Vol. 509: 181–191, 2014
doi: 10.3354/meps10880

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published August 27

Combined effects of macroalgal presence and elevated temperature on the early life-history stages of a common Caribbean coral

Kevin Olsen[1,2], Raphael Ritson-Williams[2,3], Valerie J. Paul[2], Cliff Ross[1,*]

[1]Department of Biology, University of North Florida, I UNF Drive, Jacksonville, FL 32224, USA
[2]Smithsonian Marine Station, 701 Seaway Drive, Ft. Pierce, FL 34949, USA
[3]*Present address:* Department of Biology, University of Hawaii at Manoa, 2540 Campus Road, Dean Hall R2, Honolulu, HI 96822, USA

ABSTRACT: Both global- and regional-scale stressors are contributing to the decline of corals on reefs across the planet. Even though many factors affect corals, we have very little data on the relationship between multiple co-occurring stressors and their compounding effects on these critical ecosystem engineers. We provide evidence that under short-term exposure, the presence of the brown alga *Dictyota menstrualis* has direct negative consequences for coral survival and recruitment, while seawater elevated to +3.5°C causes sub-lethal stress and compromises coral health. Three life-history stages (larvae, 6 wk-old juveniles [~0.15 cm^2], and 1–2 yr-old colonies [0.8-45 cm^2]) of the common Caribbean coral *Porites astreoides* were exposed to each stressor alone and in combination. Exposure to *D. menstrualis* reduced the survival of *P. astreoides* larvae and 6 wk-old juveniles by ~40% and caused partial mortality in 1–2 yr-old colonies. Elevated temperature did not cause mortality, but induced sub-lethal stress that was disparate among coral life-history stages. These stressors in combination predominately impacted corals in an independent manner. However, extended exposure resulted in a synergistic enhancement of oxidative damage (lipid peroxidation) in *P. astreoides* larvae. Our results suggest that benthic macroalgae can greatly inhibit coral recruitment, and this pressure combined with rising sea-surface temperatures represents a significant threat to the health of coral reef ecosystems. These results highlight the importance of local- and global-scale stressors acting in concert to impact coral demographics during critical early life-history stages.

KEY WORDS: Macroalgal presence · Thermal stress · Recruitment · *Porites astreoides*

INTRODUCTION

Coral reefs are undergoing rapid degradation, with many reef-building corals facing persistent threats and elevated risk of extinction (Pandolfi et al. 2003, Carpenter et al. 2008). The widespread decline of live coral cover has been largely attributed to the negative effects of global scale stressors acting in conjunction with regional and local scale pressures (Hughes et al. 2007, Carpenter et al. 2008). Even though particular combinations of stressors have been shown to exacerbate coral mortality (Anlauf et al. 2011, Negri et al. 2011), there is very little experimental data on how stressors of different geographic scales interact to drive marine demographic processes (Folke et al. 2004). Coral recruitment is one such process and is critical to reversing degradation on coral-depauperate reefs (Mumby 2009, Ritson-Williams et al. 2009). A better understanding of how present and future combinations of biotic and abiotic factors affect this demographic process is needed for the proper management of coral reef ecosystems (Hughes et al. 2007, Mumby & Steneck 2008).

*Corresponding author: cliff.ross@unf.edu

Competition for space is a critical factor in structuring the distribution of organisms in many habitats and has received extensive attention in the ecological literature (Jackson & Buss 1975, Chadwick & Morrow 2011). In particular, coral–algae interactions are considered fundamental to the overall status of coral reefs (Fong & Paul 2011), where macroalgal proliferation represents a significant threat to the functionality of coral reef ecosystems and the services they provide (Hughes et al. 2007, Mumby & Steneck 2008, Mumby 2009, Barbier et al. 2011). Increasing our knowledge of the consequences of algal exposure on multiple life-history stages of corals is critical to understanding the future trajectory of coral abundance on many reefs. Additionally, we have little data on the effects of macroalgal presence on the process of coral recruitment (Kuffner et al. 2006). Macroalgal presence can inhibit coral recruitment and lead to the perpetuation of alternate stable states (Mumby & Steneck 2008, Norström et al. 2009). The mechanisms involved in coral–algae competition are complex (McCook et al. 2001) and include physical factors such as abrasion and shading (Box & Mumby 2007), as well as chemical allelopathy (Rasher & Hay 2010, Paul et al. 2011) and microbial induced mortality (Smith et al. 2006). Abiotic stress associated with climate change has the potential to interact with these mechanisms of competition to exacerbate the competitive strength of macroalgae over corals (Diaz-Pulido et al. 2011). However, the impacts of climate change on competition, and other ecological interactions in general are not well understood (Harley et al. 2006).

Rising ocean temperatures associated with global climate change represent another major threat for the persistence of coral reefs on a global scale (Hoegh-Guldberg et al. 2007, IPCC 2007). Since many reef-building corals are already approaching their thermal limits, subsequent incremental temperature increases can be particularly damaging to the health of coral reef ecosystems (Hoegh-Guldberg et al. 2007). Exposure to elevated temperature can break down the mutualistic symbiosis between coral hosts and their dinoflagellate symbionts (Weis 2008), subsequently causing the expulsion of zooxanthellae (bleaching) and/or mortality of the coral holobiont (Glynn & D'Croz 1990, Lesser 1997). Temperature perturbations are also important physical factors that regulate recruitment success for coral early life-history stages (Edmunds et al. 2001, Edmunds 2004). Consequently, hyperthermal stress can have significant impacts on coral population dynamics, by causing both direct (Glynn & D'Croz 1990), and latent mortality (Hartmann et al. 2013, Ross et al. 2013), and by increasing the susceptibility of corals to the damaging effects of biotic and abiotic factors (Bruno et al. 2007, Anlauf et al. 2011, Negri et al. 2011).

In addition to mortality, contact with macroalgae and elevated temperature can severely undermine coral health (Lesser 1997, Morrow et al. 2012, Shearer et al. 2012). Exposure to environmental stressors promotes the overproduction of active oxygen such as singlet oxygen ($^1O_2$), superoxide radicals (O_2^-), and hydrogen peroxide (H_2O_2) (Lesser 1997, Lesser & Farrell 2004). Reactive oxygen species (ROS) can readily permeate cell membranes and compromise multiple intracellular targets including lipids, proteins, and nucleic acids in coral tissue (Lesser 1997). The resulting oxidative damage can be directly quantified by measuring levels of lipid peroxidation (Olsen et al. 2013, Ross et al. 2013); while the physiological response of the coral holobiont can be evaluated as a secondary means to detect sub-lethal stress. These responses include the enzymatic activity of proteins involved in the oxidative stress pathway (Lesser 2006, Olsen et al. 2013, Ross et al. 2013) and the transcriptional regulation of molecular chaperones such as heat shock proteins that facilitate protein folding and regulate apoptosis (Rodriguez-Lanetty et al. 2009, Kenkel et al. 2011). Utilizing an assemblage of cellular and molecular biomarkers can provide insight into the effects of environmental stress on the physiological condition of reef-building corals, as well as the resulting impacts on ecological and demographic processes (Downs et al. 2000).

In the Caribbean, depressed herbivory rates due to overfishing and the massive die-off of the sea urchin *Diadema antillarum* have increased macroalgal abundance and the frequency of coral–algae interactions (Hughes 1994, Rogers & Miller 2006, Mumby & Steneck 2008). Specifically, members of the brown macroalgal genus *Dictyota* are conspicuous competitors that reduce coral survival (Rasher & Hay 2010), settlement and recruitment (Kuffner et al. 2006, Box & Mumby 2007, Paul et al. 2011). Furthermore, climate change continues to impact tropical ecosystems and there is little information on how algal competition and climate change will interact to affect corals, or which life-history stage is most susceptible to these stressors. To address these critical questions, we designed controlled experiments to evaluate the independent and combined impacts of macroalgal presence and elevated temperature on the common hermatypic coral *Porites astreoides* at 3 different life-history stages: larvae, juveniles (6 wk-old), and young adults (1–2 yr-old).

MATERIALS AND METHODS

General experimental design

Porites astreoides and *Dictyota menstrualis* were chosen for this study based on their relative abundance on Caribbean coral reefs, their high frequency of interactions, and the ease of obtaining brooded coral larvae from *P. astreoides* (Rogers & Miller 2006, Green et al. 2008, Paul et al. 2011, Morrow et al. 2013). A single experimental design was used on *P. astreoides* larvae (latent treatment effects were subsequently evaluated in post-settlement recruits after 3 wk), 6 wk-old juveniles (~8 polyps, ~0.15 cm^2), and 1–2 yr-old colonies (0.8–45 cm^2) with each stressor being tested independently and in combination. Treatments included (1) ambient temperature and a plastic algae mimic (Control), (2) elevated temperature (+3.5°C) and a plastic algae mimic (Heat), (3) ambient temperature with *D. menstrualis* (Algae), and (4) elevated temperature (+3.5°C) with *D. menstrualis* (Heat+Algae). Corals were maintained under ambient salinity (~37 ppt) and covered by shade cloth allowing penetration of 10% photosynthetic active radiation (<200 µmol m^{-2} s^{-1}).

Coral husbandry and experimental treatments

Thirty colonies of *P. astreoides* as well as *D. menstrualis* were collected at 6 m depth from Wonderland reef (24° 33.62′ N, 81° 30.08′ W) and an unnamed patch reef (24° 33.14′ N, 81° 23.04′ W) in the lower Florida Keys, USA, and maintained in separate raceway tables with running seawater. Coral larvae were collected during the night of 18 May 2012 prior to the new moon (21 May) when adult colonies release brooded larvae (McGuire 1998). Adult colonies were later returned to the site of collection and reattached with Z-Spar Splash Zone Compound® underwater epoxy following experimentation.

To obtain larvae, each colony was placed in an individual 3 l Rubbermaid Grip 'n Mix® bowl supplied with continuously running seawater. The bowls were tilted so that the larvae spilled over the handles of the bowls into plastic tri-pour beakers fitted with a mesh bottom (mesh diameter: 180 µm) supported 3 cm off the raceway table bottom by 3 silicone stopper feet. The water level inside the tanks was maintained at 15 cm so the larvae remained in the tri-pour beakers until the next morning when larvae were pooled and assigned to treatments.

Forty 7 l plastic aquaria served as independent water baths and were randomly assigned to a treatment (n = 10). Aquaria were maintained at either ambient temperature (~27.5°C) or heated +3.5°C (~31.0°C) using a 75 W adjustable aquarium heater (Commodity Axis). Thirty percent seawater exchanges were conducted 4 times each day in each aquarium to prevent stagnant conditions. To assess biomarkers of stress, every aquarium contained 150 larvae housed in an acrylic treatment chamber (10 × 5.5 cm; with 180 µm mesh bottoms that contained either a floating plastic alga or floating live *D. menstrualis*; volume ~3 cm^3, surface area ~2 cm^2). Additionally, each aquarium contained a second acrylic settlement chamber containing 100 larvae and either plastic or live algae (volume ~3 cm^3, surface area ~2 cm^2) attached to the top of a single terracotta tile that had been conditioned offshore at 6 m depth for 4 wk (4.5 × 4.5 × 1 cm; Sunshine Pavers®) to test for larval settlement and survival. The amount of macroalgae utilized in replicates was selected so that *D. menstrualis* covered half of the available surface area on the settlement substrate.

Fifty larvae from the biomarker chamber were removed after 16 h of treatment for quantitative reverse transcription PCR (qRT-PCR) analysis of heat shock proteins. After 48 h, 90 and 10 larvae were removed for oxidative stress and photochemical assessments, respectively. At the 68 h time point, those larvae still swimming in settlement chambers were also used to quantify oxidative stress. Larvae removed for qRT-PCR and oxidative stress assays were flash frozen in liquid N_2 and stored at −80°C until analysis.

Larvae were allowed to settle for 68 h, after which the number of planula and those that settled and underwent metamorphosis were counted. To evaluate potential latent treatment effects on post-settlement survival, the tiles containing only new recruits (no live algae or plastic mimics) were arranged on fiberglass rods in a randomized block design and attached to a patch reef (24° 34.13′ N, 81° 22.87′ W; ~7 m depth) for 25 d.

For experiments on 6 wk-old corals, 50 preconditioned terracotta tiles were individually placed in plastic tri-pour containers containing ~200 larvae originating from ~25 colonies. The larvae were allowed to settle at ambient temperature (~27.5°C) for 1 wk, after which the juvenile corals were reared in running seawater for 5 wk. After a total of 6 wk, the 40 tiles with the greatest number of individuals on the top of the tile (varied from 5 to 32 corals) were selected for experimentation (n = 10 per treatment).

Twenty eight 1–2 yr-old colonies were raised from newly settled larvae released from parent colonies either in 2010 or 2011. Parent colonies were collected from Wonderland Reef (24° 33.62′ N, 81° 30.08′ W), Big Pine Ledges (24° 33.19′ N, 81° 31.94′ W) and a third unnamed patch reef (24° 32.91′ N, 81° 31.94′ W), located in the lower Florida Keys. At the start of the experiment each terracotta tile contained a single colony and tiles were randomly assigned to treatments (n = 7 per treatment) to control for varying colony size and age.

Treatment effects were tested on 6 wk-old and 1–2 yr-old corals simultaneously. Forty 7 l plastic aquaria served as independent water baths and were randomly assigned to one of the 4 treatments. In turn, 28 aquaria housed a tile containing 6 wk-old corals and a second tile containing a 1–2 yr-old coral. The remaining 12 aquaria housed a single tile containing 6 wk-old corals. Aquaria were maintained at either ambient temperature (~29.5°C) or heated +3.5°C (~33.0°C). Thirty percent seawater exchanges were conducted 4 times daily in each aquarium to prevent stagnant conditions. Tiles containing 6 wk-old or 1–2 yr-old corals had either a plastic algal mimic or live *D. menstrualis* (volume ~ 3 cm^3, surface area ~ 2 cm^2) held in direct but loose contact to coral tissue to simulate algal overgrowth on half of the terracotta tile (Kuffner et al. 2006). Survivorship of 6 wk-old colonies was evaluated following 7 d of treatment. Partial colony survival of each 1–2 yr-old colony following 7 d of treatment was calculated by dividing the surface area of dead and bare skeleton by the total surface area of the colony and then subtracting this ratio from 1. Surface areas were measured in duplicate using the tracing function in ImageJ® (http://nih.gov). Following treatment, 6 wk-old and 1–2 yr-old corals were biopsied (~ 2 cm^3 of tissue) directly adjacent to either the plastic algal mimic or live alga and this sample was flash frozen in liquid N_2 and stored at −80°C for subsequent qRT-PCR and oxidative stress assays. *D. menstrualis* was visually assessed following all experiments to insure that macroalgae were not compromised by physical manipulation.

Photochemistry

The photochemical efficiency of symbiotic zooxanthellae within *P. astreoides* larvae, 6 wk-olds and 1–2 yr-old corals was examined using pulse amplitude modulated (PAM) fluorometry (Diving-PAM, Walz). Changes in dark-adapted maximum quantum yield ($F_v/F_m = (F_m - F_0)/F_m'$) of PS II were assessed in larvae using the methodology of Ross et al. (2013). Larval replicates were placed in 25 µl of seawater and pipetted onto the tip of the fiber-optic cable. For analysis of 6 wk-old and 1–2 yr-old corals, the mean of 3 individual effective quantum yield (EQY) readings were taken on tissue located directly underneath the plastic or live algae.

Oxidative stress assays

Lipid peroxidation and catalase (CAT) activity were utilized as quantifiable biomarkers of oxidative stress in *P. astreoides*. Coral samples were thawed to room temperature and each replicate was extracted in 2.5 ml of buffer (50 mM potassium phosphate buffer [pH 7.0] containing 10% [w/v] polyvinylpolypyrrolidone [PVP]-40, 0.25% Triton X-100, and 1% [v/v] plant cell protease inhibitor cocktail [Sigma-Aldrich]). Samples were homogenized with a Fast Prep 24 bead homogenizer (MP Biomedicals) and centrifuged at 16000 × *g* for 10 min. The resulting supernatants were used for oxidative stress assays and normalized for protein content using the Bicinchoninic acid (BCA) Protein Assay Kit (Thermo Scientific). Lipid hydroperoxides and CAT activity were measured using the Lipid Hydroperoxide Assay Kit (Cayman Chemical) and Amplex Red (Invitrogen) respectively, as per the manufacturer's instructions.

qRT-PCR, RNA isolation

Total RNA was isolated from larvae, 6 wk old, and 1–2 yr-old corals using the TRI reagent protocol (Molecular Research Center, Cincinnati, OH, USA). Total RNA concentrations were calculated using absorbance values of 260 and 280 nm measured with a Bio-Tek® plate reader (Bio-Tek).

Quantitative 1-step RT-PCR

Porites sp. gene-specific primers were designed and validated by Kenkel et al. (2011). Gene-specific primers were diluted to 5 µM and relative standard curves for each primer set were generated using serial dilutions of total RNA encompassing 2 orders of magnitude from 0.078 to 5.0 ng. RT-PCR was performed utilizing 1 ng total RNA per reaction. The appropriate volume from each RNA sample equivalent to 1 ng total RNA was added to 1 µl forward primer, 1 µl reverse primer, 1.2 µl random hexamer primer

(Ambion Life Technologies), 12.5 µl SYBR Green Reaction Mix® (Bio-Rad) and enough nuclease-free water to sum to 25 µl of volume for each gene primer set (Hsp 16, Hsp 60, and 18S). All runs were performed on a MiniOpticon Real-Time PCR system® (Bio-Rad) using the default protocol (50°C for 10 min, 95°C for 5 min, followed by 49 cycles of 95°C for 10 s, 55°C for 30 s, then 95°C for 1 min, 55°C for 1 min and finally increments of 0.5°C for 10 s from 55.0 to 95.0°C). The expression of heat shock proteins 16 (Hsp 16) and 60 (Hsp 60) in all treatments were normalized using 18S ribosomal RNA (rRNA) as a housekeeping gene. 18S rRNA was selected as a normalizing gene on the basis that rRNA is the major component of total RNA and has commonly been utilized as a reference gene for evaluating the transcriptional abundances of genes of interest (Olsen et al. 2013). 18S rRNA was evaluated between treatments to verify its use as a normalizing agent.

Statistical analysis

Data from larval and 1–2 yr-old corals were analyzed using a 2-way ANOVA, with temperature and algal presence treated as fixed factors. The effect of juvenile density on 6 wk-old survival and EQY was evaluated as a covariate in an ANCOVA (Vermeij & Sandin 2008). In addition, since 7 of the 10 replicates in the experiment on 6 wk-old corals were maintained in aquaria containing a 1–2 yr-old colony, the presence or absence of 1–2 yr-old corals was included as a random factor in the analysis on survival and EQY. Replicates for the remaining dependent variables (oxidative stress biomarkers and Hsp regulation) were conducted in aquaria containing a 1–2 yr-old colony and were analyzed using the respective 1–2 yr-old colony surface area as a covariate. Analysis of post-settlement survival included a completely randomized block design with rod treated as a random factor. Those dependent variables with a significant interaction term were subsequently analyzed using multiple post-hoc *t*-tests and experiment-wise error was corrected for with Bonferroni's correction. Data for survival, settlement, and post-settlement survival were arcsine square root transformed prior to analysis, since they were proportions. The normality of all variables was assessed using the Shapiro-Wilk test and the equality of error variances was analyzed using Levene's test. Data that could not be transformed to meet the assumptions of ANOVA were rank transformed prior to analysis. All statistical analyses were conducted using IBM SPSS Statistics 19.

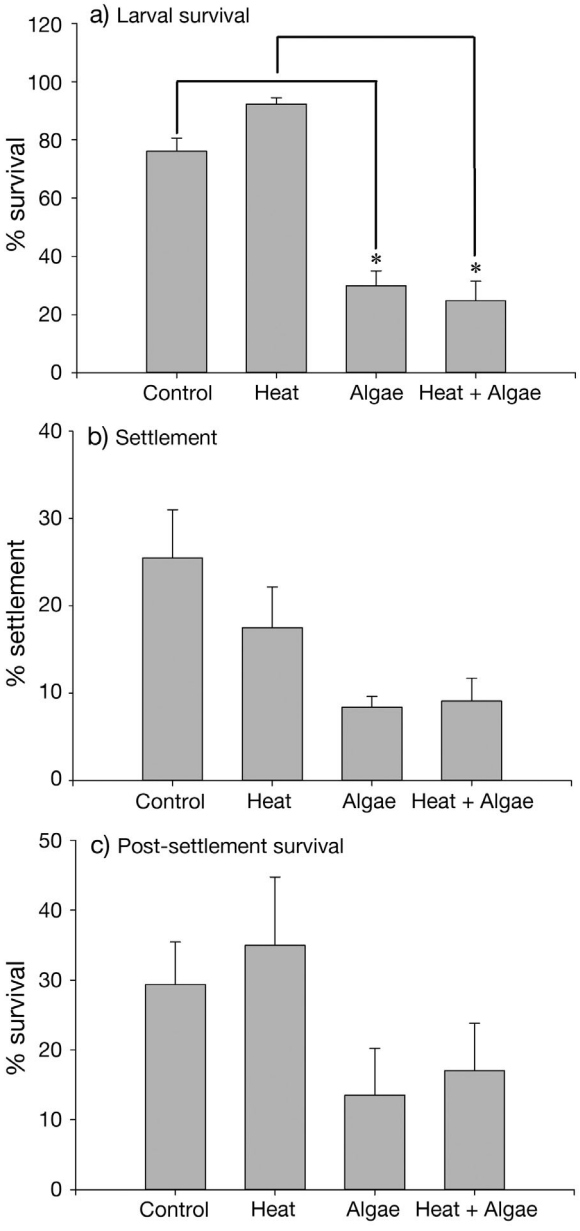

Fig. 1. (a) Percent survival, (b) settlement, and (c) post-settlement survival of coral larvae following 68 h of exposure to treatment (survival and settlement) and subsequently 25 d on a patch reef (post-settlement survival). Lines indicate a significant difference between treatments based on a post-hoc Student's *t*-test and a Bonferroni corrected significance level of $\alpha = 0.0125$ (0.05/4) (see Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m509p181_supp.pdf). Bars represent mean + 1SE

RESULTS

Exposure to +3.5°C (Heat) seawater for 68 h did not significantly affect larval survival (Fig. 1a), settlement (Fig. 1b), or post-settlement survival (Fig. 1c) (Table 1). However, the presence of live *Dictyota*

Table 1. Treatment effects on the survival and settlement of *Porites astreoides*, based on 2-way ANOVA with elevated temperature and macroalgal presence as fixed factors, significance at $p < 0.05$ (in **bold**). C: control treatment; H: heated treatment; A: algae treatment; H+A: heated and algae treatment

Life-history stage	Dependent variable	p-values of fixed factors			Sample size (n)
		Temp	Algae	Temp × Algae	
Larvae	Survival	0.304	**<0.001**	**0.026**	C=10, H=10, A=10, H+A=10
	Settlement	0.414	**0.002**	0.405	C=10, H=10, A=10, H+A=10
	Post-settlement survival	0.905	**0.041**	0.620	C=10, H=10, A=10, H+A=8
6 wk-old	Survival	0.187	**<0.001**	0.491	C=10, H=9, A=10, H+A=10
1–2 yr-old	Survival	0.130	**<0.001**	0.559	C=7, H=6, A=7, H+A=7

menstrualis (algae), significantly reduced the survival of larvae from *Porites astreoides* ($F_{1,36}$ = 110.009, $p < 0.001$; Fig. 1a), deterred their settlement and metamorphosis ($F_{1,36}$ = 10.646, p = 0.002; Fig. 1b), and reduced their post-settlement survival ($F_{1,33}$ = 5.640, p = 0.041; Fig. 1c, Table 1). A summary of the statistical results from all parameters is given in Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m509p181_supp.pdf, as well as the mean (±SE) temperatures of each treatment (Table S3 in the Supplement).

The photochemical efficiency (F_v/F_m) of *in hospite* zooxanthellae was significantly reduced by thermal stress ($F_{1,36}$ = 40.914; $p < 0.001$; Table 2, Fig. S1 in the Supplement), but was unaffected by the presence of macroalgae (Table 2, Fig. S1). Sub-lethal stress in coral larvae was also quantified using 2 biomarkers of oxidative stress (CAT activity and lipid peroxidation) following 2 time points of treatment exposure (48 h and 68 h) (Table 2, Fig. S2 in the Supplement). Exposure to elevated temperature or live *D. menstrualis* for 48 h did not significantly impact larval CAT activity (Table 2, Fig. S2a), yet those larvae exposed to the combined treatment (Heat+Algae) for 68 h underwent a ~2.5 fold increase in CAT activity compared to controls (Temp × Algae, $F_{1,30}$ = 6.679, p = 0.015; Table 2, Fig. S2c), which was not different from larvae treated with algae alone. Elevated tem-

Table 2. Treatment effects on sub-lethal stress in *Porites astreoides*, based on 2-way ANOVA with elevated temperature and macroalgal presence as fixed factors, significance at $p < 0.05$ (in **bold**). C: control treatment; H: heated treatment; A: algae treatment; H+A: heated and algae treatment. Groupings for dependent variables with a significant interaction term are based on multiple post-hoc *t*-tests and Bonferonni's correction. CAT: catalase, EQY: effective quantum yield

Life-history stage	Dependent variable	Treatment effect	p-values of fixed factors			Sample size (n)
			Temp	Algae	Temp × Algae	
Larvae	F_v/F_m	Heat reduced F_v/F_m	**<0.001**	0.267	0.135	C=10, H=10, A=10, H+A=10
	CAT activity (48 h)	No effect	0.356	0.237	0.104	C=10, H=9, A=9, H+A=10
	CAT activity (68 h)	C=H=A<H+A=A	0.603	**0.001**	**0.015**	C=8, H=10, A=8, H+A=8
	Lipid peroxidation (48 h)	Heat increased lipid peroxidation	**0.008**	0.943	**0.058**	C=6, H=4, A=5, H+A=4
	Lipid peroxidation (68 h)	C=H=A<H+A	**0.010**	0.069	**0.006**	C=7, H=6, A=7, H+A=6
	Hsp 16	Heat increased Hsp 16	**0.048**	0.092	0.589	C=5, H=5, A=5, H+A=5
	Hsp 60	No effect	0.231	0.494	0.090	C=5, H=5, A=5, H+A=5
6 wk-old	EQY	Algae reduced EQY	0.108	**<0.001**	0.957	C=10, H=9, A=10, H+A=10
	CAT activity	No effect	0.502	0.478	0.705	C=6, H=4, A=6, H+A=4
	Lipid peroxidation	Heat increased lipid peroxidation	**0.015**	0.634	0.878	C=6, H=4, A=5, H+A=4
	Hsp 16	No effect	0.235	0.698	0.055	C=5, H=5, A=5, H+A=5
	Hsp 60	No effect	0.853	0.960	0.211	C=5, H=5, A=5, H+A=5
1–2 yr-old	EQY	Heat and Algae reduced EQY	**0.016**	**<0.001**	0.340	C=7, H=6, A=7, H+A=7
	CAT activity	No effect	0.950	0.758	0.858	C=6, H=4, A=6, H+A=4
	Lipid peroxidation	No effect	0.259	0.310	0.517	C=6, H=4, A=5, H+A=4
	Hsp 16	No effect	0.821	0.446	0.243	C=5, H=5, A=5, H+A=5
	Hsp 60	No effect	0.942	0.884	0.158	C=5, H=5, A=5, H+A=5

perature significantly increased lipid peroxidation in coral larvae following 48 h ($F_{1,15}$ = 7.951, p = 0.008; Table 2, Fig. S2b). Those larvae exposed to the combined treatment for 68 h demonstrated a significant increase in lipid peroxidation compared to controls or larvae treated with heat or live algae alone (Temp × Algae, $F_{1,22}$ = 8.740, p = 0.006; Table 2, Fig. S2d), suggesting a synergistic effect of the combined stressors. The expression of Hsp 16 was elevated in larvae exposed to +3.5°C ($F_{1,16}$ = 4.573, p = 0.048; Table 2, Fig. S3a in the Supplement), but Hsp 16 expression did not change in the presence of *D. menstrualis*. Neither thermal stress nor algal exposure changed the expression of Hsp 60 (Table 2, Fig. S3b).

The survival of 6 wk-old corals was not affected by elevated temperature (Fig. 2a), but was significantly reduced in the presence of live *D. menstrualis* ($F_{1,33}$ = 64.276, p < 0.001; Table 1). The photochemical efficiency of 6 wk-old corals was not changed in +3.5°C seawater (Table 2, Fig. S4 in the Supplement), but *D. menstrualis* caused a significant reduction in EQY ($F_{1,33}$ = 149.551, p < 0.001). Neither thermal stress nor algal exposure affected CAT activity in 6 wk-old corals (Table 2, Fig. S5a in the Supplement). Seawater at +3.5°C significantly increased lipid peroxidation in 6 wk-old corals ($F_{1,14}$ = 7.732, p = 0.015; Table 2, Fig. S5b). However, direct contact with live *D. menstrualis* did not affect lipid peroxidation. Neither exposure to elevated temperature, nor algal presence significantly affected the normalized transcript abundances of Hsp 16 or 60 in 6 wk-old corals (Table 2, Fig. S6 in the Supplement).

Seawater elevated +3.5°C did not significantly affect the survival of 1–2 yr-old corals ($F_{1,23}$ = 2.472, p = 0.130; Fig 2b), whereas corals in direct contact with live *D. menstrualis* exhibited significant mortality ($F_{1,23}$ = 37.203, p < 0.001; Table 1). Both thermal stress ($F_{1,23}$ = 6.814, p = 0.016) and algal presence ($F_{1,23}$ = 55.628, p < 0.001) significantly reduced the effective quantum yield of 1–2 yr-old corals (Table 2, Fig. S7 in the Supplement). Elevated temperature and algal exposure did not significantly affect CAT activity, lipid peroxidation or the normalized expression of Hsp 16 or 60 in 1–2 yr-old corals (Table 2, Figs. S8 & S9 in the Supplement).

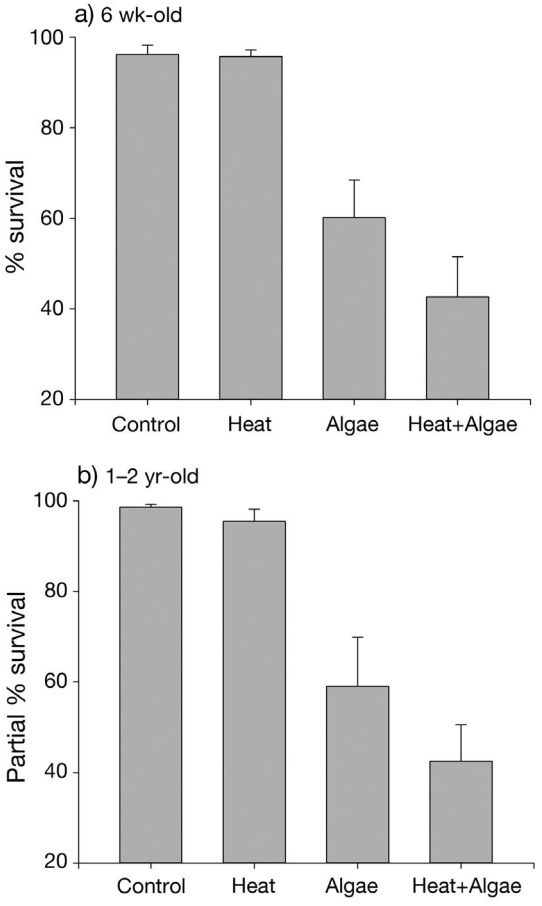

Fig. 2. Percent (a) survival of 6 wk-old colonies and (b) partial survival of 1–2 yr-old colonies following 7 d of exposure to treatments. Bars represent mean + 1SE

DISCUSSION

Modern and future coral reefs face an unprecedented combination of threats. To properly manage these habitats we need to understand how each of these stressors will interact to cause stress and mortality. Our results show that predicted increases in temperature caused sub-lethal stress to corals, while the presence of the brown alga *Dictyota menstrualis* reduced their survival and recruitment. These results highlight the distinct impacts of separate co-occurring stressors on a critical coral reef demographic process (Ritson-Williams et al. 2009).

Larval supply, settlement, and subsequent growth to maturity are key steps in the process of coral recruitment (Hughes & Tanner 2000, Ritson-Williams et al. 2009). *D. menstrualis* killed ~40% of *P. astreoides* larvae and reduced their settlement by ~20% (Fig. 1). Exposure to the macroalga also had a latent effect on post-settlement survival, further reducing the number of coral recruits. *Dictyota* spp. can reduce the survival and settlement of coral larvae (Kuffner et al. 2006), likely through allelopathic secondary metabolites (Paul et al. 2011). While we consistently see a negative effect of *Dictyota* spp. on *P. astreoides* larvae, some macroalgae have a greater impact on coral recruitment than others, and this

seems to be species-specific for each coral–algae interaction (Kuffner et al. 2006, Birrell et al. 2008). The re-establishment of coral cover on coral-depauperate reefs is greatly dependent on the ability of coral larvae to recruit in the presence of macroalgae (Hughes et al. 2007, Birrell et al. 2008, Mumby 2009). However, our results indicate that macroalgal proliferation can cause significant bottlenecks in recruiting corals, which can significantly inhibit coral replenishment and perpetuate alternate stable states (Birrell et al. 2008).

Six wk-old and 1–2 yr-old colonies held in direct contact with *D. menstrualis* also exhibited significant mortality (Fig. 2, Table 1). Tissue death was localized to areas where *D. menstrualis* was directly touching *P. astreoides*, suggesting that the deleterious effects of macroalgal exposure were induced through direct contact rather than mere proximity (Rasher & Hay 2010, Rasher et al. 2011). PAM fluorometry was utilized to supplement visual assessments of colony survival and focused on areas of coral tissue that were in direct contact with macroalgae. The EQY of 6 wk-old and 1–2 yr-old coral colonies exposed to *D. menstrualis* paralleled visual assessments of coral survival and reflected values indicative of colony mortality (Rasher & Hay 2010, Rasher et al. 2011). Plastic algal mimics had little effect on coral survival, suggesting that mortality was due to more than physical mechanisms and likely included modification of the microbiological local environment and/or allelopathic effects (Rasher & Hay 2010, Morrow et al. 2012). Abrasion and shading can also be important physical factors in coral–algae interactions, and our results likely underestimate the complete effects of macroalgal competition on recruiting corals (Box & Mumby 2007). The consistent impact of *D. menstrualis* presence on the survival of *P. astreoides* across multiple life-history stages emphasizes the degree of competitive exclusion exerted by some macroalgae on corals for space and resources (Nugues & Bak 2006).

While *D. menstrualis* exposure caused coral mortality in every life-history stage examined, we predominately detected no sub-lethal stress in *P. astreoides* as a result of macroalgal presence (Table 2). However, exposure to *D. menstrualis* for extended duration (68 h) did increase the activity of CAT in *P. astreoides* larvae. Contact with macroalgae can promote the overproduction and accumulation of harmful ROS in corals, resulting in increased enzymatic activity of proteins involved in the oxidative stress response, including glutathione-S-transferase and superoxide dismutase (Morrow et al. 2012, Shearer et al. 2012). Similarly, catalase is an enzyme that dismutates H_2O_2 into H_2O and O_2, and its activity reflects the response of the coral holobiont to elevated concentrations of ROS (Halliwell 2006, Ross et al. 2010, Olsen et al. 2013). Furthermore, the absence of cellular and molecular responses in visually compromised 1–2 yr-old colonies emphasizes the localized impact of macroalgal contact stress on corals (Rasher & Hay 2010, Rasher et al. 2011). Since areas in direct contact with *D. menstrualis* demonstrated significant mortality, tissue samples for cellular and molecular analysis were taken from areas directly adjacent to macroalgal contact. The lack of a detectable response suggests that although areas touching *D. menstrualis* exhibited significant mortality, neighboring tissue was unaffected by macroalgal presence.

Corals of the future will be exposed to a myriad of co-occurring stressors, including elevated temperature (Hoegh-Guldberg et al. 2007, IPCC 2007). Correspondingly, cellular biomarkers (Downs et al. 2002, Yakovleva et al. 2009, Olsen et al. 2013, Ross et al. 2013) and the regulation of molecular chaperones (Rodriguez-Lanetty et al. 2009, Kenkel et al. 2011) have been well characterized in corals in response to temperature perturbations. Our results indicate that temperature elevated +3.5°C impacted a variety of these cellular and molecular biomarkers and induced hyperthermal stress across multiple coral life-history stages (Table 2). These findings support the established notion that thermal stress reduces the health of the coral holobiont, rendering corals prone to large-scale disease, bleaching, and mortality events (Jokiel & Coles 1990, Bruno et al. 2007).

The present study also highlights the differing response of corals from multiple life-history stages to elevated temperature, where stress was detected predominantly in coral larvae (Table 2). Larvae of marine benthic invertebrates are often assumed to be particularly vulnerable to environmental stress compared to post-metamorphic counterparts (Pechenik 1999), but this may be misleading as the opposite has also been shown to be true (Hamdoun & Epel 2007). Furthermore few studies have compared the effects of environmental stressors on multiple life-history stages of hermatypic corals. Thermal stress reduced the photosynthetic efficiency of *in hospite* zooxanthellae, elevated lipid peroxidation, and increased the transcriptional abundance of Hsp 16 in larvae from *P. astreoides*. Comparatively, elevated temperature caused oxidative damage in 6 wk-old corals and reduced the EQY of zooxanthellae within 1–2 yr-old corals. Our results qualitatively indicate that seawater elevated +3.5°C induced hyperthermal stress that was disparate between coral life-history stages.

Similarly, Putnam et al. (2010) found that the photochemical efficiency of *Pocillopora damicornis* and *Seriatopora hystrix* larvae were reduced in comparison to adult corals exposed to identical temperature treatments. The ultimate consequences of increased larval sub-lethal stress are still unclear, but reductions in post-settlement survival (Ross et al. 2013), slower post-settlement growth and reduced immune function are all potential ecological repercussions (Monaghan et al. 2009).

Seawater elevated +3.5°C did not impact coral survivorship or parameters evaluating recruitment in any of the examined life-history stages. Temperature increases of approximately +3.0°C have not been found to induce significant mortality in *P. astreoides* larvae or to impact their settlement on pre-conditioned substrate (Olsen et al. 2013, Ross et al. 2013), but continued elevation to +5.0°C can be lethal in larvae of this species (Edmunds et al. 2001). Conversely, elevations to +4.0°C and +3.0°C can reduce the survival and settlement of larvae from *Diploria strigosa* (Bassim & Sammarco 2003), and *Favia fragum* (Randall & Szmant 2009), respectively. Discrepancies in temperature tolerance also exist in adult colonies, where *P. astreoides* has been found to be more resistant to bleaching compared to other hermatypic corals (Gates 1990). While short-term exposure to elevated temperature did not cause mortality in 6 wk-old or 1–2 yr-old corals in the present study, prolonged temperature extremes can have significant impacts on the population dynamics of juvenile and adult corals *in situ* (Glynn & D'Croz 1990, Edmunds 2004). Furthermore, the literature indicates that coral responses to rising sea-surface temperatures are species-specific, and suggest that the effects of +3.5°C on coral recruitment detected here are likely conservative compared to the impacts on more vulnerable species (Gates 1990, Edmunds 2004). The broad thermal tolerance of *P. astreoides* across multiple life-history stages may also partially explain the recent increase in relative abundance of this species detected on Caribbean reefs (Green et al. 2008).

Evidence is building that temperature can interact with multiple stressors to synergistically impact coral reef organisms (Anlauf et al. 2011, Negri et al. 2011). However, separate environmental pressures can also have adverse relationships, causing multiplicative, additive, or even antagonistic effects on coral reef ecosystems (Darling & Cote 2008, Darling et al. 2010, Dunne 2010). Thus, the impacts of compounding stressors on coral communities is a function of the specific stressors involved, as well as the temporal and spatial scale being examined (Ban et al. 2014). Our results indicate that small increases in sea-surface temperatures (+3.5°C) have sub-lethal impacts on corals, and these effects may complicate coral–algae interactions when considering complete coral life-cycles. Exposure to the combined treatment for extended duration (68 h) synergistically elevated oxidative damage (lipid peroxidation) in *P. astreoides* larvae (Table 2). The detected cellular damage suggests that coral larvae may be particularly susceptible to compounding stressors, and that exposure duration plays a key role in determining the impact of multiple environmental perturbations on coral condition.

A critical mechanism of reef recovery is coral recruitment, but many reefs are experiencing minimal larval settlement and survival (Hughes & Tanner 2000, Price 2010, Arnold & Steneck 2011). The present study indicates that exposure to *D. menstrualis* has the potential to greatly reduce coral recruitment. Local threats such as overfishing, nutrient loading and the removal of sea urchins should be managed to reduce macroalgal blooms (Mumby et al. 2007, Mumby 2009, Rasher & Hay 2010). Additionally, global threats such as elevated seawater temperatures should not be ignored because any threat that reduces the health of individuals weakens the ecosystem and its resilience (Hoegh-Guldberg et al. 2007, Mumby et al. 2007, Hofmann & Gaines 2008). Ultimately, we highlight how these stressors together have the potential to greatly reduce the ability of reef-building corals to recruit, which can inhibit their ability to recover and perpetuate alternative stable states.

Acknowledgements. We thank Dr. Eric Johnson for his advice on statistical analysis and Dr. Judy Ochrietor for assistance with qRT-PCR. We also thank Dr. Jim Gelsleichter for use of his MiniOpticon Real-Time PCR system and Maggie Vogel for field assistance. This work was conducted under permit nos. FKNMS-2010-046 and FKNMS-2011-038 issued by the Florida Keys National Marine Sanctuary and was supported by Mote Protect our Reefs Grant 2011-08 and a Link Foundation/Smithsonian Graduate Research Fellowship awarded to K.O. This is contribution no. 962 of the Smithsonian, Marine Station at Ft. Pierce.

LITERATURE CITED

Anlauf H, D'Croz L, O'Dea A (2011) A corrosive concoction: the combined effects of ocean warming and acidification on the early growth of a stony coral are multiplicative. J Exp Mar Biol Ecol 397:13–20

Arnold SN, Steneck RS (2011) Settling into an increasingly hostile world: the rapidly closing 'recruitment window' for corals. PLoS ONE 6:e28681

Ban SS, Graham NAJ, Connolly SR (2014) Evidence for mul-

tiple stressor interactions and effects on coral reefs. Glob Change Biol 20:681–697

Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2011) The value of estuarine and coastal ecosystem services. Ecol Monogr 81:169–193

Bassim K, Sammarco P (2003) Effects of temperature and ammonium on larval development and survivorship in a scleractinian coral (*Diploria strigosa*). Mar Biol 142: 241–252

Birrell CL, McCook LJ, Willis BL, Diaz-Pulido GA (2008) Effects of benthic algae on the replenishment of corals and the implications for the resilience of coral reefs. Oceanogr Mar Biol Annu Rev 46:25–63

Box SJ, Mumby PJ (2007) Effect of macroalgal competition on growth and survival of juvenile Caribbean corals. Mar Ecol Prog Ser 342:139–149

Bruno JF, Selig ER, Casey KS, Page CA and others (2007) Thermal stress and coral cover as drivers of coral disease outbreaks. PLoS Biol 5:e124

Carpenter KE, Abrar M, Aeby G, Aronson RB and others (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science 321:560–563

Chadwick NE, Morrow KM (2011) Competition among sessile organisms on coral reefs. In: Dubinsky Z, Stambler N (eds) Coral reefs: an ecosystem in transition. Springer, New York, NY, p 347–371

Darling ES, Cote IM (2008) Quantifying the evidence for ecological synergies. Ecol Lett 11:1278–1286

Darling ES, McClanahan TR, Cote IM (2010) Combined effects of two stressors on Kenyan coral reefs are additive or antagonistic not synergistic. Conserv Lett 3:122–130

Diaz-Pulido G, Gouezo M, Tilbrook B, Dove S, Anthony KRN (2011) High CO_2 enhances the competitive strength of seaweeds over corals. Ecol Lett 14:156–162

Downs CA, Mueller E, Phillips S, Fauth JE, Woodley CM (2000) A molecular biomarker system for assessing the health of coral (*Montastrea faveolata*) during heat stress. Mar Biotechnol (NY) 2:533–544

Downs CA, Fauth JE, Halas JC, Dustan P, Bemiss J, Woodley CM (2002) Oxidative stress and seasonal coral bleaching. Free Radic Biol Med 33:533–543

Dunne RP (2010) Synergy or antagonism-interactions between stressors on coral reefs. Coral Reefs 29:145–152

Edmunds PJ (2004) Juvenile coral population dynamics track rising seawater temperature on a Caribbean reef. Mar Ecol Prog Ser 269:111–119

Edmunds PJ, Gates RD, Gleason DF (2001) The biology of larvae from the reef coral *Porites astreoides*, and their response to temperature disturbances. Mar Biol 139: 981–989

Folke C, Carpenter S, Walker B, Scheffer M, Elmqvist T, Gunderson L, Holling CS (2004) Regime shifts, resilience, and biodiversity in ecosystem management. Annu Rev Ecol Syst 35:557–581

Fong P, Paul VJ (2011) Coral reef algae. In: Dubinsky Z, Stambler N (eds) Coral reefs: an ecosystem in transition. Springer, New York, NY, p 241–272

Gates RD (1990) Seawater temperature and sublethal coral bleaching in Jamaica. Coral Reefs 8:193–197

Glynn PW, D'Croz L (1990) Experimental evidence for high temperature stress as the cause of El Nino-coincident coral mortality. Coral Reefs 8:181–191

Green DH, Edmunds PJ, Carpenter RC (2008) Increasing relative abundance of *Porites astreoides* on Caribbean reefs mediated by an overall decline in coral cover. Mar Ecol Prog Ser 359:1–10

Halliwell B (2006) Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol 141:312–322

Hamdoun A, Epel D (2007) Embryo stability and vulnerability in an always changing world. Proc Natl Acad Sci USA 104:1745–1750

Harley CDG, Hughes AAR, Hultgrem KM, Miner BG and others (2006) The impacts of climate change in coastal marine systems. Ecol Lett 9:228–241

Hartmann AC, Marhaver KL, Chamberland VF, Sandin SA, Vermeij MJA (2013) Large birth size does not reduce negative latent effects of harsh environments across life stages in two coral species. Ecology 94:1966–1976

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS and others (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742

Hofmann GE, Gaines SD (2008) New tools to meet new challenges: emerging technologies for managing marine ecosystems for resilience. Bioscience 58:43–52

Hughes TP (1994) Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science 265: 1547–1551

Hughes TP, Tanner JE (2000) Recruitment failure, life histories, and long-term decline of Caribbean corals. Ecology 81:2250–2263

Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D and others (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change. Curr Biol 17:360–365

IPCC (2007) Climate change 2007. In: Solomon S, Qin D, Manning M, Chen Z and others (eds) The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Jackson JBC, Buss L (1975) Allelopathy and spatial competition among coral reef invertebrates. Proc Natl Acad Sci USA 72:5160–5163

Jokiel PL, Coles SL (1990) Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. Coral Reefs 8:155–162

Kenkel CD, Aglyamova G, Alamaru A, Bhagooli R and others (2011) Development of gene expression markers of acute heat-light stress in reef-building corals of the genus *Porites.* PLoS ONE 6:e26914

Kuffner IB, Walters LJ, Becerro MA, Paul VJ, Ritson-Williams R, Beach KS (2006) Inhibition of coral recruitment by macroalgae and cyanobacteria. Mar Ecol Prog Ser 323:107–117

Lesser MP (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs 16:187–192

Lesser MP (2006) Oxidative stress in marine environments: biochemistry and physiological ecology. Annu Rev Physiol 68:253–278

Lesser MP, Farrell JH (2004) Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. Coral Reefs 23: 367–377

McCook LJ, Jompa J, Diaz-Pulido G (2001) Competition between corals and algae on coral reefs: a review of evidence and mechanisms. Coral Reefs 19:400–417

McGuire MP (1998) Timing of larval release by *Porites astreoides* in the northern Florida Keys. Coral Reefs 17: 369–375

Monaghan P, Metcalfe NB, Torres R (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecol Lett 12:75–92

Morrow KM, Ritson-Williams R, Ross C, Liles MR, Paul VJ (2012) Macroalgal extracts induce bacterial assemblage shifts and sublethal tissue stress in Caribbean corals. PLoS ONE 7:e44859

Morrow KM, Liles MR, Paul VJ, Moss AG, Chadwick NE (2013) Bacterial shifts associated with coral–macroalgal competition in the Caribbean Sea. Mar Ecol Prog Ser 488:103–117

Mumby PJ (2009) Phase shifts and the stability of macroalgal communities on Caribbean coral reefs. Coral Reefs 28: 761–773

Mumby PJ, Steneck RS (2008) Coral reef management and conservation in light of rapidly evolving ecological paradigms. Trends Ecol Evol 23:555–563

Mumby PJ, Hastings A, Edwards HJ (2007) Thresholds and the resilience of Caribbean coral reefs. Nature 450:98–101

Negri AP, Flores F, Rothig T, Uthicke S (2011) Herbicides increase the vulnerability of corals to rising sea surface temperature. Limnol Oceanogr 56:471–485

Norström AV, Nyström M, Lokrantz J, Folke C (2009) Alternative stable states on coral reefs: beyond coral-macroalgal phase shifts. Mar Ecol Prog Ser 376:295–306

Nugues MM, Bak RPM (2006) Differential competitive abilities between Caribbean coral species and a brown alga: a year of experiments and a long-term perspective. Mar Ecol Prog Ser 315:75–86

Olsen K, Ritson-Williams R, Ochrietor JD, Paul VJ, Ross C (2013) Detecting hyperthermal stress in larvae of the hermatypic coral *Porites astreoides*: the suitability of using biomarkers of oxidative stress versus heat shock protein transcriptional expression. Mar Biol 160:2609–2618

Pandolfi JM, Bradbury RH, Sala E, Hughes TP and others (2003) Global trajectories of the long-term decline of coral reef ecosystems. Science 301:955–958

Paul VJ, Kuffner IB, Walters LJ, Ritson-Williams R, Beach KS, Becerro BA (2011) Chemically mediated interactions between macroalgae *Dictyota* spp. and multiple life-history stages of the coral *Porites astreoides.* Mar Ecol Prog Ser 426:161–170

Pechenik JA (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Mar Ecol Prog Ser 177:269–297

Price N (2010) Habitat selection, facilitation, and biotic settlement cues affect distribution and performance of coral recruits in French Polynesia. Oecologia 163:747–758

Putnam HM, Edmunds PJ, Fan TY (2010) Effect of a fluctuating thermal regime on adult and larval reef corals. Invertebr Biol 129:199–209

Randall CJ, Szmant AM (2009) Elevated temperature reduces survivorship and settlement of the larvae of the Caribbean scleractinian coral, *Favia fragum* (Esper). Coral Reefs 28:537–545

Rasher DB, Hay ME (2010) Chemically rich seaweeds poison corals when not controlled by herbivores. Proc Natl Acad Sci USA 107:9683–9688

Rasher DB, Stout EP, Engel S, Kubanek J, Hay ME (2011) Macroalgal terpenes function as allelopathic agents against reef corals. Proc Natl Acad Sci USA 108:17726–17731

Ritson-Williams R, Arnold SN, Fogarty ND, Steneck RS, Vermeij MJA, Paul VJ (2009) New perspectives on ecological mechanisms affecting coral recruitment on reefs. Smithson Contrib Mar Sci 38:437–457

Rodriguez-Lanetty M, Harii S, Hoegh-Guldberg O (2009) Early molecular responses of coral larvae to hyperthermal stress. Mol Ecol 18:5101–5114

Rogers CS, Miller J (2006) Permanent 'phase shifts' or reversible declines in coral cover? Lack of recovery of two coral reefs in St. John, US Virgin Islands. Mar Ecol Prog Ser 306:103–114

Ross C, Ritson-Williams R, Pierce R, Bullington JB, Henry M, Paul VJ (2010) Effects of the Florida red tide dinoflagellate, *Karenia brevis*, on oxidative stress and metamorphosis of larvae of the coral *Porites astreoides.* Harmful Algae 9:173–179

Ross C, Ritson-Williams R, Olsen K, Paul VJ (2013) Short-term and latent post-settlement effects associated with elevated temperature and oxidative stress on larvae from the coral *Porites astreoides.* Coral Reefs 32:71–79

Shearer TL, Rasher DB, Snell TW, Hay ME (2012) Gene expression patterns of the coral *Acropora millepora* in response to contact with macroalgae. Coral Reefs 31: 1177–1192

Smith JE, Shaw M, Edwards RA, Obura D and others (2006) Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality. Ecol Lett 9:835–845

Vermeij MJA, Sandin SA (2008) Density-dependent settlement and mortality structure the earliest life phases of a coral population. Ecology 89:1994–2004

Weis VM (2008) Cellular mechanisms of cndiarian bleaching: stress causes the collapse of symbiosis. J Exp Biol 211:3059–3066

Yakovleva IM, Baird AH, Yamamoto HH, Bhagooli R, Nonaka M, Hidaka M (2009) Algal symbionts increase oxidative damage and death in coral larvae at high temperatures. Mar Ecol Prog Ser 378:105–112

Editorial responsibility: Peter Edmunds, Northridge, California, USA

Submitted: November 7, 2013; Accepted: May 21, 2014
Proofs received from author(s): August 18, 2014