

LETTER

Thermal sensitivity in dual-breathing ectotherms: Embryos and mothers determine species' vulnerability to climate changeLyle D. Vorsatz ¹, Bruce P. Mostert,² Christopher D. McQuaid ², Stefano Cannicci ^{1,3*}, Francesca Porri ^{2,4}¹The Swire Institute of Marine Science and the School of Biological Sciences, The University of Hong Kong, Hong Kong, Hong Kong SAR; ²Department of Zoology & Entomology, Rhodes University, Grahamstown, South Africa; ³Department of Biology, University of Florence, Sesto Fiorentino, Italy; ⁴South African Institute for Aquatic Biodiversity, Grahamstown, South Africa**Scientific Significance Statement**

The thermal bottlenecks and the response to climate change in the complex life-history of intertidal ectotherms are not clearly defined. Our work tested the applicability of two major ecological and mechanistic hypotheses across life stages, latitudinal range and between respiratory media. We found that ontogenetic stage and local climate adaptations are significant factors to consider when evaluating the overall vulnerability of intertidal ectotherms to thermal stress. The evolutionary adaptation and capacity for dual breathing acquired throughout ontogeny by some intertidal ectotherms may represent an advantage in mitigating the effects of climate change in comparison to strictly water breathers.

Abstract

Understanding the life-stage specific vulnerability of ectotherms to temperature increases is crucial to accurately predicting the consequences of current and future global climate change. Here, we examined ontogeny-specific thermal vulnerability of three intertidal, bimodal (i.e., air and water) breathing crabs from tropical and warm temperate latitudes to address this issue. Spawning females and embryos of intertidal crabs from warm temperate latitudes were more vulnerable to temperature increases than tropical conspecifics, particularly in water. Our findings do not fully support the Climate Variability Hypothesis for setting upper thermal limits, but correspond with the Oxygen- and Capacity-Limited Thermal Tolerance hypothesis, suggesting ontogeny-specific aerobic capacity dictates overall species' thermal sensitivity. Bimodal breathing efficiency as an evolutionary adaptation, ontogenetic stage and local climate adaptation are therefore significant factors to consider when evaluating the vulnerability of intertidal ectotherms to temperature increases and the consequences of climate changes for intertidal organisms, populations and communities.

*Correspondence: cannicci@hku.hk**Author contribution statement:** LDV conducted the analyses and wrote the first draft of the manuscript. BPM, FP, CDM and SC conceived the study. BPM, CDM, SC and FP provided data and expert guidance. FP, CDM and SC provided the funding. All authors contributed to writing and editing the manuscript.**Data Availability Statement:** Data and metadata are available on the Open Science Framework at: https://osf.io/zpkfn/?view_only=ad146b74b8464e4abae97596e6688b1e.**Associate editor:** Rana El-Sabaawi

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The Oxygen- and Capacity-Limited Thermal Tolerance (OCLTT) hypothesis and the Climate Variability Hypothesis (CVH) are strongly supported mechanistic and conceptually unifying principles explaining the onset of metabolic stress and vulnerability to temperature increases along latitudinal gradients (Huey and Kingsolver 1989; Stevens 1989; Frederich and Pörtner 2000; Pörtner and Farrell 2008; Sunday et al. 2019). The OCLTT proposes that during short- or long-term thermal stress at the limits of an organism's thermal window (the temperature range between an organism's lower and upper thermal limits), responses in the respiration and circulatory systems fulfill the increased oxygen demands. When these systems can no longer cope with the increased demand, however, the organism's thermal tolerance is constrained by the onset of lower than normal levels of oxygen in their tissues (Frederich and Pörtner 2000; Pörtner and Farrell 2008; Verberk et al. 2016). The OCLTT predicts that the effects of thermal stress are alleviated by an increased supply of oxygen either by optimization of respiratory mechanisms or by an increased supply from the environment, thus widening the thermal tolerance window (Frederich and Pörtner 2000). Meanwhile, the CVH proposes that organisms which experience small variations of temperature in their environments are expected to have narrower thermal windows and higher upper thermal limits than those living in highly variable environments (Sunday et al. 2011; Shah et al. 2017). The OCLTT has been widely investigated (for a review see Verberk et al. 2016) and has been found to apply to aquatic rather than terrestrial animals, which is expected, due to the relationship between oxygen solubility and temperature, which strongly differs in air and water. Meanwhile, the CVH has been shown to apply to ectotherms in both the marine and terrestrial realms, but still needs validation for intertidal ectotherms that can respire in air and water (Sunday et al. 2019).

Traditionally, climate change vulnerability has been evaluated through active metabolic responses to temperature and critical thermal limits (Deutsch et al. 2008; Pinsky et al. 2019). Notwithstanding their use, these approaches overlook the sublethal effects of temperature on energy budgets and the critical limits of performance traits, where increases and peaks in oxygen consumption represent thermal optima (Kellermann et al. 2019). This is, however, not the case for standard metabolic rate, where peaks in the thermal performance curve (TPC), which describe the effects of temperature on biological process rates, represent elevated minimum costs, typically at higher temperatures than those produced by other performance traits (Kellermann et al. 2019; Shah et al. 2021). A steep increase in standard metabolic rate toward a peak is therefore considered as the onset of stress, which affects the aerobic scope for activity, that is, the energy available for other metabolically costly activities such as locomotion, growth, and reproduction (Pörtner 2001). Adaptations to local climate and dual respiratory media of some intertidal ectotherms likely involve trade-offs in their energy budgets

at different ontogenetic stages and are likely to differ from those that are exclusively water breathers.

To date, investigations into the thermal dependence of intertidal ectotherms that encompass all life stages from embryo to reproductive adults are lacking. This suggests that the potentially most thermally vulnerable stages within and among the life cycles of a large representative group of ectotherms are still unknown or continuously missed due to a lack of experimental data. Moreover, the life stages most vulnerable to temperature increases across a latitudinal gradient in different respiratory media (water or air) could result in critically different outcomes due to local thermal adaptation and species-specific cardiorespiratory functioning. Here, we address these interconnected issues by examining the stage-specific (embryo, larva, adult, and brooding adult) thermal vulnerability of three intertidal, bimodal breathing ectotherms, the mangrove crabs *Tabuca urvillei* and the congeneric *Parasesarma guttatum* and *Parasesarma capensis*, as model organisms from tropical and warm temperate regions.

Methods and materials

Model species and study area

Parasesarma guttatum and *P. capensis* are congeners endemic to the Western Indian Ocean where the distribution of the latter extends from the southernmost mangroves in South Africa to the Mozambican channel. Farther north, *P. capensis* is replaced by *P. guttatum*, which extends north to Somalia (Fratini et al. 2019). The distribution of the fiddler crab *T. urvillei* extends from South Africa to Somalia along the coasts of east African and Madagascar (Shih et al. 2018). All three species are considered to be keystone taxa in mangroves due to their bioengineering capabilities, contributing to both nutrient and chemical cycling (Cannicci et al. 2008). Two study areas were selected to represent a tropical, low latitude region (Gazi Bay, Kenya; 4°22'S, 39°30'E) and a warm-temperate, higher latitude region (Mngazana, South Africa; 31°42'S, 29°25'E; Fig. 1). See Appendix S1, SI 1 for more details on site descriptions and environmental characterization.

Animal collection, maintenance, and general experimental design

Gravid and non-gravid female *P. guttatum*, *P. capensis* and *T. urvillei* were collected by hand in Kenya and South Africa. Both non-gravid and gravid crabs brooding stage two and four embryos (Simoni et al. 2011) were maintained in individual plastic containers tilted at a 45° angle partially filled with locally collected mangrove mud and freshly aerated seawater. Females brooding stage five embryos (Simoni et al. 2011) were placed in separate holding tanks with rocks and aerated seawater. Larvae were successfully hatched from stage five embryos within 2 days of collection for ensuing experiments. All life stages were subjected to either an increasing/decreasing thermal ramp (the rate of temperature change over an interval) starting at 27°C up to 35°C or 27°C down to 19°C at a

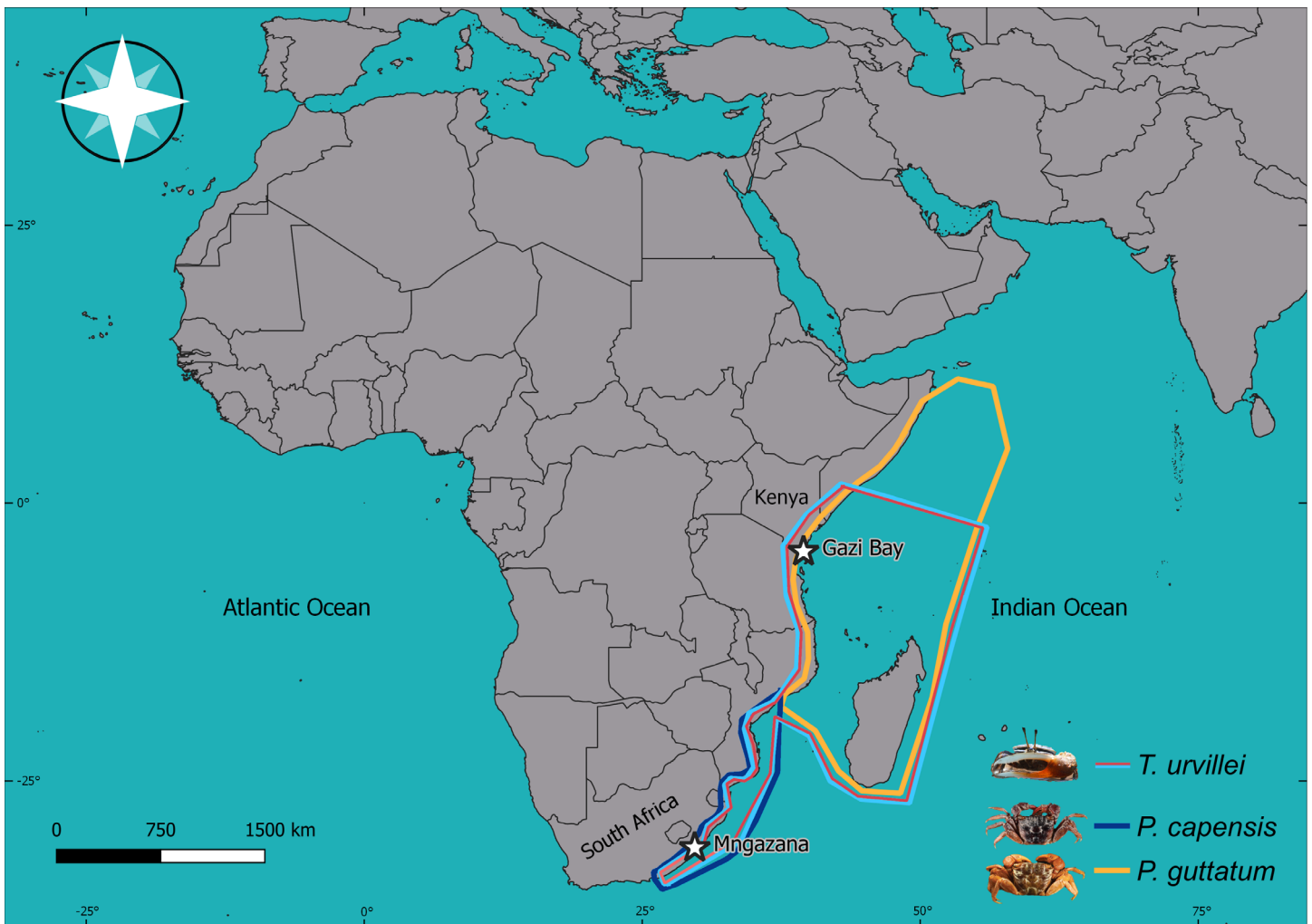


Fig. 1. Map of the latitudinal ranges of the model species (based on Shih et al. 2016; Fratini et al. 2019). Stars represent the sampling areas; double pink and pale blue lines represent *Tubuca urvillei*; dark blue line represents *Parasarsarma capensis*; orange line represents *Parasarsarma guttatum*.

rate of 1°C h^{-1} , respectively (Fusi et al. 2015). Using an intermittent flow-through respirometry technique, oxygen consumption was recorded every 2°C along the thermal ramp until at least a 5% decrease in oxygen (μmol) content of the test medium was detected. Oxygen saturation was never allowed to fall below 60% to avoid possible hypoxic effects (Schurmann and Steffensen 1992). Additionally, during each trial, a vial/chamber/syringe was left empty for aerial experiments or filled with aerated seawater to control for background respiration and oxygen consumption rates (MO_2) adjusted accordingly. New animals were used for each trial along each direction of the thermal ramp. Prior to the start of each experiment in both regions and media, all oxygen sensor types were two-point calibrated in oxygen-free and air-saturated seawater, the former containing a solution of 1% sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$). To ensure adequate mixing of water for all systems; the syringes were gently inverted

throughout trials and electro-magnetic stirrers were used for 0.5- and 2-mL vials. A minimum of eight replicates at each experimental temperature was tested for each embryonic and larval stage in both regions and respiratory media. Replicates involved individual animals except in the case of embryos and larvae; for these, replicates involved multiple individuals due to their small size. For each technical replicate, embryos and hatched larvae from the same female were used. Upon trial completion (which typically lasted 1–2 h), embryos and larvae were removed, counted and standardized with MO_2 expressed as $\text{nmol O}_2 \text{ individual}^{-1} \text{ min}^{-1}$. For more life-stage-specific experimental design details, see Appendix S1.

Data analysis

All analyses were conducted in R for computing statistics (R core team 2019). Temperature and MO_2 were assessed for normality and homoscedasticity. Natural variability in

temperature was examined using an Aligned Rank Transformation ANOVA (ARTANOVA) of the temperature data to test for differences among positions in relation to the sediment (20 cm below, 1 cm and 1.5 m above), between regions and the interaction between these two independent (fixed) variables using the “ARTool” package (Wobbrock et al. 2011). Gaussian TPCs were calculated for each of the model species, ontogenetic stages, regions, media and reproductive states and activation energies (E_a) extracted using the “*calc_params*” function in the R packages “*nls.multstart*” and “*rTPC*” (Padfield et al. 2021). A generalized linear mixed model (GLMM) was conducted using the “*lme4*” package (Bates et al. 2015) to test for differences in metabolic rate, where MO_2 was the response variable and temperature, ontogenetic stage (stage two embryos/ stage four embryos/ zoeae I), medium (water/air) and species partially nested in region were the independent fixed variables, with sample ID as a random variable (Schielzeth and Nakagawa 2013). Separate GLMM’s for each ontogenetic stage were also conducted using MO_2 as the response variable and temperature, medium (water/air) and species nested in region as independent variables to identify stage-specific patterns among media, regions and species. To test MO_2 as a proxy for the maternal effects of brooding embryos, GLMM’s were conducted using temperature, medium, reproductive state (gravid/non-gravid) and species partially nested in region as independent variables with sample ID as a random variable (Schielzeth and Nakagawa 2013). Where applicable, all post hoc tests were conducted with a Benjamini–Hochberg correction (Benjamini and Hochberg 1995) using the “*emmeans*” package. Particulars for the analysis of life-stage-specific thermal responsiveness using the Arrhenius–Boltzmann model (Clarke 2017) are detailed in Appendix S1, SI 1.

Results

There were significant differences in the temperature patterns observed in each region: Kenya and South Africa (ARTANOVA: $F_{1,38,876} = 22,415.76$, $p > 0.001$), position in the sediment: 20 cm below, 1 cm and 1.5 m above (ARTANOVA: $F_{1,38,876} = 1238.39$, $p > 0.001$) and the interaction between region and position (ARTANOVA: $F_{1,38,876} = 277.34$, $p > 0.001$; Fig. S1). Furthermore, all post hoc pairwise comparisons of temperature were significantly different with the exception of measurements taken 1.5 m above and 1 cm above the sediment surface in South Africa (Table S1).

Oxygen consumption

The metabolic responses to increasing temperature differed significantly across regions, species, ontogenetic stages and respiratory media (Fig. 2, Table S2). Metabolic rates significantly differed across species, regions and respiratory media for stage two embryos (Table 1), stage four embryos (Table 1)

and stage I zoeae (Table 2). At stage two and four of embryo development of all species examined, oxygen consumption was generally significantly greater in water than air (Figs. 2A, B, Tables S3 and S4). There were no significant differences in the MO_2 for stage two embryos among species and regions when tested in air (Fig. 2A, Table S3). Differences among species and between regions however occurred when tested in water for both stage two and four embryos (Figs. 2A,B, Tables S3 and S4). Furthermore, under increasing temperatures, the early ontogenetic stages (stage two and stage four embryos and stage I zoeae) of Kenyan populations of *T. urvillei* and *P. guttatum* exhibited significantly lower MO_2 in water than those of South African populations of *T. urvillei* and *P. capensis*. The South African populations of stage two *P. capensis* and stage four *T. urvillei* embryos showed signs of the onset of stress at temperatures above $\sim 31^\circ\text{C}$ in water, indicating a critical thermal threshold at around $\sim 29^\circ\text{C}$ (Fig. 2A, B). The Kenyan populations exhibited no such onset of thermal stress, with metabolic rate increasing gradually with temperature in both air and water for both embryos stages and zoeae. The Kenyan populations of *P. guttatum* and *T. urvillei* stage I zoeae showed no significant differences in MO_2 , whereas the South African populations of *T. urvillei* and *P. capensis* MO_2 were significantly higher than the Kenyan *P. guttatum* and *T. urvillei* (Fig. 2C; Table S5).

The MO_2 of adult crabs differed significantly between regions, species, reproductive states and respiratory media (Fig. 2D,E, Table S6). *T. urvillei* showed no significant differences between regions or reproductive states in either air or water (Table S7). In contrast, gravid females of *P. capensis* and *P. guttatum* exhibited significantly higher MO_2 than non-gravid females in both air and water, with *P. capensis* showing a marked increase in the difference between the reproductive states at temperatures above $\sim 29^\circ\text{C}$ in air and 27°C in water (Fig. 2D,E). Furthermore, in both air and water, gravid *P. capensis* showed a plateau in metabolism above 30°C , indicating that the onset of stress occurred between 27°C and 29°C before metabolic rate decreased as oxygen became limiting for the upkeep of basal metabolism (Fig. 2D,E).

Direct comparisons of thermal responsiveness revealed that slopes from the Boltzmann–Arrhenius model differed significantly for each life-stage, exemplified by the significant effects of the interaction terms in the linear mixed models for stage two and four embryos (Tables S8 and S9), stage one zoeae (Table S10), and female adult crabs (Table S11). The early ontogenetic stages (stage two and stage four embryos and stage I zoeae) of Kenyan populations of *T. urvillei* and *P. guttatum* had lower thermal responsiveness than the South African populations of *T. urvillei* and *P. capensis*, particularly in water (Fig. S2A–C; Tables S13–S16). While, gravid *P. capensis* from temperate latitudes were more thermally responsive than non-gravid females in both air and water (Fig. S2D,E; Table S17).

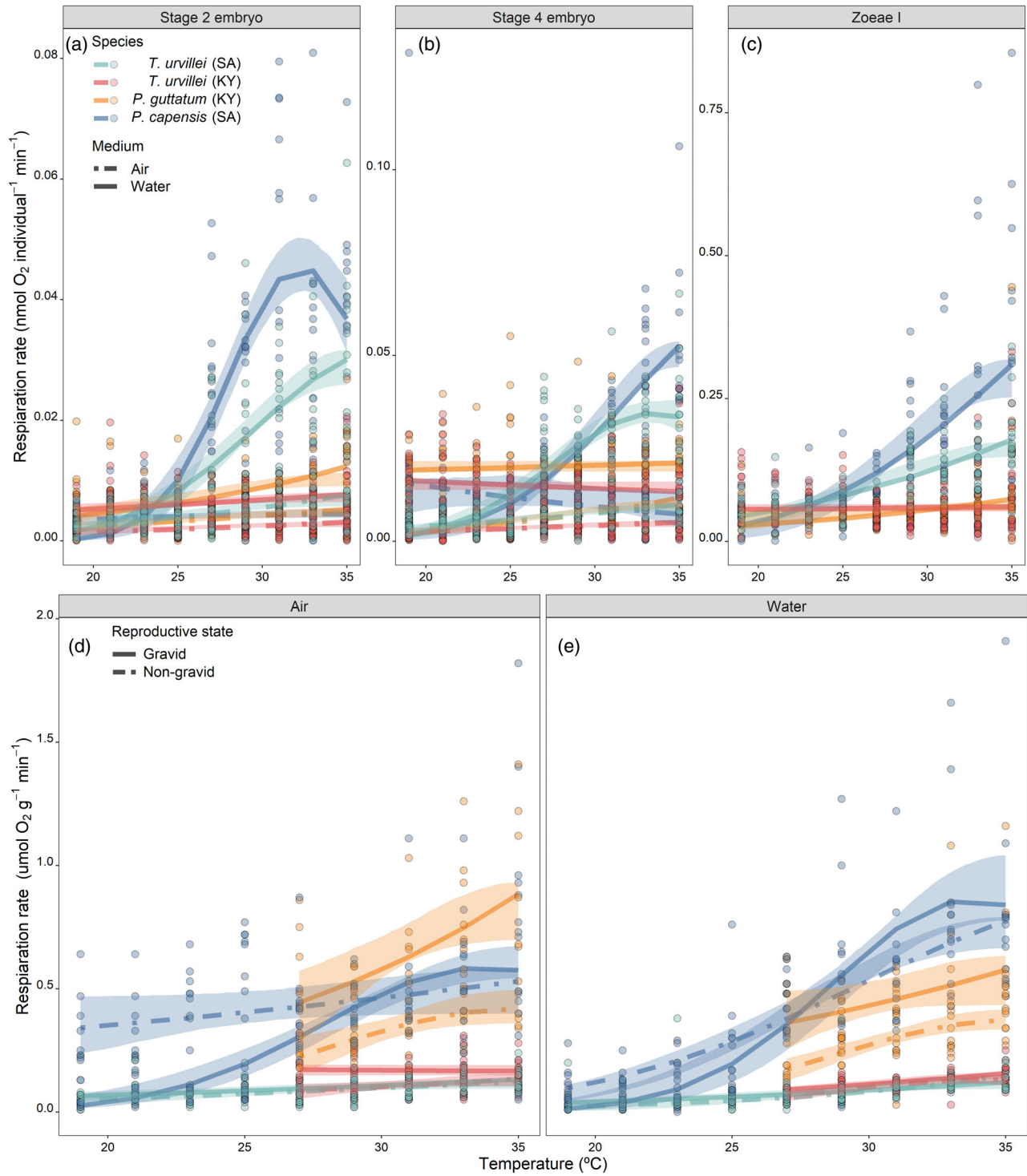


Fig. 2. Thermal performance curves of *Tubuca urvillei*, *Parasarma capensis* and *Parasarma guttatum* from Kenya (low latitude/tropical) and South Africa (higher latitude/warm temperate) in air and water across (A) stage two embryos, (B) stage four embryos, (C) zoeae I (in water only), (D) gravid and non-gravid females in air and (E) gravid and non-gravid females in water. Thermal performance curves for populations based on metabolic rate (MO_2 as a proxy) with 95% bootstrapped confidence intervals. Color coded points represent raw MO_2 per individual at a given temperature. * No measurements were recorded for gravid and non-gravid adults between 19 and 25°C in Kenya (D and E).

Table 1. Generalized linear mixed effects model (GLMM) evaluating the effect of temperature, respiratory media (water vs. air), species partially nested in region (South Africa vs. Kenya) and their interactions on the oxygen consumption (MO_2) of stage two and stage four embryos. SS, sum of squares; DF, degrees of freedom; F, Fisher's statistic; p , significance value. All significant results ($p < 0.05$) are indicated in bold. ** $p < 0.01$; * $p < 0.05$.

Effect	DF	Stage two		Stage four	
		SS	F	SS	F
Temperature	1	0.008	242.032**	0.007	115.792**
Species	2	0.002	28.502**	0.001	8.809**
Respiratory medium	1	0.003	106.545**	0.003	48.504**
Temperature \times species	2	0.003	53.313**	0.002	15.908**
Temperature \times respiratory medium	1	0.006	196.079**	0.006	105.952**
Species \times respiratory medium	2	0.002	41.812**	0.009	76.793**
Temperature \times species \times region	1	0.003	82.342**	0.001	17.355**
Temperature \times species \times respiratory medium	2	0.004	63.936**	0.009	76.314**
Temperature \times species \times region \times respiratory medium	1	0.002	1.514	0.001	5.381*

Table 2. Generalized linear mixed effects model (GLMM) evaluating the effect of temperature, species partially nested in region and their interactions on the oxygen consumption (MO_2) of stage I zoeae in water only. SS, sum of squares; DF, degrees of freedom; F, Fisher's statistic; p , significance value. All significant results ($p < 0.05$) are indicated in bold.

Effect	DF	SS	F	p
Temperature	1	0.686	159.617	< 0.001
Species	2	0.351	40.816	< 0.001
Temperature \times species	2	0.599	69.719	< 0.001
Temperature \times species \times region	1	0.221	51.288	< 0.001

Discussion

Here, we tested the ontogeny-specific responses to temperature increases among intertidal, bimodal breathing model organisms from tropical and warm temperate regions. Our results indicate that at warm temperate latitudes, embryos are the most responsive and vulnerable to temperature increases, regardless of medium, but particularly in water. Additionally, the energetic cost of carrying and brooding embryos potentially reduces performance and, possibly, survivorship in females, again regardless of respiratory medium. These findings are consistent with the OCLTT principle, which postulates that organisms in aquatic habitats are more constrained in supplying oxygen to their tissues, and thus more prone to loss of performance than animals breathing air. This theory also suggests that aerobic capacity changes throughout ontogeny (Frederich and Pörtner 2000; Pörtner and Farrell 2008; Verberk et al. 2016). Moreover, our results indicate that dual breathing intertidal ectotherms living at warm temperate latitudes are at greater risk to temperature increases than those in the tropics (Huey and Kingsolver 1989; Stevens 1989; Sunday et al. 2012). This does not fully support the predictions of the

CVH, which suggests the upper thermal limits of species are set according to temperature variability as linked to latitude, but the CVH may still be useful for predicting the width of dual breathing intertidal ectotherm thermal windows.

Stage-specific aerobic performance of decapod crustaceans reflects the development of their osmoregulatory and cardio-respiratory systems (Charmantier et al. 2002; Small et al. 2015). Embryos, particularly in aquatic habitats, are the least efficient at supplying oxygen to their tissues in the life cycle of ectotherms and have the least developed homeostatic functions (Fernandez et al. 2003; Hamdoun and Epel 2007). As shown here, warm temperate embryos were more thermally responsive and exhibited signs of oxygen limitation in water; rising temperatures resulted in an initial increase in metabolic rate, followed by an asymptote or a decline. With a high demand for oxygen, the egg membrane acts as a barrier to diffusion, potentially limiting oxygen uptake and delaying hatching (Fernandez et al. 2003). This in turn reduces the fitness of the hatched larva and causes catch-up growth, i.e., a metabolically costly convergence of growth trajectories after initial stress (Anger 2001). An immature homeostatic and

aerobic capacity in marine-dependent ectotherms exacerbates their sensitivity to temperature, salinity, hypoxia and other environmental stressors, posing a potential bottleneck for survivorship and successful development under conditions of climate change (Przeslawski et al. 2015).

Brooding females invest significant amounts of energy in oogenesis, producing large quantities of embryos that increase their metabolic load, leading to high energetic costs (Hartnoll 2006). These costs hinder the maintenance of standard metabolic functions due to the energy spent on reproduction in addition to the increase in body mass (due to carrying an egg mass) of brooding females and thus may reduce their aerobic capacity (Pörtner and Farrell 2008; Sokolova 2013). Similar observations have recently been reported for fish, in which spawning adults and fish embryos consistently present narrower thermal tolerances than larvae and nonreproductive adults, indicating that these critical stages in their life cycles can form population bottlenecks (Dahlke et al. 2020).

Our model organisms showed distinct adaptations to the local climatic conditions of each region. When tested in water, those collected at temperate latitudes exhibited higher oxygen consumption and greater thermal responsiveness at all early ontogenetic stages than those from the tropics. Here, we show that ectotherms from temperate latitudes are more vulnerable to acute heating events at all life stages, showing greater thermal responsiveness, most critically when brooding embryos in the warmer months. This vulnerability is probably due to metabolic upregulation from eurythermal adaptation that elevates standard organismal oxygen demand when compared to warmer acclimated (tropical) ectotherms at the same temperatures (Pörtner and Gutt 2016). This is a cause for concern as extreme temperature events are forecast to occur with more frequency and intensity at mid- than at low latitudes (IPCC 2014; Hayashida et al. 2020; Oliver et al. 2021). If these events coincide with the critical bottleneck stages outlined here, local extinction events are likely to occur due to the limited thermal plasticity of ectotherms (Gunderson and

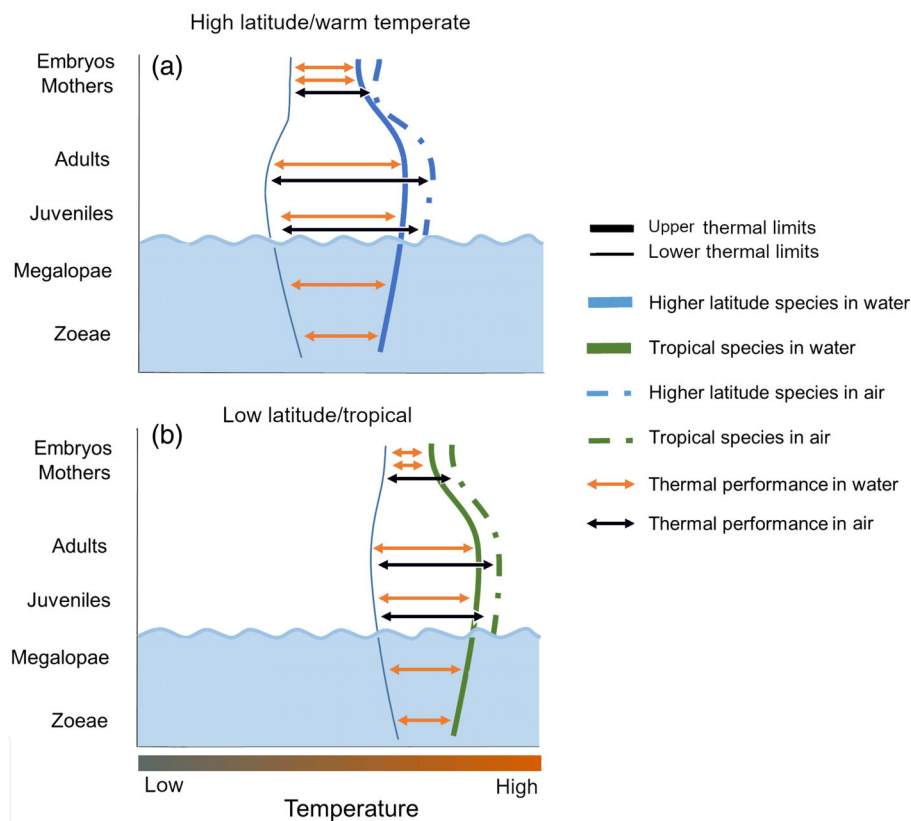


Fig. 3. Conceptual model adapted from Pörtner and Farrell (2008) illustrating the thermal sensitivity of (A) higher latitude/warm temperate and (B) low latitude/tropical brachyuran bimodal breathers throughout ontogeny. Note megalopae and zoeae only occur in water. Temperature-dependent aerobic metabolic performance is maintained throughout the local temperature range (horizontal bar). In both tropical and higher-latitude adapted bimodal breathers in water, the life cycle stages most sensitive to acute heat events are the embryo and brooding female. Tropical and higher-latitude adapted bimodal breathers are not as constrained in air and thus have a higher tolerance to acute heat events. Tropical adapted bimodal breathers in both respiratory mediums have a higher tolerance to increases in temperature than higher latitude/warm temperate adapted populations and congeners.

Stillman 2015). Decapods in the tropics have likely evolved adaptive metabolic compensation that regulates oxygen consumption at extreme upper temperatures. This will have been strongly driven by the experienced year-round climate as a mechanism to increase heat tolerance, as indicated by their lower activation energies (Frederich and Pörtner 2000). Our data indicate that bimodal breathing intertidal ectotherms in the tropics are likely to be better able to endure acute heat events, with all early life stages able to acquire sufficient oxygen with no signs of metabolic stress. This mirrors the findings of Fusi et al. (2015) on the thermal sensitivity of adult males of the same model species from the same regions. The pervasive nature of this sensitivity, from embryos through to adults, further underlines the thermal risks that higher latitude, temperate ectotherm populations are, and will be, exposed to. Thus, generalizations predicting thermal vulnerability of ectotherms along a latitudinal gradient should be made more cautiously. As shown here, such predictions may not be universally applicable and multilevel interpretation must be considered, especially for organisms already living in thermally stressful environments. Our focus on dual-breathing intertidal ectotherms like crabs, rather than the more frequently studied exclusively water-breathing animals, fills an important gap in the link between physiological vulnerability and climate change (Huey et al. 2012).

Based on our results, we propose a conceptual model to highlight the differences in thermal sensitivity among life stages, geographical regions and breathing media for intertidal ectotherms (Fig. 3). The megalopal response to temperature increases as depicted in the model is extrapolated from Vorsatz et al. (2021) for *Parasesarma catenatum* and *Neosarmatium africanum*. The juvenile response to temperature increases are extrapolated from Walther et al. (2010) for the spider crab *Hyas araneus* assuming that juveniles of brachyuran crabs regardless of primary breathing mode have a higher thermal tolerance than megalopae, but a lower thermal tolerance than adults.

The geographic-, ontogenetic-, and respiratory medium-dependent thermal response patterns unveiled in this study suggest that breathing efficiency is affected by ontogenetic stage and local climate adaptation which are significant factors to consider when evaluating the overall vulnerability of intertidal ectotherms to temperature increases. These findings highlight the need to recognize that the evolutionary adaptations of dual-breathing modes acquired by some intertidal ectotherms may be a key mechanism to ameliorate the effects of climate change. Focussing on the organisms that have acquired such mechanisms will greatly improve our understanding on the impact of climate changes on intertidal organisms, populations and communities.

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Acknowledgments

FP acknowledges use of infrastructure provided by the South African Institute for Aquatic Biodiversity Research Platform – National Research Foundation of South Africa. SC thanks the Kenya Marine and Fisheries Research Institute for the kind assistance while working at the Gazi Field station. Funds for this research were provided by the SP3- People (Marie Curie) IRSES Project CREC (no. 247514) and the South African Research Chairs Initiative of the Department of Science and Technology and the National Research Foundation (Grant number 64801).

Submitted 16 July 2021

Revised 08 November 2021

Accepted 10 November 2021