



The influence of black spot disease on the relative condition factor of *Astyanax paranae* Eigenmann, 1914 (Characiformes: Characidae) in Brazilian subtropical streams

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ABSTRACT. Environmental disturbances and their consequences require constant studies to understand how communities and their ecological relationships respond to these processes. Through analysis of the host-parasite relationships, it is observed that the effect of these disturbances is variable and can change the physiology or behavior of organisms. Black spot disease, caused by endoparasitic helminths, is a pathology observed in natural environments, however, there is not much information about the consequences of this infestation. We separated the specimens from each stream into parasitized and non-parasitized groups, which were subjected to biometric analysis. The biometrics involved cysts count and weight-length measures, which were used to analyze the average relative condition factor. Additionally, we correlate these measures with the parasitic burden of infected individuals. Finally, the parasitized individuals were submitted to histological sections to recognize the parasite. The results demonstrate a low physiological condition in the parasitized group, when compared with non-parasitized groups from the same stream and from different streams. This suggests that pollution, in addition to effects of infestation worsen the fish condition. Besides, the parasite burden was negatively correlated with the condition factor, weight and length measures. We conclude that the parasite burden negatively affects *Astyanax paranae* individuals' physiological condition and that trematodes also occur in polluted environments.

Keyword: endoparasite helminths, healthiness, biology, ecology, parasitology.

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Introduction

Natural ecosystems have been negatively impacted in recent decades due to the combined effects of urbanization and other anthropogenic activities resulting from uncontrolled population growth. Many aquatic environments, such as rivers, lakes and reservoirs, which provide essential services to humanity, have undergone a series of changes due to these process (König, Suzin, Restello, & Hepp, 2008).

Human activities' main adverse effects cause silting and homogenization of rivers and streams beds, decreased diversity, and increased artificial eutrophication in aquatic ecosystems, therefore resulting in environmental quality loss (Callisto, Ferreira, Moreno, Goulart, & Petrucio, 2002). Besides, the imbalances caused by these changes extend to different organizational levels in the impacted ecosystem.

The consequences of these disturbances, and their cumulative impacts on biota, generate the need to understand how communities and their interactive relations respond to these processes. In a host-parasite relationship, for example, the effects of stress are variable, and can change the physiology or behavior of organisms (Flores-Lopes & Thomaz, 2011). In this context, the introduction of pollutants in aquatic environments can favor the spread of parasites by excluding natural enemies, reducing host resistance and, consequently, causing damage to organisms' health (Poulin & Morand, 2005; Carvalho, Azevedo, Abdallah, & Luque, 2012).

Endoparasitic helminths, called digenetic trematodes, are known to cause black spot disease in fish. In these parasites' life cycle, which passes through gastropods, fish, birds, or mammals, fish are intermediate

hosts, carriers of metacercaria cysts (Niewiadomska, 2002; Flores-Lopes & Thomaz, 2011). As a response to the infestation, a connective tissue capsule containing melanophores is formed, which characterizes the black spots on the fish's body surface (Flores-Lopes & Thomaz, 2011).

The knowledge about the consequence of this parasitism on the hosts' physiological condition is scarce. Studies in this field are still recent and require additional research to promote understanding of the complex mechanisms of the host-parasite relationship and the environment in which they are contained (Flores-Lopes & Thomaz, 2011).

Thus, to assess the impact of black spot disease on fish health, our study aimed to evaluate the physiological condition of parasitized and non-parasitized groups of the species *Astyanax paranae* (Eigenmann, 1914) in streams with different degrees of disorders. We hypothesize that the parasite burden negatively affects the relative condition factor of this fish species.

Material and methods

The analyzed specimens came from affluent streams of the Monjolinho River, which is located in the north-central portion of the state of São Paulo (between the geographical coordinates 21° 57' - 22° 06' S and 47° 50' - 48° 05' W). This area is part of the 6th Water Resources Management Unit (UGRHi) in the state of São Paulo, located in the 13th Hydrographic Basin, called Tietê/Jacaré. It covers an area of approximately 275 km². The greater part of this basin is inserted in the district of São Carlos city. The city is located in an area of accelerated urban development and, therefore, of considerable anthropogenic impact (Espíndola, 2000).

The streams' samples were previously evaluated by Barrilli, Rocha, Negreiros, and Verani (2015) and, therefore, we reused their data for environmental classification purposes (Table 1).

Table 1. Environmental classification of the sampled streams.

Streams	RAP	TSI
Canchim (C)	Altered	Mesotrophic
Espraiado (E)	Preserved	Oligotrophic
Douradinho (D)	Altered	Mesotrophic
Ponte de Tábua (P)	Altered	Mesotrophic

RAP = Rapid Assessment Protocol; TSI = Trophic State Index.

The sampling was conducted in the months of July and August 2013: a metal wire sieve mesh (diameter = 0.75 m, mesh = 3 mm) and a net (0.50 m in diameter) were used to catch the fish fauna, with a sampling effort of one hour in each stretch (approximately 50 meters long). The specimens were fixed in 10% formalin and preserved in 70% alcohol for later identification and biometrics analyses. After taking the biometric measurements (weight and length), each fixed specimen was evaluated for the presence of metacercaria cysts, which were quantified. Some cysts were removed and processed histologically. The histological processing followed the standard methodology, including paraffin, 5 µm thick sections, and Hematoxylin and Eosin staining.

The data for weight and standard length obtained from individuals of *A. paranae* were used to calculate the length-weight relationship (Le cren, 1951). With the estimated values of coefficients -a- and -b-, the theoretically expected weights (We) for the respective values of standard length (Ls) were calculated. The relative condition factor (Kn) values were calculated by the equation $Kn = Wo/We$ for individuals sampled in each stream. Subsequently, the Kn means were compared using the nonparametric Kruskal-Wallis test (complemented by Dunn's test) at a significance level of 95% (Zar, 2010).

To test the hypothesis that infected fish have a lower condition factor when compared to non-infected fish, those containing parasites were grouped and compared to the non-parasitic groups in their respective streams. Finally, the number of parasite cysts was correlated with the standard length, total weight and Kn of fish, using Spearman's correlation analysis (Zar, 2010). The analyzes were performed using the statistical software package PAST, version 3.16 (Hammer, Harper, & Ryan, 2001).

Results

A total of 182 specimens of *Astyanax paranae* were analyzed. Approximately 84.64% of the fish collected in the Ponte de Tábua stream had their body surfaces parasitized by metacercariae of the genus *Diplostomum* (Figure 1), which corresponded to an average of 6.88 ± 3.19 cysts per individual.

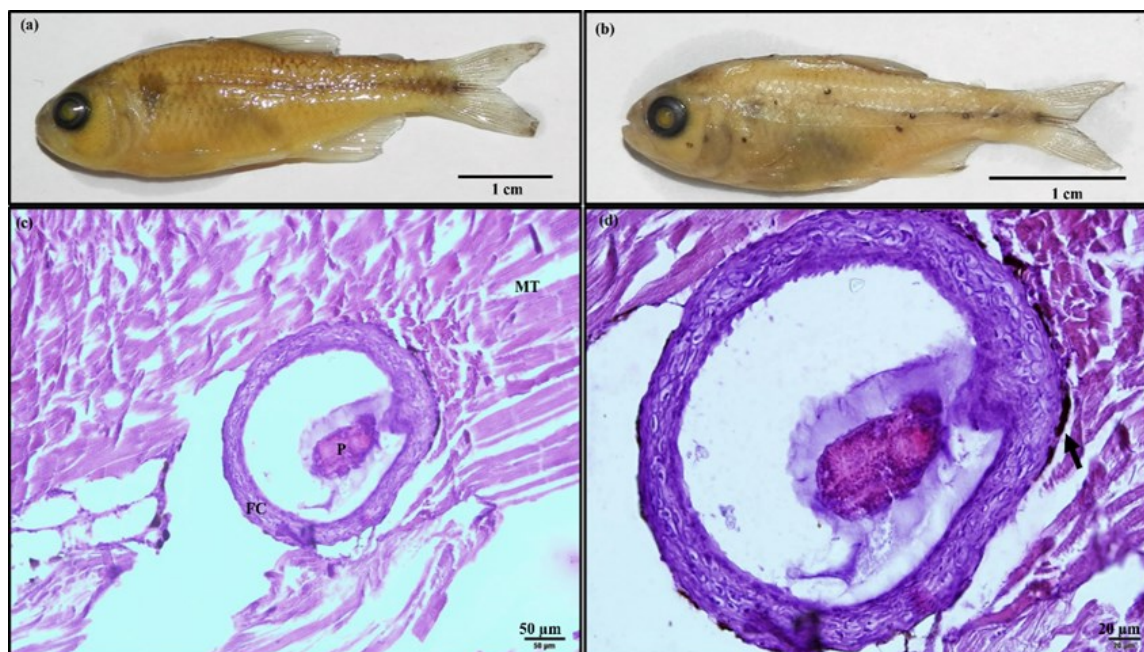


Figure 1. Black spots disease images. a) *Astyanax paranae* specimen not parasitized; b) specimen parasitized with metacercariae along the body (black spots); c) histological section showing the fibrous capsule (FC), Parasite (P) and muscle tissue (MT); d) Histological section of a metacercaria showing the melanophores (arrow).

Regarding the biometric data, the individuals of *A. paranae* varied between 1.60 to 7.45 cm (3.50 ± 1.20) in length, and from 0.07 to 14.23 grams in weight. The biometrics data for each stream are contained in Table 2.

Table 2. Biometrics data of *Astyanax paranae* between the sampled streams.

	Length (cm)			Weight (g)		
	Min	Max	Mean \pm (SD)	Min	Max	Mean \pm (SD)
C	2.40	6.81	4.32 ± 1.40	0.40	9.42	3.07 ± 2.87
D	2.02	7.45	3.51 ± 1.02	0.19	14.20	1.62 ± 2.18
E	1.74	5.69	2.87 ± 0.99	0.11	5.42	0.96 ± 1.02
P1	1.76	7.26	3.50 ± 1.31	0.12	12.00	1.75 ± 2.65
P2 (Parasitized)	1.60	6.40	4.28 ± 1.28	0.10	7.30	2.60 ± 2.24

Streams legend: C = Canchim, D = Douradinho, E = Espraiado, P1 = Ponte de Tábua (nonparasitized group) and P2 = Ponte de Tábua (Parasitized group). SD = standard deviation

According to the length-weight relationship (Figure 2), the species *A. paranae* has an allometric coefficient greater than three ($b = 3.17$), which means that its development occurs with a greater increase in the variable "weight" than in "length", featuring a positive allometric type growth.

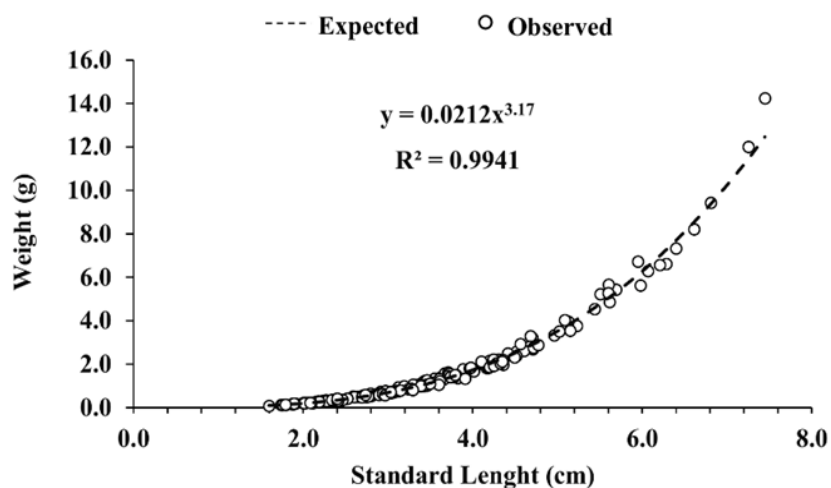


Figure 2. Length-weight relationship of *Astyanax paranae* from the sampled streams.

The averages of relative condition factor (Kn) calculated for each stream resulted in significant differences (Kruskal -Wallis; $H_c=51.36$, $p < 0.01$), in which the parasitized individuals of the P1 environment were the group with the lowest average Kn, when compared to the others (Figure 3). Among the non-parasitized groups, those belonging to the Espiraído, Canchim and Douradinho streams had higher Kn means than the groups belonging to the Ponte de Tábua stream, either from parasitized (P2) and non-parasitized (P1) groups.

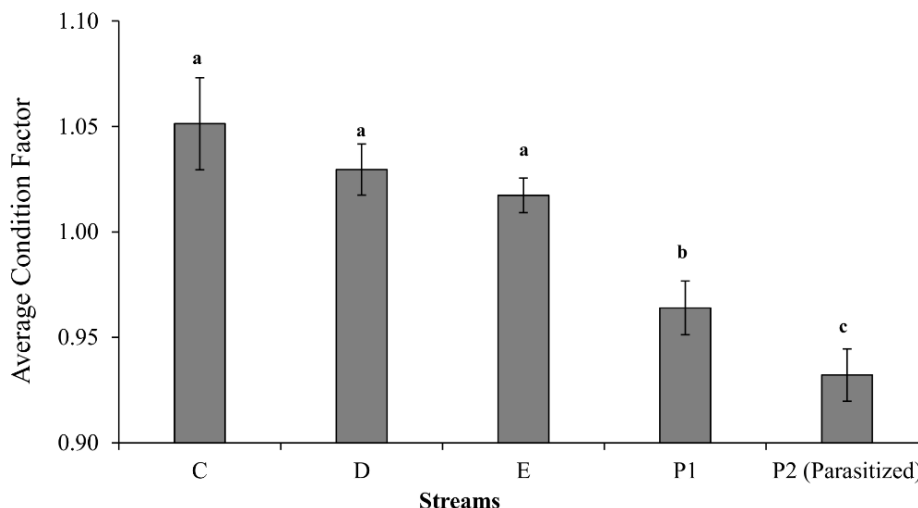


Figure 3. Mean and standard error values of the relative condition factor (Kn) of *Astyanax paranae* of the sampled streams: Espiraído (E), Canchim (C), Douradinho (D), Ponte de Tábua - (P1 = non parasitized; P2 = parasitized). Equal letters = no statistical differences for Dun's multiple comparison test, with a 95% significance level.

The data submitted to Spearman's analysis resulted in positive correlations between the number of trematode cysts, the standard length (Spearman, $p < 0.01$) and the total weight (Spearman, $p = 0.04$) of the *Astyanax paranae* species (Figure 4). However, the relative condition factor (Kn) resulted in a negative correlation with the number of cysts. (Spearman; $p < 0.01$).

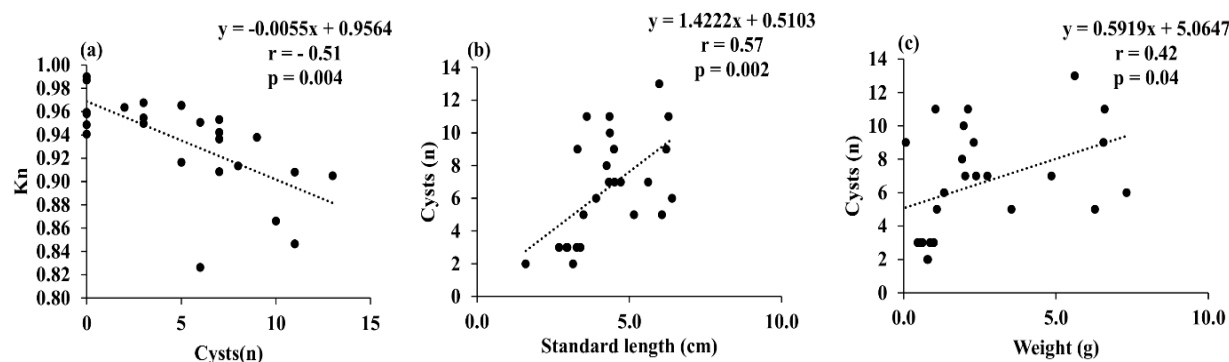


Figure 4. Correlation between the number of trematode cysts, the relative condition factor (a), the standard length (b), total weight (c) of *Astyanax paranae*. Legend: r = Spearman's correlation coefficient, p = p-value.

Discussion

Trematode metacercariae infestations are common in fish and have already been observed in many freshwater species in Brazil, resulting, in most cases, in severe injuries to the hosts (Yamada, Moreira, Ceschini, Takemoto, & Pavanelli, 2008; Flores-Lopes & Thomaz, 2011; Carvalho et al., 2012; Flores-Lopes, 2014).

Considering only the stream in which the trematodes occurred (Ponte de Tábua), most individuals (84.64%) of *A. paranae* were infested, similar to Flores-Lopes (2014) findings, which demonstrated a parasitic prevalence in all adults of the *Astyanax aff. fasciatus*. In our study, metacercariae were found throughout the entire body extension, including in juveniles of *Astyanax paranae* species.

Although the prevalence of infection for a species can be high, it can vary between different populations of the same system without a defined pattern, suggesting that the infection can occur in any fish species and at any time of the year (Ondračková, Jurajda, & Gelnar, 2002; Quist, Bower, & Hubert, 2007; Flores-Lopes, 2014).

The occurrence of trematodes in the Ponte de Tábua stream opposes the hypothesis proposed by Flores-Lopes & Thomaz (2011), which suggested that these parasites' high frequency may indicate environments with better environmental quality. In our study, the physical habitat and the stream's water where the parasites occurred were classified as low quality environments, due to anthropic origin disturbances (e.g. sewage discharge, burning, deforestation and earthworks) that take place on the site. In addition, a high rate of infestation has already been demonstrated by Galli, Crosa, Mariniello, Ortis, and D'Amelio (2001) in places with moderate levels of pollution, corroborating our study results.

Both groups of individuals (parasitized and non-parasitized) sampled in the Ponte de Tábua had a low average value in the relative condition factor (Kn) when compared to the other streams. This suggests that disturbed environments may also contribute to the establishment of parasites. Even though some fish may benefit momentarily from moderate levels of pollution, due to an increase in productivity, the intensification of pollution may compromise their physiological health (Barrilli et al., 2015). Thus, the lower average for Kn value of the parasitized group, when compared to the non-parasitized group of the same stream (Ponte de Tábua) suggests that the combined effect of pollution and parasitism contributes even to a lower condition factor of individuals *A. paranae*.

According to Poulin (1992), pollutants released into the aquatic environment can directly affect the fish's immune system, reducing the body's ability to react to parasitic action. Furthermore, some authors associate high infection of black spot disease with growth retardation, weight loss and high mortality of infected young fish, which could be a regulatory factor for the host population (Harrison & Hadley, 1982; Lemly & Esch, 1985). In this sense, the parasites that present little aggressiveness in their hosts' organism indicate a long-established association from the evolutionary point of view and, therefore, balanced, resulting in a gradual reduction of parasite virulence (Giorgio, 1995).

Our analyzes showed a negative correlation between the parasite burden and the Kn of the evaluated fish, which suggests that the increase in infection compromises the host's health, and that the parasitic relationship in the studied environment is still recent. Additionally, there was also a positive correlation of size and body weight with the number of cysts on the fish's body surface, indicating a more significant infestation in larger individuals.

Conclusion

We conclude that the parasite burden negatively affects individuals' physiological condition of *Astyanax paranae* species, and that the occurrence of trematodes is also associated with polluted environments. Studies involving parasitic infestation in environments are scarce and necessary to understand the biology of the species involved, the history and the consequences of this type of interaction.

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