UNIVERSIDAD ADOLFO IBAÑEZ FACULTAD DE INGENIERIA Y CIENCIAS



Phenotypic integration of physiological and lifehistory traits along a latitudinal gradient in intertidal ectotherms

DOCTORAL THESIS Sebastian Jorge Antonio Osores Espina

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PHENOTYPIC INTEGRATION OF PHYSIOLOGICAL AND LIFE-HISTORY TRAITS ALONG A LATITUDINAL GRADIENT IN INTERTIDAL ECTOTHERMS

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<u>Chapter 1</u>

Table 1: Generalized linear mixed model of physiological traits (cephalothorax length, heat coma, chill coma and metabolic rate) for the three crab species (significant differences at different levels: 0.001 = "**", 0.01 = "**", 0.05 = "*", 0.1 = "*").

Table 2: Number of clusters that best represent the data according to the EM algorithm (Expectation – Maximization) for the different species under study, n is the sample size, df represents degrees of freedom, BIC represents Bayesian in- formation criterion, and ICL represents Integrated Complete-data Likelihood.

Chapter 2

Table 1. Regression linear model of physiological traits (cephalothorax length, heat coma, chill coma and metabolic rate) for the three crab species.

Table 2: *Cyclograpsus cinereus.* Phenotypic matrices for each locality. Values above diagonal show the results obtained through the matrix comparison of the **Barlett test**. Values under the diagonal show the results obtained through **Jackknife-MANOVA**. Blackened values corresponds to significant differences.

Table 3: *Petrolisthes violaceus.* Phenotypic matrices for each locality. Values above diagonal show the results obtained through the matrix comparison of the **Barlett test**. Values under the diagonal show the results obtained through **Jackknife-MANOVA**. Blackened values corresponds to significant differences.

Table 4: *Petrolisthes tuberculosus.* Phenotypic matrices for each locality. Values above diagonal show the results obtained through the matrix comparison of the **Barlett test**. Values under the diagonal show the results obtained through **Jackknife-MANOVA**. Blackened values corresponds to significant differences.

Table 5: Results of matrix comparisons (Bartlett test, Jackknife-MANOVA and MCMC-MANOVA) for the populations of crabs grouped by geographic regions (north, center, south). Blackened values corresponds to significant differences. Ovaskainen D is an estimate of differences in the underlying probability distribution for two given **P**-matrices. P_{max} corresponds to the major axis of phenotypic variation in each P. The angle Between

 P_{max} follows Krzanowski (1979). Sum Volume is a method for estimating total phenotypic variance. TCI is the proportion in variation in the second locality that is explained by the major axis of variation of the first locality. With the exception of Ovaskainen D all derived metrics are presented as the difference in each metric between the two localities being compared. Bold rows are significant. For details on estimating the metrics, determining significance and further definitions, see Robinson and Beckerman (2013).

Table 6: Trait loadings onto the first axis, PC1, in each geographic region.

Chapter 3

Table 1. Physiological rates (mean ± SD) of ovigerous and non-ovigerous female of *Petrolisthes violaceus* and *Petrolisthes granulosus* along a latitudinal gradient.

Table 2. Summary results comparing ovigerous and non-ovigerous female of *Petrolisthes violaceus* metabolic rate, absorption efficiency, excretion rate, food ingestion and scope for growth by repeated measures (ANOVA) along a latitudinal gradient. Significant p-values (p <0.05) are shown in bold.

Table 3. Summary results comparing ovigerous and non-ovigerous female of *Petrolisthes granulosus* metabolic rate, absorption efficiency, excretion rate, food ingestion and scope for growth by repeated measures (ANOVA) along a latitudinal gradient. Significant p-values (p < 0.05) are shown in bold.

FIGURE CAPTIONS

Chapter 1

Figure 1: Geographic and environmental gradient of the study area and thermal tolerances of the study organisms along the southern Pacific. (A) Study sites along the Chilean coast. Mean (black points, mean ± standard deviation), maximum (red points) and minimum temperature registered (blue points) at the sampling sites. Critical temperatures of the different studied organisms; (B) *Cyclograpsus cinereus;* (C) *Petrolisthes violaceus* and (D) *Petrolisthes tuberculosus*. (blue line = Critical Thermal Minimum, red line = Critical Thermal Maximum, mean ± standard deviation).

Figure 2. Latitudinal effect on various morphological and physiological traits (cephalothorax length, heat coma, chill coma, and metabolic rate). *Cyclograpsus cinereus* (A, B, C, D), *Petrolisthes violaceus* (E, F, G, H) and *Petrolisthes tuberculosus* (I, J, K, L). Both lines model the tendency of the data, in particular the red line is a linear regression whereas the blue line is a polynomial regression adjusted to the means values of each trait.

Figure 3. Relative distance plasticity index (RDPI) estimated for each species and calculated for each trait along the latitudinal gradient. The figure shows the mean and standard deviation for the three species studied. Different letters indicate significant differences between crab species (Tukey post hoc, error 5%).

Figure 4. Correct assignments with performance measures for the different algorithms used to classify the three species: Naive Bayes (NB); Linear discriminant analysis (LDA); k nearest neighbors (knn); Neural networks (ANN). (A) Fine model; (B) Coarse model.

Chapter 2

Figure 1: Geographic and environmental gradient along the southeastern Pacific. (A) Study sites along the Chilean coast; (B) SST (mean \pm SD) registered *in situ* ;(C) Latitudinal variability of annual in situ SST (mean \pm SD) recorded at the low intertidal level of the rocky intertidal in the study sites. (D) Coefficient of variation 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.

Figure 2. Latitudinal effect on various morphological and physiological traits: (A) cephalothorax length, (B) metabolic rate, (C) chill coma and (D) heat coma in *Cyclograpsus cinereus, Petrolisthes violaceus* and *Petrolisthes tuberculosus*.

Figure 3. Subspace representation of the phenotypic variance–covariance in pairwise comparisons along the latitudinal gradient. The hulls are an ordinated (PCa) representation of the P-matrix. The size of the hull is related to estimates of total genetic variance (Table 5) while the rotation of the hulls is related to the angles separating the major axes of pheno- typic variance (Table 5 Pmax). Within and across indicate if the comparison was within or across biogeographic provinces. In each comparison, the black hull captures the phenotypic variance–covariance of the locality named first. It is also included in the north–south com- parison as reference.

Chapter 3

Figure 1: Geographic and environmental gradient along the southeastern Pacific. (A) Study sites along the Chilean coast; (B) SST (mean \pm SD) registered *in situ* ;(C) Latitudinal variability of annual in situ SST (mean \pm SD) recorded at the low intertidal level of the rocky intertidal in the study sites. (D) Coefficient of variation 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.

Figure 2: *Petrolisthes violaceus*: (A) Metabolic Rate, (B) Faeces Rate, (C) Excretion Rate; (D) Food Ingestion, along a latitudinal gradient. Blue circles represent non-ovigerous females, red squares represent ovigerous females (mean ± SD).

Figure 3: *Petrolisthes granulosus*: (A) Metabolic Rate, (B) Faeces Rate, (C) Excretion Rate; (D) Food Ingestion, along a latitudinal gradient. Blue circles represent non-ovigerous females, red squares represent ovigerous females (mean ± SD).

Figure 4: Scope for Growth (SFG) of (A) *Petrolisthes violaceus*, (B) *Petrolisthes granulosus* along a latitudinal gradient. Blue circles represent non-ovigerous females; red squares represent ovigerous females (mean ± SD).

Figure 5: Life-history traits, (A) number (blue line) and volume (red line) of egg in *Petrolisthes violaceus* (B) number (blue line) and volume (red line) of egg in *Petrolisthes*

granulosus, (C) reproductive output *P. violaceus* (D) reproductive output *P. granulosus* (mean ± SD).

ABSTRACT

In intertidal marine crustaceans, phenotypic variation in physiological and lifehistory traits is pervasive along latitudinal clines. However, organisms have complex phenotypes, and their traits do not vary independently but rather interact differentially between them, effect that is caused by genetic and/or environmental forces. One of the abiotic factors that most influences organisms is the temperature that affects practically all the biochemical and physiological processes of organisms, which has been linked to growth, survival and reproduction patterns. In this sense, these ectothermal organisms have had to adapt to different environmental conditions, modifying or adjusting their physiology and / or behavior to tolerate the extreme environmental conditions of the intertidal. This allows us to ask some questions such as: 1) Are there compromises between physiological components and life-history traits in marine ectothermal organisms?. 2) What is the role in acclimatization to different geographical gradients in physiological responses (thermal tolerances) in ectothermic marine organisms? 3) Does the integration of physiological traits responses vary between populations of different species?. To answer these questions, we evaluate the phenotypic integration of life-history and physiological traits based on geographic variations found at latitudinal and intertidal levels. This study covered a latitudinal gradient of approximately 3,000 km from the coast around Iguigue to the coast of Chiloé; we study different populations of decapod crabs that are distributed in vertical bands in the rocky intertidal zone exposing themselves to different thermal variations. Our main results reflect (1) Variations in physiological traits with latitude appear to be a plastic response controlled primarily by temperature, rather than a fixed traits of site-specific populations, therefore, population differences in physiological rates result in a differential mean in SFG of ovigerous and non-ovigerous females, which could be related to the frequency of reproductive events in those species that are distributed across wide geographical gradients. (2) The species that inhabit the intertidal-high maintain a greater integration between their physiological traits and greater thermal tolerances and present less plasticity than those that inhabit the zones of low intertidal. (3) The physiological behavior of the studied species differs along a clinal gradient, and the differentiation of the P-Matrix depends mainly on the microhabitat in which these species are distributed vertically in addition to the environment barriers such as biogeographic breaks.