Regional Studies in Marine Science 10 (2017) 107–115

Contents lists available at ScienceDirect

Regional Studies in Marine Science

journal homepage: www.elsevier.com/locate/rsma

Source location and food availability determine the growth response of *Orbicella faveolata* to climate change stressors

Erica K. Towle *, Ana M. Palacio-Castro, Andrew C. Baker, Chris Langdon

Department of Marine Biology and Ecology, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Cswy, Miami, FL 33149, United States

HIGHLIGHTS

- Local environment shapes coral physiology.
- Growth rate was highly correlated to source location, not parent colony.
- Within a single management regime, source location may determine stress response.
- Considering location effects is important for coral restoration and transplantation.
- Potential for assisted migration should be studied further.

ARTICLE INFO

Article history:
Received 27 October 2016
Received in revised form
13 January 2017
Accepted 20 January 2017
Available online 23 January 2017

Keywords:
Corals
Climate change
Florida Keys
Location effects
Restoration ecology

ABSTRACT

The local environment shapes coral physiology through acclimatization and also selects for genotypes best suited to a particular site. Both acclimatization and selection likely affect the response of corals to future climate change. The local environment is therefore an important factor to consider for restoration ecology. In this study, we exposed *Orbicella faveolata* from two different locations in Florida (Emerald Reef, near Key Biscayne in the upper Florida Keys, and Truman Harbor near Key West in the lower Florida Keys) that were common-gardened for one month prior to experimentation to four temperature, CO_2, and food availability treatments (26° C/390 ppm, 26° C/1000 ppm, 31° C/390 ppm, and 31° C/1000 ppm where each of these treatments had fed and unfed components). The goal was to determine how the same species of coral from different locations would respond to projected climate change scenarios. We found that growth (measured as changes in buoyant weight) was highly correlated to source location (i.e., whether the corals came from Emerald Reef or Truman Harbor) and not to parent colony, and growth, symbiont density, chlorophyll *a* content, and lipid content were highly correlated to feeding regime. These findings show that within a single reef tract, (i.e. the Florida Reef Tract), source location and food availability matter for the physiological outcome of a coral's stress response, and suggest that an explicit consideration of these effects may be important for management activities such as coral restoration, transplantation, and MPA placement.

1. Introduction

Global climate change threatens reef-building corals and overall coral reef ecosystem health via ocean warming and ocean acidification. Ocean warming results in coral bleaching, whereby corals expel the symbiotic algae that live within them due to their narrow thermal tolerance (Glynn, 2012). Under normal, unstressed conditions these symbionts can provide the coral with up to 95% of its daily metabolic requirements by transferring the products of photosynthesis to the coral host (Muscatine and Porter, 1977; Grottoli et al., 2006). When corals lose their symbiotic algae they lose their main source of nutrition, resulting in widespread mortality if bleaching is severe or prolonged. In addition to episodic bleaching events, the dissolution of anthropogenic CO_2 in the ocean changes its carbonate chemistry, such that the pH of seawater decreases, resulting in ocean acidification (OA). This phenomenon impairs corals' ability to build their aragonitic skeletons (Chan and Connolly, 2013) and reduces their ability to sexually reproduce (Albright et al., 2010; Albright and Langdon, 2011; Doropoulos and Diaz-Pulido, 2013). The negative effects of warming in combination with OA have been found to be additive

* Corresponding author.
E-mail address: etowle@rsmas.miami.edu (E.K. Towle).

http://dx.doi.org/10.1016/j.rsma.2017.01.007
2352-4855/

or even synergistic on a variety of processes including calcification (Reynaud et al., 2003; Albright and Mason, 2013; Kroeker et al., 2013). Coral heterotrophy, or the ability of the coral host to feed on its own from the water column on zooplankton or particulate organic matter as opposed to relying solely on photosynthetic product from its symbionts, has been shown to mitigate reductions in calcification due to thermal and/or OA stress (Grottoli et al., 2006; Cohen and Holcomb, 2010; Edmunds, 2011; Towle et al., 2015a). Other indicators of resilience to climate change stress include past history of stress exposure (Carilli et al., 2012), the identity of the coral's algal symbionts or the relative abundance of algal to coral cells (Baker et al., 2004; Cunning and Baker, 2013), and/or its parental genotype (Baums et al., 2013). Confounding these potential indicators of resilience is the observation that even within the same coral colony, coral physiological metrics such as calcification rate, lipid content, and symbiont density can vary greatly (Teece et al., 2011). This natural variation makes it challenging to determine what affects the physiological outcome of a coral to multiple stressors.

The mountainous star coral, *Orbicella faveolata*, once a very common reef-building species in the Florida Reef Tract, was listed in 2014 as threatened under the US Endangered Species Act (National Marine Fisheries Service, 2014). The decline of reef-building species in Florida and elsewhere, and the recognition that they may not be able to recover naturally without human intervention, has prompted regional efforts to restore reefs through coral propagation and transplantation (Johnson et al., 2011; Young et al., 2012). Coral restoration in Florida has focused on *Acropora cervicornis* and to a lesser extent *Acropora palmata*, but attention is now being drawn to restoring other species, including slower-growing taxa with massive or submassive morphologies as part of a coral "landscaping" (vs. coral "gardening") approach (Forsman et al., 2015). However, outplanting success is variable, with numerous factors potentially determining success such as temperature, colony size, depth at the collection and transplant sites, and predation levels at the transplant site, to name a few (Johnson et al., 2011). Many of these factors have been poorly investigated, especially outside of the genus *Acropora*.

The major aims of this study were to investigate: (1) how colonies of *O. faveolata* from two different source sites in the lower and upper Florida Keys would respond to climate change stress, with special interest given to determining the relative importance of source location versus parent colony on the physiological responses of corals to stress; and (2) whether corals that had access to food would fare better than those that did not. We tested the hypothesis that there would be no difference in the calcification rate, lipid content, symbiont density, or chlorophyll content of *O. faveolata* colonies from the two sites, i.e., the variation in physiology would be due to stress treatment, and not source location or parent colony. The results of this study will help inform coral restoration efforts regarding using corals that may be physically distant from the restored site, and have implications for the assisted colonization of corals in anticipation of climate change.

2. Materials and methods

2.1. Collection

Colonies of *Orbicella faveolata* were collected using SCUBA from a depth of five meters at two sites in April 2014. Five medium-sized colonies were collected from Truman Harbor (TH), Key West, Florida (24.332 N, 81.484 W), under permit FKNMS-2014-064, and three large colonies were collected from Emerald Reef (ER), Key Biscayne, Florida (25.674 N, 80.099 W), under Florida Fish and Wildlife Special Activities License SAL-13-1182B-SRP. The two sites are approximately 112 nautical miles distant from each other, and TH is a turbid site adjacent to an industrial port, whereas ER is a patch reef environment. Fifty cores from ER colonies (∼17 per parent colony) and fifty cores from TH colonies (∼10 per parent colony), each 2.5 cm in diameter, were made using a diamond tile drill bit (Montana MB-65207). Cores were glued to numbered plastic tiles and allowed to recover under control conditions (26 °C, 390 ppm) for one month prior to the start of the experiment.

2.2. Experimental design

Experimental corals were exposed to one of four experimental treatments: 26 °C/390 ppm (LT-LCO_2), 26 °C/1000 ppm (LT-HCO_2), 31 °C/390 ppm (HT-LCO_2), and 31 °C/1000 ppm (HT-HCO_2) for a period of eight weeks from mid-June to mid-August 2014. Each treatment was replicated twice for a total of eight independently-controlled tanks. Each tank contained 12–14 corals, for a total of 100 cores. Within each tank, half of the corals were fed and half were unfed during the experiment (see below). Cores were evenly but haphazardly distributed between tanks accounting for source, i.e., half of the fed and unfed corals were from ER and half were from TH. Temperatures in the HT treatment were increased from ambient levels of 26 °C to 31 °C at a rate of 0.5 °C per day. The target temperature (31 °C) is just above the mean local bleaching threshold in the Florida Keys (30.4 °C, Manzello et al., 2007), and the target CO_2 level (1000 ppm) is the mid-point of the range of CO_2 levels predicted for the end of the century (IPCC, 2013). Experimental corals were maintained under naturally-variable solar irradiance in a greenhouse facility at the University of Miami's Experimental Hatchery in 60 L tanks replenished by a 250 L sump tank with complete water turnover every ten minutes. Each sump tank contained a heating and cooling element connected to a temperature controller (OMEGA CN7533) with accuracy ∼0.1 °C. CO_2 levels were achieved by mixing pure CO_2 and air using mass flow controllers (Sierra Instruments model 810C) that was pumped through a Venturi injector and circulated through the sump before being pumped into experimental tanks. The sump pump delivered pressurized water to a square of 1/2″ PVC pipe with 1/8″ holes drilled every 2″ that sits within the experimental tank and surrounds the corals. Therefore, water motion in the tank was turbulent, i.e. the corals experienced jets of water from four different directions. This was as close to natural as possible without creating oscillatory flow. All tanks were connected to a HOBO U30 data logger taking measurements of CO_2, temperature, and light every five minutes. Daily PAR averaged throughout the experiment between 7:00 am and 7:00 pm was 350 μmoles m^{-2} s^{-1}. Fed corals were offered food *ad libitum* twice weekly using a powdered zooplankton diet (Ziegler's Larval AP 100) as in Towle et al. (2015a). Briefly, fed corals were placed in separate feeding bins to avoid contaminating treatment tanks containing unfed corals, and unfed corals were also put into separate bins, but without access to food, as a handling feeding bin control. Corals offered food were allowed the opportunity to feed for one hour after sunset as in Grottoli et al. (2006) and all bins had pumps to ensure adequate water motion.

2.3. Measurements

250 mL water samples were taken from each tank weekly (8 tanks × 8 weeks = 64 samples) to monitor seawater chemistry. Samples were poisoned with 100 μL mercuric chloride for dissolved inorganic carbon (DIC) analysis. CO_2 was measured using an equilibrator and LiCor CO_2 analyzer system calibrated against 700 ppm pure CO_2 gas. Salinity was measured using a YSI meter calibrated before each use against a 50,000 microSiemen standard solution. DIC was measured in duplicate using a DIC analyzer (Apollo SciTech Inc.) standardized using certified

Table 1
Mean seawater chemistry parameters during the study period presented as mean ±1 standard deviation where *L* stands for low, *H* stands for high, *T* stands for temperature in the treatment abbreviations, and DIC stands for dissolved inorganic carbon for $N = 64$ water samples. pH was measured using a total scale.

Treatment	Temperature (°C)	CO_2 (ppm)	Salinity (ppt)	pH	Ω_a	DIC (μmol kg^{-1} SW)
LT-LCO_2	26.2 ± 0.12	387 ± 21	33.9 ± 1.20	8.08 ± 0.02	3.89 ± 0.18	2060 ± 41
HT-LCO_2	31.2 ± 0.15	401 ± 28	33.9 ± 1.10	8.07 ± 0.02	4.50 ± 0.22	2034 ± 34
LT-HCO_2	26.1 ± 0.15	987 ± 123	34.0 ± 1.13	7.74 ± 0.05	2.01 ± 0.21	2216 ± 38
HT-HCO_2	31.3 ± 0.25	984 ± 117	33.9 ± 1.07	7.75 ± 0.04	2.50 ± 0.21	2203 ± 44

Table 2
Mean temperature (°C ± 1 SD) collected via HOBO temperature loggers at the two source sites during the collection month and the warmest months of the year prior to coral collection (June–September) from 2011 to 2013 where temperatures above mean local bleaching threshold (30.4 °C, Manzello et al., 2007) are bolded.

Month/Year	Emerald Reef	Truman Harbor
April 2014 (collection month)	25.30 ± 1.10	26.50 ± 1.40
June 2013	27.88 ± 0.89	29.61 ± 1.19
July 2013	28.43 ± 0.63	29.81 ± 0.93
August 2013	29.68 ± 0.30	30.34 ± 0.56
September 2013	29.32 ± 0.26	29.96 ± 0.61
June 2012	28.23 ± 0.46	29.61 ± 1.19
July 2012	29.27 ± 0.32	**30.62 ± 0.53**
August 2012	29.91 ± 0.59	**31.12 ± 1.27**
September 2012	29.19 ± 0.22	30.19 ± 0.90
June 2011	28.58 ± 0.65	30.09 ± 1.39
July 2011	29.85 ± 0.58	**31.43 ± 1.22**
August 2011	**30.54 ± 0.34**	**31.82 ± 0.55**
September 2011	30.29 ± 0.40	**31.01 ± 0.55**

reference materials obtained from Dr. A. Dickson (Scripps IO). Mean temperature, salinity, pCO_2, and DIC were used to calculate pH (using the total scale) and aragonite saturation state (Ω_a) for each treatment using the program CO_2SYS using K_1 and K_2 from Mehrbach et al. (1973) refit by Dickson and Millero (1987) per Lewis and Wallace (1998).

Calcification rates were measured biweekly as changes in coral weight in water using the buoyant weight technique according to Davies (1989). A skeletal density of 1.95 g cm^{-3}, based on four *O. faveolata* cores sacrificed at the beginning of the experiment, was used to calculate colony weight in air. Calcification rates were normalized to core surface area to permit reporting in ecologically relevant units, i.e., mg $CaCO_3$ cm^{-2} d^{-1}.

At the end of the experiment, coral tissue was removed using a Water Pik following Szmant and Gassman (1990) in order to quantify symbiont density, chlorophyll *a* content, and total lipid content. Of the total blastate volume, one mL was allocated for symbiont density, one mL was allocated for chlorophyll *a*, and five mL were allocated for total lipid content. For symbiont density, one mL was placed in a 1.5 mL Eppendorf tube with 50 µL Lugols for later quantification using two replicates per sample read on a hemocytometer (Hausser Scientific). For chlorophyll *a*, one mL was filtered onto a glass fiber filter (GF/A) and stored at −80 °C until analysis. For analysis, filters were thawed to room temperature, placed in centrifuge tubes with eight mL methanol, and returned to −80 °C for forty-eight hours following Holmhansen and Riemann (1978). After forty-eight hours, samples were read on a fluorometer (TD-700 Turner Designs) calibrated with purified chlorophyll *a* (Sigma-Aldrich catalog no. C6144). For total lipid content, five mL of blastate was filtered onto a glass fiber filter (GF/A) and stored at −80 °C until later analysis following Teece et al. (2011). Briefly, the five mL aliquot of total coral homogenate was extracted three times (four mL 1:1 dichloromethane:methanol). The resulting organic extracts were dried under a stream of nitrogen gas and weighed on an analytical balance. All parameters were normalized to core surface area.

To identify and quantify the *Symbiodinium* types hosted by the corals, a small tissue sample was taken from each core (∼one polyp) with a razor blade before blasting them. Total genomic DNA was extracted following the organic extraction protocol modified from Baker et al. (1997). Extracted DNA was analyzed with quantitative PCR (qPCR) assays that specifically quantify *Symbiodinium* in clades A, B, C, and D (Cunning and Baker, 2013; Silverstein et al., 2015; Cunning et al., 2015a,b). This was, of necessity, done at the level of clade because primers/probes for the different taxa within each clade have not yet been designed or tested for qPCR. A symbiont clade was considered present in a sample with positive amplification of two technical replicates and no amplification of no-template controls. The proportion of each clade in a coral sample was calculated and corals were classified according to their dominant clade (>90%).

2.4. Statistical analyses

Statistical analyses were completed in JMP v. 13.0.0. Data were checked for normality and homoscedasticity using a Shapiro–Wilk test and Levene's test, respectively. All data were assessed for a tank effect (random factor), which was not significant. Four-way full-factorial ANOVAs were run (temperature × CO_2 × nutrition × site) with parent colony as a random factor for calcification, lipid content, symbiont density, and chlorophyll *a*. Alpha for all tests was set at 0.05. Variations in coral physiology were also studied using multivariate techniques. A Standardized Principal Component Analysis (PCA) was performed in R v. 3.2.3 to examine the spatial structure of groups of samples depending on their calcification rate, lipid content, symbiont density, and chlorophyll *a*. Variables were centered and scaled to allow comparison between data with different units.

3. Results

All of the colonies in this study except one from Emerald Reef were dominated by *Symbiodinium* clade D (>90%). Therefore, samples from that colony that were dominated by clade B and not D were removed from the analyses to avoid changes in physiology associated with hosting a different symbiont clade and not with the treatments. In the interest of full transparency of the limitations of this study, it should be noted that removing the particular ER colony that had <90% D equated to removing 1–2 cores per tank, lowering statistical power, i.e. 10–12 cores per tank and 5–6 per feeding regime. The authors note that it would be worthwhile to repeat this study using a larger sample size, as having to remove corals from the analyses was unforeseen. To date, there has only been one type of clade D (D1a, *Symbiodinium trenchi*) found in Caribbean scleractinian corals, so there was no concern regarding further sequencing (Pettay et al., 2015). However, Denaturing Gradient Gel Electrophoresis (DGGE) analyses of subsets of these samples were run and verified that the profiles of these clade D symbionts were all the same. Although the DGGE bands were not sequenced, they were characteristic of D1a. Mean seawater chemistry parameters from the duration of the study are summarized in Table 1. Temperatures from the collection sites from the collection month and summer months from 2011 to 2013 are shown in Table 2. During the month corals were collected, temperatures at Truman Harbor were approximately 1 °C higher than at Emerald Reef. Historical data at Truman Harbor show

that in the summers of 2012 and 2011, corals at this site were exposed to temperatures above the bleaching threshold (Table 2). In contrast, the Emerald Reef site data suggest that during the previous two summers before collection, corals did not experience mean temperatures exceeding bleaching threshold (Table 2).

Calcification rates were affected by CO_2, nutrition, and site (Table 3, ANOVA, $p < 0.05$). On average, calcification rates were negative at 1000 ppm compared to positive at 390 ppm (Fig. 1(a)), negative when unfed compared to positive when fed (Fig. 1(b)), and negative at Emerald Reef compared to positive at Truman Harbor (Fig. 1(c)). Lipid content was significantly affected by nutrition, and on average unfed corals had 20% lower lipid content then fed corals (Table 3, ANOVA, $p < 0.05$, Fig. 2). Symbiont density was affected by temperature and nutrition, and on average corals had 25% fewer symbiont at 31 °C than at 26 °C, and about 38% fewer symbionts when unfed compared to when fed (Table 3, ANOVA, $p < 0.05$, Fig. 3(a), (b)). Chlorophyll *a* was affected by temperature, CO_2, nutrition, and site (Table 3, ANOVA, $p < 0.05$). On average, chlorophyll *a* was 25% lower at 31 °C than at 26 °C, 17% lower at 1000 ppm compared to 390 ppm, 43% lower when unfed compared to fed, and 30% lower at Truman Harbor compared to Emerald Reef (Fig. 4(a)–(d)).

In the multivariate analyses, symbiont density, chlorophyll *a* content, and (to a lesser extent) lipid content are reflected in PC1 ($\cos^2 = 0.91, 0.88$ and 0.60 respectively). This axis explains 61.5% of the variance and separates the fed corals (on the right of PC1, with higher scores for these three variables), from the starved corals (on the left, corresponding to lower symbiont density, chlorophyll *a*, and lipids, see Fig. 5). There was no clear separation between corals in different temperature or CO_2 treatments, suggesting that feeding was the most important condition determining these physiological characteristics. Growth rate was reflected in PC2 ($\cos^2 = 0.90$) which explains 24.3% of the variance. This second axis separated the corals from TH (on the top of the axis, with higher calcification), from the ER corals (on the bottom of PC2, with lower growth rates, see Fig. 5(a)). This result indicated that even when CO_2 significantly decreased calcification, the effect of site on growth rate was stronger than the effect of CO_2 alone.

When the PCA objects map was visualized by parent colony instead of by location to address if the location effect was a generalization of the genotype effect, we did not find any aggregation of the samples due to parent colony, temperature, or CO_2 treatment (Fig. 5(b)). These observations together demonstrate the importance of heterotrophic feeding and source location shaping coral physiology.

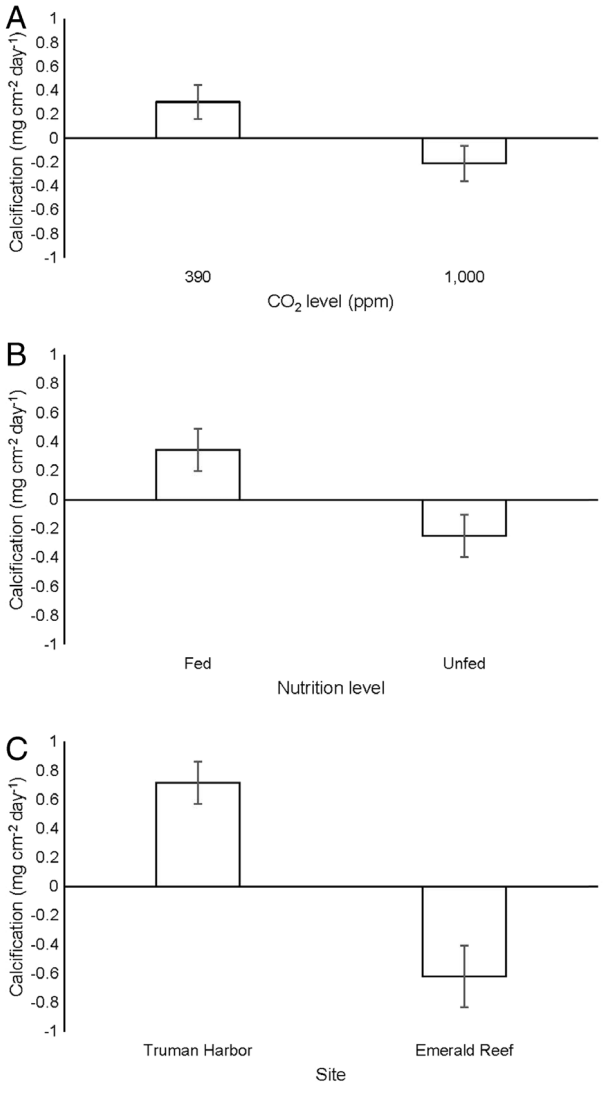

Fig. 1. Calcification rates of *O. faveolata* pooled by levels based on significant main effects from ANOVA analyses where panel A shows the CO_2 effect, panel B shows the nutrition effect, and panel C shows the site effect. Error bars represent ±1 S.E. Total N = approximately 100 cores.

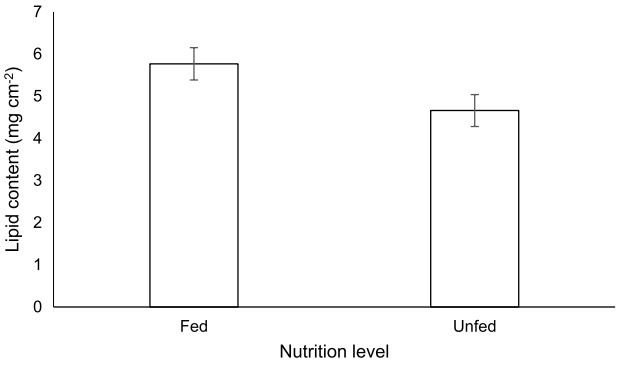

Fig. 2. Lipid content of *O. faveolata* pooled by levels based on the significant main effect of nutrition from ANOVA analyses. Error bars represent ±1 S.E. Total N = approximately 100 cores.

4. Discussion

In this study we found that in the mountainous coral (*Orbicella faveolata*) source location had a strong impact on how a coral responds to climate change stressors, even when corals were allowed to acclimate to the same conditions for a month prior to the onset of stress. This suggests that environmental history at different source locations in the Florida Reef Tract drives differences in coral physiology under stress. These effects were still detectable after one month of common gardening and two months of stress exposure. This finding has important implications for coral restoration because coral source location may play a major role in their future survivorship following outplanting.

We found that calcification rates were highly correlated with coral source location, but calcification rates in our study were lower than rates published in the field for *O. faveolata* (~2.3–2.8 mg cm^{-2} d^{-1}, Mallela and Perry, 2007; Manzello et al., 2015; Towle et al., 2015b). The lower calcification rates may be attributed to the fact that these were rates measured in a laboratory setting under projected climate change scenarios, whereas previous studies were conducted on unstressed corals. However, this does not explain why even the control group of corals from Emerald Reef had negative calcification rates, nor does it explain the differences observed between the two source locations in this study, which need to be addressed in the context of

Table 3
Effect tests for calcification rate, lipid content, symbiont density, and chlorophyll *a* for Emerald Reef and Truman Harbor where significant *p* values are bolded for $p < 0.05$ where T = temperature, CO_2 = carbon dioxide, and N = nutritional status (fed or starved).

Source	Nparm	DF	DFDen	*F* ratio	Prob > *F*
Calcification rate					
T	1	1	62.16	1.1710	0.2834
CO_2	1	1	59.55	13.9162	**0.0004***
$T * CO_2$	1	1	59.54	1.4632	0.2312
Nutrition	1	1	59.07	18.8285	<**0.0001***
$T *$ Nutrition	1	1	60.79	0.0666	0.7972
$CO_2 *$ Nutrition	1	1	59.02	0.3219	0.5726
$T * CO_2 *$ Nutrition	1	1	59.50	0.2479	0.6204
Site	1	1	3.381	27.2310	**0.0101***
$T *$ Site	1	1	62.16	0.8138	0.3705
$CO_2 *$ Site	1	1	59.55	0.4244	0.5172
$T * CO_2 *$ Site	1	1	59.54	0.2665	0.6076
Nutrition * Site	1	1	59.07	0.0746	0.7858
$T *$ Nutrition * Site	1	1	60.79	0.4382	0.5105
$CO_2 *$ Nutrition * Site	1	1	59.02	0.3740	0.5432
$T * CO_2 *$ Nutrition * Site	1	1	59.50	0.1015	0.7511
Lipids					
T	1	1	64.00	0.4419	0.5086
CO_2	1	1	61.05	1.8721	0.1763
$T * CO_2$	1	1	61.07	2.4811	0.1204
Nutrition	1	1	60.37	4.6022	**0.0360***
$T *$ Nutrition	1	1	62.14	0.0001	0.9928
$CO_2 *$ Nutrition	1	1	60.40	0.7636	0.3857
$T * CO_2 *$ Nutrition	1	1	61.58	1.2774	0.2628
Site	1	1	3.764	3.2099	0.1521
$T *$ Site	1	1	64.00	1.1827	0.2809
$CO_2 *$ Site	1	1	61.05	1.9860	0.1638
$T * CO_2 *$ Site	1	1	61.07	2.7745	0.1009
Nutrition * Site	1	1	60.37	2.1938	0.1438
$T *$ Nutrition * Site	1	1	62.14	0.2866	0.5943
$CO_2 *$ Nutrition * Site	1	1	60.40	0.0680	0.7952
$T * CO_2 *$ Nutrition * Site	1	1	61.58	0.0243	0.8766
Chlorophyll *a*					
T	1	1	63.22	13.4823	**0.0005***
CO_2	1	1	59.53	6.3284	**0.0146***
$T * CO_2$	1	1	59.52	0.0083	0.9275
Nutrition	1	1	58.81	55.9392	<**0.0001***
$T *$ Nutrition	1	1	61.05	2.1128	0.1512
$CO_2 *$ Nutrition	1	1	58.72	0.3733	0.5436
$T * CO_2 *$ Nutrition	1	1	59.46	0.6482	0.4240
Site	1	1	2.939	10.5351	**0.0490***
$T *$ Site	1	1	63.22	1.5741	0.2142
$CO_2 *$ Site	1	1	59.53	0.0002	0.9902
$T * CO_2 *$ Site	1	1	59.52	0.2665	0.6076
Nutrition * Site	1	1	58.81	1.9560	0.1672
$T *$ Nutrition * Site	1	1	61.05	1.8145	0.1829
$CO_2 *$ Nutrition * Site	1	1	58.72	2.0049	0.1621
$T * CO_2 *$ Nutrition * Site	1	1	59.46	0.8749	0.3534
Zooxanthellae density					
T	1	1	40.80	13.3886	**0.0007***
CO_2	1	1	63.13	1.6001	0.2105
$T * CO_2$	1	1	63.30	0.0491	0.8254
Nutrition	1	1	60.37	52.8440	<**0.0001***
$T *$ Nutrition	1	1	63.10	2.0640	0.1558
$CO_2 *$ Nutrition	1	1	59.73	1.6065	0.2099
$T * CO_2 *$ Nutrition	1	1	63.19	0.1713	0.6804
Site	1	1	0.753	30.6812	0.1700
$T *$ Site	1	1	40.80	2.1694	0.1485
$CO_2 *$ Site	1	1	63.13	0.0942	0.7599
$T * CO_2 *$ Site	1	1	63.30	0.0114	0.9154
Nutrition * Site	1	1	60.37	0.0806	0.7775
$T *$ Nutrition * Site	1	1	63.10	2.7342	0.1032
$CO_2 *$ Nutrition * Site	1	1	59.73	2.1953	0.1437
$T * CO_2 *$ Nutrition * Site	1	1	63.19	2.1848	0.1443

other physical and physiological parameters. Again, in the interest of full transparency of the limitations of the study, the negative calcification rates of some ER control corals suggest that one-month common gardening was not enough to alleviate the stress of coring and/or previous stress from the source site, and therefore it would be worthwhile to repeat this study to determine if the low rates were in fact representative of true responses. It also may be worth noting that the symbiont densities of unbleached *O. faveolata* in summer in this study ($\sim 1.5 \times 10^6$ cells cm^{-2}) were lower than unbleached *O. faveolata* in summer from a different

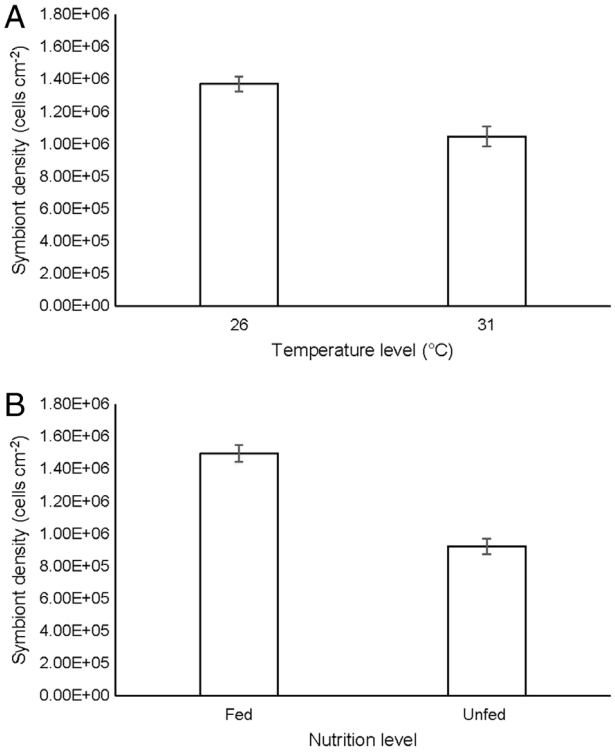

Fig. 3. Symbiont density of *O. faveolata* pooled by levels based on significant main effects of from ANOVA analyses where panel A shows the temperature effect and panel B shows the nutrition effect. Error bars represent ±1 S.E. Total N = approximately 100 cores.

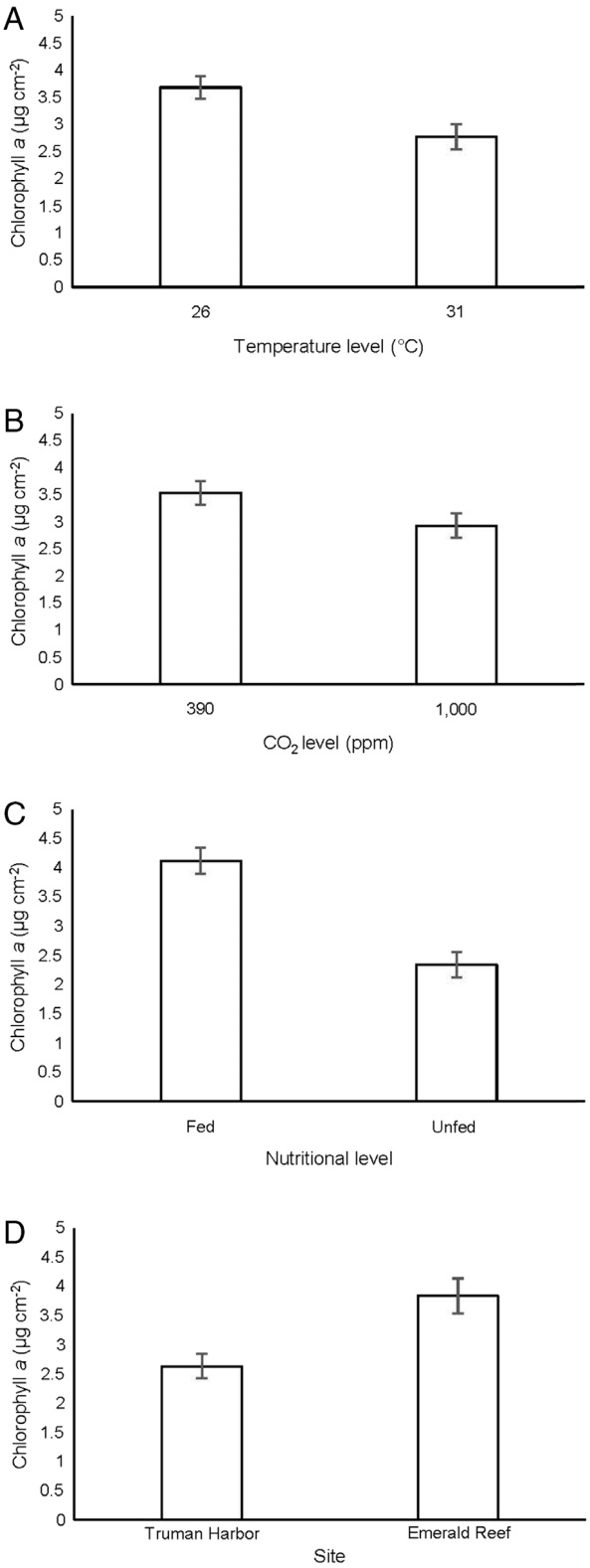

Fig. 4. Chlorophyll *a* content of *O. faveolata* pooled by levels based on significant main effects from ANOVA analyses where panel A shows the temperature effect, panel B shows the CO_2 effect, panel C shows the nutrition effect, and panel D shows the site effect. Error bars represent ±1 S.E. Total N = approximately 100 cores.

study (i.e. ~2.7 × 10^6 cells cm^{-2}, Kemp et al., 2014,) which may account in part for the lower growth rates.

Nonetheless, previous work has demonstrated that variability in physiological response to climate change stress can sometimes be explained by host heterotrophy (Grottoli et al., 2006; Rodrigues and Grottoli, 2007; Cohen and Holcomb, 2010; Edmunds, 2011; Towle et al., 2015a). While we did not assess host total biomass, i.e. total protein or ash-free dry weight, if there was a difference in host biomass between sites due to heterotrophic food availability, that could have also contributed to differences in growth rate. In this study, principle component analyses revealed that calcification, lipid content, symbiont density, and chlorophyll *a* content were highly correlated with the ability of *O. faveolata* to feed. This observation is consistent with Towle et al. (2015a), who found that feeding and lipid content were correlated in another threatened Western Atlantic/Caribbean coral species, *Acropora cervicornis*. The present study also showed that the ability of corals to feed stimulates areal symbiont density and chlorophyll *a* content, which is consistent with Ferrier-Pagès et al. (2003) and Houlbrèque et al. (2003, 2004), providing further evidence that coral feeding may be a useful physiological health metric for restoration and reef resilience. Knowing that food availability enhances coral physiology under stress should incentivize reef managers to consider the level of natural zooplankton densities as a metric for determining MPA placement sites. Additionally, coral nursery operators may want to consider implementing supplemental feeding protocols for corals pre-outplanting.

While the results of the PCA agree with previous studies on the response of corals to stress, it remains unclear exactly why the two source locations show such dramatic differences in calcification rate. One explanation is that the warmer conditions in Truman Harbor acclimatized corals to higher temperatures. The summer before the corals were collected (2013), mean temperatures in June and July at TH were more than one degree higher than June and July 2013 mean temperatures at ER. Corals from TH also experienced mean summer temperatures in July and August of 2012 and July–September of 2011 greater than the bleaching threshold in the Florida Keys (30.4 °C, Manzello

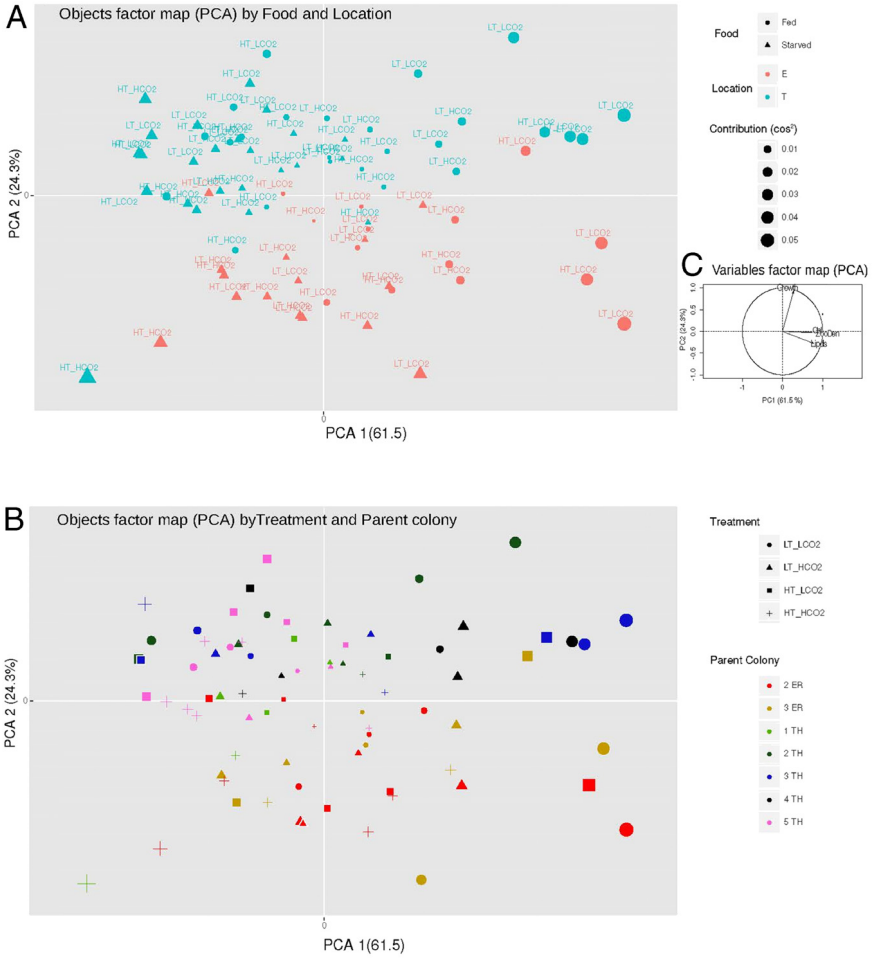

Fig. 5. Ordination of approximately 100 coral cores by principle component analysis (PCA) based on coral physiology (growth rate, chlorophyll *a* content, zooxanthellae density, and lipid content). In 5A and 5B each point represents a coral core, and proximity between the points indicates physiological similarity. Point size represents the contribution of each core to the PCA (cos^2). The legend in 2C shows the direction of the variables in the PCA in both panel A and B. The lengths of vectors indicate the contribution of the descriptors to the formation of PC1 and PC2. Chlorophyll *a* content (Chl), zooxanthellae density (ZooDen), and lipid content (Lipids) were highly correlated between them, and not correlated with growth rate (Growth).

et al., 2007), yet appear to have been unaffected. Consequently, the high temperature used in our experiment (31 °C) likely stressed ER corals more than TH corals. This hypothesis is consistent with Oliver and Palumbi (2009, 2011) who found that corals from warmer environments have higher resistance to bleaching temperatures, and can survive heat exposure that would bleach conspecifics from cooler microclimates. This may be because corals in warmer environments have higher baseline expression of genes involved in thermal stress response, such as heat shock proteins, antioxidant enzymes, and/or genes involved in apoptosis regulation and tumor suppression (Barshis et al., 2013). Variation in thermal tolerance may also be due in large part to variation in *Symbiodinium* types (Jones and Berkelmans, 2011). However, in the case of this study, all corals had >90% clade D *Symbiodinium*, and those that did not were removed from analyses, and thus we can be fairly confident that symbiont clade type had no influence on the responses we observed. It is important to note that we had not been monitoring the types of zooxanthellae in these particular colonies over multiple years, so we do not have any idea how long D-dominance has persisted in these corals (see: Thornhill et al., 2006). As previously stated, finding a different type of D (other than D1a/*S. trenchi*) would be very unusual since no other clade D symbionts have yet been found in Caribbean scleractinian corals. The idea that corals from different thermal histories have different bleaching thresholds is not novel; however, the fact that one month of acclimation to control conditions did not remove the effect of source location in *O. faveolata* suggests these findings could be the result of local selection and not acclimatization.

A second explanation, then, is that the higher mean summer temperatures at Truman Harbor selected for coral genotypes capable of dealing with relatively higher temperatures. This is supported by the fact that acclimation to 26 °C for four weeks prior to the start of the experiment did not remove the effects of source location. Local selection at the two sites may have resulted in genotypic differences (fixed effects) that cannot be removed by common gardening. These fixed effects may constrain the ability of coral populations from different sites to acclimatize to changes in their environment (Palumbi et al., 2014). The hypothesis is then that these findings may not be the result of acclimatization, but may actually be because of different genes in the two different populations. One month does not appear long enough to remove this effect, and it is unclear what timescale could have removed the effects, e.g. maybe a more appropriate time frame would be on the order of several months to a year. Nonetheless, the data show very dramatic differences that are still apparent between the two populations with one site showing positive calcification and the other negative calcification across treatments. This finding is consistent with Howells et al. (2013) who found that while acclimatization processes are important in coping with small acute and seasonal temperature fluctuations, the thermal tolerance limits of the Pacific species *Acropora millepora* were determined primarily by genetic adaptation to local thermal

regimes. Additionally, while this study is limited by the fact that sea water chemistry, turbidity, and water flow data were not available from either site, it is possible that the effects of local selection on TH corals may also allow them to calcify better under experimental CO_2 stress, possibly due to higher food availability/POM and/or carbonate chemistry factors. These physiological mechanisms of environmental accommodation to acidification (Fabricius et al., 2011; Shamberger et al., 2014) may be driven by genotypic differences among the two populations. Future work will need to assess the genotypes of *O. faveolata* at Truman Harbor and Emerald Reef, as we now have reason to believe that local selection could be driving the differences we observed in this study. While we have chosen to interpret our data as potential evidence of local selection, it should be stated that non-genetic physiological differences may be durable, in some cases even inherited, as suggested by Putnam and Gates (2015). Therefore, we cannot rule out potential acclimatization as another alternative hypothesis (other than adaptation) for the site differences found between these two sites, and follow-up work needs to be done to improve our understanding of these differences.

In conclusion, we found that source location and food availability may contribute to large variation in calcification response to climate change stressors in a threatened coral species from the Florida Reef Tract. These differences were not removed by one-month acclimation to the same control conditions, suggesting that they may be due to local selection at different sites. Because local selection is a fixed effect, source location may be a critical element in the success of long-term restoration efforts, since genotype by environment (G×E) interactions may play an important role in outplanting success. These findings also suggest that deliberate attempts to move corals from one thermal environment to another in preparation for climate change, even over short distances (i.e. within the Florida Keys) may have value, since the coral populations from different sites vary in their genetic make-up and are not simply acclimatized to different thermal regimes. However, these 'assisted colonization' (Kreyling et al., 2011) efforts should be tempered by investigations into the potential risks of genetic depression (van Oppen et al., 2014) or the introduction of pathogens or disease (Hoegh-Guldberg et al., 2008).

Acknowledgments

We are grateful to E. Bartels for help with the logistics of coral collection and for providing temperature data from the Truman Harbor site. We thank R. Pausch and D. Williams for temperature data from Emerald Reef. We also appreciate help from L. Hyder, C. Mor, and J. Fisch with tank maintenance. We thank the Florida Keys National Marine Sanctuary and the Florida Fish and Wildlife Conservation Commission for permitting this work to occur. ACB was supported by NSF OCE-1358699. Two reviewers helped improve the quality of this manuscript.

References

Albright, R., Langdon, C., 2011. Ocean acidification impacts multiple early life history processes of the Caribbean coral *Porites astreoides*. Global Change Biol. 17, 2478–2487.

Albright, R., Mason, B., 2013. Projected near-future levels of temperature and pCO_2 reduce coral fertilization success. PLoS One 8, e56468.

Albright, R., Mason, B., Miller, M., Langdon, C., 2010. Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. Proc. Natl. Acad. Sci. 107, 20400–20404.

Baker, A.C., Rowan, R., Knowlton, N., 1997. Symbiosis ecology of two Caribbean acroporid corals. In: Proc 8th Int Coral Reef Symp 2: pp. 1295–1300.

Baker, A.C., Starger, C.J., McClanahan, T.R., Glynn, P.W., 2004. Coral reefs: corals' adaptive response to climate change. Nature 430, 741.

Barshis, D.J., Ladner, J.T., Oliver, T.A., et al., 2013. Genomic basis for coral resilience to climate change. Proc. Natl. Acad. Sci. 110, 1387–1392.

Baums, I.B., Devlin-Durante, M.K., Polato, N.R., Xu, D., Giri, S., Altman, N.S., Ruiz, D., Parkinson, J.E., Boulay, J.N., 2013. Genotypic variation influences reproductive success and thermal stress tolerance in the reef building coral, *Acropora palmata*. Coral Reefs 32, 703–717.

Carilli, J., Donner, S.D., Hartmann, A.C., 2012. Historical temperature variability affects coral response to heat stress. PLoS One 7 (3), e34418.

Chan, N.C.S., Connolly, S.R., 2013. Sensitivity of coral calcification to ocean acidification: a meta-analysis. Global Change Biol. 19, 282–290.

Cohen, A., Holcomb, M., 2010. Why corals care about ocean acidification: uncovering the mechanism. In: Scott, D., Fabry, V., Balch, B., Feely, R. (Eds.), The Future of Ocean Biogeochemistry in a High CO_2 World. Oceanography 22, 118–127.

Cunning, R., Baker, A.C., 2013. Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nat. Clim. Change 3, 259–262.

Cunning, R., Silverstein, R.N., Baker, A.C., 2015a. Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. Proc. Roy. Soc. B 282, 20141725. http://dx.doi.org/10.1098/rspb.2014.1725.

Cunning, R., Vaughan, N., Gillette, P., Capo, T.R., Maté, J.L., Baker, A.C., 2015b. Dynamic regulation of partner abundance mediates response of reef coral symbioses to environmental change. Ecology 96, 1411–1420.

Davies, P.S., 1989. Short-term growth measurements of corals using an accurate buoyant weighing technique. Mar. Biol. 101, 389–395.

Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep-Sea Res. 34, 1733–1743.

Doropoulos, C., Diaz-Pulido, G., 2013. High CO_2 reduces the settlement of a spawning coral on three common species of crustose coralline algae. Mar. Ecol. Prog. Ser. 475, 93–99.

Edmunds, P.J., 2011. Zooplanktivory ameliorates the effects of ocean acidification on the reef coral *Porites* spp. Limnol. Oceanogr. 56, 2402–2410.

Fabricius, K.E., Langdon, C., Uthicke, S., 2011. Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. Nat. Clim. Change 1, 165–169.

Ferrier-Pagès, C., Witting, J., Tambutté, E., Sebens, K.P., 2003. Effect of natural zooplankton feeding on the tissue and skeletal growth of the scleractinian coral *Stylophora pistillata*. Coral Reefs 22, 229–240.

Forsman, Z.H., Page, C.A., Toonen, R.J., Vaughan, D., 2015. Growing coral faster: micro-colony-fusion as a strategy for accelerating coral cover. PeerJ 3, http://dx.doi.org/10.7717/peerj.1313.

Glynn, P.W., 2012. Global warming and widespread coral mortality: evidence of first coral reef extinctions. In: Saving a Million Species. Springer, pp. 103–119.

Grottoli, A.G., Rodrigues, L.J., Palardy, J.E., 2006. Heterotrophic plasticity and resilience in bleached corals. Nature 440, 1186–1189.

Hoegh-Guldberg, O., Hughes, L., McIntyre, S., Lindenmayer, D.B., Parmesan, C., Possingham, H.P., Thomas, C.D., 2008. Assisted colonization and rapid climate change. Science 321, 345–346.

Holmhansen, O., Riemann, B., 1978. Chlorophyll *a* determination- improvements in methodology. Oikos 30, 438–447.

Houlbrèque, F., Tambutté, E., Allemand, D., Ferrier-Pagès, C., 2004. Interactions between zooplankton feeding, photosynthesis, and skeletal growth in the scleractinian coral *Stylophora pistillata*. J. Exp. Biol. 207, 1461–1469.

Houlbrèque, F., Tambutté, E., Ferrier-Pagès, C., 2003. Effect of zooplankton availability on the rates of photosynthesis, and tissue and skeletal growth in the scleractinian coral *Stylophora pistillata*. J. Exp. Mar. Biol. Ecol. 296, 145–166.

Howells, E.J., Berkelmans, R., van Oppen, M.J.H., Willis, B.L., Bay, L.K., 2013. Historical thermal regimes define limits to coral acclimatization. Ecology 94, 1078–1088.

IPCC, , 2013. Summary for policymakers. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M., et al. (Eds.), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Johnson, M.E., Lustic, C., Bartels, E., Baums, I.B., Gilliam, D.S., Larson, E.A., Lirman, D., Miller, M.W., Nedimyer, K., Schopmeyer, S., 2011. Caribbean *Acropora* Restoration Guide: Best Practices for Propagation and Population Enhancement: pp. 1–64. http://nsuworks.nova.edu/occ_facreports/71.

Jones, A.M., Berkelmans, R., 2011. Tradeoffs to thermalacclimation: Energetics and reproduction of a reef coral with heat tolerant *Symbiodinium* type D. J. Mar. Biol. 185890.

Kemp, D.W., Hernandez-Pech, X., Iglesias-Prieto, R., Fitt, W.K., Schmidt, G.W., 2014. Community dynamics and physiology of *Symbiodinium* spp. before, during, and after a coral bleaching event. Limnol. Oceanogr. 59, 788–797.

Kreyling, J., Bittner, T., Jaeschke, A., Jentsch, A., Jonas Steinbauer, M., Thiel, D., Beierkuhnlein, C., 2011. Assisted colonization: a question of focal units and recipient localities. Restoration Ecol. 19, 433–440.

Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M., Gattuso, J.-P., 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Global Change Biol. 19, 1184–1896.

Lewis, E., Wallace, D.W.R., 1998. CO2SYS—Program developed for the CO_2 system calculations. Carbon Dioxide Inf. Anal. Center; Report ORNL/CDIAC-105. Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee.

Mallela, J., Perry, C.T., 2007. Calcium carbonate budgets for two coral reefs affected by different terrestrial runoff regimes, Rio Bueno, Jamaica. Coral Reefs 26, 53–68.

Manzello, D.P., Berkelmans, R., Hendee, J.C., 2007. Coral bleaching indices and thresholds for the Florida Reef Tract, Bahamas, and St. Croix, US Virgin Islands. Mar. Pollut. Bull. 54, 1923–1931.

Manzello, D.P., Enochs, I.C., Kolodziej, G., Carlton, R., 2015. Recent decade of growth and calcification of *Orbicella faveolata* in the Florida Keys: An inshore- offshore comparison. Mar. Ecol. Prog. Ser. 521, 81–89.
Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowicz, R.M., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18, 897–907.
Muscatine, L., Porter, J.W., 1977. Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience 27, 454–460.
National Marine Fisheries Service, , 2014. Endangered and threatened wildlife and plants: final listing determinations on proposal to list 66 reef-building coral species and to reclassify elkhorn and staghorn corals. Fed. Regist. 79 (135), 53852–54123.
Oliver, T.A., Palumbi, S.R., 2011. Do fluctuating temperature environments elevate coral thermal tolerance?. Coral Reefs 30, 429–440.
Oliver, T.A., Palumbi, S.R., 2009. Distributions of stress-resistant coral symbionts match environmental patterns at local but not regional scales. Mar. Ecol. Prog. Ser. 378, 93–103.
Palumbi, S.R., Barshis, D.J., Traylor-Knowles, N., Bay, R.A., 2014. Mechanisms of reef coral resistance to future climate change. Science 344, 895–898.
Pettay, D.T., Wham, D.C., Smith, R.T., Iglesias-Prieto, R., LaJeunesse, T., 2015. Microbial invasion of the Caribbean by an Indo-Pacific coral zooxanthella. PNAS 112, 7513–7518.
Putnam, H.M., Gates, R.D., 2015. Preconditioning in the reef-building coral Pocillopora damicornis and the potential for trans-generational acclimatization in coral larvae under future climate changes scenarios. J. Exp. Biol. 218, 2365–2372.
Reynaud, S., Leclercq, N., Romaine-Lioud, S., Ferrier-Pagès, C., Jaubert, J., Gattuso, J.P., 2003. Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. Global Change Biol. 9, 1660–1668.
Rodrigues, L.J., Grottoli, A.G., 2007. Energy reserves and metabolism as indicators of coral recovery from bleaching. Limnol. Oceanogr. 52, 1874–1882.
Shamberger, K.E., Cohen, A.L., Golbuu, Y., 2014. Diverse coral communities in naturally acidified waters of a Western Pacific reef. Geophys. Res. Lett. 41, 499–504.
Silverstein, R.N., Cunning, R., Baker, A.C., 2015. Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. Global Change Biol. 21, 236–249.
Szmant, A.M., Gassman, N.J., 1990. The effects of prolonged bleaching on the tissue biomass and reproduction of the reef coral *Montastraea annularis*. Coral Reefs 8, 217–224.
Teece, M.A., Estes, B., Gelsleichter, E., Lirman, D., 2011. Heterotrophic and autotrophic assimilation of fatty acids by two scleractinian corals, *Montastraea faveolata* and *Porites astreoides*. Limnol. Oceanogr. 56, 1285–1296.
Thornhill, D.J., LaJeunesse, T.C., Kemp, D.W., Fitt, W.K., Schmidt, G.W., 2006. Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. Mar. Biol. 148, 711–722.
Towle, E.K., Enochs, I.C., Langdon, C., 2015a. Threatened Caribbean coral is able to mitigate the adverse effects of ocean acidification on calcification by increasing feeding rate. PLoS One 10 (4), e0123394.
Towle, E.K., Carlton, R., Langdon, C., Manzello, D.P., 2015b. *In-situ* measurement of metabolic status in three coral species from the Florida Reef Tract. Reg. Stud. Mar. Sci. 2, 145–153.
van Oppen, M.J.H., Puill-Stephan, E., Lundgren, P., De'ath, G., Bay, L.K., 2014. First-generation fitness consequences of interpopulational hybridisation in a Great Barrier Reef coral and its implications for assisted migration management. Coral Reefs 33, 607–611.
Young, C.N., Schopmeyer, S.A., Lirman, D., 2012. A review of reef restoration and coral propagation using the threatened genus Acropora in the Caribbean and Western Atlantic. Bull. Mar. Sci. 88, 1075–1098.