In situ developmental responses of tropical sea urchin larvae to ocean acidification conditions at naturally elevated $p$CO$_2$ vent sites

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Laboratory experiments suggest that calcifying developmental stages of marine invertebrates may be the most ocean acidification (OA)-sensitive life-history stage and represent a life-history bottleneck. To better extrapolate laboratory findings to future OA conditions, developmental responses in sea urchin embryos/larvae were compared under ecologically relevant in situ exposures on vent-elevated $p$CO$_2$ and ambient $p$CO$_2$ coral reefs in Papua New Guinea. Echinometra embryos/larvae were reared in meshed chambers moored in arrays on either venting reefs or adjacent non-vent reefs. After 24 and 48 h, larval development and morphology were quantified. Compared with controls (mean pH$_{(T)}$ = 7.89–7.92), larvae developing in elevated $p$CO$_2$ vent conditions (pH$_{(T)}$ = 7.50–7.72) displayed a significant reduction in size and increased abnormality, with a significant correlation of seawater pH with both larval size and larval asymmetry across all experiments. Reciprocal transplants (embryos from vent adults transplanted to control conditions, and vice versa) were also undertaken to identify if adult acclimatization can translate resilience to offspring (i.e. transgenerational processes). Embryos originating from vent adults were, however, no more tolerant to reduced pH. Sea temperature and chlorophyll-a concentrations (i.e. larval nutrition) did not contribute to difference in larval size, but abnormality was correlated with chlorophyll levels. This study is the first to examine the response of marine larvae to OA scenarios in the natural environment where, importantly, we found that stunted and abnormal development observed in situ are consistent with laboratory observations reported in sea urchins, in both the direction and magnitude of the response.

1. Background

Understanding the future effect of ocean acidification (OA) on organisms within marine ecosystems is inherently complex. While much had been learned from laboratory studies on the response of marine species to elevated seawater $p$CO$_2$ [1–4], including experiments that incorporate multiple stressors [5], we cannot fully replicate experimentally the range of variables that occur within ecosystems or their inherent variability [6,7].

Some of the limitations associated with laboratory experiments can be overcome by experimenting in naturally occurring CO$_2$-vent systems, such as those found in Milne Bay, Papua New Guinea (PNG) [8,9], Ischia, Italy [10,11] and White Island, New Zealand [12,13]. Such sites provide opportunities to examine the effects of elevated $p$CO$_2$ in complex and variable marine ecosystems, and in organisms that have been exposed to elevated $p$CO$_2$ for long periods (i.e. individual lifetimes and inter-generationally). Research in such vent systems has provided greater insight into a range of OA responses, such as changes in species composition, diversity and abundance [8,9], altered sex ratios (Harvey et al. [14]), elevated metabolism, reduced growth and...
calcification [10,14,15], altered predator–prey interactions [13], settlement [16], future microbial symbiont communities [17] and the potential for extinctions in specific groups [15]. Furthermore, reciprocal transplant experiments on vent ecosystems using molluscs and polychaete worms have shown the potential for animals to acclimate or adapt to elevated pCO2 conditions in terms of metabolism [14,18,19].

In a recent study on differences in demersal zooplankton biomass among vent and non-vent sites, Smith et al. [20] noted that while there were no differences in zooplankton composition, the biomass of demersal reef zooplankton was significantly lower in vent environments. The authors proposed that differences in food quality and habitat complexity may explain the patterns observed. Importantly, they observed a group of dominant copepods thought to be relatively robust to reduced pH that were, in fact, 14 times lower in abundance in CO2-vent environments, highlighting the need for field observations to validate laboratory findings [20].

Indeed, given natural environmental complexities, responses to elevated pCO2 in situ may differ from those expected from the extrapolation of laboratory findings. For example, laboratory studies on the tropical sea urchin genus Echinometra have suggested that a lower seawater pH can induce a range of deleterious responses, including reduced calcification [21], slower somatic growth [22] and impaired reproduction [23]. By contrast, Hazan et al. [24] found no change in growth or reproduction in Echinometra viridis over an 11-month exposure to pH(89.6) 7.7, while Moulin et al. [25] found no effect on growth, respiration and test properties of Echinometra mathaei exposure to pH(7.7) 7.6 in large mesocosms over a 13-month exposure. However, growth in Echinometra sp. C was 2.3 times faster in individuals living under elevated pCO2 levels at vent populations in PNG, compared with controls [26]. In addition, this and other echinoid species were more abundant on the seep sites [9]. This unexpected result was attributed to a greater food supply through enhanced algal production at vent sites, and supports Moulin et al.’s [25] conclusions that food level may determine responses to seawater pH in E. mathaei.

Vent ecosystems also provide greater insight into adult acclimation and transgenerational plasticity, a process that is emerging as an important consideration when predicting future responses of species to OA [27]. Pespeni et al. [28] demonstrated with population genomic methods that the temperate urchin Strongylocentrotus purpuratus has the potential for adapting to OA through the selection of beneficial alleles. By contrast, in a short-term laboratory experiment on Echinometra sp. A, larvae responded similarly to OA regardless of whether adults were acclimated to reduced pH or not [22]. Natural vent systems provide a more realistic opportunity to assess these adaptive processes as vent Echinometra populations complete settlement, metamorphosis and their juvenile and adult lifespan under elevated pCO2 conditions. Hence their fundamental physiological processes, such as metabolic rate, acid–base regulation, growth and gamete production will be acclimated to OA conditions [26]. Echinoids are generally sensitive to elevated pCO2 (see reviews [3,29]), which probably reflects their high level of calcification, high-Mg skeletons and their lesser capacity to acid/base regulation [30]. As with a number of taxa, the larval stages of the sea urchin’s life cycle are especially vulnerable to reduced OA [31,32]. There have been numerous studies that show a detrimental effect of elevated pCO2 on the growth, survival, respiration, calcification and gene expression patterns of sea urchin larvae in controlled laboratory experiments [33], including those on Echinometra developmental stages [18,23]. The latter studies showed that under low pH/high pCO2 larval development is stunted, and that larvae undergo altered development patterns expressed in arm asymmetry and abnormality.

In the light of these observations, we tested the hypothesis that responses in the developmental stages of marine species to reduced seawater pH observed in laboratory exposures are consistent with in situ observations. We also tested a second hypothesis that larvae respond differentially to reduced seawater pH depending on their paternal and maternal history of low pH exposure. For the first time, these processes are tested in situ using the well-described vent system in PNG [8]. We found that, as in the laboratory, growth rates and abnormality (asymmetric growth) were correlated with environmental pH level, but a significant amount of the variance was also explained by variation in water column chlorophyll content (an indicator for food availability for larvae). Using reciprocal out-plant transplants of embryos originating from vent and non-vent adults, we found that the development of larvae depended on adult acclimation history, although not in the way that would be consistent with transgenerational adaptation to elevated pCO2. Given that embryos and larvae are key life-history stages and are often population bottlenecks in many marine species, knowing the relationship between laboratory findings and larval responses in a natural setting, and the degree of naturally occurring transgenerational acclimation, are important to predict population outcomes.

2. Material and methods

(a) Study sites

Larval responses to elevated pCO2 were tested at natural vent sites occurring on two Papua New Guinean coral reefs, namely Dobu Island and Normanby Island near Upa-Upasina (figure 1a,b). At Dobu Island, research was undertaken at Dobu A (‘control site’, 9°44.220’S, 150°51.986’E) and a vent site at Dobu B (9°44.205’S, 150°52.039’E). At the Upa-Upasina Reef, animal collections and larval out-plants were made at two sites: Upa-Upasina A (control site, 9°49.698’S, 150°49.217’E) and Upa-Upasina B (vent site, 9°49.455’S, 150°49.073’E). The physical environment and distribution of Echinometra spp. C (figure 1c) on these reef systems have been well described in previous publications [8,9,15,34].

(b) Animal collection and spawning

Echinometra sp. C (∼40–45 mm test diameter) were collected by SCUBA divers from depths between 1 and 3 m, and kept aboard the vessel MV Chertan in 60 l flow-through tanks for no longer than 1 day before spawning. For each trial, animals were spawned by a 0.5–1 ml inter-coelomic injection of 0.5 M KCl, with animals inverted over beaters containing freshly filtered seawater (FSW) at ambient pH and temperature. The number of animals spawned varied among sites and trials, ranging from 5 to 10 females and 5 to 9 males. Spawned eggs were cleaned by serial washes in FSW, and pooled into a 2 l beaker, then inseeded from five to nine males to a final sperm concentration of 10⁵ sperm ml⁻¹. Fertilization rates of more than 90% were achieved in all experiments.
Larvae in the chambers were concentrated onto a 63 μm meshed window (a pore size of 63 μm) that allowed maximum water exchange. The dimensions of the mesh windows were 45 x 6 mm and there were four windows along each falcon tube, giving a total area open to water flow of 10.8 cm² (approx. 5% of tubes’ total surface area). Based on initial counts of the fertilized eggs, we aimed to add 500 embryos into each falcon tube (10 embryos/larvae ml⁻¹).

For each site, six to eight mesh chambers were attached to moorings at a depth of 3 m, being approximately 1 m off the seafloor (figure 1d). At the end of the experiment (24 or 48 h), mesh chambers were retrieved from the moorings and placed in plastic bags containing ambient seawater for return to the research vessel.

(c) Out-planting of embryos

For out-planting in the water column, embryos at the two-cell developmental stage (1 h post-fertilization) were transferred to replicate, 50 ml plastic/meshed chambers (figure 1d) that were then moored in the water column for 24 or 48 h. The chambers, as used by Uthicke et al. [18], consisted of a falcon tube with meshed windows of a pore size (63 μm) to restrict loss of the embryos (more than 70 μm diameter) but allow maximum water exchange. The dimensions of the mesh windows were 45 x 6 mm and there were four windows along each falcon tube, giving a total area open to water flow of 10.8 cm² (approx. 5% of tubes’ total surface area). Based on initial counts of the fertilized eggs, we aimed to add 500 embryos into each falcon tube (10 embryos/larvae ml⁻¹).

For each site, six to eight mesh chambers were attached to moorings at a depth of 3 m, being approximately 1 m off the seafloor (figure 1d). At the end of the experiment (24 or 48 h), mesh chambers were retrieved from the moorings and placed in plastic bags containing ambient seawater for return to the research vessel. Larvae in the chambers were concentrated onto a 63 μm mesh filter, then transferred to 1.5 ml Eppendorf tubes and fixed with 4% buffered paraformaldehyde for later measurement. Larvae retrieved from out-planting chambers were photographed using an Olympus BX51 microscope fitted with an Olympus XC50 digital camera. The number of larvae measured within each replicate tube was typically 16, but were as many as 21.

All larvae were photographed through a dorsal–ventral orientation (figure 1e) with three measurement recorded to the nearest micrometre (total larval length and length of both post-oral arms). Larval asymmetry (the relative difference between the postoral arms expressed as a percentage) was calculated as:

\[
\text{asymmetry (\%)} = \left( \frac{\text{postoral arm 1 (\mu m)} - \text{postoral arm 2 (\mu m)}}{\text{total larval length (\mu m)}} \right) \times 100.
\]

(d) Experimental design

We conducted two specific and independent experiments (i.e. a new set of spawners, different start days) (see electronic supplementary material, table S1 for specific dates). The first (Experiment 1) was undertaken on the Dobu Island reefs and compared larval development between ambient (control) and elevated pCO₂ (vent) environments over time. The second (Experiment 2) was undertaken at the Upa-Upasina reefs and tested for evidence of local acclimation to environmental pCO₂ and evidence of transgenerational plasticity in Echinometra using reciprocal out-plants of embryos (embryos from vent-inhabiting adults transplanted to both vent and control conditions, and vice versa). Each experiment involved a number of individual replicate trials. Lastly, using the results of both experiments, we tested the overall effects of average seawater pH and food supply (Chl-a concentration) across all experiments and trials with a linear model analysis using restricted maximum-likelihood (REML).

(e) Experiment 1: larval development at 24 and 48 h for vent versus control sites in Dobu Island

Experiment 1 trials were undertaken at Dobu Island, with animals spawned for the experiment collected from the Dobu control site. Embryos from this population were out-planted at both Dobu control and vent sites, and resampled at 24 and 48 h. For each trial, 6–8 independent replicate falcon tubes were deployed for each site and sample time. At 24 and 48 h development, significant differences in larval total length and larval arm asymmetry were tested using two-way nested ANOVA (fixed factors pCO₂ (vent versus control) and time (24 versus 48 h)). The experiment was run as two consecutive trials, the first from 8 to 10 November and the second from 10 to 12 November, with the random factor ‘Trial’ nested within both fixed factors.
(f) Experiment 2: larval development and adult source
To test for evidence of local acclimation to environmental pCO2 and for evidence of transgenerational plasticity in Echinometra, reciprocal 2-day out-plants of embryos (embryos from vent-inhabiting adults transplanted to both vent and control conditions, and vice versa) were undertaken at the Upa-Upasina reefs. This experiment was run as four identical, but independent fully crossed trials, with each 2-day trial started consecutively over a one-week period (see electronic supplementary material, table S1 for specific dates). For each trial, 6–8 independent replicate falcon tubes were deployed for each site and source population. Differences in larval total length and larval arm asymmetry were tested using two-way nested ANOVA (fixed factor pCO2 [vent versus control site] and adult source location [vent versus control adults]), with the individual trials nested within main factors. A significant interaction between pCO2 and adult location would indicate that the response of larvae to pCO2 depended on adult location (i.e. evidence of local acclimation and transgenerational plasticity).

(g) Pooled analyses
To test the overall effects of average seawater pH and food supply (Chl-a concentration) across the experiments at the different reefs, we conducted a linear model analysis using REML in the model, pH (average from the SeaFET loggers) and chlorophyll were used as fixed continuous variables, and experimental trial as a random factor. To avoid inflating degrees of freedom, we conducted this analysis on data pooled for each experiment and treatment. Initial model runs indicated that including ‘location’ as a categorical factor was not appropriate because it is covarying with chlorophyll. Furthermore, temperature was barely variable among sites and times and was not included in statistical comparisons. For the REML analyses, larval lengths and asymmetry were transformed by ln(x) and arcsine (\(\sqrt{x}\)) calculations, respectively.

(h) Environmental pH, carbonate parameters, temperature and chlorophyll
Water samples (250 ml) were collected to test for dissolved inorganic carbon (DIC) and alkalinity (\(T_a\)) from each site on at least two occasions per experiment. Samples were fixed with mercuric chloride (125 µl of a 7 g l\(^{-1}\) saturated solution) and later, samples were analysed spectrophotometrically for carbonate chemistry against certified seawater standards (A. G. Dickson, Scripps Institute of Oceanography, Dixon, Batch 106). At the same time as DIC and \(T_a\) sampling, we collected water for chlorophyll concentration (\(µg\) Chl-a l\(^{-1}\)) measured fluorometrically to estimate phytoplankton abundance (i.e. food supply for the larvae) at each site.

A periodic time series of pH/temperature measurements at the Dobu Island and Upa-Upasina sites were measured using SeaFET instrumentation. Measurements were taken at a depth of 2–3 m (1 m off the sea floor), every 5 min for 35–202 h. Although each site was measured, owing to logistical constraints, the SeaFET measurements at each site were of varying lengths, and in some cases only part of the experimental period was measured. In addition, SeaFET deployment was not possible for the Upa-Upasina control site at times, and only manual measurements of pH and \(T_a\) were made. Previous long-term deployment of a SeaFET at this site (i.e. [9]), however, showed average pH at the Upa-Upasina site was consistent with our point measurements, and shows little temporal variability.

(i) Statistical considerations
All larval measurements were ln(x) transformed, while larval arm ratios were logit transformed. For all analyses, tests for normality and homogeneity of variances were made using residual plots and the Cochran’s C-test, respectively. All analyses were performed using the R software [35].

3. Results

(a) Environmental measurements
Over the periods when seawater pH\(_{(T)}\) was measured with the seaFET loggers, levels varied within and among the control and vent sites (electronic supplementary material, figure S1a). The average pH\(_{(T)}\) at the Dobu Island (Experiment 1, electronic supplementary material, figure S1a) and Upa-Upasina (Experiment 2, electronic supplementary material, figure S1a) control sites was similar at 7.92 and 7.89–7.92, respectively (electronic supplementary material, table S1). The range measured by the seaFET at the control sites was also comparable at the two locations (7.8–8.0). During Experiment 1 at the Dobu vent sites, the average seawater pH\(_{(T)}\) ranged from pH\(_{(T)}\) 7.53 to 7.72, and from pH\(_{(T)}\) 7.5 to 7.59 for the Upa-Upasina vent sites during Experiment 2 (electronic supplementary material, table S1). Variability over the sampling period was greater at the Dobu Island vent site (\(\pm\)1.43 pH units) than at the Upa-Upasina vent site (\(\pm\)0.77 pH\(_{(T)}\) units). Based on DIC and \(T_a\) measurements of water taken directly at the deployment sites, average pCO2 at control sites ranged from \(\approx\)414 to 453 ppm, and was up to \(\approx\)threefold greater at the vent sites of Upa-Upasina (\(\approx\)792–1193 ppm) and Dobu Island (\(\approx\)561–588 ppm) (electronic supplementary material, table S1). These values are also reflected in the aragonite saturation state, which ranged from 1.98 to 2.98 at vent sites and 3.40–3.50 at the control sites. Based on the water samples, pH\(_{(T)}\) was subtly lower at the Dobu vent sites (7.90–7.92) than at the controls (7.99–8.00) at the time of the sampling (8.00–9.00), while this difference was more distinct at Upa-Upasina (electronic supplementary material, table S1).

Average sea temperatures ranged from 28.25 to 28.83°C (electronic supplementary material, table S1 and figure S1), and were relatively constant among sites and over time, while average chlorophyll-a concentrations ranged from 0.12 to 0.28 µg l\(^{-1}\) (electronic supplementary material, table S1).

(b) Experiment 1: larval development at 24 and 48 h for vent versus control sites in Dobu Island
Larval development during the Dobu Island time course experiment was significantly different at control and vent sites (figure 2a). In terms of larval size (figure 2a), total length was significantly larger (\(p < 0.001\); electronic supplementary material, table S2a) in the ambient pH control at 24 h (273.6 µm ± 5.1) and 48 h (276.3 µm ± 3.6) compared with those reared in vent water at 24 h (207.2 µm ± 3.4) or 48 h 220.3 µm ± 8.9). An overall effect size of reduced seawater pH was 32% at 24 h and 25% at 48 h. The effects of pH on larval length were apparent after 24 h, with no significant difference in larval size between 24 and 48 h (\(p = 0.778\); electronic supplementary material, table S2a). A non-significant interaction term between pH and time (\(p = 0.421\); electronic supplementary material, table S2a) indicates that the response to pH was similar after 24 and 48 h. Larval asymmetry (figure 2a) was significantly greater (\(p = 0.038\);


(c) Experiment 2: larval development and adult source
At the Upa-Upasina sites, pH had no significant effect on total larval length (\( p = 0.664; \) electronic supplementary material, table S3a), although larvae on average were still smaller at the vents (mean = 269.1–280.4 \( \mu \)m) than at the controls (mean = 270.4–283.2 \( \mu \)m). There was a significant effect of adult source (i.e. vent versus control adults, \( p = 0.007; \) electronic supplementary material, table S3a) on larval total length (figure 2b). Total larval length was significantly greater in larvae originating from vent adults (280.4–283.2 \( \mu \)m) compared with those from control adults (269.1–270.3 \( \mu \)m, overall size effect = 12.1\%), with no significant interaction between pH and adult source (\( p = 0.906; \) electronic supplementary material, table S3a). Thus, larvae were larger when resulting from vent adults but showed no apparent transgenerational effect in terms of their response to reduced pH. For larval asymmetry (figure 2b; electronic supplementary material, table S3b), there was a significant effect of pH (\( p < 0.001; \) electronic supplementary material, table S3b), with larvae exposed to vent conditions having significantly greater percentage asymmetry (10.76–11.05\%) than those exposed at the control treatments (7.42–9.59\%, overall size effect = 20\%).

The effect of adult source (acclimation) on larval asymmetry was dependent on seawater pH (acclimation \( \times \) pH interaction, \( p = 0.013; \) electronic supplementary material, table S3b) and only evident in larvae exposed to ambient pH levels at the control site (figure 2b). At this site, significantly greater asymmetry (one-way ANOVA, \( F_{1,30} = 7.375, p = 0.012 \)) was observed in larvae from control site adults (9.59\% arm difference) compared with larvae from vent adults (7.42\% arm difference).

(d) Combined linear model analysis
Over all experiments, there was an increase in total larval length with increasing pH, but this was only marginally significant. However, confidence intervals of this relationship are narrow and non-overlapping at the beginning and end of the observed range (figure 3a), supporting this relationship as ‘real’. The variance in total larval length among all experimental out-plants was not significantly explained by chlorophyll concentration (\( p = 0.5894 \)). The two fixed factors explain 27\% of the proportion of variance in total length (\( R^2 = 0.2787; \) electronic supplementary material, table S4a). Among all experiments, larval asymmetry decreased with both increasing pH and decreasing chlorophyll concentration (figure 3b), with the variance in asymmetry significantly explained by differences in both pH (\( p = 0.0174 \)) and chlorophyll (\( p = 0.0213 \)). Overall, 35\% of the variance in asymmetry was explained by both factors (electronic supplementary material, table S4b).

4. Discussion
Across a series of experiments, we found that reduced seawater pH increased larval abnormality rates and decreased larval length, with the effect occurring within the first 24 h of larval development. We also found no evidence that adults growing in high \( p\text{CO}_2 \) vent environments produced...
By out-planting embryos in vent and control sites, we found that development in reduced pH environments, even for a short period (i.e., 1 day), was deleterious to embryos and larvae. This was displayed as a reduction in total size and, even more distinctly, an increase in abnormal development, as indicated by greater asymmetry of the larvae. These observations are consistent with those predicted from laboratory studies, with delayed or reduced larval development in low pH reported for a range of sea urchin species [29,32], while studies broadly report abnormal development [41,42] and specifically larval asymmetry [41,43]. Importantly, not only was the direction of the response seen in situ similar to that predicted from laboratory studies, but also the magnitude of the response was within the range expected from laboratory measurements. For example, in terms of reduced larval length, Byrne et al. [32] were able to quantify the clear relationship between experimental pH and the percentage reduction in larval arm length for 15 echinoid species, which included larvae of *Echinometra* sp. A exposed to pH 7.9 [18]. When data from our field out-plants are included, the reduction in length we observed in situ lies within the confidence intervals of the original analysis predicted from laboratory studies (electronic supplementary material, figure S3). Similarly, in terms of arm asymmetry, we observed 10–16% asymmetry at the vent sites (compared within 7–13% at control sites). These are comparable with responses seen in a range of echinoid larvae exposed to experimentally reduced pH 7.8 seawater, which typically display arm asymmetry of between 10 and 15%, whereas for larvae in control conditions, asymmetry rarely exceeds 7–10% [41,43,44]. Reductions in larval size (delayed development) and asymmetry seen in laboratory studies have been broadly explained in terms of altered larval energy budgets [45,46], impaired digestion [40], changes in metabolic rate [45] and acid/base regulation and calcification [39]. The similarity in the direction and level of responses between highly controlled laboratory conditions and those under in situ conditions suggests that larval changes seen in the field in response to reduced pH may follow similar physiological mechanisms.

Temperature has been shown to interact with pH in determining larval responses [5]. However, owing to the small variation in temperature among out-plant sites in this study, differences in larval responses between control and vent sites cannot be explained by this variable. By contrast, chlorophyll-*a* concentrations (a proxy of phytoplankton food supply) varied twofold among sites and experimental periods (although average pH and chlorophyll-*a* concentrations were not correlated ($r = 0.94, R^2 = 0.005$)). Food concentrations can strongly influence echinoid larval growth rates [46] and morphometrics [47], and variations in environmental food levels have the potential to confound the effects of environmental pH. Despite this, no significant relationship was seen between larval size and chlorophyll-*a*, suggesting it had little or no influence on larval development compared with the strong effect of pH among our experiments (figure 3b). Sea urchin larval feeding rate and digestion capacity are impaired in reduced pH [40], which may override any environmental effects of food level on larval development. In other words, in terms of nutritional status and reduced development, effects of pH are a function of endogenous factors associated with physiological changes, and this may decouple development rates from environmental variation in food concentration.

**Figure 3.** Variation in total larval length (a) and larvae post-oral arm asymmetry (b) in *Echinometra* spp. C among seven out-plant experiments as a function of seawater pH($T$) and chlorophyll concentration. Length measurements are ln(x) transformed, while percentage arm asymmetry is arcsine ($\sqrt{x}$) transformed. Fitted lines are linear for pH and chlorophyll, and grey shading is the 95% CI.

offspring better adapted to these conditions, suggesting that transgenerational effects aiding adaptation to OA and carryover effects in offspring in this species are minimal.

Developmental bottlenecks in marine species are likely to be important outcomes of environmental change in the ocean [36]. In particular, the deleterious effects of warming and OA on fertilization and embryonic/larval development have been identified potentially as key in determining future population dynamics and distributions [33]. For this reason, much laboratory research has examined the responses in developmental stages to reduced pH/increased pCO$_2$ [37]. Because of the ecological importance of sea urchins, and the abundant knowledge on their developmental stages owing to their use as models for development and ecotoxicology, sea urchins are widely used as models for examining developmental responses to pH [38–40]. To date, however, quantifying effects of reduced seawater pH on development have been restricted to the laboratory and under experimentally controlled conditions [29,31]. This study is the first to examine the response of marine larvae to OA scenarios in the natural environment.
(where faster growth and larger larvae would be expected under higher chlorophyll).

There was a significant positive relationship between larval asymmetry and chlorophyll concentration (figure 3b). This finding is harder to explain; one interpretation of this is that seawater pH determines asymmetric growth, with the difference in arm length accentuated under low pH by larvae in higher food concentration able to maintain some arm growth compared with those in lower food levels (i.e., differences in arm length increase in better fed larvae, adding to the observed effects of pH). If this is the case, it is interesting, as previous research suggests that reduced pH influences larval development through changes in energy budgets (i.e., increased costs of acid/base regulation and calcification [37,38]) and resource allocation [48], and that echinoid larvae under experimentally reduced pH have been shown to not have lower feeding rates [45]. Therefore, it might be expected that larvae in environments with greater food levels and with more available energy would have a greater metabolic capacity/scope to mitigate low pH effects, and that in situ we could expect the effects of pH to be less in areas where food availability is greater. By contrast, our observations suggest that endogenous effects of reduced seawater pH can be stronger than environmental influences on sea urchin larvae (such as food level), and that key physiological responses observed experimentally in larvae that reduce scope for metabolic capacity/scope to mitigate low pH effects, and that in situ we could expect the effects of pH to be less in areas where food availability is greater. By contrast, our observations suggest that endogenous effects of reduced seawater pH can be stronger than environmental influences on sea urchin larvae (such as food level), and that key physiological responses observed experimentally in larvae that reduce scope for growth [45] were in operation during our field exposures.

The importance of environmental variability will extend to seawater pH where, in addition to reduced seawater pH, high-frequency pH variability (i.e., on hourly scales) may be an important determinant of OA responses [49,50]. For marine algae, fluctuating pH levels in OA experiments can enhance the negative effects on photosynthesis [51,52] and growth [50,53] but can reduce the effects on calcification [52]. Little is known about the effects of fluctuating pH on marine invertebrates; in experiments on larvae of two Mussel species, Frieder et al. [54] found that fluctuating pH by ≈0.30 units around an ambient pH (7.9–8.0) and reduced pH (7.5) did not alter the response to reduced pH in terms of survival, or the pace of development. Depending on species, however, fluctuating pH eliminated the effects of reduced pH in terms of larval shell development. In our experiments, out-plants were undertaken in conditions where pH at vent sites fluctuated daily by 1.43 and 0.77 pH units at Dobu Island and Upa-Upasina, respectively. These levels of pH variability are greater than those used in laboratory experiments, and it is possible that our responses were a function not only of reduced pH at vent sites, but also of the inherent variability. Interestingly, however, as discussed previously, our estimates of reduced growth are consistent with those seen in laboratory experiments (electronic supplementary material, figure S3) where static pH reductions were applied. Therefore, we have no evidence to suggest that the fluctuating pH levels either enhanced or moderated the responses to low pH vent conditions.

Reciprocal transplant experiments on pCO2 vent systems have shown populations of polychaete worms both physiologically and genetically differentiated at high CO2 locations [20], indicating local adaptation. There is much interest in transgenerational plasticity as a mechanism of adaptation [56,57], with examples of the processes in response to reduced pH, including positive carryover effects on oyster larvae [56], positive genetic changes in sea urchin larvae [28] and reduced pCO2 stress in transgenerational copepods [58]. One drawback of the present and earlier vent studies conducted is that, owing to the small size of the vents and long larval durations (i.e., 21 days in Echinometra), it is unlikely that long-term evolutionary adaptation can be studied. Larvae of urchins settling at the vents were most likely from other locations, and larvae are probably exposed to elevated pCO2 only during the final hours prior to settlement. However, our research can test for evidence of the effects of reduced seawater pH on transgenerational adaptation and carryover effects between generations. We hypothesized that adults inhabiting vents produced offspring that were more resilient to reduced pH. In laboratory experiments on Echinometra, exposure of adults to reduced seawater pH for 10 weeks did not increase the resilience of developmental stages to reduced seawater pH [18]. Their results, based on relatively short-term experiments, are consistent with our observation that the source of the adults (whether from vents or controls) did not influence the response of the larvae to reduced pH (in terms of total length and arm asymmetry). Indeed, a lack of any apparent transgenerational adaptation in Echinometra sp. C is despite the fact that vent adults are presumed to have spent their entire lives exposed to reduced and variable pH [26].

Although we did not observe any positive carryover effects, equally important, we did not see any evidence of negative transgenerational carryover within vent populations. In fact, the larvae we observed from vent adults were significantly larger (suggesting faster growth), irrespective of the in situ pH treatment of the larvae. At the same time, under ambient pH conditions, we observed that larval asymmetry was significantly lower in larvae from vent adult compared with those from control larvae. Larger larvae with lower asymmetry would be features of fitter offspring [41] and inconsistent with the hypothesis that urchins from vents produce lower quality gametes owing to pH stress or hypercapnia, leading to less resilient larvae. In this respect, Dupont et al. [59] noted that while adult Strongylometus droebachiensis sea urchins can acclimate to elevated pCO2, negative carryover effects on larvae and juveniles could be a possible outcome under higher pCO2. Previous research on Echinometra sp. C living on the PNG vents supports Dupont et al.’s [59] observations of effective adaptation in the adults [26]; however, we did not see any negative carryover on development. In fact, within this species, there appears little response in the adults to reduced pH in terms of either reduced or enhanced development success (i.e., through changes in fecundity, egg provisioning or epigenetics), despite these mechanisms being available for sea urchins to enhance larval performance [60]. The lack of clear transgenerational adaptation or carryover effects is unlikely owing to energetic constraints on adults; Ulthicke et al. [26] showed that Echinometra adults grew faster on vent systems and had similar levels of calcification and metabolic rates.

An important limitation of our experiment was its short-term nature where we examined only responses over the first 2 (or 3) days of development. Echinometra larvae complete development in approximately three weeks, and it is likely that longer duration exposures would detect a wider range of responses. However, when responses in Echinometra adults and larvae exposed to vent environments are considered together, the widely held hypothesis that development stages are the most sensitive life-history stage, and that they may represent a population bottleneck [31,44], is supported. In this respect, while the adults living in vent environments
appear to show no apparent deleterious responses (in terms of metabolism, calcification, population size, distributions and growth [26]), their larval stages are negatively affected in terms of development. If these observed effects equate to reduced numbers of larvae reaching settlement (i.e. through delayed development and increased larval mortality), then they would represent the limiting stage for Echinometra populations in future OA.

Data accessibility. All data are archived with the Department of Marine Science, University of Otago (http://www.otago.ac.nz/marine-science/index.html).

References


Authors' contributions. M.D.L. and S.U. conceived and designed the research, M.D.L. and S.U. carried out the fieldwork, and all authors analysed larval and water samples. M.D.L. and S.U. wrote the manuscript.

Competing interests. The authors declare that they have no competing interests.

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