FLUCTUATING REEF ENVIRONMENTS MAINTAIN CORAL RESILIENCE

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ABSTRACT

The shallow back reef pools on Ofu Island, American Samoa, are highly-fluctuating environments where seawater temperature and irradiance often reach levels thought to cause bleaching of reef-building corals, yet bleaching is rare in the pools. I hypothesized that environmental fluctuations associated with the pools, and biological mechanisms associated with back reef host corals and their algal endosymbionts, both reduce the effects of elevated seawater temperatures and high irradiance levels. I tested the hypotheses with a series of water table and field experiments. Water table experiments using *Porites lobata* and *P. cylindrica* suggested that intermittent flow associated with semi-diurnal tides, and low irradiances caused by turbidity or shading, reduce photoinhibition and bleaching of back reef corals during warming events. Reciprocal transplant experiments (RTEs) of the corals *P. lobata* and *Pocillopora eydouxi* between the highly-fluctuating back reef pools and a nearby environmentally consistent forereef site demonstrated that back reef colonies of both species survived better and grew more than their forereef counterparts regardless of transplant site. For *P. lobata*, growth was more affected by source population than transplant site, suggesting effects of either developmental phenotypic plasticity or genetic polymorphism. For *P. eydouxi*, growth was only affected by transplant site, demonstrating effects of physiological phenotypic plasticity. In addition, a survey of zooxanthella genotypes of *P. lobata*, *P. eydouxi*, *Galaxea fascicularis*, and *Acropora gemmifera* colonies at the back reef and forereef sites used for the RTEs showed a higher proportion of heat-resistant Clade D genotypes in back reef colonies of all four coral species except *P. lobata*. In
conclusion, these experiments demonstrated that environmental characteristics and biological mechanisms associated with fluctuating reef environments and their corals maintain the resilience of coral populations to disturbances for three reasons. First, potentially stressful conditions (elevated seawater temperatures and high irradiance levels) are moderated by environmental characteristics such as semi-diurnally intermittent flow. Second, exposure to these conditions likely maintains selection for mechanisms of acclimatization (phenotypic plasticity) or adaptation (genetic polymorphism) within host coral populations. Third, exposure of coral colonies to diurnally and seasonally fluctuating conditions maintains zooxanthella genotype diversity in some host coral species.
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The defining characteristic of reef-building corals is their symbiosis with unicellular dinoflagellates (zooxanthellae). The symbiosis enables rapid skeletal growth in shallow, oligotrophic tropical and subtropical seas, allowing corals to outpace macroalgae and bio-eroders, thereby making coral reefs possible. As seawater temperatures drop with latitude or depth, skeletal growth slows and coral colonies are outcompeted by macroalgae. Likewise, as light diminishes with depth, skeletal growth slows and coral colonies are undermined by bio-eroders (Kleypas et al. 1999b, Veron 2000, Grigg 2006). Though warm, shallow waters provide the heat and light needed for reef-building corals to thrive, too much of either breaks down the symbiosis, resulting in coral bleaching (Fitt et al. 2001, Jokiel 2004) and disease (Sutherland et al. 2004, Rosenberg et al. 2007). Thus, global warming and other trends associated with climate change are stressing and killing reef-building corals, even on reefs little affected by other human activities (Guinnotte et al. 2003, Hughes et al. 2003, Birkeland 2004, Kleypas et al. 2006, Bruno & Selig 2007). A high conservation priority for the 21st century is identification and protection of coral communities that are most resilient to climate change (West & Salm 2003, Grimsditch & Salm 2006, Marshall & Schuttenberg 2006). Reef-building corals inhabiting highly-fluctuating habitats are frequently exposed to elevated seawater temperatures, high irradiance levels, and other abiotic extremes without ill effect, and thus may be particularly instructive on the environmental and biological factors that make corals resilient.
Brown (1997) states "Daily variations in abiotic factors in reef waters are poorly documented yet they hold an important key to the understanding of the physiological tolerances of reef organisms", and little has appeared in the literature on this topic since Brown's review. Coral populations encounter a wide range of daily temperature maxima and daily temperature fluctuations on small spatial (< 1 km) and temporal (< 1 week) scales: For example, on Samoan fringing reefs, during high temperature conditions (midday spring low tides and clear weather), daily temperature maxima and fluctuations are much greater in shallow (1 – 2 m) back reef pools than on the adjacent forereef. Within a few days, low temperature conditions (mid-day flood, ebb or high tides and cloudy weather) greatly reduce daily maxima and fluctuations (Fig. 1.1). Though reef-building corals are thought to live near their upper thermal limits and tolerate narrow temperature ranges (Coles & Brown 2003, Jokiel 2004), many back reef corals tolerate daily and annual fluctuations of > 5 °C and > 10 °C, respectively, and maxima of 5 – 10 °C above the local summer mean (Coles 1975, Brown 1997, Coles 1997).

Figure 1.1. Estimated small-scale spatial (back reef @ 1.5 m depth vs. adjacent forereef @ 10 m depth) and temporal (weekly) variability in summer-time daily temperature maxima (values) and daily temperature fluctuations (bars) on Samoan reefs (local summer mean ≈ 29½ °C; from data summarized in Chapter 6, Craig et al. 2001).
The small scale variability in temperature maxima and daily temperature fluctuations illustrated in Figure 1.1 contrasts sharply with the genetic panmixia of many populations of broadcast-spawning coral species on spatial scales of tens to thousands of kilometers (Ayre & Hughes 2000, Rodriguez-Lanetty & Hoegh-Guldberg 2002, Takabayashi et al. 2003). Each population inhabits a range of thermal regimes, from shallow highly-fluctuating back reefs to more stable off-shore or deeper forereefs and reef slopes. That is, high gene flow of these broadcast-spawning species at large spatial scales may limit genetic responses (adaptation) to the small-scale thermal regime of back reefs. The same applies to other abiotic factors that have high/low extremes and fluctuate widely in back reefs on daily or short-term temporal scales, such as irradiance, salinity, turbidity, flow and dissolved oxygen. The highly-fluctuating nature of back reef pools is described in more detail below.

Back Reef Pools: Highly-fluctuating Habitats

Back reef pools and reef flats are typically subject to rapid environmental fluctuations because of shallow water, reduced mixing, and proximity to land: Seawater temperature and irradiance levels respond quickly to sunlight or lack thereof, water velocity and dissolved oxygen greatly vary depending on the tidal cycle and weather, and salinity drops and turbidity rises when storms produce terrigenous run-off (Brown 1997, Smith & Birkeland 2003, Dahlgren & Marr 2004). The effects of such daily or short-term fluctuations in environmental conditions on reef-building corals are not well understood (Brown 1997), and may be positive, negative, or neutral. For example, frequent exposure
to large fluctuations in seawater temperature may play a positive role by maintaining a
range of physiological plasticity that would otherwise be lost, a negative role by limiting
growth during upper and lower thermal extremes, or have no effect. The back reef pools
on Ofu Island, American Samoa, are often subject to large daily or short-term
fluctuations in seawater temperature, daytime irradiance, flow velocity, salinity, turbidity
and dissolved oxygen (Smith & Birkeland 2003).

Figure 1.2. Map of study site, showing back reef pools on Ofu Island.

A series of back reef pools occurs on the south-facing coast of Ofu Island (14°S,
Fig. 1.2). During summer-time mid-day low tides, seawater temperatures and solar
irradiances reach 32 – 35 °C and 2,000 – 2,200 μmol quanta m⁻² s⁻¹, respectively, at 1 m
depth in the pools. Like many back reef systems (Dahlgren & Marr 2004), the back reef
pools are highly-fluctuating environments, with daily fluctuations commonly > 5 °C in
seawater temperature, > 1,800 μmol quanta m⁻² s⁻¹ in daytime irradiance, > 30 cm s⁻¹ in
flow velocity, and > 150 percent saturation in dissolved oxygen. In addition, high rainfall
on Ofu and Olosega Islands frequently results in reduced salinity in the back reef pools,
while occasional storms cause spikes in turbidity (Smith & Birkeland 2003). In contrast, though the nearby shallow forereef has very similar mean daily temperatures, it is a much more stable environment, with daily fluctuations almost always < 1 °C in seawater temperature (Fig. 1.3), while irradiance levels are less than in the back reef pools because of the blocking effect of breaking waves. Flow rates on the shallow forereef are consistently high because of wave exposure, and salinity and turbidity are nearly constant due to distance from shore and constant mixing (unpublished data). Small-scale spatial (back reef vs. forereef, ≈5 km from one another) and temporal (< 1 week) variability in daily temperature fluctuations demonstrate the highly-fluctuating nature of the back reef pools (Figure 1.3).

Figure 1.3. Raw data (left) of Ofu back reef vs. shallow forereef seawater temperatures taken every 30', April 1, 2004 – March 31, 2005, and spatial and temporal variability (right) of summer-time daily temperature maxima (values) and daily temperature fluctuations (bars) for the 1-year period (from data summarized in Chapter 6).

The contrast between temperatures and irradiance levels in the Ofu back reef pools vs. on the adjacent shallow forereef is especially apparent during mid-day spring low tides: The reef crest isolates the pools from ocean swell, reducing water motion in the
water column and on the surface, thereby allowing the sun to warm the still water and penetrate the flat surface, increasing temperature and irradiance and leading to large daily fluctuations in both. In contrast, low tide causes waves to break on the shallow forereef, maximizing water motion and surface turbulence, thereby moderating both temperature and irradiance and preventing major fluctuations. Coral bleaching is often much more severe in back reef pools than on the adjacent forereef at the same depth, due to higher irradiance in the wave-protected back reef (Brown et al. 1994, Anthony & Kerswell 2007).

The Ofu back reef pools are 1 – 2 m deep at low tide and support a community of ≈80 species of reef-building corals (Craig et al. 2001), most of which are broadcast spawners. Despite the regular occurrence of mid-day spring low tides during the summers that produce simultaneous elevated seawater temperatures and high irradiance levels in the back reef pools, bleaching of scleractinian corals is rare, though hydrozoan fire corals often bleach (personal observations). Continuous temperature logging since 1999 indicates that summer-time temperature maxima often exceed 33 °C at 1 – 1.5 m low tide depth (temperature data summarized in Chapter 6), and measurements taken during these periods show that irradiance maxima frequently exceed 2,200 μmol quanta m⁻² s⁻¹ (summarized in Chapter 3 appendix). Summer (Nov – Mar) spring low tides often occur at mid-day and coincide with sunny weather, resulting in several consecutive days of seawater temperatures that remain up to 5 °C above the local summer mean temperature (29.4 °C; see Chapter 2 Methods and Fig. 2.1), as well as high irradiance levels. Such simultaneous elevated seawater temperatures and high irradiance levels are known to be harmful to most reef-building corals, in the absence of environmental or biological
factors to counteract or neutralize them (Coles & Brown 2003, West & Salm 2003, Grimsditch & Salm 2006).

These observations led to the following questions: Although daily or short-term fluctuations in environmental factors produce high/low extremes in the back reef pools, environmental and/or biological characteristics of the pools appear to reduce their impacts. That is, do environmental factors associated with the fluctuations (e.g., tidal flow patterns, brevity of elevated temperatures, etc.) counteract or neutralize the potential negative effects of elevated seawater temperatures and high irradiance levels? Likewise, are there biological factors associated with back reef coral colonies (e.g., phenotypic plasticity of host corals and/or genetic diversity their symbionts) that counteract or neutralize the potential negative effects of high temperatures and irradiance levels?

**Hypothesis-testing**

Environmental and biological factors associated with highly-fluctuating back reefs were investigated for reef-building corals within the following framework of hypothesis-testing. Of particular interest were broadcast-spawning coral species whose populations presumably encompass a habitat gradient from environmentally-stable forereefs (where simultaneous elevated seawater temperatures and high irradiance levels are uncommon) to highly-fluctuating back reef pools (where simultaneous elevated seawater temperatures and high irradiance levels are common).
$H_{01}$: Semi-diurnally intermittent water motion, such as found in the back reef pools, does not affect photoinhibition and bleaching of corals exposed to elevated seawater temperatures, regardless of irradiance level;

$H_{a1}$: Semi-diurnally intermittent water motion reduces photoinhibition and bleaching of corals exposed to elevated seawater temperature when irradiance levels are high, but has no effect when irradiance levels are low.

$H_{02}$: Phenotypic response of broadcast-spawning coral colonies reciprocally-transplanted between contrasting habitats on small spatial scales ($< 5$ km) does not vary by source population or by transplant site;

$H_{a2.1}$: Phenotypic response varies by transplant site, showing physiological phenotypic plasticity;

$H_{a2.2}$: Phenotypic response varies by source population, showing either developmental phenotypic plasticity or genetic polymorphism.

$H_{03}$: There is no clade-level variability in zooxanthella genotypes found in conspecific coral colonies along a seawater temperature gradient (small back reef pool, large back reef pool, shallow forereef);

$H_{a3}$: Clade-level variability in zooxanthella genotype found in conspecific coral colonies correlates with the seawater temperature gradient (i.e., highest diversity in zooxanthella genotype found at the site with the highest daily temperature maxima and fluctuations, the small back reef pool).
To test $H_{01}$ and $H_{a1}$, a running seawater system was built on Ofu Island to provide a controlled environment for testing effects of intermittent flow and irradiance level on photoinhibition and bleaching of Ofu back reef *Porites* corals exposed to elevated seawater temperatures (Chapter 2). Water flow in many tropical back reef systems is tidally influenced, resulting in a strong semi-diurnal component to flow patterns (Kraines et al. 1998, Storlazzi et al. 2004). Constant water flow benefits coral survival (Jokiel 1978), calcification (Dennison & Barnes 1988), and photosynthesis (Lesser et al. 1994), while reducing photoinhibition and bleaching (Nakamura et al. 2005). However, effects of semi-diurnally intermittent flow on photoinhibition and bleaching of corals exposed to elevated seawater temperatures and high irradiances had not been investigated.

To test $H_{02}$, $H_{a2-1}$, and $H_{a2-2}$, two pairs of RTEs using broadcast-spawning coral species (*Porites lobata* and *Pocillopora eydouxi*) were carried out between: (1) a highly-fluctuating small back reef pool and a relatively stable large back reef pool (Chapter 3); and (2) a back reef pool and a relatively stable forereef site (Chapters 4 & 5), using skeletal growth characteristics as the phenotypic response. Zooxanthella genotypes of transplant source colonies were sampled at the beginning and end of each RTE. The RTE design provides a means of determining if the source of phenotypic variability observed in a population is physiological plasticity or developmental plasticity/genetic polymorphism (Schluter 2000, DeWitt & Scheiner 2004). The difficulty in distinguishing developmental plasticity from genetic polymorphism in RTE results is explained below (Meyers & Bull 2002).

When environmental variability is predictable within a population's range, such as the gradient from diurnally-stable forereefs to highly-fluctuating back reef pools within
the range of a broadcast-spawning coral population, phenotypic plasticity is the most common adaptive strategy. Plasticity may be physiological or developmental; the former refers to the ability of organisms to change physically or physiologically in response to changing conditions (Marfenin 1997), and the latter refers to a distinct, irreversible developmental pathway in response to local conditions during early life history stages (Meyers & Bull 2002). Thus, for RTEs of adult organisms, such as coral colony fragments, a result that resembles genetic polymorphism could actually be developmental plasticity. Because one of my RTEs indicated strong genetic polymorphism (Poc. eydouxi between back reef and forereef), this RTE was followed up with a genetic study using microsatellite markers (Selkoe & Toonen 2006) to determine whether the RTE result most likely represented genetic polymorphism or developmental plasticity (Chapter 5).

To test $H_{03}$, multi-year seawater temperature data from a small, highly-fluctuating back reef pool, a large, less fluctuating back reef pool, and a relatively stable forereef site were collected and analyzed to quantify and compare their thermal environments. Zooxanthella genotype samples were collected and analyzed from several species of reef-building corals from each site during different seasons, then patterns in zooxanthella genotype variability were correlated with patterns in the temperature data (Chapter 6). While many reef-building corals contain only one zooxanthella genotype that does not change through space or time (Thornhill et al. 2005, Goulet 2006), some genera (e.g., Acropora, Pocillopora) display spatial (LaJeunesse et al. 2004a) and temporal (Baker et al. 2004, Baker & Romanski 2007) variability. For example, Clade D is most commonly found in host colonies inhabiting shallow habitats with rapidly fluctuating (LaJeunesse et al. 2004a) or elevated seawater temperatures (Fabricius et al. 2004).
Because of the complexity of the RTE experimental design, an overview and theoretical examples are provided here: Each RTE utilized four replicate groups that were transplanted within and between two sites: From Environment 1 to Environment 1 (Native 1, N1), from Environment 1 to Environment 2 (Translocated 1, T1), from the Environment 2 to Environment 2 (Native 2, N2), and from Environment 2 to Environment 1 (Translocated 2, T2). Comparison of the Native and Translocated groups quantifies variability by transplant site (N1 vs T1, N2 vs T2) and by source population (N1 vs T2, N2 vs T1). Variability by transplant site indicates physiological plasticity, and variability by source population indicates either developmental plasticity or genetic polymorphism (molecular follow-up required to determine which is most likely). A reaction norm links a Native group to its corresponding Translocated group (N1 and T1, N2 and T2), and the two reaction norms together illustrate the interplay of the different influences on each skeletal characteristic (Schluter 2000, DeWitt & Scheiner 2004). Results and reactions norms from five theoretical RTEs are shown in Figure 1.3 to illustrate the different influences, or lack thereof, on phenotypic responses.

Though RTEs are intended to distinguish the effects of phenotypic plasticity and genetic polymorphism on phenotypic response, the use of adult organisms in RTEs (such as coral colony fragments) is potentially confounded by developmental plasticity because such plasticity is only present during early life history stages. If habitat variability is predictable within a population's range, and the state of the environment experienced by the settled larva is a good predictor of future conditions, then developmental plasticity is likely (Meyers & Bull 2002).
a. No physiological plasticity or developmental plasticity/genetic polymorphism, as indicated by flat, nearly overlapping reaction norms:

b. Physiological plasticity in phenotypic response (environmental control), as indicated by sloped, nearly overlapping reaction norms:

c. Developmental plasticity/genetic polymorphism (can’t distinguish), as indicated by flat, widely-separated reaction norms:

d. Physiological plasticity, and developmental plasticity/genetic polymorphism, as indicated by sloped, widely-separated reaction norms:

e. Physiological plasticity, and developmental plasticity/genetic polymorphism, as well as an interaction of the two, as indicated by sloped, converging reaction norms:

Figure 1.4. Results (left) and reaction norms (right) from five theoretical reciprocal transplant experiments (Schluter 2000, Trussell 2000, DeWitt & Scheiner 2004).
CHAPTER 2:

EFFECTS OF INTERMITTENT FLOW AND IRRADIANCE LEVEL ON *PORITES* CORALS AT ELEVATED SEAWATER TEMPERATURES©

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Abstract

Corals inhabiting shallow back reef habitats are often simultaneously exposed to elevated seawater temperatures and high irradiance levels, conditions known to cause coral bleaching. Water flow in many tropical back reef systems is tidally influenced, resulting in semi-diurnal or diurnal flow patterns. Controlled experiments were conducted to test effects of semi-diurnally intermittent water flow on photoinhibition and bleaching of the corals *Porites lobata* and *P. cylindrica* kept at elevated seawater temperatures and different irradiance levels. In the high irradiance experiments, photoinhibition and bleaching were less for both species in the intermittent high – low flow treatment than in the constant low flow treatment. In the low irradiance experiments, there were no differences in photoinhibition or bleaching for either species between the flow treatments, despite continuously elevated seawater temperatures. These results suggest that intermittent flow associated with semi-diurnal tides, and low irradiances caused by turbidity or shading, may reduce photoinhibition and bleaching of back reef corals during warming events.
Introduction

Coral bleaching refers to the loss of endosymbiotic dinoflagellates (zooxanthellae) from the coral host, but can also include the loss of photosynthetic pigments within individual zooxanthella. Since the 1980s, extensive coral bleaching has become increasingly common, usually as a consequence of elevated seawater temperatures in conjunction with high irradiances of solar radiation (Dunne & Brown 2001, Jokiel 2004). Exposure to high irradiance levels leads to a lower bleaching threshold temperature and quicker bleaching compared to corals exposed to lower irradiance levels (Lesser & Farrell 2004). Even in the absence of elevated seawater temperatures, supra-optimal irradiance levels reduce the function of reaction centers in Photosystem II (PS II) and cause oxidative stress (Gorbunov et al. 2001), a process known as photoinhibition (Osmond 1994). Water flow increases mass transfer of inorganic nutrients (Atkinson & Bilger 1992) and dissolved gases across the diffuse boundary layer between the water column and the coral tissue (Lesser et al. 1994), thereby reducing oxidative stress and limiting photoinhibition, which in turn may prevent or minimize coral bleaching (Nakamura et al. 2005, Finelli et al. 2006).

Tropical back reef systems occur between the reef crest and the shoreline’s upper intertidal zone, thus include a wide diversity of habitats such as coastal margins, lagoons, mangroves, seagrass beds, and others (Dahlgren & Marr 2004). Corals occurring in shallow back reef areas are often simultaneously exposed to seawater temperatures of 32 - 36 °C and supra-optimal irradiances, especially during mid-day spring low tides (Coles 1997, Craig et al. 2001). Water flow in many tropical back reef systems is tidally
influenced, resulting in a strong semi-diurnal component to flow patterns (Kraines et al. 1998, Storlazzi et al. 2004). For example, velocities dropped to zero at low tide every 12.4 hr, interspersed with maximum velocities (8 – 12 cm s⁻¹) on the rising and falling tides over a three day period in a back reef lagoon within a fringing coral reef system (Kraines et al. 1998). Constant water flow benefits coral survival (Jokiel 1978), calcification (Dennison & Barnes 1988), and photosynthesis (Lesser et al. 1994), while reducing photoinhibition and bleaching (Nakamura et al. 2005). However, effects of intermittent water flow on photoinhibition and bleaching of corals exposed to elevated seawater temperatures and high irradiances have not been investigated.

Elevated seawater temperatures and high irradiances usually occur together, and their synergistic effects on coral bleaching are well established (Jones & Hoegh-Guldberg 2001). But since seawater temperatures change more slowly than irradiance levels, warming events followed by cloudy weather or sudden increases in turbidity may result in simultaneous elevated seawater temperatures and reduced irradiances. Field observations of minimal bleaching despite warming events have been attributed to reduced irradiances from cloud cover (Mumby et al. 2001a) or turbidity (Phongsuwan 1998), and laboratory experiments have shown greater photoinhibition and bleaching of corals kept at elevated temperatures when exposed to high irradiances than low irradiances (Lesser et al. 1994). However, elevated seawater temperatures reduce photosystem function in isolated reef coral zooxanthellae kept in the dark (Warner et al. 1996) or at low irradiance (Warner et al. 1999). Effects of elevated seawater temperatures at different irradiance levels are particularly relevant to corals in shallow back reef systems, where seawater temperatures are often elevated and irradiance levels can rapidly

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fluctuate with changing weather conditions as well as the rise and fall of turbidity from runoff and tidal flushing (Dahlgren & Marr 2004). Hence, a series of experiments was designed to test effects of intermittent water flow and irradiance level on photoinhibition and bleaching of common back reef *Porites* corals exposed to elevated seawater temperatures.

**Methods**

*Field Observations, Species Selection, and Experimental System*

The fringing coral reef on the south coast of Ofu Island, American Samoa, lies mostly within the National Park of American Samoa and includes a series of back reef pools. Temperatures at 1 m low tide depth (shaded, ≈ 10 cm above substrate) have been continuously recorded every 30 minutes since 1999 with Onset Tidbit® (1999 – 2003) or Water Temp Pro® (2004 – present) loggers in a small back reef pool. During the summer, seawater temperatures are regularly 32 – 35 °C 1 m below the surface, with daily fluctuations of up to 6 °C. The local mean summer maximum seawater temperature (MSM) is the mean seawater temperature during the warmest month of the year for a locality (Jokiel 2004). Based on the 1999-2006 temperature data, MSM for the back reef pool is 29.5 °C. Maximum low tide pool depth in the back reef pool is 1.5 m and turbidity is usually low, resulting in high irradiances during sunny weather, though turbidity sporadically increases following storms (Smith & Birkeland 2003). Approximately 80 species of scleractinian corals occur in the back reef area (Craig et al. 2001), and no
major bleaching events were observed during or following the summer seasons in 2002 – 2006.

Initial field observations indicated that seawater temperature maxima result from mid-day spring low tides when isolation of the back reef pools by the emergent reef crest reduces mixing and facilitates warming. However, spring high tides result in strong flows associated with relatively great tidal range, thus observations suggested that spring tides produce a pattern of elevated seawater temperatures at the mid-day low tide followed a few hours later by strong flows at high tide. These observations were quantified by analyzing the 1999-2006 temperature data collected during summer-time spring tides, and collecting flow velocity data during a spring tide period. Mean seawater temperatures during the first spring tide of the year from 1999 to 2006 show peaks several degrees above the local MSM of 29.5 °C (Fig. 2.1). Flow velocities were measured each hour from 06:00 to 18:00 near the temperature logger by injecting fluoresceine dye 10 cm above the substrate (n = 5 dye releases within 5 minutes of the top of the hour) and timing its progress along a meter stick. Flow showed a strong semi-diurnal pattern, alternating from approximately 5 cm s\(^{-1}\) at low tide to 25 cm s\(^{-1}\) at high tide (Fig. 2.2).
Figure 2.1. Mean seawater temperatures and tidal height (thick line) at the study site during the 4-day period coinciding with first spring tide in January from 1999 to 2006 (full or new moon always between midnight and noon of Day 3).

Figure 2.2. Hourly flow speeds and tidal height (thick line) at the study site from 06:00 to 18:00 during spring tide, March 2006.

Based on observed elevated seawater temperatures, semi-diurnally intermittent water flow, and lack of coral bleaching in the back reef pools, we hypothesized that intermittent flow reduces photoinhibition and bleaching of corals during periods of elevated seawater temperatures and high irradiance levels at this site. To test the hypothesis, experiments were conducted in a running seawater system on Ofu Island.
designed to provide elevated seawater temperatures, semi-diurnally fluctuating water flow, and different irradiance levels to roughly simulate back reef conditions during summer-time spring low tides. The common back reef coral species, *Porites lobata* and *P. cylindrica*, were selected for the experiments because together they make up 39 percent of live coral cover within the back reef pools (Craig et al. 2001).

A 100-liter capacity water table 1 m in length and 60 cm in width was used for four experiments; 1) *P. lobata* at high irradiance, 1) *P. lobata* at low irradiance, 3) *P. cylindrica* at high irradiance, and 4) *P. cylindrica* at low irradiance. Each experiment lasted four days and consisted of two flow treatments, intermittent high-low flow vs. constant low flow, while elevated seawater temperatures were constantly maintained. Effects of flow treatments on photoinhibition of the coral replicates during the experiments were determined with a Walz Diving Pulse-Amplitude Modulation fluorometer (Diving-PAM®, Walz, Effeltrich, Germany). Effects of flow treatments on bleaching of the coral replicates were evaluated with visual ratings and zooxanthella densities at the end of the experiments.

*Water Flow, Seawater Temperature and Irradiance*

Intermittent high-low flow and constant low flow treatments were provided in flumes measuring 70 cm in length by 10 cm in diameter (Fig. 2.3). A 12V 2,000 liter per hour bilge pump was placed between the flumes to provide circulation throughout the water table. This pump also maintained flow speeds of 2 – 5 cm s⁻¹ at all times in the constant low flow flume, and from 18:00 – 24:00 and 06:00 – 12:00 in the intermittent high-low flow flume. A 12V 4,000 liter per hour bilge pump was placed in front of the
Figure 2.3. The water table and submerged flumes containing coral replicates used for flow treatments (arrows indicate flow). Intermittent high-low flow was provided in the left flume by switching equipment every 6 hours, as shown in the diagram, while constant low flow was provided in the right flume.

Intermittent high-low flow flume, and turned on from 24:00 – 06:00 and 12:00 – 18:00 every day to provide a flow rate of 15 – 20 cm s⁻¹ at these times. Flow speeds in the flumes were measured at each of the 10 coral replicate positions within each flume with a mechanical flow meter (Gurley Precision Instruments Model 625A Pygmy Flow Meter®), and calibrated by baffling and flume adjustments to obtain the desired flow speeds. A double layer of plastic mesh (7 mm mesh Vexar®) was placed between the flumes during high flow to prevent an increase in flow speed in the constant low flow flume (Fig. 2.3). A depth of 10 cm was maintained over the upper surfaces of the coral
replicates. A flow-through rate of approximately 100 liters per hour was maintained, with some adjustment as needed for temperature control.

When mean seawater temperatures are continuously 1 – 2 °C or more above local MSM (29.5 °C in Ofu back reef pools), corals are at risk of thermal bleaching (Jokiel 2004). Seawater temperatures were allowed to fluctuate diurnally between 30 – 36 °C in order to provide a mean temperature of 31.5 – 32.5 °C (2 – 3 °C >MSM) and to simulate natural temperatures in shallow back reef pools during conditions that are thought to lead to bleaching. Seawater temperature was controlled with a 100 watt submersible heater (Rena Cal Top Light®) and flow rate adjustments. Seawater temperatures were measured every 30 minutes in the water table with an Onset Water Temp Pro® temperature logger. Homogenous seawater temperature was maintained throughout the water table during high flow (Fig. 2.3, left) by circulation generated by the two pumps through the plastic mesh separating the two flow treatments, and during low flow by circulation generated by the small pump (Fig. 2.3, right). These design features also maintained homogenous salinity throughout the water table during rainy periods.

Maximum irradiances in the upper 1 m of coral reef waters are typically 1,800 – 2,300 μmol quanta m⁻² s⁻¹ of photosynthetically active radiation (PAR) at mid-day during clear weather (Brown 1997, Lesser & Farrell 2004). PAR was measured with the quantum sensor on the fluorometer at 10 cm depth within the flumes in the water table at noon on five sunny days before the experiment. Maximum PAR values were 1,800 – 1,950 μmol quanta m⁻² s⁻¹, thus ambient solar radiation at 10 cm depth was used for the high irradiance experiments. Knitted neutral-density polyethylene shade cloth (heavy, black, 90% EnviroCept® and 73% Cal-Pac®) was used to reduce solar radiation to
approximately 10 percent (*P. lobata*) and 27 percent (*P. cylindrica*) of ambient for the low irradiance experiments. Before the experiments, PAR was measured at each of the 20 coral replicate positions in the two flumes at 09:00, 12:00, and 15:00 p.m. to verify homogenous PAR among all positions regardless of sun aspect. During the experiments, PAR was measured within the flumes at 10 cm depth every 30 minutes during daylight hours with the quantum sensor on the fluorometer. The quantum sensor was calibrated with a light meter (Li-Cor LI-192SA®).

*Coral Replicates*

All coral replicates were obtained from colonies in the Ofu back reef pools with upward-facing surfaces at approximately 1 m low tide depth. Although the pools are dominated by massive *Porites* colonies, distinguishing among the approximately six species is difficult. Of the several dozen colonies found at the appropriate depth, only five colonies could be positively identified as *P. lobata* based on corallite skeletal characteristics (Veron 2000). For the two *P. lobata* experiments, six cores measuring 13 mm in diameter and approximately 3 cm in length were drilled from the upward-facing surface of each of the five source colonies. The 30 cylindrical cores were transferred to the water table, glued to nylon bolts with marine epoxy (Z-spar®), labeled by source colony and replicate number, and placed upright in the bottom of the water table under the same shade cloth used for the low irradiance experiment (10 percent ambient). The cores were acclimated for 2 weeks at ambient seawater temperature under the shade cloth, after which coral tissue had grown several mm down the sides of the exposed skeleton.
On the evening before Day 1 of each experiment, the 30 transplants were separated into three groups of 10 (initial, and two treatment groups) by randomly selecting two transplants from each of the five source colonies for each group. Then the initial group was preserved for laboratory analysis, the flumes were set up in the water table, the remaining two groups of experimental transplants were mounted in the flumes, and the shadecloth was removed for the high irradiance experiments. *P. lobata* replicates were mounted upright such that the tissue surface faced upward at 10 cm depth in the middle of the flumes. The position of each replicate in each flume was changed daily so replicates did not occupy the same position throughout the experiments. The same process was used for collection and preparation of *P. cylindrica*, with the following exceptions: (1) for each of the two experiments, a single 3 cm branch was taken from each of 30 source colonies to provide 30 coral transplants of roughly the same size and shape; (2) the branches were acclimated for only 3 days under 27 percent ambient shadecloth because tissue healing was not necessary (the broken base of each branch was covered with marine epoxy); and (3) branches were mounted horizontally such that tissue on one side of each branch faced upward at 10 cm depth in the middle of the flumes.

*Photoinhibition, Bleaching and Statistics*

Photochemical efficiency of zooxanthellae in the tissue of the coral replicates was assessed with chlorophyll fluorescence using PAM fluorometry (Schreiber et al. 1986). Corals were dark-adapted for 20 minutes prior to fluorometry measurements (Jones et al. 1998) using a double cover of opaque plastic and canvas that completely darkened the water table while maintaining flow treatments. The opaque plastic was then removed,
flow pumps turned off, and fluorometry measurements taken underneath the canvas cover in order to maintain reduced irradiance during measuring. Minimum ($F_o$, using 3 $\mu$s pulses of light emitting diode) and maximum ($F_m$, using a saturation pulse) fluorescence of each replicate were measured with the fluorometer (measuring intensity = 6, saturation intensity = 10, saturation width = 0.8 s, gain = 2, damping = 2, distance between diode and sample = 5 mm). $F_o$ and $F_m$ were used to calculate $F_v/F_m$, the ratio of variable fluorescence ($F_v$, where $F_v = F_m - F_o$). Dark-adapted $F_v/F_m$, or photochemical efficiency, is a measure of the maximum quantum yield of photosystem II, thus a decrease in $F_v/F_m$ in response to high irradiance indicates photoinhibition (Franklin et al. 1992, Franklin et al. 1996). Dark-adapted $F_v/F_m$ of each replicate was measured at 06:00, 09:00, 12:00, 15:00, 18:00, and 21:00 daily. Flume measuring order was randomly determined, and $F_v/F_m$ was taken with a single measurement on the upward-facing surface of each replicate.

On the evening of Day 4 of each experiment, visible bleaching of each replicate was rated as high, moderate, or none. The bleaching categories were based on the green section of a standardized coral color chart (Coral Health Chart, www.CoralWatch.org), where shades B1 & B2 = heavy bleaching, shades B3 & B4 = moderate bleaching, and shades B5 & B6 = no bleaching. The eight groups of final replicates (four experiments x two flow treatments per experiment) were preserved, along with the four initial groups, for zooxanthella densities. Only the upward-facing half of the $P.\ cylindrica$ replicates were kept for this purpose. From each of the six $P.\ lobata$ groups, one replicate from each source colony was used for zooxanthella densities, and from each of the six $P.\ cylindrica$ groups, five replicates were randomly chosen ($n = 5$ per group). Tissue was obtained for
the counts by using a 5 mm diameter cork bore to remove a 3 mm thick sample from the center of each replicate. The samples were decalcified with acetic acid, centrifuged, the supernatant discarded, and the zooxanthellae resuspended in 5 ml of filtered seawater. Eight sub-samples were taken from each sample for haemocytometer counts, and the mean normalized to the sample area to obtain an estimate of zooxanthella density per cm² for each replicate.

Statistical analyses were performed with SAS 9.1 and Minitab 13.1. For each of the two P. lobata experiments (high and low irradiance levels), a repeated measures 4-way analysis of variance (ANOVA) was used to test effects of flow treatment, source colony, day, and hour on $F_v/F_m$. For the two P. cylindrica experiments, a repeated measures 3-way ANOVA was used to test effects of flow treatment, day, and hour on $F_v/F_m$. Source colony was not a factor in the P. cylindrica experiments because a single coral replicate was collected from each colony. Paired t-tests were used to compare zooxanthella densities of transplants sampled from the intermittent high-low flow and constant low flow treatments at the end of each experiment. All data were assessed for normality and homogeneity of variances prior to testing.

Results

P. lobata photoinhibition

Mean seawater temperature over the 4-day period for the high irradiance experiment was 31.5 °C, or 2.0 °C > MSM. Seawater temperatures closely followed PAR, and photochemical efficiencies (dark-adapted $F_v/F_m$) of both treatments were
Figure 2.4. Seawater temperature and PAR (top), and \( F_v/F_m \) (bottom) of the two flow treatment groups, for the *P. lobata* high irradiance experiment.

Inversely related to PAR (Fig. 2.4), as occurs naturally on shallow reefs (Berkelmans & Willis 1999). \( F_v/F_m \) was greater for the intermittent high-low flow treatment than the constant low flow treatment over the 4-day experimental period (repeated measures 4-way ANOVA: Flow \( F_{1,424} = 109.65, p < 0.001 \), Table 2.1a, Fig. 2.4), thus indicating less photoinhibition in the intermittent high-low flow treatment than the constant low flow treatment. \( F_v/F_m \) was also affected by day and hour (Day \( F_{3,424} = 230.09, p < 0.001 \); Hour \( F_{20,424} = 131.60, p < 0.001 \), Table 2.1a), but not by source colony (Source \( F_{4,424} = 0.94, p = 0.522 \), Table 2.1a). Interactions between flow and day, and between flow and hour (Flow x Day \( F_{3,424} = 3.39, p = 0.018 \); Flow x Hour \( F_{20,424} = 3.38, p < 0.001 \), Table 2.1a), reflect greater variation in \( F_v/F_m \) between days and between hours than in \( F_v/F_m \) between the flow treatments. These effects and interactions are expected because; (1) natural variability of PAR during the 4 day period caused high variability in \( F_v/F_m \) by day; and
(2) the diurnal pattern of photosynthesis caused high variability in $F_v/F_m$ by hour (Fig. 2.4). There was no interaction between flow and source colony (Flow x Source $F_{4,424} = 0.90$, p = 0.464, Table 2.1a).

Table 2.1a. Repeated measures 4-way ANOVA (flow treatment, source colony, day, hour) for photochemical efficiency ($F_v/F_m$) of *P. lobata* at the two irradiance levels (high and low).

<table>
<thead>
<tr>
<th>Repeated Measures 4-way ANOVA</th>
<th>Df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Flow</td>
<td>1</td>
<td>0.138244</td>
<td>0.000502</td>
<td>109.65</td>
</tr>
<tr>
<td>Source</td>
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<td>0.001149</td>
<td>0.94</td>
</tr>
<tr>
<td>Day</td>
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<td>0.020862</td>
<td>230.09</td>
</tr>
<tr>
<td>Hour</td>
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<td>0.184531</td>
<td>0.009116</td>
<td>131.60</td>
</tr>
<tr>
<td>Flow x Source</td>
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<td>0.001261</td>
<td>0.001497</td>
<td>0.90</td>
</tr>
<tr>
<td>Flow x Day</td>
<td>3</td>
<td>0.004757</td>
<td>0.000243</td>
<td>3.39</td>
</tr>
<tr>
<td>Flow x Hour</td>
<td>20</td>
<td>0.004744</td>
<td>0.001122</td>
<td>3.38</td>
</tr>
<tr>
<td>Error</td>
<td>424</td>
<td>0.001402</td>
<td>0.000872</td>
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</tr>
</tbody>
</table>

When flow was changed for the intermittent flow treatment in the high irradiance experiment, $F_v/F_m$ responded within three hours, even during seawater temperature and PAR maxima between 12:00 and 15:00: After flow was changed from low to high at 12:00 each day, $F_v/F_m$ was greater for the intermittent flow treatment than the constant flow treatment at 15:00 (3 of the 4 days) and 18:00 (all 4 days). On Day 2, a rainstorm kept PAR < 200 μmol quanta m$^{-2}$ s$^{-1}$ until 13:30, and $F_v/F_m$ was not different between the treatments at 15:00. In addition, after flow was changed from high to low at 06:00 each
day, \( F_{v}/F_{m} \) was the same for the two treatments at 09:00 and 12:00. Likewise, after flow was changed from high to low at 18:00 each day, the difference in \( F_{v}/F_{m} \) between the two treatments decreased at 21:00 (Fig. 2.4). Over the 4-day period, the intermittent high-low flow treatment resulted in a mean daily loss in \( F_{v}/F_{m} \) of 43 percent from 06:00 to 15:00, compared to 55 percent for the constant low flow treatment.

![Graph showing seawater temperature and PAR (top), and \( F_{v}/F_{m} \) (bottom) of the two flow treatment groups, for the P. lobata low irradiance experiment.]

Figure 2.5. Seawater temperature and PAR (top), and \( F_{v}/F_{m} \) (bottom) of the two flow treatment groups, for the \( P. lobata \) low irradiance experiment.

Mean seawater temperature over the 4-day period for the low irradiance experiment was 31.9 °C, or 2.4 °C > MSM. \( F_{v}/F_{m} \) results from this experiment contrast sharply with those from the high irradiance experiment: Although mean seawater temperature for the low irradiance experiment was higher than for the ambient irradiance experiment, there was no difference in \( F_{v}/F_{m} \) between the two flow treatments (Flow \( F_{1,424} = 0.34, p = 0.593, \) Table 2.1a, Fig. 2.5), and no effect of source colony (Source \( F_{4,424} = 0.77, p = 0.598, \) Table 2.1a). The effects of day and hour on \( F_{v}/F_{m} \) (Day \( F_{3,424} = \)
23.91, \( p < 0.001 \); Hour \( F_{20,424} = 10.45 \), \( p < 0.001 \), Table 2.1a), and the lack of interactions, reflect natural variability by day and hour, and the absence of flow effects, respectively (Fig. 2.5).

![Graph showing seawater temperature and PAR (top), and \( F_v/F_m \) (bottom) of the two flow treatment groups, for the \( P. cylindrica \) high irradiance experiment.]

\( P. cylindrica \) photoinhibition

Mean seawater temperature over the 4-day period for the high irradiance experiment was 32.2 °C, or 2.7 °C > MSM. Results were similar to the \( P. lobata \) high irradiance experiment: \( F_v/F_m \) was greater for the intermittent high-low flow treatment than the constant low flow treatment over the 4-day experimental period (repeated measures 3-way ANOVA: Flow \( F_{1,414} = 8.16 \), \( p = 0.010 \), Table 2.1b, Fig. 2.6). \( F_v/F_m \) was also affected by day and hour (Day \( F_{3,414} = 62.89 \), \( p < 0.001 \); Hour \( F_{20,414} = 209.84 \), \( p < 0.001 \), Table 2.1b). As with the \( P. lobata \) high irradiance experiment, interactions between flow and day, and between flow and hour, are expected. On Day 4, cloudy
weather kept PAR < 700 \( \mu \text{mol quanta m}^{-2} \text{s}^{-1} \) until 14:00, and \( \frac{F_v}{F_m} \) was not different between the treatments at 15:00. As with \( P. \ lobata \), after flow was changed from high to low at 06:00 each day, \( \frac{F_v}{F_m} \) was the same for the two treatments at 09:00 and 12:00 (Fig. 2.6). Over the 4-day period, the intermittent high-low flow treatment resulted in a mean daily loss in \( \frac{F_v}{F_m} \) of 46 percent from 06:00 to 15:00, compared to 59 percent for the constant low flow treatment.

Table 2.1b. Repeated measures 3-way ANOVA (flow treatment, day, hour) for photochemical efficiency (\( \frac{F_v}{F_m} \)) of \( P. \ cylindrica \) at the two irradiance levels (high and low).

<table>
<thead>
<tr>
<th>Repeated Measures 3-way ANOVA</th>
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<th>MS</th>
<th>( F ) High</th>
<th>( F ) Low</th>
<th>P</th>
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<td>8.16</td>
<td>0.03</td>
<td>0.010</td>
</tr>
<tr>
<td>Day</td>
<td>3</td>
<td>0.080271</td>
<td>62.89</td>
<td>0.71</td>
<td>0.000</td>
</tr>
<tr>
<td>Hour</td>
<td>20</td>
<td>0.267841</td>
<td>209.84</td>
<td>17.03</td>
<td>0.000</td>
</tr>
<tr>
<td>Flow x Day</td>
<td>3</td>
<td>0.003524</td>
<td>2.76</td>
<td>0.14</td>
<td>0.042</td>
</tr>
<tr>
<td>Flow x Hour</td>
<td>20</td>
<td>0.002871</td>
<td>2.25</td>
<td>0.69</td>
<td>0.002</td>
</tr>
<tr>
<td>Error</td>
<td>414</td>
<td>0.001276</td>
<td>0.001063</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean seawater temperature over the 4-day period for the low irradiance experiment was 32.3 °C, or 2.8 °C > MSM. Once again, the \( P. \ cylindrica \) results mirror the \( P. \ lobata \) results, in that \( \frac{F_v}{F_m} \) results from the low irradiance experiment contrast sharply with those from the high irradiance experiment: Although mean seawater temperature for the low irradiance experiment was higher than for the high irradiance
experiment, there was no difference in $F_v/F_m$ between the two flow treatments (repeated measures 3-way ANOVA: Flow $F_{1,414} = 0.03$, $p = 0.867$, Table 2.1b). However, unlike the *P. lobata* low irradiance experiment, there was no difference by day in $F_v/F_m$ during the *P. cylindrica* low irradiance experiment (Day $F_{3,414} = 0.71$, $p = 0.546$, Table 2.1b), though there was by hour (Hour $F_{20,414} = 17.03$, $p < 0.001$, Table 2.1b). As with the *P. lobata* low irradiance experiment, there were no significant interactions (Table 2.1b). The effects of day on $F_v/F_m$, hour on $F_v/F_m$, and the lack of interactions, reflect natural variability by hour, a coincidental uniformity of conditions by day, and the absence of flow effects, respectively (Fig. 2.7)

![Graph](image-url)

Figure 2.7. Seawater temperature and PAR (top), and $F_v/F_m$ (bottom) of the two flow treatment groups, for the *P. cylindrica* low irradiance experiment.

**Bleaching**

The coral transplants removed after the acclimation periods (the four initial groups) did not show any visible signs of bleaching. At the end of the high irradiance
experiments for both species, more coral replicates from the constant low flow treatment were heavily bleached than from the intermittent high-low flow treatment. At the end of the *P. lobata* low irradiance experiment, some replicates were moderately bleached in both flow treatments, but most replicates in both flow treatments showed no signs of bleaching. At the end of the *P. cylindrica* low irradiance experiment, there was no sign of bleaching in any of the replicates in either flow treatment (Table 2.2). Thus, the bleaching categories suggested more zooxanthellae loss had occurred in the low flow treatment than the intermittent high-low flow treatment during the high irradiance experiments for both species, but not during the low irradiance experiments.

Table 2.2. Bleaching ratings (% per category per group) for initial and final coral replicate groups (n = 10 per group) at the two irradiance levels (high and low) for the two species. Final groups consist of replicates from the two flow treatments, intermittent high-low flow (Int) and constant low flow (Con).

<table>
<thead>
<tr>
<th>Bleaching Category</th>
<th><em>P. lobata</em> High Irradiance</th>
<th><em>P. lobata</em> Low Irradiance</th>
<th><em>P. cylindrica</em> High Irradiance</th>
<th><em>P. cylindrica</em> Low Irradiance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
<td>60</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Moderate</td>
<td>0</td>
<td>30</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Heavy</td>
<td>0</td>
<td>10</td>
<td>70</td>
<td>0</td>
</tr>
</tbody>
</table>

33
These observations were confirmed with zooxanthella densities: Initial mean zooxanthella densities for both *P. lobata* experimental experiments were between 3 and 4 million cells per cm² (Fig. 2.8, Table 2.3a). At the end of the high irradiance experiment, mean zooxanthella density was higher in the intermittent high-low flow treatment than in the constant low flow treatment (paired t-test, $p = 0.023$, Table 2.3b). Thus, less bleaching occurred in the intermittent high-low flow treatment than in the constant low flow treatment (Fig. 2.8). In the low irradiance experiment, no differences were found between the two flow treatments (paired t-test, $p = 0.323$, Table 2.3b). Similar results were found for *P. cylindrica*: Initial mean densities for both experiments were between 2.5 and 3 million cells per cm² (Table 2.3a, Fig. 2.9). At the end of the high irradiance experiment, mean zooxanthella density was higher in the intermittent high-low flow treatment than in the constant low flow treatment (paired t-test, $p = 0.013$, Table 2.3b). Thus, less bleaching occurred in the intermittent high-low flow treatment than in the constant low flow treatment (Fig. 2.9). In the low irradiance experiment, no differences were found between the two flow treatments (t-test, $p = 0.695$, Table 2.3b).

![Figure 2.8](image)

Figure 2.8. Initial and final zooxanthella densities for *P. lobata*, showing effects of intermittent high-low (Int) and constant low (Con) flow treatments ($n = 5$ per group) at high (left) and low (right) irradiance levels.
Table 2.3a. Mean zooxanthella densities (x10⁶/cm²) for initial and final groups (n = 5 per group) at the two irradiance levels (high and low). Final groups consist of replicates from the two flow treatments (intermittent high-low flow and constant low flow).

<table>
<thead>
<tr>
<th>Group</th>
<th>P. lobata High Irradiance</th>
<th>P. lobata Low Irradiance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Initial</td>
<td>3.14</td>
<td>0.31</td>
</tr>
<tr>
<td>Final: Int. High-Low Flow</td>
<td>1.97</td>
<td>0.19</td>
</tr>
<tr>
<td>Final: Constant Low Flow</td>
<td>1.08</td>
<td>0.14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>P. cylindrica High Irradiance</th>
<th>P. cylindrica Low Irradiance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Initial</td>
<td>2.81</td>
<td>0.18</td>
</tr>
<tr>
<td>Final: Int. High-Low Flow</td>
<td>2.25</td>
<td>0.16</td>
</tr>
<tr>
<td>Final: Constant Low Flow</td>
<td>1.05</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Table 2.3b. Paired t-test results for zooxanthella densities of coral replicates from intermittent high-low flow vs. constant low flow treatment groups.

<table>
<thead>
<tr>
<th></th>
<th>High Irradiance</th>
<th>Low Irradiance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>T</td>
</tr>
<tr>
<td>P. lobata</td>
<td>5</td>
<td>3.58</td>
</tr>
<tr>
<td>P. cylindrica</td>
<td>5</td>
<td>4.29</td>
</tr>
</tbody>
</table>
Figure 2.9. Initial and final zooxanthella densities for *P. cylindrica*, showing effects of intermittent high-low (Int) and constant low (Con) flow treatments (*n* = 5 per group) at high (left) and low (right) irradiance levels.

Discussion

The rates at which inorganic nutrients and dissolved gases move between the water column and the coral surface affect many physiological processes of hermatypic corals (Atkinson & Bilger 1992, Patterson 1992). These small molecules move through the boundary layer by passive diffusion down concentration gradients, a process known as mass transfer (Cussler 1984). The thickness of the diffusion-limiting boundary layer that covers coral surfaces is inversely related to water velocity, thus increasing velocity reduces boundary layer thickness, thereby increasing mass transfer rates (Nakamura & van Woesik 2001). This mechanism explains benefits of increased water flow reported for coral survival (Jokiel 1978), calcification (Dennison & Barnes 1988), and photosynthesis (Lesser et al. 1994). That is, increased flow likely resulted in quicker
diffusion of reactive oxygen species out of the coral tissue (increasing survival), calcium ions into the coral tissue (increasing calcification), and carbon into the coral tissue (increasing photosynthesis). Likewise, recent studies suggest increased flow reduces oxidative stress associated with elevated temperatures and supra-optimal irradiances by hastening dissipation of reactive oxygen species (Finelli et al. 2006), thereby minimizing bleaching (Nakamura et al. 2005), or facilitating recovery from bleaching (Nakamura et al. 2003). Thus, reduction of oxidative stress is the most likely explanation for the quicker recovery of $F_v/F_m$ in the intermittent flow treatment than the low flow treatment between noon and 18:00 (Figs. 2.4 & 2.6).

Water flow in back reef coral habitats is typically highly variable at the semi-diurnal (Kraines et al. 1998) or diurnal (Genovese & Witman 2004) temporal scales, or some combination thereof (Storlazzi et al. 2004). Back reef corals are more likely than corals in other habitats to be exposed to the dual stressors of elevated seawater temperatures and high irradiances, hence this study was conducted to investigate effects of semi-diurnally intermittent flow under these conditions on photoinhibition and bleaching of corals. The finding that intermittent water motion reduces photoinhibition and bleaching of $P. lobata$ and $P. cylindrica$, even if seawater temperatures and irradiances remain high, supports the hypothesis that “flow-mediated enhancement of mass transfer” may reduce coral mortality when such conditions occur on reefs (Nakamura et al. 2005). The experimental design may have underestimated the beneficial effect of intermittent high-low flow in the high irradiance experiments because of unrealistic duration of the low flow component of the intermittent flow treatment, and high photoinhibition in response to a sudden increase in irradiance (light shock). In the
Ofu back reef and similar systems, the duration of minimal flow periods is < 2 hours rather than the 6 hours used in the experiment. That is, a more realistic intermittent treatment would be 10 hour high flow periods interspersed with 2 hour low flow periods, which likely would have resulted in an even greater difference in photoinhibition and bleaching between the intermittent high-low and constant low flow treatments at high irradiances. Also, the use of quite low irradiance for acclimation (10% and 27% ambient) followed by high irradiances (ambient) may have resulted in severe photoinhibition, or light shock, during the high irradiance experiments. That is, these conditions may have caused severe photoinhibition regardless of flow conditions, thereby minimizing differences between the flow treatment had acclimation occurred more gradually.

There are two distinct levels of photoinhibition: Dynamic photoinhibition (photoprotection) is the reversible inactivation of reaction centers in PS II in order to dissipate excess light energy, whereas chronic photoinhibition (photoinactivation) occurs when continued high irradiance levels produce excessive reactive oxygen species that overwhelm the antioxidant defense systems, causing irreversible damage to PS II (Osmond 1994, Jones & Hoegh-Guldberg 2001, Lesser & Farrell 2004). The failure of $F_v/F_m$ to fully recover at night to previous levels is an indication that PSII may have been damaged by chronic photoinhibition (Gorbunov et al. 2001). In the high irradiance experiments, chronic photoinhibition was demonstrated by the $F_v/F_m$ and bleaching results in both flow treatments: Maximum $F_v/F_m$ occurred at 6 a.m. on Day 1, then never fully recovered to those levels at 6 a.m. on the following days (Figs. 2.4 & 2.6). Because dynamic photoinhibition is a form of photoprotection that does not cause permanent photosystem damage, it is not likely to cause bleaching (Gorbunov et al. 2001), thus the
bleaching results indicate chronic photoinhibition in both flow treatments (Fig. 2.8). However, there was less chronic photoinhibition and bleaching in the intermittent high-low flow treatments than the constant low flow treatments in both high irradiance experiments. In the low irradiance experiments, some dynamic photoinhibition is suggested by the daily reductions in $F_v/F_m$ for *P. cylindrica* (Fig. 2.7), but not for *P. lobata* (Fig. 2.5). Overall, the low irradiance experiments resulted in minimal if any photoinhibition, no bleaching, and no differences between the flow treatments.

The low irradiance experiments were carried out at mean seawater temperatures of 2.5 – 3 °C > local MSM (29.4 °C) and temperatures were continuously above MSM for the four day duration of the experiments, but no photosystem damage (Figs. 2.5 & 2.7) or bleaching (Figs. 2.8 & 2.9) occurred, regardless of flow treatment. Few studies have been done on effects of elevated temperatures on photosystem function or bleaching of corals at low irradiances. In an experiment conducted on isolated and *in hospite* zooxanthellae of five coral species kept at low irradiance (170 μmol quanta m$^{-2}$ s$^{-1}$), $F_v/F_m$ of the isolated zooxanthellae declined more at 34 °C than at 26 °C after 3 hours, but there was no difference in $F_v/F_m$ of *in hospite* zooxanthellae between the two temperature treatments (Bhagooli & Hidaka 2003). Isolated zooxanthellae from the reef coral *Oculina diffusa* kept at low irradiance (14:10 hr cycles of 90 μmol quanta m$^{-2}$ s$^{-1}$ and darkness) had approximately 20 percent lower $F_v/F_m$ than zooxanthellae held at 32 °C than those at 26 °C after four days (Warner et al. 1999), but isolated zooxanthellae are more sensitive to elevated temperatures than *in hospite* zooxanthellae (Bhagooli & Hidaka 2003). Colonies of the reef coral *Montastraea faveolata* bleached at high (maxima of ~2,000 μmol quanta m$^{-2}$ s$^{-1}$) but not low (maxima of ~550 μmol quanta m$^{-2}$ s$^{-1}$) irradiances after
eight days at elevated seawater temperatures (Lesser & Farrell 2004). In contrast, high
irradiances (maxima of ~2,000 μmol quanta m\(^{-2}\) s\(^{-1}\)) resulted in chronic photoinhibition
and bleaching of the reef coral *Stylophora pistillata* after only 48 hours at normal
seawater temperatures (Jones & Hoegh-Guldberg 2001). That is, reduced photosystem
function and coral bleaching occur more slowly in response to elevated seawater
temperatures and low irradiances than in response to supra-optimal irradiances and
normal temperatures. Thus, the four day periods used for this experiment may have been
too brief to determine if flow affects photosystem function and bleaching of corals
exposed to elevated seawater temperatures and reduced irradiances.

*Porites* species are common and often dominant members of back reef coral
communities (Veron 2000). For example, in the Ofu back reef pools, the two most
abundant reef coral taxa are *Porites cylindrica* (27 percent of live coral cover) and
massive *Porites* species (12 percent live coral cover, including *P. lobata*; Craig et al.
2001). The tissue of *Porites* species is deeper-seated in the skeleton, thus better shaded
from high irradiances than in *Acropora* species and *Pocillopora* species, a characteristic
that may contribute to relatively less bleaching of *Porites* species (Hoegh-Guldberg &
Salvat 1995). Observations of bleaching events suggest that relative bleaching rates
among taxa may also be related to colony morphology. For example, in most locations,
the 1998 bleaching event resulted in light to moderate bleaching of massive colonies,
such as massive *Porites* species and faviids, in contrast to the heavy bleaching of
branching colonies, such as branching *Porites* species, *Acropora* species, and *Pocillopora*
species (Loya et al. 2001). In the high irradiance experiments, intermittent high-low flow
reduced photoinhibition and bleaching in both the massive *P. lobata* and the branching *P.*
cylindrica. Therefore, though this experiment was conducted on two Porites species, the results may be broadly applicable to species in other genera with massive morphologies similar to P. lobata (e.g., Goniastrea and Diploastrea species), and branching morphologies similar to P. cylindrica (e.g., some Pocillopora species and Acropora species).
CHAPTER 3:

PHENOTYPIC PLASTICITY FOR SKELETAL GROWTH RATE OF POCILLOPORA EYDOUXI BUT NOT PORITES LOBATA BETWEEN BACK REEF POOLS

L.W. SMITH, H. WIRSHING, A.C. BAKER, C. BIRKELAND

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Abstract

Reciprocal transplant experiments of the corals *Pocillopora eydouxi* and *Porites lobata* were carried out for an 18-month period from Sep-04 to Mar-06 between two back reef pools on Ofu Island, American Samoa, to test environmental versus genetic effects on skeletal growth rates. Skeletal growth of *P. eydouxi* showed environmental (phenotypic plasticity) but not genetic effects, resulting in doubling of growth in Pool 300 compared to Pool 400. There were no environmental or genetic effects on skeletal growth of *P. lobata*. Pool 300 had more frequent and longer durations of elevated seawater temperatures than Pool 400, characteristics likely to decrease rather than increase skeletal growth. Pool 300 also had higher nutrient levels and flow velocities than Pool 400, characteristics that may increase skeletal growth. However, higher nutrients would be expected to increase skeletal growth in both species, but there was no difference between the pools in *P. lobata* growth. *P. eydouxi* is much more common in high energy environments than *P. lobata*, thus the higher flow velocities in Pool 300 than in Pool 400 may have positively affected skeletal growth of *P. eydouxi* while not having a detectable effect on *P. lobata*. The greater skeletal growth of *P. eydouxi* in Pool 300 occurred despite the presence of Clade D zooxanthellae in several source colonies in Pool 300, a genotype known to result in greater heat resistance but slower skeletal growth. Phenotypic plasticity for skeletal growth rate, such as higher skeletal growth of *P. eydouxi* in Pool 300, may provide a competitive advantage in shallow, high energy environments where competition for space is intense.
Skeletal growth in reef-building scleractinian corals occurs by the formation and precipitation of aragonite (Barnes 1970), a process greatly enhanced by symbiotic dinoflagellates (Symbiodinium spp.) known as zooxanthellae that provide up to 95 percent of the corals’ carbon requirements for growth, reproduction, and maintenance (Muscatine 1990). Annual skeletal growth rates, measured as linear extension, usually range from a few millimeters for massive species to several centimeters or more for branching species (Buddemeier & Kinzie 1976, Harriott 1999). Intraspecific variability in skeletal growth rate may also be considerable, even for massive species such as Porites lobata on the Great Barrier Reef (Lough & Barnes 2000) and the Hawaiian Islands (Grigg 1982). Intraspecific variability may be controlled by the environment (Foster 1979), by genetic differences between individuals or populations (Willis 1985), or by both (Via & Lande 1985). Environmental control of morphological variability is also known as phenotypic plasticity (Stearns 1989, Doughty & Resnick 2004).

Environmental vs. genetic control of phenotypic characteristics, such as skeletal growth rates, can be tested with reciprocal transplant experiments (RTEs; Doughty & Resnick 2004, Schluter 2000). RTEs of zooxanthellate corals have shown environmental control of skeletal growth rates that were attributed to different habitats (Potts 1984, Smith et al. 2007), and genetic control that was attributed to genetic differentiation of transplanted populations (Raymundo 2001). A series of shallow (1–2 m) back reef pools on the fringing reef of Ofu Island, American Samoa, support 50–80 species per pool of
reef-building corals that tolerate elevated seawater temperatures (Craig et al. 2001). The smallest pool (Pool 300, Fig. 3.1) is exposed to greater fluctuations of environmental conditions, such as higher seawater temperatures and lower salinity, than are the other pools (Smith & Birkeland 2003). While coral species diversity is lower in Pool 300 than Pool 400 (Craig et al. 2001), skeletal growth rates of some species are higher in Pool 300 (Smith 2004). Do corals grow well in the fluctuating conditions of Pool 300 because these populations have sufficient phenotypic plasticity to cope with a range of environmental conditions, or have Pool 300 corals or zooxanthellae undergone environmental selection or some other process resulting in genetic differentiation? These questions were tested with two coral RTEs between the two pools.

The spatial (LaJeunesse et al. 2004a) and temporal (Baker et al. 2004) variability of zooxanthella genotypes, and dependence of coral skeletal growth rates on them (Little et al. 2004), pose potential confounding factors for coral RTEs. Thus, zooxanthella genotypes of all source colonies were tested at the beginning and end of the RTEs. This is the first study of reef-building corals to test for environmental vs. genetic control of skeletal growth rates while accounting for zooxanthella genotype.
Methods

Study Site, Species Selection, and Experimental Design

The study site was the southeast-facing fringing reef on Ofu Island (14° S) within the National Park of American Samoa. Pool 300 and Pool 400 are approximately 1 km apart (Fig. 3.1), and separated from one another by reef flat and rubble < 0.5 m mean low tide depth. The pools were previously known as Pool A (Pool 300) and Pool B (Pool 400; Craig et al. 2001). Pool 300 is much smaller and slightly shallower (0.1 ha, 1.25 m mean low tide depth) than Pool 400 (1.5 ha, 1.5 m mean low tide depth). Seawater temperatures at 1 m low tide depth (shaded, ≈ 10 cm above substrate) have been continuously recorded every 30 minutes since 1999 in the pools. The local summer mean temperature is a useful baseline for estimating coral bleaching thresholds (Jokiel 2004). Based on the 1999-2006 temperature data, mean summer (Nov-Mar) seawater temperature in the back reef area was 29.4 °C. No streams enter the back reef and turbidity is usually low, resulting in high irradiance levels during sunny weather, though turbidity sporadically increases following storms. The reef is exposed to prevailing southeast trade winds much of the year, as well as storm swells generated in the Southern Ocean during the austral winter, frequently resulting in water velocities > 30 cm s⁻¹ in the back reef area (Craig et al. 2001, Smith 2004, Smith & Birkeland 2003).

Pocillopora eydouxi (a hermaphroditic spawner) and Porites lobata (a gonochoric spawner) were selected for RTEs because of their contrasting skeletal growth forms, growth rates, and abundances in the two pools. Massive Porites species make up a six-
fold greater proportion of total substrate cover in Pool 400 (3.1 percent) than Pool 300 (0.5 percent; Craig et al. 2001). *P. eydouxi* has the opposite pattern, making up 0.5 percent cover in Pool 300 and < 0.1 percent cover in Pool 400 (Craig et al. 2001). Each species was reciprocally transplanted between Pool 300 and Pool 400. The RTE design utilized four replicate groups per species that were transplanted within and between the two sites: From Pool 300 to Pool 300 (Native 1, N₁), from Pool 300 to Pool 400 (Translocated 1, T₁), from Pool 400 to Pool 400 (Native 2, N₂), and from Pool 400 to Pool 300 (Translocated 2, T₂). Comparison of the Native and Translocated groups quantifies variability by transplant site (N₁ vs T₁, N₂ vs T₂) and by source population (N₁ vs T₂, N₂ vs T₁). Variability by transplant site indicates environmental control (phenotypic plasticity), and variability by source population indicates genetic control, assuming the absence of confounding factors. A reaction norm links a Native group to its corresponding Translocated group (N₁ and T₁, N₂ and T₂), and the two reaction norms together illustrate the interplay of environmental and genetic control on each skeletal characteristic (DeWitt & Scheiner 2004, Schluter 2000, Trussell 2000).

**Coral Transplantation and Skeletal Growth Measurement**

For *P. eydouxi*, 14 source colonies (seven per pool) were selected to each provide two 5 cm-long branches for transplanting; one for the native site and one for the translocation site. Thus each of the four RTE groups contained seven branches, giving a total of 28 transplants for this species (see Fig. 5.2 in Chapter 5). In Pool 300, only three colonies could be positively identified as *P. lobata* based on surface morphology and corallite skeletal characteristics (Veron 2000, Fenner 2005), thus six source colonies
(three per pool) were utilized for the RTE (see Figure 4.2 in Chapter 4). A pneumatic drill was used to remove eight 35 mm diameter, 5 cm-long cores from each source colony; four cores for the native site, and four cores for the translocation site, thus providing 12 cores in each of the four RTE groups and total of 48 transplants for this species. More transplants were used for *P. lobata* than *P. eydouxi* to test source colony effects. Holes were filled with marine epoxy, and tissue grew over the epoxy within six months. All transplants were placed near the seaward edges of the pools.

To minimize confounding factors associated with variability in source colony characteristics, transplant size, transplant shape, handling stress, micro-environmental conditions, competition, predation, and disease, the following procedure was used for coral transplantation: (1) Source colonies were > 10 m from one another to reduce the likelihood of selecting clones; (2) The tops of all source colonies were at 0.75-1.25 m low tide depth, and transplant branches or cores were removed from the center portion of the tops of the source colonies; (3) Transplants were approximately the same length, weight, and shape, and were handled and transported in the same manner; (4) Transplant cores were removed from source colonies in the morning and transplanted in the late afternoon; (5) Within each pool, individual transplant attachment sites were prepared by drilling shallow 35 mm holes in dead coral substrate at 1.0 m low tide depth; (6) The two groups to be transplanted within each site (the N and T groups) were mixed, then each transplant was randomly assigned an individual attachment site; (7) Transplants were attached with Sea Goin’ Poxy Putty® marine epoxy no less than 25 cm apart, mapped, and photographed, and; (8) All transplants were surveyed for survival in September 2004, May 2005, and February 2006. Those with bleaching, overgrowth, or other tissue death
were considered mortalities and removed from the experiment because of potential effects on skeletal results. During each survey, all surviving transplants were checked for signs of competition, predation or disease.

Skeletal growth rates of the transplants were determined with the buoyant weight method to measure percentage increase in skeletal mass (Jokiel et al. 1978), and the alizarin dye method to measure upward linear extension (Barnes 1970). Transplants were removed from source colonies early in the morning, placed in plastic bags of dissolved alizarin (100 mg l⁻¹) anchored to the back reef substrate, left for six hours, transferred to a nearby weighing station, buoyant weighed (Ohaus Dial-O-Gram mechanical balance, accurate to 0.01 g), and finally transplanted near the end of the day. For each species, all transplants were removed from source colonies, stained, weighed and transplanted within 48 hours in early September 2004. In early March 2006, surviving transplants were removed without fracturing the skeleton, cleaned by removing epoxy and encrusting organisms by hand and by removing tissue with bleach, buoyant weighed, sliced with a band saw, sanded to reveal the alizarin mark, and a single measurement taken with calipers on the upper central portion of each sliced transplant to determine upward linear extension. Buoyant weight results were used to calculate increase in skeletal mass, and normalized to initial size with the equation: % mass increase = ([final weight-initial weight]/initial weight)*100.

Environmental Data Collection and Statistical Analyses

Environmental data were collected between September 2004 and April 2006 on seawater temperature, photosynthetically active radiation (PAR), water flow, turbidity,
salinity, dissolved oxygen, and dissolved nutrients from the two transplant sites. Shaded seawater temperatures were recorded simultaneously at the two sites for the duration of the 18-month experiment. The loggers were tested indoors against one another and a calibrated thermometer, deployed for six months, then retrieved and tested again. Loggers always read < 0.1°C of one another and the calibrated thermometer. Turbidity, salinity, and dissolved oxygen were recorded simultaneously at the two sites during a 10-day period near the beginning of the experiment. Seawater temperatures, turbidity, salinity and dissolved oxygen were recorded every 30 minutes at 1 m low tide depth approximately 10 cm above the substrate. PAR data were collected from the two sites during clear weather on January 20th and 21st, 2005, between 11:00 a.m. and 1:00 p.m. on the upper surfaces of coral colonies at approximately 1 m depth at low tide.

Water flow was measured at a central point within each transplant area in both pools during two 3-day periods of contrasting conditions: A calm period resulting from small surf breaking on the reef crest (average height of breaking wave faces 0.5-1.5 m), and a rough period resulting from large surf (2-3 m). During both periods, SE trade winds were 10-20 knots. Flow velocity was measured during the calm period with fluoresceine dye, and during the rough period with two mechanical flow meters. Sampling was stratified into three sampling periods per day corresponding with maximum flood, high tide, and maximum ebb tides. During the calm period, a two-person team sampled flow velocity by injecting dye at one end of a 1-m measuring rod positioned horizontally 10 cm above the substrate and timing the movement of the dye along the rod. During each sampling period, 10 measurements were made in each pool in < 10 minutes, and both pools were sampled within 30 minutes of one another. During the rough period, the flow
meters were anchored 10 cm above the substrate, and rotation counter readings were taken at the beginning and end of six 10-minute periods during each of the three sampling periods per day.

Nutrient samples were collected simultaneously in the two pools every 4 hours from the water column (50 cm above substrate) and substrate (from within sediment) for a 48-hour period. From each sample, 140 ml was drawn through a GF/F filter (0.7 μm pore size), 90 ml used to twice wash a new plastic bottle, then the final 50 ml was stored in the bottle and frozen for shipment to the laboratory for analysis. Each sample was analyzed for concentrations of dissolved inorganic nitrogen (DIN: NH₄ + NOₓ) and phosphate (PO₄).

Statistical analyses were performed with Minitab 14. All data were assessed for normality and homogeneity of variances (Levene’s test) prior to testing. For each species, a three-way ANOVA was used to test effects of transplant site, source population, and source colony on skeletal growth rate, as measured by mass increase and linear extension. Because the two measures of skeletal growth rate are dependent, p-values of < 0.05 were multiplied by a factor of two to obtain final p-values (Bonferroni correction).

Zooxanthella Sampling

To infer spatial and temporal variability in symbiont genotypes of the transplants, zooxanthella genotypes of source colonies were determined at the beginning and end of the RTE. Transplants could not be sampled because removal of skeletal material would affect skeletal growth results. Zooxanthella samples were taken using a 13 mm punch from the top of each source colony in September 2004 and March 2006, respectively.
Samples were preserved in 95% ethanol, and total DNA was extracted using established methods (Baker et al. 1997) for use in denaturing-gradient gel electrophoresis (DGGE)(LaJeunesse 2001). Using primers with a Guanine-Cytosine clamp (GC clamp) designed for DGGE, the internal transcribed spacer-2 (ITS-2) region of nuclear ribosomal DNA was amplified using polymerase chain reaction (PCR). The PCR product was then run on an acrylamide DGGE gel with a 35%-75% chemical gradient (formamide and urea) from low to high. The diagnostic bands were cut out and reamplified using PCR with primers not containing the GC clamp. The PCR products from the cut bands were then sequenced, and the edited sequences run through a Basic Local Alignment Search Tool (BLAST) search in GenBank for a *Symbiodinium* type match.

**Results**

*Survival and Skeletal Growth*

Survival of *P. eydouxi* was nearly 100 percent, with only one transplant mortality in Group N2. *P. lobata* survival was 42-50 percent for the RTE groups in Pool 300 (5/12 for N1 and 6/12 for T2), and 58-83 percent for those in Pool 400 (7/12 for N2 and 10/12 for T1). *P. eydouxi* grew more in Pool 300 than in Pool 400, whereas growth of *P. lobata* was similar between the pools (Fig. 3.2). For *P. eydouxi*, mass increase and linear extension were affected by transplant site but not by source population or source colony. In contrast, neither skeletal mass nor linear extension of *P. lobata* were affected by transplant site, source population, or source colony (Table 3.1). There were no significant interactions between transplant site and source population for either species. For each
Figure 3.2. Skeletal growth results (left) and reaction norms (right) for *Pocillopora eydouxi* (black circles) and *Porites lobata* (white circles) in, (a) mass gained, and (b) and linear extension.

species, results of the two skeletal growth measurement methods were consistent with one another.

The nearly overlapping reaction norms for each species indicate the absence of genetic effects, suggesting that the transplanted corals are part of the same population, as might be expected from broadcast spawning species in such close proximity. The strong effect of transplant site on *P. eydouxi* indicates environmental control of skeletal growth results, as illustrated by the steeply sloped reaction norms for this species (Fig. 3.2). No signs of competition, predation or disease were observed on any of the surviving
Table 3.1. Three-way ANOVAs (transplant site, source population or source colony) for *P. eydouxi* (above) and *P. lobata* (below) skeletal growth, measured by mass increase (Mss Inc, %) and linear extension (Lin Ext, mm).

<table>
<thead>
<tr>
<th>P. eydouxi</th>
<th>Df</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mss Inc</td>
<td>Lin Ext</td>
<td>Mss Inc</td>
</tr>
<tr>
<td>Transplant Site</td>
<td>1</td>
<td>706067</td>
<td>1449.23</td>
<td>11.43</td>
</tr>
<tr>
<td>Source Popn</td>
<td>1</td>
<td>41795</td>
<td>10.05</td>
<td>0.68</td>
</tr>
<tr>
<td>Source Colony</td>
<td>6</td>
<td>90898</td>
<td>29.10</td>
<td>1.47</td>
</tr>
<tr>
<td>Population x Site</td>
<td>1</td>
<td>81053</td>
<td>0.02</td>
<td>1.31</td>
</tr>
<tr>
<td>Error</td>
<td>17</td>
<td>61765</td>
<td>24.43</td>
<td></td>
</tr>
<tr>
<td>P. lobata</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transplant Site</td>
<td>1</td>
<td>19148</td>
<td>1.896</td>
<td>1.64</td>
</tr>
<tr>
<td>Source Popn</td>
<td>1</td>
<td>9428</td>
<td>11.266</td>
<td>0.81</td>
</tr>
<tr>
<td>Source Colony</td>
<td>2</td>
<td>2234</td>
<td>2.286</td>
<td>0.19</td>
</tr>
<tr>
<td>Population x Site</td>
<td>1</td>
<td>132</td>
<td>0.009</td>
<td>0.01</td>
</tr>
<tr>
<td>Error</td>
<td>22</td>
<td>11672</td>
<td>8.564</td>
<td></td>
</tr>
</tbody>
</table>

*Bonferroni corrected

transplants in 2004, 2005 or 2006, suggesting that skeletal characteristics were not affected by biotic environmental factors. Thus, abiotic environmental differences between the pools are the most likely explanation for the *P. eydouxi* skeletal growth results, assuming they were not confounded by variability in zooxanthella genotype.

Seawater temperature, PAR, turbidity, salinity and dissolved oxygen at the two transplant sites were within measurement error of one another (Appendix 3.1). However, during the summers (Nov-04 to Mar-05, Nov-05 to Feb-06), the frequency and duration
of maximum daily temperatures were greater in Pool 300 than in Pool 400 (Fig. 3.3). Flow velocities were similar between the two pools during calm conditions, but nearly twice as high in Pool 300 than Pool 400 during rough conditions. During both conditions, mean velocity was always higher in Pool 300 than Pool 400, though the difference was much greater during rough than calm conditions (Fig. 3.4). Mean DIN concentrations were greater in Pool 300 than in Pool 400, whereas mean phosphate concentrations were nearly the same in the two pools (Appendix 3.1). For all abiotic environmental factors, daily fluctuations were higher in Pool 300 than in Pool 400 (Appendix 3.1). In summary, the available data show that Pool 300 had more widely fluctuating physical environmental conditions than Pool 400, and that Pool 300 had higher summer seawater temperatures, higher flow velocities during rough conditions, and higher nutrient concentrations than Pool 400.

Figure 3.3. Frequency and duration of summer (Nov – Mar) seawater temperatures exceeding 1, 2, 3, 4, and 5 °C above the local summer mean (29.4 °C) during the RTE period.
Zooxanthella genotypes showed both spatial and temporal variability for *P. eydouxi*, but not for *P. lobata* (Table 3.2). Zooxanthella genotypes in *P. eydouxi* source colonies varied between the pools, with a mix of Clades C and D (Rowan & Powers 1991a, Rowan & Powers 1991b) in Pool 300, but only Clade C in Pool 400. A higher number of genotypes was found in Pool 300 source colonies than in Pool 400 source colonies. In Pool 300, only Source Colonies 2 and 7 had the same genotypes at the beginning and end of the RTE, while there was no change for Pool 400 genotypes. *P. lobata* source colonies in both pools all had Clade C zooxanthellae at the beginning and end of the RTE (Table 3.2).
Table 3.2. Zooxanthellae genotypes (LaJeunesse 2001, LaJeunesse et al. 2004a) found in source colonies at the beginning (9/04) and end (3/06) of the RTE.

<table>
<thead>
<tr>
<th>Source Colony</th>
<th>Pool 300 9/04</th>
<th>Pool 300 3/06</th>
<th>Pool 400 9/04</th>
<th>Pool 400 3/06</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. eydouxi</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>C1, C1c, C42, D, D1a</td>
<td>C1c, C1c, C42, D, D1a</td>
<td>C1c</td>
<td>C1c</td>
</tr>
<tr>
<td>2</td>
<td>C1, C1c</td>
<td>C1, C1c</td>
<td>C1c</td>
<td>C1c</td>
</tr>
<tr>
<td>3</td>
<td>D1a</td>
<td>C1, C1c, C42, D, D1a</td>
<td>C1c</td>
<td>C1c</td>
</tr>
<tr>
<td>4</td>
<td>C1, C1c, D1a</td>
<td>C1, C1c, C42, D, D1a</td>
<td>C1c</td>
<td>C1c</td>
</tr>
<tr>
<td>5</td>
<td>C1, C1c</td>
<td>C1, C1c, C42, D, D1a</td>
<td>C1c</td>
<td>C1c</td>
</tr>
<tr>
<td>6</td>
<td>D1a</td>
<td>C1, C1c</td>
<td>C1c</td>
<td>C1c</td>
</tr>
<tr>
<td>7</td>
<td>C1, C1c</td>
<td>C1, C1c</td>
<td>C1c, C42</td>
<td>C1c, C42</td>
</tr>
<tr>
<td>P. lobata</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>C15</td>
<td>C15</td>
<td>C15</td>
<td>C15</td>
</tr>
<tr>
<td>2</td>
<td>C15</td>
<td>C15</td>
<td>C15</td>
<td>C15</td>
</tr>
<tr>
<td>3</td>
<td>C15</td>
<td>C15</td>
<td>C15</td>
<td>C15</td>
</tr>
</tbody>
</table>

Discussion

Skeletal growth rate of *P. eydouxi* transplants responded to transplant site (Table 3.1), indicating environmental control (i.e., phenotypic plasticity). Competition, predation and disease did not appear to affect the transplants, thus growth rate variability was likely a response to abiotic differences between the two back reef pools. Abiotic factors most likely to affect skeletal growth are PAR, seawater temperature, nutrients and flow (Buddemeier & Kinzie 1976, Jokiel 1978, Lough & Barnes 2000). There were no differences in transplant depth or PAR (Appendix) at the two sites, thus the observed higher skeletal growth of *P. eydouxi* in Pool 300 than in Pool 400 was likely due to one
or more of the other three factors. The higher daily maximum seawater temperatures (Fig. 3.3) in Pool 300 than in Pool 400 would be expected to have no effect because of their short duration, or to reduce skeletal growth because temperatures were several degrees above mean summer maximum (Jokiel 2004).

Coral reef waters usually contain low levels of inorganic nutrients; typically 0.3-1.0 μmol l⁻¹ DIN and < 0.3 μmol l⁻¹ phosphorus (Crossland 1983). Zooxanthellate corals may be nitrogen limited, as shown by increased zooxanthellate (Falkowski & Dubinsky 1993) and skeletal (Meyer & Schultz 1985) growth with slight increases in DIN concentrations. However, the difference between nitrogen limitation and excess can be small, with concentrations of < 5 μmol l⁻¹ DIN often resulting in decreased skeletal growth (Ferrier-Pages et al. 2000). DIN concentrations were 0.54-0.58 μmol l⁻¹ in Pool 400, and approximately double in Pool 300 (Appendix). Thus nitrogen may be limiting in the pools, and the higher concentrations in Pool 300 may have contributed to the higher skeletal growth of *P. eydouxi*. However, if this were the case, higher skeletal growth of *P. lobata* would be expected in Pool 300 than in Pool 400, but there was no difference in growth of this species between the pools (Table 3.1, Fig. 3.2).

Velocity of water flow was higher in Pool 300 than Pool 400 during all flow sampling periods, especially during rough conditions (Fig. 3.4). Flow reduces damage by ultraviolet radiation (Kuffner 2002) and photoinhibition (Nakamura et al. 2005) from high irradiance levels, even if flow is intermittent (Smith & Birkeland 2007), as in these back reef pools. In a study of *Pocillopora meandrina*, a morphologically and ecologically similar species to *P. eydouxi*, skeletal growth of *P. meandrina* was higher in the high flow treatment (estimated at 20-40 cm s⁻¹) than the moderate (≈ 15-30 cm s⁻¹) or low (≈
5-10 cm s\(^{-1}\)) flow treatments. It was concluded that optimal water velocity for \(P.\) \textit{meandrina} skeletal growth was greater than the high flow treatment (Jokiel 1978). The higher mean flow velocities during rough conditions in Pool 300 (39.8 cm s\(^{-1}\)) than in Pool 400 (21.0 cm s\(^{-1}\); Appendix) would likely benefit \(P.\) \textit{eydouxi} skeletal growth in Pool 300. Because of tidal flow, southeast tradewinds, frequent storms, and south oceanic swell, rough conditions are the norm in the back reef pools (Smith & Birkeland 2003, 2007).

\(P.\) \textit{lobata} showed no variance in skeletal growth rate between the pools (Table 3.1), or any zooxanthella genotype differences (Table 3.2). Host or symbiont genetic differentiation was not expected between the two adjacent pools (Fig. 3.1) because \(P.\) \textit{lobata} is a broadcast spawner (Richmond & Hunter 1990), and \textit{Porites} are not known to have high spatial or temporal variability in zooxanthella genotypes (Baker et al. 2004). Due to the greater abundance of massive \textit{Porites} in Pool 400 than Pool 300 (Craig et al. 2001), and the scarcity of these species on the shallow high energy forereef (personal observation), it was thought that the relatively calm Pool 400 would be a more favorable growth environment for \(P.\) \textit{lobata} than Pool 300. However, \(P.\) \textit{lobata} survival was low, especially in the RTE groups in Pool 300 (42 and 50 percent), resulting in low sample sizes. Thus, two possible explanations for \(P.\) \textit{lobata} results are: 1) the low sample sizes and growth rates reduced statistical power below that necessary to detect variance of skeletal growth rates between the pools; or 2) the environments of the pools are not different enough to induce phenotypic plasticity of skeletal growth rate of this species.

The occurrence of Clade D zooxanthellae in Pool 300 but not Pool 400 source colonies of \(P.\) \textit{eydouxi} is consistent with previous findings that Clade D is more common
in shallow habitats with elevated seawater temperatures (Fabricius et al. 2004, van Oppen et al. 2005). Clade D increases heat tolerance but decreases skeletal growth compared to Clade C (Little et al. 2004, Berkelmans & van Oppen 2006). Thus, in the absence of environmental differences between the pools, mean growth of *P. eydouxi* transplants from Pool 300 (Groups N₁ and T₁) should be less than mean growth of transplants from Pool 400 (Groups N₂ and T₂). That is, a source population effect would be expected because of the difference in zooxanthella genotypes between the source colonies. But despite these differences (Table 3.2), source population had no effect on skeletal growth rates (Table 3.1), indicating the greater importance of environmental factors than zooxanthella genotype on skeletal growth in this experiment.

Several facets of the *P. eydouxi* zooxanthella genotype results hint at the complexity of this symbiosis. First, though a larger proportion of Clade D was found in the Pool 300 source colony samples taken in the summer (March) than in the winter (September), as expected because of elevated summer seawater temperatures, not all source colonies followed this pattern: Source Colony 6 switched from Clade D in the winter to Clade C in the summer, and Source Colonies 2 and 7 had Clade C at both sampling periods (Table 3.2). Second, zooxanthella results showed up to five genotypes per source colony in Pool 300 during the summer, suggesting that Clade D symbionts were added to, rather than switched with, existing genotypes. Third, in spite of finding multiple genotypes in some source colonies, zooxanthella diversity may have been underestimated in some or all samples because of poor resolution by the ITS-2/DGGE methodology (Apprill & Gates 2007). Though *Pocillopora* species maternally transmit zooxanthellae to larvae (Glynn et al. 1991), the genetic diversity of zooxanthellae in *P.*
eydouxi (Table 3.2) and P. meandrina (Magalon et al. 2006) suggest more complexity and flexibility in the symbiosis than has been previously thought for these species.

This study demonstrated phenotypic plasticity of P. eydouxi skeletal growth rate, with approximately doubled growth in the pool with higher water motion (Pool 300). Throughout most of its range, P. eydouxi is found in most reef environments, and is often very common where water motion is high (Veron 2000, Mumby et al. 2001b, Fisk & Birkeland 2002). Shallow, high energy reef environments provide high quality habitat (high PAR, high water motion, low sedimentation) for many sessile species, resulting in keen competition for space (Sheppard 1982, Potts 1984). Thus, increased skeletal growth with higher water motion may provide P. eydouxi a competitive advantage in shallow, high energy environments where competition for space is greatest, while still allowing adequate growth in lower energy environments where competition is reduced.
Appendix 3.1. Environmental data (MDR = maximum daily range, PAR = photosynthetically active radiation, DIN = dissolved inorganic nitrogen).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Dates</th>
<th>Periodicity</th>
<th>N/pool</th>
<th>Units</th>
<th>Error</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pool</td>
</tr>
<tr>
<td>Summer Temperatures</td>
<td>Nov-Mar</td>
<td>2x hr⁻¹</td>
<td>12,960</td>
<td>°C</td>
<td>±0.2°C</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>Winter Temperatures</td>
<td>Apr-Oct</td>
<td>2x hr⁻¹</td>
<td>13,000</td>
<td>°C</td>
<td>±0.2°C</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>PAR</td>
<td>20-21 Jan ‘05</td>
<td>4x min⁻¹</td>
<td>400</td>
<td>μmol quanta m⁻² s⁻¹</td>
<td>±5%</td>
<td>300</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>Calm Flow</td>
<td>20-22 Mar ‘06</td>
<td>30x day⁻¹</td>
<td>90</td>
<td>cm s⁻¹</td>
<td>±10%</td>
<td>300</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td>400</td>
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<tr>
<td>Rough Flow</td>
<td>22-24 Apr ‘06</td>
<td>18x day⁻¹</td>
<td>54</td>
<td>cm s⁻¹</td>
<td>±5%</td>
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<td></td>
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<td>Turbidity</td>
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<td>480</td>
<td>NTU</td>
<td>±0.1 NTU</td>
<td>300</td>
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<td>400</td>
</tr>
<tr>
<td>Salinity</td>
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<td>480</td>
<td>ppt</td>
<td>±0.01 ppt</td>
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<td>400</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>14-23 Sep ‘04</td>
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<td>480</td>
<td>% saturation</td>
<td>±1%</td>
<td>300</td>
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<tr>
<td>Water Column DIN</td>
<td>1-2 Apr ‘06</td>
<td>6x day⁻¹</td>
<td>13</td>
<td>μmol l⁻¹</td>
<td>N/A</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>Substrate DIN</td>
<td>1-2 Apr ‘06</td>
<td>6x day⁻¹</td>
<td>13</td>
<td>μmol l⁻¹</td>
<td>N/A</td>
<td>300</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>Water Column PO₄</td>
<td>1-2 Apr ‘06</td>
<td>6x day⁻¹</td>
<td>13</td>
<td>μmol l⁻¹</td>
<td>N/A</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>Substrate PO₄</td>
<td>1-2 Apr ‘06</td>
<td>6x day⁻¹</td>
<td>13</td>
<td>μmol l⁻¹</td>
<td>N/A</td>
<td>300</td>
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<td></td>
<td></td>
<td>400</td>
</tr>
</tbody>
</table>

*Instruments Used:
  1. Seawater Temperature: Onset Water Temp Pro® temperature loggers.
  2. PAR: Light sensor on a Walz Diving Pulse-Amplitude Modulation Fluorometer (Diving-PAM®).
  5. Nutrients: Laboratory analysis.
CHAPTER 4:

PHENOTYPIC PLASTICITY FOR SKELETAL GROWTH, DENSITY AND CALCIFICATION OF *PORITES LOBATA* IN RESPONSE TO HABITAT TYPE©

Lance W. SMITH, Dan BARSHIS, Charles BIRKELAND

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Abstract

A reciprocal transplant experiment (RTE) of the reef-building coral *Porites lobata* between shallow (1.5 m at low tide) back reef and forereef habitats on Ofu and Olosega Islands, American Samoa, resulted in phenotypic plasticity for skeletal characteristics. Transplants from each source population (back reef and forereef) had higher skeletal growth rates, lower bulk densities, and higher calcification rates on the back reef than on the forereef. Mean annual skeletal extension rates, mean bulk densities, and mean annual calcification rates of RTE groups were 2.6 – 9.8 mm yr⁻¹, 1.41 – 1.44 g cm⁻³, and 0.37 – 1.39 g cm⁻² yr⁻¹ on the back reef, and 1.2 – 4.2 mm yr⁻¹, 1.49 – 1.53 g cm⁻³, and 0.19 – 0.63 g cm⁻² yr⁻¹ on the forereef, respectively. Bulk densities were especially responsive to habitat type, with densities of transplants increasing on the high energy forereef, and decreasing on the low energy back reef. Skeletal growth and calcification rates were also influenced by source population, even though zooxanthellae genotype of source colonies did not vary between sites, and there was a transplant site x source population interaction for upward linear extension. Genetic differentiation may explain the source population effects, or the experiment may have been too brief for phenotypic plasticity of all skeletal characteristics to be fully expressed. Phenotypic plasticity for skeletal characteristics likely enables *P. lobata* colonies to assume the most suitable shape and density for a wide range of coral reef habitats.
The massive reef-building scleractinian coral *Porites lobata* is frequently a dominant species in back reef margins (Veron 2000; Craig et al. 2001) and fringing coral reefs (Dollar 1982, Jokiel et al. 2004) of the Indo-Pacific. Colonies may live for over 500 years, attaining diameters of > 6 m (Lough & Barnes 1997, Fenner 2005). Skeletal characteristics, such as linear extension, density, and calcification, vary along environmental gradients. Skeletal extension rates of *P. lobata* and other massive *Porites* species increase with increasing seawater temperatures along latitudinal gradients (Grigg 1982, Lough & Barnes 2000), with increasing solar irradiance along depth (Grigg 2006) or turbidity (Lough & Barnes 1992) gradients, and with decreasing water motion along hydraulic energy gradients (Scoffin et al. 1992). Generally, skeletal extension rates of *P. lobata* and other massive *Porites* species are higher in the summer than in the winter, higher in larger than in smaller colonies, and the rates of upward growth are higher than lateral growth (Lough et al. 1999, Lough & Barnes 2000). Skeletal density refers to the specific gravity of the skeletal material plus enclosed voids, also known as bulk density (Bucher et al. 1998). In *P. lobata* and other massive *Porites* species, skeletal extension rates and bulk density are inversely related (Lough et al. 1999; Lough and Barnes 2000).

Calcification rate is the mass of calcium carbonate (CaCO₃) a coral colony deposits per unit area per unit time, and is calculated as the product of skeletal extension and bulk density. Because of the inverse relationship of massive *Porites* skeletal extension rate and bulk density, only considering one or the other can lead to misleading conclusions regarding calcification rates. That is, increasing extension rates or bulk
densities do not necessarily indicate increasing calcification rates (Dodge & Brass 1984, Lough & Barnes 1992). However, in massive Porites, rates of calcification and extension are strongly linked, thus higher calcification rates are associated with higher seawater temperatures (Lough and Barnes 2000), higher solar irradiance (Lough and Barnes 1992; Grigg 2006), and lower water motion (Scoffin et al. 1992). Likewise, as with extension rates, calcification rates are typically higher on upward than lateral surfaces in these species (Lough et al. 1999; Lough and Barnes 2000).

Phenotypic variability, such as colony morphological variability in zooxanthellate corals, may be caused by the environment (Foster 1979), by genetic differences between individuals or populations (Willis and Ayre 1985), or by both (Via and Lande 1985). Phenotypic plasticity refers to environmental control of morphological variability, and a reaction norm represents the relationship between the phenotype and the environment (Stearns 1989; Doughty and Resnick 2004). Phenotypic plasticity confers broad adaptability to the range of environmental conditions encountered by sessile organisms (Bradshaw 1965). For example, in zooxanthellate corals, phenotypic plasticity across depths for colony shape in Porites sillimaniani (Muko et al. 2000) and for corallite shape in two faviids (Todd et al. 2004a) may function to maximize absorption of available light.

Though colony morphological variability of massive Porites species is well known (Dollar 1982; Grigg 1982; Scoffin et al. 1992; Veron 2000), the sources of the variability (environment, genetic, or both) have not been reported for these species. The morphology of P. lobata colonies on the reefs of Ofu and Olosega Islands, American Samoa (Fig. 4.1), varies by habitat type. In the back reef pools (< 3 m depth), colonies are hemispherical or dome-shaped until they attain 2 – 3 m in diameter, after which they
become micro-atolls up to 8 m diameter as the limit of upward growth is reached but lateral growth continues. On the shallow forereef (< 3 m depth), colonies are flat or encrusting, up to 4 m diameter but < 0.5 m thick.

Figure 4.1. Map of study area, showing back reef and forereef reciprocal transplant sites.

We hypothesized that the morphological variability of *P. lobata* at this site is produced by phenotypic plasticity for skeletal growth rate, with greater upward linear extension in the relatively low energy back reef than on the high energy forereef. To test this hypothesis, a reciprocal transplant experiment of *P. lobata* was carried out between back reef and forereef sites (Fig. 4.1), along with zooxanthellae genotyping of source colonies at the beginning and end of the experiment. The purpose of the experiment was to determine if skeletal growth rate variability of *P. lobata* at this site is environmental, genetic, or both (environmental control indicates phenotypic plasticity). Bulk densities of the transplants were also measured in order to calculate calcification rates.
Methods

Study area and species selection

The study area was a pair of small volcanic islands, Ofu and Olosega, in American Samoa (14° 11’S, 169° 40’W). The islands are separated by a channel approximately 100 m wide and 3 m deep (Fig. 4.1). Narrow fringing reefs surround the islands, with shallow (< 3 m low tide depth) back reef pools occurring where the reef is widest. Water motion in the back reef is semi-diurnally intermittent, typically alternating between > 20 cm s⁻¹ at high tide to < 5 cm s⁻¹ at low tide (Smith & Birkeland 2007). During mid-day low tides, limited mixing and high solar irradiance combine to warm the back reef waters, producing daily fluctuations in seawater temperature of up to 4 – 5 °C (Smith 2004). In addition, the smoother water surface associated with reduced water motion at low tide in the back reef increases transmission of irradiance into the water column (Kirk 1994). Frequent storms and high rainfall cause sporadically high turbidity and low salinity in the back reef (Smith and Birkeland 2003). In contrast, the shallow (< 3 m low tide depth) forereef is a more stable environment. Breaking waves create consistently very high water motion, moderating seawater temperature and irradiance transmission. The water motion, as well as distance from shore, prevents rapid changes in turbidity and salinity relative to the back reef.

Diversity of reef-building corals is highest in the larger back reef pools, dominated by massive Porites micro-atolls, Porites cylindrica, and Acropora species (Craig et al. 2001). Diversity and abundance of corals is lower on the shallow forereefs,
where robust branching species and encrusting species are prevalent (Fisk & Birkeland 2002). At least five massive Porites species occur in the study area; P. lobata, P. lutea, P. australiensis, P. mayeri and P. solida, and some skeletal growth characteristics may vary by species (Lough et al. 1999). P. lobata, a gonochoric spawner, was selected for this experiment because gross colony morphology varies between habitats (back reef and forereef), it is common elsewhere in the Indo-Pacific, and it can be distinguished from other massive Porites species by surface morphology and corallite skeletal characteristics (Veron 2000; Fenner 2005).

Reciprocal transplant experimental design

P. lobata was reciprocally transplanted between a back reef site on southeastern Ofu and a forereef site on northwestern Olosega (Fig. 4.1) for a 6-month period between August 2004 and February 2005. The reciprocal transplant experiment (RTE) design utilized four replicate groups that were transplanted within and between the two sites (Fig. 4.2): From the back reef to the back reef (Native 1, N1), from the back reef to the forereef (Translocated 1, T1), from the forereef to the forereef (Native 2, N2), and from the forereef to the back reef (Translocated 2, T2). Comparison of the Native and Translocated groups quantifies variability by transplant site (N1 vs T1, N2 vs T2) and by source population (N1 vs T2, N2 vs T1). Variability by transplant site indicates environmental control (phenotypic plasticity), and variability by source population indicates genetic control, assuming the absence of confounding factors. A reaction norm links a Native group to its corresponding Translocated group (N1 and T1, N2 and T2), and
the two reaction norms together illustrate the interplay of environmental and genetic control on each skeletal characteristic (Schluter 2000; DeWitt and Scheiner 2004).

Figure 4.2. Reciprocal transplant experimental (RTE) design, showing transplant sites, source populations, and source colonies the four RTE groups.

*Coral transplantation*

*P. lobata* source colonies were identified based on surface morphology and corallite skeletal characteristics (Veron 2000; Fenner 2005). Only three colonies could be positively identified as *P. lobata* on the shallow forereef site, thus six source colonies (three per site) were utilized for the RTE. A pneumatic drill was used to remove eight 35 mm diameter, 50 mm-long cores from each source colony; four cores for the native site, and four cores for the translocation site, thus providing 12 cores in each of the four RTE groups (Fig. 4.2). Holes were filled with marine epoxy, and tissue grew over the epoxy within six months.
To minimize confounding factors associated with variability in source colony characteristics, transplant size, transplant shape, handling stress, micro-environmental conditions, competition, predation, and disease, the following procedure was used for coral transplantation: (1) Source colonies were > 10 m from one another to reduce the likelihood of selecting clones, except for two source colonies on the forereef that were 5 m apart; (2) The tops of all source colonies were at 1 – 2 m low tide depth, and transplant cores were removed from the center portion of the tops of the source colonies; (3) Transplants were approximately the same length, weight, and shape, and were handled and transported in the same manner; (4) Transplant cores were removed from source colonies in the morning and transplanted in the late afternoon; (5) Within each transplant site (forereef or back reef), individual transplant attachment sites were prepared by drilling shallow 35 mm holes in dead coral substrate at 1.5 m low tide depth; (6) The two groups to be transplanted within each site (the N and T groups) were mixed, then each transplant was randomly assigned an individual attachment site; (7) Transplants were attached with Sea Goin’ Poxy Putty® marine epoxy no less than 25 cm apart, mapped, and photographed, and; (8) All transplants were surveyed for survival in September 2004 and February 2005. Those with bleaching, overgrowth, or other tissue death were considered mortalities and removed from the experiment because of potential effects on skeletal results. During each survey, all surviving transplants were checked for signs of competition, predation or disease.
Skeletal measurements

Skeletal growth rates of the transplants were determined with the buoyant weight method to measure percentage increase in skeletal mass (Jokiel et al. 1978), and the alizarin dye method to measure linear extension (Barnes 1970). Transplants were removed from source colonies early in the morning, placed in plastic bags of dissolved alizarin (100 mg l\(^{-1}\)) anchored to the back reef substrate, left for six hours, transferred to a nearby weighing station, buoyant weighed (Ohaus Dial-O-Gram mechanical balance, accurate to 0.01 g), and finally transplanted near the end of the day. All transplants were removed from source colonies, stained, weighed and transplanted on 20\(^{th}\) and 21\(^{st}\) August 2004. On 20\(^{th}\) and 21\(^{st}\) February 2005, surviving transplants were removed without fracturing the skeleton, cleaned by removing epoxy and encrusting organisms by hand and by removing tissue with bleach, buoyant weighed, sliced with a band saw, sanded to reveal the alizarin mark, and linear extension measured.

Both upward and lateral extension rates of the transplants were measured. Upward linear extension of each transplant was determined from the mean of four measurements taken in the central one-third of the upward facing surface of each sliced transplant. The alizarin stain mark was up to a few millimeters thick, thus the measurements were made from the upper boundary of the mark. Lateral linear extension was also determined from the mean of four measurements: Slicing each transplant dorso-ventrally revealed the well-stained, undamaged upper surface of the original core, as well as the lightly-stained straight vertical surfaces created by drilling through the skeleton. A pair of horizontal measurements was made on each corner starting from the upper and lower boundaries of the upper stain mark, and the mean of these four measurements used to calculate lateral
growth for each transplant. The results were used to estimate annual upward and lateral extension rates (mm yr⁻¹).

Bulk density (g cm⁻³) was measured by first air-drying the 35 surviving transplants for six months, then grinding each transplant to a 5 – 6 cm³ block. All alizarin-stained skeleton and post-RTE skeletal material was removed, thus the blocks were obtained from the central portion of the pre-RTE cores. The blocks were dried at 60 °C for 24 hours before weighing. To determine bulk density at the end of the RTE, dry weight was taken of each block (DW_{clean}). The blocks were dipped in molten paraffin wax kept at 110 – 115 °C to form a water-tight barrier, then dry weight was again taken of each block (DW_{wax}). Buoyant weight of each waxed block was measured in distilled water at 20 °C with specific gravity 1.00 g cm⁻³ (BW_{wax}). Total enclosed volume (V_{enclosed}) and bulk density were then calculated for each block using the equations (Bucher et al. 1998):

\[ V_{enclosed} = (DW_{wax} - BW_{wax}) \times (1.00 \text{ g cm}^{-3}) \]

\[ \text{Bulk density} = \frac{DW_{clean}}{V_{enclosed}} \]

Annual calcification rate, or the mean mass of CaCO₃ deposited per unit area per year (g cm⁻² yr⁻¹), was estimated for each surviving transplant as the product of annual linear extension and bulk density. Annual linear extension was estimated by doubling the 6-month extension results. Extension rate results were assumed to be representative of mean annual values because the 6-month RTE period was evenly split between the cool and warm seasons. Bulk density results were assumed to be representative of mean annual values because the blocks were large enough to encompass at least two years of density bands. Calcification was calculated for upward and lateral skeletal growth.
Source colony zooxanthellae typing

To infer spatial and temporal patterns in symbiont genotypes of the transplants, zooxanthellae types were determined of all source colonies at the beginning and end of the RTE. A zooxanthellae sample was taken using a 13 mm punch from the top of each source colony at the beginning and end of the experiment in August 2004 and February 2005. Samples were preserved in 95% EtOh, and total DNA extracted using established methods (Baker et al. 1997). Symbiont DNA was amplified using partial large subunit ribosomal DNA (LSU rDNA) primers 24D15F4 and 24D23R1, and the resultant products identified and assigned to clades with restriction fragment length polymorphism (RFLP) analysis using enzymes TaqI and Hhal (Baker et al. 1997, Baker 2001). In addition, denaturing-gradient gel electrophoresis (DGGE) was used for a finer-scale genotype analysis to search for spatial and temporal differences not detected by RFLP.

Statistical analysis

Statistical analyses were performed with Minitab 14. All data were assessed for normality and homogeneity of variances (Levene’s test) prior to testing. A three-way Analysis of Variance (ANOVA) was used to test effects of transplant site, source population, and source colony on six skeletal characteristics (mass increase, upward linear extension, lateral linear extension, bulk density, upward calcification, and lateral calcification) of the surviving transplants. Because the six tests are dependent, p-values of < 0.05 were adjusted by a factor of six to obtain final p-values (Bonferroni correction). Interaction of factors was tested when relative magnitudes of variable means were not uniform (Sokal & Rohlf 1981).
Results

Transplant survival was 67 - 100 percent for the RTE groups on the back reef (12/12 for N₁ and 8/12 for T₂), and 50 – 75 percent for those on the forereef (6/12 for N₂ and 9/12 for T₁). No signs of competition, predation or disease were observed on any of the surviving transplants in 2004 or 2005, suggesting that skeletal characteristics were not affected by these environmental factors. Skeletal growth rate results were consistent for skeletal mass, upward extension, and lateral extension (Fig. 4.3). Transplant site affected all three measures of skeletal growth rate, indicating environmental control or phenotypic plasticity. Source population also affected skeletal growth rate, suggesting genetic control (Table 4.1). In addition, the contrasting environmental responses in upward extension of translocated corals (T₁ decreased much more than T₂ increased) led to a transplant site x source population interaction (Table 4.1), as illustrated by the converging reaction norms on the forereef (Fig. 4.3b).

Mean bulk densities were higher on the forereef than the back reef for all RTE groups (Fig. 4.4a). Transplant site affected bulk density, but source population did not (Table 4.2), thus indicating environmental but not genetic control. The sloping, nearly overlapping reaction norms (Fig. 4.4a) illustrate that the bulk density differences between the sites were likely attributable to phenotypic plasticity alone. Extension results strongly influenced calcification estimates, with upward and lateral calcification both affected by
Table 4.1. Three-way ANOVA (transplant site, source population, source colony) for skeletal growth, measured by % mass increase (%MI), upward linear extension in mm (UpEx), and lateral linear extension in mm (LtEx).

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*Bonferroni-corrected
transplant site and source population (Table 4.2). As with linear extension, these results indicate a combination of environmental and genetic control, though in this case the converging reaction norms on the forereef for upward calcification (Fig. 4.4b) do not represent a significant interaction (Table 4.2). Because multiple transplants were obtained from each source colony, effects of source colony were included in the analyses, but none of the skeletal characteristics were affected by source colony (Tables 4.1, 4.2).

The spatial (LaJeunesse et al. 2004a) and temporal (Baker et al. 2004) variability of zooxanthella type, and dependence of coral skeletal growth rates on zooxanthella type (Little et al. 2004), pose potential confounding factors for coral RTEs. However, the zooxanthellae analyses showed no differences in zooxanthella type of the source colonies by transplant site, source population, or season: The six *P. lobata* source colonies each contained RFLP genotypes C1 and C3, and DGGE genotype C15, both at the beginning and end of the experiment. Thus, assuming source colony zooxanthella type was representative of the transplants, the patterns in skeletal growth resulting from the RTE cannot be explained by zooxanthella type.
Figure 4.4. Skeletal density and calcification results (upper) and reaction norms (lower) for *Porites lobata*, (a) bulk density, (b) upward calcification (Up Calc), and (c) lateral calcification (Lat Calc). Transplant groups: $N_1 =$ native, back reef; $T_1 =$ translocated, back to forereef; $N_2 =$ native, forereef; $T_2 =$ translocated, fore to back reef.

Table 4.2. Three-way ANOVA (transplant site, source population, source colony) for skeletal bulk density (BkDn) in g cm$^{-3}$, and upward (UpCa) and lateral calcification (LtCa) in g cm$^{-2}$ yr$^{-1}$.

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*Bonferroni-corrected
Discussion

This study demonstrates that variability in skeletal characteristics of *P. lobata* between shallow (1.5 m at low tide) habitats on Ofu and Olosega Islands is at least partially a function of phenotypic plasticity. That is, regardless of source population, skeletal growth rates, bulk densities and calcification rates of each transplant group responded to the transplant site environment (Tables 4.1, 4.2), indicating environmental control. Competition, predation and disease did not appear to affect the transplants, thus variability in skeletal characteristics was likely a response to physical differences between the back reef and forereef habitats.

Mean annual upward linear extension (Fig. 4.3) and calcification rates (Fig. 4.4) for all RTE groups were lower than reported from a density band study of 35 massive *Porites* colonies from the same latitude (14°S) collected from 3 – 5 m depth in back reefs on the Great Barrier Reef (upward extension = 13.9 mm yr\(^{-1}\), calcification = 1.64 g cm\(^{-2}\) yr\(^{-1}\)) (Lough et al. 1999). In a study of *P. lobata* skeletal growth along a depth gradient in Hawai‘i, linear extension was less at 3 m than 6 m, possibly due to high levels of solar ultraviolet radiation, increased turbidity, or episodic sedimentation (Grigg 2006). Thus, skeletal growth and calcification rates of all RTE groups were likely inhibited by physical factors associated with shallow depths. In addition, skeletal growth and calcification of RTE groups on the forereef (N\(_2\), T\(_1\)) may have been reduced by very high water motion from sporadic large ocean swells and storms. In a density band study of *P. lobata* from Hawai‘i, colonies from depths less than 10m in areas exposed to waves showed frequent interruptions in skeletal growth (Grigg 1982).
Mean bulk densities of all RTE groups were 1.405 – 1.527 g cm$^{-3}$ (Fig. 4.4). These values fall within the upper range of bulk densities of massive *Porites* from 3 – 5 m deep back reef margins on the Great Barrier Reef (Lough and Barnes 2000), and are similar to those of *P. lobata* from 10m depth in the Northwest Hawaiian Islands (Grigg 1982). Phenotypic plasticity for bulk density occurred oppositely of skeletal growth rate, with higher density associated with lower growth (Figs. 4.3, 4.4). Within individual massive *Porites* colonies, bulk density varies seasonally, producing density bands (Knutson et al. 1972). However, density bands are laid down within the tissue layer on the outermost layer of the skeleton (Barnes & Lough 1993), whereas the skeletal blocks used for density measurements in this study were at least 1 cm below the tissue layer. This is the first report of bulk density changes in the skeleton underneath the tissue of *Porites* species. Such secondary infilling occurs in the bases of many branching coral species as a skeletal-strengthening adaptation (Hughes 1987), thus the increase in bulk density of transplants placed on the forereef may function to reduce the likelihood of breakage in the face of very high water velocities.

The greater effect of source population than transplant site on skeletal growth rates, and the transplant site x source population interaction for upward linear extension (Table 4.1), suggest possible genetic differentiation of the two populations. In optimal growth environments, massive *Porites* colonies grow more quickly along the upward (vertical) than the lateral (horizontal) axis (Lough et al. 1999), a pattern displayed by transplants from the back reef source population at both transplant sites. However, transplants from the forereef source population had greater lateral than upward extension at both transplant sites (Figs. 4.3b, 4.3c). Although all source colonies and transplants
were at the same depth (1.5 m low tide), underwater irradiance may differ between the back reef and forereef because of contrasting water motion patterns. The smoother water surface associated with reduced water motion at low tide on the back reef likely allows greater irradiance transmission into the water column than on the forereef, where breaking waves and high velocities maintain a roughened water surface that reduces irradiance transmission (Kirk 1994). Upward extension at 1.5 m depth requires mechanisms to absorb, reflect or fluoresce ultraviolet radiation (Hoegh-Guldberg & Jones 1999, Corredor et al. 2000), thus selection may be occurring in the back reef for such mechanisms.

Many population structure studies of broadcast spawning corals have found panmixia at within-reef (< 10 km) spatial scales (Benzie et al. 1995, Ayre & Hughes 2000, Ridgway et al. 2001, Ayre & Hughes 2004). Others have found small-scale population structure, though evidence suggests it resulted from disturbance events, rather than selection (Hunter 1993, Whitaker 2004, Magalon et al. 2005). On Olosega Island, the north-facing forereef site is frequently hit by tropical cyclones approaching from the northwest (JTWC 2006), whereas the south-facing backreef site is relatively protected from cyclones by Ofu Island (Fig. 4.1). Thus, genetic differentiation of the two populations could occur if a large storm reduced the forereef population to a small number of individuals but did not affect the back reef population, causing a population bottleneck on the forereef (Hedrick 2005).

Alternatively, the RTE results could be falsely implying genetic differentiation between the transplanted populations when in fact they are both part of a single panmictic population because: 1) The utilization of skeletal growth to quantify phenotypic...
responses to different environments may require longer than six months to obtain accurate results; and/or 2) Source population effects could be caused by contrasting developmental phenotypic plasticity (distinct, irreversible developmental pathways in response to local conditions during early life history stages; Meyers & Bull 2002) between the back reef and forereef colonies. The difficulty in distinguishing genetic differentiation from developmental phenotypic plasticity in RTEs is describing in the General Introduction above.

This study demonstrated phenotypic plasticity of *P. lobata* for skeletal characteristics (growth rate, bulk density, calcification rate) between back reef and forereef habitats. Plasticity may contribute to colony morphological variability observed in this species, especially on small spatial scales where genetic differentiation is unlikely, such as from rounder to flatter colonies with depth (Grigg 2006) and higher hydraulic energy (this study). Such colony morphological plasticity is thought to be adaptive, as the flatter shape maximizes absorption of dwindling light at depth (Muko et al. 2000) and may increase colony stability in high water velocities. For broadcast spawning corals such as *P. lobata*, larvae settle in a wide range of environments, and plasticity provides the capacity for the colony to grow into the most suitable shape and density for that particular environment (Marfenin 1997, Warner 1997). Subsequently, *P. lobata* can grow and compete in many habitat types, likely contributing to this species’ broad range and often high abundance on Pacific coral reefs.
CHAPTER 5:

DEVELOPMENTAL PLASTICITY IN THE CORAL *POCILLOPORA EYDOUXII*?

EVIDENCE FROM A FIELD EXPERIMENT AND MICROSATELLITE SURVEY
Abstract

A 6-month reciprocal transplant experiment (RTE) of the broadcast-spawning, reef-building coral *Pocillopora eydouxi* was carried out between shallow (1.5 m at low tide) back reef and forereef habitats < 5 km from one another on Ofu and Olosega Islands, American Samoa. Back reef colonies grew at twice the rate of forereef colonies regardless of transplant site during the 6-month RTE, and all source colonies contained zooxanthella genotypes C1 and C1c at the beginning and end of the RTE. These results suggested genetic differentiation and limited gene flow between back reef vs. forereef colonies, thus the RTE was followed up with a genetic investigation using four microsatellite markers. However, low overall $F_{ST}$ (0.001) and high gene flow (11 individuals per generation) between the sampled colonies from the two sites (44-48 colonies per site) indicated that back reef and forereef *Poc. eydouxi* are part of the same population. Therefore, the RTE results may be due to developmental plasticity or balancing selection: (1) If different developmental pathways are activated by the contrasting environments after settlement, then the back reef vs. forereef adult colonies used for the RTE would be genetically indistinguishable but still have different intrinsic growth rates; or (2) if environmental heterogeneity between the back reef and forereef selects for fast-growing genotypes in the back reef and slow-growing genotypes on the forereef, then the back reef vs. forereef adult colonies used for the RTE would be genetically differentiated and have different intrinsic growth rates. Several lines of evidence suggest developmental plasticity is the most likely explanation, though balancing selection cannot be ruled out with this data set.
A reciprocal transplant experiment and microsatellite markers were used to investigate factors affecting the skeletal growth of the Indo-Pacific reef-building coral *Pocillopora eydouxi* in American Samoa. *Poc. eydouxi* is common in most coral reef environments (Veron 2000), including high energy forereefs (Mumby et al. 2001b), protected back reef pools (Craig et al. 2001), and mid-depth reef slopes (Fenner 2005). Most *Pocillopora* species, such as *Poc. eydouxi*, *Poc. verrucosa*, and *Poc. meandrina* are broadcast spawners that may spawn multiple times each summer (Richmond & Hunter 1990, Kinzie 1993), likely resulting in broad dispersal of pelagic larvae and high gene flow on large spatial scales. Though the population structure of *Poc. eydouxi* has not been studied, sister taxa *Poc. verrucosa* (Ridgway et al. 2001) and *Poc. meandrina* (Magalon et al. 2005) are panmictic across spatial scales of > 100 km.

Skeletal growth rates of hermatypic corals are highly variable, in part because growth depends on environmental factors such as irradiance level and seawater temperature, which vary greatly by depth, latitude, habitat type and other factors (Buddemeier & Kinzie 1976, Grigg 1982). For example, reported mean linear extension rates for *Poc. eydouxi* range from 8 mm yr$^{-1}$ (7.5 m depth, 21°N, reef slope) in Hawai‘i (De Villiers et al. 1994) to 50 mm yr$^{-1}$ (6 m depth, 16°N, lagoon) in Johnston Atoll (Jokiel & Tyler 1992). Variability in skeletal growth rate of hermatypic corals may be affected by genetic as well as environmental factors, as shown in reciprocal transplant experiments (Potts 1984, Raymundo 2001).
How do populations of organisms with broad dispersal respond to environmental heterogeneity within the range of a single population? Adaptation to localized environmental conditions within the population's range is precluded by high gene flow, yet phenotypic variability is necessary because of contrasting selection pressures within different environments (Schmidt & Rand 2001). Variability of a phenotypic characteristic may be maintained within the population by physiological phenotypic plasticity (Doughty and Resnick 2004), developmental phenotypic plasticity (Meyers & Bull 2002), or genetic polymorphism (Schmidt et al. 2000). Genetic polymorphism can occur within a population for a specific phenotypic characteristic when different environments favor different genotypes, a process known as balancing selection (Levene 1953).

Materials and methods

Study area and species selection

The study area is a pair of small volcanic islands, Ofu and Olosega, in American Samoa (14°S). The islands are separated by a channel approximately 100 m wide and 3 m deep (Figure 5.1). Narrow fringing reefs surround the islands, with shallow (< 3 m low tide depth) back reef pools occurring where the reef is widest. Water motion in the back reef is semi-diurnally intermittent, typically alternating between > 20 cm s^{-1} at high tide to < 5 cm s^{-1} at low tide. During midday low tides, limited mixing and high solar irradiance combine to warm the back reef waters, producing daily fluctuations in seawater temperature of up to 4 – 5 °C (Smith and Birkeland 2007). In addition, the smoother water surface associated with reduced water motion at low tide increases
transmission of irradiance into the water column (Kirk 1994). Frequent storms and high rainfall cause sporadically high turbidity and low salinity in the back reef (Smith and Birkeland 2003). In contrast, the shallow (< 3 m low tide depth) forereef is a more stable environment. Breaking waves create consistently very high water motion, moderating seawater temperature and irradiance transmission. Water motion on the forereef, as well as distance from shore, prevents rapid changes in turbidity and salinity relative to the back reef.

Figure 5.1. Back reef and forereef study sites.

Diversity of reef-building corals is highest in the larger back reef pools, dominated by massive Porites micro-atolls, Porites cylindrica, and Acropora and Pocillopora species (Craig et al. 2001). Diversity and abundance of corals is lower on the shallow forereefs, where robust branching species and encrusting species are prevalent (Fisk and Birkeland 2002). At least four Pocillopora species occur in the study area; Poc. damicornis, Poc. eydouxi, Poc. meandrina, and Poc. verrucosa (Craig et al. 2001; Fisk
and Birkeland 2002). Poc. eydouxi, a hermaphroditic broadcast spawner, was selected for this experiment because it is common on both the back reef and the forereef, it is common elsewhere in the Indo-Pacific, and it can be readily distinguished from other Pocillopora species (Veron 2000; Fenner 2005).

Reciprocal transplant experiment

Poc. eydouxi was reciprocally transplanted between a back reef site on southeastern Ofu and a forereef site on northwestern Olosega (Figure 5.1) for a 6-month period between August 2004 and February 2005. The reciprocal transplant experiment (RTE) design utilized four replicate groups that were transplanted within and between the two sites: From the back reef to the back reef (Native 1, N1), from the back reef to the forereef (Translocated 1, T1), from the forereef to the forereef (Native 2, N2), and from the forereef to the back reef (Translocated 2, T2; Figure 5.2). Comparison of the Native and Translocated groups quantifies variability by source population (N1 vs T2, N2 vs T1), and by transplant site (N1 vs T1, N2 vs T2). Variability by source population indicates genetic control, and variability by transplant site indicates environmental control (phenotypic plasticity), assuming the absence of confounding factors. Reaction norms are arrows from a Native group to its corresponding Translocated group (N1 to T1, N2 to T2), and the two reaction norms together illustrate the interplay of genetic and environmental control on each skeletal characteristic (Schluter 2000; DeWitt and Scheiner 2004).
A total of 14 source colonies (seven per site) of *Poc. eydouxi* were selected to each provide two 5 cm-long branches for transplanting; one for the native site and one for the translocation site. Thus each of the four RTE groups contained seven branches, giving a total of 28 transplants (Figure 5.2). To minimize confounding factors associated with variability in source colony characteristics, transplant size, transplant shape, handling stress, micro-environmental conditions, competition, predation, and disease, the following procedure was used for coral transplantation: (1) Source colonies were > 10 m from one another to reduce the likelihood of selecting clones; (2) The tops of all source colonies were at 1 – 2 m low tide depth, and transplant branches were removed from the center of each source colony; (3) Transplants were approximately the same length, weight, and shape, and were handled and transported in the same manner; (4) Transplants were removed from source colonies in the morning and transplanted in the late afternoon;
(5) Within each transplant site (back reef or forereef), individual transplant attachment sites were prepared by drilling shallow 35 mm diameter holes in dead coral substrate at 1.5 m low tide depth; (6) The two groups to be transplanted within each site (the N and T groups) were mixed, then each transplant was randomly assigned an individual attachment site; (7) Transplants were attached with Sea Goin’ Poxy Putty® marine epoxy no less than 25 cm apart, mapped and photographed, and; (8) All transplants were surveyed in September 2004 and February 2005. Those with bleaching, overgrowth, or other tissue death were removed from the experiment because of potential effects on skeletal growth results. All surviving transplants were checked for signs of competition, predation or disease.

Skeletal growth rates of the transplants were determined with the buoyant weight method to measure percentage increase in skeletal mass (Jokiel et al. 1978), and the alizarin dye method to measure linear extension (Barnes 1970). Transplants were removed from source colonies early in the morning, placed in plastic bags of dissolved alizarin (50 g in 4 l) anchored to the back reef substrate, left for six hours, transferred to a nearby weighing station, buoyant weighed (Ohaus triple beam balance, accurate to 0.1 g), and finally transplanted near the end of the day. All transplants were removed from source colonies, stained, weighed and transplanted on August 20th and 21st, 2004. On February 20th and 21st, 2005, surviving transplants were removed without fracturing the skeleton, cleaned by removing epoxy and encrusting organisms by hand and by removing tissue with bleach, weighed using the buoyant weight method, sliced with a band saw, sanded to reveal the alizarin mark, and a single measurement taken with calipers on the upper central portion of each sliced transplant to determine upward linear extension.
Buoyant weight results were used to calculate increase in skeletal mass, and normalized to initial size with the equation: \( \% \text{ mass increase} = \frac{[\text{final weight} - \text{initial weight}]}{\text{initial weight}} \times 100 \).

To infer spatial and temporal variability in symbiont genotypes of the transplants, zooxanthella genotypes of source colonies were determined at the beginning and end of the RTE. Transplants could not be sampled because removal of skeletal material would affect skeletal growth results. Zooxanthella samples were taken using a 13 mm punch from the top of each source colony in August 2004 and March 2005, respectively. Samples were preserved in 95% ethanol, and total DNA was extracted using established methods (Baker et al. 1997) for use in denaturing-gradient gel electrophoresis (DGGE)(LaJeunesse 2001). Using primers with a Guanine-Cytosine clamp (GC clamp) designed for DGGE, the internal transcribed spacer-2 (ITS-2) region of nuclear ribosomal DNA was amplified using polymerase chain reaction (PCR). The PCR product was then run on an acrylamide DGGE gel with a 35%-75% chemical gradient (formamide and urea) from low to high. The diagnostic bands were cut out and reamplified using PCR with primers not containing the GC clamp. The PCR products from the cut bands were then sequenced, and the edited sequences run through a Basic Local Alignment Search Tool (BLAST) search in GenBank for a *Symbiodinium* type match.

Statistical analyses for RTE data were performed with Minitab 14. All data were assessed for normality and homogeneity of variances (Levene’s test) prior to testing. A two-way Analysis of Variance (ANOVA) was used to test effects of transplant site and source population on mass increase and linear extension of the surviving transplants. Because the two tests are dependent, p-values of < 0.05 were multiplied by a factor of
two to obtain final p-values (Bonferroni correction). Interaction of factors was tested when relative magnitudes of variable means were not uniform (Sokal and Rohlf 1981).

_Microsatellite Markers_

Microsatellites are tandem repeats of 1 – 6 nucleotides found at high frequency in the nuclear genomes of most taxa. Microsatellite repeat sequences mutate frequently by slippage and proof-reading errors during DNA replication, changing the number of repeats and thereby creating numerous alleles of different repeat length. The rapid mutation rates generate the high allelic diversity necessary for genetic studies of processes acting on ecological time scales (Selkoe & Toonen 2006). Thus, microsatellite loci were used as the genetic markers for this study to determine population structure and gene flow for _P. eydouxi_ back reef and forereef sample groups.

Samples were collected in April 2006 from the back reef and forereef RTE sites. At each site, a 1 – 2 cm branch fragment was collected from 55 _Poc. eydouxi_ colonies and frozen to -80 °C. All colonies used for each sample group were within a 100 m radius of the RTE sites, and the sampled colonies were > 5 m from one another to reduce the likelihood of sampling clones. At the back reef site, all sampled colonies were 1 – 2 m low tide depth, and at the forereef site, all sampled colonies were 1 – 4 m low tide depth.

A set of 10 polymorphic microsatellite markers have been developed for _Pocillopora_ species (Magalon et al. 2004, Starger et al. Submitted). During development of these host coral markers, the absence of contamination from zooxanthellae DNA was confirmed with PCR amplifications using universal primers that always only resulted in
host coral diagnostic products (Magalon et al. 2004). Also, isolated zooxanthellae DNA never produced positive amplification at any of the 10 loci (Starger et al. Submitted).

Genomic DNA was extracted using the Qiagen DNeasy Tissue Kit®, and the 10 microsatellite loci were tested with unlabeled primers to verify that each marker amplified a single PCR product. PCR reactions (25 µL total volume) contained 1.0 µl DNA template, 1.0 µl Taq polymerase (Bioline), 2.5 µl of 10X PCR buffer, 1.0 µl MgCl2, 1.0 µl dNTPs (8 mM), 0.5 µl BSA, and 0.5 µM of each primer. The cycling protocol was: 1 X 94°C (3 min), 35 X [1 min at 94°C, 1 min 55°C and 1 min at 74°C] and 1 X 74°C (7 min). Groups of 48 samples from each site were genotyped with high resolution gel electrophoresis using fluorescent labeled primers for each of the 10 markers. Four loci, Pd3_004, Pd3_005, Pd2_006, and PV7 (Starger et al. Submitted) provided profiles that could be consistently scored using GeneMapper 3.7 software.

The observed and expected heterozygosity (\(H_O\) and \(H_E\)) and the inbreeding coefficient (\(F_{IS}\)) were calculated for each locus within each sample group (back reef and forereef). Tests of departure from Hardy-Weinberg equilibrium (HWE) were performed using exact probability tests based on a Markov chain approach (Guo & Thompson 1992). Pairwise linkage disequilibrium between loci within each sample group was used to assess independence between loci. Gene flow was estimated by calculating the absolute number of individuals exchanged between sample groups per generation (\(N_m\)) based on the private allele method. These analyses were carried out using Genepop version 3.4 (http://wbiomed.curtin.edu.au/genepop/). A pairwise \(F_{ST}\) statistic between sample groups (Weir & Cockerham 1984) was calculated with Analysis of Molecular Variance (AMOVA) using Arlequin version 2.0 (http://lgb.unige.ch/arlequin/).
Results

Transplant survival was 100 percent for the RTE groups from the back reef (7/7 for N₁ and T₁), and 86 – 100 percent for those from the forereef (6/7 for N₂ and 7/7 for T₂). No signs of competition, predation or disease were observed on any of the surviving transplants in 2004 or 2005, suggesting that skeletal growth was not affected by these environmental factors. Skeletal growth rate results were consistent for skeletal mass and linear extension: Source population affected mass increase and linear extension (p < 0.001 for both), but transplant site had no effect (p > 0.49 for both; Table 5.1), indicating genetic control of skeletal growth rate. Genetic control is illustrated in Figure 5.3, whereby the reaction norms linking the transplants from the back reef (Groups N₁ and T₁) are parallel to, and consistently higher than, the reaction norms linking the transplants from the forereef (Groups N₂ and T₂).

<table>
<thead>
<tr>
<th>a. Mass Increase (%)</th>
<th>b. Upward Extension (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N₁ T₁ N₂ T₂</td>
</tr>
<tr>
<td>X</td>
<td>42.0 45.0 23.1 19.4</td>
</tr>
<tr>
<td>SE</td>
<td>5.2 3.1 6.8 4.2</td>
</tr>
</tbody>
</table>

Figure 5.3. Skeletal growth results (upper) and reaction norms (lower) for *P. eydouxi*, a. mass increase, and b. linear extension. Transplant groups: N₁ = native, back reef; T₁ = translocated, back to forereef; N₂ = native, forereef; T₂ = translocated, fore to back reef.
Table 5.1. Two-way ANOVA (source population, transplant site) for skeletal growth, measured by % mass increase (% mass) and upward linear extension (lin ext) in mm.

<table>
<thead>
<tr>
<th>Source Population</th>
<th>Df</th>
<th>MS</th>
<th>% mass</th>
<th>F</th>
<th>% mass</th>
<th>lin ext</th>
<th>p</th>
<th>lin ext</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>3326.5</td>
<td>94.560</td>
<td>20.74</td>
<td>19.73</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Transplant Site</td>
<td>1</td>
<td>77.5</td>
<td>0.526</td>
<td>0.48</td>
<td>0.11</td>
<td>0.494</td>
<td>0.743</td>
<td></td>
</tr>
<tr>
<td>Site x Population</td>
<td>1</td>
<td>0.6</td>
<td>0.886</td>
<td>0.00</td>
<td>0.18</td>
<td>0.952</td>
<td>0.671</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>23</td>
<td>160.4</td>
<td>4.793</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Bonferroni-corrected

The spatial (LaJeunesse et al. 2004a) and temporal (Baker et al. 2004) variability of zooxanthella genotypes, and dependence of coral skeletal growth rates on zooxanthellae genotype (Little et al. 2004), pose potential confounding factors for coral RTEs. However, the zooxanthella analyses showed no differences in zooxanthella genotypes of the source colonies by source population, transplant site, or season: The 14 P. eydouxi source colonies each contained genotypes C1 and C1c, both at the beginning and end of the experiment. A single back reef source colony contained a third genotype (C42) at the beginning and end of the experiment. Thus, assuming source colony zooxanthella genotypes were representative of the transplants, the genetic control of skeletal growth shown by the RTE cannot be explained by zooxanthellae.

None of the linkage disequilibrium tests between the four loci were significant at p < 0.05, thus the loci can be considered genetically independent. The number of alleles per locus per sample group varied from four for Pd3_004 to 12 for PV7 (Table 5.2), and the total of number of alleles per locus varied from five (Pd3_004) to 12 (Pd3_005). A heterozygote deficiency was found for loci Pd3_004 in the back reef sample group, leading to the only observed departure from Hardy-Weinberg equilibrium (Table 5.2).
There was no genetic differentiation of back reef vs. forereef sample groups at any of the four loci, or overall ($F_{ST} = 0.001$, $p = 0.31$), thus no population structure between the back reef and forereef sample groups. Migration between back reef and forereef sample groups ($N_m$) was estimated at 11.3 individuals per generation (Table 5.3).

Table 5.2. Variation in each sample group for the four loci (names from Starger et al., submitted). N, sample size (scored genotypes); $n_a$, number of alleles; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity, $F_{IS}$, inbreeding coefficient.

<table>
<thead>
<tr>
<th>Sample Group</th>
<th>Loci</th>
<th>N</th>
<th>$n_a$</th>
<th>$H_O$</th>
<th>$H_E$</th>
<th>$F_{IS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Forereef</strong></td>
<td>Pd3_004</td>
<td>47</td>
<td>4</td>
<td>0.319</td>
<td>0.298</td>
<td>-0.073</td>
</tr>
<tr>
<td></td>
<td>Pd3_005</td>
<td>48</td>
<td>9</td>
<td>0.688</td>
<td>0.655</td>
<td>-0.051</td>
</tr>
<tr>
<td></td>
<td>Pd2_006</td>
<td>45</td>
<td>9</td>
<td>0.600</td>
<td>0.623</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>PV7</td>
<td>46</td>
<td>10</td>
<td>0.674</td>
<td>0.683</td>
<td>-0.006</td>
</tr>
<tr>
<td><strong>Back Reef</strong></td>
<td>Pd3_004</td>
<td>46</td>
<td>4</td>
<td>0.239</td>
<td>0.304</td>
<td>0.214*</td>
</tr>
<tr>
<td></td>
<td>Pd3_005</td>
<td>46</td>
<td>10</td>
<td>0.587</td>
<td>0.588</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Pd2_006</td>
<td>44</td>
<td>7</td>
<td>0.659</td>
<td>0.701</td>
<td>0.061</td>
</tr>
<tr>
<td></td>
<td>PV7</td>
<td>47</td>
<td>12</td>
<td>0.809</td>
<td>0.799</td>
<td>-0.012</td>
</tr>
</tbody>
</table>

*Significant at $p < 0.05$ for Hardy-Weinberg departure.

Table 5.3. Genetic differentiation ($F_{ST}$) and probability of being part of the same population ($p$) for the four loci between the two sample groups, overall $F_{ST}$ and $p$, and gene flow ($N_m$) estimates (individuals per generation between the two groups).

<table>
<thead>
<tr>
<th>Loci</th>
<th>Pd3_004</th>
<th>Pd3_005</th>
<th>Pd2_006</th>
<th>PV7</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{ST}$</td>
<td>-0.007</td>
<td>-0.006</td>
<td>0.012</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>$p$</td>
<td>0.67</td>
<td>0.77</td>
<td>0.10</td>
<td>0.32</td>
<td>0.31</td>
</tr>
<tr>
<td>$N_m$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11.3</td>
</tr>
</tbody>
</table>
Though branching corals such as *Pocillopora* species may reproduce asexually by fragmentation and other means (Highsmith 1982, Harrison & Wallace 1990), and forereef colonies at this study site are beset by very high wave energy and frequent storms (JTWC 2006), there was little indication of asexual reproduction by fragmentation or otherwise in the microsatellite data. In fact, only one locus at one site was not in Hardy-Weinberg Equilibrium (HWE) with regard to heterozygote deficiency (i.e., extensive asexual reproduction would result in the opposite), and that locus was in the back reef group (Pd3_004 back reef sample, Table 5.2). In addition, 75 of the 89 samples that were scorable at all four loci had unique genotypes (i.e., 75 genets, and 7 pairs of possible clones – analysis of additional loci could show that some or all of the possible clones are also genets), another indication of extensive sexual reproduction, further supporting the conclusion that the sampled back reef and forereef groups are part of a single sexually-reproducing population.

**Discussion**

How does a broadcast-spawning marine invertebrate population cope with environmental heterogeneity? A population that encompass a wide range of habitat types must maintain broad acclimatization capacities, especially when adults are sessile, as with corals. When environmental variability is predictable within a population's range, phenotypic plasticity is a common adaptive strategy. Plasticity may be physiological or developmental; the former refers to the ability of organisms to change physically or physiologically in response to changing conditions, and the latter refers to a distinct,
irreversible developmental pathway in response to local conditions during early life history stages (Meyers & Bull 2002; Doughty and Resnick 2004). In addition, genetic polymorphisms can occur within a population for a specific phenotypic characteristic when different environments favor different genotypes, a process known as balancing selection (Levene 1953, Schmidt et al. 2000).

_Poc. eydouxi_ is a broadcast spawner that may spawn multiple times each summer (Richmond & Hunter 1990, Kinzie 1993), likely resulting in broad dispersal of pelagic larvae and high gene flow on large spatial scales, as with broadcast-spawning congeners _Poc. verrucosa_ (Ridgway et al. 2001) and _Poc. meandrina_ (Magalon et al. 2005). Thus no population structure, high gene flow, and considerable sexual reproduction were expected between the _Poc. eydouxi_ back reef and forereef colonies used for the RTE. Indeed, the microsatellite analysis showed no genetic differentiation, high gene flow, and prevalent sexual reproduction between colonies at the two sites (Tables 5.2, 5.3). How then could intrinsic growth rates of back reef vs. forereef colonies differ, as shown by the RTE results (Fig. 5.3)?

The RTE results rule out physiological phenotypic plasticity, because such plasticity would have produced a significant transplant site effect, but transplant site had no effect on skeletal growth (Table 5.1). And the microsatellite results rule out genetic differentiation of the back reef vs. forereef colonies (Table 5.3), leaving only developmental phenotypic plasticity or balancing selection (i.e., genetic polymorphisms for skeletal growth maintained within the population by contrasting environmental selection pressures at the two RTE sites) as the most likely explanations for the combined RTE-microsatellite results.
Developmental plasticity occurs in many different forms, as illustrated by a few examples: If toad tadpoles feed on shrimp or other tadpoles, they become large-headed carnivores as adults, otherwise they become small-headed omnivores (Pfennig & Murphy 2000). Sea urchin larval morphology and length of larval stage depends on food availability, with longer arms and more extended larval stages with lower food availability (Sewell et al. 2004). A physiological example is provided by zebrafish thermal tolerances: The thermal environment of larval zebrafish induces irreversible changes in the thermal tolerances of adults (Schaefer & Ryan 2006). Developmental plasticity in *Poc. damicornis* allows newly-settled polyps to revert to the motile larval form in response to stress, thereby enhancing habitat selection ability (Richmond 1985).

Balancing selection maintains polymorphisms for a trait in a population because of contrasting environmental pressures (Levene 1953). Balancing selection for skeletal growth rates of the hermatypic corals *Acropora palifera* and *A. cuneata* was suggested by RTEs between contrasting environments, but not confirmed by genetic tests (Potts 1984). RTEs and genetic studies were combined to demonstrate that balancing selection maintains stress response polymorphisms in the barnacle *Semibalanus balanoides*, thought to be an adaptation to extreme environmental heterogeneity of this species’ intertidal habitat (Schmidt et al. 2000, Schmidt & Rand 2001).

Environmental heterogeneity between the back reef and the forereef could be maintaining fast-growth and slow-growth genotypes within the population via balancing selection: Shallow back reef margins such as the back reef study site are partially protected from storm events by the reef crest, while high water motion and irradiance levels provide a suitable environment for many hermatypic coral species, resulting in
either high coral cover, high coral species diversity, or both (Dollar 1982, Connell et al. 1997, Craig et al. 2001). Competition for space is high, and the likelihood of breakage and dislodgement is low, thus such back reef environments favor tall colonies and rapid skeletal growth rates (Jokiel & Tyler 1992, Harriott 1999). In contrast, exposed shallow forereefs such as the forereef study site are subject to daily high wave energy as well as frequent storm events, resulting in low coral cover (Dollar 1982) and diversity (Grigg 1983). In American Samoa, five tropical cyclones with wind speeds > 180 km hr⁻¹ occurred from 1987 – 2005, all approaching from the northwest, as is typical in the region (JTWC 2006). The forereef site is directly impacted by these storms, while 500 m high Mt. Tumutumu on Ofu Island partially shields the back reef site (Figure 5.1). Thus, relative to coral colonies on the back reef, colonies on the forereef face less competition for space, and greater likelihood of breakage or dislodgement, thereby reducing the benefits of rapid skeletal growth.

Since the environmental differences described above between the back reef and forereef are predictable (i.e., a larva settling in either habitat would immediately experience flow conditions that accurately predict daily conditions at that site), developmental plasticity could just as easily occur as balancing selection (Meyers & Bull 2002). In fact, several factors suggest developmental plasticity is the more likely explanation for the RTE and microsatellite results.

Balancing selection in marine invertebrates may be more likely when environmental heterogeneity is more extreme than at the back reef vs. forereef sites, such as in intertidal pools that have extreme variability in temperature, salinity and dissolved oxygen from pool to pool (Schmidt et al. 2000). Furthermore, since skeletal growth rate
is likely to be controlled by many genes (Singh & Zouros 1978, Guss & Ettensohn 1997), balancing selection for this trait would cause any microsatellite loci that coincidentally occurred near the growth genes to be out of HWE and to have low $F_{ST}$ values. However, only one locus at one site was out of HWE (Pd3_004 in the back reef), and $F_{ST}$ for this locus between the back reef and forereef was functionally zero (-0.007, Table 5.3). The lack of HWE for this locus was likely due to asexual reproduction, as suggested by heterozygote deficiency (Table 5.2), rather than selection of a nearby gene by balancing selection. Of course, it is possible that balancing selection is occurring but that none of the microsatellite loci I used happen to be near any of the growth genes. In conclusion, more information is necessary, such as genetic data from newly-settled larvae as well as adult colonies from both sites, before either developmental plasticity or balancing selection can be rejected as a likely explanation for the combined RTE-microsatellite results.
CHAPTER 6:

CORAL SYMBIONT DIVERSITY ALONG A SEAWATER TEMPERATURE GRADIENT
Abstract

The genetic diversity of endosymbiotic algae (zooxanthellae) occurring within the tissues of some Pacific reef-building coral species may vary according to environmental conditions: Most colonies of most species contain Clade C, while those occupying habitats subjected to elevated seawater temperatures or large diurnal temperature fluctuations often contain Clade D. On Ofu and Olosega Islands, seawater temperatures were logged every 30 minutes for a 1-year period at three sites along a seawater temperature gradient (all 1-1.5 m depth): A forereef site with small daily temperature fluctuations and relatively low daily temperature maxima, a large back reef pool with intermediate daily temperature fluctuations and maxima, and a small back reef pool with large daily temperature fluctuations and high maxima. The mean daily temperatures at the three sites are very similar to one another. Zooxanthella samples were collected from each site from colonies of *Acropora gemmifera, Galaxea fasicularis, Pocillopora eydouxi* and *Porites lobata*. All species except *Por. lobata* had the highest proportion of Clade D in colonies from the site with the greatest daily temperature fluctuations and highest maxima (Pool 300). In addition, all species except *Por. lobata* had more genotypes in samples from the back reef pools than in samples from the forereef. These results suggest that zooxanthella genotype diversity may increase the capacity of some reef-building coral species to tolerate the thermal conditions of shallow back reef pools. However, the less labile *Porites* species dominate the Ofu back reef coral community, suggesting this genus relies more on host coral mechanisms to cope with back reef conditions.
Introduction

Skeletal growth in reef-building scleractinian corals occurs by the formation and precipitation of aragonite (Barnes 1970), a process greatly enhanced by symbiotic dinoflagellates (*Symbiodinium* spp.) known as zooxanthellae that provide up to 95 percent of the corals’ carbon requirements for growth, reproduction, and maintenance (Muscatine 1990). Physiological differences in cultured isolates suggested genetic diversity within *Symbiodinium* (Kinzie & Chee 1979, Schoenberg & Trench 1980), providing the impetus to apply molecular systematics. The discovery of high sequence variability in nuclear small subunit ribosomal DNA (18S-rDNA) led to the classification of *Symbiodinium* into a growing number of clades (Rowan & Powers 1991a, Rowan & Powers 1991b, Baker et al. 1997, LaJeunesse 2001). The inferred phylogeny was confirmed using other molecular markers (Santos et al. 2002, Pochon et al. 2006), and eight clades are recognized, designated A – H, five of which (A – D, F) are known to form associations with scleractinian corals (Coffroth & Santos 2005, Stat et al. 2006).

Clade-level zooxanthella genotypes found in a reef-building coral species may vary spatially between colonies according to temperature maxima (Berkelmans & van Oppen 2006), irradiance level (Robison & Warner 2006), or sedimentation (Garren et al. 2006). Spatial variability also occurs within a single colony, and multiple clades may occur simultaneously within a colony (Stat et al. 2006). In the Pacific, Clade C is by far the most common genotype (LaJeunesse et al. 2004a), but Clade D is found in colonies of some coral species occupying habitats typified by elevated seawater temperatures, large temperature fluctuations, and high irradiance levels (Fabricius et al. 2004, Rowan 2004).
Clade D appears to increase the tolerances of host colonies to elevated seawater temperatures (Berkelmans and van Oppen 2006) and high irradiance levels (Robison and Warner 2006), while reducing skeletal growth rates of the host coral (Little et al. 2004).

Clade-level variability in zooxanthella genotypes can also occur temporally: Reef-building coral colonies occupying back reef habitats where seawater temperature and irradiance level rapidly fluctuate may have highly dynamic zooxanthella communities, with multiple clades and sub-clades in a state of flux (Magalon et al. 2006, Baker & Romanski 2007). Zooxanthellae density also may vary seasonally, with higher densities during cooler seawater temperatures in the winter (Stimson 1997, Fagoonee et al. 1999). Temporal variability is also demonstrated by the increased frequency of Clade D in colonies recovering from bleaching than in colonies that have not recently bleached (Baker 2001, Glynn et al. 2001, Baker et al. 2004, van Oppen et al. 2005).

Though clade-level spatial and temporal variability in zooxanthella genotypes occurs in some species of reef-building corals, it is important to note that many coral-algal symbioses remain stable both spatially and temporally as long as the external environment remains similar (Iglesias-Prieto et al. 2004, LaJeunesse et al. 2004a, LaJeunesse et al. 2004b, Thornhill et al. 2005). Furthermore, zooxanthellae variability is uncommon both spatially and temporally in certain reef-building coral genera, especially *Porites*, regardless of environmental disturbances such as warming events resulting in mass bleaching (Baker 2004, Baker et al. 2004, Fabricius et al. 2004). Yet *Porites* is one of the most common reef-building coral genera in shallow reef habitats where seawater temperatures and irradiance levels commonly reach high levels (Veron 2000) such as the back reef pools on Ofu Island, American Samoa (Craig et al. 2001).
Methods

This study was carried out at the three sites collectively used for the coral reciprocal transplant experiments described in Chapters 3 – 5 above; a shallow forereef site, a large back reef pool, and a small back reef pool (Figure 6.1). Shaded seawater temperatures were recorded simultaneously every 30 minutes at the three sites for a 1-year period from 1-Apr 2004 to 31-Mar 2005. Attempts were made to record temperatures at 1 – 1.5 m low tide depth at all three sites with Onset Water Temp Pro® temperature loggers, but extreme water motion at the forereef site repeatedly tore loose the logger at that site. However, the forereef site was originally selected in part because the National Marine Fisheries Service's Coral Reef Ecosystem Division (CRED) had been logging seawater temperatures at the site since 2004 with a Seabird 39® logger placed at 5 m low tide depth. Thus, forereef temperature data was provided by CRED's logger. At the two back reef sites, seawater temperatures were recorded with the Water Temp Pro loggers placed at 1 – 1.5 m depth. The loggers were tested indoors against one another and a calibrated thermometer, deployed for six months, then retrieved and tested again. Loggers always read < 0.1°C of one another and the calibrated thermometer. In addition, seawater temperatures at the two back reef sites have been monitored in this fashion by the National Park of American Samoa since 2000, thus back reef temperature data for a 6-year period (1 Apr-00 – 31 Mar-06) are also provided for reference.
Acropora, Pocillopora, and Porites species make up 70 percent of live coral cover in the back reef (Craig et al. 2001). Zooxanthella samples were taken from colonies of A. gemmifera, Galaxea fasicularis, Poc. eydouxi, and Por. lobata using a 13 mm punch from the top of each source colony in September 2004, near the end of the southern winter. Coral colonies were sampled in Pool 300, Pool 400 and on the shallow forereef (Figure 6.1; all colonies 1 – 3 m low tide depth) to test for spatial variability in zooxanthella genotypes within each coral species. At least 6 colonies of each species were sampled at each site, except Por. lobata, because few colonies of this species could be positively distinguished from other massive Por. species. Poc. eydouxi and Por. lobata were also sampled in March 2006 (same colonies as Sep-04 samples) during the southern summer to test for temporal variability in zooxanthella genotypes.
Samples were preserved in 95% ethanol, and total DNA was extracted using established methods (Baker et al. 1997) for use in denaturing-gradient gel electrophoresis (DGGE) (LaJeunesse 2001). Using primers with a Guanine-Cytosine clamp (GC clamp) designed for DGGE, the internal transcribed spacer-2 (ITS-2) region of nuclear ribosomal DNA was amplified using polymerase chain reaction (PCR). The PCR product was then run on an acrylamide DGGE gel with a 35%-75% chemical gradient (formamide and urea) from low to high. The diagnostic bands were cut out and reamplified using PCR with primers not containing the GC clamp. The PCR products from the cut bands were then sequenced, and the edited sequences run through a Basic Local Alignment Search Tool (BLAST) search in GenBank for a *Symbiodinium* type match.

**Results**

Pool 300 had much higher daily maxima and daily fluctuations than the forereef year-round except during stormy, well-mixed conditions (e.g., most of June and July, Figure 6.2), while Pool 400 was intermediate. Though mean annual temperatures were nearly identical at the three sites (Table 6.1), the temperature gradient suggested by the raw data (Figure 6.2) is born out by an analysis of the summer (Nov-Mar) temperature data: The local summer mean in the back reef pools was 29.4 °C for the six summers between Nov-00 and Mar-06 (Table 6.1). The frequency and duration of temperatures exceeding the local summer mean were greatest in Pool 300, intermediate in Pool 400, and lowest on the forereef (Figure 6.3, left and middle panels), a pattern also followed by maximum daily temperature fluctuations (Figure 6.3, right panel).
Figure 6.2. Seawater temperature raw data (every 30 minutes) at the Pool 300 and forereef sites during a 1-year period (1 Apr-04 to 31 Mar-05). Temperatures in Pool 400 had intermediate daily maxima and fluctuations (Figure 6.3, right panel).

Table 6.1. Temperature summaries for all three sites (1 Apr-04 – 31 Mar-05) and the back reef pools (1 Apr-00 – 31 Mar-06; MDR = maximum daily range).

<table>
<thead>
<tr>
<th>Site</th>
<th>Dates</th>
<th>n</th>
<th>Mean</th>
<th>Max</th>
<th>Min</th>
<th>Range</th>
<th>MDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool 300</td>
<td>1 Apr-04 – 31 Mar-05</td>
<td>17,520</td>
<td>28.97</td>
<td>33.76</td>
<td>26.26</td>
<td>7.50</td>
<td>5.61</td>
</tr>
<tr>
<td>Pool 400</td>
<td>&quot;</td>
<td>17,520</td>
<td>29.00</td>
<td>33.03</td>
<td>27.38</td>
<td>5.65</td>
<td>4.34</td>
</tr>
<tr>
<td>Forereef</td>
<td>&quot;</td>
<td>17,520</td>
<td>28.92</td>
<td>30.93</td>
<td>27.58</td>
<td>3.35</td>
<td>1.49</td>
</tr>
<tr>
<td>Pool 300</td>
<td>1 Apr-00 – 31 Mar-06</td>
<td>105,120</td>
<td>28.92</td>
<td>35.47</td>
<td>25.12</td>
<td>10.35</td>
<td>6.51</td>
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<td>105,120</td>
<td>28.84</td>
<td>33.03</td>
<td>26.23</td>
<td>6.80</td>
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<th>n</th>
<th>Mean</th>
<th>Max</th>
<th>Min</th>
<th>Range</th>
<th>MDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool 300</td>
<td>1 Nov-04 – 31 Mar-05</td>
<td>7,248</td>
<td>29.55</td>
<td>33.76</td>
<td>27.38</td>
<td>6.38</td>
<td>5.61</td>
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<tr>
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<td>7,248</td>
<td>29.52</td>
<td>33.03</td>
<td>28.05</td>
<td>4.98</td>
<td>4.34</td>
</tr>
<tr>
<td>Forereef</td>
<td>&quot;</td>
<td>7,248</td>
<td>29.37</td>
<td>30.93</td>
<td>28.04</td>
<td>2.89</td>
<td>1.49</td>
</tr>
<tr>
<td>Pool 300</td>
<td>1 Nov-00 – 31 Mar-06</td>
<td>43,488</td>
<td>29.50</td>
<td>35.47</td>
<td>25.94</td>
<td>9.53</td>
<td>6.51</td>
</tr>
<tr>
<td>Pool 400</td>
<td>&quot;</td>
<td>43,488</td>
<td>29.35</td>
<td>33.03</td>
<td>27.04</td>
<td>5.99</td>
<td>4.42</td>
</tr>
</tbody>
</table>
Figure 6.3. Frequency (left panel) and duration (middle panel) of summer (1 Nov-04 to 31 Mar-05) seawater temperatures exceeding 1, 2, 3, 4, and 5 °C above the local summer mean (29.4 °C) at the three sites. Also shown are maximum daily temperature fluctuations (right panel).

Zooxanthella genotypes showed clade-level spatial variability for *A. gemmifera*, *G. fasicularis*, and *Poc. eydouxi* during the winter. The maximum proportion of Clade D was found in Pool 300 colonies for all three species, though the same proportion (100%) was found in *A. gemmifera* colonies from the two back reef pools, and there was no data for *G. fasicularis* from Pool 400. Only *Poc. eydouxi* colonies in Pool 300 contained a mix of Clades C and D. Summer sampling was only done for *Poc. eydouxi* and *Por. lobata*: Zooxanthella genotypes of *Poc. eydouxi* varied temporally, having a higher number of genotypes and a larger proportion of Clade D in the summer than in the winter. In contrast, *Por. lobata* showed no spatial or temporal variability between the three sites, even at the sub-clade level (Figure 6.4, Appendix 6.1). During the winter, *A. gemmifera*, *G. fasicularis*, and *Poc. eydouxi* colonies in the back reef pools contained 1 – 3 zooxanthella genotypes, and these same *Poc. eydouxi* colonies contained 1 – 5 zooxanthella genotypes in the summer (Appendix 6.1).
Figure 6.4. Proportions of colonies with Clade C only (black), Clade D only (white), and both zooxanthella clades (gray) in *Acropora gemmifera*, *Galaxea fasicularis*, *Pocillopora eydouxi*, and *Porites lobata* at the three sites during the winter (Sep-04).

Discussion

Although the three sites had nearly identical mean seawater temperatures over the 1-year period, the differences in daily temperature maxima and fluctuations produced a
seawater temperature gradient from least-fluctuating (forereef) to most-fluctuating (Pool 300; Figure 3). Many shallow reef habitats are subjected to elevated daily maxima and large daily fluctuations in seawater temperature, such as reef flats (Glynn 1973, Brown 1997), areas that receive upwelling or advective flow of subsurface water (Coles 1997), back reef pools (Orr 1933, Craig et al. 2001), and marine lakes (Fabricius et al. 2004). Such habitats also typically experience major seasonal fluctuations in seawater temperature and other environmental variables, yet all support diverse communities of reef-building corals (Brown 1997; Coles 1997; Craig et al. 2001; Fabricius et al. 2004).

Overall, the site with highest maxima and great fluctuations in daily seawater temperatures had the highest proportion of coral colonies containing Clade D zooxanthella genotypes (Figure 4). Similarly, a study of zooxanthella genotypes in *Acropora hyacinthus* colonies from the three sites carried out during summer 2006 also showed that colonies in Pool 300 had a higher proportion of colonies containing Clade D (100%) than colonies in Pool 400 (80%) or on the forereef (70%; Tom Oliver, in preparation). Also, zooxanthella genotypes of *Poc. eydouxi* colonies in Pool 300 showed an increase in Clade D during the summer (Appendix 6.1). These results are consistent with other studies of spatial and temporal variability in zooxanthella genotypes, which have also found the highest proportion of Clade D in colonies inhabiting the warmest and/or most fluctuating environment (Baker et al. 2004; Fabricius et al. 2004). These environments typically also have very high irradiance levels, and tolerance of high irradiance may also vary by zooxanthellae genotype (Robison and Warner 2006). Thus, utilization of Clade D zooxanthellae appears to be a mechanism contributing to the tolerance of some reef-building corals to environmental conditions in the Ofu back reef.
pools, such as elevated seawater temperatures, large temperature fluctuations, and high irradiance levels.

The symbiont acquisition portion of a reef-building coral species' life history may affect spatial and temporal variability in its zooxanthella genotypes (LaJeunesse et al. 2004a): Most Pacific taxa broadcast spawn eggs and sperm that do not contain zooxanthellae, so symbionts are acquired by juvenile corals from the environments (horizontal transmitters), whereas some taxa transmit zooxanthellae from parent to offspring (vertical transmitters). For example, *Acropora* and *Galaxea* are horizontal transmitters, while *Pocillopora* and *Porites* are vertical transmitters (Richmond & Hunter 1990, Glynn et al. 1991, Kinzie 1996). In this study, *A. gemmifera* and *G. fasicularis*, both horizontal transmitters, had only Clade D in their Pool 300 colonies, while *Poc. eydouxi* and *Por. lobata*, both vertical transmitters, had either Clade C or D (*Poc. eydouxi* or only Clade C (*Por. lobata*). In addition, *Poc. eydouxi* colonies often had several zooxanthella genotypes, especially in the summer, resulting in a mix of Clades C and D (Appendix 6.1). These results suggest that horizontal transmitters more easily exchange zooxanthella genotypes (like *A. gemmifera* and *G. fasicularis*), whereas vertical transmitters either add additional genotypes as needed (like the Pool 300 *Poc. eydouxi* colonies) or do not change genotypes at all (like *Por. lobata*).

Though *Acropora* and *Pocillopora* species are important components of the Ofu back reef coral community (21% and 17% of live coral cover in the pools in 2000, respectively, while the ubiquitous *G. fasicularis* and other *Galaxea* species made up < 1 % of live coral cover in 2000), *Porites* species dominate the pools (32% of live coral cover in the pools in 2000; Craig et al. 2001). However, *Por. lobata* showed no spatial or
temporal variability in zooxanthella genotypes, even at the sub-clade level (all samples contained only genotype C15; Appendix 6.1). In addition, other Porites samples (10 samples of Por. cylindrica from each of the two pools, one or two samples of Por. lichen, Por. lutea, and Por. solida from the pools, all taken in Sep-04) also all contained only genotype C15.

Other studies have also shown that even in reef habitats with elevated seawater temperatures (Fabricius et al. 2004) or in corals recovering from bleaching (Baker et al. 2004), Porites colonies do not contain Clade D zooxanthellae (although a minority of Porites colonies contained Clade D in the study by Fabricius et al. 2004). If Clade D zooxanthella genotypes increase tolerances of reef-building corals to elevated seawater temperatures and high irradiance levels, why is a warm, shallow reef habitat like the Ofu back reef dominated by Porites, which does not appear to utilize Clade D? The high tolerances of Ofu back reef Porites species to elevated seawater temperatures and high irradiance levels (Smith and Birkeland 2007), and the lack of variability in zooxanthella genotypes in Porites, suggest that host coral mechanisms rather than zooxanthellae may be the source of high tolerances. This concept is supported by a parallel study of Por. lobata in the back reef and forereef sites, which demonstrated contrasting physiological capacities of the host corals (production of heat shock proteins and anti-oxidants) between the two sites (Barshis et al. Submitted). An alternative explanation for the tolerances of Porites species to elevated seawater temperatures is that sub-clade C15 is more similar to Clade D zooxanthellae than other C sub-clades in terms of affording the host coral high tolerances (Fabricius et al. 2004), though no data are currently available to support or rule out this hypothesis.
Appendix 6.1. Zooxanthella genotypes (LaJeunnesse 2001, LaJeunnesse et al. 2004a) detected in individual samples of *Acropora gemmifera, Galaxea fasicularis, Pocillopora eydouxi* and *Porites lobata* from Pool 300, Pool 400 and the forereef during the winter (Sep-04) and summer (Mar-06), showing proportion of Clade D genotypes (bold) for each coral species.

<table>
<thead>
<tr>
<th></th>
<th>Winter</th>
<th>Summer</th>
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<tbody>
<tr>
<td></td>
<td>Forereef Pool 400</td>
<td>Pool 300</td>
<td>Forereef Pool 400</td>
</tr>
<tr>
<td><em>Acropora gemmifera</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>D2</td>
<td>D2</td>
</tr>
<tr>
<td></td>
<td>D2 D1, D2</td>
<td>D2</td>
<td>D2</td>
</tr>
<tr>
<td></td>
<td>D2 D1, D3</td>
<td>D2</td>
<td>D1, D2</td>
</tr>
<tr>
<td></td>
<td>D2 D1, D2</td>
<td>D2</td>
<td>D2</td>
</tr>
<tr>
<td></td>
<td>D2 D1, D2</td>
<td>D2</td>
<td>D2</td>
</tr>
<tr>
<td></td>
<td>C15 D2</td>
<td>D2</td>
<td>D1, D2</td>
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<td></td>
<td>D2 D2</td>
<td>D2</td>
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<td>D2 D2</td>
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<td>D2 D2</td>
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<tr>
<td></td>
<td>D2 D2</td>
<td>D2</td>
<td>D2</td>
</tr>
<tr>
<td>Proportion w/Clade D</td>
<td>78%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><em>Galaxea fasicularis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>D1, D1a</td>
<td>D1, D1a</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>D1, D1a, D2</td>
<td>no data</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>D1, D1a, D2</td>
<td>no data</td>
</tr>
<tr>
<td>Proportion w/Clade D</td>
<td>33%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><em>Pocillopora eydouxi</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C1c</td>
<td>C1c</td>
<td>C1c, C1c, C42, D, D1a</td>
</tr>
<tr>
<td></td>
<td>C1c C1c</td>
<td>C1c, C1c</td>
<td>C1c, C1c</td>
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<tr>
<td></td>
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<td>C1c, C1c, D1a</td>
<td>C1c, C1c, C42, D, D1a</td>
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</tr>
<tr>
<td></td>
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<td>C1c, C1c, C1c</td>
<td>C1c, C1c</td>
</tr>
<tr>
<td></td>
<td>C1c C1c</td>
<td>C1c, C1c, C42</td>
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</tr>
<tr>
<td>Proportion w/Clade D</td>
<td>0%</td>
<td>0%</td>
<td>43%</td>
</tr>
<tr>
<td><em>Porites lobata</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C15</td>
<td>C15</td>
<td>C15</td>
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<td></td>
<td>C15 C15</td>
<td>C15</td>
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<td></td>
<td>C15 C15</td>
<td>C15</td>
<td>C15</td>
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<td>Proportion w/Clade D</td>
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CHAPTER SEVEN:
SUMMARY AND CONCLUSIONS

The purpose of my doctoral research was to test hypotheses on the effects of environmental characteristics and biological mechanisms associated with fluctuating reef environments and their corals on coral resilience to conditions typical of global warming. That is, do environmental characteristics of the pools counteract or neutralize the potential negative effects of elevated seawater temperatures and high irradiance levels? Likewise, are there biological mechanisms associated with reef-building coral colonies in the pools that counteract or neutralize the potential negative effects of elevated seawater temperatures and high irradiance levels? To holistically address these questions, environmental data monitoring, water table experiments (Chapter 2), field experiments (Chapters 3 – 5), and host coral and zooxanthella genetics studies (Chapters 5 and 6) were carried out on Porites and Pocillopora species of reef-building corals from the Ofu back reef pools.

The 'Hypothesis-testing' section below begins with Experimental Results by describing how each set of hypotheses was tested with at least one experiment, followed by Interpretation of RTEs to clarify and synthesize the RTE results more thoroughly than in the individual chapters, and a Summary of Hypothesis Testing. In addition, because the intent of this research effort is to provide useful resource management information for the global climate change era, the dissertation concludes with 'Implications for Coral Resilience' and 'Management Applications and Future Research' sections.
Experimental Results:

Attempts were made to test each of the three sets of hypotheses described in the General Introduction (and repeated below) using at least one *Porites* and one *Pocillopora* species, two distantly-related and morphologically-contrasting Scleractinian genera that both are common in shallow Pacific coral communities. While both genera were successfully tested for the second and third sets of hypotheses, only *Porites* species (*Por. lobata* and *Por. cylindrica*) were tested in the water table experiments used for the first set of hypotheses. The water table experiments were attempted on *Pocillopora* species (*Poc. verrucosa* and *Poc. damicornis*), but survival was inadequate. No attempts was made with *Poc. eydouxi* because this species is more difficult to maintain in water tables than other *Pocillopora* species. Similarly, only *Poc. eydouxi* was tested in the microsatellite study because of difficulty in obtaining microsatellite markers for *Porites* species. Testing of each set of null ($H_0$) and alternative ($H_a$) hypotheses are summarized below. The first set of hypotheses was:

\[ H_{01} : \text{Semi-diurnally intermittent water motion, such as found in the back reef pools, does not affect photoinhibition and bleaching of corals exposed to elevated seawater temperatures, regardless of irradiance level;} \]

\[ H_{a1} : \text{Semi-diurnally intermittent water motion reduces photoinhibition and bleaching of corals exposed to elevated seawater temperature when irradiance levels are high, but has no effect when irradiance levels are low.} \]
Controlled experiments in a water table were conducted to test $H_01$ and $H_{a1}$; i.e., effects of semi-diurnally intermittent water flow on photoinhibition and bleaching of the corals *Por. lobata* and *Por. cylindrica* kept at elevated seawater temperatures and different irradiance levels (Ch. 2). In the high irradiance experiments, photoinhibition and bleaching were lower for both species in the intermittent high – low flow treatment than in the constant low flow treatment. In the low irradiance experiments, there were no differences in photoinhibition or bleaching for either species between the flow treatments, despite continuously elevated seawater temperatures (Ch. 2 figures and tables). Thus, $H_{a1}$ was supported for the two *Porites* species by the water table experiments.

The second set of hypotheses was:

$H_{02}$: Phenotypic response of broadcast-spawning coral colonies reciprocally-transplanted between contrasting habitats on small spatial scales (< 5 km) does not vary by source population or by transplant site;

$H_{a2-1}$: Phenotypic response varies by transplant site, showing physiological phenotypic plasticity;

$H_{a2-2}$: Phenotypic response varies by source population, showing either developmental phenotypic plasticity or genetic polymorphism.
To test $H_{02}$, $H_{a2-1}$, and $H_{a2-2}$, two pairs of RTEs (each using *Por. lobata* and *Poc. eydouxi*) were carried out between: (1) two contrasting back reef pools (the highly-fluctuating Pool 300 and the less-fluctuating Pool 400; Ch. 3); and (2) a back reef pool (Pool 400) and a relatively stable forereef site (Ch. 4 & 5), using skeletal growth characteristics as the phenotypic response (i.e., growth rate, density, and calcification rate for *Por. lobata*, and growth rate for *Poc. eydouxi*). In the Pool 300 – Pool 400 *Por. lobata* RTE (Ch. 3), there was no variance by transplant site or source population (Figure 7.1, white circles), supporting the null hypothesis ($H_{02}$). However, in the Pool 300 – Pool 400 RTE of *Poc. eydouxi* (Ch. 3), transplant site strongly affected the results (indicating physiological phenotypic plasticity), with approximately double the skeletal growth rate at the more fluctuating site (Pool 300; Figure 7.1, black circles), supporting the first alternative hypothesis ($H_{a2-1}$).

In the back reef – forereef *Por. lobata* RTE (Ch. 4), bulk density was entirely controlled by transplant site (Figure 7.2a), also supporting the first alternative hypothesis ($H_{a2-1}$). But there was a stronger effect of source population than transplant site on growth (Figure 7.2b) and calcification, supporting the second alternative hypothesis ($H_{a2-2}$).

![Figure 7.1. Skeletal growth reaction norms for *Por. lobata* (white circles) and *Poc. eydouxi* (black circles; from Figure 3.2).](image-url)
Likewise, in the back reef – fore reef Por. eydouxi RTE (Ch. 5), skeletal growth was entirely controlled by source population, supporting the second alternative hypothesis ($H_{a2.2}$; Figure 7.3). The microsatellite study (Ch. 5) demonstrated high gene flow and no population differentiation between back reef and fore reef Por. eydouxi colonies (overall $F_{ST} = 0.001$, Table 5.3), indicating these colonies are part of the same population. Thus the RTE results are most likely due to developmental plasticity, although genetic polymorphism (maintained by balancing selection, see Ch. 5) cannot be ruled out. The third set of hypotheses was:

$H_{03}$: There is no clade-level variability in zooxanthella genotypes found in conspecific coral colonies along a seawater temperature gradient (small back reef pool, large back reef pool, shallow fore reef);
Clade-level variability in zooxanthella genotype found in conspecific coral colonies correlates with the seawater temperature gradient (i.e., highest diversity in zooxanthella genotype found at the site with the highest daily temperature maxima and fluctuations, the small back reef pool).

A zooxanthella survey was conducted during the summer and winter at three sites along the seawater temperature gradient to test for spatial and temporal patterns in zooxanthella genotype variability, thereby testing $H_{a3}$ (Chapter 6). Zooxanthella samples were collected from colonies of *Poc. eydouxi*, *Por. lobata*, and *Acropora gemmifera* from the three sites, *Galaxea fasicularis* from Pool 300 and the shallow forereef, and *Porites cylindrica* from the two back reef pools. Three of the four species showed the highest proportion of the heat-resistant Clade D in Pool 300 (Figure 6.4), thereby supporting $H_{a3}$.

*Interpretation of RTEs:*

Four final points may provide insight into the RTE results: 1) The highest growth occurred at the sites with the highest seawater temperatures; 2) Normalized annual growth rates were similar for the 18-month vs. 6-month RTEs in Pool 400 for both species; 3) Growth was less than reported elsewhere for both species, and 4) The contrasting distributions, abundances, and physiologies of *Por. lobata* vs. *Poc. eydouxi*.

In the two RTEs that showed physiological plasticity (Fig. 7.1, 7.2a,b), transplant survival and skeletal extension rates were higher at the sites with the highest seawater temperature maxima, which were also the most fluctuating sites (i.e., Pool 300 in the Pool 300-Pool 400 RTE, and the back reef in the back reef-forereef RTE). The temperature
maxima occurred when irradiance levels were also very high. The intermittent flows, warm temperatures, and abundant light in the back reef pools appear to provide a good growth environment most of the time. The periods of elevated seawater temperatures, high irradiance levels, and low flows only last a few hours, thus brevity of these conditions may usually prevent negative effects on survival and growth.

One pair of RTEs was three times the duration (18 months; Fig. 7.1) of the other (6 months; Fig. 7.2, 7.3). However, normalized annual extension rates for *Poc. eydouxi* in Pool 400 = 15 mm yr\(^{-1}\) for the 18-mo RTE, and 16 mm yr\(^{-1}\) in the 6-mo RTE. Likewise, normalized *Por. lobata* annual extension rates in Pool 400 were nearly identical for both RTEs (≈10 mm yr\(^{-1}\)). Thus, growth rates in both experiments appear to be representative of coral growth at these sites, despite the different experimental durations.

The normalized maximum annual extension rates in both *Por. lobata* RTEs were approximately 10 mm yr\(^{-1}\), whereas a density band study of 35 massive *Porites* colonies at 3 – 5 m depth from the same latitude (14°S) on the Great Barrier Reef reported a mean extension rate of 14 mm yr\(^{-1}\) (Lough et al. 1999). Similarly, while the normalized maximum annual extension rates of *Poc. eydouxi* in the RTEs ranged from 15 – 25 mm yr\(^{-1}\), this species has been reported to grow > 50 mm yr\(^{-1}\) in back reefs at 5 – 6 m depth (Jokiel & Tyler 1992). Thus, since the RTEs were done at 1.5 m low tide depth, skeletal growth of all RTE groups was likely inhibited by physical factors associated with shallow depths, such as high levels of ultraviolet radiation (Jokiel 1980).

*Por. lobata* is very common in the back reef pools but nearly absent on the shallow forereef (but common on the forereef at > 5 m depth). *Porites* species, including *Por. lobata*, dominate the back reef coral community (Craig et al. 2001), even without the
heat-resistant Clade D zooxanthellae. Conversely, *Poc. eydouxi* is very common on the forereef (all depths) but less so in the back reef pools, even though many back reef colonies contain the heat-resistant Clade D zooxanthellae (see Ch. 6). The greater abundances of *Porites* colonies than *Pocillopora* or *Acropora* colonies in the back reef pools support the concept that massive, slow-growing coral species are generally more tolerant of elevated seawater temperatures and high irradiance levels than branching, fast-growing corals, most likely because of more efficient host coral physiological mechanisms (Gates & Edmunds 1999, Loya et al. 2001, Barshis et al. Submitted).

Summary of Experimental Results

How do reef-building corals tolerate the elevated seawater temperatures and high irradiance levels that often occur in the Ofu back reef pools? My results indicate that semi-diurnally intermittent water motion resulting from tides reduces photoinhibition and bleaching for the dominant *Porites* species (Ch. 2), and vigorous water motion increases skeletal growth of *Poc. eydouxi* even with sporadically high temperatures and irradiances (Ch. 3). For *Por. lobata*, both physiological plasticity and genetic polymorphism of the host coral may contribute to tolerance of the highly-fluctuating conditions in the back reef, such as elevated seawater temperatures and high irradiance levels. In either case, despite occasionally challenging environmental conditions, the back reef is a superior growth environment than the shallow forereef for *Por. lobata* (Ch. 4). For *Poc. eydouxi*, developmental plasticity may contribute to tolerance of the highly-fluctuating back reef conditions. (Ch. 5). For *Poc. eydouxi* and other species (but not *Porites* species), symbiont diversity may also be important (Ch. 6).
Implications for Coral Resilience

'Resilience' is defined as the capacity of an ecosystem to absorb or recover from disturbance while maintaining its functions (Holling 1973, Gunderson 2000), and reviews abound on factors that make corals resilient to warming events (Nystrom et al. 2000, McClanahan et al. 2002, Coles & Brown 2003, Hughes et al. 2003, West & Salm 2003, Hughes et al. 2005, Obura 2005, Wooldridge et al. 2005, Bellwood et al. 2006a, Grimsditch & Salm 2006). Most attention has rightfully been placed on biological factors such as physiological mechanisms within the host coral (Lesser 1997, Brown et al. 2002b, Lesser & Farrell 2004, Robbart et al. 2004) or the symbionts (Brown et al. 1999, Jones & Hoegh-Guldberg 2001, Rowan 2004, Robison & Warner 2006) that increase temperature and irradiance tolerances, as well as ecological characteristics such as the abundance and diversity of herbivores that enhance recovery after disturbance (Bellwood et al. 2006b, Mumby et al. 2006, Hughes et al. 2007). The importance of environmental factors, such as high frequency of elevated seawater temperatures and high irradiance (thereby acclimatizing coral colonies), has also been given considerable attention (Coles & Jokiel 1978, Brown 1997, Coles & Brown 2003, Jokiel 2004). Thus, coral reef management guides now include reef habitats with frequently elevated seawater temperatures in their criteria for identifying high resilience target areas (TNC 2001, West & Salm 2003, TNC 2005, Grimsditch & Salm 2006, Marshall & Schuttenberg 2006).

The results of my dissertation research support these recommendations, and go one step further by suggesting that the rapid physical changes associated with fluctuating reef environments (not just the extremes) help maintain coral resilience. As shown in
Chapter 2, semi-diurnally intermittent water motion can reduce the adverse effects on corals of conditions typical of warming events such as simultaneous elevated temperatures and high surface irradiance. An additional environmental factor associated with highly-fluctuating habitats that may reduce the effects of warming events are the temperature fluctuations themselves. For example, laboratory studies using *Por. lobata* (Barshis et al. In preparation) and an abalone species (Hines et al. 1980) demonstrated that daily-fluctuating temperatures (compared to stable temperatures) increased thermal tolerances of both species. Similarly, daily-fluctuating temperatures (compared to stable temperatures) increase upper thermal tolerances (Feldmeth et al. 1974, Feminella & Matthews 1984, Schaefer & Ryan 2006) and production of heat shock proteins (Podrabsky & Somero 2004, Todgham et al. 2006) in fishes. Finally, implicit in a highly-fluctuating thermal environment is brevity of elevated seawater temperatures. That is, the fluctuating nature of the back reef pools results in short duration of temperatures above the local summer mean (< 5 hours; Figures 3.3 and 6.3). Such brevity of elevated seawater temperatures greatly reduces their harmful effects on corals (Jokiel 2004).

The RTEs in Chapters 3 – 5 demonstrated considerable host coral phenotypic plasticity between highly-fluctuating and relatively stable environments. Predictable environmental variability within a population's range may maintain plasticity (Meyers & Bull 2002, Doughty & Resnick 2004), especially in corals and other sessile organisms that lack the mobility to avoid environmental extremes (Marfenin 1997). For example, coral larvae predictably settle across a range of depths, thus plasticity for colony and corallite shape (Muko et al. 2000, Todd et al. 2004a) that maximizes light absorption at depth is maintained. Likewise, coral larvae predictably settle across a range of thermal
and light regimes, from highly-fluctuating to relatively stable. Highly-fluctuating habitats such as shallow back reef pools provide the warm seawater temperatures and abundant light needed for rapid skeletal growth, but also pose the threat of extreme temperatures and irradiance levels.

Host coral phenotypic plasticity within a population distributed across heterogeneous environments may be physiological, developmental, or both (Meyers & Bull 2002). The variance in skeletal growth by transplant site shown for Poc. eydouxi in Chapter 3 and for Por. lobata in Chapter 4 is a form of physiological plasticity, whereas the Poc. eydouxi skeletal growth and microsatellite results in Chapter 5 suggest that developmental plasticity may also play a role in this species. Both types of phenotypic plasticity are likely maintained in the population by the distribution each generation of individuals across various environments, including the highly-fluctuating back reef pools. That is, the occurrence of a portion of the population within this particular environment likely maintains broad plasticity, thereby increasing acclimatization capacity to elevated seawater temperatures and high irradiance levels, which in turn is beneficial for resilience to disturbances.

Panmixia of broadcast-spawning corals often occurs on spatial scales of hundreds to thousands of kilometers (Ayre & Hughes 2000, Ayre & Hughes 2004), thus it seemed unlikely that broadcast spawners like Por. lobata and Poc. eydouxi would be genetically polymorphic between the back reef and forereef sites (~ 5 km apart) used for this study. However, both RTEs between the two sites (Por. lobata in Ch. 4; Poc. eydouxi in Ch. 5) suggested genetic polymorphism of back reef vs. forereef populations. A parallel study using mitochondrial and nuclear markers in samples from the Por. lobata RTE source
colonies showed population structure between the back reef and forereef (Barshis et al. Submitted), though my follow-up study using microsatellite markers in samples from the *Poc. eydouxi* RTE source colonies did not (Ch. 5). Genetic polymorphism of broadcast-spawning corals has recently been demonstrated at spatial scales of 2 – 5 km (Magalon et al. 2005, Vollmer & Palumbi 2007), potentially contributing to adaptation of corals to environmental variability at these scales (e.g., back reef vs. forereef).

Some reef-building coral species are labile, in that they harbor a range of zooxanthella genotypes, depending on the location and environmental conditions (Baker 2003, Baker 2004, Baker et al. 2004, Fabricius et al. 2004, van Oppen et al. 2005). As shown in Chapter 6, zooxanthella genotype varied along a seawater temperature gradient. The ability of these coral species to harbor different zooxanthellae depending on the environmental conditions increases their survival of, or recovery from, warming events (Baker et al. 2004, Rowan 2004), thereby maintaining or increasing their resilience.

In summary, the environmental characteristics and biological mechanisms associated with fluctuating reef environments (such as the Ofu back reef pools) and their corals maintain the resilience of coral populations to disturbances: First, potentially stressful conditions are moderated by environmental characteristics such as semi-diurnally intermittent flow and brevity of elevated seawater temperatures, plus fluctuating temperatures may enhance thermal tolerance. Second, exposure to these conditions likely maintains selection for mechanisms of acclimatization (phenotypic plasticity) or adaptation (genetic polymorphism) within host coral populations. Third, exposure of coral colonies to diurnally and seasonally fluctuating conditions maintains zooxanthella genotype diversity in some host coral species.
Management Applications and Future Research

An important objective of my doctoral research is to provide information to the National Park of American Samoa (NPSA) that will increase their ability to conserve their coral reefs in the global climate change era. Coral reef conservation efforts should target environmental or biological features that increase coral reef resilience to global climate change, as outlined in several recent management guides (TNC 2001, West & Salm 2003, Hughes et al. 2005, TNC 2005, Wooldridge et al. 2005, Grimsditch & Salm 2006, Marshall & Schuttenberg 2006). Thus, the greater objective of my doctoral research is to contribute to the delineation of conservation target areas in American Samoa and elsewhere containing features important for coral reef resilience.

My research suggests that highly-fluctuating habitats such as the Ofu back reef pools may maintain resilience within reef-building coral populations. This and other information can be used to identify desirable criteria for conservation target areas. Reef areas meeting some or all of the criteria can be prioritized according to how many criteria they meet, thereby identifying the highest priority areas for current or future conservation efforts. Areas with the following prioritized features are particularly valuable for conservation in the climate change era because they support coral colonies that are likely to be tolerant of elevated seawater temperature and high irradiance levels, thereby increasing the resilience of the coral reef ecosystem: 1) Back reef areas adequately deep to support diverse coral communities ($\geq 1$ m depth); 2) Minimal local human disturbance; and 3) High flows (e.g., windward reefs, or near reef pass).
Areas meeting these criteria can be delineated on aerial photos or habitat maps, then ground-truthed. For example, Figure 7.4 shows all the back reef pools on Ofu and Olosega Islands meeting Criterion #1 above, identified with aerial photos and ground-truthing. Not including the four pools already encompassed by NPSA's Ofu Unit, there are at least eight additional back reef pools on these islands (Fig. 7.4). However, only two pools (North Asaga and Vaoto, Fig. 7.4) meet Criteria #2 and #3 above, by my subjective analysis: Despite a road running along its shoreline and the presence of a house or two, human impacts appear minimal in North Asaga Pool, and the pool has strong flows because of tides and proximity to a reef pass. Similarly, Vaoto Pool has an airport runway on part of its shoreline but human impacts are minimal, and the pool has strong flows because of tides and southeast tradewinds. The other six back reef pools outside of NPSA either have significant local impacts (villages, sand/gravel removal, bridge, etc.) or lack strong water flow. That certainly does not mean that these other back reef pools are unworthy of protection, only that North Asaga and Vaoto Pools are especially valuable.

Figure 7.4. Back reef pools around Ofu and Olosega Islands, American Samoa.
My final and thankfully brief topic is future research needs, with an emphasis on highly-fluctuating habitats, as well as delineation and management of coral reef conservation target areas in American Samoa and elsewhere:

1. Exposure to rapid fluctuations in physical factors, such as seawater temperature, may increase tolerances to extreme conditions. Despite relevance to shallow coral reef habitats, this topic has scarcely been studied in invertebrates (but see Coles 1975, Hines et al. 1980, and Barshis et al. In preparation);

2. Skeletal plasticity in response to environmental variability is well studied in corals, such as for corallite shape (Foster 1980, Todd et al. 2004b) and colony shape (Grigg 1982, Muko et al. 2000). Other forms of physiological plasticity may be particularly relevant to reef-building corals in highly-fluctuating habitats, such as production of compounds that minimize effects of elevated seawater temperatures and high irradiance levels. While some field studies have been done, such as those by Brown in Thailand (Brown et al. 1999, Brown et al. 2000, Brown et al. 2002a, Brown et al. 2002b, Brown et al. 2002c), these studies encompass few species at few sites;

3. Though Chapter 5 results suggest developmental plasticity, the adult microsatellite data cannot eliminate genetic polymorphism between back reef and forereef colonies as the explanation for the RTE results. One of the hypotheses could be eliminating by conducting a microsatellite study of newly-settled polyps (rather than adult colonies) in the two habitats;

4. As shown in Chapter 6, zooxanthella genotype diversity varies with environmental variability in some coral taxa (e.g., *Acropora, Pocillopora*) but not others (*Porites*).
Zooxanthella genotypes in *Porites* species should be investigated in more extreme environments, such as the perched tidepool on Nu'utele island near the Ofu harbor;

5. Acidification is now recognized as potentially the most serious climate change problem not just for corals, but for all marine calcifiers and ecosystems dependent on them (Kleypas et al. 1999a, Kleypas et al. 2006). Research is needed on what, if any, features of coral reefs counteract or reduce effects of acidification so that areas containing such features can be protected;

6. Multi-species studies of gene flow in reef-building corals have tended to focus on brooders (e.g., Ayre & Hughes 2000, 2004) even though most species are broadcast spawners, thus more information at multiple spatial scales on population structure of broadcast-spawning species is needed;

7. Many recent studies have consistently found that trophic level diversity, especially the diversity and abundance of herbivorous fishes, is crucial for coral reef resilience and recovery (Bellwood et al. 2006, Mumby et al. 2006, Hughes et al. 2007), yet NPSA and other marine protected areas (MPAs) allow subsistence fishing. Research is needed to determine if and how such fishing pressure is affecting coral reef resilience;

8. There is a strong movement in American Samoa to establish community-based MPAs by the American Samoa Department of Marine and Wildlife Resources (CRAG 2004, Oram 2005), as well as proposals by the National Park Service to increase the size and/or number of NPSA units. Proponents of these efforts should be consulted in any research effort intended to benefit MPAs in the Territory.
Literature Cited


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