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Exploring physiological plasticity and local thermal adaptation in an intertidal crab along a latitudinal cline

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ABSTRACT

Intertidal organisms have evolved physiological mechanisms that enable them to maintain performance and survive during periods of severe environmental stress with temperatures close to their tolerance limits. The level of these adaptive responses in thermal physiology can vary among populations of broadly distributed species depending on their particular environmental context and genetic backgrounds. Here we examined thermal performances and reaction norms for metabolic rate (MR) and heart rate (HR) of seven populations of the porcelanid crab *Petrolisthes violaceus* from markedly different thermal environments across the latitudinal gradient of ~3000 km. Physiological responses of this intertidal crab under common-garden conditions suggest the absence of local thermal adaptation along the geographic gradient (i.e., lack of latitudinal compensation). Moreover, thermal physiological sensitivities and performances in response to increased temperatures evidenced the existence of some level of: i) metabolic rate control or depression during warm temperature exposures; and ii) homeostasis/canalization (i.e., absence or low levels of plasticity) in physiological traits that may reflect some sort of buffering mechanism in most of the populations. Nevertheless, our results indicate that elevated temperatures can reduce cardiac function but not metabolic rate in high latitude crabs. The lack of congruence between HR and MR supports the idea that energy metabolism in marine invertebrates cannot be inferred from HR and different conclusions regarding geographic differentiation in energy metabolism can be obtained from both physiological traits. Integrating thermal physiology and species range extent can contribute to a better understanding of the likely effects of climate change on natural populations of marine ectotherms.

1. Introduction

For many marine intertidal organisms, physiological plasticity is a crucial mechanism to cope with natural fluctuations in thermal conditions (Hofmann and Todgham, 2010). This adaptive strategy allows them to maintain performance and survive periods of severe environmental stress (e.g., low tides), with temperatures at or above their heat tolerance limits (Helmuth et al., 2006, 2002; Stillman, 2002). Under these conditions, intertidal organisms adjust their metabolic rates in an attempt to balance tissue oxygenation and energy production/expenditure (McElroy et al., 2012). The scope for these adjustments depends on the functional capacity of ventilation and circulation, which is limited to a particular thermal tolerance window for a species or a population and, thus, sets limits to its geographical distribution (Pörtner, 2001). In broadly distributed species, local adaptation to different environmental regimens can lead to spatial differences in thermal tolerance and physiological plasticity among populations (e.g., Fangue et al., 2006; Gaitán-Espitia et al., 2014, 2013; Gardiner et al., 2010; Lardies et al., 2011; Pörtner, 2001). For example, in widely latitudinal distributed ectoherms, populations at high latitudes have broader thermal tolerances and live at temperatures under their physiological optima (Addo-Bediako et al., 2000; Deutsch et al., 2008; Janzen, 1967; Sunday et al., 2010), compared with their counterparts at lower latitudes (Gaitán-Espitia et al., 2014, 2013; Gaitán-Espitia and Nespolo, 2014; Naya et al., 2011; Niehaus et al., 2012). This geographic pattern of variation in thermal physiology and plasticity has profound implications in the context of climate change (Kelly et al., 2012), as elevated temperatures are likely to cause localized extinctions of many marine ectothermic species (Helmuth et al., 2002), by affecting their physiological performance and thermal

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biology (Pörtner and Knust, 2007; Skelly et al., 2007; Somero, 2010; Tomanek, 2010).

In water breathing ectotherms, such as crustaceans, a decreased capacity to perform aerobically at higher temperatures is hypothesized to be the key physiological mechanism that will determine the response of many species to climate change (Munday et al., 2009; Pörtner, 2001; Pörtner and Knust, 2007). The limited capacity of the circulatory and ventilatory systems of aquatic ectotherms to keep pace with increased oxygen demand at higher temperatures causes a reduction in the aerobic scope and sets the boundaries of whole organism thermal tolerance (Munday et al., 2009; Nilsson et al., 2009; Pörtner and Knust, 2007). Therefore, populations that can maintain their aerobic capacity at warmer temperatures have a higher thermal tolerance, and are thereby predicted to persist longer than populations that experience a decline in aerobic performance as temperature increases (Gardiner et al., 2010; Pörtner, 2001). Thus, the analysis of metabolic rates at different temperatures is crucial to understand the energetic costs of global warming, as well as disentangle the hidden reaction norms of individuals (i.e. the pattern of phenotypic expression of a single genotype across a range of environments) from different populations.

To examine variation in thermal adaptation and physiological responses to elevated temperatures, we investigated thermal performances and reaction norms for metabolic rate and heart rate in seven populations of the porcelanid crab *Petrolisthes violaceus* (Guerin, 1831) from markedly different thermal environments (Fig. 1). *P. violaceus* is commonly found in the rocky intertidal mid-low zone of the south-eastern Pacific (Viviani, 1969) along the latitudinal gradient of the Chilean coast. This region is characterized by large and meso-scale variations in the intensity of coastal upwelling (Torres et al., 2011), as well as spatial variation in sea surface temperature (SST) and air temperature (Fig. 1) (Lagos et al., 2005). Here, we: i) determine the geographic variation in phenotypic variables associated with organismal performance; ii) construct the thermal reaction norms of physiological traits at two ecologically relevant experimental temperatures; and iii) evaluate the geographic variation in the thermal sensitivity of physiological rates.

2. Materials and methods

2.1. Study populations and sites

Animals were randomly collected by hand on low tides from seven locations along the coast of Chile (ca. 3000 km) during austral spring, encompassing much of the species' latitudinal range (Fig. 1). To remove possible confounding effects of sex on metabolism (Valverde et al., 2009), only male crabs of similar size were used in the experiments. Individuals were chilled and transported to the laboratory at the Universidad Adolfo Ibañez, where they were maintained in common garden conditions at constant temperature (14 °C ± 1 °C) in artificial seawater (ASW; 33 ppm; Instant Ocean© sea salt dissolved in distilled water) for one month before the experiments. Crabs were exposed to a light/dark cycle of 12/12 h and fed with Instant Algae® and aquarium shrimp food three times a week.

2.2. Experimental setup and physiological measurements

As a mode of separating environmental effects from local differentiation among populations, common-garden experiments are the gold-standard design. We applied this approach with homogeneous laboratory acclimation to our seven populations of *P. violaceus*. A total of 185 adult crabs were sequentially acclimated (one month) at two experimental temperatures (14 °C and 20 °C ± 1 °C). These temperatures were chosen based on a high-resolution monitoring program of intertidal temperature developed along the latitudinal gradient of the Chilean coast (Gaitán-Espitia et al., 2014). Here, 14 °C falls within the mean annual in situ SST range across all sites (Fig. 1B), whereas 20 °C represents a moderate-high rise from the mean annual SST, falling within the temperature extreme window experienced in all populations along the latitudinal range (Fig. 1B-C). The 6 °C of increment in our experimental treatment is also consistent with the long-term gradual warming trend of mean annual environmental temperature expected for the year 2100 under the business-as-usual scenario (RCP8.5) (Gattuso et al., 2015). At the end of each acclimation period, metabolic rate and heart rate were measured in all individuals. Crabs were not fed 24 h prior to physiological measurements. Metabolic rate was estimated using a temperature compensated Microx optic fiber O_2-meter (Presens Inc, DE) connected to a recirculating water bath by a flow-through cell housing (Presens Inc, DE). An acrylic respirometry chamber of 113 ml was used for respirometric analysis with photosensitive, non-oxygen consumptive sensors. The optic fiber was calibrated in a solution saturated with Sodium Sulfite (Na_2O_3S, 0% air saturation) and in aerated artificial seawater (100% air saturation, ASW), checking for sensor drift before and after each trial. In addition, control chambers containing only ASW were used to quantify background microbial oxygen consumption. After calibration, oxygen availability (% air saturation) was measured in seawater for 60 min (recorded every 5 s). The first and the last 5 min were discarded in order to avoid possible disturbances when the fiber was inserted or removed. Thus, oxygen estimations are the average of the remaining 50 min of measurements. Before and after each measurement, crabs were briefly placed on a paper towel to remove excess water and then wet body mass was recorded using an analytical balance (ADAM AFA-180LC with precision ± 0.001 mg). The average of both body mass measurements was used in the statistical analyses. Slopes were calculated by the decrease of oxygen in the chamber per hour and normalized to fresh mass to get respiration rates in units of g O_2 L^{-1} h^{-1}.

Parallel to the measurements of metabolic rate, the cardiac activity was also recorded at the two experimental temperatures following Gaitán-Espitia et al. (2014). Heart rate was estimated using a heartbeat amplifier AMP 03 (Newshift Lda®) connected to an oscilloscope and the results were expressed in beats during 1 min. Measurements of cardiac activity were performed for each crab at the same period of the day to cancel the effects of a possible circadian rhythm of respiration. Animals were individually separated in plastic chambers with six subdivisions (200×200×100 mm), The first and the last 5 min were discarded in order to avoid any noise or erroneous recordings generated by animal manipulation. Finally, we calculated the ratio of metabolic rate and heart rate at the experimental temperatures (e.g., MR at 20 °C / MR at 14 °C; 6 °C interval). This ratio was used as a proxy of the temperature coefficient (Q_{10}), which is a unitless quantity that describes the change in the rate of a biological process across a 10 °C temperature interval (Chaui-Berlinck et al., 2002). In all of the physiological trials, animals were randomized in order to avoid confounding effects of time.

2.3. Statistical analyses

Physiological traits (MR and HR) were analysed using a mixed modelling approach, as we have two repeated measures on the same individual. Acclimation temperature, latitude and body mass were included as fixed effects while population and individual identifications were included as random effects. The thermal sensitivity of metabolism to temperature (a proxy of Q_{10}) was analysed in the same way but without considering individual identification. Traits were $\log_{10}$-transformed to meet normality assumptions. Hypothesis testing for both fixed (i.e., latitude and acclimation) and random (i.e. population) effects was based on a likelihood ratio test of nested models using maximum likelihood and restricted maximum likelihood, respectively. Note that the best model was found after a series of tests that compared the full model with alternative models that were simplified by exclusion of single predictors. This approach helped us to remove non-significant or redundant predictors from the best model, e.g. we expected that the