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Acclimation to low pH does not affect the thermal tolerance of *Arbacia lixula* progeny

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As the ocean warms, the thermal tolerance of marine invertebrates is key to determining their distributional change, where acclimation to low pH may impact the thermal range of optimal development. We compared thermal tolerance of progeny from a low pH-acclimated sea urchin (*Arbacia lixula*) population from the CO_2 vents of Ischia (Italy) and a nearby population living at ambient pH. The percentages of normally developing gastrulae and two-armed larvae were determined across 10 temperatures representing present and future temperature conditions (16–34°C). Vent-acclimated sea urchins showed a greater percentage of normal development at 24 h, with a larger optimal developmental temperature range than control sea urchins (12.3°C versus 5.4°C range, respectively). At 48 h, upper lethal temperatures for 50% survival with respect to ambient temperatures were similar between control (+6.8°C) and vent (+6.2°C) populations. Thus, acclimation to low pH did not impact the broad thermal tolerance of *A. lixula* progeny. With *A. lixula*'s barrens-forming abilities, its wide thermotolerance and its capacity to acclimate to low pH, this species will continue to be an important ecological engineer in Mediterranean macroalgal ecosystems in a changing ocean.

1. Introduction

The latitudinal range of marine ectotherms is closely linked to adult [1,2] and planktonic stage [3] thermal tolerance. Early developmental stages, such as embryos and larvae, often have the narrowest thermal tolerance and have been shown to predict the realized thermal niche and adult distribution [3–5]. Climate change-driven range shifts have occurred in a plethora of marine invertebrates [1,6], such as the poleward invasion of the sea urchin *Centrostephanus rodgersii* to Tasmania as waters reached temperatures favourable for larval development [3,7].

The effects of multiple stressors have been examined for marine invertebrate development, where effects of temperature on larval development can interact with other environmental variables [8,9]. Ocean warming can ameliorate the negative effects of acidification on calcification [9]. Whether adult acclimation to low pH affects the developmental thermal tolerance range of progeny is not well understood. It could be expected that temperature thresholds would be reduced as negative transgenerational effects have been observed on the survival of amphipod progeny from adults maintained at low pH [10]. For several crab species simultaneously exposed to low pH and high temperature, the thermal tolerance window was reduced [11,12]. On the other hand, a study of five echinoderm species found that thermal tolerance of early development was not impacted by decreased pH [13].

CO$_2$ vent systems are used as proxy laboratories to understand potential impacts of ocean acidification and provide insights into specific adaptations that animals might need to survive in low pH [14–16]. The sea urchin *Arbacia lixula* investigated here has occupied a low pH vent site in Ischia, Italy, with mean pH$_T$ 7.78, for approximately 30 years [15]. At these vent sites, sea urchins are similar in abundance and size to those in nearby ambient regions [17,18]. We compared the thermal range for optimal development, and the upper and lower lethal temperatures for offspring of vent-acclimated and nearby ambient *A. lixula* populations. *Arbacia lixula* adjusts its physiology to allow it to inhabit low pH vent sites [19]—whether this adjustment has flow on impacts for offspring thermal tolerance is not known. The potential that acidification may narrow the thermal range of offspring would have important consequences for species' distribution as oceans continue to change.

The Mediterranean Sea is warming 20% faster than the global average [20], with predicted warming of up to 5.8°C by 2100 [21]. Warming has favoured the expansion of native and alien thermophilic species towards northern regions of the sea [22,23]. With progressive species' shifts towards the northern winter minimum 14°C isotherm [23], there are major consequences for the biogeography of the region [24]. In conjunction with warming, pH is expected to drop between 0.25 and 0.46 units by 2100 [25].

Arbacia lixula is one of the most abundant echinoids in the Mediterranean Sea, particularly in the south basin [26]. It is a keystone engineer due to its barrens-forming abilities [27,28]. Being a species of tropical affinity [29], warming has supported an increase in *A. lixula* abundance in the Mediterranean [28]. Considering its potential to greatly impact shallow rocky reefs [26], it is important to assess whether the broad developmental thermotolerance of *A. lixula* [30] is modulated by acclimation to low pH.

For the first time, we determined the developmental thermal tolerance of a population of sea urchins residing in a low pH vent system. We compared the thermal range, the optimal and lethal temperatures of the offspring of vent-acclimated and ambient *A. lixula* populations. We hypothesized that the thermal tolerance of the embryos and larvae of vent-acclimated animals would be lower than that of the control population. These findings would have important consequences for this ecologically important sea urchin in a future ocean.

2. Material and Methods

(a) Echinoderm collection and spawning

Ten *Arbacia lixula* were collected from each of two sites in September 2017 during their spawning season (May–October, [31]). Site one at S. Pietro (40°44′46.70″ N; 13°56′40.95″ E), approximately 4 km from the vent site, had ambient pH levels (pH$_T$ 8.001). Site two at the North side of the Castello Aragonese, Ischia (40°43′55.84″ N; 13°57′52.02″ E) had decreased pH (mean pH$_T$ ± s.d. of 7.69 ± 0.23, determined through two weeks of monitoring with a SeaFET™ ($n = 296$)). Ischia seawater ranges from 14.5 to 26.0°C (Gambi MC, Lorenti M 2016, unpublished data). Ambient temperature at the time of the experiment was 24°C [32], and this was considered the control temperature. Upon collection, the urchins were kept in cool boxes with seawater from their place of origin and spawned within a few hours by injection of 2–4 ml of 0.5 M KCl, with eggs directly collected into beakers (500 ml) of fresh filtered seawater (FSW 0.22 μm) at ambient pH and temperature (24°C). Sperm was collected dry and kept on ice until use. After spawning, sea urchins were returned to their collection sites.

(b) Fertilization procedure

Three independent fertilizations were performed with urchins from the two sites, creating three individual male–female crosses per site. All fertilizations were performed at the control temperature (24°C) with ambient FSW.

For each fertilization, one male and one female were spawned. After determining egg density in counts of 100 ml aliquots from the original collection, approximately 200 000 eggs were transferred to a 2.5 l beaker filled with FSW. Sperm was activated with FSW just prior to fertilization. Haemocytometer counts were used to determine the amount of sperm required to achieve a final concentration of 10^4 sperm ml^{-1}. After 10 min, fertilization success was checked and was approximately 90–95%. The fertilized eggs were rinsed to remove excess sperm, counted and placed at a concentration of 10/mL in 30 ml glass vials, with each vial filled with ambient FSW at control temperature. For each cross, timepoint (24 and 48 h) and temperature treatment, there were 3–6 individual replicate vials.

(c) Temperature treatments

Ten temperatures were tested (16–34°C in 2°C increments; electronic supplementary material, table S1) using 10 water baths. Each bath consisted of a 15 l tray with an aquarium heater (Tronic 50 W) and aquarium pump (Askoll Micromega pump 320 L h^{-1}, 5.5 W) used to control temperature evenly throughout the bath. Temperature was checked every 4 h during the experimental period of 48 h (electronic supplementary material, table S1) using a Mettler Toledo SevenGo meter. Vials were placed in temperature treatments within 1 h of fertilization, and development was scored at 24 h (gastrulae) and 48 h (two-armed larvae). At each timepoint, 50 embryos from each vial were scored live under the microscope in a temperature-controlled laboratory (approx. 24°C) to determine the percentage of normal-developing embryos. Abnormal embryos included those that were dead, undeveloped, asymmetrical or malformed.

(d) Statistical analyses

We examined the role of temperature on developmental success using generalized linear models (GLM) with the lme4 package within R v. 3.5.0. Because separate sets of vials were used for the two timepoints, two separate GLMs were created for each timepoint (24 and 48 h). The full model for normally developing embryos included temperature as a quadratic fixed effect and urchin population (control or vent) as a fixed effect, with cross (three for each urchin population) nested within population, including the interactions between the fixed effects. Data were modelled with a polynomial binomial model with probit link. Visual inspection of model diagnostic plots showed that model assumptions were met. The GLMs were used to determine T_{opt} (temperature range with survival greater than 75%), and the lower and upper lethal temperatures with greater than 50% mortality (LT$_{50}$).

3. Results

At 24 h, temperature had a significant effect on the percentage of normally developing embryos, which was less than 25% at 32°C for both vent and control populations (table 1 and figure 1). Sea urchin population was significant, as well as the interaction between population and temperature, where vent sea urchins had a wider T_{opt} range (12.3°C) than control sea urchins (5.4°C). Additionally, the upper LT$_{50}$ of vent sea urchins was slightly higher than for control sea urchins (+6.3 versus +5.7°C, respectively; tables 1 and 2).
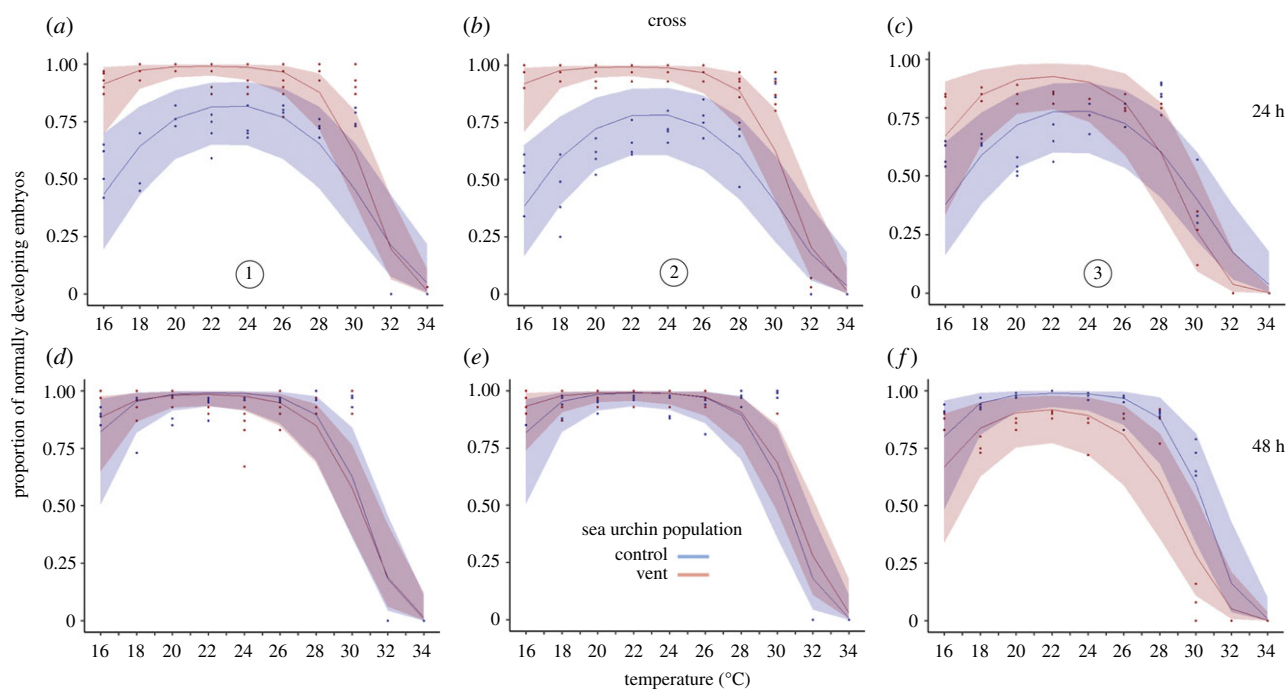
Figure 1. Thermal tolerance for normally developing *Arbacia lixula* embryos and larvae across 10 temperatures. The curves were modelled using a binomial GLM with the shading reflecting the 95% confidence interval of the fitted curves. For each population of sea urchin (vent or control), three crosses were examined, each displayed separately here. Graphs (*a*–*c*) represent the results from 24 h with (*d*–*f*) from 48 h.

Table 1. Results of the GLMs examining the roles of temperature, population and cross on developmental windows. Significant effects are indicated in italics.

factor	estimate	s.e.	Z	p
24 h				
temp	−7.8334	2.996	−3.406	0.000658
temp^2	−10.9931	2.3222	−4.452	8.5×10^{-6}
population	0.9065	0.3508	2.584	0.00976
temp * population	−10.957	3.7072	−2.956	0.003121
temp^2 * population	−4.2515	3.8659	−1.100	0.271454
control cross 2	−0.1247	0.3139	−0.397	0.691095
vent cross 2	0.0448	0.3719	0.120	0.904093
control cross 3	−0.1372	0.3157	−0.435	0.663786
vent cross 3	−0.9189	0.4102	−2.240	0.025069
48 h				
temp	−16.82079	3.09511	−0.5435	5.49×10^{-8}
temp^2	−16.90874	3.66511	−4.613	3.96×10^{-6}
population	−0.07085	0.41018	−0.173	0.86287
temp * population	−0.24803	4.14794	−0.060	0.95232
temp^2 * population	3.22267	4.69878	0.686	0.49281
control cross 2	−0.01133	0.45009	−0.025	0.97992
vent cross 2	0.29493	0.36831	0.801	0.42327
control cross 3	−0.07978	0.44400	−0.180	0.85741
vent cross 3	−0.76242	0.38657	−1.972	0.04858

For vent sea urchins, cross three performed significantly differently from the others, where the upper LT$_{50}$ was 2°C lower than the other two populations. The lower LT$_{50}$ was not reached by any vent sea urchin cross (table 1; electronic supplementary material, table S2). For control sea urchins, all crosses performed similarly to similar upper and lower LT$_{50}$'s (electronic supplementary material, table S2).

At 48 h, temperature was significant, where a reduction in the percentage of normal larvae was seen at 32°C for both vent and control populations (figure 1). At this timepoint, however, population was not significant and did not significantly

Table 2. Mean thermal optimum range (T_{opt}) with survival greater than or equal to 75% and upper temperatures with greater than 50% mortality (LT_{50}) in *A. lixula* after 24 and 48 h. Results for the three crosses per population and the lower LT_{50} (only obtained for one population at one timepoint) are shown in electronic supplementary material, Table S2.

population	T_{opt} (greater than 75%)	upper LT_{50}
24 h		
control	20.3–25.7	29.7
vent	16.2–28.5	30.3
48 h		
control	16–29.2	30.8
vent	16.3–28.2	30.2

interact with temperature (table 1). Both populations performed similarly across all temperatures examined, where the T_{opt} range was comparable for control (13.2°C) and vent (11.9°C) sea urchins with similar upper LT_{50}'s for control (+6.8°C) and vent (+6.2°C) populations (table 2).

Similar to 24 h, vent cross three performed significantly differently from the others (figure 1), where the upper LT_{50} was approximately 2°C lower than the other two populations. The lower LT_{50} was not reached for any population where all control crosses performed similarly (electronic supplementary material, table S2).

4. Discussion

We examined whether acclimation to low pH would affect the developmental thermal tolerance of *A. lixula* progeny. Although the percentage of normally developing embryos was higher in vent sea urchins than in control sea urchins at 24 h, the upper thermal limits were similar, with steep decreases in the percentage of normally developing embryos at 32°C. By the larval stage, vent and control sea urchins had similar thermal curves, with similar T_{opt} ranges and upper LT_{50}. Therefore, we found no evidence for deleterious transgenerational impacts of ocean acidification on the thermal limits of early development for this species.

Thermal tolerance can vary across life-history stages. For the control population, larvae exhibited a higher percentage of normally developing embryos in comparison to gastrulae. For vent urchins, the numbers of normally developing embryos were similar across both stages. The higher percentage of normal gastrulae for vent urchins in comparison to control urchins may be due to differences in maternal provisioning, including cellular defences that protect early embryos [33]. The egg jelly coats of vent-acclimated *A. lixula* are more resistant to low pH than control sea urchin jelly coats, reflecting a strategy used to allow embryos to inhabit low pH vents and increase fertilization success [14]. Thus, it is likely that egg constituents also support enhanced embryo resilience in *A. lixula*.

Similar to other studies, the results show that *A. lixula* larvae have a low cold tolerance, where the lower LT_{50} was not reached for the majority of crosses examined here. For *A. lixula* from the Northwestern Mediterranean, larvae were able to develop at temperatures down to 16°C [31], although research suggests its reproductive behaviour is impacted by suboptimal temperatures in this region [34]. For *A. lixula* larvae from the Southern

Mediterranean, optimal development occurs at 24°C [30], similar to our findings and coinciding with the thermal history of *A. lixula* adults during the time of our experiment. While it has been hypothesized that the range for normal development of *A. lixula* in the Mediterranean could be as large as 16–26°C [28], our results show a broader T_{opt} range of approximately 16–29°C (based on 75% success) for larvae of control populations. As the upper thermal LT_{50} of all populations, except one vent cross, was greater than the worst-case warming scenario by 2100 for the Mediterranean, i.e. +5.8°C [21], *A. lixula* will continue to thrive in a warming ocean.

For the thermophilous sea urchin *A. lixula*, decreasing pH may not represent a barrier to range expansion, where progeny show a similar thermal tolerance when parents are acclimated to low pH. With expansion not limited by gene flow [35], *A. lixula* has the potential to maintain its distribution in the Southern Mediterranean with ocean warming facilitating further expansion in the NW, especially as temperature approaches T_{opt}.

Further experiments combining the temperature treatments with pH would help determine whether resilience to low pH is transferred to progeny, as well as resilience to simultaneous changes in pH and temperature. Future work contrasting the lipid and protein profiles of eggs from the different sea urchin populations would be important in understanding long-term impacts of low pH on maternal provisioning. Lastly, examining thermal tolerance of later developmental stages and whether they match the adult thermal niche will help to more accurately model range shift patterns of *A. lixula* in a warming ocean.

These results emphasize the concerns of the negative impact of *A. lixula* on Mediterranean ecosystems, where its ability to acclimate to low pH in conjunction with a wide thermotolerance will contribute to the colonizing ability of this species in a future ocean. In particular, tolerance to acidification and warming may create an amplifying impact of the barrens created by *A. lixula*, with serious consequences for coastal habitats of the Mediterranean Sea [27].

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