Distribution of guppies *Poecilia reticulata* (Peters, 1860) and *Phalloceros caudimaculatus* (Hensel, 1868) along a polluted stretch of the Paraíba do Sul River, Brazil

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(With 3 figures)

Abstract

Cyprinodontiformes fishes (guppies) are widely distributed in Neotropical regions and use deteriorated microhabitats in rivers where few species can occur. This study was carried out in a stretch of the Paraíba do Sul River in bracketing a large urban-industrial complex. The aim was to assess eventual effects that the industrial complex could have on distribution of two closely related fish species of guppies, *Phalloceros caudimaculatus* and *Poecilia reticulata.* The area was divided into three zones: Z1, 40 km upriver of the major urban-industrial complex of Volta Redonda; Z2, just down river of the complex; and Z3, 30 km down river of the complex. Six sites (two in each zone) were sampled monthly between November 1998 and October 1999, using a standardized fishing effort with cast net throws, trays lifts and seine hauls, covering different microhabitats, that is, riffles, pools and the proximity of the river's margins. *Poecilia reticulata* was widely distributed, peaking at Z2, the most polluted area, while *P. caudimaculatus* showed the highest abundance at Z3, being almost absent in Z1. Both species occurred in high numbers throughout the year but they were scarce between April and June. Females outnumbered males for both species in most size classes and at all sites. Juveniles were more abundant than adults, with non-pregnant females outnumbering pregnant ones. Condition factor was always higher in males than females but only males *P. reticulata* showed significant difference among the three zones, with the highest values at Z2. The higher number of females confirms the expectation that these species can use very polluted areas and that availability of food provided by organic loads allowed their distribution all over the area. Although these two species have shown indication of spatial separation in the study area, their similar seasonal patterns of occurrence suggest that they respond in a similar way to changes in environmental conditions.

Keywords: pollution, distribution, Cyprinodontiformes, population structure, Paraíba do Sul River.

Distribuição dos guppies *Poecilia reticulata* (Peters, 1860) e *Phalloceros caudimaculatus* (Hensel, 1868) ao longo de um trecho poluído do rio Paraíba do Sul, Brasil

Resumo

Peixes Cyprinodontiformes (guppies) são largamente distribuídos na região Neotropical e usam microhábitats deteriorados em rios onde poucas espécies podem ocorrer. Este estudo foi realizado em um trecho do rio Paraíba do Sul compreendendo um grande complexo urbano-industrial. O objetivo foi avaliar eventuais efeitos que o complexo industrial poderia ter na distribuição de duas espécies de guppies proximamente relacionadas, *Phalloceros caudimaculatus* e *Poecilia reticulata*. A área foi dividida em três zonas: Z1, 40 km rio acima do complexo urbano-industrial de Volta Redonda; Z2, imediatamente abaixo do complexo industrial; e Z3, 30 km abaixo do complexo. Seis locais (dois em cada zona) foram amostrados mensalmente entre novembro de 1998 e outubro de 1999, usando um esforço de pesca estandardizado com tarrafas, peneiras e arrastos, cobrindo diferentes microhábitats, isto é, corredeiras, remansos e a proximidade da margem dos rios. *Poecilia reticulata* foi largamente distribuído, apresentando pico na Z2, área mais poluída, enquanto *P. caudimaculatus* apresentou a maior abundância na Z3, sendo quase ausente na Z1. Ambas as espécies ocorreram em elevados números ao longo do ano, mas foram escassas entre abril e junho. Fêmeas foram mais numerosas do que machos para ambas as espécies na maioria das classes de tamanho e em todos os locais. Jovens foram mais abundantes que adultos, com fêmeas não grávidas predominando sobre fêmeas grávidas. Fator de condição foi sempre maior nos machos do que nas fêmeas, mas somente machos de *P. reticulata* apresentaram diferenças significantes entre as três zonas, com os mais elevados valores na Z2. Maior número de fêmeas confirma as expectativas de que estas espécies podem usar áreas bem poluídas e que a disponibilidade de alimento provida pela carga orgânica permite sua distribuição ao longo de toda a área. Embora estas duas espécies tenham apresentado indicações de separação especial, seus similares padrões de ocorrência sazonal sugerem que elas respondem de maneira similar às variações das condições ambientais.

Palavras-chave: poluição, distribuição, Cyprinodontiformes, estrutura de populações, rio Paraíba do Sul.

1. Introduction

Fishes of the Poecilidae family inhabit fresh and brackish waters (Nelson, 1994) and have been introduced widely and indiscriminately in many parts of the world as mosquito control agents. The Poeciliidae family contains about 200 species in 16 genera and is widely distributed occurring in America and Africa (Parenti, 1981). *Poecilia reticulata* is native from northwestern South America, while *Phalloceros caudimaculatus* is distributed from southeastern Brazil to northern Argentina (Bisazza, 1993).

These species are characterized by viviparity and present marked sexual dimorphism (Ringuelet and Aramburu, 1967) with females being larger than males. Fertilization is internal, and males show an anal modified fin with an intermittent organ (gonopodium). Although some species are piscivorous, the majority of this family is omnivorous, feeding on invertebrates, detritus, algae and vascular plants (Meffe and Snelson, 1989).

The Paraíba do Sul basin is a very important system in southeastern Brazil, and its waters are used for industrial and domestic purposes, hydroelectric power generation, and water supply for ca. 8,000,000 people in Rio de Janeiro City (Silveira et al., 1979; Barroso, 1989). The river receives many organic and industrial effluents that affect directly the ichthyofauna (Coelho and Fonseca, 1986), especially in its middle reaches, where the major siderurgic-industrial complex is located at Volta Redonda City (Araújo, 1983; Negreiros and Wainsztain, 1978; Pinto and Araújo, 2007). Organic loads brought into these stretches are estimated in ca. 126,550 kg DBO_5/day (Bizerril et al., 1998). Urbanization, industrialization and agriculture uses are the major causes of water and habitat deterioration contributing to increase loads of untreated effluents into the river (Amorin et al., 1983, Pinto et al., 2006a).

Studies on fish population distribution in deteriorated systems can give some clues about the environmental water quality. In this scope, *P. reticulata* and *P. caudimaculatus* have been reported as indicators of poor water condition due to their ability to thrive in harsh environmental conditions (Araújo, 1983). Both species have been recorded along the Paraíba do Sul River mainly in more polluted stretches (Araújo et al., 2001; Teixeira et al., 2005; Pinto et al., 2006b; Pinto and Araújo, 2007). On the other hand, fish condition indicates health and can be influenced by environmental conditions (Vila-Gispert and Moreno-Amichi, 2001). Therefore, it seems reasonable to consider fish condition as an integrative measure of environmental conditions. This study investigates the distribution of these two species along a very polluted stretch of the Paraíba do Sul River. We compared sex rate, life stage and condition factor of fishes occupying three zones along the pollution gradient.

2. Methods

The Paraíba do Sul River basin area is 57,000 km^2 in southeastern Brazil, located between two major industrial Brazilian centres, Rio de Janeiro and São Paulo. It is 1080 km long and its watershed (Figure 1) is located between parallels 20° 26' and 23° 38' south and meridians 41° 00' and 46° 30' W. The study reaches is 70 km long, between the cities of Resende and Barra do Piraí in a single ecoregion. The ecoregion is characterized by low mountains, low nutrient soils characterized by unconsolidated and semi consolidated sand, gravel, silt and clay with basalt outcroppings, semi-deciduous seasonal rain forest with some Atlantic forest fragments and poor cropland areas. Typical winter and summer flows are 109 m^3/s and 950 m^3/s, respectively, and the mean annual flow in the study area is 318 m^3/s at 370 m above the sea level (Barroso, 1989). Rainfall ranges 1000-3000 mm annual precipitation.

The Paraíba do Sul River receives high amounts of organic and industrial effluents that directly affect the ichthyofauna by decreasing fish abundance and limiting distribution of more sensitive species (Araújo et al., 2003; Pinto et al., 2006b; Pinto and Araújo, 2007). An amount of 160 m^3/s is pumped from the study area for hydroelectric power and water supply purposes, increasing water quality concerns. Effects of habitat and water deterioration have been described by Pfeiffer et al., 1986; Carvalho and Torres, 2002; Pinto et al., 2006b, who reported large numbers of textile, chemical and food industries, and the presence of the major siderurgic industrial plant at Volta Redonda as the main source of alteration of the system. Additionally, pesticides and herbicides form agricultural activities contribute to add diffuse pollution over the extent of the river, with negative effects on the water quality (Radambrasil, 1983). According to the Rio de Janeiro Environmental Protection Agency (Feema, 1999), the Paraíba do Sul River stretch near the major industrial plant of Volta Redonda is the most used and adulterated stretch of the whole system, being classified as the most polluted. Azcue et al. (1988) consider this stretch as very polluted with heavy metals due to industrial effluents from metallurgy.

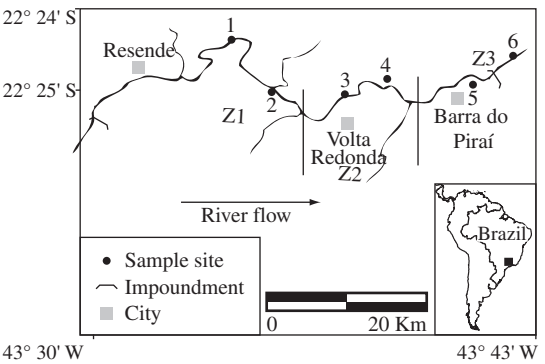

Figure 1. Study area, Paraíba do Sul River, with indications of the three studied zones and the six sampling sites.

The study area was divided into three zones: Z1 (Barra Mansa), 40 km upriver of the major urban-industrial complex of Volta Redonda; Z2 (Volta Redonda), just downriver of the complex; and Z3 (Barra do Pirai), 30 km downriver of the complex (Figure 1). Fishes were systemically collected in these three zones from November 1998 to October 1999. In each zone, two sampling sites were chosen each month. Fishes were sampled with seine nets (10 x 2 m; 5 mm mesh size), mesh trays (80 cm diameter with 1 mm mesh) and cast nets (4 m in diameter with 2 cm mesh) and seines (10 m x 2 m with 5 mm mesh). Fishing effort was standardized and the sampling unit was defined as the pooled total number of fish collected in 15 cast net throws, 15 mesh trays lifts and 1 seine haul in each site, and covering each microhabitat, that is, riffles, pools and the proximity of the river's margin.

All fishes were preserved in 10% formalin in the field, and transferred to 70% ethanol after 48 hours. Measurements were performed to total length (TL) to the nearest mm and to total weight (TW) to the nearest 0.1 g. Separation between juveniles and adults for both species were according to Aranha and Caramaschi (1999) who stated that specimens lower than 20 mm TL that did not show indication of gonopodium development or reproductive activity were assigned as juveniles. All females were examined for sexual maturation, and classified as pregnant if they had an extended coelomatic cavity due to presence of eggs or embryos, and not pregnant if they did not present these characteristics.

Sex ratio was tested for significant differences using the Chi-square test ($\alpha < 0.05$) for each species, by month, zone and size class. Mass-weight relationship equations were determined by ordinary least square, on $\log_{10}$ of the transformed raw data. The slope regression was tested for departure from 3 (isometry) by using the *t*-Student test. The condition factor was calculated according to the equation, $k = W/(L^b)$, where W is the wet weight of the fish, and L is the total length. One-way Analysis of Variance – ANOVA was used to compare conditions among zones at $P < 0.05$ (Zar, 1984).

3. Results

A total of 857 individuals of *P. reticulata* (670 females and 187 males), and 175 *P. caudimaculatus* (122 females and 53 males) were examined (Table 1). *P. reticulata* was more frequent at Z2 and *P. caudimaculatus* at Z3. Only one individual of *P. caudimaculatus* for each sex was recorded at Z1.

P. caudimaculatus females ranged from 7 to 41 mm TL while males ranged from 11 to 28 mm TL (Figure 2). *P. reticulata* females ranged from 5 to 37 mm TL, while males ranged from 8 to 25 mm TL (Figure 2). Females outnumbered males for both species in all size class, with the exception for 20-22 mm TL. The number of individuals per size class presented a more even distribution for females than for males that predominated as adults.

Females of both species outnumbered males throughout the year, with the exception of November for *P. caudimaculatus* (Figure 3). Paucity in abundance for both species was recorded from April to June. The highest abundance of *P. reticulata* was recorded in November 1998, and July/September 1999, while for *P. caudimaculatus*, in January, and August/October 1999.

Juvenile versus adult ratio changed among zones for each species. Adults of *P. reticulata* predominated at Z1, while juveniles at Z2 and Z3 (Table 2). On the other hand, juveniles *P. caudimaculatus* were more abundant at Z2, while adults at Z3. Non-pregnant females outnumbered pregnant females in all three zones for both species. The highest proportion of pregnant females (33.3%) was recorded for *P. caudimaculatus* at Z2, and 20% for *P. reticulata* at Z1 (Table 2).

Parameters of the mass-length relationship equation for *P. reticulata* and *P. caudimaculatus* are shown in Table 3. Positive allometry ($p < 0.01$) was recorded for females of both species. Negative allometry ($p < 0.01$) was found for *P. reticulata* males, while isometry ($p > 0.01$) was found for *P. caudimaculatus* males. Condition for males was higher than for females for all three zones (Table 4). Overall there are no changes in fish condition according to zone, with exception for males *P. reticulata*, which presented the highest values at Z2.

4. Discussion

High abundances of *P. reticulata* recorded in the most impacted zone of the Paraíba do Sul River (Z2) and *P. caudimaculatus* in Z3 confirms the expectation that they are very opportunistic species, which colonize poor quality habitats that are inadequate for most fish spe-

Table 1. Chi-square test for comparisons of species of Cyprinodontiformes sex ratio in three zones of the Paraíba do Sul River, between November/1998 and October/1999.

Species	*P. reticulata*			*P. caudimaculatus*			Total	
	Z1	Z2	Z3	Z1	Z2	Z3	*P. reticulata*	*P. caudimaculatus*
Females	25	485	160	1	6	115	670	122
Males	7	138	42	1	3	49	187	53
Total	32	623	202	2	9	164	857	175
Chi-Square	10.12**	193.3**	69.0**	0 ns	1.0 ns	26.5**	136.1**	27.2**

Significance level: **P < 0.01; *P < 0.05; ns = non-significant.

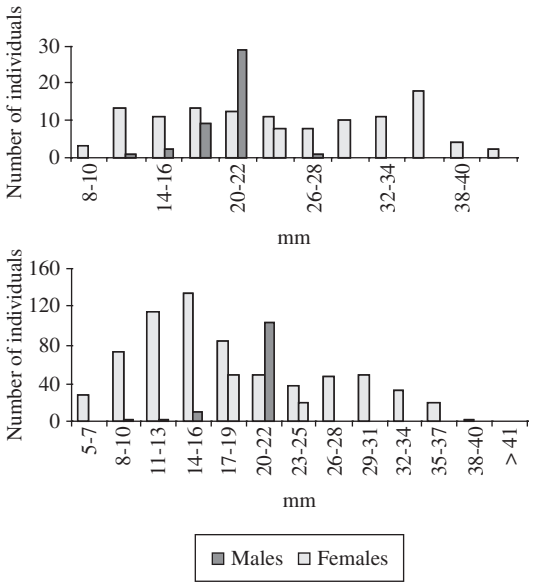

Figure 2. Length-frequency distribution (in mm) for *P. caudimaculatus* (above) and *P. reticulata* (below) in the Paraíba do Sul River from November 1998 to October 1999.

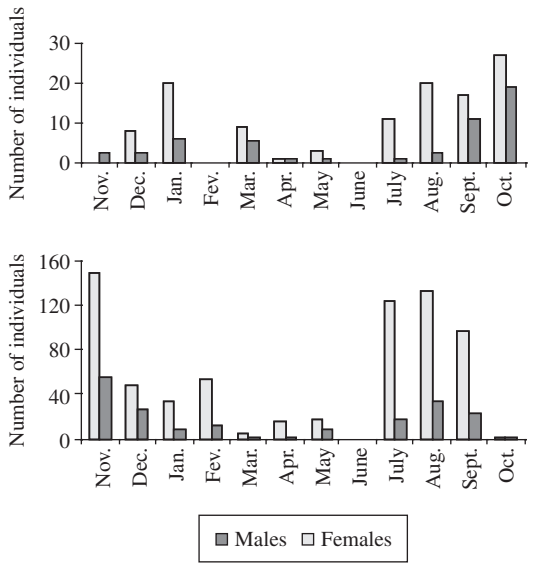

Figure 3. Monthly relative abundance for *P. caudimaculatus* (above) and *P. reticulata* (below) in Paraíba do Sul River, from November 1998 to October 1999.

cies. Fishes of the Cyprinodontiformes order are widely known for their capacity to tolerate low water quality where a large number of species have limited distribution (Araújo et al., 2003). Menezes and Caramaschi (1994) reported a long reproductive period for this group of fishes, and attributed this strategy as a biological response to undergo instable environmental constraints, where the continuous reposition of the juveniles constitutes a mechanism to maintain and reestablish the structure of populations, and this seems to be a characteristic of the whole group. Similar distribution patterns for these two species were also found by Araújo et al. (2001), with *P. reticulata* being the most abundant fish species at Z2, while *P. caudimaculatus*, ranked as the third most abundant species at Z3. The use of deteriorated areas by opportunistic fish species has been reported by several workers (Hughes and Gammon, 1987; Soto-Galera et al., 1998; Harris and Silveira, 1999; Pinto et al., 2006b; Pinto and Araújo, 2007). They have reported that the limitation of natural resources decreased specialist species which are replaced by opportunistic ones, since the latter can use the available resources and cope with the harsh environmental conditions of deteriorated areas.

The reason for this differentiated distribution found for these two studied species is difficult to explain and more detailed tests including bioassays are needed in order to have a better insight into the influence of water quality on species distribution. Hitherto, an indication of negative interaction between *Phalloceros* and *Poecilia* was reported by Aranha and Caramaschi (1999) who suggested that sympatry of *Phalloceros caudimaculatus* and *Poecilia vivipara* resulted in prejudice for the first species, since both species have similar diets. It is probable that abundant food availability at Z2 due to organic loads from municipality effluents enable the occurrence of *P. reticulata* in high numbers.

Similar seasonal variation in fish abundance was detected for both species with low numbers between April and June, when temperature and water levels are decreasing. This similar temporal variation in relative abundance suggests that both species respond in a similar way to changes in environmental parameters. During the dry period, shelter in rivers margins provided by riparian vegetation is poor, and this could also have an influence on fish occurrence. Machado et al. (2002) found that microhabitat availability, food supply and warmer temperatures may provide optimal conditions for growth of juvenile *P. caudimaculatus* in the warm season, and this coincides with the results of this work.

Females outnumbered males in all three zones as have been reported for this group of fish by several authors (Parenti, 1981; Aranha and Caramashi, 1999). Reasons for that are rather unknown. The majority of fish populations are expected to keep in equilibrium their sex rate 1:1 since the probability of occurrence for each sex should be 50%. When this trend is not followed, one should take into account that some factor is modifying this equilibrium. Henn (1916) attributed to selective predation changes in the balance between males and females, decreasing the number of males (Krumholz, 1948) or females (Britton and Moser, 1982). Narahara et al. (1985) reported that sexual proportion can be related to size of the individuals. Small sized males could be more likely to be prey for other fish species than females. Additionally, differentiation in growth rate between the sexes can cause this unbalanced proportion since the

Table 2. Percentage of juveniles and adults, pregnant and non-pregnant females *P. reticulata* and *P. caudimaculatus* in three zones of Paraíba do Sul River, between November/1998 and October/1999.

Species		Zones		
		Z1	Z2	Z3
P. reticulata	Juveniles	46.8	56.8	63.4
	Adults	53.2	43.2	36.6
	Pregnant females	20	10.8	8.75
	Non-pregnant females	80	89.2	91.25
P. caudimaculatus	Juveniles	100.0	66.6	29.1
	Adults	0	33.4	70.9
	Pregnant females	0	33.4	24.7
	Non-pregnant females	100.0	66.6	75.3

Table 3. Parameters of regression analysis (a, b) and correlation coefficients (r) from log-transformed mass-length relationship of species of Cyprinodontiformes in the Paraiba do Sul River.

Species	n	B (slope)	a (y-intercept)	r	Mean TL ± S.E. (mm)
P. reticulata					
Males	187	2.46*	–4.70	0.80	19.92 ± 0.179
Females	670	3.21*	–5.04	0.96	18.28 ± 0.303
P. caudimaculatus					
Males	53	2.87 ns	–5.01	0.91	20.24 ± 0.396
Females	122	3.18 *	–5.28	0.95	24.34 ± 0.805

*Significant departure from 3 (*t*-Student test) indicated at $P < 0.05$; ns = non-significant; n = number of individuals; S.E. = standard error.

sex that present faster growth rate will undergo the most vulnerable phase of smaller size quickly, and, therefore, diminish the predation proportion. On the other hand, the sex with slower growth rate will be more likely to undergo predation, and its abundance decreas disproportionally in the next development phases. Although aspects of growth rate were not being investigated in this study, it would be possible that females present higher growth rate than males, as indicated by their larger sizes.

Another factor that could influence sex rate is food availability. Nikolsky (1969) reported that when there is plenty of food available, females predominate over males, with the inverse situation in regions where food is limited. Feeding activity, in this case, would be influencing the metabolism through hormonal activity, resulting in changes in individual production in a given sex. Some workers (Bisazza et al., 2000; Garcia et al., 2004) reported that females require better environmental conditions than males, suffering in its development when environmental conditions are deteriorated. Moreover, there is still certain debate about which factors drive the marked sexual dimorphism in size (females being larger than males), the larger number of females in relation to males and the amplitude of reproductive season for this species. Both species are omnivorous, feeding mostly on detritus, algae, invertebrates and vascular plants. In rivers with feeding resources shortage, males outnumber females, suggesting that sexual rate could change. In this work, despite the high deterioration of the stretch of river, females were far more abundant than males, and this suggests that the environment is suitable for these species, with no evident signals of stress or food resources scarcity.

Females of both species were more evenly distributed over the size classes, while males were restricted to narrower size classes, although signals of decreasing abundances in adults classes were shown for *P. reticulata*. Aranha and Caramaschi (1999) defined adults as individuals presenting size larger than 20 mm TL. The proportion among the age groups in a given population determines the reproductive status and is an indication of the future trend in this population. Overall, an increasing fish population presents a high number of juveniles compared to a stable population, which contains a more even number of individuals among the age groups, while a decreasing population contains more adults. In the present work, the size structure for both species is an indication of stable populations of Cyprinodontiformes in the Paraíba do Sul River.

Different levels of predation per size group could also contribute to unbalanced abundance among size class. Reznick et al. (1996) reported that *Poecilia reticulata* under intense predation shows a lower reproductive size when compared with another situation when predation is

Table 4. Means ± standard error and F-values from ANOVA for *P. reticulata* and *P. caudimaculatus* condition factor comparisons among the three zones of the Paraíba do Sul River, between November/1998 and October/1999.

Species/sex	Zones			Anova	
	Z1	Z2	Z3	F	P-values
P. reticulata					
Females	6.1×10^{-6} (3×10^{-7})	7.1×10^{-6} (2.9×10^{-7})	6.3×10^{-6} (3.8×10^{-7})	1.22	0.296
Males	2.8×10^{-5} (4.4×10^{-6})	3.1×10^{-5} (7×10^{-7})	2.8×10^{-5} (1.1×10^{-6})	5.11*	0.007
P. caudimaculatus					
Females	4.1×10^{-6} (0)	7.2×10^{-6} (1.1×10^{-6})	6.1×10^{-6} (2.4×10^{-7})	0.84	0.435
Males	1.3×10^{-5} (0)	1.1×10^{-5} (4.6×10^{-7})	1.1×10^{-5} (4.0×10^{-7})	0.18	0.835

not so intense, and that predation occurs in a differentiated manner on different sized groups. This hypothesis is not corroborated in the case of the Paraíba do Sul River, especially for the females *P. caudimaculatus*, which present an even abundance pattern over size. Additionally, the number of non-pregnant females was always higher than the number of pregnant females, and this proportion was more unbalanced for *P. reticulata*, indicating different reproductive strategies between these two species. The existence of an asymmetric predation between genders due to peculiar reproductive behavior of this species was hypothesized by Bisazza et al. (2000) who reported males as less careful (compared with females) regarding avoiding predator attacks by piscivorous fishes and birds. This differential behavior between genders could result in a higher male predation rate, resulting in larger numbers of females in the population (Magurran and Nowak, 1991, Magurran and Seghers, 1994).

Positive allometry ($p < 0.01$) was found for females of both species, negative allometry ($p < 0.01$) for *P. reticulata* males and isometry ($p > 0.01$) for *P. caudimaculatus* males. This pattern is associated to greater height in females, due to their capacity to carry eggs and embryons in their coelomic cavity (viviparity). *P. reticulata* males show a "thinner" form throughout their ontongenetic development, while *P. caudimaculatus* males show uniformity in shape as they reach a larger size.

Overall condition factor did not change between sites. The only exception was found for males *P. reticulata* that presented the highest condition at Z2, when compared to the others two zones. Environments in which fishes undergo large and often erratic physical and chemical changes can potentially cause stressful or lethal conditions for resident biota (Martin-Smith, 1998, Meador and Goldestein, 2003), but Z2 did not seem to act in such a way irrespective of the proximity of the urban-industrial complex. The two studied species probably have behavioral and physiological adaptation to deal with such harsh conditions. For example, some species can increase their maximum thermal tolerance (Matthews and Maness, 1979), while others seek out cooler areas within pools (Mundahl, 1990). Additionally, riparian vegetation, local microhabitat structure could influence conditions in these areas, acting as food sources and shelter.

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