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Research Note

Report of pathogens and parasites in *Perumytilus purpuratus* from San Jorge Bay, Antofagasta, Chile

Registro de patógenos y parásitos en *Perumytilus purpuratus* en la bahía San Jorge, Antofagasta, Chile

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Abtract.- *Perumytilus purpuratus* is a mussel commonly found on the entire Chilean coastline. The knowledge of pathogens and parasites of this mollusc, as well as for many other species, inhabiting the Chilean coast remains largely unknown. This study aims to survey parasites and pathogens in *P. purpuratus* from San Jorge Bay, Antofagasta, Chile. From September to October 2009, 160 specimens of this bivalve were collected. Ciliates, virus-like, rickettsia-like organisms (RLO) and digeneans were recorded. The most prevalent parasites were metacercariae of digeneans in gonads (50.83%), gill ciliates (37.5%), and digestive gland viruses-like (37.5%). Only the prevalence of digeneans increased with host size, but any relationship between these variables was found in the other pathogens.

Key words: Ciliates, virus-like, rickettsia-like, digeneans

Introduction

Effective control of diseases in marine organisms requires a routine monitoring, which facilitates diagnosis and the implementation prevention strategies (Cáceres-Martínez & Vásquez-Yeomans 2001). In South America, studies of parasites and diseases of bivalves are scarce (Caceres-Martinez & Vasquez-Yeomans 2008), even though they are important and necessary. In Chile, major contribution about presence of parasites in molluscs have been recorded by Oliva (1984, 1992), Oliva *et al.* (1986, 1999, 2010), Franjola & Gallardo (1991), Gallardo *et al.* (1992), Oliva & Vega (1994), García-Tello *et al.* (2002), Valderrama *et al.* (2004), and pathogens by Lohrmann *et al.* (2002), Lohrmann (2009), Campalans & Lohrmann (2009). However, the knowledge of pathogens and parasites in marine organisms inhabiting San Jorge Bay (Antofagasta) and the majority of the Chilean coast remains largely unknown.

Perumytilus purpuratus (Lamarck, 1819) is a mussel (Mytilidae) that inhabits along the entire Chilean coastline. This species is also called a bioengineer because creates microhabitats for many others species in the marine rocky intertidal zone (Prado & Castilla 2006). This bivalve has been used as pollution marker for heavy metals in San Jorge Bay, north of Chile (Herrera 1997, Riveros *et al*. 2002, 2003, Salamanca *et al*. 2004) due to this bay has been polluted for many years. Besides the ecological importance of this species in the rocky coast, there are a few studies on their parasites, but none about pathogens. An increase of pathogen growth occurs in polluted environments (Gold-Bouchot *et al*. 1995, Wester *et al.* 2002), and it is also expected that pathogen growth increase with climate change (Harvell *et al*. 2002). Thus, the objective of this study was to survey and identify parasites and pathogens in the abundant mussel *P. pupuratus* from San Jorge bay, Antofagasta, Chile.

Materials and Methods

Collection of samples

From September to October of 2009, one hundred sixty specimens of *Perumytilus purpuratus* were collected from San Jorge Bay (23º43’S, 70º26’W). The specimens collected were immediately transported to laboratory, where they were measured using a vernier caliper (0.1 mm precision). Mussels were dissected and examined according to following protocols.

Histological survey

Forty specimens were utilised for histological analyses.

Gills, gonads and digestive glands were fixed (Davidson solution for 24 h); then they were dehydrated, embedded in Paraplast, sectioned at 5 µm with a Microtome Minot and stained with haematoxylin and eosin (Bancroft & Stevens 1990). The slides were then observed under light microscopy. Intensity of pathogens in gills, gonads and digestive glands was calculated as the number of pathogens observed in the whole histological mussel section, according to Svardh (1999). Intensity was categorised according to following criteria: 1-5 pathogens (range 1), 6-15 pathogens (range 2) and over 20 pathogens (range 3). Prevalence was calculated as the number of mussels infected by a particular pathogen divided by the total number of mussels examined, expressed in percentage (Bush *et al.* 1997).

Parasites '*in vivo*'

A hundred twenty specimens of *Perumytilus purpuratus* were separated, and parasites were examined *in vivo*, surveyed and counted. The prevalence of parasites and the mean intensity of infection were calculated according to Bush *et al.* (1997). Prevalence was calculated as mentioned above, and the mean intensity of infection was calculated as the mean number of a particular parasite species found in each host, considering only infected mussels.

Statistical analyses

Mussels were arbitrary categorized in seven sizes of classes as showed in Table 1. Given that data set showed non-normality and homogeneity of variances, Kruskal-Wallis test was used to compare the intensity of parasites (metacercariae) among mussel size classes. Tables of contingency 2 x 7 were used to evaluate the prevalence of parasites and pathogens, respectively, in mussels related to each size class (Zar 1999). All analyses were performed using the Statistica 6.0 software.

Results and Discussion

The sizes of examined mussels varied between 26.75 and 40.05 mm total shell length. The mussels examined were infected with two parasites (digenean and ciliates), and two pathogens (RLO and virus-like), which were recorded in gills, digestive glands and gonads (Fig. 1).

Considering all specimens sampled, the most prevalent parasite/pathogens were digeneans in gonads (50.83%), ciliates (37.5%), and viruses-like (32.5%) on gills; followed by viruses-like (37.5%) and (RLO) in digestive gland (25%). The prevalence of pathogens did not vary among mussels of different size classes. Virus-like ($G = 0.90$; df = 5; $P = 0.97$), Ciliates ($G = 4.54$; df = 4; $P = 0.34$) on gills, virus-like ($G = 0.12$; df = 3; $P = 0.72$), RLO ($G = 1.13$; df = 3; $P = 0.77$) in digestive gland.

Table 1. Prevalence of pathogens/parasites and mean intensity of metacercariae in gonads in each size classes of the mussel *Perumytilus purpuratus;* Virus-like= lesions particles virus-like in gills, Ciliates=ciliates on gills, Virus-like= lesions of presence of virus-like in digestive gland, RLO= rickettsia-like bacteria in digestive gland / Prevalencia de patógenos/parásitos e intensidad media de metacercarias en gónadas por clases de tamaño del mitílido *Perumytilus purpuratus*; Virus-like=lesiones en las partículas tipo virus en branquias, Ciliates=ciliados en branquias, Virus-like= lesiones en las partículas tipo virus en glándula digestiva, GL-RLO= bacteria tipo rickettsiales en la glándula digestiva

Groups	Size classes	N° of Mussels	Gill		Digestive gland		Gonads		
			Virus-like	Ciliates-like	Virus-like	RLO	N° of Mussels	Prevalence Metacercariae	Mean intensity
Group 1	26.75-28.65	2	0	0	100	0	10	40	6.75
Group 2	28.65- 30.55	5	40	20	0	0	26	46.15	2.58
Group 3	30.55-32.45	10	30	40	30	0	33	21.21	3.00
Group 4	32.45-34.35	8	37.5	37.5	12.5	25	16	56.25	6.89
Group 5	34.35 - 36.25	6	33.3	33.3	16.6	33.3	18	77.78	6.14
Group 6	36.25-38.15	8	25	50	12.5	12.5	14	78.57	5.72
Group 7	38.15 - 40.05	1	100	100	0	0	3	100	25.66
	Total	40					120		

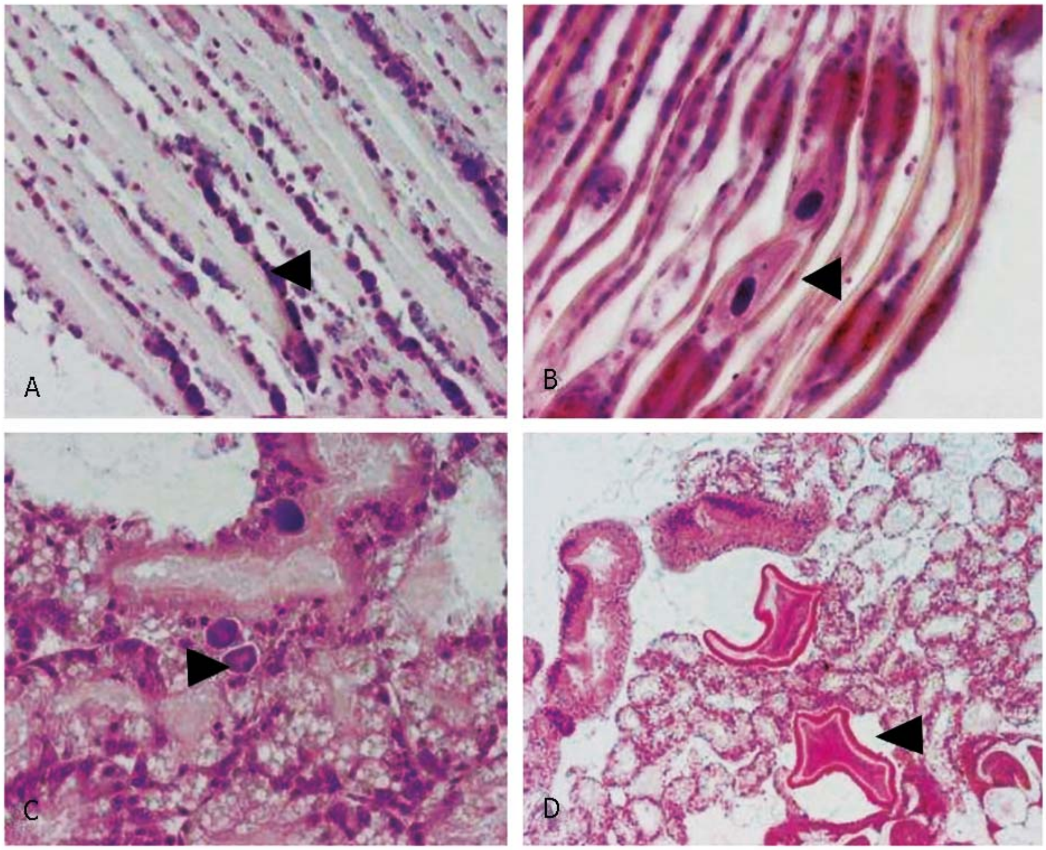

Figure 1. Histological sections showing pathogens/parasites in *Perumytilus purpuratus* from San Jorge Bay. A) lesion of virus-like in gills , B) ciliates in gills, C) rickettsia-like bacterias in digestive gland, D) metacercariae in digestive gland / Sección histológica mostrando los patógenos/parásitos en *Perumytilus purpuratus* de la bahía San Jorge. A) lesiones de tipo virus en branquias, B) ciliados en branquias, C) bacterias tipo rickettsiales en glándula digestiva, D) metacercaria en glándula digestiva

On gills, virus-like and ciliates were present in moderate (range 2) and high intensities (range 3) in mussels from the groups 2 to 6, but these pathogens were absents (range 0) in mussels of group 1 (Fig. 2). In digestive gland, virus-like showed the highest intensity in mussels from group 5 (Fig. 2) and RLO showed a high intensity in mussels from groups 4 to 6 (Fig. 2). There were not correlations in the intensity of virus-like, ciliates and RLO with sizes of mussels (on gills, virus-like: r= 0.06, N= 40, P= 0.68; Ciliates: r= 0.2, N= 40, P= 0.09; in digestive gland, virus-like: r = -0.17, N = 40, P = 0.28; RLO: r = 0.15, N = 40, P = 0.33).

Metacercariae, possibly belonging to the Renicolidae family (pers. comm., Dr. Cremonte[1], were found in gonads of *P. purpuratus* (1-49 per mussel). There were significant differences in the prevalence of metacercariae among mussel-size classes (G = 19.57, df =5, P = 0.001), varying between 21.21% in the group 3 to 100% in the group 7 (higher sizes of mussels). Mean intensity varied between 2.58 in the group 2 and 25.66 in the group 7, detecting a significantly difference between these groups ($H_{6,124}$= 29.87; P < 0.01) (Table 1). Prevalence of metacercariae was significantly correlated with mussel sizes (r = 0.34, N = 124, P < 0.01). Pathogens and parasites showed no significant differences between host sexes (G = 1.29; df = 1; P = 0.25).

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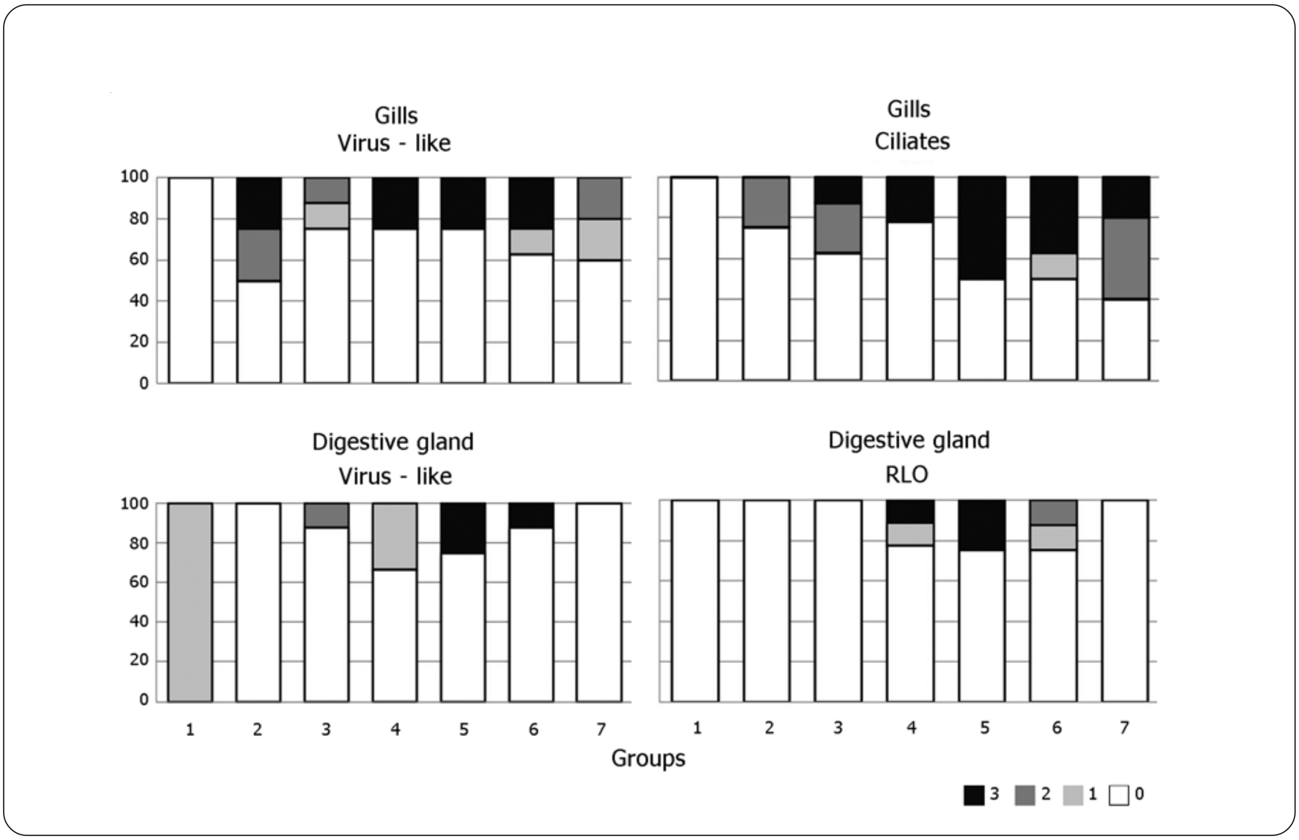

Fig 2. Intensity of pathogens/parasites in mussel *Perumytilus purpuratus*. 0 = no present, 1 = low, 2 = moderate, 3 = severe / Intensidad de patógenos/parásitos en el mitílido *Perumytilus purpuratus*. 0 = no presenta, 1 = bajo, 2 = moderado, 3 = severo

This study is the first record of microparasites such as ciliates, virus-like, and rickettsia-like organisms in *Perumytilus purpuratus* from San Jorge bay, and of the Chilean coast. According to Hatzidimitriou & Berger (1977) and Bower *et al*. (1994), ciliates can be considered as commensals rather than parasites. Villalba *et al*. (1997) recorded in mussel *Mytilus galloprovincialis* different species of ciliates, which most of them resembled to *Ancistrum mytili*. Madrazo-Garibay & Lopez-Ochoterena (1985) described *Ancistrum* genus as pyriform shape, presence of ovoid macronucleus and a spherical micronucleus. In this study, it was found an unidentified ciliate on gills (37.5% prevalence) and it is suggested that it may be a representing of this genus.

Virus-like have been reported by Jones *et al*. (1996) in New Zealand and Rasmussen (1986) in Denmark. Also, viruses-like have been reported as category 1 in Canada Fisheries, meaning that they are agents of infectious diseases, which can have serious impact on shellfish stocks, as indicated by Bower (2010). In this study, it was found lesions that could be attributed to virus-like, but ultrastructural examination and molecular analyses are needed to support this.

RLO are obligate intracellular organisms that infect the epithelial cells of the gastrointestinal tract of marine molluscs (Sun & Wu 2004). Along the Chilean coast, RLO have been previously reported in molluscs (Lohrmann *et al*. 2002, Lohrmann 2009, Campalans & Lohrmann 2009). The Rickettsia bacteria *Candidatus Xenohaliotis californiensis* have been listed by OIE (2008) as a high-risk disease for molluscs in Chile (Campalans & Lohrmann 2009). Some reports indicate that Rickettsias are directly responsible for causing mortality of marine molluscs (Sun & Wu 2004) or they play a main role in causing mortality of molluscs (Braid *et al*. 2005). In this study, the prevalence of RLO (22.2-40%) was moderate. Therefore taking account the importance of Rikettsia-like organisms as infectious agents, studies are necessary to identify RLO found in *P. purpuratus*.

In relation to digeneans, some authors have previously reported larval stages in *P. purpuratus* (Aldana *et al.* 2009, Oliva *et al.* 2010), but there is no record about metacercariae stages. Representatives of Renicolidae family have been found in molluscs (Werding 1969) and fishes (Stunkard 1932). However, representatives of this family have not previously been reported in any kind of hosts in Chile (see Muñoz & Olmos 2008). Given that morphology is not fully confident to identify this larval stage, molecular analyses can be required to support identification of metacercariae here recorded.

In the last decade, the global consumption of molluscs is increasing, so the aquaculture of molluscs is also increasing. The high density of cultivated molluscs normally put in risk of acquiring pathogens. Mussel *P. purpuratus* is an important native species due to role of bioengineering on intertidal zone and as bio-indicator of pollution in Chile, but this species also could play an important role in passing on pathogens and parasites. Therefore, clarify the specific identity of the micro-parasites here reported and life cycle of digenean (Renicolidae) is a first step to understand the potential relationships between parasites/pathogens, aquaculture, and environmental monitoring.

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