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Growth of cultured giant clams (*Tridacna* spp.) in low pH, high-nutrient seawater: species-specific effects of substrate and supplemental feeding under acidification

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Four species of giant clams, Tridacna maxima, T. squamosa, T. derasa *and* T. crocea*, were cultured in outdoor raceways for 364 days at the Waikīkī Aquarium and the Oceanic Institute on the island of O'ahu, Hawai'i, USA. Growth of each species was compared among individuals grown with and without supplemental phytoplankton feeding, and directly on the substrate or mounted on concrete plugs in low pH, high nutrient seawater. Among clams cultured with and without supplemental phytoplankton (*Chaetoceros *spp.), feeding resulted in significantly lower mortality in all species but* T. deresa*, whereas growth was significantly higher among fed clams for all species except* T. squamosa. Tridacna derasa *showed roughly a three-fold increase in growth when fed (88.5 g ± 4.4 SD) than when unfed (26.0 g ± 2.1 SD), whereas* T. maxima *growth was substantially lower, but nearly 10-fold greater in response to feeding (9.0 g ± 1.9 SD). The overall mortality rate of juvenile clams was significantly lower in the fed (44.4 ± 10.0%) than the unfed (71.8 ± 9.6%) trials, with the greatest effect observed in mortality of* T. maxima *(fed 15% versus unfed 80%) and* T. squamosa *(fed 65% versus unfed 95%). None of the* T. squamosa *remained on concrete plugs for the duration of the experiment. Among the remaining three species, there was no difference in either wet weight or shell length for* T. maxima *and for wet weight only in* T. derasa *on (186.5 g ± 16.1 SD) and off (147.0 g ± 6.0 SD) the concrete plugs. In contrast,* T. crocea *had significantly greater shell growth off the plugs (14.3 mm ± 1.0 SD versus 8.5 mm ± 1.7 SD) but significantly greater gain in wet weight on the concrete plugs (26.3 g ± 1.5 SD versus 58.5 g ± 2.5 SD). The seawater wells used for this study are well characterized with elevated levels of inorganic nutrients and higher pCO_2 relative to tropical ocean waters, roughly approximating predictions for future oceanic conditions under IPCC IS92a emission scenarios. In comparison to previous studies in natural seawater,* T. derasa *had a significantly higher shell growth rate in the high-nutrient, low-pH well water. In contrast,* T. maxima *and* T. squamosa *had significantly lower growth rates in low pH, whereas growth of* T. crocea *was not significantly different between low pH and ambient seawater. These experiments demonstrate species-specific differences with each treatment, which cautions against making broad generalizations regarding the effects of substrate type, feeding effects, nutrient enrichment, and ocean acidification on tridacnid culture and survival.*

Keywords: biodiversity, ocean acidification, climate change, variable response, Hawaii, Waikīkī Aquarium, Oceanic Institute

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INTRODUCTION

Giant clams of the family Tridacnidae are the largest living bivalves, with the largest species, *Tridacna gigas* (Linnaeus, 1758), reaching a maximum recorded size of 137 cm and weighing as much as 340 kg (Delbeek & Sprung, 1994; Knop, 1996; Fartherree, 2006). Ten extant species are known, all found in oligotrophic coral reef environments throughout the South Pacific, Indian Ocean and Red Sea (Klumpp & Griffiths, 1994; Richter *et al.*, 2008; Othman *et al.*, 2010). Giant clams are unique among bivalves not only because of their relatively large size and rapid growth rate but also due to the presence of photosynthetic symbiotic algae, commonly known as zooxanthellae, found in their mantle tissue and which provide nutrients to the host (Muscatine, 1967; Fitt & Trench, 1981; Klumpp *et al.*, 1992). Over-harvesting of giant clams for food, ornaments and the aquarium trade combined with the impacts of increased coastal development and pollution have reduced the numbers of giant clams in the wild to levels where the International Union for Conservation of Nature (IUCN) has

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listed four of the ten species as 'Vulnerable.' The remaining species fall into the 'Conservation Dependent' category, and all *Tridacna* spp. are listed in Appendix II of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) according to the IUCN Red List of Threatened Species (IUCN.org, 2010.4).

Giant clams are both an economically and culturally important resource throughout the Pacific Islands, and have been harvested since early human occupation of the Red Sea 125,000 years ago (Richter *et al.*, 2008). Interest in farming giant clams began in the 1970s when it became apparent that global populations of clams were rapidly declining (Heslinga & Fitt, 1987; Tisdell *et al.*, 1993; Ellis, 1999). In response to the collapse and in some cases extirpation of local wild stocks of giant clams, government and commercial hatcheries have been developed in most tropical Pacific Island nations where giant clams occur naturally (Ellis, 1999; Fartherree, 2006). Though most hatcheries were initially developed with the intent of replenishing or enhancing local clam populations as a food source to relieve pressure on wild stocks, many have also adapted specifically to supply ornamental clams to the global aquarium trade. Advantages of culturing clams include that they command a higher individual price and can be sold at a smaller size though only the most colourful individuals are marketable in the trade. Farming giant clams for both the ornamental and food markets has proven to be an excellent income-generating opportunity for coastal populations throughout the Pacific Islands with relatively low investments needed for infrastructure and technology with reasonable capital gains (Tisdell *et al.*, 1993; Ellis, 1999).

Despite the presence of photosynthetic symbionts which provide photosynthetic carbon to the host, giant clams are also known to filter-feed to obtain major nutrients (carbon, nitrogen and phosphorus), particularly as juveniles (Trench *et al.*, 1981; Fisher *et al.*, 1985; Klumpp *et al.*, 1992). Giant clams can also absorb dissolved nutrients directly from seawater (such as NH_3, NH_4, NO_2, NO_3 and PO_4) and several studies have documented that increased dissolved inorganic nitrogen (DIN) levels typically enhanced both the population density of zooxanthellae in the mantle and the overall growth rate of giant clams in culture (e.g. Fitt *et al.*, 1993; Ambariyanto & Hoegh-Guldberg, 1997; Grice & Bell, 1999). In addition to nutrient enrichment, much of the recent aquarium literature emphasizes the importance of phytoplankton feeding to enhance the growth of cultured giant clams (e.g. Knop, 1996; Fartherree, 2006). Most aquarists rely on logic similar to that of Knop (1996), who argues that despite photosynthetic symbionts, the presence of a fully-developed intestinal tract implies a degree of dependence on filter feeding to survive, and intentionally supplement phytoplankton to enhance clam survival and growth. Supplemental phytoplankton feeding of tridacnids has been evaluated (Estacion *et al.*, 1986; Ellis, 1998); however, few giant clam aquaculturists intentionally supplement phytoplankton during clam culture, relying instead upon fertilizers such as ammonium nitrate to enhance growth (Heslinga *et al.*, 1990; Braley, 1992; Calumpong, 1992; Hastie *et al.*, 1992; Belda *et al.*, 1993).

The well water of the Waikīkī Aquarium is well-characterized as being high in inorganic nutrients, low in organic nutrients, and higher pCO_2 relative to tropical ocean surface waters (Atkinson *et al.*, 1995; Carlson, 1999). The Waikīkī Aquarium draws water from a 14 m deep seawater well adjacent to the beach and has a peak pCO_2 level of roughly 1500 μatm with input lines to the tanks delivering between 700 and 1400 μatm resulting in a typical pH range of 7.6 to 8.0 (Atkinson *et al.*, 1995). In addition to phosphate four times higher than typical seawater surrounding coral reef habitats, and nitrogen 10 to 20 times higher, the average pCO_2 value of 1100 μatm is of similar magnitude to the $3 \times CO_2$ IPCC IS92a emission scenarios (Leggett *et al.*, 1992; Guinotte & Fabry, 2008). Thus, the seawater well makes a natural model system for studying the organismal response of giant clams to the continued nutrient and CO_2 enrichment trends predicted for coral reef environments under a wide range of future climate change scenarios (Orr *et al.*, 2005; Hoegh-Guldberg *et al.*, 2007).

Given the importance of conserving giant clams in the wild as well as the cultural and economic significance of the clams to many Pacific and Indian Ocean communities, considerable interest in maximizing the survival and growth of clams in culture has developed (Ellis, 1999). Likewise, the impacts of ocean acidification on the calcification and growth rates of these important reef species are of considerable conservation interest. The habitat preferences of each species differs, with *Tridacna crocea* (Lamarck, 1819) having a large byssal orifice and a habit of boring itself into the carbonate substrate of the reef via chemical softening (Knop, 1996; Fartherree, 2006). They are almost exclusively found on living or dead coral or solid limestone substrate, where they are typically almost entirely encased with the upper shell margin nearly even with the surface of the hole they create in the solid substrate (Knop, 1996; Fartherree, 2006). *Tridacna squamosa* (Lamarck, 1819) in contrast is found on a wide variety of comparatively sheltered habitats, has weak byssal filaments and is usually nestled loosely amongst or adjacent to live corals (Knop, 1996; Fartherree, 2006). *Tridacna maxima* (Roding, 1798) is typically found on hard calcareous substrate where it also tends to bore, though not so deeply as *T. crocea* and employs a stronger byssal attachment than *T. squamosa* (Knop, 1996; Fartherree, 2006). Finally, *Tridacna derasa* (Roding, 1798) is the largest of these four species with only a narrow gap rather than a byssal orifice and is often found on sand or coral rubble areas adjacent to coral reefs (Knop, 1996; Fartherree, 2006) rather than nestled into hard substrate as with the other species discussed above. Thus, the purpose of this study was to determine the survival and growth rates of these four species of tridacnid clams in high-nutrient, low-pH seawater approximating IPCC IS92a ocean acidification predictions with and without supplemental phytoplankton feeding and on and off substrate plugs in raceway tanks.

MATERIALS AND METHODS

All four species of giant clams used for this study, *Tridacna maxima*, *T. squamosa*, *T. derasa* and *T. crocea*, were shipped from the Marshall Islands Mariculture Farm, Majuro, the Republic of the Marshall Islands. Clams were approximately 1 to 2 years old at the time of arrival, and were split among the Oceanic Institute and Waikīkī Aquarium in raceway tanks as outlined below.

Oceanic Institute—culture conditions

Juvenile clams were divided haphazardly among two identical raceways measuring 1.85 m wide ×3.65 m long ×30 cm deep,

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one of which would receive supplemental phytoplankton and the other would not. Each raceway contained a centre baffle, held approximately 825 l, and was supplied with flow-through seawater via a spray bar at a rate of roughly 7.5 l/minute for a turn-over time of roughly 1.8 hours. To enhance the clockwise circulation of water created by the orientation of the spray bar, each system was outfitted with two airlift systems to increase water motion and maintain uniform dissolved oxygen levels. Each raceway was exposed to full natural sunlight in the middle section where clams were placed. The ends of the raceways where no clams were maintained were covered with shade cloth to minimize algal growth and reduce solar heating of the tanks. The bottom of each raceway was covered with approximately 2.5 cm of live sand (fine natural coral rubble collected from the adjacent coast) to aid in nutrient processing (Toonen & Wee, 2005). Four convict surgeonfish (*Acanthurus triostegus*, ~7.5 cm), one raccoon butterflyfish (*Chaetodon lunula*, ~2.5 cm), one humpback cowrie (*Cypraea mauritiana*, ~10 cm) and two black sea cucumbers (*Holothuria atra*, ~25 cm) were also added to each raceway to aid with tank maintenance via algal control and detrital consumption. Initial daily maintenance included cleaning tank walls of algal growth, vacuuming detrital accumulation and gentle scrubbing of individual clams with a toothbrush. The systems stabilized within two months, at which point maintenance was reduced to twice weekly.

Waikīkī Aquarium—culture conditions

Rearing tanks used at the Waikīkī Aquarium were the same as those used at the Oceanic Institute. The bottom was also covered with 2.5 cm of live sand taken from an adjacent beach. The raceway held approximately 1400 l and water input and air lifts were oriented to generate constant circulation as outlined above. Seawater from the deep wells at the Waikīkī Aquarium flowed constantly into the tank at 17 l/minute for an approximate turn-over time of 1.4 hours. Grazers added to each tank included two sea cucumbers (*Holothuria atra*, ~20 cm), two sea urchins (*Tripneustes gratilla*, ~10 cm) and one convict surgeonfish (*Acanthrus triostegus*, ~7.5 cm). Tank maintenance was performed three times a week to control algal growth and detrital accumulation. Maintenance included vacuuming detritus from the sand, cleaning algae from the walls, and scrubbing each clam with a soft toothbrush to prevent algal growth.

Both light and water parameter measurements were recorded periodically throughout the experiment. Light levels were recorded periodically using a Li-1000 data logger and Li-COR spherical underwater PAR sensor. The water temperature was recorded using a calibrated HOBO temperature logger to record the maximum, minimum, and average daily temperature, and salinity was measured using a traditional handheld refractometer. Initial water tests from the well at the Waikīkī Aquarium were performed to confirm that well water parameters were unchanged from previous characterization (Atkinson *et al.*, 1995; Carlson, 1999). Because levels were within normal ranges at the start and end of the experiment, no significant changes in water quality parameters were assumed though routine monitoring of water quality was not performed during the experimental period. The well water of the Oceanic Institute has similarly high pCO_2 and is low in organic nutrients but the primary difference is that the well water at the Oceanic Institute has NO_2 and NO_3 below detectable levels, unlike the well water from the Waikīkī Aquarium.

Oceanic Institute—phytoplankton treatment

At the Oceanic Institute, the growth and survival of individuals both with and without supplemental phytoplankton feeding was examined. A total of 31 *T. crocea* (initial mean shell length 40.6 mm; initial mean wet weight 17.0 g), 32 *T. derasa* (37.3 mm; 8.3 g), 31 *T. maxima* (45.3 mm; 16.7 g), and 32 *T. squamosa* (53.9 mm; 36.2 g) were divided at random and placed haphazardly among two treatment raceways. One raceway was then selected at random for supplemental feeding with cultured phytoplankton (*Chaetoceros* spp.) and the other raceway without supplementation. *Chaetoceros* spp. was chosen because a consistent supply was cultured and maintained on site at the Oceanic Institute, and showed better survival rate than other species of microalgae and controls in previous studies of juvenile clam culture (Ambariyanto, 2004). Thus, a dose of 1.0 l of live *Chaetoceros* spp. (~100 million cells/ml) was administered daily to the treatment raceway via a drip line connected to a flask at ~1 ml/s.

Waikīkī Aquarium—concrete plug treatment

Habitat preferences vary among the clam species, and the plug treatment had two purposes. First, concrete plugs provide substratum for the clams to attach to so that they could be easily handled and moved during cleaning without disturbing attachment points and thereby minimize stress. Second, the substratum preferences of the clams in the wild might have a growth consequence in culture, so we randomly assigned the 32 *Tridacna crocea* (initial mean shell length 36.6 mm; initial mean wet weight 19.2 g), 32 *T. derasa* (36.5 mm; 8.6 g), 37 *T. maxima* (42.9 mm; 28.6 g) and 30 *T. squamosa* (57.5 mm; 33.4 g) to each treatment and grew half the individuals on and half the individuals off of concrete plugs. Because the juvenile clams off plugs typically attached to the tank bottom via byssal threads, the threads had to be broken each time a clam was removed from a raceway for cleaning and weighing. The energy cost for reattachment and repeated stress from detachment could presumably have a negative impact on growth and survival (Delbeek & Sprung, 1994; Fartherree, 2006).

To determine the impact of routine damage to the byssal threads during maintenance and growth measurements, half of each species of clam were allowed to attach to concrete plugs rather than being placed on the raceway bottom. Each clam was mounted on a circular, concrete plug roughly 4 cm in diameter and 1.5 cm high. Clams were randomly assigned to treatments and arranged haphazardly within each raceway. Each giant clam kept at the Waikīkī Aquarium was PIT tagged for identification with a Biomark TX 1400L attached with Z-spar Splash Zone Compound A-788. A Biomark Pocket Reader EX was used for scanning individual tags and ensuring that each measurement was associated with the correct individual.

Measuring wet weight and shell size

Growth was evaluated for all clams using both wet weight in grams and maximum valve length in millimetres. At each

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facility, clams were pat dried with a paper towel before being weighed using a digital electronic scale. To minimize handling stress on experimental animals, wet weights were taken every 4–6 weeks throughout the experimental period. Shell length (mm) was measured every two weeks at the widest point of the valve using research-grade calipers. Repeated measures of growth were taken throughout the experiment. The plots of growth were relatively linear; thus, with the exception of mean daily growth rate (average of the growth measured between each measurement period), only the initial and final weights and lengths were used in the statistical analyses presented herein.

Statistical analyses

Statistical analyses were performed in JMP ver.8 (SAS institute). Differences in survival among fed and unfed clams (Figure 1) were tested using an $r \times c$ contingency table. Differences in growth among fed and unfed clams (Figure 2) were tested with a 2-way analysis of variance (ANOVA) (feeding treatment by species). Differences in growth of clams on and off the concrete plugs (Figure 3) were also tested with 2-way ANOVA (plug treatment by species). Finally, the growth rate of clams in low pH, high nutrient well water used in our study was compared to growth rates in present day seawater by exact test and plotted against the 95% confidence intervals of previously published studies (Figure 4). Data were tested for homogeneity of variance using Bartlett's test, and for those samples with a significant deviation, a Welch approximation was used to accommodate the unequal variances in place of a standard ANOVA (Zar, 1984). *Post-hoc* comparisons of means were conducted using the Tukey's honestly significant difference (HSD) test (Zar, 1984).

RESULTS

Water quality, light and temperature

The seawater drawn from the well of the Waikīkī Aquarium has been characterized in detail previously when it was shown to remain stable for roughly 5 years (Atkinson *et al.*, 1995; Carlson, 1999). Initial water quality measurements of inorganic nutrients, PO_4 (0.72 μM), NO_3 (9.48 μM), $NO_2 + NO_3$ (10.7 μM), NH_4 (2.98 μM), pH (~7.7–7.8) and salinity (35.0 ppt) were not significantly different from previous measurements conducted more than a decade ago highlighting the stability of the Waikīkī well water (Atkinson *et al.*, 1995). Likewise, ambient light level and water temperatures were similar to previously reported values (Carlson, 1999). Light fluctuated similarly among tanks with the average daily peak typically occurring between 2000 μE/m²/s to 2500μE/m²/s, and the highest reading reaching a maximum of 3000 μE/m²/s (data not shown). Water temperature was also similar between tanks and was minimized by covering the ends of the raceways with PVC covers. The average water temperature was stable throughout the experiment, between 24 and 25°C, with a peak of 28.8°C when the covers were displaced for hurricane preparation and a minimum of 23.2°C (data not shown).

The well water at the Oceanic Institute was similar in temperature but differed slightly in salinity and inorganic nutrients

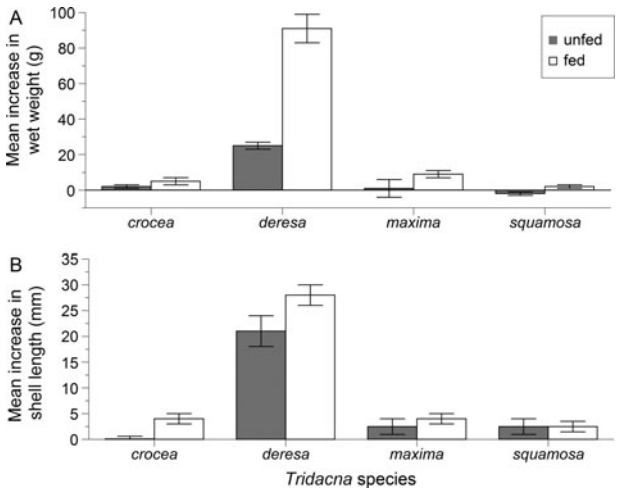

Fig. 2. Growth of giant clams (*Tridacna crocea*, *T. derasa*, *T. maxima* and *T. squamosa*) over 364 days of culture at the Oceanic Institute in fed and unfed trials: (A) wet weight (g); and (B) shell length (mm) of growth. Bars represent means ± standard errors for each treatment.

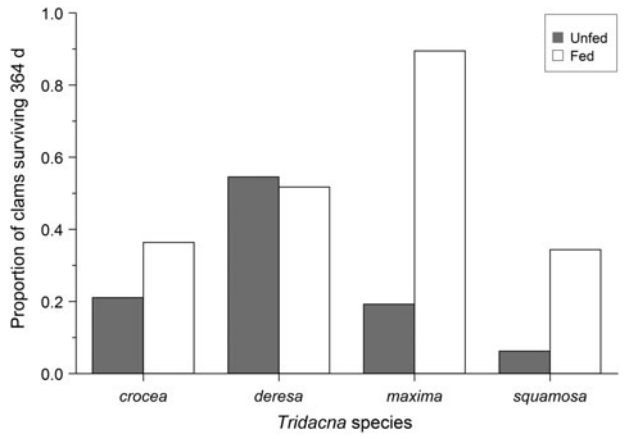

Fig. 1. Survival of giant clams (*Tridacna crocea*, *T. derasa*, *T. maxima* and *T. squamosa*) over 364 days of culture at the Oceanic Institute in fed and unfed trials.

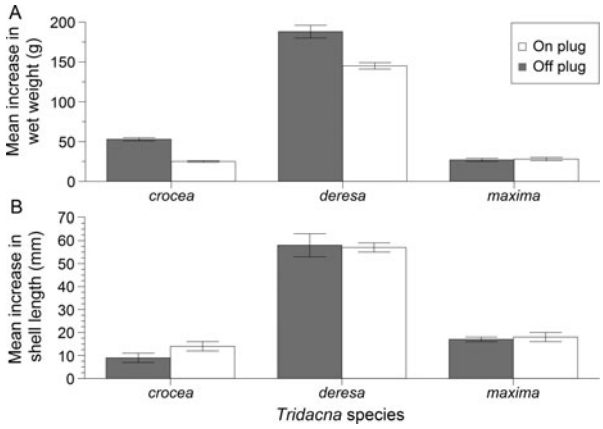

Fig. 3. Growth of giant clams (*Tridacna crocea*, *T. derasa*, *T. maxima* and *T. squamosa*) over 364 days of culture at the Waikīkī Aquarium on and off concrete plugs: (A) wet weight (g); and (B) shell length (mm) of growth. Bars represent means ± standard errors for each treatment.

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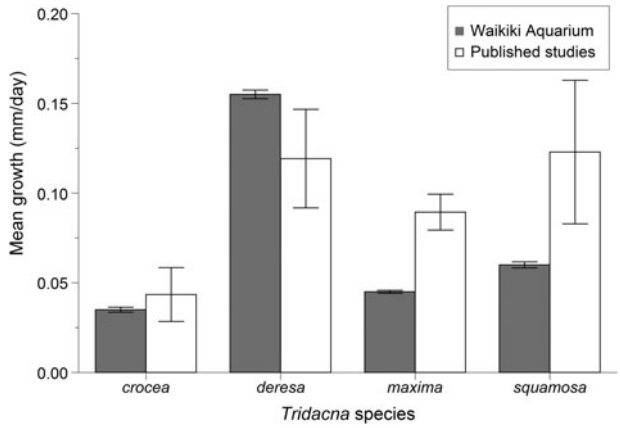

Fig. 4. Growth of giant clams (*Tridacna crocea*, *T. derasa*, *T. maxima* and *T. squamosa*) over 364 days of culture in the low pH, high nutrient seawater of Waikīkī Aquarium compared to published growth rates in oceanic seawater (see Table 1). Error bars represent means ± 95% confidence intervals for each group.

when compared to the well water at the Waikīkī Aquarium. The mean temperature of the well water from the Oceanic Institute was 24.3°C and ranged from 25.1°C to 29.0°C. The mean salinity was 32.3 ppt and ranged from a daily average of 30.3 to 35.1 ppt. Though temperature and salinity readings were taken during sunny midday, several readings were taken during heavy rain episodes. At such times the temperature did not decrease by more than a few degrees but the salinity decreased dramatically by as much as 10 ppt. Although pH levels are similar among locations, it is noteworthy that total $NO_2 + NO_3$ were below the limit of detection (1.0 μM) in the Oceanic Institute well water.

Survival

Clams at the Waikīkī Aquarium were divided between clams on and off of concrete plugs. Survival rates were high for all species: 93.5% of *T. squamosa*, 100% of *T. maxima*, 94.4% of *T. derasa* and 100% of *T. crocea* survived through to the end of the experiment.

Clams at the Oceanic Institute were divided between fed and unfed trials. Survival was substantially lower than at the Waikīkī Aquarium, with only 11 *T. squamosa*, 22 *T. maxima*, 21 *T. derasa* and 12 *T. crocea* surviving to the end of the experiment. The survival of juvenile clams in the fed trials (55.6 ± 10% SD) was significantly higher ($\chi^2 = 9.52$, df = 1, $P < 0.01$) than survival of clams in unfed (28.2 ± 9.6% SD) trials, although the impact of feeding on survival differed significantly among species ($\chi^2 = 34.91$, df = 3, $P < 0.01$). The greatest increase in survivorship was observed with *T. maxima* and *T. squamosa*, whereas no effect on survival was observed with *T. derasa*, in which one more individual survived in the unfed than the fed trials (Figure 1).

Growth

All four species at the Waikīkī Aquarium had significant increases in both wet weight and shell length throughout the 364 days of culture. Averaging across all clams in both treatments, the mean increase in wet weights throughout the year of culture were: *T. squamosa* (63.2 g ± 4.7 SD), *T. maxima* (28.9 g ± 4.1 SD), *T. derasa* (151.8 g ± 4.4 SD) and *T. crocea* (34.6 g ± 4.5 SD); and shell lengths were: *T. squamosa* (22.0 mm ± 1.2 SD), *T. maxima* (17.6 mm ± 1.1 SD), *T. derasa* (56.9 mm ± 1.2 SD) and *T. crocea* (12.8 mm ± 1.2 SD).

Although relatively high mortality rates at the Oceanic Institute resulted in low sample size and reduced statistical power, some trends in the data were evident (Figure 2). Overall the effect of feeding on shell growth (2-way ANOVA, df = 6, F = 23.04, $P < 0.01$) and wet weight (2-way ANOVA, df = 6, F = 12.34, $P < 0.01$) were significant, but differed among species (significant species × treatment interaction). *Tridacna derasa* exhibited roughly a three-fold increase in weight gain when fed (88.5 g ± 4.4 SD) than when unfed (26.0 g ± 2.1 SD), but only a slight increase in shell length between the fed (28.0 mm ± 0.64 SD) and unfed (21.0 mm ± 1.1 SD) trials. Likewise, for *T. maxima*, the wet weight gain was ten-fold higher (10.5 g ± 1.1 SD) in the fed trials than the unfed trials, whereas shell growth was not significantly higher with feeding (Figure 2). *Tridacna crocea* wet weight (6.0 g ± 2.1 SD) and shell growth (4.0 mm ± 0.35 SD) were both twice as high in the fed than the unfed trials, but the growth rate of fed clams was still less than half of that recorded at the Waikīkī Aquarium. In contrast, *T. squamosa* showed no significant response in either wet weight or shell length among fed and unfed trials (Figure 2).

None of the *T. squamosa* remained on their plug for the entire experiment. Therefore, the effect of plugs on growth was compared only with *T. maxima*, *T. derasa* and *T. crocea* (Figure 3). As with the feeding treatment, culture of clams on and off concrete plugs affected shell growth (2-way ANOVA, df = 5, F = 87.84, $P < 0.01$) and wet weight (2-way ANOVA, df = 5, F = 193.69, $P < 0.01$) significantly and differentially among species. Wet weight of *T. maxima* on the concrete plug (29.5 ± 5.3 g SD) was not significantly greater than growth of clams (22.7 ± 3.7 g SD) that were grown directly on the substrate. *Tridacna derasa* shell growth was not significantly different on or off the concrete plugs, but change in wet weight was significantly greater on (186.5 g ± 16.1 SD) than off (147.0 g ± 6.0 SD) the plugs (Figure 3). In contrast, *T. crocea* exhibited significantly greater shell growth off than on the plugs (14.3 mm ± 1.0 SD versus 8.5 mm ± 1.7 SD, respectively) but significantly greater wet weight gain on (26.3 g ± 1.5 SD) than off (58.5 g ± 2.5 SD) the concrete plugs.

The comparatively low survival of clams at the Oceanic Institute compared to the Waikīkī Aquarium prevented a direct comparison of growth rates between the two. We therefore used only the Waikīkī Aquarium clams for a comparison of growth rates of giant clams in high-nutrient, low-pH well water to those in natural seawater compiled from the literature (Table 1; Figure 4). In comparison to published growth rates, *T. derasa* had a significantly higher growth rate (0.16 mm/day ± 0.02 SD) in the low-pH well water of the Waikīkī Aquarium than values reported in the literature for clams grown in natural seawater (0.12 mm/day ± 0.06 SD). *Tridacna maxima* (0.05 mm/day ± 0.02 SD) and *T. squamosa* (0.06 mm/day ± 0.02 SD) both had significantly lower growth rates in low pH well water than those reported in the literature (0.09 mm/day ± 0.03 SD). In contrast, the growth rate of *T. crocea* (0.04 mm/day ± 0.01 SD) was not

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Table 1. Increase in shell length (mm/d) of giant clams (*Tridacna crocea*, *T. derasa*, *T. maxima* and *T. squamosa*) reported in the literature. In each study, giant clams were grown in the field, laboratory, or land-based tanks using natural seawater at ambient pH without nutrient addition.

Species	Clam culture locality	Mean growth rate (mm/d)
T. crocea	Solomon Islands	0.047
	Okinawa, Japan	0.04
T. derasa	Apo Island, Negros Oriental	0.177
	Carbin Cay, Sagay, Negros Occidental	0.187
	Silaqui Island, Pangasinan	0.120
	Land based tanks in the Solomon Islands	0.013
	Solomon Islands	0.200
	Tomasa	0.060
	Guiguiwanen	0.080
	Tomasa to Silaqui	0.110
	Guiguiwanen to Silaqui	0.120
	American Samoa	0.125
T. maxima	Solomon Islands	0.097
	Laboratory at the Bolinao Marine Laboratory, Philippines	0.070
	Silaqui	0.087
	Apo Island Reef	0.107
	Laboratory in Philippines	0.086
T. squamosa	San Juan, Siquijor	0.150
	Negros Oriental	0.093
	Silaqui Island, Pangasinan	0.077
	Papua New Guinea	0.200
	Laboratory at the Bolinao Marine Laboratory, Philippines	0.037
	Laboratory in Thailand	0.119
	Thailand	0.184

References: Gomez & Belda (1988), Solis *et al.* (1988), Ponwith (1990), Calumpong (1992), Klumpp & Griffiths (1994), Adulyanukosol (1997), Grice & Bell (1997) and Hart *et al.* (1998).

significantly different from published values of growth rates in natural seawater (Figure 4).

DISCUSSION

The conservation of giant clams is of considerable management importance due to the cultural and economic significance the clams have to the people of many Pacific and Indian Island nations. Conservation efforts have resulted in established commercial or government giant clam culture facilities across many of the tropical Pacific Island nations where giant clams occur naturally (Ellis, 1999). Techniques for both lagoon and tank-based culture of giant clams are well established and the practice has become largely successful in many locations throughout the Pacific. Although nutrient enrichment has been considered explicitly in experimental studies (Heslinga *et al.*, 1990; Braley, 1992; Calumpong, 1992; Hastie *et al.*, 1992; Belda *et al.*, 1993), the impacts of culture on and off hard substratum plugs and supplemental phytoplankton feeding of clams in culture have received far less attention. Likewise, the impacts of ocean acidification on the calcification and growth rates of these important reef species are of considerable conservation interest. To address these issues, we report the survival and growth rates of four different species of *Tridacna* clams: *T. maxima*, *T. squamosa*, *T. derasa* and *T. crocea*, on and off concrete plugs, and with and without supplemental phytoplankton feeding in high-nutrient, low-pH well water that approximates future predictions for ocean acidification as compared to current oceanic seawater conditions.

Species-specific differences in survival

Overall survival of clams was substantially higher at the Waikīkī Aquarium (97%) than at the Oceanic Institute (40%). Roughly 75% of mortality occurred in the first few months after the clams arrived, and the losses may be related to transport and relocation stress. Alternatively, poor survival of clams maintained at the Oceanic Institute may relate to frequent and heavy rainfall where salinities would become dramatically reduced during those first few months. Average annual rainfall in Waimānalo, where the Oceanic Institute is located, is 1083.8 mm with the rainy season from November to February usually accounting for about half (508 mm), whereas rainfall in Waikīkī on the leeward side of the island averages only 630.2 mm per year, with roughly a third (228.6 mm) falling during that same period (NOAA weather station data). In addition to the decreased rainfall, the ability of staff to cover the tanks prior to heavy downpours at Waikīkī Aquarium may have contributed to increased survival. Further, several studies have found increased growth of juvenile clams in nitrogen-enriched culture conditions (Heslinga *et al.*, 1990; Hastie *et al.*, 1992; Belda *et al.*, 1993; Delbeek & Sprung, 1994; Knop, 1996; Fartherree, 2006), and in addition to more stable salinity, the enhanced nutrient levels in the well water at the Waikīkī Aquarium may have further contributed to increased survivorship. Regardless, the survival of clams in the fed trials at the Oceanic Institute were roughly double (~56%) the survival rate in the unfed trials (~28%), but the benefits of supplemental feeding varied among species. The greatest increase in survivorship was observed with *T. maxima* and *T. squamosa*, a minimal effect on *T. crocea*, and the opposite effect on *T. derasa* where one more individual survived in the unfed than the fed trials, though the difference was not significant (Figure 1). These results are consistent with previous studies of the relative contribution of autotrophic nutrient production versus heterotrophic feeding reliance in giant clams studied to date (reviewed by Delbeek & Sprung, 1994; Fartherree, 2006).

Species-specific differences in growth

PHYTOPLANKTON FEEDING

Most hobby aquarists accept logical arguments for the importance of phytoplankton feeding like those of Shimek (2009) who states: 'Natural selection acts to minimize unnecessary costs. If clams from *Tridacna* or *Hippopus* species didn't need to feed, the feeding structures would be eliminated. There are a number of clams that live totally on the byproducts of symbiotic bacteria living on their gills. These clams are totally gutless. The fact that every *Tridacna* and *Hippopus* individual has a good and functional feeding

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apparatus ABSOLUTELY PROVES that they need to feed.' To date, however, experimental evidence of the impacts of phytoplankton supplementation on the growth of cultured tridacnid clams is limited and the specific role of phytoplankton feeding with tridacnids is still poorly understood (Delbeek & Sprung, 1994; Fatherree, 2006). For example, the research of Klumpp *et al.* (1992) reported ~75% of the phytoplankton (2–50 μm) passing over the Great Barrier Reef was captured and retained by giant clams (*T. gigas*) but Yonge (1975) argued that the amount of phytoplankton passing over a tropical coral reef is insufficient to meet the needs of a large clam. Klumpp *et al.* (1992) found that juvenile *T. gigas* (~4 cm) obtained ~65% of their carbon needs from filtering phytoplankton rather than from photosynthetic inputs by zooxanthellae (and under some conditions, filter feeding could provide up to 100% of their needs). However, the energy budget shifted with age where filter feeding dropped to ~34% of their total carbon needs at a length of 16–17 cm (Klumpp *et al.*, 1992). For adult clams, the proportion of their nutritional requirements met by photosynthesis as compared to filter feeding was essentially reversed—depending on the conditions under which the clam was found, output from photosynthetic symbionts provided roughly 60–100% of the carbon budget of the clam (Fisher *et al.*, 1985; Klumpp *et al.*, 1992). The ontogenetic shift from primarily heterotrophy as juveniles to primarily autotrophy as adults was most evident with *T. gigas* and *Hippopus hippopus*. In contrast, a similar study of *T. derasa* and *T. tevora* found that these species were able to meet up to 100% of their carbon needs from photosynthetic symbionts alone, even as juveniles (Klumpp & Lucas, 1994). The Waikīkī Aquarium has maintained two specimens of *T. gigas* since 1982 and 1983 respectively in exhibits that receive only well-water which is devoid of plankton. These clams are still alive at the time of this publication and have grown from juvenile to adult size in that period with no supplemental feeding.

We find that the effects of phytoplankton feeding are species-specific in terms of both survival (Figure 1) and individual growth rates (Figure 2), although the effect of feeding is more evident with wet weight gain than with shell length gain. However, *T. derasa*, one of the species reported to meet 100% of their carbon budget from photosynthesis, showed a roughly three-fold increase in wet weight when fed phytoplankton relative to the unfed individuals. Although *T. maxima* did not grow as quickly as *T. derasa*, *T. maxima* demonstrated an even greater response to feeding with roughly an order of magnitude greater wet weight gain in the fed versus unfed trials. Growth of *T. crocea* was likewise higher in fed than unfed trials but the effect is less dramatic. In contrast, no significant difference in growth between fed and unfed individuals of *T. squamosa* was observed, although the unfed clams tended to lose rather than gain weight over the course of the experiment (Figure 2). The specific response of individuals to the experimental treatments was variable both in terms of significance and magnitude of effect, although there appears to be a general trend towards enhanced survivorship and growth of juveniles with phytoplankton feeding.

CONCRETE PLUGS

The different species of giant clams have varying habitat preferences in the field, with *Tridacna maxima* and *T. squamosa* typically attached firmly to solid substrate by their byssus, *T. crocea* found primarily encased in hard substrate after having bored directly into it and *T. derasa* frequently found either loosely attached or not attached at all on sandy substrates (Delbeek & Sprung, 1994; Knop, 1996; Fartherree, 2006). We hypothesized that growing clams with a natural preference for hard substrata on concrete plugs would reduce removal stress and breaking of byssal threads in culture. Contrary to expectations based on habitat associations on the reef, the majority of *T. derasa* remained on the plugs whereas none of the *T. squamosa* remained on the plugs by the end of the experiment. A comparison of the growth rates on and off the concrete plugs between the three species for which some clams remained attached revealed substantial differences among the species with respect to relative responses. *Tridacna maxima* showed no effect on either wet weight or shell length growth when grown on the plug versus the substrate. Shell growth of *T. derasa* was not altered, but wet weight was significantly greater on than off the concrete plugs (Figure 3). In contrast, *T. crocea* showed significantly greater shell growth off than on the plugs but significantly greater wet weight gain on than off the plugs.

Tridacna crocea is well known as a boring species typically found encased within coral heads or solid limestone substrate (Delbeek & Sprung, 1994; Knop, 1996; Fartherree, 2006). Thus, it seems likely that at least some of the clams had eroded their shells while attempting to bore into the concrete plug (much harder than calcium carbonate or limestone) which would then account for greater increases in shell length for clams off the concrete plugs. No obvious explanation for the result with *T. derasa* is evident; the greater wet weight gain of clams on than off the plugs might be due to increased density of the shell when used for boring, unattached clams using more energy moving around, or the energy cost of consistently reattaching to the tank after removal for cleaning and measurement. Regardless of the specific mechanism of differential growth, the results are clearly different among the three species for which data could be collected.

Species-specific differences in acidified culture

The 2007 report of the Intergovernmental Panel on Climate Change (IPCC, 2007) cites a wide range of data, including CO_2 accumulation rate, increases in global air and ocean temperatures, widespread melting of global snow and ice, rising sea levels, and increasing frequency of extreme weather events, to conclude that anthropogenic impacts on the Earth's climate system are now 'unequivocal.' Climate change models predict that increasing atmospheric CO_2 under a variety of 'business-as-usual' scenarios will result in a loss of calcium carbonate saturation in the ocean surface waters over the next century (Orr *et al.*, 2005) and, as a result, will likely compromise growth rates of calcifying organisms such as corals, bivalves, coralline algae and some plankton (Orr *et al.*, 2005; Hoegh-Guldberg *et al.*, 2007; Kuffner *et al.*, 2008; Andersson *et al.*, 2009). The well water of the Waikīkī Aquarium is characteristically high in inorganic nutrients, low in organic nutrients, and oversaturated with CO_2 relative to tropical ocean surface waters (Atkinson *et al.*, 1995; Carlson, 1999) which results in an average 0.3 to 0.5 decrease in pH units below ambient, similar in composition to the 2100 predictions of the IS92a model of future ocean conditions (Orr *et al.*, 2005; IPCC, 2007). Thus, the

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results of this experiment also provide some insight into the growth and survival of these culturally and economically important bivalves under conditions approximating future ocean acidification scenarios.

The average rate of shell length growth recorded for each species in high-nutrient, low-pH well water used in our study was compared to previously published rates of growth for *T. squamosa*, *T. maxima*, *T. derasa* and *T. crocea* in studies where natural oceanic seawater was used. *Tridacna crocea* had no detectable difference in shell length growth rate under low pH conditions at the Waikīkī Aquarium and was comparable to the mean growth rate reported in previously published studies conducted in natural seawater. Among the remaining three species, the decreased growth of *T. squamosa* and *T. maxima* in low pH well water is opposite to the response of *T. derasa* which demonstrated a significant increase in shell length growth rate in the acidic, high-nutrient well-water (Figure 4).

Differences in survival and growth rates among field and laboratory cultures of giant clams are well documented. For example, Solis *et al.* (1988) reported three of four species of giant clams tested had lower survival but higher growth rates in the field than those maintained under laboratory conditions. Likewise, Ponwith (1990) documented increased growth rates of giant clams in the field after being transferred from an aquarium culture system. Although many factors are associated with aquarium culture of clams, our comparisons clearly demonstrate that the effects are species-specific and not easily predictable from single species studies: one species shows no effect, two have a significant decline in growth and one has a significant increase in growth relative to natural seawater.

Summary and conclusions

Overall, this study documents that each treatment (phytoplankton enrichment, substrate type and seawater acidification and eutrophication) had differential effects upon the four species tested. Supplemental phytoplankton enrichment of juvenile clams generally had significant positive effects on individual survivorship in all but *T. deresa*, whereas all but *T. squamosa* showed increased growth with supplemental phytoplankton feeding. Substrate had the greatest impact on *T. crocea* where decreased shell length and increased wet weight were observed for clams cultured on concrete plugs, but no *T. squamosa* would remain on the plugs, and *T. maxima* showed no change in growth on or off the plugs. Finally, high-nutrient, low-pH well water from the Waikīkī Aquarium, similar in profile to predictions of future ocean acidification scenarios, had highly variable impacts on the four species: *T. crocea* showed no significant difference in mean shell growth rate, whereas *T. derasa* had a significantly higher growth rate, and growth rates for *T. squamosa* and *T. maxima* were significantly depressed when cultured in acidic well water.

Our results clearly show species-specific differences in response to each treatment variable; thus, ideal culture conditions for one species of giant clam are likely suboptimal for another. Furthermore, these experiments show striking species-specific differences for each treatment that caution against broad generalizations being made about the effects of nutrient enrichment, acidification, substratum type, handling stress and phytoplankton feeding effects on tridacnid culture and survival. Such strong species-specific differences and interactions among treatment variables also caution against broad generalizations being made on community effects of ocean acidification from single-species laboratory studies.

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