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Author(s): Josué A. Yee-Duarte, Bertha P. Ceballos-Vázquez, Evgueni Shumilin, Karen A. Kidd and Marcial Arellano-Martínez

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## PARASITIC CASTRATION OF CHOCOLATE CLAM *MEGAPITARIA SQUALIDA* (SOWERBY, 1835) CAUSED BY TREMATODE LARVAE

JOSUÉ A. YEE-DUARTE,<sup>1</sup> BERTHA P. CEBALLOS-VÁZQUEZ,<sup>1</sup> EVGUENI SHUMILIN,<sup>1</sup> KAREN A. KIDD<sup>2,3</sup> AND MARCIAL ARELLANO-MARTÍNEZ<sup>1\*</sup>

<sup>1</sup>Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Av. Instituto Politécnico Nacional s/n, Col. Playa Palo de Santa Rita, C.P. 23096, La Paz, B.C.S. México; <sup>2</sup>Canadian Rivers Institute & Department of Biology, University of New Brunswick, 100 Tucker Park Road Saint John, NB, E2L4L5 Canada; <sup>3</sup>Present address: Department of Biology, McMaster University, 1280 Main Street West Hamilton, ON, L854K1 Canada

**ABSTRACT** The prevalence of parasites in gonads of the clam *Megapitaria squalida* (Sowerby, 1835) was investigated at Santa Rosalía mining port in the Gulf of California. A total of 696 gonads were histologically analyzed, observing an unusual parasitic castration caused by the development of trematode larvae within follicles. Trematode sporocysts within follicles containing germinal balls and developing cercariae were observed and associated with an inflammatory response, as evidenced by a heavy hemocytic infiltration and the formation of granulocytomas surrounding the parasite structures. Some metacercariae were observed within ovarian connective tissues suggesting that *M. squalida* could also act as a second intermediate host for digenean trematodes. Infection was age specific as juvenile clams (1–4-cm shell length) did not contain parasites and prevalence in the adult clams increased with size. Unlike males, which presented four levels of infection density, all the parasitized ovaries of females showed very high infection densities. The prevalence was significantly ( $\chi^2 = 6.99$ ;  $df = 1$ ;  $P = 0.001$ ) higher in females (30.2%) than in males (17%). The highest prevalence of parasitized clams was in the ripe stage during the summer (43.7%) when seawater temperatures were highest, whereas the lowest prevalences were observed in winter. It is possible that the polluted environment of Santa Rosalía could increase the occurrence and high infection density by trematode larvae in *M. squalida*.

**KEY WORDS:** *Megapitaria squalida*, parasitic castration, sporocysts, cercariae, bivalve, heavy metal pollution

### INTRODUCTION

Many invertebrates serve as hosts for a wide variety of parasites (Prinz et al. 2010). More specifically, marine bivalve molluscs play a key role as hosts for larval stages of digeneans, with trematodes being the main parasites both in terms of numbers and in the damage and pathological conditions they cause (Thieltges et al. 2006, Muñoz et al. 2013). These effects include the impairment of growth and reproduction, and even survival, all of which can affect species abundances and, in turn, bivalve fisheries and aquaculture (Taskinen 1998, Mouritsen & Poulin 2010). The negative effects on reproduction include parasitic castration, as trematodes partially or totally inhibit oogenesis and spermatogenesis, leading to decreased fecundity and reproductive potential (Galaktionov & Dobrovolskij 2003, Valderrama et al. 2004, Boehs et al. 2010). The severity of these effects in a population, however, depends on the prevalence and intensity of the infection, which vary in relation to a series of biotic and abiotic factors (Koprivnikar & Poulin 2009, Gilardoni et al. 2012, Müller et al. 2015). Host size and sex (Thieltges 2008, Müller et al. 2015), reproductive maturity (Taskinen & Saarinen 1999), as well as seasonality (Muñoz et al. 2013) and seawater temperature (Poulin 2006, Prinz et al. 2010) have all been reported to modulate parasitism in bivalve molluscs. Likewise, pollution can also affect, either directly or indirectly, the occurrence, prevalence, intensity, and pathogenicity of parasites (Khan & Thulin 1991, Lafferty 1997) because contaminants such as metals change the resistance of the host to infections (Sures 2008, Morley 2010).

The port of Santa Rosalía is located within the Gulf of California in the central eastern coast of the Baja California Peninsula (Mexico) and is characterized by high concentrations of heavy metals in marine sediments and soils associated with copper mining and smelting that has been undertaken for nearly a century (Huerta-Diaz et al. 2014). As a result, coastal marine sediments near this port have abnormally high levels of some heavy metals and are potentially toxic to the marine biota (Shumilin et al. 2013). This site is home to beds of the chocolate clam *Megapitaria squalida* (Sowerby, 1835), a bivalve of high commercial value in the Mexican Pacific and the Gulf of California (Arellano-Martínez et al. 2006). Given the above, the objective of the present work was to document the unusual castration caused by sporocysts and to determine the prevalence and infection density of parasites in the gonads of the venerid clam *M. squalida* inhabiting the Santa Rosalía mining port. In addition, the potential relationship with maturation stage (juvenile/adult), sex, gonad development stages, and seasonality/sea surface temperature was explored.

### MATERIALS AND METHODS

#### *Sampling and Histological Treatment*

Samples were collected monthly from May 2012 to April 2013 at the Santa Rosalía mining port (27°20' N; 112°16' W), located in the Gulf of California, in the central-eastern coast of the Baja California Peninsula, Mexico. Simultaneously, bivalve samples were obtained at San Lucas beach located 13 km south of the port (27°13' N; 112°16' W), a site used as reference because neither port nor mining operations are carried out in this location. From each site and date, an average of 30

\*Corresponding author. E-mail: marellam@ipn.mx  
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*Megapitaria squalida* specimens were collected at depths between 5 and 10 m through semiautonomous “hookah” diving. For each clam, the total shell length (maximum anteroposterior distance of the shell) ( $\pm 0.1$  mm) was registered; then, a portion of gonad tissue was removed and fixed in 10% formaldehyde in seawater.

Subsequently, each portion of tissue was dehydrated, through a sequence of alcohols of increasing concentrations, followed by clearing with Hemo-De and embedding in Paraplast-Xtra. Five-micrometer-thick sections were obtained and stained with Harris’s hematoxylin and eosin (Humason 1979). The structure of the gonads was examined under a light microscope to determine the sex, the gonad development stage of each individual, and the presence of parasites. The gonad development stage was determined using the scale by Arellano-Martínez et al. (2006) for this species (rest, development, ripe, spawning, and spent).

#### *Analysis of Parasitism*

During the histological analyses of gonads, the characteristics of any trematodes found were recognized following the work of da Silva et al. (2002). The infection density (Bush et al. 1997) was determined in males and females by calculating the area occupied by parasites and gametes relative to the total area of follicles in five histological fields of each tissue section selected at random. These areas were calculated with the software SIGMA SCAN PRO (v. 5.0, Systat Software, Inc.). Infection density values were classified according to the scale proposed by Ceuta and Boehs (2012), as follows: light <5%, moderate, 5%–25%; high, 25%–50%, and very high, >50% of the tissue occupied by parasites.

The prevalence of parasites in clams was calculated according to Margolis et al. (1982) as the number of parasitized individuals divided by the total number of individuals sampled, expressed as a percentage. The prevalence was analyzed by maturity stage (juvenile/adult) and sex, as well as by gamete development stage and season of the year (spring, summer, autumn and winter). In addition, data on sea surface temperature were obtained through MODIS Aqua satellite images to investigate any potential relationship of this variable with the prevalence of parasites.

#### *Statistical Analysis*

Differences in the frequency of parasitized and nonparasitized clams (prevalence) for each gonad development stage (rest, development, ripe, spawning, and spent) and season of the year were evaluated using a chi-square test ( $\chi^2$ ). To explore the relationship between sexes, the prevalence of parasitized females relative to parasitized males was analyzed. Correlations between prevalence and size, and between prevalence and sea surface temperature, were determined with a Spearman’s rank test (Zar 1996). Statistical analyzes were carried out using the software STATISTICA for Windows (version 6.0, StatSoft). A significance level ( $\alpha$ ) of 0.05 was set for all tests.

## RESULTS

A total of 696 gonads of *Megapitaria squalida* were histologically analyzed: 370 from the Santa Rosalía mining port and 326 from San Lucas beach. Clams with parasitized gonads were

observed in Santa Rosalía only; accordingly, only data from this site were analyzed.

#### *Histological Structure of Parasitized Gonads*

Parasitized gonads showed digenean trematode infection directly inside of follicles, but their connective tissue, in both females and males, showed the same organization relative to nonparasitized gonads; histological changes, however, were evident within parasitized follicles, including the displacement of oocytes and sperm and a heavy hemocytic infiltration process.

In ovaries, sporocysts containing germinal balls and developing cercariae in a wide range of developmental stages were detected (Fig. 1A–C). Most follicles were almost completely invaded by sporocysts showing only some gamete remnants with complete castration in most of the observed cases. A heavy hemocytic infiltration process was observed, which led to the formation of granulocytomas surrounding some parasitic structures (Fig. 1D). In testes, a similar pattern was observed, with follicles internally invaded by sporocysts with germinal balls and developing cercariae (Fig. 2A–D).

Unidentified metacercariae of digenean trematodes encysted in the connective tissue of seven ovaries were distinguished (Fig. 3). These showed the common characteristics of metacercariae: an elongated body with an acetabulum (ventral sucker) and an oral sucker, the latter continued into a pharynx; the gut caecum located in the middle of the acetabulum and the excretory pore in the posterior zone. Neither spines nor reproductive structures were present.

#### *Infection Density*

Females were heavily affected by this parasite; all the parasitized ovaries showed very high infection densities, with virtually no gamete cells within the follicles. In contrast, males showed various levels of infection density, identified by the increase in the area occupied by parasites relative to the area occupied by gametes in the testes. The mean area occupied by parasites was 3.23% ( $\pm 0.38$  SE) in the lightest density (Fig. 2A), 16.13% ( $\pm 1.17$  SE) in the moderate density (Fig. 2B), 40.26% ( $\pm 1.16$  SE) in the high density (Fig. 2C), and 72.39% ( $\pm 1.56$  SE) in the very highest density (Fig. 2D) infection. Considering the total number of parasitized clams (males and females combined), most showed a very high infection density (68.5%).

#### *Total Prevalence of Parasites by Maturity Stage, Sex, Gonad Development Stage, and Seasonality/Sea Water Temperature*

Of the 370 clams collected in Santa Rosalía, 87 were parasitized. When stage of maturity was examined (juveniles versus adults; males and females combined), none of the 20 juvenile clams (1–4 cm shell length) had parasites, but the prevalence in adults increased with size; at shell lengths of 5–6 cm, 13.9% of adults were infected whereas at shell lengths of 7–8 cm, 30.6% of all individuals had parasites. When comparisons were made between sexes, females (30.2%) were significantly more infected than males (17%) ( $\chi^2 = 6.99$ ;  $df = 1$ ;  $P = 0.001$ ).

The percentages of parasitic infection by gonad development stage and season of the year/seawater temperature are shown in Table 1. The highest prevalence of parasitized clams was observed in the ripe stage during the summer (43.7%),

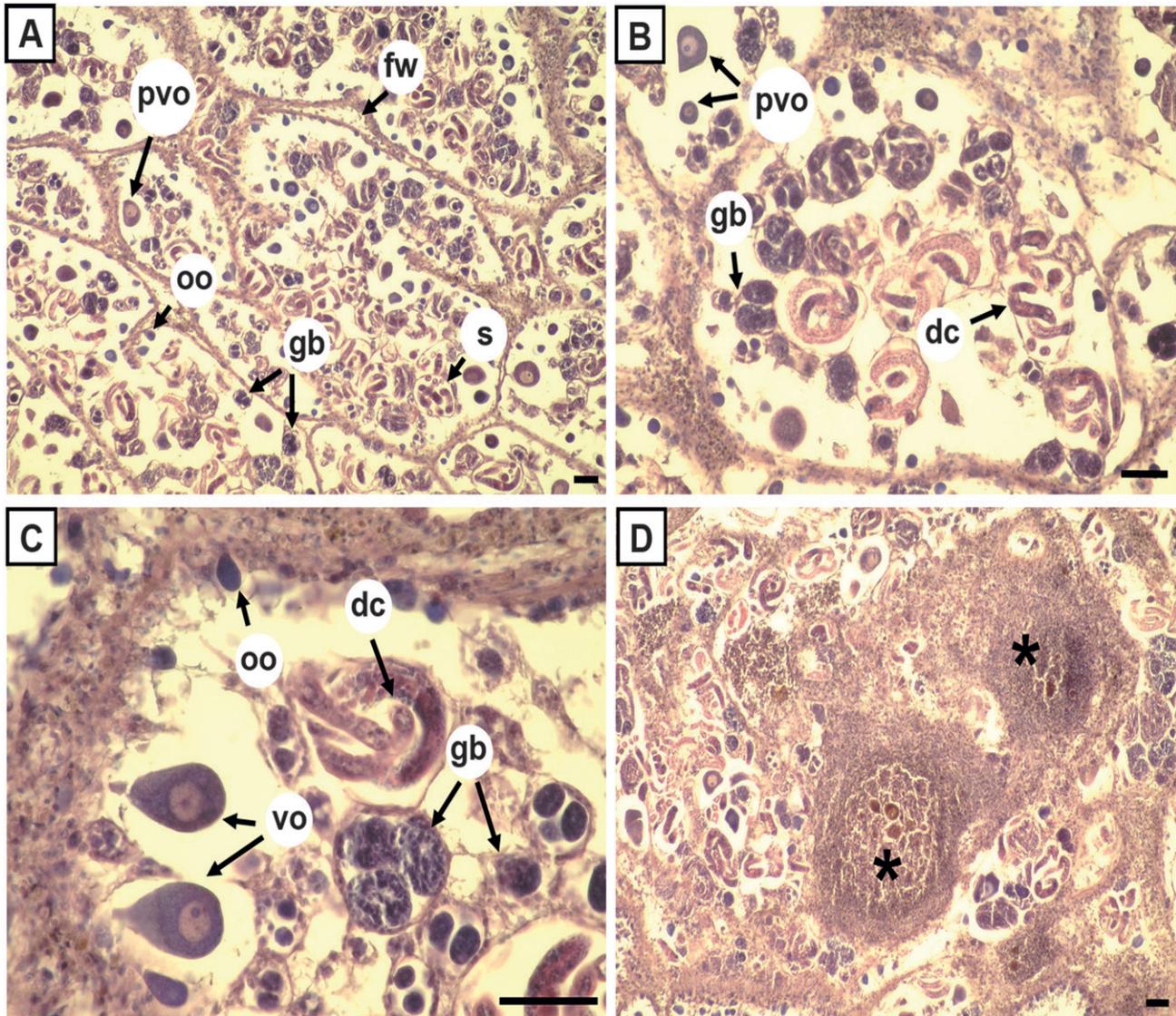


Figure 1. Photomicrographs of histological cross sections hematoxylin and eosin of *Megapitaria squalida* ovaries parasitized by trematodes. (A–C) Follicles show sporocysts (s) that contain germinal balls (gb) and developing cercariae (dc). Some oogonia (oo), vitellogenic oocytes (vo), and postvitellogenic remnants (pvo) are observed. fw, follicular wall. (D) Presence of granulocytomas (\*) in a parasitized ovary. Scale: 50  $\mu$ m.

corresponding with the highest seawater temperatures; in contrast, the lowest prevalence of infection was observed in winter (lower seawater temperature), independent of the stage of ripeness. A significant correlation was found between the percentages of infected clams and seawater temperature throughout the year ( $r = 0.582$ ,  $P = 0.046$ ) (Fig. 4). More specifically, the highest prevalence occurred in July and August (63% and 40%, respectively), when high temperatures were recorded (29°C and 30°C, respectively) and the lowest values were in November, December, and January (7%, 0%, and 0%, respectively) when seawater temperature dropped (23°C, 20°C, and 16°C, respectively).

#### DISCUSSION

In the Mexican Pacific, including the Gulf of California, a number of histological studies on the gonad tissue of

*Megapitaria squalida* have been conducted (Singh et al. 1991, Villalejo-Fuerte et al. 1996, Baqueiro-Cárdenas & Aldana-Aranda 2000, Villalejo-Fuerte et al. 2000, Arellano-Martínez et al. 2006, Romo-Piñera et al. 2009), and none of them has reported the presence of parasites. Hence, this is the first record of the occurrence of parasites, specifically of digenean trematodes, for the species.

The gonad is often the primary organ infected by larvae of digenean trematodes (Lauckner 1983), and this in turn affects the production of gamete cells and the reproductive performance of the host (Gilardoni et al. 2012, Corte 2015). The infection commonly takes place in the hemocoel space of the gonad (Cort et al. 1954, Lauckner 1983, Galaktionov & Dobrovolskij 2003); it is noteworthy, that in *Megapitaria squalida* clams from Santa Rosalía, the sporocysts were found instead inside the follicles. Terminal growth into the haemocoel spaces leads to a gradual compression of the acini by the

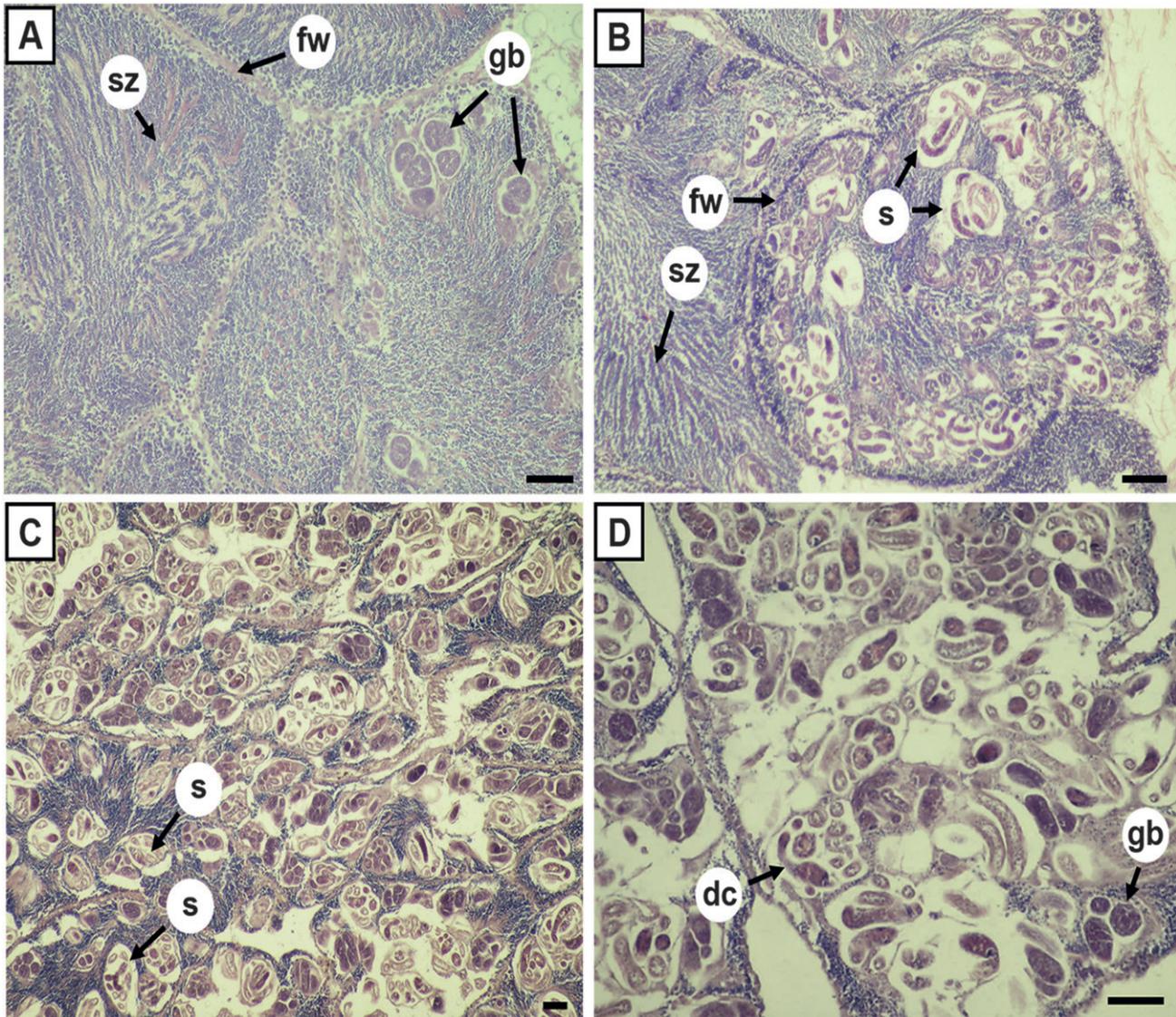
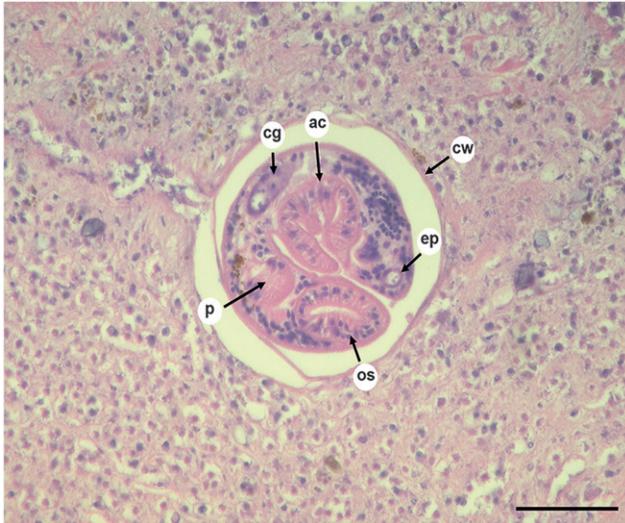


Figure 2. Photomicrographs of histological cross sections hematoxylin and eosin of *Megapitaria squalida* testes parasitized by trematodes. Different degrees of infection density are observed: light (A), moderate (B), high (C), and very high (D). (A–D) Follicles show sporocysts (s) that contain germ balls (gb) and developing cercariae (dc) among spermatozoids (sz); fw, follicular wall. Scale: 50  $\mu$ m.

sporocyst mass that results in partial or, mostly, complete parasitic castration (Lauckner 1983, Valderrama et al. 2004). Similar physical damage seems to be responsible for the castration in *M. squalida* but, instead of the acini being compressed from the outside (Lauckner 1983, Galaktionov & Dobrovolskij 2003, Valderrama et al. 2004), they are invaded from the inside, causing gradual destruction and replacement of the gametes through the accumulation of parasitic sporocysts (Oliva 1992). Histological changes within the gonad follicles of *M. squalida* from Santa Rosalía suggest that trematode larvae cause mechanical castration, because no indications of cellular alteration or disorganization were observed. Nevertheless, a chemical castration by parasites through the secretion of lytic substances and toxins that lead to the deterioration of reproductive cells (Oliva 1992) cannot be ruled out. Castration is most likely due to both mechanical damage and decomposition of tissues (Winstead et al. 2004).

Digenean trematodes may use bivalves as primary (first intermediate) or secondary (second intermediate) hosts, or—exceptionally—as definite hosts (see revisions by Lauckner 1983 and Galaktionov & Dobrovolskij 2003). In this case, the presence of sporocysts with germinal balls and developing cercariae indicates that *Megapitaria squalida* is used by trematodes as the first intermediate host, which is common in bivalve molluscs (Aldana et al. 2009, Gilardoni et al. 2011). The presence of encysted metacercariae in the connective tissue of the gonad, however, suggests that *M. squalida* could also act as a second intermediate host for digenean trematodes.

In general, the development of trematodes (sporocyst or rediae) in the first intermediate host is known to have negative effects, such as the inhibition of functions in the parasitized tissues (Corte 2015), but with very limited host reaction, if any (Lauckner 1983, Galaktionov & Dobrovolskij 2003, Boehs et al. 2010). In the current study was evident that the connective tissue



**Figure 3.** Metacercariae encysted in the connective tissue of an ovary of *Megapitaria squalida*. The cyst wall (cw), oral sucker (os), pharynx (p), acetabulum (ac), intestinal caecum (cg), and excretory pore (ep) are evident. Scale: 50  $\mu$ m.

of parasitized gonads, in both females and males, showed the same organization relative to nonparasitized gonads. Further evidence of the negative effect of parasites on the reproduction of *Megapitaria squalida* was the inflammatory response, as evidenced by a heavy hemocytic infiltration and the formation of granulocytomas surrounding parasite structures. This strong host reaction, presumably induced by sporocysts (or rediae), seems uncommon in molluscs acting as first intermediate hosts of digenetic trematodes (Cremonte et al. 2005). In bivalve molluscs, hemocytic activity is involved in several physiological functions; in the case of the immune response, these cells play a key role because they detect, encapsulate, and phagocytize foreign agents, which are subsequently removed (main cell defense mechanism) (Cima et al. 2000, Mayrand et al. 2005). The formation of granulocytomas indicates that *M. squalida*, in Santa Rosalía, has the immunological capacity to encapsulate and destroy parasites; its effectiveness, however, depends on infection density and the immunological status of the clam. In this regard, the heaviest hemocytic infiltration was observed in follicles with a very high infection density, similar to the findings reported by da Silva et al. (2002) in the mantle of mussels heavily infected by trematodes (*Bucephalus* sp.).

Unlike sporocysts, metacercariae caused no obvious negative effect on the histological structure of clams from Santa Rosalía, nor was a cellular response observed, perhaps because the infection intensity was low (one to three encysted metacercariae per specimen). In general, infection by digenetic metacercariae usually does not cause host castration (Lauckner 1983) or other serious damage or significant injury in bivalve hosts (Bower et al. 1994, Carballal et al. 2001, Winstead et al. 2004). On the other hand, when the abundance of parasites and the infection intensity are high, pathological effects are usually evident in tissues and the shell, including hypertrophy and deformity of the valves (Boehs et al. 2010), and trematode infections can be an important determinant of bivalve growth (Taskinen 1998, Thieltges 2006).

On the other hand, the analysis of prevalence by maturity stage revealed that parasites were present in the gonads of adult specimens (shell length >5 cm). This pattern agrees with reports of a higher prevalence of bucephalid infestation in larger/older mussels (Lasiak 1993, Villalba et al. 1997). Larger or older clams have been exposed to the infectious agent for a longer period, and filter higher volumes of water relative to smaller or younger individuals, hence being more susceptible to infection (Villalba et al. 1997, Boehs et al. 2010). It has also been suggested that larger hosts have more room to be parasitized and provide more available energy for parasites (Taskinen & Valtonen 1995).

Another uncommon result in this study is the higher prevalence of infection in females (30.2%) versus males (17%). Usually, both sexes of the bivalve host are affected and sterilized by bucephalids to a similar extent (Lauckner 1983). A deviation from this general rule has been observed in *Pecten alba* in Port Phillip Bay (Victoria, Australia), where up to 67% of the scallop population consisted of sterile females (Sanders 1966). In bivalve molluscs, the amount of energy allocated to reproduction is relatively high, particularly in females; hence, females may allocate less energy to immune defense, which makes them more vulnerable to parasitism (Taskinen & Valtonen 1995, Müller et al. 2015). Detailed studies to explain these discrepancies are lacking, and therefore, laboratory experiments are needed to examine the prediction that female clams are more vulnerable than males. The prevalence of parasites in these clams showed a marked seasonal pattern, with the highest prevalence in the summer, when seawater temperatures were highest. Even though, some studies have shown that high temperatures favor the maturation of trematode larvae as well as the rise in the production of

**TABLE 1.**

**Prevalence of parasites in the gonad of *Megapitaria squalida*. Seasonal variation of the total prevalence (%) by gonad development stage ( $\bar{x} \pm$  SD of shell length in cm). In addition, data of sea surface temperature ( $\bar{x} \pm$  SD).**

Seasonality	Spring (March–May)	Summer (June–August)	Autumn (September–November)	Winter (December–February)
Temperature ( $^{\circ}$ C)	20.3 $\pm$ 3.5	29 $\pm$ 1	27.3 $\pm$ 4	17.6 $\pm$ 2
Gonad development stages				
Rest (2.5 $\pm$ 0.3)	0	0	0	0
Development (7 $\pm$ 0.6)	0	6.2	0	12.5
Ripe (7.3 $\pm$ 0.7)	16.6	43.7	26.7	15.1
Spawning (7 $\pm$ 0.7)	15	16.2	16.1	12.5
Spent (6.9 $\pm$ 0.8)	0	14.2	0	0

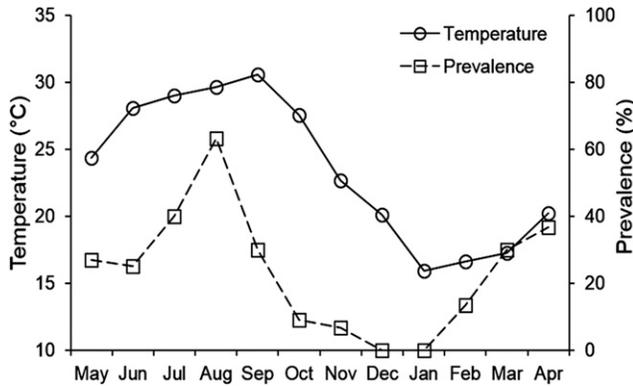


Figure 4. Monthly variation in seawater temperature and prevalence of parasites in the gonad of *Megapitaria squalida*.

cercariae (Poulin & Mouritsen 2006, Prinz et al. 2010, Muñoz et al. 2013); the cercarial emergence eventually plateaus at an optimum temperature threshold range before declining as temperatures reach high levels (Morley & Lewis 2013). Although temperature is considered one of the main parameters that governs the life cycle of many parasites, its effects may vary depending on the species (Koprivnikar & Poulin 2009).

Finally, the total prevalence of trematode larvae in the gonad of *Megapitaria squalida* from Santa Rosalía was very high (23.5%) when compared with those reported for other venerid clams in moderately contaminated or uncontaminated sites: 1.82% in *Eurhormalea lenticularis* (Valderrama et al. 2004), 6.67% in *Protothaca antiqua* (Cremonte et al. 2005), 7.43% in *Anomalocardia brasiliana* (Boehs et al. 2010) and 0.45% in *Amiantis purpurata* (Gilardoni et al. 2011). The above, together with the absence of parasites in San Lucas chocolate clams, suggest that the high prevalence of parasites in clams from Santa Rosalía is due to the abnormally high levels of some heavy metals, particularly Cu (3,390 mg kg<sup>-1</sup>), Zn (1,916 mg kg<sup>-1</sup>), Co (166 mg kg<sup>-1</sup>), Mn (6,770 mg kg<sup>-1</sup>), Pb (226 mg kg<sup>-1</sup>), and U (11.8 mg kg<sup>-1</sup>), which are toxic to marine biota (Shumilin et al. 2013). It is known that pollution-related stress and natural

environmental stress act synergistically or, rarely, antagonistically, and largely determine the response of an organism to disease-causing agents (Lauckner 1983). Similarly, organisms living in polluted sites are more susceptible to be parasitized, because pollutants negatively affect the immune system (low immunocompetence), hence lowering the resistance of the hosts to infections (Morley et al. 2006). Notwithstanding, it is also possible that the parasite has a differential distribution (no presence in San Lucas), but the proximity of both sites (13 km in coastline) makes this highly unlikely.

In conclusion, this first record of digenean trematode larvae in the gonad of *Megapitaria squalida* indicates that these bivalves serve as an intermediate host of the parasite and that high infection density causes parasitic castration, thereby affecting the reproduction of the host. Most probably, the polluted environment of Santa Rosalía favors the occurrence and high infection density of clams by trematode larvae, which increases in the warm season. Further analysis and laboratory work are needed to examine the mechanisms underlying the infections and to better understand this complex parasitism–pollution relationship.

Given the high commercial value and human consumption of the chocolate clam, it is essential to conduct further studies focused on the taxonomic identification and life cycle of its parasites (including other potential hosts). This information will be useful for the management of natural populations and the culturing of this clam, as well as for a proper health assessment of clams intended for human consumption.

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