

Role of temperature and carbonate system variability on a host-parasite system: Implications for the gigantism hypothesis

M.R. García-Huidobro^{a,b,*}, O. Varas^d, M. George-Nascimento^c, J. Pulgar^d, M. Aldana^{a,b},
M.A. Lardies^e, N.A. Lagos^a

^a Centro de Investigación e Innovación para el Cambio Climático (CiiCC), Facultad de Ciencias, Universidad Santo Tomás, Ejército 146, Santiago, Chile

^b Programa de Doctorado en Conservación y Gestión de la Biodiversidad, Facultad de Ciencias, Universidad Santo Tomás, Ejército 146, Santiago, Chile

^c Departamento de Ecología, Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Casilla 297, Concepción, Chile

^d Departamento de Ecología y Biodiversidad, Facultad de Ciencias de la Vida, Universidad Andres Bello, República 440, Santiago, Chile

^e Departamento de Ciencias, Facultad de Artes Liberales, Universidad Adolfo Ibáñez, Santiago, Chile

ARTICLE INFO

Keywords:

Environmental variability
shell
Body size
mussels
parasites
Chilean coast

ABSTRACT

Biological interactions and environmental constraints alter life-history traits, modifying organismal performances. Trematode parasites often impact their hosts by inducing parasitic castration, frequently correlated with increased body size in the host (i.e., gigantism hypothesis), which is postulated to reflect the re-allocation of energy released by the reduction in the reproductive process. In this study, we compared the effect of a trematode species on shell size and morphology in adult individuals of the intertidal mussels *Perumytilus purpuratus* (> 20 mm) collected from two local populations of contrasting environmental regimes experienced in central-southern Chile. Our field data indicates that in both study locations, parasitized mussels evidenced higher body sizes (shell length, total weight and volume) as compared with non-parasitized. In addition, parasitized mussels from the southern location evidenced thinner shells than non-parasitized ones and those collected from central Chile, suggesting geographical variation in shell carbonate precipitation across intertidal habitats of the Chilean coast. In laboratory conditions, mussels collected from a local population in central Chile were exposed to two temperature treatments (12 and 18 °C). Parasitized mussels showed higher growth rates than non-parasitized, regardless of the seawater temperature treatments. However, the metabolic rate was not influenced by the parasite condition or the temperature treatments. Our field and laboratory results support the parasite-induced gigantism hypothesis, and suggest that both the thermal environment and geographic location explain only a portion of the increased body size, while the parasitic condition is the most plausible factor modulating the outcome of this host-parasite interaction.

1. Introduction

Marine molluscs serve as hosts to a diverse group of parasites and diseases caused by agents including viruses, prokaryotes, fungi, protists, parazoans and metazoans (e.g. plathelminths) (Lauckner, 1983). Parasites, especially trematodes, are widespread in intertidal ecosystems, and usually require several hosts from the animal community to complete their life cycle (Mouritsen and Poulin, 2002). All trematode parasites use mollusc species as a first intermediate host, lodging in the gonads or digestive tissue (Lauckner, 1983; Cremonte et al., 2015). During this period, the parasite reproduces asexually and produces large numbers of infectious stages known as *cercariae* infective stages, which may occupy the volume of the host's reproductive tissue, a

phenomenon referred to as parasite-induced castration (Souza, 1983; Minchella, 1985). In some cases, this increased level of parasitic castration correlated with the growth rate of the host species (e.g. Minchella, 1985; Mouritsen and Jensen, 1994; Ballabeni, 1995; Gorbushin, 1997; Keas and Esch, 1997), led to postulate the parasite-induced gigantism in the host (Mouritsen and Jensen, 1994; Gorbushin, 1997; Gorbushin and Levakin, 1999; Sorensen and Minchella, 2001; Moore, 2002; Ebert et al., 2004).

Gigantism is frequently interpreted in terms of the redistribution of the resources made available by parasitic castration - in the same host (Baudoin, 1975). Studies suggest that resources of the infected host allocated to reproduction, are then relocated to host maintenance and growth, or deviated into parasite growth and reproduction (McCarthy

* Corresponding author. Centro de Investigación e Innovación para el Cambio Climático (CiiCC), Facultad de Ciencias, Universidad Santo Tomás, Ejército 146, Santiago, Chile.

E-mail address: mgarciahuidobro@santotomas.cl (M.R. García-Huidobro).

<https://doi.org/10.1016/j.ijppaw.2019.03.016>

Received 2 January 2019; Received in revised form 21 March 2019; Accepted 21 March 2019

2213-2244/ © 2019 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

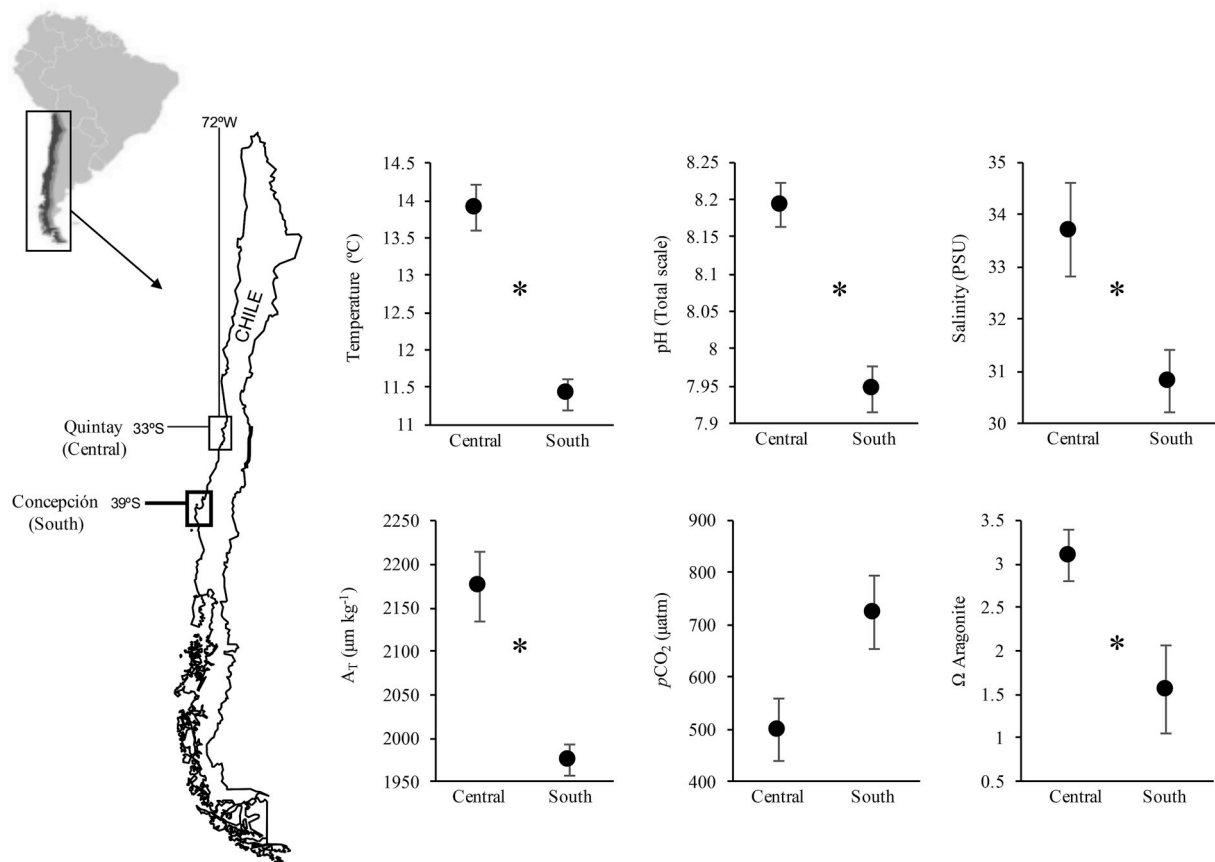


Fig. 1. Study sites along the Chilean coast (Quintay and Concepción), and variability in temperature, pH, salinity, total alkalinity, partial pressure of CO_2 , and aragonite saturation state. Bars indicate ± 1 standard error. Asterisk represents significant differences between sites ($p < 0.001$).

et al., 2004). However, the occurrence and generality of gigantism in mollusc–trematode associations have been discussed (see references in McCarthy et al., 2004). For instance, the freshwater snail *Helisoma anceps* does not show gigantism following parasitic castration by the larvae trematodes of *Halipegus occidualis*, *Echinostoma trivolis* and *Diplostomulum scheuringi* (Fernandez and Esch, 1991). On the contrary, the marine snail *Hydrobia ulvae* evidenced gigantism when infected by parasites of the Microphallidae and Heterophidae families (Gorbushin, 1997). This variability suggests that the magnitude of the reallocation of resources (and the gigantism) may depend on the life–history of the host (Sousa, 1983), food availability (Mouritsen and Poulin, 2002) and environmental conditions (Sorensen and Minchella, 2001).

Ecological theory and empirical evidence show that environmental conditions strongly influence the evolution of life–history traits of the populations (Stearns, 2002). Thus, hosts and parasites (and the result of their interaction) could be affected according to the environmental regime of the local habitat where the interaction occurs (e.g., Sorensen and Minchella, 2001). For instance, several studies have indicated that warmer environments are favourable to parasites, stimulating growth rates, reproductive capacity and infective states, and reducing the generational time (Poulin, 2006; Thieltges and Rick, 2006; Morley, 2011; Morley and Lewis, 2013). Additionally, high temperatures resulting in low oxygen levels in seawater could stress the hosts, increasing the disease susceptibility (Holmes, 1996). However, there are few studies exploring the role of environmental variability in the mollusc–parasite interaction, specifically on body size changes that could be ascribed to the occurrence of parasite-induced gigantism in marine molluscs (see Cressler et al., 2014; e.g. gastropods: Gorbushin, 1997; Gorbushin and Levakin, 1999; McCarthy et al., 2004; Chapuis, 2009; e.g. bivalves: Taskinen, 1998).

The Chilean coast is particularly suitable for the study of host–

parasite relationships across contrasting environments. For instance, along the central–southern Chilean coast, the annual average of surface seawater temperature (SST) ranges from 13.5 °C in Valparaíso (central Chile, ca. 33 °S latitude) to 10 °C in Concepción (southern Chile; ca. 38 °S latitude) (Data available for the last 10 years [www.shoa.cl/nuestros-servicios/tsm]). In addition, the mussel *Perumytilus purpuratus* (Bivalvia: Mytilidae) inhabits the south-eastern Pacific mid-intertidal rocky shores and forms dense three-dimensional matrices that provide biogenic microhabitats for several intertidal species (Prado and Castilla, 2006). This broadly distributed bivalve is the first intermediate host for, at least, three trematode species (Platyhelminthes: Trematoda) including: 1) *Proserhynchoides carvajali* (Bucephalidae); 2) *Proctoeces* sp. (Fellodistomidae); and 3) an undetermined trematode, probably of the Fellodistomidae family (Aldana et al., 2009; Oliva et al., 2010; Muñoz et al., 2013a; b). All these trematode species are found in the sporocyst stage in the reproductive tissue of adult *P. purpuratus*, whose posterior stages of the life-cycle include intertidal and subtidal fishes as definitive hosts (Aldana et al., 2009; Muñoz et al., 2013a).

Along the coast of central Chile, the undetermined trematode species show the highest prevalence in *P. purpuratus*, reaching values close to 2% (Muñoz et al., 2013b), but unpublished data show a variable prevalence throughout central–southern Chile (between 1.7% and 13.1%). Recent studies showed no effect of the undetermined trematode species upon the metabolism of *P. purpuratus* (Castro-Rojas et al., 2015). However, less attention has been given to the potential effects of this parasite on shell characteristics and the energetic budget (i.e. metabolism and growth rate) of *P. purpuratus*, and how this effect interacts with the environmental variability. In this sense, our aim was to compare shell size attributes (i.e., shell length, weight, volume, thickness, calcium carbonate content and biomass) between parasitized and non-parasitized individuals, from two adult populations of *P. purpuratus*,

geographically distant with differences in Sea Surface Temperature along the Chilean coast. In addition, we evaluated, under experimental conditions, the role of temperature on growth rates and metabolism, by exposing parasitized and non-parasitized mussels, collected from a population in Chile, to two temperature levels (12 and 18 °C). As the processes of calcification, metabolism and maintenance depend on temperature, we hypothesize that gigantism and the size variables of adult populations of *P. purpuratus* will interact with parasitism and environmental conditions in each locality.

2. Materials and methods

2.1. Animal collection

During August and September 2017, we sampled 575 adult (mature) individuals of *P. purpuratus* (Muñoz et al., 2013b) from the mid-inter-tidal zone of two locations, central (Quintay, $n = 349$) (32°43'S) and southern Chile (Concepción, $n = 227$) (37°08'S). These locations are separated by ca. 800 km, and experience contrasting patterns of natural variability in temperature occurring in the region (Fig. 1) (e.g., Gaitán-Espitia et al., 2017). Since the parasite infection occurs only upon the reproductive tissue of mature mussels, our study is based on the collection of individuals larger than 20 mm in maximum shell length (Muñoz et al., 2013b). Mussels were deposited in plastic bags, labelled, and transported to the laboratory where they were frozen (−20 °C) until their dissection to evaluate the prevalence of trematode infection and shell morphology (see below). Additionally, adult individuals of *P. purpuratus* were collected ($n = 500$) from central Chile (Quintay) and transported to the laboratory (Universidad Santo Tomás, Santiago), for 7 d of acclimation. During acclimation, the animals were maintained in several aquariums (9 L) with a natural photoperiod and aerated seawater (temperature = 15.2 ± 0.4 °C, $pH_{NBS} = 8.138 \pm 0.164$, salinity = 33.77 ± 1.22 PSU), and fed *ad libitum* with commercial food PhytoGreen-S (BrightwellAquatics®). During the first day of acclimation, the mussels were assayed for trematode infection. The procedure consists of exposing mussels to physical conditions that trigger cercarial emergence: warmed seawater (25 °C) and constant light (Castro-Rojas et al., 2015). After emergence, the trematode species were identified by examining the cercarial stage under a stereomicroscope, and comparing their morphology with published descriptions (i.e., Muñoz et al., 2013a; b). Mussels positively identified as hosting the same parasite species (i.e., undetermined Fellodistomidae), were considered as parasitized ($n = 18$). Another group of mussels ($n = 18$) that did not release cercariae (i.e., non-parasitized controls) were maintained under the same conditions mentioned above. At the end of the experiment, all the individuals were dissected to confirm the condition of parasitism. After the emergence test and dissection process, 100% of the identified parasitized mussels were found to be properly classified. All selected mussels were marked using bee-tags glued onto the anterior section of their shell (e.g., Ramajo et al., 2016).

Table 1

Environmental parameters (Mean $\pm$ SE) measured during experimental period. The values correspond to 2 discrete samples collected every week in each replicate ($n = 24$ for treatment; $n = 96$ total). Asterisk represents significant differences between groups ($p < 0.001$).

Environmental parameters	12 °C		18 °C	
	Parasitized	Non-parasitized	Parasitized	Non-parasitized
pH (25 °C)	8.15 ± 0.03	8.09 ± 0.05	8.04 ± 0.05	8.08 ± 0.04
Temperature (°C) *	11.3 ± 0.1	11.16 ± 0.07	17.6 ± 0.07	17.66 ± 0.09
Salinity (PSU)	34.4 ± 0.23	34.38 ± 0.23	34.27 ± 0.07	34.17 ± 0.07
A_T ($\mu\text{mol Kg}^{-1}$)	2254.5 ± 4.7	2268 ± 10.9	2239.2 ± 14	2268.3 ± 16.1
pCO_2 (μatm)	545.9 ± 51.6	790.2 ± 180.3	667.9 ± 121.2	590.1 ± 115.6
Ω aragonite	2.88 ± 0.15	2.72 ± 0.2	2.9 ± 0.24	3.11 ± 0.21

2.2. Experimental setup

After the acclimatization period, the mussels were randomly separated into groups of three individuals each and placed in 1 L aquaria ($n = 6$ control and 6 parasitized groups, respectively). Each group (replicate) of 3 parasitized or non-parasitized mussels, were randomly placed at two temperature treatments of 12 and 18 °C, controlled using a chiller (BOYU® model L350). These selected temperature levels represent the mean for winter and summer months (data available for the last 10 years for Valparaíso, near Quintay [www.shoa.cl/nuestros-servicios/tsm]). At the beginning of the experiment, all individuals had similar shell length (28.1 ± 2.32 mm). The mussels were maintained under these temperature treatments for 35 d. During the experiment, the seawater was renewed every day from a head-tank with constant aeration. In each aquarium, the Temperature (°C) and Salinity (PSU) were monitored every day, while pH_{NBS} and Total Alkalinity (A_T) were monitored every four days (two replicates).

2.3. Measurement of environmental variables in field and laboratory conditions

CTDO casts (Ocean Seven 304plus Logger, © Idronaut) were performed in shallow waters (~2 m in depth) in front of each rocky platform to characterize Sea Surface Temperature (SST) and salinity concentration variability at each study site in central and southern Chile. Simultaneously, three water samples were collected and processed for pH_{NBS} using a Metrohm pH-meter (model pH mobile 826), connected to a combined electrode (double junction), calibrated using Metrohm buffers (pH = 4.0, 7.0 and 9.0) at 25 °C in a temperature controlled water bath (Torres et al., 2011). At the same time, discrete samples of 500 ml of seawater were collected for Total Alkalinity (A_T) analyses, poisoned in situ using mercuric chloride (0.2 cm^3 of a 50% saturated solution), and maintained in dark conditions and ambient temperature until analysis in the laboratory. Measurements of A_T were done using potentiometric titration which the Metrohm Titrando 888 (Haraldson et al., 1997). Temperature, salinity, pH and A_T data were used to calculate the carbonate system parameters (pCO_2 , aragonite saturation [Ω_{ar}]). Analyses were performed using CO2SYS software in MS Excel (Pierrot et al., 2006) set with Mehrbach solubility constants (Mehrbach et al., 1973), refitted by Dickson and Millero (1987). Similar carbonate system measurements were performed in the seawater of replicates aquaria subjected to experimental temperature treatments. In this experiment, temperature was the only variable that presented differences between treatments, while the rest of the parameters were constant throughout the experimental period (Table 1).

2.4. Parasitism effects on body size of *P. purpuratus*

For all *P. purpuratus* collected from the field, the effect of parasitism on body size was evaluated through an allometric scaling relationship. The mussel shell length (l), width (w), and height (h) were measured with a digital caliper (± 0.1 mm), and the total weight was measured

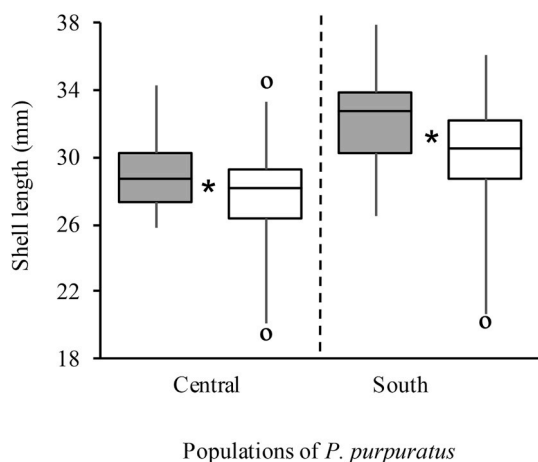


Fig. 2. Total shell length of adults parasitized (gray bars) and non-parasitized (white bars) individuals of *P. purpuratus* from central (Quintay) and southern populations (Concepción). Bars indicate ± 1 standard error. Asterisk indicates significant differences between parasitism condition ($p < 0.001$).

with an analytical balance (Mettler Toledo ± 0.0001 g). From these measurements, the volume of each mussel was approximated to an ellipsoid volume: $[(4\pi/3) \times (l \times w) \times (h)]$ (Calvo-Ugarteburu and McQuaid, 1998). Later, individuals were dissected to determine the condition of parasitism, and the soft tissue weight and shell weight were measured using the analytical balance. The shell thickness was estimated using the relationship between shell weight and shell surface area (Briones et al., 2014). The shell surface incorporated the possible effect of the valve curvature (using shell depth or width as a proxy variable) on the surface estimation, and was based on the formula of shell surface proposed by Reimer and Tedengren (1996): $[l(h^2 + w^2)^{0.5} \pi/2]$, where l , h , and w were the maximum shell length, height, and width, respectively. The CaCO_3 content of the mussel shells was estimated by calcination at 500°C for 4 h to remove the organic components of the CaCO_3 matrix (e.g., Watson et al., 2012). Calcinated shells were stored in desiccators for 1 h and then weighed.

For *P. purpuratus* exposed to temperature treatments, growth and metabolic rate (oxygen consumption) were quantified at the end of the experimental exposure. Growth rate (mm d^{-1}) of *P. purpuratus* was estimated from changes in the maximum shell length between the start and end of the experimental period (e.g., Osorio et al., 2017). The oxygen consumption was measured (as oxygen consumption, in $\text{mg O}_2 \text{ h}^{-1} \text{ g}^{-1}$) by using a Presens Mini Oxy-4 respirometer (e.g., Benítez et al., 2018). The experimental animals were individually placed in respirometer chambers filled with 70 ml of seawater and oxygen-saturation through air bubbling (15 min). The measurements were performed at a controlled temperature of 12 and 18°C using an automated temperature chiller. In each chamber, dissolved oxygen was quantified every 15 s over the course of at least 60 min. Oxygen sensors were previously calibrated in anoxic water using a saturated solution of Na_2SO_3 , and in water 100% saturated with oxygen using bubbled air. The same chambers, without animals, were used for controls, and the oxygen concentration did not decay more than 3%. Oxygen decay due to back-ground noise was deducted from the individual measurements.

2.5. Statistical analysis

Natural variation in environmental parameters was compared between study sites from southern and central Chile using a one-way ANOVA. We performed two-way ANOVA to test for differences in maximum shell length of *P. purpuratus* between parasitized condition and sites, regarded as fixed and random factors, respectively. The sites were considered as random factors because our conclusions are not limited at these levels (Bennington and Thayne, 1994). Analyses of

covariance (ANCOVA) were used to compare total weight, soft tissue weight, volume, shell weight and CaCO_3 weight between sites (Quintay and Concepción), and parasitized condition, with the maximum shell lengths used as covariates. In the case of shell thickness, ANCOVA analysis incorporated the shell weight as covariate while correcting for shell surface. Previous to these analyses, all variables were log transformed. We show the Least Square Means (LSM), the predicted response of parasitized and non-parasitized, at the mean value of the covariate for each morphological variable. The corresponding bivariate regression analyses and their parameters estimation is presented as supplementary material. Finally, growth and metabolic rate of mussels were compared between experimental temperature treatments and parasite condition using two-way ANOVA. In all cases, a Tukey post-hoc analysis was performed to determine significant differences between groups (Zar, 1996). All analyses were performed using Minitab 14[®] ver 13.3.2.

3. Results

3.1. Environmental variability at study site and laboratory experimental treatments

Both study sites exhibited contrasting environmental conditions in pH_{NBS} , SST, salinity, and Ω aragonite saturation state (Fig. 1). In general, the site of central Chile (Quintay) showed higher SST, pH, salinity, and Ω aragonite than the southern (i.e. Concepción) site. Under laboratory conditions, these environmental parameters remained stable, only allowing for significant differences in seawater temperature between treatments (Table 1).

3.2. Parasite effect on adults of *P. purpuratus*

The prevalence of undetermined trematode in *P. purpuratus* was 11.1% and 18.5% for the central and southern site, respectively. The shell length of the adult fraction of *P. purpuratus* (> 20 mm) (sexual maturity in this species is reached at 10 mm), was higher in parasitized than non-parasitized mussels, and this pattern was independent of the collection sites (see Fig. 1 in supplementary material). The mature portion of the population of *P. purpuratus* collected at both sites, also showed geographic variability patterns, in which mussels from southern Chile (Concepción) evidenced a significant increase in shell length compared to those collected from Central Chile (Quintay; Fig. 2, Table 2). Both factors operate additively, evidenced by a non-significant Site $\times$ Parasite condition ($p = 0.102$, Table 2).

Comparing morphological variables between mussels with different parasite conditions at the mean shell length (i.e., LSM value of the covariate in ANCOVA), the total weight and volume increased significantly in parasitized compared to non-parasitized mussels at both study sites (Fig. 3A, D, Table 3). In addition, the total weight and volume of the mussels were higher in the southern site than in the central site (Fig. 3A, D). The soft tissue weight was lower in parasitized than non-parasitized individuals in both study sites. Additionally, the soft tissue weight was lower in the central than in the southern population (Fig. 3B, Table 3). Furthermore, shell weight was higher in parasitized than in non-parasitized individuals at the central site, but in the southern site, the individuals did not show differences between

Table 2

Two-way analysis of variance (ANOVA) for shell length of *Perumytilus purpuratus* comparing between study sites (Central and South) and parasitized and non-parasitized conditions.

Source of variation	df	MS	F	p-value
Site (S)	1	476	71.04	< 0.001
Parasitized condition (Pc)	1	145.1	21.65	< 0.001
S $\times$ Pc	1	17.8	2.65	0.102
Error	571	6.7		

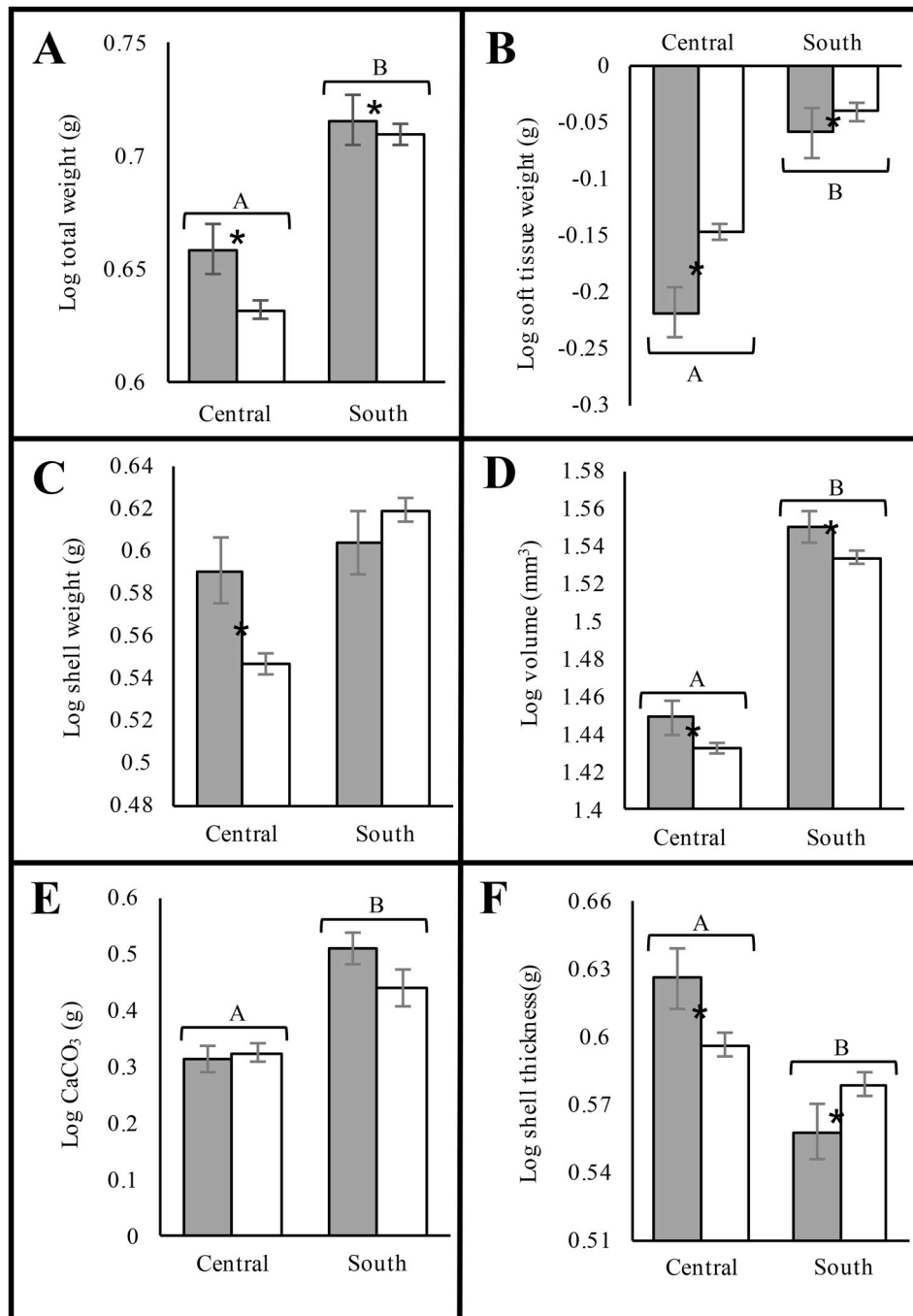


Fig. 3. Least square means (LSM), the predicted value of the response variable at the mean value of the covariate (Shell Length) in ANCOVA. Responses are (A) total weight, (B) soft tissue weight, (C) shell weight, (D) volume, (E) CaCO₃, and (F) LSM of the shell surface of *P. purpuratus* from central (Quintay) and southern populations (Concepción). Gray bars represent parasitized individuals and white bars, non-parasitized individuals. Asterisk indicates significant differences between parasitism condition, and letter indicates significant differences between sites ($p < 0.001$).

parasitized conditions (Fig. 3C). The CaCO₃ content was similar between parasitized conditions, but higher in the southern site than in the central Chile site (Fig. 3E, Table 3). The total weight, soft tissue weight, shell weight and volume correlated positively with the shell length of *P. purpuratus*, but not the CaCO₃ content (see Figs. 2 and 3 in supplementary material).

Shell thickness was higher in parasitized individuals than in non-parasitized individuals at the central site (Fig. 3F, Table 3), but an inverse pattern was recorded at the southern Chile site (Fig. 3F, Table 3). In both sites, the shell weight scaled positively with the shell surface (see Fig. 3 in supplementary material).

For the acute exposition period used in this study, the growth rate was higher in parasitized than non-parasitized mussels, and this effect was independent of temperature treatments (Fig. 4A, Table 4). However, oxygen consumption showed no difference between parasitism and experimental temperatures (Fig. 4B, Table 4).

4. Discussion

Our field and laboratory results about mussel body size and growth rates, respectively, support the parasite-induced gigantism hypothesis, but the effect of the parasite on other traits associated with the shell of

Table 3

Analysis of covariance (ANCOVA) results evaluation total weight, soft tissue weight, shell weight, volume, CaCO₃ weight and shell thickness of *Perumytilus purpuratus* between study sites (central and south) and parasitized condition.

Variable	Hypothesis $b_p = b_{Np}$	ANCOVA			
		Source of Variation	df	F	p-value
Total weight (g)	$F_{(1, 565)} = 0.55$ $P = 0.455$	Site (S)	568	60.79	0.048
		Parasitized condition (Pc)	568	3.84	0.006
		$S \times Pc$	568	1.59	0.213
		Site (S)	542	62.44	< 0.001
Soft tissue weight (g)	$F_{(1, 539)} = 1.21$ $P = 0.271$	Parasitized condition (Pc)	542	7.55	0.006
		$S \times Pc$	542	2.54	0.111
		Site (S)	541	13.57	< 0.001
		Parasitized condition (Pc)	541	1.53	0.215
Shell weight (g)	$F_{(1, 538)} = 0.09$ $P = 0.763$	$S \times Pc$	541	6.99	0.008
		Site (S)	570	220.09	< 0.001
		Parasitized condition (Pc)	570	5.98	0.014
		$S \times Pc$	570	0.001	0.974
Volume (mm ³)	$F_{(1, 657)} = 0.001$ $P = 0.974$	Site (S)	81	31.94	< 0.001
		Parasitized condition (Pc)	81	1.34	0.249
		$S \times Pc$	81	2.35	0.129
		Site (S)	538	15.96	< 0.001
CaCO ₃ weight (g)	$F_{(1, 78)} = 0.02$ $P = 0.874$	Parasitized condition (Pc)	538	0.2	0.647
		$S \times Pc$	538	7.15	0.007
Shell thickness	$F_{(1, 538)} = 0.194$ $P = 0.659$	Site (S)	538	15.96	< 0.001
		Parasitized condition (Pc)	538	0.2	0.647
		$S \times Pc$	538	7.15	0.007
		Site (S)	538	15.96	< 0.001

P. purpuratus (weight and thickness of shell) seems to be influenced by the environmental factors of the site where the interaction takes place. Thus, suggesting that the host-parasite interaction may be modulated

by seawater temperature that interplays, probably with carbonate system variability (pH, alkalinity, saturation state) in local habitats along the coast.

Trematode-induced gigantism in molluscs has been observed in laboratory studies (Rothschild and Rothschild, 1939; McClelland and Bourns, 1969; Mouritsen and Jensen, 1994). Nevertheless, field studies have failed to show gigantism unambiguously (Sorensen and Minchella, 2001; McCarthy et al., 2004). These contrasting results may emerge from the biological responses used to characterize this phenomenon. For instance, most of the studies have focused on changes in the body size, growth rate and/or the shape of the mollusc hosts (e.g. in snail: Gorbushin, 1997; Gorbushin and Levakin, 1999; McCarthy et al., 2004; Chapuis, 2009; in bivalve: Taskinen, 1998). However, less attention has been given to variables such as shell thickness, biomass, and the calcium carbonate content precipitated in the shell by the host.

Our findings agree with the phenomenon of gigantism (length, total weight and volume of the mussel shells) induced by trematodes. These results also agree with Bergmann's rule, increase in body size with increases in latitude (central vs southern site), which has been reported for free-living marine invertebrates (see Lardies and Castilla, 2001); but our results highlight the additive effect of parasitism on the host size in the latitudinal gradient. However, the shell weight and thickness of the mussels varies significantly according to site and parasitized condition. In marine molluscs, the shell is an important structure associated with growth and survival (see Marin et al., 2012), its construction is

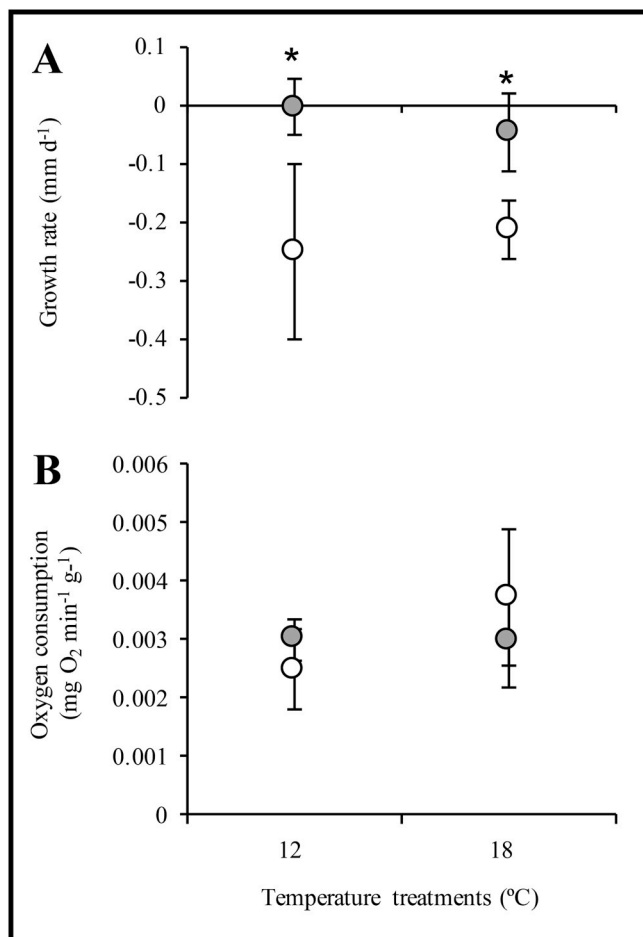


Fig. 4. (A) Growth and (B) metabolic rates of parasitized (gray bars) and non-parasitized (white bars) of *P. purpuratus* from central site (Quintay), subject to 12 and 18 °C. Bars indicate ± 1 standard error. Asterisk indicates significant differences between parasitism condition ($p < 0.001$).

Table 4

Two-way analysis of variance (ANOVA) for growth rate and oxygen consumption of *Perumytilus purpuratus* collected from Central site (Quintay) comparing between temperature treatments (12 °C vs 18 °C) and parasitized and non-parasitized conditions.

Biological Responses	Source of variation	df	MS	F	p-value
Growth rate (mm d ⁻¹)	Temperature (T)	1	0.066	2.30	0.139
	Parasitized condition (Pc)	1	0.145	5.04	0.032
	$T \times Pc$	1	0.010	0.36	0.547
	Error	31	0.028		
Oxygen consumption (mg O ₂ h ⁻¹ g ⁻¹)	Temperature (T)	1	0.009	0.01	0.938
	Parasitized condition (Pc)	1	0.080	0.54	0.466
	$T \times Pc$	1	0.064	0.43	0.514
	Error	31	0.148		

energetically expensive (Palmer, 1992), and the carbonate precipitation can be affected by environmental variability (e.g. ocean warming: Yao and Somero, 2014; ocean acidification: Kroeker et al., 2010). In this sense, environmental factors, as well as ontogenetic and evolutionary time, and the life history traits of the host and parasites could modulate the multiple responses associated with this type of biological interaction (Leung and Poulin, 2008).

Despite over 100 years of dedicated effort, the adaptive significance of gigantism to the host and/or parasite is still poorly understood (see Goater et al., 2014). There exist two hypotheses about adaptive significance of parasite-induced gigantism, which make contrasting predictions. First, the host-benefit hypothesis predicts that host size will correlate with the lifetime reproductive success of the infected host (Minchella, 1985; Ballabeni, 1995). This hypothesis requires that infected hosts have a reasonable chance to recover from the infection and resume reproduction. Our result and other reports of digenea in *P. purpuratus* do not provide evidence to support the hypothesis of Minchella (1985). The minimum infection size is approximately 20 mm of shell length (Aldana, 2007; Aldana et al., 2009; Castro-Rojas et al., 2015; Montenegro et al., 2012; Muñoz et al., 2013a; b; Lienqueo, 2008), and the sexual maturity of *P. purpuratus* is reached at 8–10 mm in shell length (Lozada and Reyes, 1981), suggesting that the infection of *P. purpuratus* is posterior to sexual maturity. Although in this study the time of permanence of a parasite in its host and gonadal regeneration during this time were not evaluated, we did not observe gonadal tissue when the mussels were parasitized. Thus, mature individuals were able to spawn before becoming completely castrated by the parasite. Later, after the spawn, molluscs lose their reproduction capacity due to the reduction of gonadal tissue, which allows them to allocate energy for growth and/or resistance to parasites (Arakelova et al., 2003, 2004).

The second hypothesis is that host gigantism is beneficial only for the parasite (Baudoin, 1975; Sousa, 1983). Here, the parasite suppresses host reproduction to make resources available for itself that the host would have used for reproduction. Gigantism is then a by-product of more energy being released by castration than the parasite can use at that time (Ebert et al., 2004). For the gigantism to be an advantage for the parasite, certain criteria must be met: (1) the energy destined for host reproduction is used in parasite reproduction, (2) there must be permanent castration of the parasite to the host, and (3) the increase in size should be associated with greater survival of the host (Mouritsen and Jensen, 1994; Sorensen and Minchella, 2001; Ebert et al., 2004; McCarthy et al., 2004).

Under this scenario, our results show an absence of differences in oxygen consumption between parasitized conditions and temperature treatments, suggesting that the energy demand imposed by parasites on the host does not generate a significant metabolic stress or the hosts are able to reallocate energy to overcome the trade-offs, or that the parasite has anaerobic metabolic pathways (Tielens, 1994; Hechinger, 2013). Similar results have been found for *P. purpuratus* – undetermined cercariae systems in the south of Chile (Concepción) (Castro-Rojas et al., 2015), and in other molluscs (see Strurock (1966) and Thorhill et al. (1986) for *Biomphalaria pfeifferi* and *B. glabrata* parasitized with *Schistosoma mansoni*, respectively). Regarding permanent castration by the parasite, as previously mentioned, that there is no evidence to indicate recovery of reproduction after the parasite is established.

Finally, continuing with the idea that gigantism is advantageous for the parasite, where the increase in size should be associated with greater survival of the host, our results indicate interesting findings. We show that in the site experiencing low temperature, pH and aragonite saturation conditions, the parasitized mussels were larger (length, total weight and volume) than non-parasitized. Although a larger host size generally brings higher rates of reproduction and survival (Roff, 2002), in the context where gigantism is advantageous for the parasite, we believe that other characteristics of the host that denote survival should

be considered, such as the environment where the host-parasite interaction develops (Sorensen and Minchella, 2001). For example, we found that the weight and thickness of the mussel's shells were greater in parasitized than non-parasitized in the central population, but in the southern population, it was the opposite. A possible explanation for this result may be related to the environment in which the calcification and other biological processes are performed. Castillo et al. (2017) showed that the mussel *Mytilus chilensis* triggers a differential gene expression in acidified conditions more than the presence of pathogen *Vibrio anguillarum*. They mentioned that environmental scenarios with lower pH and saturation states might up-regulate specific immune-related genes, promoting the transcription of several molecules against the pathogen. In this way, the energy released as a result of parasitic castration from southern individuals of *P. purpuratus* (in low pH), makes a more expensive immune response than parasitized individuals in the central zone, and this cost is reflected in the thickness of the shell.

In addition, calcification is the process through which calcifying organisms (e.g. mollusc) synthesise CaCO_3 structures from dissolved calcium (Ca^{2+}) and CO_3^{2-} ions in seawater (Weiner and Dove, 2003). In acidic waters, the mussels may reduce the rate of calcification, due to a reduction in the availability of inorganic carbon. A decrease in ambient levels of CO_3^{2-} in seawater may increase the amount of energy required by calcifiers to maintain the concentrations required for the biosynthesis of shells (Beniash et al., 2010; Melzner et al., 2011; Li et al., 2015), and thus, the energetic reassignment due to the effect of the castrating parasites only allows elongation and not thickening of the shell. The shell is a structure directly related to the survival of the mussels, for instance, to confront durophagous predators (Marin et al., 2012). From the point of view of the survival of the parasitized host, for instance, in cold waters, acidic and low carbonate saturation states (e.g. the southern site, see Fig. 1), the bivalves generate longer but thin shells (e.g., Lagos et al., 2016; Osóres et al., 2017), and the survival of the host could decrease, and thus the result of the host-parasite interaction.

Although, we did not evaluate food restrictions, this factor may also underlie our observed results. However, there is evidence that both sites are upwelling centers with similar productivity (Henríquez et al., 2007; Anguita and Simeone, 2015; Pérez et al., 2016). Thus, in field mussel populations, the trematode and environmental variables may significantly alter the shell characteristics of the host, their growth and survival. Whether or not this phenomenon leads to adaptive consequences for the host populations is a question that requires special consideration at each site where host-parasite combination takes place (Lafferty and Holt, 2003). Another enigmatic point is the physiological mechanisms of energy reallocation in the mussels-trematode system, which should be investigated in a laboratory using a multifactorial approach that combines environmental variables (e.g. pH, temperature, salinity, food, etc.).

In this study, we highlight the importance of considering the environmental context when studying the host-parasite interaction. The implications of the gigantism hypothesis can be varied. For example, at the ecosystem level, its suppose a greater demand of calcium carbonate for parasitized mussels, which could affect the carbonate budget at benthic ecosystems (e.g. Mischler et al., 2016; Vennatta and Minchella, 2018). An estimate of the abundance of *P. purpuratus* in central Chile and south-central Chile between 20 and 25 mm in length, showed 700 and 324 individuals in 0.02 m² respectively (Lagos et al., Unpublished data). If we project the prevalence recorded for each locality (11.1 and 18.5% in central and south-central Chile, respectively), yield a rough estimation of approximately 3453 and 2664 parasitized individuals, per square meter, respectively, would be sequestering more calcium carbonate than their non-parasitized counterparts of the same size. This suggest that despite the pervasiveness of parasitism in marine ecosystems, their role upon the carbonate production and cycling deserve for further attention.

Acknowledgements

We thank Josue Navarrete and Antonia Moya for their valuable assistance during laboratory and fieldwork. Much appreciation goes to Charlie Ziese for improving the English of this manuscript. This study was funded by FONDECYT grant No. 3170522 to RGH. PIA CONICYT ANILLOS ACT172037 to NAL, RGH and MAL. R.G.H., N.A.L. and M.A.L. We also appreciate the support from PCI CONICYT REDES170106 and the Millennium Nucleus Center for the Study of Multiple-drivers on Marine Socio-Ecological Systems (MUSELS) funded by MINECON NC120086. JP acknowledges the support of Universidad Andrés Bello Grants DI 16-18/R and FONDECYT grant No. 1171056. The authors would like to thank Geerat Vermeij and an anonymous reviewer for their comments that helped improve this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpaw.2019.03.016>.

References

- Aldana, M., González, K., Loot, G., Pulgar, J., Marquet, P., 2009. First intermediate host of the digenean trematode *Proctoeces lintoni* (Fellodistomidae) in Chile. *J. Parasitol.* 95, 1408–1414.
- Aldana, M., 2007. Variación espacial de la interacción hospedador-parásito: la importancia de la densidad poblacional de los hospedadores. Ph.D. dissertation. Pontificia Universidad Católica de Chile, Santiago, Chile.
- Anguita, C., Simeone, A., 2015. Influence of seasonal food availability on the dynamics of seabird feeding flocks at a coastal upwelling area. *PLoS One* 10, e0131327.
- Arakelova, E.S., Chebotareva, M.A., Zabelinskii, S.A., 2003. On adaptive changes of rate of oxygen consumption and lipid metabolism in *Littorina saxatilis* at parasitic invasions. *J. Evol. Biochem. Physiol.* 39, 519–528.
- Arakelova, K.S., Chebotareva, M.A., Zabelinskii, S.A., 2004. Physiology and lipid metabolism of *Littorina saxatilis* infected with trematodes. *Dis. Aquat. Org.* 60, 223–231.
- Ballabeni, P., 1995. Parasitic-induced gigantism in a snail: a host adaptation? *Funct. Ecol.* 9, 887–893.
- Baudoin, M., 1975. Host castration as a parasitic strategy. *Evolution* 29, 335–352.
- Briones, C., Rivadeneira, M.M., Fernández, M., Guíñez, R., 2014. Geographical variation of shell thickness in the mussel *Perumytilus purpuratus* along the southeast pacific coast. *Biol. Bull.* 227, 221–231.
- Beniash, E., Ivanina, A., Lieb, N.S., Kurochkin, I., Sokolova, I.M., 2010. Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica*. *Mar. Ecol. Prog. Ser.* 419, 95–108.
- Bennington, C.C., Thayne, W.V., 1994. Use and misuse of mixed model analysis of variance in ecological studies. *Ecology* 75, 717–722.
- Benítez, S., Lagos, N.A., Osorio, S., Optiz, T., Duarte, C., Navarro, J.M., Lardies, M.A., 2018. High pCO₂ levels affect metabolic rate, but not feeding behaviour and fitness, of farmed giant mussel *Choromytilus chorus*. *Aquacult. Environ. Interact.* 10, 267–278.
- Calvo-Ugarteburu, G., McQuaid, C.D., 1998. Parasitism and invasive species: effects of digenetic trematodes on mussels. *Mar. Ecol. Prog. Ser.* 169, 149–163.
- Castillo, N., Saaedra, L.M., Vargas, C.A., Gallardo-Escárate, C., Détreé, C., 2017. Ocean acidification and pathogen exposure modulate the immune response of the edible mussel *Mytilus chilensis*. *Fish Shellfish Immun.* 70, 149–155.
- Castro-Rojas, M., Cid-Moya, K., George-Nascimento, M., 2015. Consumo de oxígeno en *Perumytilus purpuratus* según el parasitismo por Digenea, y efectos de la temperatura del ciclo de emersión-inmersión sobre la liberación de cercarias. *Rev. Biol. Mar. Oceanogr.* 50, 315–321.
- Chapuis, E., 2009. Correlation between parasite prevalence and adult size in a trematode-mollusc system: evidence for evolutionary gigantism in the freshwater snail *Galba truncatula*? *J. Molluscan Stud.* 75, 391–396.
- Cremonte, F., Puebla, C., Tillería, J., Videla, V., 2015. Histopathological survey of the mussel *Mytilus chilensis* (Mytilidae) and the clam *Gari solida* (Psammobiidae) from southern Chile. *Lat. Am. J. Aquat. Res.* 43, 248–254.
- Cressler, E.C., Nelson, W.A., Day, T., McCaule, E., 2014. Starvation reveals the cause of infection-induced castration and gigantism. *Proc. R. Soc. B* 281, 20141087.
- Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea. Res.* 34, 1733–1743.
- Ebert, D., Carius, H.J., Little, T., Decaestecker, E., 2004. The evolution of virulence when parasites cause host castration and gigantism. *Amm. Nat.* 164, S19–S32.
- Fernandez, J., Esch, G.W., 1991. Effect of parasitism on the growth rate of the pulmonate snail *Helisoma anceps*. *J. Parasitol.* 77, 937–944.
- Gaitán-Espitia, J., Bacigalupe, L., Optiz, T., Lagos, N.A., Osorio, S., Lardies, M.A., 2017. Exploring physiological plasticity and local thermal adaptation in an intertidal crab along a latitudinal cline. *J. Therm. Biol.* 68, 14–20.
- Goater, T.M., Goater, C.P., Esch, G.W., 2014. Parasitism: the Diversity and Ecology of Animal Parasites, second ed. .
- Gorbushin, A.M., 1997. Field evidence of trematode induced gigantism in *Hydrobia* spp. (Gastropoda: Prosobranchia). *J. Mar. Biol. Assoc. U. K.* 77, 785–800.
- Gorbushin, A.M., Levakin, I.A., 1999. The effect of trematode on the growth of *Onoba aculeus*, *Littorina saxatilis* and *L. abtusata* (Gastropoda: Prosobranchia). *J. Mar. Biol. Ass. U.K.* 79, 273–279.
- Haraldson, C., Anderson, L.G., Hassellöv, M., Hulth, S., Olsson, K., 1997. Rapid, high precision potentiometric titration of alkalinity in ocean and sediment pore waters. *Deep Sea Res.* 44, 2031–2044.
- Hechinger, R., 2013. A metabolic and body-size scaling framework for parasite within-host abundance, biomass, and energy flux. *Am. Nat.* 182, 234–248.
- Henríquez, L.A., Daneri, G., Muñoz, C.A., Montero, P., Veas, R., Palma, A.T., 2007. Primary production and phytoplanktonic biomass in shallow marine environments of central Chile: effect of coastal geomorphology. *Estuar. Coast Shelf Sci.* 73, 137–147.
- Holmes, J.C., 1996. Parasites as threats to biodiversity in shrinking ecosystems. *Biodivers. Conserv.* 5, 975–983.
- Keas, B.E., Esch, G.W., 1997. The effect of diet and reproductive maturity on the growth and reproduction of *Helisoma anceps* (Pulmonata) infected by *Haliplus occidialis* (Trematoda). *J. Parasitol.* 83, 96–104.
- Kroeker, K.J., Kordas, R.L., Crim, R.N., Singh, G.G., 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol. Lett.* 13, 1419–1434.
- Lafferty, K.D., Holt, D.H., 2003. How should environmental stress affect the population dynamics of disease? *Ecol. Lett.* 6, 654–664.
- Lagos, N.A., Benítez, S., Duarte, C., Lardies, M.A., Broitman, B.R., Tapia, C., Tapia, P., Widdicombe, S., Vargas, C.A., 2016. Effects of temperature and ocean acidification on shell characteristics of *Argopecten purpuratus*: implications for scallop aquaculture in an upwelling-influenced area. *Aquacult. Environ. Interact.* 8, 357–370.
- Lardies, M.A., Castilla, J.C., 2001. Latitudinal variation in the reproductive biology of the commensal crab *Pinnaxodes chilensis* (Decapoda: Pinnotheridae) along the Chilean coast. *Mar. Bio.* 139, 1125–1133.
- Lauckner, G., 1983. Introduction: Bivalvia to Scaphopoda. In: Kinne, O. (Ed.), *Bivalvia Diseases of Marine Animals*. Biologische Anstalt Helgoland, Hamburg, pp. 477–977.
- Leung, T.L.F., Poulin, R., 2008. Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. *Vie Milieu* 58, 107–115.
- Li, S., Liu, C., Huang, J., Liu, Y., Zheng, G., Xie, L., Zhang, R., 2015. Interactive effects of seawater acidification and elevated temperature on biomineralization and amino acid metabolism in the mussel *Mytilus edulis*. *J. Exp. Biol.* 218, 3623–3631.
- Lienqueo, A.P., 2008. Parasitismo por tremátodos en dos mitilidos intermareales (*Semimytilus algosus* y *Perumytilus purpuratus*). Lic. dissertation. Universidad Austral de Chile.
- Lozada, E., Reyes, P., 1981. Reproductive biology of a population of *Perumytilus purpuratus* at el Tabo, Chile. *Veliger* 24, 147–154.
- Marin, F., Le Roy, N., Marie, B., 2012. The formation and mineralization of mollusk shell. *Front. Biosci.* S4, 1099–1125.
- McCarthy, H.O., Fitzpatrick, S.M., Irwin, S.W.B., 2004. Parasite alteration of host shape: a quantitative approach to gigantism helps elucidate evolutionary advantages. *Parasitology* 128, 7–14.
- McClelland, G., Bourns, T.K.R., 1969. Effects of *Trichobilharzia ocellata* on growth, reproduction, and survival of *Lymnaea stagnalis*. *Exp. Parasitol.* 24, 137–146.
- Mehrbach, C., Culbertson, C.H., Hawley, J.E., Pytkowicz, R.M., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol. Oceanogr.* 18, 897–907.
- Melzner, F., Stange, P., Trübenbach, K., Thomsen, J., Casties, I., Panknin, U., Gorb, S.N., Gutowska, M.A., 2011. Food supply and seawater pCO₂ impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PLoS Biol.* 6, e24223.
- Minchella, D.J., 1985. Host life-history variation in response to parasitism. *Parasitology* 90, 205–216.
- Montenegro, D., Jones, B., González, M.T., 2012. Report of pathogens and parasites in *Perumytilus purpuratus* from san Jorge Bay, Antofagasta, Chile. *Rev. Biol. Mar. Oceanogr.* 47, 345–350.
- Mischler, J., Johnson, P.T.J., McKenzie, V.J., Townsed, A.R., 2016. Parasite infection alters nitrogen cycling at the ecosystem scale. *J. Anim. Ecol.* 85, 817–828.
- Morley, N.J., Lewis, J.W., 2013. Thermodynamics of cercarial development and emergence in trematodes. *Parasitology* 121, 1211–1224.
- Morley, N.J., 2011. Thermodynamics of cercarial survival and metabolism in a changing climate. *Parasitology* 138, 1442–1452.
- Moore, J., 2002. Parasites and the Behavior of Animals. Oxford University Press, Oxford.
- Mouritsen, K.N., Poulin, R., 2002. Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology* 124, S101–S117.
- Mouritsen, K.N., Jensen, K.T., 1994. The enigma of gigantism – effect of larval trematodes on growth, fecundity, egestion and locomotion in *Hydrobia ulvae* (Pennant) (Gastropoda: Prosobranchia). *J. Exp. Mar. Biol. Ecol.* 181, 53–66.
- Muñoz, G., López, Z., Cárdenas, L., 2013a. Morphological and molecular analyses of larval trematodes in the intertidal bivalve *Perumytilus purpuratus* from central Chile. *J. Helminthol.* 87, 356–363.
- Muñoz, G., Torres, P., Valdés, J., Rodríguez, A., 2013b. Spatio-temporal variation in the prevalence of trematodes in the bivalve *Perumytilus purpuratus*. *Acta Parasitol.* 58, 155–166.
- Oliva, M., Valdivia, I.M., Cardenas, L., George-Nascimento, M., Gonzalez, K., Guíñez, R.E., Cuello, D., 2010. Molecular and experimental evidence of the life cycle of *Proctoeces lintoni* (Fellodistomidae) in Chile. *Parasitol. Res.* 106, 737–740.
- Osorio, S.J.A., Lagos, N.A., San Martín, V., Manríquez, P.H., Vargas, C.A., Torres, R., Navarro, J.M., Poupin, M.J., Saldías, G.S., Lardies, M.A., 2017. Plasticity and inter-population variability in physiological and life-history traits of the mussel *Mytilus chilensis*: a reciprocal transplant experiment. *J. Exp. Mar. Biol. Ecol.* 490, 1–12.
- Palmer, A.R., 1992. Calcification in marine molluscs: how costly is it? *Proc. Natl. Acad. Sci. U.S.A.* 89, 1379–1382.
- Pérez, C.A., Lagos, N.A., Saldías, G.S., Waldbusser, G., Vargas, C.A., 2016. Riverine

- discharges impact physiological traits and carbon sources for Shell carbonate in the marine intertidal mussel *Perumytilus purpuratus*. *Limnol. Oceanogr.* 61, 969–983.
- Pierrot, D., Lewis, E., Wallace, D.W.R., 2006. MS Excel Program Development for CO2 System Calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center. Oak ridge national laboratory, US Department of Energy, Oak ridge, TN.
- Poulin, R., 2006. Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. *Parasitology* 132, 143–151.
- Prado, L., Castilla, J.C., 2006. The bioengineer *Perumytilus purpuratus* (Mollusca: Bivalvia) in central Chile: biodiversity, habitat structural complexity and environmental heterogeneity. *J. Mar. Biol. Ass. U.K.* 86, 417–421.
- Ramajo, L., Prado, L., Rodríguez-Navarro, A.B., Lardies, M.A., Duarte, C.M., Lagos, N., 2016. Plasticity and trade-offs in physiological traits of intertidal mussels subjected to freshwater-induced environmental variation. *Mar. Ecol. Prog. Ser.* 553, 93–109.
- Reimer, O., Tedengren, M., 1996. Phenotypic improvement of morphological defences in the mussel *Mytilus edulis* induced by exposure to the predator *Asterias rubens*. *Oikos* 75, 383–390.
- Rothschild, A., Rothschild, M., 1939. Some observation on the growth of *Peringia ulvae* (Pennant) 1777 in the laboratory. *Novit. Zool.* 41, 240–247.
- Roff, D.A., 2002. The Evolution of Life Histories: Theory and Analysis. Chapman and Hall, New York.
- Sorensen, R.E., Minchella, D.J., 2001. Snail-trematode life history interactions: past trends and future directions. *Parasitology* 123, S3–S18.
- Sousa, W.P., 1983. Host life history and the effect of parasitic castration on growth: a field study of *Cerithidea californica* Haldeman (Gastropoda: Prosobranchia) and its trematode parasites. *J. Exp. Mar. Biol. Ecol.* 73, 273–296.
- Stearns, S.C., 2002. Life-history tactics: a review of the ideas. *Q. Rev. Biol.* 51, 3–47.
- Sturrock, B.M., 1966. The influence of infection with *Schistosoma mansoni* on the growth rate and reproduction of *Biomphalaria pfeifferi*. *Ann. Trop. Med. Parasitol.* 60, 187–197.
- Taskinen, J., 1998. Influence of trematode parasitism on the growth of a bivalve host in the field. *Int. J. Parasitol.* 28, 599–602.
- Thieltges, D.W., Rick, J., 2006. Effect of temperature on emergence, survival and infectivity of cercariae of the marine trematode *Renicola roscovita* (Digenea: Renicolidae). *Dis. Aquat. Org.* 73, 63–68.
- Thorhill, J.A., Jones, J.T., Kusel, J.R., 1986. Increased oviposition and growth in immature *Biomphalaria glabrata* after exposure to *Schistosoma mansoni*. *Parasitology* 93, 443–450.
- Tielens, A.G.M., 1994. Energy generation in parasitic helminths. *Parasitol. Today* 9, 346–352.
- Torres, R., Pantoja, S., Harada, N., González, H.E., Daneri, G., Frangopulos, M., Rutlant, J.A., Duarte, C.M., Rúiz-Halpern, S., Mayol, E., Fukasawa, M., 2011. Air-sea CO2 fluxes along the coast of Chile: from CO2 outgassing in central northern upwelling waters to CO2 uptake in southern Patagonian fjords. *J. Geophys. Res.* 116, C09006.
- Vennatta, J.T., Minchella, D.J., 2018. Parasites and their impact on ecosystem nutrient cycling. *Trends Parasitol.* 34, 452–455.
- Watson, S.A., Peck, L., Tyler, P., Southgate, P., Tan, K., Day, R., Morley, S., 2012. Marine invertebrate skeleton size varies with latitude, temperature, and carbonate saturation: implications for global change and ocean acidification. *Glob. Change. Bio.* 10, 3026–3038.
- Weiner, S., Dove, P.M., 2003. An overview of biomineralization processes and the problem of the vital effect. *Rev. Miner. Geochem.* 54, 1–29.
- Yao, C.-L., Somero, G.N., 2014. The impact of ocean warming on marine organisms. *Chin. Sci. Bull.* 59, 468–479.
- Zar, J.H., 1996. Biostatistical Analysis, third ed. Prentice-Hall Inc, Englewood Cliffs, NJ.