

RESEARCH ARTICLE

Thermal strategies vary with life history stage

Manuela Truebano^{1,*}, Phillip Fenner¹, Oliver Tills¹, Simon D. Rundle¹ and Enrico L. Rezende²

ABSTRACT

With both global surface temperatures and the incidence and intensity of extreme temperature events projected to increase, the assessment of species' sensitivity to chronic and acute changes in temperature has become crucial. Sensitivity predictions are based predominantly on adult responses, despite the fact that early life stages may be more vulnerable to thermal challenge. Here, we compared the sensitivity of different life history stages of the intertidal gastropod *Littorina obtusata* using thermal death time curves, which incorporate the intensity and duration of heat stress, and used these to calculate upper critical thermal limits (CT_{max}) and sensitivity to temperature change (z). Early (larval) life stages had both a lower CT_{max} and a lower z than adults, suggesting they are less good at withstanding short-term extreme thermal challenges but better able to survive moderate temperatures in the long term. This result supports the predicted trade-off between acute and chronic tolerance to thermal stress, and is consistent with the different thermal challenges that these stages encounter in the intertidal zone. We conclude that different life history stages employ different thermal strategies that may be adaptive. Our findings caution against the use of predictions of the impact of global warming that are based on only adult responses and, hence, which may underestimate vulnerability.

KEY WORDS: TDT curves, Thermal tolerance, CT_{max} , Thermal sensitivity, Trade-off

INTRODUCTION

The Earth's climate is changing rapidly, with both global surface temperatures and the incidence and intensity of extreme temperature events projected to increase (IPCC, 2014). Within this context, the assessment of species' sensitivity to elevated temperatures over different time scales is a crucial tool for modelling the effects of altered thermal conditions and developing mitigation strategies. These assessments are almost exclusively generated from adult data, despite the fact that responses can differ between life stages (Radchuk et al., 2013), with early life stages often described as more sensitive to altered environmental conditions than later stages (Delorme and Sewell, 2013; Schiffer et al., 2014; Zippay and Hofmann, 2010).

Critical thermal maxima (CT_{max}), which define the upper limit of the thermal tolerance range of organisms, have been used as proxies for predictions of the vulnerability of populations to climate change

(Huey et al., 2012). CT_{max} is typically measured as the temperature at which an organism dies upon exposure to steadily increasing temperature (Lutterschmidt and Hutchison, 1997). However, when calculated in this way, its use may be intrinsically limited, as the effect of temperature is dependent upon not just the intensity of the thermal challenge but also the duration of the exposure. As both the organism's physiology and its probability of surviving a thermal challenge vary with time, it has been argued that such endpoint temperatures should not be equated to CT_{max} , because these estimates do not control for the duration of the exposure (Castañeda et al., 2015; Santos et al., 2011, 2012; Wang et al., 2007). Thermal death time (TDT) curves address this limitation by providing an approach that incorporates both the intensity and the duration of thermal stress, and consequently can generate a more robust method of predicting such responses (Rezende et al., 2014).

The ability to tolerate extreme temperatures cannot typically be sustained for prolonged periods, and species with high CT_{max} tend to be more sensitive to longer term exposure. This trade-off between acute and chronic tolerance to thermal stress (Rezende et al., 2014) may be particularly relevant for marine invertebrates with complex life cycles. Because different life stages often inhabit different environments and thermal regimes, we predict that natural selection should favour different thermal strategies across the life cycle. Here, we tested this prediction in the marine intertidal gastropod *Littorina obtusata* (Linnaeus 1758). Adult *L. obtusata* inhabit the mid- to low-intertidal zone, where females lay egg masses (100–200 egg capsules) on seaweed (Goodwin, 1979; Williams, 1990). Embryos and larvae undergo direct development, hatching as juvenile snails. Employing TDT curves, we tested whether different life stages (early veliger larva, mid-veliger larva and adult) exhibit different sensitivities to elevated temperatures, and whether a trade-off exists between tolerance to acute versus chronic thermal challenges across life stages.

MATERIALS AND METHODS

Collection and husbandry of *Littorina obtusata*

Adult *L. obtusata* were collected from the intertidal zone at Mount Batten, Plymouth, Devon, UK (50°21'25.23"N, 4°07'37.21"W) during low tide. Upon collection, snails were placed in large plastic bags containing damp *Fucus serratus*, preventing desiccation and damage during transportation. Individuals were transported to the aquarium facilities at the Marine Biology and Ecology Research Centre at Plymouth University within 2 h of collection, and acclimated to laboratory conditions in 5 l aquaria ($n=20$ per aquarium) for at least 1 week. Each aquarium was supplied with aerated seawater (temperature $16.5\pm 0.5^\circ\text{C}$, salinity 35 ± 1 , P_{O_2} $80\pm 10\%$ air saturation, 12 h:12 h light:dark cycle). Water changes were made weekly, and snails were fed *ad libitum* on *F. serratus*.

Physiological tolerance in adults

Adult *L. obtusata* were exposed to a static thermal challenge, whereby temperature was kept constant until mortality occurred. Adult snails were placed into individual beakers containing aerated

¹Marine Biology and Ecology Research Centre, Plymouth University, Plymouth PL4 8AA, UK. ²Departamento de Ecología, Center of Applied Ecology and Sustainability, Facultad de Ciencias Biológicas, Universidad Católica de Chile, Santiago 6513677, Chile.

*Author for correspondence (manuela.truebanogarcia@plymouth.ac.uk)

 M.T., 0000-0003-2586-6524; S.D.R., 0000-0002-9570-7070; E.L.R., 0000-0002-6245-9605

seawater at each of the four treatment temperatures of 36°C ($n=16$), 38°C ($n=18$), 40°C ($n=16$) and 42°C ($n=15$). Seawater temperature was maintained constant by submersion of the beakers in a temperature-controlled waterbath. Mortality was determined by the absence of foot retraction upon disturbance with a blunt needle, manifested by the lack of movement of the operculum, at which point survival time was recorded (Sandison, 1967). Immediately after, snails were placed in seawater under control conditions for 90 min, to exclude the possibility that the animal had reached sublethal heat coma (McMahon, 1976). Individuals that exhibited retraction of the foot during the recovery period were disregarded from analysis. Individual snails were then frozen and later thawed to facilitate the extraction of soft tissue from the shell. Shells were cracked open, and soft tissue was removed, rinsed with deionised water, blotted dry and weighed.

Physiological tolerance in embryos

Thermal tolerance tests were carried out on two larval stages: early veliger (i.e. at the start of velar lobe development, approximately 4 days after the first cell division, when reared at 15°C); and mid-veliger (i.e. when the larval heart starts to beat, approximately 13 days after the first cell division at 15°C) (Bitterli et al., 2012). Larvae in their individual egg capsules at the target developmental stage were imaged with high temporal and spatial resolution at a range of temperatures for 24 h using an automated, custom-built bioimaging system for time-lapse study of aquatic larvae (Tills et al., 2013). This system comprised a machine vision camera (Pike 421B, Allied Vision Technology, Stattdroda, Germany) connected to a zoom lens (VHZ20R, Keyence, Milton Keynes, Bucks, UK), with dark-field cold illumination provided by an LED light (CD100, Keyence). The camera and lens were inverted beneath an XY motorised stage (Scan, Märzhäuser Wetzlar GmbH & Co., Wetzlar, Germany) controlled by a Tango Desktop control unit (Märzhäuser Wetzlar GmbH & Co.). A Mac Mini running Micro-Manager v. 1.4.22 was used to control and synchronise the motorised stage and camera. For a schematic drawing of the system, see Tills et al. (2013). The incubation chamber containing the embryos was mounted on the XY motorised stage above the inverted camera and optics. An image sequence (150 images, 1024×768 pixels, 7.5 frames s⁻¹) of each embryo was recorded every 5 min during the period of an experiment. Embryos at the early veliger stage were exposed to four temperature treatments: 36°C ($n=14$), 38°C ($n=12$), 40°C ($n=13$) and 42°C ($n=14$); and embryos at the mid-veliger stage were exposed to three: 38°C ($n=12$), 40°C ($n=12$) and 42°C ($n=14$) as the extended survival at 36°C led to parasitic infection before mortality occurred. The infection appears to be fungal, but we have yet to identify it. We have observed egg masses infected under field conditions but, as yet, have not investigated how embryos might be impaired by the infection. For experimental purposes, however, the movement of the parasite did not allow accurate estimation of lethal times, and therefore data were excluded. Treatment temperatures fluctuated around the target temperature by <0.5°C for up to 60 min, after which temperature fluctuations were <0.3°C for the next 23 h. A maximum of 10 individuals were analysed during each experiment. At 42°C, embryos were imaged every 90 s because of their greater sensitivity to this temperature. Mortality was assessed by visual inspection of the image sequences using the open-source image analysis software Fiji (Schindelin et al., 2012). A range of body movements were observed including muscle flexing, embryo rotation, velum cilia signals and heartbeat. Mortality was defined by the recession of movement in all of these traits.

Data analysis

Lethal time was recorded for every individual, and the median lethal time (LT₅₀) at each assay temperature was calculated. Survival probability plots for each stage were generated using the lethal endpoint times of organisms within each treatment. TDT curves were modelled from lethal times at three to four static thermal challenges for each life cycle stage. Upper thermal limits and thermal sensitivities were estimated using the following equation (Rezende et al., 2014):

$$\log_{10}t = \frac{(CT_{\max} - T)}{z}, \quad (1)$$

where t corresponds to lethal time (min), CT_{\max} is the upper critical thermal limit (°C) at 1 min ($\log_{10}t=0$), T is the assay temperature (°C) and z is the temperature change (°C) required for a 10-fold change in survival time, and therefore quantifies the sensitivity to temperature change. Here, we controlled T , and measured t as the dependent variable, so we estimated CT_{\max} and z for each developmental stage by regressing \log_{10} -transformed lethal times against three or four temperature treatments, as $CT_{\max} = -\text{intercept/slope}$ and $z = 1/\text{slope}$ (Eqn 1). A linear model was used to determine the goodness of fit. Because average survival times in each treatment closely resembled exposure times required for 50% mortality interpolated from the survival curves ($n=11$, Pearson $r=0.999$, $P=5.7 \times 10^{-13}$), here we report mean±s.d. estimates for simplicity. Data analysis was performed in R (<http://www.R-project.org/>).

RESULTS

Mortality curves

Survival times (8–904 min) differed significantly between life history stages (Fig. 1). Average survival times at 38°C were 160.3±26.4, 522.4±133.9 and 336.8±37.1 min for early veliger, mid-veliger and adult snails, respectively (mean±s.d.; $F_{2,39}=67.2$, $P=2 \times 10^{-13}$). These decreased to 12.5±5.1, 24.3±19.2 and 51.7±15.5 min, respectively, at 42°C ($F_{2,40}=27.7$, $P=2.8 \times 10^{-8}$). Adults exhibited greater survival times in response to more acute thermal challenges and reduced survival times at less extreme temperatures, which was most evident at 36°C when comparing adults against early veligers ($F_{1,28}=12.2$, $P=0.0016$). Although there were no data for the mid-veliger stage at 36°C, given that survival times in adults at this temperature were statistically indistinguishable from estimates at 38°C for mid-veliger ($F_{1,26}=0.15$, $P=0.720$), this result should hold in a complete dataset.

Differences in thermal tolerance profiles between stages

Differences in survival times across temperatures were adequately encapsulated by our semi-log model (Fig. 2). Curves exhibited high goodness of fit at all stages (adult, $R_{\text{adj}}^2=0.89$, $F_{1,63}=519.2$, $P<0.001$; mid-veliger, $R_{\text{adj}}^2=0.86$, $F_{1,36}=233.6$, $P<0.001$; early veliger, $R_{\text{adj}}^2=0.96$, $F_{1,51}=1294$, $P<0.001$). Importantly, R^2 estimates were even higher when only mean estimates of survival times per temperature were considered, which is an appropriate strategy for removing variance in survival within temperatures, because of the probabilistic nature of survival curves (Santos et al., 2011). These TDT curves revealed contrasting differences in thermal tolerance between stages, with adults exhibiting a higher CT_{\max} (52.8°C) than embryos (45.6°C for both stages) and lower sensitivity to temperature change, i.e. z values suggested that a 10-fold change in survival time resulted from a 6.01°C change in temperature for adults compared with 3.47°C and 2.85°C in early and mid-veligers, respectively. A trade-off between CT_{\max} and z is apparent because

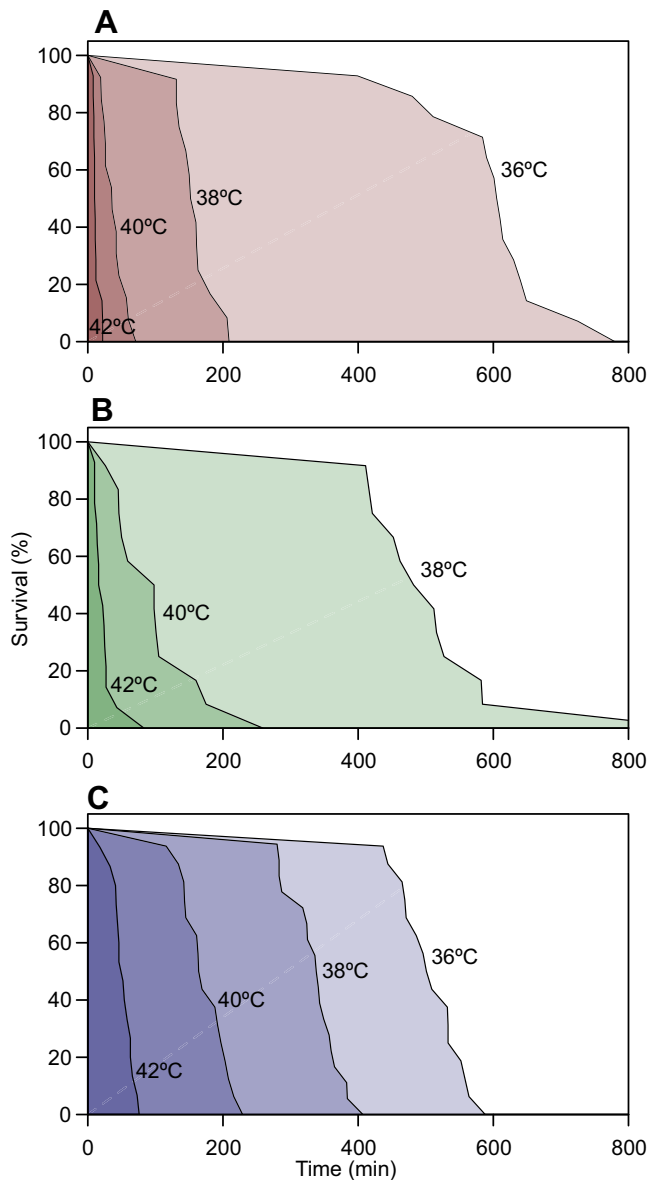


Fig. 1. Survival plots for *Littorina obtusata* at different life stages. Probability of survival with increasing exposure time at each test temperature for (A) early veliger ($n=14, 12, 13$ and 14 at $36, 38, 40$ and 42°C , respectively), (B) mid-veliger ($n=12, 12$ and 14 at $38, 40$ and 42°C , respectively) and (C) adult snails ($n=16, 18, 16$ and 15 at $36, 38, 40$ and 42°C , respectively).

adults tended to survive for longer at high temperatures, whereas early life stages survived for longer at less extreme temperatures (Fig. 2). For adults only, there was a significant effect of size on survival ($t_{62}=2.646, P=0.01$).

DISCUSSION

The relative thermal sensitivities of three life history stages of the intertidal gastropod *L. obtusata* were assessed using survival plots and TDT curves. Early life stages had both a lower CT_{max} and a lower z than adults, which suggests that they are less good at withstanding short-term extreme thermal challenges but better able to survive moderate temperatures in the long term.

Given the putative trade-off between acute tolerance and long-term survival, adaptive strategies are expected to differ between organisms experiencing different thermal regimes. Rezende et al.

(2014) indicate that low z values should be beneficial in thermally stable environments, at the expense of a high CT_{max} , whereas highly variable environments would favour a high CT_{max} at the expense of low z values. The intertidal zone is characterised by large thermal variability in both space and time, with rapid and severe fluctuations in temperature associated with the tidal cycle (Helmuth and Hofmann, 2001). The intensity and duration of the thermal stress experienced by intertidal individuals will depend on the shore height and their microhabitat. *Littorina obtusata* adults typically inhabit a range of microhabitat types on the shore, grazing epiphytes from fucoid algae and epilithic algae from rocks (Kemppainen et al., 2005). Hence, they are likely to experience greater thermal variation than embryos, which are contained within egg masses (Woods and DeSilets, 1997) glued to algal fronds or rocks and, hence, in a fixed position on the shore (Goodwin, 1979). The low acute tolerance in embryos may be associated with the protection conferred by the characteristics and positioning of the egg mass, which potentially buffers environmental insults (Woods and DeSilets, 1997). Our results support the predicted trade-off between CT_{max} and z , by which embryos, which develop in more constant environments, display lower acute thermal tolerance, but are able to survive longer exposure to less extreme temperatures than adults, which experience more variable environments.

The mechanisms employed under these contrasting thermal strategies are likely to differ. The fact that early life stages had low CT_{max} values compared with adults suggests that high upper thermal limits develop during ontogeny. It is possible that energy allocation to cellular division and rearrangements during development lead to greater susceptibility to acute stress in developing embryos than in adults (Hammond and Hofmann, 2010). Acute thermal tolerance in adults is conferred by mechanisms involved in the heat shock response, which may manifest at a lower capacity in early embryos (Brown et al., 2004; Sconzo et al., 1995; but see Hammond and Hofmann, 2010), perhaps because overexpression in early embryos inhibits development (Krebs and Feder, 1998). While embryos are equipped with defences that ensure developmental stability under different environmental conditions (Hamdoun and Epel, 2007), adaptive strategies and acute changes may defeat such defences, leading to disruptions in development and subsequent mortality. In adults, acute tolerance is higher, but cannot be sustained for long periods of exposure even to less extreme conditions. The thermal tolerance of an organism is proportional to the magnitude of the temperature variation it experiences (Deutsch et al., 2008). Ectotherms inhabiting the intertidal zone can experience large daily and seasonal temperature fluctuations, thus leading to high upper thermal limits (Stillman, 2002). The higher sensitivity to longer term exposure to less extreme temperatures could reflect, to some extent, the ability to avoid such conditions through regulating body temperature by minimising heat exposure. Behavioural plasticity of habitat use is an essential thermoregulatory strategy in ectotherms (Kearney et al., 2009; Sunday et al., 2014). Thermoregulatory behaviours have been described in intertidal gastropods (Iacarella and Helmuth, 2012; Miller and Denny, 2011; Ng et al., 2017), and can potentially ameliorate the impacts of warming temperatures (Marshall et al., 2015).

Our results also highlight the inherent complexity involved in predicting the potential impact of warming temperatures on intertidal organisms with multiple life stages. Climate change scenarios predict both a gradual increase in surface temperatures and an increase in temperature extremes (IPCC, 2014). While it is tempting to focus on the impact of temperature anomalies in adult

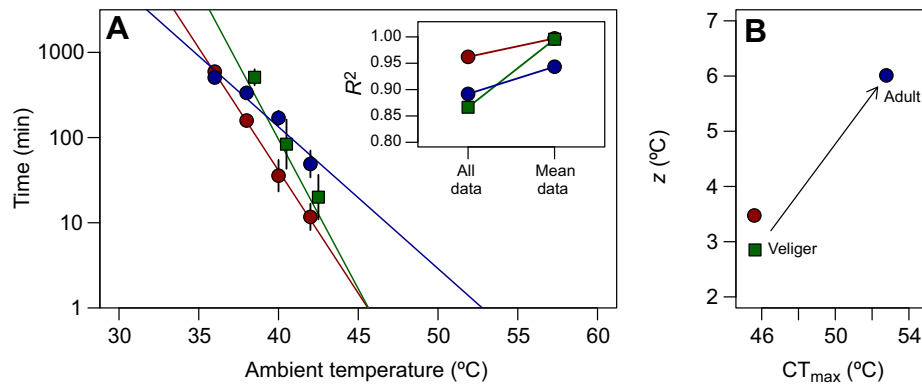


Fig. 2. Thermal death time (TDT) curves and the trade-off between upper critical thermal limits (CT_{max}) and sensitivity to temperature change (z). (A) TDT curves for adults (blue, $n=16, 18, 16$ and 15 at $36, 38, 40$ and 42°C , respectively), early veligers (red, $n=14, 12, 13$ and 14 at $36, 38, 40$ and 42°C , respectively) and mid-veligers (green, $n=12, 12$ and 14 at $38, 40$ and 42°C , respectively). The interpolation at the abscissa represents CT_{max} , or the tolerated temperature following 1 min of exposure (Eqn 1), whilst z corresponds to the reciprocal of the slope. Error bars represent standard deviations. The R^2 value obtained for these curves, as well as for linear models including mean estimates per temperature, are shown in the inset. Curves exhibited high goodness of fit at all stages (adult, $R_{adj}^2=0.89$, $F_{1,63}=519.2$, $P<0.001$; mid-veliger, $R_{adj}^2=0.86$, $F_{1,36}=233.6$, $P<0.001$; early veliger, $R_{adj}^2=0.96$, $F_{1,51}=1294$, $P<0.001$). (B) Relationship between CT_{max} and z for early veliger (red) mid-veliger (green) and adult (blue) snails. A trade-off between CT_{max} and z is apparent.

mortality because adult snails are generally exposed to more pronounced fluctuations, relatively moderate changes in water temperature could have major consequences on larval mortality should the observed differences in z between life stages hold across species. For instance, the 10-fold decrease in survival time expected with a $z=3.47^\circ\text{C}$ observed in early veligers implies a drop of 6.4% in survival time for every 0.1°C increase in temperature (i.e. t at 36.1°C corresponds to $0.936 \times t$ at 36°C , Eqn 1). A shift of this magnitude in the whole survival curve (Fig. 1) would increase mortality from 50% at a given temperature to roughly 84% at 0.1°C higher if exposure times are held constant (with 95% confidence intervals corresponding to 81.8% and 85.6% based on the intervals estimated for z of 3.29 and 3.67°C). In contrast, a similar calculation with $z=6.01^\circ\text{C}$ of adult snails results in a drop of 3.7% in survival time per 0.1°C , or an increase in mortality from 50% to 59% (95% confidence intervals between 58.9% and 59.8% given the intervals for z of 5.53 and 6.58°C), everything else being equal. A formal implementation of these calculations to estimate the impact of different thermal regimes on mortality rates constitutes work in progress (E.L.R., unpublished results).

The complexity of responses in intertidal habitats is increased by the fact that organismal physiology differs during immersion and emersion (Bjelde and Todgham, 2013; Truchot, 1990). In our experiments, measurements of thermal tolerance were made in water for both adults and early life stages to ensure that the experimental environment was standardised. It is highly likely, however, that sensitivity predictions would have been different if experiments had been performed in air. Comparisons of thermal responses between immersed and emersed adult gastropods show that thermal limits are higher in air (Bjelde and Todgham, 2013; Drake et al., 2017), which may reflect adaptations of an intertidal lifestyle that confer thermal tolerance when emersed. Increased oxygen availability in air versus water (Truchot, 1990) may mean that adult intertidal animals are able to meet oxygen demands more easily when emersed (Pörtner, 2001) and are also less reliant on anaerobic metabolism. Intertidal adults may also be able to upregulate cellular defences in response to emersion that allow greater tolerance of thermal extremes (Bjelde and Todgham, 2013) or may exhibit circatidal variation in gene expression that underpins increased thermal tolerance during emersion (Gracey et al., 2008). They may also be able to rely

partly on evaporative cooling that will be associated with exposure (Cleland et al., 1990; McMahon, 1990).

Measurements of thermal tolerance have not been made previously for encapsulated embryos and larvae of intertidal species and would require careful design because of the confounding factor of desiccation, which has a large effect on these small life stages. Under this scenario, we speculate that, in contrast to adults, larval stages would exhibit substantially lower heat tolerance in air than estimates in water. Further work on the physiological and molecular mechanisms in early life stages will be needed to unravel the capacity for these stages to tolerate thermal extremes under emersion and immersion. Nonetheless, the main take-home message is clear: in water, early life stages with low z should be more sensitive to temperature changes (see also Castañeda et al., 2015), and therefore relatively small changes in their thermal environments could have important consequences for population dynamics and ultimately resilience to climate change. Thus, while intuition suggests that in highly variable environments, such as the intertidal zone, tolerance to thermal extremes may be more important for long-term species persistence than changes in surface temperatures, our analyses suggest that the latter might be equally important.

In summary, the thermal tolerance of *L. obtusata* varies across its life cycle, with early life stages having lower acute tolerance but greater long-term tolerance to thermal challenges compared with adults. The trade-off between these two parameters may reflect different adaptive thermal strategies imposed by differential thermal challenges they are likely to encounter in the environment. Our study highlights the importance of considering different life stages if we are to make robust predictions of environmental sensitivity and the impacts of global warming on populations.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.T., P.F.; Methodology: M.T., O.T., E.L.R.; Software: O.T.; Validation: O.T.; Formal analysis: O.T., E.L.R.; Investigation: P.F.; Resources: O.T.,

S.D.R.; Writing - original draft: M.T., E.L.R.; Writing - review & editing: M.T., P.F., O.T., S.D.R.; Visualization: E.L.R.; Supervision: M.T., S.D.R.; Project administration: M.T.

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