



PII S0016-7037(01)00901-2

Effect of light and brine shrimp on skeletal $\delta^{13}\text{C}$ in the Hawaiian coral *Porites compressa*: A tank experiment

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(Received June 7, 2001; accepted in revised form December 11, 2001)

Abstract—Previous experimental fieldwork showed that coral skeletal $\delta^{13}\text{C}$ values decreased when solar intensity was reduced, and increased in the absence of zooplankton. However, actual seasonal changes in solar irradiance levels are typically less pronounced than those used in the previous experiment and the effect of increases in the consumption of zooplankton in the coral diet on skeletal $\delta^{13}\text{C}$ remains relatively unknown. In the present study, the effects of four different light and heterotrophy regimes on coral skeletal $\delta^{13}\text{C}$ values were measured. *Porites compressa* corals were grown in outdoor flow-through tanks under 112%, 100%, 75%, and 50% light conditions at the Hawaii Institute of Marine Biology, Hawaii. In addition, corals were fed either zero, low, medium, or high concentrations of brine shrimp. Decreases in light from 100% resulted in significant decreases in $\delta^{13}\text{C}$ that is most likely due to a corresponding decrease in photosynthesis. Increases in light to 112% also resulted in a decrease in $\delta^{13}\text{C}$ values. This latter response may be a consequence of photoinhibition. The overall curved response in $\delta^{13}\text{C}$ values was described by a significant quadratic function. Increases in brine shrimp concentrations resulted in increased skeletal $\delta^{13}\text{C}$ levels. This unexpected outcome appears to be attributable to enhanced nitrogen supply associated with the brine shrimp diet which led to increased zooxanthellae concentrations, increased photosynthesis rates, and thus increased $\delta^{13}\text{C}$ values. This result highlights the potential influence of nutrients from heterotrophically acquired carbon in maintaining the zooxanthellae–host symbiosis in balance. In addition, evidence is presented that suggests that coral skeletal growth and $\delta^{13}\text{C}$ are decoupled. These results increase our knowledge of how light and heterotrophy affects the $\delta^{13}\text{C}$ of coral skeletons. Copyright © 2002 Elsevier Science Ltd

1. INTRODUCTION

Recent research indicates that the stable isotope composition, and trace and minor elemental content in scleractinian coral skeletons can be used to reconstruct high-resolution paleoclimatic records from tropical latitudes (Druffel, 1997; Fairbanks et al., 1997; Gagan et al., 2000; Grottoli, 2001). Although the stable oxygen isotopic signature ($\delta^{18}\text{O}$) in reef coral skeletons has proven to be a reliable recorder of sea surface temperatures and salinities, causes of variability in the stable carbon isotope signature ($\delta^{13}\text{C}$) are only now beginning to be understood.

Corals deposit a calcium carbonate exoskeleton as aragonite that is usually depleted in ^{13}C and ^{18}O relative to ambient seawater as a result of kinetic and metabolic fractionation. Kinetic fractionation during CO_2 hydration and hydroxylation produces a simultaneous depletion of ^{13}C and ^{18}O (McConnaughey, 1986, 1989a). Metabolic fractionation produces additional changes in skeletal $\delta^{13}\text{C}$ due to changes in photosynthesis and respiration (Swart, 1983; Muscatine et al., 1989; McConnaughey, 1989a, 1989b, 1997; Allison et al., 1996; Grottoli and Wellington, 1999). Therefore, environmental variables that influence coral metabolism should also affect skeletal $\delta^{13}\text{C}$ levels.

The two main sources of carbon for corals are dissolved inorganic carbon (DIC) in the surrounding seawater (mean $\delta^{13}\text{C}$ of $\sim 0\text{‰}$) and metabolically derived CO_2 (Furla et al., 2000). The two metabolic processes that can alter the isotopic composition of the carbon pool available for skeletogenesis in reef corals are photosynthesis and heterotrophy [zooplankton

have $\delta^{13}\text{C}$ values of -14 to -21‰ or lower (Grottoli-Everett, 1998; Rau et al., 1990)]. Photosynthesis is a light-driven metabolic reaction (McConnaughey, 1989a, b; McConnaughey et al., 1997; Muscatine et al., 1989). It is believed that as the rate of photosynthesis in zooxanthellae increases, carbon isotopic fractionation decreases, and the available inorganic carbon pool becomes relatively enriched in ^{13}C , resulting in an increase in skeletal $\delta^{13}\text{C}$ levels (Swart, 1983; McConnaughey, 1989a; McConnaughey et al., 1997; Porter et al., 1989; Grottoli and Wellington, 1999; Grottoli, 2000). Decreases in skeletal $\delta^{13}\text{C}$ values in corals with depth (as light decreases) (Land et al., 1975; Weber et al., 1976; Carriquiry et al., 1994; Grottoli and Wellington, 1999) and with seasonal variation in cloud cover (Fairbanks and Dodge, 1979; Cole and Fairbanks, 1990; Klein et al., 1992, 1993; Grottoli, 1999, 2000) strongly support the idea that metabolic fractionation from photosynthesis affects coral skeletal $\delta^{13}\text{C}$. Experimentally, $\delta^{13}\text{C}$ decreases under highly reduced light conditions (Grottoli and Wellington, 1999). However, actual seasonal changes in solar irradiance levels are likely to be less pronounced than those previously tested.

Theoretically, increased heterotrophy by corals should lead to a decrease in skeletal $\delta^{13}\text{C}$ because zooplankton prey are depleted in ^{13}C relative to seawater. Corals are known to be active heterotrophs (Yonge, 1931; Coles, 1969; Pearse, 1971; Sorokin, 1973, 1981; Porter, 1974; Lewis and Price, 1975; Helmuth and Sebens, 1993; Johnson and Sebens, 1993; Sebens et al., 1996; Moberg et al., 1997; Grottoli-Everett, 1998), and coral feeding has a significant effect on skeletal linear extension in some species (Wellington, 1982). Given that zooplankton and particulate organic material in seawater have low

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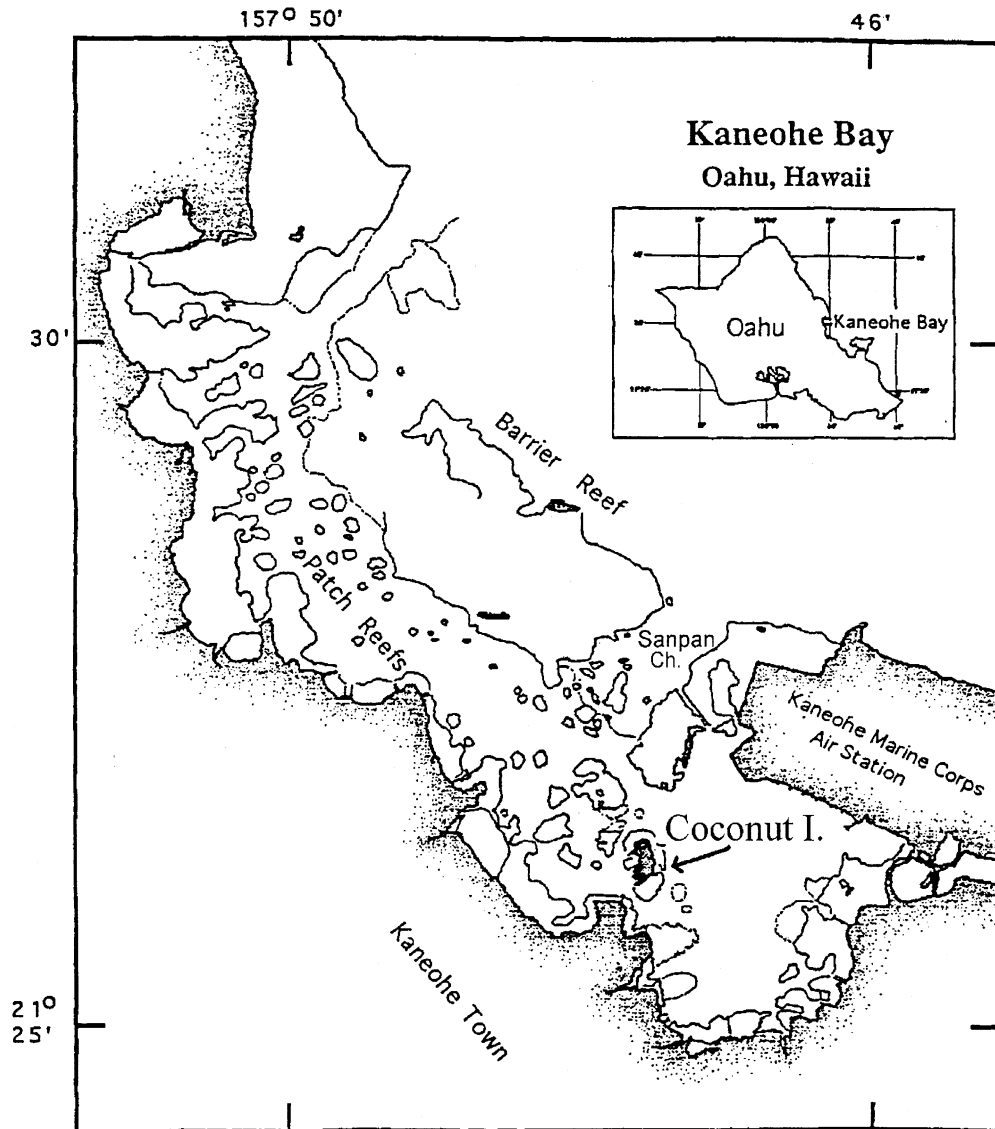


Fig. 1. Map showing the location of the coral collection site on Coconut Island (Kaneohe Bay), Hawaii.

isotopic values relative to coral skeletons, decreases in heterotrophic feeding by corals should be accompanied by increases in the $\delta^{13}\text{C}$ of coral skeletons. Grotoli and Wellington (1999) experimentally showed that $\delta^{13}\text{C}$ of coral skeletons increased when the availability of zooplankton for consumption was limited. The effect of increased consumption of zooplankton on coral skeletal $\delta^{13}\text{C}$ has not yet been experimentally evaluated.

In this study, the effect of a wide range of solar irradiance levels and heterotrophic regimens on the $\delta^{13}\text{C}$ values of coral skeletons was systematically measured by experimentally rearing corals under four different light and feeding regimes. It was hypothesized that: (1) as light levels decrease, skeletal $\delta^{13}\text{C}$ decreases, and (2) as heterotrophy increases, skeletal $\delta^{13}\text{C}$ also decreases. Understanding how light and heterotrophy together affect coral skeletal $\delta^{13}\text{C}$ will shed light on coral physiology and enhance our understanding of the natural variability in coral skeletal $\delta^{13}\text{C}$ records. Ultimately, this may increase our

ability to use $\delta^{13}\text{C}$ to reconstruct tropical paleoclimate changes related to seasonal changes in cloud cover and zooplankton-rich upwelling events.

2. MATERIALS AND METHODS

2.1. Synopsis

Fragments of *Porites compressa* corals were grown in outdoor flow-through tanks under 112%, 100%, 75%, and 50% light conditions. Within each light treatment, coral fragments were fed either zero, low, medium, or high concentrations of brine shrimp. This experiment was conducted at the Hawaii Institute of Marine Biology (HIMB), Coconut Island (Kaneohe Bay), HI, USA (21°26.18'N; 157°47.56'W) (Fig. 1) from August 8 to October 4, 1996 for a total of 57 d. Skeletal material accreted during that time was extracted and the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values measured. The results were used to evaluate the effect of a wide range of solar irradiance levels and heterotrophic regimens (brine shrimp regimens) on skeletal $\delta^{13}\text{C}$ values.

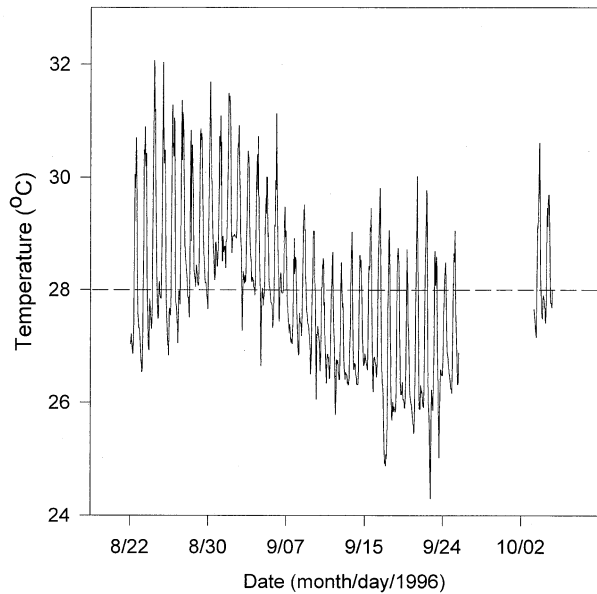


Fig. 2. Mean hourly tank seawater temperature (TST) measured by Onset Temperature Loggers in degrees Celsius. Data were not collected between September 26 and October 3, 1996 while data from the Loggers were being downloaded and reset. Mean temperature for the entire record was $27.97 \pm 0.04^{\circ}\text{C}$ (1SE) (dashed line).

2.2. Climate

Kaneohe Bay is on the windward side of the island of Oahu, Hawaii. It is a eutrophic bay, 12.7 km long by 4.3 km wide with relatively small diurnal tidal fluctuations (ranging from 0.5 to 2 m) (Bathen, 1968). The rainy season extends from mid-November to mid-April followed by a dry season from April to November. Total solar irradiance measured at 2 m depth on reef surrounding Coconut Island decreases by at least 50% during late October and November compared to summer irradiance levels (Grottole, 1999, 2000).

Tank seawater temperatures (TST) were measured every 6.5 to 10 min using temperature loggers (Onset). Calibrated data were averaged hourly and plotted for August 22 to October 4, 1996 (Fig. 2). Hourly TST averaged $27.97^{\circ}\text{C} \pm 0.06$ ($\pm 1\text{SE}$) ($n = 889$) and ranged from 24.3 to 32.1°C . On the reef flat immediately adjacent to the tanks, midday sea surface temperatures (SST) between August 8 and October 4, 1996 averaged $28.78^{\circ}\text{C} \pm 0.23$ (1SE) ($n = 24$).

2.3. Tank Conditions

Two outdoor, flow-through tanks located directly beside the reef and parallel to each other were used in this experiment. Each tank was 2.92 m long, 1 m wide, and 17 cm deep with a water height of 12 cm (350.4 L). Seawater was supplied by a flow-through system with an intake pipe located on the adjacent reef at a depth of 3 m. The inflow pipe of each tank was fitted with a 50- μm pore filter (Cole-Palmer Reusable Water Filtration Elements and housing) to remove all zooplankton and particles greater than 50 μm . The filters were cleaned twice daily to remove any accumulated sediment and particles, ensuring a stable flow rate throughout the experiment. After cleaning the filters, flow rates were set at 0.405 ± 0.024 and 0.409 ± 0.023 L/s in the first and second tanks, respectively. Over the course of 12 h, the flow rate would drop to approximately 0.13 L/s in each tank. At these rates, the water in the tanks would completely flush approximately every 14–45 min.

Both tanks were positioned along a North-South axis (i.e., at right angles to the sun's arc). SST of the adjacent reef flat and of both tanks were monitored daily with a calibrated mercury thermometer in addition to the Hobo temperature loggers from August 29 to October 4,

1996. Tanks were cleaned daily to remove any accumulated algae and sediment. In addition, even though changes in SST can have very small kinetic fractionation effects on $\delta^{13}\text{C}$ and particles smaller than 50 μm can be ingested and influence skeletal $\delta^{13}\text{C}$, SST and seawater particle size were constant across all treatments and did not interfere with the interpretation of the results. Measured $\delta^{13}\text{C}$ values in the coral skeleton were due to treatment effects alone.

2.4. The Experiment

Porites compressa is locally a very common finger-like coral ranging in color from yellow-brown to dark brown with small calices (1.5 to 2 mm in diameter). Ten individual colonies of *P. compressa* were identified on the reef at a depth of 2.0 m. The first 5 coral heads were assigned to the first tank (group 1) and the second 5 to the second tank (group 2). On August 3, 1996 seventeen coral fragments (approximately 4 cm tall) were collected from each coral head (Fig. 3A), fixed to a 2-cm by 4-cm ceramic tile using Splash Zone compound and allowed to acclimate for 5 d before random placement in 1 of 16 treatments in their respective tanks. Each treatment consisted of a combination of light (112%, 100%, 75%, or 50%) and brine shrimp concentrations (none, low, medium, or high) (Fig. 3B). The seventeenth fragment was placed back on the reef at its original depth to function as a control for transplantation to the tanks. The use of replicate genotypes across treatments reduced the effect of genotypic variation between treatments within tanks.

The four light treatments were created by placing varying layers of neutral density mesh across quarter sections of the tank (Fig. 4A). The mesh was stretched over a wooden frame to form removable screens that were anchored to the tanks with clamps. Light treatment screens were placed on an East-West axis (i.e., parallel to the sun's arc) to minimize shadowing from adjacent light treatments, and their order in each tank was randomly assigned. The intensity of light in the 100% light treatment represents the intensity of photosynthetically active radiation (PAR) measured at the 2.0 m coral collection depth at noon on the clear, cloudless day of July 13, 1996. In the experimental tanks, the 100% light treatment was achieved using one layer of 1.5 mm pore diameter neutral density mesh over one-quarter of the tank (calculations in Grottole-Everett, 1998). 75% and 50% light treatments were created using two and four layers of neutral density mesh, respectively. The enhanced light treatment (112%) was achieved using a single layer of 6 mm pore diameter neutral density screening. Within each light treatment, coral fragments were arranged along four rows (one for each brine shrimp treatment) of five columns (one of each genotype) (Fig. 4A). Fragments were rotated daily within respective light treatments to avoid positional effects (the bottom row was moved to the top, and the first column moved to the end of the row). Light treatments were rotated weekly to avoid positional effects within the tanks. Fragments were never rotated between tanks. All manipulations were made within but not between tanks.

Every evening, the screens were removed and feeding chambers were placed within the tanks. All fragments in the zero, low, medium, and high brine shrimp concentration feeding treatments were placed in the first, second, third, and fourth feeding chambers, respectively and allowed to acclimate under a low flow regime for 1 h (Fig. 4B). Several feeding trials revealed that corals fed optimally when water flow through the tanks was reduced to a minimum. To prevent brine shrimp from escaping, feeding chambers were constructed of 81-cm lengths of 16-cm-diameter polyvinylchloride piping fitted with 50- μm Nitex mesh on both ends. Corals were fed 2-day-old brine shrimp nauplii (50 μm –2 mm in size) each evening at dusk for 2 h according to the protocol outlined below. Although corals do not feed on brine shrimp in the wild, brine shrimp nauplii were selected as the heterotrophic food source because they could be easily and reliably cultured with a relatively consistent carbon isotopic signature. Such consistency in the food supply is not possible with natural zooplankton. After the feeding session, coral fragments were returned to their respective light treatments, the mesh covers replaced on the tanks, and the water flow was turned back up.

Brine shrimp cysts were hatched in 750 mL of aerated seawater in three inverted Erlenmeyer flasks for 2 d. Brine shrimp greater than 50 μm were selected by pouring the stock through a 50- μm filter, then

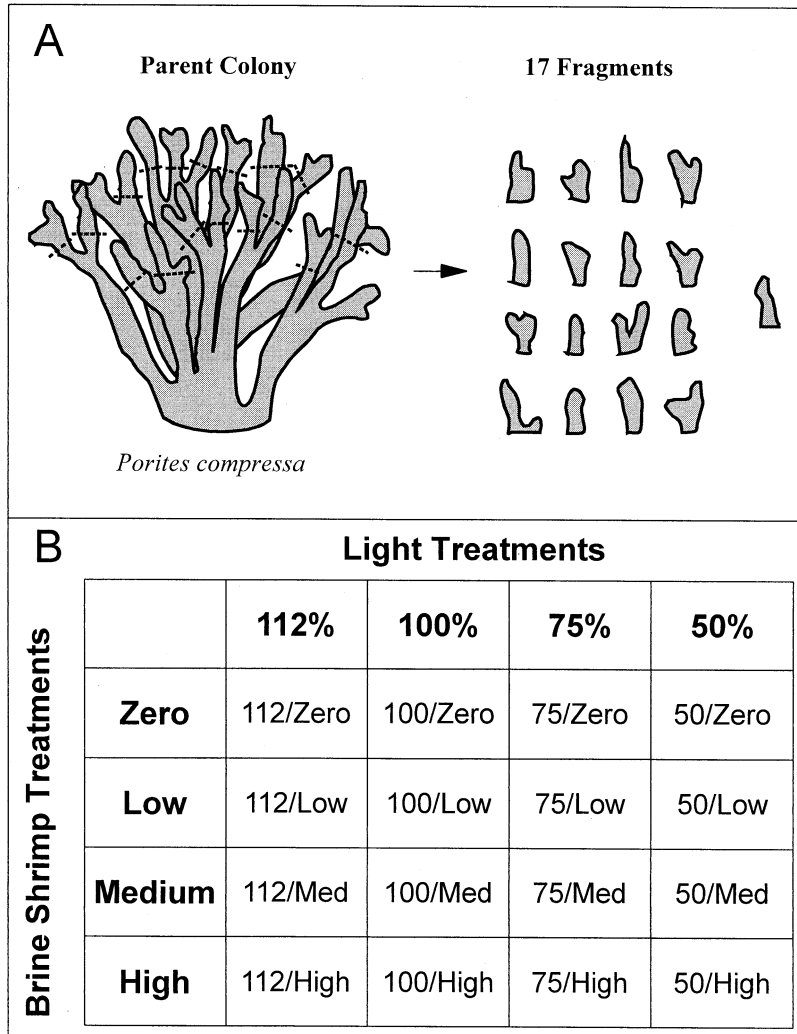


Fig. 3. Schematic representation of (A) coral collection method and (B) experimental treatments.

adding the isolated brine shrimp to 2 L of seawater to make the feeding stock. 0 mL, 50 mL, 230 mL, and 750 mL of brine shrimp stock (9.05 ± 0.57 brine shrimp/mL, $n = 42$) were added to the zero, low, medium, and high brine shrimp concentration feeding chambers, respectively. Because the volume of each chamber was 13.42 L, this yielded brine shrimp concentrations of 0, 0.73, 3.42, and 11.12 brine shrimp/mL in the zero, low, medium, and high treatments, respectively. Several feeding trials revealed that these concentrations resulted in feeding rates equivalent to 16%, 66%, and 100% in the low, medium, and high brine shrimp treatments (see Grottoli-Everett, 1998 for feeding trial details). A feeding rate of 100% meant that every polyp had at least one brine shrimp in its gut after 2 h of feeding.

Coral fragments were grown in these treatments from August 8 to October 4, 1996. All fragments were stained with Alizarin Red on August 8 (to mark the beginning of the experiment) and on October 4, 1996. Corals were kept in the tanks until November 16, 1996, then transplanted back onto the reef at 2.0 m depth where they continued to deposit skeleton beyond the last stain line until March 1, 1997. Several millimeters of skeleton was deposited beyond the October 4 stain line and little or no tissue was present in the skeleton representing the experimental period of August 8 to October 4, 1996. Thus, any possibility of tissue contamination in the skeleton that accreted during the experiment was minimized.

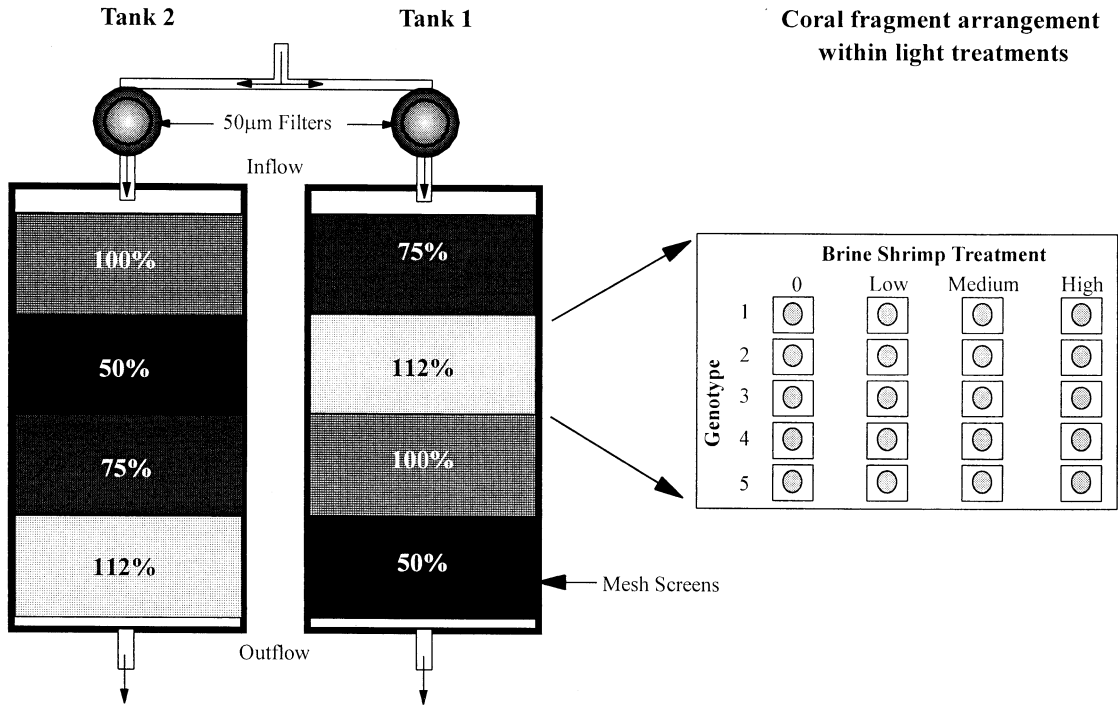
2.5. Sample Processing and Isotopic Analyses

2.5.1. Coral skeleton samples

All coral fragments were cleaned of tissue using high-pressure tap water and allowed to dry at room temperature for at least 5 d. Fragments were dry-cut in half along the major axis of growth using a circular diamond-tipped 3-mm-thick masonry blade. The cut was made slightly off-center to compensate for the thickness of the blade. This technique produced: (1) one fragment half with a face directly along the major axis of growth which was used for sample extraction and isotope analysis, and (2) one smaller fragment half which was discarded. Cut fragments were rinsed with high-pressure tap water followed by 3 min of sonication in distilled water to clean the coral surface of dust. The fragments were then dried in an oven at 60°C for 48 h. This low temperature does not affect the isotopic composition of the skeletal material (D. Mucciarone, personal communications).

The maximum linear skeletal extension (MLSE) was recorded as the distance between the two stain lines along the major axis of growth. Skeletal material was extracted for isotopic measurements by drilling a 1-mm-deep, homogenized bulk sample between the two stain lines of each coral fragment (representing growth from August 8 to October 4, 1996) using a 1-mm diamond-tipped dental bit. The skeleton material was consistently sampled along the major axis of growth. Entire bulk

A. DAYTIME AND NON-FEEDING SETUP



B. EVENING FEEDING SETUP

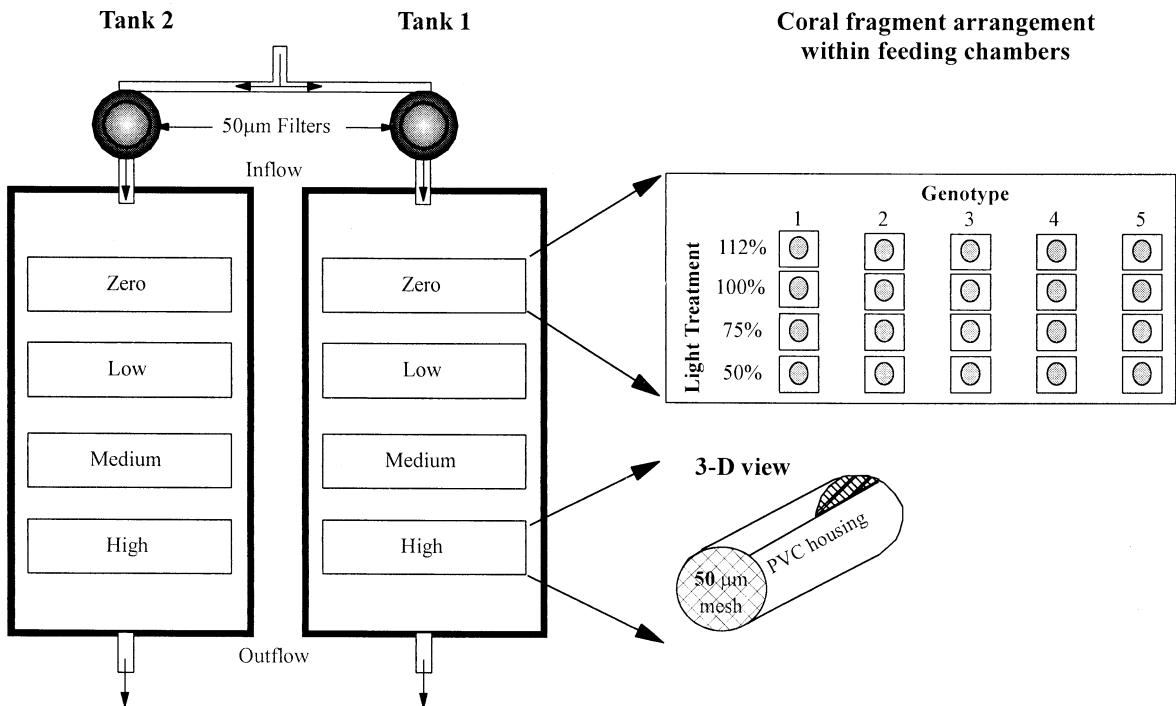


Fig. 4. Schematic aerial view of tank setup: (A) treatments and coral fragments were placed in this arrangement during the day and nonfeeding nighttime, (B) treatments and coral fragments were placed in this arrangement during evening feeding. Gray circles in small squares represent coral fragment mounted on ceramic tile.

skeletal samples were analyzed for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ with an automated carbonate device by completely dissolving samples individually in 100% H_3PO_4 (common acid bath @ 90°C), then analyzing the resulting CO_2 gas with a Finnigan-MAT 251 gas ratio mass spectrometer. The analytical precision was calculated from replicate analyses of an internal laboratory calcite standard (0.02‰ for $\delta^{13}\text{C}$ and 0.03‰ for $\delta^{18}\text{O}$). Data were corrected for the usual isobaric interferences using the equations of Craig (1957) modified for a triple collector mass spectrometer. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were reported relative to the Vienna-Pee Dee belemnite (V-PDB) standard ($\delta^{13}\text{C} =$ per mil deviation of ratio of the stable carbon isotopes $^{13}\text{C}:^{12}\text{C}$ relative to V-PDB; $\delta^{18}\text{O} =$ per mil deviation of the ratio of the stable oxygen isotopes $^{18}\text{O}:^{16}\text{O}$ relative to V-PDB).

2.5.2. Seawater and brine shrimp isotopes

The isotopic composition of the tank seawater dissolved inorganic carbon ($\delta^{13}\text{C}_{\text{DIC}}$) and brine shrimp stock was monitored weekly. Although $\delta^{13}\text{C}_{\text{DIC}}$ in seawater is known to vary over time (Swart et al., 1996), the effect was consistent across all treatments and therefore did not interfere with the interpretation of the skeletal $\delta^{13}\text{C}$ data. The same criterion applies to any variation in the brine shrimp isotopic composition over the course of the experiment. Nevertheless, seawater and brine shrimp samples were collected weekly to determine their mean relative contribution to skeletal $\delta^{13}\text{C}$ values.

Throughout the experiment, a 25-mL seawater sample was collected weekly from the inflow pipes of each tank for $\delta^{13}\text{C}_{\text{DIC}}$ analysis. Samples were fixed with a few grains of mercuric chloride and refrigerated. $\delta^{13}\text{C}_{\text{DIC}}$ was measured by reacting each water sample individually with H_3PO_4 acid under vacuum and analyzing the resulting CO_2 gas with a Finnigan-MAT 251 gas ratio mass spectrometer. The $\delta^{13}\text{C}_{\text{DIC}}$ values are reported relative to V-PDB with analytical error of ca. 0.1‰. A sample of stock brine shrimp was also collected each week. Brine shrimp were isolated by vacuum filtering approximately 100 mL of brine shrimp stock through a 2.5-cm-diameter glass fiber filter. The filter and brine shrimp were then dried for 24 h in a 60°C oven, wrapped individually in aluminum foil, and frozen. The isotopic composition of the brine shrimp organic tissue ($\delta^{13}\text{C}_{\text{ORG}}$) was determined by combusting the filter and brine shrimp sample, then analyzing the resulting CO_2 gas with a Europa 20-20 attached to an ANCA-GSL (Gas-Solid-Liquid) (precision of $\pm 0.08\text{‰}$ for $\delta^{13}\text{C}_{\text{ORG}}$).

2.6. Statistical Analyses

$\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and MLSE data sets were tested for the presence of outliers using Boxplot statistics. Within a data set, outliers were identified as data points that were more than 1.5 interquartile ranges above or below the first and third quartile (equivalent to the 1st and 99th percentile) and were removed from their respective data set. All three data sets were then tested for normality using a Shapiro-Wilk's test and statistically evaluated individually using a 3-way, Model III analysis of

Table 1. Shapiro-Wilk's test for normality of $\delta^{13}\text{C}$, C, $\delta^{18}\text{O}$, and maximum linear skeletal extension (MLSE) residuals (residuals = value – mean/cell as described in Zar, 1984).

	$\delta^{13}\text{C}$ ($n = 141$)	$\delta^{18}\text{O}$ ($n = 142$)	MLSE ($n = 142$)
W	0.986	0.989	0.983
$p < W$	0.178	0.343	0.080

W is the Wilk's statistic and ranges from 0 (non-normal distribution) to 1 (normal distribution). $p < W$ of 0.05 or greater indicates the data are normally distributed.

variance (ANOVA). A posteriori Tukey tests were used to determine which treatments significantly differed from each other. A posteriori linear and quadratic function analyses were also performed. Significant differences between controls and the 100% ambient light with zero brine shrimp treatment (most similar to field conditions) were evaluated with paired t -tests. Statistical computations were done using Statistical Analysis Systems Univariate, General Linear Model (GLM), and t -test programs (SAS Version 8, SAS Institute Inc., Cary, NC). Null hypotheses were rejected when the probability level was less than 0.05.

3. RESULTS

One hundred and sixty fragments of *Porites compressa* were reared in the experiment (4 light levels \times 4 brine shrimp levels \times 10 replicates). Sixteen of the fragments were excluded because they had indistinguishable stain lines. $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and MLSE measurements were made on the remaining 144 fragments. $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and MLSE data sets were individually tested for outliers. Seven individual data points were identified as outliers (number of outliers in each data set: $\delta^{13}\text{C} = 3$, $\delta^{18}\text{O} = 2$, and MLSE = 2) and removed from their respective data set. All three data sets ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and MLSE) were normally distributed (Table 1).

3.1. Tank Conditions

Between the two tanks, there were no significant differences in flow settings, flow rates over the course of a 12-h period (as measured by plaster of Paris clod card dissolution), nor in temperature (Table 2). There were no significant differences in temperature at the inflow and outflow ends within either tank (Table 2). Finally, there were no significant differences be-

Table 2. Comparison of flow rate and temperature among tanks, temperature within tanks, and temperature between the tanks and the reef.

Among tanks	Tank 1	Tank 2	df	t -value	$p <$
Flow setting, L/S	0.37 (0.03)	0.37 (0.03)	32	0.041	0.97
Flow rate	2.65 (0.13)	2.48 (0.07)	18	1.124	0.28
Temp (°C)	28.61 (0.14)	28.63 (0.14)	46	0.099	0.94
Within tanks	Top	End	df	t -value	$p <$
TST ₁ (°C)	28.61 (0.14)	28.51 (0.12)	42	0.510	0.58
TST ₂ (°C)	28.63 (0.14)	28.45 (0.13)	42	0.910	0.35
Tank 1 vs. RF	Tank 1	RF	df	t -value	$p <$
Temp (°C)	28.61 (0.14)	28.78 (0.23)	46	0.625	0.55
Tank 2 vs. RF	Tank 2	RF	df	t -value	$p <$
Temp (°C)	28.63 (0.14)	28.78 (0.23)	46	0.551	0.59

Flow rate = change in clod card dry weight (g) after 12 h dissolution; Temp = temperature; TST₁ = tank 1 seawater temperature; TST₂ = tank 2 seawater temperature; RF = reef flat; df = degrees of freedom.

All values are reported as means (\pm 1SE).

Table 3. Three-way model III ANOVA of light, brine shrimp and tank effects on skeletal $\delta^{13}\text{C}$, maximum linear extension, and $\delta^{18}\text{O}$ in the coral *Porites compressa*.

Source	DF	$\delta^{13}\text{C}$			Max. linear extension			$\delta^{18}\text{O}$		
		SS	F	Prob >F	SS	F	Prob >F	SS	F	Prob >F
Model	31	23.713	4.28	<0.0001	62.280	2.22	0.0014	4.631	2.57	0.0002
Light	3	4.303	8.03	<0.0001	8.164	3.00	0.0336	0.251	1.44	0.2345
Brine Shrimp	3	1.895	3.54	0.0172	14.651	5.39	0.0017	0.023	0.13	0.9406
Tank	1	14.187	79.45	<0.0001	11.107	12.26	0.0007	3.226	55.54	<0.0001
Light*BS	9	1.325	0.82	0.5952	9.205	1.13	0.3489	0.372	0.71	0.6971
Light*Tank	3	0.599	1.12	0.3448	1.1223	0.41	0.7439	0.445	2.55	0.0593
BS*Tank	3	0.2659	0.50	0.6859	3.402	1.25	0.2947	0.081	0.47	0.7066
Light*BS*Tank	9	1.4777	0.92	0.5113	13.304	1.63	0.1150	0.307	0.59	0.8051

Overall $\delta^{13}\text{C}$ model $r^2 = 0.55$, $n = 141$; Maximum linear skeletal extension model $r^2 = 0.38$, $n = 142$; $\delta^{18}\text{O}$ model $r^2 = 0.42$, $n = 142$. $F = F$ ratio; DF = degrees of freedom; SS = sum of squares; BS = brine shrimp.

tween either tank's seawater temperature (TST) and SST as measured on the reef flat adjacent to the tanks (Table 2).

3.2. Effect of Light and Brine Shrimp on Skeletal $\delta^{13}\text{C}$

A 3-way Model III ANOVA of skeletal $\delta^{13}\text{C}$ values indicated significant light and brine shrimp effects with no significant interaction effects (Table 3). Skeletal $\delta^{13}\text{C}$ values decreased significantly with decreases in light levels (Fig. 5A). A posteriori Tukey tests showed that the 50% light treatment differed significantly from the other light treatments (Fig. 5A). Further a posteriori analyses of all of the raw data revealed an additional significant overall quadratic relationship between $\delta^{13}\text{C}$ and light [$\delta^{13}\text{C} = 0.055$ (%light) - 0.0003 (%light)² - 5.084, $n = 141$, (df = 2, 139), $F = 8.03$, $p < 0.005$, $r^2 = 0.10$]. Independent analyses of the individual quadratic [$F = 7.09$, $n = 141$, (df = 1, 139), $p < 0.01$] and cubic [$F = 0.46$, $n = 141$, (df = 1, 139), $p > 0.5$] components of the model revealed only a significant quadratic component. The significance of the overall quadratic model confirms this. Within all brine shrimp feeding treatments, the quadratic response curves of the $\delta^{13}\text{C}$ across light treatments were not statistically different [$F = 1.39$ (df = 6, 129), $n = 141$, $p > 0.5$] (Fig. 5B).

Skeletal $\delta^{13}\text{C}$ values increased significantly with increases in brine shrimp feeding concentrations (Fig. 5C). A posteriori Tukey tests revealed that no feeding treatments significantly differed from one another. The response of all of the raw data was significantly linear [$\delta^{13}\text{C} = 0.086$ (brine shrimp) - 2.932, $n = 141$, $p < 0.042$, $r^2 = 0.03$, where brine shrimp concentrations of zero through high are represented by 0-3 in the linear equation] (Fig. 5C). Within the 112%, 100%, 75%, and 50% light treatments, only the slope within the 50% light treatment significantly differed from the others [$F = 5.33$, (df = 3, 133), $n = 141$, $p < 0.005$] (Fig. 5D).

3.3. Effect of Light and Brine Shrimp on MLSE

A 3-way Model III ANOVA of MLSE indicated significant light and brine shrimp effects (Table 3). MLSE decreased as light levels increased (Fig. 6A) and as brine shrimp concentrations increased (Fig. 6B). No significant interactions were detected (Table 3). A posteriori Tukey tests showed that MLSE in the 50% light treatment was significantly higher than the

MLSE in 100% and 112% light treatments, and that MLSE in the zero brine shrimp treatment was significantly higher than in the medium and high brine shrimp treatments (Fig. 6B). A posteriori analyses revealed a significant negative correlation between MLSE and light treatments [MLSE = -0.0118 (%light) + 4.387, $n = 142$, $r^2 = 0.07$, $p < 0.0015$] and between MLSE and brine shrimp feeding concentrations [MLSE = -0.253 (brine shrimp) + 3.742, $n = 142$, $r^2 = 0.07$, $p < 0.0017$, where brine shrimp treatment levels of 0-3 in the linear equation represent zero through high treatments]. Additional quadratic components to the models were not significant.

3.4. Effect of Light and Brine Shrimp on Skeletal $\delta^{18}\text{O}$

Because oxygen isotopes are not believed to be fractionated metabolically in corals, light and brine shrimp were not expected to have any significant effect on the skeletal $\delta^{18}\text{O}$ composition of *Porites compressa*. However, examination of each individual effect within the 3-way Model III ANOVA indicated a strong difference between tanks (Table 3). Because temperature did not differ between tanks (Table 2), the difference is most likely a result of coral genotype grouping (see "Discussion").

3.5. Controls

The effect of rearing corals in a tank versus the field was assessed by comparing the control fragments to the coral fragments reared in the tanks under 100% of ambient light with zero brine shrimp. It was assumed that the light and feeding regime of this treatment would most resemble the natural conditions on the reef. Paired t -test detected significant differences in $\delta^{13}\text{C}$ and MLSE between controls and the 100% light with zero brine shrimp treatment ($t = 2.41$, df = 8, $p < 0.04$ and $t = 2.34$, df = 8, $p < 0.04$, respectively). $\delta^{18}\text{O}$ did not significantly differ between the controls and the 100% of ambient light with zero brine shrimp treatment ($t = 1.72$, df = 8, $p < 0.12$).

3.6. Isotopic Composition of Brine Shrimp and Seawater

The carbon isotopic composition of the organic fraction in brine shrimp ($\delta^{13}\text{C}_{\text{ORG}}$) used in the experiment averaged

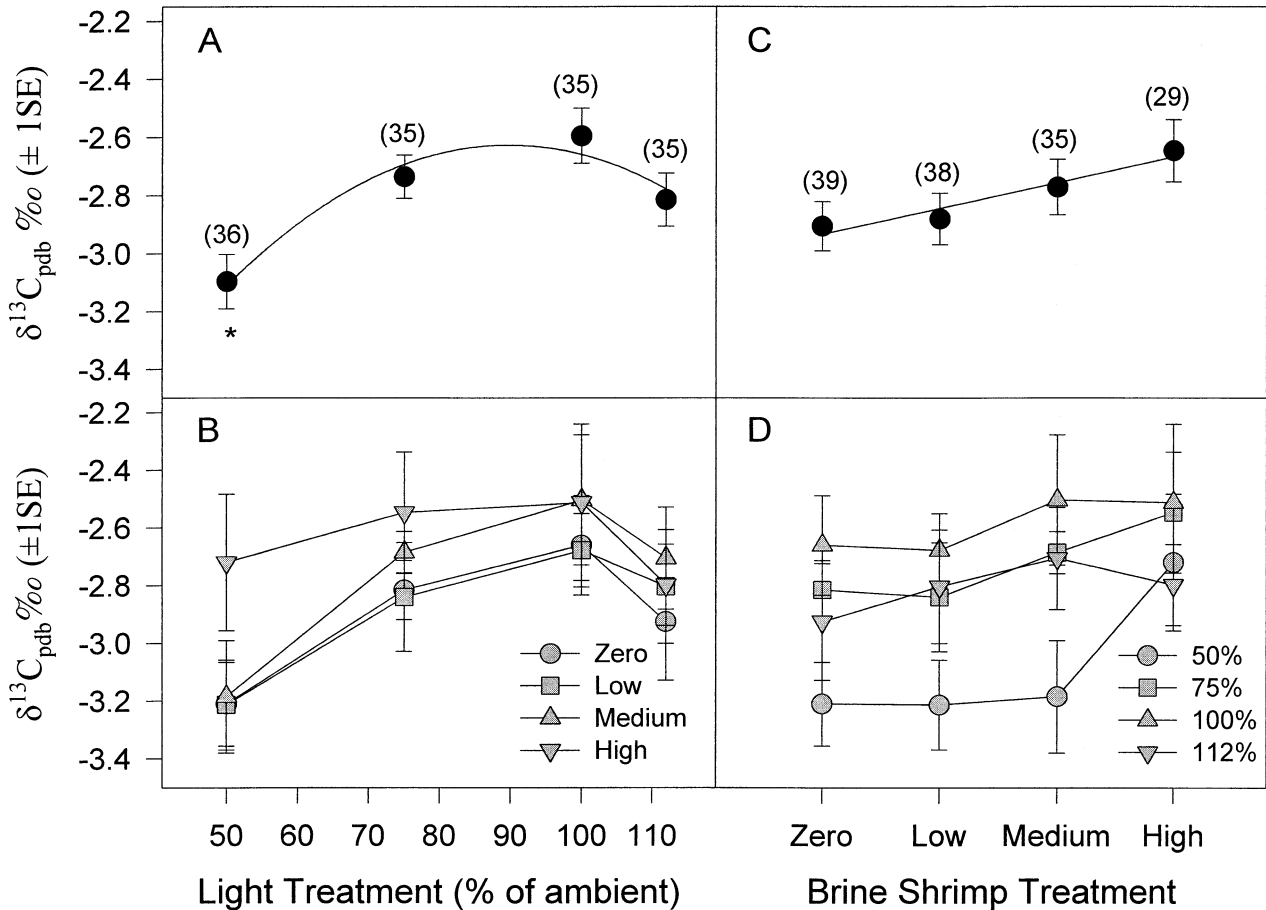


Fig. 5. The mean skeletal $\delta^{13}\text{C}$ in *Porites compressa* reflecting changes in light and brine shrimp concentrations. In all cases, means were calculated using pooled data from both tanks and error bars represent $\pm 1\text{SE}$ of each mean plotted. (A) Mean skeletal $\delta^{13}\text{C}$ for each light treatment. Sample size of each mean is given in brackets. Light has a significant effect on skeletal $\delta^{13}\text{C}$ (3-way Model III ANOVA). Asterisk indicates that the 50% light treatment significantly differs from the others according to the a posteriori Tukey test. Solid line represents the best-fit quadratic equation for all 141 raw data points (raw data not shown) where $\delta^{13}\text{C} = 0.055(\% \text{light}) - 0.0003 (\% \text{light})^2 - 5.08$. (B) Within brine shrimp treatments, mean skeletal $\delta^{13}\text{C}$ for each light treatment. The quadratic response curves do not significantly differ from each other. (C) Mean skeletal $\delta^{13}\text{C}$ for each brine shrimp treatment. Brine shrimp feeding had a significant effect on skeletal $\delta^{13}\text{C}$ (3-way Model III ANOVA). Sample sizes are in brackets above each mean. Solid line represents the best-fit linear equation of all 141 raw data points (raw data not shown) where $\delta^{13}\text{C} = 0.086 (\text{brine shrimp}) - 2.932$. Brine shrimp treatment levels of 0–3 in the linear equation represent zero through high treatments, respectively. (D) Within light treatments, mean skeletal $\delta^{13}\text{C}$ for each brine shrimp treatment. The slope of the linear response in the 50% light treatment significantly differed from the other slopes. For (B) and (D) lines are drawn for visual purposes only and sample sizes of the means ranges from 7 to 10.

$-20.29\% \pm 0.12$ (1SE) ($n = 13$) with a range of -20.95% to -19.53% . The isotopic composition of brine shrimp was very consistent throughout the experiment. Tank seawater samples collected in 1996 were improperly fixed and unusable for DIC measurements. Seawater samples collected weekly over the same time period in 1997 had average DIC $\delta^{13}\text{C}$ values of $0.55\% \pm 0.10$ (1SE) ($n = 9$).

3.7. The Tank Effect

A significant difference in $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and MLSE among tanks was detected (Table 3). The response in $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and MLSE to both changes in light and brine shrimp concentrations was similar in both tanks with a slight and consistent offset (as indicated by the lack of any significant interaction terms, Table

3). Because none of the measured environmental variables differed between tanks (Table 2) and the seawater in both tanks came from the same source and was equally filtered, differences between tanks are not likely attributable to physical factors associated with the tanks themselves. Genotypic differences between the corals assigned to each tank are probably responsible for the measured tank effect. Corals were sequentially identified and labeled 1 through 10 along a horizontal 30-m transect at 2 m depth on the reef. Flow rates measured along the first 5 m of the transect were significantly higher by 44% than at the end of the transect (measured at approximately 25 m) (t -test $t = 4.40$, $df = 8$, $p < 0.01$). Corals of genotype 1 through 5 assigned to tank 1, and genotypes 6 through 10 assigned to tank 2, were from different flow regions centered

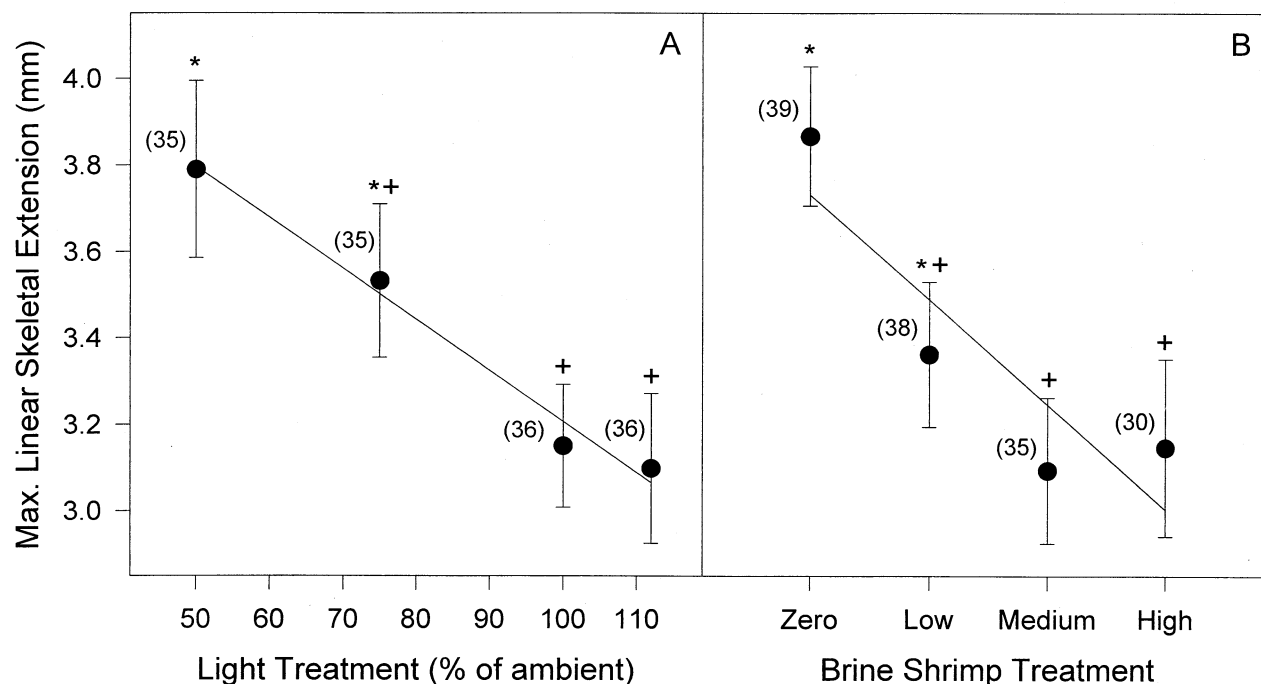


Fig. 6. MLSE of *Porites compressa*. (A) Mean MLSE for each light treatment. Light had a significant effect on MLSE (3-way Model III ANOVA). Solid line represents the best-fit linear equation of all 142 raw data points (raw data not shown) where $MLSE = -0.0118 (\%light) + 4.387$. (B) Mean MLSE for each brine shrimp treatment. Brine shrimp feeding had a significant effect on MLSE (3-way Model III ANOVA). Solid line represents the best-fit linear equation of all 142 raw data points (raw data not shown) where $MLSE = -0.2531(brine\ shrimp) + 3.742$. Brine shrimp treatment levels of 0–3 in the linear equation represent zero through high treatments, respectively. Within (A) and (B), means were calculated using pooled data from both tanks and error bars represent $\pm 1SE$ of each mean plotted. Different symbols (* or +) above the error bar indicate significant differences among means as determined by a posteriori Tukey tests. Sample sizes of each mean are indicated in brackets.

approximately around 8 m and 20 m from the beginning of the transect, respectively.

Given the genotypic composition of each tank and the consistent lack of any differences in measured physical factors among the tanks, it is likely that the significant tank effect on $\delta^{13}C$, $\delta^{18}O$, and MLSE is largely a result of the genotypic composition of each tank. These results highlight the importance of genotypic differences among corals of the same species and how genotype can affect skeletal $\delta^{13}C$, $\delta^{18}O$, and MLSE. Nevertheless, because the trends in both tanks were always in the same direction, the overall interpretation of the data remains unchanged.

4. DISCUSSION

Carbon isotopic composition in reef corals is believed to be predominantly affected by metabolic fractionation (McConnaughey, 1989a, b; McConnaughey et al., 1997; Muscatine et al., 1989; Grottoli and Wellington, 1999). Therefore, the magnitude of physiologic processes such as photosynthesis (light-driven) and heterotrophy may be predicted to alter the skeletal carbon isotopic composition. Results from the manipulative field experiment by Grottoli and Wellington (1999) confirm that both light and zooplankton contribute significantly to the skeletal $\delta^{13}C$ composition. However, their experiment only examined the effect of excluding zooplankton larger than 95

μm and reducing ambient light to 5%. In the present study, the effects of intermediate changes in light, increased light, and increases in zooplankton were addressed experimentally. By growing corals under controlled tank conditions and manipulating only light and brine shrimp levels, the individual as well as the interactive effects of both variables on skeletal $\delta^{13}C$ could be quantitatively assessed. Skeletal carbon isotopes may also vary due to changes in the isotopic composition of (1) DIC in the seawater (Swart et al., 1996), (2) dissolved organic matter and bacteria (Muscatine and Porter, 1977; Sorokin, 1973), or (3) spawning (Gagan et al., 1994, 1996). However, these factors were constant across all treatments and therefore did not interfere with the interpretation of the present data.

4.1. Effect of Light on Skeletal $\delta^{13}C$

Skeletal $\delta^{13}C$ values in tank-reared *Porites compressa* corals significantly decreased as light levels decreased. An a posteriori Tukey test showed that the mean $\delta^{13}C$ in the 50% light treatment differed significantly from the other light treatment means (Fig. 5A). This is consistent with the hypothesis that skeletal $\delta^{13}C$ decreases as light levels decrease and with the results reported by Grottoli and Wellington (1999). In addition, the response was significantly nonlinear. Skeletal $\delta^{13}C$ values decreased as light levels were reduced from 100%

and when light levels were increased to 112% (Fig. 5A). The quadratic equation fully describes the relationship between $\delta^{13}\text{C}$ and light in *P. compressa*. This response lends support to the hypothesis that light levels above normal lead to photoinhibition (decreased photosynthesis) (Barnes and Taylor, 1973; Erez, 1978; Huston, 1985; Falkowski et al., 1993) and decreases in skeletal $\delta^{13}\text{C}$ values. The decrease in mean sunlight intensity recorded in situ at 2 m depth on the fringing reef of Coconut Island from August to November 1996 is directly correlated with increases in cloud cover during the fall rainy season and with decreases in coral mean monthly skeletal $\delta^{13}\text{C}$ observed in field-collected *Porites compressa* (Grottoli, 2000). The combined results of both the field and tank observations support the hypothesis that $\delta^{13}\text{C}$ should reflect seasonal changes in cloud cover. With current analytical precisions of 0.02‰ or lower, small changes in $\delta^{13}\text{C}$ are easily detectable. Therefore, mean seasonal changes in cloud cover could have a significant and detectable effect on the mean skeletal $\delta^{13}\text{C}$ of corals.

The overall quadratic response in $\delta^{13}\text{C}$ to changes in light intensity was observed within all of the brine shrimp treatments (Fig. 5B). Though not a statistically significant departure, it is interesting to observe that within the high brine shrimp treatment, $\delta^{13}\text{C}$ did not vary as much between light treatments. Under 50% light, $\delta^{13}\text{C}$ in the high brine shrimp treatments was greater than in the other brine shrimp treatments (though not significantly: 1-way ANOVA $F = 1.63$, $p < 0.20$, $n = 36$). Under low light levels with high feeding, decreases in $\delta^{13}\text{C}$ appeared to be offset by the increasing $\delta^{13}\text{C}$ effect of high brine shrimp levels (see below) (Fig. 5B). This offset between light and heterotrophic effects was also observed in *Pavona* spp. by Grottoli and Wellington (1999).

4.2. Effect of Brine Shrimp on Skeletal $\delta^{13}\text{C}$

Skeletal $\delta^{13}\text{C}$ in tank-reared *Porites compressa* corals significantly increased as brine shrimp concentrations increased (Table 3, Fig. 5C). Mean $\delta^{13}\text{C}$ level increased by 0.25‰ between the zero and high brine shrimp feeding treatment. Within each of the 50%, 75%, 100%, and 112% light treatments, an increase in skeletal $\delta^{13}\text{C}$ values with increasing brine shrimp concentrations was also observed (Fig. 5D). These results were not consistent with the second hypothesis. Grottoli and Wellington (1999) showed that in field-reared *Pavona* spp. corals feeding on naturally occurring zooplankton, skeletal $\delta^{13}\text{C}$ values decreased when coral were reared under ambient versus reduced zooplankton levels. Felis et al. (1998) also showed a decrease in coral skeletal $\delta^{13}\text{C}$ levels during upwelling periods with high zooplankton concentrations in the Red Sea. Based on both studies, it was hypothesized that increases in brine shrimp concentrations would result in decreases in skeletal $\delta^{13}\text{C}$ values. However, the opposite response was observed in the present study.

Weekly measurements of the carbon isotopic composition of the brine shrimp stock indicated that they had a low $\delta^{13}\text{C}$ composition [average $\delta^{13}\text{C}$ of $-20.29\text{‰} \pm 0.12$ (1SE)] compared to the skeletal $\delta^{13}\text{C}$ and therefore could not have caused the increase in skeletal $\delta^{13}\text{C}$. One possible explanation for the unexpected increase in skeletal $\delta^{13}\text{C}$ is that brine shrimp may be a rich source of nitrogen. Increases in nitrogen (as dissolved

nitrate, ammonia, or dissolved inorganic nitrogen) lead to increases in zooxanthellae density (Muscatine and Weiss, 1992; Falkowski et al., 1993; Cook et al., 1994; Marubini and Davies, 1996; Stimson, 1997; Titlyanov et al., 2000), increases in photosynthesis (Falkowski et al., 1993; Cook et al., 1994; Marubini and Davies, 1996), decreases in the amount of fixed carbon being translocated from zooxanthellae to host (Falkowski et al., 1993), and decreases of ~50% in skeletogenesis (Marubini and Davies, 1996). Furthermore, brine shrimp fed corals show increased carbon fixation rates and increased zooxanthellae concentrations equivalent to corals supplemented with ammonia, suggesting a high nitrogen concentration in brine shrimp (Cook et al., 1994; Titlyanov et al., 2000). It has been hypothesized that zooxanthellae concentrations in corals are nitrogen limited (Falkowski et al., 1984, 1993; Marubini and Davies, 1996). When additional nitrogen is supplied and zooxanthellae populations increase, the diffusion-limited supply of CO_2 from surrounding seawater is used preferentially by the enlarged zooxanthellae population for photosynthesis, thereby reducing the availability of dissolved inorganic carbon for calcification (Marubini and Davies, 1996). Under this scenario, one would predict skeletal $\delta^{13}\text{C}$ to increase as nitrogen supply increased (leading to increased photosynthesis and increases in $\delta^{13}\text{C}$ levels).

In the tank experiment, as brine shrimp feeding concentration increased, skeletal $\delta^{13}\text{C}$ levels increased and MLSE decreased (Table 3, Fig. 6B). It was noted that coral fragments in the high brine shrimp feeding treatment were darker brown in color than fragments in the zero brine shrimp treatment under similar light conditions. Also, the length and density of filamentous algae growing on the ceramic tile supporting coral fragments were visibly higher in the high brine shrimp treatments compared to the low brine shrimp treatment. Even though nitrogen levels in brine shrimp were not directly measured, the measured increase in skeletal $\delta^{13}\text{C}$ accompanied by the decrease in MLSE, observed darkening in color (implying increases in zooxanthellae density), and increased filamentous algal growth in the high brine shrimp treatment compared to the low brine shrimp treatment, supports the idea that the brine shrimp were serving as a supplementary source of nitrogen to the corals. Furthermore, these results suggest that heterotrophically acquired nutrients can play a large role in regulating zooxanthellae concentrations in coral hosts.

Why do increases in brine shrimp result in higher skeletal $\delta^{13}\text{C}$ values in tank-reared *Porites compressa*, while increases in zooplankton $>95 \mu\text{m}$ in size in the field-reared *Pavona* spp. corals result in lower skeletal $\delta^{13}\text{C}$? There are three possible hypotheses. First, the concentration of brine shrimp in the low feeding treatment (0.73 brine shrimp/mL) was still six times higher than the average concentration of naturally occurring zooplankton on the surrounding reef (mean number of zooplankton on the reef/mL = 0.122). Perhaps the nutrient enrichment effect of brine shrimp is only effective once heterotrophy passes a certain "nutrient threshold" (somewhere between the zero and low brine shrimp treatment concentrations). A second hypothesis is that naturally occurring zooplankton $>95 \mu\text{m}$ in size are a poor nitrogen source. If so, increases in zooplankton $>95 \mu\text{m}$ in the diet would not promote increases in zooxanthellae density and skeletal $\delta^{13}\text{C}$ values would decrease. However, the ratio of carbon to nitrogen in zooplankton collected on

the reef (mean C:N = 4.23 ± 0.26 [1SE], $n = 4$) and brine shrimp (mean C:N = 4.83 ± 0.45 [1SE], $n = 3$) are not significantly different ($F = 1.50$, $p < 0.28$, $n = 7$). If anything, zooplankton might have greater nitrogen content than brine shrimp. Third, coral physiologic responses to heterotrophic input may somehow be altered when reared in tanks versus field conditions. This is supported by the fact that skeletal $\delta^{13}\text{C}$ levels in the controls did significantly differ from the corresponding tank treatment (100% light with zero brine shrimp). However, the field control corals were exposed to unfiltered seawater that contained plankton larger than $50 \mu\text{m}$ and thus not perfect controls for the tank corals. Due to this limitation, it is not possible to say with certainty that the physiology of tank and field-reared corals is different, but one can only suggest this as a possibility.

It is clear that heterotrophy significantly influences coral skeletal $\delta^{13}\text{C}$ levels. In field-reared corals, increases in zooplankton resulted in decreases in $\delta^{13}\text{C}$ levels (Grottoli and Wellington, 1999). In tank-reared corals, increases in brine shrimp resulted in increases in $\delta^{13}\text{C}$ levels. Furla et al. (2000) have shown that in corals, 70–75% of the inorganic carbon used for skeletogenesis is derived from metabolic CO_2 . It is therefore not surprising that heterotrophy affects skeletal $\delta^{13}\text{C}$ levels. The difference in the effect of heterotrophic input on coral skeletal $\delta^{13}\text{C}$ levels in the field versus in tanks is most likely due to one or both of the following factors: (1) Under field conditions, zooplankton abundance is much lower than brine shrimp abundances under tank conditions. As such, field-reared corals feed on zooplankton below the “nutrient threshold.” (2) Corals reared under tank conditions physiologically may respond differently to heterotrophic input than do field-reared corals.

Changes in heterotrophic input can be viewed as a source effect on skeletal $\delta^{13}\text{C}$. Changes in nutrient input affect the rate of photosynthesis (and carbon fractionation) and thus the amount of ^{13}C in the pool of carbon available for skeletogenesis. This can be viewed as a nutrient effect on skeletal $\delta^{13}\text{C}$. Because feeding intensity under natural field conditions was so much lower than in the tanks, it seems most likely that under natural field conditions, corals feed on zooplankton below the proposed “nutrient threshold.” Therefore, under natural conditions the $\delta^{13}\text{C}$ of coral skeletons appeared to be influenced predominantly by heterotrophy (a source effect). Under tank conditions the $\delta^{13}\text{C}$ of coral skeletons appeared to be affected primarily by nutrient-related effects and the corresponding changes in ^{13}C fractionation in the available carbon pool. Further research is needed to decouple the nutrient versus zooplankton effects on skeletal $\delta^{13}\text{C}$ in field- and tank-reared corals.

4.3. Effect of Light and Brine Shrimp on Maximum Linear Skeletal Extension

As skeletal $\delta^{13}\text{C}$ values increased with increasing light or brine shrimp, MLSE significantly decreased (Table 3, Fig. 6A,B). MLSE was negatively correlated with $\delta^{13}\text{C}$ [$\delta^{13}\text{C} = -0.89$ (MLSE) + 0.92, $n = 139$, $r^2 = 0.22$, $p < 0.0001$]. These negative relationships between MLSE and light, and MLSE and $\delta^{13}\text{C}$, were unexpected because previous observational data indicated that MLSE decreased as light levels de-

creased with changes in depth (Goreau, 1959; Goreau and Goreau, 1959; Highsmith, 1979; Huston, 1985; Logan and Tomascik, 1991; Klein et al., 1993; Meesters et al., 1994; Grottoli and Wellington, 1999; Marubini et al., 2001) and decreased with seasonal increases in cloud cover (Buddemeier, 1974; Glynn, 1977; Guzmán and Cortés, 1989; Grottoli and Wellington, 1999). Experimental evidence by Wellington (1982) showed significantly higher MLSE under ambient light compared to reduced light treatments for Panamanian *Pavona clavus*, *P. gigantea*, and *Pocillopora damicornis* corals. However, unlike many corals studied in the literature, *Porites compressa* in situ on the Hawaii study site reef do not exhibit a positive correlation between light and MLSE across depth. A survey of *P. compressa* across depth at two locations in Kaneohe Bay showed a nonsignificant peak in MLSE at mid-depth accompanied by a decrease in skeletal $\delta^{13}\text{C}$ values over the entire depth range (Grottoli, 1999). The negative correlation between light and MLSE in surveyed corals shallower than 8.3 m, coupled with a positive correlation between light and skeletal $\delta^{13}\text{C}$ detected in *P. compressa* surveyed in the field is consistent with the results of the tank-reared *P. compressa* fragments. Since the decreases in $\delta^{13}\text{C}$ with increasing depth and decreasing light are consistent irrespective of MLSE, there seems to be some evidence that skeletal extension rate and metabolic processes in corals might be decoupled. Furla et al. (2000) and Dubinsky and Berman-Frank (2001) have also reported data that suggest that skeletal and metabolic processes in corals are decoupled.

4.4. Implications for Coral Climate Records

Cloud cover plays a major role in the global heat budget yet is one of the primary unknowns in climate models. There is currently no mechanism for including long-term paleoclimate variability in cloud cover into global climate models. The development of a method for reconstructing relative changes in solar irradiance could be extremely useful. Stable carbon isotope records in coral skeletons represent a promising cloud-cover proxy with monthly resolution in tropical regions. So far, past research in combination with the present research suggests that skeletal $\delta^{13}\text{C}$ levels in shallow corals from nonupwelling regions reflect changes in solar intensity and may be optimally suited for recording paleoclimate variation in cloud cover. Further research is needed to fine-tune coral skeletal $\delta^{13}\text{C}$ as a cloud-cover proxy and to isolate the contribution of heterotrophy to $\delta^{13}\text{C}$ particularly in corals from upwelling regions.

In addition, the natural variability in $\delta^{13}\text{C}$ and MLSE between corals grown under the same environmental conditions (i.e., within the same treatment) is apparent in this study. The standard error about each mean plotted is a result of the variation that exists between different coral individuals within each treatment. These results reinforce the fact that different coral individuals of the same species can have slight differences in their $\delta^{13}\text{C}$ and MLSE values under identical conditions (see Wellington et al., 1996 for a full discussion of natural variability in $\delta^{18}\text{O}$). This has significant implications for paleoclimate reconstruction. When attempting to reconstruct climate from any coral proxy record, the natural variation that exists between corals can be minimized by measuring multiple

coral cores and averaging the proxy. The average proxy signal of many corals can enhance the rigor of climate interpretation.

4.5. Summary

This work represents the first attempt to experimentally examine the effects of variable light and heterotrophy on coral skeletal $\delta^{13}\text{C}$ under controlled tank conditions. The results show that $\delta^{13}\text{C}$ is significantly influenced by heterotrophy and light levels. As light levels decrease, photosynthesis decreases, metabolic fractionation increases, the carbon pool becomes relatively enriched in ^{12}C , and skeletal $\delta^{13}\text{C}$ values decrease. Decreases in light led to a statistical decrease in mean skeletal $\delta^{13}\text{C}$ in this study and in Grotto and Wellington (1999). In the absence of large fluctuations in heterotrophic input, mean changes in solar irradiance levels due to seasonal differences in cloud cover can be detectable in the coral skeletal $\delta^{13}\text{C}$ signature of some corals. For example, high-resolution analysis of the intra-annual variation in skeletal $\delta^{13}\text{C}$ composition of shallow *Porites compressa* corals in Hawaii indicates that skeletal $\delta^{13}\text{C}$ responds predominantly to seasonal changes in sunlight (Grotto, 1999). Our ability to acquire a paleo-record of solar irradiance levels can be optimized by choosing shallow corals from a nonupwelling region.

Coral skeletal $\delta^{13}\text{C}$ is significantly influenced by heterotrophic input. Increases in brine shrimp feeding concentration appear to enhance the nitrogen content of corals leading to enhanced zooxanthellae concentrations, increased photosynthesis, and increased $\delta^{13}\text{C}$ levels. This result highlights the potential influence of nutrients from heterotrophically acquired carbon in maintaining the zooxanthellae–coral symbiosis in balance. However, increases in zooplankton in field-reared corals resulted in decreases in skeletal $\delta^{13}\text{C}$ levels (Grotto and Wellington, 1999). The interesting differences between the tank and field experiment support the idea that carbon isotopic composition in coral skeletons is highly influenced by metabolic fractionation. Further research is needed to decouple the nutrient versus zooplankton effect on skeletal $\delta^{13}\text{C}$. Due to the complexity associated with nutrients and heterotrophy, the $\delta^{13}\text{C}$ record from upwelling region corals is still difficult to resolve. Overall, this work increases our knowledge of how light and heterotrophy affect coral physiology and the $\delta^{13}\text{C}$ of coral skeletons.

Acknowledgments—Special thanks go to G. Wellington, E. Druffel, R. Dunbar, W. Ewens, and P. Jokiel for valuable discussion and comments on various aspects of this manuscript. Many thanks to N. Ostrom, F. Podosek, and three anonymous reviewers; field assistants C. Grotto and S. Lamont; dive buddies I. Kuffner, R. Sahaghian, K. Longenecker, K. Sherwood, J. Collier, and J. Fender; P. Jokiel and all the faculty, students, and staff at the Hawaii Institute of Marine Biology; P. Swart for his generosity with the isotope analyses; A. Saied and all the members of the Swart lab. I thank Alfred and Louise Grotto for their support and encouragement. This work complies with the current laws of the country in which it was performed and was supported primarily by the Environmental Protection Agency STAR Graduate Fellowship (U-914955-01-0) and the Environmental Institute of Houston, and also in part by Sigma Xi Student Research Grant, Seaspac Student Fellowship, and the Dreyfus Postdoctoral Fellowship in Environmental Chemistry.

Associate editor: N. E. Ostrom

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