

Intraspecific geographic and seasonal physiological variability in an intertidal fish, *Girella laevis*, along a climatic gradient

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Metabolic rate and condition factor (K) of juvenile *Girella laevis* were highest in southern populations along the Chilean coast. Since adults of *G. laevis* complete their life cycle in subtidal waters, the results suggest a metabolic cold adaptation in juveniles of this species and physiological compensation that enable them to move early to subtidal environments for reproduction

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Studies of geographically separated populations are important in understanding how selective processes affect physiological characters. Diversity and variation are key characteristics of life (Vitousek *et al.*, 1997), however, few studies exist concerning the effects of physical habitat variations on physiological diversity and physiological responses of animals and plants in time and space, *i.e.* from variability within and between individuals, populations and species (Spicer & Gaston, 1999). A new approach to understanding patterns of biodiversity and of the mechanisms underlying those patterns is one where physiological variability is examined from within the hierarchical structure of biodiversity (from genes to ecosystems). This approach is an attempt to explore the ecological implications of the diversity of physiological patterns, focusing mainly on variation in latitude and altitude, limits of tolerance and physiological limits of geographical distribution (Barry *et al.*, 1995; Spicer & Gaston, 1999; Hughes, 2000). For example, the metabolic cold adaptation hypothesis (MCA) predicts higher metabolic rates of ectotherms from cold environments than those of their more temperate counterparts (Krogh, 1916; Scholander *et al.*, 1953; Lardies *et al.*, 2004). The predictions are that any change in temperature will affect physiological processes of these ectotherms with implications on loss of biodiversity and local extinctions

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(Hughes, 2000). In this vein, ecologists have always believed that physiological mechanisms are at the base of ecological patterns of biodiversity in time and space. Nevertheless, their importance has been at best underestimated and at worst ignored, with physiological diversity being either dismissed or treated as random noise or error (Spicer & Gaston, 1999).

Intertidal rockpools are a system characterized by their discrete nature in time and space, as well as by their daily and seasonal variability in temperature, oxygen concentration and salinity (Newell, 1970; Stephenson & Stephenson, 1972; Truchot & Duhanel-Jouve, 1980; Metaxas & Scheibling, 1993). These variations, particularly in temperature, depend on the vertical position (elevation) of the rockpool along the intertidal gradient, which determines the time that a rockpool remains isolated from the subtidal system (Gibson, 1972, 1982; Horn & Gibson, 1988; Horn *et al.*, 1999). High intertidal rockpools in Chile are characterized by high thermal variability with thermal extremes reaching 30° C in the northern coast (Antofagasta) and near 16° C in the south (Pulgar *et al.*, 2005). This pattern of thermal conditions among localities along the Chilean coast suggests that fishes inhabiting high intertidal rockpools are subjected to extreme thermal variability, which has greater effects on their energy budgets (Pulgar *et al.*, 2005). *Girella laevis* (Tschudi) is the most abundant transitory intertidal fish along the Chilean coast (Varas & Ojeda, 1990), being found primarily in high intertidal rockpools (most distant to the subtidal zone), and presenting a wide geographical distribution from 18 to 40° S (Ojeda *et al.*, 2000). These characteristics of *G. laevis* and geographic variability in high intertidal pool thermal conditions make this an ideal study model to evaluate this species' responses to spatial variability in climatic and thermal conditions.

Oxygen consumption (as a proxy for metabolic rate) and condition factor (K) of *G. laevis* from three localities spanning 10° of latitude along the Chilean coast were measured. Metabolic rate represents the cost of life associated to particular habitat conditions (Schmidt-Nielsen, 1997). The K , on the other hand, indicates the degree of robustness (muscular tissue) of the fish (Moyle & Cech, 1982). Thus, differences in K of fishes exposed to different environmental conditions will shed light on how the latter may alter fish energetics, performance and, in consequence, fitness.

Specimens were captured from high tidepools at three localities along the Chilean coast, Antofagasta (23°20' S, 70°38' W), Carrizal Bajo (28°04' S, 71°08' W) and Las Cruces (33°26' S, 71°41' W), between June 2001 and January 2002. At each locality, tide pool seawater thermal variations were measured (see Table I; Pulgar *et al.*, 2005). Specimens were transported to Las Cruces Coastal Marine Research Station (ECIM) where they were maintained under laboratory conditions with constantly circulating filtered sea water and aeration and were supplied mussels *Mytilus chilensis* daily for *ad libitum* feeding.

Fish total length (L_T) and fish body mass (M) were recorded using a caliper (± 0.1 cm) an electronic balance (± 0.1 g), respectively and K was calculated from: ML_T^{-3} (Wootton, 1991). To avoid differences in the body mass of fish sampled among localities and seasons of the year (winter and summer), K was only evaluated in pre-reproductive fish, *i.e.* fish < 7 cm L_T (Stepien, 1990).

To measure metabolic rate, all *G. laevis* from Antofagasta ($n = 16$), Carrizal Bajo ($n = 13$) and Las Cruces ($n = 13$) were maintained during 45

TABLE I. Seawater temperature registered in high intertidal pools sampled in three geographic localities and two seasons of the year

Localities	Season	Mean \pm s.e. seawater temperature ($^{\circ}$ C)	
		Minimum	Maximum
Antofagasta	Summer	13.40 \pm 0.91	33.80 \pm 2.42
	Winter	12.50 \pm 0.60	26.50 \pm 2.71
Carrizal Bajo	Summer	13.22 \pm 1.62	30.08 \pm 3.41
	Winter	9.26 \pm 1.11	24.88 \pm 3.73
Las Cruces	Summer	11.20 \pm 1.75	25.02 \pm 1.77
	Winter	11.49 \pm 0.92	17.22 \pm 0.89

days, with *ad-libitum* food (mussels) and aeration in sea water held at 25 $^{\circ}$ C, with a 12L : 12D photoperiod. In order to avoid digestion metabolic costs, all fish were starved for 48 h before measuring oxygen consumption (V_{O_2}). To avoid endogenous cycles of intertidal individuals (Horn *et al.*, 1999), all measurements were conducted between 1200 and 1600 hours and in animals captured in both seasons.

Each fish was deposited in dark metabolic chambers for 2 h before measuring V_{O_2} oxygen saturated sea water at constant temperature (25 $^{\circ}$ C). Later, the metabolic chamber was sealed and an O₂ electrode (Strathkelvin model 1302) was connected to a Strathkelvin model 781 oxygen meter. This instrument recorded the decrease in percentage of O₂ inside the closed metabolic chamber. Metabolic rate was expressed as mg O₂ g⁻¹ min⁻¹.

Differences in K values among localities and season of the year were tested by a two-way ANOVA. Differences between levels were evaluated with *a posteriori* Scheffé test (Zar, 1996). Oxygen consumption among localities was evaluated using ANCOVA with body size as a covariate (Zar, 1996). Locality and season of the year were considered fixed factors, because both represent endpoints of thermal variability in the geographic range studied.

Significant interactions between locality and season of the year indicated that fish collected in winter from Las Cruces, had significantly higher mean K values than fish collected from other localities during summer and winter (two-way ANOVA, $F_{2, 702}$, $P < 0.001$; Fig. 1 and Table II). Mean K -values of fish collected from other localities did not differ (*a posteriori* Scheffé test $P > 0.05$; Fig. 1).

On the other hand, V_{O_2} was higher in fish inhabiting at Las Cruces (ANCOVA $F_{2, 41}$, $P < 0.05$). After an *a posteriori* Scheffé test, the V_{O_2} of fish from Carrizal Bajo and Antofagasta was shown not to differ ($P > 0.05$; Fig. 2).

Physiological processes of ectotherms, in general, and fishes in particular, strongly depend on environmental temperature (Fry, 1971; Pulgar *et al.*, 1999), which coupled with the rate of food consumption, determines the rate at which fishes grow. In this sense, fishes may be particularly vulnerable to climate variability because their physiological processes are directly affected by water temperature of their habitat (Hill & Magnuson, 1990; Mora & Ospina, 2001). Despite this, animals and particularly fishes, do exhibit physiological and behavioural compensation to their different habitat conditions (Pulgar *et al.*, 1999;

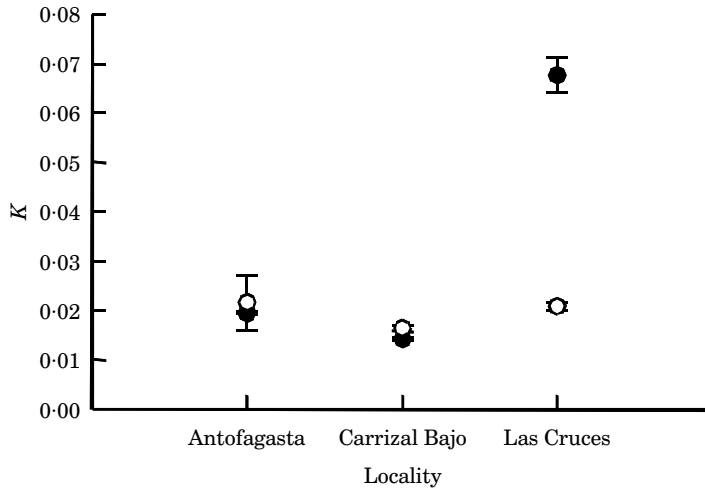


FIG. 1. Mean \pm s.e. condition factor of *Girella laevisfrons* sampled from three localities on the Chilean coast in winter (●) and summer (○).

Spicer & Gaston 1999; Hernandez *et al.*, 2002; Pulgar *et al.*, 2003). Fish from Las Cruces had highest K values in winter (Fig. 1 and Table II), which is the season and locality with the lowest temperature and the highest food availability (Table I; Santelices & Marquet, 1998; Aldana *et al.*, 2003; Pulgar *et al.*, 2005).

On the other hand, *G. laevisfrons* increases their metabolic rate in the most southern locality (Fig. 2). The classical studies of Krogh (1916) suggest that southern ectotherms would have higher metabolic rates in comparison to temperate ectotherms when exposed to the same temperature. Moreover, Scholander *et al.*, (1953) suggest that cold water fishes tended to have a relatively higher metabolic rate than temperate and warm water species. As pointed out earlier the MCA hypothesis predicts an increase in the metabolic rate of ectotherms from cold environments compared with their more temperate counterparts. This hypothesis is one the most polemical in comparative physiological ecology, where evidence comes principally from the meta-analysis of data sets of arthropods. An important approximation for testing MCA is to carry out comparisons over a large latitudinal scale, because mean annual temperature decreases towards high latitudes. Furthermore, few studies have conducted intraspecific

TABLE II. Two-way ANOVA to evaluate the effects of locality (Antofagasta, Carrizal-Bajo, Las Cruces) and season (summer or winter) on the condition factor (K) in *Girella laevisfrons*

Source of variation	d.f.	Mean square	F	P
Locality	2	0.27	79.38	0.0001
Season	1	0.018	54.98	0.0001
Locality \times season	2	0.025	66.22	0.0001
Error	702	0.0003		

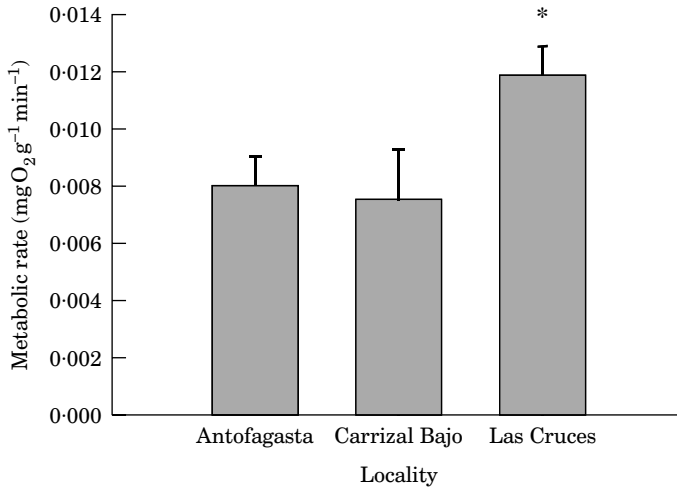


FIG. 2. Mean \pm S.E. metabolic rate of *Girella laevisfrons* sampled from three study localities. *, $P < 0.05$.

comparisons of metabolic rates along a latitudinal gradient as were done here (Lardies *et al.*, 2004). Interestingly, the present results seem to support the metabolic cold adaptation hypothesis (Fig. 2), that is, fish from Las Cruces present the highest metabolic rate in comparison to similar sized fish from northern localities (Fig. 2). High metabolic rates has been interpreted as advantageous in ectotherms, especially in insects inhabiting high latitude or altitude environments, because it enables them to metabolize food and develop more rapidly, and thus to complete their entire life cycle earlier (Chown & Gaston, 1999; Gotthard *et al.*, 2000).

Along the Chilean coast, only small juveniles of *G. laevisfrons* occupy the high intertidal rockpools, which represents nursery areas that are protected from predators invading at high tide for this and other littoral fish species (Varas & Ojeda, 1990). As they grow, they move into subtidal waters where they complete their life cycle and reproduce. In this sense, high metabolic rate of *G. laevisfrons* in cold water (Las Cruces) enable them to grow faster than in warm waters, which means that they can move sooner to subtidal environments and reproduce. Therefore, the high metabolic rate observed in southern fish may well represent a physiological advantage and possibly a selective benefit to complete their life cycle.

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