Thermal strategies vary with life history stage.

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Abstract

With both global surface temperatures and the incidence and intensity of extreme temperature events projected to increase, the assessment of species' sensitivities 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 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.

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' sensitivities to elevated temperatures over different timescales 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 not dependent upon just the intensity of thermal challenge, but also the duration of the exposure. As both the organism's physiology and its probability to survive 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; Santos et al., 2012; Wang et al., 2007). Thermal death time (TDT) curves address this limitation by providing an approach that incorporates both the intensity and 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 test this prediction in the marine intertidal gastropod *Littorina obtusata* (Linnaeus, 1758). Adult *L. obtusata* inhabit the mid to low intertidal 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 if a trade-off exists between tolerance to acute versus chronic thermal challenges across life stages.

Methods

Collection and husbandry of *Littorina obtusata*

Adult *Littorina 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* (Linnaeus, 1753), 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 one week. Each aquarium was supplied with aerated sea water (temperature = 16.5 ± 0.5°C, salinity = 35 ± 1, PO_2= 80 ± 10% air saturation, 12h:12h L:D 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 sea water at each of the four treatment temperatures of 36 (n=16), 38 (n=18), 40 (n=16) and 42°C (n=15). Sea water temperature was maintained constant by submersion of the beakers in a temperature controlled water

bath. 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 sea water 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, 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, Statdtroda, Germany) connected to a zooming lens (VHZ20R, Keyence, Milton Keynes, Buckinghamshire, UK), with dark-field cold illumination provided by an LED light (CD100, Keyence, Milton Keynes, Buckinghamshire, UK). 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., Wetzlar, Germany). A Mac Mini running MICROMANAGER v. 1.4.22 was used to control and synchronise the motorised stage and camera. For a schematic 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. Embryos were recorded by recording an image sequence (150 images, 1024 x 768 pixels, 7.5 frames s^{-1}) of each embryo every 5 min during the period of an experiment. Embryos at the early veliger stage were exposed to four temperature treatments 36 (n=14), 38 (n=12), 40 (n=13) and 42°C (n=14) and embryos at the mid veliger stage were

exposed to three 38 (n=12), 40 (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 identified it. We have observed eggs 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 period temperature fluctuations were <0.3°C for the next 23 h. A maximum of ten individuals were analysed during each experiment. At 42°C embryos were imaged every 90 s due to greater sensitivity at 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. Thermal death time (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):

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

(Eqn1)

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 times, 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}= - intercept/slope and z = 1/slope (Eq1). 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 = 5.7×10^{-13}), here we report mean ± SD estimates for simplicity. Data analysis was performed in R (R Development Core Team, 2017).

Results

Mortality curves

Survival times (8-904 min) differed significantly between life history stages (Figure 1). Average survival times at 38°C were 160.3 ± 26.4 min, 522.4 ± 133.9 min and 336.8 ± 37.1 min for early veliger, mid veliger and adult respectively (mean ± SD; $F_{2,39} = 67.2$, P = 2×10^{-13}). These decreased to 12.5 ± 5.1 min, 24.3 ± 19.2 min, and 51.7 ± 15.5 min, respectively, at 42°C ($F_{2,40} = 27.7$, P = 2.8×10^{-8}). Adults exhibited higher survival times in response to more acute thermal challenges and reduced survival times at less extreme temperatures, which was most evident at 36ºC when comparing adults against early veliger ($F_{1,28} = 12.2$, P = 0.0016). Although there were no data for the mid veliger stage at 36ºC, given that survival times in adults at this temperature were statistically indistinguishable from estimates at 38ºC 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 (Figure 2). Curves exhibited high goodness-of-fit at all stages (adult, R^2_{adj} = 0.89, $F_{1,63} = 519.2$, P <0.001; mid veliger, R^2_{adj} = 0.86, $F_{1,36} = 233.6$, P <0.001; early veliger, R^2_{adj} = 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, due to 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 times 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 adults tend to survive for longer at high temperatures, whereas early life stages survive for longer at less extreme temperatures (Figure 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 (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 both in space and time, with rapid and severe fluctuations in temperature associated with the tidal cycle (Helmuth and Hofmann, 2001). The intensity and time that intertidal individuals experience thermal stress will depend on the shore height and microhabitat they inhabit. *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 insult (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 to 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 compared to 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 embryo (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 defenses that ensure developmental stability under different environmental conditions (Hamdoun and Epel, 2007), adaptive strategies and acute changes may defeat such defenses, leading to disruptions in development and subsequent mortality. In adults, acute tolerance is higher, but cannot be sustained for long periods of exposure to even less extreme conditions. The thermal tolerance of an organism is proportional to the magnitude of temperature variation it experiences (Deutsch et al., 2008). Ectotherms inhabiting the intertidal 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 mortality because adult snails are generally exposed to more pronounced fluctuations, relatively moderate changes in water temperature could have major consequences in larval mortality should the observed differences in *z* between life stages hold across species. For instance, the 10-fold decrease in survival times expected with a z = 3.47ºC observed in early veliger implies a drop of 6.4% in survival times for every 0.1ºC increase in temperature (i.e., t at 36.1ºC corresponds to 0.936 × t at 36ºC, Eqn1). A shift of this magnitude in the whole survival curve (Figure 1) would increase the mortality from 50% at a given temperature to roughly 84% at 0.1ºC 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 3.67ºC). In contrast, a similar calculation with *z* = 6.01ºC of adult snails results in a drop of 3.7% in survival times per 0.1ºC, or an increase in mortality from 50% to 59% (95% CI between 58.9% and 59.8% given the intervals for z of 5.53 and 6.58ºC) 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 standardized. 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 defenses in response to emersion that allow greater tolerance of thermal extremes (Bjelde and

Todgham, 2013) or may exhibit circatidal variation in gene expression that underpin 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 due to 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 to population dynamics and ultimately resilience to climate change. Thus, while intuition suggests that in highly variable environments, such as the intertidal, 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 to 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

No competing interests declared.

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References

Bitterli, T. S., Rundle, S. D. and Spicer, J. I. (2012). Development of cardiovascular function in the marine gastropod *Littorina obtusata* (Linnaeus). *J. Exp. Biol.* **215**,.

Bjelde, B. E. and Todgham, A. E. (2013). Thermal physiology of the fingered limpet *Lottia digitalis* under emersion and immersion. *J. Exp. Biol.* **216**, 2858–2869.

Brown, H. M., Briden, A., Stokell, T., Griffin, F. J. and Cherr, G. N. (2004). Thermotolerance and Hsp70 profiles in adult and embryonic California native oysters, *Ostreola conchaphila* (Carpenter, 1857). *J. Shellfish Res.* **23**, 135–142.

Castañeda, L. E., Rezende, E. L. and Santos, M. (2015). Heat tolerance in *Drosophila subobscura* along a latitudinal gradient: Contrasting patterns between plastic and genetic responses. *Evolution (N. Y).* **69**, 2721–2734.

Cleland, J. D., McMahon, R. F. and Morton, B. (1990). Upper thermal limit of nine intertidal gastropod species from a Hong Kong rocky shore in relation to vertical distribution and desiccation associated with evaporative cooling. *Proc. Second Int. Mar. Biol. Work. Mar. Flora Fauna Hong Kong South. China, 2-24 April. 1986*.

Delorme, N. J. and Sewell, M. A. (2013). Temperature limits to early development of the New Zealand sea urchin *Evechinus chloroticus* (Valenciennes, 1846). *J. Therm. Biol.* **38**, 218–224.

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C. and Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 6668–72.

Drake, M. J., Miller, N. A. and Todgham, A. E. (2017). The role of stochastic

391 thermal environments in modulating the thermal physiology of an intertidal
392 limpet, *Lottia digitalis*. *J. Exp. Biol.* **220**, 3072–3083.
393 **Goodwin, B. J.** (1979). The egg mass of *Litorina obtusata* and *Lacuna pallidula*
394 (Gastropoda : prosobranchia). *J. Molluscan Stud.* **45**, 1–11.
395 **Gracey, A. Y., Chaney, M. L., Boomhower, J. P., Tyburczy, W. R., Connor, K.**
396 **and Somero, G. N.** (2008). Rhythms of gene expression in a fluctuating
397 intertidal environment. *Curr. Biol.* **18**, 1501–1507.
398 **Hamdoun, A. and Epel, D.** (2007). Embryo stability and vulnerability in an always
399 changing world. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 1745–50.
400 **Hammond, L. M. and Hofmann, G. E.** (2010). Thermal tolerance of
401 *Strongylocentrotus purpuratus* early life history stages: mortality, stress-induced
402 gene expression and biogeographic patterns. *Mar. Biol.* **157**, 2677–2687.
403 **Helmuth, B. S. T. and Hofmann, G. E.** (2001). Microhabitats, thermal
404 heterogeneity, and patterns of physiological stress in the rocky intertidal zone.
405 *Biol. Bull.* **201**, 374–384.
406 **Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M. and**
407 **Williams, S. E.** (2012). Predicting organismal vulnerability to climate warming:
408 roles of behaviour, physiology and adaptation. *Philos. Trans. R. Soc. Lond. B.*
409 *Biol. Sci.* **367**, 1665–79.
410 **Iacarella, J. C. and Helmuth, B.** (2012). Body temperature and desiccation
411 constrain the activity of *Littoraria irrorata* within the *Spartina alterniflora* canopy.
412 *J. Therm. Biol.* **37**, 15–22.
413 **IPCC** (2014). *Climate Change 2014: Synthesis Report. Contribution of Working*
414 *Groups I, II and III to the Fifth Assessment Report of the Intergovernmental*
415 *Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer*
416 *(eds.)]. IPCC, Geneva, Switzerland,*.
417 **Kearney, M., Shine, R. and Porter, W. P.** (2009). The potential for behavioral
418 thermoregulation to buffer cold-blooded animals against climate warming. *Proc.*
419 *Natl. Acad. Sci. U. S. A.* **106**, 3835–40.
420 **Kemppainen, P., Nes, S. van, Ceder, C. and Johannesson, K.** (2005). Refuge
421 function of marine algae complicates selection in an intertidal snail. *Oecologia*
422 **143**, 402–411.
423 **Krebs, R. A. and Feder, M. E.** (1998). Hsp70 and larval thermotolerance in
424 *Drosophila melanogaster*: how much is enough and when is more too much? *J.*

425 *Insect Physiol.* **44**, 1091–1101.
426 **Lutterschmidt, W. I. and Hutchison, V. H.** (1997). The critical thermal maximum:
427 history and critique. *Can. J. Zool.* **75**, 1561–1574.
428 **Marshall, D. J., Rezende, E. L., Baharuddin, N., Choi, F. and Helmuth, B.** (2015).
429 Thermal tolerance and climate warming sensitivity in tropical snails. *Ecol. Evol.*
430 **5**, 5905–5919.
431 **McMahon, R. F.** (1990). Thermal tolerance, evaporative water loss, air-water oxygen
432 consumption and zonation of intertidal prosobranchs: a new synthesis.
433 *Hydrobiologia* **193**, 241–260.
434 **Miller, L. P. and Denny, M. W.** (2011). Importance of behavior and morphological
435 traits for controlling body temperature in littorinid snails. *Biol. Bull.* **220**, 209–23.
436 **Ng, T. P. T., Lau, S. L. Y., Seuront, L., Davies, M. S., Stafford, R., Marshall, D. J.**
437 **and Williams, G. A.** (2017). Linking behaviour and climate change in intertidal
438 ectotherms: insights from littorinid snails. *J. Exp. Mar. Bio. Ecol.* **492**, 121–131.
439 **Radchuk, V., Turlure, C. and Schtickzelle, N.** (2013). Each life stage matters: the
440 importance of assessing the response to climate change over the complete life
441 cycle in butterflies. *J. Anim. Ecol.* **82**, 275–285.
442 **R Development Core Team, R.** (2017). R: A Language and Environment for
443 Statistical Computing. *R Found. Stat. Comput.*
444 **Rezende, E. L., Castañeda, L. E. and Santos, M.** (2014). Tolerance landscapes in
445 thermal ecology. *Funct. Ecol.* **28**, 799–809.
446 **Sandison, E. E.** (1967). Respiratory response to temperature and temperature
447 tolerance of some intertidal gastropods. *J. Exp. Mar. Bio. Ecol.* **1**, 271–281.
448 **Santos, M., Castañeda, L. E. and Rezende, E. L.** (2011). Making sense of heat
449 tolerance estimates in ectotherms: lessons from Drosophila. *Funct. Ecol.* **25**,
450 1169–1180.
451 **Santos, M., Castañeda, L. E. and Rezende, E. L.** (2012). Keeping pace with
452 climate change: what is wrong with the evolutionary potential of upper thermal
453 limits? *Ecol. Evol.* **2**, 2866–80.
454 **Schiffer, M., Harms, L., Pörtner, H., Mark, F. and Storch, D.** (2014). Pre-hatching
455 seawater pCO_2 affects development and survival of zoea stages of Arctic spider
456 crab *Hyas araneus*. *Mar. Ecol. Prog. Ser.* **501**, 127–139.
457 **Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch,**
458 **T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al.** (2012). Fiji: an

459 open-source platform for biological-image analysis. *Nat. Methods* **9**, 676–682.
460 **Sconzo, G., Ferraro, M. G., Amore, G., Giudice, G., Cascino, D. and Scardina, G.**
461 (1995). Activation by heat shock of hsp70 gene transcription in sea urchin
462 embryos. *Biochem. Biophys. Res. Commun.* **217**, 1032–1038.
463 **Stillman, J. H.** (2002). Causes and consequences of thermal tolerance limits in
464 rocky intertidal porcelain crabs, Genus Petrolisthes. *Integr. Comp. Biol.* **42**, 790–
465 796.
466 **Sunday, J. M., Bates, A. E., Kearney, M. R., Colwell, R. K., Dulvy, N. K.,**
467 **Longino, J. T. and Huey, R. B.** (2014). Thermal-safety margins and the
468 necessity of thermoregulatory behavior across latitude and elevation. *Proc. Natl.*
469 *Acad. Sci. U. S. A.* **111**, 5610–5.
470 **Tills, O., Rundle, S. D. and Spicer, J. I.** (2013). Parent-offspring similarity in the
471 timing of developmental events: an origin of heterochrony? *Proc. R. Soc. B Biol.*
472 *Sci.* **280**, 20131479–20131479.
473 **Truchot, J. P.** (1990). Respiratory and ionic regulation in invertebrates exposed to
474 both water and air. *Annu. Rev. Physiol.* **52**, 61–74.
475 **Wang, S., Tang, J. and Hansen, J. D.** (2007). Experimental and simulation methods
476 of insect thermal death kinetics. In *Heat treatments for postharvest pest control :*
477 *theory and practice* (ed. Tang, J.), p. Wallingford, Oxfordshire, UK ; Cambridge,
478 MA.
479 **Williams, G.** (1990). The comparative ecology of the flat periwinkles, *Littorina*
480 *obtusata* (L.) and *L. mariae* Sacchi et Rastelli. *F. Stud.* **7**, 469–482.
481 **Woods, H. A. and DeSilets, R. L.** (1997). Egg-mass gel of *Melanochlamys*
482 *diomedea* (Bergh) protects embryos from low salinity. *Biol. Bull.* **193**, 341–349.
483 **Zippay, M. L. and Hofmann, G. E.** (2010). Effect of pH on gene expression and
484 thermal tolerance of early life history stages of red abalone (*Haliotis rufescens*).
485 *J. Shellfish Res.* **29**, 429–439.
486
487 **Figure legends**
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489 **Figure 1. Survival plots.** Probability of survival with increasing exposure time at
490 each test temperature for A) early veliger (n=14, 12, 13 and 14 at 36, 38, 40 and
491 42°C respectively), B) mid veliger (n=12, 12 and 14 at 38, 40 and 42°C respectively)
492 and C) adult snails (n=16, 18,16 and 15 at 36, 38, 40 and 42°C respectively).

Figure 2 Thermal death time curves and the trade-off between CT_{max} and z. A) Thermal death time (TDT) curves for adults (blue, n=16, 18, 16 and 15 at 36, 38, 40 and 42°C respectively), early veliger (red, n=14, 12, 13 and 14 at 36, 38, 40 and 42°C respectively) and mid veliger (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 (Eqn1), whilst *z* corresponds to the reciprocal of the slope. Errors bars represent standard deviations. The R-square obtained for these curves, as well for linear models including mean estimates per temperature, are shown in the inset. Curves exhibited high goodness-of-fit at all stages (adult, R^2_{adj} = 0.89, $F_{1, 63}$ = 519.2, P <0.001; mid veliger, R^2_{adj} = 0.86, $F_{1,36}$ = 233.6, P <0.001; early veliger, R^2_{adj} = 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.