Mar Biol
DOI 10.1007/s00227-012-2129-9

ORIGINAL PAPER

The physiological and molecular responses of larvae from the reef-building coral *Pocillopora damicornis* exposed to near-future increases in temperature and pCO_2

H. M. Putnam · A. B. Mayfield · T. Y. Fan · C. S. Chen · R. D. Gates

Received: 24 August 2012 / Accepted: 12 November 2012

Abstract Given the threats of greenhouse gas emissions and a changing climate to marine ecosystems, there is an urgent need to better understand the response of not only adult corals, which are particularly sensitive to environmental changes, but also their larvae, whose mechanisms of acclimation to both temperature increases and ocean acidification are not well understood. Brooded larvae from the reef coral *Pocillopora damicornis* collected from Nanwan Bay, Southern Taiwan, were exposed to ambient or elevated temperature (25 or 29 °C) and pCO_2 (415 or 635 µatm) in a factorial experiment for 9 days, and a variety of physiological and molecular parameters were measured. Respiration and rubisco protein expression decreased in larvae exposed to elevated temperature, while those incubated at high pCO_2 were larger in size. Collectively, these findings highlight the complex metabolic and molecular responses of this life history stage and the need to integrate our understanding across multiple levels of biological organization. Our results also suggest that for this pocilloporid larval life stage, the impacts of elevated temperature are likely a greater threat under near-future predictions for climate change than ocean acidification.

Communicated by H.-O. Pörtner.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-012-2129-9) contains supplementary material, which is available to authorized users.

H. M. Putnam (✉) · R. D. Gates
Hawaii Institute of Marine Biology, University of Hawaii, PO Box 1346, Kaneohe, HI 96744, USA
e-mail: hputnam@hawaii.edu

A. B. Mayfield · T. Y. Fan
National Museum of Marine Biology and Aquarium, 2 Houwan Rd., Checheng, Pingtung, Taiwan, ROC

T. Y. Fan
Institute of Marine Biodiversity and Evolution, National Dong-Hwa University, Hualien, Taiwan, ROC

C. S. Chen
Graduate Institute of Marine Biotechnology, National Dong-Hwa University, Hualien, Taiwan, ROC

C. S. Chen
Taiwan Coral Research Center (TCRC), National Museum of Marine Biology and Aquarium, Checheng, Pingtung 944, Taiwan, ROC

C. S. Chen
Department of Marine Biotechnology and Resources, National Sun Yat-Sen University, Kaohsiung, Taiwan, ROC

Introduction

Future predictions of anthropogenic greenhouse gas emissions and climate change represent global environmental factors that result in detrimental effects on marine organisms. These include two major physical drivers in marine ecosystems: (1) elevated seawater temperature and (2) increased atmospheric CO_2 (van Vuuren et al. 2008, 2011). Increased temperature has well-documented negative impacts on organismal physiology, specifically above organisms' thermal optima (Hofmann and Todgham 2010). Ocean acidification (OA), the "other CO_2 problem" (Doney et al. 2009b), occurs when CO_2 is taken up by the oceans, which shifts the carbonate buffering system increasing hydrogen ion concentration and decreasing pH, an environmental setting that is also detrimental to many marine organisms (reviewed by Doney et al. 2009a, Dupont et al. 2010a, b; Kroeker et al. 2010).

Published online: 29 November 2012

Relative to preindustrial values, near-future temperature predictions include increases from 1.5 to 3.5 °C (Meinshausen et al. 2011). Increased temperatures have negative impacts on marine organisms that include changes in the success rates of fertilization and development (Negri et al. 2007), shifts in ecological range (Parmesan 2006), and mortality (Fitt et al. 2001). Additionally, greenhouse gas emission scenarios of representative concentration pathways (RCPs) predict that near-future (2075) atmospheric CO_2 will range from ~440 to 700 ppm (van Vuuren et al. 2011) and reach 490, 650, 850, and >1,370 ppm by 2100, based on IMAGE, GCAM, AIM, and MESSAGE models, respectively (Moss et al. 2010). Ocean acidification affects marine organism reproduction (reviewed by Byrne 2011a, b), and while it can be beneficial in some systems as characterized by enhanced development and growth rates (Dupont et al. 2010a, b, 2012), it can also result in detrimental effects such as malformation during development (Kurihara 2008; Talmage and Gobler 2010), declines in adult calcification and growth (Kroeker et al. 2010), and loss of discriminatory capacity for environmental cues (Munday et al. 2009). Together, elevated temperature and OA have the potential to greatly perturb the stability and net accretion of marine ecosystems, particularly those based on calcifying organisms (Hoegh-Guldberg et al. 2007; Fabry et al. 2008; Hofmann et al. 2010). Of specific interest in this context, is the response of reef-building corals, the calcifying structural engineers of highly productive and diverse ecosystems. Corals are symbiotic organisms that contain single-celled dinoflagellates of the genus *Symbiodinium* within their gastrodermal cells. These photosynthetic symbionts produce and translocate the majority of their fixed carbon to the coral host (Muscatine et al. 1984) and are responsible for the high productivity and high rates of calcification of coral reef ecosystems. These framework-building corals are especially sensitive to environmental changes, and the synergistic impacts of elevated temperature and OA have the potential to drive coral reef ecosystems past functional thresholds toward alternate ecosystem states (Pandolfi et al. 2005; Hoegh-Guldberg et al. 2007; Veron 2011).

Reef-building corals are of particular concern due to their sensitivity to elevated temperature, which can cause bleaching, and in severe cases, bleaching-related mortality (Coles and Brown 2003). Likewise, OA has been shown to result in decreased coral calcification (Langdon and Atkinson 2005). To date, the severe declines in the health of coral reef ecosystems that have been predicted under climate change scenarios (Hoegh-Guldberg et al. 2007; Veron 2011) have predominantly been based on studies documenting adult coral responses (reviewed by Lesser 2011; Erez et al. 2011). However, factors such as interspecific variability, location-specific responses, physical synergisms and antagonisms, the potential for adaptation and acclimatization (Chauvin et al. 2011; Edmunds 2011; Fabricius et al. 2011; Pandolfi et al. 2011), and the importance of reproduction and recruitment (Albright 2011) have now been recognized as being critical considerations in determining the impacts of disturbance regimes on corals and coral reefs.

The maintenance of coral reefs demands the continuous supply of new propagules, recruitment into the population, and persistence of these juveniles within the community. Larval tolerance may present a bottleneck for this process (Byrne 2011b), and as such, there is an urgent need to better understand the capacity of this early life history stage to respond to temperature and CO_2 regimes expected to characterize reefs over the next 50–100 years (Kurihara 2008; Byrne 2011a, b). Elevated temperature can affect the larval response by modulating settlement choice (Putnam et al. 2008) and can also reduce photosynthetic rates (Edmunds et al. 2001), settlement success (Randall and Szmant 2009), and survivorship (Edmunds et al. 2001; Bassim and Sammarco 2003; Yakovleva et al. 2009). Likewise, larvae of reef-building corals have been shown to exhibit shifts in fertilization success (Albright et al. 2010), settlement (Albright and Langdon 2011; Nakamura et al. 2011b), metabolic demands (Albright and Langdon 2011; Nakamura et al. 2011b), and survival (Nakamura et al. 2011b) when exposed to elevated CO_2.

Despite the importance of this early life history stage, few studies have tracked the response of coral larvae across multiple biological scales. This reflects the fact that early life history stages of corals are only very intermittently available and difficult to work with due to size and other factors, so most studies focus either on whole-organism physiological characteristics, such as respiration, survival, and settlement (e.g., Edmunds et al. 2001; Anlauf et al. 2011; Nakamura et al. 2011b), or solely on molecular parameters, such as gene expression (e.g., Rodriguez-Lanetty et al. 2009; Aranda et al. 2011; Meyer et al. 2011). Here, with the goal of attaining a more comprehensive mechanistic understanding of the phenotypic responses of coral larvae, we examined the effects of increased temperature and OA on several aspects of both whole-organism physiology and the sub-cellular response (Fig. 1) of brooded *Pocillopora damicornis* larvae.

We selected three physiological response variables to assess larval performance under exposure to elevated temperature and pCO_2 (Fig. 1). First, we examined the photochemical efficiency of PSII (F_V/F_M), in which a decline indicates potential damage to, or photoinactivation of PSII, a documented precursor to the bleaching cascade (Jones et al. 2000; Fitt et al. 2001). Second, we assessed holobiont metabolism via larval dark respiration measurements, with the expectation that metabolic performance

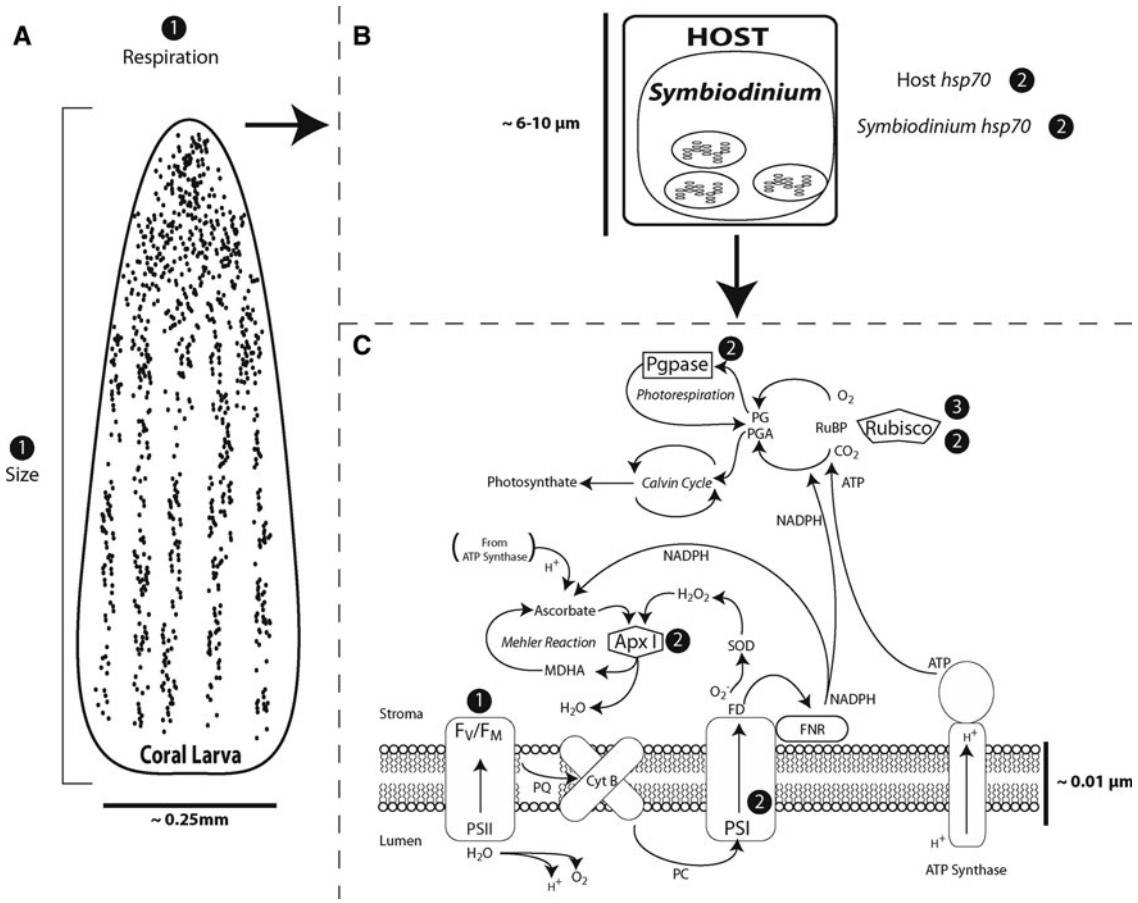

Fig. 1 Generalized schematic summarizing physiological and molecular assays for **a** the whole organism, **b** cellular response, and **c** photosynthetic pathway. *Numbers* within the *circles* indicate the biological scale of measurement: *1* physiology, *2* gene expression, *3* protein expression

would decline under thermal and hypercapnia stress (Byrne 2011a; Pörtner 2008, but see Stumpp et al. 2011b). Thirdly, we measured larval size as an indicator of dispersal potential (Isomura and Nishihira 2001), and more generally, fitness.

In addition to the physiological variables, we measured the expression of two broad categories of genes and proteins hypothesized to be important in coral acclimation to altered environments using real-time quantitative PCR (QPCR) and western blotting, respectively. The first group included four photosynthesis-related genes (Fig. 1); photosystem I (*psI* subunit III), phosphoglycolate phosphatase (*pgpase*), ribulose-1,5-bisphosphate carboxylase/oxygenase (rusbisco, *rbcL*), and ascorbate peroxidase (*apx1*). Additionally, the expression of the rubisco protein, RBCL, was measured with western blotting. We hypothesized that the expression of the first three genes and the RBCL protein would decrease in larvae exposed to elevated temperature and CO_2, indicating potential damage to the photosynthetic machinery, and the latter gene, *apx1*, would increase to detoxify reactive oxygen species (ROS) generated by damage to the photosynthetic pathway (e.g., Lesser 1997; Venn et al. 2008). The second group included the molecular chaperone heat shock protein 70 (*hsp70*) gene in both the host coral and *Symbiodinium* and the holobiont HSP70 protein. Expression of both *hsp70* orthologs and HSP70 was expected to increase in larvae exposed to elevated temperature and CO_2, as environmental stress has previously been shown to necessitate repair of damaged protein (Downs et al. 2000).

Materials and methods

Manipulative experiment

To obtain brooded larvae for experimental use, adult *P. damicornis* colonies were collected from Nanwan Bay, Southern Taiwan (21°56.179′N, 120°44.851′E), on March 12, 2010. The lunar cycle of larval release for brooding *P. damicornis* in Southern Taiwan is well documented (Fan et al. 2002), and peak release follows the new moon (lunar

days 6–10). Adult *P. damicornis* colonies were held in larval collectors (described in Putnam et al. 2008, 2010) under ambient light (~200 μmol photons m^{-2} s^{-1}) and temperature (~25 °C). Swimming larvae were collected from the tank outlet in mesh beakers via flowing seawater as described in Putnam et al. (2010) near peak larval release on lunar day 9. The larvae from multiple colonies were pooled and randomly subdivided into groups of eighty larvae that were placed in plankton mesh cylinders (~100 ml volume, 170 μm mesh). Cylinders containing larvae were then suspended in each of sixteen 40-l tanks representing four treatments ($N = 4$ tanks per treatment).

Larvae were exposed for 9 days (March, 23–April 01, 2010) to one of four treatments of temperature and CO_2 (two levels each) in a factorial crossed design as follows: (1) 25 °C, ambient CO_2 (ATAC), (2) 25 °C, high CO_2 (ATHC), (3) 29 °C, ambient CO_2 (HTAC), and (4) 29 °C, high CO_2 (HTHC). These treatments used spring-time ambient temperature and the partial pressure of CO_2 (pCO_2) of Nanwan Bay, Taiwan, as well as near-future predictions of ~600 ppm CO_2 (RPC 8.5, rising emissions scenario for the years 2050–2075; Riahi et al. 2011; van Vuuren et al. 2011) during a bleaching stress event scenario of ~ambient + 4 °C (Donner 2009; Meinshausen et al. 2011). While larvae were not fed during the experiment, *P. damicornis* larvae are released with a full complement of photosynthetic symbionts (~3,000–7,000 cells $larva^{-1}$; Putnam et al. 2010) and therefore have capacity to sustain themselves autotrophically (Muscatine et al. 1981). In addition, the incoming seawater was sand filtered, allowing for natural seawater bacteria and DOM to pass through into the experimental aquaria and contribute to larval energetic demands.

Tanks were illuminated with metal halide lamps (MH-150 W), and irradiance (PAR) measurements were taken multiple times daily in each tank using a cosine-corrected Li-Cor sensor (192-SA, Li-Cor). Average daily light levels did not differ between tanks ($F_{(15,111)} = 1.0739$, $p = 0.39$) and treatments ($F_{(3,123)} = 1.8452$, $p = 0.14$), and the average irradiance was 176 ± 2 μmol photons m^{-2} s^{-1}, (mean ± standard error [SE]). Within the experimental tanks, temperature was controlled using submerged heaters (AZOO 300w, Taikong Corporation) and external chillers (Aquatech) connected to recirculating pumps for each treatment tank. CO_2 control was created by the addition of either ambient or high premixed CO_2 concentrations bubbled into each tank using an automated feedback CO_2 control system (Qubit Systems; see Edmunds 2011 for details).

Treatment temperatures were assessed several times each day using a certified thermometer (15-077-8, accuracy 0.05 °C, resolution 0.001 °C, Control Company). Sampling for seawater chemistry was carried out as described below, including the use of the recommended best practices for OA research and reporting (Riebesell et al. 2010), and certified reference materials (CRMs) for total alkalinity (TA) and pH standards obtained from the laboratory of Andrew Dickson (UCSD). In short, tanks were monitored for temperature, salinity, TA (potentiometric titrations, Dickson et al. 2007, SOP 3B), and pH [total scale, m-cresol dye method, Dickson et al. 2007 SOP 6B, with modifications to a 1-cm path length (Fangue et al. 2010)]. From these measurements, the carbonate chemistry parameters of pCO_2 (μatm), HCO_3^-, CO_3^{2-}, DIC (μmol kg^{-1} sw), and Ω_a (aragonite saturation state) were calculated with CO2SYS (Pierrot et al. 2006) using the dissociation constants for carbonic acid by Mehrbach et al. (1973) refit by Dickson and Millero (1987).

Physiological parameters

Following 9 days of exposure to the treatments, the presence or absence of larvae in each tank, in relation to initial larval sample size, was used to quantify percent survivorship. In addition, groups of larvae were sampled from replicate tanks of each treatment for response measurements. First, one group of 13 larvae was used to assess the dark-adapted yield, or photochemical efficiency of PSII (F_V/F_M), of the *Symbiodinium* within the coral larvae with a Diving-PAM fluorometer (Walz GmbH) as described in Putnam et al. (2008, 2010). Second, larval size was assessed as planar surface area measured from photographs of 10 larvae per tank (Putnam et al. 2010) using ImageJ software (NIH, http://rsb.info.nih.gov/nih-image/). Third, dark respiration was measured as oxygen consumption (nmol O_2 $larva^{-1}$ min^{-1}) using a fiber optic oxygen sensor (FOXY-R Ocean Optics) and Ocean Optics spectrophotometer (USB-2000, Ocean Optics) as described in Edmunds et al. (2011). Briefly, six larvae were placed in 2 ml seawater in sealed glass vials and held in the dark for ~2 h. Oxygen concentrations were measured in the treatment seawater immediately prior to sealing the vials and prior to any air contact after the dark incubations at each of the treatment temperatures and CO_2 conditions (ambient = 25.35 ± 0.06 °C, high = 29.17 ± 0.02 °C). Prior to use, the probe was calibrated to 0 and 100 % saturation at each treatment temperature. To avoid measurement artifacts from oxygen-dependent effects, all measurements were completed at >80 % O_2 saturation. All larval respiration rates were corrected by subtracting the oxygen change in treatment water vials containing no larvae under the same conditions.

Molecular assays

Samples were assayed with real-time quantitative PCR (QPCR) for mRNA expression of photosynthesis and stress

response genes, including photosystem I (*psI* subunit III), phosphoglycolate phosphatase (*pgpase*), ribulose-1,5-bisphosphate carboxylase/oxygenase (rusbisco, *rbcL*), ascorbate peroxidase (*apx1*), and heat shock protein 70 (*hsp70*, for both host and *Symbiodinium*). In addition, rubisco (RBCL) and HSP70 protein expression were assayed from the same samples using SDS-PAGE and western blotting. One group of 13 larvae from each tank was collected for molecular analysis, placed in 500 µl TRIzol™ (Invitrogen), and immediately frozen at −80 °C. Preliminary titration of DNA, RNA, and protein concentration as a function of the number of larvae revealed that extraction of groups of ≥10 larvae resulted in ~3–4 µg RNA and DNA, and ~140 µg of protein. These quantities were more than sufficient for the molecular analyses.

Nucleic acid and protein extractions

RNA, DNA, and protein were extracted from a group of 13 larvae from each of 15 of the 16 treatment tanks; due to a spill, there were insufficient larvae in one tank of the HTAC treatment to conduct these analyses. Larvae were pulverized with a micropestle in 500 µl TRIzol in a microcentrifuge tube, and when completely homogenized, an additional 500 µl TRIzol was added. RNAs were extracted as in Mayfield et al. (2009) except that after precipitation, RNA pellets were solubilized in Lysis Buffer A of the GeneMark® Plant RNA miniprep purification kit (Hopegen Biotechnology). RNAs were re-purified according to the manufacturer's instructions, including the 15-min on-column DNase digestion, and eluted in 30 µl DEPC-treated water. The quantity and quality of the RNA were assessed using a Nanodrop Spectrophotometer (Infinigen) and formaldehyde–agarose (0.8 % TBE) gels post-stained with ethidium bromide, respectively.

DNAs from the same samples were extracted as in Mayfield et al. (2010) with two modifications. First, the DNA pellets were dissolved in Buffer PCR-A of the Axyprep™ PCR clean-up kit after precipitation (Axygen Biosciences). Second, the DNAs were dried for an additional 5 min at 65 °C to evaporate residual ethanol, as recommended by the manufacturer. DNAs were eluted into 30 µl of the manufacturer's elution buffer, and quantity and quality were assessed using the Nanodrop spectrophotometer and native agarose gels (0.8 % TBE) post-stained with ethidium bromide, respectively. Proteins were extracted from the organic phase of the same samples as in Mayfield et al. (2011) and quantified with the 2-D Quant® kit (Amersham Biosciences) according to the manufacturer's instructions. RNA/DNA and protein/DNA ratios were calculated for each sample to estimate total gene and protein expression, respectively.

Reverse transcription and QPCR

RNAs (200 ng) were reverse transcribed with the High Capacity® cDNA synthesis kit (Applied Biosystems) supplemented with a 1× Solaris® RNA spike (Thermo-Scientific) following the respective manufacturer's protocols. Prior to QPCR, cDNAs were diluted threefold in DEPC-treated water. Triplicate PCRs were conducted for each sample and primer set, and a serial dilution of a random cDNA sample was run on each 96-well plate to estimate the PCR efficiency of each primer set [~98–102 % (data not shown)]. QPCR was carried out using 1× EZ-TIME® SYBR® Green I mastermix with ROX passive reference dye (Yeastern Biotech. Co., LTD) and 2 µl of cDNA in 20 µl reaction volumes. Target gene expression was conducted with the primer concentrations, annealing temperatures, and cycle numbers presented in Table 1, and each thermocycling profile consisted of an initial 10-min incubation at 95 °C followed by cycling at 95 °C for 15 s and then 60 s at the respective annealing temperature (Table 1). A melt curve was conducted after each run to ensure specificity of the respective primer sets for all genes.

QPCR standardization/normalization

In order to standardize all assays, equal amounts of DNA, RNA, and protein were loaded into PCRs, reverse transcription (RT) reactions, and SDS-PAGE gels, respectively. Therefore, the data from the DNA, RNA, and protein parameters are presented on a relative expression change basis, and so are not influenced by any larval size differences. To control for potential differences in RT efficiency between the samples, QPCR amplification of the exogenous Solaris spike was conducted using the kit primers (200 nM), but not probes (see details of SYBR® Green I mastermix above), according to the manufacturer's recommendations. A melt curve analysis ensured that the Solaris primers were specific to the spike amplicon. Target gene expression was first normalized to recovery of the Solaris RNA spike, thereby ensuring expression patterns were not influenced by differential RT efficiency.

A DNA-based normalization was used (sensu Mayfield et al. 2011) to standardize for potential differences in biological composition among samples (i.e., the proportion of host and endosymbiont material in each sample). Host and *Symbiodinium hsp70* genome copies were each amplified in triplicate using 20 ng DNA (10 ng μl^{-1}), 1× EZ-TIME SYBR Green mastermix, and 500 nM each primer in 20 µl reaction volumes (Table 1). Thermocycling was conducted at 95 °C for 15 min for one cycle, followed by 35 cycles of 95 °C for 15 s and 59 °C/62.5 °C for 60 s for the host and *Symbiodinium* ortholog, respectively. A melt curve analysis was conducted after every reaction.

Table 1 Primers and PCR conditions for normalization controls and response genes

Gene (length)	Compartment	NCBI accession #	Forward primer (5′–3′) or reference	Reverse primer (5′–3′) or reference	Primer (nM)	Annealing temp. (°C)	Cycle #
Solaris™ spike	Exogenous	NA	TGCAAAGCCAATTCCCGAAG	CCATTGTAGTGAACAGTAGGAC	125	63	35
hsp70 (62 bp)	Coral	AB201749	ATCCAGGCAGCGGTCTTGT	TCGAGCAGCAGGATATCACTGA	300	60	35
hsp70 (86 bp)	*Symbiodinium*	EU476880	CTGTCCATGGGCCTGGAGACT	GTGAACGTCTGTGCCTTGTTGGTT	500	62.5	33
apx1 (107 bp)	*Symbiodinium*	HM156698	GCCAAGTTCAAGGAGCATGTA	AGCTGACCACATCCCAACT	150	61	40
rbcL (126 bp)	*Symbiodinium*	AAG37859	CAGTGAACGTGGAGGACATGT	AGTAGCACGCCTCACCGAAA	200	60	30
psI/III (136 bp)	*Symbiodinium*	HM156699	GTGGAGTTGACATTGACTTGGA	TGCTGCTTGGTGGTCTTGTA	500	59	35
pgpase (100 bp)	*Symbiodinium*	EU924267	Crawley et al. (2010)	Crawley et al. (2010)	200	60	35

Previously published primer sets were included when PCR conditions were not previously specified, or when certain reaction and/or thermocycling parameters were altered

NA not applicable

The Ct values were used to calculate host and *Symbiodinium* genome copy proportions (GCPs, Mayfield et al. 2011), and host and *Symbiodinium* target gene expression were normalized to the host and *Symbiodinium* GCP, respectively, to control for biological composition differences between samples.

Protein expression

Rubisco (RBCL) protein expression was assessed via SDS-PAGE and western blotting. In addition to the experimental samples (~20 µg/assay), a 50 µg protein loading control extracted from 50 *P. damicornis* larvae was added to a lane on each SDS-PAGE gel to control for the variation between the two gels required to analyze all 15 samples. Proteins were electrophoresed through 4–12 % Tris–glycine SDS-PAGE gels and transferred onto PVDF membranes at 100 V for 75 min at 4 °C. The membranes were blocked with 5 % skim milk in Tris-buffered saline with 0.05 % Tween 20 (TBST) for 1 h at room temperature and then incubated with a 1:2,000 dilution of a polyclonal rubisco (RBCL) antibody (Abcam ab62391) at room temperature for 2 h. For HSP70 protein expression, both monoclonal (Stressgen Cat. #822, 1:2,000 dilution) and polyclonal (Stress Marq Cat. SPC-103, 1:2,000) HSP70 antibodies were used on the same protein samples. After three washes in TBST, the membrane was incubated in an anti-rabbit secondary antibody conjugated to horseradish peroxidase (1:10,000 dilution in TBST) for 1 h. They were again washed three times in TBST and then overlaid with 400 µl ECL (SuperSignal® West Pico Chemiluminescent Substrate, Thermo-Scientific). Membranes were visualized with chemiluminescence in a Fusion-SL (Vilber Lourmat) gel doc after exposure for 2–5 min. Individual sample band densities were assessed with NIH's ImageJ and normalized to the intensity of the loading control band. To control for differences in the biological composition of samples, *Symbiodinium* RBCL protein expression was also normalized to the *Symbiodinium* GCPs as described above.

Statistical analyses

A two-way ANOVA was used to examine the effects of temperature and pCO_2 (2 levels each, fixed factors) on the larval response for the three physiological response variables, nucleic acid and protein ratios, GCP, and gene and protein expression. Post hoc analyses of significant results were carried out using Tukey's HSD. To compare the correlation between host and *Symbiodinium hsp70* expression, as well as between *rbcL* gene and RBCL protein expression, we checked for separate slopes among the four treatments using ANCOVA, and when slopes were homogenous, regression analysis was conducted. Visual checks of heteroscedasticity and goodness of fit tests to a normal distribution were carried out to assess the appropriateness of the data for parametric analyses, and response variables were transformed where necessary to meet the assumptions prior to completing the statistical tests. When transformations were needed, the back-transformed means and SEs were presented in the figures. All statistical analyses were carried out using JMP 8.0 (SAS).

Results

Seawater chemistry and temperature treatments were stable throughout the experiments (Table 2). Larval survivorship was high (~80 %) over the 9 days of treatment exposure except for in one HTAC treatment tank where most larvae were accidentally lost by spilling; there were insufficient larvae to conduct physiological and molecular analyses in this tank. Survivorship showed a significant interaction between temperature and pCO_2, but there were no effects of the main factors (Fig. 2; Table 3).

Physiological parameters

Larval photophysiology (Fig. 2a) was unaffected by temperature, pCO_2, or their interaction, with F_V/F_M averaging 0.72 ± 0.01 (mean ± SE, Table 3). Larval respiration ranged from 0.035 to 0.129 nmol O_2 larva^{-1} min^{-1} and was significantly effected by temperature ($F_{(1,10)} = 8.092$, $p = 0.017$), with an average reduction of 32 % at 29 °C compared to 25 °C (Fig. 2b). On average, respiration was 13 % lower at high pCO_2; however, there was no significant effect of pCO_2 or the temperature–pCO_2 interaction on larval dark respiration (Table 3). Size was significantly higher at high pCO_2, with larvae being on an average of ~10 % larger in high versus ambient pCO_2 treatments. However, post hoc comparisons (Tukey's HSD) of CO_2 levels at each temperature did not detect any pair-wise significant differences. Larval size was not affected by temperature or the interaction between temperature and pCO_2 (Table 3).

Molecular assays

On average, a single *P. damicornis* larva yielded ~230 ng RNA, ~240 ng DNA, and ~15 µg protein, which corresponds to RNA/DNA and protein/DNA ratios of ~1 and 62.5, respectively. Neither RNA/DNA (Fig. 3a) nor protein/DNA (Fig. 3b) ratios varied in response to temperature, pCO_2 or their interaction (Table 3). Similarly, from the DNA phase exclusively, neither *Symbiodinium* (Fig. 3c) nor host (Fig. 3d) *hsp70* GCP varied in response to either of the treatment factors, or their interaction (Table 3), demonstrating that these conditions did not cause a significant change in the biological composition of these larvae among treatments with respect to the ratio of host and *Symbiodinium*. However, there were differences in the levels of GCP variance among treatments (Fig. 3), so both gene and protein expression data were normalized to the GCP of the respective target compartment to control for inter-sample differences in biological composition.

The expression of a series of genes from both the host coral and the endosymbiotic dinoflagellates was measured using QPCR. The expression of *Symbiodinium psI* (subunit III, Fig. 4a), *pgpase* (Fig. 4b), and *apx1* (Fig. 4c) did not vary significantly in response to temperature, pCO_2, or their interaction (Online Resource 1). It is of note, however, that had the expression of *Symbiodinium apx1*, as well as *hsp70* (described below), been normalized to only total RNA, as is commonly the case in studies of corals, a significant interaction effect would have been detected for these genes (Online Resource 2), driven by the variance in biological composition of the holobiont among samples.

The mRNA expression of both host coral (Fig. 5a) and *Symbiodinium* (Fig. 5b) *hsp70* was unaffected by temperature, pCO_2, or their interaction (Table 3). There was, however, a statistically significant, positive relationship in the expression of this homolog between the host and *Symbiodinium* ($r^2 = 0.327$, $F_{1,13} = 6.3279$, $p = 0.0258$, Fig. 5c). The expression of the *rbcL* gene (Fig. 6a) did not vary significantly in response to either treatment or their interaction; however, the protein it encodes, RBCL (Fig. 6b), responded significantly in larvae exposed to elevated temperature ($F_{(1,11)} = 16.92$, $p = 0.005$), showing a 2.6-fold decrease (see post hoc letter groups of Fig. 6b). There was no statistically significant relationship between *rbcL* gene and RBCL protein expression ($r^2 = 0.142$, $F_{1,13} = 2.1519$ $p = 0.1662$, Fig. 6c). Finally, HSP70 proteins failed to be detected in the majority of samples (12/15).

Table 2 Carbonate chemistry parameters (±SE) calculated from measurements of temperature, salinity, pH, and TA in the treatment tanks ($N = 24$, 24, 18, and 30 for 25 °C, 415 µatm [ATAC]; 25 °C, 635 µatm [ATHC]; 29 °C, 415 µatm [HTAC]; and 29 °C, 635 µatm [HTHC], respectively)

Treatment	Temp. (°C)	pCO_2 (µatm)	pH	TA (µmol kg^{-1})	HCO_3 (µmol kg^{-1})	CO_3 (µmol kg^{-1})	DIC (µmol kg^{-1})	Ω_{Arag}
ATAC	25.28 ± 0.09	420 ± 13	8.029 ± 0.011	2,223 ± 17	1,729 ± 13	200 ± 5	1,941 ± 14	3.20 ± 0.08
ATHC	25.47 ± 0.07	607 ± 15	7.900 ± 0.009	2,249 ± 8	1,856 ± 10	160 ± 3	2,033 ± 9	2.55 ± 0.04
HTAC	29.01 ± 0.15	413 ± 16	8.052 ± 0.015	2,350 ± 8	1,739 ± 18	249 ± 7	1,998 ± 12	4.02 ± 0.11
HTHC	29.14 ± 0.10	656 ± 18	7.878 ± 0.010	2,285 ± 14	1,851 ± 17	177 ± 3	2,044 ± 16	2.86 ± 0.05

Measurements were made on each of 6 days during the 9-day exposure. Unequal sample sizes result from one a priori HTAC tank that grouped with more closely to the high CO_2 treatment, and therefore was included in the HTHC treatment for chemistry measurements and all biological response variables

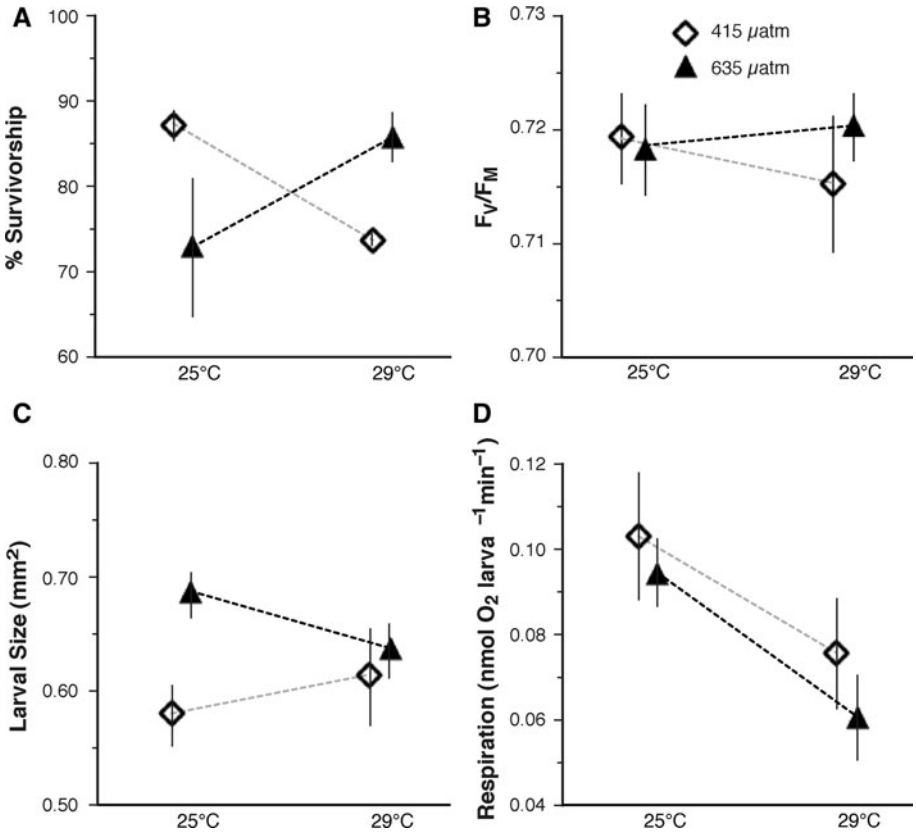

Fig. 2 Larval physiological response measured at the completion of the experiment in each of the four treatments. The ambient (415 µatm) and high (635 µatm) CO_2 treatments are denoted by *open* and *filled symbols*, respectively, and *error bars* represent standard error (SE). Data points on the x-axis were offset for clarity. Larval survivorship (**a**) was assessed as described above. Dark-adapted yield of PSII (**b**) was calculated from groups of 13 larvae from each tank ($N = 4, 4, 3$, and 5 tanks in treatments ATAC, ATHC, HTAC, and HTHC, respectively). Larval size (**c**) was determined by image analysis of planar surface area in 10 larvae per replicate tank, except for one tank in HTHC, where only 9 were measured ($N = 3, 4, 3$, and 5 tanks in treatments ATAC, ATHC, HTAC, and HTHC, respectively). Dark respiration rates (**d**) were measured by oxygen flux from groups of 6 larvae from each tank ($N = 3, 4, 3$, and 4 tanks in treatments ATAC, ATHC, HTAC, and HTHC, respectively)

Discussion

A continuous supply of propagules from corals whose larvae can survive the physical bottleneck created by climate change is critical to the maintenance and persistence of coral reefs. This study investigated the response of brooded larvae from the common coral *P. damicornis* exposed to elevated temperature and OA. Examination of the larval response was documented across biological scales ranging from organism physiology to gene and protein expression (Fig. 1). The results suggest that coral larvae are more strongly influenced metabolically and biochemically by elevated temperature than CO_2 (Table 4). This is evidenced in particular by respiration and the expression of a protein critical to photosynthesis and carbon fixation, rubisco (RBCL), which were both significantly depressed in larvae exposed to high temperature. In contrast, overall larval size responded more strongly to high pCO_2. While expression was equal across both treatments for a suite of stress response genes, *hsp70* gene expression was tightly correlated between the host and *Symbiodinium* compartments, identifying a concerted response. Conversely, there was no connection between *rbcL* gene and RBCL protein expression. Collectively, these findings lay the groundwork for identifying the cellular and physiological mechanisms of acclimation to future temperature and OA conditions in a critical life history stage of this broadly distributed coral species.

Physiological response

Our assays were chosen to target a suite of responses, including genes in the photosynthetic pathway, cellular stress response, and whole-organism metabolism and fitness (Fig. 1). Chronic exposure to elevated temperature has been repeatedly shown to result in declines in coral biomass (e.g., Szmant and Gassman 1990; Grottoli et al. 2006; Ainsworth et al. 2008) and changes in metabolic demands (Coles and Jokiel 1977; Lesser 1997). Prior work has found that the cascade of response typically involved with thermal stress is initiated within the photosynthetic and photoprotective

Table 3 Results of two-way ANOVAs for physiological measures and biological composition parameters

Source of variation	*df*	MS	*F*	*p*
% Survivorship				
Temperature	1	2.58×10^{-4}	0.020	0.889
pCO_2	1	1.40×10^{-4}	0.011	0.918
Temperature × pCO_2	1	0.096	7.498	**0.019**
Error	11	0.0127		
F_V/F_M				
Temperature	1	5.300×10^{-7}	0.004	0.925
pCO_2	1	1.139×10^{-5}	0.244	0.662
Temperature × pCO_2	1	3.862×10^{-5}	0.686	0.425
Error	10	5.600×10^{-5}		
Respiration (nmol O_2 $larvae^{-1}$ min^{-1})				
Temperature	1	3.367×10^{-3}	8.092	**0.017**
pCO_2	1	4.810×10^{-4}	1.156	0.308
Temperature × pCO_2	1	2.978×10^{-5}	0.072	0.795
Error	10	4.161×10^{-2}		
Size (mm^2)				
Temperature	1	0.002	0.064	0.801
pCO_2	1	0.149	5.173	**0.024**
Temperature × pCO_2	1	0.062	2.169	0.143
Error	145	0.029		
RNA/DNA				
Temperature	1	0.329	1.565	0.237
pCO_2	1	0.218	1.037	0.330
Temperature × pCO_2	1	0.113	0.537	0.479
Error	11	0.211	1.127	
Protein/DNA				
Temperature	1	421.6	0.334	0.575
pCO_2	1	416.8	0.331	0.577
Temperature × pCO_2	1	764.7	0.607	0.453
Error	11	1260.8	0.381	
Symbiodinium genome copy proportion				
Temperature	1	0.0421	2.873	0.118
pCO_2	1	0.0004	0.024	0.879
Temperature × pCO_2	1	0.0020	0.136	0.720
Error	11	0.0146	0.960	
P. damicornis (host) genome copy proportion				
Temperature	1	0.0420	2.888	0.117
pCO_2	1	0.0003	0.024	0.880
Temperature × pCO_2	1	0.0020	0.135	0.721
Error	11	0.0145	0.965	

Significant differences are highlighted in bold font

pathways of the *Symbiodinium* (reviewed by Venn et al. 2008; Lesser 2011) and that this cascade may be exacerbated by OA (Anthony et al. 2008). This stress response cascade includes photoinhibition of PSII, followed by generation of reactive oxygen species (ROS) and oxidative stress within the *Symbiodinium* and host. High ROS concentrations cause cellular damage and can result in endosymbiotic breakdown (coral bleaching) and mortality (reviewed by Venn et al. 2008). Neither photoinhibition nor more generally a host or *Symbiodinium* cellular-level stress response was evident in coral larvae exposed to elevated temperature or pCO_2. We did, however, document metabolic suppression via a significant reduction in respiration upon exposure to high temperature, a phenomenon that is potentially linked to a significant reduction in RBCL protein expression in these same specimens.

Larval metabolism in our study was similar to previously documented rates for *P. damicornis* (0.07–0.18 nmol $larva^{-1}$ min^{-1}, reviewed by Edmunds et al. 2011). Q_{10} expectations ($Q_{10} = 2$ within a natural temperature range, Hochachka and Somero 2002) for respiration rates at 29 °C in comparison with ambient (25 °C) predict an increase of ~20 %; however, respiration at 29 °C was reduced by 32 %. The pattern of decreased larval respiration at high temperature in our study is in agreement with the parabolic response of metabolism as a function of temperature in larval corals (Edmunds et al. 2011). The reduction at high temperature is likely the result of crossing the optimal temperature threshold and can be coincident with the onset of thermal damage to proteins and normal biochemical processes (Hochachka and Somero 2002).

Increased CO_2 has previously been documented to depress metabolism due to the pH sensitivity of enzymatic reactions such as protein conformation and enzyme–substrate interactions (Hochachka and Somero 2002). The majority of the literature documents metabolic suppression (Byrne 2011a); however, the opposite has also been documented in larval sea urchins, where high CO_2 results in metabolic stimulation (Stumpp et al. 2011b). Within the coral literature, the predominant signal documented to date is metabolic suppression as a function of increasing CO_2 concentration. This pattern is observed in *Porites astreoides* larvae exposed to increased CO_2 for several hours (Albright and Langdon 2011). Additionally, *Acropora digitifera* larvae exposed to low pH for several days (Nakamura et al. 2011b) exhibited slightly, though not significantly, lower respiration rates than controls. Exposure of *P. damicornis* larvae to high pCO_2 treatments in our study resulted in a similar response, where larvae had on average 13 % lower respiration rates at elevated pCO_2, although these results were also not significant. The trend for depressed metabolism seen on short time scales (24–48 h, Albright and Langdon 2011) does not appear to manifest as strongly over more extended exposures (3 and 7 days in Nakamura et al. 2011b; 9 days in this study) and may be a result of compensatory acclimation to the high CO_2 conditions. As such, differences in coral larval response to OA among studies may be driven in part by the duration of the stress and the degree to which acclimatory processes have been initiated (Pörtner 2008).

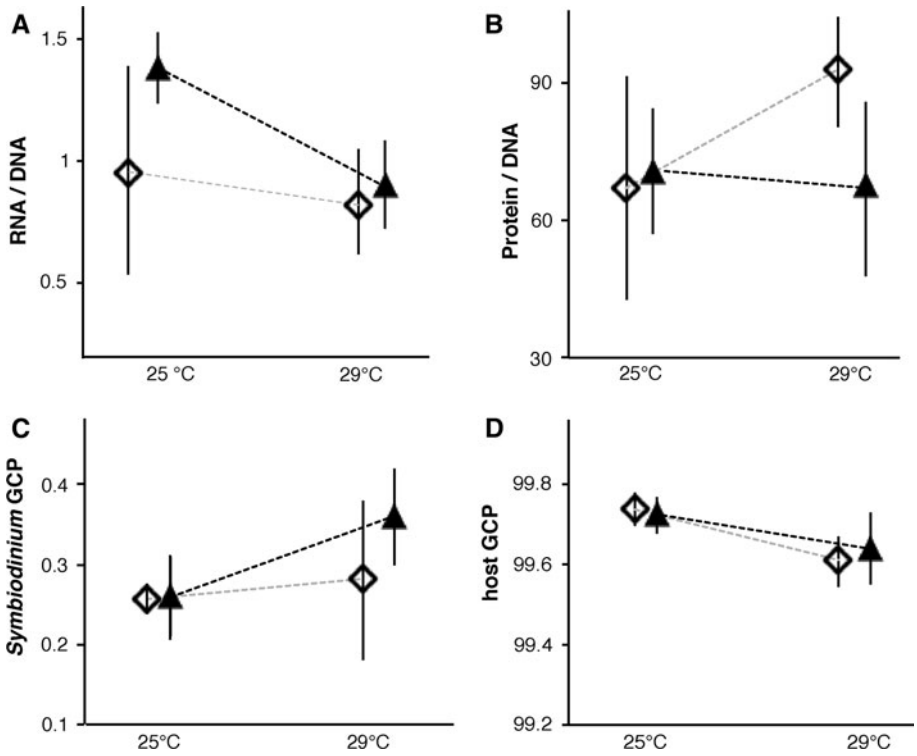

Fig. 3 Larval biological composition measured at the completion of the experiment in each of the four treatments. The ambient and high CO_2 treatments are denoted by *open* and *filled symbols*, respectively, and *error bars* represent standard error (SE). Data points on the *x* axis were offset for clarity. RNA/DNA (**a**) and protein/DNA (**b**) ratios (unitless) were calculated for each sample, and the DNA phase was used as the template in QPCR to calculate both the *Symbiodinium hsp70* genome copy proportion (GCP, **c**) and the host *hsp70* GCP (**d**). The *y* axes of the panels **c**, **d** represent the percentages calculated from the respective GCPs. For molecular analyses, $N = 3$, 4, 3, and 5 tanks for ATAC, ATHC, HTAC, and HTHC, respectively

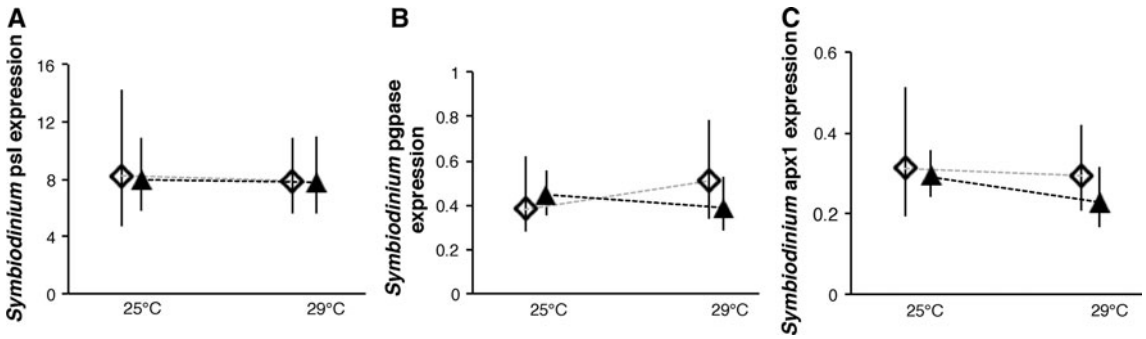

Fig. 4 *Symbiodinium* gene expression in larvae sampled at the completion of the experiment in each of the four treatments. The ambient and high CO_2 treatments are denoted by *open* and *filled symbols*, respectively, and *error bars* represent standard error (SE). Data points on the x-axis were offset for clarity. mRNA expression of photosystem I (*psI* subunit III, **a**), phosphoglycolate phosphatase (*pgpase*, **b**), and ascorbate peroxidase (*apx1*, **c**) was measured with QPCR. For molecular analyses, $N = 3$, 4, 3, and 5 tanks for ATAC, ATHC, HTAC, and HTHC, respectively

The tendency of marine calcifiers to display decreased larval size with increasing CO_2 is prevalent in the literature (reviewed by Byrne 2011a); however, the results in our study counter this trend. One potential explanation for the discrepancy is the calcification status of the coral larvae upon measurement. The majority of experiments conducted on marine calcifiers focus on organisms calcifying from early in development (Byrne 2011a, b) and in some instances, those depositing a significantly more soluble form of calcium carbonate (Kurihara 2008). In contrast, the coral larvae in our experiment had not settled and were not secreting skeletons. This suggests that under high pCO_2, *P. damicornis* larval size may be less constrained by the energetic expense of modifying the composition and pH of intercellular space and the production of proteins involved in calcification, as compared to more immediate calcifiers such as clams, scallops, and oysters (Talmage and Gobler 2010, 2011), and more advanced stages of juvenile corals (Albright and Langdon 2011). In addition, prior to settlement, larval morphology of corals can display plasticity, and therefore does not necessarily exhibit a linear link to tissue biomass. These results highlight the importance of tracking organism size throughout settlement and early juvenile stages to identify shifts in ontogenetic sensitivity of coral size, biomass, and growth to high pCO_2.

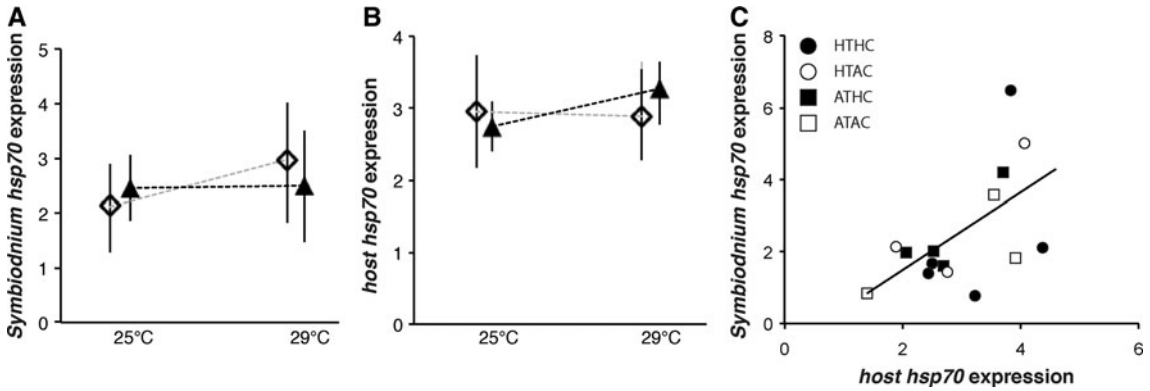

Fig. 5 Host coral and *Symbiodinium* heat shock protein 70 (*hsp70*) gene expression in larvae sampled at the completion of the experiment in each of the four treatments. In panels **a** and **b**, the ambient and high CO_2 treatments are denoted by *open* and *filled symbols*, respectively, and *error bars* represent standard error (SE). Data points were offset for clarity on the x-axis. mRNA expression of both the *Symbiodinium* (**a**) and host (**b**) orthologs of the gene encoding the HSP70 protein was measured with QPCR (see text for normalization details). *Symbiodinium hsp70* gene expression was plotted as a function of host *hsp70* gene expression (**c**) in each of the four treatments, with the ATAC, ATHC, HTAC, and HTHC treatments denoted by *open squares*, *filled squares*, *open circles,* and *filled circles*, respectively

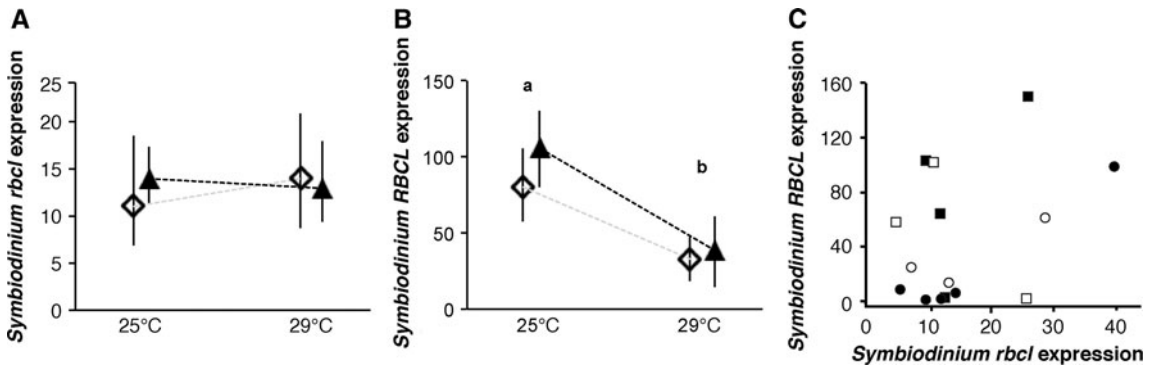

Fig. 6 Ribulose-1,5-bisphosphate carboxylase/oxygenase gene (*rbcL*) and protein (RBCL) expression in *Symbiodinium* within larvae sampled at the completion of the experiment in each of the four treatments. In panels **a**, **b**, the ambient and high CO_2 treatments are denoted by *open* and *filled symbols*, respectively, and *error bars* represent standard error (SE). mRNA (**a**) expression was normalized to both recovery of the Solaris™ RNA spike and the *Symbiodinium* genome copy proportion (GCP, Fig. 3), while protein (**b**) expression was normalized only to the *Symbiodinium* GCP. Results of Tukey's HSD post hoc temperature comparisons letter groups are also displayed in panel **b**, where temperatures with different letters document a significant difference. *Symbiodinium* RBCL expression was plotted as a function of *Symbiodinium rbcL* expression (**c**) across each of the four treatments, with the ATAC, ATHC, HTAC, and HTHC treatments denoted as in Fig. 5. The abscissa was shifted below zero in its intersection with the ordinate to enable the visualization of samples with low RBCL protein expression

Linking physiology to cellular processes

Phenotypic outcomes such as metabolism can be mechanistically examined at lower levels of biological organization, with rates of protein expression and turnover directly responsible for overall organism response (Hochachka and Somero 2002). Consequently, RBCL protein expression was examined because it is important in carbon fixation, a pathway previously shown to be sensitive to environmental stress (Leggat et al. 2004). We observed a 2.6-fold decrease in RBCL expression in larvae at 29 °C, and this is most likely linked to the reduced respiration documented in the same samples. Rubisco is the rate-limiting enzyme of the Calvin cycle (Falkowski and Raven 2007). While it is possible that a reduction in RBCL protein expression could ultimately lead to a reduction in respiration due to a diminished translocation of organic carbon from *Symbiodinium* to the coral host, neither the maximum dark-adapted quantum yield (F_V/F_M) nor the expression of *apx1* appears to suggest photoinhibition or photodamage, which should appear following damage to the dark reactions. Another possibility is that the decrease in respiration could have led to a decrease in intracellular pCO_2 in the holobiont (Leggat et al. 1999), which could suppress the expression of

Table 4 Summary of physiological and molecular response variables measured in *P. damicornis* larvae exposed to elevated temperature and ocean acidification conditions

Response variables	Elevated temperature	Ocean acidification
Survivorship	ns	ns
F_V/F_M	ns	ns
Dark respiration	Negative	ns
Larval size	ns	Positive
GCP	ns	ns
psI	ns	ns
apx1	ns	ns
pgpase	ns	ns
rbcL	ns	ns
Host *hsp70*	ns	ns
Symbiodinium hsp70	ns	ns
RBCL protein	Negative	ns

"Negative" denotes a decrease in response, and "Positive" denotes an increased in response in comparison with the control treatment

ns not significant

RBCL. This is feasible given that the dinoflagellate form II Rubisco is very sensitive to the pCO_2:O_2 ratio (Leggat et al. 1999) and metabolic depletion of intercellular CO_2 and diffusion limited external CO_2 acquisition has been documented in corals (Muscatine et al. 1989).

While RBCL protein expression was significantly reduced in *Symbiodinium* from larvae exposed to elevated temperature, expression of the respective gene, *rbcL*, was similar among the four treatments. A lack of correlation between rubisco gene and protein expression has also been documented in 16 plant species (Moore et al. 1998) and strongly suggests that regulation of rubisco takes place through post-transcriptional modifications (Parry et al. 2008). As such, the RBCL protein appears to be a superior candidate as a biomarker for thermal stress in *Symbiodinium* than the *rbcL* gene.

Expression patterns

Our results revealed no significant induction of genes from either the photosynthetic (*psI* subunit III, *pgpase, apx1*, and *rbcL*) or stress response (host and *Symbiodinium hsp70*) categories. In contrast, Crawley et al. (2010) document a decrease in *pgpase* expression ($\sim$45–50 %) under high pCO_2 conditions. A decline in *pgpase* expression could mean loss of conversion of phosphoglycolate and thus a decrease in the efficiency in carbon fixation, or a decline in the need for conversion of phosphoglycolate due to elevated affinity for rubisco carboxylation at high pCO_2 concentrations (Drake et al. 1997). While we found no difference in *pgpase* expression, it is difficult to make a direct comparison between the two studies, as Crawley et al. (2010) normalized gene expression to total RNA, while here we normalized to control for RT efficiency and the biological composition differences between samples.

Leggat et al. (2011) also show little change in *Symbiodinium* gene expression under thermal stress, with expression levels of *Symbiodinium* only $\sim$20 % of those of the host. However, these authors saw changes in adult *Acropora aspera hsp70* expression in response to natural environmental variation, as well as during a simulated thermal bleaching event (Leggat et al. 2011). Expression of *hsp70* in *Acropora millepora* was also shown to vary with the diel cycle, peaking at 16:00, in concert with a cluster of molecular chaperones (Levy et al. 2011). Unlike the response to temperature stress, adult *A. digitifera* exposed to OA conditions did not display differential *hsp70* expression in comparison with the controls (Nakamura et al. 2011a). While this stability of *hsp70* expression documented in *Acropora* exposed to increased CO_2 matches that seen in our experiment (Fig. 5), the lack of induction in response to elevated temperature in our study is in contrast with both Leggat et al. (2011) and Levy et al. (2011).

This lack of differential *hsp70* expression under elevated temperatures and OA could occur for several reasons. First, acclimation of the coral larvae could have occurred over the course of our 9-day experiment, which is a longer exposure period than the previous work. While larvae are likely outside their optimum temperatures and may be suffering thermal damage to proteins and biochemical processes, the continuous exposure to high temperature may have resulted in acclimation of inducible *hsp70* gene expression. This pattern of acclimation is consistent with a shift in induction threshold documented due to seasonal and laboratory variation in thermal history of various marine invertebrates (reviewed by Hofmann et al. 2002). Secondly, it is possible that the two *hsp70*s targeted in this study only encode constitutively expressed, and not inducible, HSP70 proteins (Mayfield et al. 2011), and as such, would not be predicted to be differentially expressed under stress. While alignment of the *Symbiodinium hsp70* gene indicates homology with both inducible and constitutive paralogs (Mayfield et al. 2011), the host *hsp70* sequence from which primers were designed was previously specified to encode an inducible HSP70 (Hashimoto et al. 2004), making the constitutive paralog hypothesis less likely. Lastly, due to logistical constraints, our study has the limitation of low sample size and high variability in molecular response, and it is possible a significant effect might be detected with greater sampling effort. Given the strong response to temperature seen in respiration and protein expression, however, it is not very likely that significant changes in gene expression escaped detection.

Despite the fact that there was no difference in *hsp70* expression in either host or *Symbiodinium* following exposure to treatment, our results reveal a positive relationship between the expression of *hsp70* in host and

Symbiodinium compartments (Fig. 5). This correlation, which has also been observed in adult *Seriatopora hystrix* and their *Symbiodinium* exposed to acute thermal stress (Mayfield et al. 2011), may be due to the conserved nature of HSP expression under stress in a wide range of taxa (Feder and Hofmann 1999), where increased expression occurs in both partners in more sensitive holobiont samples. While it is unclear here if there is a directed or synchronized response of gene expression between partners (e.g., inter-partner signaling, Ganot et al. 2011), they appear to be presenting concerted positive expression. However, to date, few studies have simultaneously assessed the same gene(s) in multiple symbiotic partners, and a more thorough meta-transcriptomics endeavor is warranted to further identify relationships between host and *Symbiodinium* gene expression. Given the lack of treatment effect, it does not appear that the *hsp70* gene targeted here is a useful biomarker for detecting environmental stress in *P. damicornis* larvae. We were unable to measure the HSP protein, but if, like RBCL, it is regulated via post-transcriptional modifications (Parry et al. 2008), this molecule may still prove to be a suitable candidate biomarker for environmental stress detection in corals.

Standardization in molecular assays

The endosymbiotic nature of corals and their documented sensitivity to environmental changes results in rapid changes in the host/*Symbiodinium* biomass ratio (i.e., bleaching; Coles and Brown 2003). As such, there is a compelling rationale for utilizing a normalization method that controls for the biological composition of larval corals in parallel with controls for other methodological discrepancies that can occur during the QPCR process. It is noteworthy that had *Symbiodinium hsp70* and *apx1* been only normalized to total RNA, a significant interaction effect driven by the slight differences in biomass ratios would have been reported (Online Resource 2). The gene expression normalization strategy used here ensures that expression of, for instance, a *Symbiodinium* gene in a bleached coral with very few *Symbiodinium* and a large amount of host material can be compared to *Symbiodinium* from a healthy coral with high densities of *Symbiodinium* and hence a lower proportion of nucleic acids attributable to the host. This study represents the first on endosymbiotic coral larvae that incorporates molecular methods designed and optimized for measuring and controlling for differences in both biological composition and methodology-induced differences across samples.

Future research needs

Currently, a large portion of our knowledge of the effects of ocean acidification on corals comes from relatively short exposure times (reviewed by Edmunds et al. 2012) and single point sampling. While the goal of this study was to address pre-settlement response and as such, represents a snapshot of response, future work will benefit from increased sampling frequency across exposure time (Stumpp et al. 2011a) to detect changes occurring due to developmental state (Stumpp et al. 2011b). In addition to increased sampling frequency, including a multi-stressor approach with ecological overlays will be critical to extrapolating results of laboratory studies to natural settings. As such, there is a pressing need to understand the effects of OA in conjunction with factors such as increased temperature, nutrients concentration, heavy metal toxicity, UV irradiance, as well as organism phenotypic plasticity due to genetics and epigenetics.

To date, the potential for adaption and acclimatization to global climate change and OA has been considered low. Recent work, however, examining the potential for evolutionary adaptation to OA using breeding studies in sea urchins and mussels has revealed species-specific differences in evolutionary response (Sunday et al. 2011), and highlights the need for understanding the potential for differences in genetic variation to modulate future population demographics. As corals are comprised of a large number of species and employ varying life history strategies, assessing evolutionary adaptation through quantitative genetic experiments is essential. Another promising area of research includes the examination of acclimatization to OA (Melzner et al. 2009) and increased temperature (Brown et al. 2002). Previous work in non-brooding systems suggests positive carry-over effects in oysters (Parker et al. 2012), which could have substantial implications for future population response. The brooding coral *P. damicornis* used here provides an excellent model to approach the topic of parental effects, and we are currently examining the potential for modulation of larval sensitivity through exposure of the adult *P. damicornis* colonies during their brooding cycle, and analysis of their subsequently released larvae (Putnam et al. unpublished data).

Conclusions

Studies at a single biological scale can be extremely informative with regard to pathways and mechanisms of response. However, it is often difficult to link ecological implications to cellular-level changes. Here, we have documented metabolic suppression in larvae exposed to high temperature, an outcome possibly linked to a reduction in RBCL protein expression. Together, the reduction in metabolic rate and decrease in a key enzyme for photosynthate production signals the potential for a larval energetic debt under continued exposure to stress, which

has significant ecological ramifications for settlement and survivorship. This work highlights the need to integrate our understanding across biological scales within experiments (Pörtner et al. 2006), as not all levels of biological organization respond in concert. While the detrimental effects of OA can be clearly identified in adult stages and result in constriction of thermotolerance (e.g., Anthony et al. 2008; Pörtner and Farrell 2008), it appears that temperature stress may be a more important consideration for the near-future response of the brooded pocilloporid coral larval life stage.

Acknowledgments We are grateful to the staff and students of the NMMBA and technical assistance from Okay Chan, Yao-Hung Chen, Yi-Yuong Hsiao, Peter Edmunds, Vivian Cumbo, and Aaron Dufault. We thank Gretchen Hofmann and her program for seawater chemistry protocols (work supported by the United States National Science Foundation [NSF] awards OCE-1040960 and ANT-0944201 to GEH). We would also like to thank three anonymous reviewers for their comments, which have greatly improved the manuscript. This study was supported by grants from NSF (BIO-OCE 08-44785 to PJE and OCE-0752604 to RDG), and funding from the International Society for Reef Studies, the Ocean Conservancy, and the American Fisheries Society to HMP. ABM was funded by an NSF international postdoctoral research fellowship (OISE-0852960). In addition, this research was developed under STAR Fellowship Assistance Agreement no. FP917199 awarded by the U.S. Environmental Protection Agency (EPA). This manuscript has not been formally reviewed by the EPA, and the views expressed are solely those of the authors. The EPA does not endorse any products or commercial services mentioned in this manuscript. This manuscript is HIMB contribution number 1531 and SOEST contribution number 8795.

References

Ainsworth TD, Hoegh-Guldberg O, Heron SF, Skirving WJ, Leggat W (2008) Early cellular changes are indicators of pre-bleaching thermal stress in the coral host. J Exp Mar Biol Ecol 364:63–71. doi:10.1016/j.jembe.2008.06.032

Albright R (2011) Reviewing the effects of ocean acidification on sexual reproduction and early life history stages of reef-building corals. J Mar Biol Article ID 473615, pp 14. doi:10.1155/2011/473615

Albright R, Langdon C (2011) Ocean acidification impacts multiple early life history processes of the Caribbean coral *Porites astreoides*. Glob Change Biol 17:2478–2487. doi:10.1111/j.1365-2486.2011.02404.x

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 USA 107:20400–20404. doi:10.1073/pnas.1007273107

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. doi:10.1016/j.jembe.2010.11.009

Anthony KRN, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O (2008) Ocean acidification causes bleaching and productivity loss in coral reef builders. Proc Natl Acad Sci USA 105:17442–17446. doi:10.1073/pnas.0804478105

Aranda M, Banaszak AT, Bayer T, Luyten JR, Medina M, Voolstra CR (2011) Differential sensitivity of coral larvae to natural levels of ultraviolet radiation during the onset of larval competence. Mol Ecol 20:2955–2972. doi:10.1111/j.1365-294X.2011.05153.x

Bassim KM, Sammarco PW (2003) Effects of temperature and ammonium on larval development and survivorship in a scleractinian coral (*Diploria strigosa*). Mar Biol 142:241–252. doi:10.1007/s00227-002-0953-z

Brown BE, Dunne RP, Goodson S, Douglas AE (2002) Experience shapes the susceptibility of a reef coral to bleaching. Coral Reefs 12:119–126

Byrne M (2011a) Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. Oceanogr Mar Biol Ann Rev 49:1–42

Byrne M (2011b) Global change ecotoxicology: identification of early life history bottlenecks in marine invertebrates, variable species responses and variable experimental approaches. Mar Environ Res 76:3–15. doi:10.1016/j.marenvres.2011.10.004

Chauvin A, Denis V, Cuet P (2011) Is the response of coral calcification to seawater acidification related to nutrient loading? Coral Reefs 30:911–923. doi:10.1007/s00338-011-0786-7

Coles SL, Brown BE (2003) Coral bleaching-capacity for acclimatization and adaptation. Adv Mar Biol 46:183–223

Coles SL, Jokiel PL (1977) Effects of temperature on photosynthesis and respiration in hermatypic corals. Mar Biol 43:209–216

Crawley A, Kline D, Dunn S, Anthony K, Dove S (2010) The effect of ocean acidification on symbiont photorespiration and productivity in *Acropora formosa*. Glob Change Biol 16:851–863. doi:10.1111/j.1365-2486.2009.01943.x

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

Dickson A, Sabine C, Christian J (2007) Guide to best practices for ocean CO_2 measurement. In: Dickson A, Sabine C, Christian J (eds) PICES Special Publication 3

Doney SC, Balch WM, Fabry VJ, Feely RA (2009a) Ocean acidification: a critical emerging problem for the ocean sciences. Oceanography 22:16–25

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009b) Ocean acidification: the other CO_2 problem. Ann Rev Mar Sci 1:169–192

Donner SD (2009) Coping with commitment: projected thermal stress on coral reefs under different future scenarios. PLoS One 4(6):e5712. doi:10.1371/journal.pone.0005712

Downs CA, Mueller E, Phillips S, Fauth JE, Woodley CM (2000) A molecular biomarker system for assessing the health of coral (*Montastraea faveolata*) during heat stress. Mar Biotechnol 2:533–544. doi:10.1007/s101260000038

Drake BG, Gonzalez-Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO_2? Annu Rev Plant Physiol Plant Mol Biol 48:609–639. doi:10.1146/annurev.arplant.48.1.609

Dupont S, Dorey N, Thorndyke M (2010a) What meta-analysis can tell us about vulnerability of marine biodiversity to ocean acidification? Estuar Coast Shelf Sci 89:182–185. doi:10.1016/j.ecss.2010.06.013

Dupont S, Lundve B, Thorndyke M (2010b) Near future ocean acidification increases growth rate of the lecithotrophic larvae and juveniles of the sea star *Crossaster papposus*. J Exp Zool (Mol Dev Evol) 314B:382–389

Dupont S, Moya A, Bailly X (2012) Stable photosymbiotic relationship under CO_2-induced acidification in the acoel worm *Symsagittifera roscoffensis*. PLoS One 7(1):e29568. doi:10.1371/journal.pone.0029568

Edmunds PJ (2011) Zooplanktivory ameliorates the effects of ocean acidification on the reef coral *Porites* spp. Limnol Oceanogr 56:2402–2410. doi:10.4319/lo.2011.56.6.2402

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. doi:10.1007/s002270100634

Edmunds PJ, Cumbo V, Fan TY (2011) Effects of temperature on the respiration of brooded larvae from tropical reef corals. J Exp Biol 214:2783–2790. doi:10.1242/jeb.055343

Edmunds PJ, Brown D, Moriarty V (2012) Interactive effects of ocean acidification and temperature on two scleractinian corals from Moorea, French Polynesia. Glob Change Biol 18:2173–2183. doi:10.1111/j.1365-2486.2012.02695.x

Erez J, Reynaud S, Silverman J, Schneider K, Allemand D (2011) Coral calcification under ocean acidification and global change. In: Dubinsky Z, Stambler N (eds) Coral reefs: an ecosystem in transition. Springer, Berlin, pp 151–176

Fabricius KE, Langdon C, Uthicke S, Humphrey C, Noonan S, De'ath G, Okazaki R, Muehllehner N, Glas M, Lough JM (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. Nat Clim Change 1:165–169. doi:10.1038/nclimate1122

Fabry VJ, Seibel BA, Feely RA, Orr JC (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. ICES J Mar Sci 65:414–432. doi:10.1093/icesjms/fsn048

Falkowski PG, Raven JA (2007) Aquatic photosynthesis, 2nd edn. Princeton University Press, Princeton pp 484

Fan TY, Li JJ, Ie SX, Fang LS (2002) Lunar periodicity of larval release by pocilloporid corals in southern Taiwan. Zool Stud 41(3):288–294

Fangue NA, O'Donnell MJ, Sewell MA, Matson PG, MacPherson AC, Hofmann GE (2010) A laboratory-based, experimental system for the study of ocean acidification effects on marine invertebrate larvae. Limnol Oceanogr Methods 8:441–452. doi:10:4319/lom.2010.8.441

Feder ME, Hofmann GE (1999) Heat shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu Rev Physiol 61:243–282

Fitt WK, Brown BE, Warner ME, Dunne RP (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral Reefs 20:51–65. doi:10.1007/s003380100146

Ganot P, Moya A, Magnone V, Allemand D, Furla P, Sabourault C (2011) Adaptations to endosymbiosis in a cnidarian-dinoflagellate association: differential gene expression and specific gene duplications. PLoS Genet 7(7):e1002187. doi:10.1371/journal.pgen.1002187

Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. Nature 440:1186–1189. doi:10.1038/nature04565

Hashimoto K, Shibuno T, Murayama-Kayano E, Tanaka H, Kayano T (2004) Isolation and characterization of stress-responsive genes from the scleractinian coral *Pocillopora damicornis*. Coral Reefs 23:485–491. doi:10.1007/s00338-004-0410-1

Hochachka PW, Somero GN (2002) Biochemical adaptation. Oxford University Press, Oxford pp 466

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742. doi:10.1126/science.1152509

Hofmann GE, Todgham AE (2010) Living in the now: physiological mechanisms to tolerate a rapidly changing environment. Annu Rev Physiol 72:127–145. doi:10.1146/annurev-physiol-021909-135900

Hofmann GE, Buckley BA, Place SP, Zippay ML (2002) Molecular chaperones in ecothermic marine animals: biochemical function and gene expression. Integr Comp Biol 42:808–814

Hofmann GE, Barry JP, Edmunds PJ, Gates RD, Hutchins DA, Klinger T, Sewell MA (2010) The effect of ocean acidification on calcifying marine organisms in marine ecosystems: an organism to ecosystem perspective. Annu Rev Ecol Evol Syst 41:127–147. doi:10.1146/annurev.ecolsys.110308.120227

Isomura N, Nishihira M (2001) Size variation of planulae and its effect on the lifetime of planulae in three pocilloporid corals. Coral Reefs 20:309–315. doi:10.1007/s003380100180

Jones RJ, Ward S, Amri AY, Hoegh-Guldberg O (2000) Changes in quantum efficiency of photosystem II of symbiotic dinoflagellates of corals after heat stress and of bleached corals sampled after the 1998 Great Barrier Reef bleaching event. Mar Fresh Res 51:63–71. doi:10.1071/MF99100

Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol Lett 13:1419–1434. doi:10.1111/j.1461-0248.2010.01518.x

Kurihara H (2008) Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. Mar Ecol Prog Ser 373:275–284. doi:10.3354/meps07802

Langdon C, Atkinson MJ (2005) Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. J Geophys Res 110:C09S07. doi:10.1029/2004JC002576

Leggat W, Badger MR, Yellowlees D (1999) Evidence for an inorganic carbon-concentrating mechanism n the symbiotic dinoflagellate *Symbiodinium* sp. Plant Physiol 121:1247–1255

Leggat W, Whitney S, Yellowlees D (2004) Is coral bleaching due to the instability of the zooxanthellae dark reactions? Symbiosis 37:137–153

Leggat W, Seneca F, Wasmund K, Ukani L, Yellowlees D, Ainsworth TD (2011) Differential responses of the coral host and their algal symbiont to thermal stress. PLoS One 6(10):e26687. doi:10.1371/journal.pone.0026687

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

Lesser MP (2011) Coral bleaching: causes and mechanisms. In: Dubinsky Z, Stambler N (eds) Coral reefs: an ecosystem in transition. Springer, Berlin, pp 405–420

Levy O, Kaniewska P, Alon S, Karako-Lampert S, Bay LK, Reef R, Rodriguez-Lanetty M, Miller DJ, Hoegh-Guldberg O (2011) Complex diel cycles of gene expression in coral-algal symbiosis. Science 331:175. doi:10.1126/science.1196419

Mayfield AB, Hirst MB, Gates RD (2009) Gene expression normalization in a dual-compartment system: a real-time quantitative polymerase chain reaction protocol for symbiotic anthozoans. Mol Ecol Res 9:462–470. doi:10.1111/j.1755-0998.2008.02349.x

Mayfield AB, Hsiao YY, Fan TY, Chen CS, Gates RD (2010) Evaluating the temporal stability of stress-activated protein kinase and cytoskeleton gene expression in the Pacific corals *Pocillopora damicornis* and *Seriatopora hystrix*. J Exp Mar Biol Ecol 395:215–222. doi:10.1016/j.jembe.2010.09.007

Mayfield AB, Wang LH, Tang PC, Fan TY, Hsiao YY, Tsai CL, Chen CS (2011) Assessing the impacts of experimentally elevated temperature on the biological composition and molecular chaperone gene expression of a reef coral. PLoS One 6(10):e26529. doi:10.1371/journal.pone.0026529

Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol Oceanogr 18:897–907

Meinshausen M, Smith SJ, Calvin K, Daniel JS, Kainuma MLT, Lamarque J-F, Matsumoto K, Montzka SA, Raper SCB, Riahi K, Thomson A, Velders GJM, van Vuuren DPP (2011) The RPC greenhouse gas concentrations and their extensions from 1765 to

2300. Clim Change 109:213–241. doi:10.1007/s10584-011-0156-z
Melzner F, Gutowska MA, Langenbuch M, Dupont S, Lucassen M, Thorndyke MC, Bleich M, Pörtner H-O (2009) Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences 6:2313–2331
Meyer E, Aglyamova GV, Matz MV (2011) Profiling gene expression responses of coral larvae (*Acropora millepora*) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. Mol Ecol 20:3599–3616. doi:10.1111/j.1365-294X.2011.05205.x
Moore BD, Cheng S-H, Rice J, Seeman JR (1998) Sucrose cycling, Rubisco expression, and prediction of photosynthetic acclimation to elevated atmospheric CO_2. Plant Cell Environ 21:905–915
Moss RH, Edmonds JA, Hibbard KA, Manning MR, Rose SK, van Vuuren DP, Carter TR, Emori S, Kainuma M, Kram T, Meehl GA, Mitchell JFB, Nakicenovic N, Riahi K, Smith SJ, Stouffer RJ, Thomson AM, Weyant JP, Wilbanks TJ (2010) The next generation of scenarios for climate change research and assessment. Nature 463:747–756. doi:10.1038/nature08823
Munday PL, Dixson DL, Donelson JM, Jones GP, Pratchett MS, Devitsina GV, Døving KB (2009) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. Proc Natl Acad Sci USA 106:1848–1852. doi:10.1073/pnas.0809996106
Muscatine L, McCloskey LR, Marian RE (1981) Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol Oceanogr 26:601–611
Muscatine L, Falkowski PG, Porter JW, Dubinsky Z (1984) Fate of photosynthetic fixed carbon in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. Proc R Soc Lond B 222:181–202
Muscatine L, Porter JW, Kaplan IR (1989) Resource partitioning by reef corals as determined from stable isotope composition. Mar Biol 100:185–193
Nakamura M, Morita M, Kurihara H, Mitarai S (2011a) Expression of *hsp70*, *hsp90* and *hsf1* in the reef coral *Acropora digitifera* under prospective acidified conditions over the next several decades. Biol Open BIO2011036. doi:10.1242/bio.2011036
Nakamura M, Ohki A, Suzuki A, Sakai K (2011b) Coral larvae under ocean acidification: survival, metabolism, and metamorphosis. PLoS One 6:e14521. doi:10.1371/journal.pone.0014521
Negri AP, Marshall PA, Heyward AJ (2007) Differing effects of thermal stress on coral fertilization and early embryogenesis in four Indo Pacific species. Coral Reefs 26:759–763. doi:10.1007/s00338-007-0258-2
Pandolfi JM, Jackson JBC, Baron N, Bradbury RH, Guzman HM, Hughes TP, Kappel CV, Micheli F, Possingham HP, Sala E (2005) Are US reefs on the slippery slope to slime? Science 307:1725–1726. doi:10.1126/science.1104258
Pandolfi JM, Connolly SR, Marshall DJ, Cohen AL (2011) Projecting coral reef futures under global warming and ocean acidification. Science 333:418–422. doi:10.1126/science.1204794
Parker LM, Ross PM, O'Connor WA, Borysko L, Raftos DA, Pörtner H-O (2012) Adult exposure influences offspring response to ocean acidification in oysters. Glob Change Biol 18:82–92. doi:10.1111/j.1365-2486.2011.02520.x
Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annu Rev Ecol Evol Syst 37:637–669. doi:10.1146/annurev.ecolsys.37.091305.110100
Parry MAJ, Keys AJ, Madgwick PJ, Carmo-Silva AE, Andralojc PJ (2008) Rubisco regulation: a role for inhibitors. J Exp Bot 59:1569–1580. doi:10.1093/jxb/ern084
Pierrot D, Lewis E, Wallace DWR (2006) MS Excel program developed for CO_2 system calculations. ORNL/CDIAC-105a
Pörtner H-O (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Mar Ecol Prog Ser 373:203–217. doi:10.3354/meps07768
Pörtner H-O, Farrell AP (2008) Physiology and climate change. Science 332:690–691. doi:10.1126/science.1163156
Pörtner H-O, Bennett AF, Bozinovic F, Clarke A, Lardies MA, Lucassen M, Pelster B, Schiemer F, Stillman JH (2006) Trade-offs in thermal adaptation: the need for molecular to ecological integration. Phys Biochem Zool 79:295–313. doi:10.1086/499986
Putnam HM, Edmunds PJ, Fan TY (2008) Effect of temperature on the settlement choice and photophysiology of larvae from the reef coral *Stylophora pistillata*. Biol Bull 215:135–142
Putnam HM, Edmunds PJ, Fan TY (2010) Effect of a fluctuating thermal regime on adult and larval reef corals. Invertebr Biol 129:199–209. doi:10.1111/j.17447410.2010.00199.x
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. doi:10.1007/s00338-009-0482-z
Riahi K, Rao S, Krey V, Cho C, Chirkov V, Fischer G, Kindermann G, Nakicenovic N, Rafaj P (2011) RCP 8.5 A scenario of comparatively high greenhouse gas emissions. Clim Change 109:33–57. doi:10.1007/s10584-011-0149-y
Riebesell U, Fabry VJ, Hansson L, Gattuso J-P (eds) (2010) Guide to best practices for ocean acidification research and data reporting. European Commission, Brussels. doi:10.2777/58454
Rodriguez-Lanetty M, Harii S, Hoegh-Guldberg O (2009) Early molecular responses of coral larvae to hyperthermal stress. Mol Ecol 18:5101–5114. doi:10.1111/j.1365-294X.2009.04419.x
Stumpp A, Dupont ST, Thorndyke MC, Melzner F (2011a) CO_2 induced seawater acidification impacts sea urchin larval development II: gene expression patterns in pluteus larvae. Comp Biochem Physiol A 160:320–330. doi:10.1016/j.cbpa.2011.06.023
Stumpp A, Wren J, Melzner F, Thorndyke MC, Dupont ST (2011b) CO_2 induced seawater acidification impacts sea urchin larval development I: elevated metabolic rates decrease scope for growth and induce developmental delay. Comp Biochem Physiol A 160:331–340. doi:10.1016/j.cbpa.2011.06.022
Sunday JM, Crim RN, Harley CDG, Hart MW (2011) Quantifying rates of evolutionary adaptation in response to ocean acidification. PLoS One 6(8):e22881. doi:10.1371/journal.pone.0022881
Szmant AM, Gassman NJ (1990) The effects of prolonged "bleaching" on the tissue biomass and reproduction of the reef coral *Montastrea annularis*. Coral Reefs 8:217–224
Talmage SC, Gobler CJ (2010) Effects of past, present and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish. Proc Natl Acad Sci USA 107:17246–17251. doi:10.1073/pnas.0913804107
Talmage SC, Gobler CJ (2011) Effects of elevated temperature and carbon dioxide on the growth and survival of larvae and juveniles of three species of Northwest Atlantic bivalves. PLoS One 6(10):e26941. doi:10.1371/journal.pone.0026941
van Vuuren DP, Meinshausen M, Plattner GK, Joos F, Strassmann KM, Smith SJ, Wigley TML, Raper SCB, Riahi K, de la Chesnaye F, den Elzen MGJ, Fujino J, Jiang K, Nakicenovic N, Paltsev S, Reilly JM (2008) Temperature increase of 21st century mitigation scenarios. Proc Natl Acad Sci USA 105:15258–15262. doi:10.1073/pnas.0711129105
van Vuuren DP, Edmonds J, Kainuma M, Riahi K, Thomson A, Hibbard K, Hurtt GC, Kram T, Krey V, Lamarque J-F, Masiu T, Meinshausen M, Nakicenovi N, Smith SJ, Rose SK (2011) The representative concentration pathways: an overview. Clim Change 109:5–31. doi:10.1007/s10584-011-0148-z

Venn AA, Loram JE, Douglas AE (2008) Photosynthetic symbioses in animals. J Exp Bot 59:1069–1080. doi:10.1093/jxb/erm328

Veron JEN (2011) Ocean acidification and coral reefs: an emerging big picture. Diversity 3:262–274. doi:10.3390/d3020262

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. doi:10.3354/meps07857