

Environmental versus genetic influences on growth rates of the corals *Pocillopora eydouxi* and *Porites lobata*

L.W. Smith^{1,*}, H. Wirshing², A.C. Baker², C. Birkeland¹

¹Hawai‘i Cooperative Fishery Research Unit, Zoology Department, University of Hawai‘i at Mānoa, Honolulu, HI 96822 USA

²The Rosenstiel School of Marine and Atmospheric Science, 4600 Rickenbacker Causeway Miami, FL 33149-1098 USA

* Corresponding author: lancesmi@hawaii.edu, TEL (808) 221-8297, FAX (808) 956-4238

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 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. Increased skeletal growth rates in higher water motion may provide *P. eydouxi* a competitive advantage in shallow, high energy environments where competition for space is intense.

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). 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 (reviewed in Buddemeier and 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 and 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 and Ayre 1985), or by both (Via and Lande 1985).

Environmental vs. genetic control of phenotypic characteristics, such as skeletal growth rates, can be tested with reciprocal transplant experiments (RTEs; Doughty and 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. In press), 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, Figure 1) is exposed to

greater fluctuations of environmental conditions, such as higher seawater temperatures and lower salinity, than are the other pools (Smith and 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 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. 2004) 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 (Figure 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 long-term mean summer 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

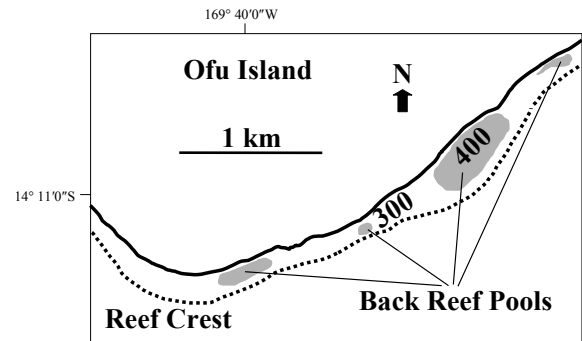


Figure 1. Map of study site, showing the two pools.

velocities $> 30 \text{ cm s}^{-1}$ in the back reef area (Craig et al. 2001, Smith 2004, Smith and 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, N_1), from Pool 300 to Pool 400 (Translocated 1, T_1), from Pool 400 to Pool 400 (Native 2, N_2), and from Pool 400 to Pool 300 (Translocated 2, T_2). Comparison of the Native and Translocated groups quantifies variability by transplant site (N_1 vs T_1 , N_2 vs T_2) and by source population (N_1 vs T_2 , N_2 vs T_1). Variability by transplant site indicates environmental control, 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 (N_1 and T_1 , N_2 and T_2), and the two reaction norms together illustrate the interplay of environmental and genetic control on each skeletal characteristic (DeWitt and 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. 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. 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' Pox 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: $\text{NH}_4 + \text{NO}_x$) and phosphate (PO_4).

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 (ITS2) 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 (Figure 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 1). There were no significant interactions between transplant site and source population for either species. For each 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 (Figure 2). No signs of competition, predation or disease were observed on any of the surviving 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). 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 (Figure 3). Flow velocities were similar between the two pools during

Table 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).

	Df	MS		F		p	
		Mss Inc	Lin Ext	Mss Inc	Lin Ext	Mss Inc	Lin Ext
<i>P. eydouxi</i>							
Transplant Site	1	706067	1449.23	11.43	59.32	0.008*	0.001*
Source Population	1	41795	10.05	0.68	0.41	0.422	0.530
Source Colony	6	90898	29.10	1.47	1.19	0.246	0.357
Population x Site	1	81053	0.02	1.31	0.00	0.268	0.975
Error	17	61765	24.43				
<i>P. lobata</i>							
Transplant Site	1	19148	1.896	1.64	0.22	0.214	0.643
Source Population	1	9428	11.266	0.81	1.32	0.379	0.264
Source Colony	2	2234	2.286	0.19	0.27	0.827	0.768
Population x Site	1	132	0.009	0.01	0.00	0.916	0.974
Error	22	11672	8.564				

*Bonferroni corrected

a. Mass Increase (%)				
	N ₁	T ₁	N ₂	T ₂
<i>P. eydouxi</i> ●				
\bar{x}	865	428	459	675
SE	145	76	50	110
<i>P. lobata</i> ○				
\bar{x}	346	403	437	385
SE	35	32	25	62
b. Linear Extension (mm)				
	N ₁	T ₁	N ₂	T ₂
<i>P. eydouxi</i> ●				
\bar{x}	37.8	36.6	23.0	21.7
SE	2.4	1.7	1.1	2.4
	24.2	14.3	15.2	24.9
<i>P. lobata</i> ○				
\bar{x}	13.6	14.1	15.4	15.0
SE	1.3	1.1	0.8	1.0
Yr ⁻¹	9.1	9.5	10.3	10.0

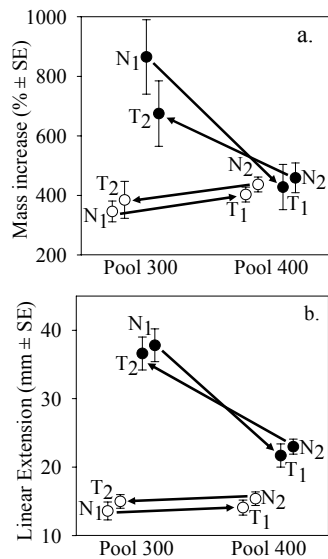


Figure 2. Skeletal growth results (left) and reaction norms (right) for *Pocillopora eydouxi* (black circles) and *Porites lobata* (white circles) in, (a) mass gained, and (b) and linear extension.

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 (Figure 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). For all abiotic environmental factors, daily fluctuations were higher in Pool 300 than in Pool 400 (Appendix). 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.

Zooxanthella genotypes showed both spatial and temporal variability for *P. eydouxi*, but not for *P. lobata* (Table 2). Zooxanthella genotypes in *P. eydouxi* source colonies varied between the pools, with a mix of Clades C and D (Rowan and Powers 1991) 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 2).

Discussion

Skeletal growth rate of *P. eydouxi* transplants responded to transplant site (Table 1), indicating environmental control. 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 and Kinzie 1976, Jokiel 1978, Lough and 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 (Figure 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

Table 2. Zooxanthella genotypes (LaJeunesse 2001, LaJeunesse et al. 2004) found in source colonies at the beginning (9/04) and end (3/06) of the RTE.

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

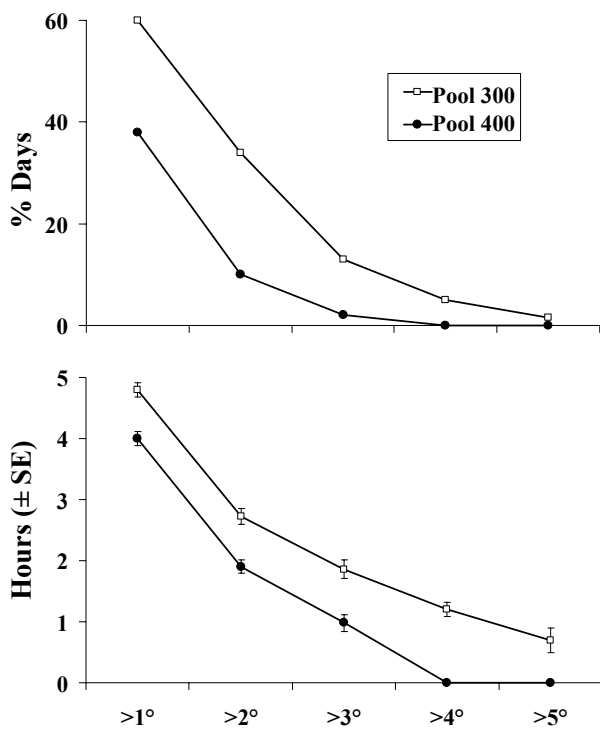


Figure 3. Frequency and duration of summer (Nov – Mar) seawater temperatures exceeding 1, 2, 3, 4, and 5 °C above the local summer mean (29.4 °C) during the RTE period.

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 $\mu\text{mol l}^{-1}$ DIN and < 0.3 $\mu\text{mol l}^{-1}$ phosphorus (Crossland 1983). Zooxanthellate corals may be nitrogen limited, as shown by increased zooxanthellate (Falkowski et al. 1993) and skeletal (Meyer and Schultz 1985) growth with slight increases in DIN concentrations. However,

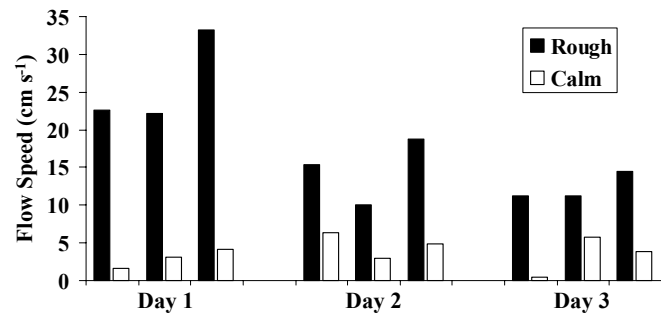


Figure 4. Difference in mean flow speed between the two pools (300 – 400) at max flood (F), high (H), and max ebb (E) tides during 3-day periods of rough (4/22-24/06) and calm (3/20-22/06) conditions.

the difference between nitrogen limitation and excess can be small, with concentrations of < 5 $\mu\text{mol l}^{-1}$ DIN often resulting in decreased skeletal growth (reviewed in Ferrier-Pages et al. 2000). DIN concentrations were 0.54-0.58 $\mu\text{mol l}^{-1}$ 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 1, Figure 2).

Velocity of water flow was higher in Pool 300 than Pool 400 during all flow sampling periods, especially during rough conditions (Figure 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 and 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 ($\approx 15\text{-}30 \text{ cm s}^{-1}$) or low ($\approx 5\text{-}10 \text{ cm s}^{-1}$) flow treatments. It was concluded that optimal water velocity for *P. 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. 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 and Birkeland 2003, 2007).

P. lobata showed no variance in skeletal growth rate between the pools (Table 1), or any zooxanthella genotype differences (Table 2). Host or symbiont genetic differentiation was not expected between the two adjacent pools (Figure 1) because *P. lobata* is a broadcast spawner (Richmond and Hunter 1990), and *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 *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. lobata* than Pool 300. However, *P. 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. 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 produce variance in skeletal growth rates of this species.

The occurrence of Clade D zooxanthellae in Pool 300 but not Pool 400 source colonies of *P. 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 (Berkelmans and van Oppen 2006, Little et al. 2004). Thus, in the absence of environmental differences between the pools, mean growth of *P. eydouxi* transplants from Pool 300 (Groups N_1 and T_1) should be less than mean growth of transplants from Pool 400 (Groups N_2 and T_2). 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 2), source population had no effect on skeletal growth rates (Table 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 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 ITS2/DGGE methodology (Aprill and Gates In press). Though *Pocillopora* species maternally transmit zooxanthellae to larvae (Glynn et al. 1991), the genetic diversity of zooxanthellae in *P. eydouxi* (Table 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 environmental control 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 common in most reef environments and often dominant where water motion is high, such as shallow forereefs (Fisk and Birkeland 2002, Mumby et al. 2001, Veron 2000). 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 (Potts 1984, Sheppard 1982). 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.

Acknowledgements

We thank P. Craig of the National Park of American Samoa for the seawater temperature data and many different forms of assistance, D. Barshis, L. Basch, R. Bradley, G. DiDonato, G. Garrison, C. Hawkins, M. Malae, G. Piniak, M. Speicher, C. Squair, F. Tuilagi, and J. Zamzow for logistical support and field assistance, and the U.S. Geological Survey's Global Climate Change Program, the National Park of American Samoa, the American Samoa Coral Reef Advisory Group, and the American

Museum of Natural History's Lerner-Gray Fund for financial support.

Literature Cited

- Apprill, A. M., and R. D. Gates. In press. Recognizing diversity in coral symbiotic dinoflagellate communities. *Mol. Ecol.*
- Baker, A. C., R. Rowan, and N. Knowlton. 1997. Symbiosis ecology of two Caribbean acroporid corals. *Proc. 8th Inter. Coral Reef Symp., Panama.* 2:1295-1300.
- , C. J. Starger, T. R. McClanahan, and P. W. Glynn. 2004. Coral reefs: Corals' adaptive response to climate change. *Nature* 430:741.
- Barnes, D. J. 1970. Coral skeletons: An explanation of their growth and structure. *Science* 170:1305-1308.
- Berkelmans, R., and M. J. H. van Oppen. 2006. The role of zooxanthellae in the thermal tolerance of corals: a "nugget of hope" for coral reefs in an era of climate change. *Proc. R. Soc. Lond. B Biol. Sci.* 273:2305-2312.
- Buddemeier, R. W., and R. A. Kinzie, III. 1976. Coral growth. *Oceanogr. Mar. Biol. Annu. Rev.* 14:183-225.
- Craig, P., C. Birkeland, and S. Belliveau. 2001. High temperatures tolerated by a diverse assemblage of shallow-water corals in American Samoa. *Coral Reefs* 20:185-189.
- Crossland, C. J. 1983. Dissolved nutrients in coral reef waters. Pp. 56-68. *In* D. J. Barnes, ed. *Perspectives in coral reefs.* AIMS Publ. 200.
- DeWitt, T. J., and S. M. Scheiner. 2004. Phenotypic Variation from Single Genotypes. Pages 1-9 *in* T. J. DeWitt, and S. M. Scheiner, eds. *Phenotypic Plasticity: Functional and conceptual approaches.* Oxford University Press, New York.
- Doughty, P., and D. N. Resnick. 2004. Patterns and Analysis of Adaptive Phenotypic Plasticity in Animals. Pages 126-150 *in* T. J. DeWitt, and S. M. Scheiner, eds. *Phenotypic Plasticity: Functional and Conceptual Approaches.* Oxford University Press, New York.
- Fabricius, K. E., J. C. Mieog, P. L. Colin, D. Idip, and M. J. H. van Oppen. 2004. Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Mol. Ecol.* 13:2445-2448.
- Falkowski, P. G., and Z. Dubinsky. 1993. Population control in symbiotic corals. *Bioscience* 43:606-611.
- Fenner, D. 2005. *Corals of Hawai'i.* Mutual Publishing, Honolulu.
- Ferrier-Pages, C., J. P. Gattuso, S. Dallot, and J. Jaubert. 2000. Effect of nutrient enrichment on growth and photosynthesis of the zooxanthellate coral *Stylophora pistillata*. *Coral Reefs* 19:103-113.
- Fisk, D., and C. Birkeland. 2002. Status of coral communities in American Samoa: A re-survey of long-term monitoring sites. *Dept. Mar. and Wildl. Res., Pago Pago.*
- Foster, A. B. 1979. Phenotypic plasticity in the reef corals *Montastrea annularis* and *Siderastrea sideria*. *J. Exp. Mar. Biol. Ecol.* 39:25-54.
- Glynn, P. W., N. J. Gassman, C. M. Eakin, J. Cortes, D. B. Smith, and H. M. Guzman. 1991. Reef coral reproduction in the east Pacific: Costa Rica, Panama, and the Galapagos Islands (Ecuador). I. Pocilloporidae. *Mar. Biol.* 109:355-368.
- Grigg, R. W. 1982. Darwin Point: A threshold for atoll formation. *Coral Reefs* 1:29-34.
- Harriott, V. J. 1999. Coral growth in subtropical eastern Australia. *Coral Reefs* 18:281-291.
- Jokiel, P. L. 1978. Effects of water motion on reef corals. *J. Exp. Mar. Biol. Ecol.* 35:87-97.
- . 2004. Temperature stress and coral bleaching. Pages 401-425 *in* E. Rosenberg, and Y. Loya, eds. *Coral Health and Disease.* Springer-Verlag, Heidelberg.
- , J. E. Maragos, and L. Franzisket. 1978. Coral growth: buoyant weight technique. Pages 529-541 *in* *Coral Reefs: Research Methods.* UNESCO, Paris.
- Kuffner, I. B. 2002. Effects of ultraviolet radiation and water motion on the reef coral *Porites compressa* Dana: a transplantation experiment. *J. Exp. Mar. Biol. Ecol.* 270:147-169.
- LaJeunesse, T. C. 2001. Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search of a 'species' level marker. *J. Phycol.* 37:866-880.
- , R. Bhagooli, M. Hidaka, L. M. De Vantier, T. J. Done, G. W. Schmidt, W. K. Fitt, and O. Hoegh-Guldberg. 2004. Closely related *Symbiodinium* spp. differ in relative dominance in coral reef host communities across environmental, latitudinal, and biogeographic gradients. *Mar. Ecol. Prog. Ser.* 284:147-161.
- Little, A. F., M. J. H. van Oppen, and B. L. Willis. 2004. Flexibility in algal symbioses shapes growth in reef corals. *Science* 304:1492-1494.

- Lough, J. M., and D. J. Barnes. 2000. Environmental controls on the massive coral *Porites*. *J. Exp. Mar. Biol. Ecol.* 245:225-243.
- Magalon, H., E. Baudry, A. Huste, M. Adjeroud, and M. Veuille. 2006. High genetic diversity of the symbiotic dinoflagellates in the coral *Pocillopora meandrina* from the South Pacific. *Mar. Biol.* 148:913-922.
- Meyer, J. L., and E. T. Schultz. 1985. Tissue condition and growth rate of corals associated with schooling fish. *Limnol. Oceanogr.* 30:157-166.
- Mumby, P. J., J. R. M. Chisholm, A. J. Edwards, C. D. Clark, E. B. Roark, S. Andrefouet, and J. Jaubert. 2001. Unprecedented bleaching-induced mortality in *Porites* spp. at Rangiroa Atoll, French Polynesia. *Mar. Biol.* 139:183-189.
- Muscatine, L. 1990. The role of symbiotic algae in carbon and energy flux in reef corals. *Coral Reefs* 25:1-29.
- Nakamura, T., R. van Woesik, and H. Yamasaki. 2005. Photoinhibition of photosynthesis is reduced by water flow in the reef-building coral *Acropora digitifera*. *Mar. Ecol. Prog. Ser.* 301:109-118.
- Potts, D. C. 1984. Natural selection in experimental populations of reef-building corals (Scleractinia). *Evolution* 38(5):1059-1078.
- Raymundo, L. J. 2001. Mediation of growth by conspecific neighbors and the effect of site in transplanted fragments of the coral *Porites attenuata* Nemenzo in the central Philippines. *Coral Reefs* 20:263-272.
- Richmond, R. H., and C. L. Hunter. 1990. Reproduction and recruitment of corals: Comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. *Mar. Ecol. Prog. Ser.* 60:185-203.
- Rowan, R., and D. A. Powers. 1991. Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). *Mar. Ecol. Prog. Ser.* 71:65-73.
- Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press.
- Sheppard, C. R. C. 1982. Coral populations on reef slopes and their major controls. *Mar. Ecol. Prog. Ser.* 7:83-115.
- Smith, L. W., and C. Birkeland. 2003. *Managing NPSA's Coral Reefs in the Face of Global Warming: Research Project Report for Year 1*. Hawai'i Cooperative Fishery Research Unit, Zoology Department, University of Hawai'i at Mānoa, Honolulu.
- . 2004. Influence of water motion on resistance of corals to high temperatures: Evidence from a field transplant experiment. *Proc. 10th Inter. Coral Reef Symp., Okinawa, Japan.* 1:724-728.
- and C. Birkeland. 2007. Effects of intermittent flow and irradiance level on back reef *Porites* corals at elevated seawater temperatures. *J. Exp. Mar. Biol. Ecol.* 341:282-294.
- , D. Barshis, and C. Birkeland. In press. Phenotypic plasticity for skeletal growth, density and calcification of *Porites lobata* in response to habitat type. *Coral Reefs*.
- Trussell, G. C. 2000. Phenotypic clines, plasticity, and morphological trade-offs in an intertidal snail. *Evolution* 54:151-166.
- van Oppen, M. J. H., A. J. Mahiny, and T. J. Done. 2005. Geographic distribution of zooxanthellae types in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event. *Coral Reefs* 24(3):482-487.
- Veron, J. E. N. 2000. *Corals of the World, Volumes 1-3*. Australian Institute of Marine Science. Odyssey Publishing.
- Via, S., and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39:505-522.
- Willis, B. L., and D. J. Ayre. 1985. Asexual reproduction and genetic determination of growth form in the coral *Pavona cactus*: Biochemical genetic and immunogenic evidence. *Oecologia* 65:516-525.

Received: 10 November 2006

Accepted: 31 January 2007

Appendix. Environmental data (MDR = maximum daily range, PAR = photosynthetically active radiation, DIN = dissolved inorganic nitrogen).

Factor*	Dates	Periodicity	N/pool	Units	Error	Results				
						Pool	Mean	SD	Total Range	MDR
Summer Temperatures	Nov-Mar	2x hr ⁻¹	12,960	°C	±0.2°C	300	29.46	0.90	26.77-34.31	5.29
						400	29.34	0.69	27.19-33.03	4.34
Winter Temperatures	Apr-Oct	2x hr ⁻¹	13,000	°C	±0.2°C	300	28.48	0.71	25.33-32.67	5.07
						400	28.53	0.60	26.11-31.51	3.01
PAR	20-21 Jan '05	4x min ⁻¹	400	µmol quanta m ⁻² s ⁻¹	± 5%	300	1,396	333	104-2,250	2,146
						400	1,411	324	224-2,120	1,896
Calm Flow	20-22 Mar '06	30x day ⁻¹	90	cm s ⁻¹	± 10%	300	17.8	7.7	8.1-41.7	30.5
						400	14.2	6.0	5.7-31.3	24.0
Rough Flow	22-24 Apr '06	18x day ⁻¹	54	cm s ⁻¹	± 5%	300	39.8	11.6	21.0-68.9	38.3
						400	21.0	6.3	12.0-35.8	25.8
Turbidity	14-23 Sep '04	2x hr ⁻¹	480	NTU	±0.1 NTU	300	0.14	0.26	0.0-2.0	2.0
						400	0.10	0.15	0.0-1.9	1.9
Salinity	14-23 Sep '04	2x hr ⁻¹	480	ppt	±0.01 ppt	300	35.65	0.28	32.90-35.84	2.43
						400	35.66	0.14	34.44-35.81	1.90
Dissolved Oxygen	14-23 Sep '04	2x hr ⁻¹	480	% saturation	±1%	300	100.9	36.6	23.2-212.3	184.4
						400	101.3	30.2	34.4-187.2	147.8
Water Column DIN	1-2 Apr '06	6x day ⁻¹	13	µmol l ⁻¹	N/A	300	1.07	0.71	0.54-2.73	2.00
						400	0.58	0.18	0.32-0.97	0.64
Substrate DIN	1-2 Apr '06	6x day ⁻¹	13	µmol l ⁻¹	N/A	300	1.06	0.56	0.34-2.11	1.50
						400	0.54	0.16	0.31-0.85	0.40
Water Column PO ₄	1-2 Apr '06	6x day ⁻¹	13	µmol l ⁻¹	N/A	300	0.22	0.04	0.13-0.29	0.16
						400	0.21	0.03	0.11-0.25	0.13
Substrate PO ₄	1-2 Apr '06	6x day ⁻¹	13	µmol l ⁻¹	N/A	300	0.22	0.04	0.16-0.28	0.10
						400	0.22	0.02	0.18-0.25	0.07

*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[®]).
3. Rough Flow: General Oceanics Model 2030R[®] flow meter with standard rotor.
4. Turbidity, Salinity, Dissolved Oxygen: YSI Sonde[®] 6600 water quality loggers.
5. Nutrients: Laboratory analysis.