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RESEARCH ARTICLE

Preconditioning in the reef-building coral *Pocillopora damicornis* and the potential for trans-generational acclimatization in coral larvae under future climate change conditions

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ABSTRACT

Coral reefs are globally threatened by climate change-related ocean warming and ocean acidification (OA). To date, slow-response mechanisms such as genetic adaptation have been considered the major determinant of coral reef persistence, with little consideration of rapid-response acclimatization mechanisms. These rapid mechanisms such as parental effects that can contribute to trans-generational acclimatization (e.g. epigenetics) have, however, been identified as important contributors to offspring response in other systems. We present the first evidence of parental effects in a cross-generational exposure to temperature and OA in reef-building corals. Here, we exposed adults to high (28.9°C, 805 µatm P_{CO_2}) or ambient (26.5°C, 417 µatm P_{CO_2}) temperature and OA treatments during the larval brooding period. Exposure to high treatment negatively affected adult performance, but their larvae exhibited size differences and metabolic acclimation when subsequently re-exposed, unlike larvae from parents exposed to ambient conditions. Understanding the innate capacity corals possess to respond to current and future climatic conditions is essential to reef protection and maintenance. Our results identify that parental effects may have an important role through (1) ameliorating the effects of stress through preconditioning and adaptive plasticity, and/or (2) amplifying the negative parental response through latent effects on future life stages. Whether the consequences of parental effects and the potential for trans-generational acclimatization are beneficial or maladaptive, our work identifies a critical need to expand currently proposed climate change outcomes for corals to further assess rapid response mechanisms that include non-genetic inheritance through parental contributions and classical epigenetic mechanisms.

KEY WORDS: Ocean acidification, Temperature, Parental effects, Epigenetics, Stress

INTRODUCTION

The current rates of climate change are extreme and in the next 100 years modelers forecast that temperature will increase by up to 4°C (IPCC, 2014) and atmospheric carbon dioxide will double or treble (van Vuuren et al., 2011), based on representative concentration pathway estimates, RCPs. The interactive impacts of temperature shifts and ocean acidification (OA) have been, and will continue to be, profoundly damaging to coral reefs, through high temperature-induced bleaching and OA-driven declines in coral calcification and productivity. Together, these negative biological responses highlight a critical need to better understand the intrinsic capacity that corals possess to respond to high rates of environmental change, which will ultimately dictate whether the structural and biological integrity of these valuable tropical ecosystems can be maintained near current levels (Pandolfi et al., 2011).

With the growing awareness of the vulnerability of coral reefs to climate change, research has focused on variability in biological responses in the system (i.e. resistance or sensitivity of different coral taxa and the potential for adaptation) (Pandolfi et al., 2011), including a growing body of literature on acclimatization processes in corals (Brown and Cossins, 2011). Evidence of parental effects such as trans-generational acclimatization in other marine organisms suggests that the thermo-tolerance of offspring is substantially influenced by parental thermal history (Donelson et al., 2012). Additionally, with the heightened concern regarding OA impacts in the marine realm, the study of trans-generational acclimation associated with OA stress is becoming an active area of research (Kurihara, 2008; Donelson et al., 2012; Dupont et al., 2013; Miller et al., 2012; Parker et al., 2012; Allan et al., 2014; Munday, 2014). This early OA work has primarily focused on organisms with relatively short generation times (i.e. copepods, oysters, urchins and fish), but has been predominantly conducted in the absence of multiple stressors (Miller et al., 2012). Remarkably, no studies have yet examined the potential for trans-generational acclimatization to the simultaneous threats of temperature and OA on critical ecosystem engineers such as reef-building corals, likely due to the longer generation times of reef corals.

An ideal model system to test the contributions of parental effects and the potential for cross-generational acclimatization to influence offspring health and fitness (Bollati and Baccarelli, 2010) is found in animals that brood their offspring internally. Here, the environment of larval development is controlled by the external environment and parental modifications, and variation in parental investment throughout the brooding period is therefore possible. To date, the examination of acclimation and acclimatization in corals has been limited to a single generation. For example, corals have been shown to acclimatize to temperature through preconditioning within a generation (i.e. corals with a history of bleaching show resistance to subsequent thermal stress events; Brown et al., 2002). This focus on different life stages in isolation fails to capture the feedback loops that exist among multiple life stages or generations of organisms (Marshall and Morgan, 2011) that sequentially channel responses through an environmental and ontogenetic funnel. It is this interplay of life history, ontogeny, acclimatization and adaptation that creates the potential for synergistic and antagonistic outcomes with positive and/or negative fitness implications (Marshall and Morgan, 2011; Bonduriansky and Day, 2009). The examination of the effects of environmental

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stress through multiple life stages via parental effects, or epigenetic mechanisms such as trans-generational acclimatization (Bossdorf et al., 2008; Bonduriansky and Day, 2009; Schlichting and Wund, 2014), are critical to fully understanding the evolutionary trajectories and responses of reef corals to climate change.

We tested whether preconditioning of larvae to high temperature and OA inside parental polyps during development resulted in parental effects that may contribute to trans-generational acclimation in the newly released larvae. Here, we report evidence of such acclimation in coral larvae when the brooding adult *Pocillopora damicornis* (Linnaeus 1758) is exposed to high temperature and OA (28.9°C and 805 µatm P_{CO_2}; hereafter 'high') in comparison to ambient controls (26.5°C and 417 µatm P_{CO_2}; hereafter 'ambient').

RESULTS

Adult response

In a trans-generational approach, adults of the brooding coral *P. damicornis* were exposed to the high or ambient experimental treatments for 1.5 months, which represents the majority or entirety of the internal larval development period (~1 month; Stoddart and Black, 1985). Towards the end of this period, ~55% of the adult colonies (N=22) released larvae over the course of 4 days during the September 2011 lunar peak of release. Of the 12 colonies releasing larvae, release was split between the ambient tanks (seven colonies, 64%) and high tanks (five colonies, 45%). There was no indication of acceleration of release due to exposure to the high treatment (supplementary material Fig. S1). Adult colonies in the high treatment displayed significant reductions in productivity of 23.5% in photochemical efficiency (F_V/F_M, t=4.9995, d.f.=17.854, P<0.0001), 40% in gross photosynthetic rate (P_G, t=4.9615, d.f.=17.099, P<0.001), 79% in net photosynthetic rate (P_N, t=4.1607, d.f.=15.222, P<0.001), and 2.1 times lower photosynthesis to respiration rate ratios (P:R, t=3.4789, d.f.=11.369, P<0.01) than those in ambient conditions (Fig. 1). There was no difference in dark respiration rate (R_D, t=−0.7463, d.f.=16.561, P>0.05, log transformed) or calcification rate (t=−0.2039, d.f.=14.449, P>0.05) between treatments (Fig. 1).

Table 1. Results of statistical analyses

Source of variation	d.f.	MS	F	P
Size (mm^2)				
History	1	0.847	16.076	**0.001**
Secondary	1	0.588	11.147	**0.004**
History×secondary	1	0.007	0.139	0.714
Error	15	0.053		
Respiration (nmol O_2 larva^{-1} min^{-1})				
History	1	5.444×10^{-3}	20.563	**0.0004**
Secondary	1	5.000×10^{-7}	0.0018	0.967
History×secondary	1	8.014×10^{-4}	3.0273	0.102
Error	15	2.647×10^{-4}		
Size-normalized respiration (nmol O_2 mm^{-2} min^{-1})				
History	1	1.308×10^{-4}	0.345	0.567
Secondary	1	2.709×10^{-3}	7.139	**0.018**
History×secondary	1	2.643×10^{-3}	6.964	**0.019**
Error	14	3.795×10^{-4}		

Larval response variables were analyzed with two-way ANOVA.
Bold indicates a significant difference.

Larval response

Larvae collected following the parental exposure were pooled within each treatment, reciprocally allocated to offspring environmental treatments for 5 days (Fig. 2A), and subsequently sampled for R_D and size. Size was significantly reduced on average by 33% ($F_{1,15}$=16.076, P=0.001) and R_D by 43% ($F_{1,15}$=20.563, P=0.0004) in larvae brooded by parents in high temperature and OA environments (Fig. 2B,C; Table 1). When the larvae were exposed to high temperature and OA, the size of larvae from parents exposed to ambient and high parental environments declined by 22% and 38%, respectively (P=0.004; Table 1). However, when normalized to account for size differences, respiration rates displayed a significant interaction effect ($F_{1,14}$=6.964, P=0.019; Table 1), such that the response of larvae to the secondary treatment varied depending on whether larvae were brooded in parents from the ambient or high environments. Thus, in the ambient offspring environment, size-normalized respiration for larvae brooded under

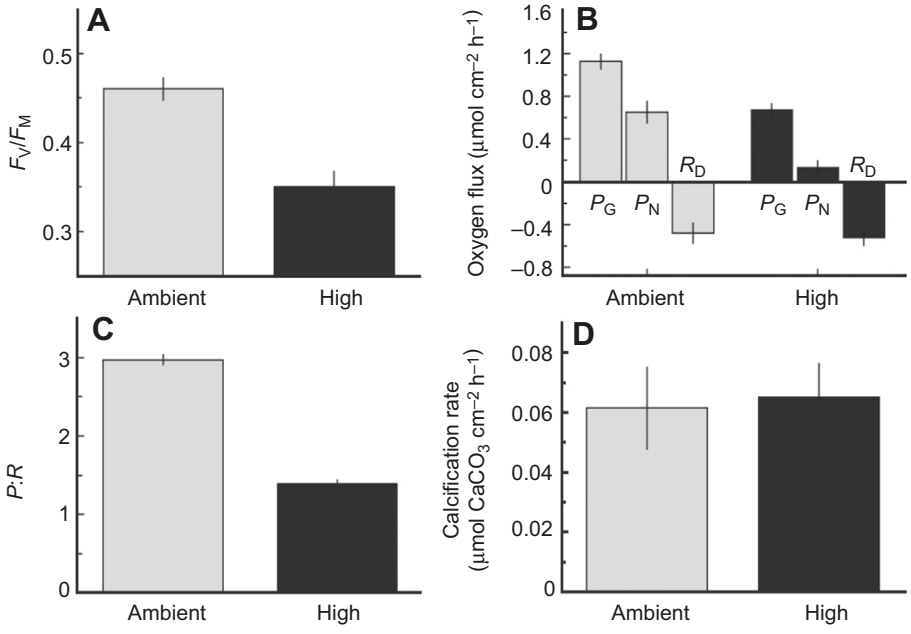

Fig. 1. Adult coral responses to ambient and high temperature and ocean acidification (OA) treatments following preconditioning period. Adult physiology measurements were taken at the end of exposure to ambient and high treatments (means±s.e.m.). These include: (A) maximum quantum yield of PSII of the *Symbiodinium* within the adult coral (F_V/F_M); (B) oxygen flux measurements of gross photosynthesis (P_G), net photosynthesis (P_N) and dark respiration (R_D); (C) photosynthesis to respiration rate ratio (P:R); and (D) calcification rates measured by the alkalinity anomaly technique. Significant reductions under high treatment were present in F_V/F_M (A), P_G and P_N (N=10 per treatment; B) and P:R (N=10 per treatment; C), but no change was detected in R_D (N=10 ambient, N=11 high; B) or calcification (N=8 ambient, N=10 high; D).

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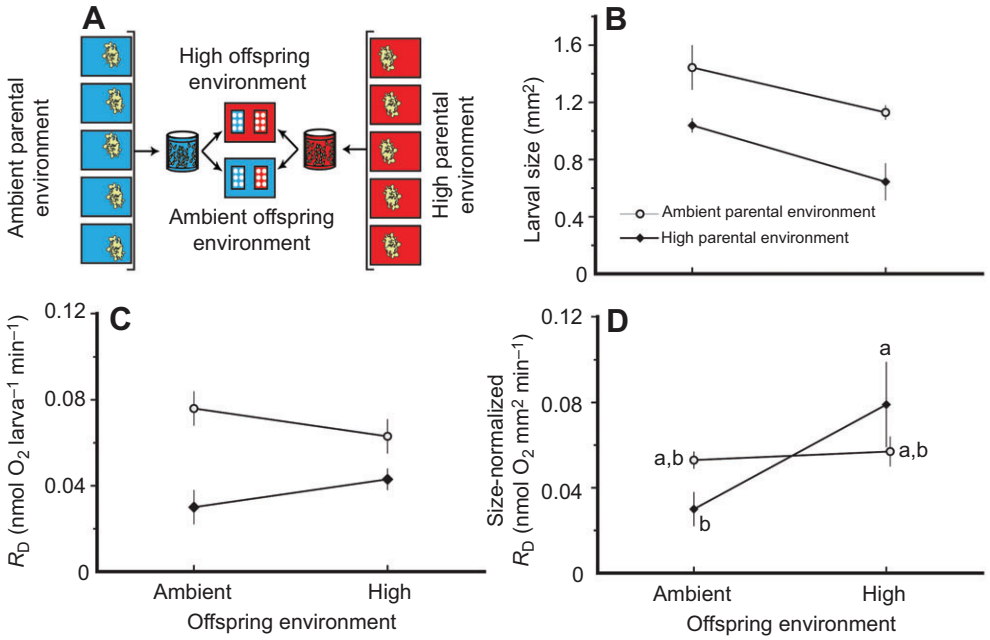

Fig. 2. Response of larvae to stress following preconditioning. Larvae were pooled based on preconditioning to either ambient or high conditions (N=5 adults per treatment) for 1.5 months. (A) Larvae were exposed to a secondary treatment in a reciprocal fashion for 5 days, then assessed for: (B) larval size; (C) R_D rate per larva; and (D) R_D rate normalized to larval size (N=5 groups of larvae per treatment, except high–high where N=4 for size, N=4 for R_D rate per larva and N=3 for R_D rate normalized to larval size; means±s.e.m.). A significant effect of parental exposure was detected in B and C, with declines in both responses due to adult conditioning in high treatments. Larval size (B) from both parental environments was reduced in the high offspring condition. For size-normalized R_D rates (D), a significant interaction was present [different letters indicate significantly different groups based on Tukey's honest significant difference (HSD) test].

the high parental treatment was significantly lower compared with larvae brooded under ambient conditions and increased significantly when exposed to the high treatment. In comparison, the respiration of larvae brooded under ambient conditions did not change between the ambient and high offspring environments (Fig. 2D).

DISCUSSION

It has frequently been suggested that coral adaption will not keep pace with the rate of environmental change and corals are severely threatened by increasing climate change (e.g. Hoegh-Guldberg et al., 2007; Veron et al., 2009). There is, however, evidence of rapid acclimatory processes in biological systems (e.g. maternal effects, non-genetic inheritance, symbiotic shuffling) that interact to drive organism and evolutionary responses in non-linear ways and the links between acclimatization, phenotypic plasticity and evolutionary responses are currently underexplored (Bonduriansky and Day, 2009; Ho and Burggren, 2010; Munday et al., 2013; Schlichting and Wund, 2014; Sunday et al., 2014; Fig. 3). Here, we provide the first evidence for trans-generational acclimatization as a process with the potential to influence the response of reef-building corals to a changing climate. The exposure of brooding adults to warmer, more acidic seawater resulted in acclimation of the larvae when exposed to these conditions post-release. While still in its infancy for corals, research examining parental effects and trans-generational acclimatization has the potential to transform our understanding of the response of coral ecosystems to a rapidly changing environment, as it can identify that the rate and direction of organismic response may be much different than previously understood (Fig. 3).

Many reef systems have shown strong declines in coral cover over the past several decades (Gardner et al., 2003; Bruno and Selig, 2007) despite the potential contributions of trans-generational mechanisms, which questions the importance of such mechanisms. The lack of information on the implications of trans-generational acclimatization in corals, despite the large amount of literature on positive and negative epigenetic effects in model systems, was a motivating factor in our study. There are many factors that contribute to reef decline, some acute and some chronic, and not all of these will elicit trans-generational acclimatization. Specifically, acute disturbances such as predation driven by overfishing, mass corallivory by crown-of-thorns sea stars and destruction by hurricanes, for example, result in rapid mortality that does not provide the same preconditioning as chronic stressors (e.g. increasing temperatures and increasing ocean acidification). There are already examples of intra-generational acclimatization ameliorating the stress response (i.e. bleaching) in terms of temperature stress. In the field, multiple bleaching events have resulted in less negative effects in subsequent events in *Goniastrea* (Brown et al., 2002), *Porites* (Carilli et al., 2012) and *Oculina* (Armoza-Zvuloni et al., 2011). Additionally, laboratory exposure to a simulated thermal bleaching event resulted in less symbiont loss and an increase in symbiont photoprotective mechanisms in *Acropora aspera* (Middlebrook et al., 2008). This work on intra-generational acclimatization suggests that for chronic environmental stressors such as temperature at least, acclimatization has an important role. The current approach of exposing naive corals to environmental change is likely overestimating the negative effects. We can identify this with preconditioning experiments such as ours and this is a critical area of research to inform forecasts of future reef maintenance.

There are several potential mechanisms that may explain our trans-generational acclimatization results. First, the larval acclimation may result from parental effects through a directed shift in allocation of resources to the offspring when brooded in a stressful environment. In this scenario, parental effects such as energetic and protective provisioning through maternal soma (e.g. Hamdoun and Epel, 2007), epigenetic mechanisms such as DNA methylation (Feil and Fraga, 2012), and/or control of the brooding space (e.g. Agostini et al., 2012) may prepare the larvae, resulting in acclimatization to high temperature and OA upon re-exposure. One mechanism by which positive acclimatization could be achieved in corals is a potential adaptive tradeoff for production of smaller larvae that are more metabolically tuned for their predicted environment. Our data identify higher size-normalized metabolic rates in larvae exposed to the high treatment when their parents were conditioned in that treatment. Temperature coefficient (Q_{10}) effects alone would suggest larvae from both parental conditionings would increase their respiration rate

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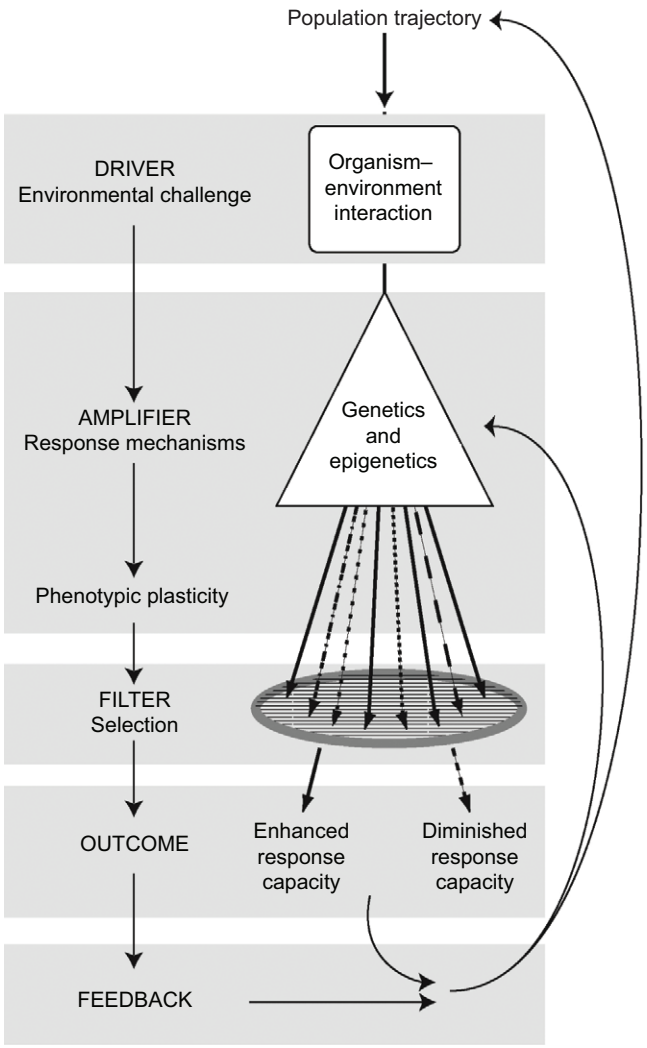

Fig. 3. Feedback loop connecting organismic response to the environment to population trajectory. Organisms respond to environmental drivers through the organism×environment interaction, but in addition to the classical genetic amplifier, consideration should also be given to another amplifier, the epigenetic response mechanism, for its capacity to generate phenotypic plasticity. Plasticity generated by both of these mechanisms is filtered by selection, resulting in outcomes that may either enhance or diminish response capacity to environmental change. This capacity is then fed back into the population across generations to influence the future population trajectory. In the case of reef corals, this plasticity provides a potential buffer to the rapid rate of climate change.

when exposed to the high offspring environment (Edmunds et al., 2011; Hochachka and Somero, 2014), whereas a typical response to OA in marine organisms is metabolic depression (Portner, 2008; Nakamura et al., 2011). Here, however, we did not see either parallel response patterns expected of Q_{10} effects or acidification-driven metabolic suppression across the two preconditioning environments in response to the secondary (offspring) environment, suggesting adaptive mechanisms are at play as a result of the preconditioning. In adult corals, higher metabolic rates are linked to increased protein turnover and energy allocation to fitness-related traits, which potentially drives a greater ability to respond to stress (Gates and Edmunds, 1999). High respiration rates may reduce pelagic larval duration and therefore hasten settlement and growth. Given the necessary energetic demands of metamorphosis and settlement (Edmunds et al., 2013), and the need to rapidly progress through life stages to escape mortality and competition, the increased supply of ATP generated by a higher respiration rate would likely be beneficial (Edmunds et al., 2011). While high respiration rate can create a substrate deficit in lecithotrophic larvae, given that *P. damicornis* is a vertical transmitter of *Symbiodinium* to the coral larvae, food resources from translocation of photosynthate should be more than adequate to sustain these high metabolic rates.

Under stressful conditions that limit productivity in adult corals, but are environmentally predictable (i.e. high temperature and CO_2), smaller larvae may be produced as an adaptive means for a metabolic benefit in the offspring. Adaptive plasticity of parental modulation of larval size under environmental change has been documented in a number of taxa (Marshall et al., 2008; Burgess and Marshall, 2011). For example, polychaetes collected from CO_2 vent areas following life-long exposure to high P_{CO_2} conditions had smaller body sizes and higher metabolic rates than those from outside the vent areas (Calosi et al., 2013). Similarly, in a marine bryozoan in which parents were preconditioned at high and low temperatures, offspring of parents from high temperatures are smaller and more variable in size with higher rates of metamorphosis than those produced by parents conditioned at low temperatures (Burgess and Marshall, 2011). Bet-hedging for eggs or offspring size based on environment is also documented in other marine invertebrates (reviewed by Crean and Marshall, 2009). Furthermore, adaptive trans-generational acclimation has been demonstrated following exposure to predation in aphids, where parents shifted production to a greater number of offspring with wings, and the shift in offspring type has population implications for the F2 generation (Mondor et al., 2005). Together, these studies support a large role for the adaptive potential of trans-generational plasticity under environmental change.

Our research suggests parental effects may be one avenue of rapid change for reef-building corals, but it is necessary to also extend this work to later life stages to examine larval settlement, juvenile survivorship, and recruitment and fecundity in the next generation with long-term studies. For example, the effect of small egg size was manifest as lower post-metamorphosis survivorship and growth (Emlet and Hoegh-Guldberg, 1997) in sea urchin larvae. It is difficult to compare the results of externally fertilized, lecithotrophic larvae to those of the brooding coral larvae that are provisioned immediately by their symbionts. Work in three brooding Pocilloporids, however, revealed that larval size was positively related to survivorship (Isomura and Nishihira 2001), suggesting that carry-over effects from a small larval size such as those seen in our study could result in cascading consequences in future life stages. This is true in the case of the Olympia oyster, where exposure to ocean acidification during development resulted in smaller shell sizes, and the latent effect of small size persisted for more than a month in ambient common garden conditions (Hettinger et al., 2012). It appears for corals, however, that small size is not always a certain predictor of negative performance, as large and small larvae exposed to temperature and salinity stress had the same propensity for latent effects in the coral *Orbicella faveolata* (Hartmann et al., 2013). These variable post-metamorphosis responses in marine taxa highlight the need to track the duration and magnitude of trans-generational acclimatization beyond the larval stage, through recruitment and into the next generation.

A second hypothesis underlying the larval acclimation is that larval phenotype may be driven by physical environmental influences, or constraints, on developmental rate. In this case, developmental rate and survival are influenced directly, thereby selecting for smaller larvae developed more quickly under high temperature and CO_2, which perform better upon re-exposure. Therefore, the outcome is not

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dictated by parental effects per se, but a selection or weeding out of the weak phenotypes during the brooding period, resulting in positive performance of the remaining larvae. While this is a possible mechanism underlying the performance of the larvae developed in the high treatment, the lack of a difference in the timing of larval release between treatments during the peak period (supplementary material Fig. S1) suggests that development times were not substantially influenced and that it is likely 'true' parental effects were responsible for our results.

A third hypothesis for our results is that the acclimatization is due simply to the conditioning of the larvae to temperature and CO_2 during development in the parental coelenteron. Although it is not possible to fertilize these brooded larvae and raise them in the absence of their parents to fully test this hypothesis, the next closest comparison is exposure of larvae immediately following release. Exposure of *P. damicornis* larvae to high temperature (29°C, as in this study) and CO_2 (635 µatm) immediately upon release resulted in a decline in larval respiration, not an increase (Putnam et al., 2013). This suggests direct larval acclimatization may not be responsible for our results, although Putnam and co-authors only exposed the larvae for ∼64% of the minimum time that it takes from fertilization to development (∼14 days minimum; Stoddart and Black, 1985), whereas here larvae were exposed for the entire expected duration of fertilization and development. Regardless of the exact underlying mechanism(s) of response, however, the outcome is the same. Exposure to high treatments across a generation and at the developing stage within the parent has different impacts from preconditioning to ambient. This potential for 'stress' preconditioning has implications that need to be considered to better understand the response of reefs to chronic environmental stressors (i.e. temperature and OA) and to better predict the trajectory of future reef demographics.

In contrast to positive acclimatization, it is possible that the reported negative effects of increasing temperature and OA response of corals (Hoegh-Guldberg et al., 2007) may be amplified through trans-generational acclimation, resulting in maladaptive outcomes. In our work, adult corals exposed to future conditions displayed reduced photosynthetic rates and energetic scope in comparison to control conditions (Fig. 1). These reductions in energy availability may signal the necessity for trade-offs in energy allocation. As calcification and metabolic rate were both maintained at the same levels in adults exposed to future and current conditions (Fig. 1), it is possible that this constrained the amount of energy allocated to reproductive output, with negative consequences for offspring performance. While not contextualized trans-generationally at the time, previous ecological research examining adult corals after a thermal stress event reveals significant declines in both tissue lipids and the number of eggs (Jones and Berkelmans, 2011), which suggests strong adult controls on the offspring characteristics and performance. Additionally, recent trans-generational work examining the locomotion and cognition responses of coral reef fish to OA reveals the potential for trans-generational acclimation to vary in magnitude and direction, being complete (positive response rescue), partial (slight response amelioration) or absent (negative performance), across the different traits examined, when compared with offspring of parents from ambient conditions (Allan et al., 2014). It is therefore necessary to conduct further research for corals to identify the magnitude and direction of acclimatization responses associated with parental effects. It will also be critical to interpret the results of trans-generational work with an eye towards the implications of either positive or negative consequences for corals and search for a greater understanding of the mechanistic basis for these effects (e.g. classical epigenetic mechanisms such as DNA methylation).

While identifying the exact mechanism of the acclimatization response seen in our study is not possible, soft inheritance mechanisms such as epigenetics or those outside changes in DNA sequence are known to play pivotal roles in environmental response in a diversity of organisms such as humans, fruit flies and plants (Feil and Fraga, 2012). A diversity of non-genetic inheritance factors have been identified, such as RNA, soma, cytoplasm, proteins, methylation, hormones, metabolites, nutrients, waste, symbionts and, more broadly, the physical conditions surrounding the organism controlled by the parents, and the behavior of the parents themselves (Bonduriansky and Day, 2009; Ho and Burggren, 2010; Greer and Shi, 2012). The interplay between traditional genetic adaptation and epigenetics is a framework championed in the theoretical literature (Badyaev and Uller, 2009; Schlichting and Wund, 2014) and in well-studied model systems (Feil and Fraga, 2012). Indeed, parental effects and other epigenetic mechanisms have come to the forefront in a diversity of research fields. For example, there are documented epigenetic effects such as consequences of famine in humans exposed in the prenatal environment (Heijmans et al., 2008), identified as differential methylation patterns in the offspring as long as 60 years later. There are also epigenetic consequences of a parental methylated diet that influences health and lifespan in offspring phenotype of mice (Wolff et al., 1998). Epigenetic marks are also found in flowering plants (De Lucia et al., 2008), where temperature-driven vernalization occurs, with repressive chromatin maintained through time. Additionally, invertebrates display epigenetics effects from environmental stress on heat shock protein (HSP)-related interactions with chromatin linked to developmental genes in *Drosophila* (Gendall et al., 2001), resulting in an abnormal eye disk development that is heritable within the next generation. These epigenetic phenomena have both deepened our understanding of, and highlighted complexities in predicting biological responses to the environment, and provide a compelling rationale to examine these processes in corals (Fig. 3).

It is undisputed that the contributions of adaptation and acclimatization processes have significant implications for our understanding of reef systems (e.g. Donner et al., 2005; Pandolfi et al., 2011; Logan et al., 2014). What is less clear, however, is the direction of response these processes may facilitate, potentially resulting in stressor amplification or amelioration (Fig. 3). While parental effects are an understudied area and epigenetic mechanisms have been overlooked in corals to date, perhaps reflecting the biological and experimental challenges of studying these long-lived, marine organisms, they are critical areas of future research for an ecosystem stressed by unprecedented rates of environmental change. Non-genetic mechanisms play a major role in evolutionary trajectories by accelerating the rate of phenotypic change beyond genotypic adaptation, thereby potentially ameliorating perceived adaptive constraints associated with the rapid rates of anthropogenic environmental change. The evidence we present here suggests that non-genetic, trans-generational acclimatization processes (e.g. epigenetics) have the potential to contribute to the trajectories of coral populations in a rapidly changing climate. The nature and duration of these processes for corals remains to be discovered.

MATERIALS AND METHODS

Experimental design

Corals were collected on 4 August 2011 from the fringing reefs of Kaneohe Bay, Hawaii (Special Activities Permit 2011 Hawaii DAR) and experiments were carried out from 4 August to 17 September 2011 at the Hawaii Institute

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Table 2. Experimental seawater chemistry

Treatment	Temperature (°C)	P_{CO_2} (µatm)	pH	TA (µmol kg^{-1})	HCO_3^- (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})	Ω_a
Ambient	26.53±0.06	417±12	8.011±0.010	2160±2	1667±10	198±4	3.16±0.06
High	28.93±0.09	805±37	7.779±0.016	2163±2	1815±11	140±4	2.27±0.07

Carbonate chemistry parameters (means±s.e.m.) were calculated from measurements of temperature, salinity, pH and total alkalinity (TA) in the treatment tanks (N=55 per treatment, 11 tanks by 5 replicates). Ω_a, aragonite saturation state. See Materials and methods for details.

of Marine Biology (Kaneohe, HI, USA). Temperature records from the collection site were recorded for the 2 weeks prior to collection (UA-002-08, Onset Computer Corporation, Bourne, MA, USA; accuracy ±0.5°C, precision 0.1°C) and revealed a mean of 26.9±0.02°C and average daily range of 2.8±0.1°C (means±s.e.m.). The experimental system consisted of a custom-built, flow-through mesocosm array of 22, ∼50 l insulated tanks (Aqualogic, San Diego, CA, USA). Temperature was controlled independently in each of the tanks by recirculating seawater via heat exchangers mounted on the back of the array. For continuous records, temperature was logged hourly in each tank using Hobo underwater temperature loggers (UA-002-08, Onset Computer Corporation, Bourne, MA, USA) and provided natural daily fluctuations (supplementary material Fig. S2). Incoming water flow was maintained in the tanks with a rate of ∼360 ml min^{-1}, which resulted in tank water turning over ∼10 times daily. The tanks were lit with overhead metal halide lights (14K bulbs, 250w IceCap Inc., Hamilton, NJ, USA) on a 12 h:12 h photoperiod, with light from 06:30 h to 18:30 h. Light levels in the tanks were measured at ∼396±4 µmol quanta m^{-2} s^{-1} (mean±s.e.m., N=305) using a cosine corrected photosynthetically active radiation (PAR) sensor (LI-COR 192, LICOR Biosciences, Lincoln, NE, USA).

Adult *P. damicornis* were exposed to either ambient or high treatment conditions (26.5°C and 417 µatm, or 28.9°C and 805 µatm P_{CO_2}, respectively, N=11 per treatment; Table 2) for 1.5 months prior to peak larval release in September 2011. The combination of these factors was chosen specifically to simulate the most likely future scenario of simultaneous temperature and P_{CO_2} increase, with the goal of maximizing the number of adult corals in independent tanks for larval release at the end of the exposure period, not to mechanistically test each factor separately. Each of 22 replicate, 50 l tanks contained one adult colony for a total of 11 replicate colonies per treatment.

Seawater carbonate chemistry

Carbon dioxide control was achieved through mixing of scrubbed air and pure CO_2 that was metered using pairs of Mass Flow Controllers (MFCs; Sierra Instruments, Monterey, CA, USA). The concentration of mixed gas flowing out of the MFCs was measured with an infrared CO_2 analyzer (Qubit IR S151, Qubit Systems, Kingston, ON, Canada) calibrated with certified gas mixes (Airgas West, Northridge, CA, USA). The pre-mixed CO_2 was injected into four header tanks filled with incoming seawater using venturi injectors (MK-484, Mazzei Injector Company LLC, Bakersfield, CA, USA) connected to recirculating pumps (700gph Magnetic Drive, Danner Manufacturing Inc., Islandia, NY, USA).

Carbonate chemistry of the tanks was tracked by measuring total alkalinity and pH. For rapid checks of the system, pH was measured in each tank using a hand-held pH meter and showed significant differences across the experiment of 0.2 units (NBS scale; ambient=8.08±0.01, high=7.88±0.01, N=307). In addition, water samples from all tanks (N=22) were collected for assessment of carbonate chemistry weekly throughout the experiment (Table 2) after determination of the high stability of temperature and total alkalinity in preliminary experiments (Putnam, 2012). Temperature (°C) and salinity of each of the treatment tanks were measured simultaneously with water sample collections for pH (total scale) and total alkalinity (µmol kg^{-1} seawater). Temperature was measured using a traceable certified digital thermometer (15-077-8, accuracy 0.05°C, resolution 0.001°C; Control Company, Friendswood, TX, USA) and salinity was measured with a YSI sonde (YSI 63, Yellow Springs Instruments, Yellow Springs, OH, USA).

Samples were titrated using certified reference materials (CRM) including calibrated acid titrant (∼0.1 mol kg^{-1}, ∼0.6 mol kg^{-1} NaCl; Dickson et al., 2007), and analyses were quality controlled with oceanic carbon dioxide standards (Dickson Lab CO_2 CRM Batch 99). Total alkalinity was calculated via a non-linear, least-squares procedure of the Gran approach (SOP 3b; Dickson et al., 2007) and reported in units of µmol kg^{-1} seawater.

Spectrophotometric determinations of pH (total scale) were made using an *m*-Cresol Purple dye indicator on a temperature-controlled spectrophotometer (Spectramax M2, Molecular Devices, Sunnyvale, CA, USA) on duplicate 3 ml samples held at 25°C in the dark. In addition, quality control was assessed by measuring Tris standards (Dickson Lab Tris Standard Batch 4). All carbonate chemistry measurements were made based on the standard operating procedures for analysis of carbonate chemistry (Riebesell et al., 2010; Dickson et al., 2007), with dissociation constants for carbonic acid according to Mehrbach et al. (1973), and subsequent refitting following Dickson and Millero (1987) in the program CO2SYS (Pierrot et al., 2006) to calculate the remaining parameters of P_{CO_2} (µatm); HCO_3^-, CO_3^{2-} and DIC (µmol kg^{-1} seawater); and aragonite saturation state, Ω_a.

Larval reciprocal exposure

During the anticipated peak of larval release (9–12 September; *sensu* Jokiel, 1985), adult corals were each placed in a larval collection apparatus within the treatment tanks, such that flowing water flushed the buoyant larvae into a collection beaker with plankton mesh sides. The number of adult colonies releasing larvae was counted for 4 days over the release period. Larvae were collected each day, pooled within each treatment, and held in glass beakers containing 0.2 µm filtered seawater from origin treatment conditions that was refreshed two times daily. At the end of the 4 days of release, larvae were reallocated in a reciprocal fashion to either the treatment of parental origin or the opposite treatment (Fig. 2A). Replicate groups of larvae (N=5 groups) were exposed to the high or ambient treatments for 5 days in plankton mesh wells with continuous free exchange of treatment water within the wells, and full flushing of the wells with treatment water twice per day.

Dark respiration of groups of larvae was measured as oxygen flux with a fiber optic oxygen electrode (FOXY-R Ocean Optics, Dunedin, FL, USA; Edmunds et al., 2011) for five replicate groups of six larvae per treatment in 2.15 ml glass vials held in the dark, with seawater-only vials as controls for each treatment. After respiration measurements, the groups of larvae were fixed in 10% formalin and photographed on a compound microscope. The planar surface area of the larvae was measured (mm^2) from image analysis of microscopic photographs of the fixed larvae using ImageJ (NIH; Schneider et al., 2012). Size-normalized respiration was calculated by dividing the respiration rate of a replicate group by their average size.

Adult measurements

Following larval release, the response of the adult corals to 1.5 months of treatment conditions was measured. Photophysiology of the *Symbiodinium* was measured as maximum quantum yield of PSII (dark-adapted F_V/F_M) using a pulse amplitude-modulated fluorometer (Diving-PAM, Walz GmbH, Effeltrich, Germany). Corals were measured between 19:50 h and 20:30 h following a period of 80 min of dark adaption. The PAM was set for a measuring intensity of 10 and gain of 7, a damp of 2, a saturating intensity of 8 and a saturating width of 0.8. Photosynthetic and dark respiration rates were measured in 1.9 l respiration chambers with rotating stir bars using a fiber optic oxygen electrode (FOXY-R Ocean Optics). Temperature was controlled in the chambers by submerging them in a waterbath of the same temperature as the treatment condition. The measurements were carried out in closed chambers to accurately assess oxygen flux, thus there was no exchange of water during the incubations. Measurement of pH (NBS scale) prior to and following the light incubations resulted in mean (±s.d.) differences from the starting water pH of 0.19±0.10 (N=22) in high, 0.12±0.09 (N=20) in ambient, 0.03±0.03 (N=3) in ambient blanks and −0.04±

0.04 (N=4) in high blanks. These differences are less than the differences in measured pH values between treatments 0.2 pH units (NBS scale). Calcification rates were measured in the same chambers simultaneously with photosynthesis measurements using the total alkalinity anomaly technique (Chisholm and Gattuso, 1991) on chamber water prior to and following the incubation period (~30–45 min). All incubations were carried out in treatment water at the same treatment temperatures and light (~30–45 min) or dark (~90 min) conditions for the appropriate treatment that the samples were held in for the initial 1.5 months. Rates were calculated with the subtraction of blank seawater control chambers for both oxygen flux and alkalinity anomaly.

Statistics

Adult data were analyzed with t-tests between the two treatments. Larval data were analyzed with a two-way ANOVA with the fixed factors of parental environment and offspring environment and their interaction, and variables with a significant interaction were followed by Tukey's honest significant difference (HSD) test to determine differences among each group. Assumptions of t-tests and ANOVA were tested through graphical analysis of the residuals and goodness of fit tests of the standardized residuals to a normal distribution as well as the Bartlett test and Shapiro–Wilk test and Levene's test. Data were transformed for analysis as necessary. Statistical analyses were carried out in R (R Core Team, 2014) using the Companion to Applied Regression package (Fox and Weisberg, 2011).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

H.M.P. designed and executed the experiments and analysed the data. H.M.P. and R.D.G. interpreted the findings, and wrote the paper.

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Supplementary material

Supplementary material available online at
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