



ORIGINAL ARTICLE

## The fit between parasites and intermediate host population dynamics: larval digeneans affecting the bivalve *Neolepton cobbi* (Galeommatoida) from Patagonia

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### Abstract

Temporal variations in prevalence and intensity of infection of larval trematodes in their second intermediate host, the bivalve *Neolepton cobbi*, were studied relative to the host population dynamics and season in the intertidal zone of Patagonian rocky shores. Two larval gymnophallid metacercariae of *Gymnophallidae* sp. and of *Bartolius* sp. were found parasitizing *N. cobbi*. The parameters of infection were related to the size structure of the host population. The high values of prevalence of *Gymnophallidae* sp. and *Bartolius* sp. found between late winter and early austral summer coincided with the dominance of intermediate and larger size classes in the host population. Conversely, the lower prevalence in midsummer coincided with the dominance of small size bivalves (mean size:  $0.7 \pm 0.1$  mm length) after the turnover of the host population. For metacercariae of *Gymnophallidae* gen. sp., the prevalence was related positively to host size. Mean intensity of infection (mean number of metacercariae per infected individual in a sample) of *Gymnophallidae* sp. showed no defined seasonal pattern, with more than 60% of bivalves showing low intensities ( $\leq 5$ ) on each sampling occasion. Intensity of infection was significantly higher in bivalves  $\geq 1.4$  mm length compared to smaller sizes, but showed a tendency to decrease in bivalves  $\geq 2.4$  mm length. For metacercariae of *Bartolius* sp., higher values of prevalence were found in bivalves with lengths between 1.4 and 2.4 mm. Prevalence was lower in larger size classes. Intensity of infection was not related to host size.

**Key words:** Bivalve hosts, *Gymnophallidae*, larval trematodes, seasonality of infection

### Introduction

Many invertebrates of the littoral macrozoobenthos act as intermediate hosts in the life cycle of a variety of digenean trematodes (Lauckner 1983), and the infection levels of these parasites is frequently governed by seasonal fluctuations in environmental parameters and the population dynamics of intermediate and definitive hosts (Thieltges 2007; Thieltges et al. 2008; Studer & Poulin 2012).

Digenean trematodes are parasites with complex life cycles, where invertebrates (usually molluscs, but also crustaceans and polychaetes) act as first and/or second intermediate hosts. In the first host, usually gastropods, parasites reproduce asexually, producing sporocysts and cercariae (the infective stage). When the cercariae leave the first intermediate host, they

may either infect a vertebrate, the definitive host, or infect a second intermediate host (often belonging to the same species as the first intermediate host or to a different species). In the second intermediate host, the cercariae develop into metacercariae, a larval stage (encysted or not encysted) that waits until transmission to an appropriate definitive host, a vertebrate, in which the adult parasites reproduce sexually and lay eggs (Pechenik 2000).

Trematode parasites can potentially affect key life-history traits of their hosts. Most literature refers to the impact of trematodes on first intermediate hosts. Sporocysts have been reported to cause castration, increased mortality and alteration of growth rates in the first intermediate hosts, frequently resulting in severe consequences for the affected population

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(Lauckner 1980; Lafferty 1993; Mouritsen & Poulin 2002; Fredensborg et al. 2005; Fredensborg & Poulin 2006). However, either acting directly or synergistically with other factors, the growth, behaviour and survival of the second intermediate host may also be affected. The detrimental effects caused by metacercariae to the second intermediate hosts include a decrease in their growth rates and survival (Lim & Green 1991; Wegeberg & Jensen 1999, 2003; Desclaux et al. 2002, 2004; Fredensborg et al. 2004; Thieltges 2006).

The parameters of infection by digenean trematodes in their second intermediate hosts, such as prevalence (% of infected individuals) and intensity (number of parasites per infected individual), usually exhibit temporal patterns in relation to biotic and abiotic environmental factors and their interplay (Meissner 2001; Thieltges et al. 2008). Regarding biotic factors, density of potential hosts, host condition, predation on infective stages and other biological interactions have been reported to affect transmission and infection processes (Mouritsen et al. 2003; Thieltges 2007; Thieltges et al. 2008). Parameters of infection in the second intermediate host were found to vary seasonally in relation to host, population dynamics and infection dynamics of the first intermediate host (Meissner 2001). Regarding abiotic factors, temperature can affect parasites, as well as the interactions with their hosts (Paull & Johnson 2011). Temperature affects the emergence, survival and infectivity of cercariae (Poulin 2006; Thieltges & Rick 2006; Studer et al. 2010) and may affect the physiology of the second intermediate host, enhancing the infection success (Thieltges & Rick 2006). Jensen et al. (2004) reported that infections by microphallid metacercariae in the isopod *Cyathura carinata* (Krøyer 1847) peaked in autumn and winter months, coinciding with rising bird abundance and a short period of appropriate temperature. Studer & Poulin (2012) linked abundance of the first intermediate host and rising temperature with increasing infection parameters in the second intermediate host.

The aim of the present study was to describe the temporal variations in the population dynamics of two larval trematode species, namely Gymnophallidae sp. and *Bartolius* sp. parasitising the bivalve *Neolepton cobbi* (Cooper & Preston, 1910) as the second intermediate host, in relation to the population dynamics of *N. cobbi*. To achieve this goal, we investigated the following topics: (1) the temporal variation in the prevalence and intensity of infection of the two larval gymnophallids parasitizing *N. cobbi* as metacercariae; (2) the timing of the variations in prevalence and infection intensity in relation to host population dynamics, host density and surface seawater temperature; and (3) the shell size/prevalence and

shell size/intensity relationships of both trematode species and *N. cobbi*.

Infections of marine bivalves with larval trematodes are common along Patagonian coasts; however, they have not been the subject of study until recently (Cremonte 2001 and references therein). Most of their life cycles remain unknown, and the interaction between parasites and their hosts (at cellular and individual levels) have been studied only in a few cases (Ituarte et al. 2001, 2005, 2009; Cremonte & Ituarte 2003). The results of the present study represent the first contribution towards the understanding of the seasonal dynamics of host–parasite systems in the Southwestern Atlantic.

## Materials and methods

The study was based on field data resulting from the monthly or bimonthly sampling of a *Neolepton cobbi* population over a 10-month period at Puerto Deseado (47°45'S, 65°55'W), Santa Cruz province, Argentina. The littoral at the study site is an extended rocky flat with shallow depressions of various depths, usually around 20 cm (referred to as tide pools), that retain water during low tides. The site shows a marked stratification of physical and biotic conditions from the land to the sea. Bivalves were collected in the intertidal zone during the lowest tides, monthly or bimonthly, from August 2009 to May 2010. Based on preliminary information indicating the existence of a spatial size segregation of the population of *N. cobbi*, the study area was divided into three zones along the intertidal: high, medium and low. Five samples were taken haphazardly from tide pools from each zone and in each sampling event. A plastic sampling box (15 × 15 cm) was placed over the shallow tide pools, and the thin layer of very fine sediment was extracted with a manual vacuum pump, washed through a sieve ( $\approx 400 \mu\text{m}$  mesh size) and preserved in plastic flasks with 5% formalin. The shell length of bivalves (the maximum distance from anterior to posterior end in mm) was measured under a stereoscopic microscope with an ocular micrometer. Mean length/size is given with standard deviation (SD). From the length measurements, size–frequency distributions corresponding to each sampling event were calculated, and polymodal frequency distributions were analysed using the Bhattacharya method (Sparre & Venema 1997) with the aid of the free software FISAT II Computer program. The mean size, standard deviation and separation index (SI) were obtained for the age groups (cohorts) identified. Age groups showing a SI greater or equal to 2 were considered significantly separated. The bivalves collected in each sampling event were counted and their density calculated.

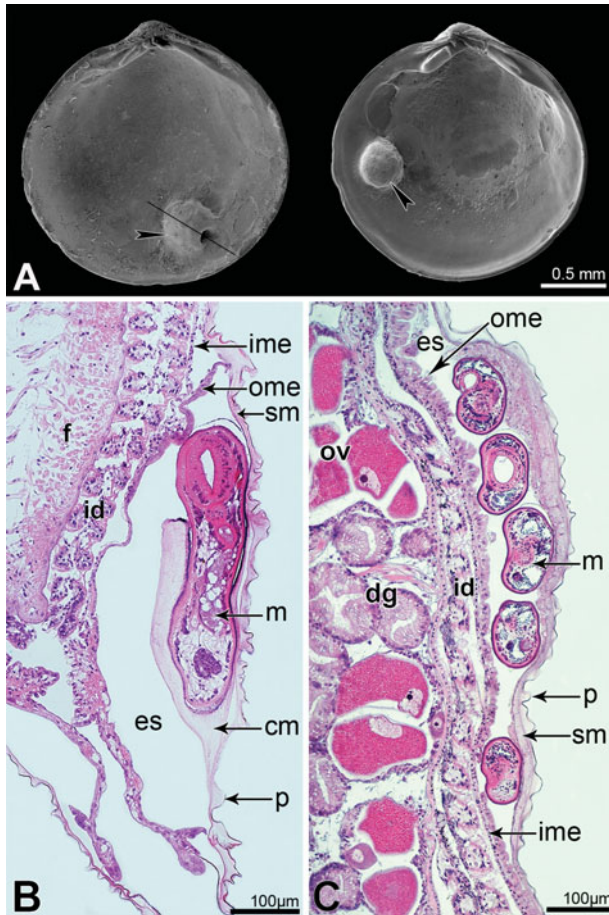


Figure 1. Metacercariae of Gymnophallidae parasitizing *Neolepton cobbi*. **A**, Reaction elicited by *Bartolius* sp. Inner view of two right valves with igloo-shaped coverings at different sites (arrows). **B**, Longitudinal section (at level of section depicted in **A**) of a metacercaria of *Bartolius* sp. showing the mantle reaction. **C**, Transverse section of a bivalve showing a cluster of metacercariae of Gymnophallidae sp. and the outer mantle epithelium reaction. Abbreviations: cm, organic matrix of igloo-shaped covering; dg, digestive gland; es, extrapallial space; f, foot; id, inner demibranch; ime, inner mantle epithelium; m, metacercaria; ome, outer mantle epithelium; ov, ovary; p, periostacum; sm, shell organic matrix.

Data on mean monthly surface seawater temperature at Puerto Deseado were obtained from the National Naval Hydrographic Service.

A subsample of 40 specimens of *N. cobbi* representative of each size range, calculated for each sampling occasion, was measured and decalcified in a 10% formalin solution with 5% acetic acid. This procedure allowed for counting metacercariae larvae by transparency. The prevalence (percentage of infected individuals), intensity of infection (number of metacercariae per infected bivalve) and mean intensity of infection (mean number of metacercariae per infected bivalve in a sample) were calculated for Gymnophallidae sp. and *Bartolius* sp. To facilitate the analysis of the temporal variations in the intensity of infection for the metacercariae of Gymnophallidae

sp., four intensity categories were defined as follows: low intensity,  $\leq 5$ ; moderate intensity,  $> 5$  and  $\leq 10$ ; high intensity:  $> 10$  and  $\leq 20$ ; and very high intensity,  $> 20$  larvae per host.

Based on the observed prevalence of larvae of the genus *Bartolius* according to size class and to obtain more reliable information on prevalence in the larger size classes (underrepresented in the original subsamples), additional specimens larger than 1.4 mm in length were studied. These specimens were taken from the primary samples.

The statistical analyses were performed with InfoStat/E software. The results were considered significant at  $p \leq 0.05$ . The required assumptions of homogeneity of variance and/or normality of data sets were considered after data transformation, and nonparametric tests were used. The correlation between the prevalence and intensity of Gymnophallidae sp. and the size of the bivalve host was analysed with a Spearman rank correlation coefficient. Differences in the intensity of Gymnophallidae sp. in bivalves with sizes below and above 1.4 mm were tested with a Wilcoxon test. To compare the monthly prevalence of Gymnophallidae sp. in bivalves below and above 1.7 mm in length (approximately half of the size range observed in *N. cobbi*), the Difference of Proportions Test (a test based on the Fisher Exact Test) was used. The values of prevalence of *Bartolius* sp. relative to host size were examined with a chi-squared analysis of contingency tables and a Tukey-type multiple comparisons test (for the latter test, the proportions were transformed using a modification of Freeman and Tukey 1950's transformation (Zar 1996)). In an attempt to evaluate the interactions between the considered variables in determining the patterns of infection found, we tried an analysis using Generalized Linear Models; however, the best predictive models obtained were of very low weight, so no conclusion could be drawn. Consequently, we only present the results of the seasonal dynamics in the parameters of infection observed and the relationship with each of the variables studied.

## Results

### *The parasites and their pathology*

Metacercariae of the two different Gymnophallidae, one belonging to an unidentified genus and species (Gymnophallidae sp.) and a second belonging to the genus *Bartolius*, were found occupying the extrapallial space and parasitizing *Neolepton cobbi* throughout the study period. Both species were usually found together in a single bivalve specimen, each species eliciting a different host reaction (Figure 1A–C).

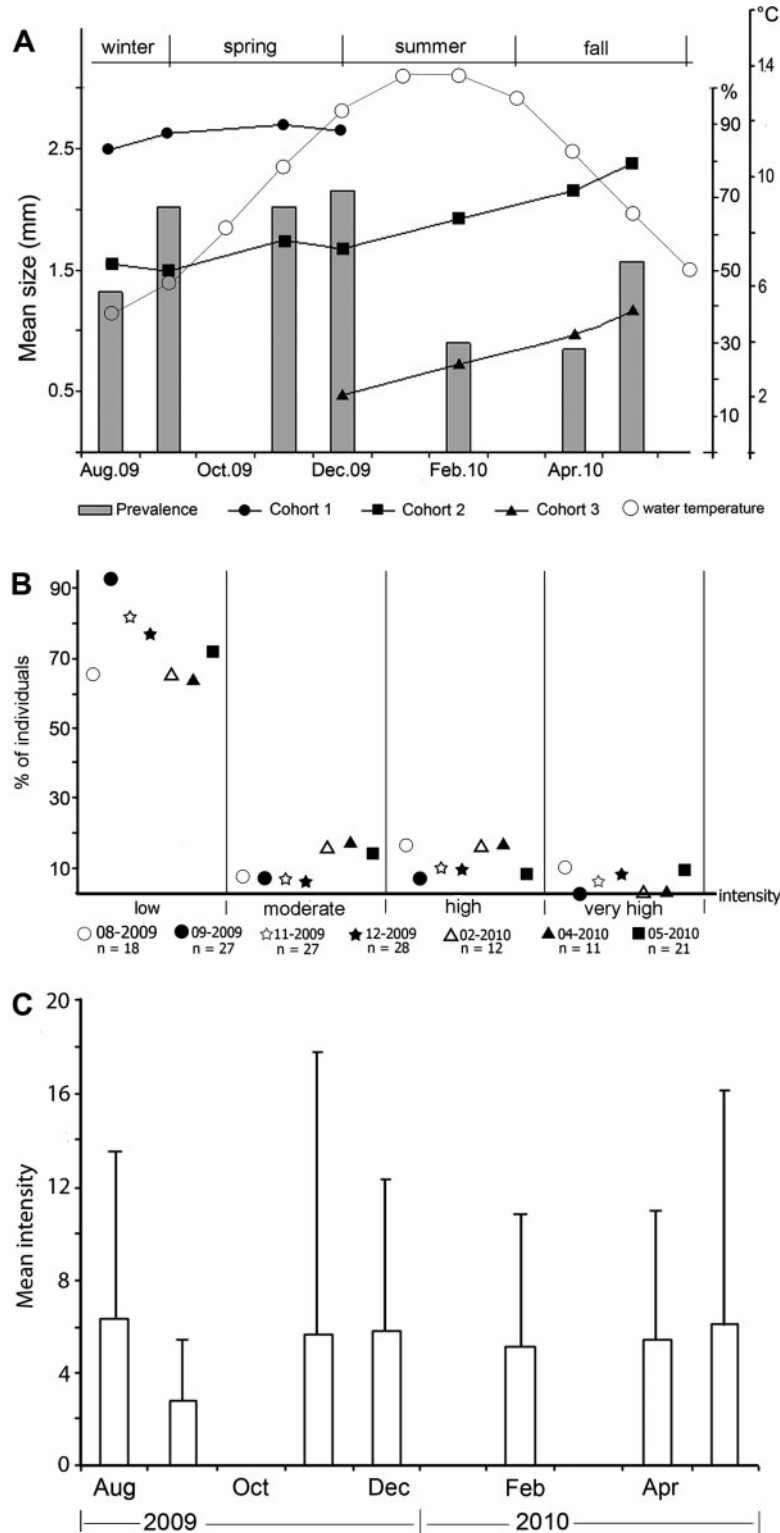


Figure 2. Measures of infection by metacercariae by *Gymnophallidae* sp. **A**, Temporal variation of prevalence related to shifts of mean values of size–frequency distributions of *Neolepton cobbi*, and annual variation of mean surface water temperature at Puerto Deseado. **B**, Percentage of individuals with different categories of intensity of infection in each sampling event (date format: mm-yyyy). **C**, Temporal variation of mean intensity of infection (mean + SD).

Metacercariae of *Gymnophallidae* sp. (mean size:  $198.8 \pm 18.5$   $\mu\text{m}$  length,  $132.9 \pm 14.0$   $\mu\text{m}$  width,  $n=17$ ) occurred in clusters of up to 41 larvae in

the extrapallial space, eliciting hyperplasia and metaplasia of the outer mantle epithelium (Figure 1C). Metacercariae of *Bartolius* sp. (mean

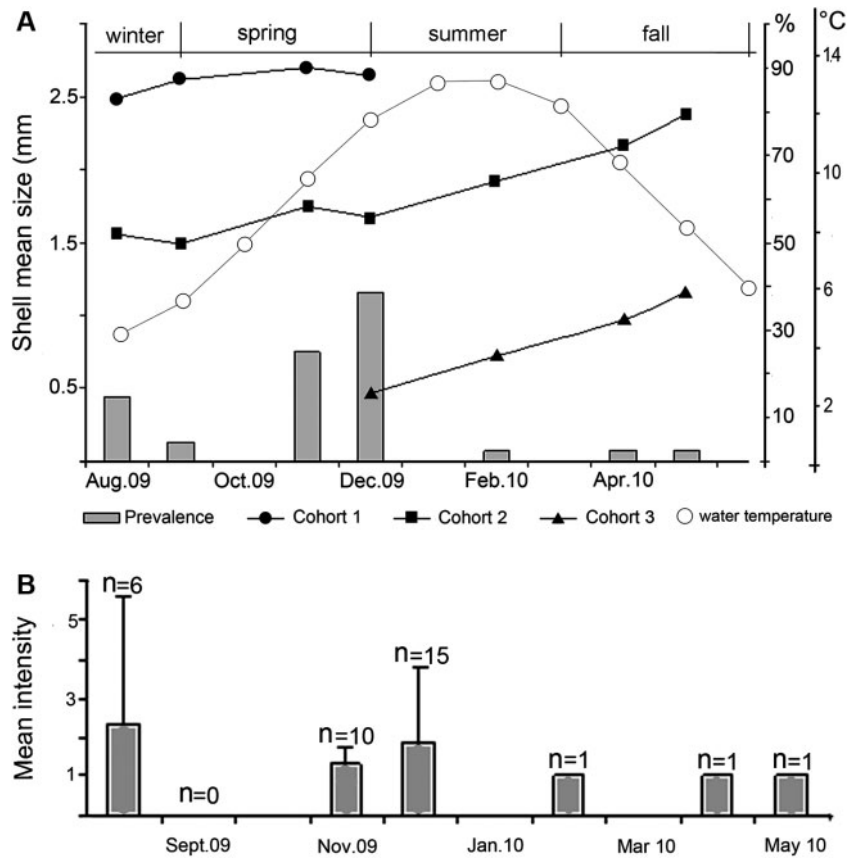


Figure 3. Measures of infection by metacercariae of *Bartolius* sp. **A**, Temporal variation of prevalence related to shifts of mean values of size–frequency distributions of *Neolepton cobbi*, and annual variation of mean surface water temperature at Puerto Deseado. **B**, Temporal variation of mean intensity of *Bartolius* sp. (mean + SD).

size:  $351.5 \pm 41.0$   $\mu\text{m}$  length,  $118.0 \pm 24.1$   $\mu\text{m}$  width,  $n = 10$ ) usually occurred as solitary parasites, provoking hyperplasia and metaplasia of the outer mantle epithelium and the alteration of the deposition of calcium carbonate to form an igloo-shaped covering partly surrounding each metacercaria (Figure 1A, B). If two or more larvae were found in the same host individual, each larva generated a shell–mantle complex reaction.

#### Temporal variation of prevalence and intensity of infection

The prevalence of metacercariae of *Gymnophallidae* sp. varied between 28.2% and 71.7%. Prevalence increased from the late austral winter (August), peaked at the beginning of summer (December), then decreased to 30%, remaining at this level from midsummer to the end of autumn (May 2010), when it reached 52.5% (Figure 2A).

The intensity of infection by *Gymnophallidae* sp. varied between 1 and 123 metacercariae per host individual. Within each sample, bivalves showing low-, moderate- and high-intensity degrees of infection were found (Figure 2B). Very high intensities

were only found between late winter and early summer (August–December 2009) and in late autumn (May 2010). The percentage of individuals with low-intensity infection was always greater than 60% (Figure 2B). Despite the substantial variation of intensity values, the mean intensity of infection by *Gymnophallidae* sp. was  $\sim 6$  larvae per bivalve host, with the exception of spring 2009 (September), when the values were less than 3 (Figure 2C).

The prevalence of metacercariae of *Bartolius* sp. varied seasonally between 2.5% and 38.4%, with the highest values between late winter and early summer (August–December 2009). By midsummer (February 2010), prevalence fell to 2.5%. Subsequently, the values showed no major change through May (Figure 3A). Overall, the intensity of infection was low, with means varying between 1 and 2.3 larvae per host, without a defined seasonality (Figure 3B).

#### Temporal pattern of host population

The size–frequency distributions of *Neolepton cobbi* were bimodal for most of the study period (Figure 4). From late winter to late spring (August–November), the host population structure was dominated by

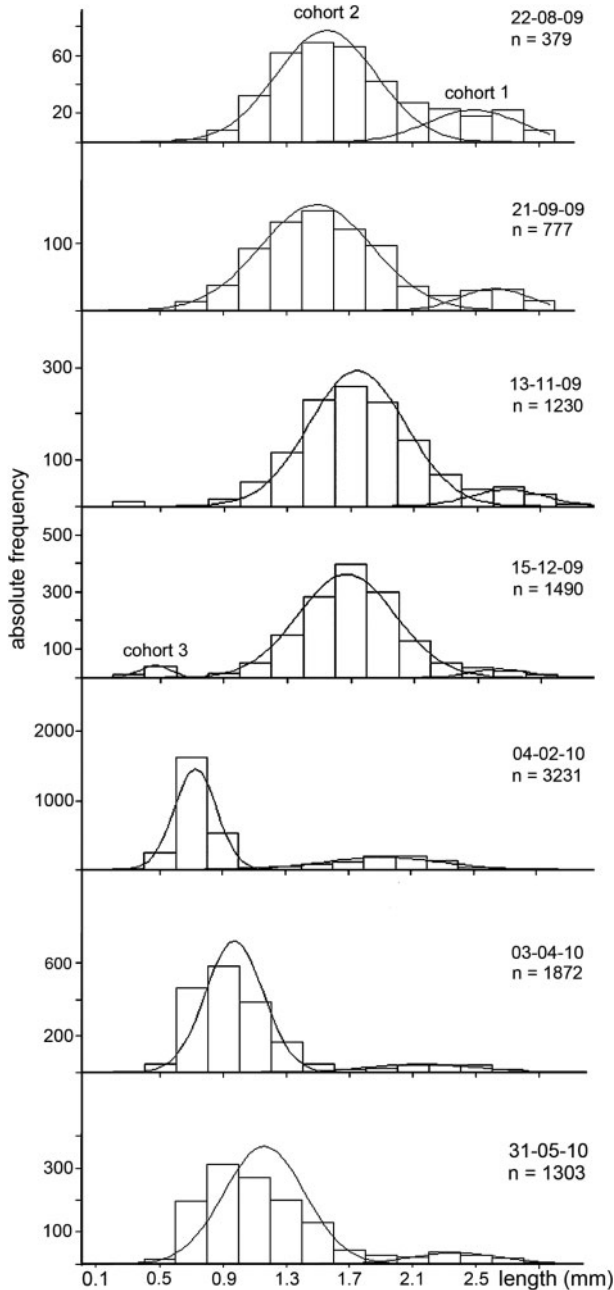


Figure 4. Size–frequency distributions of the bivalve *Neolepton cobbi* at Puerto Deseado during the study period (August 2009–May 2010). Date format: dd-mm-yy.

intermediate- and large-sized specimens (cohorts 1 and 2). By December 2009, at the start of the austral summer, a third cohort (mean size:  $0.46 \pm 0.09$  mm), whose first individuals settled in November, joined the population. By February 2010 (austral midsummer), the size peak of cohort 3 shifted to 0.7 mm length (SD: 0.1 mm), the dominant size class in the population. Thereafter, the individuals of cohorts 1 and 2 represented less than 25% of the monthly size frequencies. This population turnover occurred dur-

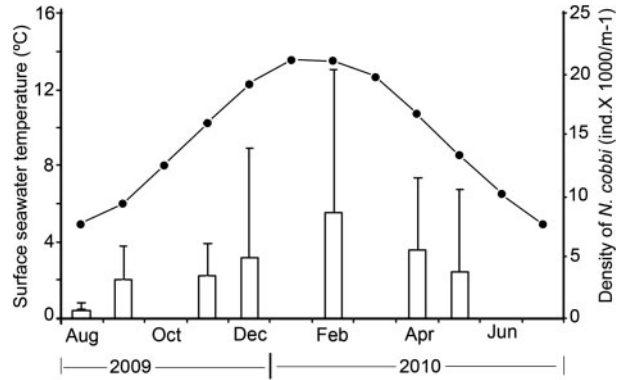


Figure 5. Mean density of the bivalve *Neolepton cobbi* during the study period (August 2009–May 2010; bars) and annual variation of mean surface water temperature at Puerto Deseado (continuous line).

ing the austral summer (December 2009 and January 2010; Figure 4).

The mean density of bivalves varied between 623.8 and 8594.8 individuals  $m^{-2}$ . The variations in the mean density of *N. cobbi* followed the variations in surface seawater temperature (Figure 5). The mean density of bivalves increased from late winter to late spring/early summer (August–December) and decreased from midsummer to late autumn (May). Similarly, the surface seawater temperature showed a consistent increase from the late austral winter to late spring/early summer (August–December), with a peak in midsummer (January–February) ( $\approx 13^{\circ}C$ ) and the lowest values in autumn/winter ( $\approx 4^{\circ}C$ ; Figures 2A, 3A, 5).

#### Correlation between parasites and host population dynamics, and host size

The parameters of infection were generally related to the size structure of the host population. The high values of prevalence of *Gymnophallidae* sp. and *Bartolius* sp. found between late winter and early summer (August–December 2009) coincided with the period in which intermediate and larger size classes of *N. cobbi* were dominant in the population structure. Conversely, the lower prevalence in midsummer coincided with the dominance of cohort 3 (mean size:  $0.7 \pm 0.1$  mm length) after the turnover of the host population (Figures 4, 2A, 3A).

Metacercariae of *Gymnophallidae* sp. were found affecting individuals of *Neolepton cobbi* between 0.6 and 3.5 mm in length. A positive correlation between prevalence and host size was found ( $\rho = 0.84$ ,  $p < 0.001$ , Spearman correlation coefficient; Figure 6A). The monthly prevalence found in individuals less than 1.7 mm in length (approximately half of the size range observed in *N. cobbi*) was significantly less than that found in bivalves  $> 1.7$  mm length ( $p < 0.05$  for

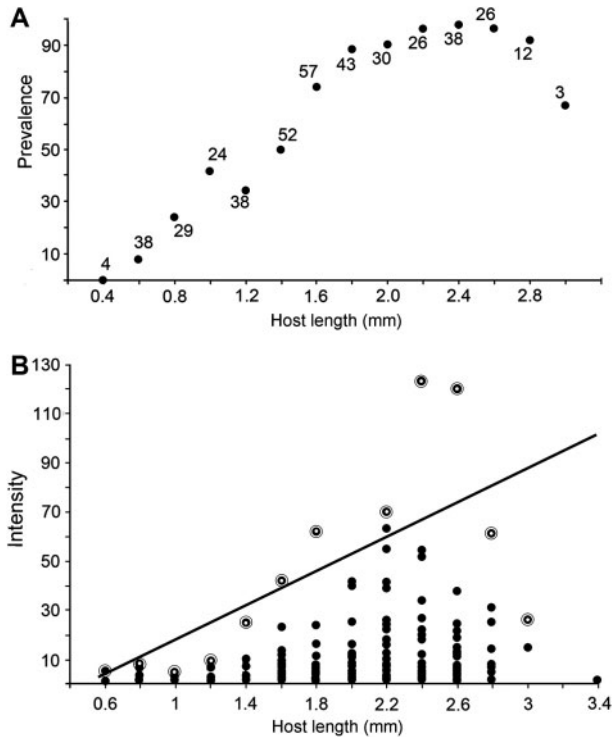


Figure 6. Measures of infection by metacercariae of Gymnophallidae sp. **A**, Relationship between prevalence and host length (numbers beside data points indicate  $n$  for each size class). **B**, Relationship between intensity of infection and host shell length (the line in the figure corresponds to the linear correlation between maximum values of intensity (dots with white centre) found within each size class studied).

all months studied, Difference of Proportions Test). The intensity of infection with metacercariae of Gymnophallidae sp. was positively correlated with host size ( $p=0.42$ ,  $p<0.001$ , Spearman correlation coefficient). The maximum values of intensity observed within each size class studied were positively correlated with host size ( $\rho=0.75$ ,  $p=0.01$ , Spearman correlation coefficient; Figure 6B). Bivalves between 0.6 and 1.4 mm in length had low- and moderate-intensities of infection, whereas high and very high intensities of infection were found in bivalves  $\geq 1.4$  mm in length. The intensity in bivalves  $\geq 1.4$  mm in length was significantly higher than in bivalves of sizes  $< 1.4$  mm length ( $W=2441$ ,  $p<0.001$ , Wilcoxon test). Extreme values of intensity ( $> 120$ ) were found in individuals between 2.4 and 2.8 mm in length. However, with the exception of these extreme values, the intensity values in size classes greater than 2.4 mm consistently showed a tendency to decrease with host size (Figure 6B).

*Bartoliuss* sp. was found infecting bivalves  $> 1$  mm in length. Prevalence was not independent of host size (Pearson chi-squared = 37.63,  $p<0.001$ ). The prevalence in bivalves between 1.4 and 2.4 mm in length was significantly higher than in bivalves with

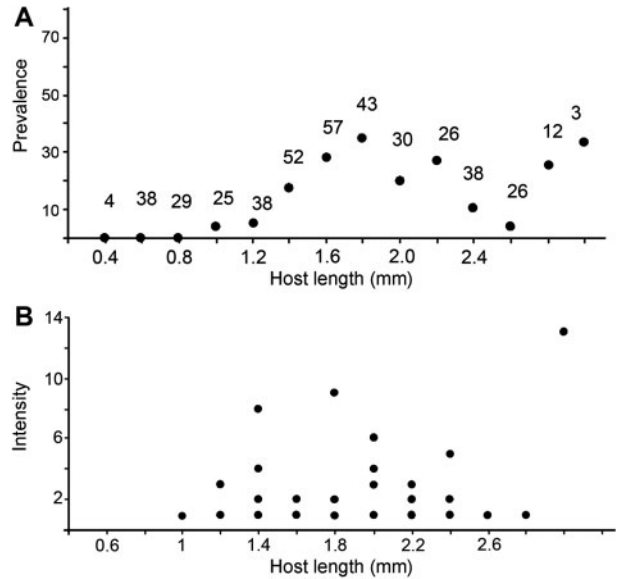


Figure 7. Measures of infection by metacercariae of *Bartoliuss* sp. **A**, Relationship between host length and prevalence for each size class at Puerto Deseado (numbers above data points indicate sample size). **B**, Relationship between host length and intensity of infection.

sizes above and below this range ( $q=9.44$  and  $q=3.90$ , respectively,  $p<0.05$ , Tukey multiple comparison test). In bivalves 1.4–2.4 mm in length, prevalence was higher than 17%, whereas in bivalves  $< 1.4$  mm and between 2.4 and 2.8 mm, prevalence was always less than 10% (the values of the prevalence of bivalves greater than 2.8 mm in length might be overestimated due to the limited data available) (Figure 7A). Intensity of infection by *Bartoliuss* sp. varied between 1 and 13 metacercariae per bivalve host. However, the values in most of the cases studied were 1 or 2 ( $n=65$ ), irrespective of the size of the host (Figure 7B).

## Discussion

The 10-month survey of larval digenean infections in the bivalve *Neolepton cobbi* at Puerto Deseado showed that the infection parameters varied seasonally. A high prevalence of infection for both larval gymnophallids studied was observed from the late austral winter to the late spring/early summer, decreasing markedly in midsummer/autumn after the host population turnover, when bivalves less than 1 mm in length were dominant in the population structure.

The host population structure also showed a seasonal pattern consistent with changes in surface seawater temperatures at Puerto Deseado during the study period. The shift in the size–frequency distributions of the host population closely followed the variations in surface seawater temperature. A con-

sistent increase in temperature from late winter to late spring/early summer coincided with the dominance of growing cohorts 1 and 2. The temperature peak in January–February marked the population turnover (Figure 4). The recruitment period of *N. cobbi* was relatively long, extending from late spring to summer. This observation is consistent with the presence of brooding females of *N. cobbi* (i.e. females bearing egg capsules adhering to the anteroventral shell margin) from August 2009 (late winter) to February 2010 (midsummer) (unpublished data).

The turnover of the host population represented a major event that strongly affected the dynamics of the parasite populations supported by the bivalve. The fluctuations in prevalence were correlated with the pattern of temporal variation of the size structure of the population of *N. cobbi*. The overall values of prevalence and intensity by infection by Gymnophallidae sp. increased with host size. However, the values of infection intensity showed a consistent decrease above a size of 2.4 mm. In contrast, the infection intensity of *Bartolius* sp. was not correlated with host size (most likely masked by low values of prevalence). The prevalence of *Bartolius* sp. also showed a different pattern of variation with host size. The values increased up to a length of 1.8 mm, decreasing in larger specimens (except in the size range of 2.8–3.0 mm, where only a few observations were made). Based on host size/prevalence and/or intensity relationships, several authors have postulated that the decrease in prevalence and/or intensity values in larger bivalves could indicate that the parasites represent a source of differential mortality for the affected population (de Mountaudouin et al. 2000; Desclaux et al. 2004; Jensen et al. 2004).

Temperature is one of the environmental factors generally considered to play a determining role in the modulation of marine invertebrate life histories (Giese & Pearse 1974). Temperature also affects the emergence, survival and infectivity of cercariae after leaving the first intermediate host, with increasing number and infectivity up to an optimum temperature level (Thieltges & Rick 2006; Studer et al. 2010). An increase in the number and infectivity of cercariae will affect the second intermediate host population, increasing prevalence and infection intensity. However, our seasonal field study at Puerto Deseado showed that most of the parasite transmission from the still-unknown first intermediate host to *N. cobbi* occurred from the late austral winter to late spring, where higher values of prevalence were observed; in contrast, during the warmest summer months (January and February) prevalence showed a marked decrease (Figures 2A, 3A). Although we cannot provide conclusive evidence, this result could indicate that temperature, in the case studied, is not

a directly determining factor of the temporal pattern of transmission. Rather, transmission appears to be more closely linked to the size/age of the potential hosts. This hypothesis is also supported, in the case of Gymnophallidae sp., by the high percentage of bivalves with a low-intensity category of infection ( $\leq 5$  larvae per bivalve host) in all months surveyed, which could reflect a permanent incorporation of infective stages (cercariae) by the second intermediate host. This outcome could only be possible if infective stages emerge from the first intermediate host throughout the year. The intensity of infection increased with increases in the size of the bivalve host. This result could be explained by a longer time of exposure to a continuous flux of infective stages in the environment and the consequent accumulation of infections over the lifespan of the older bivalves. Regarding host density, which is recognized to affect biotic interactions such as the transmission processes of parasites (Mouritsen et al. 2003; Studer & Poulin 2012), in the case of the larval gymnophallids studied here, variation of host densities were highly correlated with the temporal pattern of surface seawater temperature. The maximum values of density of *N. cobbi* occurred in summer months (February) coinciding with the decrease of prevalence values. Studer & Poulin (2012) have demonstrated a different pattern of transmission rates, higher during warm summer months, of the trematode *Maritrema novaezealandense* (Martorelli et al., 2004) from its first intermediate host, the snail *Zeacumantus subcarinatus* (G.B. Sowerby III, 1855), to its second intermediate host, the amphipod *Paracalliope novizealandiae* (Dana, 1852). Their study was conducted at a southern latitude similar to that of the site of the present study. Meissner & Bick (1997) also found increasing prevalence and intensities of infection of microphallid metacercariae in a population of the amphipod *Corophium volutator* (Pallas, 1766) over the summer, with the highest values in late summer and autumn. Interestingly, in that case the infection dynamics were mainly explained by the population dynamics of the amphipod, as we found in *N. cobbi*, and its coincidence with the infection dynamics of the first intermediate host.

The occurrence of maximum values of intensity of Gymnophallidae sp. specific to each host size class considered (the dots with a white centre in Figure 6B) may suggest that a mechanism exists to limit the parasite load for different host sizes (i.e. each host size allows a particular limited number of larvae to be lodged in the extrapallial space). Similar limitations in the parasite load originating in the space available for larvae were reported in the isopod *Cyathura carinata* (Krøyer, 1847) parasitized by



microphallids (Jensen et al. 2004). Cremonte & Ituarte (2003) discussed different alternatives allowing escape from this limitation of space in the bivalve *Darina solenoides* (King & Broderip, 1832) parasitized by metacercariae of the gymnophallid *Bartolius pierrei* Cremonte, 2001. According to Bartoli (1981), the mode of recruitment of metacercariae in their hosts (limited or unlimited) depends on both the volume of space in the host available for lodging larvae and the characteristics of the environment where the parasite system develops. If the physical environmental conditions and the dynamics of adult parasites and definitive host populations determine a low density of infective larvae, unlimited recruitment is considered an adaptive condition, favouring the concentration of infective stages in the intermediate host. If the number of infective stages is consistently high over time, as could be the case for larvae of *Gymnophallidae* sp. affecting *N. cobbi*, limited recruitment serving to preserve the minimum space required for developing larvae is considered a positive trait ensuring that the available space is adequate for the normal development of the larvae.

Note that the population of *N. cobbi* investigated in this study suffers a certain degree of environmental stress due to exposure to organic effluents leaking into the littoral environment and originating from the waste pipelines of fish-processing industries. Such locally concentrated organic enrichment would favour the development of the population of *N. cobbi* at this site (Martin et al. 2009). Thieltses et al. (2008) considered that anthropogenic pollutants may affect the conditions experienced during transmission and infection. For this reason, it would be of interest in the future to undertake comparative parasitological studies on populations from habitats with different levels of environmental disruption and on the life history characteristics of parasites affecting them.

In summary, our results indicate that the population dynamics of the parasites in the host–parasite system examined in this study are strongly related to the variation in the population structure of the second intermediate host. The variation of the parameters of infection showed a well-defined seasonal pattern during the study period, with increasing infection levels until the turnover of the bivalve host population in early austral summer.

An important limitation for the analysis of seasonality in the patterns of infection lies in the lack of knowledge of the life cycle of the parasites studied. This prevented us from considering relevant variables affecting the dynamics of infection parameters, such as the density of the first intermediate host that provides the infective stages and the phenology of the definitive host birds, as for all gymnophallids. Despite this, it was

possible to describe strong patterns of seasonality in infection parameters and their relationship with second host population dynamics.

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