

Spatio-temporal variation in the prevalence of trematodes in the bivalve *Perumytilus purpuratus*

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Abstract

Perumytilus purpuratus is an abundant bivalve located in the intertidal rocky zone of South America that has been considered as a key species of the ecosystem. There are few studies of the host-parasite relationship of this bivalve; thus, this research aims to analyse the spatial and temporal variation in the prevalence of trematodes in *P. purpuratus*. Bivalves were collected from three localities (El Tabo, Las Cruces and Montemar) of central Chile (33°S, 71°W) during different seasons of 2010. The bivalves were also collected every metre, from the lowest to the highest level of the intertidal rocky zone, to determine the parasite distribution within the localities. Three species of trematodes as sporocyst stages were found: *Prosoerhynchoides carvajali*, *Proctoeces* sp. and an undetermined fellodistomid species. Of the 37,692 bivalve specimens collected, 2.68% were parasitised. The undetermined fellodistomid species was the most prevalent parasite observed (1.69%). There were little detected differences in the prevalence of some trematode species between seasons. The prevalence of *P. carvajali* varied between localities, being most prevalent at Montemar. The distribution of trematodes along the rocky zone within the localities was variable, with *P. carvajali* being more prevalent in the mid-lowest level of the intertidal zone and the undetermined fellodistomid species being more prevalent in the mid-highest level. Both the abundance of definitive hosts and the environmental conditions likely result in different levels of infection by trematodes in *P. purpuratus* between and within the localities.

Keywords

Perumytilus purpuratus, sporocysts, Bucephalidae, Fellodistomidae, intertidal rocky zone, Chile

Introduction

All species, whether sessile or mobile, are heterogeneously distributed across space and time. Many of them have distribution patterns that are related to dispersal, behaviour, physiology and interactions with other species (Krebs 2001). Parasite distribution is determined by the same factors, but they are also strongly affected by the presence and abundance of their hosts (Vázquez *et al.* 2005). Therefore, the abundance and prevalence of parasites usually shows spatial and seasonal variation, which may result in local or regional patterns (e.g., Poulin 1998).

Molluscs have various parasites, the most common of which are trematodes (digeneans) that are present at the larval stages. A single species of mollusc can harbour several trematode species, which are unevenly distributed in space and time. Generally, only some of the parasite species are found in a given host individual or even in a sample from a single time or place (e.g., Fernandez and Esch 1991; Fredensborg *et al.*

2005; Olmos and George-Nascimento 1997; Smith 2007). Thieltges *et al.* (2009), however, showed that the prevalence has a low coefficient of variation among sites, whether the locations are close together or separated by many km, whereas trematode intensity was very variable between sites that were over 100 km apart. As in the parasite communities of vertebrates (e.g., Simková *et al.* 2001; Thieltges *et al.* 2009), the prevalence of bivalve parasites may be more similar on a small scale (e.g., sites, habitats) than on large scales (e.g., localities, regions). At the small scale, several variables influence parasite infections in some way, but a few of these can lead to marked local variation in the prevalence of parasites. In contrast, at a large scale, there are strong factors affecting the life cycles and distributions of hosts and parasites that can better explain the distribution and infection load of the parasites.

The present study is focused on the small bivalve *Perumytilus purpuratus* (Lamarck, 1819), which is common in the intertidal rocky zone of South America (Zagal and Hermosilla 2001). This bivalve is distributed along the Pacific

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coast from Ecuador to Estrecho de Magallanes (52–52°S) in southern Chile (Osorio and Bahamonde 1968) and along the Atlantic coast from La Lobería (41°S) to Santa Cruz (52°S) in Argentina (Prado and Castilla 2006; Zagal and Hermosilla 2001). It is abundant and forms conglomerated matrices over rocks, thereby affecting habitat conditions and the local diversity of invertebrate species (Prado and Castilla 2006). Moreover, *P. purpuratus* represents an important component of the food chain for many species (Prado and Castilla 2006; Zagal and Hermosilla 2001), including fish (Martin and Bastida 2008; Muñoz and Zamora 2011) and birds (Bertellotti *et al.* 2003). Therefore, *P. purpuratus* has been considered a key species of the intertidal ecosystem (Prado and Castilla 2006). In addition, it is also an important host for parasites, as it is the first intermediate host of at least three trematode species (Platyhelminthes: Trematoda): *Prosohrynchoides carvajali* Muñoz and Bott, 2011 (Bucephalidae), *Proctoeces* sp. (Fellodistomidae) and an undetermined species (possibly a member of the Fellodistomidae) (Aldana *et al.* 2009; Lasiak 1992; Muñoz *et al.* 2012; Oliva *et al.* 2010). These three trematode species are found in *P. purpuratus* as sporocyst larvae (Muñoz *et al.* 2012), and their life cycles include intertidal and subtidal fish as definitive hosts (Aldana *et al.* 2009; Muñoz *et al.* 2012), although the secondary and definitive hosts for the undetermined fellodistomid are still unknown (Muñoz *et al.* 2012).

Trematodes that infect *P. purpuratus* have different distributions between locations because the intertidal zones differ with respect to their length, slope, the type of rocks and the species diversity (Sibaja-Cordero and Vargas-Zamora 2006). Thus, it is possible that tidal ebbing and surging in a rocky zone that is long or highly sloped may take more time compared to sites with rocky zones with contrary features because the high intertidal sites are less exposed to water, which may influence the larval stage of trematodes. First, the miracidia have to swim to infect a mollusc, and second, the bivalves exposed to desiccation for longer periods have different internal chemical conditions than bivalves that are permanently submerged (Montecinos *et al.* 2009). There have been a few studies on trematodes of *P. purpuratus* (Aldana *et al.* 2009; Loot *et al.* 2005, Muñoz *et al.* 2012; Oliva *et al.* 2010), but none have attempted to document the distribution of parasites over time and space. Therefore, the objective of this study was to determine the distribution of trematode species in time and space by observing their prevalence in the bivalve *P. purpuratus* between seasons and localities and within 3 localities in central Chile.

Materials and methods

Study area and collection of bivalve samples

Perumytilus purpuratus samples were collected from 3 coastal localities of the central zone of Chile (~33°S, 71°W): Las

Cruces (33°S30'S), El Tabo (33°27'S) and Montemar (32°57'S). These sites exhibited different physical characteristics of length and slope. The lengths of the intertidal rocky zones (in metres) were related to the presence of *P. purpuratus*. El Tabo had a length of 21 m, Las Cruces had a length of 23 m, and Montemar has a length of 45 m. The slope of the intertidal rocky zone also differed among localities. The slope percentage from the highest to lowest level of the intertidal zone of each locality was 7.4% for El Tabo, 3.4% for Las Cruces and 6.2% for Montemar.

Seasonal sampling of the bivalves was performed over the course of one year, from January to November 2010. All sampling was performed manually during low tide. Because of the heterogeneity of the mollusc distribution within the intertidal zone, three replicate samples were collected per metre from the low water level up to the highest level of the intertidal zone where the bivalve was present (Fig. 1a). To guide sampling, three parallel lines (Fig. 1b) that were marked every metre (Fig. 1c) were placed 3 m apart. A sample was then randomly collected for each metre, from within 1 m of the line. When possible, at least 10 specimens with a total valve length (VL) greater than 0.5 mm were collected at each metre for each locality and season.

Most bivalves were frozen at –10°C until the time of examination. Some samples per metre (~10% of the total samples) were dissected while they were fresh so that the parasites could be collected immediately. The VL of the bivalves (from the umbo to the edge of the valve) was measured for each individual, and both the gonads and mantle were examined for parasites. The collected parasites were then fixed in 5% buffered formalin.

Trematode identification

Three trematode species (one bucephalid and two fellodistomid species) have been reported for *P. purpuratus*; however, taxonomical identification of the two fellodistomid species has not been successful, although molecular techniques have been applied (Muñoz *et al.* 2012, Oliva *et al.* 2010). Therefore, the *Proctoeces* sp. has yet to be described (Muñoz and Zamora 2011), and the DNA of the undetermined fellodistomid is not similar to the other fellodistomid species found in Chile or those available in public databases (Muñoz *et al.* 2012). Therefore, we refer to them at the lowest taxonomic level known.

The morphology of the sporocysts and cercaria larvae of the trematodes allowed us to distinguish among species. *P. carvajali* (Bucephalidae) is characterised by sporocysts in the form of long tubes and cercaria larvae bearing two long filaments, *Proctoeces* sp. (Fellodistomidae) produce oval sporocysts and oval larval cercariae with tiny tails (although the tail is not always visible), and the unidentified fellodistomid species (Oliva *et al.* 2010) presents rounded-oval sporocysts and oval cercaria larvae with a rounded reddish tail that is longer and wider than the body of the cercaria (Muñoz *et al.* 2012).

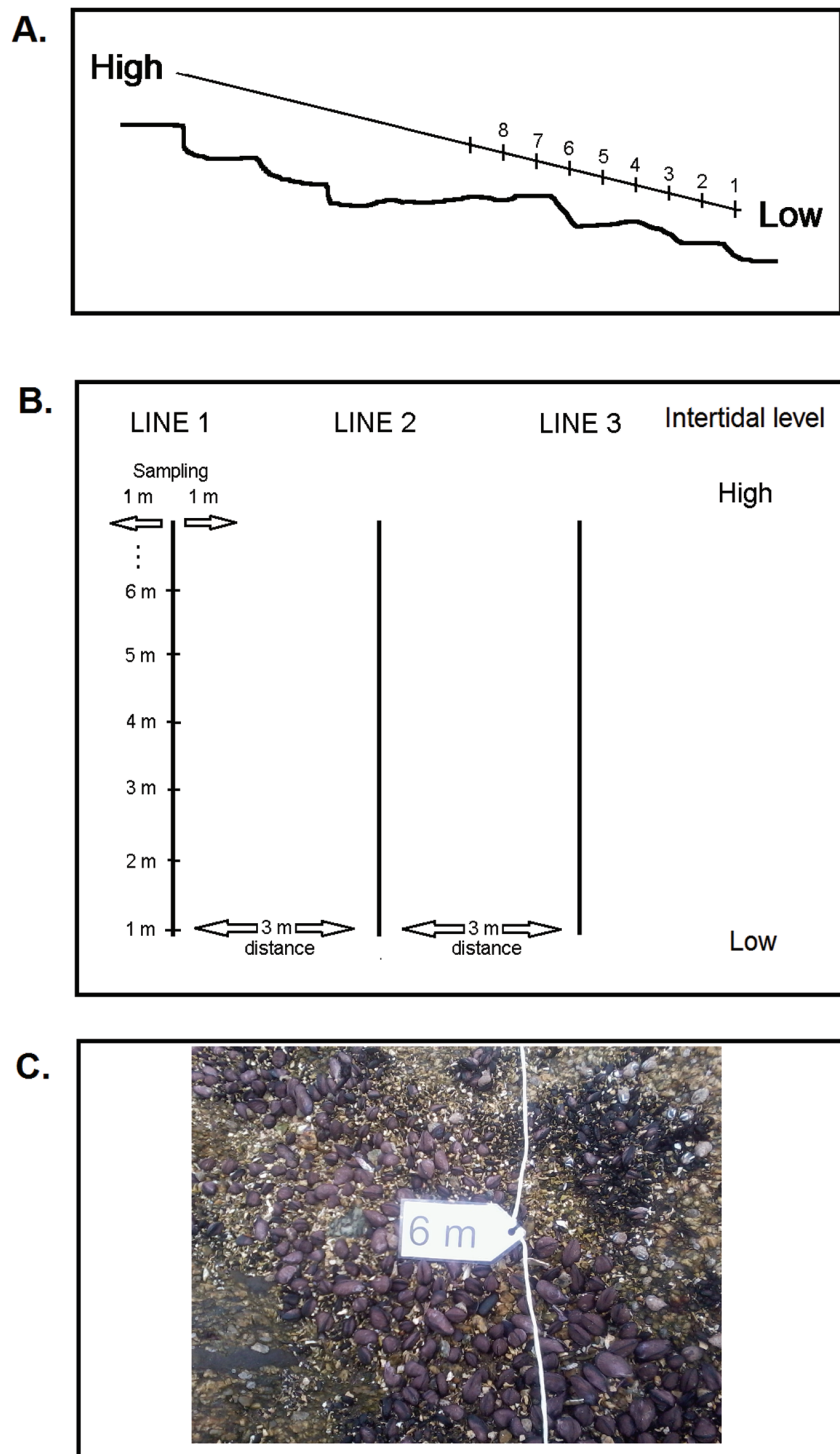


Fig. 1. Method of sampling the bivalve *P. purpuratus* in the intertidal rocky zone of central Chile. Bivalves were collected from the low water level up to the highest level of the intertidal zone (A.), along three parallel lines (B.), which were marked every metre (C.)

Statistical analyses

The prevalence of the parasites (percentage of bivalves parasitised in a sample) was calculated for each locality, season, trematode species and metre above the low water mark. The frequencies of the parasitised and non-parasitised bivalves

identified between the localities and trematode species were compared using 2 x 3 and 2 x 2 contingency tables and Chi-squared tests (Zar 1996).

The total valve length (VL) was compared among localities and seasons by an analysis of variance (ANOVA) after the normality of the distribution was tested using a Kol-

mogorov-Smirnov test and the homogeneity of variance with Levene's test. The three replicate samples per metre were averaged per locality and season, and the average values were used for the parametric statistical analyses. The spatio-temporal differences between the trematodes were determined by comparing the prevalence of each trematode species in each locality and season using a General Linear Model (GLM) and considering the valve length and the sample size of the bivalves, separately, as covariates (see Zar 1996). To determine the trend of the prevalence within the rocky zone (i.e., along the sampling distance determined in metres), Pearson's correlation was applied to the species and locality data. When there were groups detected in the prevalence distribution within the rocky zone, the contingency tables were used to compare the frequency of the parasitised and non-parasitised bivalves between these groups. The prevalence was arcsine transformed to achieve normality and homogeneity of variance of the data, thereby allowing for GLM and ANOVA calculations. Tukey HSD tests were used for multiple comparisons of the trematode prevalence between the localities and seasons (Zar 1996). Statistical analyses were performed using STATISTICA software.

Results

Prevalences of the trematodes species according to different variables

A total of 37,692 *P. purpuratus* individuals were collected during the sampling year. Of these specimens, 2.68% were parasitised with trematodes. Only three trematode species were found, *P. carvajali*, *Proctoeces* sp. and one undetermined fel-

lodistomid species (Muñoz *et al.* 2012; Oliva *et al.* 2010). No other trematode species was found. The three trematode species were sporocysts (not redia stages found). Moreover, the sporocysts of *Proctoeces* sp. and the undetermined fel-lodistomid were observed at different development levels throughout the year.

Most of the parasitised bivalves were infected with only one trematode species. Multiple infections were infrequent (0.005%), with only two host individuals containing two trematode species each (Table I). The undetermined fellodistomid trematode was the most prevalent (1.69%) of the three species, while prevalences of 0.7% and 0.29% were observed for *P. carvajali* and *Proctoeces* sp., respectively (Table I). *P. carvajali* and *Proctoeces* sp. were significantly more prevalent at Montemar than at the other localities (Table I).

The VL of the bivalves ranged from 3.0 to 44.5 mm. Parasitised bivalves exhibited VLs from 5.5 mm to 39.4 mm. The average VL of the non-parasitised bivalves varied between the localities ($F_{(2, 36672)} = 4,325.7$, $P < 0.001$); the bivalves from Montemar were the largest and those from Las Cruces were the smallest (Tukey HSD test, $P < 0.01$, Table II). The average VL of the bivalves among seasons also varied ($F_{(3, 36672)} = 1,028.8$, $P < 0.001$); the bivalves collected during the summer were larger than those observed at all other seasons (Table II). The non-parasitised bivalves were smaller than those parasitised with any trematode species ($F_{(1, 37691)} = 243.6$, $P < 0.001$).

P. carvajali was observed in bivalves from 16.6 mm of VL. The VL of the bivalves parasitised with this trematode did not exhibit any differences between localities and seasons (Table II). *Proctoeces* sp. was found in the bivalves from 12.6 mm in VL. The VL of the bivalves harbouring this parasite did not show any differences between localities and seasons (Table II). The undetermined fellodistomid species was found in the bi-

Table I. Prevalence of trematodes found in *P. purpuratus* from three localities in central Chile. The results of the contingency tables comparing the prevalence of trematodes among localities using Chi-squared tests are displayed

Locality	Trematode prevalences				Chi-squared test		
	El Tabo (n = 7,834)	Las Cruces (n = 6,788)	Montemar (n = 23,070)	Total sample (n = 37,692)	χ^2	df	P
Unique infection							
<i>Prosorhynchoides carvajali</i>	0.23	0.37	0.94†	0.70	55.62	2	<0.001
<i>Proctoeces</i> sp.	0.24	0.12	0.34†	0.29	31.32	2	<0.001
Undetermined fellodistomid	1.63	1.62	1.73	1.69	0.56	2	0.754
Morphotype indetermined*	0	0	<0.01	<0.01			
Multiple infections							
<i>Proctoeces</i> sp. and <i>P. carvajali</i>	0	0	<0.01	<0.01			
Undetermined fellodistomid and <i>P. carvajali</i>	0	0	<0.01				
<0.01							
Prevalence per locality	2.19	2.15	3.12	Total: 2.67%			

*Sporocysts were not identified due to their premature developmental stages; †Prevalence of trematodes that was significantly greater than at the other localities (based on 2 x 2 contingency tables, significant differences at $P < 0.05$).

Table II. Average valve length (X) and the standard error (SE) of bivalves, which were either non-parasitised or parasitised with trematodes, according to locality and season samplings

Locality	Non-parasitised bivalves		<i>Proserhynchoides carvajali</i>		<i>Proctoeces</i> sp.		Undetermined Fellodistomid	
	X	SE	X	SE	X	SE	X	SE
El Tabo	20.76 ± 0.06 ^b		25.75 ± 0.99 ^a		22.73 ± 1.26 ^a		22.55 ± 0.45 ^{a,b}	
Las Cruces	17.13 ± 0.08 ^a		27.59 ± 1.01 ^a		19.20 ± 1.14 ^a		20.93 ± 0.61 ^a	
Montemar	23.54 ± 0.04 ^c		26.77 ± 0.27 ^a		23.94 ± 0.57 ^a		25.71 ± 0.22 ^b	
	F	4,325.7	1.21		2.94		60.63	
	P	< 0.001	0.299		0.057		< 0.001	
Season								
Spring	20.81 ± 0.07 ^c		26.53 ± 0.38 ^a		24.42 ± 0.93 ^a		23.42 ± 0.47 ^b	
Summer	23.51 ± 0.05 ^a		27.36 ± 0.46 ^a		24.30 ± 0.83 ^a		25.61 ± 0.36 ^a	
Autumn	21.01 ± 0.06 ^b		25.50 ± 0.66 ^a		21.13 ± 1.25 ^a		23.52 ± 0.39 ^b	
Winter	20.62 ± 0.07 ^{a,b,c}		27.13 ± 0.60 ^a		21.79 ± 0.87 ^a		24.17 ± 0.46 ^{a,b}	
	F	1,028.8	2.28		1.98		9.87	
	P	< 0.001	0.079		0.121		< 0.001	

Different superscript letters indicate the groups of bivalves which showed significant differences in VL (according to *a posteriori* test, $P < 0.01$). For statistical analyses, locality and season were considered separately.

valves from 8.0 mm of VL. The average VL of the bivalves parasitised with this trematode varied significantly between locality and season (Table II); the differences were detected between all localities and between autumn and summer (Tukey HSD test, $P < 0.01$) (Table II). There was no relationship between prevalence and the host bivalve length, although the bivalves parasitised with *P. carvajali* were larger than those parasitised with the other trematode species at the three localities ($F_{(2, 995)} = 26.26$, $P < 0.001$; Tukey HSD, $P < 0.05$) (Table II).

When comparing prevalences of each trematode species, through contingency tables, among seasons for each locality, the frequency of *P. carvajali* in the bivalves was greater in sum-

mer than in the other seasons, in El Tabo ($X^2 = 12.21$, f.d. = 3, $P = 0.0311$) and Las Cruces ($X^2 = 16.75$, f.d. = 3, $P = 0.0007$) (Fig. 2), but not in Montemar ($X^2 = 5.69$, f.d. = 3, $P = 0.127$). Whereas the frequency of *Proctoeces* sp. was greater in spring than in the other seasons in Montemar ($X^2 = 11.56$, f.d. = 3, $P = 0.009$) (Fig. 2), but not in the other localities ($3.47 < X^2 < 7.62$, f.d. = 3, $P > 0.05$). The frequency of the undetermined fellodistomid species did not showed any difference among seasons in each locality ($3.47 < X^2 < 4.03$, f.d. = 3, $P > 0.305$). Because the VL and sample sizes of bivalves differed among localities and seasons (Tables I and II), these were considered as covariates in the following statistical results.

Table III. Results of the General Lineal Model (GLM) that compare the prevalence of trematodes between localities and seasons, using the total valve lengths (VL) and the sample sizes of the bivalves as covariates

Trematodes and variables	VL as covariate			Sample size as covariate		
	FD	F	P	FD	F	P
<i>P. carvajali</i>						
Locality	2	9.297	< 0.001*	2	15.961	< 0.001*
Season	3	1.456	0.227	3	0.564	0.639
Locality * Season	6	0.323	0.925	6	0.583	0.744
Error	309			309		
<i>Proctoeces</i> sp.						
Locality	2	0.299	0.742	2	0.270	0.763
Season	3	0.602	0.614	3	0.246	0.864
Locality * Season	6	1.173	0.321	6	1.210	0.301
Error	309			309		
Fellodistomidae UD						
Locality	2	3.289	0.038*	2	0.243	0.784
Season	3	1.682	0.171	3	0.313	0.816
Locality * Season	6	1.568	0.156	6	1.102	0.361
Error	309			309		

The prevalence of *P. carvajali* varied significantly between localities, with the highest prevalence found at Montemar (Tukey HSD test, $P < 0.05$) (Fig. 2), with or without considering the bivalve VL as covariable (Table III). There was no difference in the prevalence of *P. carvajali* between seasons and between locality*seasons (Table III). When considering the host sample size as covariate, the prevalence of this trematode was still different between localities (Table III). The prevalence of *Proctoeces* sp. was less than 1% per locality and season, thereby suggesting that there were no differences between localities, seasons or both locality*seasons (Table III, Fig. 2). However, the prevalence of the undeter-

mined fellodistomid species varied between the localities, as the bivalves from El Tabo displayed a higher prevalence of this species than those found at the other localities (Tukey HSD test, $P < 0.05$), but this difference was affected by the bivalves sample sizes (Table III). This trematode species did not show any significant differences in prevalence between seasons (Table III, Fig. 2).

Distribution of trematodes within localities

The distribution of trematodes within localities was variable along the rocky zone. The parasite prevalence ranged from 0

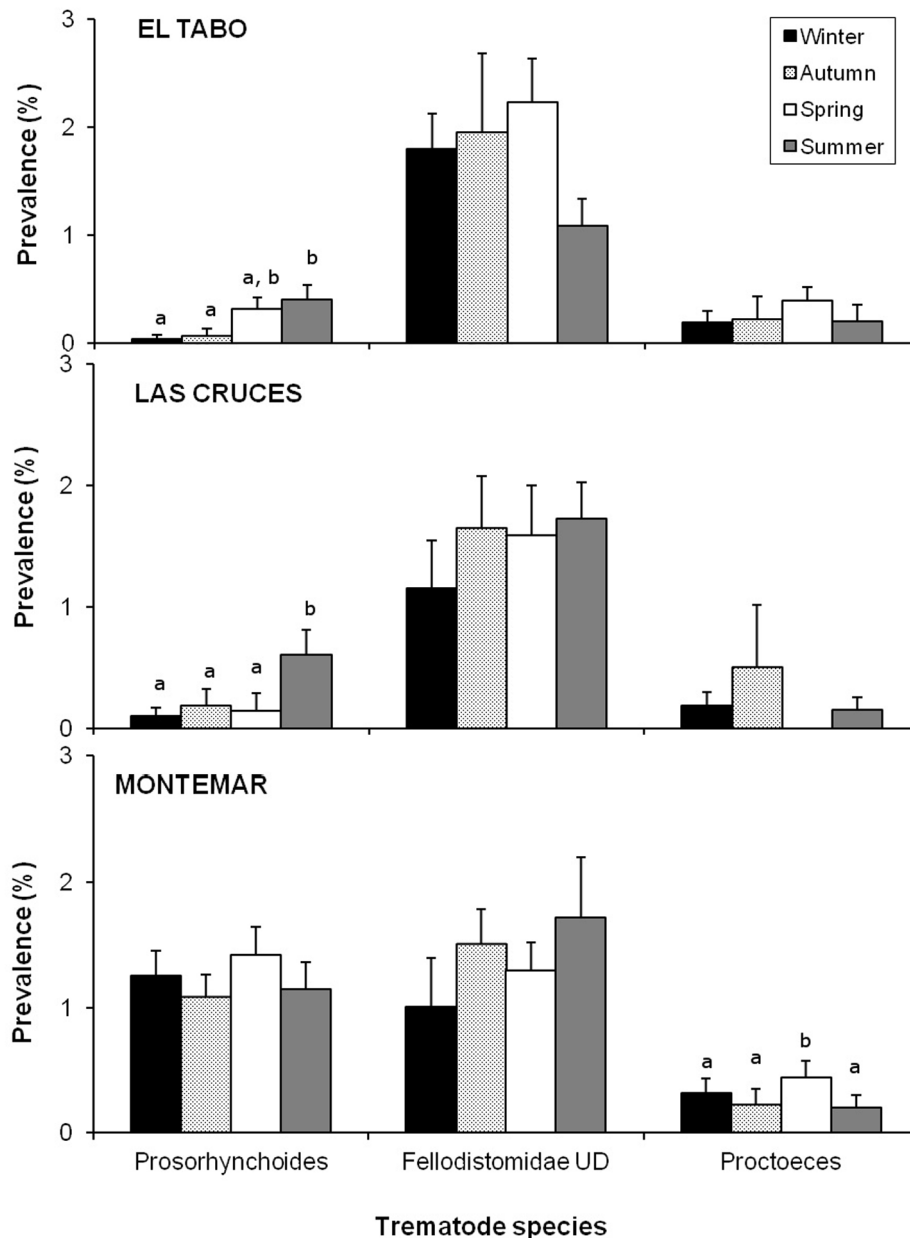


Fig. 2. Average prevalence (%) and the standard error of the three trematode species in the bivalve *P. purpuratus* by locality and season. Different letter on the bars indicate the seasons where the frequency of trematodes were significantly different (through Tables of Contingency, $P < 0.05$)

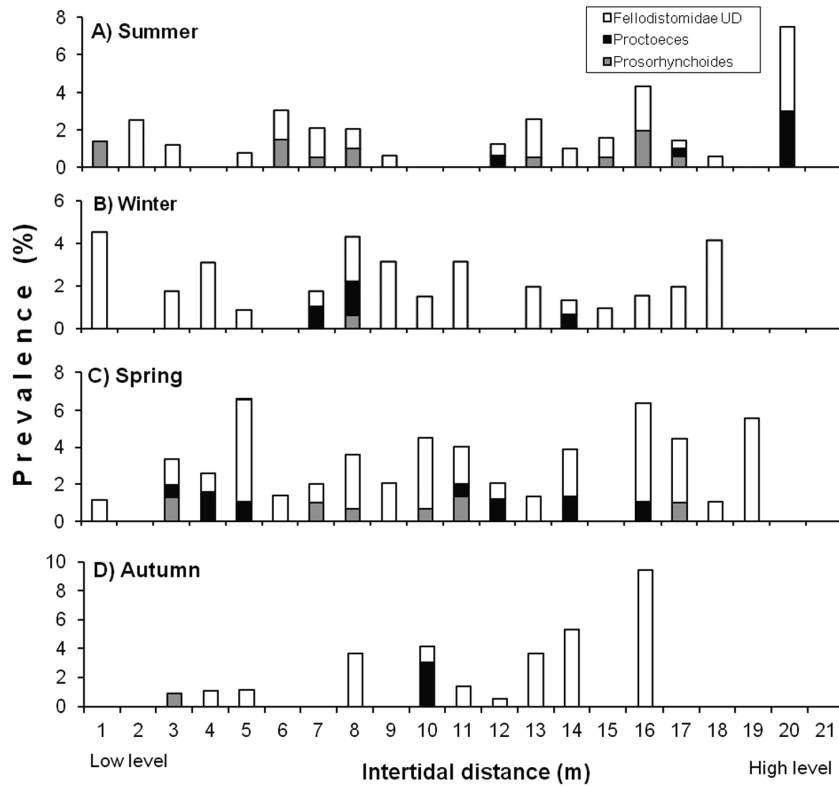


Fig. 3. Prevalence of trematodes between seasons and along the intertidal rocky zone from the lowest to the highest level (metres) in *P. purpuratus* from El Tabo

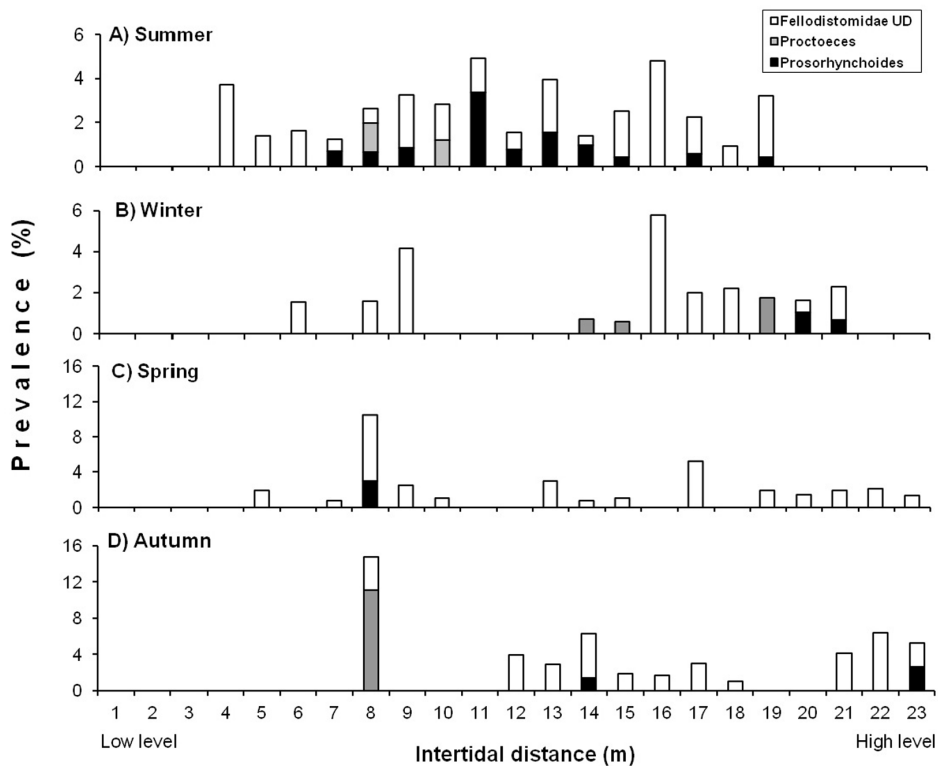


Fig. 4. Prevalence of trematodes between seasons and along the intertidal rocky zone from the lowest to the highest level (metres) in *P. purpuratus* from Las Cruces

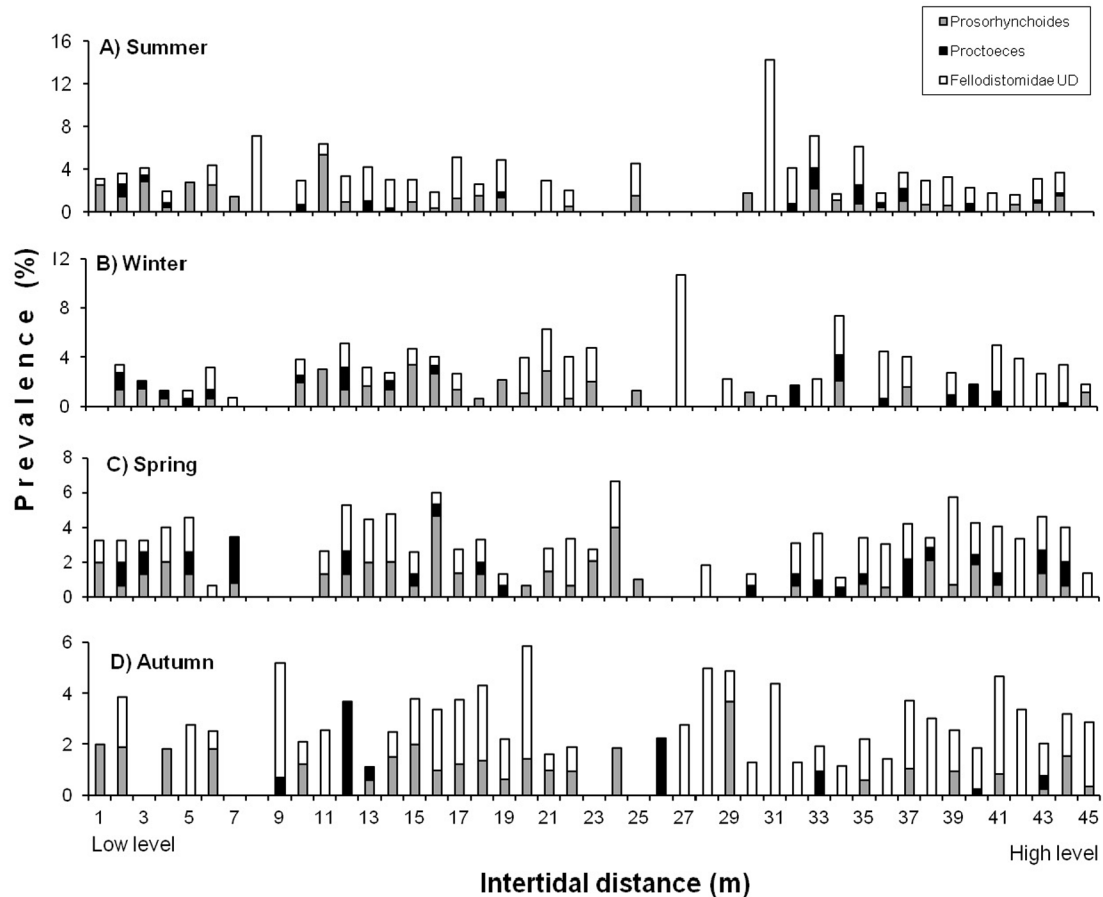


Fig. 5. Prevalence of trematodes between seasons and along the intertidal rocky zone from the lowest to the highest level (metres) in *P. purpuratus* from Montemar

to 14% for the undetermined fellodistomid species, 0 to 12% for *Proctoeces* sp., and 0 to 6% for *P. carvajali* (Figs 3–5). The prevalence of the three trematodes varied greatly between one sampling site (metre) and the next. Despite this, the prevalence of the undetermined fellodistomid species positively correlated with distance (from lower to higher intertidal levels) at both Las Cruces ($r = 0.35$, $P < 0.05$, Fig. 4) and Montemar ($r = 0.28$, $P < 0.05$, Fig. 5), which was more frequent between the middle and highest level of the intertidal zone. In contrast, the prevalence of *P. carvajali* negatively correlated with the intertidal level distance only at Montemar ($r = -0.31$; $P < 0.05$, Fig. 5), which was more frequent in the lower to middle levels of the intertidal zone. At Montemar, where the rocky zone was longer than at the other localities, there was a short natural break with few rocks and sand at 26 m that was not considered in the division between the lowest and highest level when comparing the prevalence of trematodes. *P. carvajali* was prevalent at 1–25 m in all seasons ($5.19 < X^2 < 14.55$, $P < 0.02$) except summer ($X^2 = 2.98$, $P = 0.08$), whereas the undetermined fellodistomid species was more prevalent at 27–45 m of the rocky zone during winter and autumn ($5.84 < X^2 < 12.21$, $P < 0.05$) but not during spring and summer ($0.12 < X^2 < 3.09$, $P > 0.05$) (Fig. 5).

Discussion

The prevalence of the trematode species observed in *P. purpuratus* varied spatially rather than temporally during the short time period analysed in this study, thereby resulting in great differences in the prevalence of trematodes between and within localities. The prevalence of *P. carvajali* and *Proctoeces* sp. was higher at Montemar than at El Tabo and Las Cruces, whereas the distribution patterns of *P. carvajali* and the undetermined fellodistomid showed certain preferences for the low and high levels of the intertidal zone, respectively, particularly at Montemar.

Montemar differs physically in several ways when compared with the other localities. The length of the intertidal rocky zone represents one of these differences measured in the study. Montemar exhibited a longer intertidal zone in which bivalves were found (45 m) compared with El Tabo (21 m) and Las Cruces (23 m); consequently, the abundance of the bivalve was greater at Montemar than at the other localities (Table I). The source of infection is important for the prevalence of parasites in molluscs; in areas where the definitive hosts are abundant, there may be a high number of eggs released by the parasites, which suggests that infection rates can

be greater in molluscs than in areas where there are less definitive hosts (e.g., Fredensborg *et al.* 2005; Kube *et al.* 2002). Some studies focusing on directly transmitted parasites have found a positive relation between host abundance and the abundance or prevalence of parasites (Arneberg *et al.* 1998), while others have established a negative relationship between these variables (Stanko *et al.* 2006). However, studies that have demonstrated an association between the prevalence of trematodes in gastropods and the abundance of their definitive hosts have shown different relationships, which may depend on whether the definitive hosts are birds or fish as well as the complexity of the parasite life cycle (Kube *et al.* 2002). To put the relationship between the trematodes and the bivalves of this study into context, it is likely that the bivalve abundance was positively associated with the prevalence of trematodes. This is because intertidal fish are the definitive host for two of the trematode species (*P. carvajali* and *Proctoeces* sp.), and the intertidal fish normally remain in rocky pools during ebbing. The fish faeces, which contain trematode eggs, are released into pools where the miracidia emerge, and miracidia actively infect the bivalves, thereby suggesting that there may be a great chance to infect the bivalves within the pools.

The average VL of the bivalves represented another difference found among the localities; the bivalves from Montemar were larger than the specimens from other localities (Table II). However, the prevalence of the trematode *P. carvajali* was different at Montemar, despite the differences in average VL and sample sizes (Table III). The average VL may represent different growth rates of the bivalves between these localities which can be important for parasitic prevalence. The prevalence of some trematodes species were related to the bivalve VL and the trematodes were present throughout the year and exhibited relatively regular prevalence, there may be several trematode infection events throughout a year. There is evidence that the miracidium of some trematodes, e.g., *Schistosoma mansoni* (Sambon, 1907), cannot re-infect a host mollusc for several weeks after the first infection (Sire *et al.* 1998). However, in the present study, we found that the sporocysts of both *Proctoeces* and the undetermined fellodistomid were present at different developmental levels in a bivalve specimen, suggesting that bivalves were infected by miracidia at different times. Moreover, sporocysts may be able to accumulate in the bivalve while it ages. A similar finding was observed in the bivalve *Anodonta piscinalis* (Nilsson, 1823), which displayed early developmental stages of sporocysts and cercariae of two species of *Rhipidicotyle* throughout one year (Taskinen *et al.* 1994). Alternatively, sporocysts are long-lived stages and may be present in the host for a long time.

In the present study, we observed little differences of prevalences of trematodes among seasons; there was a weak seasonal tendency of prevalence as greater prevalences occurred in warm seasons (summer or spring), but it was not consistent among localities and trematode species (Fig. 2). These results can be explained because the development of

sporocysts and cercariae emergence can be triggered by factors associated with the season, such as temperature (Koprivnikar and Poulin 2009; Thieltges 2006) and water salinity (Koprivnikar and Poulin 2009), especially at intertidal rocky pools (Rees 1948). However, each trematode species has its own transmission strategy to infect the host; therefore, there are many other factors that are not associated with the season that affect egg and cercaria development (Koprivnikar and Poulin 2009; Lei and Poulin 2011; Pietrock and Marcogliese 2003; Rees 1948). Consequently, seasonal differences in the prevalence or abundances of trematodes in molluscs have been documented in some studies (Brown *et al.* 1988; Fermer *et al.* 2010; Olmos and George-Nascimento 1997; Tonn *et al.* 1964), whereas others have not observed any seasonal patterns (Cannon 1979; Taskinen *et al.* 1994). Taskinen *et al.* (1994) have demonstrated seasonal infections based on indirect evidence, such as the frequency of the recent infection stages of sporocysts and the presence of miracidia in the aquatic media. However, they did not observe seasonal differences based on prevalence. The little temporal variation in the prevalence may be due to the longevity of the sporocysts in molluscs; if this stage lasts more than a year, then there would not be great seasonal variations in prevalence (Cribb 2005). Additionally, in at least two trematode species observed in *P. purpuratus* displayed multiple miracidia re-infections by the same trematode species in a bivalve, and the bivalves are therefore permanently parasitised.

Prosorhynchoides carvajali showed three specific infecting characteristics when compared with the other two sporocyst species, particularly at Montemar; this species displayed a high prevalence, they were found more commonly in larger bivalves of at least 16.6 mm VL, and they exhibited a distinct distribution along the intertidal rocky zone. The high prevalence of *P. carvajali* sporocysts can be explained by the longer VL of the hosts at Montemar compared to other localities. Moreover, the bivalves parasitised with this trematode species were larger than the non-parasitised bivalves (Fig. 1). Altogether, it is possible that *P. carvajali* requires larger energetic resources than the other trematode species. Larger bivalves are mature and present with a greater gonadic mass than small bivalves (Oyarzún *et al.* 2010), thereby providing more available tissue for this parasite. Moreover, a phenomenon commonly called “gigantism” may also be associated with the requirement of the trematodes for larger hosts. The somatic growth increase of the host is affected because its reproduction is negatively affected by these parasites through castration (e.g., Curtis *et al.* 2000; Chapius 2009; Negovetich and Esch 2007).

The trematode distributions differed along the rocky zone within the study localities. On the other hand, the intertidal rocky zones exhibit different characteristics among localities and from lower to higher levels within a locality. Differences in slope and length of the rocky shore, abundances and species diversity and dynamic physical conditions (e.g., waves and winds) can produce highly heterogeneous habitats within the

intertidal zone. Larval parasite dispersion is dependent on these variables (Blower and Roughgarden 1988, Smith 2001), thereby creating different spatial distributions of parasites among the habitats. The undetermined fellodistomid was mainly distributed on the upper level of the intertidal zone, this was in Las Cruces and Montemar, both localities with different length. Whereas *P. carvajali* was mainly distributed in the lower zone of the intertidal zone, but this result was found only in Montemar. It is difficult to infer what kind of variable affect the prevalence of these trematodes along the intertidal zone, it could be due to the environmental conditions but also to the abundance of the definitive hosts, as it was suggested above.

The parasites may influence host-behaviour associated with the movement to different sites, as it has been documented in gastropods (e.g., Curtis 1987, 1993) and mobile bivalves (e.g., Thomas and Poulin 1998). For example, parasitised molluscs can be present in sites where they are more visible to be preyed on by the next host; therefore, the transmission to the next host is likely to be successful in those sites (Curtis 1987, 1993, 2009). However, *P. purpuratus* is a sessile bivalve, which becomes parasitised when it is fixed on a substratum; therefore, differential distribution of this host due to parasitism cannot occur. Additionally, parasites increase their chances to be transmitted to the next host by being more prevalent in certain sites where contact can be kept with other hosts. To analyse this phenomenon, it is important to understand the life cycles of the parasites to determine which biological or ecological characteristic of the next host (secondary intermediate or definitive hosts) may contribute to the spatial infection differences of the trematodes. Unfortunately, there are few antecedents regarding the life cycle of trematodes in Chile. *P. carvajali* requires labrisomid fish (*Auchenionchus* spp.) as the definitive host (Muñoz and Bott 2011) and intertidal fish as the secondary host (Muñoz *et al.* unpublished data); therefore, trematode larvae may be more exposed to intertidal fish if they are distributed in the lowest rocky zone. For the undetermined fellodistomid species, there are few biological antecedents as the identity of this trematode is unknown (Muñoz *et al.* 2012) and the host species has not been identified. Therefore, it is not possible to suggest an explanation for the different patterns of parasite infection detected in different habitat conditions. It is important to note that Montemar has been a protected marine area since 1941, and fishing activities (fish and invertebrates) are not allowed. Therefore, it is possible that some species, e.g., fish, molluscs and birds, are more abundant in this area than in open areas, such as Las Cruces and El Tabo, resulting in the observed differences in the rates of infection with trematodes in *P. purpuratus*.

Contrary to other systems in which abundant and widely dispersed molluscs can harbour several trematode species (e.g., Cannon 1979; Curtis 2009; Kube *et al.* 2002), *P. purpuratus* harboured only three species (*P. carvajali*, *Proctoeces* sp. and an undetermined fellodistomid) that have already been

reported in other studies (Aldana *et al.* 2009; Muñoz *et al.* 2012; Oliva *et al.* 2010), despite the great abundance of the bivalve in the central rocky zone of Chile. Some trematode families are adapted to infect mytilid bivalves. Bucephalidae (e.g., Francisco *et al.* 2010; Lasiak 1992) and Fellodistomidae (e.g., Lang and Dennis 1976, Muñoz *et al.* 2012) are the most common trematode groups infecting this family of bivalves, and Gymnophallidae can be found in mytilids but are also common parasites of other mollusc groups (Cremonte *et al.* 2008; Fermer *et al.* 2009; Pekkarinen 1987). Moreover, only two intertidal bivalves have been examined for the parasites in Chile; *Brachidontes granulatus* (Hanley, 1843) has not been shown to be parasitised with trematodes (Aldana *et al.* 2009), and *Semimytilus algosus* (Gould, 1850) can be parasitised with an undetermined bucephalid species (Lasiak 1992). Thus, the trematodes *P. carvajali*, *Proctoeces* sp. and an undetermined fellodistomid can be considered significantly host-specific parasites of *P. purpuratus*.

In conclusion, the spatial variations in the prevalence of the three trematode species observed in *P. purpuratus* may be determined by various factors, including host abundances (intermediate and definitive), environmental conditions and host body size. *P. purpuratus* exhibited size-selective parasitism by *Prosohynchoides carvajali*, which may be due to the host mass available to the parasite or possibly some physiological host condition that favours parasite infection. However, castration by these parasites may affect the host's somatic growth, thereby resulting in larger infected bivalves compared with uninfected bivalves. Despite the evidence of the negative effects on the gonads induced by each of these species of trematodes, the low prevalence (2.68%) of these parasites may have little effect on the population dynamics of *P. purpuratus*.

Acknowledgements. Financial support by the research grant project DIPUV 12-2008 granted to GM.

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(Accepted February 07, 2013)