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RESEARCH ARTICLE

Developmental carryover effects of ocean warming and acidification in corals from a potential climate refugium, the Gulf of Aqaba

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ABSTRACT

Coral reefs are degrading from the effects of anthropogenic activities, including climate change. Under these stressors, their ability to survive depends upon existing phenotypic plasticity, but also transgenerational adaptation. Parental effects are ubiquitous in nature, yet empirical studies of these effects in corals are scarce, particularly in the context of climate change. This study exposed mature colonies of the common reef-building coral *Stylophora pistillata* from the Gulf of Aqaba to seawater conditions likely to occur just beyond the end of this century during the peak planulae brooding season (Representative Concentration Pathway 8.5: pH −0.4 and +5°C beyond present day). Parent and planulae physiology were assessed at multiple time points during the experimental incubation. After 5 weeks of incubation, the physiology of the parent colonies exhibited limited treatment-induced changes. All significant time-dependent changes in physiology occurred in both ambient and treatment conditions. Planulae were also resistant to future ocean conditions, with protein content, symbiont density, photochemistry, survival and settlement success not significantly different compared with under ambient conditions. High variability in offspring physiology was independent of parental or offspring treatments and indicate the use of a bet-hedging strategy in this population. This study thus demonstrates weak climate-change-associated carryover effects. Furthermore, planulae display temperature and pH resistance similar to those of adult colonies and therefore do not represent a larger future population size bottleneck. The findings add support to the emerging hypothesis that the Gulf of Aqaba may serve as a coral climate change refugium aided by these corals' inherent broad physiological resistance.

KEY WORDS: Phenotypic plasticity, Early life history, Ocean acidification, Trans-generational, Parental effects, Planulae

INTRODUCTION

Coral reefs face severe threats from human activities, including anthropogenic CO_2 emissions that cause increasing sea surface temperatures (SSTs) and decreasing ocean pH (ocean acidification, OA) (IPCC, 2014). In 2016, anomalously high SSTs caused the longest and most widespread global coral bleaching event on record (Hoegh-Guldberg et al., 2017; Hughes et al., 2018a). As SST continues to rise, coral bleaching events have and will continue to increase in frequency and severity (Hughes et al., 2018b), dramatically reducing the vital recovery time between such events (Schoepf et al., 2015; Neal et al., 2017). Consequently, even under the lowest projected greenhouse gas emission scenario, most warm-water coral reefs are expected to be lost by the year 2050 (Hoegh-Guldberg et al., 2017; Hughes et al., 2018a).

However, coral reefs display geographical variation in their susceptibility to climate change. A commonly applied model predicts that significant bleaching is expected after 4-degree heating weeks (DHW) or if the SST increases by 1–2°C above the local average annual temperature maximum (Liu et al., 2014). Indeed, these bleaching threshold criteria have successfully predicted coral bleaching in a number of recent events (Liu et al., 2003; Hughes et al., 2017; Kayanne, 2017). At the same time, there are also cases where corals do not comply with this bleaching model. Some such regions have even been suggested as coral refugia, where reef biodiversity may persist through climate change (Keppel et al., 2012). An example of a proposed coral refugium is the Gulf of Aqaba (GoA) (Fine et al., 2013) in the northern Red Sea region, where, despite a local warming rate exceeding the global average (Osman et al., 2017), mass coral bleaching is absent. Multiple coral species in the GoA appear to be living at least 5°C below their bleaching threshold (Bellworthy and Fine, 2017) and, contrary to the common paradigm, after extensive experimental heat exposure of 11.2 DHW at pH 7.8, *Stylophora pistillata* fragments exhibited enhanced per-cell chlorophyll concentration, photosynthetic efficiency, as well as elevated net oxygen productivity (Krueger et al., 2017). Such physiological resistance to ocean warming and acidification (OWA) [i.e. resisting conditions beyond the most pessimistic end-of-century Representative Concentration Pathway (RCP 8.5) scenario] is unique among corals and therefore warrants further investigation (Grottoli et al., 2017; Krueger et al., 2017; Osman et al., 2017). The suggested refugium encompasses 1800 km of coastline of the northern Red Sea (Osman et al., 2017). In the central Red Sea, reefs remain vulnerable and have suffered severe bleaching (Monroe et al., 2018). It should be noted that corals in the GoA and northern Red Sea region are of course still vulnerable to local stressors, such as increased nutrient loads from agricultural run-off and aquaculture (Fishelson, 1973; Loya et al., 2004; Hall et al., 2018; Hozumi et al., 2018).

The perceived OWA resistance in this geographical region is currently primarily based on reef-scale monitoring observations and adult coral eco-physiology experiments. In order for the northern Red Sea to serve as a future climate change coral refugium, parental and trans-generational effects must not have a negative impact on

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population persistence and growth, and early life stages of corals must also be resistant to OWA, even as parental colonies become exposed to future ocean conditions. Multi-generational exposure (four generations) of the marine polychaete *Ophyrotrocha labronica* to temperatures 3°C above ambient resulted in multiple changes in the organisms, including increased reactive oxygen species production and decreased fecundity in the F5 population (Gibbin et al., 2017). Parental effects occur when the offspring's phenotypic trait(s) vary dependent upon parental condition and environment alone rather than offspring environment (Salinas et al., 2013). In the case of brooding coral species, internally brooded planulae complicate the distinction between parental effects and developmental acclimation of offspring that may occur within the parental tissues during brooding (Putnam et al., 2018preprint). Therefore, the term 'carryover effects', which could denote true parental effects and/or developmental acclimation, has recently been preferred when discussing exposure over a single reproductive period in brooding corals (Putnam et al., 2018preprint).

Although carryover effects are likely ubiquitous in nature (van den Heuvel et al., 2016), they represent a significant knowledge gap in coral ecology, not only in relation to potential refugia populations. Of the few studies that address carryover effects in corals, most use natural environmental gradients for comparative physiology studies. For example, *Porites astreoides* colonies from inshore warmer local environments produce offspring that have a higher growth rate under elevated temperature compared with those of cooler offshore origin (Kenkel et al., 2015). Similarly, *Acropora millepora* larvae showed up to 10-fold higher survival under heat stress if their parents originated from a warmer lower-latitude location (Dixon et al., 2015). Conversely, consistent gamete investment, irrespective of the parental environment, has also been demonstrated. Antioxidant defense, photosynthetic pigments and biochemical composition of eggs from *Montipora capitata* from Hawaii were not significantly different between reef sites with contrasting light and temperature regimes (Padilla-Gamiño et al., 2013), but note that the adult condition was also not different between sites in that study.

To date, only two cross-generational empirical studies have addressed the issue of climate change in corals and both were conducted on the same species and in the same location. The most recent of these showed positive effects on growth rate and survival following parental acclimation to OA in isolation (Putnam et al., 2018preprint). The second study, also using *Pocillopora damicornis* from Hawaii, exposed colonies to elevated temperature and P_{CO_2} during the larval brooding period. The resulting planulae showed metabolic acclimation to future ocean conditions compared with planulae from ambient condition colonies (Putnam and Gates, 2015). Clearly, it is crucial to consider parental effects when predicting the fate of corals under climate change.

In corals, broadcast spawning species are believed to be less likely to feature parental effects and more likely to produce offspring with greater developmental plasticity and high phenotypic variation compared with brooding species (Torda et al., 2017). This is because greater larval dispersal distances render the local parental environment less determinant (Torda et al., 2017). Thus, over multiple generations, adaptive parental effects are more likely to be observed in species with an internal brooding reproductive mode. *Stylophora pistillata* is an internally brooding species abundant on GoA reefs, releasing planulae between December and August each year (Rinkevich and Loya, 1979a,b). Its reproductive cycle (Rinkevich and Loya, 1979a,b) and adult response to OWA in field populations is well characterized in this region (Bellworthy and Fine, 2017; Krueger et al., 2017). Thirty-year records indicate that during the peak planulae release months for *S. pistillata* [March–June (Shefy et al., 2018)], the warming rate in the Eilat location has been between 0.21 and 0.36°C per decade (Krueger et al., 2017). Average local temperatures are therefore ca. 1°C higher than 30 years ago. Assuming that the reproductive timing remains the same, peak planulae release for this dominant species will occur at temperatures ca. 3°C above present-day temperatures by the end of this century. This change will expose both parents and planulae to a different thermal environment during this critical phase of the coral life cycle.

The present study, conducted in the GoA, aimed to investigate: (1) whether adult *S. pistillata* from a potential refugium population retain OWA resistance during peak brooding season, (2) whether carryover effects occur when adult *S. pistillata* are exposed to OWA during brooding and (3) whether planulae exhibit physiological resistance to OWA during early stages of development. Results may be used to more thoroughly assess the potential of the northern Red Sea as a coral reef refugium, and carryover effects in corals in general. This work may also contribute to assisted evolution projects that aim to enhance the resilience of corals to future ocean conditions (van Oppen et al., 2017).

MATERIALS AND METHODS

Experimental set-up

The experiment was conducted at the Interuniversity Institute (IUI) for Marine Sciences in Eilat, Israel. *Stylophora pistillata* Esper 1797 were sampled from the adjacent reef where, in 2017, average daily maximum temperatures during the coldest month (February) were 21.57±0.22°C and during the warmest month (August) were 27.55±0.45°C (means±s.d.; National Monitoring Program, Eilat). Annual reef seawater pH (measured monthly) averaged 8.19±0.01 (mean±s.d.; National Monitoring Program, Eilat, 2015). Colonies of *S. pistillata* ('parent'; n=19) were collected in early March 2017 from the coral nursery between 6 and 15 m water depth. Only colonies with a radius >8 cm were collected (range ca. 8–15 cm radius) to maximize the likelihood that they contained both male and female gonads (Rinkevich and Loya, 1979b). Colonies were maintained in a flow-through tank supplied with seawater directly from the collection site (temperature 21.3°C, pH 8.04, salinity 41) for 2 weeks after collection to allow for recovery prior to initiation of the experiment. All colonies were transferred into experimental aquaria with ambient water conditions (as above, n=11 total aquaria) in the Red Sea Simulator at the IUI (Bellworthy and Fine, 2018).

Ten parent colonies were assigned to the treatment condition and the remaining nine were assigned to the control condition. Colonies of different sizes were distributed equally between treatment aquaria and further acclimated for 9 days. Following aquaria acclimation, seawater pH was decreased daily by 0.1 units and the temperature simultaneously increased by 1°C per day for four consecutive days in treatment aquaria (n=6), while the remaining control parent colonies were kept at ambient seawater temperature and pH in control aquaria (n=5) for the duration of the experiment (Fig. 1). The treatment condition of ∼25°C and pH 7.7 is hereafter defined as OWA. The pH, measured on the pH_{TOTAL} scale (pH_T), was controlled by the addition of pure CO_2 into mixing tanks before flowing into experimental aquaria (for further technical details of the system, see Bellworthy and Fine, 2018). Two mixing tanks were used for the low pH treatment and two for ambient pH to reduce the pseudo-replication arising from the use of a single mixing tank. Temperature in each experimental aquarium was independently controlled by titanium heat exchangers connected to every

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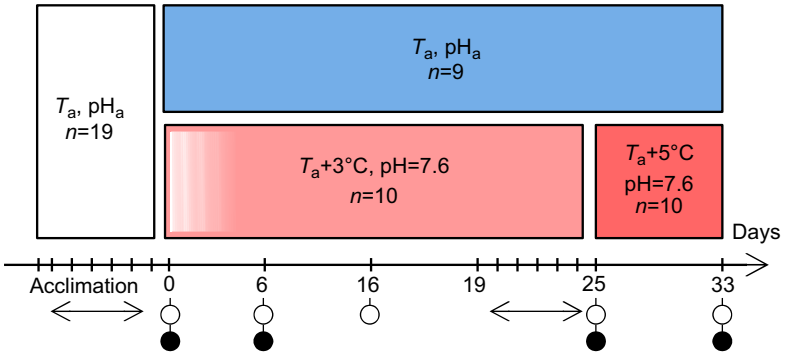

Fig. 1. Experimental treatment and sampling schematic. The horizontal time line represents experimental days. Open and filled circles indicate planulae and parent colony sampling days, respectively. *n* represents the number of parent colonies in each treatment: acclimation (white) *n*=19, ambient (blue) *n*=9, experimental (red) *n*=10. T_a, ambient seawater temperature; pH_a, ambient seawater pH. Double-headed arrows indicate nights where total planulae output was counted.

individual aquarium. Temperature and pH were manipulated at a constant offset from incoming seawater, thereby maintaining natural environmental fluctuations. Temperature and pH were monitored quasi-continuously in experimental aquaria by a sensor-carrying robot. Data were reported in real time and used to automatically adjust system settings to user-defined values (Bellworthy and Fine, 2018). On days 15 and 16 of the experiment there was a fault with the CO_2 valve in one of the mixing tanks. This meant that the pH returned close to ambient levels (up to 8.1 for ca. 24 h) in two of our six treatment aquaria and results in larger pH variation in this period (Table 1). The remaining treatment aquaria were unaffected. During this period (phase one) the mean temperatures in ambient and treatment aquaria were 21.9±0.5°C and 24.9±0.6°C, respectively. This 3°C positive temperature anomaly represents predicted end-of-century seawater temperature for the peak reproductive season based on the past 30-year record (Krueger et al., 2017). Seawater pH_T was 8.12±0.04 in ambient aquaria and 7.66±0.12 in treatment aquaria, corresponding to projected conditions just beyond the end of this century (RCP 8.5, likely range; IPCC, 2014). On day 26 (i.e. after 26 days of OWA treatment), temperature was increased by a further 2°C in treatment tanks for 7 days (phase two). In total, the experimental incubation lasted 33 days. Corals were not given supplementary food during the experiment. Maximum ambient photosynthetically active radiation (PAR) during the experimental period was 1820 µmol m^{-2} s^{-1}. An overhead shade reduced PAR levels in the aquaria to one-sixth of ambient levels (Fig. 2), corresponding to a field depth of ca. 15 m (Dishon et al., 2012).

Sampling

Corals were sampled for physiological assessments at multiple time points during the experiment (Fig. 1). In order to conduct physiological assessments on parent colonies, a 3 cm fragment was taken from each colony at each sampling point. There was no observed bleaching, disease or algae growth resulting from this sampling, and these lesions were observed to heal within 1 week. All parental colonies survived the duration of the experiment and at least 1 year beyond (after out-planting in the IUI field nursery). Planulae were collected using a Pasteur pipette between sunset and midnight, when the large majority of planulae shedding occurs (Rinkevich and Loya, 1979b). To retain planulae within experimental aquaria, a mesh cover was placed over the overflow drain (Fig. S1). In addition to on experimental days 19–25 (from 3 to 9 April), the total number of planulae released from colonies in each treatment condition was counted (Fig. 1) prior to the initiation of OWA conditions (acclimation period, from 5 to 11 March). All parent colonies released planulae during the counting periods; the number of planulae released ranged from 0 (8% of the time) to 459 from a single colony on a single night (mean±s.d. per colony/night=26±24). The first physiological sampling of both parents and planulae occurred on day 0, after acclimation to experimental aquaria and before manipulation of seawater conditions. Subsequently, parents were sampled on day 6 (once final OWA conditions were reached), day 25 (before final temperature ramping) and day 33 (after 1 week of additional temperature ramping; i.e. August peak temperatures). Planulae were collected on the same days as parent colonies, and in addition on experimental day 16. At each sampling point, planulae from all colonies in a given parent treatment were pooled. During collection and until all physiological measurements were completed, planulae were kept in seawater in the same conditions as the parent colonies. Duplicate samples of 20 planulae from each treatment pool were collected into an Eppendorf tube and stored at −80°C for later physiological analyses. Additional live planulae were used for photochemical and settlement assessments (see below).

Settlement and mortality

At each sampling point, 30 planulae from each treatment pool (*n*=10 in triplicate per treatment) were placed in a six-well plate (Fig. S1) containing conditioned terracotta ceramic tiles of similar shape and size (ca. 3 cm^2). Tiles were held in flow-through ambient seawater from the adjacent reef for 6 months prior to the experiment to allow the development of natural biofilm and crustose coralline algae settlement cues. Well plates (six-well, 15 ml water volume) were filled with water from the respective treatment aquaria, sealed with Parafilm, and held in experimental aquaria of the corresponding pH and temperature. Planulae were incubated in the same treatment

Table 1. Seawater temperature and pH_T (means±s.d.) in experimental aquaria during different phases of the experiment

Variable	Acclimation	Phase one	Phase two
Control temperature	21.3±1.0°C (204)	21.9±0.5 (1267)	22.0±0.8°C (525)
Treatment temperature	21.7±0.4°C (802)	24.9±0.6°C (139,060)	26.6±1.1°C (42,839)
Control pH_T	8.04±0.06 (207)	8.12±0.04 (1210)	8.15±0.04 (399)
Treatment pH_T	8.03±0.04 (194)	7.66±0.12 (1232)	7.71±0.18 (463)

Acclimation period: 9 days, before adjustment of the conditions; phase one: treatment phase excluding temperature ramping period, days 7–25; phase two: elevated treatment phase, days 26–33. Numbers in brackets indicate *n* measurements. (The number of measurements in each treatment is not identical because the software records a data point only when a >0.01 value change is detected.)

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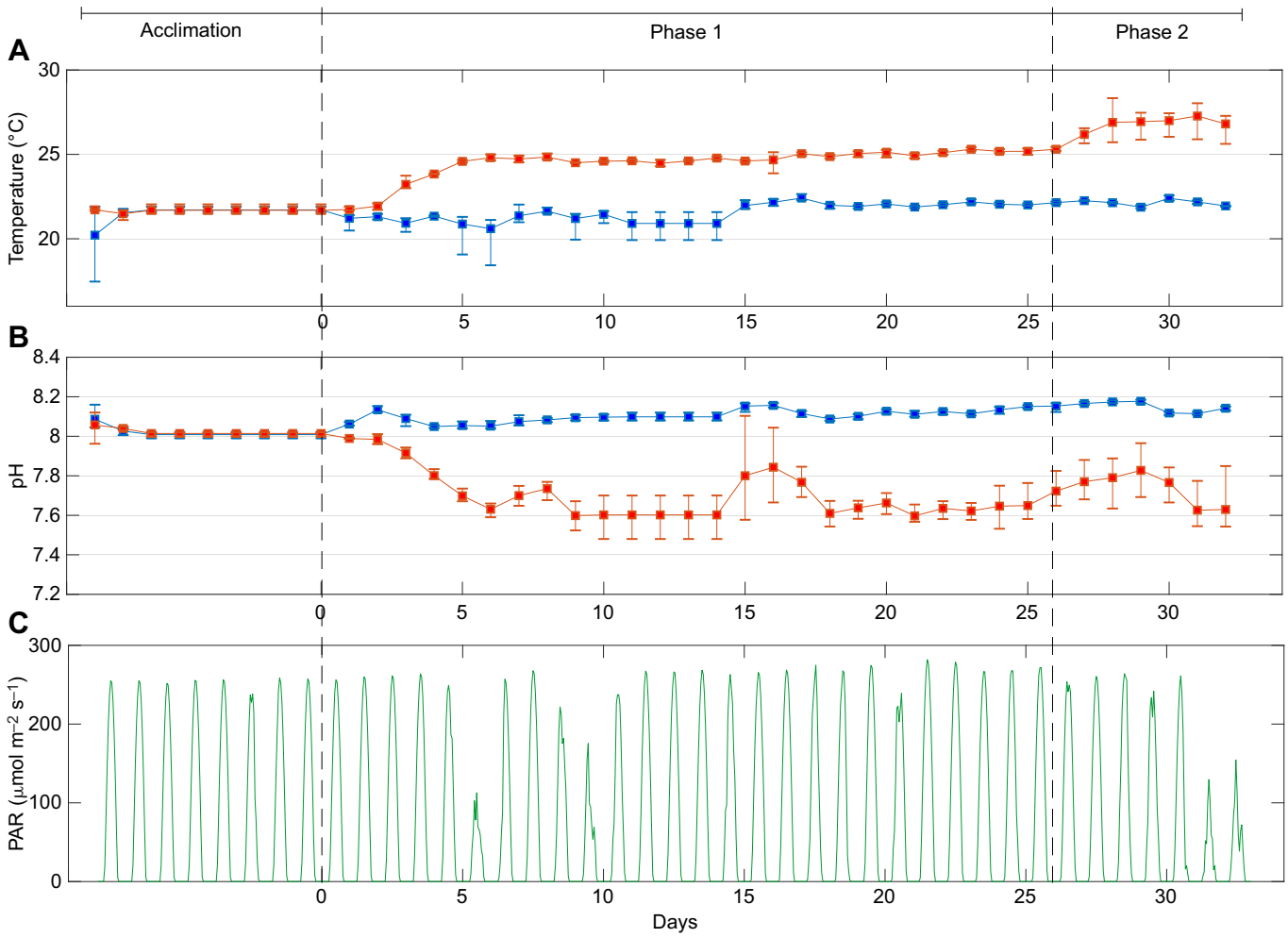

Fig. 2. Abiotic conditions in aquaria during the acclimation and experimental phases. (A) Mean±s.e.m. daily aquaria temperature, (B) mean±s.e.m. aquaria pH and (C) photosynthetically active radiation (PAR) at aquaria surface. Blue points represent ambient control aquaria and red points represent treatment aquaria. Phases of the experiment are indicated by the upper *x*-axis and separated by a dashed vertical line: tank 'acclimation' phase in ambient seawater, 'phase one' treatment parent colonies (n=10) at +3°C and pH 7.6, and 'phase two' treatment parent colonies (n=10) at +5°C and pH 7.6.

conditions as their parent colonies; this experimental design allows the assessment of carryover effects (treatment-induced changes in offspring), but a full factorial design would be required to assess whether these are adaptive parental effects (Uller et al., 2013). In order to assess the average time to settlement and percentage mortality, the number of planulae swimming and settled (post metamorphosis) were counted at 12, 24 and 36 h post-release. Because dead planulae degrade quickly, those absent from the count were recorded as dead (Graham et al., 2008) whilst those still visible but unattached were assumed alive ('swimming') but not settled. A two-thirds water change to the wells was performed at each monitoring point. Change in seawater pH between these points was <0.05 units. Based on published respiration rates for *S. pistillata* (Titlyanov et al., 1998; Edmunds et al., 2011), we estimate that there is likely to have been <3% change in oxygen saturation between water changes (12 h).

Physiological analyses

After 15 min dark acclimation, photochemical parameters were measured using an Imaging Pulse Amplitude Modulation fluorometer (PAM, Walz GmbH, Germany). For parent colonies, the Maxi version was employed to generate rapid light curves (RLCs; measuring intensity 2, frequency 1, saturating intensity 9, gain 2; 13 steps up to 700 µmol m^{-2} s^{-1}, 20 s time interval between light pulses). The Microscopy PAM version was used to measure RLCs for planulae (n=10 planulae/treatment/sampling time: measuring intensity 5, measuring frequency 8, saturating intensity 9, gain 12; eight steps up to 200 µmol m^{-2} s^{-1}, 20 s time interval between light pulses). Measurements were performed mid-morning for parent colonies and between 20:00 and 23:00 h for newly shed planulae. Maximal dark-adapted photochemical efficiency (F_v/F_m) was recorded to assess any changes in quantum efficiency as an indication of stress resulting in photoinhibition. Light curve data were processed using SigmaPlot v. 12.3 to extract three parameters describing the shape of the curve: relative initial photosynthetic rate (α_r; indicates the corals' ability to maximize yield before the onset of saturation), relative maximum electron transport rate through photosystem II ($rETR_{max}$) and compensation point (I_k; i.e. $rETR_{max}$ divided by α_r; gives an indication of the irradiance at which absorbed quanta become dissipated through non-photochemical quenching), from the general equation of Ralph and Gademann (2005). Substantial reductions in these parameters are indications that the algae are experiencing stress, leading to modifications in quantum pathways and efficiency, resulting in reduced energetic transfer to the coral (Maxwell and Johnson, 2000; Hill et al., 2004). After photochemical measurements, all fragments were frozen at −80°C pending further analysis.

Tissue was removed from frozen parent fragments using an airbrush and ice-cold buffer solution (100 mmol l^{-1} sodium phosphate, 0.1 mmol l^{-1} EDTA, at pH 7.0, 4°C). Skeletons were retained for surface area determination using the wax dip technique (Stimson and Kinzie, 1991). Tissue samples of both parents and planulae were electrically homogenized for 30 s (DIAX 100, Heidolph Instruments, Germany). Samples were centrifuged to separate host and symbiont tissues. A sub-sample (100 µl) of the supernatant was taken to determine host protein concentration by a colorimetric method (Bradford, 1976) using a multi-scan spectrum spectrophotometer (595 nm, 450 nm, Biotek HT Synergy plate reader) and bovine serum albumin (BSA) as a standard (Zor and Seliger, 1996). Protein concentration was used as a biomass and normalization index for the coral fragments and planulae. Further centrifugation and washing with filtered seawater was performed to isolate symbiont cells for cell counts (hemocytometer or flow cytometer) and photosynthetic pigment extraction; a reduction in areal cell number or cell pigmentation is diagnostic of bleaching. Pigments were extracted for 24 h in 100% acetone at 4°C in the dark and chlorophyll (chl) *a* and c_2 concentrations were determined using a spectrophotometer (Ultrospec 2100 pro) following the equations of Jeffrey and Humphrey (1975).

Statistical analyses

All parent data were analyzed with a linear mixed model with a restricted maximum-likelihood approach (REML) using time, treatment and their interactions as fixed factors and replicate ID as random factor. Time was treated as an ordinal factor. Non-significant interaction terms were removed to create the minimal adequate model. Raw data were transformed if necessary to achieve a normal distribution as tested with a Shapiro–Wilk test. *Post hoc* results are derived from Tukey's HSD test. Planulae data were analyzed with a two-way ANOVA, except for the planulae PAM data. This dataset was analyzed via univariate non-parametric Wilcoxon/Kruskal–Wallis tests for each factor, because normality could not be achieved for a multivariate parametric test. *Post hoc* results for the time factor are based on non-parametric comparisons for each pair using the Wilcoxon method. Human error meant that on each sampling day, the replicate sample number for symbiont density cell counts was reduced to two for one treatment. Therefore, although some data relating to this parameter are presented, statistical analyses could not be conducted. All analysis was performed with the software JMP v.11.2.1 (SAS Institute, Cary, NC, USA).

The number of planulae released was summed for each treatment and this number was compared between the two discrete counting periods. Data were square-root transformed. A mixed model was applied with month and treatment set as nominal factors, and aquarium number as a random factor.

RESULTS

Treatment response

Parent colonies

All photochemical parameters significantly decreased with time from the first to the last sampling (F_v/F_m=6.7%, $rETR_{max}$=72%, α_r=6.6%, I_k=69.5%; Table 2, Fig. 3). None of these parameters were significantly impacted by treatment as a main factor. OWA treatment significantly affected parent colonies on day 25, in the form of decreased F_v/F_m, I_k and $rETR_{max}$, compared with ambient colonies (Fig. 3). These interactions of time and treatment resulted in significant interdependence in all photochemical parameters except α_r. The total number of planulae released significantly increased ($F_{1,9}$=10.061, P=0.0113) between the first counting period in March (131±132 planulae per aquaria) and April (490±431 planulae per aquaria). This increase occurred independent of treatment (ambient: 309±363 planulae per aquaria; treatment: 313±379 planulae per aquaria; $F_{1,9}$=0.013, P=0.91). Symbiont cell abundance normalized to surface area was significantly lower in the OWA treatment (22% lower, $F_{3,53,65}$=4.394, P=0.01) and displayed a 36% decrease with time across all colonies ($F_{1,17,29}$=6.903, P=0.02; Table 2, Fig. 3). Chlorophyll concentration did not statistically change with time (day 0: 7.52±1.02 pg total chl $cell^{-1}$; day 33: 5.63±0.60 pg total chl $cell^{-1}$) or between treatments (ambient: 6.72±1.11 pg total chl $cell^{-1}$; OWA: 7.49±1.36 pg total chl $cell^{-1}$; Table 2, Fig. 3). Host protein concentration also did not significantly change with time (day 0: 0.90±0.07 mg protein cm^{-2}; day 33: 0.91±0.10 mg protein cm^{-2}) or between treatments (ambient: 0.91±0.12 mg protein cm^{-2}; OWA:

Table 2. Statistical output of parent colony physiology parameters

Parameter	Time	Treatment	Time×Treatment	Transformation
F_v/F_m	$F_{3,51}$=25.375 P<0.0001*	$F_{1,67.81}$=0.017 P=0.8973	$F_{3,51}$=3.655 P=0.0183*	–
$rETR_{max}$	$F_{3,51}$=157.833 P<0.0001*	$F_{1,61.87}$=0.873 P=0.3537	$F_{3,51}$=4.246 P=0.0094*	3rd root
α_r	$F_{3,54}$=8.990 P<0.0001*	$F_{1,17}$=4.301 P=0.0536	N/A	–
I_k	$F_{3,51}$=254.209 P<0.0001*	$F_{1,58.29}$=0.767 P=0.3848	$F_{3,51}$=7.141 P=0.0004*	Square root
Planulae output	$F_{1,9}$=10.061 P=0.0113*	$F_{1,9}$=0.0132 P=0.9110	$F_{1,9}$=0.2345 P=0.6387	Square root
Symbiont cm^{-2}	$F_{3,53.65}$=4.394 P=0.0078*	$F_{1,17.29}$=6.903 P=0.0175*	N/A	3rd root
Host protein cm^{-2}	$F_{3,51.29}$=0.675 P=0.5712	$F_{1,15.49}$=1.262 P=0.2785	N/A	3rd root
Total chl/symbiont	$F_{3,53.23}$=2.353 P=0.0825	$F_{1,17.04}$=0.5153 P=0.4826	N/A	3rd root
Chl *a*/chl c_2	$F_{3,48.56}$=1.637 P=0.1930	$F_{1,64.17}$=0.545 P=0.4633	$F_{3,48.56}$=4.874 P=0.0048*	–

Sample *n* is 8–10 per time and treatment. Asterisks indicate statistically significant results (*P≤0.05). N/A denotes the removal of non-significant interaction terms for a minimal adequate model. In the case of planulae output, time represents the two counting periods in March and April. F_v/F_m, maximal photochemical efficiency; $rETR_{max}$, relative maximum electron transport rate; α_r, initial photosynthetic rate under light-limited conditions; I_k, compensation point; chl, chlorophyll.

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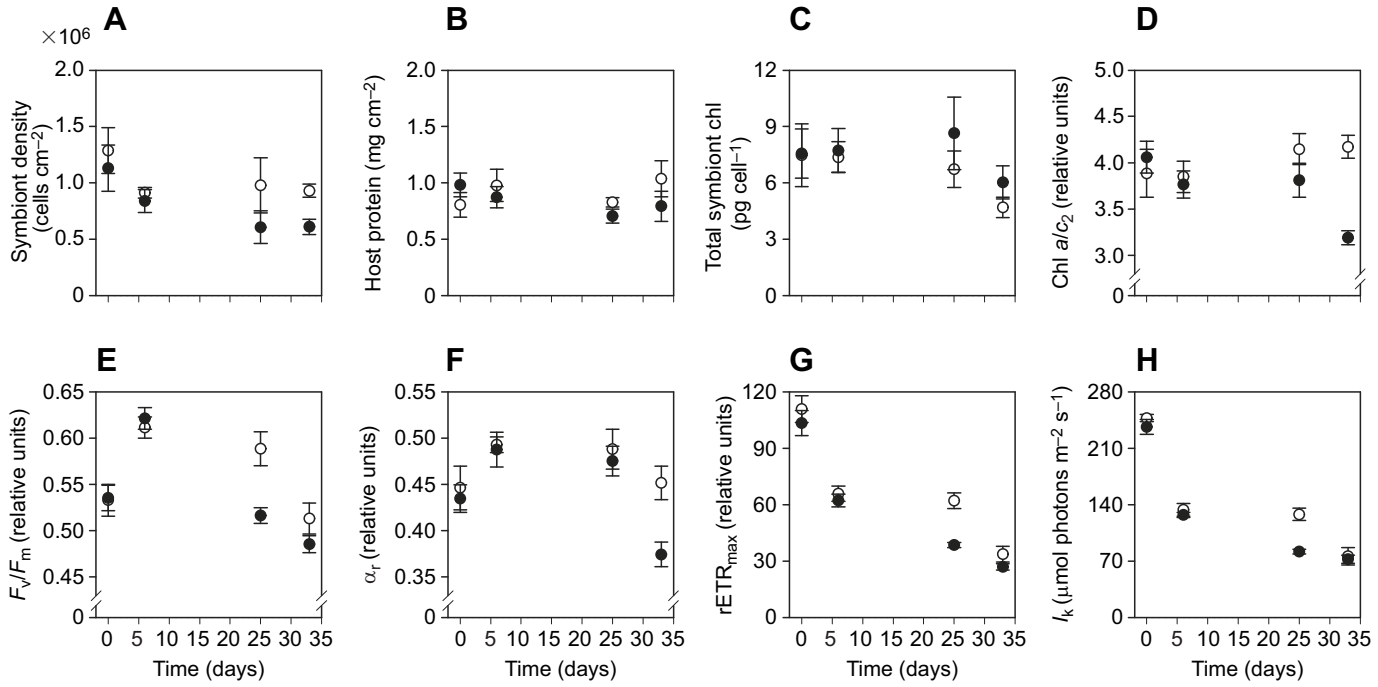

Fig. 3. Physiological parameters repeatedly measured in all parent colonies on each sampling day (days 0, 6, 25 and 33). Data are means±s.e.m., *n*=8–10 colonies. Open circles represent ambient control aquaria and closed circles represent treatment aquaria. (A) Symbiont cell density, (B) host protein concentration, (C) total symbiont chlorophyll (chl *a* plus chl c_2), (D) ratio of chl *a* to chl c_2, (E) maximal dark-adapted photochemical efficiency (F_v/F_m), (F) initial photosynthetic rate under light-limited conditions (α_r), (G) relative maximum electron transport rate ($rETR_{max}$) and (H) compensation point (I_k).

0.84±0.10 mg protein cm^{-2}). There were no significant effects on host protein content or symbiont chlorophyll content. The ratio of chl *a*:c_2 was significantly lower in OWA colonies compared with ambient colonies on day 33 (Fig. 3).

Planulae

Maximal photochemical efficiency of planulae only varied significantly between OWA and ambient treatments on days 6 and 25 (Fig. 4, Table 3). There were no other treatment effects on the remaining photosynthetic parameters assessed in the planulae. Individual planula protein content decreased with time, but this was a consistent and not significantly different trend in both treatments (ambient: 27% decline; OWA: 36% decline). The total chlorophyll concentration normalized to symbiont cell number (total chl per cell: ambient: 8.7±0.5 pg; OWA: 8.9±0.2 pg; time factor: ambient: 5.9% increase; OWA: 19.2% increase) and the ratio of chl *a*:c_2 pigments did not appear to vary with treatment or time (chl *a*:c_2: ambient: 3.4±0.2; OWA: 3.3±0.1; time factor: ambient: 10.2% increase; OWA: 11.2% increase), but replicate numbers were low for these data sets. Symbiont cell number per planula was not significantly different between treatments (ambient: 5135±505; OWA: 6285±6678) or through time (ambient: 16% decline; OWA: 28% decline).

Time to settlement was not significantly different between treatments ($F_{1,28}$=1.962, P=0.1328) or over time ($F_{4,25}$=0.534, P=0.47). Including all time points and treatments, planulae settled in an average time of 16 h 20 min, although many were observed to have settlement competency immediately upon release and 22% were still swimming at the end of the 36 h incubation. Sixty-one percent of planulae settled within 36 h in the ambient treatment and 66% in the OWA treatment. Settlement most often occurred on the ceramic tile, with 16% of total settlement also occurring on the well plate. Of those that settled during the observation period (36 h), the majority of planulae settled within the first 12 h (83%). Average mortality was significantly higher on day 16 (56.6±3.3%) compared with day 0 in the OWA treatment (0.0±0.0%), but this was the only treatment effect upon settlement and mortality (Fig. 5). Because planulae mortality was not further increased on later sampling days 25 and 33, we are currently unable to resolve the reason for this observation with a supported hypothesis. The majority of total mortality occurred within the first 12 h post-release (51%), with the percentage of new mortalities declining at 24 h (30%) and 36 h (19%).

DISCUSSION

Following 5 weeks of exposure to present-day ambient and future OWA conditions, brooding colonies and planulae of *S. pistillata* from the GoA exhibited few significant sub-lethal treatment-induced differences. Thus, it has been shown that at predicted end-of-century temperature and acidification levels, carryover effects are not sufficiently strong to cause significant changes in GoA *S. pistillata* planulae. The data also show, for the first time, that these planulae have physiological tolerance to OWA. Therefore, at two short but potentially critical bottleneck life stages in terms of population number and recruitment success – namely, reproduction and juvenile development (Donelson et al., 2018; Richmond et al., 2018) – *S. pistillata* in the GoA appears to be resistant to OWA conditions predicted to occur at the start of the next century (Krueger et al., 2017).

Parent colony response

OWA conditions caused a significant treatment effect in parent colonies' photochemistry only on day 25 (time×treatment effect), namely, 12%, 36% and 38% drops in F_v/F_m, I_k and $rETR_{max}$, respectively, whereas these parameters remained constant in corals maintained under ambient conditions between days 0 and 25

Journal of Experimental Biology (2019) 222, jeb186940. doi:10.1242/jeb.186940

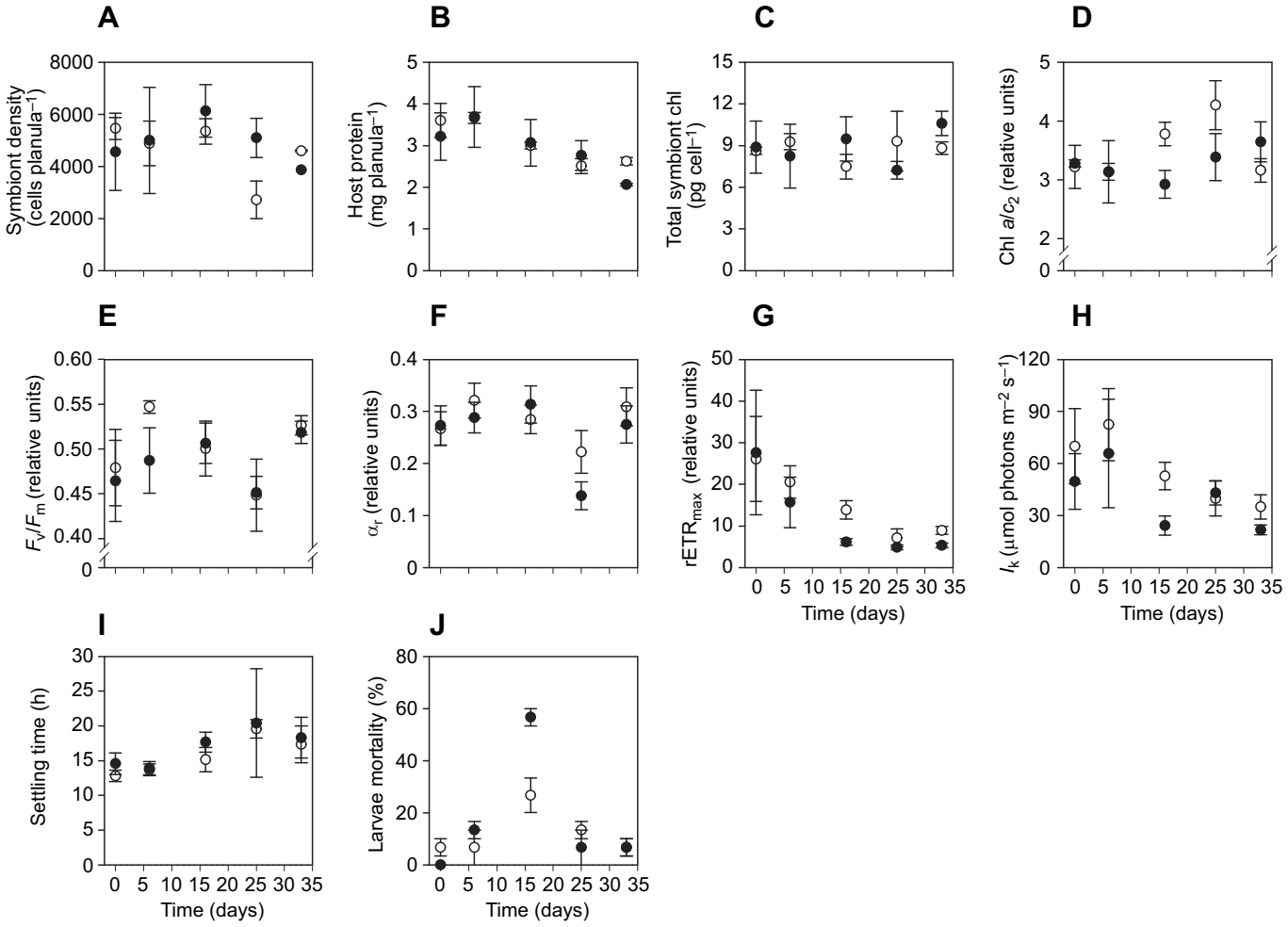

Fig. 4. Physiological parameters measured on newly released planulae on each sampling day (days 0, 6, 16, 25 and 33). Open and closed circles represent planulae from parents in ambient control and treatment aquaria, respectively. Data are means±s.e.m., n=2–3. (A) symbiont cell density, (B) host protein concentration, (C) total symbiont chlorophyll (chl *a* plus chl c_2), (D) ratio of chl *a* to chl c_2, (E) maximal dark-adapted photochemical efficiency (F_v/F_m), (F) initial photosynthetic rate under light-limited conditions (α_r), (G) relative maximum electron transport rate (rETR$_{max}$), (H) compensation point (I_k), (I) time to settlement (h) and (J) percentage total mortality 36 h post-release.

(Fig. 3). If declines in these parameters were to persist as a result of OWA, the likely result would be decreased energy available to the coral colony, leading to overall reductions in growth and reproductive output. However, this treatment difference is lost on day 33 because ambient colonies also decline to the same levels as the OWA colonies (Fig. 3), indicating photoacclimation. This change, dependent on the experimental day, serves to highlight the importance of multiple sampling time points in manipulative studies. An experiment of similar duration with another pocilloporid species from Hawaii (*Pocillopora damicornis*) reported a significant reduction of approximately 25% in F_v/F_m after 45 days of treatment during the brooding phase (Putnam and Gates, 2015). The former experiment used seawater pH (7.78±0.02) similar to that in the present study and elevated temperature by 2.4°C. After 33 days in our study, the temperature difference was 5°C and significant treatment differences in F_v/F_m were absent, attesting to the relative resistance of this pocilloporid GoA coral to OWA. Furthermore, changes to planulae output, protein and chlorophyll concentrations were not significantly dependent upon treatment effect alone.

Jiang et al. (2018) conducted a thermal stress test on coral planulae from *P. damicornis* where the experimental temperatures were 1.8°C above ambient at the time of the experiment but within the considerable annual range (19.9–33.4°C). A positive growth response to increased temperature was suggested to result from existing phenotypic plasticity owing to variable field conditions (Jiang et al., 2018). In the present experiment, treatment temperatures were also within the annual temperature range for the GoA, and it could be considered that both adult corals and planulae (see below) were already adapted to these temperatures, although they exhibit physiological resistance rather than plasticity. Annual temperature in the GoA ranges from ca. 21 to 28°C, and daily temperatures at the collection site can vary by more than 1°C (Fig. S1). Whilst recent high-resolution *in situ* pH data are lacking for the GoA (although see Silverman et al., 2007), values of 7.6 as used in the present experiment are likely beyond the present typical range (Fig. S2; Silverman et al., 2007), reinforcing the conclusion of OWA resistance and refugium potential.

At the termination of the present experiment (day 33), chl a:c_2 and α_r were lower in the OWA treatment, yet the mechanism as to how OWA impacts these parameters is unknown. In addition, symbiont density was significantly lower (by 27%) in the OWA treatment on day 33 compared with ambient conditions (Fig. 3). This may be a sign of initial bleaching or continuing acclimation; a

Journal of Experimental Biology (2019) 222, jeb186940. doi:10.1242/jeb.186940

Table 3. Statistical outputs of planulae physiology parameters

Parameter	Time	Treatment	Time×Treatment
F_v/F_m	X^2 (4, N=98)=14.636 P=0.0055*	X^2 (1, N=98)=3.055 P=0.0805	N/A
$rETR_{max}$	X^2 (4, N=98)=18.209 P=0.0011*	X^2 (1, N=98)=9.796 P=0.0017*	N/A
α_r	X^2 (4, N=98)=13.748 P=0.0081*	X^2 (1, N=98)=1.260 P=0.2616	N/A
I_k	X^2 (4, N=96)=3.729 P=0.4440	X^2 (1, N=96)=5.464 P=0.0194*	N/A
Host protein/planula	$F_{4,25}$=4.348 P=0.0087*	$F_{1,28}$=0.301 P=0.5883	N/A
Total chl/cell	$F_{3,20}$=0.421 P=0.7402	$F_{1,22}$=0.029‡ P=0.8675	N/A
Chl *a*/chl c_2	$F_{3,20}$=1.527 P=0.2316	$F_{1,22}$=1.309‡ P=0.3007	N/A
Time to settlement	$F_{4,25}$=0.534 P=0.4720	$F_{1,28}$=1.962 P=0.1328	N/A
Mortality	$F_{4,4}$=24.111 P≤0.0001*	$F_{1,1}$=1.111 P=0.3044	$F_{4,4}$=5.778 P=0.0029*

Asterisks indicate statistically significant results (*P≤0.05). N/A denotes where non-significant interaction terms were removed to create the minimal adequate model. No data transformations were required. F_v/F_m, maximal photochemical efficiency; $rETR_{max}$, relative maximum electron transport rate; α_r, initial photosynthetic rate under light limited conditions; I_k, compensation point; chl, chlorophyll; mortality, average number of planulae dead after 36 h incubation. ‡Day 0 excluded from the analysis owing to low *n*.

longer experiment would be required to resolve this point. Significantly reduced symbiont density contrasts with other 4-week experiments on this species from the GoA, which have not shown significant effects of up to 6°C warming on adult coral physiology (Bellworthy and Fine, 2017). Additionally, a previous 6-week OWA combination treatment (2°C above long-term summer maximum, pH 7.8) during peak reproductive season did not alter symbiont density or F_v/F_m of the same GoA species (Krueger et al., 2017). Therefore, the observed photochemical and symbiont density changes in this case more likely represent positive physiological acclimation to seawater temperature increase (Suggett and Smith, 2011). Nevertheless, if decreasing cell density represents the onset of bleaching here, it may have significant impacts on reproductive output (Baird and Marshall, 2002) seen only with multi-year experiments. Furthermore, there are other fundamental physiological processes in corals such as nutrient uptake (Godinot et al., 2011), calcification and respiration, as well as changes in gene expression (Kaniewska et al., 2012; Kurihara et al., 2018), that may significantly impact corals under OWA, yet these were not measured in the present study.

Planulae response

At no time point did we observe significant treatment-dependent differences in planulae physiology or time to settlement. This contrasts with *Porites astreoides* planulae from Florida for which elevated temperature (33°C) significantly increased the median number of planulae that metamorphosed within 24 h post-release from 0% to 7% (Edmunds et al., 2001). Reduced time to settlement would reduce reef connectivity because planulae would settle closer to their origin (Figueiredo et al., 2014). In the present study, average time to settlement was not significantly affected by OWA. There was a trend towards increased average time to settlement towards the end of the experiment, but this was not significant and occurred in both treatments (Fig. 4), suggesting this is a natural trend towards the seasonal peak. In contrast to modeling efforts (Figueiredo et al., 2014), the lack of treatment-induced changes implies that reef connectivity would not change as a result of future OWA. The range of connectivity of the GoA refugium to more southern reefs specifically warrants further research.

During phase two, under more extreme OWA conditions, trends towards decreased protein and symbiont cell number per planula

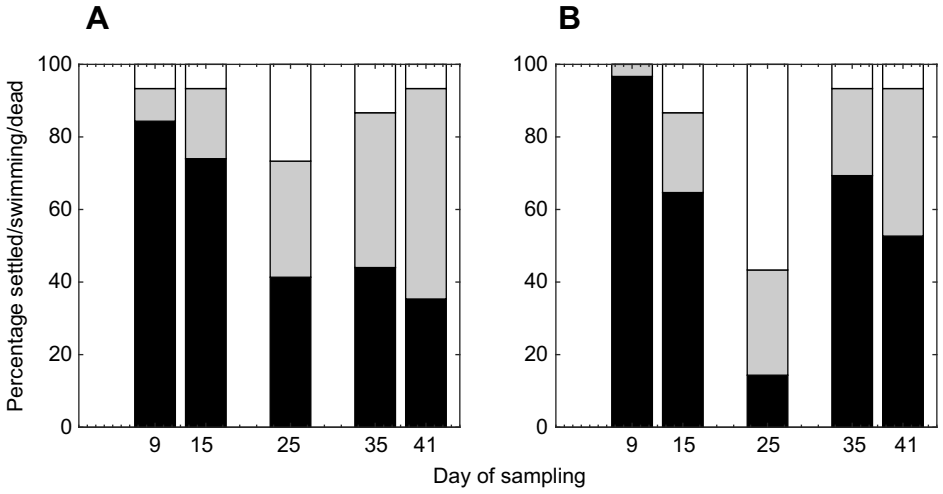

Fig. 5. Total cumulative percentage of planulae settled (black), swimming (gray) and absent/dead (white) after 36 h incubation immediately upon release. (A) Ambient control; (B) ocean warming and acidification treatment. Data represent total % from three replicates per time point.

Journal of Experimental Biology (2019) 222, jeb186940. doi:10.1242/jeb.186940

together with lowered $rETR_{max}$ and I_k can be seen in Fig. 4. Although these results are not significant, longer exposure to OWA may increase the magnitude of these differences so that they become biologically relevant with downstream impacts on early development such as linear growth or polyp budding, as seen in *P. damicornis* from Luhuitou reef, South China Sea (Jiang et al., 2018). On the time scale of the present experiment, retention of planulae size (protein concentration), symbiont cell number and pigment concentration indicates sustained parental investment under OWA. Changes in parental investment in terms of the aforementioned resources have been found in response to environmental conditions in other species. For example, eggs produced by the coral reef damselfish *Acanthochromis polyacanthus* were 19% smaller and significantly shorter and lighter at hatching when the parents were maintained at +3°C above ambient compared with the control (Donelson et al., 2018). Despite the few statistically significant results, the current data do indicate initial changes that may decrease parental energy budget if they persisted, i.e. OWA decreased symbiont density and F_v/F_m, which would eventually reduce the energy available for reproduction and therefore parental investment.

In this experiment, the general lack of systematic and significant treatment effects in planulae leads to the conclusion that the coral planulae did not show physiological plasticity and essentially resisted 'stressful' OWA conditions. A lack of significant physiological changes in response to future ocean conditions has previously been shown in adult corals in the GoA (Fine et al., 2013; Bellworthy and Fine, 2017). However, this is the first empirical evidence that planulae also display similar environmental robustness. The average treatment seawater temperature during phase two (26.6±1.1°C) exposed corals to temperatures highly uncharacteristic for the peak reproductive period and spring season when the experiment was conducted. Even though few *S. pistillata* planulae are still released at these temperatures in July in the GoA (Shefy et al., 2018), the recruitment success during the summer is as yet unknown and the shift in thermal regime may yet prove to be detrimental. Therefore, although the null results of this study further highlight the resistance of GoA corals to acute OWA, the chronic and post-settlement impacts of environmental change may play a significant role in population dynamics. For example, *P. astreoides* planulae from the Caribbean settled under heat stress had significantly higher post-settlement mortality when returned to ambient conditions compared with control planulae (Ross et al., 2013). This result occurred despite no initial effect of warmer temperatures on metamorphosis, photochemical efficiency or pre-settlement survival (Ross et al., 2013). In addition, slower linear extension under reduced pH puts new coral recruits at higher predation risk for longer until they move beyond particular size thresholds, indirectly increasing mortality as a result of OA (Doropoulos et al., 2012).

It is important to assess the range of phenotypes produced, but such comparisons are difficult because most studies only report the mean phenotype (Donelson et al., 2018). Variation in planulae physiological parameters ranged from 2- to 6-fold in this study. For example, chl *a* per symbiont cell ranged from 4.38 to 12.64 pg, a 2.9-fold difference. In addition, symbiont cell counts varied 5.7-fold and F_v/F_m varied 3.8-fold. This observation indicates the use of a bet-hedging strategy, i.e. producing offspring with various phenotypes to increase likelihood of survival and recruitment in an unpredictable environment (Crean and Marshall, 2009; Chamberland et al., 2017), as has been reported for other coral species (Gaither and Rowan, 2010; Cumbo et al., 2013). Specifically, *S. pistillata* in the GoA is found across a large depth range from 0 to 70 m and behaves like an opportunistic 'r-selected' species, colonizing environments where other coral species do not (Loya, 1976). Furthermore, the GoA is a high-latitude reef with a ca. 7°C annual temperature range, and planulae are released across almost this entire temperature gradient. Therefore, it is hypothesized that *S. pistillata* uses bet-hedging, producing a wide range of offspring phenotypes to minimize the risks associated with an unpredictable environment. This may, in part, contribute to the success of this species in this region in terms of abundance and environmental plasticity.

Carryover effects of OWA

Owing to the lack of treatment-induced changes in planulae physiology, recruitment and reproductive output, carryover effects were absent in this study for the assessed biological parameters. Therefore, it can be postulated that these parameters will not contribute significant population bottlenecks (number of planulae and recruits) under OWA in this region. However, it must be borne in mind that these processes can be affected by a number of other factors expected to change in future oceans. For example, the chemical cues required for metamorphosis may be blocked by sedimentation, and reduced water quality (pollutants) can impact reproductive and recruitment success (Richmond et al., 2018 and references within). It is therefore vital to remove local-scale impacts in order to enable the maintenance of recruitment rates under OWA. Furthermore, in order to draw well-informed conclusions concerning carryover effects and potential bottleneck stages, future experiments should include survivorship in later life stages, time to sexual maturity, growth rate, and reproduction and fecundity in the subsequent generation(s).

Adaptive parental effects are more likely to occur where the parental environment is well correlated with the offspring environment (Marshall and Uller, 2007; Burgess and Marshall, 2011, 2014; Uller et al., 2013). For offspring likely to disperse into a considerably different environment, high phenotypic diversity between individuals is more likely to be selected for ('bet-hedging'; Burgess and Marshall, 2014). This is particularly relevant in the Red Sea, where the latitudinal temperature gradient is steep. For the present study, seawater pH and temperature data from the time of parent colony collection are known (Fig. S2). However, it is acknowledged that, at present, model predictions of coral planulae dispersal ranges are inaccurate (Bode et al., 2018). Therefore, understanding the autocorrelation between parental and coral planulae environments poses a severe challenge for which more data pertaining to the biophysical characteristics of planulae and hydrodynamic models must first be acquired (Bode et al., 2018). This knowledge may then be incorporated into dispersal models, which will also be of high importance in identifying other climate change reef refugia.

In contrast, it has also been suggested that acute environmental perturbations are unlikely to result in parental effects (Galloway, 2005). Such unpredictable, random events are unlikely to select for phenotypic change in offspring (Galloway, 2005) because altering offspring phenotype to increase fitness during acute environmental changes may well have a negative effect when the former environment returns (Galloway, 2005). This may, in part, explain the lack of carryover effects in the present study because increased temperature and lowered pH_T would appear as a relatively short-term, anomalous event. Alternatively, it can also be suggested that because the current *S. pistillata* phenotype persists under this level of OWA, there is no demand to alter planulae phenotype. It is

therefore required that experiments with long-term (years) acclimation to future ocean conditions become a research priority. With ongoing climate change, increased frequency of marine heat waves (Frölicher et al., 2018) and decreasing global pH (IPCC, 2014), the cue for parental effects in successive generations may present itself.

Temporal changes

Significant changes in physiology were measured with time through the experiment, particularly in the photochemistry. These changes occurred in both treatment and ambient conditions and therefore are not OWA effects. Although not measured, it is assumed that the shallow aquaria had higher UV levels compared with the collection depth. The corals appear to continuously acclimate to the higher UV level during the experiment, resulting in the photochemical changes through time. In order to avoid this confounding influence, longer acclimation periods are required than used here (9 days). However, isolating brooding colonies long term in individual experimental aquaria will likely lead to decreased planulae number (decreased output and reduction in experimental material) and/or a concurrent increase in self-fertilization.

Increased number of planulae coincided with progression towards peak release season in field colonies (Shefy et al., 2018). Typical seasonal increase was also observed under future ocean conditions and observed in a previous ocean acidification study on this species (Grinblat et al., 2018). In the present study, there was an associated significant decline in per planulae protein content towards the timing of peak planulae production. Assuming that biochemical composition of planulae is consistent across seasons, as has been reported for the soft coral *Heteroxenia fuscescens* (Ben-David-Zaslow and Benayahu, 2000), this represents a decrease in per offspring investment. In the present study, declining protein content did not affect settlement success or survival rates. However, owing to reduced reserves, smaller *S. pistillata* planulae have a shorter pelagic survival time (Isomura and Nishihira, 2001), and this may thereby lead to more localized planulae settlement during the peak of the season. The full seasonal change in planulae biochemistry and settlement behavior is yet to be investigated in this species, which releases planulae from December to August, encompassing the full ambient temperature in the GoA.

Release-day-dependent differences in individual growth rate and organic content have been reported in the barnacle *Semibalanus balanoides* (Jarrett, 2003). In addition, release-day-dependent differences in mortality, F_v/F_m and respiration rates in response to elevated P_{CO_2} have been observed in coral planulae (Cumbo et al., 2013). We suggest that this would be a fruitful direction for future research, elucidating the energetic demands required to reproduce in this way.

A note on experimental design

Cross-generational experiments are not new to ecology, but they are relatively new in coral species. As such, the experimental design implemented should face strict standardization (Donelson et al., 2018; Torda et al., 2017). Firstly, in order to make inferences about population trends, measures of absolute and relative fitness are needed (Burgess and Marshall, 2011). Furthermore, directional maternal effects are more strongly selected for when parental colonies are able to predict the offspring environment (Torda et al., 2017); where the environment is variable, producing a range of phenotypes (bet-hedging) instead may increase fitness (Burgess and Marshall, 2014). In order to better predict parental/carryover effects, the correlation between the parental and offspring environment should be known (Burgess and Marshall, 2011). For coral studies, this not only means the collection of *in situ* data, but also first requires improving the accuracy of dispersal models (Bode et al., 2018).

Typically (present study included), coral planulae are pooled from multiple parental genotypes (Putnam and Gates, 2015; Ritson-Williams et al., 2016; Jiang et al., 2018; Bergman et al., 2018; Puisay et al., 2018). This not only removes the direct traceability of parental origin and therefore colony condition, but also may result in significant cohort differences with time or treatment simply because different parent colonies contribute more or less planulae at discrete sampling points. The reasons for pooling planulae are likely due to the variable, unpredictable and limited planulae output. Possible solutions include increasing the size and number of parent colonies sampled and thereby increasing the likelihood that enough colonies will produce sufficient planulae for all analyses. In addition, it may be necessary to increase the number of sampling nights in order to achieve the required level of reproducibility (n). This suggestion is, however, complicated by studies that conclude there are significant differences in planulae phenotype on consecutive release nights (e.g. Cumbo et al., 2013).

One fundamental caveat remains for many biochemical measurements of coral planulae and gametes: current methods often do not permit the physiological assessment of single larvae and thus do not report the true range of individual physiological plasticity. For most studies, assessed biochemical variables consequently represent averaged values (e.g. n=10 in this study) and will thus be already closer to the overall population mean, limiting the assessment of the full range of individual responses.

Conclusions

This experiment did not find significant positive (i.e. acclimation) or negative carryover effects in the coral *S. pistillata* in the GoA under end-of-century predicted levels of OWA. Furthermore, planulae from this population showed similar physiology irrespective of the environment, with a wide phenotypic range within a single cohort. Specifically, *S. pistillata* planulae were resistant in terms of their settlement, physiology and survival, even when parents were exposed to severe OWA during the gamete maturation, fertilization and brooding period. The findings are likely an indirect result of the selection process that took place in the warm Southern Red Sea when corals repopulated the region after the last glacial maximum. Our findings therefore add support to the emerging hypothesis that the GoA may serve as a climate change coral refuge (Fine et al., 2013).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.B., T.K., M.F.; Methodology: J.B., T.K.; Formal analysis: J.B., M.M., T.K.; Investigation: J.B., M.M.; Resources: M.F.; Data curation: J.B., T.K.; Writing - original draft: J.B., M.M.; Writing - review & editing: J.B., T.K., A.M., M.F.; Supervision: T.K., A.M., M.F.; Funding acquisition: M.F.

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Supplementary information

Supplementary information available online at
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