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Skeletal growth of four scleractinian corals is not enhanced by *in situ* mesozooplankton enrichment

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ABSTRACT: The importance of heterotrophy in enhancing the skeletal growth of corals in transparent, shallow tropical waters is unclear. The impact of food availability on coral growth was examined by enriching the abundance of naturally occurring mesozooplankton (>200 µm in width) *in situ* nightly for 1 mo around transplanted fragments of 4 coral species (*Pocillopora verrucosa*, *Acropora nasuta*, *Porites rus*, *Montipora* sp.) in a lagoon of Moorea, French Polynesia. Zooplankton were attracted to the corals throughout each night using light from gallium nitride light-emitting diodes tuned to 1 of 4 wavelengths (590 nm [amber], 525 nm [green], 470 nm [blue], 400 nm [near UV, hereafter called violet]). An unlit light source was used as control. Mean total zooplankton abundance and biomass were significantly enriched above ambient concentrations by a factor of 3 to 14 times under green, blue, and violet wavelengths; amber did not enrich abundance or biomass. The mean abundance of specific taxa known to be favored for ingestion by corals (e.g. amphipods, mysids, polychaetes, crustacean larvae) was significantly enriched by as much as 100-fold. Despite sustained increases in the availability of mesozooplankton, none of the 4 coral species experienced higher skeletal growth under zooplankton enrichment compared to those exposed to ambient zooplankton concentrations. This pattern supports the conclusion that skeletal growth of healthy corals living in shallow, light-replete habitats may be adapted for greater reliance on autotrophy. The low ambient flux of zooplankton appears sufficient to meet the heterotrophic needs of the coral species investigated.

KEY WORDS: Coral skeletal growth · Mesozooplankton · Coral feeding · Zooplankton enrichment · Coral heterotrophy

INTRODUCTION

Hermatypic scleractinian corals are both autotrophic, through their relationship with their endosymbiotic algae, and heterotrophic, ingesting organisms ranging from bacteria to macrozooplankton (see Houlbrèque & Ferrier-Pagès 2009 for review). Mesozooplankton in particular are frequently consumed by a diverse group of corals (Porter 1974, Sebens et al. 1996, Palardy et al. 2005, 2006, 2008), and their consumption can enhance growth (Ferrier-Pagès et al. 2003, Houlbrèque et al. 2004a) and enhance photosynthesis by increasing nutrient availability, algal symbiont (*Symbiodinium*) density, and chlorophyll concentration (Muscatine et al. 1989, Dubinsky et al. 1990, Houlbrèque et al. 2003, 2004a). Consumption of mesozooplankton and other particulate matter may be particularly important under stressful conditions that impair the functionality of *Symbiodinium*, or in shaded habitats where light can be limiting for photosynthesis. Heterotrophy can mitigate the negative nutritional impacts of bleaching

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(Grottoli et al. 2006, Connolly et al. 2012), shading (Anthony & Fabricius 2000), elevated temperature and greater depth (Palardy et al. 2005, Ferrier-Pagès et al. 2010), and ocean acidification (Edmunds 2011) in some coral species. Thus, depending on environmental conditions, zooplankton consumption promotes coral growth and survival.

However, while laboratory studies show that zooplankton consumption can increase tissue synthesis and protein levels in corals (Sebens & Johnson 1991, Al-Moghrabi et al. 1995, Anthony & Fabricius 2000, Ferrier-Pagès et al. 2003, Houlbrèque et al. 2003, 2004a), feeding can disproportionately enhance growth of the host colony's symbiotic algae relative to its own (animal) cells (Houlbrèque & Ferrier-Pagès 2009). Calcification is disproportionately supported by photoautotrophically acquired carbon in some species (Hughes et al. 2010). The effect of zooplankton feeding on coral skeletal growth appears to be highly species- and situation-specific. For example, in laboratory studies, skeletal growth and calcification rates of the coral *Stylophora pistillata* increased significantly when the coral was fed high concentrations of zooplankton (Ferrier-Pagès et al. 2003, Houlbrèque et al. 2003); similarly, *Goniastrea retiformis* more than doubled its rate of feeding at high particle concentrations, leading to a gain in skeletal mass despite being shaded (Anthony & Fabricius 2000). By contrast, *Fungia scutaria, Montipora verrucosa*, and *Porites compressa* grew equally fast in 1 µm filtered and unfiltered seawater (Johannes 1974), *P. compressa* had decreased skeletal extension rates with increased feeding under laboratory conditions (Grottoli 2002), and *P. cylindrica* was unable to compensate for shading with increased particle consumption and lost tissue mass, even at enhanced food concentrations (Anthony & Fabricius 2000).

Field studies addressing zooplanktivory by corals are scarce and often show little effect of zooplankton availability on coral performance. *Pocillopora damicornis* and *Pavona clavus* grew independently of zooplankton supply at ambient light conditions during a year-long field study during which zooplankton density was reduced below ambient conditions in some treatments (Wellington 1982), and while *Pavona gigantea* growth was dependent on both light and zooplankton, this species was unable to compensate for effects of shading via zooplankton consumption (Wellington 1982). Moreover, overnight capture rates of *Porites compressa, Porites lobata*, and *Montipora capitata* in the field did not increase when the corals were offered higher than ambient zooplankton concentrations (Palardy et al. 2008). Clearly, corals exhibit species-specific plasticity in nutritional dependence between heterotrophy and autotrophy (Anthony & Fabricius 2000, Palardy et al. 2008, Houlbrèque & Ferrier-Pagès 2009, Ferrier-Pagès et al. 2011). The role of zooplanktivory in determining the growth rate of corals in nature, especially for shallow (i.e. <10 m depth), light-dependent species, is not well understood.

The possibility that long-term enhanced mesozooplankton availability might increase the growth and survival of corals *in situ* is of interest to conservationists and restoration ecologists. The decline of coral reefs in recent decades has led to an increase in restoration efforts including transplantation of coral colonies or fragments (Rinkevich 2005). Survival and success of these transplants depend primarily on the extent of handling stress, size of the transplanted fragments, and coral growth rates (Rinkevich 1995, Laydoo 1996, Yap et al. 1998, Edwards & Clark 1999, Kopjes & Quinn 2001). One means of increasing the growth rates and health of transplanted corals might be through the enhancement of zooplankton availability in the field. If successful, this technique could increase the survival of corals by accelerating skeletal growth of vulnerable size classes.

The developing technology of light-emitting diode (LED) lights, which are both energy efficient and wavelength specific, provides a potentially powerful method for investigating the impact of zooplankton enhancement on coral performance in the field. The narrow wavelengths of these lights attract mesozooplankton at night without the confounding potential increase in symbiont photosynthesis produced by white light (see Witting 1999). We investigated the impact of sustained nocturnal enhancement of naturally occurring mesozooplankton on the skeletal growth rates of 4 species of Pacific corals *in situ* on the north shore of Moorea, French Polynesia. The study also provides insight into the potential role of new technologies in increasing the growth and survivorship of corals in reef restoration efforts.

MATERIALS AND METHODS

Overview

We conducted a multi-factor field experiment in the lagoon on the north shore of Moorea, French Polynesia (17° 28′ 52″ S, 148° 58′ 59″ W), in the austral winter of 2006 using transplanted fragments of 4 coral species. Fragments (hereafter called nubbins) consisting of approximately 3 cm long tips of coral branches were placed near LEDs of 4 wavelengths and an unlit

control for 1 mo to explore whether increased mesozooplankton availability would enhance coral performance. Mesozooplankton abundance was determined throughout the night and over the lunar cycle using automated *in situ* plankton pumps.

Study site

The study site, located in the lagoon approximately 800 m behind the reef crest, was 2.5 to 3 m deep with a reflective, light-colored sand and rubble bottom separating small, dispersed coral heads and larger coral conglomerates known as bomboras or bommies. Temperature ranged from 26.7 to 27.6°C during the experimental period (June through August 2006). Mean flow at the study site during the austral winter ranges from 10 to 14.5 cm s^{-1}, with maxima during storms of 31 to 76 cm s^{-1} (Holbrook et al. 2008). The seawater had very high clarity and low nutrient and particle content. Concentrations of nutrients measured in the lagoon during the study were 0.09 to 0.15 µM PO_4, 0.18 to 0.81 µM SiO_3, and 0.47 to 0.53 µM $NO_2 + NO_3$ (Alldredge & Carlson 2013).

Nocturnal light manipulations

Five replicate experimental plots were illuminated nightly from 18:00 to 04:00 h using commercially available hand-held dive lights (Tek-Tite), each refitted with 20 (AlInGa)N and (AlInGa)P LEDs (Holbrook et al. 2007); dive lights that were left unlit served as controls. Because of their small size and the power efficiency of (AlInGa)N and (AlInGa)P LED emitters, each flashlight could be powered on for periods exceeding 24 h using 6 rechargeable C-cell nickel metal hydride batteries. A small circuit board in the head of the flashlight allowed the lights to be programmed for day-of-the-week specific on and off times and controlled the intensity of the LEDs. Lights were programmed with a palmtop computer running a custom application written for the Palm operating system. We used 4 different wavelengths: 590 nm (amber), 525 nm (green), 470 nm (blue), and 400 nm (near UV, hereafter called violet). These very narrow wavelengths were chosen to avoid peaks in the photosynthetic action spectra of *Symbiodinium* within the corals (Scott & Jitts 1977) and eliminate any possible confounding effects of light on coral growth through enhancement of algal photosynthesis. White light was not tested because of its possible impact on coral symbiont photosynthesis.

Responses of zooplankton to LED wavelengths

Previous studies demonstrate that nighttime zooplankton abundance and composition on the north shore of Moorea are highly sensitive to the lunar phase (Alldredge & King 2009). Therefore, zooplankton >200 µm in width were quantitatively sampled nightly at the experimental site on 10 nights over the lunar cycle in late June and throughout August 2006 using 200 µm mesh nets in 5 programmable *in situ* plankton pumps. One pump was placed in each of the 5 experimental plots and the 5 pumps, with their attached lights (4 test wavelengths and an unlit control), were rotated among the experimental plots on different nights to account for any possible across-site variability in zooplankton abundance. Zooplankton sampling was conducted immediately before and after the July coral growth experiment to avoid confounding the coral feeding results by disturbing the corals and diluting prey around them. Long-term monitoring of mesozooplankton in July in the Moorea lagoon shows similar abundances to those observed in June and August in this study (Alldredge 2012), and scuba divers observed high abundances of mesozooplankton around the lights and corals at night throughout the July experiment. Thus, we assumed that mesozooplankton enrichment measured immediately before and after the coral feeding experiment was similar in magnitude to that experienced during the experiment.

Each plankton pump was switched on for four 20 min periods at 20:00, 22:00, 24:00, and 02:00 h each night, collecting the 4 samples in the same cod end to produce an estimate of mean mesozooplankton abundance throughout that night. Each total sample represented the zooplankton in 3.5 to 6 m^3 of seawater. The pumps also contained inverted funnels in the cod ends and one-way flaps in the intake pipes to inhibit escape of zooplankton. Upward-facing LED lights were attached to the intake pipes of the pumps 15 cm below the intake opening, a distance from the lights identical to that experienced by the transplanted coral nubbins, described below.

The total volume of each concentrated zooplankton sample from each pump, ~20 ml, was measured in a small graduated cylinder. Approximately 1/3 was removed with a wide-bore 10 ml pipette while stirring constantly, and the volume removed was noted. This subsample was then filtered onto a preweighed 25 mm nucleopore filter (5 µm pore size), rinsed with filtered fresh water, dried overnight at 65°C, and reweighed, and the total dry mass was determined by subtraction of the filter weight. The remaining

zooplankton sample was rinsed into a vial, preserved in 2% formalin, and later counted under a dissecting microscope using a zooplankton counting wheel (40 ml volume; Aquatic Research Instruments). When necessary, samples were split with a Folsom plankton splitter prior to counting. *In situ* zooplankton concentration and mass were calculated based on subsample proportion and the volume filtered by the *in situ* plankton pump.

We measured the length of 5 to 25 individuals of each taxon from samples representative of each lunar phase and light treatment with an ocular micrometer under the microscope. The mean zooplankton lengths from the various lunar phases were then averaged to obtain an estimate of the mean size of each taxon available at each wavelength throughout the month. This approach prevented bias toward any particular lunar phase. The mean size of all the zooplankton captured in each pump sample was calculated using the following formula where i = an individual taxon, n = total number of taxa, L_i = mean length of taxon i in the sample, No_i = number of individuals of taxon i in the sample, and No = total number of individuals in the sample:

$$\text{Mean length in total sample} = \frac{\sum_{i=1}^{n}(L_i \times \mathrm{No}_i)}{\mathrm{No}} \quad (1)$$

The mean zooplankton lengths of all the samples collected at each particular wavelength were then averaged to obtain a grand mean length for all the zooplankton captured at that wavelength. An average enrichment factor (EF) was also calculated for each taxon in each treatment on each night as the ratio of the treatment relative to the control concentration (no. ind. m^{-3}):

$$\mathrm{EF} = \frac{\text{conc. of taxon } i \text{ in light treatment } x}{\text{conc. of taxon } i \text{ in corresponding control}} \quad (2)$$

One-way ANOVA and the Tukey-Kramer test for multiple comparisons were used to assess differences among wavelength treatments in the absolute abundance and total mass of zooplankton, the EFs of these parameters, and the EFs of selected individual taxa. Total mesozooplankton abundance, biomass, and enrichment were ln-transformed to meet assumptions of homogeneity of total variance as determined with Levene's test. Enrichment data for individual taxa did not require transformation. Differences in the proportional taxonomic composition of zooplankton attracted to different wavelengths were tested using standard chi-square analysis and an expected proportional distribution of taxa equal to that observed in the unlit controls. Finally, the difference in zooplankton sizes (ln-transformed) among the light treatments was tested with one-way ANOVA and the Holm-Sidak method for multiple comparisons.

Coral performance

Experimental corals consisted of small fragments of 4 species of corals commonly found within the Moorea lagoon: *Pocillopora verrucosa, Acropora nasuta, Porites rus,* and *Montipora* sp. Because coral growth could vary among colonies (i.e. potentially different genotypes) within the same species, we used nubbins from 4 different colonies of all species except *Montipora*, whose individuals often spread over large areas, making it difficult to identify discrete colonies. Coral nubbins (branch tips ~3 cm in length and 1.5 to 2.5 cm in diameter) were collected in the lagoon near, and at the same depth as, the experimental site and transported to the laboratory submerged in seawater from the site. In the laboratory, nubbins were buoyantly weighed (Davies 1989) and mounted on small (3 × 3 cm) squares of plastic using marine epoxy. Nubbins were kept in the laboratory on outdoor water tables with flow-through seawater for 24 h following mounting. Individual nubbins from each source colony of each species were then attached inverted to a thin wire mesh rack (2.5 cm mesh openings) 30 cm from the (sandy) reef substrate and 15 cm above 2 upward-facing lights of 1 of the 5 treatments (e.g. unlit control, amber, green, blue, violet). Since zooplankton aggregated above the lights, nubbins were inverted directly above the lights to provide a more even distribution of zooplankton around the corals and to ensure that the corals were positioned within the zone of enrichment. Wire mesh cages (2.5 cm mesh) placed over the nubbins prevented predation by corallivorous fishes. Each rack contained 4 nubbins (one from each source colony) of each of the 4 coral taxa, for a total of 16 nubbins. The 5 racks, each receiving a different light treatment, were situated 10 m apart at each of 5 replicate study sites (25 racks and 400 initial corals total). The nearest racks between adjacent study sites were 30 m apart.

Four of the 5 racks at each study site received one of the LED wavelength treatments, and one rack per site had an unlit light. LEDs were programmed to turn on after dusk and remained on for 10 h (18:00 to 04:00 h local time). For each treatment, we obtained (1) the change in skeletal mass of each coral colony (using the buoyant weighing method; Davies 1989)

and (2) an estimate of nubbin survivorship within each species and colony combination. The experiment ran for 1 lunar month in July 2006.

Statistical tests for the effects of wavelength, genotype, and position (rack) on daily nubbin growth were performed using a multiple ANOVA (PROC GLM in SAS ver. 9.2 for Windows; SAS Institute 2008). Skeletal growth of coral nubbins over the experimental period was significantly correlated with initial nubbin weight and varied among the 4 coral species. We used initial nubbin weight as a covariate in all analyses that involved the growth of coral nubbins as a response variable to account for the effect of initial size. Because we detected no significant effect of genotype in any of our analyses ($p > 0.05$ in all cases), coral genotype was dropped as a factor, and all nubbins of the same species (but differing genotypes) were assumed to be replicates. To avoid issues of pseudo-replication, all subsequent analyses of nubbin growth used the mean value of nubbin growth calculated from the 4 nubbins representing a given species (1 nubbin representing each of the 4 genotypes) on a given rack as the unit of replication. Coral mass data were ln-transformed to meet assumptions of ANOVA.

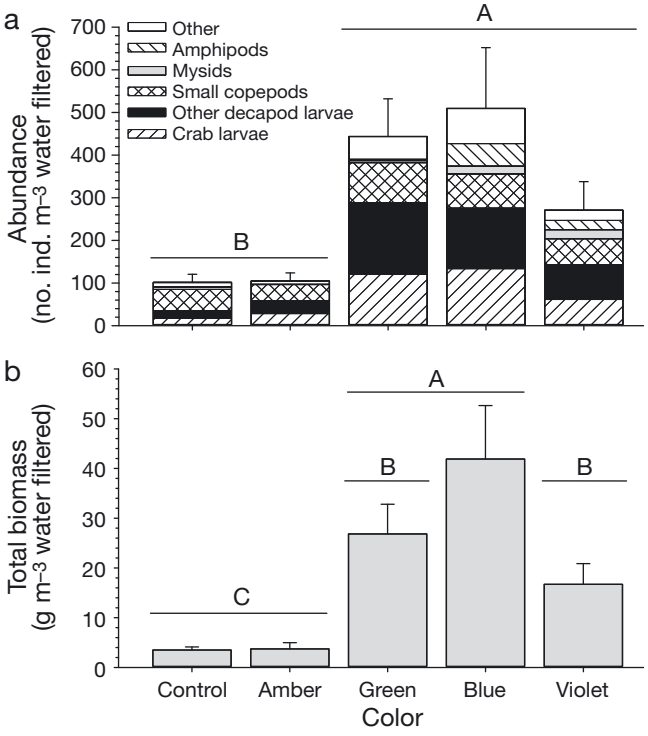

Fig. 1. Mean (+1 SE) (a) abundance and (b) dry mass of total mesozooplankton attracted to each of 5 light treatments around corals; (a) also displays the major taxonomic composition of the zooplankton. Treatments sharing the same letter are not significantly different from each other ($p \geq 0.05$)

RESULTS

Responses of zooplankton to LED wavelengths

Day-to-day variation in mesozooplankton abundance and composition was high. However, despite this variation, the wavelength treatments attracted significantly different assemblages of zooplankton. Over the entire sampling period, the mean (±1 SE) ambient (unlit) nighttime mesozooplankton abundance was 101 (±19) animals m^{-3} and 3.5 (±0.6) mg m^{-3} dry mass. This differed sharply from abundance and biomass in samples taken under LED illumination (Fig. 1a). Pumps lighted with blue, green, and violet wavelengths consistently captured 3 to 5 times more total zooplankton (mean ± SE: 509 ± 142, 444 ± 88, and 271 ± 67 animals m^{-3}, respectively) than amber (105 ± 19 animals m^{-3}) and the unlit control. Zooplankton mass showed a similar pattern to that of abundance with the exception that among the 3 longer wavelengths, the total biomass of zooplankton attracted to green was significantly less than that for blue and violet but still significantly higher than that for the control and amber (Fig. 1b). Both abundance and biomass represent minimum enrichment values, since the original concentrations above the lights became increasingly diluted as animals were removed during each 20 min pumping cycle.

Chi-square tests of zooplankton taxonomic composition indicated that the community composition of all 4 light treatments was significantly different from that of the (unlit) control ($p < 0.001$ for all treatments). Ambient zooplankton in the control was dominated by small copepods, crab larvae, and other decapod larvae (Fig. 1a). Other light treatments also captured crab larvae and other decapod larvae but in higher numbers and higher proportions. Mysids and the predatory copepod *Labidocera* sp. (included in the 'other' category in Fig. 1) were rare in the control and amber treatments but abundant in violet and blue treatments, making up to 20% of some samples. Amphipods were especially abundant in blue and violet treatments.

The general pattern of enrichment of both total zooplankton abundance (Fig. 2a) and biomass (Fig. 2b) as expressed by the EF followed a similar pattern to the trends reported for total abundance and biomass (Fig. 1). Total zooplankton abundance was significantly enriched ($p < 0.01$) an average of 3 times above ambient in violet and 5 times above ambient in blue and green treatments. The amber treatments did not

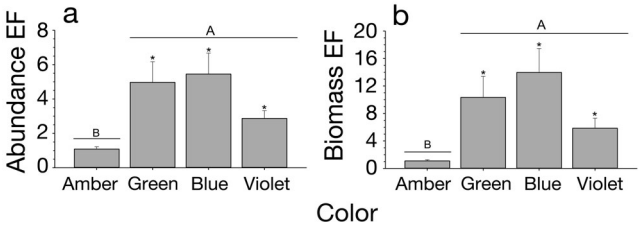

Fig. 2. Mean enrichment factors (EFs) (+1 SE) of total mesozooplankton at the 4 light treatments based on (a) abundance and (b) dry mass. EF is the ratio of the treatment to the control. Values >1 indicate enrichment above the control. *Significantly different from the control at $p < 0.05$. Letters over the bars as in Fig. 1

differ from the control ($p > 0.5$). Biomass was also significantly enriched ($p < 0.01$) an average of 6 times above ambient for violet, 14 times above ambient for blue, and 10 times above ambient for green treatments. Biomass captured in the amber treatment was not different from the control ($p > 0.5$). The EFs of the blue, green, and violet treatments were not significantly different from each other for either total abundance or biomass because of high temporal variability.

All the major taxa except gastropod veliger larvae were substantially enriched above ambient (control) abundances (Fig. 3), although the mean abundances of copepods (excluding *Labidocera* sp.), while enriched, were not significantly different for any light treatment. *Labidocera* sp. (data not shown), amphipods, crab zoea, and crab megalops were all enriched above ambient in 3 treatments (green, blue, and violet), while mysids, other decapod larvae, and polychaetes were significantly enriched in 2 treatments. The level of enrichment was often substantial, being, for example, as much as 102, 57, and 74 times above ambient for crab megalops, amphipods, and other decapod larvae, respectively (in the blue treatment). Polychaetes, a favored food by some corals, were significantly enriched an average of 20 times above ambient in the blue treatment.

Many individual taxa, especially larval forms, occurred sporadically over the lunar cycle and were not abundant on all nights. This generated high variability among sample nights and confounded statistical comparisons of enrichment among the 4 light treatments themselves. Only mysids were significantly enriched in blue and violet above amber and green treatments, and amphipods differed in enrichment between amber and blue treatments (Fig. 3).

With the exception of mysids, the mean body lengths of individual taxa were similar among the light treatments and not significantly different from the control (Table 1). Mysid species attracted to the violet and blue treatments were significantly larger

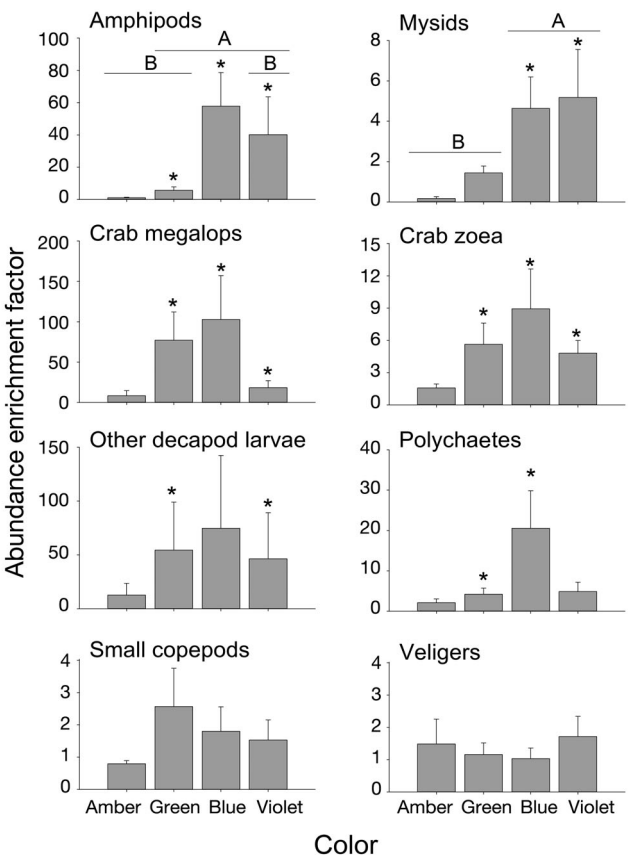

Fig. 3. Mean enrichment factors (EF) (+1 SE) of abundance of the dominant zooplankton taxa. EF values >1 indicate enrichment above the control. *Significantly different from the control at $p < 0.05$. Letters over the bars as in Fig. 1

than those captured by the control and the amber treatments, and this accounted for the observed difference in body size for this taxon. However, because larger taxa in general were attracted to the light treatments, the overall mean zooplankton size for these treatments was significantly larger ($p < 0.01$ for all) than in the control and also resulted in biomass being more enriched than abundance in the blue, green, and violet treatments. Mean zooplankton length ranged from 1.52 to 1.84 mm in the light treatments, about 1.5 times larger than in the control (1.14 mm) (Table 1).

Coral performance

Survival rates of the transplanted nubbins were high; >95% survived for all species except *Porites rus*, a small number of which became infested with predatory *Phestilla* nudibranchs, and one source colony (genotype) of *Acropora nasuta* that suffered 70% mortality early in the experiment (probably

Table 1. Mean length (±1 SE) of various individual taxa in mm. 'All zooplankton' represents the mean size of all zooplankton attracted to each light color. *Significantly larger than in the control at $p < 0.01$ (except mysids in the blue treatment, where $p < 0.05$). *Labidocera* is a genus of large copepods abundant at the study site

Light color	All zooplankton	*Labidocera*	Other copepods	Amphipods	Mysids	Crab zoea	Crab megalops	Decapod larvae	Veligers
Control	1.14 ± 0.23	2.0 ± 0.4	0.8 ± 0.1	0.8 ± 0.1	2.6 ± 0.5	1.1 ± 0.1	1.9 ± 0.3	2.0 ± 0.2	0.3 ± 0.2
Amber	1.52 ± 0.25*	1.8 ± 0.2	0.9 ± 0.1	0.9 ± 0.6	1.5 ± 0.1	1.0 ± 0.1	2.3 ± 0.1	2.0 ± 0.1	0.2 ± 0.1
Green	1.68 ± 0.30*	1.9 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	3.2 ± 0.7	1.2 ± 0.1	2.4 ± 0.3	2.2 ± 0.1	0.2 ± 0.1
Blue	1.79 ± 0.29*	1.9 ± 0.1	0.9 ± 0.1	1.9 ± 0.5	4.8 ± 1.2*	1.2 ± 0.1	2.2 ± 0.1	2.0 ± 0.3	0.2 ± 0.2
Violet	1.84 ± 0.52*	1.9 ± 0.3	0.8 ± 0.1	2.0 ± 0.2	5.6 ± 0.4*	1.2 ± 0.1	3.1 ± 0.7	2.0 ± 0.1	0.3 ± 0.1

Table 2. Initial coral nubbin skeletal weight (g) for the 4 coral species. N = no. of samples

Light color	*Acropora nasuta*			*Montipora* sp.			*Pocillopora verrucosa*			*Porites rus*		
	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N
Control	4.017	0.463	14	1.310	0.117	20	5.070	0.284	20	3.401	0.365	14
Amber	3.975	0.402	15	1.384	0.201	20	5.781	0.314	20	2.384	0.246	13
Green	3.562	0.389	14	1.154	0.069	20	5.642	0.496	20	2.597	0.188	10
Blue	4.028	0.483	15	1.804	0.196	20	5.765	0.478	20	3.061	0.475	9
Violet	4.534	0.703	15	1.469	0.133	20	5.806	0.428	20	3.219	0.377	14

from handling). All nubbins from this *Acropora* colony were eliminated from our analyses, as were any *P. rus* nubbins suffering >15% tissue loss. There was no pattern of survivorship of any coral species among the treatments.

Within each coral species, the initial buoyant weight of nubbins did not differ statistically among treatments and ranged between a mean of 1.42 g for *Montipora* sp. to 5.61 g for *Pocillopora verrucosa* (Table 2). All individual nubbins increased in buoyant weight from 5 to >25% of their initial skeletal mass over the month. *Montipora* sp. grew the fastest, adding 13 to 15% to its initial buoyant weight across all treatments (0.26 to 0.34 g nubbin^{-1}), followed by *Porites rus* at 9.1 to 10.6% (0.66 to 0.83 g nubbin^{-1}), *Pocillopora verrucosa* at 5.8 to 8% (0.58 to 0.85 g nubbin^{-1}), and *Acropora nasuta* at 5.5 to 7.5% of initial weight (0.54 to 0.73 g nubbin^{-1}).

While all species grew in all treatments, there was no evidence for enhancement of skeletal growth relative to the control in any of the light treatments (Fig. 4). In 2 cases (*Montipora* sp. and *Porites rus*), skeletal growth did not differ significantly among the treatments. In a third case (*Pocillopora verrucosa*), growth varied among treatments, but the control nubbins performed as well as the other treatments (Fig. 4). Lastly, for *Acropora nasuta,* skeletal growth was significantly lower under violet light than in the control, amber, and green treatments (Fig. 4). When growth data from all coral species were combined, coral skeletal growth was inversely

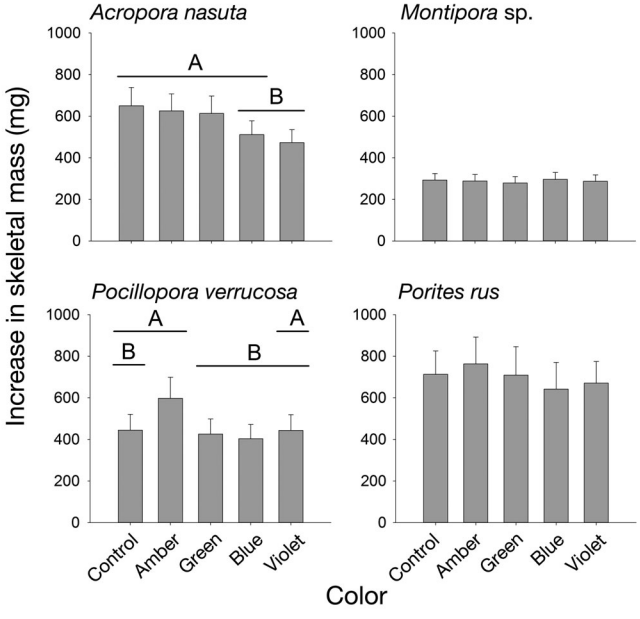

Fig. 4. Mean growth (+1 SE) of nubbins of the 4 coral species in each treatment over 1 mo after adjusting for effects of initial nubbin size. Letters over the bars as in Fig. 1. Treatments were not significantly different for *Montipora* sp. or *Porites rus*

related to increasing zooplankton enrichment measured as either abundance or biomass (Fig. 5). Enrichment of zooplankton abundance explained 28% of the variance in nubbin growth as determined from the value of r^2, while enrichment of biomass explained 38%.

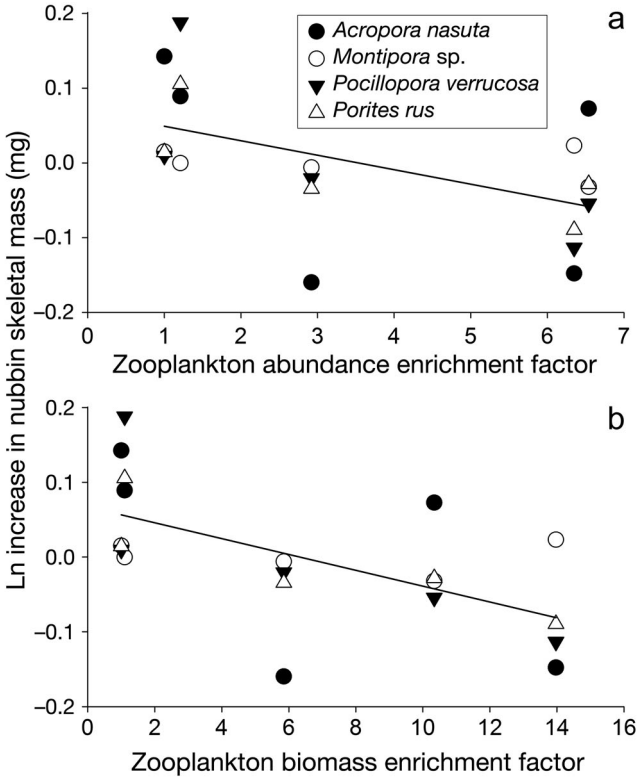

Fig. 5. Mean growth of all nubbins of each coral species (after adjusting for any effects of initial nubbin size) as a function of the mean total zooplankton enrichment factor (EF). Plotted data represent the average residual value of the ln-transformed nubbin growth obtained from a linear regression of the ln-transformed nubbin growth against initial nubbin size. Enrichment based on zooplankton (a) abundance ($p = 0.02$, $r^2 = 0.28$) and (b) dry mass ($p < 0.01$, $r^2 = 0.38$). EF values of 1 represent the (unlit) control treatments

DISCUSSION

This is the first study to investigate the impacts of enrichment of natural zooplankton on coral skeletal growth *in situ* under controlled wavelengths of light and the first to investigate the impacts of zooplankton availability on the growth of *Pocillopora verrucosa*, *Acropora nasuta, Porites rus*, and *Montipora* sp. Our results indicate that for these 4 coral species in the location we tested, zooplankton enrichment did not increase coral skeletal growth; indeed, growth for one species, *A. nasuta,* was lower at higher levels of enrichment. This latter result is similar to that found under laboratory conditions for *Porites compressa*, which experienced decreased skeletal extension rates with increases in feeding stimulated by plankton concentrations that were higher than ambient on the reef (Grottoli 2002). Moreover, it is consistent with Palardy et al. (2008), who found that the proportionate effects of species, depth, and bleaching treatments on nightly coral feeding rates of *P. compressa, Porites lobata*, and *Montipora capitata* were not significantly different between those experiencing ambient and enhanced zooplankton concentrations *in situ* off Hawaii.

One potential explanation for the lack of an effect of zooplankton enrichment on growth of nubbins in our trials is that it arose because of our experimental setup, specifically, the deployment of nubbins upside down during the austral winter. However, we believe it highly unlikely that the inverted orientation of our experimental nubbins affected the outcome for 2 reasons. First, the open lattice of the wire mesh to which the nubbins were attached, together with the highly reflective sand below, exposed coral nubbins to light levels similar to upright nubbins. Midday downwelled light at the study site averaged 745 ± 13 µmol photons m^{-2} s^{-1}, while reflected light of similar spectral quality averaged 705 ± 25 µmol photons m^{-2} s^{-1} (Colvard 2010). Second, our inverted control nubbins of *Pocillopora verrucosa* grew an average of 14.3 ± 1.5 mg d^{-1}, a rate that is similar to the 12.8 ± 1.5 mg d^{-1} and 13.8 ± 1.0 mg d^{-1} growth of upright nubbins in control treatments of a nearly identical sister species, *P. eydouxi,* measured at the same study site in August 2005 (Holbrook et al. 2008) and 2008 (Holbrook et al. 2011), respectively. Moreover, the *P. verrucosa* nubbins in our experiments during the austral winter grew considerably faster than upright nubbins of *P. verrucosa* measured during the austral summer at our study site by Colvard (2010). Together, these findings support the conclusion that nubbin position and the season in which we conducted our trials did not alter the outcome of the enrichment experiment.

Two biological hypotheses may explain why greatly enhanced mesozooplankton levels did not result in increased skeletal growth during our study. First, the corals we used may display species-specific adaptation for a proportionately greater reliance on photosynthesis than on zooplankton consumption. Heterotrophically acquired carbon is clearly a significant contribution to coral carbon budgets, accounting for as much as 46% of the daily requirements of some species when healthy and 100% when bleached (Palardy et al. 2008, Houlbrèque & Ferrier-Pagès 2009). However, our study suggests that the ambient flux of zooplankton in the shallow lagoons of Moorea is sufficient for the heterotrophic needs of the shallow-water species investigated. The lack of increased skeletal growth under zooplankton enrichment observed here is consistent with studies indicating that

healthy corals primarily use photoautotrophically acquired carbon for calcification (skeletal growth) and short-term metabolic demands and heterotrophically assimilated carbon for support of coral tissue and endosymbiotic algae (Ferrier-Pagès et al. 2003, Hughes et al. 2010).

Natural mesozooplankton abundance in Moorea is low, averaging <200 animals m^{-3} both day and night, although abundances can reach 400 animals m^{-3} near the surface and at full moon (Alldredge & King 2009). These ambient abundances are 2 to 5 orders of magnitude lower than zooplankton or particle abundances used in most laboratory studies (Sebens & Johnson 1991, Al-Moghrabi et al. 1995, Anthony & Fabricius 2000, Ferrier-Pagès et al. 2003, Houlbrèque et al. 2003, 2004a) and, although similar to other Pacific reefs, are considerably lower than abundances reported for Caribbean reefs (see summary comparisons in Heidelberg et al. 2004). Under shallow reef conditions of high water clarity and low zooplankton abundance, coral species in Moorea may rely primarily on photosynthesis for growth, a conclusion also drawn by Wellington (1982) for 3 eastern Pacific coral species. His study found no effect of reduced zooplankton supply on the growth of the shallow water corals *Pocillopora damicornis* and *Pavona clavus* over a year at ambient light conditions in the field, although growth of a third species, *Pavona gigantea*, was negatively affected by low zooplankton supply. Heavy reliance of corals on *Symbiodinium* symbionts rather than their own prey capture also has been supported by studies investigating the shift from autotrophy to heterotrophy. The ability to switch between these 2 nutritional modes appears to be species specific, and only certain coral species show heterotrophic plasticity (Houlbrèque & Ferrier-Pagès 2009). Heterotrophy appears to be particularly important in response to stress including turbidity/light reduction (Anthony 2000, Anthony & Fabricius 2000), depth (Palardy et al. 2005), and bleaching (Grottoli et al. 2006, Palardy et al. 2008, Connolly et al. 2012).

A second, perhaps less likely, biological hypothesis for the lack of a skeletal growth response to enhanced zooplankton in our study is that light treatments did not attract optimum food items. DNA analysis (see Leray 2012 for methods) of the guts of 3 fragments of *Pocillopora* sp. collected at our study site revealed consumption of a variety of taxa, especially polychaetes; the large, predatory copepod *Labidocera*; chaetognaths; and planktonic larvae such as those of crabs, decapods, and gastropods (M. Leray pers. obs.). All these taxa were enriched in our study.

While *Pocillopora verrucosa* is the only one of the 4 corals investigated for which food identity is available, this species did not grow faster despite being offered enriched concentrations of taxa it clearly consumes. Many previous studies document that corals feeding *in situ* on ambient plankton consume a diversity of taxa including amphipods, isopods, crab zoea, and polychaetes with occasional high capture rates of ostracods, mysids, and shrimp larvae; copepods, usually the most abundant taxon in the mesozooplankton, are only rarely captured by corals (Sebens et al. 1996, Heidelberg et al. 1997, Palardy et al. 2005, 2006, 2008). Amphipods, crustacean larvae, polychaetes, and mysids were highly enriched in our light treatments. The lights attracted the same taxonomic groups as present naturally but in higher numbers than ambient. Thus, it is unlikely that the lights in our study did not attract at least some of the types of zooplankton preferred by the coral species tested.

Our findings are also unlikely to be the result of the polyp sizes of the coral species selected. The size of zooplankton captured is not well related to polyp size: Both large and small polyps are capable, within limits, of capturing very large zooplankton including amphipods, isopods, crab zoea, and polychaetes (Sebens et al. 1996, Palardy et al. 2005, 2006), and branching corals with small polyps can capture more zooplankton per unit size than mounding corals with much larger polyps (Sebens et al. 1996, Palardy et al. 2005, 2006).

Finally, the coral species we used may depend more on particulate organic matter and microzooplankton too small to be collected by our 200 µm mesh nets, including crustacean nauplii, which are known to be consumed by corals (Houlbrèque et al. 2004b, Houlbrèque & Ferrier-Pagès 2009). The lagoon on the north shore of Moorea has very low levels of particulate and dissolved organic matter (Nelson et al. 2011), but little is known about the concentrations of microzooplankton. The role of microheterotrophy may be significant in this environment, and additional studies would be needed to clarify its role.

To the extent that nubbin growth differed among enrichment treatments, the tendency was for skeletal growth rates to be lower when zooplankton were most enhanced. One possible explanation for this is that high mesozooplankton concentrations interfered with coral feeding. Zooplankton were stimulated by the lights, and divers observed them swarming around them. High concentrations of rapidly swimming, relatively large taxa such as *Labidocera* sp., mysids, amphipods, decapod larvae, polychaetes, and shrimp continually bumping up against the coral

polyps may have elicited withdrawal of the polyp tentacles and inhibited food capture. Blue, green, and violet wavelengths attracted significantly more of the very large taxa. However, we consider feeding interference due to high abundance unlikely, primarily because previous laboratory studies with natural plankton used zooplankton concentrations of 10^7 animals m^{-3}, 4 orders of magnitude higher than our average enriched concentrations, with no ill effects on feeding (Ferrier-Pagès et al. 2003). Moreover, large taxa such as polychaetes, amphipods, and decapod larvae are the preferred zooplankton food for many coral species (Sebens et al. 1996, Palardy et al. 2005, 2006). While Palardy et al. (2005, 2006) reported maximum consumption of zooplankton in the size range of 200 to 400 µm, they derived zooplankton sizes by sieving the plankton. Their sizes represent minimum widths, not lengths, of zooplankton. Since amphipods, isopods, mysids, polychaetes, and most decapod larvae are usually 3 or more times longer than wide, corals in their studies were also feeding on mesozooplankton in the range of 500 to >1200 µm, similar to the sizes observed here. Behavioral studies of coral polyps feeding in the presence of high abundances of large, active zooplankton will be required to determine if high concentrations of naturally occurring zooplankton can, in fact, inhibit coral feeding.

The larger-bodied zooplankton attracted by our light treatments may have competed with corals for smaller prey. Corals are known to consume detrital particles (Anthony 2000, Anthony & Fabricius 2000), phytoplankton, and protozoans (Houlbrèque & Ferrier-Pagès 2009), and many of the larger zooplankton taxa enriched in the light treatments are also known to consume suspended particles, phytoplankton, mesozooplankton larval stages, and microzooplankton not captured by our 200 µm nets. If these smaller organisms were also preferred by the corals, the high numbers of zooplankton feeding near the outplanted nubbins may have depleted some of the coral food, resulting in the lower growth rate with higher zooplankton enrichment

Varying degrees of mortality and transplantation success have been reported for transplanted corals and coral fragments during restoration efforts (Rinkevich 2005). In response, Rinkevich (1995) suggested a 2-step strategy of 'gardening coral reefs', starting with *in situ* nurseries in sheltered areas where different types of coral could be grown to adequate size, followed by transplantation of these spats, nubbins, fragments, or small colonies to degraded reef sites. In such a 2-step process, practical methods to enhance coral growth and health during the 'nursery stage' could increase their success when later transplanted to the reef. However, our results suggest that enhancing zooplankton availability to coral fragments using artificial lights at night is unlikely to increase outplant skeletal growth unless heterotrophy is a growth-limiting process. However, we did not investigate other possible impacts of enhanced zooplankton abundance on the long-term survival of outplants including improved coral tissue health, greater resistance to environmental insult (e.g. temperature excursions), enhanced injury healing and attachment success, or possible detrimental effects such as greater exposure to zooplankton-borne pathogens. Additional research will be needed to evaluate the efficacy and practicality of this technology for reef restoration.

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