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The embryonic thermal environment has positive but weak effects on thermal tolerance later in life in the aquatic invertebrate *Gammarus chevreuxi*

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ABSTRACT

Recent evidence suggests that the adult phenotype is influenced by temperatures experienced in early life. However, our understanding of the extent to which the embryonic environment can modulate thermal tolerance later in life is limited, owing to the paucity of studies with appropriate experimental designs to test for this form of developmental plasticity. We investigated whether the thermal environment experienced during embryonic development affects thermal limits in later life. Embryos of the estuarine amphipod *Gammarus chevreuxi* were incubated until hatching to 15 °C, 20 °C and 25 °C, then reared under a common temperature. Using thermal ramping assays, we determined upper thermal limits in juveniles, four weeks post-hatch. Individuals exposed to higher temperatures during embryonic development displayed greater thermal tolerance as juveniles (acclimation response ratio ≈ 0.10–0.25 for upper lethal temperature). However, we suggest that the degree of developmental plasticity observed is limited, and will provide little benefit under future climate change scenarios.

1. Introduction

Phenotypic plasticity is an important mechanism increasing resilience of ectotherms to environmental change (Bush et al., 2016; Gunderson et al., 2017; Huey et al., 2012; Morley et al., 2019; Seebacher et al., 2015). However, research to date has focused largely of adults, with less emphasis on the importance of developmental plasticity (Spicer et al., 2018). In the face of rapid environmental change, aquatic animals with complex life cycles will experience different environmental conditions at different life stages, and there is increasing evidence that different life stages have different sensitivities (Davison, 1969; Kingsolver et al., 2011; Krebs and Loeschcke, 1995; Truebano et al., 2018a) and acclimatory capacities (*sensu* Stillman, 2003; Kingsolver and Huey, 1998; Marais and Chown, 2008; Truebano et al., 2018b). While conditions experienced during early life can affect survival and fitness (Lindström, 1999), the potential consequences of pre-exposure during early development, on the ability to cope with stress later in life has received little attention (Pottier et al., 2022).

Thermal acclimation can occur during early development and then be carried over into later stages (Maynard Smith, 1957). This form of developmental plasticity can lead to remodeling of the phenotype with consequences later in life (Pottier et al., 2022), and thus could help some

ectotherms survive the thermal extremes predicted under current climate change scenarios. Exposure to elevated temperatures during early development can result in increased thermal tolerance and/or performance in later life in a range of ectotherm species (Scott and Johnston, 2012; Seebacher and Grigaltchik, 2014; Sfakianakis et al., 2011; Slotsbo et al., 2016; van Heerwaarden et al., 2016). However, other species show little evidence of such plasticity (Abayarathna et al., 2019; Carey and Franklin, 2009; Gunderson et al., 2020; Terblanche and Chown, 2006), or even suffer maladaptive effects (Dayananda et al., 2017), when individuals are then reared under common garden conditions. Overall, few studies have focused on the extent to which the embryonic environment can affect thermal performance in later life. A recent meta-analysis (Pottier et al., 2022) suggested a tendency towards reduced thermal tolerance in individuals that had been exposed to elevated temperatures as embryos, and then tested in later life stages. However, they also highlighted the paucity of studies with an appropriate experimental design to test for this form of developmental plasticity.

Therefore, we investigated whether the thermal environment experienced during embryonic development affected thermal limits later in life through developmental plasticity. Here, we use the term 'thermal limits' to refer to both lethal and sub-lethal thresholds, whereas 'thermal

tolerance' is used to refer to lethal thresholds only. Newly fertilized eggs were exposed to a range of temperatures and, upon hatching, reared under a common temperature for four weeks. After this time, thermal ramping assays were performed using juveniles, during which we determined sub-lethal and lethal thresholds. Previous work in our laboratory found that amphipods developing under higher temperatures have greater acclimatory capacity for some physiological traits as adults. Therefore, we predicted that embryos developing at higher temperatures would show increased thermal limits later in life. The amphipod *Gammarus chevreuxi* was used as a model aquatic invertebrate as it is lab-hardy, with well-characterized reproduction (Sexton, 1928), and direct development (Truebano et al., 2020). Gammarid amphipods are abundant in coastal and estuarine areas, which experience high fluctuations in environmental parameters (Collins et al., 2022).

2. Methods

2.1. Amphipod collection and husbandry

Amphipods were collected using a kick net (mesh size = 500 μm) from the Plym estuary, Devon (50° 23′ 24″ N, 4° 5′ 7″ W) during low tide in January 2020. The specific site is in a brackishwater (tidal) stream, that experiences variable temperature, oxygen and salinity conditions, both seasonally and daily (Collins et al., 2019, 2022). At the time of collection, temperature and oxygen in the main stream were approximately 8.5 °C and 100 % air saturation respectively. Salinity varied daily from 2 to 29. Individuals were transferred in some of the water they were collected from to a temperature-controlled environment where they were sorted into three stock aquaria (volume = 8 L) supplied with a substratum of aquarium gravel to provide shelter. Aquaria contained dilute sea water, temperature = 15 °C, salinity = 15, O_2 = 100 % air saturation) and experienced a 12 h:12 h Light:Dark cycle. Amphipods were held in the stock aquaria in pre-exposure conditions for a minimum of one week, during which period any pairs were discarded. Amphipods were fed carrot *ad libitium*.

2.2. Experimental conditions

After a one week pre-exposure period, pre-copula pairs were separated over a period of two weeks between three aquaria (volume = 3 L) each containing approximately 70 pairs. Environmental conditions were maintained as per the pre-exposure period. Pairs were visually examined daily and any females that had left amplexus, and carried eggs in the brood pouch, were transferred into one of three temperature treatments (temperature = 15, 20 or 25 °C, three aquaria per treatment, n = 3–7 females per aquarium) within 17 h post fertilization. Embryos were left to develop in the brood pouch until hatching. Within 17 h of leaving the brood pouch, offspring were returned to pre-exposure conditions (15 °C), where they were reared for four weeks under common garden conditions, as described for the adults above. Offspring were maintained in three aquaria per treatment, each containing a variable number of offspring (n = 5 to 100 per tank). After this time, haphazardly selected individuals were presented with a thermal challenge (N = 20 amphipods per treatment). The number of breeding females used varied between treatments (N = 18, 12 and 14 for 15 °C, 20 °C and 25 °C respectively) and it was not possible to establish how many of these females contributed offspring that were later used in thermal assays. However, the experimental design ensured that each treatment contained individuals from a minimum of three mothers.

2.3. Determination of sub-lethal and lethal thresholds

Thermal tolerance was determined using ramping assays by exposing juveniles (four weeks post-hatching) to gradually increasing temperatures at a rate of 1 °C min^{-1} from a starting temperature of 15 °C using a computer-controlled water bath (TXF200 Grant Instruments Cambridge

Ltd). This rate of change was selected based on previous work in our laboratory (Calosi et al., 2013), and supported by the rationale presented in Rezende et al. (2011). Juveniles were placed individually into one well of a 24 well-plate, partly submerged in the water bath. Juveniles were left to settle for 15 min in their wells before starting the ramping. A maximum of seven individuals were examined at any time. The temperature at which each of the thresholds was reached was measured within a well (minus amphipod) using a thermocouple (HH806AV; Omega Engineering Inc.). All wells were filled with the same volume of water to ensure heat was distributed homogeneously, and early work in our laboratory confirmed that all the wells utilized experienced the same rate of change and remained normoxic for the duration of the trials (Calosi et al., 2013). Twenty juveniles from each embryonic temperature treatment were assayed across nine plates. Once thermal assays were completed, individuals were removed from the wells, blotted dry and weighed to the nearest microgram (Electronic high-precision scale, PF-203; Fisher Scientific UK Ltd.)

The onset of circular swimming (CS) and "impaired tail flexion" (ITF) were used as sub-lethal thresholds as per (Calosi et al., 2013). CS was defined as individuals swimming rapidly in circles around the edge of the well for 5 s or more, a behaviour which was interpreted as an escape response. ITF was defined by the juveniles having a delay in tail flexion response to prodding in excess of 2 s and interpreted as impaired physiological capacity. Upper lethal temperature (ULT) was recorded when the individual showed no response to prodding. This end-point was considered "death" based on preliminary trials showing that individuals did not recover once this state was reached. The three end-points were recorded in every individual.

2.4. Statistical analyses

Linear mixed effect models were used to test the effects of development under different temperatures on lethal and sub-lethal responses using the *lme* function in the *nlme* R package. In the maximal model, developmental temperature (15, 20 and 25 °C) was specified as a fixed factor, mass as a continuous covariate, and tank and plate identity as random terms. A stepwise model reduction based on likelihood ratios (using the *anova* function in R) and AIC values was used for model simplification. There was no significant effect of mass and no significant effect of the random terms, tank or plate, for any of the measured responses, thus these factors were removed from the model. Therefore, three separate one-way ANOVA were used to test for differences in mean temperatures between developmental treatments on CS, ITF and ULT, with any differences between levels identified by Tukey's Honest significance tests. All analyses were performed using R version 4.2.0 (R Development Core Team, 2021). Data were tested for normality and variance homogeneity by visual inspection of the model residuals. Statistical significance was assigned as P ≤ 0.05. Acclimation response ratios (ARR) for each pairwise comparison (15–20 °C, 20–25 °C, 15–25 °C) were calculated as the magnitude of increase in thermal tolerance per every 1 °C increase in developmental temperature (Claussen, 1977). The 95% confidence intervals for ARR were calculated using the sample variances as derived in Pottier et al. (2022).

3. Results

Thermal developmental environment significantly affected the temperature at which the onset of circular swimming occurred (CS, $F_{2,57}$ = 5.19, p = 0.008) and tail flexing became impaired (ITF, $F_{2,57}$ = 11.08, $p < 0.001$), as well as the upper lethal temperature (ULT, $F_{2,57}$ = 11.50, $p < 0.001$) of juveniles. Mean temperature of CS onset in juveniles was significantly greater in individuals developed at 25 °C (CS = 19.63, 95% CI = 18.93, 20.33), compared to those developed at 15 °C (CS = 18.29, 95% CI = 17.69, 18.89) (p = 0.008; ARR = 0.134, 95% CI = 0.113–0.155). ITF occurred at a greater mean temperature in juveniles developed at both 20 °C (ITF = 33.08, 95% CI = 32.41, 33.74; p = 0.022;
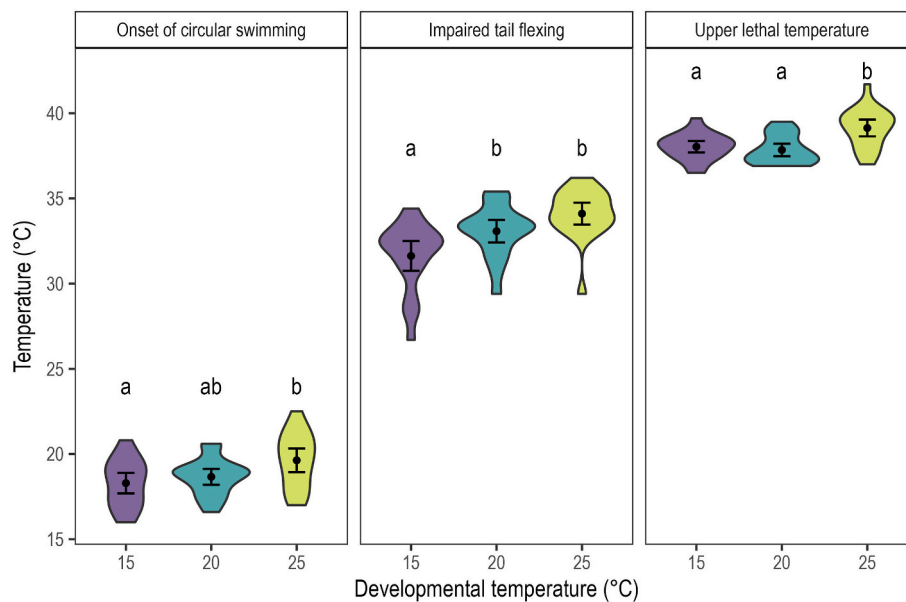
Fig. 1. Temperature (˚C) for the onset of circular swimming (left), impaired tail flexing (middle) and upper lethal temperature (right) in amphipods developed at 15 ˚C (purple, N = 20), 20 ˚C (blue, N = 20) and 25 ˚C (green, N = 20), and reared under a common temperature (15 ˚C) for four weeks from hatching. Violin plots indicate approximate frequency of data points at each temperature, with mean ± 95% confidence intervals inside the violins. Letters indicate significant differences between the temperature treatments for each of the responses.

ARR = 0.290, 95% CI = 0.241–0.339) and 25 ˚C (ITF = 34.11, 95% CI = 33.46, 34.75; $p < 0.001$; ARR = 0.248, 95% CI = 0.224–0.272) compared to 15 ˚C (ITF = 31.63, 95% CI = 30.75, 32.50). Mean ULT was greater in individuals developed at 25 ˚C (ULT = 39.14, 95% CI = 38.64, 39.63) compared to those developed at 15 ˚C (ULT = 38.03, 95% CI = 37.69, 38.34; $p = 0.001$; ARR = 0.111, 95% CI = 0.097–0.124) and 20 ˚C (ULT = 37.84, 95% CI = 37.47, 38.21; $p < 0.001$; ARR = 0.259, 95% CI = 0.231–0.287). Across the traits measured, the ARR was approximately 0.1–0.3 (Fig. 1).

4. Discussion

Here we demonstrate that the thermal environment experienced during embryonic development has significant, though weak effects on both lethal and sub-lethal responses to elevated temperatures later in life in the estuarine amphipod, *Gammarus chevreuxi*. When assessed as juveniles using ramping assays, individuals exposed to higher developmental temperatures exhibited an escape response, impaired physiological function and reached their upper thermal limits at greater temperatures than those developed under cooler conditions. However, the magnitude of this plasticity was small.

Our results support the conclusion of a recent meta-analysis, where exposure of ectotherms to higher temperatures during early life was associated with weak increases in thermal tolerance later in life, with ARR values for aquatic invertebrates estimated at 0.199 (95% CI = −0.055, 0.454), similar to those presented in our study (Pottier et al., 2022). This means that for every degree increase in developmental temperature, thermal tolerance later in life only increases by approximately 0.2 °C. In this comprehensive analysis, ARR was further reduced, when only some forms of developmental plasticity were considered. For example, when including only persistent changes in tolerance such as those observed here (i.e. those that persist following a period of exposure under control temperatures), estimated ARR in aquatic invertebrates decreased to 0.136. When only embryonic stages incubated at higher temperatures and raised in control conditions after hatching were considered, ARR decreased even further to negative values (i.e. reduced tolerance, ARR = −0.082; 95% CI = −0.248, 0.085), albeit differences were not significant, and the conclusions were based on the

limited number of studies available to date (seven studies of six species across taxa). We did not observe reduced thermal tolerance in our study, but a positive, weak effect, comparable to that estimated when all forms of developmental plasticity are considered. The slightly greater ARR values observed here could simply reflect the lack of studies with appropriate methodology to test for this form of developmental plasticity, and/or be associated with taxonomic differences in thermal histories. Aquatic amphipods are able to acclimate to elevated temperatures as adults, likely as a result of the highly variable nature of their thermal environments (Campbell et al., 2020; Collins et al., 2020, 2022). Animals inhabiting areas that experience such temporal thermal variability across generations tend to have greater developmental plasticity (Cooper et al., 2012). In this species, females carry developing eggs in their brood pouch and, while there is potential for behavioral thermoregulation, animals often remain in shallow pools during low tide, experiencing extremes in a range of physico-chemical parameters (Collins et al., 2019). Exposure to environmental extremes may have selected for greater plastic responses throughout the life cycle. An alternative hypothesis is that the temperatures used here were not stressful to embryos of this species, so that the mechanisms leading to negative carry-over effects later in life are not at play. However, this is unlikely as females of this species carry their eggs in the brood pouch, where they are exposed to the same thermal environment as adults. We may therefore predict embryos have, at best, similar thermal tolerance and acclimation capacities as their parents, whom we have previously shown to experience a marked increase in metabolic rate at 20 °C, and are unable to fully acclimate after 3 weeks at this temperature (Collins et al., 2022).

Whether the weak beneficial effect persists into adulthood and across generations, and whether there are costs associated with this plasticity remains to be determined. Ectotherms are most plastic when tested immediately following exposure (Pottier et al., 2022). Here, we kept hatchlings under common garden conditions for 4 weeks into the juvenile stage (i.e. before reaching sexual maturity). The time taken to reach sexual maturity in this species varies with environmental temperature (Sexton, 1928) and is between 5 and 8 weeks at the tested temperatures (Truebano, pers. comm.), thus it is possible that the observed thermal tolerance weakens further as individuals reach the adult stage.

Acclimation of brooding females and their offspring through to adulthood (i.e. spanning several life stages) in the marine amphipod, *Sunamphitoe parmerong* led to increased thermal tolerance in individuals acclimated to 23 °C compared to 17 °C, but the magnitude of the effect was similar to that observed here at ARR ≈0.17 (Campbell et al., 2020). Considering both species inhabit highly variable thermal environments and have similar life histories, we suggest that developmental plasticity is limited in this group, irrespectively of exposure lengths and windows. The potential for any effects to be reversed over subsequent generations remains (Jarrold et al., 2019).

Given amphipods were raised in a common environment post-hatching, any differences in thermal tolerance are likely the result of the differences in their embryonic environment. It was not possible to determine in this experiment whether mortality of hatchlings was higher at the higher developmental temperatures, thus differential selection, alone or in combination with developmental plasticity, cannot be excluded. Disentangling the effects of developmental plasticity and thermal selection requires a more targeted experimental design, with measurements of survival and inter-/intra-brood variability in thermal tolerance, and assessment of genetic composition in sub-populations exposed to different thermal environments during development. Some of these factors are currently under investigation in our laboratory.

Overall, our study supports the view that exposure to elevated temperatures during embryonic stage can lead to increased thermal limits later in life. In this species, a 10 °C increase in developmental temperature results in a 1.1 °C increase in thermal tolerance in the juvenile stage. Increases in global temperatures are very likely to exceed 2.7 °C by the end of the century, and this will be accompanied by increased frequency and severity of extreme heat waves (Arias et al., 2021). Given these predictions, we suggest that developmental plasticity alone will provide little benefit to these animals under the rapid changes predicted under climate change.

CRediT authorship contribution statement

Honor Haskett: Conceptualization, Formal analysis, Investigation, Writing – original draft. **Luke Gill:** Conceptualization, Methodology. **John I. Spicer:** Supervision, Writing – review & editing. **Manuela Truebano:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are available at DOI: 10.5281/zenodo.10462701.

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