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RESEARCH ARTICLE

Geographic variation in thermal physiological performance of the intertidal crab *Petrolisthes violaceus* along a latitudinal gradient

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ABSTRACT

Environmental temperature has profound effects on the biological performance and biogeographical distribution of ectothermic species. Variation of this abiotic factor across geographic gradients is expected to produce physiological differentiation and local adaptation of natural populations depending on their thermal tolerances and physiological sensitivities. Here, we studied geographic variation in whole-organism thermal physiology of seven populations of the porcelain crab *Petrolisthes violaceus* across a latitudinal gradient of 3000 km, characterized by a cline of thermal conditions. Our study found that populations of *P. violaceus* show no differences in the limits of their thermal performance curves and demonstrate a negative correlation of their optimal temperatures with latitude. Additionally, our findings show that high-latitude populations of *P. violaceus* exhibit broader thermal tolerances, which is consistent with the climatic variability hypothesis. Interestingly, under a future scenario of warming oceans, the thermal safety margins of *P. violaceus* indicate that lower latitude populations can physiologically tolerate the ocean-warming scenarios projected by the IPCC for the end of the twenty-first century.

KEY WORDS: Reaction norm, Thermal sensitivity, Heart rate, Thermotolerance, Thermal safety margin, Latitudinal gradients

INTRODUCTION

For ectotherms, environmental temperature (T_a) is perhaps the most important abiotic factor that affects their body temperature (Hochachka and Somero, 2002; Young et al., 2011) influencing a variety of organismal processes (Angilletta et al., 2002) with profound implications on their performance, physiology and fitness (Cano and Nicieza, 2006; Huey and Berrigan, 2001; Kingsolver and Huey, 2008). T_a also influences large-scale processes such as the biogeographical distribution and the habitat preferences of many ectothermic species (Hofmann and Todgham, 2010; Pörtner, 2002; Sunday et al., 2011), depending on their thermal tolerance and physiological sensitivity (Schulte et al., 2011; Sunday et al., 2014). Most physiological processes in ectotherms operate within the bounds of lethal temperature extremes, with the performance of a physiological trait gradually increasing with T_a from a critical minimum (CT_{min}) to an optimum (T_{opt}) before dropping

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precipitously as T_a approaches a critical maximum (CT_{max}) (Kingsolver, 2009; Wilson, 2001). This effect of T_a on performance is usually described by a continuous nonlinear reaction norm (i.e. thermal performance curve, TPC) (Huey et al., 1999). Variation in the parameters of the TPCs (i.e. T_{opt}, CT_{min}, CT_{max}, maximum performance and thermal breadth) has been used to describe mechanistically the variation of thermal sensitivities among natural populations of diverse ectothermic species (see Gaitán-Espitia et al., 2013; Kingsolver and Gomulkiewicz, 2003; Lachenicht et al., 2010). The evidence indicates that these parameters usually co-vary with geographic clines (e.g. latitude), reflecting at least partial adaptation of ectotherms to their environments (Castañeda et al., 2004; Gaitán-Espitia et al., 2013).

Phenotypic plasticity and/or local adaptation to thermal clines can cause different patterns of thermal performance across the geographic range of an ectothemic species (Gardiner et al., 2010; Huey and Kingsolver, 1989; Knies et al., 2009; Schulte et al., 2011; Yampolsky et al., 2014). These patterns can be explained by at least four theoretical models of thermal evolution (Angilletta et al., 2002; Gardiner et al., 2010). The models contain implicit assumptions about the existence of evolutionary trade-offs acting on thermal physiology as a result of negative genetic correlations between performance and T_a (Angilletta et al., 2002; Huey and Kingsolver, 1993). For instance, the counter-gradient variation model explains that populations from cooler environments (high latitudes) tend to exhibit higher maximum performance (μ_{max}) than those from warmer environments (low latitudes) at all temperatures. This pattern is predicted when genetic and environmental influences on performance are negatively associated across the thermal gradient (Angilletta, 2009; Gardiner et al., 2010). In contrast to this, if genetic and environmental influences on performance are positively associated, the pattern of thermal performance would be related to the co-gradient variation model in which populations from warmer environments (low latitudes) tend to exhibit higher μ_{max} and T_{opt} than populations from cooler environments (high latitudes) (Conover et al., 2009; Gardiner et al., 2010). However, the local adaptation model states that if thermodynamic constraints do not limit adaptation to temperature, then adaptation to warmer and colder environments is expected to result in equal μ_{max} in their corresponding T_{opt} (Angilletta, 2009). Finally, if populations do not acclimate to the local thermal environment and high gene flow between populations restricts local adaptation to a thermal gradient, then no differences in TPCs would be expected between populations (Gardiner et al., 2010).

In addition, theory suggests that the thermal tolerance and sensitivity of many organisms are proportional to the magnitude of variation in T_a they experience (Addo-Bediako et al., 2000; Calosi et al., 2010; Gaitán-Espitia et al., 2013), a characteristic of climate that also increases with latitude (Ghalambor et al., 2006; Janzen, 1967; Naya et al., 2011). Therefore, it is expected that individuals at higher

latitudes require broader tolerance ranges (i.e. greater physiological plasticity) than individuals inhabiting lower latitudes (i.e. climatic variability hypothesis, CVH) (Chown et al., 2004; Ghalambor et al., 2006; Janzen, 1967; Naya et al., 2011; Stevens, 1989). This is particularly interesting in the case of organisms that inhabit highly variable habitats, such as rocky intertidal regions, because these habitats are characterized by a wide range of thermal conditions as a result of the tidal cycle (Helmuth and Hofmann, 2001; Helmuth et al., 2002). During low tide, the intertidal region exhibits a steep thermal stress gradient which increases with shore height (Helmuth et al., 2006). In these habitats, intertidal invertebrates experience temperatures at or above their heat tolerance limits during times when low tide occurs simultaneously with a heat wave (Helmuth et al., 2002; Helmuth et al., 2006; Stillman, 2003).

Here, we investigated the geographic variation in thermal sensitivity and thermal tolerance of physiological traits in seven populations of the intertidal crab *Petrolisthes violaceus* Guerin 1831, spanning a latitudinal gradient of ca. 3000 km along the Chilean coast. Our hypothesis was that individuals of low latitude populations live closer to their thermal limits compared with populations of central or high latitudes. Thus, the objectives of this study were: (i) to characterize the TPCs at the intraspecific level in seven local populations of *P. violaceus* along a latitudinal gradient of ca. 3000 km on the Chilean coast; (ii) to determine the geographic variation in their thermal physiology and organismal performance.

RESULTS

Latitudinal variability of *in situ* seawater temperature

The high-resolution monitoring program of intertidal temperature showed a clear latitudinal cline in annual mean and absolute minimum *in situ* sea surface temperature (SST) along the Chilean coast (Fig. 1A–C). Lower values and greater variation (~5°C) of annual *in situ* mean and absolute minimum SST were observed at high latitudes (Fig. 1B,C). Annual absolute minimum and maximum *in situ* SST showed two marked thermal breaks at 30°S and 40°S (Fig. 1B,C). These breaks were also evident for the coefficient of variation of daily mean *in situ* SST (Fig. 1D). However, there were no latitudinal trends for heating or cooling days (i.e. days where exposure was over or under the CT_{max} and CT_{min}, respectively). Signals of heating days were evidenced only for Antofagasta and Talcaruca at low latitude, whereas cooling days were detected only in Chiloe, at high latitude (supplementary material Table S1).

Thermal performance curves

The best-fit models used to describe the TPCs of *P. violaceus* populations (Table 1), revealed the typical left-skewed shape of TPC curves (Fig. 2). The upper and lower limits of temperature at which the heart rate decreased (CT_{min} and CT_{max}) were similar among populations (Table 2), with no statistical differences along the latitudinal gradient (one-way ANOVA, CT_{min}: $F_{6,94}=2.09$, $P=0.06$; CT_{max}: $F_{6,94}=1.37$, $P=0.23$). However, there were differences in the thermal optimum (T_{opt}), thermal breadth (T_{br}) and maximal performance (μ_{max}) among populations (Table 2; one-way ANOVA, T_{opt}: $F_{6,94}=17.26$; T_{br}: $F_{6,94}=7.63$; μ_{max}: $F_{6,94}=8.35$, $P<0.05$).

Overall, critical thermal limits showed no signature of latitude across the geographic range of the species (CI 95 for latitude: $CT_{max}=−0.047$, 0.096; $CT_{min}=−0.016$, 0.021) (Fig. 3D,E). In addition, neither the height of the TPCs (CI 95 for latitude: $\mu_{max}=−2.34$, 0.791) nor their amplitude (CI 95 for latitude: $T_{br}=−0.550$, 0.019) changed across latitude (Fig. 3B,C). Similar results were found for these four TPC parameters when the climatic variables related to SST (mean, maximun, minimum, coefficient of
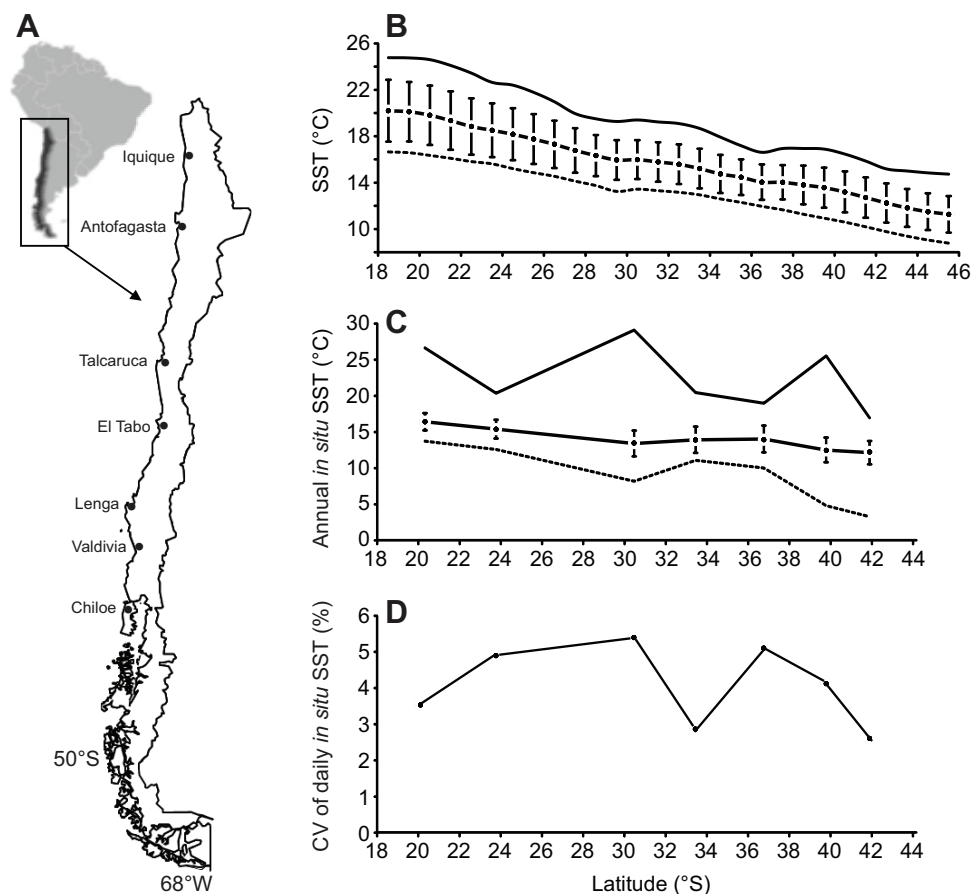

Fig. 1. Geographic and environmental gradient along the Chilean southeastern Pacific coast. (A) Study sites along the Chilean coast. (B) Latitudinal variability in sea-surface temperature (SST) (means ± s.d.) from 18 to 46°S estimated using 5 years of historical records from July 1999 to July 2004 (data available at http://www.podacc.gov). (C) Latitudinal variability of annual *in situ* SST (means ± s.d.) recorded at the low intertidal level of the rocky shore platform in the study sites. (D) Coefficient of variation (CV) of daily mean *in situ* SST. Absolute maximum (solid line) and minimum (dashed line) *in situ* SST values recorded for the corresponding time period are also showed in each graph.

Table 1. Comparison of functions used to describe the thermal performance curves of *Petrolisthes violaceus* populations across the latitudinal gradient, using Akaike's information criterion (AIC)

Population	Function	K	AIC	λ_i	w_i	r^2
Iquique	**Gaussian**	4	51.22	0.00	0.48	0.96
	Lorentzian	4	51.43	0.21	0.43	0.96
	Weibull	5	54.51	3.29	0.09	0.96
Antofagasta	**Gaussian**	4	55.36	0.00	0.38	0.87
	Lorentzian	4	55.38	0.02	0.37	0.87
	Weibull	5	56.21	0.85	0.25	0.90
Talcaruca	**Lorentzian**	4	38.52	0.04	0.47	0.97
	Gaussian	4	38.47	0.00	0.48	0.97
	Weibull	5	43.20	4.73	0.05	0.96
El Tabo	**Weibull**	5	32.88	0.00	0.78	0.99
	Lorentzian	4	36.72	3.84	0.11	0.98
	Gaussian	4	36.86	3.99	0.11	0.98
Lenga	**Gaussian**	4	38.13	0.00	5.24	0.98
	Lorentzian	4	38.14	0.01	5.20	0.98
	Weibull	5	41.44	3.31	1.00	0.98
Valdivia	**Weibull**	5	39.07	0.00	0.44	0.98
	Gaussian	4	39.94	0.87	0.28	0.96
	Lorentzian	5	39.94	0.87	0.28	0.96
Chiloé	**Gaussian**	4	44.87	0.56	0.40	0.89
	Lorentzian	4	44.31	0.00	0.53	0.90
	Weibull	5	48.25	3.93	0.07	0.90

The function with the lowest AIC is the one that best describes the data. K, number of parameters in the function; λ_i, difference between a given model's AIC and the lowest AIC; w_i, Akaike weight. Models in boldface were selected for analyses.

variation and heating/cooling days) were included in the analysis (results not shown). However, T_{opt} was negatively influenced by the geographic gradient (CI 95 for latitude: T_{opt}=−0.543, 0.130; slope b=−0.335±0.108; mean ± s.e.) (Fig. 3A).

Critical thermal resistance

Chill and heat coma (critical thermal resistances), using roll-over speed as a proxy of the relationship between organismal performance

and environmental temperature, were different among populations in the latitudinal gradient (Kruskal–Wallis test, chill coma: H_6=78.03; heat coma: H_6=57.05, P<0.05). From these indices of thermal tolerance, only the critical thermal resistance to low temperatures (CTR_{min}) was influenced by latitude (Fig. 4A; CI 95 for latitude: CTR_{min}=−0.543, 0.130; slope b=−0.063±0.01; mean ± s.e.).

Warming tolerances and thermal safety margins

Although warming tolerance (WT) and thermal safety margin (TSM) showed differences among populations (one-way ANOVA, WT: $F_{6,94}$=11.79; TSM: $F_{6,94}$=7.02, P<0.05), both indices showed opposite trends along the latitudinal gradient (Table 2). WT of the species increased towards higher latitudes (CI 95 for latitude: WT=0.142, 0.332; b=0.237±0.05) whereas TSM showed no clear pattern with latitude (CI 95 for latitude: TSM=−0.319, 0.073) (Fig. 5A,B).

DISCUSSION

In this study, we assessed the geographic variation in whole-organism thermal physiology of seven populations of the porcelain crab *P. violaceus* across a latitudinal gradient of ca. 3000 km, characterized by different local thermal conditions. Our study found that populations of *P. violaceus* exhibit differences in thermal sensitivities (i.e. different patterns of thermal performance curves) and thermal tolerances. These findings are consistent with the climatic variability hypothesis, in which populations at higher latitudes exhibit broader tolerance ranges than populations inhabiting lower latitudes with more stable environments (Janzen, 1967; Levinton, 1983; Naya et al., 2011). The strong association between latitude and climate does not permit separation of these factors to establish to what extent higher physiological tolerance at higher latitudes is due to the direct effect of climatic variability or to other factors associated with latitude (Ghalambor et al., 2006).

Latitudinal variation in habitat thermal conditions

The marine intertidal zone along the Chilean coast is characterized by a thermal gradient with lower values of annual *in situ* mean and
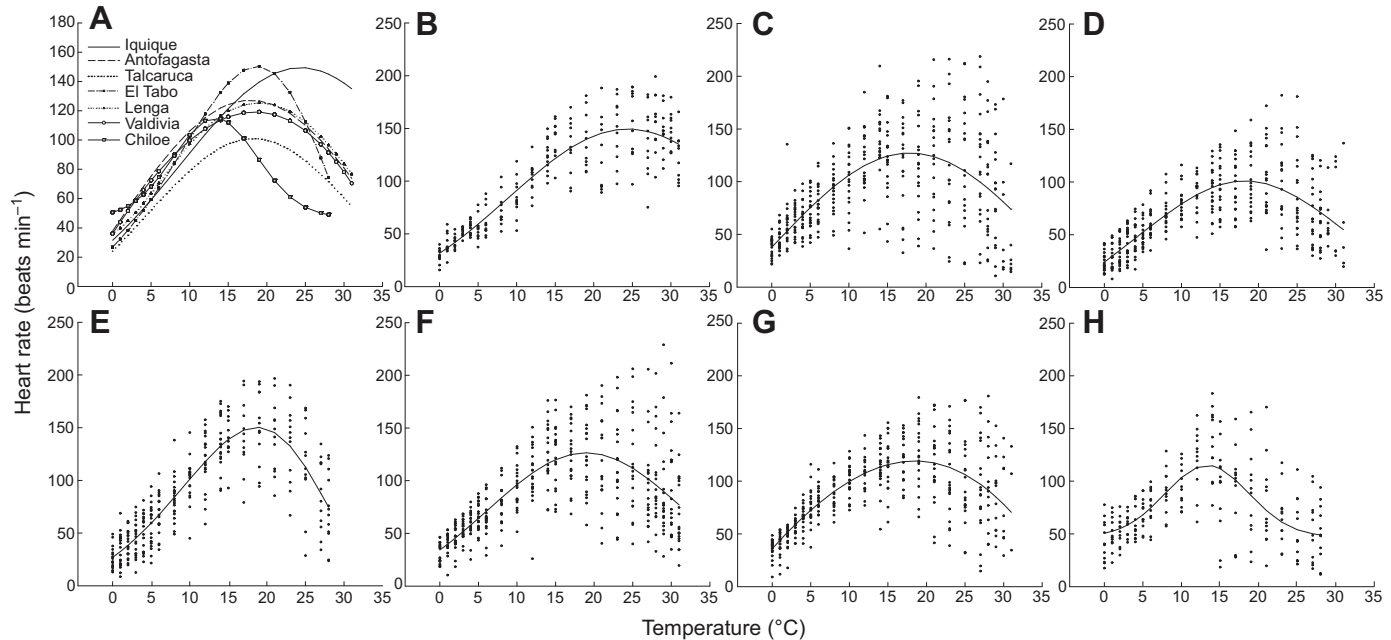

Fig. 2. Thermal sensitivity of the intertidal crab *Petrolisthes violaceus*. (A) Latitudinal comparison of thermal performance curves (TPCs). Single TPCs for populations from (B) Iquique (20°S), (C) Antofagasta (23°S), (D) Talcaruca (30°S), (E) El Tabo (33°S), (F) Lenga (36°S), (G) Valdivia (39°S) and (H) Chiloé (40°S). Black dots indicate more than one data point.

Table 2. Parameters of thermal performance curves in populations of the porcellanid crab *Petrolisthes violaceus*

Population	Latitude	CT_{min} (°C)	CT_{max} (°C)	T_{opt} (°C)	μ_{max}	T_{br}	WT	TSM
Iquique	20°19′S, 70°15′W	−0.74±0.33	32.31±0.74	24.43±0.87	149.93	28.74	15.49	7.61
Antofagasta	23°46′S, 70°40′W	−0.75±0.24	33.46±0.75	19.08±0.46	127.51	27.81	17.06	2.69
Talcaruca	30°29′S, 71°41′W	−0.68±0.18	32.90±0.68	18.34±0.27	100.85	26.89	17.39	2.83
El Tabo	33°27′S, 71°66′W	−1.13±0.27	31.99±0.74	18.43±0.27	148.18	21.22	17.15	3.59
Lenga	36°45′S, 73°10′W	−0.84±0.21	34.23±0.82	18.98±0.23	133.24	27.51	19.54	4.29
Valdivia	39°46′S, 73°23′W	−0.52±0.13	34.31±0.52	16.98±0.36	118.31	26.59	21.72	4.39
Chiloé	40°52′S, 73°59′W	−0.68±0.17	34.38±0.52	13.32±0.40	118.52	19.65	22.12	1.06

Thermal optimum (T_{opt}), thermal breadth (T_{br}), maximal performance (μ_{max}), critical temperatures (CT_{min} and CT_{max}), warming tolerance (WT) and thermal safety margin (TSM). Data are means ± s.e.m.

absolute minimum seawater temperature at high latitudes. This clinal change of SST along the southeastern Pacific is related to a conspicuous gradient in solar irradiation (Broitman et al., 2001), the influence of atmospheric circulation and rainfall variability (Barros and Silvestri, 2002) and the effect of other oceanographic processes (Thiel et al., 2007). Our results revealed that SST was much more variable at higher latitudes (30–42°S) than at lower latitudes (20–24°S), which has also been shown in previous studies along the Chilean coast (Broitman et al., 2001; Lagos et al., 2005).

In the southeastern Pacific coast of Chile, there are two biogeographic breaks located in areas with sharp discontinuities in upwelling regimes (30–32°S) and with strong influence of the Antarctic circumpolar current (40–42°S) (Camus, 2001; Thiel et al., 2007). These geographic breaks are in accordance with the thermal breaks that we found at 30°S and 40°S for all of the variables related to SST (mean, maximun, minimum and coefficient of variation). Some authors have shown that these environmental discontinuities influence the temporal and spatial dynamics of benthic and pelagic communities (Rivadeneira et al., 2002; Thiel et al., 2007), the species composition (Meneses and Santelices, 2000), the larval dispersal and gene flow of many marine species (Brante et al., 2012; Haye et al., 2014; Sánchez et al., 2011; Thiel et al., 2007) and the phenotypic (co)variation of fitness-related traits (Barria et al., 2014).

However, in the case of the intertidal crab *P. violaceus*, the evidence indicates that these biogeographic breaks do not affect the gene flow or genetic structure of its populations, probably as a result of its long dispersal capacity (larvae spend 15 days or more in the water column) (Haye et al., 2014).

Thermal physiological variation in natural populations

According to theoretical models of thermal evolution, the absence of genetic structure and high gene flow between populations may constrain local adaptation to a thermal gradient (Angilletta, 2009; Gardiner et al., 2010). This, in turn, would be reflected in a lack of differences in all of the parameters of the TPCs among populations (Gardiner et al., 2010). Our results offer partial support to this idea. Overall, *P. violaceus* populations along the latitudinal gradient exhibited differences in their thermal sensitivities that are explained by three parameters of the TPCs (optimal temperature, T_{opt}; thermal breadth, T_{br}; and maximal performance, μ_{max}). Nonetheless, our results indicate that the upper and lower limits of temperature at which the heart rate decreased (CT_{max} and CT_{min}) are similar among populations, without an effect of the climatic variables or the geographic gradient. These findings are possibly explained by the limited capacities of ectotherms to adapt the CT_{min} and CT_{max} in the face of variation in T_a (Faulkner et al., 2014; Hoffmann et al., 2013;
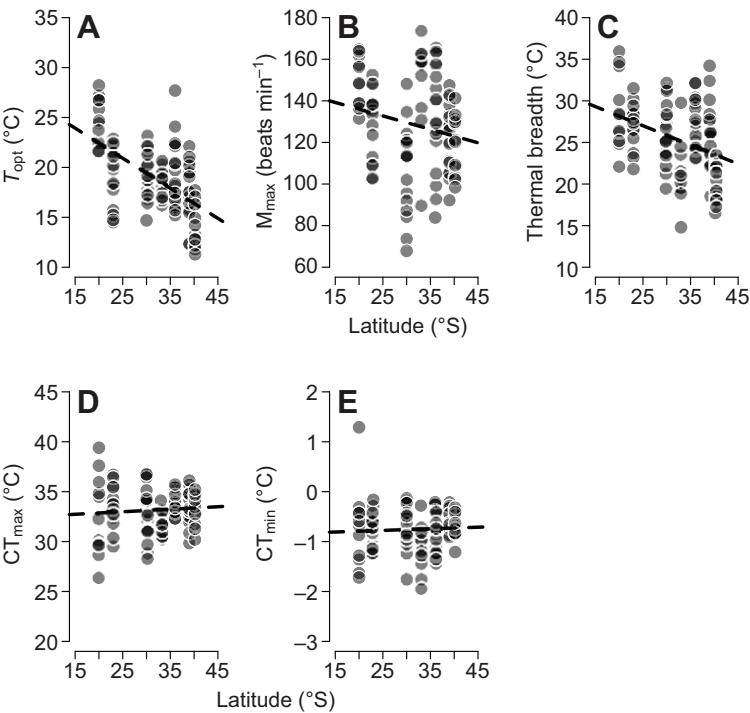

Fig. 3. Latitudinal variation in *Petrolisthes violaceus*. The temperature optimum (A), maximum performance (B), thermal breadth (C), critical minimum temperature (D) and critical maximum temperature (E). The effect of latitude (fixed effect, dashed lines) was evaluated through confidence intervals computed from the likelihood profile (Bates et al., 2013). Black dots indicate more than one data point.
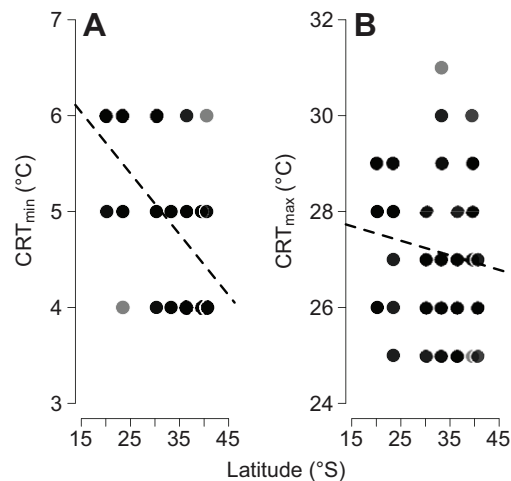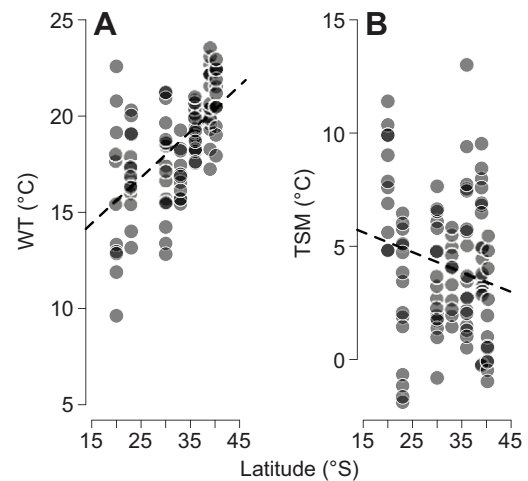
Fig. 4. Latitudinal variation in thermal coma in *Petrolisthes violaceus.* (A) Chill coma. (B) Heat coma. The effect of latitude (fixed effect, dashed lines) was evaluated through confidence intervals computed from the likelihood profile (Bates et al., 2013). Black dots indicate more than one data point.

Fig. 5. Geographic variation in warming tolerance and thermal safety margins of *Petrolisthes violaceus.* (A) Warming tolerance (WT) and (B) thermal safety margins (TSMs) along a latitudinal gradient of ca. 3000 km along the Chilean coast. Black dots indicate more than one data point.

Stillman, 2003). Moreover, it has been documented that the upper and lower critical temperatures are controlled by biophysical and thermodynamic constraints (Hoffmann et al., 2013; Somero, 2005) that influence the shape and limits of the TPCs (Angilletta, 2009). Our critical temperatures for heart rate were, on average, beyond maximum and minimun SST along the Chilean coast, and are close to the limits of the ranges described for other species of porcelanid crabs of the genus *Petrolisthes* (CT_{max}, 28.4–41.7°C and CT_{min}, −1.3–11.3°C) (Stillman, 2003). In these species, inter-population differences in the thermal cardiac performance are evidenced between seasons and across latitudinal gradients in accordance with their habitat temperature differences (Stillman and Tagmount, 2009) and their local thermal microhabitat conditions (Stillman, 2003; Stillman, 2004). For example, porcelanid crabs from warmer habitats exhibit higher CT_{max} compared with species from cooler habitats, whereas the opposite pattern is described for CT_{min} (Stillman, 2003).

From the three TPC parameters that differed among populations of *P. violaceus*, only T_{opt} showed signals of geographic influence (negative correlation with latitude). This pattern of thermal performance follows some of the expectations of the co-gradient variation model in which populations from warmer environments (low latitudes) tend to exhibit higher μ_{max} and T_{opt} than populations from cooler environments (high latitudes) (Conover et al., 2009; Gardiner et al., 2010). The analyses of μ_{max} and T_{br} between the most extreme populations of *P. violaceus* in Chile are consistent with this model of thermal evolution. However, the effect of the geographic and climatic gradient on these TPC parameters is eroded by the values of μ_{max} and T_{br} in populations that inhabit close to the southern limit of the thermal and biogeographic break located between 30 and 32°S. For some porcelanid species in the northern hemisphere, the evidence suggests that in addition to latitude, the habitat temperature range or fluctuation is a more important environmental factor in setting thermal performance than mean habitat temperature (Stillman and Tagmount, 2009). This pattern was not consistent with our findings probably because of the smaller differences in temperature ranges along the latitudinal cline in the Chilean coast compared with the northern hemisphere.

Thermal tolerance and thermal safety margins

Thermal tolerance is closely related to the biogeographic distribution of marine especies along latitudinal and vertical clines in coastal ecosystems (Pörtner, 2002; Somero, 2005). It is known that heat-tolerance limits of ectothermic species vary little with latitude whereas cold-tolerance limits decline steadily with increasing latitude (Sunday et al., 2014). In our study, populations of *P. violaceus* along the Chilean coast differed in their critical thermal resistances to high (CTR_{max}) and low (CTR_{min}) temperatures. However, from these indexes of thermal tolerance, only the CTR_{min} showed a geographic influence that suggests adaptation to local environmental conditions (Somero, 2005) in which the lower CTR_{min} exhibited by crabs form high latitude populations is perhaps the result of natural selection favoring a more cold-adapted populations, which has been observed in other ectotherms (Keller and Seehausen, 2012; Pörtner et al., 2000; Stitt et al., 2014).

In addition to the cold-tolerance findings, the average amount of environmental warming that populations of *P. violaceus* can tolerate before performance drops to fatal levels (i.e. warming tolerance) (Deutsch et al., 2008), points out the existence of a positive correlation of this thermal tolerance index with latitude. This pattern has been described for other ectotherms, suggesting that at low latitudes rising T_a, which is associated with global warming, is likely to have the most deleterious consequences because ectothermic species in these geographic areas are relatively sensitive to temperature change and are currently living very close to their T_{opt} (Deutsch et al., 2008; Stillman, 2002; Sunday et al., 2014). Interestingly, our results on thermal safety margins (the differential between an organism's T_{opt} and its current T_a) (Deutsch et al., 2008) of *P. violaceus* crabs, which are primary marine ectotherms, indicate that populations at high latitudes are closer to their physiological optima than those populations from low latitudes. These results suggest that low-latitude populations of *P. violaceus* might be able to physiologically tolerate the increase in temperature projected by the IPCC for the end of the twenty-first century (~4°C in the RCP8.5 scenario) (IPCC, 2013). Nonetheless, in the case of the southeastern Pacific, a departure of the the general ocean warming trend described by the IPCC has been described (Aravena et al., 2014; Falvey and Garreaud, 2009). Indeed, recent studies have

found a cooling of SST (~−0.2°C per decade) along the Chilean coast related to a Niña-like intensification of the South Pacific anticyclone (Aravena et al., 2014; Falvey and Garreaud, 2009). This SST cooling might play an important role in the geographic variation of the thermal resistances to low temperatures (CTR$_{min}$) that we found in our study.

In summary, because the geographical range of *P. violaceus* covers a broad latitudinal gradient of climate conditions, we expected to find signals of physiological differentiation of natural populations as well as adaptation to local T_a (Ghalambor et al., 2006; Janzen, 1967; Kuo and Sanford, 2009; Levinton, 1983). Indeed, we observed differences in thermal sensitivities that are explained by partial combination of two models of thermal evolution characterized by the lack of differences in the limits of the TPCs and the negative correlation of T_{opt} with latitude. Additionally, high-latitude populations of *P. violaceus* exhibited broader thermal tolerances, which is consistent with the climatic variability hypothesis (Ghalambor et al., 2006; Janzen, 1967). Finally, under a future rising T_a scenario, our findings on TSM indicate that lower-latitude populations can physiologically tolerate future warming, at least that projected by the IPCC for the year 2100.

MATERIALS AND METHODS

Study populations and sites

Petrolisthes violaceus is a free-living porcellanid crab commonly found in the rocky intertidal low zone of the south-eastern Pacific (Vargas et al., 2010; Viviani, 1969) and distributed in a latitudinal gradient along the Chilean coast, characterized by large and meso-scale variations in the intensity of coastal upwelling (Thiel et al., 2007; Torres et al., 2011), as well as spatial variation in SST and air temperature (Fig. 1) (Lagos et al., 2005). Animals were collected randomly by hand on low tides during 2011 and 2012 from the rocky intertidal area of Iquique (20°19′ S, 70°15′ W), Antofagasta (23°46′ S, 70°40′ W), Talcaruca (30°29′ S, 71°41′ W), El Tabo (33°27′ S, 71°66′ W), Lenga (36°45′ S, 73°10′ W), Valdivia (39°46′ S, 73°23′ W) and Chiloé (40°52′ S, 73°59′ W). These seven locations along the southeastern coast of Chile (ca. 3000 km), encompass much of the latitudinal range of the species (Fig. 1). To remove possible confounding effects of sex, only male crabs were used in the experiments and physiological measurements. Individuals were chilled and transported to the laboratory at the Universidad Adolfo Ibañez, where they were maintained in common conditions, at constant temperature (14°C) in artificial seawater (ASW; 33 ppm; Instant Ocean© sea salt dissolved in distilled water) for 1 month. Crabs were exposed to a 12 h:12 h light:dark cycle and fed with Instant Algae® and aquarium shrimp food three times a week.

A high-resolution monitoring program of intertidal temperature was developed along the latitudinal gradient in the Chilean coast. Trends in intertidal temperature (SST; annual and daily mean and variance) and other environmental variables such as heating/cooling days (i.e. days where exposure was over/under the CT$_{max}$ and CT$_{min}$, respectively) were monitored between 2011 and 2013. Temperature loggers (TibdiT®, Onset Computer Corp., MA, USA) were deployed in the low intertidal level (~0.2–0.3 m above mean lower low water, MLLW) of each location. The intertidal logger recorded both seawater and air temperature (during extreme low tides), which are significantly correlated (Menge et al., 2008), thus recording overall fluctuations in temperature experienced by the species at each local intertidal habitat. All experiments were conducted according to current Chilean law.

Thermal performance curves

Thermal effects on physiological performance were estimated for populations of *P. violaceus* along the latitudinal gradient in the Chilean coast. Here we used heart rate (HR; cardiac activity) and roll-over speed (RS; the speed at which animals change from the inverse to the upright position) as proxies of the relationship between organismal performance and T_a (Bruning et al., 2013; Gaitán-Espitia et al., 2013; Schulte et al., 2011). A

total of 210 adult individuals (30 crabs for each population) were selected for analyses of thermal sensitivities (TPCs). TPCs for HR were described in terms of four parameters: (1) the optimal temperature (T_{opt}); (2) the thermal breadth (T_{br}); (3) the maximal performance (μ_{max}); and (4) the upper and lower limits of temperature at which the HR decrease (CT$_{min}$ and CT$_{max}$) (Angilletta, 2009). At each temperature, animals were exposed separately in plastic chambers with six subdivisions (200×200×100 mm), installed in a thermo-regulated bath at constant sea water temperature (±0.5°C, LWB-122D, LAB TECH) for 30 min. We randomized the order of temperature trials for each individual and ensured at least 24 h of rest between trials. Experimental temperatures for TPCs were chosen between 0 and 31°C. In extreme temperatures of the thermal treatment (i.e. 0–6°C and 26–31°C), HR was measured every 1°C, whereas at intermediate temperatures (i.e. 6–26°C), it was measured every 2°C. HR was estimated using a heartbeat amplifier AMP 03 (Newshift Lda®) connected to an oscilloscope and the results were expressed in beats min^{-1}. Measurements of cardiac activity were performed at the same period of the day to cancel the effects of a possible circadian and tidal rhythm of respiration.

The mean HR for each crab at each experimental temperature was calculated with the aim of estimating the TPCs for each population. We used the TableCurve2D curve-fitting software (version 5.01; Systat Software, Inc.) for model fitting. TPC parameters (μ_{max}, T_{opt}, CT$_{min}$ and CT$_{max}$) were extracted from the best models (see below for details). The ecophysiological characteristics of critical thermal maximum (CT$_{max}$) and minimum (CT$_{min}$) were derived numerically as the intersection points of the resulting thermal performance curve with the temperature axis (μ=0). Temperature breadth (T_{br}) for each population was calculated with the mean values of HR for each experimental temperature using the following equation (Gilchrist, 1996):

$$T_{br} = \sqrt{\sum_{i=1}^{N}\left[\frac{\mu_i(T_i - T_{opt})}{\mu_{max}}\right]^2},\qquad(1)$$

where N is the number of temperatures and μ_i is the mean HR at temperature T_i.

Thermal coma and thermal tolerances

Critical thermal resistance to high and low temperatures (CTR$_{min}$ and CTR$_{max}$) were estimated in a total of 185 adult individuals of *P. violaceus* using the thermal coma methodology (see Gaitán-Espitia et al., 2013). Overall, thermal comas were defined as the lack of ability to achieve an upright position, or lack of movement of structures and/or appendices within a pre-defined time period (e.g. Bacigalupe et al., 2007; Castañeda et al., 2005; Gaitán-Espitia et al., 2013; Lutterschmidt and Hutchison, 1997). This measure is sub-lethal and is useful for a broad range of ectothermic invertebrates and large sizes samples (Huey et al., 1992). Animals were exposed separately in plastic chambers with six subdivisions (200×200×100 mm), installed in a thermo-regulated bath at constant sea water temperature (±0.5°C, LWB-122D, LAB TECH), for 30 min. In the last 15 min, crabs were turned over and if an individual was not in a thermal coma, it responded by returning to an upright position. At extreme temperatures, some individuals had zero performance. Those animals that exhibited a complete loss of righting response under extreme temperatures were considered to be in a thermal coma (e.g. Castañeda et al., 2005; Gaitán-Espitia et al., 2013; Lutterschmidt and Hutchison, 1997). Tolerance ranges were determined experimentally *a priori*.

Warming tolerance and thermal safety margin

At the collection sites of *P. violaceus*, mean habitat temperature (T_{hab}) and variance (σ_t) were extracted from a high-resolution temperature data-logger as the mean and s.d. of monthly SST. Theoretically, seasonality (variance) is a strong predictor of warming tolerance (WT=CT$_{max}$−T_{hab}) and also of thermal safety margins (TSM=T_{opt}−T_{hab}) (for details, see Deutsch et al., 2008). These correlations simply reference the position of performance curves to local climate conditions.

Statistical analysis

Prior to analysis, we tested for normality and homoscedasticity for all variables using the Lilliefors and Levene tests, respectively. Data was

transformed either by $\log_{10}$ or by square root to fulfill the requirements for parametric tests. The fit of several functions (e.g. Gaussian, Lorentzian, Weibull) that could describe organismal performance as a function of temperature was analyzed using the Akaike Information Criterion (Angilletta, 2006). The AIC represents a balance between the likelihood explained by the model and the number of model parameters, with the best model minimizing AIC (Kingsolver and Massie, 2007). Thermal physiological traits obtained from the TPCs (i.e. μ_{max}, T_{opt}, T_{br}, CT_{min} and CT_{max}) and WT and TSM indexes were analyzed using a mixed-modelling approach, with locality as a random grouping factor. The effect of latitude (fixed effect) was evaluated through confidence intervals computed from the likelihood profile (Bates et al., 2013). Critical thermal resistance (i.e. thermal coma) was analyzed using the Kruskal–Wallis test. In all cases, when differences in the means were significant at the $P<0.05$ level, they were also tested with a posteriori Tukey's test (HSD). Statistical analyses were performed with R 3.0.2 software and the package lme4 (Bates et al., 2013).

Competing interests
The authors declare no competing financial interests.

Author contributions
M.A.L. and N.A.L. conceived the study. T.O. and T.T. developed the experiments and collected the data. J.D.G.E., L.B., and M.A.L. analysed and interpreted the data and wrote the manuscript.

Supplementary material
Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.108217/-/DC1

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Table S1. Environmental variables along the latitudinal gradient in Chile.

Locality	Heating days	Cooling days	Daily *in situ* SST (°C)					
			Minimun	Maximun	Mean	Desvest	Variance	Coefficient of variance
Iquique	0	0	15.70036702	17.90562234	16.76035971	0.597836509	0.454406181	3.555574245
Antofagasta	13	0	15.25421884	18.22968698	16.4350029	0.827145183	1.106006466	4.951327771
Talcaruca	5	0	12.32934383	15.03471913	13.40820546	0.712412122	0.841047431	5.17242414
LasCruces	0	0	12.90477927	14.15957873	13.50792121	0.385366907	0.23877249	2.725839739
Lenga	0	0	12.99883051	15.14961017	14.07293863	0.629295514	0.498657444	4.614779097
Valdivia	0	0	11.79230682	13.56028788	12.57676094	0.498563506	0.655121349	3.897123956
Chiloe	0	12	11.64237805	12.61520427	12.15611015	0.285740077	0.141969621	2.382552046