Review

The stunting effect of a high CO_2 ocean on calcification and development in sea urchin larvae, a synthesis from the tropics to the poles

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The stunting effect of ocean acidification on development of calcifying invertebrate larvae has emerged as a significant effect of global change. We assessed the arm growth response of sea urchin echinoplutei, here used as a proxy of larval calcification, to increased seawater acidity/pCO_2 and decreased carbonate mineral saturation in a global synthesis of data from 15 species. Phylogenetic relatedness did not influence the observed patterns. Regardless of habitat or latitude, ocean acidification impedes larval growth with a negative relationship between arm length and increased acidity/pCO_2 and decreased carbonate mineral saturation. In multiple linear regression models incorporating these highly correlated parameters, pCO_2 exerted the greatest influence on decreased arm growth in the global dataset and also in the data subsets for polar and subtidal species. Thus, reduced growth appears largely driven by organism hypercapnia. For tropical species, decreased carbonate mineral saturation was most important. No single parameter played a dominant role in arm size reduction in the temperate species. For intertidal species, the models were equivocal. Levels of acidification causing a significant (approx. 10–20+%) reduction in arm growth varied between species. In 13 species, reduction in length of arms and supporting skeletal rods was evident in larvae reared in near-future (pCO_2 800+ μatm) conditions, whereas greater acidification (pCO_2 1000+ μatm) reduced growth in all species. Although multi-stressor studies are few, when temperature is added to the stressor mix, near-future warming can reduce the negative effect of acidification on larval growth. Broadly speaking, responses of larvae from across world regions showed similar trends despite disparate phylogeny, environments and ecology. Larval success may be the bottleneck for species success with flow-on effects for sea urchin populations and marine ecosystems.

1. Modern climate-driven ocean change

Marine organisms and their pelagic propagules are experiencing oceans simultaneously warming and decreasing in pH [1–5]. These changes to the ocean are directly linked to anthropogenic carbon dioxide (CO_2) emissions, with current atmospheric CO_2 levels approximately 30% higher than over the last 2 Myr [6]. Changes in mean global ocean pH/pCO_2, due to uptake of anthropogenic CO_2, will reduce pH (ca −0.3 to 0.5 units/500+ μatm), and global warming will contribute to increased sea surface temperature (+1.1 to 6.4°C), by 2100 [1–4,7]. These changes are occurring at a rate faster than that evident in the fossil record for the past 200–300 Myr [2,8,9].
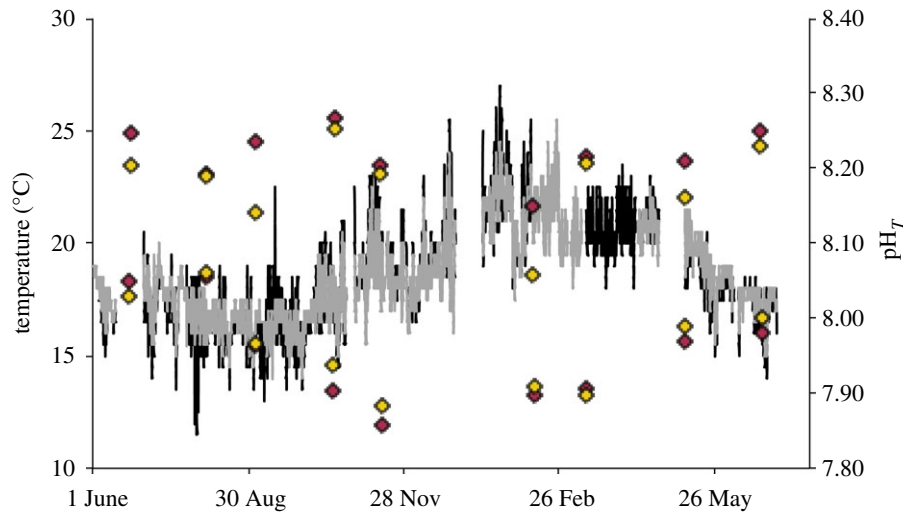
Figure 1. Temperature and pH variation in the shallow subtidal habitat (grey line) of the sea urchin *Heliocidaris tuberculata* (Lamarck, 1816) and the low intertidal tide pool habitat (black line) of the sea star *Meridiastra calcar*, Sydney, Australia (June 2011 – June 2012), showing the seasonal cycle and the warm/cold temperature spikes experienced by these species. Tide pool temperature as recorded by *in situ* loggers (Thermodata iBCod G, data collected every 20 min) can change daily by $8-10°C$. The gaps are when loggers were lost [25,26]. Seawater chemistry at pre-dawn and pre-sunset low spring tides indicates extreme conditions in the shallow subtidal (yellow (grey) diamonds; pH_t (total scale) range: $7.88-8.25$; pCO_2: $219-614$ μatm) and low intertidal (red (black) diamonds; pH_t range: $7.86-8.27$; pCO_2: $209-659$ μatm). pH_t and pCO_2 were determined from analysis of total alkalinity (measured by potentiometric titration) and dissolved inorganic carbon (measured by coulometry) using CO2SYS [27]. (Online version in colour.)

Ocean acidification is accompanied by reduced availability of carbonate minerals, with negative effects on skeletogenesis, and by increased pCO_2 that causes hypercapnia (increased organism pCO_2) and altered metabolism [10–12]. The ability of calcifying marine invertebrates, a great diversity of species, to make their shells and skeletons may be compromised by ocean acidification [13–16]. This has been demonstrated in time-series studies of coral community calcification [17], and reduced calcification in marine communities has been associated with natural CO_2 seeps [18,19]. The impacts of hypercapnia on organism physiology, and interactive effects with increased temperature and other stressors, are less understood, but are likely to alter individual performance through alteration of aerobic scope and energy budget [10,11,20–22].

Owing to the natural spatio-temporal variation in pH, temperature and carbonate conditions and differences in ocean circulation, the rate of acidification and warming differs locally and regionally [7,23,24]. This has been shown through use of *in situ* loggers [24–26] and by manual determination of water chemistry (figure 1). In parallel, the increase in air temperatures will exacerbate levels of thermal stress on coastal shallow water biota [7], with concerns that temperature spikes may exceed tolerance levels. Air temperatures in some regions (e.g. Australia and Antarctic Peninsula) are warming much faster than the global average [7,28].

Natural spatial and temporal variability in the pH/pCO_2 conditions experienced by marine populations provide the potential for different levels of adaptation (genetic) and acclimatization (phenotypic) to decreased pH and elevated pCO_2, emphasizing the importance of understanding physiological variation existing along natural environmental gradients (macrophysiology) in understanding global change impacts [29]. For instance, owing to natural variability in habitat temperature and pH, animals living in intertidal and estuarine environments may have some resilience to increased temperature and/or acidification [21,22,25,26,30–32]. In these habitats, seawater pH fluctuates markedly in association with diurnal cycles of respiration and photosynthesis (figure 1).

Organisms living in areas where upwelling of cool, low pH water occurs (e.g. Eastern Pacific and Baltic Sea) may be acclimatized/adapted to acidification [31–34], whereas those living in areas with strong warming (e.g. Eastern Australia) may be acclimatized/adapted to increased temperature [7,35]. Polar regions have naturally low carbonate mineral saturation owing to increased CO_2 solubility in cold water and upwelling of cold, CO_2-rich seawater, whereas warmer tropical waters have comparatively higher mineral saturation and lower pCO_2 [23,36–38]. Thus, it is essential to consider the conditions in study species' habitats when applying global change scenarios to experimental designs, and in assessing how local baseline levels of temperature, pH/pCO_2 and carbonate mineral saturation may change in a future ocean [24,26,39]. For benthic invertebrates with a pelagic life stage, it is important to understand the conditions that adults experience in the benthos, and those that the larvae experience in the plankton, to place stress tolerance levels in context with projected future change. Global change will shift current baseline conditions of pH and temperature, as well as those of other stressors (e.g. hypoxia, precipitation patterns/salinity), challenging the physiological capacity of resident biota [5,10,11,22].

Among the most fragile skeletons produced by marine organisms are those made by calcifying invertebrate larvae, and there has been considerable interest in the vulnerability of these larvae to changing ocean conditions [13–16], with many studies of sea urchin echinoplutei (table 1 and figure 2). The cellular, genetic regulatory network and molecular mechanisms underlying calcification in sea urchin development are well understood [60], and these, together with the availability of a sequenced genome, provide an important model system for investigating the impacts of ocean change on calcifying larvae [61]. Because of the important ecological role of sea urchins in driving transitions between alternative kelp forests and 'barrens' habitat free of foliose seaweed and their numerical dominance in many habitats [62], it is important to understand the responses of sea urchin life stages to climate change stressors.

Table 1. Impacts of ocean acidification (pH/pCO_2, µatm) conditions on development of echinoplutei from tropical to polar regions and stressor levels resulting in significant negative effects in statistical analyses. Ambient seawater pH$_{NIST}$ (National Institute of Standards and Technology) is most commonly reported (*pH$_t$—total scale) and ranged from pH 8.0 to 8.3 depending on region (see references). Only data from the pre-feeding pluteus stages were considered to avoid the confounding influence of arm growth plasticity in response to food regime [40,41]. IT, intertidal; ST, subtidal.
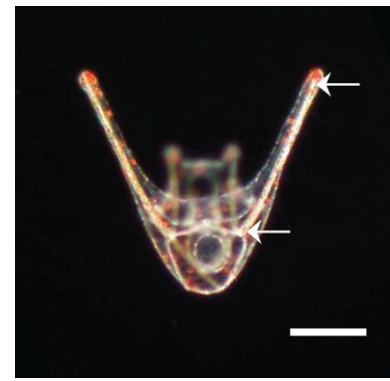
species (family)	habitat	stage placed in experimental conditions	pH/pCO_2—significant effect on larvae (decrease pH units with respect to ambient)	reference
tropical				
Arachnoides placenta (Linnaeus, 1758) (Arachnoididae)	IT	from fertilization	7.65/1891 (0.5) smaller larvae	[42]
Echinometra mathaei (Blainville, 1825) (Echinometridae)	ST	from fertilization	7.7–7.9/785 (0.3–0.4) smaller larvae, abnormal, altered allometry, altered gene expression	[43,44]
Tripneustes gratilla (Linneaus, 1758) (Toxopneustidae)	ST	from fertilization or hatched larvae	7.8/1119 (0.35–0.4) smaller larvae, abnormal, altered allometry	[45,46]
Anthocidaris crassispina (A. Agassiz, 1863) (Echinometridae)	IT	embryos	7.86/614 (0.14) smaller larvae	[47]
temperate				
Centrostephanus rodgersii (A. Agassiz, 1863) (Diadematidae)	ST	from fertilization	7.8/1100 (0.5) smaller larvae	[48]
Dendraster excentricus (Eschscholtz 1829) (Clypeasteridae)	IT	gastrula (10 h)	7.75/1000 (0.3) no effect on postoral arm length altered allometry	[49]
Evechinus chloroticus (Valenciennes, 1846) (Echinometridae)	ST	hatched larvae	7.7/1320 (0.4) smaller larvae	[45]
Heliocidaris tuberculata (Lamarck, 1816) (Echinometridae)	ST	from fertilization	7.6/1190 (0.5) smaller, abnormal larvae	M. Byrne (2003, unpublished data)
Hemicentrotus pulcherrimus (A. Agassiz, 1863) (Strongylocentridae)	ST	from fertilization	7.6/1000 (0.2) smaller larvae, change in gene expression	[47,50,51]
Lytechinus pictus (Verrill, 1867) (Toxopneustidae)	ST	zygote	540 smaller larvae, altered allometry	[52]
Paracentrotus lividus (Lamarck, 1816) (Echinidae)	IT	from fertilization or zygote—20 min	7.6/1040 (0.5) slower cleavage; 7.4–7.5/1690–1900 (0.6–0.7) abnormal—smaller larvae, change in gene expression	[30*,53]
Pseudechinus huttoni (Benham, 1908) (Echinidae)	ST	hatched larvae	7.7/1280 (0.4) smaller larvae	[45]
Strongylocentrotus franciscanus (A. Agassiz, 1863) (Strongylocentridae)	IT	from fertilization or gastrulae (48 h)	7.9/570 (0.4) smaller larvae, reduced gene expression after thermal shock	[54,55]
Strongylocentrotus purpuratus (Stimpson, 1857) (Strongylocentridae)	IT	embryos (ca less than 1 h)	7.63–7.7/1000 (0.4–0.5) smaller larvae, changed gene expression	[31,32,39,56]*
polar				
Arbacia dufresnei (Blainville 1825) (Arbaciidae)	ST	embryos (3–4 h)	7.4/1882 (0.6) smaller larvae	[57*]
Sterechinus neumayeri (Meissner, 1900) (Echinidae)	ST	from fertilization or hatched larvae	7.6–7.8/897–1380 (0.2–0.4) smaller larvae, abnormal, altered allometry	[58,59*]
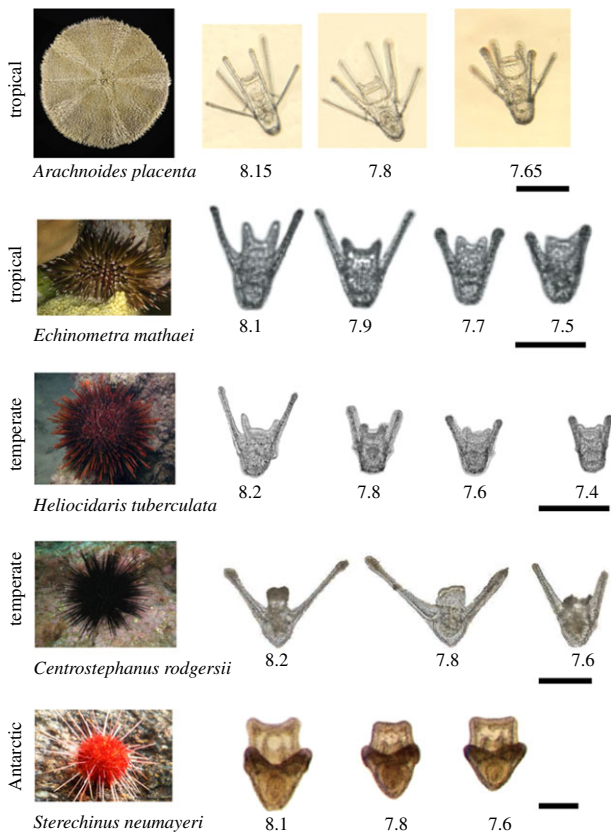
Figure 2. Echinoplutei from tropical, temperate and polar sea urchins reared in control and acidification treatments are smaller, and in some cases asymmetrical with arms differing markedly in length. All species were reared following similar methods [42,43,48,58]. Scale bars, 200 µm.

Figure 3. Early echinopluteus of *Tripneustes gratilla* (Linneaus, 1758) in polarized light showing the postoral skeletal rod (between the arrows), supporting the arm. Scale bar, 100 µm. (Online version in colour.)

investigated in three species [67]. The responses of echinoplutei to simultaneous exposure to these parameters are compared with that documented for other invertebrate larvae, calcifying and non-calcifying.

The responses of sea urchin larvae to ocean acidification may be influenced by habitat type, and may also be species specific, leading to the suggestion that some species are more robust to ocean change stressors than others (i.e. 'winners versus losers') [68]. In this synthesis, we address four hypotheses: (i) owing to similar cellular and molecular mechanisms of calcification and growth in echinoplutei, there is an overall similarity in the response of larval growth to ocean acidification conditions; (ii) the larvae of urchins from different latitudinal regions differ in their sensitivity to these stressors; (iii) the larvae of intertidal and subtidal species differ in their sensitivity to these stressors; and (iv) near-future ocean warming (approx. 2–4°C) reduces the negative effect of decreased pH on larval growth though stimulation of physiological processes, especially calcification. Finally, we consider potential mechanisms underlying responses of larvae to changing ocean conditions.

A recent meta-analysis indicated a significant negative effect of ocean acidification on calcifying and non-calcifying echinoderm larvae ($n = 26$ studies) [63]. Here, we synthesize data from recent studies on the impacts of ocean acidification on development of the calcifying larvae (echinoplutei) of sea urchin species from tropical to polar regions (22 studies, 15 species). In these studies, the smaller size of larvae reared in ocean acidification conditions indicates an overall negative effect on calcification (table 1 and figure 2). We focused on echinoplutei to gain an understanding of where sensitivities of larval growth and calcification lie, with regard to particular levels of acidification/pCO$_2$ and carbonate mineral saturation. We addressed questions on the vulnerability of sea urchin larvae to changes in ocean conditions in species from a range of environments (intertidal/ subtidal) and latitudes (tropical to polar).

Carbonate chemistry parameters (e.g. pH$_{NIST}$, pCO$_2$ and Ω_{ca}) covary, so we used multiple linear regression models incorporating these highly correlated parameters to explore their effects on larval growth. We also consider each of these parameters individually because their relative influence on metabolism, growth and calcification are likely to be through different physiological mechanisms [12]. For instance, as echinoderms transport bicarbonate (not carbonate) at the site of calcification and use respiratory CO$_2$ as a source of inorganic carbon [64,65], carbonate mineral saturation state may not be as important as pH/pCO$_2$. In addition, as pH levels are strongly influenced by total alkalinity, strong buffering by the coastal water can maintain high pH despite very high pCO$_2$ levels [66]. The interactive effects of increased acidification and temperature on echinopluteal development have been

2. Material and methods

Our synthesis included 15 species in 22 studies for which data on the effects of parameters associated with CO$_2$-driven ocean acidification (pH, pCO$_2$, Ω_{ca} and Ω_{ar}) on echinopluteal arm growth are available (table 1). The levels of pH/pCO$_2$ reported to result in a statistically significant reduction in larval size and other significant developmental effects are indicated in table 1. Data from independent experiments were used, and for several species there is more than one study.

To standardize comparisons between species and studies, we used data on the percentage decrease in growth of the postoral arms in four-armed pre-feeding larvae, reared in acidification treatments, compared with larvae reared in control pH (figure 2). Postoral arm length is often used as a metric of echinopluteal growth and data are available in most studies. This measure is a proxy of larval calcification, because arm length is determined by the length of its supporting skeletal rod, with just a thin (approx. 10 µm) epithelial cover (figure 3). Where the percentage change in postoral arm length was not stated, the data were determined from the published figures using Digitizelt v. 1.5. We analysed data on arm length prior to commencement of feeding because after food is introduced to sea urchin larval cultures, comparisons between studies are confounded by the natural phenotypic plasticity in arm growth due to a feedback mechanism linking nutritive

environment (food levels and larval density) and arm length, the expression of which differs among echinoid families [40,41].

Most studies report pH on the pH_{NIST} (National Institute of Standards and Technology) scale, which is approximately 0.1 units lower than the pH_t (total) scale ([69], see also, [26,58]). To compare all data on the NIST scale, we added 0.1 pH units to the pH_t values provided by studies that used this scale. For studies where levels of Ω_{ca} and Ω_{ar} were not stated, these parameters were calculated from data provided on salinity, temperature, pH, total alkalinity and/or pCO_2 using CO2SYS [27]. Data for pCO_2 were plotted and analysed on log-scaled data to be comparable to the (logarithmic) pH scale.

As the data were sourced from 15 species across eight families, we performed phylogenetic comparative analyses to investigate whether phylogenetic relatedness influenced species' response. There are no published echinoid phylogenies that included all the species analysed here. To generate a tree (see the electronic supplementary material, figure S1), we were able to retrieve COI sequences for 12 species from GenBank. We then estimated a phylogenetic tree from these sequences with RAxML [70] using a GTR + G model of sequence evolution with the topology constrained to match the genus-level relationships presented in the most recent echinoid phylogeny [71]. We performed a phylogenetic comparative analysis for each regression on reduction in arm length with respect to pH_{NIST}, pCO_2 and Ω_{ca} using the R package MCMCglmm [72]. For each predictor variable, we fitted two generalized linear mixed models, one containing a phylogenetic effect and the other missing this effect. MCMCglmm estimates model parameters using Markov chain Monte Carlo (MCMC) sampling. For each combination of model and predictor, we ran three MCMCs and checked for convergence among these chains using the methods implemented in CODA [73]. We used a single MCMC (chosen at random) to estimate parameters for each model.

We investigated the effect of variation in pCO_2, Ω_{ca} and pH_{NIST} on postoral arm growth by fitting multiple linear regression models using the R statistical language [74]. The strong correlation of these variables, a condition known as multicollinearity, decreases the power of statistical analyses to detect significant variables and can lead to incorrect parameter estimates. Thus, we fitted models containing all combinations of our explanatory variables and compared these models using the Akaike information criterion (AIC), a statistic that is not affected by multicollinearity [75,76]. We analysed the total dataset ($n = 78$) and in subsets for tropical (latitude: $0-30°$, $n = 20$), temperate (latitude: $30-60°$, $n = 44$), polar (latitude: $60-90°$, $n = 14$), intertidal ($n = 31$) and subtidal ($n = 47$) data points. The best five models for each of these datasets are presented.

To illustrate the data for each parameter, the relationships between the percentage decrease in postoral arm length and pH_{NIST}, pCO_2, Ω_{ca} and Ω_{ar}, as independent parameters, were also analysed by linear models in R. The statistics for these regressions are in the electronic supplementary material, table S1.

3. Marine propagules in a changing ocean

(a) Effects of ocean acidification on echinopluteal growth

There was little phylogenetic signal in the arm length data (see the electronic supplementary material, table S2). In models containing pH_{NIST}, pCO_2 and Ω_{ca} as fixed effects, the phylogenetic heritability [77], a measure of the variation in a given trait that can be explained by phylogenetic relatedness, is very low (less than 0.01). Although phylogenetic models produced a marginally better fit to the data than

non-phylogenetic models (increasing the conditional R^2 of models by five percentage points), parameter estimates acquired from phylogenetic and non-phylogenetic models are only marginally different. As phylogenetic relatedness explained such a small proportion of the variation in these data, and phylogenetic comparative methods require us to exclude data from three species that could not be placed in a phylogeny, we did not include phylogenetic effects in subsequence analyses.

When the combined data for all (i) climatic regions and (ii) habitats are considered, pCO_2 emerges as a major parameter influencing the decrease in growth of postoral arms (table 2). No single model is strongly supported by the data, but the four best-fitting models, which make up 99% of the AIC-weight, all contain an effect for pCO_2. This overall similarity in the response across all species in the global context supports hypothesis no. 1. Models containing effects for Ω_{ca} and pH_{NIST} provided marginally better fits to the data than a model with pCO_2 as the only explanatory variable ($\Delta AIC = 4.1$). This suggests that there may be some additional influence of Ω_{ca} and pH_{NIST}.

In 13 species, a significant reduction in length of arms and supporting skeletal rods was evident in larvae reared in near-future (pCO_2 800+ µatm, ca pH 7.8) conditions (table 1, figures 2 and 4a,b). The exceptions were two intertidal sand dollars, where this pCO_2 level did not cause a reduction in arm growth (table 1). At greater levels of acidification ($pCO_2 \geq 1000$ µatm, pH ≤ 7.6), there is a sharp decline in arm length in all echinoplutei investigated (table 1 and figure 4a; the electronic supplementary material, table S1). Larval arm length also decreased as Ω_{ca} and Ω_{ar} approached undersaturation (figure 5; the electronic supplementary material, table S1).

When data on percentage decrease in postoral arm growth are analysed by region, there are contrasting patterns. As with the global dataset, the models that best explained the data from polar species had very similar AIC values, meaning a single best model cannot be identified. It is clear however that variation in pCO_2 has a substantial impact on arm growth in polar species. Models containing this variable make up 86% of the AIC-weight for these data. By contrast, Ω_{ca} appears to be the most important for the tropical species. Among the models explaining this subset of the data, those that contain Ω_{ca} make up 93% of the AIC-weight. No single parameter appears to play a dominant role in the temperate dataset. The five best-fitting models for these data contain varying combinations of parameters and produce similar fits to the data (the five best models having $\Delta AIC < 3$). Thus, there were some differences between regions, supporting hypothesis no. 2.

The greatest decrease in growth response to near-future Ω_{ca} is in the tropical species (figure 4; the electronic supplementary material, table S1). The polar species appeared better abled to calcify compared with temperate and tropical species as Ω_{ca} declined below 3. Once the saturation falls to less than $1-2$, and at pH < 7.6, growth of the larvae of polar species also declines (figures 4a and 5).

With regard to habitat type, no single model best explains the data for either the intertidal or subtidal dataset, as reflected by small differences in AIC scores for models of each subset of the data (table 2). It is clear however that the model comparisons indicated that pCO_2 has a major influence on the decrease in postoral arm length in the larvae of subtidal species. The
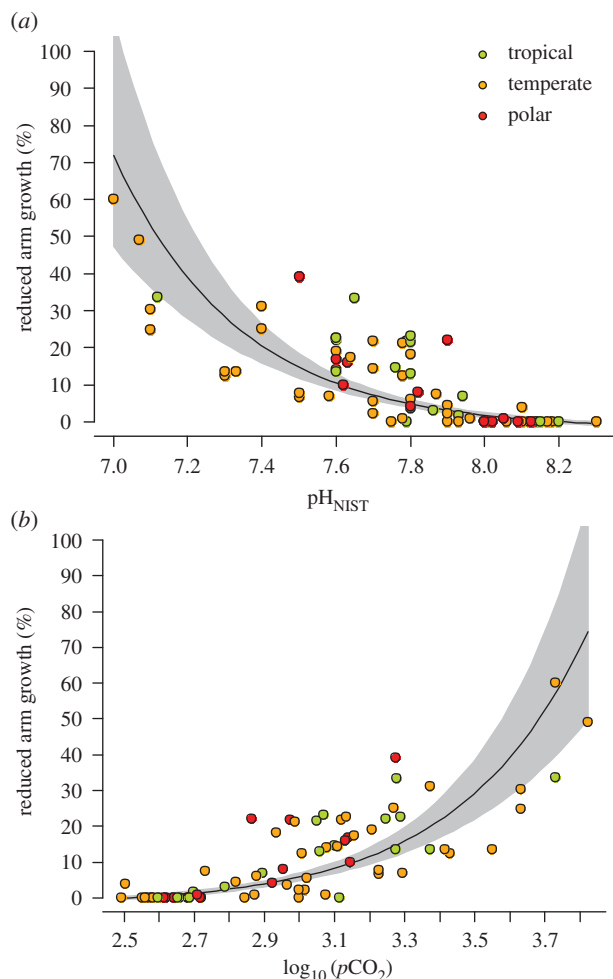
Table 2. Model selection to estimate factors influencing the decrease in postoral arm growth in echinoplutei reared in ocean acidification conditions. In each case, 'predictors' refers to the combination of explanatory variables included in the model, ΔAIC is the difference in AIC between this model and the best-fitting one and AIC_{wt} to the AIC-weight (the probability that this model represents the best-fitting model among those considered). 'Sum of AIC_{wt}' refers to the sum of AIC_{wt} scores for the given model and all better-fitting models. Parameters with a significant slope are highlighted in bold. For each dataset, the five best models are presented.

dataset	predictors	AIC	ΔAIC	AIC_{wt}	sum of AIC_{wt}	R^2
global	**$p\text{CO}_2 + \Omega_{ca}$**	149.8	0	0.61	0.61	0.69
	$p\text{CO}_2 + \text{pH} + \Omega_{ca}$	151.5	1.7	0.26	0.87	0.69
	$p\text{CO}_2$	153.9	4.1	0.08	0.95	0.67
	$p\text{CO}_2 + \text{pH}$	155.4	5.6	0.04	0.99	0.67
	pH	158.6	8.8	0.01	1.0	0.64
tropical	pH $+ \Omega_{ca}$	39.6	0	0.31	0.31	0.75
	Ω_{ca}	39.8	0.2	0.27	0.58	0.74
	$p\text{CO}_2 + \text{pH} + \Omega_{ca}$	40.1	0.5	0.24	0.82	0.75
	$p\text{CO}_2 + \Omega_{ca}$	41.7	2.1	0.11	0.93	0.72
	$p\text{CO}_2$	43.2	3.6	0.05	0.98	0.69
temperate	**$p\text{CO}_2 + \Omega_{ca}$**	83.6	0	0.31	0.31	0.71
	pH $+ \Omega_{ca}$	84.2	0.5	0.24	0.55	0.7
	pH	85.1	1.4	0.15	0.7	0.69
	$p\text{CO}_2 + \text{pH} + \Omega_{ca}$	85.2	1.5	0.15	0.85	0.7
	$p\text{CO}_2 + \text{pH}$	86.3	2.7	0.08	0.93	0.69
polar	**$p\text{CO}_2$**	22.4	0	0.33	0.33	0.78
	$p\text{CO}_2 + \text{pH}$	22.7	0.3	0.29	0.62	0.79
	$p\text{CO}_2 + \Omega_{ca}$	24.3	1.9	0.13	0.75	0.76
	$p\text{CO}_2 + \text{pH} + \Omega_{ca}$	24.6	2.2	0.11	0.86	0.77
	pH	25.7	3.3	0.06	0.92	0.72
intertidal	**pH**	49.6	0	0.28	0.28	0.68
	$p\text{CO}_2$	50.4	0.9	0.18	0.46	0.67
	$p\text{CO}_2 + \Omega_{ca}$	50.8	1.2	0.16	0.62	0.68
	pH $+ \Omega_{ca}$	50.8	1.3	0.15	0.77	0.68
	$p\text{CO}_2 + \text{pH}$	50.9	1.4	0.14	0.91	0.67
subtidal	**$p\text{CO}_2 + \Omega_{ca}$**	64.1	0	0.37	0.37	0.78
	$p\text{CO}_2$	64.4	0.3	0.31	0.68	0.77
	$p\text{CO}_2 + \text{pH} + \Omega_{ca}$	65.4	1.3	0.19	0.87	0.78
	$p\text{CO}_2 + \text{pH}$	66.4	2.3	0.12	0.99	0.77
	pH	75.7	11.6	0.00	0.99	0.71

five best-fitting models for this dataset contained this variable and together contributed 99% of the AIC-weight. There is no such clear pattern in the models for the larvae of intertidal urchins. The five best-fitting models for these data all produced approximately equal fits (ΔAIC ranging from 0.9 to 1.4 compared with the best model). These models also explained substantially less of the variation in the dataset than that for the subtidal models (maximum $R^2 = 0.68$ for intertidal models and $R^2 = 0.78$ for subtidal). Among the five best-fitting models, those containing pH_{NIST} and $p\text{CO}_2$ contributed 57% and 48% of the AIC-weight, respectively. Thus, there was not a clear difference in the response of echinoplutei of intertidal and subtidal urchins to rearing in ocean acidification conditions, with an equivocal assessment of hypothesis no. 3.

Overall, multiple regression analyses of the global dataset across climatic regions and habitats showed the strong influence of $p\text{CO}_2$ on larval growth. However, separate analysis of the data for tropical species indicated that these larvae were more sensitive to Ω_{ca}, whereas the analyses of the temperate and intertidal data were equivocal.

For the tropical species, sensitivity to Ω_{ca} may reflect the naturally higher levels of carbonate mineral saturation in warm water and perhaps is also influenced by skeleton mineralogy. The mineralogy of the adult echinoid skeleton shows a strong trend of decreasing Mg^{2+} content towards higher latitudes [78]. For example, the tropical species *Echinometra mathaei* (Blainville, 1825) has a higher Mg^{2+} content in the test than that of the polar species *Sterechinus neumayeri* (Meissner, 1900) (13–16% versus 6%) [78,79].

Figure 5. Reduced growth of the postoral arm in echinoplutei in response to changes in calcite and aragonite saturation. The solid line is the regression line and the shaded area is 95% CI. Regression parameters are given in the electronic supplementary material, table S1. Different colours represent geographical regions (see legend). Percentage growth reduction (natural log transformed for curve fits) was back transformed for graphic representation.

Figure 4. Reduced growth of the postoral arm in echinoplutei in response to changes in (a) pH_{NIST} (National Institute of Standards and Technology) and (b) pCO_2 (log_{10} transformed). The solid line is the regression line and the shaded area is 95% CI. Regression parameters are given in the electronic supplementary material, table S1. Different colours represent geographical regions (see legend). Percentage growth reduction (natural log transformed for curve fits) was back transformed for graphic representation.

As the vulnerability of the skeleton to reduced carbonate mineral saturation increases with Mg^{2+} content [80], tropical sea urchins appear to be the most vulnerable to ocean acidification. The test of *S. neumayeri* has one of the lowest Mg^{2+} contents known for the sea urchin skeleton [78] and so would be comparatively more resilient to ocean acidification. Although we do not have data on the mineralogy of the larval skeleton across global climatic regions, the average weight percentage of magnesium carbonate (wt% $MgCO_3$) in skeletal elements in the larvae of tropical (*Tripneustes gratilla* (Linneaus, 1758)) and temperate (*Evechinus chloroticus* (Valenciennes, 1846), *Pseudechinus huttoni* (Benham, 1908)) species indicate that the tropical larvae have a higher Mg^{2+} content (M. Lamare 2009, unpublished data). In addition, scanning microscopic examination of the arm skeleton of echinoplutei (figure 6) indicates that the structural integrity of the skeletal rods of *S. neumayeri* larvae was not affected at pH 7.5, whereas the skeleton of temperate *E. chloroticus* larvae was pitted in pH 7.7 treatments [45]. Given the strong relationship between temperature and the Ca : Mg ratio of the adult skeleton, it seems likely that a parallel trend characterizes the larval skeleton. Comparative data on the mineralogy of echinoid larval skeletons in species from the tropics to the poles
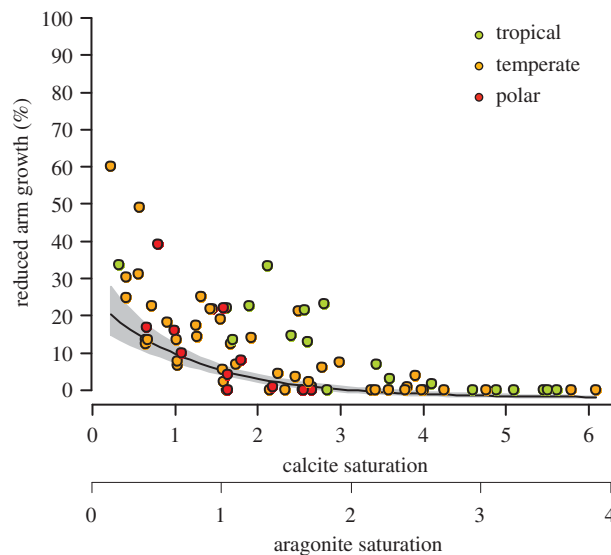
are needed to determine whether there is a similar latitudinal trend in Mg^{2+} content.

(b) Effects of ocean acidification on echinopluteal form, function, survivorship and fitness

In addition to being smaller than larvae reared in ambient control conditions (figure 2), echinoplutei reared in ocean acidification conditions also exhibit altered body morphometry, including increased abnormality, increased left–right asymmetry and a change in the relationship between arm and body size (table 1). This has been reported for *T. gratilla*, *S. neumayeri*, *Dendraster excentricus* (Eschscholtz 1829), *Strongylocentrotus purpuratus* (Stimpson, 1857), *Lytechinus pictus* (Verrill, 1867) and *E. mathaei* [42,46,52,58] but not for *Paracentrotus lividus* (Lamarck, 1816) and in a different study of *S. purpuratus* [53,81]. A recent study on the effects of acidification in the tropical sand dollar *Arachnoides placenta* (Linnaeus, 1758) to the eight-arm echinopluteus, a facultative planktotroph that does not require larval feeding to achieve metamorphosis, showed that in addition to being smaller at pCO_2 1900 μatm (pH 7.6), with long-term rearing, the larvae started to decrease in size [42]. This indicated that their growth would not catch up given time. The larvae of *A. placenta* may have arrested development, ceased growing or may have been resorbing their skeleton.

The inability of plutei to grow their skeleton in a timely manner, resulting in smaller larvae with a longer planktonic duration, will increase susceptibility to predation, decreasing chances of survival and recruitment [82,83]. The arm rods are essential for feeding, swimming and protection from predation, and feeding success is related to arm length [40,41,83,84]. Echinoplutei with an altered body profile in ocean acidification treatments are poor eaters [49]. Owing to plasticity in allocation of growth to arms and the juvenile rudiment, well-fed echinoplutei often develop small arms [40,41,84]. Therefore, it is hard to predict whether the arms of well-fed larvae reared in acidification conditions would be
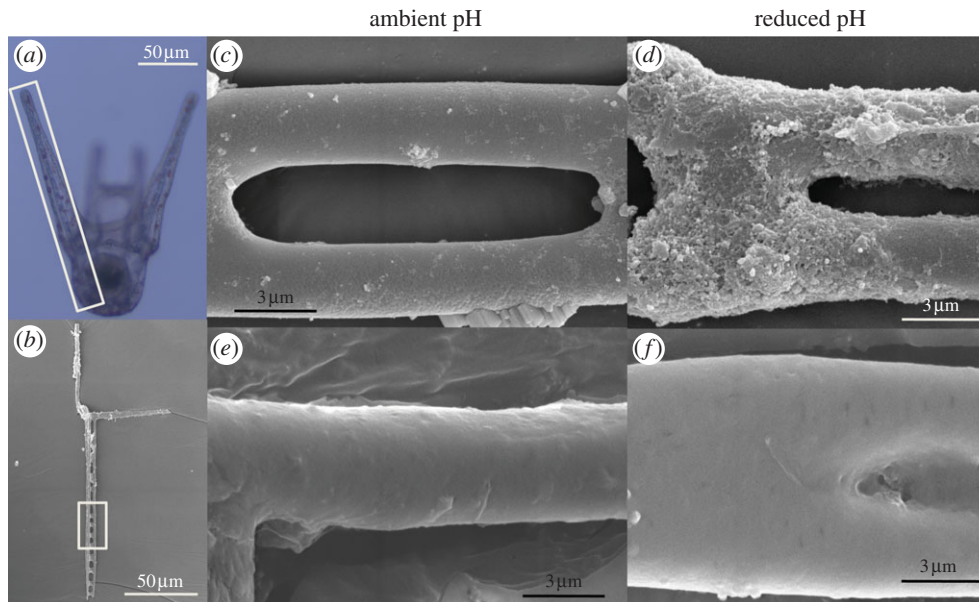
Figure 6. Effects of reduced seawater pH_{NIST} on larval skeleton surface features. (*a,b*) *Evechinus chloroticus* (Valenciennes, 1846) larva showing postoral arm and skeletal rod (in box) in the living larvae and isolated for scanning electron microscopy. (*c,d*) Scanning view of the surface of the postoral arm rod of *E. chloroticus* reared in ambient (pH 8.1) and reduced seawater pH (pH 7.7) conditions, the latter showing surface etching and abnormal deposition. (*e,f*) The postoral arm rod of *Sterechinus neumayeri* (Meissner, 1900) reared in ambient (pH 8.0) and reduced seawater pH (pH 7.5) conditions with no changes evident [49]. (Online version in colour.)

even smaller in the presence of food, or if enhanced nutrition would reduce the negative effects of acidification [34]. Our analysis of growth in pre-feeding echinoplutei covers just a small portion of the planktonic life stage. It is important to extend investigations of the impacts of ocean acidification through the feeding larval stage, especially for polar species that have a very long planktonic phase. Most importantly, in these investigations, it will be essential to monitor larval density to ensure that the larva–algal food ratio remains constant. This is required to discern between growth plasticity in response to food regime and changes in growth owing to experimental treatments. It is also important to consider the impact of acidification on algal condition. In microcosms where grazers and algae were reared in ocean acidification and warming conditions, consumption by grazers was reduced and algae was less palatable to grazers [85,86].

Reduced larval calcification in near-future ocean acidification conditions is also evident for the calcifying larvae of molluscs [87,88]. Non-calcifying planktotrophic bipinnaria larvae of asteroids also exhibit decreased growth and altered morphology in ocean acidification conditions [89,90]. By contrast, growth in the non-calcifying lecithotrophic asteroid brachiolaria and coral planula larvae are not affected by increased pCO_2 [26,91,92]. In addition to changing larval size and form, ocean acidification has a negative effect on survivorship of echinoplutei, with a 10–20% increase in mortality at pCO_2 1000+ μatm [42]. Overall, the negative impact of ocean acidification on the size and survivorship of echinoplutei, and other larvae, will lower their performance, compromising success of the pelagic life stage.

## (c) Mechanisms underlying reduction of echinopluteal growth in ocean acidification conditions

Changes in echinopluteal morphometry, metabolism and gene expression in response to ocean acidification (table 1) indicate fundamental disruptions to mechanisms of development. Our analysis with the global dataset indicated that reduction in larval size (and perhaps other responses– metabolism, gene expression and larval profile) is largely driven by increased pCO_2.

Organism hypercapnia (increased body pCO_2) has a strong influence on metabolism [10,11]. The responses of sea urchin larvae have varied, with some studies showing that elevated CO_2 suppresses larval metabolism in the larvae of *S. purpuratus* [93] whereas others indicate elevated metabolism in the same species with diversion of energy to acid/base regulation away from growth and so causing developmental delay [94]. Other studies found no change in the metabolism of the larvae of *S. purpuratus* in ocean acidification conditions [32]. More data are needed to understand variation in metabolic responses even within the same species. Thus far, evidence for delayed development to the pluteus stage is equivocal with no change in the embryonic cell cycle, gastrulation and first appearance of skeleton under ocean acidification conditions, although a delay in hatching is reported [59,95]. In addition, a recent study showed that the larvae of *S. purpuratus* reared in ocean acidification conditions actually develop faster than those reared in control conditions and have a shorter time to metamorphosis [31].

The presence of smaller calcifying larvae in ocean acidification experiments is generally thought to be due to physiological stress on calcification systems caused by reduced Ω_{ca}. Therefore, it is interesting to note that Ω_{ca} did not exert a strong influence on larval arm growth in the global dataset. Calcification in sea urchin development initially involves the production of amorphous calcium carbonate (ACC) by primary mesenchyme cells [96], using Ca^{2+} and HCO_3^- from seawater (60% of HCO_3^- requirements), and the conversion of respired CO_2 to HCO_3^- (40% of requirements) [64]. For this reason, a stronger correlation of arm growth with pCO_2 rather than with carbonate mineral saturation (estimated from the saturation states of CO_3^{2-} and Ca^{2+}), in our global data analyses, reflects the requirement of HCO_3^- for calcium carbonate production and
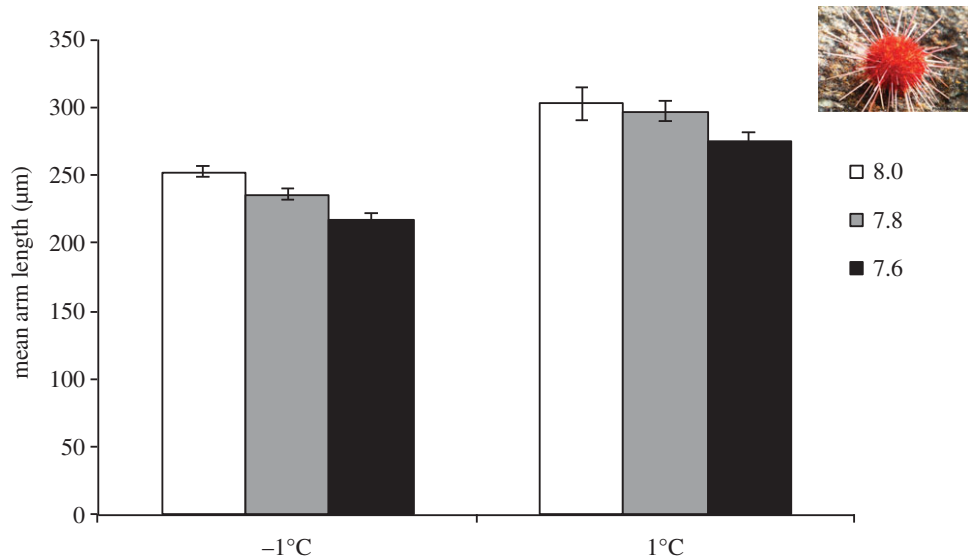
Figure 7. Effects of simultaneous exposure to increased temperature and reduced seawater pH_{NIST} on length of the postoral arm skeleton in the larvae of the Antarctic sea urchin *Sterechinus neumayeri*. Increased temperature increased larval growth in low pH treatments ($n = 12$, with 30 larvae measured per replicate [58]). (Online version in colour.)

the changes that occur in seawater with acidification (i.e. increases in HCO_3^- and CO_2 concentrations and decreases in pH and CO_3^{2-}). Thus, as shown by Stumpp *et al.* [65], the process of ACC precipitation is dependent on H^+ and HCO_3^- concentrations in the cytosol rather than seawater Ω states. The detailed understanding of the molecular biology of the cells involved in larval calcification in the sea urchin model [97] provides an opportunity to achieve a mechanistic understanding of why larvae are smaller and produce less skeleton in ocean acidification treatments.

The reduction in growth and calcification in echinoplutei reared in elevated pCO_2 appears primarily to be an energetic problem owing to regulation of internal pH and transport costs, rather than a physiochemical problem as observed in other metazoans [11,21,65,94]. Therefore, lower carbonate mineral saturation may not directly impair calcification. Stumpp *et al.* [65] suggest that the metabolic costs associated with pH_i regulation will increase larval mortality, and, although not examined in this review, several papers point to the positive relationship between increased pCO_2 and mortality in echinoderm development [42,89,90]. The strong stunting effect of ocean acidification on growth in non-calcifying feeding larvae (e.g. asteroid bipinnaria), for which carbonate mineral saturation is not likely to be a major direct issue, also shows the strong influence of pCO_2 [89,90].

(d) Interactive effects of warming and acidification to echinopluteal development

While ocean acidification/hypercapnia reduces the size of echinoplutei (table 1 and figure 2), increased temperature has the opposite effect, resulting in faster growth, larger larvae and skeletons and enhanced metabolism in warmer conditions, up to thermotolerance limits when developmental impairment or failure occurs [14,67]. As characteristic of many ectotherms [98], echinopluteal growth is likely to have a nonlinear response to increasing temperature, with initial elevation of metabolic rates and increasing fitness until a thermal threshold is reached, beyond which deleterious effects occur. A 4°C warming above ambient approaches the developmental thermal limit of many larval

echinoids, although some species tolerate much greater levels of temperature increase [99–101]. For the tropical sand dollar *A. placenta*, larval size increases with warming, but as the boundary of optimal temperature for development is approached and exceeded larvae are smaller [101]. For asteroid brachiolaria, near-future warming also stunted larval growth [26].

Understanding the potential interactive effect of concurrent ocean acidification and warming remains a significant challenge. The positive effect of temperature on growth may reduce the negative effects of ocean acidification on skeletogenesis, or it may act synergistically in a negative way, when temperature thresholds are breached [14,46]. Acidification and warming may also have additive deleterious effects, with both exerting a negative influence on larval growth [67]. Thus far, the interactive effects of near-future ocean warming and acidification, on marine invertebrate development have been investigated for 23 species (three corals, nine molluscs, six echinoderms and five crustaceans) including three sea urchin species [67]. In several studies, near-future warming, not acidification, is the more important stressor, especially with regard to survivorship of early embryos [26,102]. For those embryos that develop through the early bottleneck of mortality to reach the larval stage, the effects of simultaneous exposure to warming and acidification differ depending on species and stressor levels used [67]. Temperature is also the more important stressor to diatoms [103], the major food for plutei.

For the echinoplutei of *T. gratilla* that tolerate 3°C above ambient, in the subtropical part of its range, the stunting effect of increased pCO_2 on larval growth and calcification is mitigated by this level (ca near-future) of warming [46]. A similar response is evident for growth in the post-metamorphic juvenile sea urchins to 2°C above ambient [88]. For the larvae of *S. neumayeri*, a 2°C warming reduced the magnitude of the effect of acidification on growth (figure 7), however a 3°C warming is deleterious to development. Thus, hypothesis no. 4, that near-future warming reduces the negative effect of decreased pH on larval growth in echinoplutei, is, broadly speaking, supported; however, more data from cross-factorial studies are needed to more fully understand the interactions between these two stressors.

4. Conclusion

Despite variation in habitat, latitude and methods across studies, trends are emerging with respect to the effects of ocean acidification on echinopluteal growth and where sensitivities lie. Ocean acidification has a general stunting effect on echinoplutei, with a particularly significant relationship between reduced arm growth and increased pCO_2. The similar responses in echinoplutei across regions, including smaller arms, changes in morphometry and increased abnormality and mortality are likely owing to the interactive effects of this metabolic stressor (hypercapnia) and the toxic effects of acidification on regulation of development and body patterning.

There were some differences in the strength of the reduced arm growth response across regions, indicating potential differences in species' vulnerability in a future ocean. Interestingly, tropical species may be more vulnerable to reduced Ω_{ca}, whereas polar species (albiet only two in the dataset) were more resilient (but see [59]). This may reflect the naturally lower (polar) and higher (tropical) Ω_{ca} for these regions and the naturally lower (polar) and higher (tropical) Mg^{2+} content of the skeleton. Polar species are likely to be adapted to the stable and naturally low carbonate ion concentrations characteristic of these waters for millions of years [36]. More data for polar echinoplutei are needed, especially data for the larvae of Northern Hemisphere high-latitude sea urchins.

Our synthesis included larvae derived from sea urchins from tropical–temperate transition zones. There is a great diversity of sea urchins with the planktotrophic-echinopluteus life history in the tropics that warrant investigation on the effects of ocean change stressors to development. Many sea urchin species, for instance *T. gratilla*, have broad tropical–temperate distributions, and others, for instance the temperate *Centrostephanus rodgersii* (A. Agassiz, 1863), have their northern limit in subtropical regions [62]. Insights will be generated by investigation of the responses of the larvae of broad-range species across their tropical–temperate distribution.

The larvae of *S. purpuratus* from the Eastern Pacific upwelling system are comparatively more resilient to near-future acidification projections, exhibiting a low decrease in arm growth in high pCO_2 [31,32,56]. Populations of *S. purpuratus* from this region have experienced upwelling of CO_2-rich water for thousands of years and so may be acclimatized/adapted to seawater acidification [31,32,56]. However, the shifting baseline of increasing acidification may move pH spikes during upwelling to levels beyond present day threshold tolerances, as indicated by failure of spat in bivalve hatcheries in the region [33]. The *S. purpuratus* larvae were also generated from adults in intertidal rock pools that experienced regular hypercapnic conditions, and the comparative resilience of their larvae to ocean acidification is suggested to be influenced by environmental conditions in the adult habitat [31,32]. Larvae of the European intertidal species *P. lividus* are also comparatively more resilient to seawater acidification, and this is influenced by tide pool of origin of the adults [30]. Intertidal sand dollar larvae exhibited the greatest tolerance to ocean acidification conditions, with respect to the arm growth response. Living in the intertidal zone may convey some resilience to the larvae of rock pool and sandy beach echinoids, as is also the case for the early metamorphic juveniles of intertidal sea urchins [104]. However, the potential drivers of the growth response of larvae from intertidal urchins to seawater acidification were

not clear in our analyses. There is an indication that intertidal and subtidal urchins may differ for some parameters (see the electronic supplementary material, table S2); however, more data are needed to explore potential trends in the differences in responses of the larvae of intertidal and subtidal urchins.

Despite the pervasive effect of temperature on development, ocean warming is rarely considered in studies of climate change impacts on marine larvae. Both warming and acidification have negative impacts on sea urchin development. Increased temperature is particularly deleterious to early developmental (prelarval) stage and is the more important stressor for some echinoderm, coral and crustacean larvae [26,67,88,102] and for their algal food [103]. Especially for regions with appreciable ocean warming, more multi-stressor studies are needed, because acidification may interact with high temperature (and other climate-related stressors) to narrow stress tolerance ranges across life stages [10,11,21,67,102,105].

The ocean will change over coming decades more gradually than in laboratory experiments, and it is likely that changing ocean conditions may result in production of more resistant sea urchin larvae, through phenotypic buffering and natural selection. The presence of normal larvae reared in near-future warming (ca +2−3°C) and decreased pH (ca pH 7.8) conditions in several studies (table 1) indicate that a tolerant subset of embryos/echinoplutei are resilient to near-future ocean change conditions. Acclimatization (phenotypic plasticity) of urchins and oysters to moderately elevated pCO_2 can result in trans-life cycle enhancement of larval and juvenile tolerance of reduced pH in some species ([87,106], but see [43]). Similar transgenerational phenotypic resilience to increased temperature has also been shown for sea urchins [107,108]. Adaptation through natural selection over coming decades may also facilitate persistence in a changing ocean, especially for species from warmer latitudes that have a comparatively shorter generation time compared with polar species. Recent quantitative genetics and genomic studies with echinoids indicate the presence of traits to facilitate resilience and adaptation (genetic) to ocean acidification [31,32,54,109], ocean warming and both stressors [110].

As yet, there are few data on species' response across the complete life cycle of marine invertebrates to ocean acidification in isolation, and combined with (cf. [31]) warming and other climate-related stressors, to assess transgenerational adaptive potential to changing ocean conditions [67]. This will require long-term rearing in multi-stressor conditions, from fertilization through planktonic development to maturation into adults, which are then used to generate F_1 progeny for new generations. These studies are challenging and are more feasible for organisms with short generation times, but are possible for sea urchins, particularly tropical species. Long-term microcosm experiments are providing insights into potential outcomes with regard to seasonal change and acclimatization adaptation [85,111,112]. Multi-stressor studies on sea urchin development have incorporated most life-history stages [67], and the next step is to investigate the complete bentho−pelagic life cycle in changing ocean conditions.

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