Title
Thermal sensitivity of the crab Neosarmatium africanum in tropical and temperate mangroves on the east coast of Africa.

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Abstract

Mangrove forests are among the tropical marine ecosystems most severely affected by rapid environmental change and the activities of key associated macrobenthic species contribute to their ecological resilience. Along the east coast of Africa, the amphibious sesarmid crab *Neosarmatium africanum* (=meinerti) plays a pivotal role in mangrove ecosystem functioning through carbon cycling and sediment bioturbation. In the face of rapid climate change, identifying the sensitivity and vulnerability to global warming of this species is of increasing importance. Based on a latitudinal comparison, we measured the thermal sensitivity of an tropical and a temperate population of *N. africanum*, testing specimens at the centre and southern limit of its distribution, respectively. We measured metabolic oxygen consumption and haemolymph dissolved oxygen content during air and water breathing within a temperature range that matched the natural environmental conditions. The results indicate different thermal sensitivities in the physiological responses of *N. africanum* from tropical and temperate populations, especially during air breathing. The differences observed in the thermal physiology between the two populations suggest that the effect of global warming on this important mangrove species may be different under different climate regimes.

Keywords

Sesarmidae; Decapods; Tropical and Temperate wetlands; Oxygen Consumption; Haemolymph; Physiology, Populations.
Introduction

Recent integrative frameworks propose that the vulnerability of species to environmental changes depends on their capacity to individually adapt their physiology and behaviour in response to the changes they effectively experience (Williams et al., 2008; Huey et al., 2012; Rezende et al., 2014). These frameworks thus suggest that the vulnerability of a species to climate change should be assessed through a mechanistic approach, based on the integration of data from its biological traits (such as behaviour, thermal physiology and metabolism) with environmental data (Gaston et al., 2009; Kearney & Porter, 2009; Sih et al., 2010; Kearney et al., 2012). Among physiological traits, respiration and respiration control provide one of the most accurate proxies of a species’ thermal sensitivity, defined as the physiological response (such as metabolic oxygen consumption and haemolymph oxygen content) to changes in its thermal environment (Sinclair et al., 2016; Verberk et al., 2016). Furthermore, it is becoming evident that the assessment of thermal sensitivity has to be determined throughout a species entire distributional range, since conspecific populations subject to different environmental conditions can respond in different ways (Eliason et al., 2011; Sunday et al., 2011; Baldanzi et al., 2015; Fusi et al., 2015).

Mangrove forests are amongst the most vulnerable and endangered ecosystems in the world (Duke et al., 2007; Hoegh-Guldberg & Bruno, 2010) and are heavily threatened by sea-level rise as a consequence of global warming (Gilman et al., 2008; Lovelock et al., 2015). Their exposure to factors related to climate change, such as rising temperature, is, however, still debated amongst ecologists (Gilman et al., 2008) and few data are available on the relevant ecological traits of key benthic species that play a critical role in mangrove ecosystem functioning (Lee, 2008). In east African mangrove forests, the large burrowing sesarmid crab Neosarmatium africanum (namely=meinerti, Ragionieri, Fratini & Schubart, 2012), can occur at densities of over 20 individuals per square meter (Andreetta et al., 2014). N. africanum is a semi-terrestrial species and is the African representative of a complex of four sister species colonizing the Indian Ocean and East Australia regions (Ragionieri et al., 2010, 2012). It occupies the landward fringe of mangrove forests (Macnae, 1968; Hartnoll, 1975), which is inundated only during spring tides and is frequently dominated by Avicennia marina trees (Forssk.) Vierh. (Tomlinson, 1986). N. africanum provides crucial ecological functions for the entire ecosystem such as burrowing (Micheli et al., 1991; Berti et al., 2008) and a contribution to carbon burial and storage (Andreetta et al., 2014), among the others. By burrowing, N. africanum also contributes to modification of sediment topography and the distribution of sediment grain size (Warren & Underwood, 1986), reduces pore water salinity (Ridd, 1996; Stieglitz et al., 2000), creates microhabitats for other fauna (Bright & Hogue, 1972; Dittmann, 1996; Tack et al., 2001), contributes to secondary production (Lee, 1997) and increases nutrient levels while decreasing sulfide concentrations in the sediment (Smith et al., 1991; Kristensen, 2008).
Despite such an array of multiple and critical functions, information on the sensitivity of *N. africanum* to environmental changes is lacking. The landward edge of the *A. marina* zone is a particularly harsh environment for intertidal species as it is subject to acute fluctuations in both salinity and temperature (Macnae & Kalk, 1962). Gillikin et al. (2004) showed that these crabs are highly effective hyper/hypo-osmoregulators, able to survive a range of 16–65 ppt of salinity, but no data are available concerning this species’ thermal responses. Given the accumulating evidence that allopatric conspecific populations may exhibit important differences in their metabolic responses to stress, we asked whether *N. africanum* individuals belonging to tropical and temperate populations may have a different thermal sensitivity. We addressed this question by examining physiological responses of *N. africanum* to temperature in the laboratory and coupling this result with the temperatures they experience in the field. Since a population comparison is fundamental to a reliable assessment of species sensitivity (Eliason et al., 2011; Fusi et al., 2015), we carried out these experiments on specimens at the center (Kenya) and the southern limit (South Africa) of *N. africanum* distribution.

Materials and methods

Study areas (Fig. 1)

The study was performed during the hottest season at each study site, November/December in Kenya as the tropical site, January/February in South Africa as the temperate site.

Tropical site: Kenya – Gazi Bay (4° 22’ S, 39° 30’ E)

Gazi bay is a semi-enclosed shallow coastal system located about 40 km south of Mombasa, Kenya. The climate is typically monsoonal, with moist southeast monsoons from March to September and dry northeast monsoons from October to February; rain occurs from March to May and, to a lesser extent, during October and November (Kitheka et al., 1996). Average annual maximum temperature value is around 27-30°C throughout the year.

Temperate site: South Africa – Mngazana estuary (31°42’ S, 29°25’ E)

The Mngazana River is situated about 250km south-west of Durban, on the southeast coast of South Africa. The estuary measures 5.3km in length and is permanently open to the sea. There are two creeks, which support the main populations of mangroves. Rainfall occurs throughout the year but especially during summer (November– January) (Rajkaran & Adams, 2012) Temperatures vary from an average maximum values of 30-33°C in summer to 10-14°C in winter. The mangrove forest at the Mngazana estuary is one of the southerly in the world (Quisthoudt et al., 2013).
Thermal and tidal series

To determine the temperature range experienced by natural populations of *N. africanum*, temperature was recorded in the field for approximately two months during the summer period (49 days in Kenya, 43 days in South Africa) in 2011/2012. Forty temperature loggers (Maxim integrated product, ColdChain Thermodynamics) were placed in areas inhabited by the focal species, avoiding direct exposure to the sun; 20 were positioned about 3 cm above the sediment surface and 20 approximately 20 cm beneath the sediment surface close to the burrows of the animals in order to record the temperature to which the animals were directly exposed (Edney, 1961). Loggers were waterproofed using silicon cases that do not affect the accuracy of measurements (Roznik & Alford, 2012). Four additional loggers were used to measure air temperature and humidity. These were attached to branches under the canopy, about 4 m from the ground to avoid tidal submersion, and were protected from rain with plastic covers. All loggers were set up to measure temperature (±0.1°C) at five minute intervals for 15 days, after which data were downloaded and the loggers reset and re-deployed for a total of 4 times in Kenya, 3 in South Africa. Data were downloaded with Cold Chain Thermodynamics software (version 4.9 - Fairbridge Technologies) and average temperature was calculated for every hour of all recorded days.

Tidal series data were retrieved by Wtide software version 3.1.7 (www.wtide.com) taking as reference points Kilindini and East London in Kenya and South Africa, respectively, corrected with the delay recorded for our study sites. The tides were therefore correlated hourly with the temperature.

Crab sampling for laboratory experiments

Sixteen adult male *N. africanum* of similar size (approximately 40mm carapace width) were collected at each site. For acclimation, they were held for two days in aquaria prior to the start of the experiments. They were kept in filtered seawater (35‰ salinity) at 27 ± 0.5°C, under a 12h/12h light/dark cycle. In Kenya, the animals were kept at the laboratory of KMFRI (Kenya Marine and Fisheries Research Institute) in Gazi, while in South Africa at the Coastal Research Group Laboratory, Rhodes University, Grahamstown.

Oxygen consumption

Oxygen consumption (MO$_2$) approximating the routine metabolic rate, was measured in air and water for 8 adult males for each site using an intermittent flow respirometer equipped with eight parallel darkened Perspex chambers placed in a temperature controlled water bath. An oxygen sensor (Sensor Type PSt3 PreSens, Regensburg, Germany), glued to the inside wall of the chamber and connected to a single channel oxygen transmitter Fibox 3 (PreSens, Regensburg, Germany) through an optical sub miniature fiber, was used to measure the partial pressure of oxygen in air and water. Data were recorded using the FibSoft v.1.0 software (Loligo Systems ApS). Prior to measurements, sensors were calibrated in air-
equilibrated seawater (100% oxygen saturation) and in sodium thiocyanate saturated seawater (0% oxygen). During trials, oxygen concentration was not allowed to fall below 60% in order to avoid exposing the animals to severe hypoxic conditions (Schurmann & Steffensen, 1992). The limited movements of individuals inside the experimental chambers were adequate to ensure stirring of the water and MO$_2$ was determined by measuring the linear decline in oxygen saturation. An empty chamber was used during each trial as a control, to account for background oxygen depletion, which was less than 2% of the animals’ consumption in water and negligible in air. Prior to ramping of temperature, individuals were placed in the chambers and allowed to recover from handling stress overnight at 27 ± 0.5°C. From an initial measurement performed at 27°C, MO$_2$ was determined at every two degrees of temperature across the increasing range 27 to 37°C, raising temperature at the rate of 1°C h$^{-1}$ (Terblanche et al., 2011). Differences in the variability of MO$_2$ were not caused by differences in behaviour between the two populations (personal observation). Following each experiment, every animal was individually weighed and its volume calculated by immersing it in a graduated cylinder and measuring the water displacement. All experiments lasted less than 24 h to avoid interference with the metabolic rate by other factors such as starvation and during air respiration humidity was kept at 90% to avoid desiccation (Terblanche et al., 2011). Since the Q$_{10}$ coefficient is an integrated measure of biochemical reactions with physical processes in relation to increases in temperature, it provides a good proxy for thermal performance. Q$_{[27-37°C]}$ were therefore calculated for each treatment following Baldanzi et al. (2015).

**Haemolymph dissolved oxygen content**

Dissolved oxygen content in arterial and venous (sensu Greenaway & Farrelly, 1984) haemolymph was measured with fiber-optic oxygen microsensors (PreSens GmbH) connected to an oxygen meter (MicrOx-TX, PreSens GmbH) with integrated signal processing software. Sensors were calibrated before each experiment using a two-point calibration in oxygen-free (addition of sodium dithionite) and air-saturated seawater. Animals were acclimated overnight at 27°C and the oxygen dissolved in haemolymph was estimated between 27°C and 38°C (accuracy ± 1°C), using the protocol described for the MO$_2$ experiment.

Arterial haemolymph was withdrawn from the pericardial sinus through a 0.2 mm hole drilled through the carapace (Frederich & Pörtner, 2000). Venous blood was withdrawn from the sinus below the arthrodial membrane, at the base of the fourth or fifth pereiopod (Greenaway & Farrelly, 1984; Giomi & Pörtner, 2013). In both cases, a small amount of haemolymph (less than 20 µl) was collected through capillary action using a manually sharpened Pasteur pipette with a pre-inserted oxygen sensor close to the tip. Because of instrumental failure the measurement of haemolymph oxygen content was only possible in Kenya.
Statistical analysis

For the environmental data, a Permutational Analysis of Variance was performed with the null hypothesis of no differences for environmental temperatures and humidity across Regions (Kenya KY, South Africa ZA), and Sampled Zones (Above ground, Below ground and Air), defined as fixed and orthogonal. Further, a PERMDISP was performed to test the similarity of the variances among temperatures and humidity; whenever the variances proved heterogeneous, log-transformation was applied prior to proceeding with PERMANOVA analysis. These analyses were performed using PERMANOVA+ routines for PRIMER 6 (Anderson et al., 2008).

To test for statistical differences in MO$_2$ between populations and medium of respiration, an ANCOVA was performed using a linear mixed model (lme4). MO$_2$ was set as the continuous response variable, temperature as a continuous explanatory variable, and Region (Kenya, South Africa) and Medium (water, air) were set as fixed categorical explanatory variables. Prior to statistical tests, MO$_2$ data were log transformed and the normality for each group of data was tested using the Shapiro-Wilk test. Levene’s test indicated homogeneity of variances in the data (d.f.=3, 69; F=2.6; p=0.5883). Since measurements across the temperature ramp were made on the same individuals, they were not independent. Consequently, we treated Individual ID is as a random factor in the mixed model to account for multiple observations (Bates, 2010).

The best fitted model obtained as described above, was then used to calculate the potential daily MO$_2$ experienced by the two populations at neap and spring tide, feeding the model with the hourly average temperatures above and below ground recorded in the field.

The same analysis was performed for haemolymph dissolved oxygen content, the only difference being that the explanatory variable Region was excluded since PO$_2$ measurements were performed only in Kenyan and we included the explanatory categorical variable Haemolymph (levels: Arterial, Venous) (Levene’s Test; d.f.=3, 38; F=3.34; p=0.649). The response Haemolymph oxygen content was previously square root transformed for normality. These statistical analyses were carried out in R (R Development Core Team, 2014).

Results

Tidal and thermal series

The tidal range during the observation periods differed between the two study regions (Figs. 2 and 3). In Kenya, maximum tidal range was 4 m during spring tides and 2.5 m during neap tides. In South Africa, the range was 2 m during spring tides and 0.5 m during neap tides. Either in Kenya or South Africa, during almost all the duration of neap tides, sea level did not reach the area occupied by N. africanum (Fig. 6).
Thermal regimes also differed significantly between regions: Kenya experienced less variable (PERMDISP, t=5.67, p<0.01) and hotter (PERMANOVA p-hpt, t=20.39, p<0.001) temperatures than South Africa. Average temperatures in Kenya ranged between 23-39°C above the sediment surface, 25-34°C below the surface and air temperatures were 22-39°C. These values were significantly different from those for South Africa: 16-37°C above ground, 23-30°C below ground and 15-36°C for air (PERMANOVA p-hpt, t=7.74, p<0.001 in each case).

Humidity measurements were not significantly different between regions (PERMDISP, t=1.79, p=0.16; PERMANOVA p-hpt, t=1.56, p=0.15) with values in both localities ranging between 50% and almost 100%.

The interaction among temperature, medium and region was highly significant (Fig. 4; F<sub>4,170</sub>= 57.266, p<0.0001; ANCOVA), indicating that MO<sub>2</sub> differed significantly between the two respiratory media (i.e. air and water) for both populations. In both cases, metabolic rate was higher in air than in water, but the difference was markedly greater for Kenya than South Africa. The best significant model that described the MO<sub>2</sub> of <i>N. africanum</i> was represented by an exponential relationship between temperature and oxygen consumption. For the Kenyan population, the best fitted model during air respiration was <i>y = 0.018e^{0.06294κ}</i> with a Q<sub>10</sub> of 1.8, while for respiration in water it was <i>y = 0.00513e^{0.06916κ}</i> with a Q<sub>10</sub> of 1.9. For the South African population, the best models were <i>y = 0.0102e^{0.04358κ}</i> with a Q<sub>10</sub> of 1.2 for air and <i>y = 0.000856e^{0.11070κ}</i> with a Q<sub>10</sub> of 1.9 for water.

Haemolymph dissolved oxygen content

The oxygen content of venous and arterial haemolymph differed significantly between air and water along the temperature ramp (Fig. 5; significant interaction among temperature, medium, haemolymph ANCOVA, F<sub>4,139</sub>=99.648; p < 0.001). In both media, <i>N. africanum</i> showed a low ability to saturate arterial haemolymph. With increasing temperatures, oxygen saturation was significantly more affected in water than in air, dropping to almost 0 kPa at 33°C. During air respiration, haemolymph was saturated until 34°C, dropping to 0 kPa around 37°C. Similar patterns were observed for venous haemolymph, though with lower saturation levels than for arterial haemolymph.

Discussion

The thermal environment experienced by the two populations of <i>Neosarmatium africanum</i> differed significantly between the two sites, with wider above ground temperature fluctuations in South Africa than in Kenya. This difference was less marked for below ground temperatures, presumably because soil buffers temperature variation. Although the natural thermal environment was monitored for a relatively short time, a consistent difference in temperature between latitudes
reflects different thermal niches across the distributional range of *N. africanum*. Above ground temperature variability suggest that burrows can play a fundamental role as stable thermal refugia from the heat (Edney, 1961) that crabs of both populations experience when active above ground (Sunday et al. 2014). Regardless of local differences in tidal regime, both populations experience prolonged periods of emersion during neap tides, during which they are exposed to fluctuations of air temperature for extended periods. Conversely, at spring tide, when the inhabited zones are flooded, animals experience more stable temperature in both regions. Despite a higher variable regime of pronounced temperature fluctuation, the South African population of *N. africanum* revealed a limited capability for sustaining high metabolic costs under increasing temperatures. Similarly, when submerged, the crabs from the Kenyan population showed no compensatory capacity for the temperature-induced increase of metabolic costs. On the contrary, while breathing in air, the Kenyan crabs showed the potential to adjust their metabolism during temperature rise. It should be noted that natural temperature fluctuations are more rapid and abrupt than those experimentally simulated in our protocol, and that these even more severe thermal regimes may further exacerbate the different metabolic responses of the two populations. The ability for such metabolic adjustment can be explained by the fact that the thermal responses are deeply influenced by the biogeographic effects (Pörtner, 2001; Gaitán-Espitia et al., 2014; Baldanzi et al., 2015) that involve different thermal histories for conspecific populations (Giomi et al., 2016). The tropical Kenyan population is subject to more constant, if higher, temperatures than the South African population, which experiences a wider temperature range and notably low temperatures during winter (Quisthoudt et al., 2013). Q_{10} values were similar for tropical specimens in air and water and South African specimens in water (Q_{10(27.37°C)} = 1.8, 1.9 and 1.8 respectively), but markedly lower for South African specimens in air (Q_{10(27.37°C)} = 1.2). While a Q_{10} around 2 is relatively common in marine ectotherms (Clarke & Fraser, 2004) and reflects a fairly normal response to increasing temperature, a Q_{10} of 1.2 indicates reduced thermal sensitivity.

We propose two opposing explanations for these results. The first is that, during air respiration, the animals from the South African population are able to moderate its metabolic response to warming as observed in the in tropical high shore snail, *Echinolittorina malaccana* (Philippi, 1847) (Marshall & McQuaid, 2011). Alternatively, we can interpret these data as a sign that the South Africa population is unable to endure such acute thermal stress (Verberk et al., 2015), exhibiting an inefficient metabolic response to increased temperature. This second interpretation would indicate that the temperate population is more vulnerable to global warming and to heat events, in agreement with similar finding for the closely related species *Perisesarma gutatum* (A. Milne Edwards, 1869) (Fusi et al., 2015).

The analyses of haemolymph dissolved oxygen content for the Kenyan population showed an overall decrease in oxygen levels as temperature increases, with a markedly lower level of oxygen during respiration in air than water. This pattern may indicate that animals from this population adjust the metabolic rate to endure increased temperatures in air (Hochachka, 1991).
By integrating the results for temperature-dependent routine metabolic rates with the thermal data series recorded in the field and the tidal regimes, we developed a diagram that predicts the daily metabolic requirements of the two study populations (Fig. 6). The temperature recorded by loggers was used as body temperature of animals when above and below ground during neap and spring tides and the graph shows MO₂ calculated for those temperatures. The results indicate that, theoretically, Kenyan individuals are able to mobilise a wider thermal response than South African conspecifics (Fig. 6). Importantly, the figure highlights the fact that, although individuals from Kenya exhibit a pronounced increase of metabolic rate in air, they can rely on the cooler environment of their burrows (recorded as below ground temperature), especially during spring tides, enabling them to buffer the heat load accumulated during above ground activity (Edney, 1961). *N. africanum* is fully active only when its habitat within the mangrove forest is uncovered by water at spring and neap tide (Micheli et al., 1991; Fratini et al., 2011). Diurnal low tides, especially during neap tides, often correspond to the hottest hours of the day, maximizing the risk of thermal stress and desiccation (Porter & Gates, 1969; Kearney et al., 2012; Sunday et al., 2014). In contrast, the South African population has a weaker thermal response, exhibiting similar oxygen consumption above and below ground.

We interpret the results of this study as an indication that the thermal sensitivities of the two populations differ and that the temperate population in South Africa is likely to be more vulnerable to heat events, suggesting a weak capacity to tolerate climate warming. In contrast, the tropical, Kenyan population shows the ability to endure heat stress by increasing its respiratory rate to meet heat-induced increases in oxygen demand (Verberk et al., 2015). Indeed, tropical and tropical thermal specialists may maximise their fitness within a narrow thermal niche and minimize maintenance costs, showing a residual capacity for phenotypic plasticity and acclimation responses (Verberk et al., 2015). Other studies have confirmed that environmental temperatures can shape thermal physiology, and that higher temperature variability increases the thermal sensitivity of species (e.g. Paaijmans et al. 2013). This may be one reason why the Kenyan population has evolved a more variable thermal response with a marked ability to endure higher temperatures in air than the South African population. Further studies that involve other proxies such as Heat Shock Protein production, behavioural assays and lactate/succinate production could confirm this (i.e. Marshall et al., 2011, 2013).

**Conclusion**

Our results indicate that the findings of earlier studies that tropical species are more vulnerable to climate warming than temperate species is an oversimplification when considering species that span a wide range of latitudes (Deutsch et al., 2008; Sunday et al., 2012) and display bimodal breathing strategy (Fusi et al., 2016). The vulnerability of species is more complex and goes beyond explanations derived from general temperature envelope models based on latitudinal gradients.
and climate, especially when species’ ranges are broad so that different populations are exposed to a wide range of climatic conditions. Our data show that some tropical ectotherms can show adaptation of their physiology that makes them more resilient to global warming than temperate ones. This study adds data on the thermal sensitivity of intertidal tropical and temperate species (Poloczanska et al. 2013) which are still largely overlooked in the estimation of the community temperature index (CTI, Stuart-Smith et al. 2015), a recent and potentially powerful instrument to assess ecosystem thermal vulnerability. Resilience to either heat events or chronic heating can also be highly modified by behaviour. In the case of *N. africanum* this includes modulating its activity below and above ground, balancing its foraging time and burrow occupancy (Sih et al., 2010; Nemeth et al., 2013) to buffer thermal stress.
Acknowledgements

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Ethical approval:

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Furthermore, all procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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Figures

Fig. 1. Study sites along the east coast of Africa. The tropical one at Gazi, Kenya, and temperate one, Mngazana, South Africa.
Fig. 2. Environmental temperatures and humidity of the area colonized by *Neosarmatium africanum* in Kenya during the period 31 October to 19 December 2011. The dashed line is the daily average of the variable described in each graph, while the solid line is the hourly average.
**Fig. 3.** Environmental temperature and humidity of the area colonized by *Neosarmatium africanum* in South Africa during the period 16 January to 5 March 2012. The dashed line is the daily average of the variable described in each graph, while the solid line is the hourly average.
Fig. 4. MO$_2$ of *Neosarmatium africanum* from Kenya in air (a) and water (c) and from South Africa in air (b) and water (d). The significant best fitted models (see results section for the equations) are represented with continuous black lines for each population and medium.
Fig. 5. Haemolymph dissolved oxygen content of Kenyan *Neosarmatium africanum* during water (A) and air (B) respiration; open circles arterial haemolymph, black circles venous haemolymph. Significant regressions are plotted: arterial (*y* = 96.016*e*^−0.129κ* for water and 176.49*e*^−0.0185κ* for air respiration) shown in dotted-grey line, while venous (*y* = 10.516*e*^−0.076κ* for water and 6058.2*e*^−0.0532κ* for air respiration) shown in solid-black line.
Fig. 6. Hourly MO₂ predicted for *Neosarmatium africanum* during Neap Tide (A) and Spring Tide (B) on the basis of temperatures measured above ground and below ground (see the legend in the graph). Shaded bars indicate the hours flooded at both sites during spring tides.