

# Spatio-temporal variations in larval digenean assemblages of *Heleobia parchappii* (Mollusca: Cochliopidae) inhabiting four human-impacted streams

## Research Paper

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## Abstract

In the last years, a growing number of studies have focused on the search for biological indicators of human impact in freshwater environments. Given their susceptibility to different types of impact, larval digeneans have been considered as useful tools for the study of temporal variations in parasite communities. However, few studies have been carried out in lotic environments. For that reason, the spatio-temporal variations of the larval digenean assemblages parasitizing the gastropod *Heleobia parchappii* were analysed in four human-impacted streams, located on the coast of Argentina. In total, 4800 specimens of *H. parchappii* were collected seasonally, and 12 digenean species belonging to eight families were registered. Three of the streams showed similar species presence but, during spring and summer, the streams located within the city presented lower species presence than the streams located in the urban periphery. By contrast, the spatial prevalence values evidenced a higher variation between the environments. Streams located in the urban periphery showed higher prevalence values throughout the year, compared to those calculated for streams located within the city. The spatio-temporal variations of larval digenean assemblages parasitizing *H. parchappii* seem to be mainly influenced by the diversity and vagility of definitive hosts, the types of digenean life cycles and habitat characteristics. However, environmental disturbances derived from anthropogenic activities are highlighted as the probable main factors that may affect the composition and dynamic of these parasite assemblages.

## Introduction

Freshwater ecosystems contain about 9.5% of all described animal species and can be considered among the most endangered ecosystems in the world (Dudgeon *et al.*, 2006; Balian *et al.*, 2008). Declines in biodiversity in freshwater environments are far greater than in the most affected terrestrial ecosystems, and streams stand out as biodiversity hotspots (Domisch *et al.*, 2015). The settlement of human populations around these environments, with the combination of land use, habitat fragmentation and climate change, contribute to intensifying the vulnerability of animal species inhabiting freshwaters ecosystems (Carpenter *et al.*, 1992; Dudgeon *et al.*, 2006).

In the last years, a growing number of studies have focused on the search for biological indicators of human impact in aquatic environments. Taking into account that the abundance and virulence of parasites can increase or decrease under the influence of human activity, parasites rank among the most sensitive bioindicators of environmental disturbances (Lafferty, 1997; Marcogliese & Cone, 1997; Marcogliese & Price, 1997; Valtonen *et al.*, 1997; Huspeni & Lafferty, 2004; Blanar *et al.*, 2009; Di Giorgio *et al.*, 2016). Understanding how parasites respond to climate and anthropogenic pressures can provide important information about the stability of the ecosystems (Morley, 2007). For that reason, to elucidate how parasite–host systems could change in response to natural or human disturbance, it is necessary to identify temporal patterns in infection dynamics.

Among the parasites, the digeneans (Platyhelminthes: Trematoda) have been used as study models for the investigation of temporal variations in host–parasite systems (Gérard 2001; Yurlova *et al.*, 2006; Herrmann & Sorensen 2009; Studer & Poulin 2012; among others). These parasites usually have three-host life cycles, which include free-living infective stages (miracidia and cercariae). These free-living stages of digeneans are exposed to variations of environmental factors (natural or human-induced) that influence their development and mobility, and that can interfere with the contact between hosts, hindering the completion of parasite life cycles (Pietrock & Marcogliese, 2003).

Knowledge about the composition and diversity of helminth parasite assemblages in naturally poor in abundance ecosystems is limited, and streams, in particular, are not frequently considered for patterns in biodiversity, probably because of their low species diversity (Hernandez *et al.*, 2007). Argentina has a significant number of streams that cross through populated or rural areas, most of them with null or incomplete treatment (Feijoó & Lombardo, 2007), and are thus very vulnerable ecosystems and different from many streams of the world (Giorgi *et al.*, 2005). However, there is little information on the ecological characteristics of these environments (Giorgi *et al.*, 2005). The snail *Heleobia parchappii* (d'Orbigny, 1835) (Gasteropoda: Cochliopidae) is one of the most representative species of the genus in freshwater bodies (streams, rivers and shallow lakes) in Argentina, Uruguay and Brazil (Gaillard & De Castellanos, 1976; Castellanos & Landoni, 1995). From a parasitological point of view, this host is very interesting since it serves as host for at least 25 digenean species in Argentina, which make up species-rich digenean assemblages (Merlo, 2013; Merlo *et al.*, 2019). Moreover, the study of Merlo *et al.* (2019), carried out in human-impacted shallow lakes, made it possible to identify several factors determining the temporal variations of the digenean assemblages of *H. parchappii*.

Considering that no attempts have been undertaken to analyse the temporal variation of the larval digenean assemblages of this molluscan host in lotic environments of Argentina, the main achievement of this study was to analyse the spatio-temporal variations of the digenean assemblage of *H. parchappii* in four streams, located along 65 km of the Argentine coast, and to evaluate how local factors can influence these assemblages in lotic environments.

## Materials and methods

### Study area

The four streams selected for this study are located on the southeastern coast of Argentina: Tapera stream (TS: 37°56'S, 57°32'W), Corrientes stream (CoS: 38°05'S, 57°33'W), Lobería stream (LS: 38°09'S, 57°38'W) and Carolina stream (CaS: 38°20'S, 57°59'W) (fig. 1). These Pampean streams are characterized by running waters with slow current due to the low slope, high nutrient levels, variable turbidity, riparian vegetation dominated by grassland and by absence of dry periods or extreme temperatures that favour the development of dense and rich macrophyte communities (Giorgi *et al.*, 2005; Feijoó & Lombardo, 2007). They are also heavily impacted by human disturbances mainly derived from agricultural activities and urbanization (Giorgi *et al.*, 2005).

### Sample collections

A total of 4800 specimens of *H. parchappii* were collected from one sampling trip per season during 2014 in each stream (1200 per season and per stream). Five replicate samples per sampling trip and per stream were taken. Snails were collected from the stream coast along a 120-m transect with the aid of sieves (0.1 mm × 0.1 mm) and placed into plastic containers of 1.5 l capacity for transportation. The physical parameters of each stream were measured at every sampling trip per season: water temperature, depth, width, water velocity and turbidity. The water temperature was measured using a digital multi-thermometer (range -50°C to 150°C), a metric aluminium rod was used to

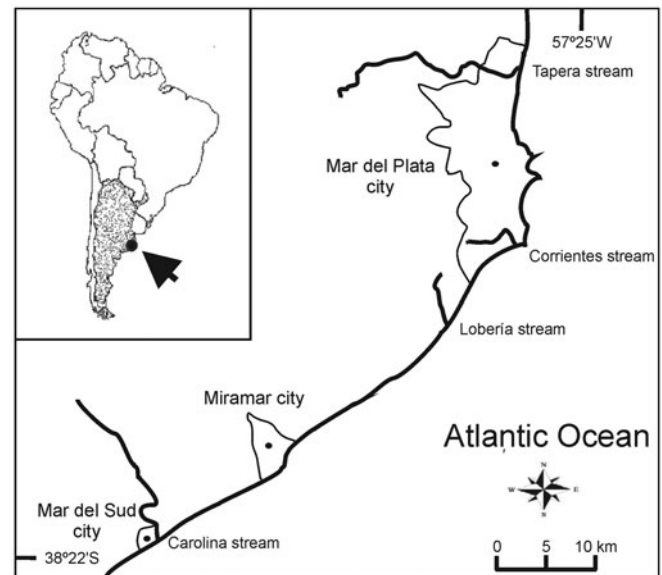


Fig. 1. Map showing locations of streams on the Argentinean coast, surrounding Mar del Plata, Argentina.

measure the height of the water column and the width of each stream, the water velocity was measured by recording the time taken for a float to travel a known distance along the channel and the turbidity was measured with a Secchi disk. The seasonally relative abundance of *H. parchappii* was estimated by reference to a standardized search effort (number of snails caught per hour) and conducted by the same person to avoid sampling bias. This method for quantifying relative abundance was adopted due to the turbidity of the streams, which made it impossible to either view the substratum or collect all of the snails in a given area (Martín *et al.*, 2001; Prepelitchi, 2009).

In the laboratory, molluscs were placed individually in plastic containers until further analysis. The shell length of each *H. parchappii* was measured to the nearest 0.05 mm using a Leica DM 500 stereomicroscope (Leica S.A., Buenos Aires, Argentina). Randomly selected molluscs were isolated individually and exposed to a 100-watt incandescent lamp for 48 h to stimulate the shedding of cercariae. Finally, all gastropods were dissected under a stereomicroscope and all organs and tissues were examined to detect the presence of sporocysts, rediae, developing cercariae and metacercariae (Curtis & Hubbard, 1990). Emerged cercariae were studied alive, under a light microscope, and were identified according to Szidat (1956), Yamaguti (1975), Martorelli (1986, 1989, 1990), Martorelli & Etchegoin (1996), Etchegoin & Martorelli (1998) and Merlo *et al.* (2014).

### Statistical analyses

To analyse and compare the temporal and spatial dynamics of the community of larval digeneans in *H. parchappii* in the four streams, two indices were used: seasonal overall prevalence as the number of parasitized snails/the number of collected snails × 100 (Lafferty *et al.* 1994); and prevalence by species as the number of snails parasitized by that species/the number of collected snails × 100.

The differences in community structure between streams were checked with two permutational multivariate analysis of variance

**Table 1.** Detailed list of species or morphological types of larval digeneans parasitizing *Heleobia parchappii* in four streams detected in this study.

Family	Species/morphological type	Stream	Ref.	Definitive host
Notocotylidae	Notocotylidae sp. 1	TS-CoS-LS-CaS	1	Birds and mammals
Echinostomatidae	Echinocercaria sp. 1	CoS	2	Birds, mammals and fishes
Heterophyidae	Pleurolophocercaria VI	CaS	3	Birds and mammals
	<i>Ascocotyle (L.) hadra</i>	TS-CoS-LS-CaS	4; 2	Birds and mammals
	<i>Pygidiopsis crassus</i>	TS-LS-CaS	5	Birds and mammals
	Cercaria Heterophyidae sp. 8	TS-LS-CaS	6	Birds and mammals
Microphallidae	<i>Microphallus similimus</i>	TS-CoS-LS-CaS	2	Birds and mammals
	<i>Microphallus szidati</i>	TS-CoS-LS-CaS	7; 2	Birds and mammals
Acanthostomidae	Cercaria Acanthostomidae sp. 1	TS-CoS-LS-CaS	8	Fishes, reptiles and amphibians
Haploporidae	Cercaria Haploporidae sp. 1	TS	2	Fishes
Psilostomidae	<i>Psilochasmus oxyurus</i>	TS-CoS-LS-CaS	9	Birds and mammals
Hemiuridae	<i>Genarchella genarchella</i>	TS-LS-CaS	10	Fishes

TS, Tapera stream; CoS, Corrientes stream; LS, Lobería stream; CaS, Carolina stream. Ref.: 1, Etchegoin & Martorelli (1998); 2, Etchegoin (1997); 3, Martorelli & Etchegoin (1996); 4, Ostrowski de Núñez (1992); 5, Martorelli & Etchegoin (1996); 6, Parietti (2011); 7, Martorelli (1986); 8, Merlo (2013); 9, Szidat (1957); 10, Szidat (1956).

(PERMANOVA) based on the Bray–Curtis and Jaccard similarity index, using the function *adonis* from *vegan* package for R (Anderson, 2001; Oksanen *et al.*, 2019). Since permutational analysis of variance is sensitive to differences in multivariate dispersion between groups (*sensu* homogeneity of variances), the same models were tested for differences in dispersion using multivariate homogeneity of group dispersions (PERMIDISP) (Anderson *et al.*, 2006). We performed this analysis using the *betadisper* and *permutest* function of the *vegan* package for R (Oksanen *et al.*, 2019). We performed pairwise comparisons after significant interactions of both PERMANOVA (Bray–Curtis and Jaccard) with the *pairwise.perm.manova* function of the *RVAideMemoire* package (Herve, 2018). Similarity percentages (SIMPER) based on the Bray–Curtis and Jaccard index were calculated between samples to determine which species contributed largely to the average similarity between parasite assemblages in every stream.

To visualize patterns of component community level, ordination was performed by non-metric multidimensional scaling ordination (NMDS) and Canonical correspondence analysis (CCA) using the Bray–Curtis index. The NMDS ordinations were obtained using the *metaMDS* function and the fit of NMDS was quantified by a value of stress (<0.05 provides excellent representation in reduced dimensions, <0.1 is great, <0.2 is good/ok and stress <0.3 provides a poor representation). In the CCA analysis, we started with the full model (including all environmental and biological variables) and then performed a backward elimination of non-significant variables using the step function. Also, to test the correlation between similarity of digenean community and similarity of environmental and biological variables' matrix distances among sites, the Mantel test was performed. The calculations were performed using the *vegan* package (Oksanen *et al.*, 2019) in R.

To evaluate the spatio-temporal variation in the parasite prevalence and the possible influence of biotic and abiotic factors, a parametric analysis of variance of two fixed factors with interactions was designed. A linear model was performed using the following model: prevalence ~ depth + water temperature + turbidity + width + water velocity + mollusc abundance + mollusc size + season × stream. The fixed factors are seasons with four

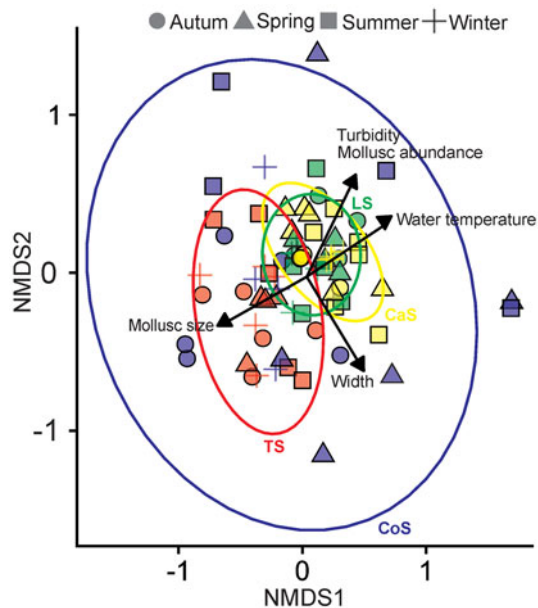
factors (autumn, winter, spring, summer) and streams with four factors (Tapera, Corrientes, Lobería, Carolina). Depth, water temperature, turbidity, width, water velocity and mollusc abundance and size were used as the covariates. The Tukey honestly significant difference (HSD) test was used to test differences among individuals. Analyses were done using the statistical programming language R, version 2.15.2. (R Development Core Team, 2018).

## Results

A total of 12 digenean species belonging to eight families were recorded in the four streams in the study (table 1). In Tapera stream, ten species were found, seven species in Corrientes, nine species in Lobería and ten in Carolina. Definitive hosts for these digenean species were detailed in table 1. Most digenean species found in *H. parchappii* (ten species – 83%) display allogenic life cycles, using mostly birds as definitive hosts. Only *Cercaria Haploporidae* sp. 1 and *Genarchella genarchella* had autogenic life cycles and fishes as definitive hosts.

The results of PERMANOVA on digenean prevalence data (Bray–Curtis similarity index) showed a strong effect of sites and seasons on the parasite community structure and mollusc size effect (mollusc size:  $F = 9.75$ ,  $P(\text{perm}) < 0.01$ ; season × stream:  $F = 3.78$ ,  $P(\text{perm}) < 0.01$ ). Pairwise comparisons showed significant differences in most pairs of samples (all  $P(\text{perm}) < 0.01$ ). However, three compositional groups were identified: (1) Tapera and Corrientes; (2) Lobería; and (3) Carolina. A proportion of these differences can be attributed to differences in the dispersions of parasite communities in the streams (PERMIDISP:  $F = 20.16$ ,  $P(\text{perm}) < 0.01$ ). According to SIMPER analysis, four digenean species contributed with more than 80% of the similarity between sites: *Cercaria Acanthostomidae* sp. 1, *Psilochasmus oxyurus*, *Microphallus szidati* and *Notocotylidae* sp. 1.

The results of PERMANOVA on presence/absence data (Jaccard similarity index) showed a strong effect of sites and seasons on the parasite community structure ( $F = 2.73$ ,  $P(\text{perm}) < 0.01$ ). Pairwise comparisons showed significant differences in all pairs of samples (all  $P(\text{perm}) < 0.01$ ). However, the test indicated two compositional groups: samples from Carolina



**Fig. 2.** Non-metric multidimensional scaling (NMDS) ordination plot based on Bray–Curtis similarity on parasite prevalence and Canonical correspondence analysis (CCA) in four streams located on the Argentinean coast. Value of stress: 0.17. Abbreviations: TS, Tapera stream; CoS, Corrientes stream; LS, Lobería stream; CaS, Carolina stream.

and Lobería showed greater similarity in species composition compared to samples from Tapera and Corrientes. A proportion of these differences can also be attributed to differences in the dispersions of parasite communities in the streams (PERMIDISP:  $F = 20.47$ ,  $P(\text{perm}) < 0.01$ ). SIMPER analysis identified *Cercaria Acanthostomidae* sp. 1 and *P. oxyurus* as the species that contributed to more than 70% of the similarity between sites.

In the multidimensional ordination of parasite prevalence based on the Bray–Curtis similarity (NMDS), axis 1 and axis 2 explained, respectively, 46.2% and 12.9% of the variance in species composition. Samples from Lobería and Carolina evidenced similar internal variance and similar species composition, and were grouped together in all seasons. Tapera presented higher variance and different species composition with respect to Lobería–Carolina. Finally, Corrientes showed the highest internal variance and the highest variability in species composition throughout the year. The stress level (0.17) indicated a community structure substantially different from random. In the CCA, several variables explained the ordination observed in the NMDS. The first ordination axis was positively associated with the mollusc's abundance, water temperature, width and turbidity, and negatively associated with the mollusc size ( $P < 0.001$ , for all cases) (fig. 2). Nevertheless, no correlation was detected in the matrix distances among sites between similarity in species composition and similarity in environmental and biological variables (Mantel test:  $r = 0.05$ ,  $P = 0.166$ ). The physical parameters of every stream and the molluscs' relative abundance and sizes are detailed in table 2.

Significant effects of all the variables analysed (physical parameters and host's biotic characteristics) on the prevalence values, and an interaction between sites and seasons ( $P < 0.001$ , for all cases) were found. Prevalence values were higher in Carolina and Lobería, compared to Tapera and Corrientes, in all seasons. However, each stream showed seasonal differences in parasite prevalence: Corrientes showed the lowest prevalence values during winter, Tapera during autumn and spring, Lobería during autumn/

winter and Carolina during autumn. Finally, the highest prevalences throughout the study were recorded in Carolina (fig. 3 and table 3).

## Discussion

The number of digenean species parasitizing *H. parchappii* was similar in three of the streams studied while, in Corrientes stream, a lower number of species was observed. With respect to stream locations, streams running inside city limits (Tapera and Corrientes) showed a lower number of digenean species, and lower values of species prevalence than streams located in the southern outskirts of Mar del Plata city (Lobería and Carolina). By contrast, the spatial prevalence values evidenced a higher variation between the environments. Streams located in the urban periphery (Lobería and Carolina) showed higher prevalence values throughout the year, compared to those calculated for streams located within the city (Tapera and Corrientes). Concerning seasonal variations, all the streams showed the highest values of prevalence during the spring.

Lotic and lentic ecosystems differ in their local habitat characteristics such as water flow, water residence time, 'stream hydraulics', etc. Streams and rivers, in particular, may have differences in water flow and depth along their channels, determining slower and faster-moving sections (pools and riffles). In turn, lentic habitats are more productive with a higher diversity in macroinvertebrates and aquatic plants (Yang, 1971, Williams *et al.*, 2003; Germano & Bury, 2009; Rosset *et al.*, 2017). All these local characteristics can influence the presence and transmission of parasite species in molluscan hosts (Wetzel & Shreve, 2003; Tolley-Jordan & Owen, 2008). In the case of *H. parchappii*, only 40% of the potential digenean species that can parasitize this mollusc in lentic environments were registered in the coastal streams of the same geographical area. This is probably due to a difference in the composition of host fauna between lotic and lentic environments, and to a combination of biotic and abiotic factors that could determine the absence of some of these digenean species in the streams studied here.

There are a variety of factors that determine diversity at different spatial scales. On a larger scale, animal diversity can be affected by factors such as environmental fragmentation, environmental diversity, urbanization and human impact or the climate (Didham *et al.*, 1996; James & Shine, 2000; Cane, 2001; Steffan-Dewenter *et al.*, 2002; Dauber *et al.*, 2003; Kruess, 2003; Krauss *et al.*, 2004; Opdam & Wascher, 2004; Zanette *et al.*, 2005). On a smaller scale, diversity could be influenced by habitat type, the habitat area and inter- or intra-specific interactions (Ritchie & Olf, 1999; James & Shine, 2000; Veech *et al.*, 2002; Kruess, 2003; Weibull *et al.*, 2003; Krauss *et al.*, 2004; Summerville & Crist, 2004). In the case of parasites with complex life cycles, variables such as distribution and abundance of definitive and intermediate hosts in the environment must also be taken into account (Hechinger & Lafferty, 2005; Fredensborg *et al.*, 2006), together with the dispersive capacity of hosts. In the case of digeneans, definitive hosts with a higher vagility should make a greater contribution to the dispersion of parasites than intermediate hosts. Thus, as the dispersive capacity of hosts grows, the digenean species replacement rate will decrease (Thieltges *et al.*, 2009; Locke *et al.*, 2012). Besides, autogenous parasites (which use fish to complete their life cycles in freshwater environments) have a lower dispersal capacity than allogenic parasites (which use birds, mammals or reptiles to complete their life

**Table 2.** Composition of samples of *Heleobia parchappii* and environmental variables in the four streams and the four seasons (mean (standard deviation)).

		Depth (cm)	Water temperature (°C)	Turbidity (cm)	Width (m)	Velocity (m/s)	Mollusc size (mm)	Mollusc abundance (ind/hr)
Autumn	Tapera	22 (2.8)	16.2 (0.1)	14.6 (0.5)	3.1 (0.2)	0.05 (0.01)	5.2 (0.1)	748 (38.7)
	Corrientes	34 (4.2)	12.6 (0.2)	35.2 (0.4)	7.4 (0.2)	0.02 (0.01)	4.1 (0.2)	1580 (593)
	Loberia	25 (3.5)	13.9 (0.5)	71.6 (2.3)	3.3 (0.5)	0.24 (0.01)	4.6 (0.2)	2160 (481.4)
	Carolina	23 (2.7)	12.3 (0.3)	60.2 (0.4)	8.7 (0.6)	0.26 (0.01)	5.2 (0.4)	370 (14.1)
Winter	Tapera	25 (1.6)	7.1 (0.2)	14.8 (0.4)	3.2 (0.3)	0.05 (0.01)	5.3 (0.1)	1044 (222.9)
	Corrientes	15 (0.7)	17.2 (0.1)	35.2 (0.4)	7.4 (0.2)	0.02 (0.01)	4.5 (0.1)	1680 (268.3)
	Loberia	29.8 (4.9)	16.2 (0.1)	72 (2.7)	3.3 (0.2)	0.24 (0.01)	4.6 (0.2)	1164 (410.5)
	Carolina	23.2 (6.3)	14.4 (0.1)	60.4 (0.9)	8.3 (0.3)	0.26 (0.01)	5.6 (0.2)	1032 (477)
Spring	Tapera	35.4 (9.5)	18.3 (0.1)	20.4 (0.5)	5.2 (0.5)	0.46 (0.01)	5.5 (0.3)	300 (60)
	Corrientes	26 (6.5)	19.6 (0.6)	20.2 (0.5)	5.8 (0.5)	0.02 (0.01)	3.9 (0.1)	984 (210.4)
	Loberia	30 (5)	19.5 (0.1)	34.4 (0.9)	3.4 (0.5)	0.17 (0.01)	4.4 (0.2)	220 (28.3)
	Carolina	20 (5)	20.8 (0.3)	24.8 (0.5)	7.7 (0.3)	0.25 (0.01)	5.2 (0.4)	1860 (894)
Summer	Tapera	16 (3.9)	30.4 (0.1)	4.6 (0.6)	2.7 (0.3)	0.01 (0.01)	4.7 (0.2)	511.7 (132.5)
	Corrientes	20.6 (2.6)	20.5 (0.5)	24.8 (0.5)	3.7 (0.5)	0.04 (0.01)	4.1 (0.1)	750.9 (160)
	Loberia	26 (4.2)	19.8 (0.2)	25.4 (0.5)	2.7 (0.3)	0.15 (0.01)	3.5 (0.3)	366 (53.7)
	Carolina	8.8 (1.1)	20.38 (0.2)	7.6 (0.5)	3.1 (0.2)	0.01 (0.01)	4.0 (0.3)	4096.7 (1486.4)

**Table 3.** Seasonal prevalence values for *Heleobia parchappii* in the different sampled sites and Tuckey HSD comparisons.

	Tapera stream			Corrientes stream			Loberia stream			Carolina stream		
Autumn	28%	A	+–	9%	A	–x	32%	A	x	41%	A	+–x
Winter	17%	AB	+	8%	B	–	27%	A	+	61%	A	x
Spring	21%	C	+	7%	A	–	36%	B	x	82%	B	x
Summer	12%	B	+	8%	A	+	26%	B	x	56%	A	x

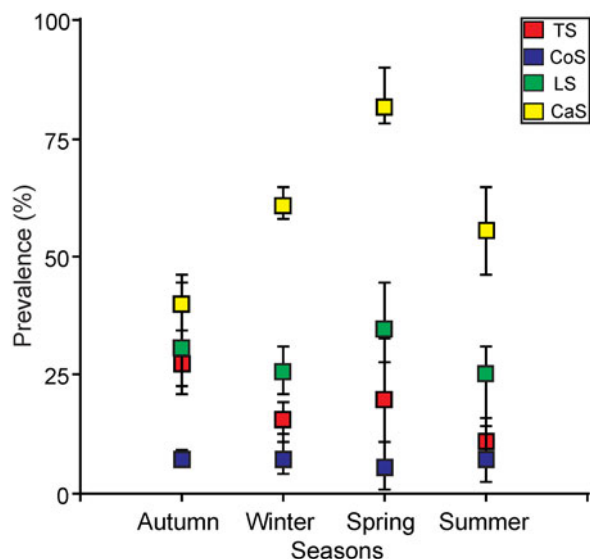
The same letters indicate no differences in prevalence values between seasons within every stream ( $P > 0.05$ ) and the different symbols indicate differences between streams within every season ( $P < 0.05$ ); considering that sharing at least one letter or one symbol, depending on the case, indicates the absence of significant differences.

cycles, usually outside water bodies) (Esch *et al.*, 1988; Criscione & Blouin, 2004; Thieltges *et al.*, 2011). In the larval digenean assemblages of *H. parchappii*, most digenean species had allogenic life cycles that can contribute to the species' dispersal in the area and, consequently, may be responsible for the similarity in species richness recorded between environments.

Many authors have indicated that the first intermediate host can also influence the composition of digenean assemblages through their relative abundances (e.g. Kuris & Lafferty, 2005; Rifkin *et al.*, 2012; Buck & Lutterschmidt, 2017). In the case of *H. parchappii*, it was three times more abundant in coastal streams than in nearby shallow lakes (Merlo, 2013; Merlo *et al.*, 2019). Differences in relative abundances of *H. parchappii* between lotic and lentic environments could be influenced by the hydrological characteristics of the lotic water bodies. The water currents could transport the molluscs and deposit them in the 'point bars', the internal curvatures of the stream, generating high densities in certain points of the bed and low densities in others.

Concerning human impact, the streams studied in this work are located in the most populated province of the country, with

15 million inhabitants representing 47.7% of the total population of Argentina. This province is also characterized by industrial activities (56% of the country's industries are established there) and by intensive agriculture (INDEC, 1994). Precisely, the expansion of intensive agriculture has increased the human impact on lotic and lentic bodies, resulting in elevated nutrients, increased organic and inorganic contaminants and altered biotic assemblages (Walsh *et al.*, 2007). Lafferty & Kuris (1999) mentioned that parasitism may increase or decrease in response to anthropogenic activities like pollution or habitat alterations. Also, several authors had registered greater cercarial release in non-agricultural environments (Lafferty *et al.*, 2005; Koprivnikar *et al.*, 2007), but others studies have suggested that agricultural areas have, for example, higher trematode infection in larval amphibians because of increased exposure to agrochemicals and eutrophication (Johnson & Chase, 2004; Rohr *et al.*, 2008). The area where the streams are located is heavily impacted by recreational activities, especially during summer. In the warmer months, Mar del Plata city receives a great number of visitors (e.g. 1,400,000 tourists only during January 2014) (MGP, 2019). Recreational activities together with the visitor increase could interfere in the



**Fig. 3.** Seasonal prevalence values for *Heleobia parchappii* in the four streams. Boxes represent means and vertical bars denote maximum and minimum values. Abbreviations: TS, Tapera stream; CoS, Corrientes stream; LS, Lobería stream; CaS, Carolina stream.

contact between birds (definitive hosts) and snails (first intermediate hosts) of the digenean life cycles, affecting the transmission of the infectious stages of the parasite (Lafferty, 1997; Etchegoin *et al.*, 2012; Parietti *et al.*, 2013). These perturbations, resulting from human activities, could be responsible, at least partly, for the variability in species composition observed within and between streams.

Among the four streams studied, Tapera and Corrientes seem to be the most affected environments, with the lowest values of prevalence in all seasons, and lower species presence in spring and summer. Both streams flow through urban zones located in the northern and southern borders of Mar del Plata city, with permanent human populations that can triple during summer months. As a result of human activities and the absence of official sewage dumps in domestic residences a high concentrations of faecal coliforms were detected in these sites (Isla *et al.*, 1998), and the presence of organochlorine pesticides derived from agricultural practices was also registered in Tapera stream (Miglioranza *et al.*, 2004). Thus, urbanization and human disturbances, derived from agricultural and recreational activities, could affect the dynamic of digenean assemblages in these coastal environments. In the Tapera stream, Taglioretti *et al.* (2018) also reported a similar negative effect of land use on the parasite populations and assemblages of the poeciliid fish *Cnesterodon decemmaculatus*. The other two streams (Lobería and Carolina), located in the urban periphery, might be less impacted by seasonal visitor increase, and by contaminants derived from untreated urban sewage and agricultural practices.

The composition and dynamics of larval digenean assemblages parasitizing *H. parchappii* in the four streams studied seem to be mainly influenced by a series of factors: diversity and vagility of definitive hosts, types of digenean life cycles, habitat characteristics, molluscan host size and abundance and anthropogenic disturbances. Several studies have already emphasized the influence of these factors on the structure of parasite assemblages at different spatial scales (see Blanar *et al.*, 2016). However, it should be mentioned that human disturbances derived from urbanization,

land use or recreational activities seem to play an important role in the spatio-temporal variations of larval digenean assemblages in streams, as they do in shallow lakes of the area (Merlo *et al.*, 2019). Our results reinforce the idea that parasites can be key indicators to gauge the impact of environmental disturbances on animal communities. Understanding how they respond to these disturbances may contribute to the implementation of measures for the conservation of environments.

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**Ethical standards.** The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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