

ORIGINAL ARTICLE

Latitudinal variation in the aerial/aquatic ratio of oxygen consumption of a supratidal high rocky-shore crab

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Abstract

The colonisation of the terrestrial environment by crustaceans is more apparent in tropical latitudes because of the high diversity of semi-terrestrial and terrestrial crabs. However, in temperate regions there are also great numbers of crustaceans that inhabit ecological niches at the water–air interface. Grapsidae crabs (Decapoda) are especially important in studies of water-to-land transition as the family contains species occupying the intertidal and adjacent regions. A way to evaluate the ability of intertidal invertebrates to breathe air is to measure the aerial/aquatic oxygen consumption ratio. The objective of this study was to test the effect of thermal variation on the aquatic and aerial metabolism. We selected as study model the decapoda crab *Cyclograpsus cinereus* Dana and utilised five populations of the species spread over 2000 km along the Chilean coast. To determine the compensation capacity in respiration with respect to latitude, we evaluated metabolic rate at the same temperature in a common garden design in the laboratory, to examine the extent to which variation in crab physiology is environmentally determined. Whereas in our study, m_b (body mass) varied significantly with latitude, the difference in mass-independent metabolism both in air and water persisted, indicating that observed differences in MR (Metabolic Rate) were not an effect of differences in body size. We demonstrated that *C. cinereus* is able to breath oxygen from air and water as expected for an amphibious crab. Almost all the studied populations of *C. cinereus* show a aerial/aquatic metabolism ratio near 1. The pattern found indicates an increase in metabolic rate, both aerial and aquatic, in low latitudes and therefore does not support the latitudinal compensation hypothesis for temperate habitats. Finally, these kinds of studies are required to make the necessary link between ecological physiology and macroecology and to help develop a global understanding of organismal function in marine systems.

Introduction

A great number of animals, particularly invertebrates, inhabit the water–land interface and are able to thrive, at least temporarily, in both media. Intertidal organisms,

during periods of low tide, are exposed to terrestrial or semi-terrestrial conditions that may impose physiological stresses (Hofmann & Somero 1995; Stillman & Somero 1996; Somero 2002; McGaw 2003). The transition from aquatic to aerial life is a major event that probably took

place several times in animal evolution, but is often far from entirely understood (Truchot 1990). Among crustaceans the adoption of terrestrial habits seems to be a comparatively recent evolutionary event (Greenaway 1999; Morris 2002).

Bimodal breathing crustaceans represent a transitional stage in the invasion of the terrestrial habitat by aquatic forms, they also possess and/or lack specific morphological, behavioural, physiological, and biochemical mechanisms that set them apart from their fully aquatic and terrestrial counterparts (McMahon & Burggren 1988; Truchot 1990; Henry 1994; Stillman 2000). Furthermore, in various phyla of marine invertebrates, the limited capacities of both ventilatory and circulatory performance set the borders of the thermal tolerance window (Pörtner *et al.* 2004).

Oxygen uptake is considerably more expensive in water because the medium's density, viscosity and low capacitance for O₂, resulting in high ventilation requirements (O'Mahoney & Full 1984). Nevertheless, oxygen uptake by amphibious species is often similar in both media (DeFur *et al.* 1988). Bimodal breathing has been well documented in a wide variety of crustacean species (see Henry 1994; Greenaway 2003). Grapsidae crabs (Decapoda) are especially important in studies of water-to-land transition as the family contains species occupying the intertidal and adjacent regions (Innes *et al.* 1986; Morris 2002; Katsanevakis *et al.* 2007). A way to evaluate the ability of intertidal invertebrates to breathe air is to measure the aerial/aquatic oxygen consumption ratio. Previous studies found that low shore species have low aerial/aquatic ratios compared with high shore species (Truchot 1990). Low shore species have low aerial/aquatic ratios and correspondingly accumulate more anaerobic end products when exposed to air. Conversely, many high-shore animals do not rely on anaerobic metabolism and are able to breathe in air as well as in water during exposure periods. Also, temperature has a variety of effects on respiration gas exchange and acid-base regulation in crustaceans, as described by Truchot (1987), Whiteley *et al.* (1997), McGaw (2003). Temperature gradients are commonplace in nature and are broadly recognised to exert a major effect on patterns of variation within species, including physiological traits (Ikeda 1985; Whiteley *et al.* 1997). Previous studies of bimodal breathing have been conducted with populations of different species inhabiting dissimilar habitats (for examples see Barlow 1961; Eshky *et al.* 1995; Whiteley *et al.* 1997; Gillikin *et al.* 2004); nevertheless, bimodal breathing variation between several populations along a geographical gradient has never been studied in crustaceans.

We studied different populations of *Cyclograpsus cinereus* Dana (Decapoda: Grapsidae) over a latitudinal gradi-

ent of 18° along the Chilean coast. *Cyclograpsus cinereus* inhabit the supratidal high rocky-shore, at this level, crabs are only immersed briefly by the tide and spend the majority of the tidal cycle in air; during neap tides and low wave action, many individuals may rest on dry sand under boulders (Bahamonde & López 1969). *Cyclograpsus cinereus* has a geographical distribution, from Ancón, Perú to Calbuco, Chile (Bahamonde & López 1969). According to Thiel *et al.* (2007) the sea surface temperature (SST) decreases gradually from northern towards southern Chile and the life-history traits of populations of marine crustaceans, including *C. cinereus*, show consistent variations along the Chilean coast (Lardies *et al.* 2008, 2010; Lardies & Castilla 2001). We hypothesize that intertidal crabs inhabiting low latitudes (*i.e.* high air temperatures) have other immediate physiological and reproductive costs when they are exposed to air compared with their temperate counterparts, and consequently we predict that the aerial/aquatic ratios of oxygen consumption for crabs from high latitudes will be lower when compared with crabs from low latitudes. Consequently, the objective of this work was to test the effect of latitudinal variation in environmental temperature on the aquatic and aerial rates of metabolism using *C. cinereus* as a model species.

Material and Methods

Animals and study site

Specimens were collected in the intertidal zone from the following locations on the coast of Chile: Antofagasta (n = 14), La Serena (n = 13), El Tabo (n = 14), Valdivia (n = 14), and Puerto Montt (n = 13) (Table 1, Fig. 1). The latitudinal range sampled spanned approximately 2000 km. Individuals were collected randomly by hand on low tides during the spring of 2005 and 2006 using two transects (at different tidal heights) as sampling methodology during two different times in 2 days. Crabs were placed in refrigerated containers at each study site

Table 1. Latitudinal gradient information for the five study sites along the Chilean coast.

locality	latitude	SST (°C)	AT (°C)
Antofagasta	23°31' S	17.5 (15.6, 20.1)	17.0 (13.3, 22.9)
Coquimbo	29°56' S	15.6 (13.5, 17.6)	14.8 (11.2, 18.9)
El Tabo	33°24' S	13.6 (12.0, 15.0)	14.0 (9.8, 17.7)
Valdivia	39°46' S	12.8 (10.5, 14.9)	11.0 (6.1, 16.9)
Puerto Montt	41°29' S	12.6 (10.4, 13.9)	10.1 (6.4, 14.9)

Sea surface temperature (SST) and air temperature (AT) during the period 1980–2005. Temperature is presented as the annual average, with annual average minimum and maximum temperatures in parentheses.

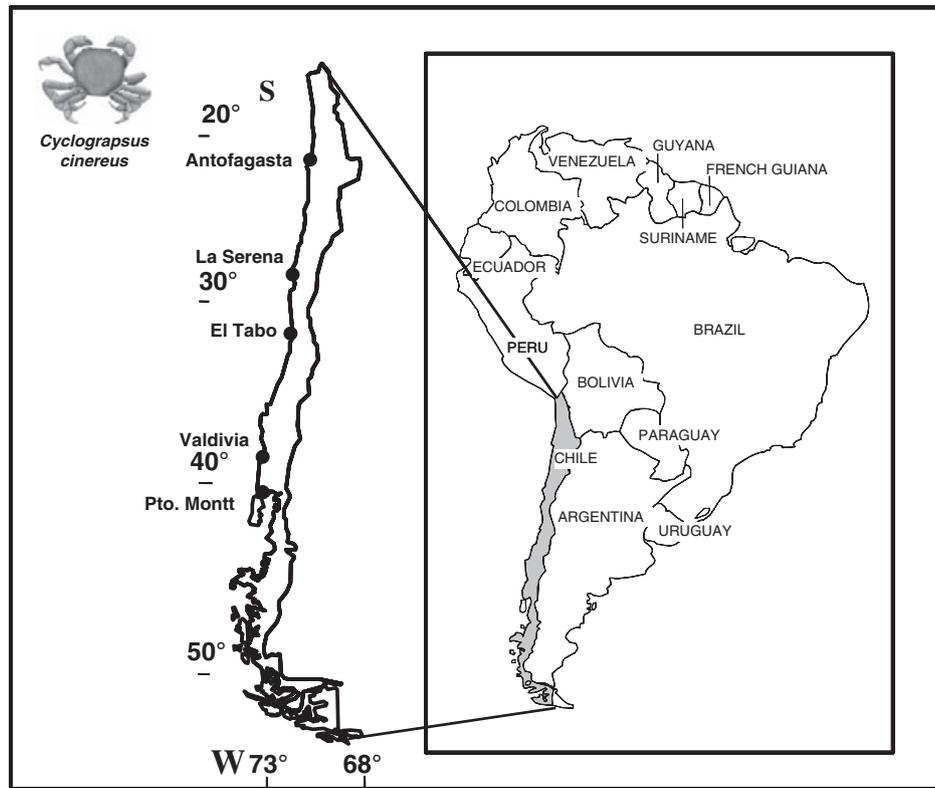


Fig. 1. Map of South America and the coast of Chile showing the locations of the sampling sites for *Cyclograpsus cinereus*.

and immediately transported to the terrestrial laboratory in Santiago ($33^{\circ} 27' S$), where they were sexed and, after an acclimation period, the aerial metabolic rate (MR) records were taken. Subsequently, crabs were transported with food in oxygenated seawater to the marine laboratory in Puerto Montt ($41^{\circ} 28' S$) for aquatic MR records. To remove possible confounding effects of sex in the variables measured, we used only males (see McMahon 2001; Taylor & Lelepiyanart 2001).

Aerial and aquatic metabolism

To determine the capacity of compensation in MR with latitude we evaluated it at the same temperature (T_a), in a common garden design. All animals were acclimated for 2 weeks at $T_a = 20 \pm 1^{\circ} C$ in filtered seawater with food *ad libitum* (i.e. edible mussel *Mytilus edulis chilensis*). During the acclimation period, the individuals were allowed to move freely into aquariums and through a platform to emerge from or submerge in seawater. Prior to metabolism measures, the specimens were starved for 5 days. Before and after each measurement in air and water, body mass (m_b) was recorded (0.01 mg), and an average of both m_b measurements was used in the statistical analyses.

For aerial metabolism we use carbon dioxide production (VCO_2) which was measured in a computerized open-flow respirometry system (Sable Systems, Henderson, NV, USA), using a metabolic chamber of 10 cm^3 , at $T_a = 20.0 \pm 0.5^{\circ} C$. The metabolic chamber received air at a rate of $200 \text{ ml} \cdot \text{min}^{-1}$ from mass flow controllers (Sable Systems). Air passed through CO_2 absorbent granules (Baralyme®) before entering the metabolic chamber. CO_2 production was monitored four times per second during a 40-min period. Baseline measurements for each run were made using the same experimental chamber both at the beginning and at the end of each measurement period using certificated gas standards. Each record was automatically transformed by a macro program and recorded in DATACAN software (Sable Systems). We estimated metabolic rate as the average of the 5–8 min steady-state VCO_2 production during periods of inactivity. Animal activity inside the metabolic chamber was determined directly by visual observation.

The same individuals of the different populations were used to measure oxygen consumption (VO_2) in water at the ecophysiology laboratory at the Universidad Austral, Puerto Montt, Chile. Oxygen consumption in water was measured by closed respirometry. Crabs were incubated individually in a respirometric chamber with a known

volume (227 ml) of filtered seawater (0.45 μm) and sterilised for 2 h with UV light. Chambers with crabs were placed in a thermoregulated bath at 20 ± 1 °C, controlled by means of a Thermo Neslab RE7 heater-cooler. Respirometric chambers without crabs were used as controls, running in parallel during each experimental determination. Oxygen concentration in the experimental chambers and controls was quantified by means of oxygen meter Microx TX3 from Optical Technology (PreSens, GmbH, Germany) and a flow optode calibrated with a solution of $\text{Na}_2\text{O}_3\text{S}$ at 5% and aerated water for the values 0% and 100% air saturation, respectively.

To estimate aerial/aquatic ratios in different populations, VCO_2 production (air) and VO_2 consumption (water) metabolism values were transformed into energy units. To make the relative fraction of fats, proteins and carbohydrates uniform, we fed crabs during each acclimation and transport period with the same diet, the edible mussel *Mytilus edulis chilensis*. Carbon dioxide production was converted into energetic units, using $23.32 \text{ J}\cdot\text{ml}^{-1} \text{ CO}_2$ (Hill *et al.* 2004). In water, we followed Gnaiger (1983) for energy conversion, where 1 mg O_2 is equivalent to 14.06 Joules.

Statistical analysis

Differences in m_b and in the ratio air:water respiration between populations were evaluated using one-way ANOVAs. Changes in all remaining dependent variables (MR in air and water) were evaluated separately by one-way ANCOVAs, using m_b as the covariate. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance using Kolmogorov–Smirnov and Levene tests, respectively. Interactions between covariates and factors (parallelism test) were also tested. Differences between groups (*post hoc* comparisons) were evaluated by Tukey HSD tests with Bonferroni correction. Values given throughout the text are means \pm SE. Statistical significance was established at the 0.05 level. All of the analyses were performed using the General Linear Models module of the statistical package STATISTICA® version 6.0 (StatSoft, Tulsa, OK, USA) for the Windows® operating system.

Results

Body mass

Body mass of males of *C. cinereus* (Fig. 2) was significantly different among sites (one-way ANOVA $F_{4,132} = 17.272$; $P < 0.0001$). An *a posteriori* Tukey (HSD) test showed that mean male m_b was significantly smaller in Antofagasta (0.50 ± 0.053 g) and Puerto Montt ($0.44 \pm$

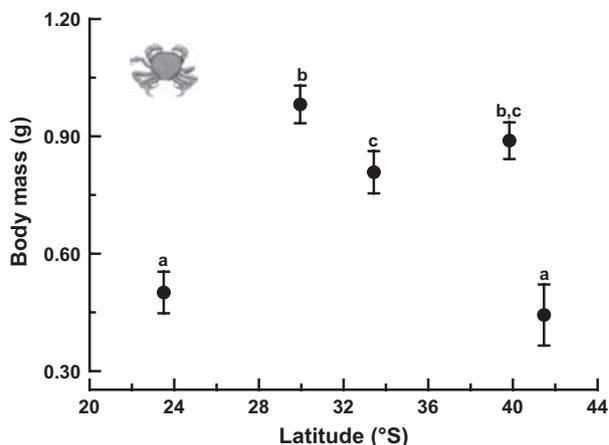


Fig. 2. Male body mass of *Cyclograpsus cinereus* in different populations along the coast of Chile. The mean \pm SE is indicated. Significant differences are highlighted by different letters.

0.078 g) populations than at the other three sites studied ($P < 0.0001$). In Puerto Montt and Antofagasta populations, the m_b was not significantly different (Tukey test; $P = 0.0024$). The largest males were found in Coquimbo and Valdivia, exhibiting an average mass of 0.982 ± 0.048 g and 0.889 ± 0.047 g, respectively. The smallest crabs were found at the northern and southern extremes of the sampled latitudinal range, with large crabs found at intermediate latitudes.

Air metabolism

Aerial MR decreased gradually towards high latitudes (Fig. 3). There were significant differences in VCO_2 among the studied populations (one-way ANCOVA

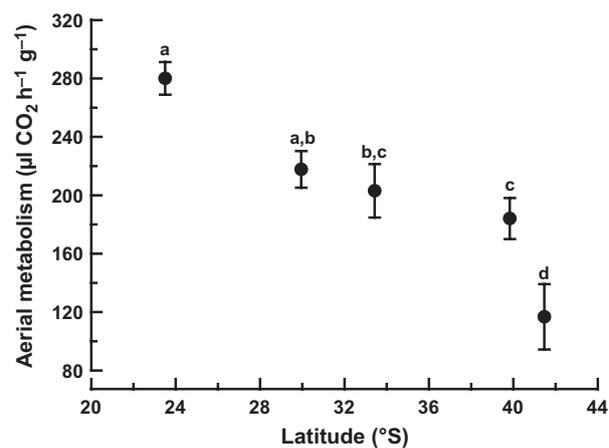


Fig. 3. Mass-specific rate of CO_2 production (VCO_2 in $\mu\text{l CO}_2\cdot\text{h}^{-1}\cdot\text{g}^{-1}$) in air by *Cyclograpsus cinereus* from different populations along the coast of Chile. Mean \pm SE is indicated. Significant differences are highlighted by different letters.

$F_{4,64} = 9.8713$; $P < 0.0001$). Crabs from Puerto Montt exhibited significantly lower values of VCO_2 compared to other localities (*a posteriori* Tukey HSD, $P = 0.0019$). The highest MR values were found in Northern-Central Chile, at Antofagasta, Coquimbo and El Tabo, where the average MRs were 280.06 ± 11.15 , 217.72 ± 12.53 , $203.044 \pm 18.33 \mu\text{l CO}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$, respectively. Crabs from central-southern populations (*i.e.* Coquimbo, El Tabo and Valdivia) showed similar intermediate values (*a posteriori* Tukey HSD, $P = 0.0874$).

Aquatic metabolism

Water MR values varied significantly among localities (one-way ANCOVA $F_{4,64} = 4.013$; $P < 0.005$). Minimum values were found in populations at the extremes of the latitudinal range studied, with Antofagasta and Puerto Montt exhibiting comparable values of 285.13 ± 22.98 and $315.45 \pm 40.27 \mu\text{g O}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$, respectively (Fig. 4). Central-southern populations (*i.e.* Coquimbo, El Tabo and Valdivia) exhibited similar values with no significant differences in VO_2 (*a posteriori* Tukey HSD, $P = 0.179$, $P = 0.084$ and $P = 0.102$ for paired comparisons). Crabs from Antofagasta and Puerto Montt exhibited similar VO_2 values ($P = 0.0784$) and, except Valdivia, were significantly different from all the other studied populations (*a posteriori* Tukey HSD, $P < 0.005$) (see Fig. 4).

Respiration ratio

The individual aerial/aquatic MR ratio decreased gradually towards high latitudes in the populations studied (Fig. 4). The MR ratio of *Cyclograpsus cinereus* was

significantly greater (one-way ANCOVA $F_{4,64} = 4.009$; $P < 0.005$) in Antofagasta (2.02 ± 0.23) in comparison to populations from El Tabo (1.02 ± 0.21), Valdivia (0.99 ± 0.17) and Puerto Montt (0.88 ± 0.19) (Tukey HSD, $P < 0.005$). However, no significant differences in the MR ratios from Antofagasta and Coquimbo were found (Tukey HSD, $P = 0.085$).

Discussion

Cyclograpsus cinereus displays consistent geographic variations in body mass, air and water MR and MR ratios over a latitudinal gradient along the Chilean coast. Bahamonde & López (1969) remark that ‘individuals of *C. cinereus* live under stones in the rocky intertidal and tend to form aggregations in areas of high humidity which allow them to take advantage of atmospheric oxygen’. We demonstrated that, indeed, *C. cinereus* was able to breath oxygen from air and water as an amphibious crab. From the viewpoint of weight-specific gill surface areas, *C. cinereus* can be considered a bimodal crustacean following the classification of Henry (1994). *Cyclograpsus cinereus* is therefore an amphibious crab equipped with gills stabilised for air breathing (see Schmitz & Harrison 2004). Using the Greenaway (1999) classification for anomuran crustaceans, *C. cinereus* is a member of the T₂ group, representing crabs which are voluntarily active out of water for substantially long periods of time. Corrected metabolism (using body mass as covariable) shows strong intra-specific latitudinal variation, both in air and in water.

Body mass

Cyclograpsus cinereus exhibits a mid-latitude peak in m_b , decreasing towards high and low latitudes. Body mass values fit within the ranges reported for this species along the Chilean coast (Bahamonde & López 1969). Interestingly, body size in El Tabo was smaller in comparison with crabs sampled 50 years ago at the same site, from maximum sizes of 1.60 g (Bahamonde & López 1969) to maximum sizes of 1.00 g. Indeed, the average m_b of *C. cinereus* are lower than those of the congeneric species *Cyclograpsus lavauxi* from New Zealand, which exhibits sizes of between 2.0 and 7.5 g (Waldron *et al.* 1986).

This kind of latitudinal pattern (mid-latitude peak) has been observed in marine bivalves (Roy & Martien 2001) and cladocerans (Gillooly & Dodson 2000). Correlations with latitude were weaker, as individuals both at higher and lower latitudes tended to be smaller. A likely explanation is that both northern and southern sites correspond to the geographical and ecological boundaries of the species distribution where the environment conditions are limiting, resulting in smaller body sizes. The north

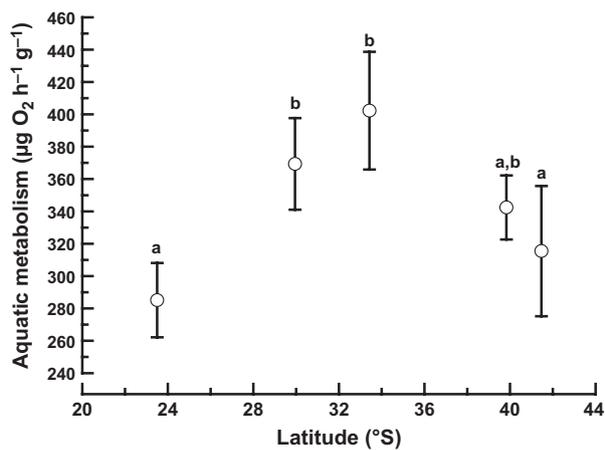


Fig. 4. Mass-specific rate of oxygen uptake (VO_2 in $\mu\text{g O}_2 \cdot \text{h}^{-1}$) in water by the crab *Cyclograpsus cinereus* at the five study sites along the Chilean coast. Mean \pm SE is indicated. Significant differences are highlighted by different letters.

boundary of the geographical distribution of *C. cinereus* was not studied, however, it is improbable that this would change the observed results. This pattern is of particular importance given that body size is correlated with many organismal and ecological traits (Peters 1983; Brody & Lawlor 1984; Blanckenhorn 2000; Roy *et al.* 2000; Lap-tikhovskiy 2006). In general, this pattern also agrees with that observed in some ectotherms, which tend to be larger in colder environments (Atkinson & Sibly 1997; Lardies & Castilla 2001; Robinson & Partridge 2001; Blanckenhorn & Demont 2004). Optimal resource-allocation models may explain the temperature–size rule. Latitudinal clines in body size with an established genetic basis have been reported in several ectotherms (Imasheva *et al.* 1994; Chown & Gaston 1999; Billerbeck *et al.* 2000), and many ecological variables could be selective agents, with temperature being the key selective pressure (Fischer & Fiedler 2002). Taken together, these correlations suggest a process of size-selection of body mass with thermal environment (see Roy *et al.* 2000). The difference in air and water temperature experienced by southern populations of *C. cinereus* (Valdivia and Puerto Montt, see Table 1) is small but individuals m_b are significantly different, which strengthens the idea that Puerto Montt is close to the ecological limit of distribution in this species.

Respiration in air–water

The marine water/terrestrial air breathing crabs have constant access to a reliable, fixed source of water at all times of the year. *Cyclograpsus cinereus* shows a relatively constant VO_2 consumption and CO_2 production in water and air, respectively. This result is consistent with the published observations of O'Mahoney & Full (1984) for *Cardisoma guanhumi* Latreille, 1825. A constant metabolic rate in air and water has also been reported for other crustaceans such as the shore crab *Carcinus maenas* (Linnaeus, 1758) and the freshwater crayfish *Austropotamobius pallipes* (Lereboullet, 1858) (Taylor & Butler 1973; Taylor & Wheatly 1980). Grapsids with aerial respiration and bimodal breathing utilise both the branchiostegal walls (simple folds and lobulated projections that increase the respiratory surface area) and gills for gas exchange during air exposure (Halperin *et al.* 2000).

Almost all studied populations of *C. cinereus* show an air/water ratio of metabolism near 1 (see Fig. 5). Thus, resting metabolic rates in air and water are very similar in *C. cinereus* and comparable to the New Zealand grapsid *Cyclograpsus lavauxi* Milne Edwards, 1853 (Innes *et al.* 1986). In *C. lavauxi*, when forced into activity, maximum rates of metabolism are much greater in water, showing that the uptake mechanisms are primarily used in water (see Innes *et al.* 1986). In general, amphibious crabs

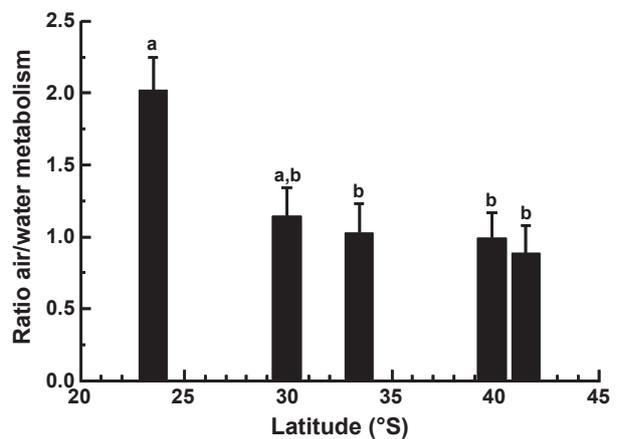


Fig. 5. *Cyclograpsus cinereus*. Ratio of air/water respiration at the five study sites along the coast of Chile. The mean and \pm SE is indicated. Significant differences are highlighted by different letters.

maintain a store of water in the branchial chamber that covers the gills and allows for simultaneous gas exchange between the two media (air and water). There is also a partitioning of gas exchange, with oxygen uptake occurring preferentially from air across the branchiostegites, and carbon dioxide excretion occurring across the gills into the branchial water (Henry 1994). Previous physiological studies in *Cyclograpsus lavauxi* (Innes *et al.* 1986; Waldron *et al.* 1986) demonstrated a number of features which seems to be adaptive (increased survival) to its high-shore habitat. It is not especially resistant to water loss, but is able to tolerate higher fractional losses of total body water than are crabs occupying lower shore positions. The respiratory system allows bimodal gas exchange and, in air, was able of delivering oxygen to the tissues at moderately high rates during activity, even when the gills and whole body were desiccated. During short periods of submaximal locomotion, a considerable acidosis develops and, in the absence of water, recovery is slow and incomplete (Waldron *et al.* 1986). Thus, *C. cinereus*, like *C. lavauxi*, could be capable of moderate activity during periods of exposure to air, within the limits of its aerobic scope and desiccation tolerance, which is supported by observation of individuals in the rocky intertidal during low tides (M.A. Lardies, personal observation).

Aerial respiration in *C. cinereus* from central Chile falls within the interspecific regression analyses parameters, aerial oxygen uptake *versus* live weight reported for nine air-breathing crabs from Trinidad, West Indies (Innes & Taylor 1986). Although in our study m_b varied significantly with latitude, the difference in mass-independent metabolism both in air and water persisted, indicating that observed differences in MR were not an effect of differences in body size. In this study,

differences in metabolic rate between crab populations could have been due simply to phenotypic flexibility. Given that in marine crabs, realised rates of acclimation to temperature are especially fast (Vernberg 1962; Somero 2002; McGaw 2003), it is likely that observed differences in aerial and aquatic metabolic rate are the end-product of local adaptation, and are probably a heritable characteristic. Crustaceans subjected to marked seasonal differences in temperature, as in temperate regions, have developed corresponding life cycles. Growth and reproduction are restricted to the warmer months when conditions are the most favourable (high temperature, long days and food availability) (Bauer 1992). Some crustaceans show marked physiological differences between winter and summer, with seasonal adaptation (Whiteley *et al.* 1997). In our study we are unable to avoid this problem; however, individuals of different populations were sampled during the same season (spring) and acclimated in the laboratory at the same temperature. There is a possible problem with the estimation of aerial rates of oxygen consumption because grapsid crabs are bimodal breathers and use gills for CO₂ production and the branchiostegal lining for O₂ uptake. Therefore, CO₂ production is dependent on branchial chamber water being in contact with gills. As a result, CO₂ production can vary over time with activity rate and probably temperature (see Halperin *et al.* 2000). To decrease this probable bias (*i.e.* CO₂ production and water quantity in branchial chamber) the body mass used in experimental individuals (within each population) were similar, and aerial metabolism measurements were realised during the same season.

Geographic variation in metabolism

Some studies have addressed latitudinal trends in routine oxygen consumption in marine invertebrates (*e.g.* Houlihan & Innes 1984; Ikeda 1985). Interestingly, the effect of latitude on differences in respiratory physiology of populations from the same species has received little attention, usually considering only two populations (Vernberg 1959; Vernberg & Vernberg 1964; Whiteley *et al.* 1997). In general, individuals from lower latitude populations have elevated rates of aerobic metabolism when compared with conspecifics at the same experimental temperature (Vernberg 1959; Osovitz & Hofmann 2007). Previous observation is confirmed by the trend found in *C. cinereus* for aerial metabolism (see Fig. 3). As far as we are aware, this research is the first to record the air/water metabolism ratio of *C. cinereus* for populations along a latitudinal gradient. The pattern found was an increase in the metabolic ratio towards low latitudes. Consequently, our working hypothesis, that intertidal crabs inhabiting low

latitudes have additional immediate physiological costs when they are exposed to air compared with their temperate counterparts, is supported. The latitudinal pattern found in *C. cinereus* for the metabolic ratio mimics the pattern found when low-shore species are compared with high-shore species (Truchot 1990). Because of the pervasive effect of temperature on physiological systems, the association between environmental temperature and trait variation patterns is to be expected, especially for ectothermic species (Pörtner *et al.* 2004; Clarke 2003; Sokolova & Pörtner 2003); that is to say, the latitudinal and vertical patterning of intraspecific trait variation commonly reflects gradients or discontinuities in environmental temperature. This is a logical addition to the comments of Somero (2002) on species distributions.

The considerable information available indicates that many marine ectothermic animals living in polar or colder waters have higher metabolic rates than we would expect based on their metabolic rate-temperature curves (Dêmeusy 1957; Clarke 1993; Sokolova & Pörtner 2003), or compared to close species or individuals from the same species living at different latitudes. This cold acclimation, or latitudinal compensation hypothesis, has attracted significant research into this singular pattern of metabolic rate and its mechanisms and evolutionary implications (Sommer *et al.* 1997; Dittman 2003). Many different physiological functions can be responsible for temperature-dependent growth rate differentiation, including duration of feeding activity, metabolic rate, digestive efficiency, allocation of resources to growth *versus* reproduction (Hochachka & Somero 1973; Prosser 1986; Lonsdale & Levinton 1989; Present & Conover 1992). Very few studies have tested the metabolic cold adaptation hypothesis, considering the oxygen consumption of marine invertebrate populations of a widely distributed species in a temperate zone (more than 2000 km) where noticeable environmental gradients exist. Nevertheless, oxygen consumption measurements for invertebrates, including crustaceans (Luxmoore 1984; Whiteley *et al.* 1997; Spicer & Gaston 1999), have shown that these animals have low MRs at low temperatures, suggesting low requirements for standard metabolism and other energy-demanding processes. The general pattern indicates an increase in aerial MR towards low latitudes, not supporting the latitudinal compensation in temperate habitats. The same conclusion was reached from a latitudinal comparison of the metabolism of the terrestrial isopod in temperate habitats across Chile (Lardies & Bozinovic 2006).

At least two questions arise from our study, as noted by Vernberg (1962) more than 40 years ago. One is the question of whether physiological variation is genotypic or phenotypic. The second is the demonstration of the

physiological mechanism involved in this variation. These kinds of studies will allow us to make the necessary link between ecological physiology and macroecology (macro-physiology) to develop a global understanding of organismal function in the marine system. Macrophysiology compares physiological features between individuals separated geographically (Chown *et al.* 2004), which was the approach we followed in this study, but also using principally the intrinsic physiological properties of organisms to predict their responses to climatic change (Rodríguez-Serrano & Bozinovic 2009). In this vein, considering that one of the important consequences of global change will be an increase in temperature in the future (Tebaldi *et al.* 2006), our prediction is that the respiration ratio (air/water) of *C. cinereus* could increase, causing the crabs to expend more energy on aerial respiration.

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