Effects of thermal stress and nitrate enrichment on the larval performance of two Caribbean reef corals

[1]Xaymara M. Serrano
[2]Margaret W. Miller
[3]James C. Hendee
[4]Brittany A. Jensen
[4]Justine Z. Gapayao
[4]Christina Pasparakis
[4]Martin Grosell
[4]Andrew C. Baker

[1]Cooperative Institute of Marine and Atmospheric Studies, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL USA

[2]National Oceanic and Atmospheric Administration-Southeast Fisheries Science Center, Miami, FL USA

[3]National Oceanic and Atmospheric Administration-Atlantic Oceanographic and Meteorological Laboratory, Miami, FL USA

[4]Department of Marine Biology and Ecology, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL USA

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Abstract

The effects of multiple stressors on the early life stages of reef-building corals are poorly understood. Elevated temperature is the main physiological driver of mass coral bleaching events, but increasing evidence suggests that other stressors, including elevated dissolved inorganic nitrogen (DIN), may exacerbate the negative effects of thermal stress. To test this hypothesis, we investigated the performance of larvae of *Orbicella faveolata* and *Porites astreoides*, two important Caribbean reef coral species with contrasting reproductive and algal transmission modes, under increased temperature and/or elevated DIN. We used a fluorescence-based microplate respirometer to measure the oxygen consumption of coral larvae from both species, and also assessed the effects of these stressors on *P. astreoides* larval settlement and mortality. Overall, we found that (1) larvae increased their respiration in response to different factors (*O. faveolata* in response to elevated temperature and *P. astreoides* in response to elevated nitrate) and (2) *P. astreoides* larvae showed a significant increase in settlement as a result of elevated nitrate, but higher mortality under elevated temperature. This study shows how microplate respirometry can be successfully used to assess changes in respiration of coral larvae, and our findings suggest that the effects of thermal stress and nitrate enrichment in coral larvae may be species specific and are neither additive nor synergistic for *O. faveolata* or *P. astreoides*. These findings may have important consequences for the recruitment and community reassembly of corals to nutrient-polluted reefs that have been impacted by climate change.

Keywords: *Orbicella faveolata*, *Porites astreoides, e*utrophication, nutrients, climate change, bleaching

Introduction

Elevated sea surface temperature is the main physiological driver behind mass coral bleaching events worldwide (e.g., Hughes et al. 2003). However, new evidence suggests that poor water quality may have strong additive or synergistic effects, to the extent that corals that regularly experience poor water quality conditions may be less thermotolerant than corals elsewhere (Wooldridge 2009; Wooldridge and Done 2009; Negri et al. 2011; Negri and Hoogenboom, 2011; Wiedenmann et al. 2013). One of the mechanistic explanations for this interaction has centered on the potential for dissolved inorganic nitrogen (DIN) to promote excessive algal symbiont growth rates (D'Angelo and Wiedenmann 2014), resulting in phosphate starvation (Wiedenmann et al. 2013) or CO_2 limitation (Wooldridge 2013) and higher production of reactive oxygen species in corals with more symbionts (Cunning and Baker 2013). However, evidence of clear and direct negative impacts of nutrient enrichment on corals is relatively scant, as the mechanisms by which dissolved inorganic nutrients can affect corals and their algal symbionts are still subject to debate (reviewed in D'Angelo and Wiedenmann 2014). Mixed effects reported in the literature are also confounded by different concentrations and/or inorganic form used (e.g., ammonium vs. nitrate; see Fabricius 2005), as well as different experimental conditions and lack of control of actual nutrient uptake (Langdon and Atkinson 2005). Therefore, a better understanding of the impacts of nutrients (particularly when they act in combination with heat or light stress) is critical to understanding coral reef resilience in nutrient-polluted coastal areas.

Many characteristics of the physiology of coral larvae (e.g., pelagic duration, depletion of maternal reserves, immature cellular defenses and smaller biomass, and higher surface-to-volume ratios compared to adults) may render them more susceptible than adult corals when

exposed to the same stressors (e.g., Putnam et al. 2010). Most research on coral larvae to date has focused on the effects of elevated temperature and has reported effects on performance and/or survival (Edmunds et al. 2001; Nozawa and Harrison 2007; Putnam et al. 2008; Randall and Szmant 2009a, 200b; Yakovleva et al. 2009; Hartmann et al. 2013; Olsen et al. 2013), competency periods and dispersal distances (O'Connor et al. 2007; Figueiredo et al. 2014). However, the effects of multiple stressors on early life stages of coral are poorly understood, with only few studies available (Negri et al. 2011; Negri and Hoogenboom 2011; Cumbo et al. 2013a, 2013b; Putnam et al. 2013; Rivest and Hofmann 2014; Putnam and Gates 2015; Humanes et al. 2016), and only one study focused on a Caribbean coral species (*Diploria strigosa*; Bassim and Sammarco 2003). The limited data available suggest that coral larvae and recruits are highly dependent on clean water and low sedimentation (reviewed in Fabricius 2005 and Jones et al. 2015), but only two studies have investigated interactions of nutrients and temperature (Bassim and Sammarco 2003; Humanes et al. 2016), reporting additive effects. To our knowledge, no studies have yet investigated the effects of these stressors on coral larvae from a brooding species, or compared responses among coral species with different reproductive and algal transmission modes.

The aim of this study was to investigate the performance of coral larvae from two important Caribbean reef coral species (*Orbicella faveolata* and *Porites astreoides*) under elevated seawater temperature and/or nitrate enrichment. We tested whether these stressors can affect the respiration, settlement and/or survival of coral larvae, and hypothesized additive or synergistic effects regardless of species. *Orbicella faveolata* (Ellis and Solander, 1786) is an important Caribbean reef-building coral (Villinski 2003), recently listed as threatened under the US Endangered Species Act. It undergoes annual mass spawning, typically 6–8 d after the full

moon in August and/or September (Szmant 1986, 1991). In addition, algal symbionts are not present in the eggs (Szmant 1991) and must be acquired from the water column, usually several days after settlement (e.g., Coffroth et al. 2001). In contrast, *P. astreoides* (Lamarck, 1816) is a brooding species that appears to be one of the few scleractinian coral species increasing in relative cover on shallow Caribbean coral reefs (Green et al. 2008). *Porites astreoides* undergoes internal fertilization and releases mature planulae monthly from January to September (Szmant 1986; McGuire 1998), in addition to having an unusual mixed breeding system in which colonies may be hermaphroditic or female (Chornesky and Peters 1987). Finally, algal symbionts are present in brooded larvae when released (Chornesky and Peters 1987), and there appear to be strong patterns of depth zonation in types hosted at most locations examined to date (e.g., Bongaerts et al. 2015; Serrano et al. 2016).

Materials and Methods

Larval collection

Porites astreoides

A total of 50 adult colonies of *P. astreoides* were collected from two patch reefs near the Keys Marine Laboratory in Layton, Florida (24°44.004′N, 80°49.728′W and 24°44.023′N, 80°49.770′W), at depths < 10 m, one day prior to the new moon (night of 17 May 2015). To obtain larvae, colonies were placed in a holding tank in individual Rubbermaid Grip ‘N Mix bowls supplied with continuous seawater flow through a sprinkler system outfitted with individual inflow hoses. Each bowl was tilted so that the larvae would travel through the channel in the handle and into a plastic Ziploc Twist ‘N Loc round container modified with 120-µm nylon mesh at the bottom to allow water to flow out but larvae to remain inside. The water level inside the holding tank was adjusted so that the positively buoyant larvae would remain inside

the containers until the following morning. Twenty-five different colonies released larvae on the first night, and these larvae were pooled and transported to the University of Miami's Marine Technology and Life Science Seawater (MTLSS) building on Virginia Key for experiments. Finally, adult colonies were returned to their site of collection and reattached using underwater epoxy or a mixture of cement and plaster.

Orbicella faveolata

Orbicella faveolata larvae were collected and cultured as described in Szmant and Miller (2006) and Miller (2014). Gamete bundles were collected seven nights after the full moon (night of 6 August 2015) from colonies that spawned at two reefs in the upper Florida Keys (Horseshoe and Sand Island reefs: 25°08.313′N, 80°17.690′W and 25°01.093′N, 80°22.096′W, respectively). Once the bundles broke up, equal volumes of gametes from 3 to 4 parental genets were combined for fertilization. Resulting embryos were maintained in water collected from their natal reef (either in static bins with regular water changes or in a recirculating system with mesh-floored bins and a constant drip input) and cultured for ~ 2 d so that unfertilized eggs would disintegrate, leaving behind only developing larvae. Embryos were kept in a shaded area under ambient light conditions and a mean temperature of 29°C. Larvae were then transferred to the University of Miami's MTLSS building to begin experiments. However, due to inadequate supply of *O. faveolata* larvae we were only able to carry out Experiment 1 (see below).

Experimental set-up

A high-temperature treatment of 31.5°C was chosen in this study because it represents an increase of ~ 1.5°C above the current mean temperature for August (when spawning occurs) in the Florida Keys (30°C; Kuffner et al. 2015) and ~1°C above the bleaching threshold (> 30.5°C)

reported for corals in the same region (Manzello et al. 2007). Conversely, a temperature of ~ 28°C was chosen as a control for both species, as Kuffner et al. (2015) have also shown that corals in the Keys experience average temperatures of ~ 28°C in June and average temperatures > 29°C during July through September (and although the majority of larval release by *P. astreoides* occurs in April and May, smaller numbers of larvae have also been observed in June through September; McGuire 1998). Finally, target nitrate concentrations of ~ 3 μM (low treatment) and ~ 12 μM (high treatment) were also chosen to encompass and exceed real values measured from reef waters globally [0.22–8.04 μM total DIN (NO_2 + NO_3); D'Elia and Wiebe 1990], without exceeding those reported in groundwater along the Florida Keys (up to 67 μM DIN; Shinn et al. 1994). Because we were primarily interested in the effects of elevated nitrate, phosphate and nitrite were kept at ambient levels (see electronic supplementary material, ESM, Table S1).

A factorial design was used, in which the four experimental treatments were targeted as: 28°C / ~ 3 μM NO_3 (low nitrate and temperature, considered the control treatment); 28°C / ~ 12 μM NO_3 (elevated nitrate); 31.5°C / ~ 3 μM NO_3 (elevated temperature); and 31.5°C / ~ 12 μM NO_3 (elevated nitrate and temperature). Experimental treatments were in eight 5-gallon aquaria (two replicate aquaria per treatment), randomly allocated to two tanks which served primarily as a water bath to hold temperature constant. Temperature was continuously regulated using a cold–heat exchanger (Aqualogic, USA), while additional heating to 31.5°C was achieved using individual 300 Watt ViaAqua titanium submersible heaters placed inside aquaria. Small water powerheads (Azoo model 600, USA) provided constant water motion, and temperatures were continuously monitored using a HOBO logger (Onset Computer, USA) set to a sampling frequency of 10 min (ESM Fig. S1). Seawater was filtered and UV-sterilized throughout the

duration of the experiment to reduce microbial growth. Full water changes were done every 2 d, and sodium nitrate ($NaNO_3$, EMD Millipore, USA) was added to maintain target nitrate concentrations. Preliminary tests were conducted to assess the appropriate frequency needed for water changes and avoid ammonia buildup (data not shown). To validate nutrient treatments, water samples were collected from individual aquaria 1 d after each water change for analyses of nitrate, nitrite and phosphate using an autoanalyzer (Lachat QuickChem 8500, USA). Experiments were conducted using custom spectrum LED lights (Build My LED, USA) set to an intensity of ~ 90 µmol m^{-2} s^{-1} PAR and diel cycling of 12:12 h; light levels were measured with an in-water cosine irradiance radiometer (Biospherical Instruments, USA) placed in the center of the bottom of aquaria so that the sensor was adjacent to corals. These light levels, although low, were used to resemble the light conditions at which embryos and larvae were reared prior to bringing them to the University of Miami's MTLSS building for experiments. Finally, the same experimental set-up was used for both species even though experiments were carried out at different times of the year because of different reproductive seasons.

Experiment 1: effects on larval respiration

Five-day-old (*P. astreoides*) or 12-day-old (*O. faveolata*) larvae were randomly assigned to one of the four treatments. Larvae were held in Ziploc Twist 'N Loc round containers fitted with a 120-µm nylon mesh (to allow for water flow) clamped to a wall of each aquaria. Two hundred larvae of each species were placed in each aquarium. Due to differences in larval size, they were divided into two flow-through containers for *P. astreoides* (N = 100 each) or kept in one for *O. faveolata*. After 72-h treatment exposure, larvae were taken at random for measurements of respiration rates using a sealed glass 200-µL microplate developed by Loligo Systems (Denmark) and described elsewhere (e.g., Pasparakis et al. 2016; Yashchenko et al.

2016). This instrument allows the measurement of respiration rates simultaneously in each of 24 independent wells using an integrated optical fluorescence-based oxygen sensing technology (SDR SensorDish Reader, PreSens, Germany). The system comes pre-calibrated with data sets provided specifically for the reader used, and oxygen solubility is continuously calculated with oxygen sensors (optrodes) attached to the bottom of each well. These sensors have several important features (e.g., very small, signal does not depend on the flow rate of the sample, suitable for noninvasive measurements), which make them ideal for measuring respiration rates in very small mobile organisms.

In our experiments, two different runs were conducted per species: one at the lower temperature (28°C) and one at the elevated temperature (31.5°C). In each run, we randomly filled half of the wells with UV-sterilized low nitrate water (~ 3 μM NO_3) and the other half with elevated nitrate (~ 12 μM NO_3) and placed either three (*P. astreoides*) or eight (*O. faveolata*) larvae per well, with 8–10 replicate wells per treatment. Four additional control wells without any larvae were used in each plate to measure any background respiration in the treatment water. Larvae were visually inspected for normal swimming behavior prior to each run. After each plate was prepared, wells were inspected to make sure there were no air bubbles and an oxygen-impermeable seal created using a silicone membrane covered with parafilm and a compression block. To maintain the target temperature, the microplate was placed inside a temperature-controlled flow-through water bath (included as part of the system), in addition to conducting the experiments in a temperature- and light-controlled incubator. The plate was then placed in a titer shaker at a low speed to maintain constant water motion. Finally, measurements of oxygen concentrations were taken every 15 s throughout the duration of the run and recorded using the SDR version 4.0.0 software (PreSens, Germany). Preliminary trials were conducted to assess

whether respiration rates measured in individual coral larvae from these species were comparable with other studies (e.g., Albright et al. 2008) and to determine the appropriate number of larvae needed per well to yield linear declines in oxygen concentration comparable in duration for both species (ESM Fig. S2). Oxygen concentration was plotted as a function of time for individual wells, and the first 10–20% (pO_2 in percentage air saturation) linear decreases in oxygen were used to calculate oxygen consumption rate. (Note that any portion of the slope that dipped below 70% air saturation was not used for analysis.) Finally, respiration rates were corrected for background respiration and calculated as nM O_2 $larva^{-1}$ min^{-1}.

Experiment 2: effects on *P. astreoides* larval settlement and mortality

A total of four replicate containers (two per aquarium; $N = 100$ *P. astreoides* larvae placed in each) were used to quantify larval settlement and mortality per treatment. Inside each container, a ceramic plug (Boston Aquafarms, USA) was added, after preconditioning it on a reef adjacent to the site of larval collection for ~ 1 month. This was done to allow enough time for crustose coralline algae and bacterial biofilms to develop on the substrates and act as settlement cues (Erwin et al. 2008). Each substrate was then placed on a piece of polystyrene egg crate to elevate it from the bottom of the container and allow larvae to access both the top and bottom surfaces. The substrate was removed from each container after 2, 4 and 5 d exposure to treatments. The number of larvae swimming (still in planula phase) and the total number settled and metamorphosed on the substrate (top, sides or bottom) and on the walls of the container were counted. Metamorphosis was visually confirmed either using a stereoscope or magnifying lens. After each count of survivorship/settlement, larvae remaining in each container were transferred to another container with a new substrate and then returned to the aquaria until the next time point. Aquaria were cleaned and reallocated among treatments at the same time. This was done

to remove any potential bias or settlement cues caused from using the same container, substrate or aquarium as in the previous time. Finally, to determine the cumulative percentage of larval settlement, the total number of metamorphosed spat at each time point was divided by the initial number of larvae added to each container ($N = 100$). Similarly, the cumulative loss of swimming larvae due to mortality was calculated by dividing the total number of swimming larvae + metamorphosed spat at each time point by the initial number of larvae added to each container.

Statistical analyses

Larval respiration data for both species were analyzed using a two-way ANOVA after natural log transformation (statistical comparisons used data obtained independently from each of the 24 wells of the microplate reader). *Porites astreoides* settlement and mortality data were arcsine square root-transformed and analyzed using a generalized linear mixed-effect model (GLMM) with fixed effects of nitrate, temperature and time (exposure days), and aquarium as a nested random factor (since each aquarium held duplicate containers with larvae). In the final GLMM, nonsignificant fixed effects were eliminated by backwards selection using F tests (e.g., Kuznetsova et al. 2015). Assumptions of normality were evaluated with the Shapiro–Wilk test. All statistical analyses used the software JMP Pro version 12 (SAS Institute Inc., USA).

Results

Inorganic nutrient measurements (nitrate, nitrite and phosphate) for all treatments are provided in ESM Table S1. After 72 h of exposure to the four treatments, elevated temperature significantly increased the respiration of *O. faveolata* (two-way ANOVA, $F = 2.5812$, $p = 0.009$; Fig. 1a), but not of *P. astreoides* larvae ($p > 0.05$; Fig. 1b). Conversely, elevated nitrate

conditions significantly increased the respiration of *P. astreoides* (two-way ANOVA, $F = 1.9731$, $p = 0.027$; Fig. 1b), but not of *O. faveolata* larvae ($p > 0.05$; Fig. 1a).

Settlement and mortality were scored for *P. astreoides* larvae after 2, 4 and 5 d of exposure to treatments (Figs. 2a, 2b). Elevated nitrate conditions had a significant positive effect on larval settlement (final GLMM: $p = 0.0382$; Table 1a; Fig. 2a), but no significant effect on larval mortality ($p > 0.05$). Elevated temperature, on the other hand, had no significant effect on settlement ($p > 0.05$) but significantly increased larval mortality over time (final GLMM: $p = 0.0036$; Table 1b; Fig. 2b). Time (exposure days) had a significant effect on both larval settlement (final GLMM: $p < 0.0001$; Table 1a; Fig. 2a) and mortality (final GLMM: $p < 0.0001$; Table 1b; Fig. 2b), as well as a significant interaction with temperature on larval mortality (final GLMM: $p = 0.0005$; Table 1b; Fig. 2b). There were no other significant interaction effects.

Discussion

In this study, we investigated the performance of coral larvae from two important Caribbean reef corals (*O. faveolata* and *P. astreoides*) under elevated temperature and/or nitrate conditions. We hypothesized that additive or synergistic effects of these two stressors would result in reduced respiration, settlement and/or survival of coral larvae, regardless of species. However, our findings did not support this hypothesis and illustrated species-specific differences, namely: (1) only temperature increased larval respiration of *O. faveolata* and only nitrate of *P. astreoides*; (2) nitrate showed a significant improvement of larval settlement of *P. astreoides*; and (3) temperature increased larval mortality of *P. astreoides*. Contrary to our expectations, these findings suggest that the effects of thermal stress and nitrate enrichment are neither additive nor synergistic for *O. faveolata* or *P. astreoides* larvae. We hypothesize that the different reproductive strategies and algal transmission modes of *P. astreoides* and *O. faveolata*

may account for some of these species-specific responses; for example, *P. astreoides* larvae may be more susceptible to nutrient effects than *O. faveolata* because they have algal symbionts. However, further work should include a broader survey of coral species to test the generality of this pattern. Together, these findings may have important consequences for the recruitment potential of these species and suggest that variability among species may affect trajectories of coral community reassembly on nutrient-polluted reefs that have been impacted by climate change.

Effects on coral respiration

To our knowledge, only one study has assessed the effects of elevated temperature on the respiration of larvae from a broadcast-spawning coral species. Rodriguez-Lanetty et al. (2009) assessed the effects of three temperatures (24, 28 and 31°C) on gene expression and respiration of larvae of the coral *Acropora millepora* after 3- and 10-h exposure. Respiration significantly increased in the elevated temperature treatment (31°C) after as little as 3-h exposure, along with increased expression of heat-shock proteins, suggesting an increase in the metabolic demand of larvae. In our study, *O. faveolata* larvae had similar respiratory responses to those of *A. millepora* after 72-h exposure to elevated temperature, although the onset of the response and potential increase in gene expression of heat-shock proteins were not addressed. It is possible that the increase in metabolic rate in *O. faveolata* larvae provides increased energy resources to ameliorate cellular stress at elevated temperatures, but we were unable to assess this. In fact, Polato et al. (2010) assessed the effects of a 48-h exposure to elevated temperatures (1–2°C above summer mean temperatures) on gene expression in *O. faveolata* aposymbiotic embryos and found variation in genes associated with key processes such as apoptosis, cellular structure, and energy and protein metabolism, leading the authors to hypothesize that *O. faveolata* embryos

may possess the potential to cope rapidly with thermal stress. In addition, a more recent study (Humanes et al. 2016) assessed the cumulative effects of nutrient enrichment and elevated temperature in the early life stages of the broadcast-spawning Pacific coral *A. tenuis*. Larval survivorship and settlement decreased in response to elevated temperature, despite little effect of nutrient enrichment in the larval stage compared to earlier life stages (i.e., fertilization and embryogenesis). In the case of *O. faveolata*, further work could investigate whether nutrient effects might be more pronounced at the embryonic stage (as observed in Humanes et al. 2016) or after larvae settle and symbiosis is established.

For brooding corals, the effects of elevated temperatures on respiration of coral larvae appear to vary depending on the day larvae are released (e.g., Edmunds et al. 2001; Cumbo et al. 2013a, 2013b; Rivest and Hofmann 2014), exposure duration (e.g., Cumbo et al. 2013a, 2013b; Olsen et al. 2013) and/or species studied. Previous work with *P. astreoides* suggested no significant increases in respiration after 24 h of exposure to 30°C (Ross et al. 2012) or 33°C (Edmunds et al. 2001) compared to control conditions, even though photosynthesis was significantly reduced in the latter (Edmunds et al. 2001). A more recent study (Olsen et al. 2013) found significant increases in larval respiration, oxidative damage and antioxidant enzyme activity on *P. astreoides* larvae after just 4-h exposure to 30.8°C. Our study, however, did not find significant changes in larval respiration even after 72 h of exposure to 31.5°C and low nitrate levels, consistent with findings from Edmunds et al. (2001) and Ross et al. (2012). It is possible, however, that different responses in studies of *P. astreoides* resulted from physiological differences between sequential broods of larvae (see Edmunds et al. 2001; Cumbo et al. 2013a, 2013b; Rivest and Hofmann 2014), different parental genotypes or different methods.

To our knowledge, this is the first study to report increased respiration rates for coral larvae exposed to increases in DIN. Since aposymbiotic *O. faveolata* larvae did not exhibit this response, we hypothesize that increased respiration in *P. astreoides* larvae could be due to the presence of algal symbionts. As elevated DIN has been shown to promote increases in algal symbiont densities (reviewed in Fabricius 2005), its influence on symbiont populations could be more significant than that of temperature (Edmunds et al. 2005), potentially accounting for increased larval respiration rates in the present study. (Unfortunately, we did not assess algal symbiont densities to test this hypothesis.) Alternatively, increased respiration rates in nutrient-enriched *P. astreoides* larvae could be the coral's stress response to potential photo-physiological damage to their algal symbionts, given that imbalances in DIN have been shown to result in coral bleaching (Wiedenmann et al. 2013; D'Angelo and Wiedenmann 2014). Finally, it is possible that increases in metabolic activity in *P. astreoides* resulted from larvae more actively seeking a favorable substrate to settle as result of nitrate enrichment (Fig. 2a).

Effects on *P. astreoides* larval settlement and mortality

In this study, *P. astreoides* larvae did not exhibit significant changes in respiration or settlement rates in response to elevated temperature (Figs. 1b, 2a), yet they exhibited significant increases in mortality (Fig. 2b), consistent with findings from Edmunds et al. 2001 (but see Ross et al. 2012; Olsen et al. 2013). We acknowledge that the elevated temperature treatment (31.5°C) used here may be more extreme for *P. astreoides* larvae than for *O. faveolata*, as larvae in the Florida Keys are generally exposed to mean temperatures of 24–27.5°C during planulation periods (McGuire 1998). However, since *P. astreoid*es adult colonies have been shown to release mature planulae monthly from January to September (Szmant 1986; McGuire 1998), larvae could be exposed to a wide temperature range during this period. In addition, brooded larvae

have been shown to have competency periods > 100 d (e.g., Harii et al. 2002), which suggests that *P. astreoides* larvae could experience high temperatures if they have a long pelagic duration. Furthermore, even though we were unable to assess the effects of thermal stress on the settlement and mortality of *O. faveolata* larvae, our respiration data suggest that symbiotic brooded larvae may respond differently to elevated temperatures than aposymbiotic larvae of broadcast-spawning coral species. In one study, Yakovleva et al. (2009) suggested that maternal inheritance of symbionts may come at a cost to larval mortality if temperatures are elevated (but see Baird et al. 2006); larvae of *A. intermedia* inoculated with algal symbionts had lower survival than those that lacked symbionts when exposed to high temperature (26 vs. 32°C). The authors hypothesized (1) that these responses likely resulted from oxidative stress in the algal symbionts and (2) that higher mortality of symbiotic larvae can make coral species with maternal inheritance of symbionts more susceptible to the effects of global warming.

While nutrient enrichment has been shown to impact various stages in the reproduction of broadcast-spawning species (such as fecundity, fertilization success and embryonic development; e.g., Ward and Harrison 2000; Harrison and Ward 2001; Lam et al. 2015; Humanes et al. 2016), studies focusing on the effects of DIN on larval settlement and/or survival are more limited. These studies report negative consequences for the broadcast-spawning coral species studied (reviewed in Fabricius 2005), and the few data available for brooding coral species come primarily from field studies (e.g., Ward and Harrison 1997), making comparisons with our study difficult. Regardless, in this study, even though nutrient enrichment significantly increased *P. astreoides* larval settlement rates in both temperature treatments (contrary to effects reported for broadcast-spawning species *D. strigosa* and *A. tenuis*; Bassim and Sammarco 2003; Humanes et al. 2016), we hypothesize that elevated nitrate conditions may either (1) induce an earlier

settlement response in *P. astreoides* larvae (resulting in higher rates of respiration; Fig. 1b) or (2) come at a metabolic cost which may be offset by larvae settling more rapidly.

Ecological consequences and management implications

The ability to predict the success of organisms in their environment, particularly in early life stages, is essential for understanding the effect of environmental change on species distributions (Woods et al. 2016), estimating the potential for adaptation and designing effective management strategies (Figueiredo et al. 2014). In this study, increases in metabolic activity of *O. faveolata* and *P. astreoides* larvae as a result of different factors (elevated temperature or DIN, respectively) suggest that larvae are using their maternal reserves faster than in optimal scenarios. Consequently, larvae of these species may have to settle closer to their natal reef to avoid mortality (thereby decreasing their potential for long-distance dispersal and connectivity; e.g., Foster et al. 2012; Serrano et al. 2016; but see Holstein et al. 2014). This is of particular concern for threatened species such as *O. faveolata*, already showing poor recruitment in the Caribbean (Brainard et al. 2011; Edmunds et al. 2011; Miller 2014). Furthermore, the physiological plasticity necessary to tolerate a range of environments is energetically costly, and broadcast-spawning species with potentially long pelagic durations may have a greater likelihood of encountering a wider range of environments than short-dispersing brooded larvae (Hartmann et al. 2013), making the effects on their dispersal harder to predict (e.g., Figueiredo et al. 2014).

The effects of thermal stress and nitrate enrichment in this study appear to be species specific for *O. faveolata* or *P. astreoides* coral larvae, two important Caribbean reef coral species with contrasting reproductive and algal transmission modes. These findings suggest that management aimed at minimizing the effects of multiple stressors on corals may need to specifically target particular species of interest, or use a precautionary approach by protecting the

most vulnerable species. Higher local retention rates suggest that populations are likely to be more responsive to local conservation actions (Figueiredo et al. 2014), and our findings could be used to help establish adequate local thresholds for nutrient pollution, especially during expected reproductive seasons when larvae are more likely to be affected. Further work should focus on (1) modeling dispersal and recruitment success under different water quality scenarios (such as those for the Great Barrier Reef; Wooldridge 2009) to identify sensitive locations, (2) including a broader survey of coral species and (3) comparing adult and larval responses, as evidence is accumulating that suggests that the effects of thermal stress and/or ocean acidification may be exacerbated by nutrient enrichment, at least in adult corals (e.g., Renegar and Riegl 2005; Langdon and Atkinson 2005; Wooldridge 2009; Cunning and Baker 2013; D'Angelo and Wiedenmann 2014).

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Table 1. Results of the final generalized linear mixed-effect model for *Porites astreoides* (a) larval settlement and (b) mortality of swimming planulae (shown in Figs. 2a, 2b).

Source	Num d*f*	Den d*f*	*F*	*p* (> F)
a. Larval settlement				
Nitrate	1	5.7	7.171248	0.0382
Time	2	14.3	21.40488	<0.0001
b. Mortality of swimming planulae				
Temperature	1	6	21.27123	0.0036
Time	2	12	370.6209	< 0.0001
Temperature * time	2	12	15.66213	0.0005

The model incorporated fixed effects of nitrate, temperature and time (exposure days), and aquaria as a nested random factor. Nonsignificant fixed effects were eliminated by backwards selection using *F* tests.

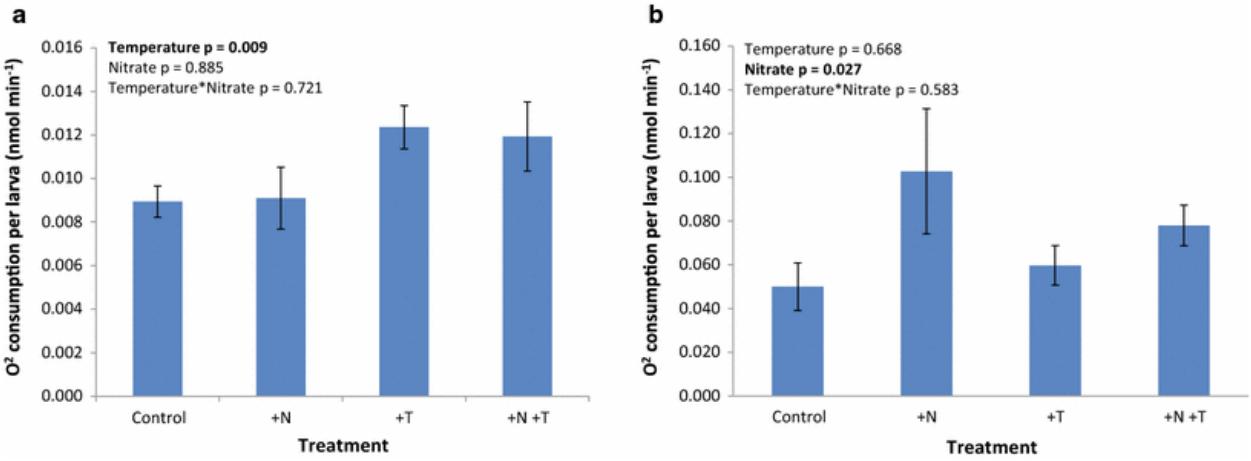

Fig.1. Average respiration rates (± SE) measured in (a) *Orbicella faveolata* and (b) *Porites astreoides* coral larvae after 72-h exposure to low nitrate and temperature (control treatment), elevated nitrate (+ N), elevated temperature (+ T) or elevated nitrate and temperature (+ N + T). A total of $N = 8–10$ replicate wells were used per treatment. Note difference in scale of y-axis for species. Results from a two-way ANOVA are enclosed in each figure.

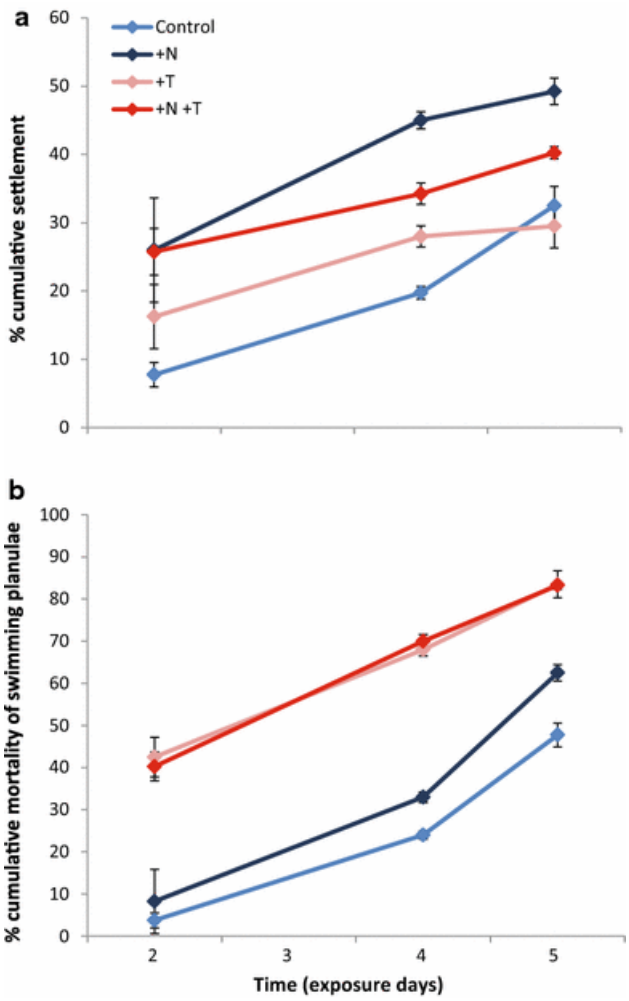

Fig. 2. Cumulative percentage of (a) larval settlement and (b) loss of swimming planulae due to mortality (± SE) in *Porites astreoides* over time. Results from the final GLMM model are given in Table 1.