and to align the chain to travel around the driving system and rotating platform (Figure 2.1, Figure2. 4).

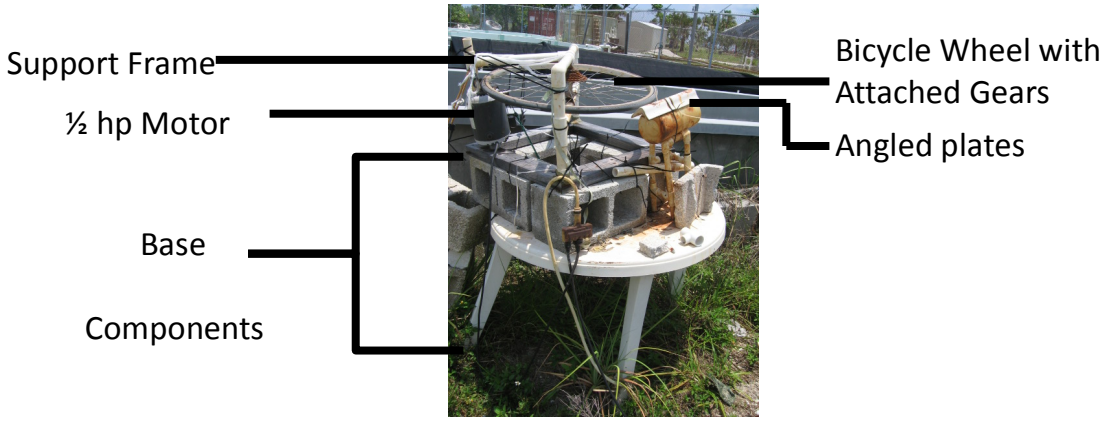

Figure 2.5: Coral-sel motor and gear driving components.

Figure 2.6: Close-up of the driving component of the coral-sel with its attached gears.

The in-tank component consisted of a 104 cm high piece of 4 inch (10 cm) diameter PVC pipe (Figure 2.3). Five 189 cm long pieces of 1 inch (2.54cm) diameter PVC pipe were passed through the central column to form five levels (with one arm of equal length on each side of the central column) at randomly staggered angles around the column with 14 cm spacing between levels, beginning 12.5 cm from the base of the

central column (Figure 2.3). Each arm had three mounting sites at set distances of 15 cm, 50 cm, and 85 cm from the central axis of rotation (Figure 2.4). The sites allowed tiles to be mounted on the rotating arms using nylon nuts and bolts. The central column was placed within a supporting frame that allowed it to spin freely while being held in a constant position relative to tank walls and the driving mechanism (Figure 2.3, Figure 2.4).

The chain loop ran through two angled 1" PVC pipes connected to the supporting frame (Figure 2.3). These pipes controlled the chain's position as it entered and exited the channel that encompassed the "in tank" bicycle wheel (Figure 2.3). The channel was coated with rubber and hot glue to create a frictional surface that allowed the chain to drive the motion of the "in tank" component (Figure 2.3, Figure 2.4). This frictional material was replaced regularly throughout the length of the experiment.

The gearing system (Figure 2.6) stepped down the motor's rotational energy, allowing the motor's spindle which spun at 1725 rotations per minute (rpm) to turn the submerged component at the desired rate of 3rpm. This rotational speed caused the attached corals to move at speeds of 4.7cm/s, 15.7cm/s, and 26.7 cm/s depending on the coral's distance from the center of rotation (15, 50, and 85 cm respectively).

Testing the system

After construction, the system was tested to ensure that attachment sites equal in distance from the center of rotation experienced flow rates that were similar to one

another, but differed from positions at other distances. This was accomplished using the clod card technique (Doty 1971). According to this method, plaster blocks that are equal in size and mass, placed under conditions of varying water movement will lose mass in relation to the amount of water movement around them. Therefore, if blocks of equal size and mass were placed in specified positions on the rotating structure, changes in their mass would indicate how the flow conditions produced at each related to the others (i.e. did all attachment sites 15cm from the center of rotation experience equal flow and did that vary significantly from the flow seen at positions 50cm and 85 cm from the center).

Plaster blocks of equal size, shape, and mass were fabricated using 500g of plaster of paris powder combined with 335 mL of freshwater. The plaster was formed into uniform blocks using standard ice cube trays (bottom base=4.9cm x 2.7cm, top base = 3.15cm x 1cm, height = 3cm). The blocks were allowed to set for at least 1 hour before they were removed from the molds and allowed to dry completely on the counters of a dry office space for at least one week. After drying, a razor blade and sandpaper were used to shave and smooth the largest side of the blocks, creating a set of blocks of equal size and mass (Figure 2.7).

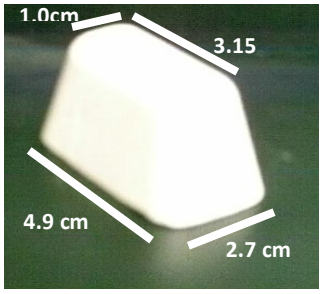

Figure 2.7: Sample "clod card" plaster block before exposure to coral-sel treatments. Blocks were created using ice cube trays to create trapezoidal blocks with consistent dimensions (indicated by scale bars).

The blocks were affixed to the coral-sel at each of the 30 mounting sites (n=10/treatment) using two rubberbands stretched around the block and ceramic plate at the mounting site. The bands allowed the blocks to be attached to and removed from the system without requiring additional modifications of the blocks (through use of adhesives or screws). In addition, the elasticity of the rubberbands allowed them to remain snug around the blocks even as their sizes decreased due to dissolution. The system with attached blocks was run for 24 hours. The blocks were removed from the system and allowed to dry completely by exposing them to air in a dry room for 3-7 days. Drying ovens were not utilized because pilot studies revealed that the blocks were compromised by exposure to their extreme conditions. Rapid drying in the ovens caused the blocks to quickly lose structural integrity upon introduction to salt water. Each block's change in mass (Figure 2.8) was determined using a balance. The changes in mass related directly to the water flow speeds they experienced (Doty 1971). These values were analyzed and used to verify that mounting positions set at different distances from the center of rotation experienced flow speeds that varied significantly from each other (ANOVA, $p<0.001$) (Figure 2.9).

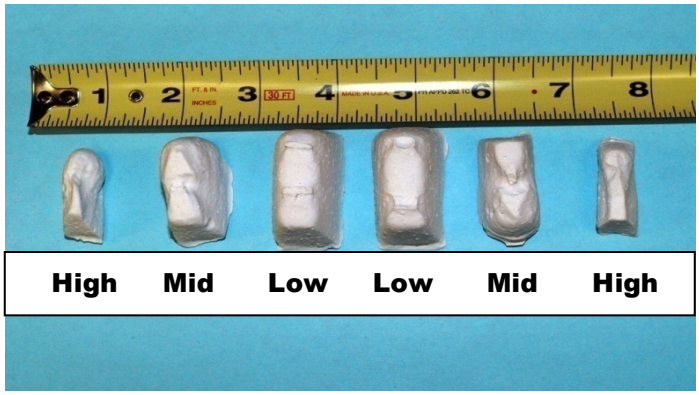

Figure 2.8: A subset of plaster blocks used to test the difference in flow speed experienced at each position on the coral-sel.

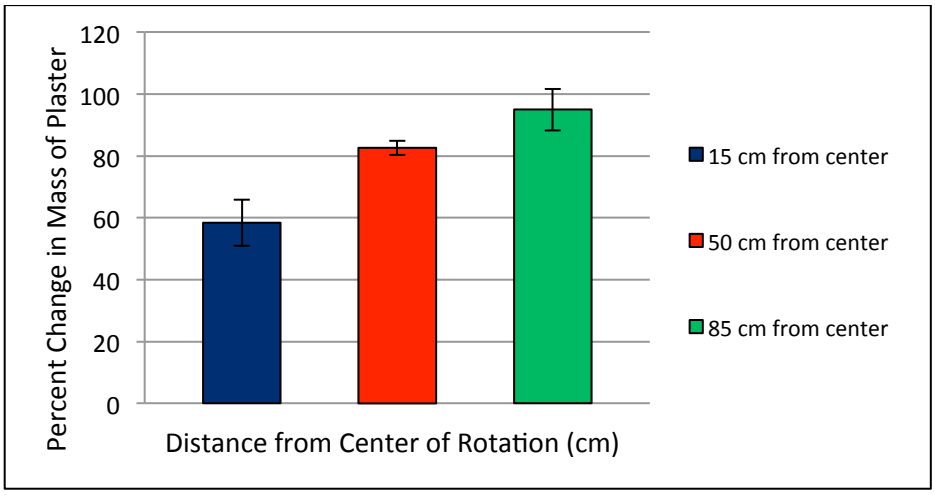

Figure 2.9: Mean percent change in the mass of plaster blocks attached to the coral-sel and exposed to experimental flow conditions for 24hours. Error bars denote one standard deviation from the mean (n=10 units/ treatment). The percent change in mass varied significantly between all distance/flow treatments. (ANOVA, $p<0.001$)

C) Methodology

Each experimental unit (Figure 2.10) consisted of a 10.8cm x 10.8cm x 0.6cm ceramic tile with a 7.5cm x 7.5cm x 0.5cm limestone block secured to the non-glazed side. The limestone block had a 2.5cm diameter hole drilled in its center. A 2.5cm

diameter “plug” of *Montastraea faveolata* was inserted into each hole, with its live tissue surface level with the surrounding limestone (Figure 2.10). Previous observations of mounted *M. faveolata* fragments have shown the tendency of this species to grow laterally over available adjacent surface areas (pers. obs.). Epoxy was used to secure each plug in place and fill the minimal space that existed between the plug and the surrounding limestone plate to allow uninterrupted lateral expansion.

All fragments were taken from a single parental colony of *Montastraea faveolata.* This ensured that any differences seen in the growth rates and other monitored responses were only due to experimental influences and not genetic differences, varying zooxanthellae communities, or previous “life experiences” of multiple colonies. The parental colony originated in Key West, but had been maintained in the outdoor tanks of the University of Miami’s Aplysia Resource Center for several years prior to the beginning of this experiment. Experimental “plugs” were created using a drill press with a diamond tip hole saw with an internal diameter of 2.52cm to produce experimental colonies of identical dimensions.

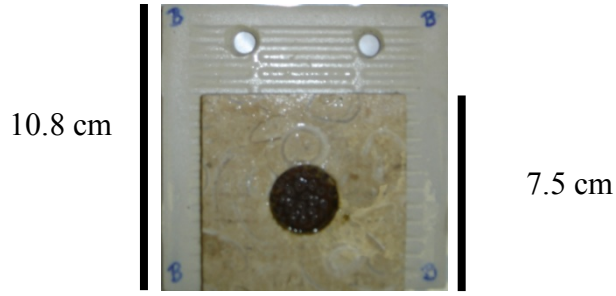

Figure 2.10: Experimental Unit for coral-sel experiment consists of a plug of *Montastraea faveolata* inserted into a tile of limestone affixed to the unglazed side of a standard bathroom tile. Drilled holes at the top of the tile allowed it to be secured to the coral-sel using nylon bolts.

Each experimental unit was photographed with scale and color standards (red, green, and blue). These standards allowed the photographs to be utilized to determine changes in fragment size and pigmentation. The photographs were analyzed using Image J software to determine initial surface area for each colony. Initial masses were determined with a top-loading electronic balance.

Bi-weekly, all tiles were removed from the coral-sel and cleaned of all fouling plant, animal, and sediment material. Tiles were then dried to remove excess water that could be a source of variation during weighing. Masses were determined and digital pictures of each sample were taken, using the same scale and color standards throughout the course of the experiment.

Fluorometry

Beginning in December 2005, measurements of the photosynthetic capacity of the symbiotic dinoflagellates within the experimental colonies were taken using a WALZ Diving-Pulse Amplitude Modulated fluorometer. Two types of fluorometric data were collected. Data concerning the maximum quantum efficiency (Fv/Fm) of the zooxanthella communities were collected bi-weekly at least one hour after nightfall. The fiberoptic light source with the "masking attachment" was placed over an area of live tissue near the center of each colony. The fluorometer then was used to measure photosynthetic yield. Sites at the center of the colony were chosen to minimize possible

influences of the growing edge or the supporting tile on the photosystem or the fluorometer.

Photosynthesis vs. Irradiance (P-I) curves were constructed using a series of light pulses of increasing intensity from the PAM fluorometer with average strengths of 2.5, 8.0, 15.9, 26.2, 39.1, 53.7, 78.9, and 103.5 μmole quanta $m^{-2}s^{-1}$. These measurements were taken in the morning beginning 2-3 hours after sunrise.

At the conclusion of the experiment, each plate was cut in two using a standard table saw. One portion was utilized for further analyses. The other portion was returned to the tank. These "reserve" pieces were available for use as parental material for possible coral nursery culturing.

Zooxanthella Community

An airbrush with filtered sea water was used to remove all of the tissue from the non-reference half plate. This tissue was sampled to determine the dominant symbiont clades within each colony. The blastate samples were extracted using a modified organic protocol (Baker et al. 1997). The genetic identity of the *Symbiodinium* within each sample was then determined by analysis of Restriction Fragment Length Polymorphisms (RFLP) in large sub-unit rDNA (Baker et al. 1997).

Corallite Morphology

A reflex microscope was used to digitize the three dimensional Cartesian coordinates (x-y-z) of 25 distinct landmarks on the calical surfaces. The landmarks chosen reflect the shape of the uppermost growing edge (the septal margin) and the costal extension between the corallites (Figure 2.11) (Klaus *et al.*2007).

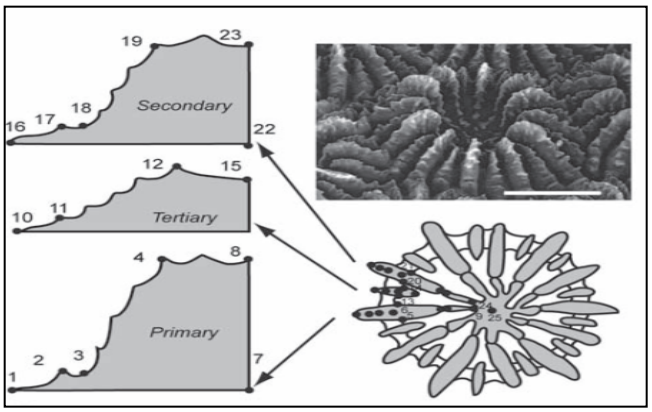

Figure 2.11: Schematic diagrams of landmarks used in morphometric analyses. (A) Scanning electron micrograph of corallite and schematic diagrams (left, vertical profiles of costosepta; right, calical surface) showing landmark positions used in three-dimensional analyses. Points 10, 25, and 12 were used as a baseline. Scale bar in photograph is 1.5 mm. Schematic diagrams are not to scale. (*Diagram taken from Klaus et al. 2007)*

The topographies of three adjacent costoseptae (primary, tertiary, and secondary) were digitized for six corallites in areas of new growth from each experimental plate. In cases where six appropriate corallites had not developed, measurements were taken only for corallites that fit the proper qualifications (new growth and fully developed). Corallites within the area of the original "plug" were excluded to remove the effects of prior physical conditions on corallite construction from the analysis of corallite morphology.

Size and shape coordinates (Bookstein 1991) were calculated from the data using the computer program GRF-ND (Generalized rotational fitting of n-dimensional

landmark data, DE Slice 1994,). Shape coordinates were calculated using 23 triangles formed from triplets of the 25 points, in which the same landmarks (#25 and #10) served as a fixed baseline positioned at coordinates (0,0) and (0,1) respectively, within a plane oriented relative to the highest point (#12) in the z-dimension. Each of the 23 triangles was translated, rotated and rescaled relative to the baseline. The three dimensional shape coordinates were calculated by translating the triangles so that one vertex was situated on the origin, rigid rotation and scaling about the y and z axes placed the second point at (1,0,0), and rotation placed the third point in the first quadrant of the x-y plane. The x-y-z coordinates of the 23 unfixed third points, (three coordinates x 23 points = 69; minus the fixed z point #12 = 68 total points), termed "shape coordinates," served as the variables that were analyzed statistically (Klaus *et al.*2007).

Variables that begin with the letter X refer to the distance of the given point in the x dimension (parallel to the septa), while variables starting with the letter Y and Z respresent distances in the Y dimension (septal relief) (Klaus *et al.*2007).

The results were plotted using the Morpheus Program (D. Slice). Plots of the primary septa of colonies under each of the flow speeds were plotted and compared using spline plots. In a spline plot, each representative diagram of the primary septa is compared to a reference plot. In the coral-sel study, the form of an average primary septum formed under low flow conditions was used as the reference for other comparisons. The grid behind the plot is warped/ altered to reflect the ways that the

reference plot would need to be manipulated in order to plot the reference points onto the "target" plot.

D) **Results and concern with pseudoreplication**

The coral-sel was designed with the express purpose of keeping all other factors constant, while exposing the corals to different flow rates. The goal of this first experiment was to determine if the coral-sel method could produce flow rates for attached animals that varied enough to produce different flow related growth rates. The system was successful in accurately simulating these flow conditions.

Random variation can never be completely eliminated. Using a single parental colony to produce all of the experimental fragments for this study removed genetic variation of the host as a source of variation. The parental colony had a semi-planar morphology which received comparable amounts of light over its entire surface. Fragments from the top surface were expected to have very similar symbiont communities. The need to test the system caused a necessary situation of pseudoreplication in this experiment. Limitations based on tank space, availability of coral, and supplies also played a role in the decisions that led to a pseudoreplicated experiment.

This situation must be acknowledged, so that the experimental results can be taken in context, and the data from pseudoreplicated studies can serve their intended purpose. In this case, they can be used to draw initial conclusions about the viability of

the coral-sel method as a research tool, provide an indication of how flow may affect *M. faveolata,* and supply useful information that may be utilized in future studies of this species.

Chapter 3:

Influence of Water Flow on the Growth and Morphology of *Montastraea faveolata*

A) Overview

Abiotic factors such as water flow rate and temperature can have significant influences on the growth and survival of many coral species. It is difficult to isolate individual physical factors in *in situ* studies, but laboratory techniques may be utilized to study them. The "coral-sel" method was employed to examine the effect of three water flow rates (4.7, 15.7, and 26.7 cm/s) on the massive coral *Montastraea faveolata*. Numerous metrics of holobiont growth and health, including changes in mass and surface area, the number of polyps formed, and zooxanthellae activity were monitored for 18 months. Flow rate had a significant influence on coral growth rates (both tissue surface area and whole colony mass) as well as morphological and photophysiological responses. Surface area and mass exhibited seasonal growth patterns, showing positive correlations with increasing temperature, while changes in mass/surface area showed a negative correlation with temperature. The cladal make-up of the symbiont community was not changed by the flow environment. Growth of *M. faveolata* was highest at 4.7 cm/s.

B) Introductory Remarks

Flow can have significant influences on coral growth and survival. It can influence how dissolved gases (Dennison and Barnes 1988, Patterson *et al.* 1991, Patterson 1992, Atkinson *et al.* 1994, Lesser *et al.* 1994, Shashar *et al.* 1996, Bruno and

Edmunds 1998), inorganic nutrients (Stambler *et al.* 1991, Atkinson and Bilger 1992, Thomas and Atkinson 1997), and carbon (Lesser *et al.* 1994) reach the tissues of the animal. Flow can influence the rates of prey encounter and capture (Helmuth and Sebens 1993, Johnson and Sebens 1993, Sebens1991, Sebens *et al.* 1997; 1998). In addition to the effects on the coral host, water flow also impacts the holobiont through its effects on the algal symbionts (*Symbiodinium* spp.) of corals (Mass *et al.* 2010).

Along with changes to the physiological activities of corals, flow can also influence the overall morphology of the organism. The morphology of an organism represents aspects of the relationship between the organism and its environment. The concept of ecomorphology is based on the premise that the phenotype provides useful information about this relationship (Ricklefs and Miles 1994). Much of the morphological variation in a species is attributable to differences among groups in the state of some environmental variable experienced during sensitive periods of development (Travis 1994).

Morphological plasticity is an integral property of many scleractinian species. Phenotypic plasticity of morphological features can be highly adaptive (Travis 1994). Minor morphological characteristics can change in response to environmental stimuli in the direction that would enhance performance under the environmental conditions indicated by those stimuli (Travis 1994). The overall morphology of a coral colony can influence its abilities to exchange gases and acquire nutrients from the waters surrounding them, as well as their abilities to capture zooplankton (Sebens and Johnson 1991, Helmuth and Sebens 1993).

While the morphological development of corals is heavily influenced by water flow regimes, a coral with a form suited to one set of flow conditions may not be limited in its ability to adapt to other conditions. Colonies exposed to a new regime may have plasticity in their physiological and biochemical activities which allows them to enhance the carbon delivery to assimilation sites for biological processes (Lesser *et al.*1994). In addition to general morphology being influenced by environmental conditions, the symmetry of colonies can be an indication of the conditions around the colony (Mass and Genin 2008).

Morphological plasticity includes both large-scale colony morphology and small-scale corallite morphology. There is a wide range of variation for corallite shape and size. Differences in general corallite morphology can be used to classify corals into broad groups based on the size and shape of the corallite (round, ellipsoid, meandroid etc.) Many corals, like *Montastraea faveolata,* have circular corallites, while other corals, like *Dichocoenia stokesii,* have corallites with a typically elongated elliptical shape.

Corallites can be classified based on size or shape. The internal diameter of a corallite is measured across its center. Corallites can then be classified into size categories: those that have diameters smaller than 1.5 mm, diameters between 1.5 and 10 mm, diameters between 10 and 20 mm), and finally, corallites with diameters greater than 20 mm. The general size and shape of the corallites are characteristic of a species and can be used for taxonomic identification. Some species have small corallites like the lettuce coral (*Pavona decussata*), while others, like *Fungia fungites* have very large corallites.

While general features of a species' corallites remain constant within a species, at a microscale level the components of the corallites can vary in a number of ways including the height and length of the septae and costae and the relationships between the sizes of these features. These microscale variations may be the result of the corals making adjustments to optimize their fitness in the environment that they occupy during the period when the corallites are formed. For instance, corals that are exposed to physical stress can decrease their porosity, thus increasing their density, by enlarging and thickening their thecal walls, septa, and other structural components (Chamberlain 1978).

Corallites with similar shapes have general features that are constant across a species. While the idea of "shape" can often be very loosely defined, it can be made operational and useful as a descriptive characteristic through the use of a number of these features and their relationships to one another (Bookstein *etal*.1985). These features can serve as landmarks – loci with both names and locations which can be measured and compared between individuals (Bookstein 1991). These landmarks can be used to quantify and qualify differences between species and the effects of a variety of influences on individuals of a single species.

A colony's overall morphology develops in order to suit the biotic and abiotic conditions at the site that it occupies. Different morphologies can be induced by flow and light through their effects on respiration, production, calcification and prey capture (Mass and Genin 2008). This may involve the flattening of a colony in low light in deeper waters in order to maximize light uptake in deeper waters, the spacing of branches in various water flow conditions (Bruno and Edmunds 1997, 1998), or the thickness of

branches/density of the skeleton based on flow conditions. These morphological modifications can have a direct influence on the metabolic activities of the corals (Bruno and Edmunds 1998).

Morphological variation is caused by the combined effects of genetic polymorphism and phenotypic plasticity, but the effects of the two components can be difficult to separate (Knowlton and Budd 2001, Budd and Pandolfi 2004, Fukami et al 2004).

Corals have adapted to different environments by evolving unique morphological and/or physiological features. There are hundreds of coral species, many with different colors, growth forms, and colony sizes. A classification system has been developed to distinguish among different species often based on characters such as a coral's morphology and/or individual corallite (skeleton) shape and size (e.g., see http://nmita.geology.uiowa.edu/florlist.htm). Although the general properties of morphology are constant within a species, and may be used to define it, environmental conditions can cause alterations to the "details" of these generalized growth forms.

Abiotic factors that may influence the morphology of a colony in terms of overall growth form and corallite size, shape, and arrangement include water flow and light availability (light level and directionality). Increasing water flow rates cause decreases in boundary layer thicknesses, which can increase the diffusive exchange of gases and nutrients (Patterson and Sebens 1989, Patterson *et al.*1991, Patterson 1992 Bruno and Edmunds 1997, Gardella and Edmunds 1999; 2001, Kuffner 2001). Biotic factors

include the presence of algae, sponges, and other organisms that will compete with the coral for substrate.

In addition, the coral hosts may adjust their manner of growing under various environmental conditions. They may adjust their overall growth form, allowing the colony to flatten in areas of low diffuse light, or remain more rounded in areas of high light (Fricke and Schumacher 1983). They may allot more energy to horizontal growth and less into thickening of the skeleton in areas of low physical stress and more into creating a robust skeleton in areas of high physical disturbance. In some areas and species, the depth gradient is also characterized by an increase in skeletal porosity. As depth increases (often accompanied by a decrease in light and physical disturbance) coral skeletons become more fragile and porous while not showing marked decreases in surface area (Fricke and Schumacher 1983). In other regions, in which deeper areas were characterized by low light and low prey concentrations, the massive corals present were characterized by very dense skeletons. The dense skeletons were hypothesized to be related to the very low growth rates of these corals (due in part to low light and/or nutrient availability) and the resulting proximity of the growth bands within the skeleton (Holstein pers. comm).

The basic unit of coral colony construction is the corallite, which is the skeleton of a single individual polyp within a colony. The architecture of corallites is fundamentally similar across species, but minor differences in form and structure form a basis of coral taxonomy (Veron 2000). Each corallite is surrounded by a vertical wall or theca. There are vertically arranged partitions, called septa within each of the corallites.

The septa may be classified as primary, secondary, or tertiary based on when they formed during the corallite's development. Costae are extensions of the septa beyond the corallite wall. An axial structure, known as the columella, may occupy the center of the corallite. The general arrangement of these structures and their features remain constant within a species. This allows landmarks to be defined within the corallite structure. A landmark is a point that has both a name and Cartesian coordinates (Bookstein 1991). These landmarks can be mapped and their relationships to one another can be used to define the shapes of corallite components and determine if the morphology of the corallite differs in the presence of a variety of environmental conditions or genetic influences.

A coral colony can acclimate to a variety of environmental conditions including changing temperatures (Reynaud *et al.* 2003, Carricart-Ganivet 2004,D'Croz and Mate 2004, Rodolfo-Metalpa *et al,* 2006; 2008, Edmunds 2009, Carpenter *et al* 2010), light levels (Falkowski and Dubinsky 1981, Titlyanov 1991, Marubini *et* al. 2001, Titlyanov *et* al 2001, Treigner *et al.* 2008*,* Edmunds 2009, Hoogenboom and Connelly 2009, Reynaud *et* al 2009, Lesser *et al.* 2010), water flow rates(Jokiel 1978, Patterson *et al.* 1991, Stambler 1991, Helmuth and Sebens 1992, Johnson and Sebens 1993, Sebens *et al.* 1997, Carpenter and Patterson 2007, Mass and Genin 2008, Suzuki *et al.* 2008, Arkema 2009, Hoogenboom and Connelly 2009, Schutter *et al.* 2010, Carpenter *et al* 2010, Purser *et al.* 2010), competing organisms(Lapid *et al.* 2004, Idjadi and Karlson 2007), and predators (Gochfeld 2004, Lenihan and Edmunds 2010).

The corals may acclimate to changing environmental conditions in multiple ways including adjustments within their symbiont communities (Baker 1999; 2001). These adjustments may include changing the composition of the zooxanthella communities within their tissues. The zooxanthellae themselves may exhibit changes in the amount of chlorophyll and accessory pigments within their cells that could optimize their use of available light or change the arrangement of chloroplasts and other pigments within their cells (Titlyanov *et al.* 2001).

With the environmental changes that are projected to occur in the coming decades, it is imperative that scientists explore how corals will grow under the predicted future conditions as well as gain a thorough understanding of how they grow under current natural and controlled conditions. With this knowledge, scientists will be better able to predict the impacts of changing climate on corals of different areas as well as determine how to culture corals to help restore areas that have been negatively impacted by both natural and anthropogenic disturbances. This study sought to determine how flow conditions influenced the growth of the massive species *Montastraea faveolata*.

C) Methods*:*

1) Growth

The coral-sel system (Margolin 2008) was utilized to determine the effects of water flow as an isolated variable on the growth of the massive coral, *Montastraea faveolata.* Fragments of a single parental colony, originally collected from Key West, FL and maintained in an outdoor tank at the University of Miami's Experimental Hatchery for

>12 months were produced using a 25 mm hole saw bit. The circular fragments were then trimmed to 1 cm heights using a table saw. The fragments were returned to the outdoor tanks for a 5-week recovery period. Healthy fragments were then secured to individual tiles with their surface tissue set flush with the calcium carbonate tile around them (Figure 3.1).

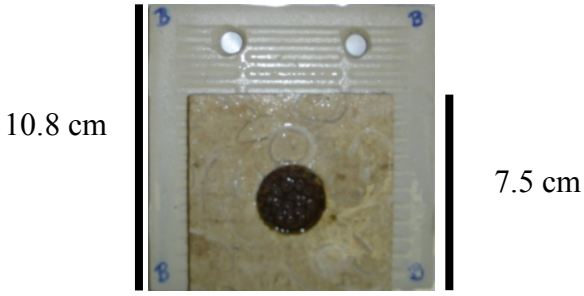

Figure 3.1: Experimental Unit for coral-sel experiment consists of a plug of *Montastraea faveolata* inserted into a tile of limestone affixed to the unglazed side of a standard bathroom tile. Drilled holes at the top of the tile allowed it to be secured to the coral-sel using nylon bolts.

Identical experimental units containing 2.5 cm diameter fragments of a single parental colony were connected to the coral-sel and exposed to one of three flow rates (4.7, 15.7, or 26.7 cm/s) for 18 months (September 2005 - March 2007) in an outdoor tank (~4 m in diameter) at the University of Miami's Experimental Hatchery. Experimental units were removed from the coral-sel bi-weekly, cleaned to remove all settling organisms and sediment, weighed, and photographed.

During the experiment, coral growth was monitored using digital photographs of each unit with size and color standards. Images were analyzed using Image-J computer software to monitor changes in surface area over the course of the experiment.

Experimental units were also weighed on an electronic balance (precision 0.01g), after excess water was removed.

2) Photosynthetic efficiency

Beginning in December 2005, three and a half months after the experiment began, the photosynthetic abilities of the symbiotic dinoflagellates within the experimental colonies were measured using a WALZ Diving-Pulse Amplitude Modulated fluorometer. Two types of fluorometric data were collected. Maximum quantum efficiency (Fv/Fm) of the zooxanthellae communities were measured bi-weekly at least one hour after nightfall. The fiberoptic light source with the "masking attachment" was placed over an area of live tissue near the center of each colony. The fluorometer was then used to measure photosynthetic yield. Sites at the center of the colony were chosen to minimize possible influences of the growing edge or the supporting tile on the photosystem or the fluorometer.

Photosynthesis vs. Irradiance (P-I) curves were constructed using a series of light pulses of increasing intensity from the PAM fluorometer with average strengths of 2.5, 8.0, 15.9, 26.2, 39.1, 53.7, 78.9, and 103.5 μmoles m^{-2} s^{-1}. These measurements were taken in the morning beginning 2-3 hours after sunrise.

The photosynthetic efficiency of the zooxanthellae was also measured. The coral samples were dark adapted in order to allow all of the electron acceptors of the electron transport chain to "open" and become available to accept electrons (Schreiber *et al.* 1995). The fluorescence is measured when all of the acceptors are open. Then, the

sample is exposed to a pulse of light that saturates the electron transport chain and causes all of the electron acceptors to close. Any remaining light that is absorbed is given off as fluorescence which is measured by the fluorometer. The difference between the initial fluorescence (F_0) and the maximum fluorescence (F_M) is known as variable fluorescence (F_V) ($F_V = F_M-F_0$). Variable fluorescence (F_V) normalized to maximum fluorescence (F_M), (F_V/F_M) is a measure of the photosynthetic efficiency of photosystem II. This PSII quantum yield is often used as a proxy measurement of photosynthesis (Maxwell and Johnson 2000). Fluorescence yield is the parameter that carries direct information about photosynthesis (Schreiber 2004).

The efficiency with which light energy is used is a function of the photosynthesizer's health. When cells are under stress, the photosystem becomes saturated more easily and will not process light as efficiently. Variable fluorescence can change relatively rapidly in cells that are under stress. For this reason, this parameter is often considered as one of the most sensitive indicators of physiological stress.

3) Zooxanthella Community

At the conclusion of the study, all of the living tissue was removed from each colony using an airbrush. Total genomic DNA was extracted from each sample using a modified organic protocol (Baker et al. 1997). Final elution was in 100mL of TE buffer.

4) *Corallite Morphology*

The morphology of the calices of polyps formed during the experiment was examined to determine if water flow alone could have a significant impact on the small scale growth patterns of *Montastraea faveolata.* A reflex microscope was used to digitize the three dimensional Cartesian coordinates (x-y-z) of 25 distinct landmarks on the calical surfaces. The landmarks chosen reflect the shape of the uppermost growing edge (the septal margin) and the costal extension between the corallites (Figure 3.2) (Klaus *et al.*2007).

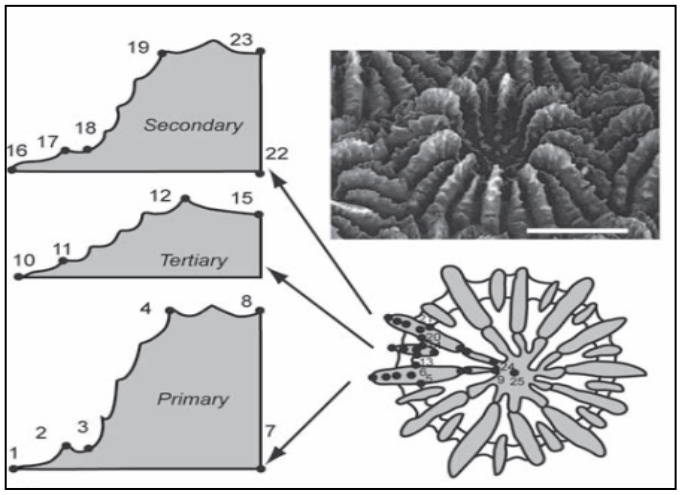

Figure 3.2: Schematic diagrams of landmarks used in morphometric analyses. (A) Scanning electron micrograph of corallite of *M. annularis* and schematic diagrams (left, vertical profiles of costosepta; right, calical surface) showing landmark positions used in three-dimensional analyses. Points 10, 25, and 12 were used as a baseline. Scale bar in photograph is 1.5 mm. Schematic diagrams are not to scale.(*Diagramtaken from Klaus et al. 2007)*

The topographies of three adjacent costoseptae (primary, tertiary, and secondary) were digitized for six corallites in areas of new growth from each experimental plate. In cases where six appropriate corallites had not developed, measurements were taken only for corallites that fit the proper qualifications (new growth and fully developed).

Corallites within the area of the original "plug" were excluded to remove the effects of prior physical conditions on corallite construction from the analysis of corallite morphology.

Size and shape coordinates (Bookstein 1991) were calculated from the data using the computer program GRF-ND (Generalized rotational fitting of n-dimensional landmark data, DE Slice 1994,). Shape coordinates were calculated using 23 triangles formed from triplets of the 25 points, in which the same landmarks (#25 and #10) served as a fixed baseline positioned at coordinates (0,0) and (0,1) respectively, within a plane oriented relative to the highest point (#12) in the z-dimension. Each of the 23 triangles was translated, rotated and rescaled relative to the baseline. The three dimensional shape coordinates were calculated by translating the triangles so that one vertex was situated on the origin, rigid rotation and scaling about the y and z axes placed the second point at (1,0,0), and rotation placed the third point in the first quadrant of the x-y plane. The x-y-z coordinates of the 23 unfixed third points, (three coordinates x 23 points = 69; minus the fixed z point #12 = 68 total points), termed "shape coordinates," served as the variables that were analyzed statistically (Klaus *et al.*2007).

Variables (Table 1) that begin with the letter X refer to the distance of the given point in the x dimension (parallel to the septa), while variables starting with the letter Y and Z represent distances in the Y dimension (septal relief) (Klaus *et al.*2007).

The results were plotted using the Morpheus Program (Slice 1994). Plots of the primary septa of colonies under each of the flow speeds were plotted and compared using spline plots. In a spline plot, each representative diagram of the primary septa is

compared to a reference plot. In the coral-sel study, the form of an average primary septum formed under low flow conditions was used as the reference for other comparisons. The grid behind the plot is warped/ altered to reflect the ways that the reference plot would need to be manipulated in order to plot the reference points onto the "target" plot.

D) Results

1) Growth

All of the colonies used in this experiment were identical in shape and surface area at the outset of the study. The change in surface area of *M. faveolata* fragments showed a clear trend of decreasing growth rate with increasing water flow speed (Figure 3.3). Flow rates caused significant differences in the change in surface area over the course of the study (Final surface area-Original Surface Area) (ANOVA, $p<0.0001$). The lowest flow conditions (4.7 cm/s) produced the highest increase in surface area (mean increase 28.31cm^2) over the course of the 18 month study. These colonies showed significantly greater growth than those under mid flow (15.7 cm/s) conditions (ANOVA $p = 0.0332$) and those under high flow conditions (26.7 cm/s) (ANOVA, $p<0.0001$). Colonies exposed to 15.7 cm/s of water flow also showed significantly greater increases in surface area compared to the high flow treated corals (ANOVA, $p=0.0087$).

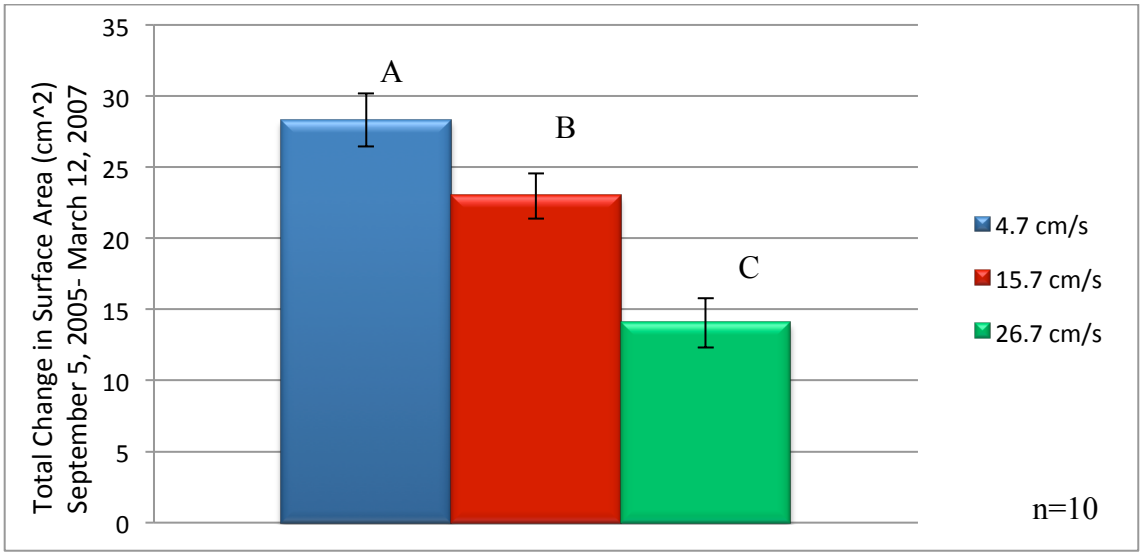

Figure 3.3: Mean surface area growth (+/- 1 SE) of experimental colonies of *Montastraea faveolata* as a function of water flow speed over the entire experimental period, Sept. 5, 2005-Mar. 12, 2007.

The relationship of changing surface area to flow rate was apparent throughout the entire course of the experiment, with growth trajectories showing patterns of differentiation after only 2-3 months (Figure 3.4). Colonies exposed to the lowest flow rate (4.7 cm/s) showed the highest rate of area growth at all times, with smaller increases at each increasing flow speed.

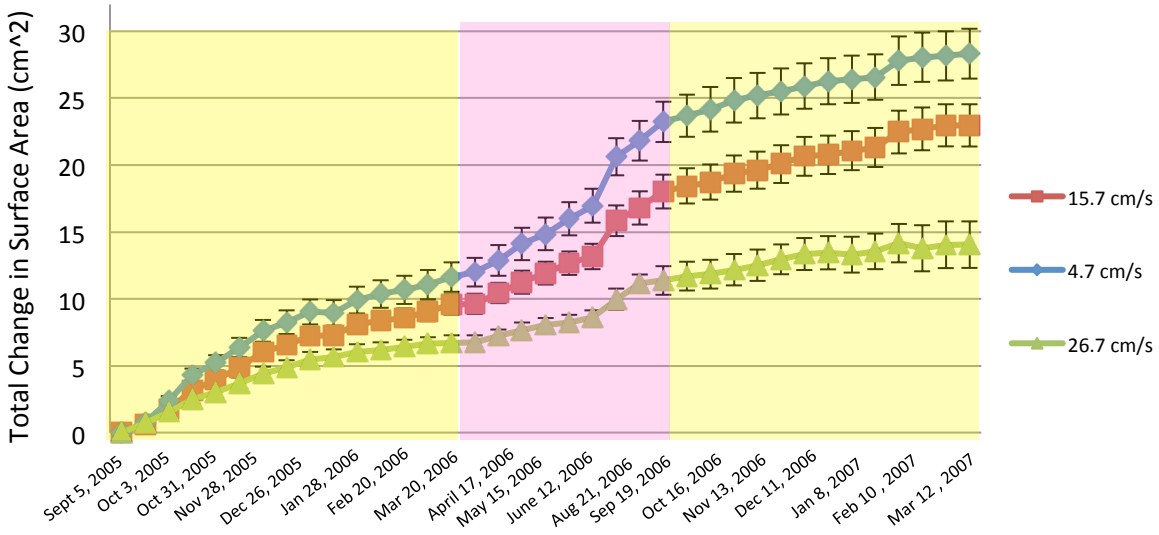

Figure 3.4: Mean surface area (+/- 1 SD) of *Montastraeafaveolata* as a function of flow speed over an eighteen month period. Yellow shaded areas indicate cooler months. Pink shaded areas indicate warmer months.

Changing surface area is only one measurement of growth. Corals also grow by adding material to their tissues and skeletons, increasing their mass. Greater increases in surface area do not necessarily coincide with greater increases in mass. Corals may show large increases in surface area with minimal increases in mass if they extend their tissue over new areas, but do not add proportional amounts of material to their skeletal structures. Over the course of this experiment, the colonies in the 15.7cm/s flow treatment showed a substantially greater increase in mass compared to both the low (ANOVA, $p<0.0001$) and high flow treatments (ANOVA, $p=0.0028$) (Figure 3.5). Colonies exposed to flow rates of 26.7cm/s also showed significantly greater increases in mass than corals under 4.7 cm/s of flow (ANOVA, $p<0.0001$) despite the fact that corals under the 4.7cm/s treatment showed greater increases in surface area (Figure 3.3 and Figure 3.4). This pattern in mass accumulation was apparent throughout the study, though

mass accumulation by the high flow treated corals appeared to slow down during the latter half of the study.

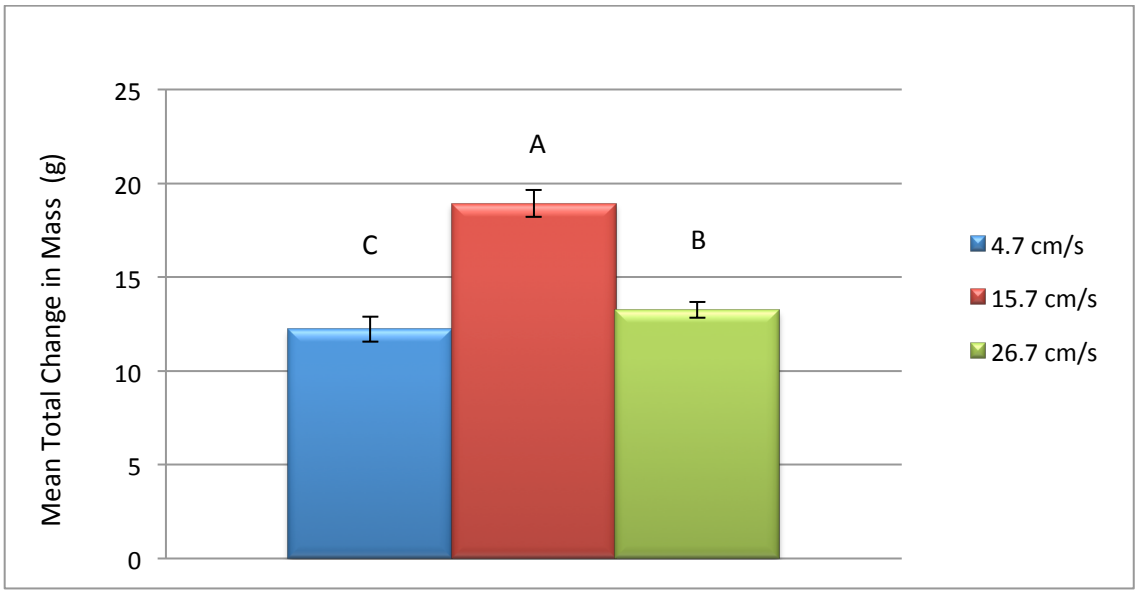

Figure 1.5: Total change in mass (+/- 1 SE) Sept. 5, 2005-Mar. 12, 2007. Significant differences in treatment effects are indicated by different letters.

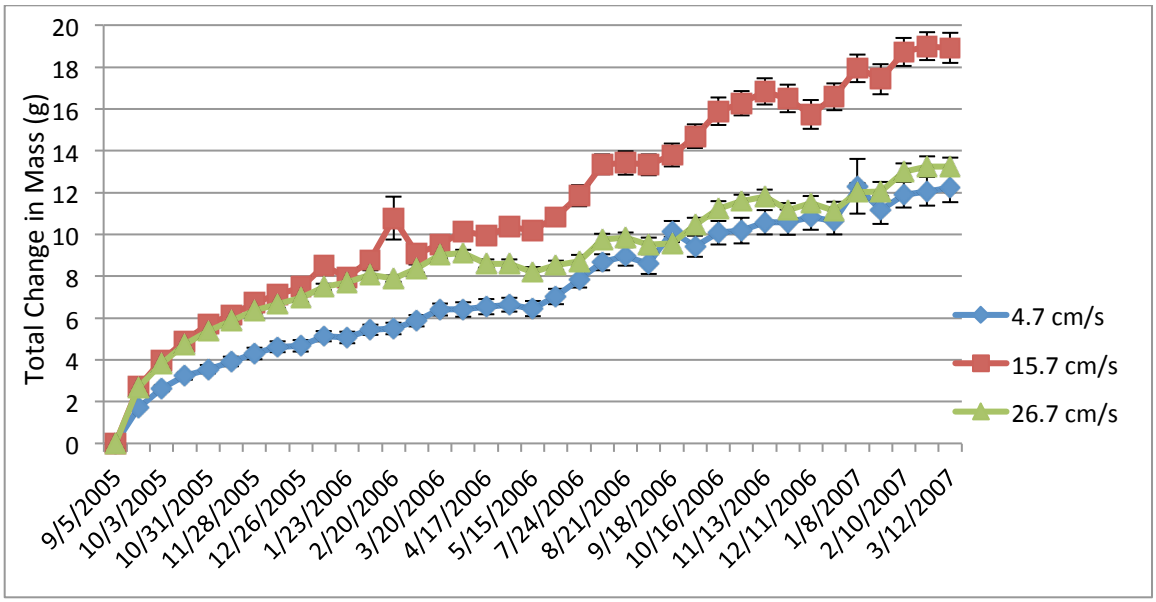

Figure 3.6: Mean change in mass (+/- 1 SE) over the course of the experiment Sept. 5, 2005 – Mar. 12, 2007.

Although there were distinct differences in area and mass growth over the course of the study, the increase in mass per unit of increased surface area only differed significantly between the mid and high flow rates (ANOVA, p=0.0285) (Figs. 3.7, 3.8). The low flow and mid flow treated corals showed similar changes in mass per unit area ((final mass-initial mass)/surface area) (ANOVA, p=0.5642). Although the corals under low and high flow appeared to have some differences in their mass/area growth rates, they did not vary significantly (ANOVA, p=0.1284).

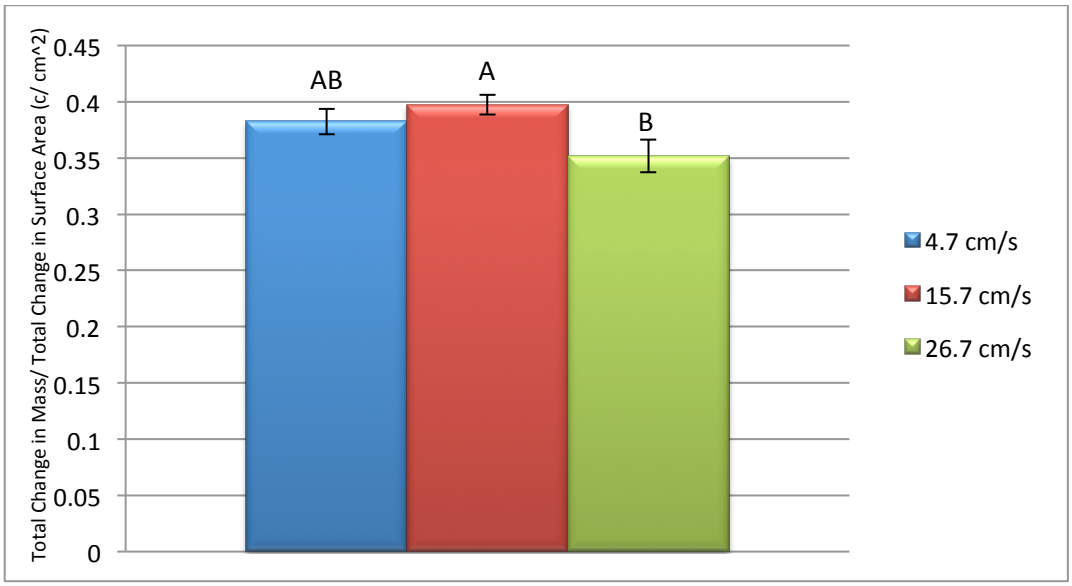

Figure 3.7: Total change in mass per change in surface area (+/- 1 SE) Sept. 5, 2005- Mar. 12, 2007. Treatments marked with the same letter do not vary significantly from one another.

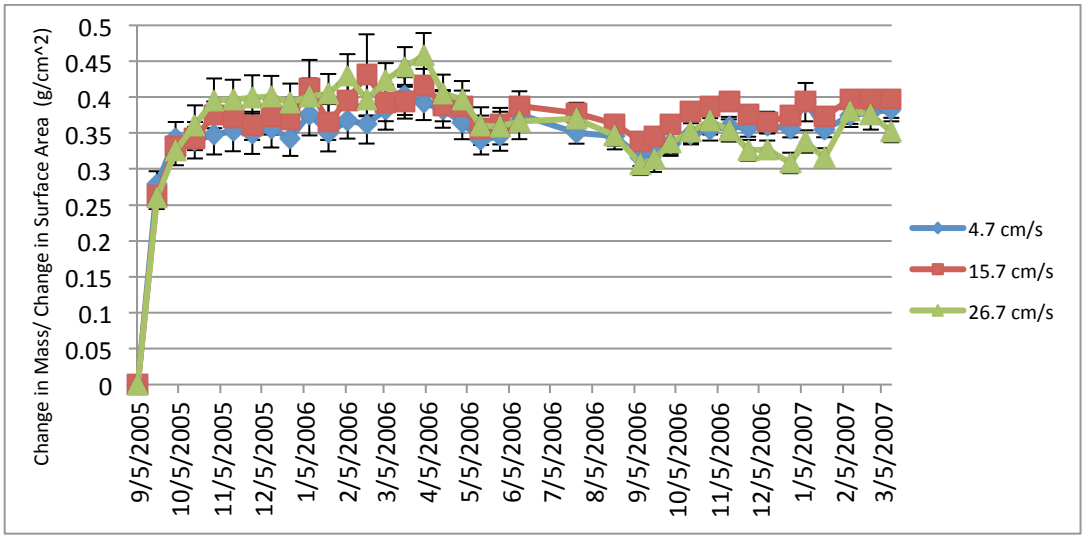

Figure 3.8: The mean cumulative change in mass per change in surface area (g/cm^2) (+/- 1 SE)
Sept. 5, 2005-Mar. 12, 2007

No significant differences in the number of polyps per unit of area that developed during this experiment were observed (Figure 3.9)(ANOVA, all $p>0.15$). Similarly, the changes in mass per polyp and changes in mass per unit area (Figure 3.10) were similar across flow treatments over the course of this experiment (ANOVA, all $p>0.71$). The fact that there were not significant changes in wall thickness or corallite diameter is not unexpected. If there had been significant changes in corallite structure and relationships, the coral's ability to feed, compete for space, and clear sediment from its surface could also have been influenced. In addition, changes to the size and structure of gonads within the corallites could have occurred (Hughes 1987).

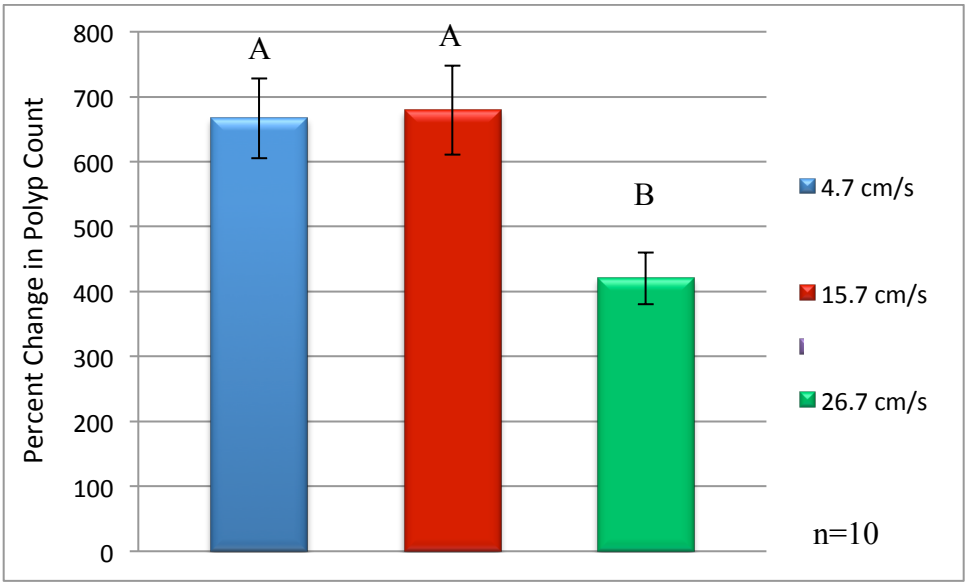

Figure 3.9: The mean percent change in the number of polyps (+/- 1 SE) after 18 months as a function of flow rate. Treatments are identified by the same letter do not differ significantly from one another.

Exposure to high water flow rates produced a smaller percent increase in the number of polyps in each colony than both the low flow (ANOVA, p= 0.0068) and mid flow treatments (ANOVA, p=0.0083). The low and mid treatments did not produce significant differences (ANOVA, p=0.9018) (Figure 3.9). The density of new polyps per unit of new area did not vary significantly with flow treatment in this experiment (ANOVA, p = 0.4920, p=0.5480, p=0.1595) (Figure 3.10). In addition, the amount of mass added by each polyp was not significantly impacted by water flow rate (ANOVA, low vs. mid p =0.9424, low vs. high p=0.7122, mid vs. high p=0.7672) (Figure 3.11). Therefore, the major influences of flow on the development of these colonies were related to changes in mass and surface area, but the density of the polyps in the new growth areas or on the amount of material that each polyp added to the colony's mass were not significantly affected. The overall linear expansion of the colonies appeared

symmetrical, with no differences between their upstream and downstream portions. The small size and planar nature of the experimental colonies likely contributed to this pattern. Differences in growth rate between the upstream and downstream portions would be more likely in colonies with greater topographic complexity.

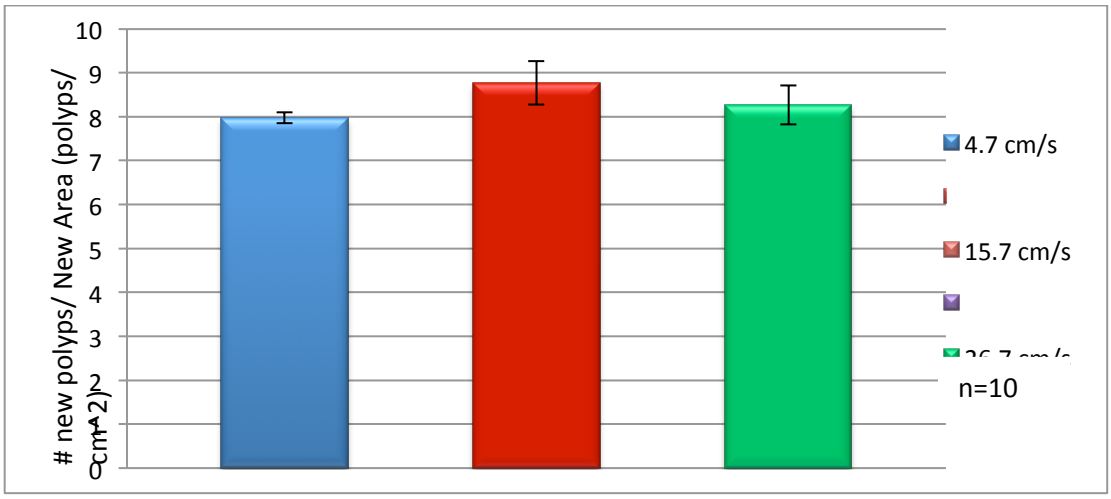

Figure 3.10: The mean change in the number of polyps/cm^2 of newly formed area (+/- 1 SE) as a function of flow rate. Flow rate did not have a significant influence on the number of polyps that formed per unit of newly formed area.

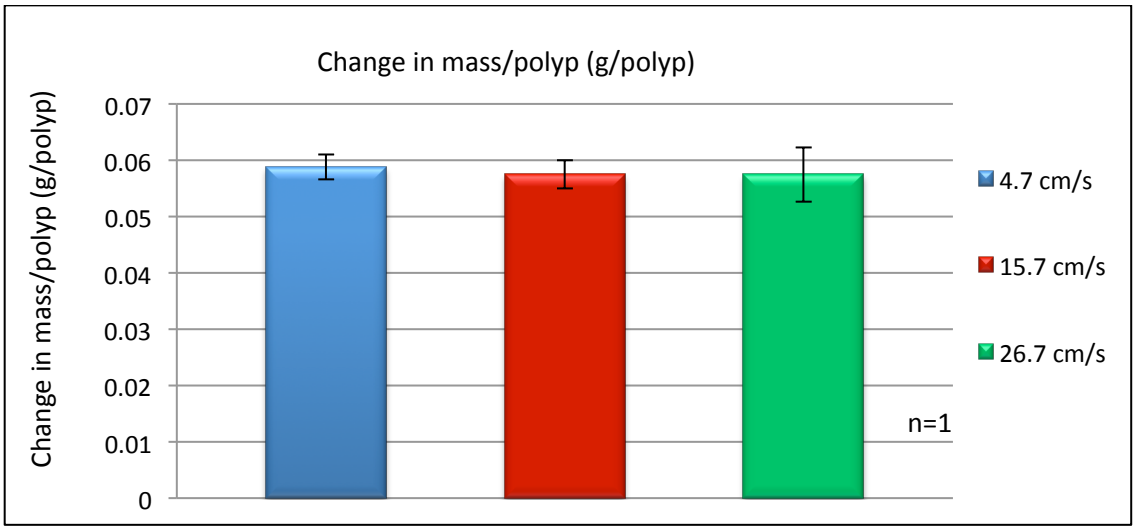

Figure 3.11: The mean change in mass per polyp (+/- 1 SE) of *Montastraea faveolata* as a function of flow rate. Flow rate did not significantly influence the change in colony mass contributed by each polyp in the experimental conditions.

In addition to simple patterns of overall growth and growth over time, growth rates can be seen to have seasonal patterns that appear to depend, at least in part, on thermal conditions.

2) Temperature/seasonal patterns

Thermal conditions have long been recognized as an important factor determining the growth rates of corals. The variation in local temperatures throughout the experiment was monitored and the temperature anomalies were plotted along with the anomalies of the various growth metrics. Patterns revealed that increases in mass and area both appeared to be directly related to temperature variations which varied seasonally. Anomalies of temperature and growth metrics were calculated as: Anomaly = single measurement – mean measurement.

The lowest local air and water temperatures in Miami generally occurred in January-March and the highest temperatures occurred in June-August. Lower area growth rates corresponded with lower temperatures while increased growth tended to occur during periods of increased temperature. Overall, growth trends reflected patterns of temperature anomalies over the course of the experiment. Growth rates followed temperature trends which followed the course of the seasons, and the association between temperature anomalies and anomalies in growth rate were consistent across flow rates (i.e. all colonies exhibited similar growth responses to changes in temperature with no apparent difference in response based on flow regime) (Figure 3.12, Figure 3.13).

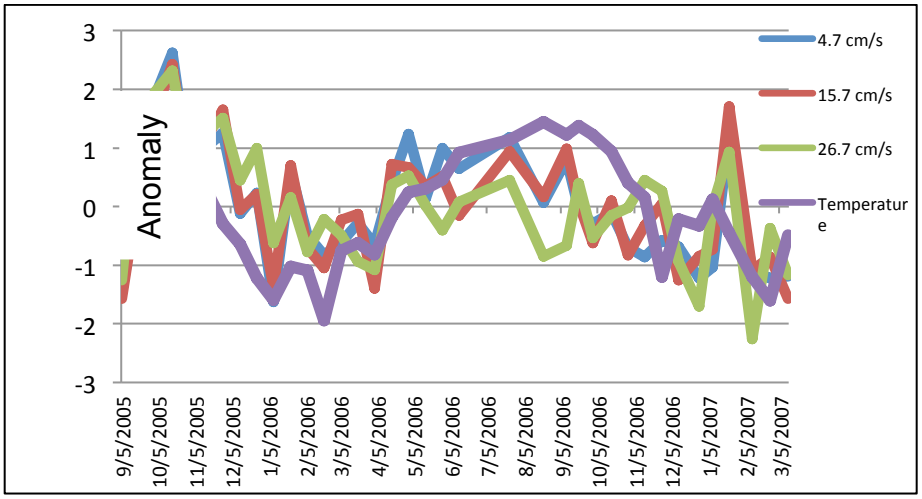

Figure 3.12: Rolling averages of the anomalies (difference from the mean) of changes in area during the previous time step and the rolling average of temperature anomalies during the same time period

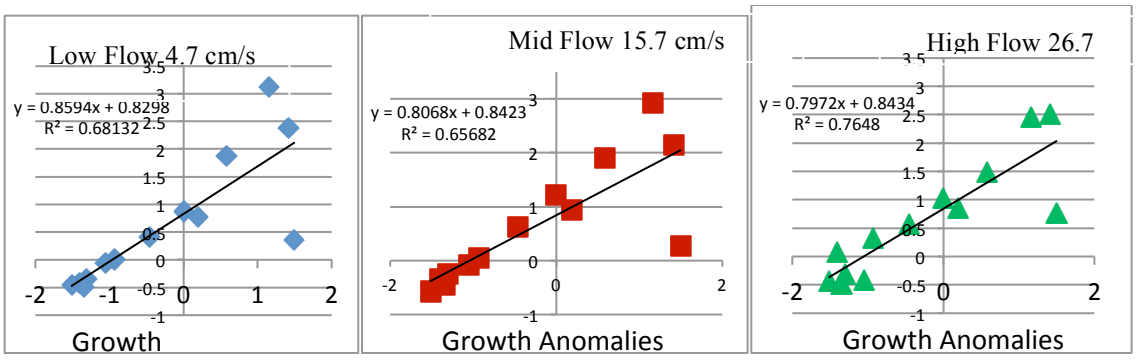

Figure 3.13: Rolling averages of the anomalies in surface area growth during each time step (x-axis) regressed against rolling averages of temperature anomalies (y-axis) over the same time period for each flow rate during the first six months of the experiment, Sept. 5, 2005 - Mar 6, 2006).

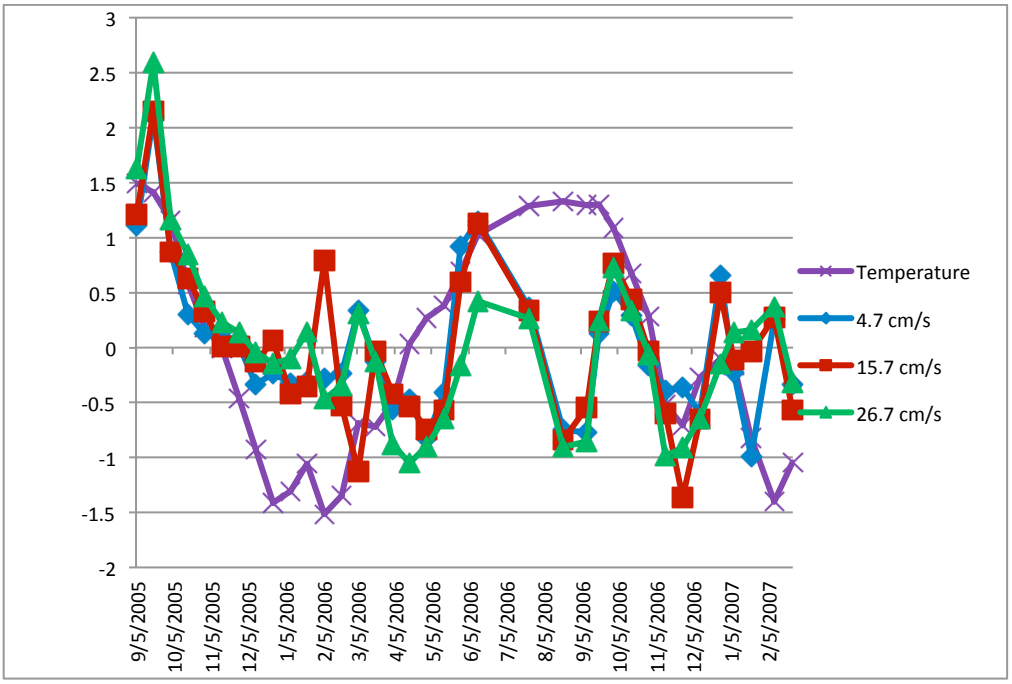

Figure 3.14: Anomaly of the rolling average change in mass during the last time step under each flow treatment as well as the rolling average of the temperature anomalies over the same time period.

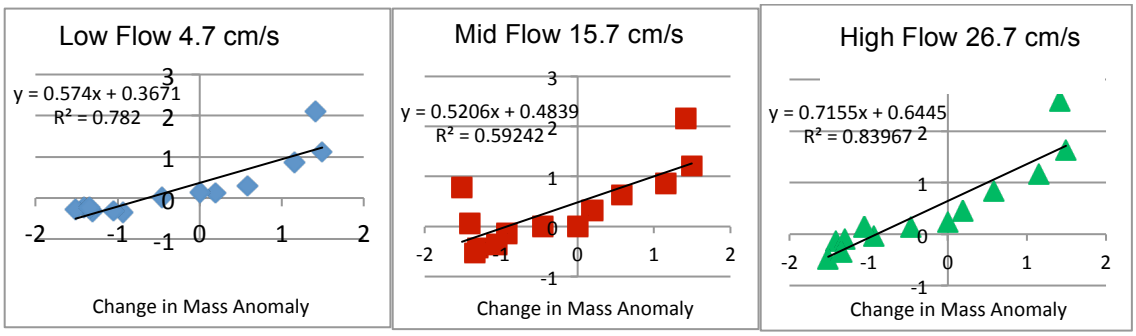

Figure 3.15: Rolling averages of the anomalies in the change in mass (x-axis) regressed against rolling averages in temperature anomalies (y-axis) during each step for the first six months of the experiment, Sept. 5, 2005- Mar. 6, 2006.

Both changes in area and mass appeared to be directly correlated with temperatures over the first six months of the experiment (Figs. 3.12 – 3.15). However the changes in mass gained per unit area appeared to be negatively correlated with temperature anomalies, with more mass added per area during cooler periods (Figs. 3.16, 3.17). Due to this apparent relationship, anomalies in the change in mass exhibited by

corals during each time step (change in mass during time step (x) – mean change in mass for that treatment over the course of the experiment), were plotted against the "negative anomaly"

(negative temperature anomaly = -(temperature measurement – average temperature).

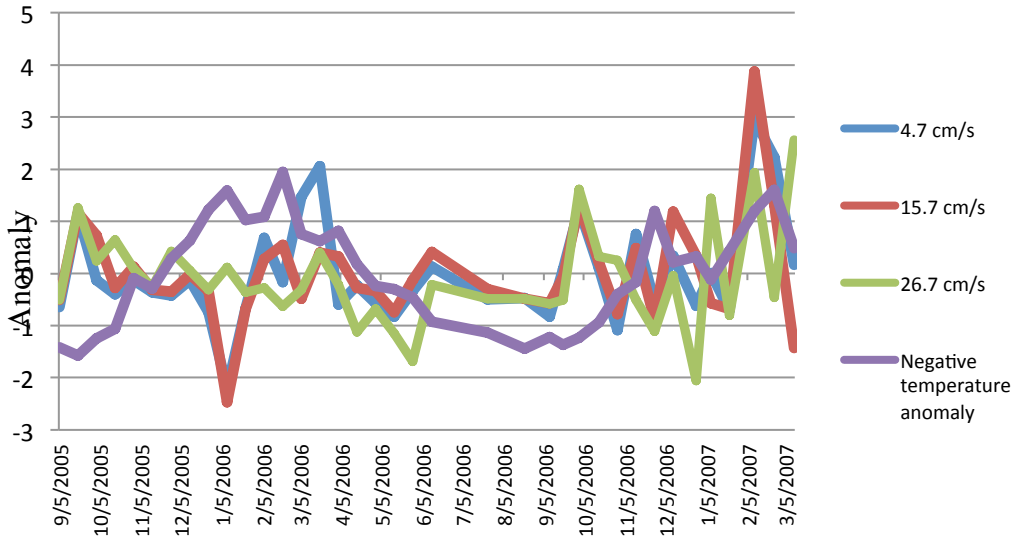

Figure 3.16: Rolling average of the anomaly of daily change in mass/daily change in area during previous two week time step for each flow treatment over time and the negative of the thermal anomaly over the course of the experiment, Sept. 5, 2005- Mar. 12, 2007.

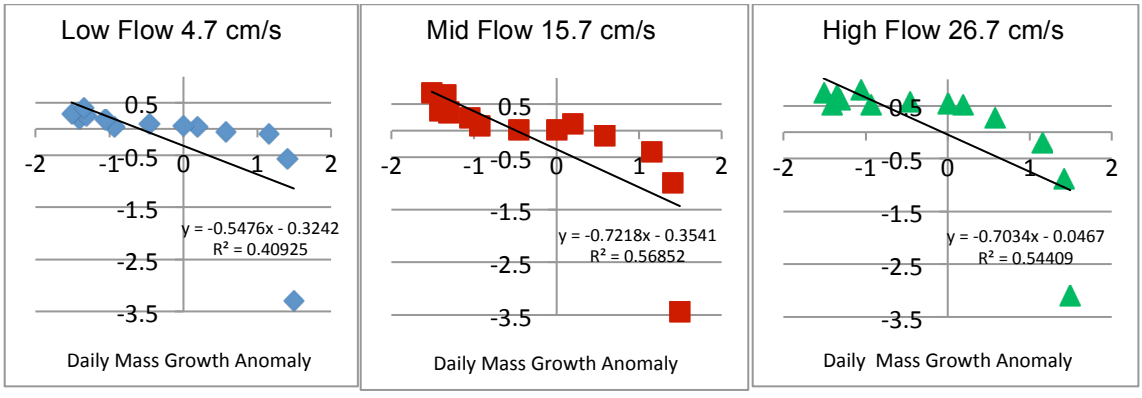

Figure 3.17: Rolling averages of the anomalies in the change in mass/unit area during each two week time step regressed against temperature anomalies for the first six months of the experiment. Sept. 5, 2005-Mar. 6, 2006.

3) Photosynthetic Activity

In general, water flow speed did not cause significant differences in the average photosynthetic yield of the zooxanthellae communities within *Montastraea faveolata.* Significant differences between treatments were found on only two occasions during the experiment, on 8/2/2006 ($p = 0.0481$) and 9/29/2006 ($p = 0.0136$) (Figure 3.17). On these dates, the symbionts in corals experiencing low flow (4.7 cm/s) conditions showed higher average photosynthetic yield than those under mid flow (15.7 cm/s) conditions. On 9/29/06, high flow (26.7 cm/s) zooxanthellae also showed higher yields than those in mid flow treatments.

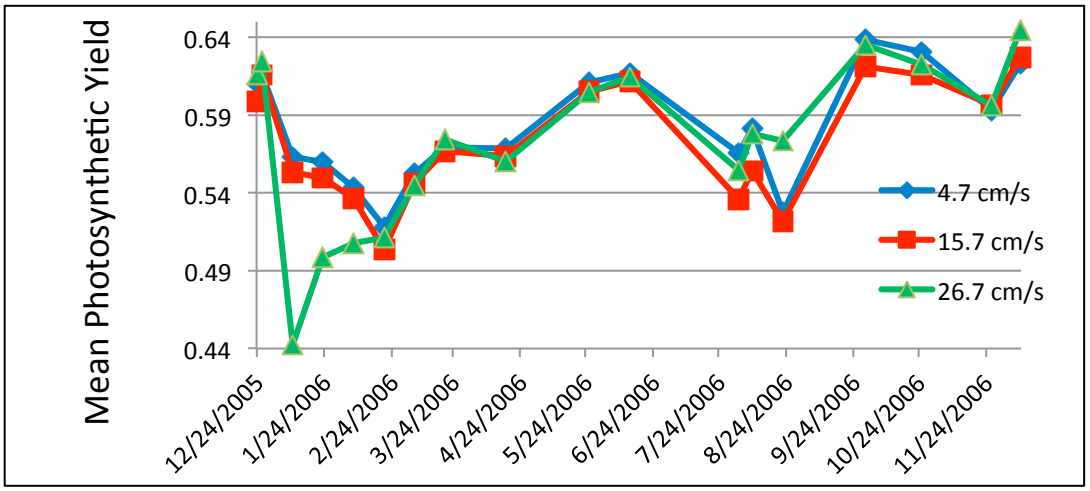

Figure 3.18: Mean photosynthetic yield (F') of *Montastraea faveolata* measured using PAM after dark adaptation. **Note, these readings began three months after the experiment began and do not include the original "acclimation period".

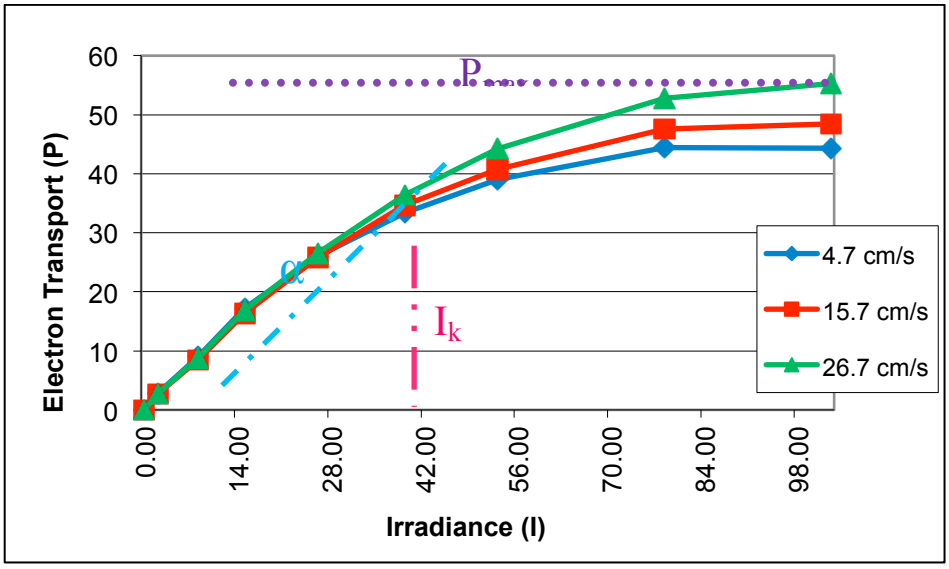

Figure 3.19: A standard example of the P-I curves yielded from coral-sel samples. Dashed lines indicate the metrics calculated from the high flow curve. Purple line indicates the maximum electron transport rate (P_{max}) for the high flow treatment. The blue dashed line indicates the initial slope of the rapid light curve, an indication of the efficiency of the photosynthetic mechanism (α). The pink dashed line indicates the light saturation point (I_k) for these treatments.

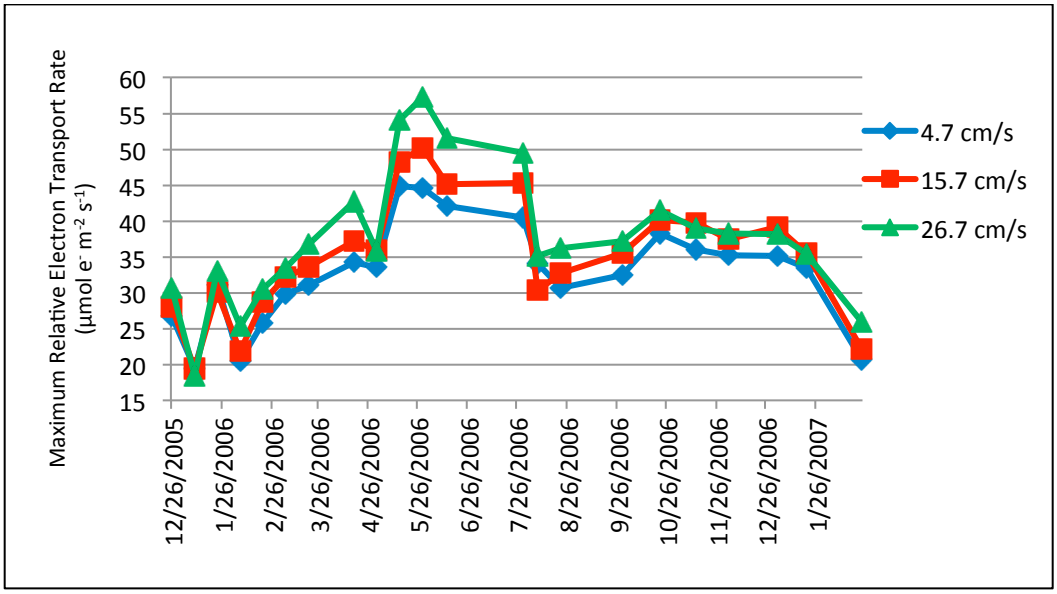

Figure 3.20: Mean maximum relative electron transport rates (P) determined from rapid light curves

The maximum relative electron transport rates (ETR) were determined from P-I curves produced for each sample and averaged for each flow treatment. Measurements

used to create P-I curves were taken on 22 occasions between December 2005 and March 2007 (Figure 3.19). Significant differences in maximum ETR between treatments were found on 19 of these occasions (Figure 3.20). On each of these 19 occasions, zooxanthellae under the highest flow conditions (26.7 cm/s) showed significantly greater average ETR values than those experiencing low flow conditions. On 12 of these occasions, the high flow symbionts had ETRs significantly higher than those in the mid flow (15.7 cm/s) treatments as well. On two of the original 19, the mid flow (15.7 cm/s) treatments showed significantly higher ETR values than the low flow (4.7 cm/s) treatments. All of these findings support the hypothesis that the zooxanthellae of corals experiencing higher flow conditions have higher rates of electron transport than those in corals that experience low flow conditions. This increase in photosynthesis at higher flow rates can be attributed, in part, to increased effluxes of oxygen under higher flow rates which have been found to increase the RuBisCO and the zooxanthellae's affinity for CO_2 (Mass *et al.* 2010).

In addition to the relationship with flow, electron transport rates were positively correlated with thermal conditions. Maximum electron transport increased under conditions of higher temperature and decreased when temperatures fell (Figs. 3.21, 3.22).

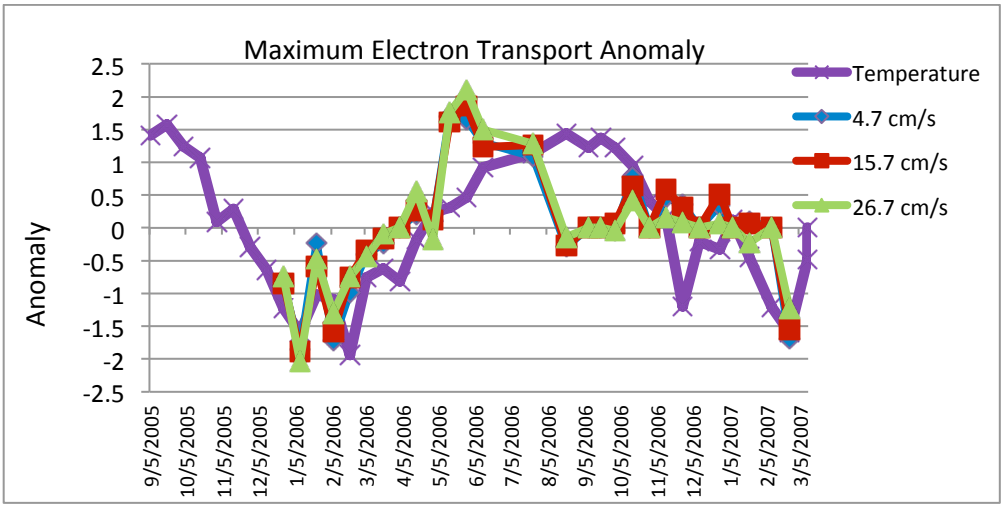

Figure 3.21: Mean anomaly of the maximum electron transport rate over time as well as the temperature anomalies over time.

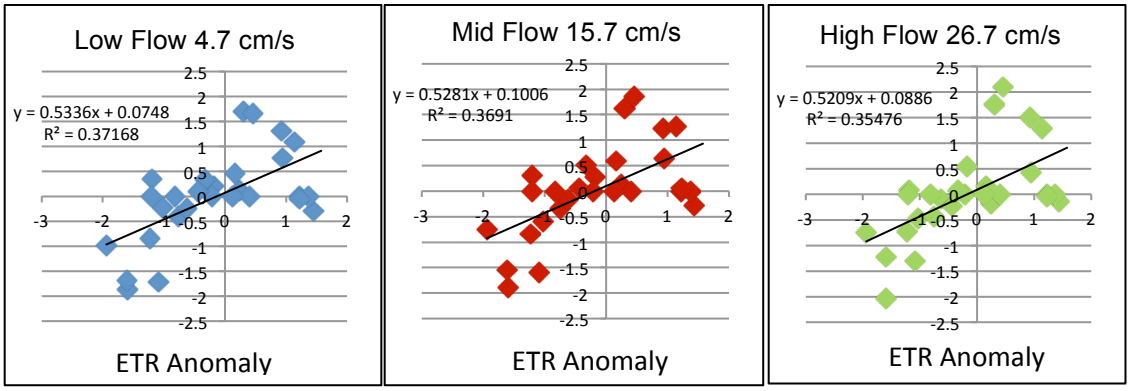

Figure 3.22: The rolling average of the anomaly of maximum ETR regressed against temperature anomalies over the course of the entire experiment, Dec. 26, 2005 – Mar. 12, 2007.

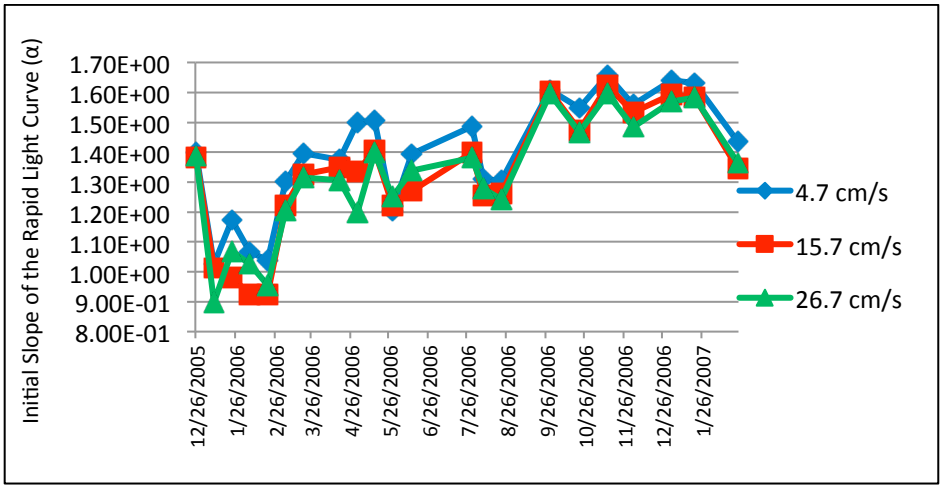

Figure 3.23: The initial slopes of the rapid light curves measured using the Diving Pulse Amplitude Modulated Fluorometer

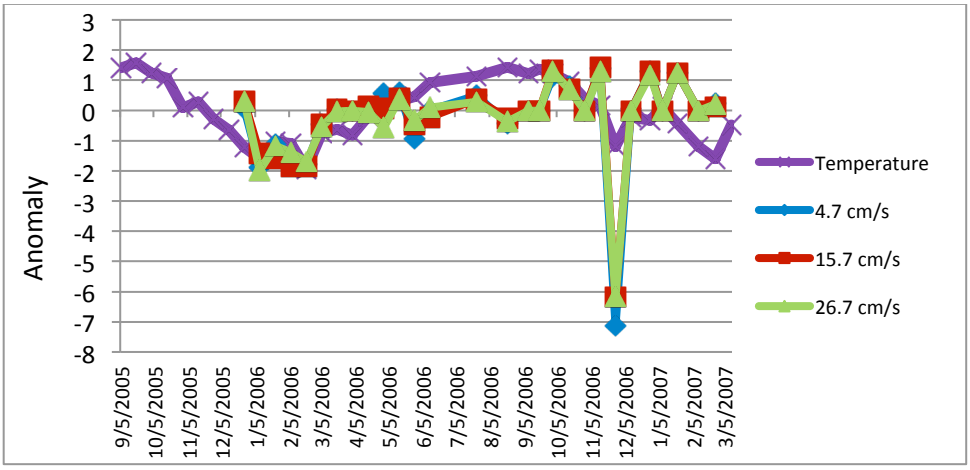

Figure 3.24: Average anomaly of (α) the initial slope of the rapid light curve over time as well as the temperature anomalies over the course of the experiment, Dec. 26, 2005- Mar. 12, 2007.

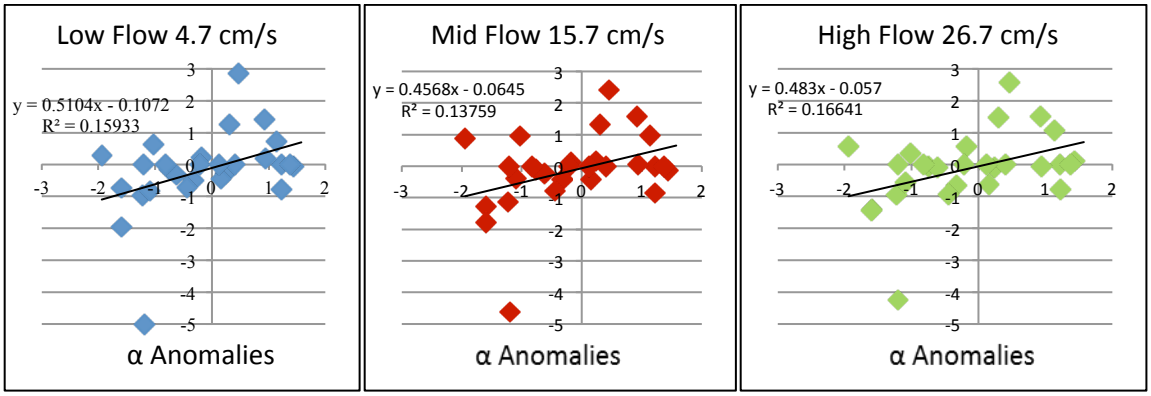

Figure 3.25: Regression of anomalies of α against thermal anomalies over the course of the experiment, Dec. 26, 2005-Mar. 12, 2007.

The initial slope of the rapid light curve (α) is a measurement of the efficiency of photosystem II. Of the 22 days that light curves were assessed, flow treatments showed significant effects on the alpha values on 10 of them (Figure 3.23). On these occasions, the low flow (4.7 cm/s) treatments had the highest values for the initial slope of the P-I curve, therefore showing the greatest photosynthetic efficiency of PSII. On 8 of these occasions, the values of α for zooxanthellae under low flow conditions were significantly higher than those exhibited by zooxanthellae under high flow (26.7 cm/s) conditions. The majority of these occasions occurred during winter months. On only one occasion, did the zooxanthellae under mid flow (15.7 cm/s) treatments exhibited α values that were even notably (though still not significantly) greater ($p=0.0709$) than those measured under high flow conditions, but not significantly different from those in low flow treatments. The photosynthetic efficiencies exhibited under low flow conditions were significantly higher ($p<0.05$) than those measured under mid flow treatments on two occasions. Photosynthetic efficiency had only a slight positive correlation with

temperature (Figure 3.24 and Figure 3.25), and all flow treatments had similar responses to thermal conditions over the course of the experiment.

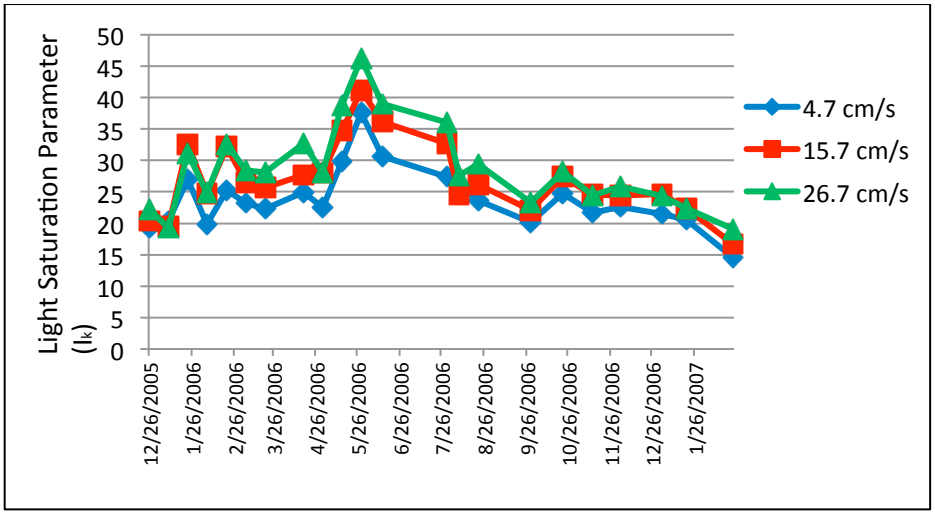

Figure 3.26: The mean light saturation point based on flow speed measured using the diving PAM fluorometer between December 2005 and March 2007.

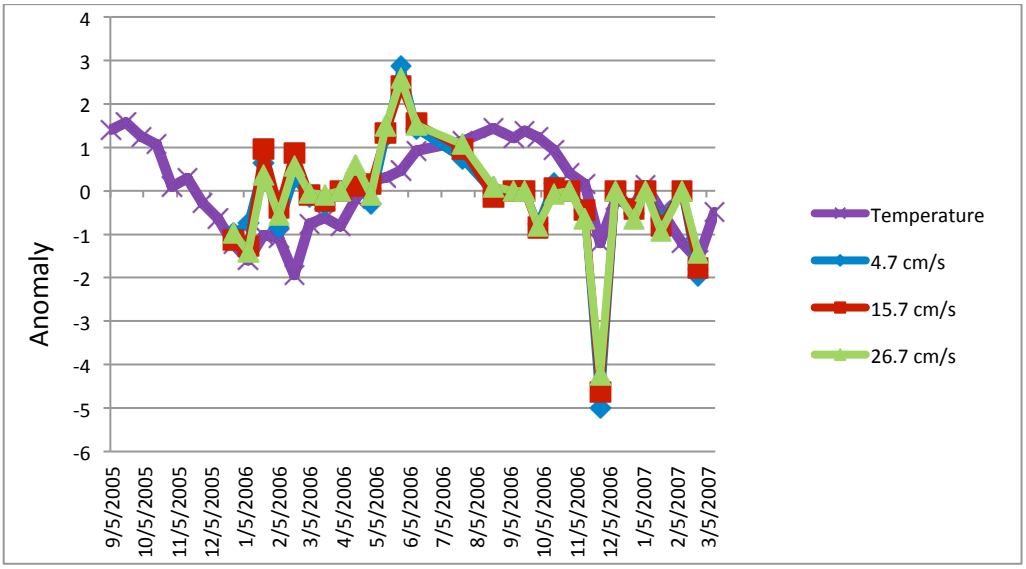

Figure 3.27: Mean anomaly of the light saturation point (I_k) December 2005-March 2007 along with the temperature anomalies over time.

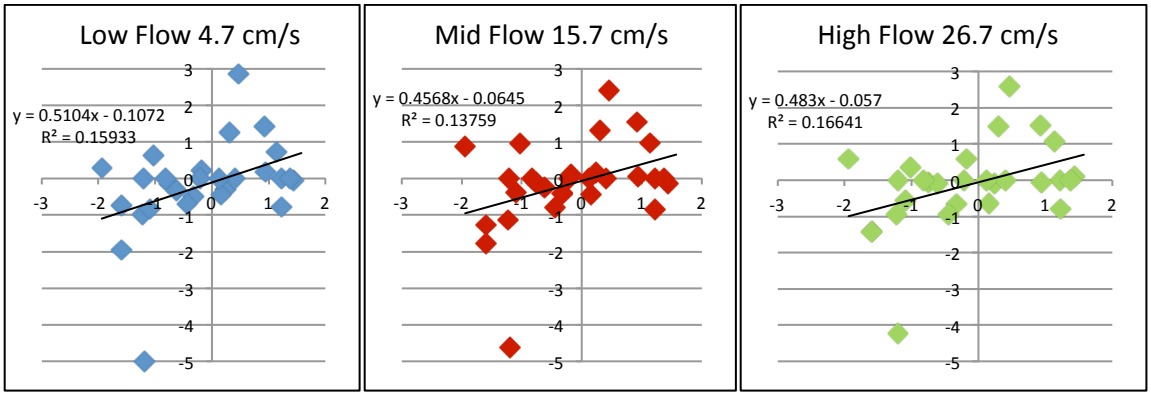

Figure 3.28: Regression of anomalies of I_k against temperature anomalies, Dec. 26, 2005- Mar. 12, 2007.

The average light saturation point (I_k) was calculated for the coral-sel samples on 22 separate occasions (Figure 3.26). Flow rate was found to have a significant influence on the light saturation point on 18 of them. On each of these occasions, the zooxanthellae in high flow treatments had the highest values, which were significantly higher (ANOVA, $p<0.05$) than those for zooxanthellae under low flow treatments. Of these, the values for light saturation point seen under high flow conditions were also significantly higher than those seen under the mid flow treatments on six occasions. The values found under mid flow conditions also showed significantly higher light saturation point values than those seen under low flow conditions on five occasions (Figure 3.26). Light saturation point was only loosely associated with thermal anomalies and corals under all flow treatments appeared to be effected in similar ways by thermal conditions (Figure 3.27 and Figure 3.28).

4) Corallite construction

Analysis showed that the positions of a number of shape coordinate landmarks varied significantly between treatments (Table 3.1, Figure 3.28). This indicates that the structures of a number of corallite components can be influenced by water flow conditions. The landmarks that varied significantly in position between flow rates included structural elements of both septae and costae of *M. faveolata* corallites.

Landmark	**Corallite Features**	**Influence of Flow**
Y4	Primary costoseptum	Higher under mid flow than under low flow
Y19	Secondary costoseptum	Higher under mid flow than under low flow
	Secondary septum	Higher under mid flow than under low flow
Y12	Outside edge of tertiary septum	Higher under mid flow than under low flow
Y15	Inside edge of tertiary septum	Higher under mid flow than under low flow
Y7	Height of the base of the outer edge of primary septa above the central columella	Lower under high flow than under low flow
Y22	Height o the base of the outer edge of the secondary septa above the central columella	Lower under high flow than under low flow
Y20	Height of the walls compared to the height of the outer theca adjacent to the secondary septum	Higher under mid flow than under high flow
Y21	Height of the walls compared to the height of the outer theca adjacent to the secondary septum	Higher under mid flow than under high flow
X4	Length of the primary costae	Longer under low flow than under high flow
X6	Length of the primary costae	Longer under low flow than under high flow
X23	Length of the secondary septa	Longer under low flow than under high flow, but not significantly so
X19	Length of the secondary costae	Longer under low flow than under high flow
X12	Height of the tertiary septa	Higher under low flow than under high flow

Table 3.1: Summary of corallite landmarks whose positions differed significantly between flow treatments along with the morphological properties they describe and their response to flow rate.

There was a surprising morphological difference between the mean height of the columellae that developed in corallites that grew under the 15.7 cm/s and 26.7 cm/s flow speeds. The mean height of the wall compared to the heights of the outer theca, when measured on either side of the secondary septum (Y20 and Y21) was higher under the 15.7 cm/s conditions than under the higher flow conditions.

In general, corallites that developed under low flow conditions had a wider, more dispersed morphology than those that developed under high flow treatments. The length of the primary costae (X4, X6) were greatest under low flow conditions, and were significantly longer than those that developed under high flow treatments. The secondary septa (X23) that developed under low flow conditions were longer than those grown under high flow conditions, though not quite significantly so ($p=0.0684$). The secondary costae (X19) that developed under low flow conditions were significantly longer than those that grew under either of the higher flow speeds. In addition to the lengths of the septa, there were also significant differences (ANOVA, $p<0.05$) between the heights of tertiary septa (X12) that were developed under the various flow regimes, with tertiary septa developed under low flow conditions growing significantly higher (ANOVA, $p<0.05$) than those that grew under high flow conditions.

Figures 3.30, 3.32, and 3.34 are simplified representations of the primary septa of corallites formed under each of the tested flow conditions. Figures 3.31, 3.33, and 3.35 are thin plate splines of the same septa. These compare the silhouettes of the primary septa formed under each of the flow conditions to a “reference” plot of the primary septa formed under low flow conditions. The grid of the spline is deformed, or “bent” to

illustrate how the reference plot would have to be changed in order to be identical in shape and size to the reference plot. Greater deformation of the grid indicates greater differences in the morphologies of the septa being compared.

Examination of the thin plate splines (Figs 3.31, 3.33, and 3.35) reveals the similarity between the generalized form of the primary septa grown under mid and low flow treatments. The grid shows that only minor adjustments would be needed in order to align the silhouettes. Alternatively, they also illustrate the differences between the forms of the septa grown under low and high flow treatments. Mid flow conditions lead to taller septa than low flow conditions (also taller than high flow conditions, but not significantly so). Low flow conditions lead to corallites with longer septa and costasepta and lead to wider (more dispersed corallites) compared to those formed under higher flow rates. Low and mid flow rates cause corallites to form with septal base heights that are higher (compared to the columella) than those found in corallites formed under high flow conditions. These results are similar to those found in previous studies in which some acroporids were seen to produce corallites with reduced features, thicker theca, and synapticulae when they were located in exposed areas with higher water motion (Brown *et al.* 1985)

Since the number of corallites per unit of new area did not differ between treatments, the changes in corallite morphology may be related to metrics that were significantly impacted by water flow rates. Wider corallites that formed under low flow conditions would have less skeletal material between them. These differences in corallite width and associated modifications to skeletal structure of the colony could be related to

the rapid area growth rates of colonies in low flow treatments. The corallite features that developed with higher relief and therefore higher fine scale rugosity under flow rates of 15.7 cm/s compared to those that developed under flow rates of 26.7 cm/s may increase turbulence of flow over the corallite tissues under this lower flow. Increased turbulence breaks down boundary layers and increases the rates of nutrient and gas exchange between the organism and surrounding waters.

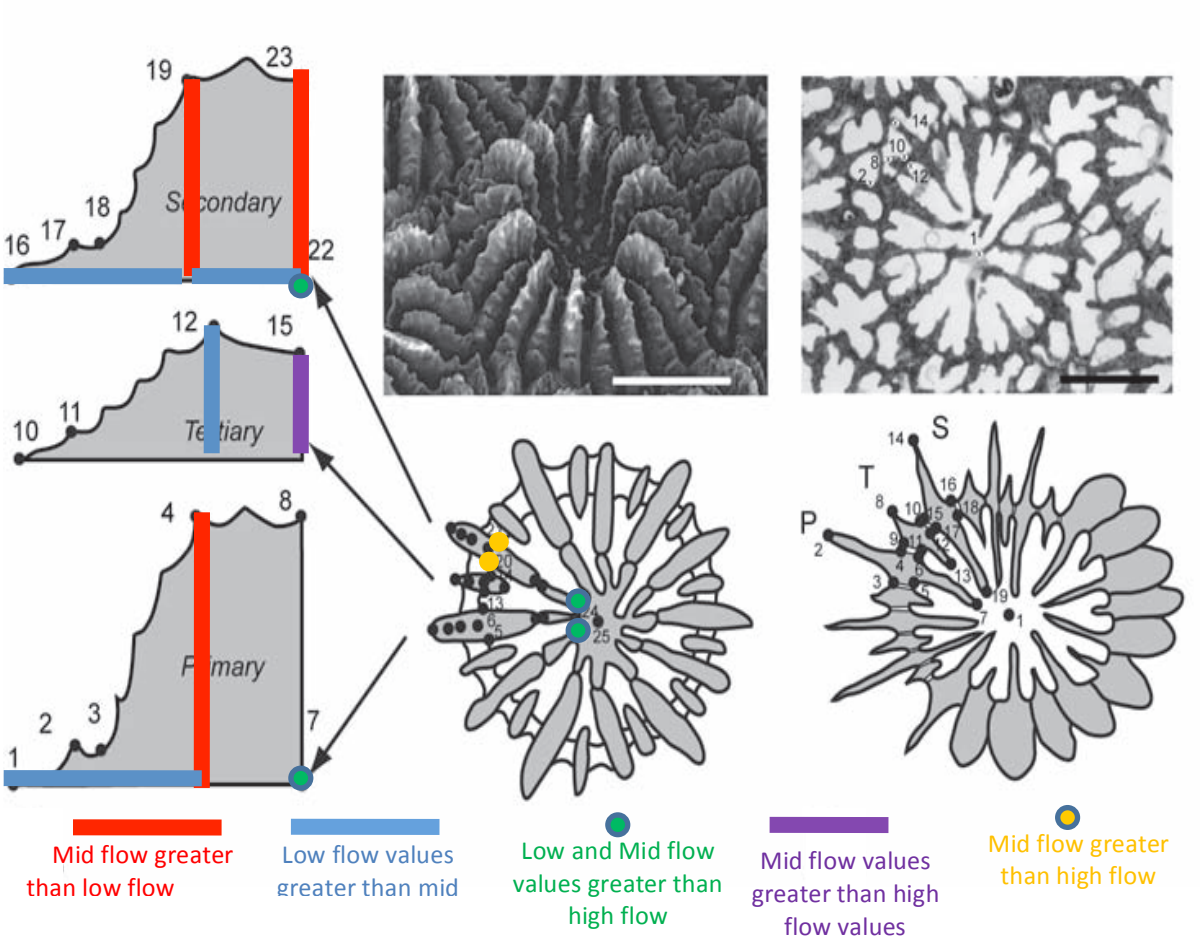

Figure 3.29: Results of morphological analyses: Components of corallites that varied significantly with water flow speed are indicated with colored lines or dots. Features that had significantly greater values in mid flow corals than low flow corals are indicated by red lines. Those in which low flow corals show greater values than mid flow corals are indicated by blue lines. Features that have higher values in the mid flow corals than high flow corals are indicated by purple lines or yellow dots. Features that have higher values in low or mid flow corals than high flow corals are indicated by green dots. Figure based on diagrams of *M. annularis* from Klaus et al. (2007).

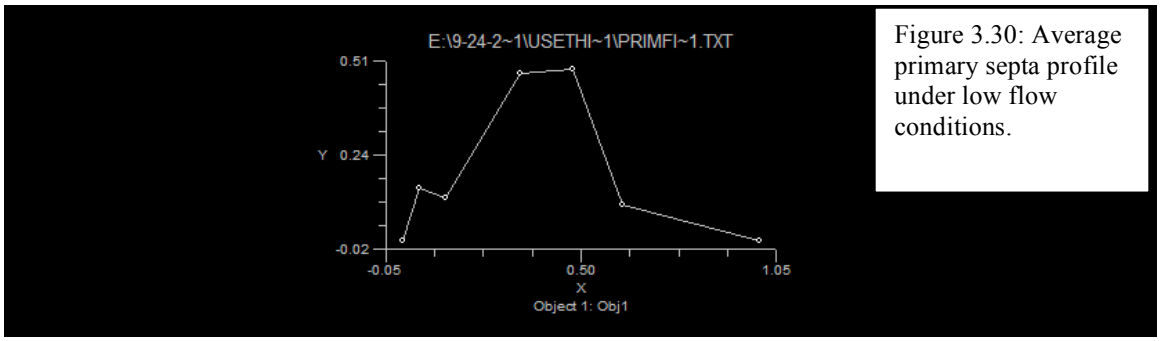

Figure 3.30: Average primary septa profile under low flow conditions.

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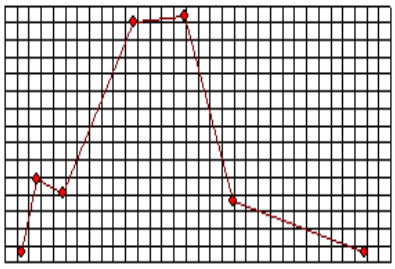

Obj1 -> Obj1

Figure 3.31: Profile of a primary septum under low flow conditions plotted on a "spline" plot. All spline plots will use this low flow plot as a reference.

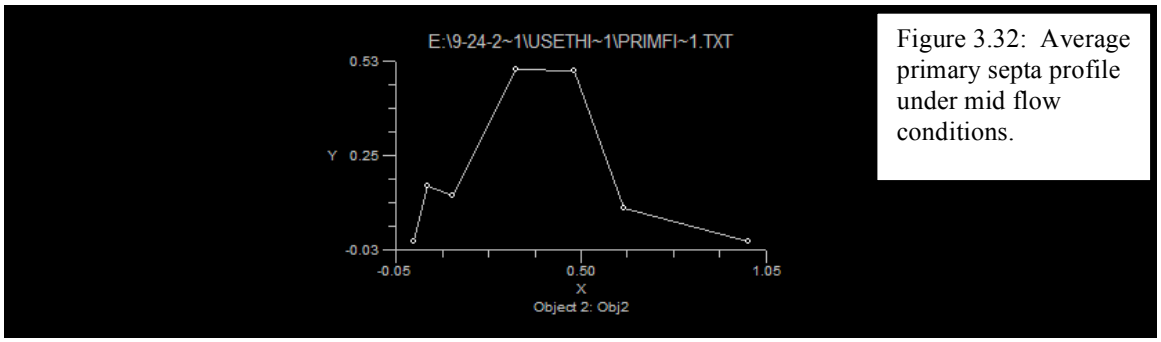

Figure 3.32: Average primary septa profile under mid flow conditions.

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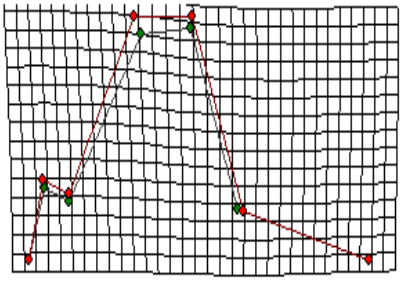

Obj1 -> Obj2

Figure 3.33: Profile of a primary septum under mid flow conditions plotted on a "spline" plot (red). The green is the reference plot. The grid shows how the reference plot would have to change in order to map those points onto the "target" plot.

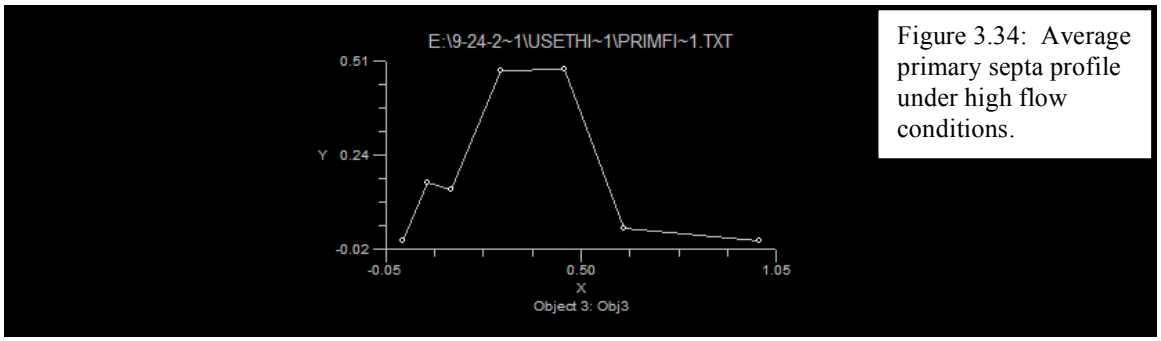

Figure 3.34: Average primary septa profile under high flow conditions.

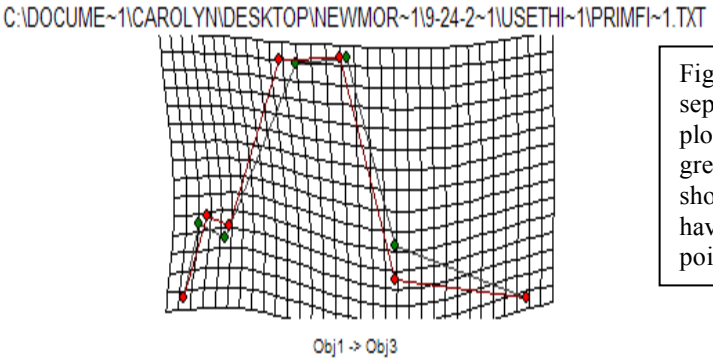

Figure 3.35: Profile of a primary septum under high flow conditions plotted on a "spline" plot (red). The green is the reference plot. The grid shows how the reference plot would have to change in order to map those points onto the "target" plot.

5) Zooxanthella community

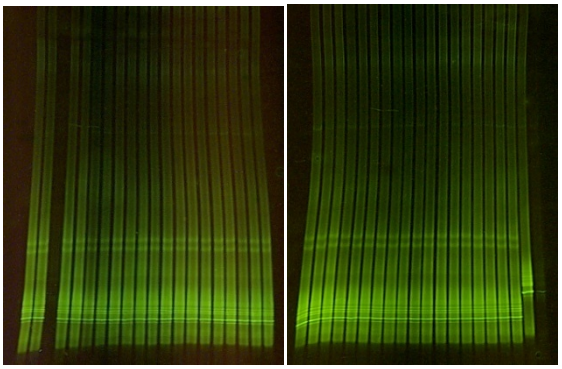

Figure 3.36:DGGE gels based on the ITS2 region of the symbionts isolated from the coral-sel colonies.

Zooxanthellae play an integral role in the overall health and growth of their host colonies. The properties of the symbionts, in terms of their sensitivity to environmental conditions (temperature etc.), can have substantial impacts on the growth of the holobiont.

At the conclusion of the experiment, all colonies were dominated by *Symbiodinium* in the common D1 clade (Figure 3.36). Differences in water flow rates did not show any influence on the cladal composition of the zooxanthella communities contained in *Montastraea faveolata* colonies. Since the final cladal community make-up did not differ between flow treatments, the differences in growth patterns could not be attributed to differences in the symbiont communities present in the colonies in each flow treatment. The "blasted" surface areas and volume of liquid used to blast the tissue varied between individuals and was not measured with enough precision to ensure accurate measurements to compare zooxanthellae densities in each colony. Information on the original cladal make-up of the original community was not available. However, numerous studies carried out on *M. faveolata* colonies from the same source population, held at the same facility, and in the same tanks have all shown similar cladal communities when tested.

E) Discussion

In natural environments, physical factors interact with one another as they influence organisms. It can be difficult to isolate individual factors and determine how they alone are influencing the organisms. Understanding the effects of individual

physical factors can provide researchers with important information about organisms in the natural environment as well as how they will grow within controlled environments. The results of this study show how that flow alone can have a significant influence on some growth metrics for *Montastraea faveolata.* The change in mass/area can be considered as a proxy for changing density. Under mid flow (15.7 cm/s) conditions, the density of the new growth area of the *M. faveolata* was higher than the new growth formed under high flow conditions. These results disagreed with previous studies (Bottjer 1980, Schumacher and Plewka 1981, Scoffin *et al.*1992, Smith *et al.* 2007, Schutter *et al.* 2010), which all showed increases in skeletal density of numerous coral species with increasing water flow rates or hydraulic energy. Patterns of higher density, and increased branch thickness (for branching species) under higher flow exposure occur even within colonies. Under high flow conditions colony branches on the "upstream" side of the colony have greater thickness and densities compared to downstream branches (Nakamura and Yamasaki 2005, Suzuki 2007, Mass and Genin 2008). It appears that under conditions of higher physical stress (higher flow), when there may be a greater risk of breakage due to physical "disruption" (due to impacts of debris carried in the water, or physical strains caused by the water motion itself), corals put more resources into increasing skeletal density in order to resist damage from stresses related to water motion. Conversely, individuals that have a minimal risk of damage from these sources dedicate a greater portion of their growth effort to expanding their surface areas.

The patterns in surface area growth that were observed, with more rapid increases under lower flow conditions than high flow conditions agree with previously observed

patterns in which rapidly growing corals have been found to have lower density skeletons than slower growing ones (Holstein pers. comm.). Growth and calcification rates vary between genera and species. Skeletal density is important for the structural integrity of corals, particularly those with branching or foliaceous morphologies. However, the principal mechanisms controlling density are likely common across groups and relate to calcification rates and the differences in the allocation of resources among growth parameters. The primary driver of differences in calcification rate in *Porites* appears to be variations in linear extension rate (e.g. Scoffin *et al*.1992, Lough *et al*. 1999,Elizalde-Rendon *et al*.2010). For *Montastraea*, differences in skeletal density exert the greatest control on calcification rates (Carricart-Ganivet, 2004, Davalos-Dehullu *et al*., 2008; Carilli e*t al*., 2010). Linear extension of species within this genus exceeded the skeletal density contribution to calcification rates, as seen in *Montastraea faveolata* from the Mesoamerican reef. In general, calcification rates reflect the resources available in the environment for the active deposition of the calcareous material (Fang *et al* 1989). The density and extension rates of each coral are the result of the manner in which the animal uses the material to construct its skeleton (Carricart-Ganivet and Merino 2001). *M. faveolata* colonies in the Florida Keys were observed to maintain skeletal extension rates under suboptimal growth conditions by sacrificing skeletal density (Cook *et al*. 2002). This response has been termed "stretch modulation of skeletal growth" (Carricart-Ganivet and Merino 2001, Cook *et al*. 2002) and may allow colonies to be competitive in sub-optimal environmental conditions. In areas of extremely low

light, mounding corals have been observed to have very low growth rates and very dense skeletons (Holstein pers. comm.).

Together, these previous observations shed light on the patterns seen in this experiment. In areas with sufficient light, surface area continues to increase even when conditions are not optimal, but growth is dependent on the availability of resources. Therefore, while both the low and high flow conditions may be "suboptimal" for this species, the low flow treated corals were able to maintain their areal growth rates, while the high flow treated corals showed decreased growth.

The digital images were also examined to determine changes in the number of polyps present within the colony. This information was used to determine if the density of polyps developing in newly formed areas is dependent upon flow rate. The density of a colony's skeleton can be influenced by the spacing and size of corallites due in part to the ratio of wall thickness to corallite diameter. When all other growth variables are equal, the skeletons of corals with polyps spaced farther apart would be denser than those of corals with closely spaced corallites (Highsmith 1981). The concentration of corallites in an area will influence the overall porosity of a coral skeleton. Denser skeletons are expected to develop when the coral experiences greater possibilities of physical damage. Skeletons of branching colonies are often more dense than massive growth forms since they are more likely to experience physical breakage (Chamberlain 1978). It would therefore be expected that if the density of polyps per unit area varied between colonies of the same species exposed to different flow treatments, that skeletal density would vary similarly between treatments.

In this study, the increases in the surface area and mass of the *M. faveolata* fragments were positively correlated with temperature. However, the amount of mass added per unit of new surface area was negatively correlated with temperature. This is similar to patterns seen by other researchers (Cruz-Piñón *et al*. 2003) in which this species maintained high extension rates even in lower temperatures, but decreased the amount of material laid down within these new areas.In addition to high summer temperatures, the marked decrease in mass growth rate during August-September 2006 was hypothesized to be related to the nearly daily rainstorms that occurred during this period. The experimental system was located outside and was subjected to the influences of local weather conditions. A combination of the regular influxes of fresh water, and decreased light availability due to these frequent storm conditions may have played an additional role in causing decreased mass growth rates during this period.

Much of the existing research on salinity effects on corals was carried out in natural systems where changes in salinity were coupled with changes in nutrients caused by stream or river inputs or runoff due to precipitation. It is difficult to uncouple these variables in natural systems. While there are few previous studies that have examined the impacts of decreased salinity alone on coral, some species including *Siderastrea siderea* (Muthiga and Szmant 1987), *Porites lutea*, and *Pocillopora damicornis* (Moberg *et al.* 1997) have been shown to have adverse reactions to changes in salinity. These previous studies found decreases in photosynthetic production under extreme decreases in salinity. However, changes that are likely to occur in natural reef environments were not seen to have measurable whole organism effects in *Siderastrea siderea.* While daily

precipitation did cause regular influxes of freshwater to the experimental tanks, the minimal decreases in salinity that likely resulted from these episodes were unlikely to have made marked contributions to the differences in mass deposition rates seen during these months.

The photosynthetic activity of the symbiotic zooxanthellae also showed some seasonal patterns. While this study did not explicitly measure light availability in the tanks over the course of the 18 month experiment, photosynthetic activity did show an observable association with temperature which may also be associated with light. These responses were similar to those seen by Warner *et al.* (2002). They hypothesized that these shifts in photosynthetic capacity are most likely due to biochemical processes within the dinoflagellates that lead to alterations of photoprotection and photodamage.

F) Recommendations for Aquaculture

Based on the findings of this experiment, a number of recommendations can be made to aquaculturists that are seeking to culture *Montastraea faveolata* for scientific, restoration, and aquarium trade purposes. If the intention is to produce the maximum amount of living surface area/ number of polyps for experimental purposes or as "brood stock" for later culture, the colonies should be affixed to plates that allow simple lateral growth rather than on "pegs" which gives minimal opportunity for lateral growth, but encourages skeletal thickening and vertical growth.

In addition, when the intention of culturing is the production of the greatest amount of living surface area, colonies affixed to tiles (or similarly flat surfaces) should be placed in low flow conditions. According to the findings in this study, low flow

conditions (of approximately 4.7 cm/s) are most conducive to the rapid lateral growth of tissue in this species. This would be the preferred approach for aquaculturists producing coral tissue for experimental purposes and the aquarium trade. It produces the greatest amount of healthy coral tissue surface area, since under these conditions corals apportion the majority of their resources toward lateral growth rather than vertical extension or strengthening/thickening of the basal skeleton. Corals cultured under this method would be ideal for low stress environments like ornamental aquaria and simple experimental conditions. It may be that these low flow conditions are sub-optimal for this species, since previous researchers have noted that some species will continue extension at high rates while decreasing calcium carbonate deposition causing decreases in skeletal density (Cruz-Pinon *et al.* 2003). Although this manner of growth would not be ideal under most natural conditions, it is advantageous when the intent of the culturing process is to produce the maximum area of living coral tissue.

Alternatively, if the intended use of the cultured colonies is reef restoration, the site of proposed restoration must be taken into account before culturing begins. Restoration areas that have flow rates less than approximately 20cm/s and less chance of algal overgrowth can be restored with colonies that have been grown under conditions of approximately 15 cm/s on flattened plates. This culturing method will allow the greatest amount of living coral tissue to be produced for transplantation to the reef in the shortest amount of time. The flattened form of the cultured colonies would be ideal for transplantation to areas where algal overgrowth and similar competition is minimal.

Areas that experience higher flow rates and have more algal competition are likely to benefit most from restoration with corals that have been grown in mid flow (~15.7cm/s) conditions, since they had high growth rates in terms of both surface area and mass per unit of area. If the area to be restored is regularly threatened by algal overgrowth, cultured colonies that are intended for implantation on the reef that are grown on "peg-like" bases (rather than flattened tiles) could have a greater likelihood of survival and future growth. Colonies grown on these raised bases would give them the best opportunity to survive. They could then serve as sources of gametes and larvae for the restored reef and nearby areas since they would be less endangered by algal overgrowth during the early stages of their transplantation when they are most vulnerable. The "peg-like" bases would encourage the colonies to put resources into skeletal calcification and vertical growth rather than lateral expansion. This allows them to resist disturbances from physical impacts and high water motion as well as algal overgrowth since the colony base is slightly raised above the substrate that would be most impacted by the effects of both algal overgrowth and abrasion. This would give transplants the greatest likelihood of becoming long-term residents and ecological contributors to the reef community.

Chapter 4: Flow, Light, and Changing Water Chemistry: Their Impacts on CnidarianGrowth

A) History of multifactor controlled studies

An organism's "fundamental niche" is the range of environmental conditions under which it can survive and reproduce in the absence of biotic interactions (Hutchinson 1957). The shape of the niche will relate to how the organism will react to a suite of environmental conditions including, water flow rates, light levels, nutrient availability, and temperature. Niche also depends upon species, location, and symbiont condition, and the interactions of these factors. The way that these niches will change as the global climate is altered could play a critical role in determining the areas that will be viable habitat for many species of interest in the future.

Traditionally, biological research studies have examined the effects of differences in a single independent variable on survival, growth, behavior, and other quantifiable metrics of living organisms. In nature, however, variables rarely change one at a time. Instead, environmental variables interact, creating many different environmental conditions that organisms may be exposed to. It is the combination of environmental variables present that determines the conditions in which organisms will function. Single factor studies are very common and in the last 20 years, two factor studies have become slightly more common. The most commonly studied factors (for corals) include: temperature (Rodriguez *et al.* 2010, Donner 2011, Edmunds *et al.* 2011, Oliver and Palumbi 2011), light (Frade *et al.* 2008, Rodolfo-Metalpa *et al* 2008, Banaszak and Lesser 2009, Hoogenboom *et al.* 2009 , flow (Bruno and Edmunds 1998, Sebens *et al.* 2003, Nakamura and Yamasaki 2005, Carpenter *et al.* 2010*,* Jimenez *et al.* 2011, Mass *et*

al. 2011, , nutrients (Shimoda *et al.* 1998, Littler *et al. 2006,* Haas *et al.* 2009, Tanaka *et al.* 2010, , and carbonate related factors (Gattuso *et al.* 1999, Schneider and Erez 2006, Hoegh-Guldberg *et al.* 2007, Jury *et al*. 2010, dePutron *et al.* 2011, Anthony *et al.*2011). However, the manipulation of multiple factors in a controlled study is still rather rare (Edmunds *et al.* 2012) compared to the plethora of studies examining single variables. Rather than investigating the effects of a single variable, it is imperative that the interactive influences of multiple variables be examined for species and habitats that scientists wish to study.

Most of the research in coral biology has focused on the effect of temperature, light, water flow, nutrients and pH on coral growth, survival, and reproduction. However, the manipulation of multiple factors in controlled studies is rare compared to studies examining single variables.

As global warming becomes an increasing concern, many of the recent multi-factor studies that have been carried out have examined the effects of temperature in concert with another variable, for example light (Glynn *et al.* 1992, Goulet *et al.* 2005) or pCO_2 (Lesser 1996, Reynaud *et al* 2003, Wissman 2003, Langdon and Atkinson 2005, McClanahan *et al.* 2005, Rodolfo-Metalpa *et al.* 2008a, Rodolfo-Metalpa *et al* 2008b, Schönberg *et al.* 2008, Edmunds 2009, Edmunds and Lenihan 2010). Since it had been observed that both increased temperature and light exposure often appeared to be stressful to many species of coral, the combined effects of light (both visible and UV radiation) and temperature on corals is one of the most commonly studied pairings (Glynn *et al.* 1992, Lesser 1996, Wissman 2003, Goulet *et al* 2005, Rodolfo-Metalpa *et*

al 2008, Schönberg *et al.* 2008, Edmunds 2009). While elevated temperature has a greater negative impact upon coral health than increased UV radiation (Glynn *et al.* 1992), the combined influence of heightened temperature and UV radiation was larger than increased temperature alone (Glynn *et al.* 1992, Lesser 1996, Wissman 2003).

Increased temperatures combined with varying light levels have been examined by a number of researchers (Rodolfo-Metalpa *et al.* 2008, Schönberg *et al*. 2008, Edmunds 2009). At constant temperatures (14˚C and 30˚C) changes in light did not influence the concentration of chlorophyll or zooxanthellae density within the tissues of *Cladocera caespitosa*. When both low temperature (14 ˚C) and low light (30µmol m^{-2} S^{-1}) co-occurred, chlorophyll concentrations increased. This increase was presumed to allow sufficient level of autotrophy under these conditions (Rodolfo-Metalpa *et al.* 2008).

Other studies of light and temperature have been hindered by variation within samples. A study of the response of sponges and algal symbionts to heat and light by found decreases in the photochemical efficiency of zooxanthellae of corals exposed to increased heat and light levels, but the decreases were not significant due to the variability within samples (Schönberg et al. 2008).

Biomass change in the coral, *Porites irregularis* varies based on the temperature and light levels they are acclimatized to as well as the new conditions to which they are exposed (Edmunds 2009). Acclimatization of corals to ambient temperatures and shade conditions had an ameliorating effect on the detrimental influence of increased temperatures. Conversely, corals that were acclimatized to shaded conditions and low temperatures showed greater negative impacts upon exposure to high temperatures.

When temperature and water flow rates were coupled in the analysis of recovery from sub-lethal damage in juvenile *Porites* colonies, these factors and their interactions had a variety of effects on the experimental colonies. (Edmunds and Lenihan 2010). Growth and healing was retarded by increased temperatures, but were unaffected by flow rates. However, photochemical efficiency of damaged corals increased under flow rates of 21 cm s^{-1} and decreased under flow rates of 4 cm s^{-1}. Under higher water flow rates, photochemical efficiency was 11% lower under higher temperatures (29.6˚C) compared with the photochemical efficiency at 26.7 ˚C. Under low flow rates (6 cm s^{-1}), temperature did not significantly influence the photochemical efficiency of the colonies. Tissues of damaged *Porites* colonies showed the most rapid return to normal appearance under low flow and low temperature conditions.

The effects of water flow can also interact with the concentration of food particles to influence the heterotrophic success of azooxanthellate corals, such as *Lophelia pertusa* (Purser *et al.* 2010). This species was observed to have the greatest capture success under conditions combining very low flow rates (2.5 cm/s) with high food density. This combination of conditions appears to produce the most advantageous balance of high encounter rates and tentacle capture capabilities. The high encounter rates are the result of high food densities. The capture capabilities are high due to both the tentacles' abilities to remain extended with little deformation under low flow conditions, and the fact that low flow does not provide the captured particles with sufficient momentum to escape the mucus that covers the tentacles. Although *L. pertusa* often lives in areas known for generally high flow conditions, flow rates are greatly decreased near the

substrate, and further slowed by the complex thickets formed by these branching colonies.

Light and water flow rates can interact to influence changes in buoyant weight, surface area, and skeletal growth rate. In *Galaxea fascicularis*, flow velocity becomes more important to coral growth as irradiance levels increase (Schutter *et al.* 2011). Increased flow allows for increased photosynthetic rates by the symbiotic zooxanthellae. Changes in buoyant weight showed an interactive effect of light and flow rate, with flow becoming more influential at higher light levels. This may be due in part to the fact that under lower flow rates, colonies need greater amounts of metabolically costly photoprotective mechanisms and/or compounds (Schutter *et al.* 2011).

In addition to the readily apparent changes in growth and coloration, environmental conditions can influence more subtle changes in the affected corals. Light availability and feeding rates have been seen to affect the fatty acid and sterol composition of some species, with corals exposed to higher light possessing higher concentrations of fatty acids and sterols, but smaller amounts of phytols than shaded corals. The light levels that a coral experience have also been seen to influence how the coral will utilize the energy gained from heterotrophic feeding. Fed corals in high light conditions are more likely to direct their energy toward increasing calcification, lipid, and protein content while corals in low light direct energy toward increasing their growth rates, lipid storage, saturated fatty acids, and membrane constituents (Treigner *et al*. 2008). Light and feeding can also impact the nitrogen isotopic composition of both hosts and/or zooxanthellae (Reynaud *et al.* 2009). Feeding influences the nitrogen

composition of the colony, with starved corals exhibiting higher levels of $\delta^{15}N$ than fed corals. This is due to the recycling of nitrogen within the coral holobiont, and preferential excretion of lighter nitrogen (Reynaud *et al.* 2009). Light and feeding were found to influence the fatty acid and sterol content of both zooxanthellae and host tissues in *Turbinaria reniformis*. Corals that experienced higher light exposure showed lower relative phytol content, but higher concentrations of fatty acids and sterols, compared to those that were shaded. Light exposure also influenced the way that acquired nutrients were utilized in colony growth and maintenance. Under low light conditions, corals that were fed exhibited increased growth rates, and more stored lipids, including saturated fatty acids and membrane constituents. Under higher light conditions, the energy gained from heterotrophic feeding was used to increase calcification, chlorophyll content, and protein content (Treigner *et al.* 2008).

The influence of light exposure on the calcification abilities of *Porites compressa* under various carbonate ion concentrations has also been investigated (Marubini *et al.* 2001). Decreases in the aragonite saturation state of seawater have been seen to cause decreases in the calcification rates of corals (Langdon and Atkinson 2005). Decreases were proportional to the growth rates observed under control pH conditions. For instance, growth rates of *P. compressa* increases with increasing light. However, as aragonite saturation state decreases, growth rates of this species fall in proportion to the growth rate expected under "normal" water chemistry conditions. This means that under low pH conditions, growth rates at higher light levels will be more depressed than growth rates at lower light levels. (Marubini *et al.* 2001).

These responses of the interaction between water chemistry and temperature do not follow the same patterns of decreased growth with increases in both temperature and light. One study observed an increase in chlorophyll *a* content and a 50% decrease in calcification when corals were exposed to both high temperatures and high CO_2 conditions. Under lower temperatures (25˚C compared to 28˚C), changing water chemistry conditions did not significantly influence calcification rates (Reynaud *et al* 2003).

These studies are important because, while changes in oceanic pH will have global effects, the related physical and biological changes that will result will occur on smaller local scales that are subject to different flows, light levels, and nutrient levels. Increases in nitrate concentration reduce calcification in some studies (Marubini and Davies 1996, Marubini and Thake 1999), but not in others (Marubini and Atkinson 1999) perhaps because the difference in response was due to differing water chemistry between the studies (Marubini and Atkinson (1999). A moderate addition of nutrients to seawater has been shown to help mitigate the negative effects of decreased pH on calcificiation (Langdon and Atkinson 2005, Holcomb *et al.* 2010). This supports the idea that there are conditions that can alleviate the stress placed on corals due to changing water chemistry.

Langdon and Atkinson (2005) also examined the influence of changing light levels on corals along with temperature and pCO_2 interactions. This study that aragonite saturation state affects calcification rates and carbon production, though there was no interaction between aragonite saturation levels and the seasonal changes in light or temperature. When water with decreased aragonite saturation was supplemented with

additional nutrients, the experimental corals showed increases in carbon production and decreases in calcification rates. These results supported the hypothesis that the decreases in calcification that are often seen in response to increases in pCO_2 may be caused by competition between photosynthesis and calcification for limited amounts of dissolved organic carbon within the colony.

Other studies have attempted to examine the interactive effects of more than two factors (McClanahan *et al.* 2005, Rodolfo-Metalpa *et al.* 2008, Hoogenboom and Connolly 2009, Lesser *et al.* 2010). In some cases, the factors studied included the genetic traits of the species being examined (Lesser *et al.* 2010). In some areas of Mauritius, members of the dominant *Acropora* and *Montipora* taxa were more susceptible to increased temperatures, and corals in areas of high flow rates appeared more susceptible to bleaching due to high temperature than members of the *Galaxea,Goniopora, Favites,* and *Pavona* genera (McClanahan *et al.* 2005). It was suspected that the decreased tolerance for higher temperatures in high flow areas was due to the role of water flow in minimizing background variability in stress conditions. The presence of established high flow conditions was attributed with decreasing the tolerances of the local corals to thermal variation (McClanahan *et al.* 2005). The large scale patterns of flow related tolerance to heat stress witnessed in Mauritius may also be related to the structure of coral communities that developed under the observed flow regimes. Areas characterized by high flow conditions are often inhabited by large communities of *Acropora,* which is known to be temperature sensitive (Loya *et al*. 2000). The taxonomic identity of the corals found in particular flow environments should also be

considered in determining the bleaching susceptibility of corals in areas of interest (van Woesik *et al.* 2005).

A study of the interactions of light, temperature, and heterotrophy on a temperate species, *Cladocera caespitosa* again found that temperature was the major factor influencing coral growth. There was an interaction between temperature and heterotrophic capacity. At lower temperatures, feeding success had a greater influence on coral health. High feeding rates at low temperatures led corals to possess higher densities of zooxanthellae and chlorophyll which contributed to greater photosynthetic efficiency. Although, this supposed influence on photosynthetic efficiency was influenced by temperature and feeding, there was no significant impact of irradiance on the experimental colonies (Rodolfo-Metalpa *et al.* 2008).

As the previous study demonstrated, characteristics of the living organism can interact with environmental factors to alter individual responses of growth and metabolic activities. Light levels and water flow rates exist in related gradients along coral reefs. These conditions together can act synergistically to have strong influences on both tissue biomass and reproductive output in numerous species (Hoogenboom and Connolly 2009). The responses of impacted colonies depend in part on the morphological characteristics of the colonies, including shape and size. Branching colonies and larger colonies can maintain positive growth rates under a wider range of environmental conditions relative to those of smaller mounding or foliaceous morphologies (Hoogenboom and Connolly 2009).

Previous research studies have examined the interactions of several physical factors on coral growth. While light and water motion have been examined in the past, no prior studies have inspected how both of these factors interact with changing water chemistry. An understanding of these interactions will become increasingly important as ocean acidification continues in the coming decades. This information will be of particular importance to individuals designing marine protected areas intended to preserve coral reef habitats in the future. I sought to expand the current understanding of interactive influences on cnidarian species by examining the impacts of changing water chemistry, water flow rates, and light levels in a controlled environment. This necessitated the construction of a laboratory based system where the variables under study could be controlled and outside influences could be minimized.

B) Methods:

1) Semi-recirculating flume system

To examine the effects of changing water chemistry, flow rate, irradiance, and their interactions on tropical cnidarians, two identical flume tanks were constructed. Each tank was a "semi-recirculating" system with a constant inflow and outflow of filtered seawater. Each system held approximately 120L of seawater, exchanged at a rate of ~325 ml per minute. The relatively high turnover rate (~6 h) allowed replenishment of gases and nutrients throughout the experiments.

a) Flow treatments

Each experimental flume system (Figs. 4.1 and 4.2) was constructed using a rectangular open-top polyethylene tank (122 cm x 61cm x 30.5 cm). Clear plexiglass dividers were used to provide three raceways of equal width (20 cm) along the length of the tank. The raceways allowed the separation of the treatment flow speeds. The dividers were secured to the upstream end of the tank and the bottom of the tank using marine epoxy. The dividers extended 101.5 cm from the inflow wall along the length of the tank to an egg-crate divider that spanned the entire width of the tank. The remaining 20.5 cm long area extended the entire width of the tank. This was the "mixing area" where the water flowing through the separate raceways could merge and become re-distributed through the pumps and different flow treatments in that flume. Within the "mixing" section, a weir of plexiglass was positioned to encourage water mixing. Each of the raceways had a flow straightener consisting of eight staggered layers of "egg-crate" positioned approximately 8 cm from the site of water inflow.

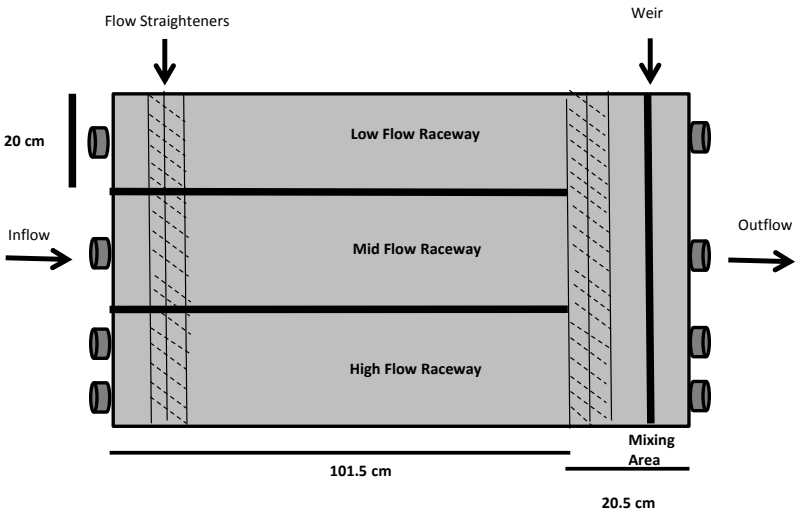

Figure 4.1: Diagram of semi-recirculating flume tank as seen from above.

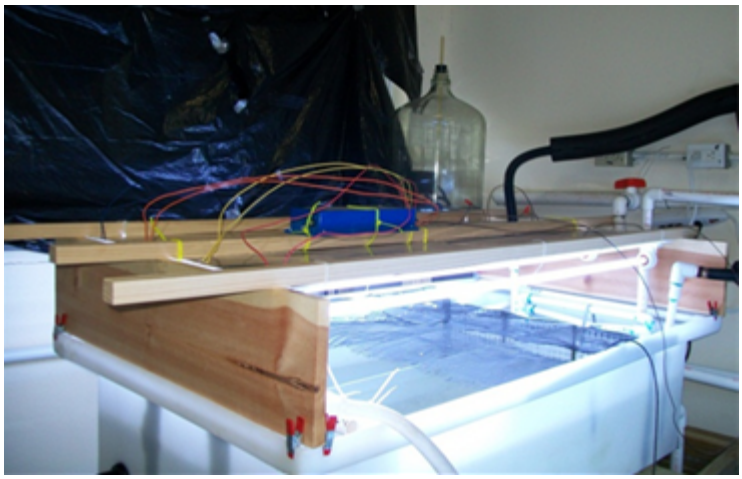

Figure 4.2: View of a semi-recirculating flow tank with lights and shade cloths in place. The acid reservoir can be seen in the back of the image.

The varying flow rates were controlled using electric water pumps. The low flow treatments were each fed by a single Pentaire Quiet One 5000 pump. The mid flow treatments were each fed by a single Mag Drive 36 utility pump. The high flow treatments were produced by two Mag Drive 36 pumps flowing into a single raceway. The flow treatments tested were: low flow = <12 cm/s, mid flow = ~15 cm/s, and high flow = ~27 cm/s. Flow speeds in each raceway were estimated using a General Oceans Model 2030 flow meter. Flow speeds are listed as "approximate" because flow decreased along the length of each raceway (due to distance from the site of water inflow). The majority of the low flow raceway experienced flow speeds much slower than 12 cm/s, but the minimum sensitivity of the flow meter prevented a more precise measurement from being made.

b) Light Treatments

The light was provided by an array of 4 Aquablue light bulbs placed above the flume (Figure 4.2). The differing light treatments were produced using shade cloth stretched across segments of the tank. The high light treatments received direct light and had an average light exposure of 170 µmol quanta s^{-1} m^{-2}. The mid light treatment was created by stretching a single layer of shade cloth over a section of the flume (spanning all three raceways) producing light levels of 85 µmol s^{-1} m^{-2} at the level of the corals. The lowest light levels 50 µmol s^{-1} m^{-2} were attained by stretching a double layer of shade cloth across the raceways. Sources of external light were removed by blocking all laboratory windows with opaque material and keeping all other lights turned off throughout the entirety of the experiment.

c) Temperature Control

Tank temperatures were controlled by a two component system. The first was an array of ½ inch PVC pipes with chilled water running through them positioned in the mixing area of each tank. The second chiller component was a ¼ hp (183.87 W) chiller that was fed by one of the "high flow treatment" pumps (Figure 4.3). The cooled water was returned to the high flow raceway in front of the flow straighteners.

Figure 4.3: A semi-recirculating tank showing the connection of the electronic chiller to one of the high flow pipes. Note: Before the initiation of the experiment, the hose returning the chilled water to the tanks was repositioned to the "inflow" end of the tank to allow chilled water to be fed through the system before immediately being returned to the chiller.

d) Water Chemistry Treatments

The decreased aragonite saturation states that approximated those expected to occur in natural systems in the next 50-100 years were produced in one tank by adding a constant drip of a weak hydrochloric acid solution into the mixing area. The addition of hydrochloric acid reduces the total alkalinity in the system while leaving the amount of dissolved inorganic carbon (DIC) in the tank constant. The impact of the addition of chloride ions to the system is assumed to be insignificant because this ion is already a dominant component in seawater. The acid additions cause decreases in pH, and increases in CO_{2aq} and pCO_2 in the treated tank. Discrete water samples from each tank were taken twice weekly and fixed using mercury chloride.

Water samples were analyzed for total alkalinity (TA) and pH. TA was determined in duplicate (30-40 ml analyses) using an automated, open-cell Gran titration (Dickson et al. 2007, SOP3b), and accuracy was checked against certified seawater reference standards (A. Dickson, Scripps Institute of Oceanography). pH was determined

on the total scale using an Orion Ross combination pH electrode calibrated at 25˚C against a seawater TRIS buffer (Dickson et al. 2007*,* SOP6). Total DIC, pCO_2, concentrations of HCO_3^-, CO_3^{2-}, CO_2, and Ω_{arag} were computed from TA, pH, temperature, and salinity using the program CO2SYS (E. Lewis,Brookhaven National Laboratory), with dissociation constants for carbonate determined by Mehrbach et al. (1973), as refit by Dickson & Millero (1987) and dissociation constant for boric acid determined by Dickson (Dickson 1990). pH is reported on the total scale, the scale on which K1 and K2 were determined.

2) Study Species

Four cnidarian species were studied using the paired systems. The effects of decreased pH, and varying light levels on the zooxanthellate scleractinian corals *Montastraea faveolata* and *Pocillopora damicornis*, the azooxanthellate scleractinian *Tubastraea coccinea*, and the zooxanthellate anemone *Aiptasia pallida*.. Fragments of *M. faveolata* and *P. damicornis* were acquired from the University of Miami's coral culture facility at the National Resource Center for *Aplysia* on Virginia Key. Individual *A. pallida* were collected from the outdoor tanks of the same facility. *T. coccinea* colonies were collected by volunteer divers from the exterior of the Aquarius Habitat near Key Largo in November 2010 (Permit #FKNMS-2010-125）.

In order to minimize the influence of flow baffling by colony structure (Sebens *et al.*1997), coral fragments of similar morphology were used within each species. *Montastraea* fragments were all of similar size and "hemispherical" in shape mounted on

the top of labeled sand filled eppendorf tubes using hot glue (Figure 4.4). Due to the limited availability of *Montastraea faveolata* at the outset of this experiment only four water chemistry-flow-light treatments were investigated. Given the results of the coral-sel study regarding the benefits of mid-flow rates, the available colonies were all placed under mid flow (15 cm/s) conditions. They were divided among the two separate water chemistry treatments and the high and low light conditions. Surface area was determined from pictures of each colony analyzed using the Image J processing program.

Pocillopora fragments with only one or two major branching points were selected for the experiment. This uniform morphology minimized variation in the general flow rate within and between fragments. The base of each of *Pocillopora* fragments was embedded in a hot glue "plug" on the top of a sand-filled eppendorf tube (Figure 4.5). In order to minimize effects of position within the raceway (based on distance from the tank input or the walls), light treatments (the shade cloths that created the light treatments and the corals selected for each treatment) were rotated along the length of the raceways twice each week and animals were shuffled within their assigned treatments on each of those occasions. Fragments were grown under either current water chemistry conditions (pH 8.187, pCO_2 300.77), or decreased pH and aragonite saturation state (pH 7.87, pCO_2 602.7).

Figure 4.4: An experimental colony of *Montastraea faveolata* utilized in the semi-recirculating flume study. The colony is cemented to a sand-filled eppendorf tube.

Figure 4.5: An experimental colony of *Pocillopora damicornis* utilized in the semi-recirculating flume study. The base of the colony is cemented to a sand-filled eppendorf tube.

Tubastraea coccinea colonies utilized in this study were more variable in size and shape (due to collection limitations). Colonies were not trimmed down to single polyps to avoid causing additional stress to the colonies. Efforts were made to randomize colony assignments so that the various treatment conditions had "populations" that were similar at the outset of the experiment. Each colony was secured to a ReefBase pedestal which allowed them to be individually labeled, positioned within the flume, and moved without damaging the colony (Figure 4.6).

A. pallida polyps were allowed to settle in individual rectangular open-top translucent plastic containers (~2cm x 1 cm x 0.75cm). The containers were then secured

to the top of pedestals using rubber bands which allowed the containers to be removed from the bases for weighing purposes (Figure 4.7).

Figure 4.6: A *Tubastraea coccinea* colony utilized in the flume experiment. The colony is cemented to the ceramic pedestal.

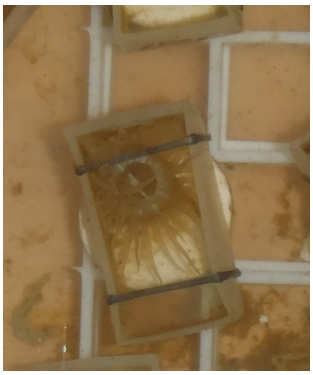

Figure 4.7: An *Aiptasia pallida* polyp utilized in the flume experiment. The anemone attached to the plastic container using natural processes. The container was attached to the pedestal using rubberbands.

All experimental corals were fed with ZoPlan (minimum 51% protein, 6.5% fat, and 5.2% fiber), or similar powdered food, twice each week. In addition, *Tubastraea coccinea* polyps were offered mysid shrimp as well as powdered food. Feedings were carried out in separate aerated tanks to standardize access to supplemental food between treatments and to minimize fouling of the experimental system. The powdered food was sprinkled over the entire feeding tank, and aerators (air stones) used to promote water and food circulation. The powdered food was offered as a source of nutrition as well as a method of encouraging the polyps to extend, increasing their opportunity and ability to

consume the mysids which were distributed over the colonies 30 minutes after the powdered food was added. Colonies were then given approximately 1 hour to feed on the combination of mysids and powdered supplements.

The *Aiptasia* were not supplementally fed. Pilot studies indicated that multiple disturbances to this species increased the likelihood that they would detach from their experimental mounts resulting in mortality or loss from the experimental system.

3) Data Collection

a) Growth

Several metrics of health and growth were measured for each research species. Corals grown under current water chemistry conditions (pH 8.04-8.18), and decreased pH and aragonite saturation state (pH 7.85-7.87) were compared. This will allow an assessment of the likely effects of changing ocean chemistry on corals in each of the tested light and flow conditions.

Mass measurements were recorded bi-weekly. Two different types of mass measurements were taken for each coral during each data collection session. Prior to weighing, all corals and bases were delicately cleaned of any diatoms and filamentous algae using a soft bristled toothbrush.

Buoyant masses were taken using a Sartorius electronic balance (precision = 0.001g) with a small wire loop suspended from the weighing pan using nylon line. The loop and line allowed the coral to be suspended and completely submerged in a container of saltwater beneath the balance. The saltwater in the container was allowed to acclimate

to room temperature for at least an hour before weighing began and care was taken to minimize air movement in the room.

In addition to buoyant masses, "traditional" mass measurements were recorded for each sample as well. In order to minimize variance in measurement due to excess water, the base of each sample was dried thoroughly and the sample was given several vigorous "flicks" to remove excess water. The sample was then weighed using the top-loading function of the balance.

The *M. faveolata* colonies utilized were small rounded colonies on loan from the coral aquaculture team. All measurements were made using non-destructive methods. Their surface areas were estimated by analyzing digital images using the Image J computer program. Repairs and alterations to the buoyant weighing system prevented buoyant masses to be measured during the sixth and eighth weeks of the experiment. Only air masses were measured on these occasions.

Nutritionally, *T.coccinea* is heterotrophic. The "relative active area" (the total area of the calyx openings of the living polyps) of these colonies was estimated by measuring polyp calices in two perpendicular axes after the tissue was removed. Measurements were made using calipers (precision = 0.005cm).

The oral disc area of each anemone was measured by analyzing digital images using the Image J program. The images were recorded after the anemones relaxed for >30 minutes in still water to encourage maximum expansion.

Due to the highly irregular growth form of *Pocillopora damicornis*, determining surface area through conventional measurement methods is very difficult. Since all

growth measurements must be normalized to living surface area, another method had to be employed. In this study, the surface area of each sample was determined using the wax dipping method.

This method utilizes the fact that the mass of wax that will cling to an object dipped into a pool of melted wax is directly related to the surface area of that object. The paraffin wax utilized in this study was melted and held at temperatures of 68-72˚C using a water bath set to 90˚C. A regression of increased mass vs. surface area was created using a set of wooden dowels of known surface area. The length and diameter of each dowel was measured using calipers accurate to 0.005cm. These measurements were used to calculate the surface area of the dowels that would be dipped into the melted wax. Each dowel was weighed, mounted on a pin (to allow dipping without compromising the surface area being dipped). Each dowel was dipped for one second and "flicked" downward to remove excess wax. The dowels were allowed to dry/harden for >10 minutes. They were then weighed using an electronic balance (precision= 0.001g). Each dowel was dipped again for 5 seconds. The dowel was again flicked to release excess wax. The dowels were dried and re-weighed.

The mass of wax deposited on each smooth wooden dowel during the dipping process was plotted against their measured surface areas (Figure 4.8). A regression line was fit to the plotted points revealing that 0.0989g of wax were added to each square centimeter of dipped surface area.

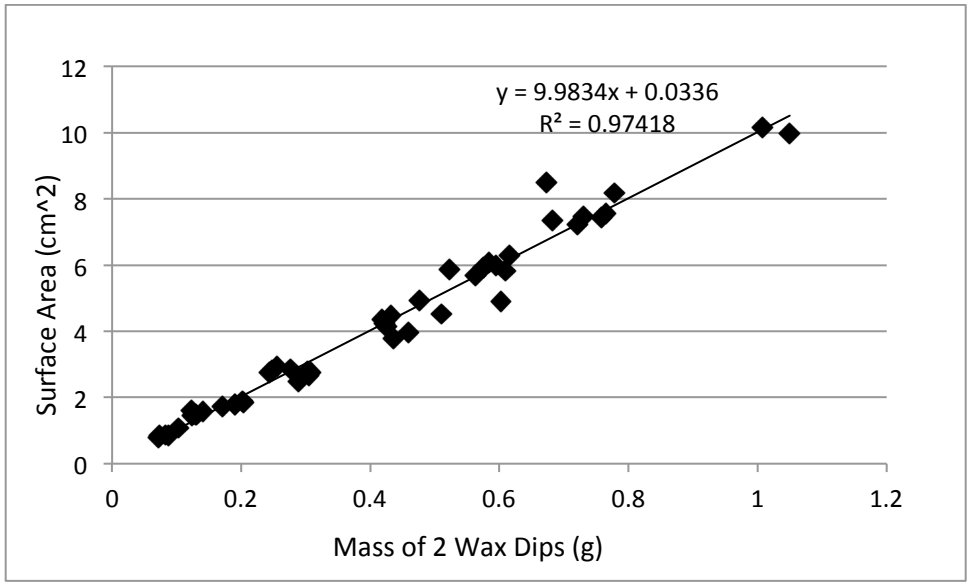

Figure 4.8: Sample of regressions utilized to determine the surface area of colonies based on the mass adhering to them after the "two dip method" described above.

Each experimental colony was wax dipped using the same technique described above. The regression relationship determined using the dowels was utilized to determine the surface area of the irregularly shaped *Pocillopora damicornis* colonies. A new regression, with new wax was produced each day that colonies were dipped to correct for any possible differences in dipping speed or wax consistency that might arise from multi-day testing.

Since the majority of the experimental colonies exhibited partial tissue mortality over the course of the experiment, an additional step was taken in order to properly utilize the wax dip data in further calculations. The proportion of living to dead surface area at the conclusion of the experiment had to be determined before the wax dip data could be used to calculate the "final" living surface area for each colony. Digital photographs were taken of each coral from multiple angles at the conclusion of the experiment. The

Image J computer program was utilized in order to determine the percent of the coral skeleton that still possessed living tissue at the experiment's termination. These percentages, along with the total surface areas (determined through wax dipping) were used to calculate the surface area of surviving coral tissue. All growth calculations were made using these values for live surface area.

Pocillopora damicornis, as a branching species, not only grows from the major branch tips, but also increases in complexity by producing new "growing points" at numerous positions along the branches. These were counted on multiple occasions over the course of the experiment and these changes in complexity were monitored and calculated for each colony.

Tubastraea polyps vary greatly in size. The total size of polyps may also influence the impact that different abiotic conditions may have upon members of this species. Although it was impossible to accurately measure the sizes of the extended polyps, the total area of each calyx bearing a polyp was calculated and used as another metric to normalize growth rates for this species.

b) Photosynthetic Activity

The flume based experiment allowed both Imaging- Pulse Amplitude Modulation (I-PAM) and Diving PAM fluorometry methods to be utilized. The I-PAM allowed the activity of zooxanthellate species to be measured by exposing colonies to a series of light pulses of increasing intensity and measuring the fluoresced light. These measurements allowed the estimation of photosynthetic efficiency as well as the construction of "light

curves" and the metrics that are calculated from them (including I_k, P_{max}, and α). The diving PAM was used to take readings of the photosynthetic efficiency of the Photosystem II (Fv/Fm) of the *Pocillopora damicornis* fragments. Readings were taken after approximately 1 hour of dark adaptation. The probe was placed at the major branching point of each fragment to minimize possible influences of new growth points and tissues. The Imaging PAM fluorometer was used to examine the photosynthetic activity of the anemones bi-weekly. All analyses were taken after >30minutes of dark adaptation.

c) Tubastraea skeleton analysis

After the experiment concluded, the colonies were submerged in fresh water for several days and the dead or loosened flesh was removed using a waterpik. Variability in the coloration of the skeletons was noted. The skeletons were air-dried before further analysis was carried out. The color of each skeleton was characterized by comparison with a "Reef Watch" color card (Figure 4.9). Skeletons could generally be characterized as having red, orange, brown, or green tints.

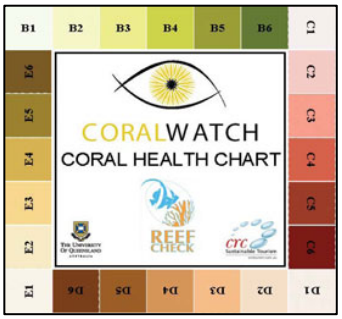

Figure 4.9: Coral Watch card, distributed by The University of Queensland, utilized for skeleton coloration classification.

d) Aiptasia protein bioassay

At the conclusion of the final experiment, each anemone was removed from its container and was gently blotted with a paper towels for approximately one minute. The blotted organism was then weighed on an electronic balance (precision = 0.0001g). The blotted tissue was then homogenized in 2ml of purified water for 20 seconds. Aliquots of known volume of the homogenized sample were then added to two cuvettes and the total volume brought up to 100 µL using purified water. 5ml of Bradford reagent was added to each cuvette and vortexed thoroughly. The reaction was allowed to progress for 15 minutes before analyzing its absorbance with a spectrophotometer. The average absorbance of each sample was then compared to a standard absorbance curve (produced using bovine serum albumin (BSA) solutions of known concentrations) to determine the protein concentration of each tested solution. This information was used to determine the total protein content of each of the anemones.

e) *Aiptasia zooxanthella density*

One drop of the original homogenate from each anemone was placed on a hemocytometer with a 5 x 5 cell square grid imprinted on it. A microscope camera was used to take a digital photograph of the grid. Five cells on each grid were randomly selected and the total number of zooxanthellae within them recorded in order to estimate relative densities of zooxanthellae within each anemone. Two drops were analyzed for each anemone.

C) Results

1) Pocillopora damicornis

Many of the experimental corals experienced partial tissue mortality. The portion of live coral tissue was determined at the conclusion of the experiment. The effect of decreased pH, flow rate, and light levels on the mean percent live tissue coverage was examined using a 3-way ANOVA (Table 4.1) . The average value for each light/flow treatment was compared for the water chemistry treatments (Figure 4.10). In every case, colonies in water of decreased pH and aragonite saturation state showed higher percent live tissue than their counterparts in control water. This difference was only significant however, when low light conditions were combined with low flow conditions ($p < 0.001$) or high flow ($p = 0.0029$).

The simple "air mass" (standard, non-buoyant mass) of each of the experimental corals was also measured. Both "air" and buoyant masses were measured to improve the possibility of detection of growth over short time scales. The overall change in mass normalized to final living surface area was compared between the control and decreased pH treatments for each flow/light combination (Figure 4.11). Generally, the change in air mass per surface area decreased with increasing flow speed. Change in standard mass/surface area was only significantly affected by changing water chemistry under low flow-mid light and high flow-mid light conditions. There was no significant influence of water chemistry on this metric under any of the mid flow (15 cm s^{-1}) treatments. In fact, under mid flow treatments, high and low light treatments showed slightly higher growth values under lower pH conditions.

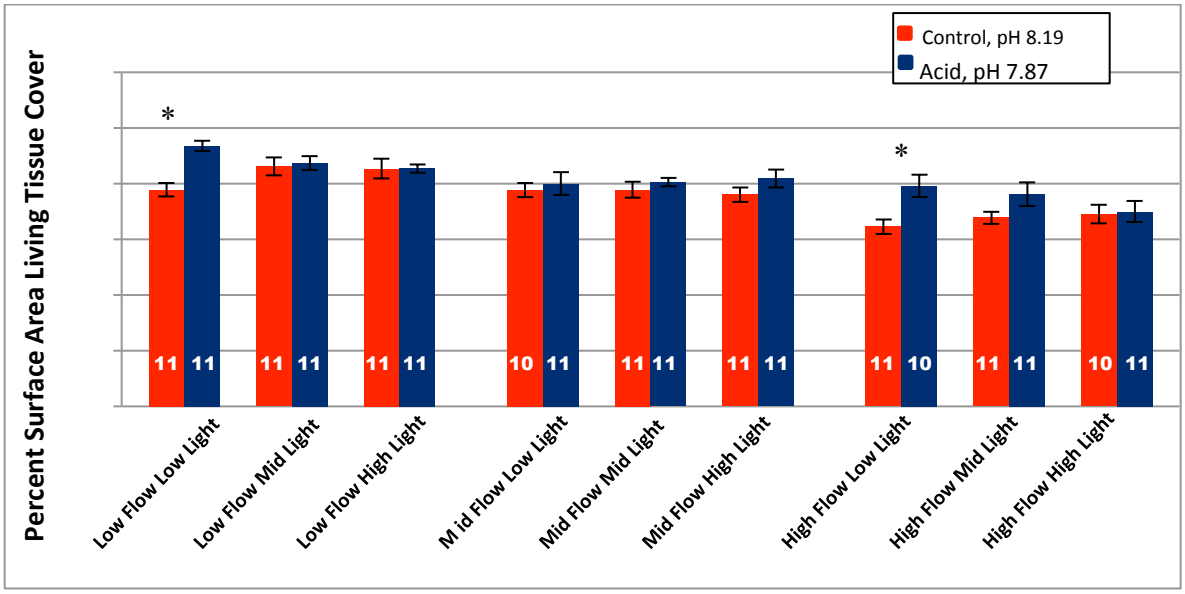

Figure 4.10: The mean percent of the skeletal surface area covered with live tissue at the conclusion of the experiment. Error bars indicate +/- 1 SE. Asterisks indicate treatments in which the control and acidified treatments differ significantly ($p < 0.05$). White numbers denote the final n of each treatment.

Table 4. 1: ANOVA results comparing the percent of live surface area remaining on *Pocillopora damicornis* fragments exposed to two water chemistry conditions (control and acidified), by flow rates (~4, 15, 25 cm/s), and three light levels (50, 85, 180 µmol photons $m^{-2}s^{-1}$). Measurements were taken after 12 weeks of exposure. Fixed factors were Water Treatment, Flow, and Light. Significant differences were explored by Tukey's test or Student's T-test when only two conditions were compared.

Factor	DF	F	Sig.	Post-hoc Results
Water Treatment	1	16.4089	<0.0001	Acid > Control
Flow	2	36.5829	<0.0001	Low > Mid > High
Light	2	0.3139	0.7310	
Water Treatment x Flow	2	0.7668	0.4661	Acid Low Flow > Acid Mid Flow, Acid High Flow > Control Mid Flow, Control High Flow Control Low Flow > Control High Flow, Acid High Flow Control Mid Flow, Acid Mid Flow > Control High Flow Acid High Flow> Control High Flow
Water Treatment x Light	2	3.4250	0.0347	Acid Low Light>Control Low Light, Control High Light Acid Mid Light> Control Low Light
Flow x Light	4	0.1188	0.9757	All Low Flow> All High Flow Treatments
Water Treatment x Flow x Light	4	1.7895	0.1330	Control Low Flow Mid Light > Control High Flow Mid Light Control Low Flow High Light> Control High Flow High Light

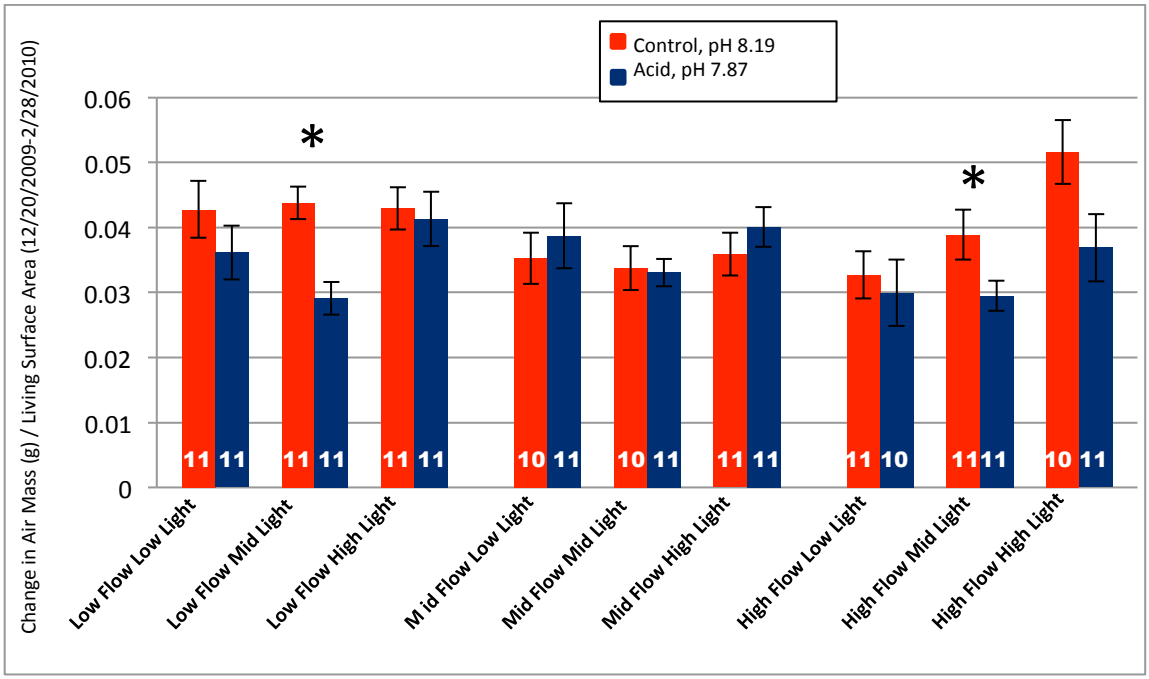

Figure 4.11: The mean change in air mass (g)/cm^2 of living tissue Dec 12, 2009-Feb. 28, 2009. Single asterisks indicate treatments in which the control and acidified treatments differ significantly ($p < 0.05$). White numbers denote the final n of each treatment. Error bars indicate +/- 1 SE.

Table 4.2: ANOVA results comparing the changes in the mass normalized to the live surface area of *Pocillopora damicornis* fragments exposed to two water chemistry conditions (control and acidified), by flow rates (~4, 15, 25 cm/s), and three light levels (50, 85, 180 µmol photons $m^{-2}s^{-1}$). Measurements were taken after 12 weeks of exposure. Fixed factors were Water Treatment, Flow, and Light. Significant differences were explored by Tukey's test or Student's T-test when only two conditions were compared.

Factor	DF	F	Sig.	Post-hoc Results
Water Treatment	1	6.9082	0.0093	Control > Acid (Student's t test)
Flow	2	1.2392	0.2922	
Light	2	5.3476	0.0056	High Light> Mid Light, Low Light
Water Treatment x Flow	2	3.8120	0.0240	Control Low Flow > Acid High Flow
Water Treatment x Light	2	1.0851	0.3401	Control High Light > Acid Mid Light
Flow x Light	4	1.3796	0.2429	High Flow High Light > High Flow Low Light
Water Treatment x Flow x Light	4	0.9362	0.4443	Control High Flow High Light > Acid High Flow Low Light > Acid High Flow Mid Light > Acid Low flow Mid Light

The overall changes in buoyant mass normalized to final area of live tissue were compared between treatments using a 3-way ANOVA (Figure 4.12, Table 4.3). Under low and high flow treatments, values were generally higher under current water chemistry conditions when compared with those under decreased aragonite saturation

conditions. These differences were only significant under the high flow-mid light treatment ($p = 0.0388$). Under mid flow treatments, values appeared to increase with decreasing pH, but not significantly.

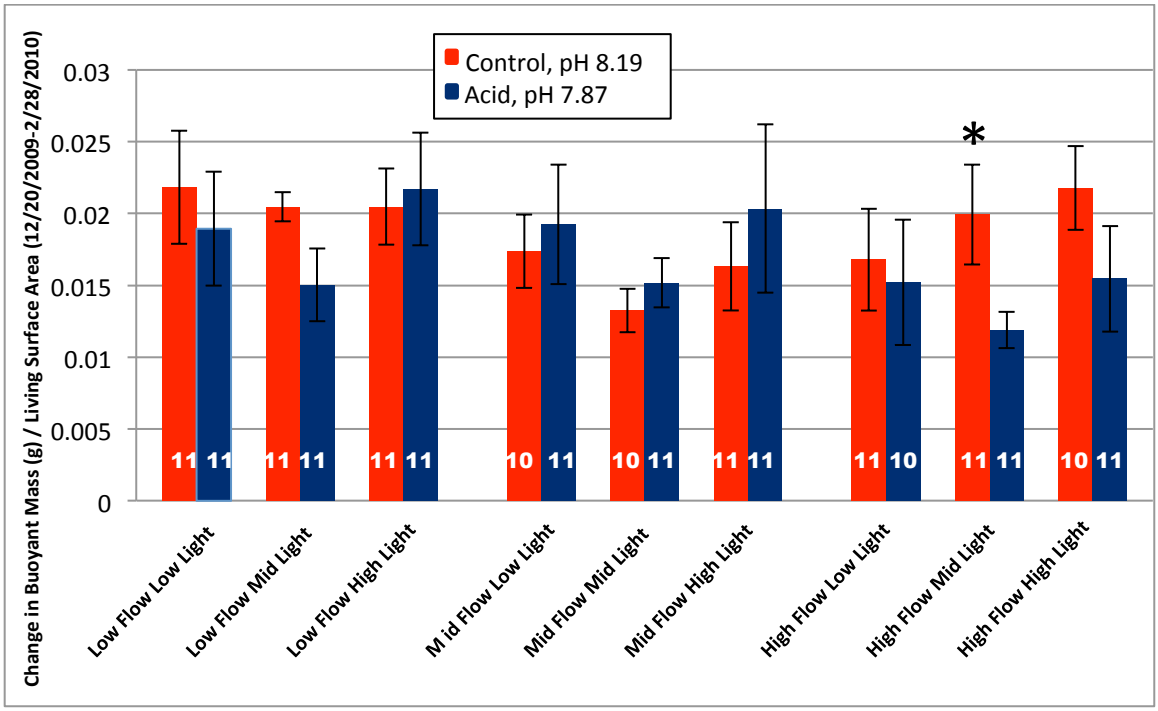

Figure 4.12: The mean change in buoyant mass (g)/cm^2 of living tissue Dec. 20, 2008 – Feb. 28, 2009. Single asterisks indicate treatments in which the control and acidified treatments differ significantly ($p < 0.05$). White numbers denote the final n of each treatment. Error bars indicate +/- 1 SE from the mean.

Table 4.3: ANOVA results comparing the changes in the buoyant mass normalized to the live surface area of *Pocillopora damicornis* fragments exposed to two water chemistry conditions (control and acidified), by flow rates (~4, 15, 25 cm/s), and three light levels (50, 85, 180 µmol photons $m^{-2}s^{-1}$). Measurements were taken after 12 weeks of exposure. Fixed factors were Water Treatment, Flow, and Light. Significant differences were explored by Tukey's test or Student's T-test when only two conditions were compared.

Factor	DF	F	Sig.	Post-hoc Results
Water Treatment	1	0.7015	0.4034	
Flow	2	1.2789	0.2810	
Light	2	2.2387	0.1097	
Water Treatment x Flow	2	2.9766	0.0536	
Water Treatment x Light	2	07989.	0.4515	
Flow x Light	4	0.2503	0.9092	
Water Treatment x Flow x Light	4	0.3687	0.8307	

The photosynthetic yield measured by the I-PAM is an indicator of the health of photosystem II mechanisms within the endosymbiotic dinoflagellates. Not surprisingly, light demonstrated a positive influence on the photosynthetic yield of the zooxanthellae within *Pocillopora* fragments in this experiment (Figure 4.13). Increasing light caused a decrease in mean photosynthetic yield, particularly under controlled conditions. The symbionts demonstrated slightly higher photosynthetic yields under conditions of decreased pH. The influence of low pH was significant ($p<0.05$) only under low flow and high light conditions. At $p< 0.10$, low pH had a significant effect under high light conditions at all flow rates.

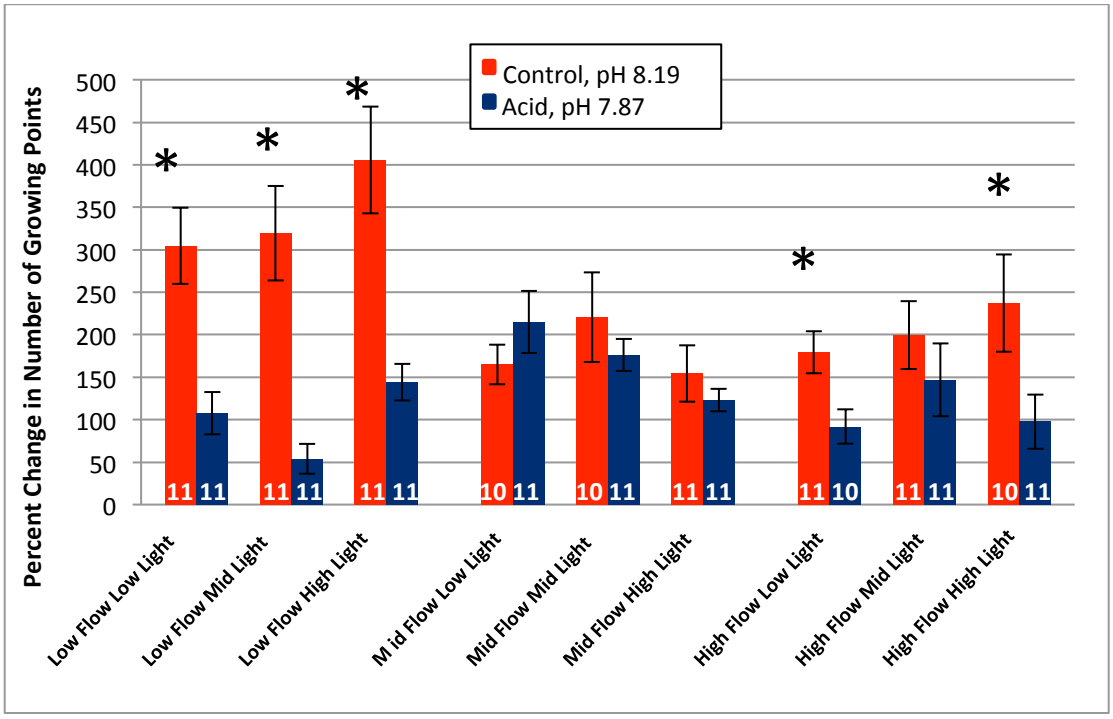

Figure 4.13: Figure depicts the mean percent change in the number of growing points of the experimental colonies. Single asterisks indicate treatments in which the control and acidified treatments differ significantly ($p < 0.05$). White numbers denote the final n of each treatment. Error bars indicate +/- 1 SE from the mean.

Table 4.4: ANOVA results comparing the percent changes in the number of growing points of *Pocillopora damicornis* fragments exposed to two water chemistry conditions (control and acidified), by flow rates (~4, 15, 25 cm/s), and three light levels (50, 85, 180 µmol photons $m^{-2}s^{-1}$). Measurements were taken after 12 weeks of exposure. Fixed factors were Water Treatment, Flow, and Light. Significant differences were explored by Tukey's test or Student's T-test when only two conditions were compared.

Factor	DF	F	Sig.	Post-hoc results
Water Treatment	1	44.6493	<0.0001	Control > Acid (Student's t test)
Flow	2	5.0100	0.0077	Low Flow > High Flow
Light	2	0.4183	0.6588	
Water Treatment x Flow	2	13.2302	<0.0001	Control Low Flow > all other treatments Control High Flow > Acid High Flow, Acid Low Flow
Water Treatment x Light	2	1.3091	0.2727	Control High Light, Control Mid Light > All Acid Treatments Control Low Light > Acid High Light
Flow x Light	4	2.5243	0.0427	Low Flow high Light > Mid Flow High Light, > High Flow Low Light
Water Treatment x Flow x Light	4	0.6607	0.6201	Control Low Flow High Light > All Acid Treatments >Control High Flow Mid Light > Control High Flow Low Light >Control Mid Flow Low Light > Control Mid Flow High Light Control Low Flow Low Light > Acid Low Flow Low Light >Acid Low Flow Mid Light >Acid High Flow High Light >Acid High Flow Low Light Control Low Flow Mid Light > Acid Low Flow Low Light >Acid Low Flow High Light >Acid Mid Flow High Light >Acid High Flow Low Light >Acid High Flow High Light

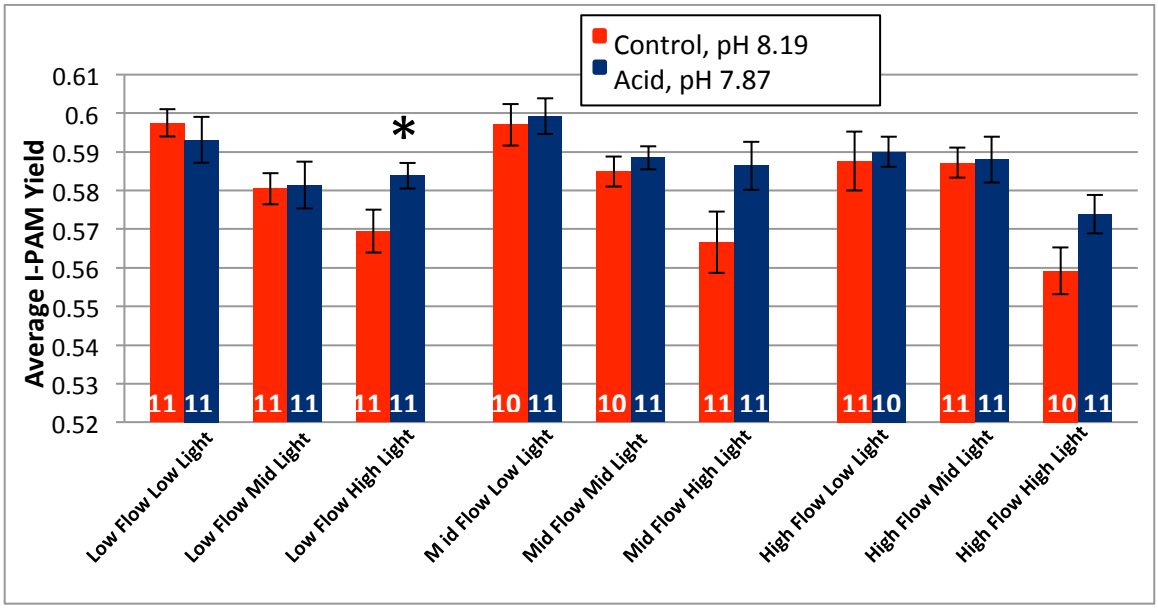

Figure 4.14: Figure depicts the mean photosynthetic yield of measured using an Imaging Pulse Amplified Modulated Fluorometer at the conclusion of the experiment. Single asterisks indicate treatments in which the control and acidified treatments differ significantly ($p < 0.05$). White numbers denote the final n of each treatment. Error bars indicate +/- 1 SE from the mean.

Table 4.5: ANOVA results comparing the fluorescence responses of the zooxanthellae within *Pocillopora damicornis* fragments exposed to two water chemistry conditions (control and acidified), by flow rates (~4, 15, 25 cm/s), and three light levels (50, 85, 180 µmol photons $m^{-2}s^{-1}$). Measurements were taken after 12 weeks of exposure. Fixed factors were Water Treatment, Flow, and Light. Significant differences were explored by Tukey's test or Student's T-test when only two conditions were compared.

Factor	DF	F	Sig.	Post-hoc Results
Water Treatment	1	3.6116	0.0590	
Flow	2	0.3847	0.6812	
Light	2	1.3064	0.2734	
Water Treatment x Flow	2	1.3894	0.2520	
Water Treatment x Light	2	0.6949	0.5005	
Flow x Light	4	1.0355	0.3904	
Water Treatment x Flow x Light	4	0.9664	0.4274	

Several metrics show evidence that changes in water chemistry will cause differences in the growth of *Pocillopora damicornis* colonies living under either high or low flow rates. It must be noted that no metric showed significant differences (alpha = 0.05) between corals growing under today's water chemistry conditions and those

exposed to projected future water chemistry conditions. In fact, a number of metrics show indications of improved growth at decreased pH under mid flow conditions.

The zooxanthellae populations were tested at the conclusion of the experiment. DGGE and sequencing showed that all of the tested colonies were dominated by type C1 zooxanthellae. Neither light, flow, nor pH conditions showed any influence on the identity of the zooxanthellae community members.

2) *Montastraea faveolata*

Over the course of the experiment, the overall change in mass/area was not found to vary significantly due to decreased pH (control -pH 8.06, acidified pH – 7.88) or aragonite saturation state (control 3.98Ω, acidified treatment – 2.65 Ω). Light however, was found to have significant effects on change in mass/area (Figure 4.15, Table 4.6, Table 4.7). Growth was higher under high light conditions than low light conditions in both water chemistry conditions. The differences were apparent throughout the course of the entire experiment (Figure 4.16). Light appears to have a slightly greater influence on growth under conditions of decreased pH and aragonite saturation state ($p=0.0032$) than under current water chemistry conditions ($p=0.0180$).

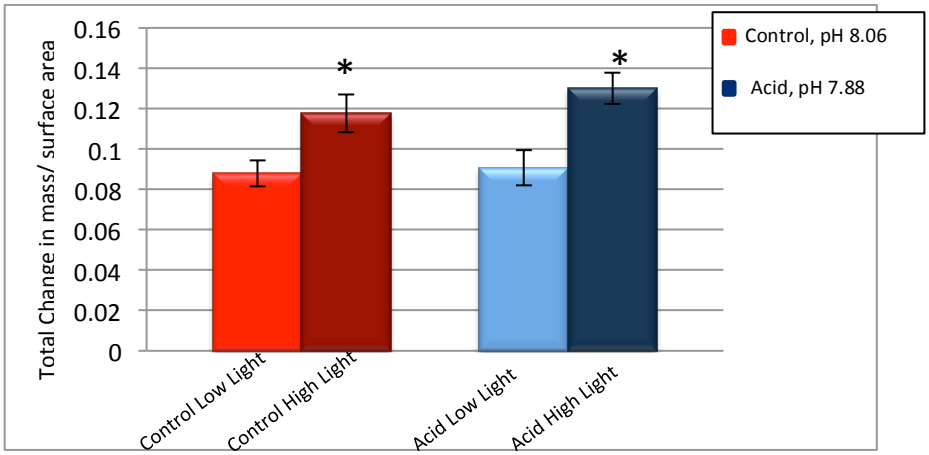

Figure 4.15: The total change in mass/total surface area of *Montastraea faveolata* exposed to varying light and pH conditions. Growth was significantly higher under high light conditions than under low light conditions under both experimental water chemistry conditions. Error bars indicate +/- 1 standard error from the mean.

Table 4.6: ANOVA results comparing the changes in the mass normalized to the live surface area of *Montastraea faveolata* fragments exposed to two water chemistry conditions (control and acidified), and two light levels (50 and 180 µmol photons $m^{-2}s^{-1}$). Measurements were taken after 12 weeks of exposure. Fixed factors were Water Treatment and Light. Significant differences were explored by Tukey's test or Student's T-test when only two conditions were compared.

Factor	DF	F	Sig.	Post-hoc Results
Water Treatment			0.3589	
Light			0.0001	High > Low
Water Treatment x Light			0.5512	Acid High Light> Acid Low Light, Control Low Light

Table 4.7: ANOVA results comparing the changes in the buoyant mass normalized to the live surface area of *Montastraea faveolata* fragments exposed to two water chemistry conditions (control and acidified), and two light levels (50 and 180 µmol photons $m^{-2}s^{-1}$). Measurements were taken after 12 weeks of exposure. Fixed factors were Water Treatment and Light. Significant differences were explored by Tukey's test or Student's T-test when only two conditions were compared.

Factor	DF	F	Sig.	Post-hoc Results
Water Treatment			0.7373	
Light			<0.0001	High> Low
Water Treatment x Light			0.3728	Acid High Light> Control Low Light, Acid Low Light Control High Light > Acid Low Light

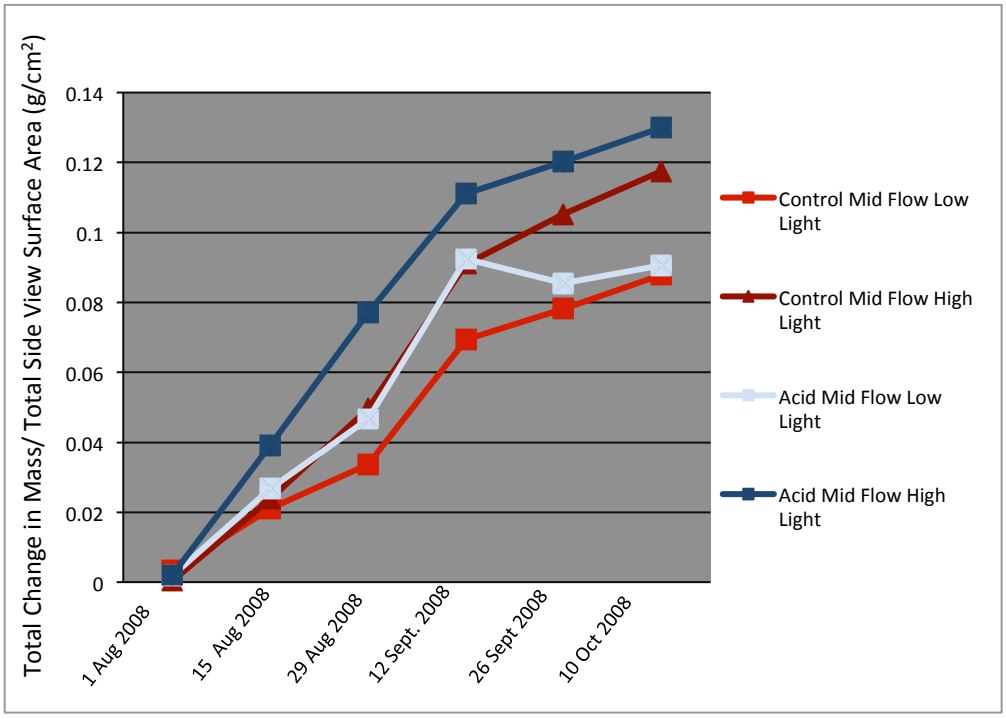

Figure 4.16: The change in mass/total surface area of *Montastraea faveolata* exposed to varying light and pH conditions.

3) Tubastraea coccinea

Tubastraea coccinea is an azooxanthellate species whose primary feeding activities are carried out heterotrophically. Since feeding and major nutrient acquisition occurs only through the polyps rather than over the entire surface area of the colony, calculations of growth were normalized to metrics related to polyp number and size (see methods).

In general, *T. coccinea* showed very little response to changes in water chemistry (control pH 8.04, Ω_{Ar} – 3.83 acidified – pH 7.88, Ω_{Ar} – 2.74). The changes in mass, normalized to the number of polyps present at the outset of the experiment, were not significantly influenced pH under any light or flow combinations. The highest mean growth per polyp occurred under high flow and high light conditions under both

examined water chemistry conditions. Under high flow in current water chemistry conditions, there was a trend of increasing mean growth/polyp with increasing light but this trend was not seen under any other conditions.

The observed growth in *T. coccinea* occurred in the form of new polyp development. Calculations of growth normalized to the number of new polyps formed yielded several noteworthy findings. Some colonies exhibited partial mortality, which caused the observed decreases in polyp count. In 8 of 9 flow and light combinations, water chemistry did not significantly influence this metric of growth. In 7 of those 8 conditions, colonies in the control treatment showed greater increases in mass per newly formed polyp than colonies in water with decreased pH. Under high light conditions in flow rates of 15 cm/s, corals under low pH conditions showed a significant decrease in changing mass/new formed polyp ($p = 0.0314$) compared to growth under current conditions (Figure 4.17). Similar decreases were observed under high light – high flow conditions, but the decreases were marginally non-significant ($p = 0.0554$).

When compared with colonies exposed to either low or high flow rates and low pH conditions, colonies exposed to current water chemistry in low flow or high flow conditions showed larger increases in mass per new polyp. These differences in growth between water treatments were evident, but not statistically significant. There were no apparent differences in the generation of new polyps under flow rates of 15 cm/s between the two water treatments (Figure 4.18). When flow treatments within each light treatment were pooled and compared, water chemistry appeared to impact the amount of mass acquired per newly formed polyp under mid light ($p = 0.0983$) and high light

conditions (p = 0.0563) at non-significant levels (Figure 4.17). With higher sample sizes, decreases in variation might allow the presence or absence of significant relationships to be determined with more certainty.

Within the control (current water chemistry treatments), colonies under high flow (25 cm/s) showed significantly larger gains of mass per new polyp than those exposed to lower flow rates (p=0.0184) (Figure 4.17). While the effects of light on this metric of growth were not significant for corals exposed to control chemistry conditions, *T. coccinea* colonies exposed to the highest light levels showed larger gains of mass per new polyp than those exposed to lower light levels (p=0.0591).

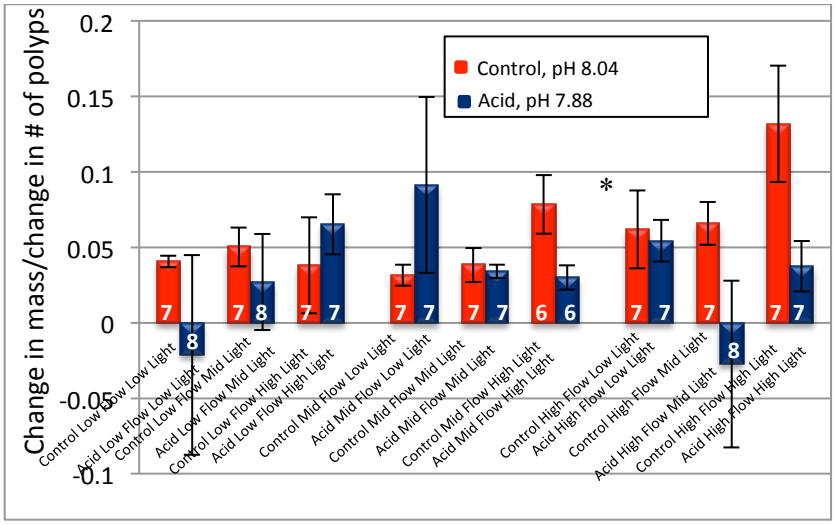

Figure 4.17: Mean change in buoyant mass normalized to the change in the number of polyps. Flow and light combinations that were significantly ($p<0.05$) impacted by water chemistry are indicated by a single asterisk (*). Error bars indicate +/- 1 standard error from the mean. White numbers denote the final number of colonies in each treatment.

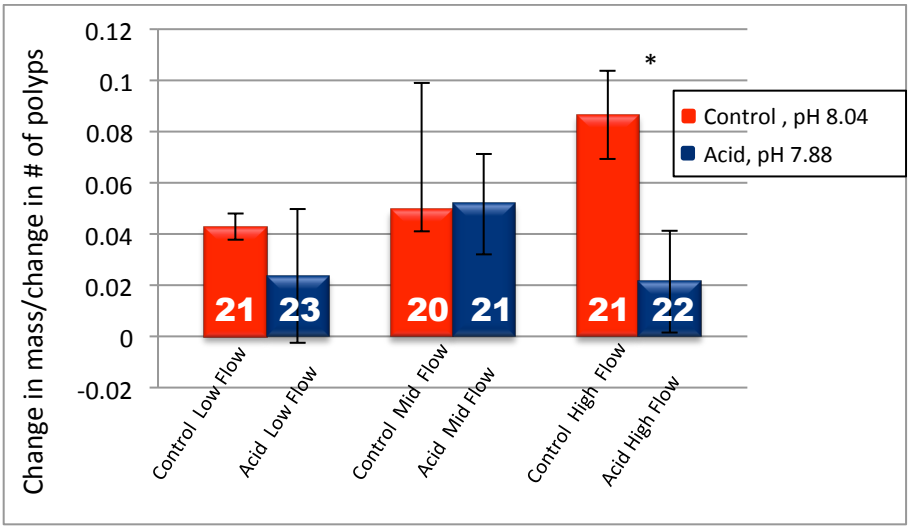

Figure 4.18: Mean change in buoyant mass normalized number of polyps present. Asterisks (*) indicate light and flow conditions which are significantly impacted by water chemistry. Error bars indicate +/- 1 standard error from the mean. White numbers denote the final number of individuals in each treatment.

Changes in the number of living polyps present in colonies revealed several patterns. Under low flow conditions, mean percent increase in number of polyps was always higher at current pH conditions compared with projected "acidified" conditions, though not significantly so. (Figure 4.19, Table 4.8). Under higher flow conditions, polyp counts were similar among treatments except at high light levels. Under high light conditions and 15cm/s flow rates, there was a significantly greater increase in polyp count at decreased pH compared to changes under current water chemistry conditions ($p=0.0249$) (Figure 4.19).

When light treatments were pooled across flow treatments, polyp counts under current water conditions were significantly greater than under decreased aragonite saturation ($p=0.0100$) (Table 4.8, Figure 4.20). *T. coccinea* colonies in control conditions added fewer polyps with increasing flow rates, while colonies at lower pH added more polyps with increasing flow rates. No significant trends or differences in polyp additions were apparent when flow treatments were pooled and the effects of light and its interactions with water chemistry were tested (Table 4.8).

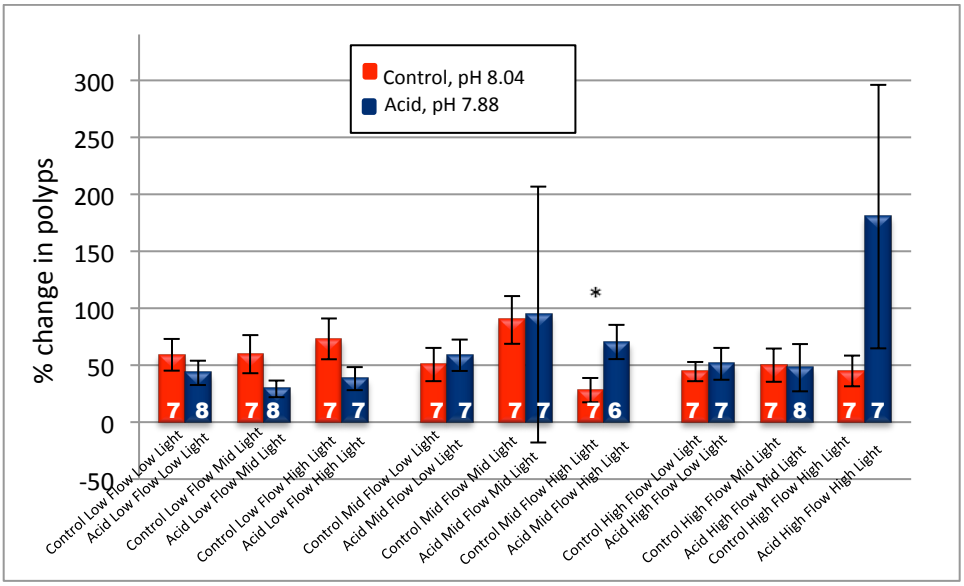

Figure 4.19: Mean percent change in number of polyps present within colonies over course of experiment. Flow and light combinations that were significantly ($p<0.05$) impacted by water chemistry are indicated by a single asterisk (*). Error bars indicate +/- 1 standard error from the mean. White numbers denote the final number of individuals in each treatment.

Table 4.8: ANOVA results comparing the percent change in the number of polyps present within each colony of *Tubastraea coccinea* colonies exposed to two water chemistry conditions (control and acidified), by flow rates (~4, 15, 25 cm/s), and three light levels (50, 85, 180 µmol photons $m^{-2}s^{-1}$). Measurements were taken after 12 weeks of exposure. Fixed factors were Water Treatment, Flow, and Light. Significant differences were explored by Tukey's test or Student's T-test when only two conditions were compared.

Factor	DF	F	Sig.	Tukey's
Water Treatment	1	0.7654	0.3834	
Flow	2	0.6648	0.5163	
Light	2	0.7177	0.4900	
Water Treatment x Flow	2	2.2220	0.1130	
Water Treatment x Light	2	1.5045	0.2264	
Flow x Light	4	1.7321	0.1476	
Water Treatment x Flow x Light	4	0.9581	0.4334	

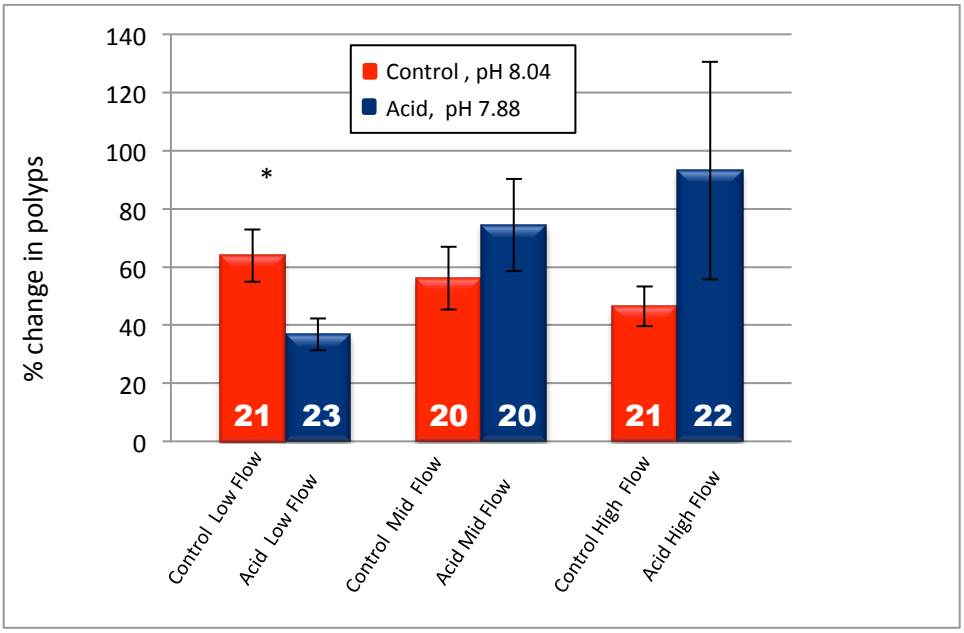

Figure 4.20: Mean percent change in number of polyps colony^{-1} over the course of the experiment. Asterisks (*) indicate light and flow conditions which are significantly impacted by water chemistry. Error bars indicate +/- 1 standard error from the mean. White numbers denote the final number of individuals in each treatment.

There was no significant effect of pH on the buoyant mass normalized to calyx area in any of the flow rate, light level combinations examined.

At the conclusion of the experiment, all tissue was removed from each *T. coccinea* colony and the remaining skeletons were air dried. This species possesses a typically "white" skeleton. When examined closely, the skeletons were seen to be slightly tinted. The skeletal tints could be classified into "red", "orange", "brown", or "green" categories. Each dried skeleton was compared with a Coral Watch Coral Health Chart (Figure 4.9) and categorized by the standard color scheme indicated along the card's edges. Under control conditions, there was an overall increase in red coloration and decrease in green skeletal coloration with increasing flow rates (Figure 4.21). In contrast, colonies under decreased pH showed a decrease in red with increasing flow

(Figure 4.21). Under equal flow conditions, corals showed increases in red and brown coloration and decreasing orange hues with increasing pH/ aragonite saturation.

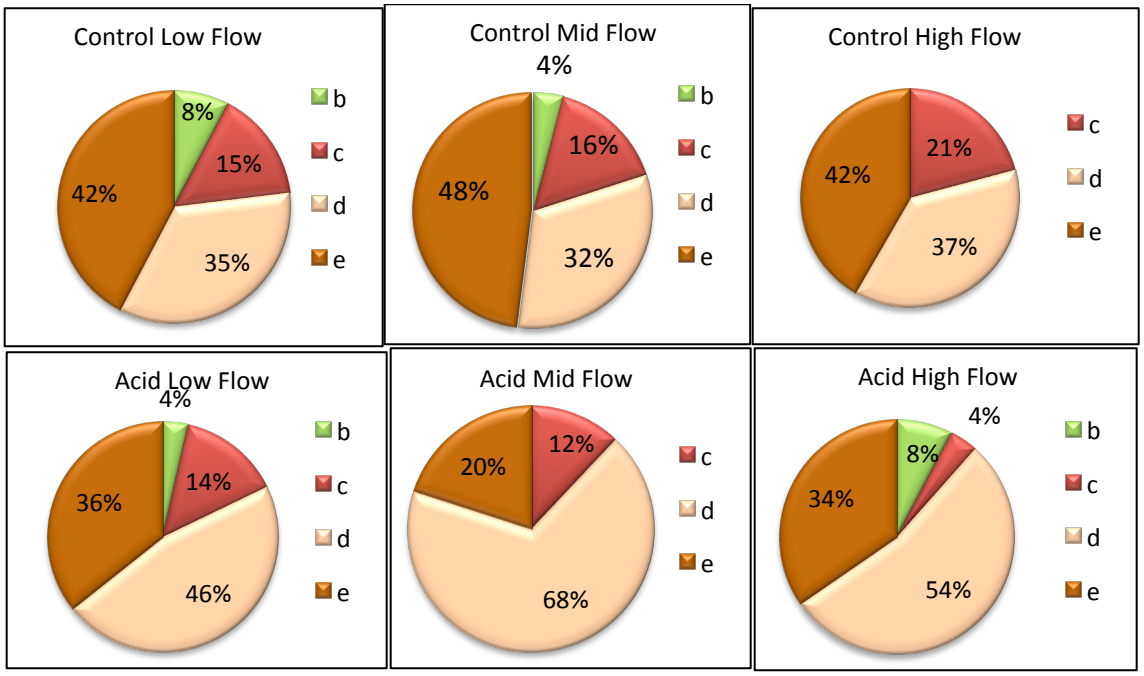

Figure 4.21: Charts denote the proportion of the skeletons under the designated conditions that were characterized by the indicated colors (b= green, c = red, d= peach/orange, e = brown). Letters refer to the color families on the Coral Watch cards.

When flow treatments were grouped within experimental light conditions, other patterns emerged. Under current water chemistry conditions, colonies exhibit increasing amounts of brown and decreasing amounts of red coloration within their skeletons with increasing light level (Figure 4.22). Skeletons of colonies kept under decreased pH conditions showed more orange coloration and less red coloration with increasing light levels. Under equal light treatments, colonies under current chemistry conditions showed more brown and red coloration and less orange coloration than their counterparts in water

with lowered pH levels (Figure 4.23). Further investigation of the causes of differential skeletal coloration should be made.

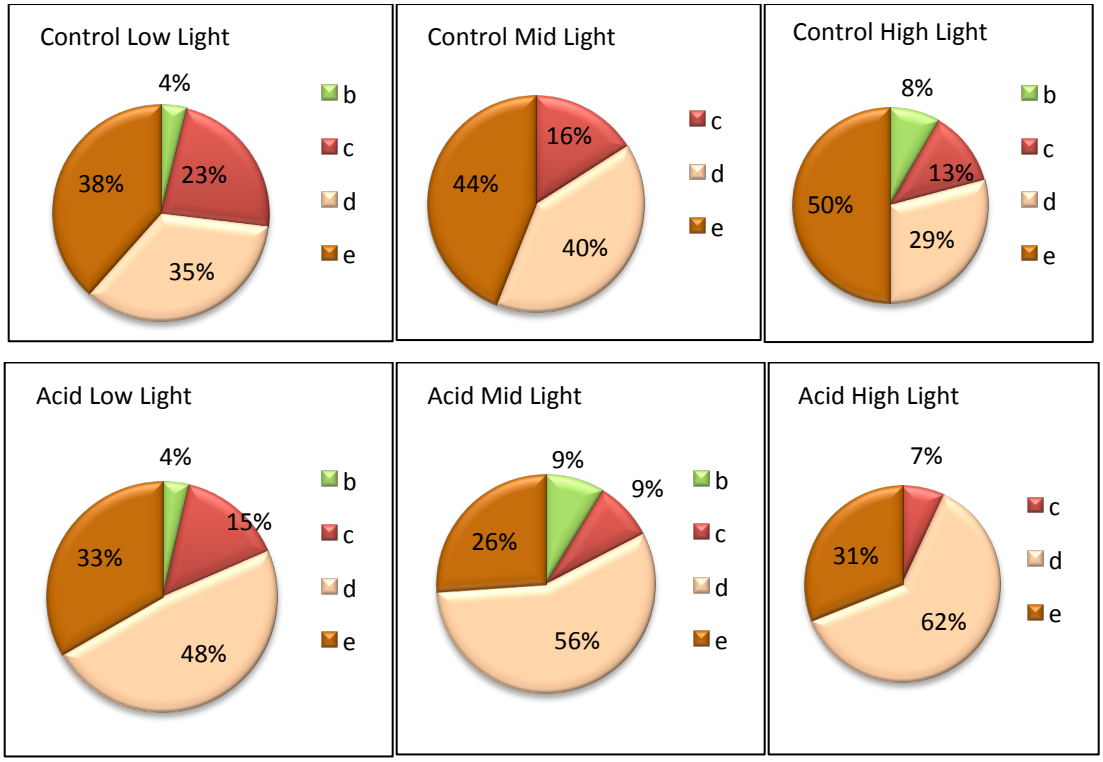

Figure 4.22: Proportions of discolored skeletons under the pH and light treatments (b= green, c = red, d= peach/orange, e = brown). Letters refer to the color families on the Coral Reef Watch cards.

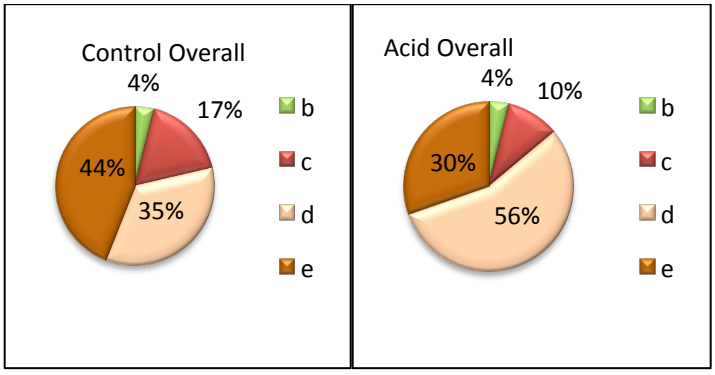

Figure 4.23: Charts denote the portion of the skeletons under the designated conditions (b= green, c = red, d= peach/orange, e = brown). Letters refer to the color families on the Coral Reef Watch cards.

4) Aiptasia pallida

The final species examined in this study was *Aiptasia pallida*, a common actinian in South Florida. This species was included in the study since it is zooxanthellate, but non-calcifying. There are concerns that simple shell-less invertebrates will be particularly susceptible to the effects of ocean acidification since they cannot fully regulate their internal pH (Petkewich 2009). During pilot studies, anemones were not able to withstand the highest generated flow rates (approximately 26 cm/s). Therefore, the anemones(Figure 4.24) were exposed to tolerable low flows of <12 cm/s and high flows of 15 cm/s in each of the studied water chemistry conditions (control pH – 8.04, Ω_{Ar} – 3.47, acidified pH – 7.85, Ω_{Ar} – 2.28).

The effects of pH, flow rate, and light levels were measured in several ways including, changing mass, oral disc area, protein concentration, and zooxanthellae density and activity. The change in the area of the oral discs over the six week study was significantly influenced by water chemistry ($p<0.0001$) with control anemones growing larger than those under decreased pH. Effects were significant under both low flow ($p = 0.0003$) and high flow ($p=0.0209$) (Figure 4.25, Table 4.9) as well as under all experimental light regimes (low light, $p=0.0394$, intermediate light, $p=0.0054$, and high light, $p=0.0331$) (Figure 26, Table 4.9). The only flow-light combination that showed significantly higher growth in oral disc area under current conditions when compared to its low pH counterpart was the combination of low flow and intermediate light ($p=0.0098$).

Figure 4.24: Anemones in their experimental units.

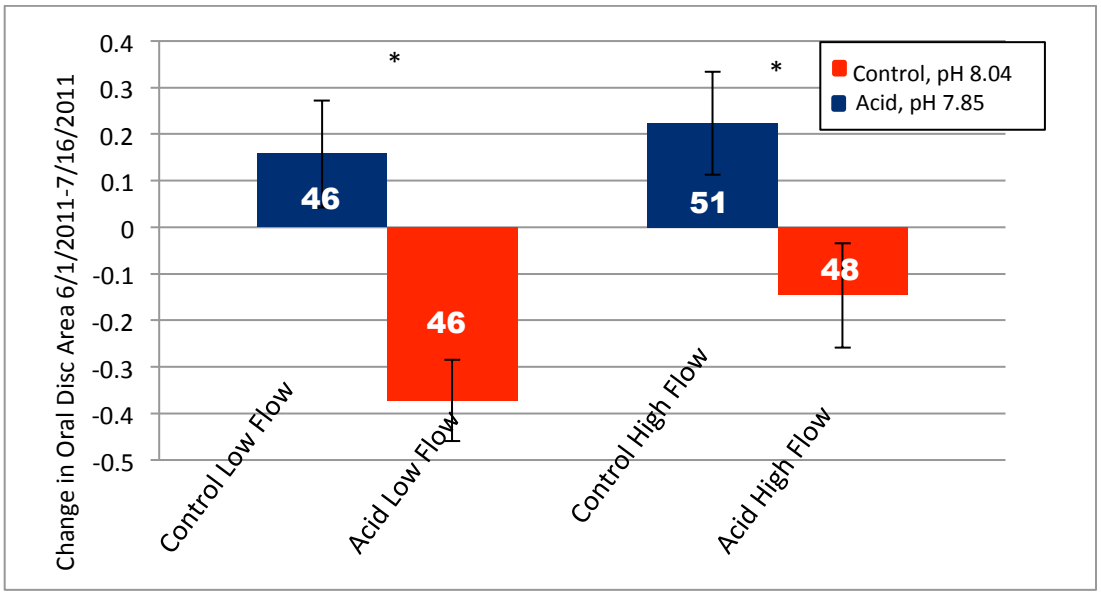

Figure 4.25: The mean change in oral disc area 6/1/2011-7/15/2011 of *Aiptasia* under the indicated conditions. Asterisks (*) indicate flow conditions in which zooxanthellae densities were significantly ($p<0.05$) impacted by changes in water chemistry. Error bars indicate +/- 1 standard error from the mean. White numbers denote the final number of individuals in each treatment.

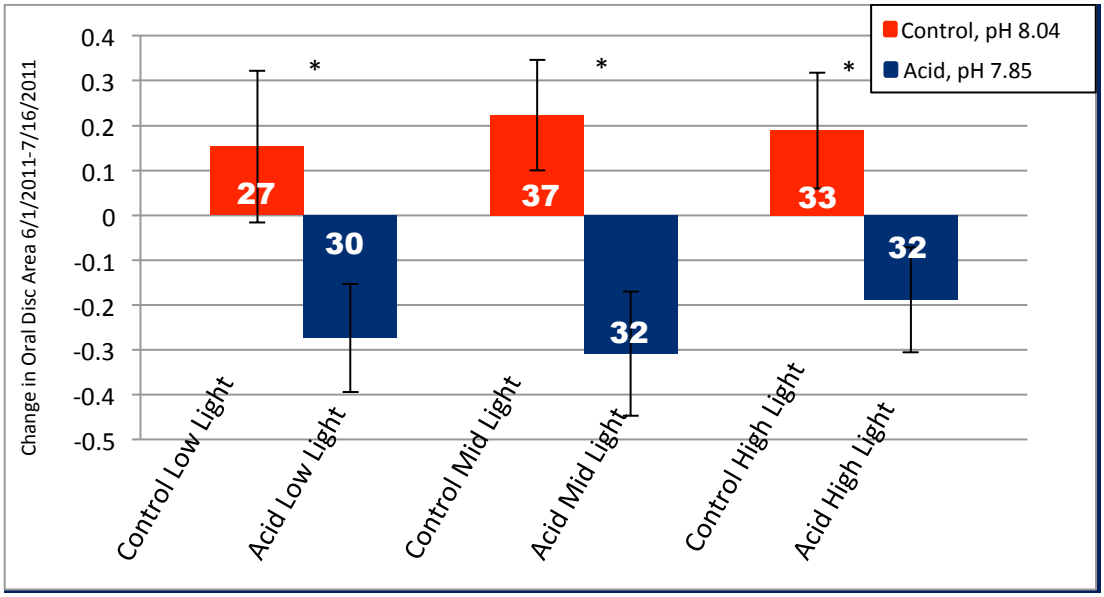

Figure 4.26: The mean change in oral disc area 6/1/2011-7/15/2011 of the anemones kept under the indicated conditions. Asterisks (*) indicate flow conditions in which zooxanthellae densities were significantly ($p<0.05$) impacted by changes in water chemistry. Error bars indicate +/- 1 standard error from the mean. White numbers denote final number of individuals in each treatment.

Table 4.9: ANOVA results comparing the change in the oral disc area of *Aiptasia pallida* exposed to two water chemistry conditions (control and acidified), by flow rates (~4 and 15 cm/s), and three light levels (50, 85, 180 µmol photons $m^{-2}s^{-1}$). Measurements were taken after 12 weeks of exposure. Fixed factors were Water Treatment, Flow, and Light. Significant differences were explored by Tukey's test or Student's T-test when only two conditions were compared.

Factor	DF	F	Sig.	Tukey's
Water Treatment	1	12.4659	0.0005	Control>Acid
Flow	1	1.5196	0.2193	
Light	2	1.4679	0.2332	
Water Treatment x Flow	1	1.0339	0.3107	Control Low Flow > Acid High Flow, Acid Low Flow
Water Treatment x Light	2	1.9501	0.1454	Control Mid Light > Acid Mid Light, Acid Low Light
Flow x Light	2	0.0115	0.9885	
Water Treatment x Flow x Light	2	1.8224	0.1647	Control High Flow Mid Light > Acid Low Flow High Light > Acid High flow Mid Light > Acid Low Flow Low Light > Acid High Flow Low Light

The anemones showed significant differences in response to the two experimental water chemistry conditions in 5 of the 6 flow rate-light level treatments. Decreased pH yielded anemones with more protein per wet weight than the treatment with present day pH (Figs. 4.27). Only anemones exposed to the combination of flow rates of 15 cm/s and

approximate light levels of 100 µmol s^{-1} m^{-2} did not show significant effects from changing water chemistry.

When the three flow regimes across the tested light treatments were pooled, significant effects of changing water chemistry on the amount of protein per wet weight were observed in all light treatments. Under low light conditions, the protein was significantly greater for anemones kept in conditions of decreased pH compared to those in current water chemistry conditions under low light ($p<0.0001$), mid light ($p=0.0063$) and high light ($p<0.0001$) conditions (Figure 4.28, Table 4.10).

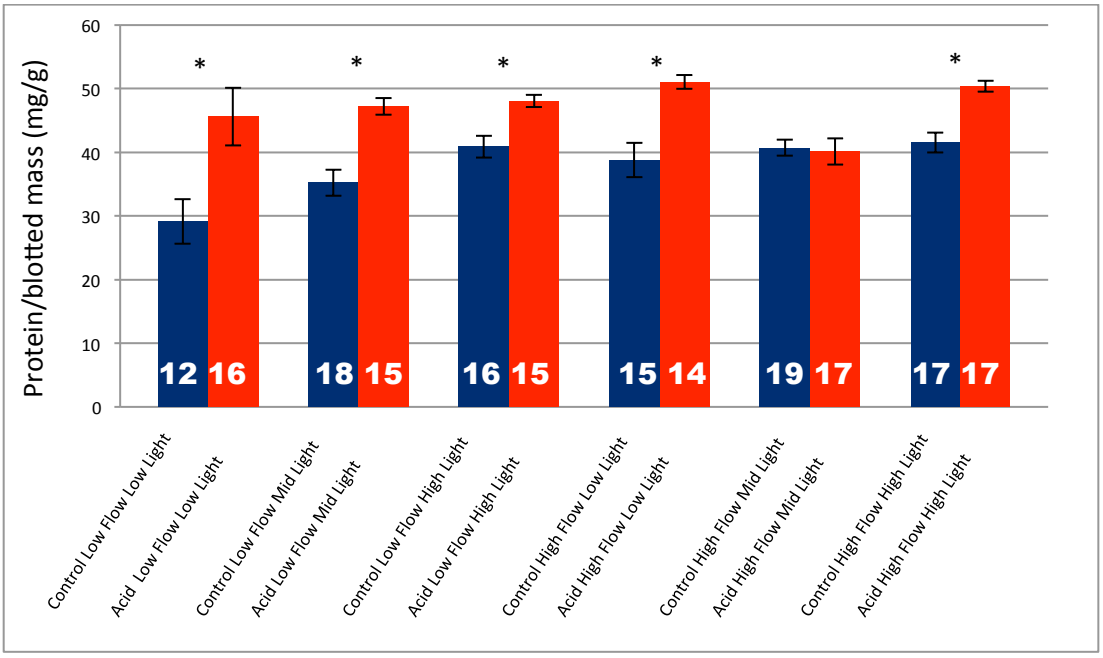

Figure 4.27: Mean protein/blotted mass of *Aiptasia* under the indicated treatments. Asterisks (*) indicate flow and light combinations that are significantly impacted by changes in water chemistry. Error bars indicate +/- 1 standard error from the mean. White numbers denote the final number of individuals in each treatment.

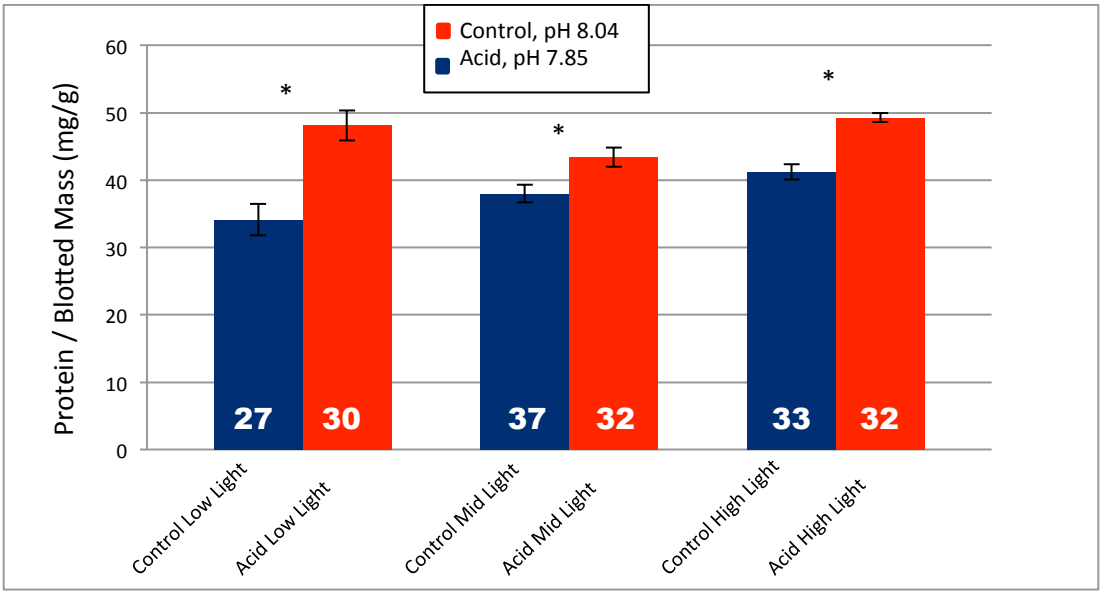

Figure 4.28: Mean wet weight of anemones under the indicated light treatments. Asterisks (*) indicate light conditions in which anemone masses were significantly impacted by changes in water chemistry. Error bars indicate +/- 1 standard error from the mean. White numbers denote the final number of individuals in each treatment.

Table 4.10: ANOVA results comparing the amount of protein/total blotted mass present at the conclusion of the experiment normalized to the final blotted mass in *Aiptasia pallida* exposed to two water chemistry conditions (control and acidified), by flow rates (~4 and 15 cm/s), and three light levels (50, 85, 180 µmol photons $m^{-2}s^{-1}$). Measurements were taken after 12 weeks of exposure. Fixed factors were Water Treatment, Flow, and Light. Significant differences were explored by Tukey's test or Student's T-test when only two conditions were compared.

Factor	DF	F	Sig.	Tukey's
Water Treatment	1	54.9387	<0.0001	Acid > Control
Flow	1	4.7478	0.0306	High Flow >Low Flow
Light	2	4.5113	0.0123	High Light> Low Light, Mid Light
Water Treatment x Flow	1	3.9282	0.0490	Acid High Flow, Acid Low Flow > Control High Flow>Control Low Flow
Water Treatment x Light	2	4.2785	0.0153	Acid High Light, Acid Low light > all control treatments Acid Mid Light, Control High Light> Control Low Light
Flow x Light	2	3.6869	0.0270	High Flow High Light, High Flow Low Light, Low Flow High Light> Low Flow Low Light
Water Treatment x Flow x Light	2	3.2613	0.0460	Acid High Flow Low Light, Acid High Flow High Light>Acid High Flow Mid Light, Control High Flow Low Light, Control Low Flow Mid Light, Control Low Flow Low Light Acid Low Flow High Light, Acid Low Flow Mid Light, Acid Low Flow Low Light> Control Low Flow Mid Light, > Control Low Flow Low Light

It is possible that the observed differences in oral disc size and protein per unit of blotted mass are influenced by behavioral differences under the tested pH conditions. If the low pH conditions are somehow "irritating" to anemone tissues, the anemone may

minimize the surface area that it puts in contact with the surrounding waters. This may be accomplished by failing to expand to its maximum possible diameter, thus decreasing the observed oral disc area. In addition, the anemone may retain less water within its body than it would under higher pH conditions. This would also lead to higher proportions of protein/blotted mass under lower pH conditions compared to anemones under control/higher pH conditions.

Physical conditions can influence all taxa of marine holobionts. In the case of *A.pallida*, the endosymbiotic zooxanthellae could also be affected. In general, the number of zooxanthellae within the anemone decreased with increasing flow rate, but not significantly so (T-test, $p=0.0798$). Decreases in the number of zooxanthellae present within anemones exposed to increasing amounts of light may be caused by the differential expulsion of zooxanthellae (Wissman 2003). Differential expulsion may also be responsible for the patterns seen in this study.

The number of zooxanthellae present per unit of mass was generally lower in anemones kept under current water chemistry conditions than in those kept in water of decreased pH (T-test, $p=0.0033$). The differences between zooxanthellae density per biomass were significant under some light and flow combinations, (Figure 4.29, Table 4.11). When results were pooled, zooxanthellae densities were higher under decreased pH conditions under both low flow ($p= 0.0287$) and high flow ($p=0.0061$) conditions (Figure 4.31, Table 11). In addition, densities of zooxanthellae were higher under low pH conditions under low ($p<0.0001$) and high light ($p=0.0149$) conditions (Figure 4.30, Table 11). In studies of well fed anemones, zooxanthellae were thought to benefit from

the enhanced CO_2 that results from host feeding (Davy and Cook 2001). While the anemones in this experiment were not supplementally fed, the low pH treated individuals would likely have experienced higher concentrations of available carbon compared to the control anemones. This may have contributed to the ability of these individuals to maintain higher densities of zooxanthellae than the control anemones.

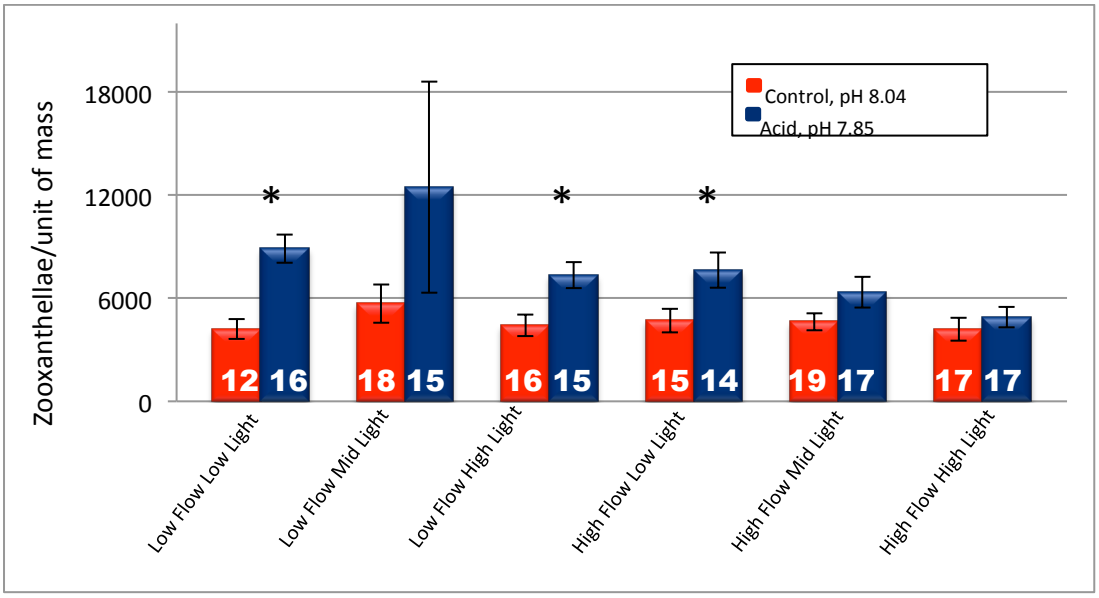

Figure 4.29: The mean number of zooxanthellae/per unit of blotted mass at the conclusion of the experiment under the indicated conditions. Error bars indicate +/- 1 standard error from the mean. White numbers denote the final number of individuals in each treatment.

Table 4.11: ANOVA results comparing the number of zooxanthellae normalized to the final blotted mass of *Aiptasia pallida* exposed to two water chemistry conditions (control and acidified), by flow rates (~4 and 15 cm/s), and three light levels (50, 85, 180 µmol photons $m^{-2}s^{-1}$). Measurements were taken after 12 weeks of exposure. Fixed factors were Water Treatment, Flow, and Light. Significant differences were explored by Tukey's test or Student's T-test when only two conditions were compared.

Factor	DF	F	Sig.	Tukey's
Water Treatment	1	9.4301	0.0025	Acid > Control
Flow	1	2.7246	0.1006	
Light	2	1.3130	0.2711	
Water Treatment x Flow	1	1.9791	0.1612	Acid Low Flow > Control Low Flow, Control High Flow
Water Treatment x Light	2	0.5064	0.6035	
Flow x Light	2	0.8039	0.4492	
Water Treatment x Flow x Light	2	0.2419	0.7854	

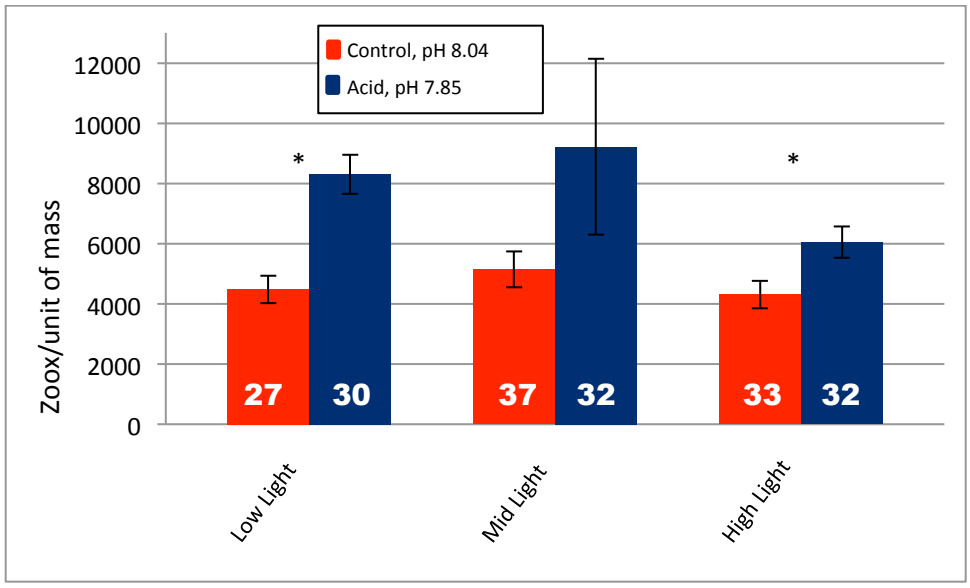

Figure 4.30: The mean number of zooxanthellae normalized to the blotted mass of the anemones at the conclusion of the experiment under the indicated conditions. Error bars indicate +/- 1 standard error from the mean. White numbers denote the final number of individuals in each treatment.

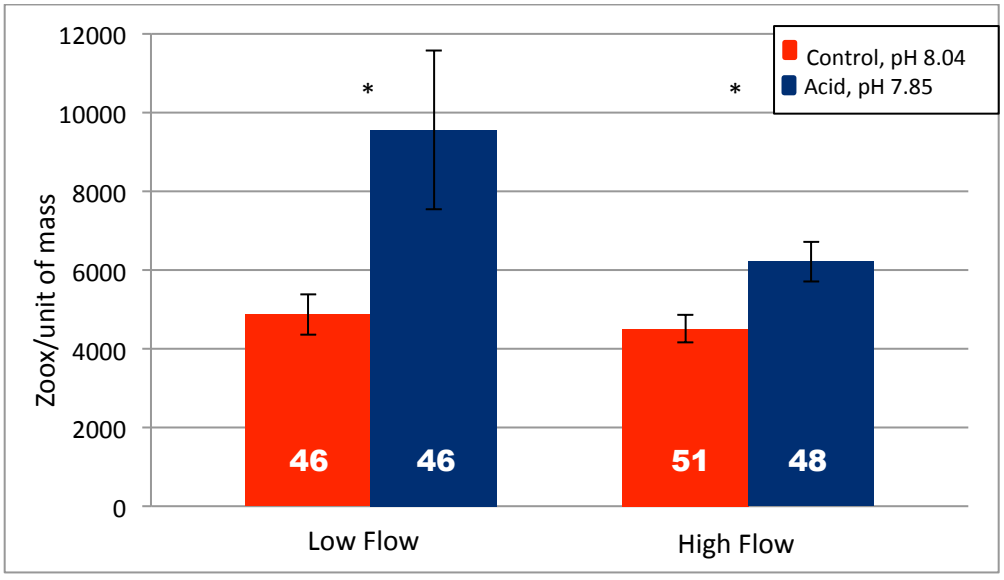

Figure 4.31: The mean number of zooxanthellae present normalized to blotted mass of the anemones at the conclusion of the experiment under the indicated conditions. Asterisks (*) indicate flow conditions in which zooxanthellae densities were significantly ($p<0.05$) impacted by changes in water chemistry. Error bars indicate +/- 1 standard error from the mean. White numbers denote the final number of individuals in each treatment.

The photosynthetic activity of the zooxanthellae was significantly influenced by water chemistry conditions, particularly within the low flow treatments. The Fv/Fm was significantly higher for anemones kept under low flow and current water chemistry conditions than those that experienced low flow rates in waters with decreased pH levels ($p<0.0001$). When data from different flow rates were combined, symbiotic zooxanthellae exposed to high ($p=0.0013$) and low light ($p=0.0007$) levels also manifested differential photosynthetic activity based on water chemistry conditions (Figs. 4.32-4.34).

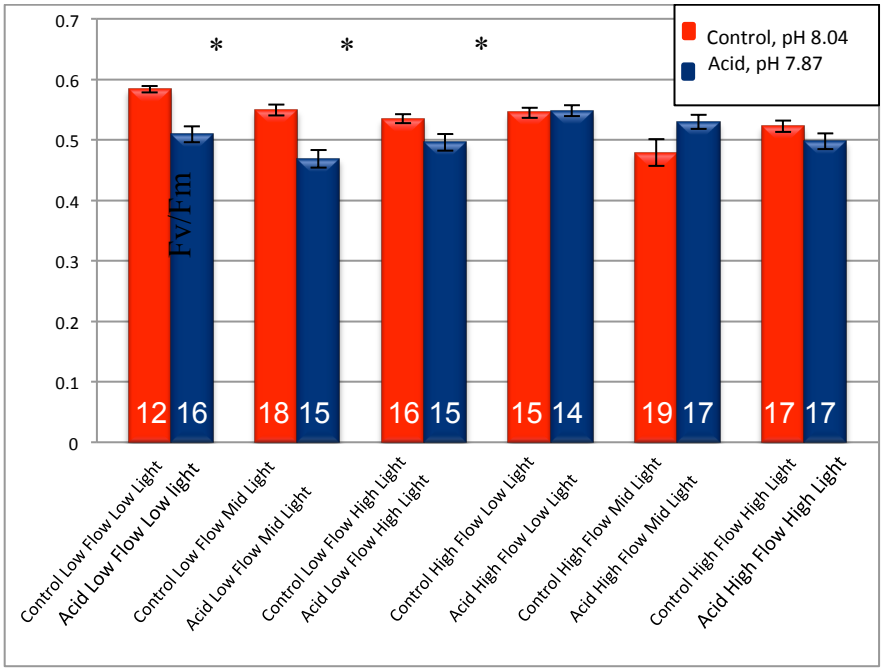

Figure 4.32: The Fv/Fm of the symbiotic zooxanthellae at the conclusion of the experiment under the indicated conditions. Asterisks (*) indicate conditions in which the photosynthetic activity of the zooxanthellae were significantly impacted by changing water chemistry. Error bars indicate +/- 1 standard error from the mean. White numbers denote the final number of individuals in each treatment.

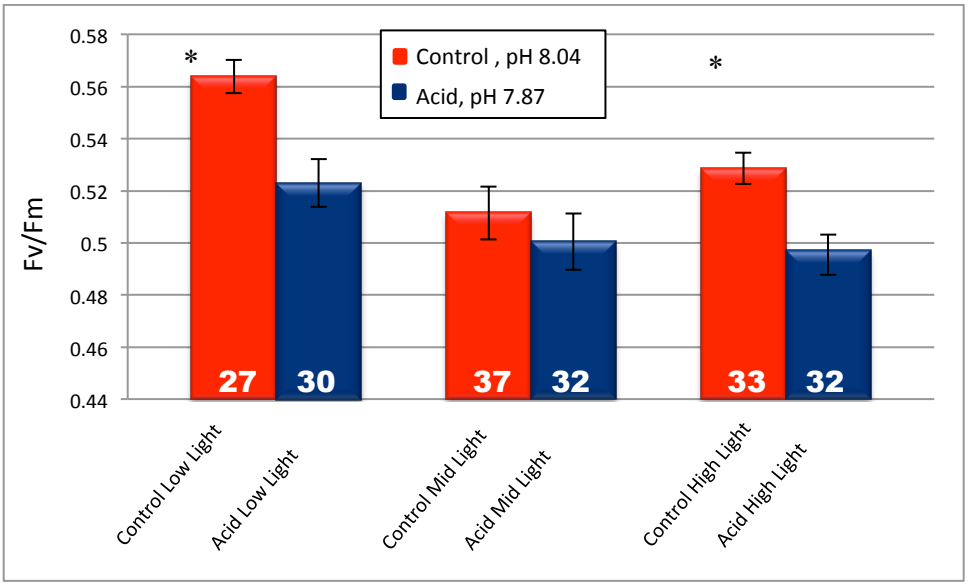

Figure 4.33: The Fv/Fm of the symbiotic zooxanthellae at the conclusion of the experiment under the indicated conditions. Asterisks (*) indicate conditions in which the photosynthetic activity of the zooxanthellae were significantly impacted by changing water chemistry. Error bars indicate +/- 1 standard error from the mean. White numbers denote the final number of individuals in each treatment.

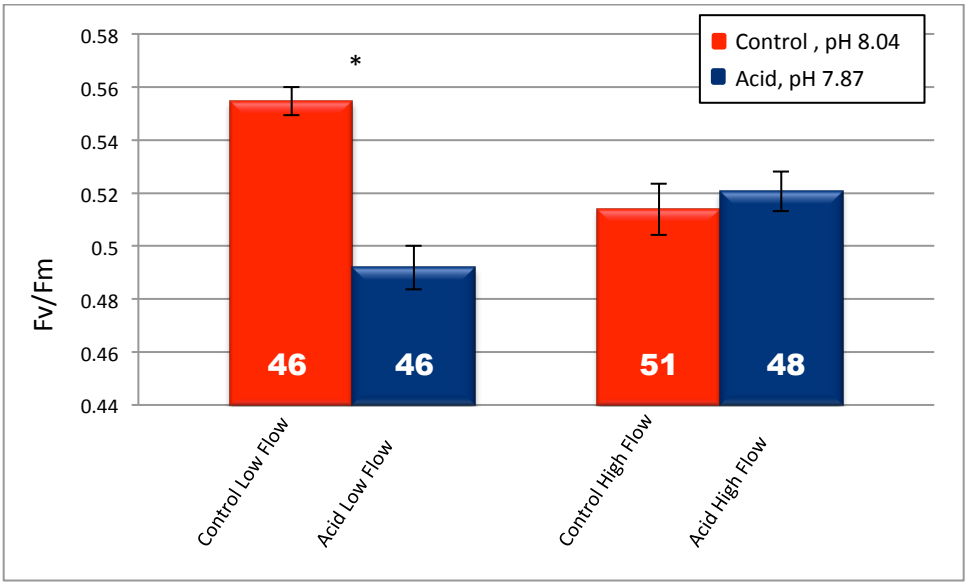

Figure 4.34: The Fv/Fm of the symbiotic zooxanthellae at the conclusion of the experiment under the indicated conditions. Asterisks (*) indicate flow conditions in which the photosynthetic activity of the zooxanthellae were significantly impacted by changing water chemistry. Error bars indicate +/- 1 standard error from the mean. White numbers denote the final number of individuals in each treatment.

D) Discussion

In this experiment, the responses of the experimental species to changes in pH were strongly influenced by species and morphology. Negative impacts were most common in the branching species *Pocillopora damicornis.* These impacts were more pronounced when low pH is combined under conditions that are additional sources of stress to the colonies, such as particularly low light and low flow. For numerous metrics, *P.damicornis* showed no significant negative responses when the low pH was combined with flow rates of 15 cm/s. In fact, the some metrics showed improved growth under the low pH conditions.

The *P. damicornis* colonies in this study did not exhibit significant decreases in growth due to low pH under 7 of the 9 tested light and flow combinations. The ability of *P. damicornis* to add mass may simply not be sensitive to pH effects, or the individuals in this study were less sensitive to the stress due to the fact that they were well fed. In other zooxanthellate corals, like *Astrangia poculata*, high nutrient availability appeared to offset the possible negative impacts of increasing CO_2 on calcification (Holcomb *et al.* 2010). Since the *P. damicornis* colonies in this study were given regular access to high nutrient food as well as being exposed to low pH conditions, the overall trend of slight, but insignificant differences in mass with changing water chemistry gives further support to the hypothesis that high nutrient availability may minimize the negative impacts of CO_2 increases in the future. If this is the case, in the future corals in areas of lower pH may become nutrient limited (Holcomb *et al.* 2010). Therefore, areas with naturally high nutrient availability should be targeted as potential marine protected areas for these

species. Care should be taken to choose areas that are naturally high in zooplankton and similar food sources, areas that possess natural controls that balance these nutrient levels. Areas that have artificially increased amplified nutrient levels should be avoided as they may lack the appropriate natural checks and balances to maintain a healthy system in the faces of stress. If an area has artificially amplified nutrient levels, zooxanthellae densities may be artificially increased, causing a greater bleaching response when stressed.

Branching zooxanthellate *Oculina arbuscula* have also been seen to decrease calcification under aragonite saturation states (Ω_A) of 0.8 or below due to reduced aragonite accretion. This species was not seen to modify its accretion activities to deposit calcite rather than aragonite under low Ω_A conditions. The responses of *O. arbuscula* to Ω_A levels of 0.8 or less are expected to be both abrupt and severe since their abilities to deposit aragonite under those conditions are highly diminished and they are unable to utilize an alternative carbonate form (Ries *et al.* 2010). When these observations are expanded to an entire reef community, changing acidity has little impact on the net production of the community because the photosynthetic organisms (including zooxanthellae and algae were utilizing HCO_3^- (Leclerq *et al.* 2002). If the zooxanthellae continue to utilize HCO_3^- to carry out photosynthesis in times of decreased pH, then this mechanism will continue to interfere with the calcification process, causing more apparent decreases in the calcification and growth processes of the coral hosts, while corals without zooxanthellae would show a far smaller effect.

The changing complexity of *P. damicornis* exhibited greater increases in complexity in control treatments than in low pH conditions except when exposed to the

combination of mid flow rates and low light levels. These differences are significant under all low flow treatments and high flow-low light and high flow-high light treatments. There were no significant differences in the mid flow treatments. Growth of the arborescent species *Montipora capitata* has also shown decreases in skeletal growth extension, as well as decreases in calcification under conditions of increased CO_2 in some experiments (Jokiel *et al.* 2008). Previous studies near Ishigaki Island, Japan have shown significant effects of water flow rate on the growth of *Pocillopora damicornis* in terms of both colony size and buoyant mass. Corals in 20cm/s flow conditions increased in size and buoyant weight significantly faster than those in still water (Suzuki *et al.*2008).

The photosynthetic yield measured by the I-PAM is an indicator of the health of photosystem II mechanisms within the endosymbiotic dinoflagellates. Not surprisingly, light has demonstrated a positive influence on photosynthetic yield. Increasing light caused a decrease in mean photosynthetic yield, particularly under controlled conditions. The symbionts demonstrated slightly higher photosynthetic yields under conditions of decreased pH. The influence of low pH was significant ($p<0.05$) only under low flow and high light conditions. The generally minimal/non-significant influence of decreased pH/aragonite saturation state on photosynthetic activity agrees with findings of Leclerq *et al.* (2002) and Langdon *et al.* (2003). In each of these studies, dinoflagellate photosynthetic activity was not observed to change as a function of changing CO_2. pCO_2 levels of 600-790ppm caused enhanced photosynthetic activity in the symbionts of the branching coral *Acropora formosa* (Crawley *et al.* 2010). Acroporids have also been

seen to exhibit enhanced photosynthetic productivity under mildly enhanced CO_2. pCO_2 levels of 600-790ppm caused enhanced photosynthetic activity in the symbionts of the branching coral *Acropora formosa* (Crawley *et al.* 2010). Acroporids have also been seen to exhibit enhanced photosynthetic productivity under mildly enhanced CO_2 conditions by Anthony *et al.* (2008).

Several metrics show evidence that changes in water chemistry will cause differences in the growth of *Pocillopora damicornis* colonies living under either high or low flow rates. It must be noted that no metric showed significant differences between corals growing under today's water chemistry conditions and those exposed to projected future water chemistry conditions. In fact, a number of metrics show indications of improved growth at decreased pH under mid flow conditions.

Unlike the branching species, *P. damicornis,* the mounding species, *Montastraea faveolata,* showed no significant effect of low pH on its growth. These findings agree with those of Helmle *et al.* (2011) which found that the growth rates of *M. faveolata* in Florida had not been impacted by climate changes up to the time of their collection in 1996. Together, this supports the hypothesis that this species has not been /will not be heavily impacted by global climate change.

This response of a massive species to low pH conditions is similar to patterns observed near CO_2 seeps near Papua New Guinea (Fabricius *et al.* 2011). Coral cover was constant across pH conditions of 7.8-8.1, however, the species and morphological diversity of areas of different pH was not. As pH decreased, massive *Porites* species

established dominance over structure building species which contributed to the stable level of coral cover despite changes in pH.

These findings that coral communities change with decreasing pH are consistent with observations that have been made of fossils from historical periods of low oceanic pH. Fossils from these eras show transitions to more mounding species. Then during periods of extremely low pH, evidence of hard corals is completely absent.

The absence of fossils from geological periods of low oceanic pH lend support to the theory that low pH inhibits the calcification and survival of hard corals. Scleractinians first appeared in the fossil record during the Paleozoic era, though records of them were lost during the Permian period. Fossil records show nine or ten families of hard corals present during the Middle Triassic (Deng and Kong 1984). Hard corals were absent during the previous 14 million years when carbonate suppression worldwide were suppressed (Stanley 1988). There is a theory that corals did not go extinct during that time, but instead existed during the 14 million year gap as non-calcifying, soft bodied animals. The early Triassic oceans were characterized by anoxic, deep ocean turnover, elevated CO_2 and shifts in global ocean chemistry that decreased the deposition of calcium carbonate (Knoll *et al.* 1996). When ocean chemistry once again shifted to favorable conditions in the Middle Triassic, some of these soft-bodied animals began to secrete calcium carbonate skeletons (Stanley and Fautin 2001). There is molecular evidence that the ability to secrete a calcium carbonate skeleton has evolved four times (Romano and Cairns 2000). This could indicate that the ability to produce a skeleton

may be lost and regained depending on environmental conditions. (Fautin and Lowenstein 1994).

If the hypothesis that corals survived as skeleton-less "naked corals" (Stanley and Fautin 2001) during past periods of low pH, it may be possible that modern corals may undergo similar morphological shifts in order to survive the current changes in ocean chemistry. Experiments by Fine and Tchernov (2007a) have shown that under extremely low pH conditions (7.3-7.6) some coral species can survive while experiencing complete dissolution of their skeletons. In this way, some coral species may be able to survive the predicted periods of low oceanic pH, however, since the corals will no longer be building solid, complex structures, the corals will no longer contribute to reef growth and the reefs will then cease to provide many of the services that they do currently (Fine and Tchernov 2007b).

Reef communities have recovered after being decimated by numerous climatic events that affected marine biota in the past, but those recoveries have taken 4-100 million years. During the recovery periods, framework building species were largely absent (Newell 1971). Therefore, during these periods a number of reef services were lost as well. Anthropogenic disturbances, including the climate change related stresses that are impacting today's reefs, and will continue to stress them in the coming decades, have preconditioned the reefs in a way that may have decreased their resilience to further perturbations (Hughes *et al.* 2003, Pandolfi *et al.* 2006). In addition, the rates and magnitude of current climate change is unprecedented in millions of years (Pandolfi 2011).

Unlike changing pH, which had no significant effect on the growth of *M. faveolata*, changes in available light did impact each colony's ability to add mass. The colonies in this study showed greater growth under higher light levels. Increases in light (below bleaching thresholds) have been seen to enhance productivity, growth, and calcification in numerous species (Juillet -Leclerc and Reynaud 2010). Very low irradiance has been seen to impair calcification of a number of hermatypic corals (Fricke and Schumacher 1983). The patterns seen in this species indicate that even the low relatively low irradiances present in this study were sufficient to support growth in *Montastraea faveolata* colonies and growth increased with increasing levels of irradiance and that within the levels tested, this pattern is unlikely to change with changing water chemistry.

Growth trajectories of colonies of *M. faveolata* under high light conditions were consistently higher than their low light counterparts. The faster increase in growth per unit of surface area under high light levels compared to lower light levels are consistent with observations of *Stylophora pistillata* by Moya *et al.* (2008). The zooxanthellae in the *S. pistillata* increased the calcification of the corals through the process of "light-enhanced calcification". It appears that the pattern of enhanced calcification with increased light is common among zooxanthellate species. This pattern is unlikely to change under conditions of decreasing pH.

Given these results and the results of the coral-sel experiments *Montastraea faveolata* colonies demonstrated highest biomass growth under conditions of high light (approximately 170 μmol s^{-1} m^{-2}) and flow rates of approximately 15cm/s. These data

also indicate that the changes in water chemistry predicted to occur in the next few decades are unlikely to have significant effects on *M. faveolata* populations. This may indicate that reef areas with structures dominated by this, and similarly behaving species, will maintain their structure, as well as their ability to grow and support complex biological communities in the future unlike areas dominated by *P. damicornis* and similarly affected species. Therefore, these *M. faveolata* dominated reefs may be capable of serving as refuges for other reef species during periods of ecological transition.

The azooxanthellate species, *T. coccinea,* showed little if any response to changing water chemistry while the zooxanthellate *Pocillopora damicornis* did. This dichotomy may be explained, in part, by the hypothesis that mechanisms of calcification and photosynthesis compete for a shared pool of dissolved inorganic carbon (Langdon and Atkinson 2005). Recent work by Holcomb *et al.* (2012) gives further support to the hypothesis that azooxanthellate corals will show smaller growth responses to ocean acidification than corals that are dependent on zooxanthellae as a source of nutrition. In their study, significant declines in growth and calcification were observed in zooxanthellate individuals of *Astrangia poculata* at temperatures associated with photosynthesis, but not at lower temperatures and not in azooxanthellate individuals of the same species. In addition, spawning females showed a greater response to pCO_2 levels than non-spawning females or males. This may be due to the allocation of carbon and energy to eggs rather than growth. If this observation holds true for other corals, then many species that are strongly dependent on the photosynthetic activity of symbionts will show greater impacts of changing pCO_2 than those that are azooxanthellate or those

that are less reliant upon zoooxanthellae. The increased impacts on colonies producing eggs could lead to imbalances in gender distribution of these species in the future, with a shortage of successful egg producing females, which could lead to low future recruitment. Further experimentation with azooxanthellate species and reproductive zooxanthellate colonies will be needed before definitive conclusions and predictions can be made.

The minimal impact of changing water chemistry on growth of *T. coccinea* seen in this experiment is similar to the patterns seen in *Astrangia poculata*. When *A. poculata* colonies were exposed to lowered pH conditions combined with high nutrient levels, their rates of growth and calcification were not significantly different from corals in ambient conditions. This suggests that high CO_2 conditions may lead to nutrient limitation (Holcomb *et al.* 2010). These species have similar growth morphologies with large polyps, though *A. poculata* is zooxanthellate while *T. coccinea* lacks symbionts. This suggests that species and colony morphology likely play a role in determining the extent to which some corals will be impacted by changing water chemistry in the coming years.

Few scientists have attempted to analyze the coloration of coral skeletons or how it is influenced by environmental conditions. There is some support for the hypothesis that coral skeletons contribute to the UVR absorption capabilities of corals (Reef *et al.* 2009). The color of the skeleton is likely due to traces of chemicals deposited along with the calcium carbonate by the polyp as the coral grows. The deposition of these chemicals is influenced by both water conditions, such as temperature and pH (Fallon *et al.* 2003,

Shirai *et al.* 2008) and physiological processes (de Villiers *et al.* 1995, Brown *et al.* 1991). The luminescent properties of the skeletons have been shown to be dependent on skeletal density (Barnes and Taylor 2005). Alternatively, the skeleton may be inhabited by endolithic algae which can cause the skeleton to appear tinted with one of several colors depending on the algal pigments. The endolithic community of coral skeleton is composed of photosynthetic microorganisms with a wide diversity of pigments for capturing wavelengths that pass through the coral tissue and are transmitted through the skeleton (Ralph *et al.* 2007). Some endosymbiotic algae can form distinct bands parallel to the skeleton's surface (*Ostreobium queketti*) (Lukas 1974). Other endolithic organisms include cyanobacteria, such as *Plectonema tenebrans*, some stages of red algae, fungi, and bacteria (Lukas 1974; Le Campion-Almusard *et al*. 1995).

The "black" skeleton of *Oulastrea crispata* exhibits variation in pigment concentration. The levels of pigment concentration present did not show consistent patterns with geographic distribution. Corals that were cross-transplanted between areas took on the coloration patterns of colonies from the regions they were relocated to. Their coloration was therefore not genetically fixed, but was environmentally influenced (Yamashiro 2000).

Overall patterns observed in *Aiptasia pallida* indicate that under conditions of low pH, anemones become more "compact," decreasing in oral disc size and concentrating the protein they do possess within a smaller amount of overall blotted mass. In addition, the mean number of zooxanthellae present within each anemone increases with decreased pH.

Physical conditions can influence all taxa of marine holobionts. In the case of *A. pallida*, the endosymbiotic zooxanthellae may also be affected. The number of zooxanthellae present within the anemones was significantly lower under current pH conditions than under conditions of decreased pH. The number of zooxanthellae present decreased with increasing light levels, particularly under conditions of low pH. The observed decreases in the number of zooxanthellae present within anemones exposed to increasing amounts of light may be caused by the differential expulsion of zooxanthellae (Wissman 2003).

The observed differences in the number of zooxanthellae present per unit of mass in anemones kept under different water chemistry may be a consequence of differing CO_2 availability. In studies of well fed anemones, zooxanthellae were thought to benefit from the enhanced CO_2 that results from host feeding (Davy and Cook 2001). While the anemones in this experiment were not supplementally fed, the low pH treated individuals would likely have experienced higher concentrations of available carbon compared to the control anemones. This may have contributed to the ability of these individuals to maintain higher densities of zooxanthellae than the control anemones while also influencing their photosynthetic activities. It is possible that the observed differences in oral disc size and protein per unit of blotted mass are influenced by behavioral differences under the tested pH conditions. If the low pH conditions are somehow "irritating" to anemone tissues, the anemone may minimize the surface area that it puts in contact with the surrounding waters. This may be accomplished by failing to expand to its maximum possible diameter, thus decreasing the observed oral disc area. In addition,

the anemone may retain less water within its body than it would under higher pH conditions. This would also lead to higher proportions of protein/blotted mass under lower pH conditions compared to anemones under control/higher pH conditions.

E) **Summary**

The series of flume studies described in this chapter indicated that *P.damicornis* colonies located in areas that experience approximately 15 cm/s of flow will not be significantly impacted by changes in water chemistry. *M. faveolata* colonies living in similar flow conditions will also continue to grow at similar rates to those seen today even as water chemistry changes. *T. coccinea* will see little impact of changing water chemistry on any of the metrics measured in this study. Colonies in areas with low flow conditions may experience a small decrease in growth, but those in flow rates of 15cm/s and higher light levels may see some increases in growth in waters with low pH. Areas with this flow rate may serve as refugia for numerous species. While some corals will likely exhibit decreases in growth with changing water chemistry, the results of the *T. coccinea* experiments show that not all corals will be impacted identically. Some species will show smaller effects and may help maintain reef structure providing habitats for numerous reef organisms despite the changes in water chemistry expected to occur in the coming decades.

Chapter 5: Implications for future reefs, conservation, and restoration efforts

A) Conservation and the role of Marine Protected Areas

Historically, disturbances to marine systems that have been studied were localized in terms of source, affected area, or in terms of time scale. Even "large scale" disturbances, such as those related to the recent Deep Horizon Oil Spill are still, in a sense, localized to areas directly influenced by the waters contained in a particular area of the ocean and individual current systems. Global climate change is, as the name suggests, a global phenomenon. Instead of eliminating entire swaths of marine life in "small" local areas, ocean acidification will instead place stresses on particular marine species over the breadth of the entire ocean. These species must therefore adapt to or succumb to the new conditions that result from these changes (Cooley *et al.*2009). In addition, the species may move or alter their possible ranges to encompass areas where chemical and physical properties are more conducive to the continued growth and survival of those species.

The changing trophic and geographic relationships that will develop as each species reacts to the environmental changes may alter marine ecosystems from their present states. These changes are likely to alter the relationships not only between each of the organisms in affected areas, but also the relationships that these animals and systems have to human populations that depend on them for numerous ecosystem services (Cooley *et al.*2009). In order to maximize the possibility of marine environments adapting to climate change conditions and increase the probability of them continuing to provide the ecosystem services that are depended upon by so many humans

and communities, anthropogenic pressures on oceanic ecosystems must be decreased (Cooley *et al.*2009).

Coral reefs possess a particular combination of attributes, creative power and fragility, resilience and sensitivity, productivity and vulnerability to overexploitation. This amalgamation of properties makes the management of coral reef systems a special challenge to science (Birkeland 1996) and therefore management. The development of Marine Protected Areas (MPAs) that will be able to fulfill their missions into the future, despite the impacts of global climate change is by necessity, a multidisciplinary approach. It must combine governmental policy makers, reserve managers, scientific experts in a multitude of disciplines, government agencies, conservation organizations, and local communities in order to be successful (Salm *et al.*2006). Strategically placed and managed MPAs seem to offer the most viable means of protecting and conserving key species and habitats in perpetuity (Salm *et al.*2006) as well as one of the "easiest" ways to manage overexploited fisheries (Bohnsack 1993, Roberts and Polunin 1993, Roberts 1997). The managers of MPAs play an invaluable role in ensuring that the areas are fulfilling their intended purposes. They can perform and/or oversee monitoring activities within the park areas and are well positioned to keep scientists and other stakeholders informed of changes within the park (Marshall and Schuttenberg 2006). Monitoring should include both ecological monitoring focused on the physical and biological parameters of the MPA as well as socio-economic monitoring which investigates the ways that humans use and interact with the reserve areas (Wilkinson *et al.* 2003).

The establishment of MPAs can protect marine communities from direct human impacts like overfishing, but cannot prevent organisms within them from experiencing the physical influences of global climate change (Hughes *et al.*2003). Efforts must therefore be made to ensure that the areas that are chosen as MPAs are either in areas that have natural buffers to climate change influences (such as natural upwelling) or contain features and organisms that will not exhibit significant decreases in health due to these influences. The abilities of these areas, and the communities they contain, to enhance the survival of other species should also be considered. Of the species examined in this study, *Montastraea* showed the most potential as a structure building species that would continue to provide this service as water chemistry changes. *Pocillopora damicornis* is a major framework builder on some Pacific reefs, but showed significant negative responses to decreased pH. Informed management decisions require an understanding of each area's potential ecological and evolutionary responses to climate changes and how those changes, as well as anthropogenic activities, will interact to influence the community (Baskett *et al.*2010).

There are currently more than 6000 declared MPAs, more than 1700 in the US alone (National Marine Protected Areas Center 2012), but of those, many have "unknown management" and are therefore limited in their effectiveness (McClanahan 1999). Although some MPAs become paper parks, or proposed sites do not make it through all stages of implementation and management, those that do are often extremely successful both ecologically and economically (McClanahan 1999, Halpern 2003, Vandeperre *et al.*

2011). While the goal of numerous parks is related to preservation and improvement of commercial fisheries, the effects of an area being protected are not restricted to simply increasing the abundance of large commercial fishes. Protection of an area can have both direct and indirect influences on the community in the MPA itself and nearby areas (Menge 1995, Vandeperre *et al*. 2011), including non-commercial effects such as influencing the calcium carbonate balances of a reef area (McClanahan 1995).

B) Marine Protected Area Selection and Design

The design of marine parks has evolved from the philosophy of total protection of small areas, to one allowing multiple uses of large areas with an integrated management system that takes account of all resource management goals including total protection (Kelleher, 1993). MPAs that include no-take areas can help restore reef communities that have become degraded by supporting higher abundances of fish as well as individuals of greater mass within the designated areas (Halpern and Warner 2002).

Marine Protected Areas have become an integral part of coral reef conservation efforts (Salm *et al.*2006). Managers today are faced with the daunting task of designing MPAs that will protect coral reefs that are increasingly at risk from numerous influences that they have no control over (Buddemeier *et al.* 2004). The factors that must be considered include: increasing temperature (Hoegh-Guldberg 1999), rising sea levels (Done 1999), increasing levels of ultra-violet light exposure (Gleason and Wellington 1993, 1995; Hoegh-Guldberg 1999; Siebeck 1988), and changing ocean chemistry (Gattuso *et al.*1998; Kleypas *et al.*1999, Langdon *et al.*2000, 2003; Leclerq *et al.*2000;

Marubini *et al.*2001). MPA designers and managers may feel that these large scale environmental factors are beyond their sphere of influence when they see that even well managed, protected areas, far from *direct* human influence still show signs of decline (Salm *et al.*2006).

Unless there are dramatic changes in fossil fuel use, the related projected increases in ocean acidification will continue at rates that have been unprecedented in tens of millions of years (Doney *et al.*2009). Natural processes including deep sea mixing and dissolution of marine carbonate sediments will take hundreds to thousands of years to remove the excess carbon dioxide from the atmosphere (Doney *et al.*2009). As ocean acidification proceeds, the number of species that are affected will increase both directly and indirectly. Calcifying organisms are influenced directly and the other organisms whose lives are intertwined with them in terms of nutrition and habitat are influenced more indirectly. Organisms like seagrasses are predicted to be "winners", showing improved growth and fitness. Calcifying organisms, including coral,are predicted to be "losers", showing decreases in growth and fitness, as ocean acidification continues. As increasing numbers of species become affected, regime shifts due to portions of ecosystems collapsing become likely (Kleypas and Yates 2009).

Although large scale factors relating to global warming and ocean acidification will continue to impact protected areas, there are ways to mitigate the negative impacts on the overall survival of reef ecosystems and biodiversity. By understanding the responses of organisms within a target area to current conditions, anthropogenic impacts, and climate change, management plans may be developed that could alleviate the impacts

of global climate change on and area (Heller and Zaveleta 2009). First, sites should be chosen that naturally possess conditions that are favorable for "general" survival and may have increased resistance to temperature related declines. These sites should then be protected from anthropogenic influences through the creation of marine reserves and protected areas. Second, these sites should be chosen in areas that maximize the likelihood that they can serve as both refugia for coral and sources of larvae to other areas (Salm *et al.*2001). In this way a reserve will protect not only the organisms within its borders, but may also assist surrounding areas in retaining or rebuilding their biodiversity. West and Salm (2003) clustered factors that reduce temperature stress and could therefore increase the protective properties of a reserve. They were 1) physical factors that reduce temperature stress, 2) physical factors that enhance water movement and flush toxins, 3) physical factors that decrease light stress, and 4) factors that confer stress tolerance to the corals themselves. Areas that possess these qualities will make the best candidates for effective marine protected areas.

1) Physical factors to consider during MPA selection and design

Physical factors that reduce temperature stress often include large scale water motion elements. Water motion at various scales can reduce temperature stresses that could otherwise lead to coral bleaching. Vertical mixing of cooler deep waters into shallow areas can diminish stresses on shallow corals (Skirving *et al.* 2006). Even in warm water conditions, strong currents can relieve temperature stress to some degree by

working to remove free radicals and other harmful materials from the vicinity of corals (Nakamura and van Woesik 2001) and their associated communities.

Light can be a double edged sword for corals. Under ideal conditions, many coral species thrive under high light levels (Jokiel and Coles 1990). In these experiments, *Montastraea faveolata* was again seen to have improved growth under high light conditions (180 µmol photons $m^{-2}s^{-1}$) compared to its growth under low light conditions (50 µmol photons m^{-2} s^{1}). This pattern was true under both control conditions and when pH was decreased. Therefore, when designing marine protected areas that contain reefs whose primary structure builders are montastrids, the sites chosen should be in areas with high light penetration. Alternatively, areas with large areas of shallow *Montastraea* reefs should be targeted for protection. If however, the corals are simultaneously experiencing another form of stress, such as increased temperature, high light can become an additional stressor (Salm *et al.*2006). When the effects of these stressors are combined, corals may bleach, and in extreme cases die. Bleaching and mortality can be decreased in areas that are shaded by islands or suspended sediment (Goreau 1998a, Goreau 1998b).

2) Biological factors that influence MPA selection and design

Corals show improved stress tolerance, particularly to heat stresses when they host a diverse array of *Symbiodinium* clades, particularly when they host individuals in clade D which has been shown to be more heat tolerant than most other forms (Baker et al 2004). If managers can select areas that host a diverse assortment of *Symbiodinium* clades they will improve the likelihood that the hermatypic corals within them will

survive stressful conditions and events. For many hermatypic corals, the ability to successfully calcify at rates in which accretion is greater than erosion is related to the photosynthetic activities of their symbiotic zooxanthellae (Al-Horani *et al.* 2005).

A major tipping point in determining the survival of a reef community is the threshold where the coral reef shifts from the net production and accretion of calcium carbonate to the net dissolution and erosion of reef structures. The tipping point will vary greatly from reef to reef based on numerous factors including the community composition, seasonal variations in calcification and dissolution rates, variations in sediment composition, mixing rates of water masses in each area, and the amount of biological control on calcification and dissolution mechanisms (Kleypas and Yates 2009). It is predicted that almost all coral reefs will cross the threshold and be in a state of net dissolution once atmospheric CO_2 reaches 560 ppm (Silverman *et al.*2009). The water chemistry conditions examined in this study are expected to be reached within the next 50-60 years.

Coral communities may be able to respond to climate change through shifts in community composition, acclimatization, and genetic adaptation (Baird *et al.*2007, Brown 1997, Hughes *et al.* 2003, Baker 2004, Hoegh-Guldberg *et al.* 2007). Most research has been focused on responses to thermal change, however other changes, like acidification, must be considered as well. Changes in coral community may mean shifts from domination by fast growing branching species, which appear to be more sensitive to environmental changes, to domination by slow growing species like *Montastraea* and *Tubastraea* which exhibit a smaller response to changing water chemistry.

The coral community that is the "target of protection" will also play a role in determining the level of jeopardy that a particular reef area is in. Coral species with lower growth rates, thicker tissues, and massive growth forms tend to be more bleaching resistant than those that have high growth rates, thin tissues, and branching growth forms (Gates and Edmunds 1999, Loya *et al.*2001). Fast growing, branching species like *Pocillopora damicornis* are more sensitive to changes in water chemistry under numerous conditions. Under conditions of low flow (<12 cm/s) and high flow (>27cm/s) decreases in pH and aragonite saturation state are expected to cause decreases in growth and complexity. Under flow rates of approximately, 15 cm/s, changes in water chemistry are predicted to show no noticeable effects. Therefore, if managers seek to preserve reef communities dominated by this species, they should target areas that regularly experience flow rates of 15-20 cm/s, but are not usually subjected to very high or low flow conditions. Under these flow conditions, the light treatments that were tested did not have a significant impact on growth of this species.

If the reserve is targeting corals that are more bleaching resistant such as massive corals including *Favites, Favia, Goniastrea, Astreopora* and *Turbinaria* (Marshall and Baird 2000, Floros *et al.* 2004, McClanahan *et al*. 2004) the marine reserve designers can allow for a wider range of physical conditions within the reserve and still have a reasonable expectation that the targeted species will find conditions that allow them to survive over time. There are several reasons why slower growing, massive corals, (particularly those with thicker tissues) are more resistant to bleaching. The high rates of respiration and protein metabolism of these corals may allow the rapid flushing of

harmful oxygen radicals from their tissues. The thicker tissues may also provide some protection to the zooxanthellae against UV radiation (Loya *et al.* 2001)

Faster growing species like *Acropora, Seriatopora, Stylophora, Millepora* and *Pocillopora* are often more susceptible to bleaching mortality *Turbinaria* (Marshall and Baird 2000, Floros*et al.* 2004, McClanahan *et al*. 2004). If these species are the targets of protection within the reserves, more care must be taken in choosing the protected sites to ensure that the areas contain conditions that minimize bleaching stress. By understanding the conditions that will improve the success of the targeted species, the likelihood of unwanted regime shifts within preserved sites can be decreased.

However, it must be kept in mind that, as is so often the case, there is a trade-off. These massive corals may survive conditions that will induce bleaching in most coral species, but their slow growth rates make areas whose primary structure builders are massive species slow to recover after a destructive event, particularly those that involve large amounts of structural erosion. Therefore, efforts should be made to preserve areas whose primary structures consist of a mixture of both branching and massive species. This will increase the likelihood of the overall survival and recovery of the reef structure during and following a variety of disturbance events.

By increasing the likelihood that the structure will survive, we can increase the probability that the fish, invertebrates, and other organisms that depend upon the structure of the reef for habitat and other resources will continue to thrive during and after stress events. This structure is important for dozens to hundreds of species of endolithic, epiphytic, free living, and cryptic organisms. By taking steps to preserve the continued

existence of reef structure after a stress event, we are not only preserving the coral species present and the structures they create, but the myriad species that depend on them for their survival through trophic and other ecosystem interactions.

This study revealed variations in the coloration of the skeletons of the *Tubastraea* colonies related to water chemistry, flow rate, and light exposure. One possible cause of this variation is the make-up and activities of the endolithic bacteria and fungi within the skeletons. If the persistence and/or metabolic activities of endolithic species are differently impacted by changes in abiotic conditions (temperature, light, acidity, etc.), then the endolithic communities within an area must also be considered during MPA design. Further examination of these communities and their abilities to improve the growth and survival of their host colonies and/or the stability of their structure over time should be undertaken by researchers to determine if they merit consideration of how coral communities will respond to future environmental changes.

3) MPA design – Considerations of Size, Shape, and Connectivity

There are other steps that can be taken during MPA design that can improve the likelihood that the reserve(s) will be successful in aiding a reef community's survival through resistance and resilience in the face of global climate change and all of the associated modifications to oceanic conditions (Table 5.1). Researchers have begun to prioritize indicators that are most likely to be useful predictors of reef resilience in a changing climate (McClanahan *et al.* 2012). They determined that a small number of indicators will likely have a large impact on the resilience of an area. One of the

strongest indicators is the presence of resistant coral species. The research presented in this dissertation along with experiments by other researchers will help identify the resistant species that should be targeted.

MPAs should be designed that provide coverage to the entire spectrum of habitat types and species that represent the biodiversity of the area in question. The size and shape of the protected area must also be taken in to account. If an individual reserve is being designed, larger reserves can encompass greater proportions of critical habitats, populations, and species. In addition, large areas can support larger populations and possible maintain higher genetic diversity (Almany *et al.* 2009). While many small areas may include a large number of habitats, they are also subject to greater influences of "edge effects". The mobile organisms in these areas would be likely to travel beyond the borders of the MPA where they would be directly exposed to harvest and other human activities (Moustakas and Silvert 2010). All organisms (both mobile and sessile) would be subjected to the influences of numerous physical and biological factors from areas beyond the reserve. Other than the size and shape of the preserve, designers must also determine how individual protected areas should be chosen in relation to one another.

Resistance to coral bleaching.	*Resistance Factor Reliability**
Physical factors that reduce temperature stress	
exchange (warm water replaced with cooler oceanic water)	high
upwelling	high
areas adjacent to deep water	high
wind-driven mixing	low
Physical factors that enhance water movement and flush toxins	
fast currents (eddies, tidal and ocean currents, gyres)	high
topography (peninsulas, points, narrow channels)	high
high wave energy	low
tidal range	low
wind	low
Physical factors that decrease light stress	
shade (high land profile, reef structural complexity)	high
aspect relative to the sun	high
slope	high
turbidity	low
absorption/CDOM	low
cloud cover	low
Factors that correlate with bleaching tolerance	
temperature variability	high
emergence at low tide	high
Indirect indicators of bleaching tolerance	
broad size and species distributions	high
areas of greatest remaining coral cover	high
history of corals surviving bleaching events	high

*Reliability *refers to whether the factor is considered predictable and persistent in its operation and thus of high value as a predictor of survivability.*

Consolidated list of factors that may contribute to resilience of a coral community.	*Resilience factor Reliability**
Intrinsic resilience factors	
availability and abundance of local larvae	high
recruitment success	high
low abundance of bioeroders, corallivores, diseases	high
diverse well-balanced community to prepare substratum for coral settlement (e.g., herbivorous fishes)	high
Extrinsic resilience factors	
good potential for recovery because of effective management regime	high
connectivity by currents (larval transport from other source reefs)	low
concentration of larval supply (e.g.,concentration and settlement in eddies)	low

*Reliability *refers to whether the factor is considered predictable and persistent in its operation and thus of high value as a predictor of recovery potential.*

Table 5.1: Factors that contribute to the resistance or resilience capacities of corals and coral communities. Adapted from West and Salm (2003)

C) Geographically Distinct Responses to Ocean Acidification as a consideration for MPA design

Different ocean basins and regions are likely to have varying reactions to ocean acidification both chemically and biologically depending on the types of organisms present and the construction of the food webs in the area (Cooley and Doney 2009). In addition, ocean waters at different latitudes will experience different degrees of change due to this acidification owing to temperature, salinity, and other physical properties (Fabry *et al.* 2008). Areas at high latitudes are expected to see the greatest *relative* changes in chemical properties related to acidification (Fabry *et al. 2008*). While it is therefore tempting to assume that tropical waters,which are predicted to remain supersaturated with respect to aragonite, are "safer" than those areas at higher latitudes with regards to acidification related dangers, that is not necessarily the case. Although numerically the chemical changes in the water itself are "relatively" small in tropical regions, they can have a disproportionately large effect on the biological processes of the organisms living within them (Cooley *et al.*2009). It is possible that many calcifying organisms are already living in areas that are close to their optimal chemical conditions. Therefore, even small changes in water chemistry are likely to have larger effects than would be predicted based solely on the magnitude of "numerical" changes in the chemical conditions.

Pacific and Atlantic areas whose food webs and harvests are highly dependent on calcifying organisms like clams, oysters, and scallops may see declines of millions of tons of animals and billions of dollars due to acidification effects (Cooley and Doney 2009). Changes in these calcifying populations may also cause changes to the

populations of predators and other reef dwellers, which will alsoalter global harvests (Cooley *et al.*2009)

D) Human activities and responses to changing conditions

There are a number of factors can contribute to the resilience of a reef area. Areas that have: reduced temperature stress (due to upwelling or proximity to cool waters), increased water movement (to aid in toxin removal), decreased radiation stress (island or reef shelf shading and turbidity), a predisposition to resist temperature damage (previously demonstrated resistance to regularly occurring thermal swings, exposure at low tides, history of bleaching event survival), strong potential for recovery if damage did occur (high larval abundance or recruitment), connectivity via larval transport, and the ability to maintain a favorable substrate for growth and recruitment offer greater probabilities that they will be successful MPAs (reviewed in Grimsditch and Salm 2006).

E) Recommendations for conservation efforts

An important aspect of park design is the division of the area into zones for different uses. The application of zoning will depend on the physical and biological characteristics of each potential park area. One zoning system that has proved workable is the placement of a central zone of high conservation value inside a buffer zone that will protect the core from outside disturbance (Hodgson 1997).

With these ideas in mind, marine protected areas that are intended to protect reef systems with *Pocillopora damicornis* as the primary framework builder should be

designed to encompass large areas that include numerous types of ecosystems, both marine and terrestrial. They should be large enough to incorporate concentric areas of decreasing permitted usage surrounding a central area of maximal protection that possesses mid flow (~15 cm/s) conditions. Of these mid flow areas, MPA designers should aim to choose regions that are dominated by low light conditions caused by shading from land, underwater outcroppings and overhangs, or possibly turbidity (though the types of suspended material must be taken into account). In general, MPA designers should strive to avoid areas that are likely to experience inordinately high temperature and/or solar radiation levels regularly (Salm et al 2006). In addition, choosing large areas that encompass segments that predominantly possess the characteristics listed above, but also include areas that experience stressful events (to a limited extent), often enough for the organisms they contain to tolerate short term exposure to stressful conditions, may improve the overall long term success of the MPA. With a present, but limited exposure to moderately stressful conditions, organisms may develop resistance mechanisms that will allow them to survive in the event of a high stress event. When organisms are kept naïve in relation to stresses (such as temperature) they may fail to develop any effective resistance mechanisms that might allow them to survive when/if they encounter a high stress condition (McClanahan *et al.* 2005).

Corals that experience moderately stressful conditions (i.e. moderately high temperatures) periodically, exhibit stress responses to them, such as bleaching. Although the short term effects of these stresses appear negative, the long term effect of these stresses and associated responses may be beneficial to the overall coral community. This

benefit is born of the fact that the periodically stressed corals have the opportunity to adapt to deal with the stresses. These corals are then better prepared to cope with more intense stresses when they occur. In contrast, coral communities that have been shielded from moderate thermal stresses by the presence of high water flow regimes (or other protective conditions) show higher mortality than the moderately stressed areas when a high stress event occurs (McClanahan *et al.*2005). In order to improve an MPA's ability to preserve the overall survival of its coral community, it should contain both areas that are generally protected from stresses as well as areas that see moderate disturbance, but are therefore more likely to survive more intense disturbances when they occur. The low disturbance areas can serve as source populations under normal circumstances and time spans, while the moderately disturbed areas may serve as refugia during intense stress events, like anomalously high thermal periods. After these high stress events, the communities that survive within the moderately disturbed areas could then serve as sources to help re-seed areas that experienced high mortality due to the stress event.

Based on the findings of Rowan (2004) and Baker (2003) it is also important that the selected areas host a diverse assortment of *Symbiodinium*. This is important for areas in which resistance or resilience to bleaching are of particular concern. There have been a number of *Symbiodinium* distribution patterns noted in different oceanic regions. In the Caribbean, there is a predictable pattern of symbiont distribution with depth in major reef builders including *Montastraea faveolata and M. annularis*. Shallower colonies contained clades A, B, C, and D. Deeper colonies were dominated by clade C individuals while very deep colonies of *M. franksi* also contained clade D symbionts (reviewed in

Baker 2004). In the Pacific, colonies are dominated by clades C and D, with some depth patterns within clade C (reviewed in Baker 2004). The ability of clade D to persist and thrive under conditions unsuited to other clades, and its tendency to dominate corals that have suffered thermal stresses makes it imperative that regions chosen for preservation as MPAs host a diversity of symbionts, particularly those within clade D.

In addition, areas that have the potential to provide additional heterotrophic nutrition for the corals should be considered as potential MPAs, or portions of MPAs. A coral's calcification is often limited by the availability of nutrients. Increasing the coral's nutritional status (the amount of food available to the coral) can increase the coral's ability to grow (Houlbreque *et al.* 2004) as well as influence its ability to reproduce (Sere *et al.* 2010). If corals are living in water with a low aragonite saturation state, the coral's ability to calcify may be maintained or improved by enhanced heterotrophic feeding or the increased availability of inorganic nutrients such as nitrates and phosphates. The corals in these experiments were fed supplementally to improve health and survival. This likely helped offset the influence of the changing water chemistry on those colonies. The *Tubastraea* and *Montastraea* species studied showed little response to these changes under fed conditions, but these responses may be magnified under conditions of low nutrient availability. *Pocillopora* colonies showed decreases in growth under low and high flow conditions that might become even more severe when additional sources of nutrition are not available. These species should be further studied under both fed and starved conditions to determine if heterotrophic feeding does indeed influence the extent to which water chemistry impacts coral growth. These facts must be kept in mind as we

consider the information gained from this study and how it may be utilized in the selection of suitable marine protected areas in the future. The observations made in this study could best be used to make predictions about the possible growth of colonies in areas with high abundances of zooplankton and other dissolved organic material.

Under elevated CO_2 conditions, nutrient additions can support increased zooxanthellate photosynthetic processes without reducing the total amount of carbon available for calcification. The increase in photosynthesis could provide more energy for the coral to produce their skeletons. Therefore, the increase in photosynthetic products coupled with the increase in nutrients could offset the negative effects of increasing CO_2 alone (Cohen and Holcomb 2009).

The impacts of natural and human activities influence marine systems in multiple, type specific ways. Human influences are often chronic and persistent (i.e. overfishing and nutrient enrichment). While human activities may be carried out by individuals, they often occur so frequently that there is little to no recovery time for the marine system between events (Nystrom *et al.*2000). Managers can use maps based on human impacts on reefs to make informed decisions on management and surveillance priorities based on threats and predicted impacts, guide permitting decisions based on these impacts, and choose areas to monitor for climate change effects (Selkoe *et al.*2009). All of the information gained from this research can and should be applied by scientists and conservationists to contribute to reef conservation and rehabilitation efforts.

The results of these experiments point toward the benefits of choosing areas that have a high percentage of reef areas that experience mid flow rate conditions (approximately 15 cm/s). These areas are unlikely to experience major changes in growth of reef building species like *Pocillopora, Montastraea,* or *Tubastraea* due to changing oceanic pH and decreasing aragonite saturation states over the next 100 years.

Areas that rely on *Montastraea faveolata* as a major structure building species should also be selected based on light penetration at the depths of target reef areas. Coral-sel studies showed that *M. faveolata* are likely to grow well under flow rates of 15cm/s and based on the flume study, its growth is not significantly impacted by decreases in pH. The flume studies indicated that higher light levels (180 μmol m^2 s^{-1})will yield higher growth and more robust corals in the years to come than any of the other light and flow combinations tested. They are unlikely to suffer significant changes in growth due to ocean acidification. Therefore, it is imperative that these areas be identified and designated as marine protected areas now to ensure that they will remain productive reefs that can continue to serve as healthy habitats for myriad species in the future.

In addition, the results of these studies indicate that conservationists, aquaculturists, and scientists seeking to produce colonies of *M. faveolata* for experimental purposes, the aquarium trade, or restoration efforts will be able to produce the greatest amount of biomass in tanks with flow rates of ~15 cm/s and higher light levels (at least 180-200 μmol m^2 s^{-1}). If the corals grown are intended for lab work, the aquarium trade, or restoration of areas with few fast growing competitors for space, the

colonies should be mounted on flat plates that will allow them maximal surface area to extend horizontally. If the colonies are intended for transplantation to areas with multiple fast growing competitors for space or grazing animals that are likely to cause damage to colonies that are entirely prostrate with little vertical extension, cultured corals grown on peg-like bases would be better suited to transplantation than those without any vertical distance from the substrate.

Many areas that are dominated by *Pocillopora damicornis* as the major reef building species are likely to see significant changes in growth in the next 50-100 years due to the effects of ocean acidification. Not all areas will be equally affected. Those that have flow rates of approximately 15 cm/s around the reef will see far smaller changes in their growth and complexity than those in areas characterized by higher (24 cm/s) or lower (<12 cm/s) flow rates due to changing water chemistry. In fact, there may even be slightly higher growth of *Pocillopora* colonies in areas with 15 cm/s flow rates under slightly acidified conditions. Areas characterized by low flow conditions (<12 cm/s) are likely to show the greatest impacts on reef complexity, with decreased complexity under all light conditions. Areas with high flow rates (~25 cm/s or more) will show decreases in growth and complexity under most conditions, but under low light levels the changes may not be significant.

Pocillopora is an important structure building species in many areas of the Pacific Ocean. As such, they serve as major habitat builders on reefs. They will continue to provide both structural niches and sources of food and other resources far into the future if we take steps to preserve reef areas that possess the physical properties that will

encourage reef growth and survival despite the large scale changes occurring in the ocean.

Under current water chemistry, low flow conditions (<12 cm/s) are most conducive to increases in biomass and increases in complexity. Therefore, scientists and aquaculturists seeking to produce the greatest amount of coral biomass for research, transplantation, or the aquarium trade should keep their colonies under low flow conditions and higher light levels (though light had little impact on growth under this flow regime).

Tubastraea coccinea is not considered a major structure builder on most reefs and is in fact an invasive species in the Caribbean. According to these experiments, *T. coccinea* will experience very minimal response to changing water chemistry in areas that are dominated by the light conditions between 50-180 μmol m^2 $s^{-1}$and flow conditions of <12 – 27 cm/s.

This minimal response could have important consequences both for the *T. coccinea* and numerous reef dependent species. While other coral species are expected to exhibit marked decreases in growth rate and complexity as ocean acidification progresses, the fact that colonies of this species will continue to thrive (and likely reproduce and spread) could allow them to act as "life boats" for other species that depend upon corals and coral structures for survival. Their presence may provide structures that will allow some species to survive until other framework builders either adapt to new conditions or colonize areas with more amenable conditions in which to establish a healthy community.

T. coccinea is an invasive species in numerous parts of the world including the waters of Florida and Brazil. Members of the genus *Tubastraea* are adept at colonization for numerous reasons. These include their relatively rapid growth (Vermeij 2005), early reproductive age (Fenner and Banks 2004, Glynn *et al.* 2008), as well as their ability to produce planulae that can remain viable in the plankton for up to 100 days (Ayre and Resing 1986, Fenner and Banks 2004, Glynn *et al.* 2008). Observations in Brazil have shown that the presence of *T. coccinea* in a community causes increased species diversity, richness, and evenness compared to areas lacking the species (Lages *et al.* 2011). If *T. coccinea* colonies are capable of supporting diverse communities on structures that they have colonized, it is possible that they will be able to continue to support diverse communities in coming years as other species lose some or all of their ability to serve this pivotal function for these other organisms. They may be able to serve as interim refugia, supporting the continued existence of multiple species as other coral communities adapt or adjust to the new conditions or until water chemistry changes again. This species is not usually considered a major structure builder, and is more often considered a minor cryptic species, but its ability to persist through stressful conditions and the influences it and other similarly resilient species will have on reef communities should be considered.

Additionally, by colonizing new areas, fast growing, invasive species may create new structural safe zones for organisms driven from their former recognized ranges by environmental changes. These new areas may serve as new permanent habitats, or they may serve as stepping stones allowing the supported species to continue to expand their

ranges to exploit areas beyond their traditional habitats, or to reach areas that have not been as severely impacted as their original habitats. These colonized areas might be best considered as temporary habitats, since invasive species have been seen to lead to short term increases in diversity and richness, but after extended periods, they may outcompete native species leading to later decreases in diversity (Lages *et al.*2011).

The responses of *Aiptasia pallida* to long term exposure to seawater with decreased pH are decreases in oral disc area, protein and zooxanthellae present per unit of mass, as well as the photosynthetic activity of the zooxanthellae within. These anemones appear to be smaller and denser in terms of protein content compared to individuals that grew in unaltered seawater. Changes in the growth of these anemones due to changes in water chemistry will have effects that extend to the animals that feed upon these animals. These species include some butterflyfishes, filefishes, puffer fish, and hermit crabs. Peppermint shrimp (*Lysmata* sp.) (Rhyne *et al.* 2004) and *Berghia* sea slugs (Carroll and Kempf 1990) may be more intensely affected since they depend heavily on anemones (particularly *Aiptasia*) as a food source (). If other anemone species are impacted by changing water chemistry in similar ways to *Aiptasia* the effects will be seen in animals like clownfish (*Amphiprion percula*) that depend on anemones for both protection and habitat (Allen 1972, Fautin and Allen 1992). If anemones decrease in overall size with decreasing seawater pH, animals that depend upon them for protection and shelter will be detrimentally affected by these declines as they lose both shelter and protection.

If anemones are able to maintain their reproductive and settlement rates despite changes in water conditions, these changes may have a slight positive side effect for animals that feed on the anemones. The anemones observed in this study showed higher concentrations of protein per area under acidified conditions. This could mean that the consumers would obtain higher amounts of protein per unit volume of anemone that they consume. The consumers would therefore need to consume less total material than they currently do in order to attain an equal amount of nutrition.

As global warming and ocean acidification continue in the years to come, the preservation of marine resources will become increasingly important and more difficult as more areas are damaged by both natural and anthropogenic influences. The information gained from this research and similar studies can be utilized by scientists and conservationists to maximize the success of marine protected areas in preserving marine habitats and species. In addition, this information can assist aquaculturists in choosing the conditions they will maintain their stocks in in order to produce the greatest amount of material in the proper forms for research and transplantation purposes.

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