Urbanization effects on the host/parasite relationship in fishes from tributary streams of Pirapó River, Paraná State: assessment of potential environmental bioindicators

Luis Henrique de Aquino Moreira, Ricardo Massato Takemoto and Gilberto Cezar Pavanelli

ABSTRACT. A parasitological study was conducted with 131 fishes belonging to the following species: *Poecilia reticulata* and *Rhamdia quelen* species. Samplings occurred in tributary streams of Pirapó River, located in the urban and rural area on the surrounding of Maringa city, State of Paraná. The aim of the study was to evaluate the use of fish endoparasites to determine the environmental quality of streams. There was a high similarity between parasites species considering both sets of environments. No influence of streams groups (urban and rural) on the parasites (*Sebekia oxycephala* and *Cucullanus (Cucullanus) pinnai*) prevalence and abundance was found. The Relative Condition Factor (Kn) was not different in urban and rural environments for *P. reticulata* species, but in *R. quelen* it was significantly higher in urban environments. The parasitism does not interfere in Kn, but was statistically significant in females of *P. reticulata* when evaluated separately by environment (urban and rural). In urban environments, the parasite *S. oxycephala* becomes more pathogenic resulting in lower Kn in *P. reticulata* females. In these, the parasite abundance resulted in negative correlation with Kn in urban streams, suggesting parasite potential damaged action in the anthropized streams. It was verified that the parasite species *S. oxycephala* and *Cucullanus (Cucullanus) pinnai* are inadequate as bioindicators of environmental quality.

Keywords: relative condition factor (Kn), endoparasites, *Sebekia oxycephala*, *Cucullanus (Cucullanus) pinnai*, urban and rural streams, IndVal.

Introduction

Parasitism, along with predation and competition, an important factor of population control, ensuring ecological balance (VANDERGRIFT et al., 2008). The simple presence of a parasite species in a host may indicate the presence of other organisms in the environment (heteroxenous parasite), environmental quality, or diet and biology of the host. (SILVA-SOUZA et al., 2006).
Parasites are sensitive to changes that occur in the environment, either directly on them or indirectly (on intermediate hosts). This feature makes them suitable for use in organisms as bioindicators (Lafferty, 2008).

Disordered human development and population growth that occurs in cities often compromises hydric resources, threatening biodiversity. Unfortunately, there are a lot of cases in which the environment is completely changed without previous records of the original dynamic or even of the organisms which lived in that site (Silva-Souza et al., 2006).

Although at rural areas, the streams still suffer due to influence of anthropogenic activities (agriculture and livestock), it is minor when compared to what happens in the urban area of cities. In urban streams occurs deforestation of riparian vegetation, soil sealing, release of sewage and industrial contaminants changing the aquatic biota (Ternus et al., 2011).

The relation parasite / host, especially in fish, may be changed (prevalence, abundance, relative condition factor of the host) with the effects of urbanization, especially by sewage release (Thilakaratne et al., 2007). These changes can be used in the bioindication. The advantage of fish parasites use as bioindicators is to detect the impact of environmental changes, before the degradation/pollution expresses its lethal form or that it has already been strongly affecting the entire water community. Thus, the bioindicators not only detects changes in the environment, but can also be used as a tool to direct government management actions and environmental protection (Silva-Souza et al., 2006).

Thus, this study aimed to analyze the relationships parasite / host in tributaries of the river Pirapó, including urban and rural streams with bias in parasite bioindicators.

**Material and methods**

The hosts' capture occurred in six tributaries of the river Pirapó, Paraná State. The Ribeirão Maringá (23°22’28.09”S, 51°58’8.00”W) and Córrego Mandacaru (23°23’5.01”S, 51°56’49.16”W) which cross the urban area of Maringá city, suffering the effects of urbanization (deforestation of riparian vegetation, soil sealing, release of sewage). This streams received effluent from a Sewage Treatment Plant (besides unlawful liberation) (Neves; Souza, 2013). According to the studies of Otsuschi (2000), Schneider et al. (2011), Netto et al. (2012) and Freire et al. (2012; 2013), these streams were monitored and it could be verified that limnological parameters (nitrogen forms, total phosphorus and metals) were above those permitted by the Conama Resolution 357/2005 (Brasil, 2005). According to Freire et al. (2013) critical stretches of water pollution were observed in Maringá and Mandacaru streams exceeding limits allowed by law. Thus, these streams under strong anthropogenic influence of urbanization (urbanized) were included in the urban group. On the other side, rural streams, without urbanization effect (distant from the urban area), are Ribeirão Zaúna (23°23’41.99”S, 51°50’55.11”W), Ribeirão Granada (23°18’5.26”S, 51°45’39.95”W), Ribeirão Remo (23°21’27.09”S, 52°1’0.75”W) and Ribeirão Água da Roseira (23°21’2.26”S, 51°54’50.45”W) (Figure 1). Due to less influence of the urban area, the streams were classified within the rural group.

The hosts’ capture were performed by using electrofishing equipment (500 volts and 2 amperes). Electrofishing relies on two electrodes which deliver current into the water to stun fish in the sampled sites. The fishes were colected and placed in separate vials (sampled sites) containing 4% formaldehyde.

Data on the prevalence, mean abundance, mean intensity and range of parasites species found were surveyed in both groups of streams. These concepts were used according to Bush et al. (1997). The Jaccard Similarity Index was used to evaluate the similarity of fish parasite between groups of environments. We performed a Canonical Discriminant Analysis (DCA) to check for equal distribution of parasite species in investigated streams.
Host/parasite relationship in fishes from streams

To check the influence for both groups (urban and rural streams) in the abundance and prevalence of each parasite species the tests used were Mann-Whitney U and G ('log-likelihood'), respectively (ZAR, 1996).

In order to assess the impact of urbanization on the ‘well-being’ (Kn) (LE CREN, 1951) of the host populations belonging to urban and rural streams, it was used Mann-Whitney U test (ZAR, 1996). Mann-Whitney U test was used to investigate influences on the welfare of fish (Kn) between parasitized and non-parasitized hosts. In order to investigate possible correlations of parasite abundances and relative condition factor (Kn) of the hosts, we used Spearman's rank correlation coefficient (ZAR, 1996). In P. reticulata the Kn was calculated separately for males and females, due to the sexual dimorphism observed in the species, which resulted in total length and weight difference between sexes.

The Indicator Value Index (IndVal) described by Dufrêne and Legendre (1997) was adapted (host used as a replica) and tested to assess the distribution of fish parasites in response to different environmental variables (identification of parasite bioindicator species). The IndVal test is calculated individually by parasite species, and it is carried out a statistical analysis of each parasite and host (VENANCIO et al., 2010). The level of statistical significance adopted was p ≤ 0.05.

### Results and discussion

It was collected 131 hosts, belonging to species *Poecilia reticulata* (77 females and 31 males) and *Rhamdia quelen* (23 not sexed), 78 fishes in urban and 53 in rural streams. The values of prevalence, mean abundance, mean intensity, amplitude and parasite species are presented in Table 1, for both groups of streams.

The infection site of all parasites identified was the intestine of the host. The cestode Pseudophyllidea was the only species found only in urbanized streams. The nematode *Cacullanus (Cacullanus) pinnai* and the pentastomid *Sebekia oxycephala* were found in both streams groups showing similar parasitic levels.

### Table 1. Host fish and species of intestinal parasite, prevalence (Prev.), mean abundance (MA), mean intensity (MI) and amplitude (Ampl.) in *Poecilia reticulata* and *Rhamdia quelen* collected in urban and rural streams, tributaries of the Pirapó River, Paraná State.

<table>
<thead>
<tr>
<th>Host/Parasite</th>
<th>Urban Streams</th>
<th>Rural Streams</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Poecilia reticulata</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cestode</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudophyllidea</em></td>
<td>1.61</td>
<td>0.016</td>
</tr>
<tr>
<td>Pentastomid</td>
<td>Sebekia oxycephala</td>
<td>15.22</td>
</tr>
<tr>
<td><em>Rhamdia quelen</em></td>
<td>Nematode</td>
<td><em>Cacullanus (Cacullanus) pinnai</em></td>
</tr>
</tbody>
</table>

The prevalence values of *S. oxycephala* are low in both environments, but are similar to those observed in other studies with this parasite (ALMEIDA et al., 2010; VICENTIN et al., 2013). *Cacullanus (Cacullanus) pinnai* was the most prevalent parasite in both analyzed environments groups, being found with higher prevalence in *R. quelen* that had already been found in other studies with the same parasite and host (VENANCIO et al., 2010). Thus, apparently in both groups of streams the parasite finds suitable conditions to complete its parasitic cycle.

Parasites of this genus have been reported being favored in degraded / polluted places due to the site provide better conditions for the development of their hosts (SULGUSTOWSKA; STYCZYŃSKA-JUREWICZ, 1996 apud DZIKA; WYZLIC, 2009-2010). However, there was no difference between the prevalence of *Cacullanus (C.) pinnai* in streams with effluents inputs (urbanized streams) compared to rural areas. Possibly crustaceans, the first intermediate hosts of this parasite, present similar distribution between both groups of studied streams, resulting in similar levels of prevalence.
The low parasite diversity found in analyzed hosts can be assigned to The River Continuum Concept (RCC) (VANNOTE et al., 1980). In the beginning (Headwaters - order 1-3), where the research was conducted, predominate heterotrophic organisms, lower primary productivity, resulting in lower biodiversity, when compared to Midreaches (order 4-6) and sites of flood pulses (JUNK et al., 1989). The low organisms biodiversity found in this environments restricts the hosts possibilities to parasites life cycles resulting in low parasite diversity in headwaters.

The Jaccard Similarity Index (JSI) indicated a high similarity between species of endoparasites present in urban and rural environments, considering the host species P. reticulata (JSI = 0.25) and R. quelen (JSI = 0.33). Thus, we can say that although the sites have different levels of impact related to urbanization, they were not enough to modify parasite species of the investigated fish, leaving a large similarity between them. Possibly, the factors involved in parasitism, such as existence of infective forms of the parasite, the occurrence of intermediate hosts, host susceptibility to the parasite, environmental toxicity, are similar in both places.

The result of the Discriminant Canonical Analysis (DCA) (λ Wilks = 0.94268, F (12,325) = 0.61238, p < 0.8317) pointed to an equal distribution in the abundance of the parasites species from each one of the streams analyzed so there were no relationships between the parasites and the collection sites. Possibly the differences caused by urbanization in analyzed streams were not able to provide conditions that favor or disfavor the parasite species found, making the distribution of these independent of the stream analysis.

The concept that altered environments (degraded / polluted) provide increased levels of parasitism (host stress) is not always true. Pollution can act in several ways on parasitism. Negatively on the host (toxic compounds, damage for the correct nutrition, stress) increasing individual susceptibility to infectious diseases, thus favoring the parasitism (GOPAL et al., 1992). Positively on the host when it induces an increase of hosts (intermediate / final) used by the parasite to complete its life cycle (positive to parasitism). However, in many situations pollution can negatively impact the parasitism. When it causes mortality of host or of free-living parasitic forms (THIELTGES et al., 2008), sometimes can even completely exclude parasitic populations of certain environments (THOMAS et al., 2005; SILVA-SOUZA, et al., 2006; LAFFERTY, 2008). There is a tendency in anthropogenic environments to be more harmful to parasites of heteroxenic cycle (indirect effect on the host). In these sites prevail parasites of direct cycle with a low specificity to host and high tolerance to disturbance (MARCOGLIESE; CONE, 1996).

No statistically significant differences were found in the relative condition factor (Kn) in P. reticulata, between urban and rural environments, females $Z (U) = 0.8517, p = 0.1972$; males, $Z (U) = 0.5929, p = 0.2766$. In R. quelen significant difference was found in Kn between the two investigated sites ($Z (U) = 2.4054, p = 0.0081$). Thus, R. quelen hosts belonging to urbanized streams had better development conditions compared to rural streams.

Possibly the largest organic load and other disorders in urbanized streams have not influenced the diet of P. reticulata, which basically contains detritus, zooplankton, insect larvae and benthic algae (FROESE; PAULY, 2011). However, in R. quelen Kn values were higher in urbanized streams. Given that the species feeds on fish, crustaceans, insects, plant debris, and organic debris, possibly the environmental enrichment by nutrients has favored an increase of their food items, resulting in better Kn.

Piaia et al. (1999) demonstrated the best development of the host (R. quelen) in areas of higher turbidity when compared to well-lit environments, which may have happened in urban streams due to the input of organic matter with consequent decrease in water transparency.

There was no statistically significant influence on the type of the analyzed environment (urban and rural) on the prevalence of S. oxycephala or Cucullanus (C.) pinnai, according to Table 2. Due to the low prevalence of the tapeworm Pseudophyllidea (only one specimen) it was not used in statistical inferences. There was no significant influence among the study sites (urban and rural) on parasite abundance of S. oxycephala or Cucullanus (C.) pinnai (Table 3).

### Table 2. Results of the loglihood G and Mann-Whitney U test for verifying the influence of environments groups (urban and rural), respectively, in the prevalence and abundance of parasites Sebekia oxycephala and Cucullanus (Cucullanus) pinnai collected in urban and rural streams, tributaries of the Pirapó river, Paraná State.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>G</th>
<th>Mann-Whitney U Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td>p</td>
</tr>
<tr>
<td>Sebekia oxycephala</td>
<td>0.1176</td>
<td>0.7517</td>
</tr>
<tr>
<td>Cucullanus (Cucullanus) pinnai</td>
<td>0.0583</td>
<td>0.8091</td>
</tr>
</tbody>
</table>

The lack of difference between groups of streams (urban and rural) and prevalence and abundance of parasite species suggest that behavioral and
biological changes don’t occur in the fish between the groups. This fact results in the similarity of fish feed, making it an equal risk of infection from both sites, resulting in similar parasitic levels.

*P. reticulata* feeds on detritus, which are the infective forms of *S. oxycephala* similarly in both environments. Apparently in this study changes from urbanization (pollution, deforestation) do not interfere in the distribution of intermediate host of *Cicullanus (C.) pinnai*, in the locations studied, since the parasite was found in both locations with similar parasitic levels.

No statistically significant difference between the Kn of parasitized and non-parasitized fish was found, considering the parasitism of *S. oxycephala* in females of *P. reticulata* (*Z (U) = 0.6868 and p = 0.2461*) and *Cicullanus (C.) pinnai* in *R. quelen* (*Z (U) = 0.3150 and p = 0.3764*) without evaluating the effect of the environment in which the fish inhabits (urban or rural). Few males of *P. reticulata* were infested (2), and the test was not performed with them.

Subsequently, the same analysis was performed separately for each of the two investigated groups of environments. No statistically significant difference was found between the Kn of fish *R. quelen* parasitized and non-parasitized in the urban environment (Table 3).

<table>
<thead>
<tr>
<th>Host</th>
<th>Urban Streams</th>
<th>Rural Streams</th>
<th>Z(U)</th>
<th>p</th>
<th>Z(U)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. reticulata</em> females</td>
<td></td>
<td></td>
<td>1.6809</td>
<td>0.0464</td>
<td>0.3000</td>
<td>0.3821</td>
</tr>
<tr>
<td><em>P. reticulata</em> males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rhamdia quelen</em></td>
<td></td>
<td></td>
<td>0.2169</td>
<td>0.4141</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant.

*Rhamdia quelen* develops itself in the urban environment regardless of whether or not parasitized by *Cicullanus (C.) pinnai*. It is believed that the fish species is not sensitive to degradation caused by urbanization (shows no decrease immune resistance), naturally fighting parasitic infections without affecting its development (Kn) (SILVA-SOUZA et al., 2006). Females of *P. reticulata* did not show significant differences in Kn between parasitized and non-parasitized fish in the rural environment, but were significant in the urban environment, according to Table 4. Thus females *P. reticulata* collected in urbanized environments showed values of Kn significantly lower in infected individuals compared to non-parasitized.

Due to the small number of infected male hosts of *P. reticulata* it was not possible to perform the test, as well as *R. quelen* from the rural streams.

In urban streams, where conditions of host stress (change in water quality) are present, the parasite *S. oxycephala* could become more pathogenic to females of *P. reticulata*. The parasite favored by stress and immunosuppression fish damages the same promoting decrease in relative condition factor of fish (Kn) (HOUGE; PARIS, 2002), Thilakaratne et al. (2007) corroborate this hypothesis stating that the effects of parasitic infections are more serious about the fish exposed to contaminants compared to unexposed.

No correlations were found between the relative condition factor of the fish and parasite abundance, considering the parasitism by *S. oxycephala* in *P. reticulata* females (*rs = -0.0803, p = 0.4874*), males (*rs = 0.2225 and p = 0.2289*) and by *Cicullanus (C.) pinnai* in *R. quelen* (*rs = -0.0294, p = 0.8941*) without evaluating the effect of the environment which the fish inhabits. The separated analysis for each one group of environments is presented in Table 4.

Females of *P. reticulata* were the only hosts that presented negative correlation between Kn and parasite abundance of *S. oxycephala* in the urban streams (Table 4). This fact occurs because the hosts are already with their immune systems weakened, due to the action of pollution in that environment, increasing the susceptibility of fish to parasitism. With the encouragement of parasitism, *S. oxycephala* reaches higher abundance, which results in greater impairment of the host and consequently lower values of Kn (Figure 2).

*Thilakaratne et al. (2007)* suggests that the interaction between pollution and the stress produced by the parasitism are more harmful when combined, increasing its effect, than when we consider their individual effects.
In rural environments susceptibility of *P. reticulata* females to the parasite is smaller, thus it can grow and gain weight regardless of parasitism by *S. oxycephala*, which does not cause higher pathogenicity to the host. *Rhamdia quelen* no correlation between the relative condition factor (Kn) and parasite abundance, suggesting that the fish has resistance to parasitism in both rural and urban environments.

Often hosts may harbor high parasite load without affecting their relative condition factor (Kn) (DIAS et al., 2004). One factor that enables the lack of correlation is the low pathogenic parasite that does not influence in a negative correlation of Kn with parasitism, like what happens with highly virulent parasites (POULIN, 1998). *Rhamdia quelen* gained weight and grew independently of parasitism by *Cucullanus (C.) pinnai*, suggesting little damage action of this parasite on the fish and wispy effect of pollutants directly on the host. Kn analysis pointed to better conditions of *R. quelen* in urbanized environments, possibly as an indirect result of improving your diet in these environments. This nutritional improvement may ensure a better resistance to parasitism, even in conditions of pollution/parasitism.

*Poecilia reticulata* males showed no correlation between the relative condition factor (Kn) and parasite abundance (independent development of parasitism) in rural and urban streams. Thus, it points to a differential susceptibility to parasitism between genders in *P. reticulata*. The combination of urban environmental pollution and parasitism acts negatively in females development, but causes no change in males. Possibly males present a distinct physiological/immune response to combination pollution/parasitism, tolerating fluctuations (increase / decrease) in parasite abundance without influence in their Kn.

In an attempt to identify a parasite species with potential to be used as the bioindicator IndVal test was used (Table 5). The results obtained in *S. oxycephala* and *Cucullanus (C.) pinnai* showed that both species are not good for this purpose. The value of the index value indicators found were low, not statistically significant in the two studied environments and their use as bioindicators was discarded. Despite urban streams suffer strong anthropogenic influence, as characterized by Schneider et al. (2011), Freire et al. (2013) and Neves and Souza (2013), these were not sufficient to reflect as bioindicators in endoparasites species found.

Due to tolerance of parasites species found to urbanization effects, there were no significant variations in their presence and abundance between the groups investigated, which impairs their use as bioindicators. The IndVal values are low, according to Table 5, considering the maximum would be 100 (all individuals of a particular group would be parasitized and parasite occurring only in this group of host). Therefore, in this research, due to the characteristic of parasites involved (pollution tolerance), we observe the inability to use IndVal to determine bioindicators species in the assessed environments.

<table>
<thead>
<tr>
<th>Endoparasites</th>
<th>Environment</th>
<th>IndVal</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sebekia oxycephala</em></td>
<td>Rural</td>
<td>8.2</td>
<td>0.525</td>
</tr>
<tr>
<td><em>Cucullanus (C.) pinnai</em></td>
<td>Urban</td>
<td>6.1</td>
<td>0.446</td>
</tr>
</tbody>
</table>

The techniques for bioindicator are based on the reflection of environmental changes, usually of anthropogenic origin (pollutants) on groups of susceptible organisms (GALLI et al., 2001). In general, in polluted environments it is checked a larger diversity of organisms, larger evenness and lower dominance of species (DZIKA; WYZLIC, 2009-2010).

The fish species chosen for hosts as bioindicators were those framed as ideal according to Lafferty (1997) and Vidal-Martínez et al. (2009) for its characteristics (abundant, cosmopolitan, easy sampling, capture and triage, interact with sediment). However the low parasitic levels and diversity differentiated among surveyed environments, negatively interfered in the bioindication of this research.
Both parasites found are considered generalists (favored in disturbed habitats), suggesting that perhaps even rural environments, where we still have a higher environmental integrity, are still under an effect of anthropogenic impact. The scarcity of previous studies with fish parasites in the streams investigated also complicates the proof of altered / loss in parasites species richness due to the anthropic impact. However, low parasite richness found suggests, as previously reported by Silva-Souza et al. (2006), the occurrence of negative anthropic effects not only in the parasite community, but on a whole group of organisms in the areas surveyed. Nevertheless, other studies become necessary to confirm this hypothesis, since the homogeneity of niches in headwaters streams (order 1-3) can be restricting greater parasite biodiversity.

**Conclusion**

High similarity was observed in parasite levels and between species identified in both groups of streams (urban and rural). Thus, there were no differences in the prevalence and abundance of fish belonging to urban and rural streams. The DCA showed similar distribution of endoparasites abundance between different sampled streams.

The fish species *R. quelen* showed higher relative condition factor (Kn) in urban streams compared to rural, indicating it is better adapted to these environments. The combination of parasitism with changes/degradation (pollution, deforestation) in urban streams making parasite *S. oxycephala* more pathogenic to *P. reticulata* females (fish stress, immunosuppression), resulting in lower values of Kn. In urbanized streams a negative correlation was found between Kn of *P. reticulata* and abundance of parasite *S. oxycephala*. This way, the higher the parasite abundance, the greater becomes the harmful action of parasite on its host, resulting in lower Kn values. Due to low parasite diversity found, the absence of significant differences in parasitic levels between urban and rural streams and the fact of the species found are generalists, tolerant to urbanization effects, it was not feasible to use the endoparasites observed as bioindicators of environmental quality.

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**References**


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