ELSEVIER

Original Articles

Fish-trematode systems as indicators of anthropogenic disturbance: Effects of urbanization on a small stream

Verónica Taglioretti*, María A. Rossin, Juan T. Timi

Laboratorio de Ictioparasitología, Instituto de Investigaciones Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Funes 3350, (7600) Mar del Plata, Argentina

ABSTRACT

Parasites have proven to display high responsiveness to anthropogenic stress, having, therefore a considerable value as bioindicators. We analysed the variability in the structure of parasite populations and assemblages of the poeciliid *Cnesterodon decemmaculatus* caught at partially urbanized stream along a unidirectional gradient with the aim of evaluating their utility as indicators of the effect of urbanization. A total of 209 fish were examined for parasites; some physical parameters of the stream, including the percentage of urbanization (UR) were also recorded. Data were analyzed at the levels of parasite populations and assemblages discriminated by host sex. Fish condition factor was negatively affected by UR, possibly related to flashing hydrology, but it was not related to parasite abundance. Different combinations of variables explained the distribution of parasite populations and assemblages along the creek, with UR being recurrently identified among the best predictors of parasite loads at all levels of analyses. Whereas densities of mollusc first intermediate hosts were considered of little relevance for parasite distribution, distribution of definitive hosts, as well as the presence of pollutants and increased flashiness in urbanized sections of the creek were retained as possible explanations to the observed patterns. As these factors can act simultaneously, the mechanisms driving differences in parasite dynamics of urban streams are difficult to individualize, but the effect of urbanization on both hosts and parasites was clearly identified. Parasites found, with abundances clearly related to UR, can be considered as good ecological indicators, since they are broadly distributed in the region, easy to observe and count, their prevalence and abundance allow reliable quantitative comparisons and are sensitive to variations of environmental stress and/or ecological conditions at small spatial scales. Consequently the susceptibility of fish-trematode systems to variations in both biotic and abiotic stressors makes them a promising tool for detecting urbanization-related disturbances in streams.

1. Introduction

Urbanization is a dominant demographic trend and an important component of global land transformation (Pickett et al., 2011). It impacts dramatically on ecological systems and biodiversity (Alberti, 2015; Gaston, 2010; McKinney, 2008), as has been shown for several taxa (McKinney, 2002, 2008). Among components of urban landscapes, hydrology can be drastically modified compared to agricultural and wild lands (Walsh et al., 2005). Indeed, natural catchments can be severely impacted by urbanization, resulting in alterations of their physical traits and certain ecosystem functions that include elevated nutrients, increased organic and inorganic contaminants, increased hydrologic flashiness, and altered biotic assemblages (Walsh et al., 2007). Whereas considerable amount of work has analysed urban impacts at the scale of stream reaches, there has been less conceptual and

empirical work focusing on longitudinal impacts along urban river continua (Pickett et al., 2011).

A significant proportion of studies on stream ecology are based on the River Continuum Concept (RCC), proposed by Vannote et al. (1980), which predicts that the structural and functional characteristics of stream communities follow a longitudinal gradient in response to downstream changes in hydrologic and geomorphic properties. Nevertheless, the ideal assumptions of the RCC are not always met, particularly in the case of urban watersheds, due to heterogeneous effects of anthropogenic conditions across space and time (Pickett et al., 2017). Therefore, the unidirectional gradients determined by the stream flow should facilitate the identification of those deviations from expected clines, which in turn could be indicatives on the effect of different urbanization-related factors.

The complex nature of urban land use can influence local

---

biodiversity in a diversity of ways, resulting of processes acting at different temporal and spatial scales (Allan, 2004). That complexity requires to be explored from different perspectives and on different components of biota. Among a variety of organisms used as bioindicators of environmental impact produced by urbanization, parasites are increasingly utilised as shown by recent research on different host taxa, particularly on stream fishes (Blanar et al., 2016; Bhuthimethee et al., 2005; Chapman et al., 2015; de Aquino Moreira et al., 2015; Iwanowicz et al., 2016; Machut and Limburg, 2008), possibly based on the demonstrated responsiveness of parasites to anthropogenic stress and their applied bioindication value in an environmental sense (Sures et al., 2017).

As for research on streams in general, most studies on parasites of stream fishes as bioindicators has also dealt with comparisons between urbanized and natural areas, or along urbanization clines represented by different localities, but little effort has been made on understanding urban impacts along a urban river continuum (Barger and Esch, 2001; Blasco-Costa et al., 2013). To the best of our knowledge, only Blanar et al. (2016) have evaluated changes on parasites assemblages along an upstream–downstream gradient of anthropogenic disturbance in a single stream. One of the main findings of Blanar et al. (2016) was that landscape scale differences in land use were significant drivers of fish parasites community structure. This pattern was detected as discontinuities in parasite component community structure along the unidirectional gradient, related to sediment hydrocarbons content and anthropogenic land use.

All these studies are, explicitly or not, framed by RCC and their predicted longitudinal gradients. Nevertheless, the global applicability and utility of the RCC is uncertain as shown by a number studies in different biomes, where the structure and functioning of streams do not fit to those assumed by the RCC (Feijoó and Lombardo, 2007; Greathouse and Pringle, 2006).

Here, we carried out a study on the variability in the structure of parasite assemblages of a common fish from La Tapera creek, along an urbanization gradient. Pampean streams are different from other lotic systems in the world. In these environments, dense and rich macrophyte communities generate habitat heterogeneity affecting the development of other communities. These systems are very vulnerable to the discharge of pollutants and to modifications in stream morphology and hydrology (Giorgi et al., 2005).

The aim of this work is to evaluate the utility of parasites of the poeciliid *Cnesterodon decemmaculatus* as indicators of the effect of urbanization along a unidirectional gradient in La Tapera creek. Analyses were performed on parasite populations and assemblages, as well as on the host condition factor.

2. Study area and dataset

2.1. The stream

La Tapera creek is a Pampean stream typical of lowlands in central Argentina, characterized by low current velocities, high nutrient levels, lack of riparian forest vegetation and absence of dry periods or extreme temperatures, which favours the development of dense and rich macrophyte communities (Giorgi et al., 2005; Feijoó and Lombardo, 2007).

This stream originates at Los Padres lake, a natural eutrophic lagoon located at 37° 56′ S and 57° 44′ W (Gonzalez Sagrario and Balseiro, 2003) (Fig. 1), its flow being controlled by a floodgate at its origin. The creek has a length of 30 km, and before its outfall it passes through horticultural areas and an urban zone along the north side of Mar del Plata city (Miglioranza et al., 2004). Both the lake and the creek are embedded in an intensive horticultural agroecosystem (Miglioranza et al., 2004; Romanelli et al., 2014). On the other hand, along its urban portion, the creek receives the discharge of drain storms, draining large areas of the city.

It is noteworthy that during a preliminary survey of sampling sites

carried out before this study, the section of the creak comprised between its origin and site 1 was completely dry, this site being consequently different from the rest of sites surveyed downstream.

2.2. The fish

Cnesterodon decemmaculatus (Jenyns, 1842) (Cyprinidontiformes, Poeciliidae) is a small freshwater non-migratory and ovoviviparous teleost endemic of the Neotropical Region, distributed throughout the Pampean region (Lucinda, 2005). This species has a poor swimming capacity and is unable to disperse actively across long distances (Ramos-Fregonezi et al., 2017). It can be found in pristine as well as in severely degraded habitats (Hued and Bistoni, 2005); often being the most abundant and sometimes the sole species present in small watercourses. This species has broad ranges of tolerance to many environmental parameters, characteristics that makes them suitable as test organism for pollution and toxicity studies (Baudou et al., 2017; Menéndez-Helman et al., 2015). As a member of Poeciliidae, *C. decemmaculatus* exhibits a marked sexual dimorphism, with females (45 mm) being bigger than males (25 mm) and displaying different behaviour and habitat preferences.

2.3. Samples collection

A total of 209 fish were sampled from 7 locations along the creek (Fig. 1, Table 1). Fish were collected with hand nets (net mesh 0.28 mm) along creek shoreline sections (maximum 15 m long) until reaching a total of 30 specimens per sample. As physical and chemical conditions of Pampean streams vary greatly depending on rainfall (Arreghini et al., 2005; Giorgi et al., 2005), all samples were collected the same day (April 24th, 2016). Fish were sacrificed by spinal cord severing with the aid of dissecting needles under a stereoscopic microscope and placed in individual vials containing 5% formalin. Each host was measured (TL), weighed and sexed before dissection. Then, each fish was weighted after dissection and viscera and metacercariae contained in the abdominal cavity were removed. Fulton's condition factor (K) (Ricker, 1975) was calculated for eviscerate fish. All organs and tissues were examined for parasites. Additional samples were used to obtain living parasites, which were processed following conventional protocols and identified to the lowest possible taxonomic level.

2.4. Environmental variables

Physical parameters of the stream (Table 1) were measured at each sampling site (VE was square root-transformed as it had skewed distribution). The coverage by riverine vegetation on the stream (CO) was estimated as the average distance between the border of vegetation and the shoreline at five points in each sampling site. The degree of urbanization (UR) was estimated within squares of 1 km^2 disposed upstream of each sampling site. The area was limited to 1 km^2 to diminish overlap among sites. Free satellite images from Google Earth Pro (2017) were utilized, since they provide the latest satellite imagery, having spatial resolution less than 1 m (Malarvizhi et al., 2016). Urban land use was estimated considering surface of built-up areas as polygons, which were imported into ArcGIS 10.1 software and transformed to percentages. Moreover, latitude (LA) and longitude (LO) were included as geographical references and the upstream–downstream linear distance from the initial site was also recorded.

3. Statistical analysis

All multivariate analyses were implemented in PERMANOVA+ for PRIMER7 package (Anderson et al., 2008; Clarke and Gorley 2015). Since the creek was dry from los Padres Lake to Site 1 during a period previous to the study, it is assumed that fish colonized it for a short period, harbouring parasite communities brought from other places
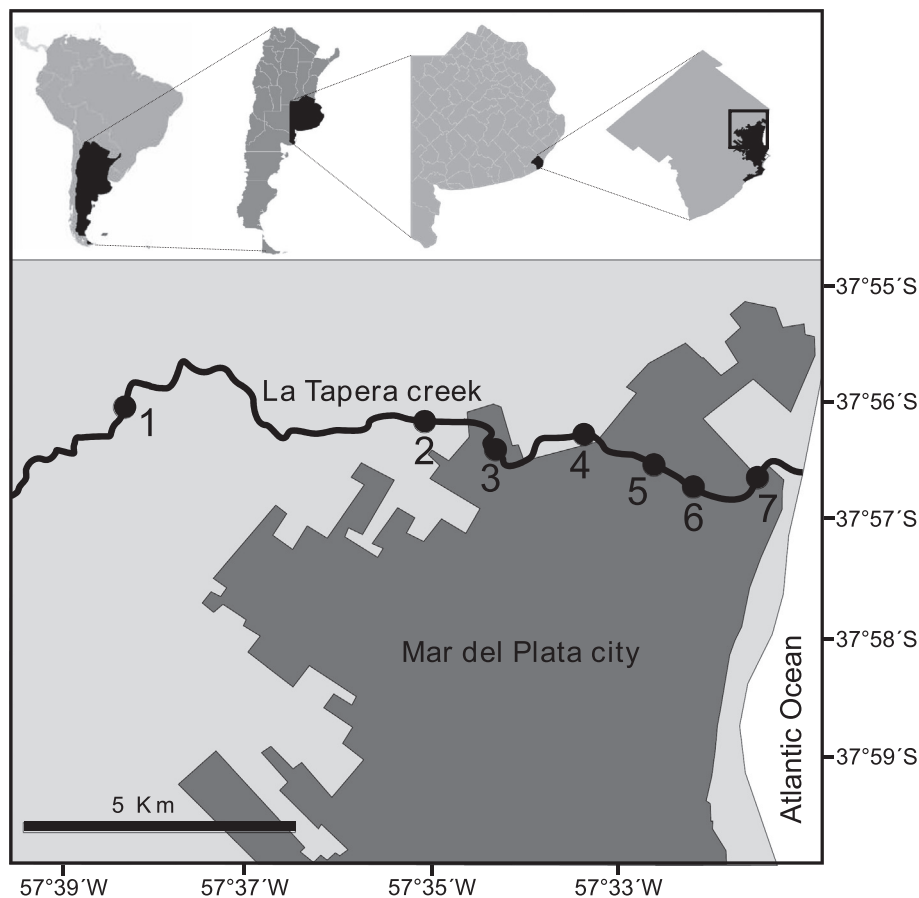
Fig. 1. Map showing sampling sites along La Tapera creek, Mar del Plata city, Argentina.

(lake or downstream), or developed in-situ for a shorter period. Consequently, and based on the observed differences of parasite burdens for Site 1 (see Sections 4.3 and 4.4), it was excluded from all models involving parasite data as variables.

3.1. Upstream-downstream environmental gradients

The RELATE routine (Clarke and Gorley, 2015) was used under seriation model to assess whether observed changes in environmental features were the result of an orderly linear sequence determined by an upstream–downstream gradient and not merely determined by the distance separating sites. Permutation tests (9999 permutations) were used to evaluate the significance of Spearman's correlations between the serial model and the Euclidean distance of environmental variables, including normalized data of MD, MW, VE, CO and UR.

3.2. Host features along the stream

To determine if host features (TL and K) varied among sampling sites or between host sexes, two-way crossed permutational analyses of variance (PERMANOVA), considering host sex and sample site as factors, were performed based on Euclidean distances and tested for main effects and interactions (9999 permutations) using permutation of residuals under an unrestricted model and partial sum of squares. When differences were detected, pair-wise comparisons were used to determine which samples differed (Anderson et al., 2008).

In order to analyze the relative contribution of environmental parameters on fish characteristics, distance-based multiple linear models (DistLM, Anderson et al., 2008) were applied with significance testing based on 9999 permutations. Euclidean distance matrices of TL and K of individual hosts were used as response variables and environmental variables (MD, MW, VE, CO, UR, LA and LO) as predictors. In the case of the K, abundances of individual parasite species were also

Table 1
Composition of samples *of Cnesterodon decemmaculatus* and environmental variables along La Tapera creek, Argentina.

Sample	N	F/M	Latitude S (LA)	Longitude W (LO)	Distance (Km)	MD (m)	MW (m)	VE (m/s)	CO (cm)	UR
1	30	13/17	37.94	57.65	0	0.11	4.87	0.06	30	1.97
2	30	7/23	37.94	57.60	5.88	0.58	3.66	0.05	52	7.49
3	30	12/18	37.94	57.59	7.21	0.18	2.90	0.06	0	40.66
4	30	12/18	37.94	57.57	8.80	0.11	2.27	0.22	89	25.67
5	29	8/21	37.94	57.56	9.99	0.38	4.00	0.04	80	48.27
6	30	11/19	37.95	57.55	10.89	0.22	3.25	0.06	82	68.80
7	30	13/17	37.94	57.54	11.81	0.13	1.42	0.33	27	64.82

N: number of examined hosts; F/M: females/males; MD: maximum depth; MW: maximum width; VE: velocity; CO: coverage of riparian vegetation along shoreline; UR: percentage of urbanization in surrounding area (1 km²).

Table 2

Two-factor PERMANOVA results comparing length, condition factor and square root-transformed infracommunity parasite abundance of *Cnesterodon decemmaculatus* across sampling localities and host sex along La Tapera creek.

Response variable	Source	d.f.	SS	MS	Pseudo F	P (perm)
Host length	Sampling site	6	1.71	0.28	2.03	> 0.05
(Euclidean distance)	Host sex	1	0.89	0.89	6.35	< 0.05
	Site x sex	6	0.23	0.04	0.27	> 0.05
	Residual	195	27.41	0.14		
	Total	208	30.78			
Host condition factor	Sampling site	6	0.55	0.09	9.84	< 0.01
(Euclidean distance)	Host sex	1	1.07	1.07	117.20	< 0.01
	Site x sex	6	0.12	0.02	2.15	> 0.05
	Residual	195	1.76	0.01		
	Total	208	3.93			
Infracommunity abundance	Host size	1	37,008	37,008	55.72	< 0.01
(Bray-Curtis dissimilarity)	Sampling site	6	87,787	14,631	22.03	< 0.01
	Host sex	1	4368.3	4368.3	6.58	< 0.01
	Site x sex	6	5697.3	949.55	0.08	> 0.05
	Residual	195	$1.2884\ e^5$	664.13		
	Total	208	$2.64\ e^5$			

Table 3

Prevalence of parasites of *Cnesterodon decemmaculatus* in seven sampling sites along La Tapera creek.

Host sex	Females							Males						
Sampling site	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Sample size	17	23	18	18	21	19	17	13	7	12	12	8	11	13
A. (L.) hadra	82.3	100	100	100	100	100	100	100	100	100	100	100	100	100
Ascocotyle (P.) sp.	23.5	65.2	66.7	77.8	52.4	52.6	47.1	38.5	57.1	58.3	58.3	87.5	54.5	30.7
Ascocotyle. (A.) sp.	11.8	52.2	27.8	16.7	0	0	17.6	53.8	28.6	25.0	0	0	0	7.7
Pygidiopsis sp. 1	17.6	87.0	72.2	77.8	52.4	57.9	52.9	23.1	100	16.7	41.7	37.5	72.7	30.8
Pygidiopsis sp. 2	5.9	47.8	0	0	0	0	0	0	14.3	0	0	0	0	0
P. nanum	5.9	78.3	66.7	55.6	57.1	52.6	29.4	0	71.4	50.0	33.3	75.0	45.4	23.1
A. gnerii	23.5	95.6	77.8	94.4	80.9	57.9	52.9	61.5	85.7	50.0	66.7	62.5	72.7	38.5
Stephanoprora sp.	100	87.1	94.4	100	90.5	100	76.5	100	100	91.7	100	87.5	90.9	69.2
Cyclophyllidean larva	0	21.7	0	5.5	0	10.5	0	0	0	0	8.3	12.5	0	0

included as predictors. Due to the observed differences between sexes in these response variables (see Section 4.2.), models were fitted for each sex separately. The relative importance of environmental conditions and parasites were estimated by grouping creek characteristics and parasite abundances as indicators (environmental and parasitological indicators, respectively). Draftsman plots and correlation matrices were used to check for multicollinearity in predictor variables, excluding one of those providing redundant information.

Models including all possible combinations of predictor variables (or indicators in the case of K) were generated using the BEST procedure. Additionally, a theoretic approach based on Akaike's Information Criterion (AIC) was used to identify the best model (Symonds and Moussalli, 2011). The contribution of each predictor variable was analysed by mean of multiple partial correlations (ρ) between predictor variables, included in the fitted model, and distance-based redundancy analyses (dbRDA) coordinate axes. The difference (Δ_i) between the AIC value of the best model and each of the other models was calculated; and those models with Δ_i between 0 and 2 were considered as having a substantial level of empirical support, being therefore as good as the best model (Burnham and Anderson 2002). For such cases, the Akaike weights (w_i) (Burnham and Anderson, 2002) were calculated to estimate the relative importance of each predictor variable (predictor weight). For each predictor, the w_i of all the models (with $\Delta_i < 2$) containing that predictor were summed and that values were interpreted as its relative importance (Symonds and Moussalli, 2011).

Similar analyses where performed on averaged data of K and on sex ratio per sample. In these cases, the modified Akaike's Information Criterion (AICc) was used due to the small number of samples (Anderson et al., 2008).

3.3. Parasite populations along the stream

Prevalence (percentage of parasitized hosts) and mean abundance (average number of parasites per sample) of each parasite species in each sample, discriminated by fish sex, were calculated following Bush et al. (1997). Pearson's correlations were performed to test for relations between mean abundance values of different pairs of species across sampling sites.

For each parasite species, Euclidean distance matrices were computed at level of infrapopulation (all individual parasites in a single host) and component population (all individual parasites in a host sample) (Bush et al., 1997), on abundance and mean abundance values, respectively. To assess which environmental variables could explain the variations in parasite abundance, DistLM analyses were performed in a similar way than for host features, including fish size as an additional predictor for analyses of infrapopulations.

3.4. Parasite assemblages along the stream

Analyses of assemblage structure were performed at the level of infracommunity (all infrapopulations in a single host) and component community (all component populations in a host sample) (Bush et al., 1997) based on Bray-Curtis similarity matrices, previous square root transformation of data in order to down-weight the importance of very abundant species role in determining similarity among samples (Clarke and Gorley, 2015).

As in the case of environmental features, the RELATE routine under seriation model was applied on Bray-Curtis similarity matrices.

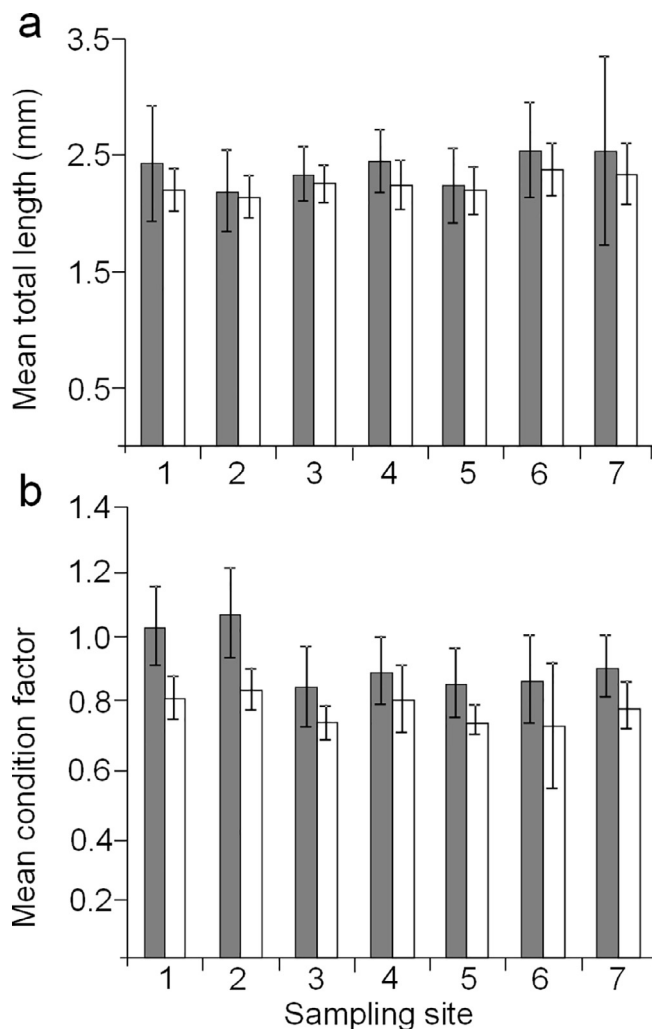In order to assess whether or not sampling sites can be differentiated

Fig. 2. Mean total length (cm) (a) and mean condition factor (b) of female (black columns) and male (white columns) *Cnesterodon decemmaculatus* across seven sampling sites along La Tapera creek.

based on the abundance values of their parasites, non-metric multi-dimensional scaling (nMDS) (Clarke and Gorley, 2015) of the Bray-Curtis matrix was performed between all individual hosts and their centroid differences were visualized by means of bootstrap averaging (Clarke and Gorley, 2015), based on repeated resampling (with replacement, 50 iterations) from the original dataset. The average values were then visualized in a nMDS using as many dimensions as needed to closely match the original distance matrix (correlation coefficient of rho = 0.99). Parasite infracommunity differentiation was further tested using a two-way crossed PERMANOVA, in a similar way than in the case of host features, including host size as covariate.

To assess which environmental variables could explain the variations in the structure of parasite assemblages at infracommunity (multivariate abundances) and component community (multivariate mean abundances) levels, two statistical approaches were performed. First, DistLM analyses were performed in a similar way than for host features, including size of individual fish as an additional predictor variable for infracommunities. Second, as a complementary approach, BEST procedures were performed at both community levels in order to search which variable best matches with multivariate parasite data (Clarke and Ainsworth, 1993). For this, Euclidean distances were calculated from normalized explanatory variables, including host size for analysis at infracommunity level. This routine was run using Spearman rank correlations to determine the strength of association between

biological and environmental matrices, with significance determined after 9999 permutations.

4. Results

4.1. Upstream-downstream environmental gradients

RELATE procedure under seriation model indicated that there was no tendency for physical characteristics of the creek (maximum depth, maximum width, velocity, percentage of urbanization) and coverage of riparian vegetation to be similar at nearby localities, therefore the combination of these features did not follow a significant upstream–downstream gradient (RELATE, $\rho = 0.25$, $P > 0.05$).

4.2. Host features along the stream

PERMANOVA results (Table 2) showed that host size differed significantly between males and females (Fig. 2a), with females attaining larger sizes in all samples; however, no differences of fish length between sites or interaction between factors were observed for each sex. Condition factor also differed significantly between fish sexes (Table 2), with females attaining larger values in all samples (Fig. 2b). In this case a significant effect of site was observed, but no significant interaction between sex and sample site was detected. Pair-wise tests indicated that K of females was similar between sites 1 and 2 (*P(perm)* > 0.05), and these two samples differed from the rest (all *P(perm)* < 0.01) which, in turn were similar each other (all *P(perm)* > 0.05). Pair-wise tests for males evidenced significant differences (*P(perm)* 0.01 < *P* < 0.05) between some pairs of samples involving also Sites 1 and 2 (1-3, 1-5, 2-3 and 2-5).

Draftsman plots and correlation matrices, among environmental predictors, indicated that LA and LO were both highly correlated with UR ($R^2 = 0.95$ and $R^2 = 0.88$, respectively), consequently these variables were excluded from subsequent analyses.

Results from DistLM, indicated that no individual explanatory variables or subset of them explained more than 8% of the total variation in TL of individual fish ($R^2 = 0.06$ for females and $R^2 = 0.08$ for males).

In the case of individual female fish, DistLM analysis indicated that the 33.2% of the total variation of K ($AIC = -484.44$) was explained by environmental conditions. Alternative models (including parasitological indicators) resulted with $\Delta_i > 8$, indicating that female K did not significantly depend on parasite abundances. Among components of the environmental indicators, UR was the descriptor that best explained the observed variation of K for females (*partial* $\rho = |0.78|$), showing a negative relationship between these variables (Fig. 3a), followed in importance by MW (*partial* $\rho = |0.51|$). The best model explaining K of males also contained only the environmental indicator ($R^2 = 0.19$; $AIC = -333.46$), being the MW (*partial* $\rho = |0.85|$) and UR (*partial* $\rho = |0.45|$), the most important variables in determining this pattern, despite the low explanatory power of the model, K decreased with increasing UR (Fig. 3b). Non alternative models with $\Delta_i < 2$ were obtained. Based on these results, the parasitological indicator was excluded from analyses of mean K across sampling sites. In this case, the predictor UR explained 56.5% ($AICc = -34.28$) and 57.4% ($AICc = -43.43$) of the total variation of mean K for females (Fig. 4a) and males (Fig. 4b), respectively. In the case of females, no subsequent models attained a $\Delta_i < 2$. For males, a single model with $\Delta_i < 2$ ($AICc = -43.27$; $R^2 = 0.84$) was composed by two predictors, summing VE to UR. For both sexes, UR affected negatively K.

The proportion of females in each sampling site was explained by MD and CO (Table 5). An alternative model with $\Delta_i < 2$ ($AICc = 12.89$, $R^2 = 0.95$) was composed only by MD.
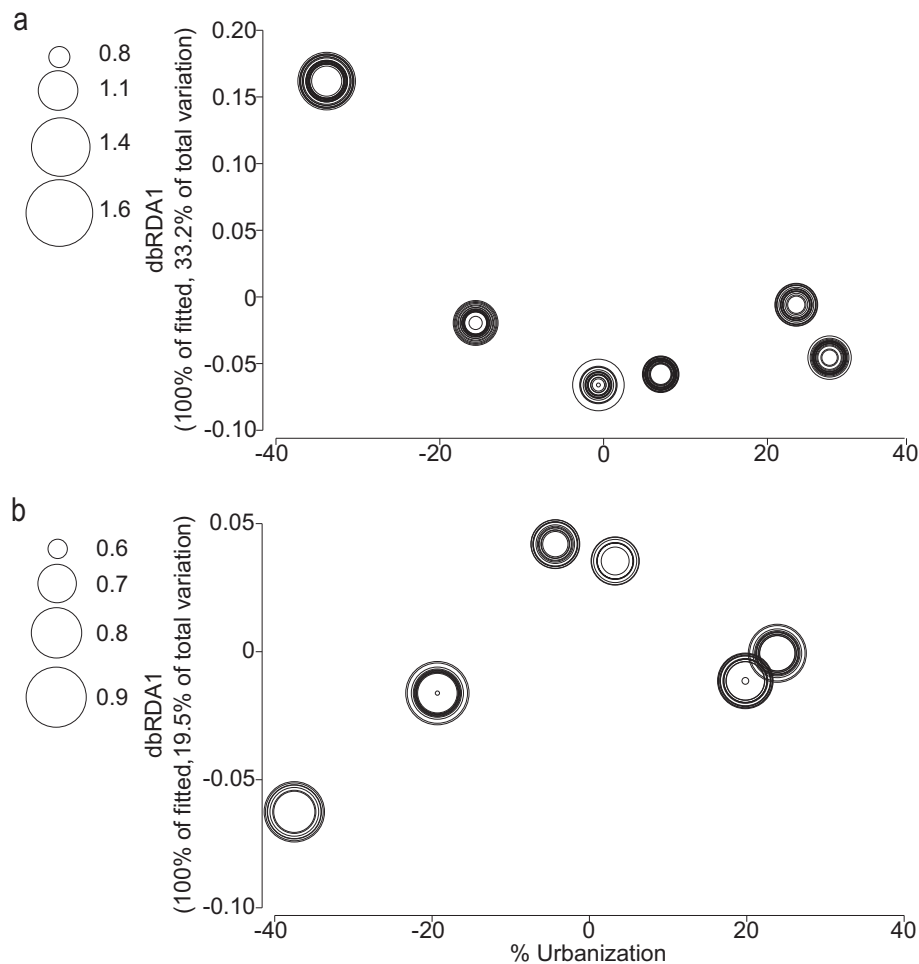
Fig. 3. dbRDA analyses of condition factor values of female (a) and male (b) *Cnesterodon decemmaculatus* along six sampling sites at La Tapera creek. Circle diameters scaled according the condition factor of each individual fish. Numbers indicate sampling sites.

4.3. Parasite populations along the stream

Fish harbored high parasite burdens at La Tapera creek. All hosts were found parasitized by at least one of eight species of larval digeneans, with a total of 47,726 metacercariae being counted in the whole sample. These larvae included specimens of Heterophyidae (*Ascocotyle (Leighia) hadra,* Ostrowski de Nuñez 1992, in the body cavity and mesenteries, *Ascocotyle (Ascocotyle)* sp. in arterial heart bulb, *Ascocotyle (Phagicola)* sp. in gill filaments, *Pygidiopsis* sp. 1 in scales and *Pygidiopsis* sp. 2 in mesenteries), Diplostomidae (*Posthodiplostomum nanum* Dubois, 1937 in the body cavity and mesenteries), Acanthostomatidae (*Acanthostomum gnerii* Szidat, 1954 in scales) and Echinostomatidae (*Stephanoprora* sp.) in gill filaments. Additionally, larval cyclophyllidean cestodes were occasionally found in the mesenteries at low prevalence (Table 3), these larvae also were at very low abundance and were excluded from quantitative analyses.

Parasite prevalence (Table 3) and mean abundance (Fig. 5) were variable across sampling sites and host sexes. Site 1 showed, in general, lower values of parasitism. Regarding the rest of sites, female hosts showed two patterns of mean abundance, one for *A. (L.) hadra* and *Stephanoprora* sp. which reached higher values in central sites, whereas *P. nanum, A. gnerii, Ascocotyle (A.)* sp. and *Pygidiopsis* sp. 1 and sp. 2 were more abundant in the origin (site 2) and at site 4, which agrees with significant correlations (all $P < 0.05$) between the mean abundances of these species (Table 4). For males, mean abundance of *Pygidiopsis* sp. 1, *Pygidiopsis* sp. 2, *Ascocotyle (A.)* sp., *A. gnerii* and *P. nanum* also showed a similar pattern, although no as consistent as for females

(Table 4).

DistLM analyses on individual parasite species (Table 5) showed that those models that best explained the variation in parasite abundances in female fish included a positive effect of TL and a negative effect of UR for 6 and 7 taxa, respectively. Some of these models explained a low proportion of the total variation of parasite abundance (6–17%), but for most of them the percentage explained was $\geq 20\%$; MD was identified as explanatory variable for 4 taxa, MW for 3 taxa, VE for 2 taxa and CO for only 1 taxon. Since alternative models were obtained with $\Delta_i < 2$ in several analyses, w_i were calculated (Table 5). These results also showed that TL and UR were present in all alternative models for 6 and 5 taxa, respectively. For males, the percentages of variability explained by the models were more variable than for females. As in females, TL was included in the best model for 6 species, but UR only for 3 species. Akaike weights showed that TL was the predictor variable included in all alternative models for 3 taxa, with UR being a relevant predictor for 2 taxa.

When mean abundance of each parasite species across samples was analyzed, results indicated that UR was the most important predictor for female hosts as it was included in models of 5 taxa, in that models the percentage of the total variation explained by predictors ranged between 22 and 87% (Table 6). The major percentages of mean abundance variation explained by urbanization were obtained for *P. nanum*, *Acanthostomum* sp. and *A. tenuicollis*. For males, the percentages of variation explained, as well as the predictor variables, varied greatly among taxa, including UR, CO, MV and MD (Table 6).
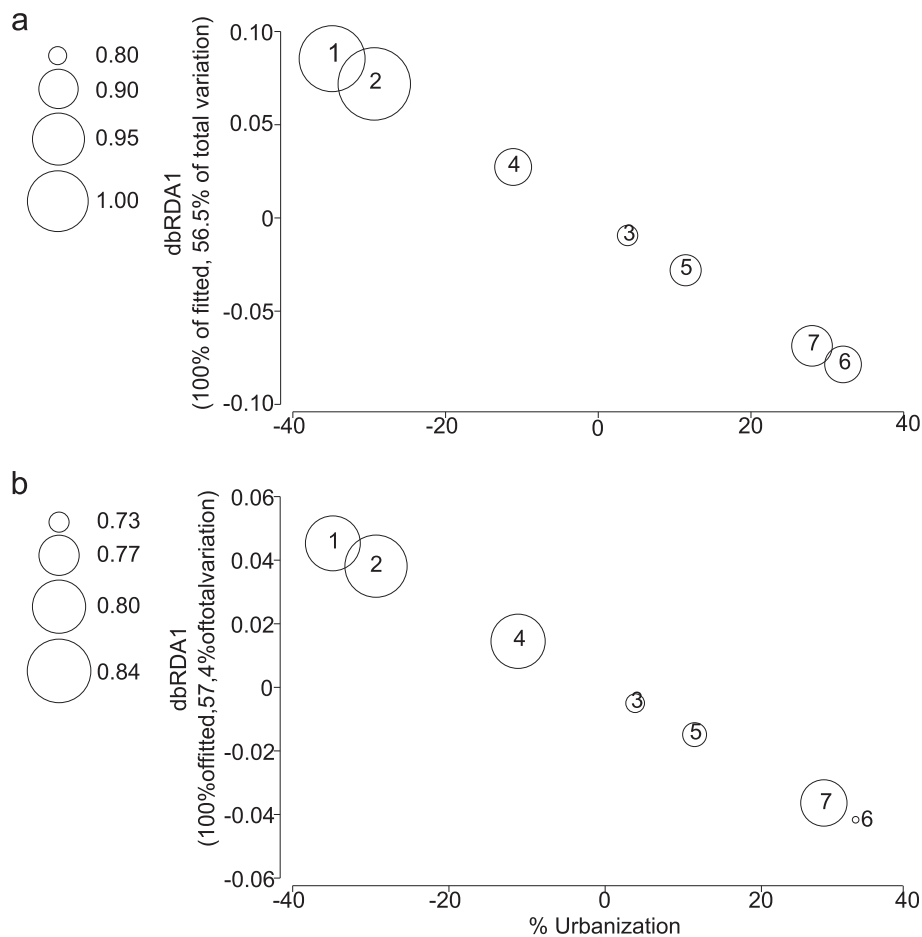
Fig. 4. dbRDA analyses of mean condition factor values of female (a) and male (b) *Cnesterodon decemmaculatus* along seven sampling sites at La Tapera creek. Circle diameters scaled according to mean condition factor in each sample. Numbers indicate sampling sites.

4.4. Parasite assemblages along the stream

Only the 14% and 12% of the total variation in infracommunity abundance for female and male fish, respectively, were significantly explained by the trend determined by the water flow (RELATE, $\rho = 0.14$ for females and $\rho = 0.12$ for males, $P < 0.01$ for both).

The bootstrap-average-based nMDS ordination of both females (Fig. 6a) and males (Fig. 6b) showed an apparent pattern of separation between samples, with a low level of stress (0.06 and 0.07, respectively). Indeed, the parasite assemblages from site 1 were clearly separated from the rest. Among the latter, central sites apparently composed a single group with samples 2 and 7 located to higher distance from the rest in the bi-dimensional space, in the case of females also sample 4 was located apart from the central group.

PERMANOVA analyses (Table 2) showed a significant effect of host length on the response variables. Furthermore, taking into account the variations among samples due to fish size, significant variability was detected among the parasite assemblages both across sampling sites and between fish sexes, but there were no interaction between these factors. Pairwise tests showed that, for both sexes, assemblages in sites 1, 2 and 7 were significantly different from those in any other sample (Table 7). Furthermore, for females only samples 3 and 5 were similar. On the other hand, for males samples from the central sites of the creek were similar in terms of multivariate parasite abundances.

DistLM results indicated that MD, MW, UR and TL were significant predictor of infracommunity similarity for both sexes, accounting for 45 and 40% of total variance for females and males respectively (Table 5). Although there were 5 and 6 alternative models with $\Delta_i < 2$ for females and males, respectively, TL and UR were always included as

predictors in all likely models for both sexes and MD was also always included for females. At component community level (Table 6) UR lonely accounted for 34% for mean abundance in females; for males, the 46% of variability in mean abundance was explained by MD, but there were 2 likely alternatively models with $\Delta_i < 2$, one including only UR and the other only MW.

Similarly, for female fish, the BEST routine indicated that the subset of environmental data that best matched ($\rho = 0.4$, $P < 0.01$) infracommunity similarity were UR, TL and MW. Moreover, UR, VE and MW were the predictors that best matched with values of component community ($\rho = 0.79$, $0.01 < P < 0.05$). For males, TL and MW were the predictors that best matched infracommunity similarity ($\rho = 0.35$, $P < 0.01$) and UR, MD and MW were the predictors that best matched with component community similarity ($\rho = 0.74$, $0.01 < P < 0.05$).

5. Discussion

The present findings showed that both fish condition factor and parasite assemblages were negatively affected by the degree of urbanization. Despite the mechanisms driving differences in parasite dynamics of urban streams are difficult to individualize, the observed susceptibility of fish-trematode systems to variations in both biotic and abiotic stressors makes them a promising tool, as ecological indicators, for detecting urbanization-related disturbances in streams.

At present, few studies have evaluated the influence of urbanization on fish condition factor, obtaining contrasting results (de Aquino Moreira et al., 2015; Mikaelian et al., 2002). In fact, contrasting results have been obtained for K in *C. decemmaculatus* in response to environmental stressors. For example, reduced K has been observed for
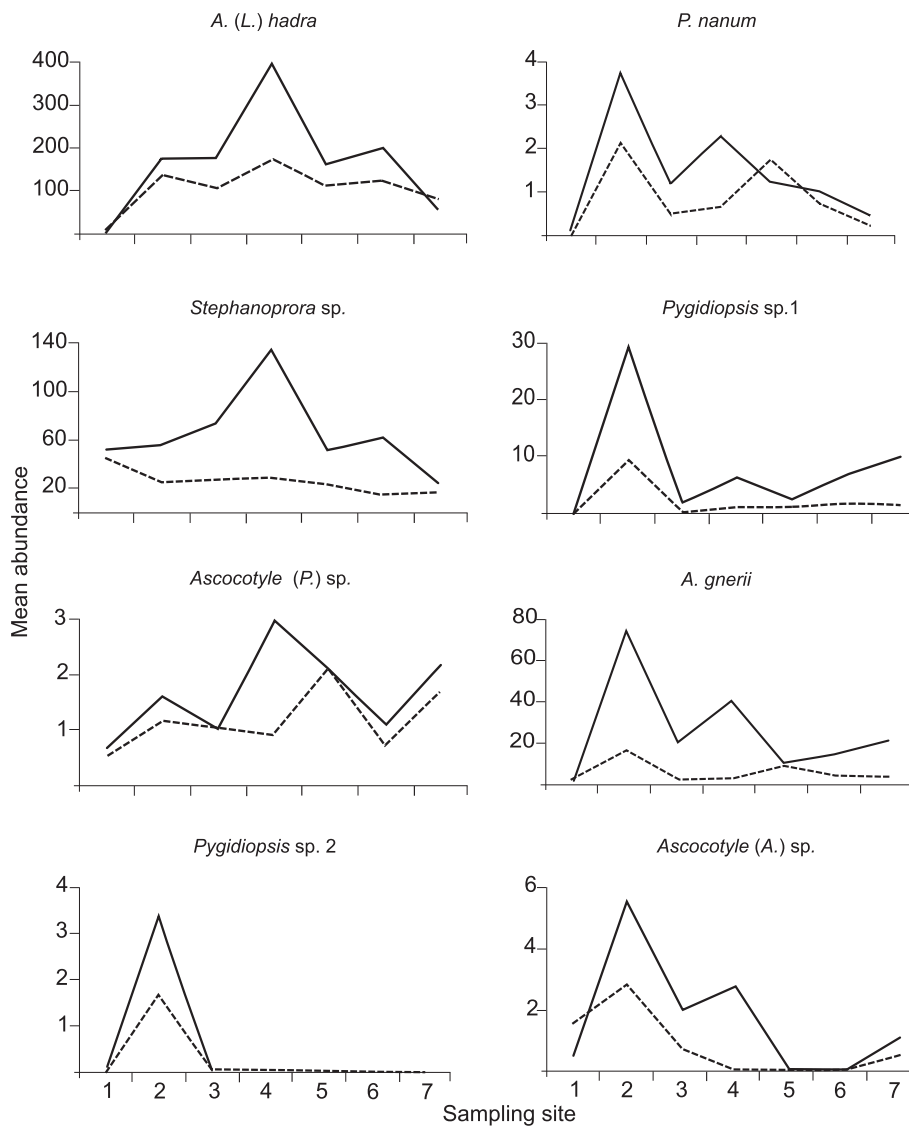
Fig. 5. Mean abundance of eight parasite species of female (solid lines) and male (dotted lines) *Cnesterodon decemmaculatus* across seven sampling sites along La Tapera creek.

Table 4
Pearson's correlations coefficients (significant correlations, $P < 0.05$ in bold) between mean abundances of parasites of *Cnesterodon decemmaculatus* across seven sampling sites along La Tapera creek. Data on male and female fish below and above the diagonal, respectively.

	Ah	Ap	Aa	P1	P2	Pn	Ag	S
A. (L.) hadra (Ah)	–	0.64	0.34	0.12	0.02	0.57	0.45	**0.89**
Ascocotyle (P.) sp. (Ap)	0.17	–	0.21	0.19	−0.05	0.35	0.35	0.45
Ascocotyle (A.) sp. (Aa)	−0.29	−0.25	–	**0.82**	**0.85**	**0.89**	**0.95**	0.27
Pygidiopsis sp. 1 (P1)	0.29	0.02	**0.76**	–	**0.90**	**0.79**	**0.91**	−0.13
Pygidiopsis sp. 2 (P2)	0.22	−0.02	**0.84**	**0.99**	–	**0.82**	**0.86**	−0.12
P. nanum (Pn)	0.51	0.45	0.33	0.73	0.71	–	**0.95**	0.38
A. gnerii (Ag)	0.26	0.33	0.63	**0.91**	**0.90**	**0.92**	–	0.26
Stephanoprora sp. (S)	−0.55	−0.46	0.35	−0.21	−0.08	−0.33	−0.23	–

individuals exposed to some pollutants, such as some herbicides or heavy metals (Baudou et al., 2017; Bonifacio et al., 2016), but no changes in this index were observed after exposure to other compounds (Bonifacio et al., 2016; Carriquiriborde et al., 2007). Indeed, de la Torre et al. (2005) found lack of significant alterations in *C. decemmaculatus* collected from a peri-urban polluted area and pristine artificial ponds.

Although some features of the stream (width and velocity) were identified between the main drivers of K in this study, UR was present as a relevant determinant in all analyses affecting negatively the values of K, especially for females. The extent of impervious surrounding surfaces can be a major determinant of hydrologic conditions in urbanized lands (Allan, 2004; Roy et al., 2005). In fact, the amount of impervious surface in watersheds has been identified as the best measure predicting changes in fish communities (Wang et al., 2001).

At its urban section, La Tapera creek undergo strong pulses of increased flow after rains due to ground runoff and the discharge of large storm drains, increasing significantly its caudal, flow velocity and turbulence for variable periods of time in comparison with its headwater

Table 5
Results of DistLM analyses of environmental/host variables on populations and infracommunities of parasites of *Cnesterodon decemmaculatus* across six sampling sites along La Tapera creek.

Host sex	Response variable	AIC	R^2	Explanatory variables (ρ)						Models	w_i					
				VE	MD	MW	CO	UR	TL	$\Delta_i > 2$ (n)	VE	MD	MW	CO	UR	TL
Females	*A (L.) hadra*	1221.8	0.31	−0.09	−0.5	–	0.42	−0.33	0.68	2	0.59	1	0.57	1	1	1
	Ascocotyle (P.) sp.	194.2	0.33	0.37	–	−0.08	–	−0.09	0.92	5	1	0.31	1	0.44	0.55	1
	Ascocotyle. (A.) sp.	439.6	0.07	–	–	–	–	−1	–	7	0.17	0	0.19	0.1	1	0.18
	Pygidiopsis sp.1	609.1	0.33	–	0.52	−0.08	–	−0.46	0.71	3	0.13	1	1	0.14	0.67	1
	Pygidiopsis sp.2	352.0	0.06	–	1	–	–	–	–	9	0.23	0.86	0.29	0.28	0.36	0.05
	P. nanum	245.1	0.17	–	–	–	–	−0.82	0.56	5	0.14	0.34	0.16	0.22	1	1
	A. gnerii	998.4	0.20	–	–	–	–	−0.56	0.83	5	0.12	0.33	0.24	0.12	1	1
	Stephanoprora sp.	999.4	0.32	–	−0.52	−0.03	–	−0.27	0.81	3	0.43	1	0.78	0.47	1	1
	Infracommunity	759.0	0.45	–	−0.31	0.10	–	−0.38	0.86	5	0.54	1	0.74	0.44	1	1
Males	*A (L.) hadra*	566.4	0.22	–	–	–	0.43	−0.39	0.81	4	0.13	0.16	0.13	0.77	1	1
	Ascocotyle (P.) sp.	110.1	0.07	–	–	–	–	–	1	4	0.33	0.18	0.13	0.12	0.13	1
	Ascocotyle. (A.) sp.	123.5	0.05	–	1	–	–	–	–	12	0.2	0.85	0.31	0.29	0.34	0.05
	Pygidiopsis sp.1	105.2	0.65	−0.2	0.75	0.07	0.08	−0.51	0.36	–	–	–	–	–	–	–
	Pygidiopsis sp.2	11.9	0.17	–	−0.95	−0.31	–	–	–	–	0.34	0.95	0.51	0.32	0.38	0.13
	P. nanum	18.0	0.25	–	0.98	–	–	–	0.2	9	0.16	1	0.17	0.34	0.24	0.8
	A. gnerii	296.7	0.18	–	0.93	–	–	–	0.36	5	0.17	1	0.15	0.11	0.14	0.85
	Stephanoprora sp.	399.6	0.20	–	–	–	–	−0.39	0.92	4	0.15	0.15	0.15	0.15	1	1
	Infracommunity	397.8	0.40	–	−0.1	0.4	–	−0.43	0.8	6	0.49	0.88	0.74	0.38	1	1
–	Male-female proportion	12.30	0.98	–	0.92	–	0.38	–	–	1	–	1	–	0.57	–	–

VE: velocity; MD: mean depth; MW: mean width; CO: riverine vegetation coverage; UR: percentage of urbanization; TL: host total length.

Table 6
Results of DistLM analyses of environmental variables on component populations and communities of parasites of *Cnesterodon decemmaculatus* across six sampling sites along La Tapera creek.

Response variable	Female hosts							Male hosts						
			Explanatory variables (ρ)							Explanatory variables (ρ)				
	AICc	R^2	V	MD	MW	CO	UR	AICc	R^2	V	MD	MW	CO	UR
A. (L.) hadra	21.5	0.23	–	–	–	–	−1	8.0	0.35	–	–	–	–	−1
Ascocotyle (P.) sp.	−10.9	0.34	1	–	–	–	–	−11.2	0.06	–	–	–	−1	–
Ascocotyle. (A.) sp.	−0.6	0.68	–	–	–	–	−1	0.1	0.31	–	–	–	–	−1
Pygidiopsis sp.1	7,7	0.23	–	1	–	–	–	−0.2	0.60	–	1	–	–	–
Pygidiopsis sp.2	−2.3	0.67	–	1	–	–	–	−22.2	0.67	–	1	–	–	–
P. nanum	−15.4	0.87	–	–	–	–	−1	−16.8	0.86	–	1	–	–	–
A. gnerii	7.2	0.73	–	–	–	–	−1	−12.4	0.99	−0.08	0.99	–	–	–
Stephanoprora sp.	14.8	0.22	–	–	–	–	−1	−3.9	0.57	–	–	–	–	−1
Component community	36.9	0.34	–	–	–	–	−1	30.7	0.45	–	1	–	–	–

VE: velocity; MD: mean depth; MW: mean width; CO: riverine vegetation coverage; UR: percentage of urbanization.

in rural areas. These frequent episodes makes the channel an unstable habitat for fishes, which usually tend to avoid environments with large fluctuations in velocity, since turbulent flows can cause shear stress on a fish's body (Hockley et al., 2014). Therefore, variations in hydrodynamic drag in high flow and turbulent waters can be responsible of the reduced K observed for *C. decemmaculatus*. Since trade-offs involving body shape and swimming performance of fish vary in relation to flow conditions (Vogel, 1994), the sexual dimorphism shown by this species could explain the observed differences in the relationship between urbanization and K between males and females. The complex sexual courtship of poeciliids also results in different swimming capabilities, mobility, shoaling behaviour and habitat preferences between sexes (Hockley et al., 2014; Richards et al., 2010), which is also possibly related to the observed preference of females by deeper waters.

On the other hand, K was not significantly affected by parasite abundance. Indeed, although urbanization affect as much to K as to parasite burdens (as shown in Sections 4.3 and 4.4), such influence seems to act independently on both variables in this host-parasite-stream system. Parasites are often expected to reduce fish body condition, with their effect depending of many variables, such as level of infection, parasite size, life stage, parasite taxa and the tissue or organ affected (Lagrue and Poulin, 2015). However, in the case of trematode

metacercariae, once established and the cysts are formed, individual parasites probably impose little energetic demands upon the host (Lemly & Esch, 1984), especially when larvae are incorporated gradually over the fish life time, instead as mass infections (Lagrue and Poulin, 2015).

Longitudinal patterns of parasite loads were more evident for females, probably due to the comparatively lower number of males caught, but other possible causes are discussed below. For females, two patterns were observed, with *P. nanum*, *Pygidiopsis* sp. 1, *A. gnerii* and *Ascocotyle (A.)* sp. showing abrupt increases of mean abundance in less urbanized sites (2 and 4), whereas *A. (L.) hadra* and *Stephanoprora* sp. showed a marked peak only in site 4. Due to the complex life-cycles displayed by digeneans, their abundance is strongly associated with host densities (Blasco-Costa et al., 2013; de Montaudouin and Lanceleur, 2011). Most species of larval trematodes found in *C. decemmaculatus* display allogenic life cycles, using birds as definitive hosts; the unique exception is *A. gnerii*, with an autogenic life cycle and a siluriform fish as definitive hosts (Ostrowski de Núñez, 2007). Regarding first intermediate hosts, with the exception of *P. nanum* that parasitizes the ancylid *Uncancylus concentricus* (Ostrowski de Núñez, 1973), the rest of species share the same species of snail hosts (*Helobia parchappei* and *H. piscium*) (Ostrowski de Núñez, 1999, 2007), being *H.*
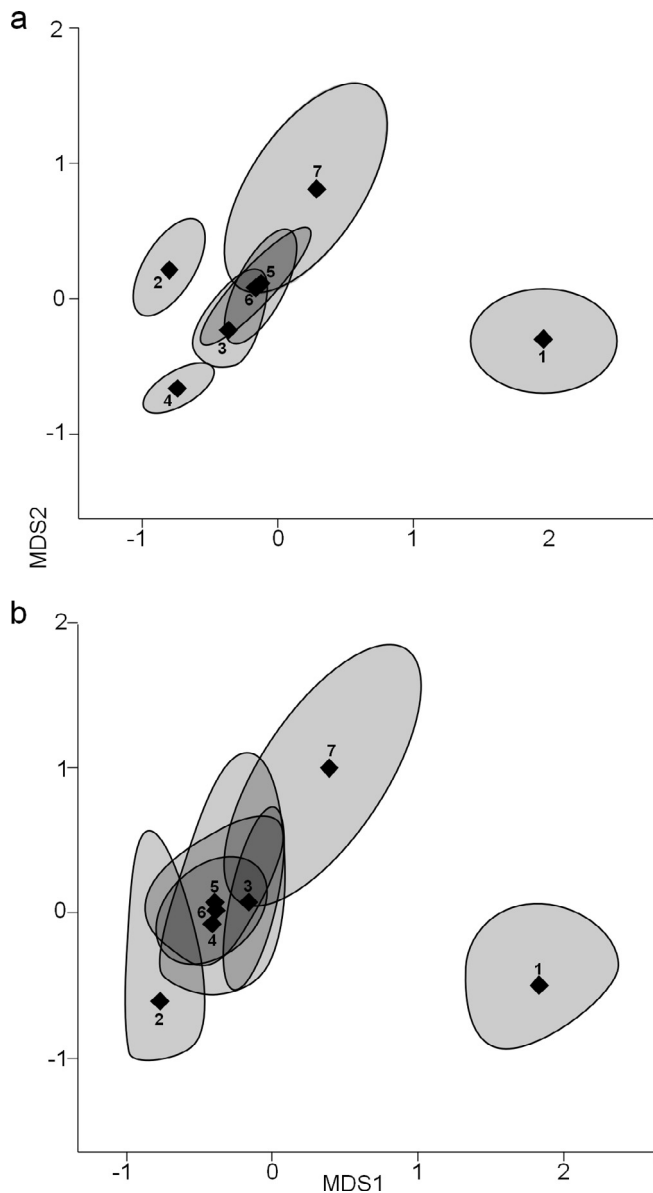
Fig. 6. nMDS plot of bootstrap averages of parasite infracommunities of female (a) and male (b) *Cnesterodon decemmaculatus* along seven sampling sites at La Tapera creek. Symbols represent the overall centroids across all repetitions. Boundary lines represent 95% confidence regions. Numbers indicate sampling sites.

Table 7

Values of t-scores of two-factor PERMANOVA post hoc pair-wise comparisons of square-root transformed abundance of parasites of *Cnesterodon decemmaculatus* in seven samples along La Tapera creek. Results of female and male hosts above and below the diagonal, respectively. Non-significant differences ($P > 0.05$) in bold.

Site	1	2	3	4	5	6	7
1	–	6.067	5.78	6.53	5.17	4.95	4.10
2	3.71	–	2.589	2.53	2.85	3.90	4.39
3	3.53	2.65	–	1.81	**0.97**	2.73	4.21
4	3.93	1.80	**1.04**	–	1.93	3.61	4.91
5	3.50	1.86	**1.23**	**0.82**	–	2.16	3.45
6	3.43	2.78	**1.24**	**1.28**	1.68	–	2.48
7	3.20	3.44	2.89	2.97	2.77	2.01	–

parchappei the only member of the genus present in Pampean streams (Darrigan, 1995; Tietze, 2011).

Therefore, if snail density was a relevant driver of parasite abundance, all species sharing *H. parchappei* should display similar patterns, which is not the case. On the other hand, *P. nanum*, transmitted by another snail behaved like most of them. Otherwise, distribution of birds could play a role on the variations of parasite burdens along the creek. Indeed, Los Padres lake is a natural reserve harbouring a rich waterbird community, many of which have shown to be vulnerable to disturbance by human activities avoiding the presence of people (Cardoni et al., 2008) and this behavior could explain the decreased parasite loads in more urbanized sections of the creek.

However, quantitative results of the effect of urbanization showed that the drivers of such patterns must be also searched among other biological and environmental factors. The identification of those determinants of parasite distribution depended on the level of analyses (host sex, population or community level, and individual host or host sample). In general, these drivers were more heterogeneous across species for male fish, leading, however, to more homogeneous assemblages in the central portion of the stream. The smaller number of males caught could play a role in these differences; this unbalanced sampling was due to the female-biased sex ratio characteristic of poeciliids (Pandian, 2010), but the higher homogeneity of parasite communities in males is most probably a consequence of the marked sexual dimorphism displayed by this species. Poeciliid males display mating and courtship behaviour and male-male competence (Conradsen and McGuigan, 2015; Leris et al., 2013; Plath et al., 2004) that possibly results in a higher vagility or broader home ranges, which could explain the observed patterns and the comparatively lower influence of urbanization on their parasite loads.

Different combinations of variables explained the distribution of parasite populations and assemblages along the creek, among them, TL and UR were recurrently identified as the best predictors, or even the only ones, determining the distribution at all levels of analyses. The observed significant and positive effect of TL on parasite burdens is expectable, given the possibility of larger hosts to accommodate more parasite species and sustain a greater absolute number of parasites than small ones, as well as to display larger surface areas for parasite attachment (Poulin, 2000; Timi et al., 2010; Timi and Lanfranchi, 2013). Moreover, for larval parasites that persist in the host for long periods, cumulative patterns result of longer periods of exposure for older fish (Braicovich et al., 2016).

The effect of UR on parasite loads indicates that local factors are more important in structuring parasite communities than water drift, contrarily to the observed for other stream-host-parasite systems (see Blasco-Costa et al., 2013), since in our system the linear gradient of samples along the creek only explained a low percentage of the total variation of infracommunities.

In the present study, urbanization affected negatively parasite loads of *C. decemmaculatus*. The negative effect also observed for fish K and its lack of relationship with parasite abundance, may indicate that host health or immunological status are not involved in the observed patterns. These results also cast doubts on the hypothesis of decreased levels of parasitism due to increased mortality of highly parasitized fishes subjected to urban stressors (Lafferty and Kuris, 1999). Therefore, it is more probable that urbanization-related stressors are affecting negatively survival or transmission of parasite free living stages (Pietrock and Marcogliese, 2003).

Both Los Padres lake and the headwaters of La Tapera creek receive diverse pollutants from surrounding horticultural areas (Miglioranza et al., 2004; Romanelli et al., 2014), therefore a differential detrimental effect on parasites at urban sections should imply the input of other compounds. La Tapera stream is not connected with official sewage dumps, however, Isla et al. (1998) reported high concentrations of faecal coliforms attributed to domestic residential inputs which could be sources for other kinds of chemicals. Although high concentrations

of faecal coliforms have been related to lower parasite burdens in fish from urban areas (Krause et al., 2010; Schmidt et al., 2003), they have been also considered as a surrogate of eutrophication, leading to increased parasite richness in other fish communities (Blanar et al., 2011) and therefore cannot be considered as a reliable driver of parasite burdens without consideration or measurement of concentrations of other compounds.

Finally, alteration of physical traits such as increased frequency and rapidity of short term changes in stream drift, especially during runoff events, can also affect the abundance and prevalence of trematodes, causing a decrease through a reduced transmission rates in turbulent or rapid currents (Janovy et al., 1997; Marcogliese, 2001; Marcogliese et al., 2016). As in the case of K, urban related flashiness could also produce a decrease of parasitism by larval trematodes.

6. Conclusions

Anthropogenic-induced environmental perturbations on parasitic organisms at both the population and the community level include domestic and industrial sewage, eutrophication, acidification, pesticides, thermal stress, hydrological changes and ultraviolet light (Marcogliese, 2005). Indeed, most of them are normally related to urban development and can act simultaneously; consequently, the mechanisms driving differences in parasite community dynamics are often not well understood (Chapman et al., 2015). Therefore, although it may be possible to determine the degree of impairment accurately, it is difficult achieving the same level of certainty regarding the causes and, consequently, matching a response to the responsible stressor can be very difficult (Allan, 2004). Otherwise, the effects of these mechanisms on hosts and parasites may vary depending on the type and intensity of the stressor, the parasite life cycle and exposure time (Marcogliese, 2005; Sures et al., 2017). Similarly, in this study the causes of the observed patterns cannot be determined with certainty, but the effect of urbanization, either direct or indirect on both hosts and parasites was clearly identified and observed at the level of both parasite assemblages and populations. Among the latter, *P. nanum*, *A. gnerii* and *Ascocotyle* (*A*) sp. were identified as promissory tools for further studies since UR explained the highest percentages of their variability. Furthermore they comply with the requisites for being good ecological indicators, since they are broadly distributed across streams in the region, are easy to observe and count, their prevalence and abundance allow reliable quantitative comparisons and are sensitive to variations of environmental stress and/or ecological conditions at small spatial scales.

Human actions at the landscape scale are a principal threat to the ecological integrity of lotic ecosystems, impacting habitat, water quality, and the biota via numerous and complex pathways (Allan, 2004). These systems are currently among the most threatened environments in the world and are degrading more rapidly than terrestrial ecosystems (Chapman et al., 2015). Consequently, there is a peremptory need to monitor and mitigate their degradation in an increasingly urbanized world. Humans act as selective agents determining changes in population and community structure of stream biota, which can be reliable indicators of that habitat degradation. In this scenario, the susceptibility of fish-trematode systems to undergo variations in response to both biotic and abiotic stressors makes them a promising tool for detecting urbanization-related disturbances in streams.

Declaration of interest

The authors declare that they have no conflict of interest.

Acknowledgements

References

Alberti, M., 2015. Eco-evolutionary dynamics in an urbanizing planet. Trends Ecol. Evol. 30, 114–126. http://dx.doi.org/10.1016/j.tree.2014.11.007.

Allan, J.D., 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annu. Rev. Ecol. Evol. Syst. 35, 257–284. http://dx.doi.org/10.1146/annurev.ecolsys.35.120202.110122.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK.

Arreghini, S., Cabo, L.D., Seoane, R., Tomazin, N., Serafini, R., De Iorio, A.F., 2005. Influence of rainfall on the discharge, nutrient concentrations and loads of a stream of the "Pampa Ondulada" (Buenos Aires, Argentina). Limnetica 24, 225–236.

Barger, M.A., Esch, G.E., 2001. Downstream changes in the composition of the parasite community of fishes in an Appalachian stream. J. Parasitol. 87, 250–255.

Baudou, F.G., Ossana, N.A., Castañé, P.M., Mastrángelo, M.M., Ferrari, L., 2017. Cadmium effects on some energy metabolism variables in *Cnesterodon decemmaculatus* adults. Ecotoxicology 26, 1250–1258. http://dx.doi.org/10.1007/s10646-017-1850-z.

Bhuthimethee, M., Dronen, J.N.O., Neill, W.H., 2005. Metazoan parasite communities of sentinel bluegill caged in two urbanizing streams, San Antonio, Texas. J. Parasitol. 91, 1358–1367. http://dx.doi.org/10.1645/GE-3463.1.

Blanar, C.A., Marcogliese, D.C., Couillard, C.M., 2011. Natural and anthropogenic factors shape metazoan parasite community structure in mummichog (*Fundulus heteroclitus*) from two estuaries in New Brunswick, Canada. Folia Parasitol. 58, 240–248.

Blanar, C.A., Hewitt, M., McMaster, M., Kirk, J., Wang, Z., Norwood, W., Marcogliese, D.J., 2016. Parasite community similarity in Athabasca River trout-perch (*Percopsis omiscomaycus*) varies with local-scale land use and sediment hydrocarbons, but not distance or linear gradients. Parasitol. Res. 115, 3853–3866. http://dx.doi.org/10.1007/s00436-016-5151-x.

Blasco-Costa, I., Koehler, A.V., Martin, A., Poulin, R., 2013. Upstream-downstream gradient in infection levels by fish parasites: a common river pattern? Parasitology 140, 266–274. http://dx.doi.org/10.1017/S0031182012001527.

Bonifacio, A.F., Cazenave, J., Bacchetta, C., Ballesteros, M.L., Bistoni, M.A., Amé, M.V., Bertrand, L., Hued, A.C., 2016. Alterations in the general condition, biochemical parameters and locomotor activity in *Cnesterodon decemmaculatus* exposed to commercial formulations of chlorpyrifos, glyphosate and their mixtures. Ecol. Indic. 67, 88–97. http://dx.doi.org/10.1016/j.ecolind.2016.02.011.

Braicovich, P.E., Ieno, E.N., Sáez, M., Despos, J., Timi, J.T., 2016. Assessing the role of host parasites as drivers of the abundance of long-lived parasites in fish stock assessment studies. J. Fish Biol. 89, 2419–2433. http://dx.doi.org/10.1111/jfb.13127.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference, second ed. Springer, New York.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., et al., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83, 575–583.

Cardoni, D.A., Favero, M., Isacch, J.P., 2008. Recreational activities affecting the habitat use by birds in Pampa's wetlands, Argentina: implications for waterbird conservation. Biol. Conserv. 141, 797–806. http://dx.doi.org/10.1016/j.biocon.2007.12.024.

Carriquiriborde, P., Díaz, J., Mugni, H., Bonetto, C., Ronco, A.E., 2007. Impact of cypermethrin on stream fish populations under field-use in biotech-soybean production. Chemosphere 68, 613–621. http://dx.doi.org/10.1016/j.chemosphere.2007.02.051.

Chapman, J.M., Marcogliese, D.J., Suski, C.D., Cooke, S.J., 2015. Variation in parasite communities and health indices of juvenile *Lepomis gibbosus* across a gradient of watershed land-use and habitat quality. Ecol. Indic. 57, 564–572. http://dx.doi.org/10.1016/j.ecolind.2015.05.013.

Clarke, K.R., Ainsworth, M., 1993. A method of linking multivariate community structure to environmental variables. Mar. Ecol. Prog. Ser. 92, 205–219.

Clarke, K.R., Gorley, R.N., 2015. Getting Started with PRIMER v7. PRIMER-E, Plymouth Marine Laboratory, Plymouth.

Conradsen, C., McGuigan, K., 2015. Sexually dimorphic morphology and swimming performance relationships in wild type zebrafish *Danio rerio*. J. Fish Biol. 87, 1219–1233. http://dx.doi.org/10.1111/jfb.12784.

Darrigan, G.A., 1995. Distribución de tres especies del género *Heleobia* Stimpson, 1865 (Gastropoda, Hydrobiidae) en el litoral argentino del Río de La Plata y arroyos afluentes. Iheringia Sér. Zool. 78, 3–8.

de Aquino Moreira, L.H., Takemoto, R.M., Pavanelli, G.C., 2015. Urbanization effects on the host/parasite relationship in fishes from tributary streams of Pirapó River, Paraná State: assessment of potential environmental bioindicators. Acta Sci. Biol. Sci. 37, 319. http://dx.doi.org/10.4025/actascibiolsci.v37i3.27201.

de la Torre, F.R., Ferrari, L., Salibián, A., 2005. Biomarkers of a native fish species (*Cnesterodon decemmaculatus*) application to the water toxicity assessment of a periurban polluted river of Argentina. Chemosphere 59, 577–583. http://dx.doi.org/10.1016/j.chemosphere.2004.12.039.

de Montaudouin, X., Lanceleur, L., 2011. Distribution of parasites in their second intermediate host, the cockle *Cerastoderma edule*: community heterogeneity and spatial scale. Mar. Ecol. Prog. Ser. 428, 187–199. http://dx.doi.org/10.3354/meps09072.

Feijoó, C.S., Lombardo, R.J., 2007. Baseline water quality and macrophyte assemblages in Pampean streams: a regional approach. Water Res. 41, 1399–1410. http://dx.doi.org/10.1016/j.watres.2006.08.026.

Gaston, K.J., 2010. Urbanization. In: Gaston, K.J. (Ed.), Urban Ecology. Cambridge University Press, Cambridge, pp. 10–34.

Giorgi, A., Feijóo, C., Tell, G., 2005. Primary producers in a Pampean stream: temporal variation and structuring role. Biodivers. Conserv. 14, 1699–1718. http://dx.doi.org/10.1007/s10531-004-0694-z.

Gonzalez Sagrario, M.D.L.A., Balseiro, E., 2003. Indirect enhancement of large zooplankton by consumption of predacious macroinvertebrates by littoral fish. Arch. Hydrobiol. 158, 551–574.

Greathouse, E.A., Pringle, C.M., 2006. Does the river continuum concept apply on a tropical island? Longitudinal variation in a Puerto Rican stream. Can. J. Fish. Aquat. Sci. 63, 134–152.

Hockley, F.A., Wilson, C.A.M.E., Brew, A., Cable, J., 2014. Fish responses to flow velocity and turbulence in relation to size, sex and parasite load. J. R. Soc. Interface 11, 20130814. http://dx.doi.org/10.1098/rsif.2013.0814.

Hued, A.C., Bistoni, M.A., 2005. Development and validation of a Biotic Index for evaluation of environmental quality in the central region of Argentina. Hydrobiologia 543, 279–298. http://dx.doi.org/10.1007/s10750-004-7893-1.

Isla, F.I., Perez Guzzi, J., Zamora, A., Folabella, A., 1998. Aportes de coliformes a la costa de Mar del Plata (Argentina) por vías naturales e inducidas. Thalassas 14, 63–70.

Iwanowicz, D., Black, M.C., Blazer, V.S., Zappia, H., Bryant, W., 2016. Effects of urban land-use on large scale stonerollers in the Mobile River Basin, Birmingham, AL. Ecotoxicology 25, 608–621. http://dx.doi.org/10.1007/s10646-016-1620-3.

Janovy Jr., J., Snyder, S.D., Clopton, R.E., 1997. Evolutionary constraints on population structure: the parasites of *Fundulus zebrinus* (Pisces: Cyprinodontidae) in the South Platte River of Nebraska. J. Parasitol. 83, 584–592.

Lagrue, C., Poulin, R., 2015. Measuring fish body condition with or without parasites: does it matter? J. Fish. Biol. 87, 836–847. http://dx.doi.org/10.1111/jfb.12749.

Lemly, A.D., Esch, G.W., 1984. Effects of the trematode *Uvulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis macrochirus*: ecological implications. J. Parasitol. 70, 475–492.

Krause, R.J., McLaughlin, J.D., Marcogliese, D.J., 2010. Parasite fauna of *Etheostoma nigrum* (Percidae: Etheostomatinae) in localities of varying pollution stress in the St. Lawrence River, Quebec, Canada. Parasitol. Res. 107, 285–294.

Lafferty, K.D., Kuris, A.M., 1999. How environmental stress affects the impacts of parasites. Limnol. Oceanogr. 44, 925–931. http://dx.doi.org/10.4319/lo.1999.44.3_part_2.0925.

Leris, I., Sfakianakis, D.G., Kentouri, M., 2013. Are zebrafish *Danio rerio* males better swimmers than females? J. Fish Biol. 83, 1381–1386. http://dx.doi.org/10.1111/jfb.12210.

Lucinda, P.H.F., 2005. Systematics of the genus *Cnesterodon* Garman, 1895 (Cyprinodontiformes: Poeciliidae: Poeciliinae). Neotrop. Ichthyol. 3, 259–270.

Machut, L.S., Limburg, K.E., 2008. *Anguillicola crassus* infection in *Anguilla rostrata* from small tributaries of the Hudson River watershed, New York, USA. Dis. Aquat. Org. 79, 37–45. http://dx.doi.org/10.3354/dao01901.

Malarvizhi, K., Vasantha Kuma, S., Porchelvan, P., 2016. Use of high resolution Google earth satellite imagery in landuse map preparation for urban related applications. Procedia Technol. 24, 1835–1842.

Marcogliese, D.J., 2001. Implications of climate change for parasitism of animals in the aquatic environment. Can. J. Zool. 79, 1331–1352.

Marcogliese, D.J., 2005. Parasites of the superorganism: are they indicators of ecosystem health? Int. J. Parasitol. 35, 705–716. http://dx.doi.org/10.1016/j.ijpara.2005.01.015.

Marcogliese, D.J., Locke, S.A., Gélinas, M., Gendron, A.D., 2016. Variation in parasite communities in spottail shiners (*Notropis hudsonius*) linked with precipitation. J. Parasitol. 102, 27–36. http://dx.doi.org/10.1645/12-31.

McKinney, M.L., 2002. Urbanization, biodiversity, and conservation. BioScience 52, 883–890.

McKinney, M.L., 2008. Effects of urbanization on species richness: a review of plants and animals. Urban Ecosyst. 11, 161–176.

Menéndez-Helman, R.J., Ferreyroa, G.V., dos Santos Afonso, M., Salibián, A., 2015. Circannual rhythms of acetylcholinesterase (AChE) activity in the freshwater fish *Cnesterodon decemmaculatus*. Ecotox. Environ. Saf. 111, 236–241. http://dx.doi.org/10.1016/j.ecoenv.2014.10.017.

Miglioranza, K.S., de Moreno, J.E.A., Moreno, V.J., 2004. Land-based sources of marine pollution: organochlorine pesticides in stream systems. Environ. Sci. Pollut. R. 11, 227–232. http://dx.doi.org/10.1065/espr2004.03.192.

Mikaelian, I., De Lafontaine, Y., Harshbarger, J.C., Lee, L.L., Martineau, D., 2002. Health of lake whitefish (*Coregonus clupeaformis*) with elevated tissue levels of environmental contaminants. Environ. Toxicol. Chem. 21, 532–541.

Ostrowski de Núñez, M., 1973. Sobre el ciclo biológico experimental de *Posthodiplostomum nanum* Dubois, 1937 (Trematoda, Diplostomatidae). Physis 32, 121–132.

Ostrowski de Núñez, M., 1999. Fishes as definitive or intermediate hosts of

Opisthorchioid trematodes in South America. Wiad Parazytol. 45, 329–336.

Ostrowski de Núñez, M., 2007. Life cycle of *Stephanoprora uruguayense* (Digenea: Echinostomatidae) in Argentina. J. Parasitol. 93, 1090–1096.

Pandian, T.J., 2010. In: Sexuality in Fishes. CRC Press, pp. 189.

Pickett, S.T., Cadenasso, M.L., Grove, J.M., Boone, C.G., Groffman, P.M., Irwin, E., Kaushal, S.S., Marshall, V., McGrath, B.P., Nilon, C.H., Pouyat, R.V., Szlavecz, K., Troy, A., 2011. Urban ecological systems: scientific foundations and a decade of progress. J. Environ. Manage. 92, 331–362.

Pickett, S.T.A., Cadenasso, M.L., Rosi-Marshall, E.J., Belt, K.T., Groffman, P.M., Grove, J.M., Irwin, E.G., Kaushal, S.S.M., LaDeau, S.L., Nilon, C.H., Swan, C.M., Warren, P., 2017. Dynamic heterogeneity: a framework to promote ecological integration and hypothesis generation in urban systems. Urban Ecosyst. 20, 1–14. http://dx.doi.org/10.1007/s11252-016-0574-9.

Pietrock, M., Marcogliese, D.J., 2003. Free-living endohelminth stages: at the mercy of environmental conditions. Trends Parasitol. 19, 293–299.

Plath, M., Arndt, M., Parzefall, J., Schlupp, I., 2004. Size-dependent male mating behaviour in the Cave molly, *Poecilia mexicana* (Poeciliidae, Teleostei). Subterr. Biol. 2, 59–64.

Poulin, R., 2000. Variation in the intraspecific relationship between fish length and intensity of parasitic infection: biological and statistical causes. J. Fish Biol. 56, 123–137. http://dx.doi.org/10.1111/j.1095-8649.2000.tb02090.x.

Ramos-Fregonezi, A.M.C., Malabarba, L.R., Fagundes, N.J.R., 2017. Population genetic structure of *Cnesterodon decemmaculatus* (Poeciliidae): a freshwater look at the Pampa Bioma in southern South America. Front. Genet. 8, 214. http://dx.doi.org/10.3389/fgene.2017.00214.

Richards, E.L., van Oosterhout, C., Cable, J., 2010. Sex-specific differences in shoaling affect parasite transmission in guppies. PLoS One 5, e13285. http://dx.doi.org/10.1371/journal.pone.0013285.

Ricker, W.E., 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Canada 191, 1–382.

Romanelli, A., Massone, H.E., Lima, M.L., Esquius, K.S., 2014. Spatial decision support system for assessing lake pollution hazard: southeastern pampean shallow lakes (Argentina) as a case study. Wetlands Ecol. Manage. 22, 247–265. http://dx.doi.org/10.1007/s11273-013-9327-1.

Roy, A.H., Freeman, M.C., Freeman, B.J., Wenger, S.J., Ensign, W.E., Meyer, J.L., 2005. Investigating hydrologic alteration as a mechanism of fish assemblage shifts in urbanizing streams. J. North Am. Benthol. Soc. 2, 656–678. http://dx.doi.org/10.1899/04-022.1.

Schmidt, V., Zander, S., Körting, W., Steinhagen, D., 2003. Parasites of the flounder *Platichthys flesus* (l.) from the german Bight, North sea, and their potential use in ecosystem monitoring. Helgol. Mar. Res. 57, 236–251.

Sures, B., Nachev, M., Selbach, C., Marcogliese, D.J., 2017. Parasite responses to pollution: what we know and where we go in 'Environmental Parasitology'. Parasit. Vectors 10, 65. http://dx.doi.org/10.1186/s13071-017-2001-3.

Symonds, M.R.E., Moussalli, A., 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike'sinformation criterion. Behav. Ecol. Sociobiol. 65, 13–21. http://dx.doi.org/10.1007/s00265-0101037-6.

Tietze, E. 2011. Distribución de Heleobia parchappii parchappii en ambientes dulceacuícolas de la Región Pampeana (Argentina). Amici Molluscarum (N° Special Issue) 73–75.

Timi, J.T., Lanfranchi, A.L., 2013. Ontogenetic changes in heterogeneity of parasite communities of fish: disentangling the relative role of compositional versus abundance variability. Parasitology 140, 309–317. http://dx.doi.org/10.1017/S0031182012001606.

Timi, J.T., Luque, J.L., Poulin, R., 2010. Host ontogeny and the temporal decay of similarity in parasite communities of marine fish. Int. J. Parasitol. 40, 963–968. http://dx.doi.org/10.1016/j.ijpara.2010.02.005.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedel, J.R., Cushing, C.E., 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37, 130–137.

Vogel, S., 1994. Life in Moving Fluids, second ed. Princeton University Press, Princeton, NJ.

Walsh, C.J., Fletcher, T.D., Ladson, A.R., 2005. Stream restoration in urban catchments through redesigning stormwater systems: looking to the catchment to save the stream. J. N. Am. Benthol. Soc. 24, 690–705.

Walsh, C.J., Waller, K.A., Gehling, R., MacNally, R., 2007. Riverine invertebrate assemblages are degraded more by catchment urbanization than by riparian deforestation. Freshwater Ecol. 52, 574–587.

Wang, L., Lyons, J., Kanehl, P., Bannerman, R., 2001. Impacts of urbanization on stream habitat and fish across multiple spatial scales. Environ. Manage. 28, 255–266. http://dx.doi.org/10.1007/s002670010222.