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K. BOWLER (U.K.)		Editors		J. E. HEATH (U.S.A.)
CONTENTS				
Maritza Sepúlveda, Marcela A. Vidal, José M. Farfán and Pablo Sabat	141	Seasonal and geographic variation in thermal biology of the lizard <i>Microlophus atacamensis</i> (Squamata: Tropiduridae)		
J.L.P. Muñoz, P.A. Camus, F.A. Labra, G.R. Finke and F. Bozinovic	149	Thermal constraints on daily patterns of aggregation and density along an intertidal gradient in the periwinkle <i>Echinolittorina peruviana</i>		
S. Bhasari, I.B. Hearne, D.E. Spiers, W.R. Lamberson and E. Antoniou	157	Transcriptional profiling of mouse liver in response to chronic heat stress		
Jennifer Ward, Dominic J. McCafferty, David C. Houston and Graeme D. Ruxton	168	Why do vultures have bald heads? The role of postural adjustment and bare skin areas in thermoregulation		
J.M. Kapfer, M.J. Powers, D.M. Reinecke, J.R. Coggins and R. Hay	174	Environmental, behavioral, and habitat variables influencing body temperature in radio-tagged bullsnakes, <i>Pituophis catenifer snyderi</i>		
Tadeusz Wlóstowski, Elżbieta Bonda and Alicja Krasowska	180	Effect of cold on lipid peroxidation in the brown adipose tissue and liver of rats		
<i>(continued on outside back cover)</i>				
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# Thermal constraints on daily patterns of aggregation and density along an intertidal gradient in the periwinkle *Echinolittorina peruviana*

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## Abstract

Variations in environmental temperature have both direct and indirect effects that affect organisms at levels ranging from intra-cellular physiological processes to ecological patterns. These variations are especially important for intertidal marine ectotherms such as littorinids since they alternate between periods of immersion in seawater, and must also experience long periods of emersion. In central Chile, *Echinolittorina peruviana* is one of the most conspicuous species on rocky intertidal shores, occurring at high tidal levels and in the splash zone. The species is known to resist direct exposure to the sun for long periods, although juveniles tend to be restricted to protected microhabitats. Adults show seasonal variations in abundance and vertical distribution and may form aggregations that have been shown to help reduce water loss and body temperature. In this study, we evaluate the relationship between daily thermal variations throughout the vertical distribution of this species and how these affect the patterns of density and aggregation. Our results suggest that one of the leading determinants of the spatio-temporal variation of density in *E. peruviana* may be operative temperature ( $T_O$ : the amount of stored heat resulting from the balance between heat fluxes into and out of the body, measured with taxidermic mounts mimicking heat transfer properties of the snail).  $T_O$  showed a strong negative relationship with density and a strong positive relationship with aggregation in the highest intertidal level monitored. The strength of these relationships decreased in importance at lower levels. While  $T_O$  alone cannot explain the abundance of *E. peruviana* throughout its range of distribution, our results show that it does have a strong influence that should be considered in addition to other ecological factors affecting the density of intertidal littorinids.

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**Keywords:** Operative temperature; Rocky intertidal; Littorinids; Chile

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## 1. Introduction

Variations in environmental thermal regime are of utmost importance to ectothermic animals since temperature has both direct and indirect effects on physiological and ecological processes (e.g., Huey and Stevenson, 1979; Johnston and Bennett, 1996; Pörtner, 2002; Zippay et al., 2004). Thermal constraints are particularly important in determining patterns of activity and behavior

of marine invertebrates inhabiting intertidal environments, where the animals are periodically exposed to terrestrial conditions with the cyclic rise and fall of tides (e.g., Widdows et al., 1979). During periods of low tides, intertidal organisms are exposed to terrestrial or semi-terrestrial conditions that impose physiological challenges on them, which approach the limits of physiological tolerance (Hofmann and Somero, 1995; Stillman and Somero, 1996; Somero, 2002; Stillman, 2002). Micro-climatic variation is high in the intertidal and is dependent largely on exposure to solar radiation (Helmuth, 1998, 1999; Jones and Boulding, 1999). Thus, intertidal species are expected to select suitable thermal patches from the environment and display

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behaviors that permit them a stabilization of their body temperature (Johnston and Bennett, 1996).

Several studies have investigated the roles of biotic and abiotic factors in determining the patterns of distribution and abundance of prosobranch gastropods from the family Littorinidae on rocky intertidal shores (Gendron, 1977; Boulding and Van Alstyne, 1993; Chapman and Underwood, 1996; Soto and Bozinovic, 1998; Sokolova and Pörtner, 2003). The main environmental factors affecting their distribution include predation, dislodgement by waves and heat load (Atkinson and Newbury, 1984; Garrity, 1984; Chapman and Underwood, 1992, 1994; Rochette and Dill, 2000). However, since littorinids must experience long periods of emersion, their behavior and physiology are thus especially affected by temperature and desiccation. In some species, mean body temperature during low tide may differ by 10 °C between high- and low-shore individuals (Sokolova et al., 2000). Consequently, such factors appear closely related with the patterns of aggregation, habitat selection, morphology and pigmentation of many littorinid species (e.g., McMahon, 1990; Britton, 1995; Chapman and Underwood, 1996; Jones and Boulding, 1999; Sokolova and Berger, 2000). Aggregations or clusters are thus potentially important to reduce evaporative water loss, since small individuals are more affected by solar radiation than larger ones (Vermeij, 1973; Lalli and Parsons, 1993; Chapman, 1998; Soto and Bozinovic, 1998; Rojas et al., 2000).

The periwinkle *Echinolittorina peruviana* (Lamarck) is distributed along the southeastern Pacific seaboard from Panama to central Chile (Guzmán et al., 1998), thereby facing a wide variety of thermal regimes encompassing tropical and temperate regions. In central Chile, *E. peruviana* is one of the most conspicuous species on rocky intertidal shores (Santelices, 1980), occurring at high tidal levels and above in the splash zone. This species is known to resist direct exposure to the sun for long periods, although juveniles tend to be restricted to protected microhabitats and adults show characteristic seasonal variations in abundance and vertical distribution (Santelices et al., 1986; Guzmán et al., 1998). In this regard, Soto and Bozinovic (1998) and Rojas et al. (2000) showed that the spatial distribution of *E. peruviana* can be affected by variations in water, rock substrate and air temperatures. While extreme rock temperatures can regularly exceed 30 °C and occasionally reach 45 °C, field and laboratory observations showed that this species preferentially selects substratum temperatures between 12 and 20 °C and is most active nocturnally. The above suggests that thermal variation in the intertidal habitat may have a strong impact on the abundance and spatial distribution of *E. peruviana*, although such effects have not been adequately assessed in the field. Therefore, in this study we set out to evaluate the relationship between daily thermal variations in the intertidal environment and the patterns of density and aggregation of *E. peruviana* along a vertical gradient.

## 2. Material and methods

Thermal variation and its effects on the patterns of spatial distribution were studied in a population of *E. peruviana* from the Coastal Station for Marine Research of the Pontificia Universidad Católica de Chile in Las Cruces, central Chile (33°35'S; 71°38'W), during austral spring and summer 2004. Las Cruces is located near the southern limit to the distribution of *E. peruviana*, in a temperate region where thermal conditions are presumably less stressful than those at lower latitudes.

### 2.1. Thermal variation

Samplings were conducted during 3 days presenting clear skies and similar tides, where thermal variations (simultaneous measurements of air temperature and operative temperature) were recorded each day. Shaded air temperature was measured using a thermistor, placed 20 cm above the surface under a white hood, and located at 3 m above lower low water datum. Signal conditioning and data recording were provided by a LI-COR LI-1400 datalogger programmed to register temperature every 20 min. The analysis of thermal variability in *E. peruviana* and its effects on the spatial distribution of individuals was based on operative body temperature (Bakken et al., 1985; Bakken, 1992), which has proved to be an important tool in the study of ectothermic organisms. Operative temperature ( $T_O$  hereafter) refers to the amount of heat energy stored in the organism's body after it has achieved a balance between the fluxes of heat into and out of the body, from which its body temperature can be estimated (Helmuth, 2002). We therefore constructed "taxidermic" mounts of *E. peruviana* to serve as  $T_O$  models, as defined by Chappell and Bartholomew (1981) and applied by Helmuth (2002) to intertidal invertebrates, to mimic heat transfer properties of snails (Wills and Beaupre, 2000). The snail body was thus removed from the shell to eliminate the potential effects of evaporation and metabolic heat production (Dzialowski, 2005), and a Cu–constantan thermocouple was inserted into a hollowed out shell (mean maximum length of  $1.35 \pm 0.27$  cm). These mounts may also account for the effects on heat fluxes derived from the size, morphology (e.g., shape, color pattern) and radiative properties of *E. peruviana* shells, improving the estimation of  $T_O$  (Bakken et al., 1985; Helmuth, 2002). Our  $T_O$  models did not include filling materials to approach the specific heat of tissue, thus excluding the possible dampening effect of thermal inertia on  $T_O$ . However, the small size of *E. peruviana* models should minimize potential biases because smaller organisms show faster responses to thermal environmental changes, as Helmuth (2002) demonstrated with  $T_O$  models of intertidal mussels filled with silicone, where the smallest models were 42 mm in length (ca. four times larger than ours).

Thus, three equivalent  $T_O$  models, one for each tidal zone, were mounted in a natural posture on a sheets of

granite with dental cement, and the thermocouples connected to a digital logger (Digi-Sense DuaLogR) programmed to register temperature every 12 min. Both air temperature and  $T_O$  were recorded continuously between 3:00 and 21:00 on each day. These measurements were then divided into six 3 h bins: (1) between 3:00 and 6:00, (2) 6:01 and 9:00 (3) 9:01 and 12:00; (4) 12:01 and 15:00; (5) 15:01 and 18:00; and (6) 18:01 and 21:00. Bins 1 and 6 correspond to night periods. All temperature records for a given time interval (i.e., a single bin) were averaged and expressed as the mean value for that interval.

## 2.2. Density and spatial distribution of *E. peruviana*

Nine permanent transects were installed along the vertical distribution range of *E. peruviana*, to determine its density and distribution with respect to thermal variability. These transects were perpendicular to the line of coast, located on rocky intertidal outcroppings of similar substratum texture, and marked with an epoxy resin for recognition and separation of three tidal sectors: (a) a high (spray) zone, dominated by chthamaloid barnacles; (b) a medium zone, with shared dominance of mussels and barnacles; and (c) a low zone with shared dominance of mussels and macroalgae. The low zone was located immediately above the intertidal belt of the brown kelp *Lessonia nigrescens* Bory, which marks approximately the mean low water level in Chilean rocky shores.

To evaluate the effect of the diurnal thermal variation on the density and degree of aggregation, digital photographs (3.2 mega pixels, Sony cyber-shot DSC-P72) were taken of one 0.25 m<sup>2</sup> quadrat (covering all its surface) at each tidal height along all nine transects during low tides, and during each of the six time intervals defined above. These photographs were analyzed with the software Image Tool 2.0 (University of Texas Health Science Center, San Antonio, TX, USA). We defined aggregation as the degree of spatial isolation of individuals within each quadrant. Aggregation was measured as the proportion between the number of individuals separated from all the others by at least one body length, and the total number of individuals present. Values range from 0 to 1, where values approaching unity indicate a greater tendency towards individuals being isolated, and lower values indicate that the individuals present in a quadrant are more frequently aggregated. Notwithstanding, values of this index approaching zero reflect high aggregation but they do not discriminate between maximum proximity (separation by less than one body length) and direct physical contact between individuals. Thus, zero values represent a threshold below which all individuals will be highly aggregated, although not necessarily reaching their maximum aggregation level.

## 2.3. Statistical analyses

The effects of tidal height and time of the day on the observed variations in density, proportion of isolated

individuals and  $T_O$  of *E. peruviana* were independently analyzed by fixed, two-way ANOVA models. Density data were normal and homoscedastic after logarithmic transformation. When significant differences were detected, *a posteriori* comparisons were performed using Tukey's test. Associations among variables were assessed by Pearson ( $r_P$  in the text) or Spearman ( $r_S$ ) correlation coefficients, depending on the normality and/or linearity of their relationship. All analyses were performed using Statistica<sup>®</sup> 6.0 (2001).

## 3. Results

### 3.1. Thermal variation

Environmental (air) temperature showed a clear daily fluctuation throughout the observation window, varying on average from ca. 15 °C at the interval 03:00–06:00 AM to ca. 23 °C at 12:00–15:00 PM (Fig. 1A). Likewise,  $T_O$

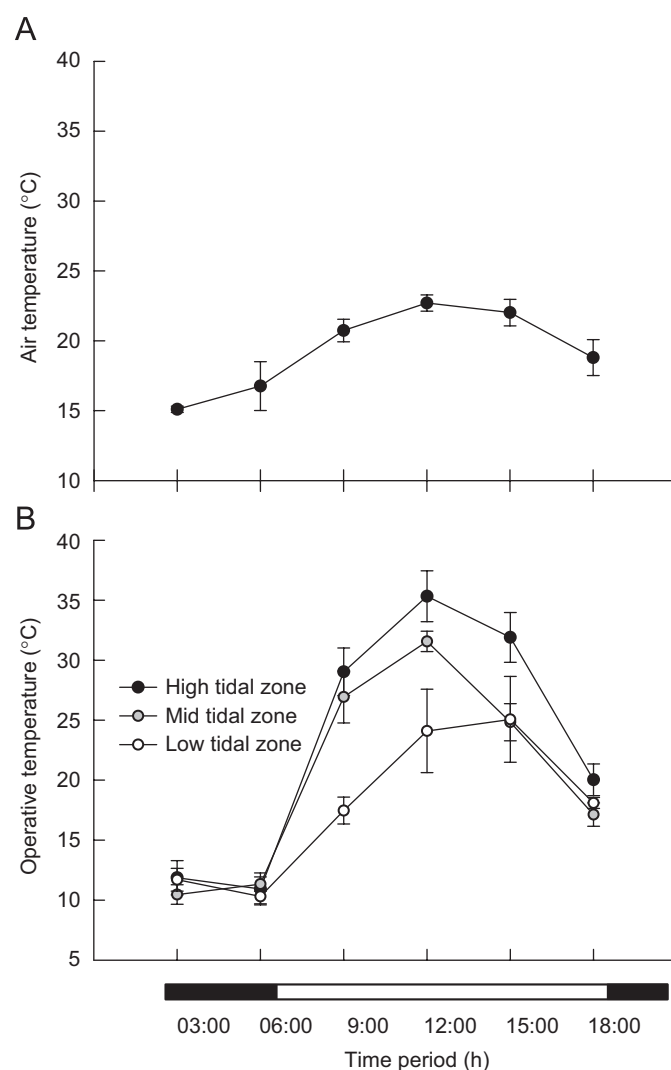


Fig. 1. Daily thermal fluctuations recorded in Las Cruces. (A) Air temperature at the high intertidal zone; (B) operative temperature for *E. peruviana* models located at three intertidal zones.

exhibited a strong daily oscillation at each tidal height (Fig. 1B), a trend that was highly correlated among the three intertidal zones ( $0.852 < r_P < 0.979$ ;  $0.010 < p < 0.031$ ), and resulted in positive correlations between  $T_O$  and air temperature at each tidal level ( $0.894 < r_P < 0.962$ ;  $0.020 < p < 0.016$ ). In addition, the amplitude of the mean  $T_O$  oscillation increased with tidal height: 16, 21 and 25 °C for the low, mid and high intertidal zones, respectively. Similarly, the ranges of the mean  $T_O$  values were 26–10, 32–11 and 36–11 °C for the low, mid and high zones, respectively, evidencing the critical effect of heating during emersion. It is worth pointing out that while maximum  $T_O$  was 10 °C warmer in the high zone in comparison to the low zone, minimum  $T_O$  differed by only 1 °C.

Overall, during sunlight hours the high and mid intertidal zones had greater thermal ranges and appear to be more stressful thermal environments when compared with the lower zone. At night, however,  $T_O$  was more homogeneous across the whole intertidal zone (Fig. 1B) and closely followed air temperature (Fig. 1A). Beyond the obvious statistical differences among the heights of each single factor in the ANOVA, the differences just described are emphasized by the significant interaction between tidal height and time of the day affecting  $T_O$  (Table 1A).

### 3.2. Variations in density and aggregation

The density of *E. peruviana* was highly variable across tidal heights and with time of the day (Fig. 2). While showing pronounced daily fluctuations, on average, density showed a significant variation with tidal height (Table 1B), increasing from higher to lower intertidal zones. Mean density ranges were 4–20, 18–37 and 33–47 individuals  $m^{-2}$  for the high, mid and low intertidal zones, respectively (Fig. 2A–C). Both in the mid and high zones the highest densities occurred at night periods and the lowest density around midday. Density in the lower zone was more variable, showing no clear pattern throughout the day. Such contrasting trends among tidal zones resulted in non-

significant effects of time of the day on density, and a lack of interaction among the main effects (Table 1B). Nonetheless, the fluctuation of mean density values was highly correlated between the high and mid zones ( $r_P = 0.883$ ,  $p = 0.020$ ), just like the trend recorded for  $T_O$ .

On the other hand, there were significant differences in aggregation with tidal heights and time of the day, and the interaction term between the two factors was also significant (Table 1C). The aggregation index exhibited no common trend among intertidal zones, showing similar values at night periods and strong differences during the day between 9:00 and 12:00 h. *E. peruviana* exhibited a significant increase in aggregation (i.e., lowest index values) in the high intertidal zone during midday (Fig. 2A; Tukey's test,  $p < 0.05$ ), although no clear patterns were observed in the mid and low zones.

Despite the aforementioned, the proportion of isolated individuals decreased significantly (i.e., individuals were more aggregated) with increasing  $T_O$  in the high and mid zones (mean values per time period:  $r_s = -0.829$ ,  $p = 0.042$ , in both cases). While the density of *E. peruviana* was not correlated with  $T_O$ , density did show a significant positive association with the proportion of isolated individuals in the high intertidal zone (mean values per time period;  $r_s = 0.899$ ,  $p = 0.015$ ), meaning that aggregation decreased with increasing density in the most thermally stressful habitat.

## 4. Discussion

Thermal variations in the environment throughout the day exerted differential influences on the patterns of density and aggregation of *E. peruviana* across a vertical gradient in the rocky intertidal zone. However, the density of this species was not directly associated with its aggregation responses to prevailing thermal conditions.

The periodic heating and cooling of rocky substrata was clearly reflected in  $T_O$  values, which showed a marked and similar daily oscillation at the three intertidal levels,

Table 1  
Two-way ANOVA results for the effects of tidal height (TH) and time of the day (TD) on the operative temperature ( $T_O$ ), density and proportion of isolated individuals of *Echinolittorina peruviana*

Variable	Source	d.f.	M.S.	F	p
$T_O$	Tidal height	2	131.02	42.38	<0.001
	Time of the day	5	611.00	197.65	<0.001
	TH*TD	10	27.68	8.95	<0.001
	Error	36	3.09		
Density	Tidal height	2	4.184	15.859	<0.001
	Time of the day	5	0.195	0.740	0.594
	TH*TD	10	0.255	0.967	0.472
	Error	402	0.264		
Proportion of isolated individuals	Tidal height	2	0.414	3.411	0.033
	Time of the day	5	0.491	4.044	0.001
	TH*TD	10	0.249	2.059	0.026
	Error	402	0.121		

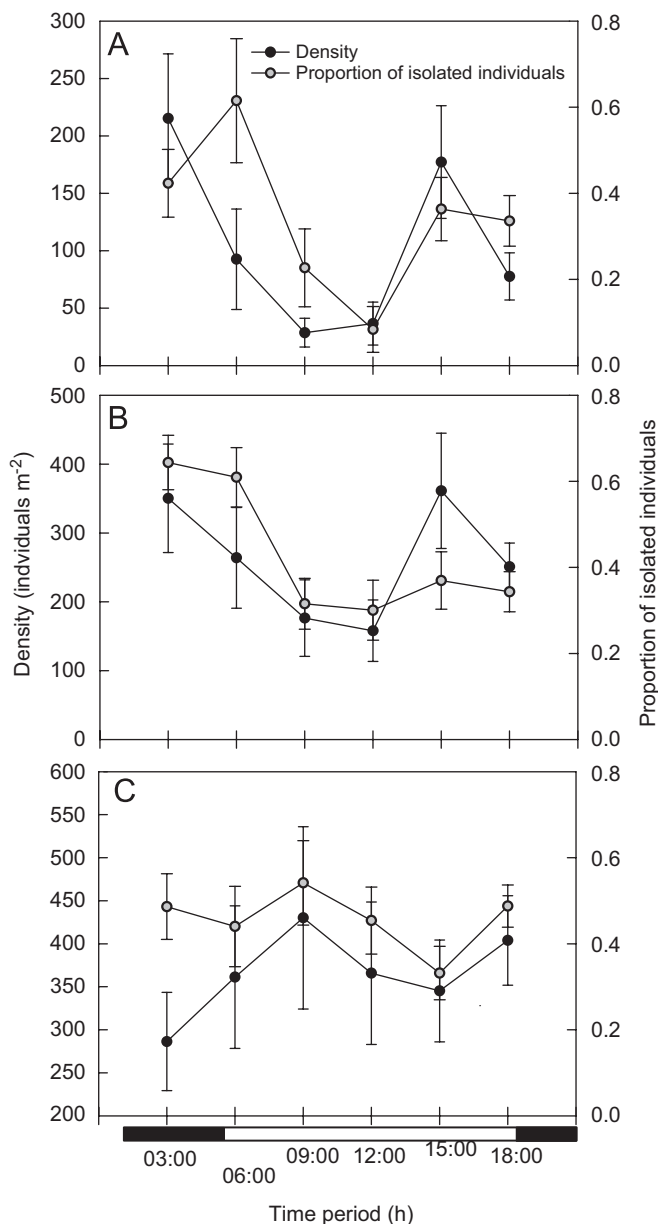


Fig. 2. Daily fluctuations of density and aggregation of *E. peruviana* at three intertidal zones in Las Cruces. (A) High zone, (B) mid zone, (C) low zone.

peaking at midday in correlation with air temperature. At night, the whole intertidal environment appeared thermally homogeneous, reaching average minimum  $T_O$  around 11–12 °C. During the day, however,  $T_O$  sharply contrasted over tidal levels, allowing the differentiation of two thermal environments: the low (more benign) vs. the mid–high (more thermally extreme) intertidal zones. Maximum mean  $T_O$  at noon was approx. 36 °C in the high intertidal zone, approaching, and in many point cases exceeding, the thermal tolerance limit of *E. peruviana* (approx. 41 °C when emersed; Muñoz et al., 2005).

In this regard, our results suggest that  $T_O$  may be one of the leading determinants of the spatio-temporal variation in the density of *E. peruviana*. Although density values per

intertidal level did not change in direct response to  $T_O$  during the day, both variables showed an overall negative association along the vertical gradient and between contrasting thermal periods.  $T_O$  alone cannot explain the abundance of *E. peruviana*, but it does have a strong influence which should be investigated in connection with previously known thermoregulatory responses, such as the active selection of rocky substrata with lower temperatures (Soto and Bozinovic, 1998), the formation of large aggregations which reduce body temperature and the rate of water loss (Rojas et al., 2000), and photokinetic responses with respect to the position of the sun to reduce the heat gain (Muñoz et al., 2005). Nonetheless, we lack precise information on a number of ecological factors that are known to affect the density of intertidal littorinids, such as food availability and competition (e.g., Branch and Branch, 1981; Petraitis, 2002), wave exposure (e.g., De Magalhaes, 1998), abundance of other species such as barnacles, limpets, algae and decapods (e.g., Underwood and McFadyen, 1983; Svensson, 1997; Carlson et al., 2006; Williams, 2006), and complexity or heterogeneity of the rocky substratum (e.g., Raffaelli and Hughes, 1978; Chapman, 1994; Reid and Williams, 2004; Carlson et al., 2006).

Due to the limited ecological knowledge on *E. peruviana*, there are no conclusive interpretations for parts of our results, for example the average increase in density from higher to lower tidal levels matched by a decreasing average  $T_O$ . While this pattern may reflect a response to thermal constraints, there may be other factors involved. We lack information on possible vertical migrations of individuals across tidal levels, as have been shown in other littorinid species during mating (e.g., Takada, 2003) or spawning (e.g., Ohgaki, 1988) seasons, independent of thermal conditions. However, other observations provide evidence supporting our hypothesis regarding thermal tolerance. For instance, in the more thermally variable mid and high zones *E. peruviana* attained its highest densities and lowest  $T_O$  at night, and lowest densities and highest  $T_O$  at noon; it showed no such trends in the more thermally homogeneous low zone. Likewise, during sunlight hours in the mid and high zones, density decreased steadily until noon (when average  $T_O$  reached 32 and 36 °C, in mid and high zones, respectively) and increased thereafter. In the low zone, where mean  $T_O$  reached a mere 26 °C at noon, density also decreased but its minimum value was displaced to the afternoon period. We suspect that since the low zone is much closer to breaking waves, wave splash and spray may help to attenuate heating by directly moistening the habitat with cooler seawater more frequently than higher levels.

Similarly, the aggregation (i.e., lower values of isolation) of *E. peruviana* individuals showed a strong positive relationship with  $T_O$  at mid and high tidal levels, with the highest degree of aggregation occurring at noon. However, the correlation vanished at the lower level where the highest aggregation occurred after noon, suggesting

that this environment allows littorinids not only to delay their aggregation responses but also to shorten the period during which they experience intense heating. Furthermore, aggregation did not covary with density in the mid and low zones, but there was a significantly negative relationship between them in the high zone, where *E. peruviana* showed its lowest density. This latter fact seemed counterintuitive, but it suggests that the thermal hostility of the high zone keeps the number of individuals down, and those that are present are forced to aggregate for thermoregulation more frequently. However, this cannot explain why snails at higher densities were less aggregated under similar thermal conditions, considering that aggregation is known to reduce the water loss and body temperature of *E. peruviana* (Rojas et al., 2000), and that at high numbers it would be easier for snails to follow the more abundant mucus trails of others to reach the aggregation (e.g., Chapman, 1998; but see Edwards and Davies, 2002), or to use the available topographic complexities to form aggregations (e.g., Raffaelli and Hughes, 1978). On the other hand, the significant negative correlation between aggregation and density in the high zone may have been influenced by the extreme situation at noon, when the maxima in  $T_O$  and aggregation coincide with the minimum density. Thus, aggregation and density would be strongly related only at the periods of greater thermal stress, but they may lack a general underlying relationship as observed in other littorinids (e.g., Chapman, 1995).

The literature shows that aggregation behavior varies among species and habitat types, in association with diverse factors that may or may not interact with the physiology of snails, such as mate search, reproductive stage, following of mucus trail, feeding, topographic complexity, predation or contrasting hydrodynamic conditions between emersion and immersion periods (e.g., Chapman, 1995; Chapman and Underwood, 1996; Erlandsson et al., 1999; Boulding et al., 2001; Edwards and Davies, 2002; Stafford and Davies, 2005; Pardo and Johnson, 2006). Our results suggest that  $T_O$  may likely be a leading signal to display behavioral thermoregulatory responses in *E. peruviana*. However, prior results (Rojas et al., 2000) showed that the thermal tolerance and water economy of this species may exhibit geographical variations under contrasting climatic conditions, affecting the relative effectiveness of aggregation as a thermoregulatory mechanism. Thus, it is clear that aggregation behavior in *E. peruviana* is not explained solely by thermal stress, but apparently no single factor would account for aggregation in other littorinids as well, which explains the elusiveness of a general explanation. We thus suggest that littorinid clustering is not a specific mechanism, but rather an emergent phenomenon, and in some cases an epiphenomenon, resulting from a suite of distinct proximal causes. With respect to *E. peruviana*, previous works (Soto and Bozinovic, 1998; Rojas et al., 2000; Muñoz et al., 2005) and our present results point to environmental temperature

(through its impact on periwinkle's physiology) as one of such causes in central Chilean populations, although its importance relative to the other causes remains to be assessed.

From the above, the observed behavior of *E. peruviana* in regard with  $T_O$  suggests that, on a local scale, thermal physiological tolerance to a significant extent controls the vertical distribution and daily fluctuations in density of this species. However, thermal constraints would play a different role at much larger scales, as this species occurs in tropical, subtropical and warm-temperate regions along the eastern Pacific, but it does not penetrate into the cold-temperate region south of approx. 42°S (Guzmán et al., 1998; Camus, 2001). Moreover, its southern endpoint of distribution has contracted from 42° to 37°10'S since 1962, in close relation with a marked cooling trend of air temperature in the area during the last century (Rivadeneira and Fernández, 2005). Along the Chilean coast, therefore, upper thermal tolerance would be a key factor in the behavior of *E. peruviana* on small spatial scales in the short term, while lower thermal tolerance could control its large-scale distribution in the long term.

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