

# Infection levels of *Austrodiplostomum compactum* (Digenea, Diplostomidae) metacercariae in *Plagioscion squamosissimus* (Teleostei, Sciaenidae) from the Nova Avanhandava reservoir, São Paulo State, Brazil

J.V.K. Paes<sup>1†</sup>, E.D. Carvalho<sup>2‡</sup> and R.J. da Silva<sup>3\*</sup>

<sup>1</sup>Pós-graduação em Ciências Biológicas (Zoologia), Instituto de Biociências, Universidade Estadual Paulista (UNESP) – Botucatu, Distrito de Rubião Júnior s/nº, Botucatu, 18618-00 São Paulo Brazil:

<sup>2</sup>Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil; <sup>3</sup>Departamento de Parasitologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil

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## Abstract

This study was aimed at evaluating the infection levels of *Austrodiplostomum compactum* metacercariae in 378 specimens of *Plagioscion squamosissimus* ('corvina' or 'pescada branca') from the Nova Avanhandava reservoir, low Tietê River, São Paulo State, Brazil. High prevalence, mean intensity of infection and abundance were observed in *P. squamosissimus* during most of the study, with the exception of March 2004. The relative condition factor (Kn) did not differ between parasitized and non-parasitized fish. A statistically significant positive correlation was observed between host standard length and intensity of infection. The analysis of biotic and abiotic variables showed that no abiotic variable correlated with parasitic infection levels. Moreover, *P. squamosissimus* status was demonstrated to be unaffected by *A. compactum* metacercariae infection.

## Introduction

Digenetic trematodes of the genus *Austrodiplostomum* Szidat & Nani, 1951 have been reported infecting several vertebrate species (Yamaguti, 1971; Kohn *et al.*, 1995; Ramos-Ramos, 1995). The metacercariae of these parasites can infect the vitreous humour (Scholtz *et al.*, 1995; Silva-Souza, 1998; Amato *et al.*, 2001) and, more rarely, the brains of the fish (intermediate hosts)

(Ostrowski-Nunez, 1982; Silva-Souza, 1998; Amato *et al.*, 2001). The life cycle of this diplostomid is completed in the intestines of birds, such as 'biguá' *Phalacrocorax olivaceus* (Humboldt, 1805) (definitive host) (Rietschel & Werding, 1978) (Ostrowski-Nunez, 1982; Ramos-Ramos, 1995; Flowers *et al.*, 2004; Machado *et al.*, 2005). The genus *Austrodiplostomum* comprises 40 digenetic helminth species, including adults and metacercariae, and can be found in 125 host species (Eiras, 1994; Niewiadomska, 1996) distributed across all continents, but most commonly in Eurasia and North America, and occasionally in South America (Niewiadomska, 1996; Niewiadomska & Laskowski, 2002).

\*E-mail: reinaldo@ibb.unesp.br

†E-mail: jvkpaes@gmail.com

‡E-mail: carvalho@ibb.unesp.br

*Austrodiplostomum compactum* (Lutz, 1928) Dubois, 1970, is widely distributed in the Neotropical region, where several fish species from Brazilian freshwater rivers are parasitized by metacercariae, including: *Cichla ocellaris* Bloch & Schneider, 1801 ('tucunaré' or 'tucunaré açu'); *Cichla monoculus* Spix & Agassiz, 1831 (tucunaré or tucunaré açu); *Crenicichla britskii* Kullander, 1982 ('joaninha'); *Hoplias malabaricus* (Bloch, 1794) ('traíra' or 'lobó'); *Plagioscion squamosissimus* (Heckel, 1840) ('corvina' or 'pescada branca'); and *Satanoperca pappaterra* (Heckel, 1840) ('cará', 'acará' or 'porquinho') from the Itaipu reservoir and the Upper Paraná River floodplain, State of Paraná (Kohn *et al.*, 1995; Silva-Souza, 1998; Pavanelli *et al.*, 2000; Santos *et al.*, 2002; Machado *et al.*, 2005; Yamada *et al.*, 2007); *Geophagus brasiliensis* Quoy & Gaimard, 1824 (cará or acará) from Barra Bonita (middle Tietê River, State of São Paulo) (Novaes *et al.*, 2006); and *P. squamosissimus* from Nova Avanhandava, lower Tietê River, State of São Paulo (Paes *et al.*, 2003) and from the Volta Grande reservoir,

State of Minas Gerais (Martins *et al.*, 1999, 2002). In Venezuela, Cuba, Colombia and Mexico, other metacercariae of the genus *Diplostomum* have also been recorded infecting *Rhamdia quelen* (Quoy & Gaimard, 1824) ('bagre' or 'jundiá'), *Oreochromis aureus* (Steindachner, 1864) ('tilapia' or 'tilapia azul') and *Oreochromis mossambicus* (Peters, 1852) (tilapia or 'tilapia de mozambique') (Pérez-Ponce de Leon *et al.*, 1992; Garcia *et al.*, 1993; Aragort *et al.*, 1997; Aguirre-Macedo *et al.*, 2001).

*Plagioscion squamosissimus* is a widespread fish species from the Amazonian basin. Because of its economical importance, it was introduced in the Paraná basin, mainly in great reservoirs such as Itaipu, Promissão and Marimbondo (Torloni *et al.*, 1993; Moretto *et al.*, 2008), where it is now the major fish product in the south-east of Brazil (Graça & Pavanelli, 2007; Vidotto & Carvalho, 2007).

Considering the previous occurrence of *A. compactum* metacercariae in the Tietê River (Paes *et al.*, 2003), the aim

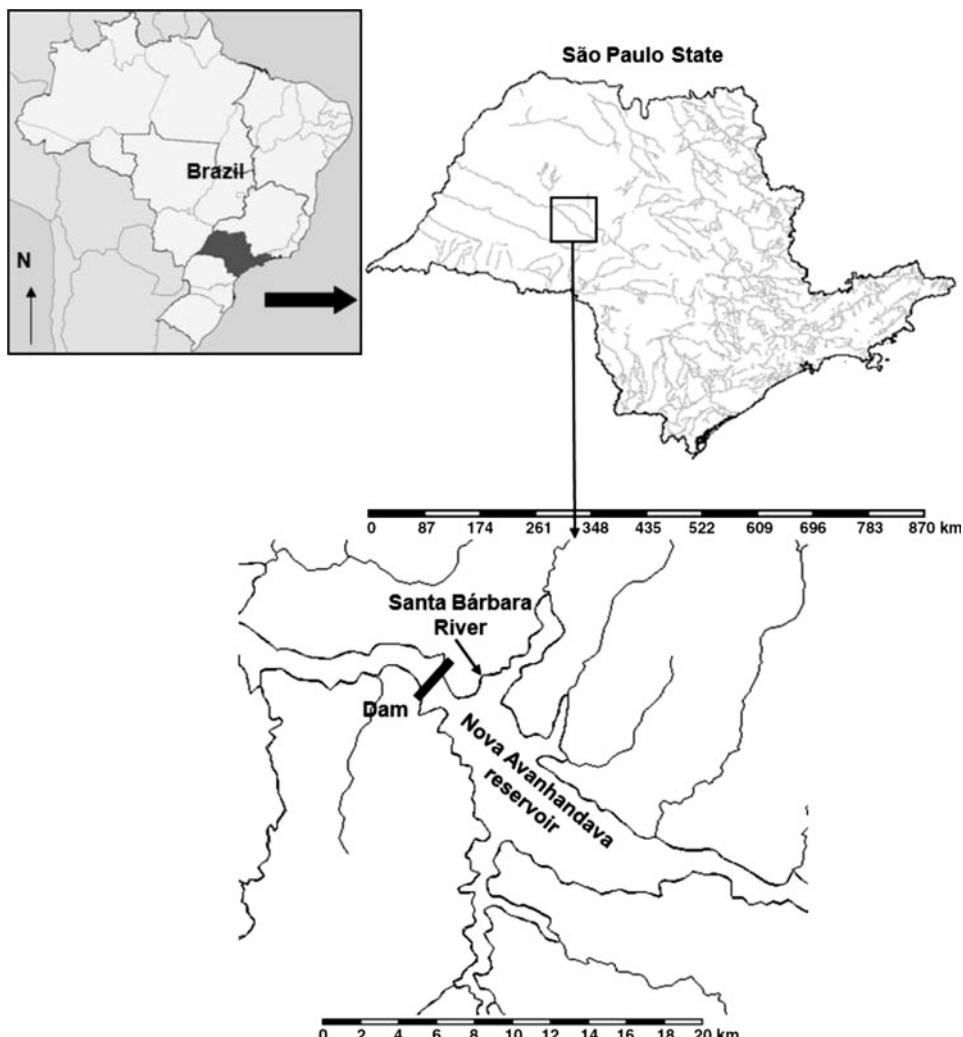


Fig. 1. Map of Brazil highlighting São Paulo State and the study area at the mouth of the Santa Bárbara River, in the Nova Avanhandava reservoir (lower Tietê River).

of this study was to determine the prevalence, intensity of infection, abundance, seasonality and host status of *A. compactum* metacercariae infection. In addition, the existence of possible correlations between biotic factors (population and parasitic) and some abiotic variables were assessed.

## Materials and methods

### Study area and parasitological procedures

This study was conducted in the Santa Bárbara River (municipality of Buritama, São Paulo State, Brazil), a major tributary of the Nova Avanhandava reservoir ( $21^{\circ}07'S$  and  $50^{\circ}17'W$ ), and the penultimate of the middle Tietê River cascade of reservoirs (fig. 1). Nova Avanhandava is the second largest hydroelectric plant in operation on the Tietê River, with an installed power capacity of 347.40 MW. The reservoir has an area of  $210\text{ km}^2$ , total water volume of  $2720 \times 10^6\text{ m}^3$ , mean discharge rate of  $688\text{ m}^3/\text{s}$ , mean depth of 13 m and water permanence time of 46 days (Torloni et al., 1993; CESP, 1998; Rodghe et al., 2002).

*Plagioscion squamosissimus* specimens were collected using nylon monofilament gill nets with mesh sizes of 3–14 cm (not opposite knot lengths) from six sites on the Santa Bárbara River. Nets were placed at 17.00 h and removed by 07.00 h the following day, total exposure 14 h, during the months of July, September, October and December of 2003 and February, March, April and June of 2004. All samples were collected from the same sites with the same network area (standardized effort =  $1200\text{ m}^2$  of nets per month). The total weight (in grams) and standard length (distance from the anterior extremity of the head to the end of the hipural bone, in centimetres) of each specimen were recorded.

Three to six hours after collection, the eyes of all individuals were analysed under a stereomicroscope. Prevalence (number of hosts infected with one or more individuals of a particular parasite species), mean intensity of infection (mean number of parasites of a particular species found in the infected hosts of a particular species) and mean abundance (total number

of individuals of a particular parasite species in a sample of a particular host divided by the total number of examined hosts of that species) were calculated according to Bush et al. (1997). Voucher helminths were deposited in the Coleção Helmintológica do Departamento de Parasitologia, Instituto de Biociências (CHIBB), Universidade Estadual Paulista, municipality of Botucatu, São Paulo State, Brazil.

### Data analysis

The relative condition factor ( $\text{Kn}$ ) was employed to determine the status of *P. squamosissimus* infected by *A. compactum* (Andrade-Talmelli et al., 1999; Dias et al., 2004; Yamada et al., 2008). Data on the weight and length of *P. squamosissimus* specimens were pooled to establish the species weight-length relationship (LeCren, 1951). A total of 377 specimens (non-parasitized = 20; parasitized = 357) were used to determine the parameters of the equation:

$$W_{\text{exp}} = L^b \times a,$$

where  $W_{\text{exp}}$  is the expected weight;  $L$  is the fish length;  $a$  is the intercept;  $b$  is the slope.

Host status was assessed using the 'relative condition factor' ( $\text{Kn}$ ), estimated by dividing the observed weight of an individual by the expected weight for a given length ( $\text{Kn} = W/W_{\text{exp}}$ ). The non-parametric Mann-Whitney statistical test was used to compare  $\text{Kn}$  between parasitized and non-parasitized fish. Intensity of infection in all samples was compared by the Kruskal-Wallis test, and multiple comparisons were performed by Dunn's method. Significance level was set at 5% in all statistical analyses.

Spearman's rank correlation coefficient ( $r_s$ ) was used to test the relationship between host length and intensity of infection (Abdallah et al., 2005) in order to check whether the infection process is cumulative (Takemoto et al., 2005). The distribution of mean intensity of infection according to host length in parasitized specimens was also evaluated (Sturges, 1926).

Table 1. Parasitic infection data and abiotic variables monitored by collection during the study period.

Date	CHIBB	Parasitic infection data						Abiotic variables					
		N	$N_i$	$N_m$	P	MII $\pm$ SE (R)	MA $\pm$ SE (R)	Pr	Tw	pH	K	T	DO
Jul 03	511	31	30	635	96.8	21 $\pm$ 5.4 (1–129)	20 $\pm$ 5.2 (0–129)	9.2	0.8	8.3	154.0	23.4	7.6
Sep 03	512	55	53	1161	96.4	22 $\pm$ 3.7 (1–167)	21 $\pm$ 3.7 (0–167)	15.1	0.9	8.5	163.4	22.6	8.5
Oct 03	520	53	50	1067	94.3	21 $\pm$ 3.7 (1–140)	20 $\pm$ 3.5 (0–140)	101.9	1.5	7.8	176.5	25.5	9.3
Dec 03	970	63	57	1124	90.5	20 $\pm$ 3.3 (1–159)	18 $\pm$ 3.2 (0–159)	1.0	1.2	8.5	149.7	29.0	7.8
Feb 04	459	75	73	1807	97.3	25 $\pm$ 3.6 (1–159)	24 $\pm$ 3.6 (0–159)	146.7	2.4	6.8	178.9	29.2	6.8
Mar 04	561	38	31	196	81.6	6 $\pm$ 1.3 (1–33)	5 $\pm$ 1.2 (0–33)	53.3	2.0	7.0	201.8	28.4	6.2
Apr 04	476	40	39	1275	97.5	33 $\pm$ 7.2 (1–232)	32 $\pm$ 7.1 (0–232)	116.6	2.4	7.2	203.2	27.2	6.1
Jun 04	485	23	23	455	100.0	20 $\pm$ 3.4 (1–55)	20 $\pm$ 3.4 (1–55)	39.6	1.4	8.1	204.2	22.4	9.0

CHIBB, Coleção Helmintológica do Departamento de Parasitologia, Instituto de Biociências, Universidade Estadual Paulista; N, number of collected specimens;  $N_i$ , number of parasitized specimens;  $N_m$ , total number of metacercariae; P, prevalence (%); MII, mean infection intensity; R, range; SE, standard error; MA, mean abundance; Pr, precipitation (mm); Tw, water transparency (m); K, conductivity ( $\mu\text{S}/\text{cm}$ ); T, water temperature ( $^{\circ}\text{C}$ ); DO, dissolved oxygen (mg/l).

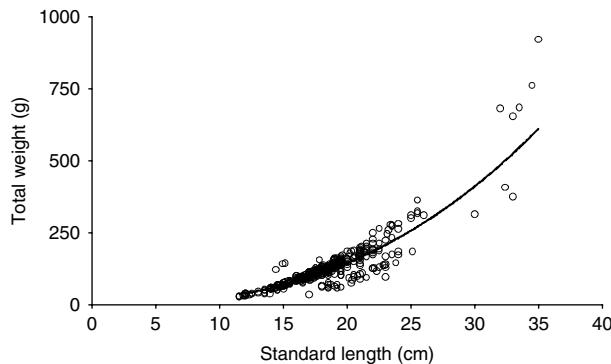


Fig. 2. Weight-length relationship in *Plagioscion squamosissimus* specimens ( $n = 377$ ) captured in the study area during the study period. Equation  $y = 0.0649x^{2.5732}$ ;  $R^2 = 0.8171$ .

Concomitantly, Spearman's correlation test was used to check the existence of correlations between parasitic infection levels and some abiotic variables, such as accumulated monthly precipitation (data provided by the AES Tietê Company), water transparency (measured with a Secchi disk) and pH, electrical conductivity, water temperature and dissolved oxygen, measured with Horiba U-22 (Paes, 2006).

## Results

A total of 378 *P. squamosissimus* specimens were analysed. The mean prevalence observed was  $94.3 \pm 2.1\%$ . The number of metacercariae collected ranged from 1 to 232 parasites. Mean intensity of infection and mean abundance were  $21.6 \pm 1.5$  and  $20.2 \pm 1.6$ , respectively (table 1).

During the study period, the prevalence of *A. compactum* metacercariae infection remained above 90%,

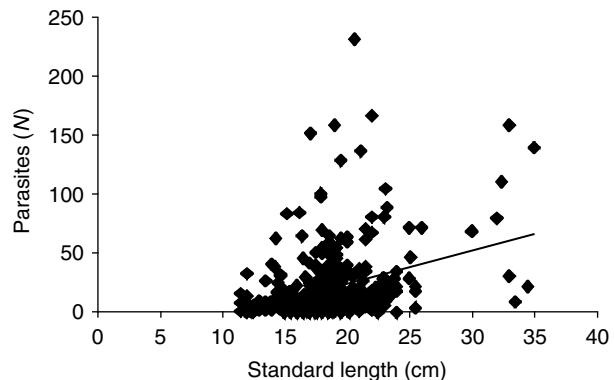


Fig. 4. Correlation between standard length and abundance of *Austrodiplostomum compactum* from the Nova Avanhandava reservoir, between July 2003 and June 2004. Linear regression  $y = 2.8292x - 32.004$ ;  $R^2 = 0.1201$ .

except in March 2004, when it was 81.6%. Mean intensity of infection and mean abundance also showed low variation, except in March 2004, when a significant reduction in these parameters was observed ( $P \leq 0.001$ ) (table 1).

Host fish length and weight were  $18.5 \pm 3.5$  (11.5–35) cm and  $131.4 \pm 91.8$  (27.7–920.6) g, respectively. The weight-length relationship (fig. 2) was established and  $K_n$  was calculated for parasitized and non-parasitized specimens (fig. 3). No significant difference in  $K_n$  was observed between parasitized fish ( $1.03 \pm 0.2$ ; 0.44–2.1) and non-parasitized fish ( $1.0 \pm 0.1$ ; 0.86–1.09;  $T = 2913$ ;  $P = 0.068$ ).

The number of parasites significantly correlated with host length ( $r_s = 0.32$ ,  $P < 0.001$ ) and host weight ( $r_s = 0.36$ ,  $P < 0.001$ ) (figs 4 and 5). Therefore, a greater intensity of infection (78 parasites/individual) was observed in the larger specimens ( $L_s = 26.6 \pm 31.5$  cm) (fig. 6).

The analysis of biotic and abiotic variables (table 1) revealed that no abiotic variable correlated with parasitic infection level ( $P > 0.05$ ).

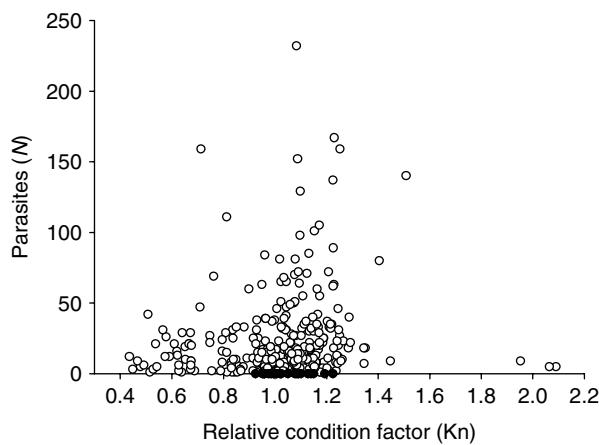


Fig. 3. Change in condition factor values ( $K_n$ ) in *Plagioscion squamosissimus* around the 1.0 default, according to the total weight of parasitized (white circle;  $n = 357$ ) and non-parasitized (black circle;  $n = 20$ ) specimens.

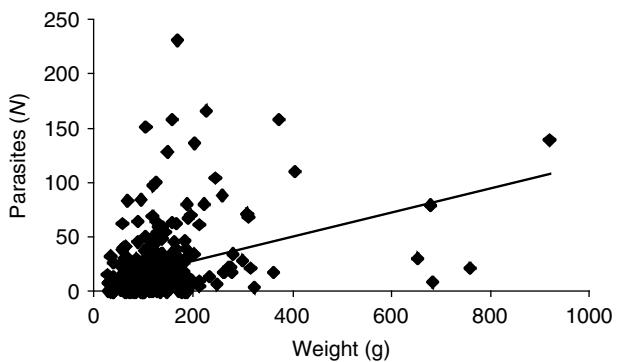


Fig. 5. Correlation between standard length and abundance of *Austrodiplostomum compactum* from the Nova Avanhandava reservoir, between July 2003 and June 2004. Linear regression  $y = 0.1114x + 5.8046$ ;  $R^2 = 0.1264$ .

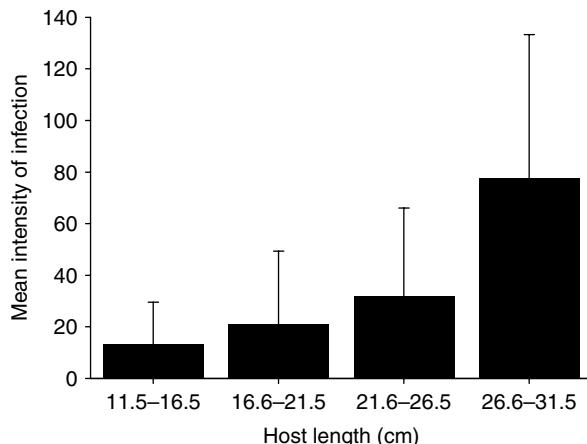


Fig. 6. Mean *Austrodiplostomum compactum* infection intensity and standard deviation in *Plagioscion squamosissimus* according to host length.

## Discussion

In this study, a high prevalence of *A. compactum* metacercariae infection in *P. squamosissimus* was observed. This fish species from the Amazon Basin was introduced in São Paulo Power Company (CESP) reservoirs between 1966 and 1973 (Torloni *et al.*, 1993). According to Pojmanska & Chabros (1993), the prevalence of diplostomids in allochthonous fish (species from other Neotropical basins) is higher than that of autochthonous fish (species from the Upper Paraná). This was also observed by Machado *et al.* (2005), who found higher rates of infection in the fish introduced into the floodplain of the upper Paraná River, where *P. squamosissimus* species showed the highest prevalence and intensity of infection. Thus, it is possible that *A. compactum* metacercariae utilize native fish as intermediate hosts, and that their adaptation to these native hosts is the explanation for the low prevalence and intensity of infection in these fish (Machado *et al.*, 2005).

According to Pavanello *et al.* (2000), the occurrence of *A. compactum* metacercariae in several fish species and in different aquatic ecosystems (rivers, lakes and dams) shows that while this trematode may have been introduced with *P. squamosissimus*, it appears to have found appropriate ecological conditions for its parasitic cycle because, in addition to fish (second intermediate hosts), it also found snails (first intermediate hosts) (Machado *et al.*, 2005; Ruiz & Aguilar, 2005) and birds (definitive host) (Silva-Souza, 1998; Machado *et al.*, 2005) in many of these ecosystems. Moreover, the low level of specificity of the metacercariae associated with the presence of intermediate hosts allows metacercariae to spread and infect other species of autochthonous fish. Specifically, in the Nova Avanhandava reservoir, the intermediate hosts, i.e. gastropod molluscs *Biomphalaria glabrata* (Say, 1818) and *Biomphalaria intermedia* (Paraense & Deslandes, 1962) (França *et al.*, 2007), as well as birds *Phalacrocorax brasiliianus* (Gmelin, 1789) (Branco, 2003) and those of the genus *Casmerodus* (J.V.K. Paes, pers. obs.) were also observed.

The analysis of *A. compactum* infection in *P. squamosissimus* showed that infection levels (prevalence, mean intensity of infection and abundance) were high during most parts of the study period, with the exception of March 2004. Dörücü & Ispir (2001), studying *Diplostomum* sp. metacercariae infection in *Acanthobrama marmid* Heckel, 1843, also noted a seasonal variation in the intensity of infection. Silva-Souza (1998) observed variation in monthly prevalence. However, rather than a clear seasonal pattern, the author found only a trend to higher *A. compactum* prevalence in *P. squamosissimus* in the driest months. These findings differ from those reported by Santos *et al.* (2002), who found a trend towards an increase in infection levels with the increase in precipitation and temperature.

The relative condition factor, however, did not differ significantly between parasitized and non-parasitized fish, indicating a good general fish status, irrespective of the presence of the parasite. In support of this hypothesis, studies on fish composition have shown that *P. squamosissimus* is dominant in number and biomass among the species recorded in Nova Avanhandava (Paes, 2006; Vidotto & Carvalho, 2007). This indicates that *A. compactum* metacercariae parasitism does not exert a substantially negative influence on the host population. Nonetheless, lower condition factors in *P. squamosissimus* individuals parasitized by *A. compactum* metacercariae as compared to non-parasitized fish were observed in the Tibagi River, Paraná State by Silva-Souza (1998).

An increase in food supply might be a compensatory factor that minimizes the negative effect of parasitism. Indeed, this may have occurred in Nova Avanhandava, where *P. squamosissimus* preferably consumed aquatic insects, especially Odonata and Ephemeroptera (Vidotto, 2005) and Libelulidae and Gomphidae (Ramos *et al.*, 2008), and shrimps (*Macrobrachium* sp.) (Vidotto, 2005; Ramos *et al.*, 2008), which are very abundant in the reservoirs located on the middle and lower Tietê River (Pereira *et al.*, 2002). In the Tibagi River, however, the main food item for this species was fish (*Astyanax altiparanae* Garutti & Britski, 2000 and *P. squamosissimus* young) (Bennemann & Shibatta, 2002), which require a greater effort (and tactics) to catch in comparison with shrimps and aquatic insects.

Differences in the period of introduction and establishment in ecological aquatic ecosystems, as well as different limnological features may also explain the discrepancy between *P. squamosissimus* data obtained in the Tibagi River (Silva-Souza, 1998) and those found in the present study in Nova Avanhandava (Bittencourt-Oliveira, 2002; Shibatta *et al.*, 2002; Yabe & Gimenez, 2002; Paes, 2006; Vidotto & Carvalho, 2007). According to some reports, *P. squamosissimus* was equivocally introduced in the Tietê River during the 1960s (Torloni *et al.*, 1993), while in the Tibagi River, Paraná State, it was introduced more recently, in the early 1990s (Bennemann & Shibatta, 2002). This information suggests that the longer the time of *A. compactum* metacercariae interaction with *P. squamosissimus*, the smaller the negative effects on the host.

In this study, the significant correlation found between the length of parasitized specimens and mean intensity of infection indicates that *A. compactum* metacercariae

infection is a cumulative process. Aragort *et al.* (1997) also found a positive correlation between length and intensity of infection in a study of the parasitic fauna in tilapia (*O. mossambicus* and *O. aureus*) of the Valencia Lake (Venezuela). They observed that intensity of infection was higher in the larger fish (in weight), and suggested that the relationship between intensity of infection and length might be due to the fact that larger specimens have a greater body surface for infection. The present study and that of Aragort *et al.* (1997) corroborate the island biogeography theory (Kuris *et al.*, 1980).

*Austrodiplostomum compactum* metacercariae most commonly parasitize the vitreous humour, but some metacercariae can also be found in the aqueous humour (Garcia *et al.*, 1993) and the brain of their hosts (Ostrowski-Nunez, 1982; Conroy *et al.*, 1985; Pineda-López, 1985; Osorio-Sarabia *et al.*, 1987; Silva-Souza, 1998). In this study, however, although the brains or other organs of the specimens were not examined, only free metacercariae were found in the vitreous humour, and no eye lesions were observed macroscopically.

According to Martins (1998), digenetic trematodes are more pathogenic when a fish is acting as an intermediate host because the parasite is more aggressive to the host at this metacercaria stage than in its adult forms. Migration to the host tissues in order to reach other infection sites (eyes, brain, among others) can cause injury and, if encysted, tissue changes. The fact that metacercariae cause diseases and weaken hosts is an important ecological characteristic. Weakened fish can be easily preyed and, as a consequence, the parasite cycle can be completed in the predator (bird), the definitive host (Pavanelli *et al.*, 2006).

Evans *et al.* (1976) and Eiras (1994) showed that, depending on host size, 40 metacercariae/eye may cause blindness, cataracts or undermined vision. Garcia *et al.* (1993) found that the presence of *A. compactum* metacercariae in the eyes (aqueous and vitreous humour) and brain of tilapia *O. aureus* and *O. mossambicus* from the Amela Lake in Colima (Mexico) caused injuries in the cornea and conjunctiva. Silva-Souza (1998) did not find any injury or inflammatory reaction resulting from infection in *P. squamosissimus*. The same (no damage, cataracts or blindness) was observed in this study despite the record of up to 136 metacercariae in a single eyeball. It is noteworthy that metacercariae of other species of the Diplostomidae family have caused mortality and decreases in condition factors in catfish *Ictalurus punctatus* (Rafinesque, 1818) at fish farms in the USA (Overstreet & Curran, 2004).

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