SEASONALITY OF *Eustrongylides sp.* (NEMATODA: DIOCTOPHYMATIDAE) LARVAE IN FISHES FROM PARANÁ RIVER, SOUTH-WESTERN BRAZIL

Maurício Laterça MARTINS [1, 4]; Rosemeire de Souza SANTOS [2]; Newton Garcia MARENGONI [2]; Haroldo Kazuyuki TAKAHASHI [2]; Eduardo Makoto ONAKA [3]

ABSTRACT

This work evaluated the infection with larvae of *Eustrongylides* sp. (Nematoda: Dioctophymatidae) in *Cichla piquiti*, *Plagioscion squamosissimus* and *Hoplias malabaricus*, collected in Paraná River, near Presidente Epitácio City, São Paulo State, Southwestern Brazil. Fishes were captured monthly with net and hook from September 2000 to August 2001 for parasitological analyses. The data were analysed by season. Prevalence and mean intensity of infection were higher in *H. malabaricus* than in *C. piquiti* in winter. The mean parasite abundance in *H. malabaricus* was also higher in *P. squamosissimus*. Females of *H. malabaricus* showed higher prevalence rate than males. The most frequent occurrence in hosts was from 1 to 4 parasites located in muscle and mesentery. This work was able to demonstrate *H. malabaricus* as the most susceptible fish to larvae of *Eustrongylides* sp. in this region of Paraná River.

Keywords: *Cichla ocellaris*; *Eustrongylides*; *Hoplias malabaricus*; infection; *Plagioscion squamosissimus*; seasonality

SAZONALIDADE DE LARVAS DE *Eustrongylides sp.* (NEMATODA: DIOCTOPHYMATIDAE) EM PEIXES DO RIO PARANÁ, SUDESTE DO BRASIL

RESUMO

Este estudo avaliou a infecção com larvas de *Eustrongylides* sp. (Nematoda: Dioctophymatidae) em *Cichla piquiti*, *Plagioscion squamosissimus* e *Hoplias malabaricus*, capturadas no Rio Paraná, próximo à Cidade de Presidente Epitácio, Estado de São Paulo, Sudoeste do Brasil. Os peixes foram mensalmente capturados com rede e anzol entre setembro de 2000 e agosto de 2001 para exame parasitológico. Os dados foram analisados por estação. No inverno, a taxa de prevalência e a intensidade média foram mais altas em *H. malabaricus* do que em *C. ocellaris*. A abundância media de parasitos em *H. malabaricus* também foi maior do que em *P. squamosissimus*. Fêmeas de *H. malabaricus* apresentaram taxa de prevalência mais significativa do que machos. A ocorrência mais frequente nos hospedeiros foi de 1 a 4 parasitos no músculo e mesentério. Este estudo demonstrou que *H. malabaricus* foi mais susceptível à parasitose por larvas de *Eustrongylides* sp. na região do Rio Paraná.

Palavras-chave: *Cichla ocellaris*; *Eustrongylides*; *Hoplias malabaricus*; infecção; *Plagioscion squamosissimus*; sazonalidade

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[1] *Laboratório AQUOS - Sanidade de Organismos Aquáticos, Departamento de Aquicultura, Centro de Ciências Agrárias (CCA), Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brasil.*

[2] *Centro de Aquicultura, UNOESTE, Presidente Prudente, SP, Brasil.*

[3] *Instituto de Pesca, APTA-SAA, São José do Rio Preto, SP.*

[4] *Endereço/Address: Departamento de Aquicultura, CCA, UFSC. Rod. Admar Gonzaga, 1346 - CEP: 88040-900 – Florianópolis – SC - Brasil. e-mail: mlaterca@cca.ufsc.br.*

INTRODUCTION

The *Eustrongylides* larvae can be found in proventriculus, guts, liver, kidney, peritoneum, gall bladder or muscles of piscivorous birds. In birds, the released larvae L_1 are ingested by an aquatic oligochaete (MEASURES, 1988a) in which develops L_3 infective larvae (MEASURES, 1988b). The susceptible fish ingests the infected oligochaete (COYNER *et al*., 2001) originating larvae L_4 that remain encysted until its consumption by the definitive host bird. Their pathogenic action is discussed, but sometimes causes fibrosis around cyst (EIRAS and REGO, 1989). The presence of *Eustrongylides* larvae was mentioned in *Cichla ocellaris* from Panamá (VASQUEZ and ROGERS, 1992); in *Thoracocharax sternicla* and *Aequidens pulcher* from the small lakes of Suripá River, Venezuela (MORAVEC *et al*.. 1997); in *Cichlasoma managuense* from Mahogany River, Nicaragua (AGUIRRE- MACEDO *et al*., 2001) and in *Salmo trutta* from Norway (HAUGEN *et al*., 2008).

In Brazil, TRAVASSOS *et al*. (1928) reported for the first time *Eustrongylides* larvae in *Callichthys callichthys*, *Pimelodus clarias*, *Symbranchus marmoratus*, *Salminus maxillosus*, *Pseudoplatystoma* sp., *Acesthrorhamphus* sp., *Tetragonopterinae* sp. and *Poecilia vivipara*. Later in *Pseudoplatystoma corruscans* from Piracicaba River, SP (LORDELLO and MONTEIRO, 1959), in *S. maxillosus*, *Cynopotamus hureralis* and *Steindachneridion parahybae* from Mogi-Guacu River, Pirassununga, SP (TRAVASSOS and KOHN, 1965); in *Auchenipterus nigripinnis*, *Serrasalmus nattereri*, *Pirinampus pirinampu*, *Rhaphiodon vulpinus*, *Pseudoplatystoma fasciatum* and *S. marmoratus* from Cuiabá River, MT (REGO and VICENTE, 1988a); in *Hoplias malabaricus* from Pirassununga - SP (REGO and VICENTE, 1988b); in *Paratrygon* sp., *Pygocentrus nattereri* and *P. corruscans* from Cuiabá River - MT (EIRAS and REGO, 1988) and in characoid fishes (SARAIVA *et al*., 2006).

The influence of host sex in fish from Paraná River parasitized by larvae of nematodes was reported by MACHADO *et al*. (1994, 2000). MOREIRA *et al*. (2005) observed that 80% of examined fish were parasitized by, at least, one species of parasite. In the studies of MARTINS *et al*. (2003), the parasitic level was influenced by weight classes in piscivorous fishes from Paraná River in São Paulo State. A high intensity of parasites was also observed in *H. malabaricus* from Northern Brazil (MARTINS *et al*., 2005).

The present work studied the seasonality of *Eustrongylides* sp. larvae in *Cichla piquiti* Kullander and Ferreira, 2006 (*sin. part. Cichla ocellaris* 'sensu' KULLANDER and FERREIRA, 2006) (Cichlidae), *Plagioscion squamosissimus* HECKEL 1840 (Sciaenidae) and *Hoplias malabaricus* BLOCH 1794 (Erythrinidae), collected from Paraná River, in Presidente Epitácio city, South-western Brazil.

METHODOLOGY

This work was developed at the Aquaculture Center of São Paulo West University (UNOESTE) ,situated at the shores of Paraná River, near Presidente Epitácio city, São Paulo State (21º 45′ 48″ S, 52º 06′ 56″ W). Net and hook were used to collect 120 *C. piquiti*, 101 *P. squamosissimus* and 109 *H. malabaricus* from September 2000 to August 2001, analysed by season and maintained in ice to be examined at the laboratory. A total of 81 fishes in spring, 69 in summer, 90 in autumn and 90 in winter were analysed. The fishes were divided in size classes for analysis of total prevalence in the period.

Water quality was measured in each month when fishes were collected. Dissolved oxygen, temperature, pH, electric conductivity, alkalinity and transparency were measured by YSI 55, phmeter VDSF F-1002 (Bernauer), F-1000 (Bernauer), Hach model FF-1A and Secchi disc, respectively. After fishe's sacrifice, nematodes were carefully dissected from their cysts into muscles or peritoneum, maintained in 0.65% saline solution, fixed in AFA 65ºC and preserved in a solution of 70% alcohol and 10% glycerine. Then, nematodes were identified according to ANDERSON and BAIN (1982). Prevalence, mean parasite intensity and mean parasite abundance were calculated according to BUSH *et al*. (1997).

The variance analysis was obtained when data were transformed in arcsine SQRT (x + 0.50) and by average comparison by Tukey test. Rainfall, temperature and dissolved oxygen, regarding nematode larvae, were analyzed with a 0.05 significance rate (JOHNSON and WICHERN, 1992). Results were compared among hosts despite the season and between season in each host.

RESULTS

Table 1 shows rainfall and water quality in each season of sampling. Rainfall was proportional to the three months mean in relation to each season. The weight classes of fishes and the respective prevalence are on Table 2. *Cichla piquiti* and *P. squamisissimus* with 600-2,500g showed the highest prevalence rate (P<0.05). On the other hand, in *H. malabaricus,* high prevalence was between 425-550g and 800-1,650g.

Table 1. Mean values ± standard deviation and amplitude of variation in parentheses of rainfall and aquatic parameters recorded in Paraná River, Presidente Epitácio city, São Paulo state, Brazil.

Season	Rainfall (mm)	Oxygen (mg/l)	Temperature (°C)	Electric conductivity (µS/cm)	pH	Alkalinity (mg/l)	Transparency (meters)
Spring	134.9±45.3 (67.0-195.4)	7.2±0.5 (6.4-8.0)	26.0±2.3 (22.6-28.7)	62.4±12.9 (49.0-81.8)	7.2±0.1 (7.1-7.3)	27.4±0	2.4±0
Summer	253.3±38.2 (215.1-291.6)	6.2±0.05 (6.2-6.3)	28.0±0.3 (27.7-28.4)	52.4±2.6 (49.8-55.1)	6.8±0.4 (6.4-7.3)	27.4±0	2.4±0
Autumn	140.8±109.5 (57.2-140.8)	6.5±5.6 (3.7-6.5)	28.8±0.5 (27.1-28.2)	56.5±0.2 (56.5-57.0)	6.4±0	27.4±0	2.4±0
Winter	44.9±8.7 (31.8-52.9)	5.5±1.1 (3.8-6.7)	24.4±2.1 (22.0-27.6)	56.7±0.2 (56.5-57.0)	6.5±0.1 (6.4-6.7)	32.6±5.6 (27.4-41.0)	2.4±0

Table 2. Weight classes (g) and prevalence rate (%) of *Eustrongylides* sp. larvae in fishes from Paraná River, Presidente Epitácio city, São Paulo state, Brazil. Different letters indicate significant difference in the same column (P<0.05).

Cichla piquiti	Prevalence (%)	*Plagioscion squamosissimus*	Prevalence (%)	*Hoplias malabaricus*	Prevalence (%)
200 to 550 g	18.9 c	250 to 450 g	25.7 c	150 to 400 g	20.0 c
600 to 850 g	50.0 a	475 to 750 g	39.1 b	425 to 550 g	72.7 a
900 to 1,150 g	38.9 b	800 to 1,510 g	69.0 a	600 to 750 g	56.5 b
1200 to 2,500 g	41.4 b			800 to 1,650 g	76.2 a

Cichla ocellaris and *P. squamosissimus* showed a significant increase (P<0.05) in the prevalence rate in spring, summer and autumn (Table 3). On the other hand, *H. malabaricus* showed high prevalence in winter (Table 3). The mean intensity of infection and mean abundance were similar among these three fish species. Except for *H. malabaricus,* collected in winter that showed mean intensity of 4 to 11.2 parasites per host; in other hosts, there was no difference nor at the mean intensity neither at abundance.

Table 3. Mean values of the parasitological data ± standard deviation and amplitude of variation between parentheses of fish infected by *Eustrongylides* sp. in the Paraná River, Presidente Epitácio, São Paulo, Brazil. IF/EF: infected fish/examined fish; P: prevalence; MI: mean intensity; MA: mean abundance. Different letters indicate significant difference between the fishes (P<0.05)

Season	*Cichla piquiti*				*Plagioscion squamosissimus*				*Hoplias malabaricus*			
	IF/EF	P (%)	MI	MA	IF/EF	P (%)	MI	MA	IF/EF	P (%)	MI	MA
Spring	14/30	46.7±5.8 a (40-50)	2.1±0.7 (1.2-2.6)	1.0±0.4 (0.5-1.3)	8/22	40.0±14.1 b (30-50)	1.7±1.0 (1.0-2.5)	0.7±0.6 (0.3-1.2)	17/29	68.0±1.7 b (67-70)	2.2±0.7 b (1.7-3.0)	0.8±0.2 b (0.5-1.0)
Summer	15/30	50.0±17.3 a (30-60)	2.5±0.8 (1.7-3.3)	1.2±0.3 (1.0-1.5)	15/19	69.3±31.5 a (33-89)	2.6±0.4 (2.3-3.0)	1.7±0.6 (1.0-2.2)	10/20	58.0±2.1 c (57-60)	3.7±1.0 b (3.0-4.4)	1.9±0.3 b (1.7-2.2)
Autumn	12/30	40.0+26.4 a (10-60)	3.7+2.3 (1.8-6.3)	1.2+1.1 (0.3-2.5)	13/30	43.3+20.8 b (20-60)	3.0+1.5 (1.5-4.6)	1.1+0.7 (0.3-1.5)	21/30	70.0+10.0 b (60-80)	3.0+1.7 b (1.2-4.5)	1.8+1.0 b (0.7-2.7)
Winter	8/30	26.7±20.8 b (10-50)	3.1±1.4 (1.5-4.0)	0.9±0.9 (0.3-1.9)	3/30	10.0±17.3 c (0-30)	0.8±1.3 (0-2.3)	0.2±0.4 (0-0.7)	27/30	90.0±10.0 a (80-100)	7.1±3.7 a (4.0-11.2)	6.0±2.7 a (3.5-9.0)
Total	49/120	40.8±15.6 b	2.8±1.3 ab	1.1±0.7 b	39/101	38.6±20.9 b	2.0±1.0 b	1.1±0.6 b	75/109	68.8±5.9 a	4.0±1.8 a	2.6±1.0 a

When analysed by fish species, *H. malabaricus* showed higher (p<0.05) mean intensity (4.0) than that one observed in *C. ocellaris* (2.8). Once more, the parasitic mean abundance in *H. malabaricus* was significantly higher (2.6) than in *P. squamosissimus* (1.1). Although such fish species, there was no difference (p>0.05) on prevalence rate. Mean intensity and mean abundance were found in spring (53.0±14.2; 2.0±0.7; 0.8±0.4 respectively), summer (59.4±21.2; 2.8±0.8; 1.6±0.5 respectively), autumn (51.1±22; 3.2±1.6; 1.4±0.9 respectively) and winter (42.2±39.3; 3.6±3.4; 2.4±3.1 respectively).

The sex of *C. ocellaris* and *P. squamosissimus* did not affect the infection rate. On the other hand, females of *H. malabaricus* were significantly (p<0.05) more parasitized than males (Table 4). A significant increase (p<0.05) in prevalence rate and mean intensity was found in *H. malabaricus* collected in winter (Table 3). The highest frequency distribution of larvae was at one to four parasites in the muscle and mesentery (Table 5). It can be observed that only in *H. malabaricus* the number of parasites raised from 10 to 15 and from 16 to 26.

Table 4. Mean values of prevalence (P) ± standard deviation and amplitude of variation of males and females, infected by *Eustrongylides* sp. in Paraná River, Presidente Epitácio city, São Paulo state, Brazil. Different letters showed significant difference between sexes of each host (P<0.05).

Species	Cichla piquiti		*Plagioscion squamosissimus*		*Hoplias malabaricus*	
Season	P (%) males	P (%) females	P (%) males	P (%) females	P (%) males	P (%) females
Spring	42.3±12.8 (33-57)	46.7±12.3 (33-57)	29.0±25.9 (0-50) b	16.7±28.9 (0-50)	39.0±33.8 (0-60)	91.7±14.3 (75-100) a
Summer	48.7±17.1 (29-60)	53.3±17.9 (33-60)	58.0±26.6 (33-86) a	33.3±(57.7 (0-100)	26.7±23.1 (40-80)	60.0±52.9 (0-100) b
Autumn	49.7±33.5 (11-71)	25.3±22.5 (0-43)	54.7±4.0 (50-57) a	37.3±27.7 (12-67)	66.7±23.1 (40-80)	73.3±11.5 (60-80) ab
Winter	15.0±13.2 (0-25)	33.0±29.7 (12-67)	0 c	14.3±24.8 (0-43)	58.3±52.0 (0-100)	94.3±9.8 (83-100) a
Total mean	38.9±19.1 **a**	39.6±20.6 **a**	35.4±14.1 **a**	25.4±34.8 **a**	47.7±33.0 **b**	79.8±22.1 **a**

Table 5. Frequency distribution and location of *Eustrongylides* sp. in fishes from Paraná River, Presidente Epitácio city, São Paulo state, Brazil.

	Cichla piquiti (Parasite number)										
Organ	1	2	3	4	5	6	7	8	9	10-15	16-26
Muscle	22	10	7	6	2	0	1	1	0	1	0
Mesentery	3	1	1	0	0	0	0	1	0	0	0
Total	25	11	8	6	2	0	1	2	0	1	0
	Plagioscion squamosissimus										
Muscle	10	12	8	3	2	0	0	0	0	0	0
Mesentery	5	1	2	0	1	0	0	0	0	0	0
Total	15	13	10	3	3	0	0	0	0	0	0
	Hoplias malabaricus										
Muscle	19	13	12	10	4	1	1	4	3	3	4
Mesentery	7	11	3	2	4	2	0	0	1	2	4
Total	26	24	15	12	8	3	1	4	4	5	8

DISCUSSION

The influence of environmental quality, natural susceptibility to parasite and number or infection capacity must be considered. Parasites may be usually present in hosts or environment, but if balance among host, parasite and environment is broken, some diseases may occur, culminating in death (COUTANT, 1998).

Since *H. malabaricus* is the most parasitized fish, this could probably be explained by its piscivorous habit of ingesting smaller infected fish and oligochaetes, as suggested by MEASURES (1988c).

This work corroborated the findings of KENNEDY and LIE (1976) and VASQUEZ and ROGERS (1992), who associated the preference of *Eustrongylides* larvae for female brown trout and female *C. ocellaris*, respectively. The preference of *Eustrongylides* for female *H. malabaricus* was probably due to physiological or diet differences as also supported by KENNEDY and LIE (1976).

According to GOMIERO e BRAGA (2004), the preys consumed by *C. piquiti* may vary according to the environment. After 80 mm length, the diet of such fish is composed by crustaceans, insects and small fishes. There was no difference concerning feeding between sexes in *Cichla* spp (GOMIERO and BRAGA, 2003). In studies with *C. piquiti* and *C. monoculus* at Volta Grande reservoir, GOMIERO and BRAGA (2003) observed scarceness of preys in winter due to their reproductive behaviour. In this work, as in *C. piquiti*, the prevalence rate in winter was lower than other seasons and this can be explained due to a decrease in their reproductive behaviour and feeding (GOMIERO and BRAGA, 2003). This situation could also happen to *P. squamosissimus* in winter.

In the region of Paraná River, MARTINS *et al.* (2003) pointed out *H. malabaricus,* 600 to 750 g of weight, as the most parasitized fish by larvae of *Contracaecum*. This study revealed that larger fish presented the highest prevalence of *Eustrongylides*, corroborating the observations of VASQUEZ and ROGER (1992).

The overall prevalence of larval *Eustrongylides* was 40.8% in *C. ocellaris*; 38.6% in *P. squamosissimus* and 68.8% in *H. malabaricus*. On the other hand, MACHADO *et al.* (1994) reported lower prevalence (1.8%) of *Eustrongylides* in *P. corruscans* collected in the same River. During two years, 100% prevalence of *Contracaecum* larvae in *Cichla monoculus* was also registered by MACHADO *et al*. (2000) in Paraná River.

According to HAHN *et al*. (1999), several piscivorous predator fishes show increased feeding activity in summer and autumn. The first hypothesis to higher infection in *H. malabaricus* in winter and summer is that, possibly, such increased feeding activity in autumn and spring was responsible for accumulation of larvae in the subsequent seasons. The lack of seasonality on *Eustrongylides* occurrence in *P. squamosissimus* can be associated to the way they grab their preys, as commented by HAHN *et al*. (1999). (RABELO and ARAUJO-LIMA, 2002). Piscivorous fishes change their feeding when they increase in size and fish may constitute the main source of an infection. By this fact, this habit makes easy the transmission of larvae from one fish to another. Another hypothesis is that during the drought or winter the reduced space in the environment may favour piscivorous habit of *H. malabaricus*, as suggested by MERONA and BITTENCOURT (1993).

The frequency distribution, here observed, differed in part from the findings of MEASURES (1988c). This author found out the main frequency distribution, situated between one to two parasites, while in this study, such frequency varied from one to four larvae. The frequency distribution of *Eustrongylides* larvae may be related to diet and environmental status as commented by MEASURES (1988c). In the studies of MOREIRA *et al.* (2005), prevalence of fish parasitized by at least one parasite species was 80%, similar to the one presented in this trial.

Hence, the present results corroborated the findings of MARTINS et al. (2003), who reported *H. malabaricus* as the most parasitized fish in Paraná River. Moreover, at least in this region of Paraná River, *H. malabaricus* females were more susceptible than males, supporting the findings of GUIDELLI *et al.* (2003). It is supported by the hypothesis of males and females inhabit different ecological environment in any time of their lives cycles. MACHADO *et al.* (1994) found no differences between male and female nor in

Pseudoplatystoma corruscans neither in *Schizodon borelli*. The possibility of human infection by *Eustrongylides* larvae is rare when compared to the anisakid nematodes (MORAES e MARTINS, 2004). Still, when observing patients that had eaten raw fish (sushi, sashimi), the parasite caused human infection (WITTNER *et al*., 1989). The diagnosis of anisakiasis is usually by endoscopy techniques and the treatment by chemicals like tiabendazole, flubendazole and mebendazole (UBEIRA *et al.,* 2000). Lately, the consumption of 'sushi' and 'sashimi' has contributed to the spread of this disease. The consumption of 'sashimi' from *C. piquiti* or other kind of susceptible fish is not recommended.

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