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Obligate ectosymbionts increase the physiological resilience of a scleractinian coral to high temperature and elevated pCO$_2$

Steve S. Doo[1] · Robert C. Carpenter[1] · Peter J. Edmunds[1]

Abstract Invertebrate ectosymbionts within the coralla of scleractinians enhance host fitness through protection from corallivores and nutrient addition. Here, we explore the ectosymbiotic relationship between the coral *Pocillopora verrucosa* and the crab *Trapezia serenei* and the shrimp *Alpheus* spp., to test for effects on coral calcification under contrasts of seawater temperature (27.7 °C and 29.9 °C) and pH (ambient, 8.0 and reduced, 7.7). Regardless of temperature, ectosymbionts depressed calcification by 55% (vs without ectosymbionts) at ambient pH; however, ectosymbionts only depressed calcification under ambient pH but not at reduced pH. These results suggest that *P. verrucosa* grows fastest at ambient pH without ectosymbionts, but when ectosymbionts are present, colonies are protected from further declines in calcification at reduced pH. This implies that there may be a change from a currently parasitic ectosymbiont–coral relationship to a commensal relationship that could increase fitness advantages for corals hosting crustacean ectosymbionts under ocean acidification conditions.

Keywords Coral · Climate change · Ocean acidification · Ectosymbiont · Ocean warming · Physiological buffering

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Introduction

A high diversity of ectosymbiotic organisms are typically found in the coralla of living corals (Stella et al. 2011; Leray et al. 2012) and within the interstices among branches and plates of select coral species (Coles 1980; Zann 2002). Regardless of whether the functional interactions between ectosymbionts and their scleractinian hosts are parasitic (e.g., vermetid snails), commensal (e.g., shrimps), or mutualistic (e.g., damselfish), their effects on the coral host usually have implications for the entire coral community (Zann 2002; Stella et al. 2011). Such community-level effects are likely to be particularly striking for ectosymbionts that modify coral growth and corallum morphology, which could be mediated through consumption of coral polyps (Abelson et al. 1991), thereby altering the availability of habitats to other taxa (Nash et al. 2013).

In colonies of the common branching Indo-Pacific corals *Pocillopora verrucosa* and *Acropora hyacinthus*, coralla are often inhabited by ectosymbiotic *Trapezia* spp. crabs and *Alpheus* spp. shrimps (Stella et al. 2011; Leray et al. 2012). Ectosymbiotic crabs protect their coral hosts against the corallivorous sea star *Acanthaster planci* (Pratchett 2001) and the parasitic vermetid snail *Dendropoma maximum* (Stewart et al. 2013). Additionally, coral hosts benefit through clearing of sediments from their tissue surfaces (Stewart et al. 2006) and increased growth rates (Glynn 1983). For *T. sereni* living in colonies of *P. meandrina*, the association is an obligate mutualistic symbiosis (Castro et al. 2004), because the adult crabs cannot survive outside of the coral host.

While association between corals and ectosymbionts has been documented on shallow coral reefs worldwide (Stella et al. 2011; Nogueira et al. 2014), the physiological interactions between these taxa remain poorly described.

Understanding these interactions is important to elucidating the causal basis of the mutualistic symbioses that are ubiquitous on tropic reefs (e.g., Muscatine et al. 1984), but they may also have relevance to the response of coral reefs to global climate change (GCC) and ocean acidification (OA). To date, laboratory experiments have been used to test for the physiological mechanism driving the organismic responses of corals to changing environmental conditions, including rising temperature and declining seawater pH (e.g., Edmunds and Burgess 2016), but such analyses often lack ecological relevance, such as interactions between species (Gaylord et al. 2015).

In this study, we quantified how the calcification rate of *Pocillopora verrucosa* is affected by the ectosymbionts *Trapezia serenei* and *Alpheus* spp. under combinations of two temperatures and two seawater pH conditions. These physical and chemical conditions provide relevance to understanding the implications of GCC and OA for ectosymbiont interactions within coral reef communities (Hoegh-Guldburg et al. 2007).

Methods

Colony collection and treatments

In January 2017, 48 colonies of *Pocillopora verrucosa* (Ellis and Solander 1786) were collected randomly on scuba from 5 m depth on the north shore of Mo'orea, French Polynesia (17°28′33″S, 149°49′20″W). Colonies $\sim$ 15 cm diameter containing ectosymbiont crustaceans were collected and transported to the Richard B. Gump South Pacific Research Station where they were acclimated in a flow-through tank (1000 L volume) and maintained in conditions similar to the ambient treatment described below.

Following 5 d of acclimation, 24 of the corals were selected randomly for removal of all trapeziid crabs and alpheid shrimps by probing with a wooden stick (3 mm diameter). Crabs and shrimp were left in the other 24 corals, which were subjected to a procedural control in which they were probed with a wooden stick. Hereafter, these two groups of corals are referred to as "plus-ectosymbiont" and "minus-ectosymbiont." Ectosymbiont density in the plus-ectosymbiont corals was adjusted so that each colony contained similar numbers of crabs and similar numbers of shrimps. Plus-ectosymbiont corals were carefully inspected for ectosymbionts, and if they contained < 2 crabs and < 1 shrimp, densities were increased through the addition of crabs, shrimps or both, which were randomly selected from crustaceans freshly isolated from the "minus-ectosymbiont" corals. The densities of ectosymbionts in *P. verrucosa* colonies freshly collected in

the field were 2.33 ± 0.14 (mean $\pm$ S.E., $n = 24$) for *Trapezia* sp. In the few cases ($n = 6$), where corals naturally contained a larger number of crabs and shrimp, ectosymbionts were not removed to prevent further disturbance to the corals. With this procedure, the density of crabs and shrimps in the plus-ectosymbiont corals was not identical among colonies, but any experimental bias introduced by this effect was addressed through the random allocation of plus-ectosymbiont corals to different treatments.

Incubation setup and experimental design

Twelve mesocosm tanks (150 L volume with sand-filtered seawater pumped from 14 m depth in Cooks' Bay and supplied to the tanks at $\sim$ 200 mL min^{-1}) were used in this experiment (Fig. 1a), with four colonies tank^{-1} in a split-plot design contrasting plus-ectosymbiont ($n = 2$ colonies tank^{-1}) and minus-ectosymbiont ($n = 2$ colonies tank^{-1}) corals. *In situ*, it is common for crabs and shrimps to move among adjacent coral colonies (Castro 1978), and corals were individually enclosed in cages (25 cm diameter) made of nylon net (1 mm aperture) that surrounded the base of each coral and extended 1 cm above the surface of the seawater to prevent this behavior. The mesh of the cages was supported on a cylindrical polypropylene fence (1-cm mesh; Fig. 1b) that ensured that the net did not contact the coral branches (Fig. 1b). The four corals within their cages were repositioned daily in each tank (Fig. 1b), and the cages were cleaned every 3–4 d to remove mucus and algae. Inspection of the corals during the day and night verified that neither crabs nor shrimps moved among colonies within each tank.

Light was provided to each tank using LED lamps (Sol White 75 W Aqua Illumination) operating on a 12:12 h light/dark photoperiod, with a 4-h ramp-up and ramp-down period, at the start and end of each day. At maximum power, the lamps supplied $\sim$ 500 µmol quanta m^{-2} s^{-1} as measured underwater and in the center of each mesh cage with a Li-Cor LI-1400 m and 4π LI-193 sensor. The maximum irradiance was lower than maximum irradiance *in situ* (5 m depth) when the experiment was conducted (January 2017), where maximum PAR was 800 µmol quanta m^{-2} s^{-1} (measured with a Li-Cor LI-1400 m and LI-193 sensor). The lower maximum irradiance in the tanks reduced the risks of bleaching in the high-temperature treatment (Abrego et al. 2008), while likely ensuring that *Symbiodinium* in the corals were able to photosynthesize at their maximum rate. The maximum irradiance in the tanks was greater than the sub-saturation irradiance (I_k) expected for corals living at $\sim$ 5 m depth on a tropical coral reef (Anthony and Hoegh-Guldberg 2003).

Fig. 1 Photograph and schematic of the experimental setup: **a** six of 12 tanks used in the experiment, **b** one tank containing four cages within which one colony of *Pocillopora verrucosa* was positioned, and **c** schematic of the experimental design showing the factorial combinations of ectosymbiont treatment (plus and minus), two pH treatment, and two temperatures
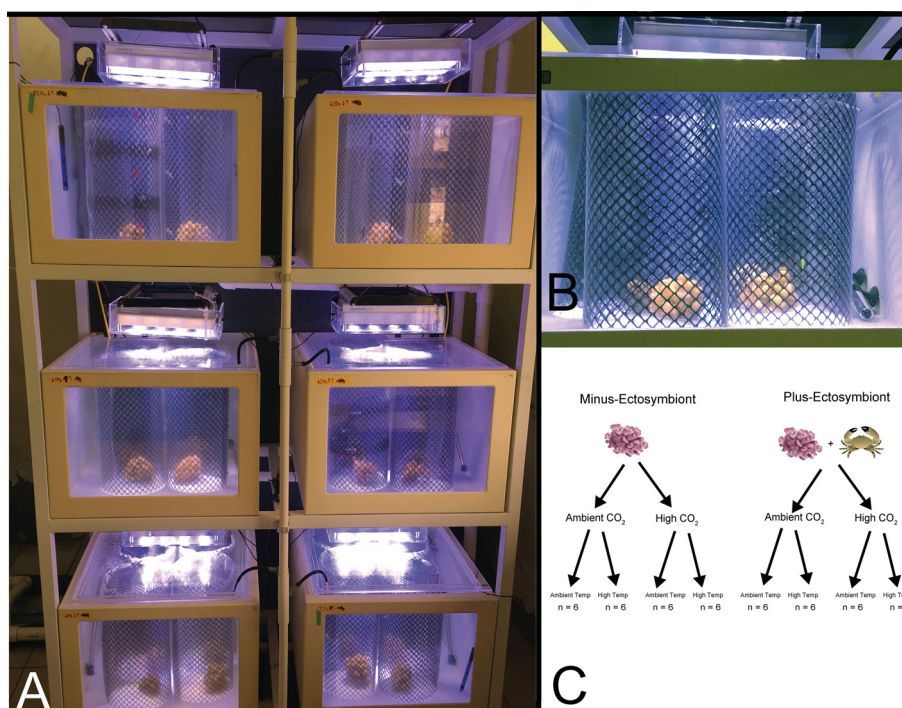
Seawater manipulation

The 12 tanks were set to either 27.7 °C or 29.9 °C that were maintained as in Comeau et al. (2014), and temperatures were measured daily with a calibrated Fisher Scientific Traceable Digital Thermometer (Model 15-077, $\pm$ 0.05 °C). CO_2 treatments were controlled using an Apex Neptune Systems Aquacontroller that operated solenoids supplying CO_2 gas to the treatment tanks to maintain a pH that was 0.3 pH below the ambient 8.02 pH treatment. This pH treatment corresponded to an atmospheric pCO_2 of $\sim$ 1000 µatm, which is projected to occur in seawater by the end of the current century assuming moderate RCP 6.0 estimates (IPCC 2013). Seawater pH and total alkalinity were measured every second day using spectrophotometric and potentiometric methods, respectively (after Dickson et al. 2007), with accuracy and precision evaluated using Certified Reference Materials (CRMs; Batch 151 from A. Dickson Laboratory, Scripps Institute of Oceanography). Salinity was measured every 2 d using a benchtop conductivity meter (Thermo Scientific Orion Star A212).

Experimental duration and calcification measurements

Corals were incubated in treatment conditions for 21 d, with an initial 3-d ramp-up (both temperature and CO_2) prior to the onset of the experiment, and calcification rates were measured by determining the change in mass at the beginning and end of the experiment through buoyant weighing (after Spencer-Davies 1989). The change in buoyant weight was converted to mass of $CaCO_3$ using the density of aragonite (2.93 g cm^{-3}), and an empirical determination of seawater density, and normalized to the surface area of coral tissue and time. Surface area of living tissue was quantified at the end of the experiment by wax dipping and was used to normalize calcification (Stimson and Kinzie 1991).

Statistical analysis

A four-way, split-plot ANOVA was used to test for treatment effects on coral calcification (dependent variable), in which pH and temperature were main effects, tank was a random effect nested within pH and temperature, and the "ectosymbiont" was a split-plot effect within tank. The tank effect was dropped from the model when it was not significant at $P > 0.25$ (Underwood 1997), and the analysis was repeated as a three-way ANOVA. The statistical assumptions of ANOVA, normality and homoscedasticity, were tested through visual inspection of residuals and Bartlett's test, respectively. All analyses were performed using R statistical software.

Results and discussion

Net calcification of *Pocillopora verrucosa* ranged from $-$ 0.018 to 0.556 mg cm^{-2} d^{-1}. The interactive effect of ectosymbionts and pH caused a 55% decline in mean net

calcification of corals compared to colonies with ectosymbionts incubated in ambient pH conditions, the effect was absent at low pH, and this interactive response was unaffected by temperatures (Table 1; Fig. 2). Overall (i.e., averaged across pH and temperature), ectosymbionts caused a 38% decline in mean calcification of *P. verrucosa*; no other main effects or interactions were significant (Table 1), and tank was dropped from the model (*P* = 0.58). The mean calcification rates of *P. verrucosa* reported here are ~ 50% lower than for the same species incubated under similar conditions in previous studies conducted in Mo'orea (Comeau et al. 2014; Edmunds and Burgess 2016), but this discrepancy likely reflects differences in the environmental conditions preceding the experiments. When the present study was completed in January 2017, *Pocillopora* on the outer reef had been exposed to ~ 4 months of warm seawater associated with the 2016 El Niño, and earlier in 2016, had experienced minor bleaching (Edmunds 2017). In contrast, Edmunds and Burgess (2016) conducted their experiments in April and May of 2014 and 2015 and Comeau et al. (2014) completed their experiments in August–October of 2012. Therefore, it is reasonable to expect that the present calcification rates were depressed by a combination of thermal stress (Cantin and Lough 2014) and the discrepancy in seasons in which the experiments were conducted (Bahr et al. 2017).

As OA is expected to intensify over the next century (Hoegh-Guldberg et al. 2007), results from our study suggest that invertebrate ectosymbionts in pocilloporid corals could reduce the susceptibility of calcification to declining pH by reducing the magnitude of the OA effect on calcification. Although we cannot evaluate the independent contributions of skeletal dissolution versus accretion to this outcome, ectosymbionts reduced calcification under ambient pH. A parsimonious explanation for the subsequent resistance of calcification to reduced pH is that
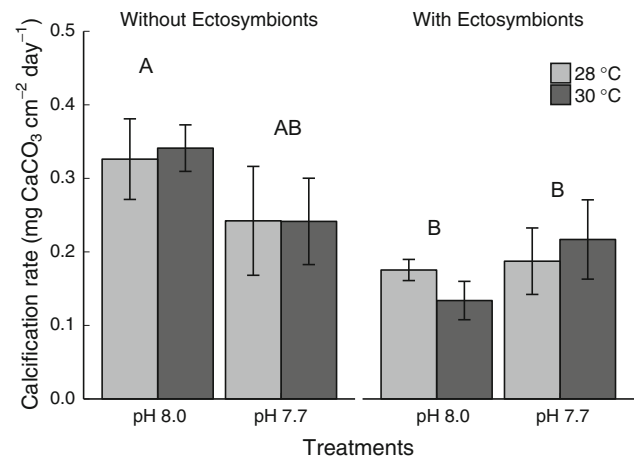

Fig. 2 Effects of pH (8.0 vs 7.7) and temperature (27.7 °C vs 29.9 °C), and ectosymbionts (plus and minus) on surface area normalized calcification (mg cm^{-2} d^{-1}) based on a mass increment measured over a 21-d incubation. Bars show mean ± SE (*n* = 6); letters indicate statistically significant Tukey post hoc results (*P* < 0.05)

slower calcifiers are less affected by OA (Comeau et al. 2014) and physiologically modify seawater to tightly regulate the pH at the site of calcification (Comeau et al. 2014). This interpretation of our results suggests that a 55% depression of coral calcification in the presence of crabs could reduce the sensitivity of calcification in *Pocillopora* to low pH (Fig. 2). However, it is premature to exclude the possibility that the ectosymbionts explicitly prevented impairment of calcification at low seawater pH.

Our results suggest that the association between pocilloporid corals and their ectosymbiotic crustaceans is more complex than an obligate parasitism (as in Knudsen 1967; Castro et al. 2004). An obligate parasitism between the coral host and their ectosymbionts is consistent with the outcome observed in the present study at pH 8.0, where coral calcification was depressed by ectosymbionts, and in return, the ectosymbionts presumably benefited from shelter and food. At pH 7.7, however, the ectosymbionts probably benefited from shelter and food, but host calcification was unaffected by ectosymbionts, suggesting that the association had shifted from host impairment (i.e., parasitism) to a null interaction for both organisms (i.e., commensalism). This outcome raises the possibility that the functional relationship between pocilloporid corals and their ectosymbiotic crustaceans is dynamic, transitioning from parasitic to commensal as environmental conditions change. As such, on future coral reefs subject to OA, impairment of calcification rates of *P. verrucosa* is not expected to be accentuated further impaired by crustacean ectosymbionts. Our study suggests that associations between corals and crustacean ectosymbionts appear to have the capacity to modulate the implications of OA to

Table 1 ANOVA table for a three-way split-plot design in which net calcification normalized to surface area was the dependent variable

Effect	df	MS	F	P
pH	1	5.88E−3	0.308	0.594
Temperature	1	< 0.001	0.001	0.989
Ectosymbionts	1	0.144	11.190	0.003
Ectosymbionts × pH	1	0.058	4.547	0.049
pH × temperature	1	2.30E−3	0.163	0.738
Temperature × ectosymbionts	1	5.2E−4	0.040	0.842
pH × temperature × ectosymbionts	1	5.65E−3	0.440	0.512
Residuals	32	0.014		

Temperature, pH and ectosymbionts were fixed effects, with tank treated as a random effect nested in pH and temperature; tank was dropped from the model as it was not significant (*P* = 0.46)

coral communities, while still providing other ecological benefits to corals, such as protection from corallivorous predators.

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