

Nematodes in *Hoplerytrinus unitaeniatus*, *Hoplias malabaricus* and *Pygocentrus nattereri* (pisces characiformes) in Marajó Island, Brazil

Nematóides em *Hoplerytrinus unitaeniatus*, *Hoplias malabaricus* e *Pygocentrus nattereri* (pisces characiformes) na Ilha de Marajó, Brasil

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Received July 4, 2011

Accepted October 6, 2011

Abstract

The aim of this study was to evaluate the tegument, musculature and mesentery of 102 specimens of *Hoplerytrinus unitaeniatus*, 104 of *Hoplias malabaricus* and 101 of *Pygocentrus nattereri*, from Arari Lake, Marajó Island, State of Pará, Brazil. Were identified the nematodes *Contracaecum* sp., *Eustrongylides* sp. and *Procamallanus* sp. *Contracaecum* sp. was the most prevalent, with rates of 84.31% (*H. unitaeniatus*), 95.19% (*H. malabaricus*), and 89.11% (*P. nattereri*). The highest prevalences of *Eustrongylides* sp. occurred in *H. unitaeniatus* (56.86%) and *H. malabaricus* (53.84%). *Procamallanus* sp. was only collected in the mesentery. Specimens of *Eustrongylides* sp. collected from the musculature were 91.9% of its population. Among the nematodes found in the mesentery, 98.34% were *Contracaecum* sp. with a mean intensity (MI) of 7.92 ± 8.11 (*H. unitaeniatus*), 8.49 ± 8.34 (*H. malabaricus*) and 7 ± 6.40 (*P. nattereri*). *Contracaecum* sp. presented the highest MI (8.49 ± 8.34) and mean abundance (8.09 ± 8.34). The highest MI values were observed in the mesentery. *Eustrongylides* sp. presented MI of 2.65 ± 3.21 (*H. unitaeniatus*), 3.41 ± 3.27 (*H. malabaricus*) and 2.17 ± 1.18 (*P. nattereri*). Nematodes with zoonotic potential that were found with high prevalence, shows the importance of actions by the health authorities.

Keywords: Nematoda, Fish, hygienic-sanitary importance, Marajó Island, Brazil.

Resumo

Examinou-se o tegumento, mesentério e musculatura de 102 espécimes de *Hoplerytrinus unitaeniatus*, 104 de *Hoplias malabaricus* e 101 de *Pygocentrus nattereri*, do Lago Arari, Ilha do Marajó, Estado do Pará, Brasil. Foram identificados os nematóides *Contracaecum* sp., *Eustrongylides* sp. e *Procamallanus* sp. nas três espécies de peixes. *Contracaecum* sp. foi o mais prevalente, com índices de 84,31% (*H. unitaeniatus*), 95,19% (*H. malabaricus*) e 89,11% (*P. nattereri*). As maiores prevalências de *Eustrongylides* sp. foram observadas em *H. unitaeniatus* (56,86%) e *H. malabaricus* (53,84%). *Procamallanus* sp. só foi coletado no mesentério, sendo o sítio de infecção mais parasitado. Na musculatura, foram coletados espécimes de *Eustrongylides* sp., representando 91,9% de sua população. Dos nematóides coletados no mesentério, 98,34% foram *Contracaecum* sp. com intensidade média (IM) de $7,92 \pm 8,11$ (*H. unitaeniatus*), $8,49 \pm 8,34$ (*H. malabaricus*) e $7,0 \pm 6,40$ (*P. nattereri*). *Contracaecum* sp. apresentou maior IM ($8,49 \pm 8,34$) e abundância média ($8,09 \pm 8,34$). Os maiores valores de IM foram obtidos no mesentério. *Eustrongylides* sp. apresentou IM de $2,65 \pm 3,21$ (*H. unitaeniatus*), $3,41 \pm 3,27$ (*H. malabaricus*) e $2,17 \pm 1,18$ (*P. nattereri*). Nematóides com potencial zoonótico, encontrados com alta prevalência, demonstram a importância para ações das autoridades sanitárias.

Palavras-chave: Nematoda, peixes, importância higiênico-sanitária, Ilha de Marajó, Brasil.

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The increasing popular interest in consuming raw or poorly cooked fish has induced sanitary authorities and researchers to pay more attention to this trend, taking into account that it has already been well established that bioagents with zoonotic potential exist in different aquatic environments. According to Souza (2003), such agents can promote alterations in fish production, with a strong impact on the safety and quality of the fish.

Fish can be contaminated with parasites that may be potentially harmful to humans. According to González et al. (2001), the pathogens can be classified into two groups: firstly, parasites of public health interest that may be harmful to consumers; and secondly, agents that only affect fish by inducing lesions in the hosts and thus altering their commercial value. Such lesions may even, based on sanitary and hygiene standards, lead to rejection of these fish because of the ensuing repugnant appearance of the flesh.

Most occurrences of parasites of public health interest are related to consumption of raw, poorly cooked, inadequately salted and cold-smoked fish. Human cases of parasitism by helminths due to consumption of fish processed under these conditions have been reported in Brazil and are associated with the species *Ascoctyle (Phagicola) longa* (Ransom, 1920) (CHIEFFI et al., 1990, 1992; ALMEIDA-DIAS; WOICIECHOVSKI, 1994), *Diphyllbothrium latum* (Linnaeus, 1758) (EDUARDO et al., 2005a, b; SANTOS; FARO, 2005; TAVARES et al., 2005; EMMEL et al. 2006; LACERDA et al., 2007; CAPUANO et al., 2007; LLAGUNO et al., 2008), *Clonorchis sinensis* (Cobbold, 1875) (LEITE et al., 1989; DIAS et al., 1992) and *Gnathostoma* sp. (DANI et al., 2009).

Among the nematodes of zoonotic potential, anisakids are prominent and, although no human cases have yet been recorded in Brazil, they have already been reported in marine and freshwater fish (PADOVANI et al., 2005). Human anisakiasis is considered to be the most severe infection caused by consumption of contaminated fish harboring third-stage larvae of nematodes of the genera *Anisakis* (Dujardin, 1845), *Pseudoterranova* (Mozgovoy, 1950) and *Contracaecum* (Railliet & Henry, 1912). Infections with the species *A. simplex* (Rudolphi, 1809) and *P. decipiens* (Krabbe, 1878) are the most important occurrences, according to Ishikura et al. (1993) and Adams et al. (1997). *Contracaecum* larvae mostly occur in the visceral organs and mesentery of fish, thus limiting their zoonotic threat, which may explain why human cases are rare even considering that mammals are susceptible to experimental infection with these larvae (VIDAL-MARTINEZ et al. 1994; BARROS et al., 2004).

Another nematode group with zoonotic potential is Dioctophymatidae, represented by larvae of the genera *Dioctophyma* (Collet-Meygret, 1802), *Eustrongylides* (Jägerskiöld, 1909) and *Hystrichis* (Dujardin, 1845), which can infect freshwater fish. These fish are essential hosts only in the life cycle of eustrongylids, considering that the third-stage larvae of this parasite develop into the fourth stage in fish on which piscivorous birds feed to complete the cycle. Moreover, predatory fish can act as paratenic hosts by ingesting other infected fish with fourth-stage larvae (COLE, 1999). Cases of accidental human infection can occur if raw or poorly cooked fish is consumed, and there have been further reports of symptoms associated with the abdominal location of

the nematodes (EBERHARD et al., 1989; SCHANTZ, 1989; WITTNER et al., 1989; NARR et al., 1996).

The data available from the Amazon region on the parasitic biota of economically important fish species are very limited, and there is a lack of information about parasites of zoonotic potential. The aim of the present study was to analyze the parasitic profile of nematodes with hygiene and sanitary relevance that infect fish of commercial value, caught in the Ararí Lake, Marajó Island, State of Pará, Brazil.

Between August and December 2009, 102 specimens of gold wolf fish, *Hoplerysternus unitaeniatus* (Spix & Agassiz, 1829), of weight 107-376 g and standard length 15.4-25 cm, 104 specimens of thraira, *Hoplias malabaricus* (Bloch, 1794), of weight 110-530 g and standard length 17.8-27.2 cm and 101 specimens of red-bellied piranha, *Pygocentrus nattereri* (Kner, 1858), of weight 111-276 g and standard length 12.5-16.5 cm were randomly purchased from fishermen, at the time of anchorage, in Ararí Lake, municipality of Santa Cruz do Arari, Marajó Island, State of Pará, Brazil (0° 39' 48" S 49° 10' 30" W).

The fish specimens were kept in insulated containers with ice and were processed in the Animal Parasitology Laboratory, Federal Rural University of the Amazon, Belém campus, State of Pará. The tegmental surface of the fish was firstly inspected and their sex was determined. To recover visceral parasites, the specimens were gutted and the organs and abdominal musculature were transferred to individual Petri dishes with 0.65% NaCl solution to be examined under a stereoscopic microscope. The filleted flesh, which was obtained by means of an incision from near the opercula to the insertion of the caudal fin, was observed on a candling table for better evaluation and recovery of tissue parasites. The larvae and adults were processed in accordance with the methodology of Amato et al. (1991) and Eiras et al. (2006). The nematodes were identified based on Hartwich (1974), Moravec (1994) and Vicente and Pinto (1999).

Prevalence, mean intensity, range of infection and mean abundance were obtained in accordance with Bush et al. (1997). To compare the prevalences of nematodes in different hosts and at different infection sites, the chi-square test was applied. Linear associations between infection intensity and variables relating to weight and total length were evaluated by means of Pearson's linear correlation coefficient. Considering that the intensity and mean abundance of infection did not present Gaussian distribution, Kruskal-Wallis analysis of variance was applied with Tukey post-test analysis, as recommended by Ayres et al. (2007), for comparing mean intensity and mean abundance of parasitism, in relation to the nematode species and infection sites. Alpha = 0.05 was the level preset for rejection of the null hypothesis. The statistical procedures were performed using the BioEstat 5 software.

Representative specimens from each nematode species were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, State of Rio de Janeiro, Brazil.

From the three fish species collected (*H. unitaeniatus*, *H. malabaricus* and *P. nattereri*), 2,578 nematodes belonging to three species were obtained: *Contracaecum* sp. (2,152), *Eustrongylides* sp. (395) and *Procamallanus* sp. (31). *H. malabaricus* was the most infected fish species, with 1037 worms, following by *H. unitaeniatus* and *P. nattereri* with respectively 839 and 702 worms.

The data indicated that in the three fish species examined, larvae of *Contracaecum* sp. and *Eustrongylides* sp. and adults of *Procamallanus* sp. appeared with patterns of either single or concomitant infections, except for *Procamallanus* sp. in the samples of *H. unitaeniatus* and *H. malabaricus* (Table 1).

Parasitism occurred at high prevalence rates in the three fish species: greater than 92% overall; and reaching 96.15% in the specimens of *H. malabaricus*. *Contracaecum* sp. was the most prevalent parasite genus, since 84.29% of the specimens of *H. unitaeniatus*, 95.19% of *H. malabaricus* and 89.11% of *P. nattereri* were infected with these parasites, whereas representatives of *Procamallanus* sp. appeared at the lowest rates: 3.9%, 1.92% and 10.89% respectively, in the three fish species considered. The highest infection rates relating to *Eustrongylides* sp. were 56.86% in *H. unitaeniatus* and 53.84% in *H. malabaricus*. Analysis on the parameters referring to single and associated infections revealed that the rate for *Contracaecum* sp. was significantly higher (58.42%) in *P. nattereri*. The rates in *H. unitaeniatus* and *H. malabaricus* were similar to what was observed in co-infections with *Eustrongylides* sp. ($p < 0.05$) (Table 1).

Parasitism occurred at five infection sites (musculature, mesentery and serosa of the stomach, kidneys and ovaries), singly or concomitantly. The musculature only showed specimens of *Eustrongylides* sp., while *Procamallanus* sp. only occurred in the mesentery. This site had the highest frequencies of nematodes:

78.74% in *H. unitaeniatus*, 81.77% in *H. malabaricus* and 92.17 in *P. nattereri* (Table 2). Correlations between weight, total length and intensity of infection were significant only in specimens of *H. malabaricus* ($p = 0.0003$).

Significantly, *Contracaecum* sp. appeared with higher mean infection (MI) and mean abundance (MA) rates: respectively, 8.49 ± 8.34 and 8.09 ± 8.34 , obtained in *H. malabaricus*. However, these were not statistically different from those observed in *H. unitaeniatus* and *P. nattereri* ($p > 0.05$) (Table 2). Regarding the infection sites, the highest MI values were observed in the mesentery, irrespective of the host. There was greater intensity of infection in fish parasitized with *Contracaecum* sp., with higher values for samples recovered from *H. malabaricus* (8.41 ± 8.35 ; 841 worms). However, these values were not statistically different from those obtained in the other hosts ($p > 0.05$): respectively 7.65 ± 8.05 (658 worms) and 6.91 ± 6.34 (622 worms) from *H. unitaeniatus* and *P. nattereri*.

The MI values for *Eustrongylides* sp. in the musculature, i.e. the site with highest prevalence of parasitism (91.9% or 363 out of the 395 specimens collected from the fish examined), were 2.43 ± 3.11 in *H. unitaeniatus*, 3.38 ± 3.14 in *H. malabaricus* and 2.19 ± 1.15 in *P. nattereri*, and these did not differ significantly ($p > 0.05$).

The high prevalence of nematodes of hygiene and sanitary interest in these three species of commercially important fish has been correlated with the high infective capacity of *Contracaecum* sp. and

Table 1. Prevalences (%) of the nematodes *Contracaecum* sp., *Eustrongylides* sp. and *Procamallanus* sp. recovered from specimens of the fish species *H. unitaeniatus*, *H. malabaricus* and *P. nattereri* that were caught from Arari Lake, Marajó Island, State of Pará, Brazil, between August and December 2009.

Fish species	<i>H. unitaeniatus</i>		<i>H. malabaricus</i>		<i>P. nattereri</i>	
	Infecteds	%	Infecteds	%	Infecteds	%
<i>Contracaecum</i> sp. (C)	35	34.31	43	41.35	59	58.42*
<i>Eustrongylides</i> sp. (E)	8	7.84	1	0.96	1	0.99
<i>Procamallanus</i> sp. (P)	-	-	-	-	2	1.98
Single infections	43	42.15	44	42.31	62	61.39
C + E	47	46.08	54	51.92	22	21.78
C + P	1	0.98	1	0.96	9	8.91
C + E + P	3	2.94	1	0.96	-	-
Concomitant infections	51	50	56	53.84	31	30.69
Total of infections	94	92.15	100	96.15	93	92.08

*Showing a significant difference ($p < 0.05$)

Table 2. Prevalence (P), mean intensity (MI), range of infection (RI), mean abundance (MA), site of infection (SI) and CHIOC deposit numbers of the nematodes *Contracaecum* sp., *Eustrongylides* sp. and *Procamallanus* sp. recovered from the fish species *Hopleryttrinus unitaeniatus* (Hu), *Hoplias malabaricus* (Hm) and *Pygocentrus nattereri* (Pn) that were captured in Arari Lake, Marajó Island, State of Pará, Brazil, between August and December of 2009.

Nematode species	<i>Contracaecum</i> sp.			<i>Eustrongylides</i> sp.			<i>Procamallanus</i> sp.		
	Hu	Hm	Pn	Hu	Hm	Pn	Hu	Hm	Pn
P (%)	84.31	95.19	89.11	56.86	53.85	22.74	3.92	1.92	10.89
MI	7.92 ± 8.11	8.49 ± 8.34	7 ± 6.40	2.65 ± 3.21	3.41 ± 3.27	2.17 ± 1.18	1 ± 0.20	2.5 ± 0.40	2 ± 0.73
RI	1-50	1-44	1-41	1-26	1-20	1-8	-	1-4	1-4
MA	6.68 ± 8.11	8.09 ± 8.34	6.24 ± 6.40	1.51 ± 3.21	1.84 ± 3.27	0.50 ± 1.18	0.04 ± 0.20	0.05 ± 0.40	0.22 ± 0.73
SI	me, sl, so, ss	me, sl, ss	me, sl, ss	me, ss, mu	me, sl, mu	me, ss, mu	me	me	me
CHIOC	35759	35760	35761	35762	35763	35764	35765	35766	35767

me = mesentery, mu = musculature, sl = serosa of liver, so = serosa of ovaries, ss = serosa of stomach.

Eustrongylides sp., considering that findings of adult *Procamallanus* sp. in the mesentery are uncommon, since the adults of these nematodes parasitize the stomach and intestine of freshwater and marine fish (MORAVEC et al., 2000). The three fish species captured in Arari Lake, which is located in the eastern hydrographic basin and is considered to be the largest depression on Marajó Island, were highly susceptible to infection, with heavy parasitism due to nematodes of the genera *Contracaecum* sp. and *Eustrongylides* sp.

The data indicate that in the case of *Contracaecum* sp., crustaceans and piscivorous birds are also implicated in the food chain in this aquatic environment, the former acting as intermediate hosts and the latter as the definitive hosts. Fish can act as the second intermediate hosts by feeding on parasitized crustaceans or on paratenic hosts, in the case of predatory fish. In both cases, fish may be a source of infection for piscivorous birds and marine mammals (KOIE; FAGERHOLM, 1995; KOIE et al., 1995; ANDERSON, 2000). In parasitism involving *Eustrongylides* sp., the definitive hosts are piscivorous birds; while in infections with *Contracaecum* sp., fish participate as the second intermediate hosts and contamination occurs after ingestion of parasitized oligochaetes (COLE, 1999; COYNER et al., 2001).

Brazilian data (KOHN et al., 1988; MARTINS et al. 2003, 2005, 2009; MADI; SILVA, 2005; BARROS et al., 2006, 2007, 2010; PARAGUASSÚ; LUQUE, 2007; VICENTIN, 2009; RODRIGUES, 2010) show that to date, most investigations dealing with parasites of hygiene and sanitary importance in *H. unitaeniatus*, *H. malabaricus* or *P. nattereri* have been conducted on specimens caught in aquatic environments of the Brazilian Amazon region. The only other investigations have been the studies by Martins et al. (2005) dealing with *H. unitaeniatus* and *H. malabaricus* from the swamplands of the State of Maranhão; Barros et al. (2006) with *P. nattereri* from the metropolitan area of Cuiabá, State of Mato Grosso (MT); Barros et al. (2007) with *H. malabaricus* from the municipality of Santo Antonio do Leverger, MT; and Barros et al. (2010) with *P. nattereri* in the Cuiabá river, municipality of Barão de Melgaço, MT. The most compatible previous data for comparisons with our findings are from Martins et al. (2005), who recovered samples of *Contracaecum* sp. from 80% of the specimens of *H. unitaeniatus* and from 100% of *H. malabaricus*.

Concerning *H. malabaricus*, other reports in Brazil also add data relating to the high infectivity of specimens of *Contracaecum* sp. in this fish species (KOHN et al., 1988; MADI; SILVA, 2005; MARTINS et al., 2005; PARAGUASSÚ; LUQUE, 2007). Taking into account the parasitism with *Eustrongylides* sp., only the lowest rate (58%) observed by Martins et al. (2005) is close to what was obtained in the present study. Lower rates were also detected in the State of Rio Grande do Sul by Rodrigues (2010) (32%), and in the State of São Paulo by Martins et al. (2003) (31.69%).

The fact that the highest parasite prevalence and intensities occurred in the mesentery of the three fish species, thus reflecting the significantly high mean intensity, was directly related to the marked tropism of *Contracaecum* sp. larvae, which accounted for 98.34% of the nematode specimens at this site. This characteristic behavior was also documented by Barros et al. (2010) in specimens of *P. nattereri* infected with *Contracaecum* sp. larvae, which presented 99% prevalence. Moreover, absence of these larvae in the musculature of *P. nattereri* has already been reported by

Barros et al. (2006), Vicentin (2009) and Barros et al. (2010) and in *H. malabaricus* by Barros et al. (2007) and Rodrigues (2010). Nevertheless, occurrences of *Contracaecum* sp. larvae in the musculature of *Hoplias malabaricus* were observed in Brazil by Martins et al. (2003) in the State of São Paulo and Martins et al. (2005) in the State of Maranhão, and also in Colombia by Olivero-Verbel et al. (2006) and Pardo et al. (2008).

The marked tropism of *Eustrongylides* sp. larvae towards the musculature confirms data from Barros et al. (2010) relating to specimens of *P. nattereri* in which the larvae presented 82.6% prevalence. However, Vicentin (2009) did not observe tropism of *Eustrongylides* sp. towards the musculature in 152 specimens of *P. nattereri*.

In the present investigation, the mean intensity of *Contracaecum* sp. in *H. malabaricus* was lower than the values reported in Brazil by Martins et al. (2003) (11.24), Madi and Silva (2005) (15.56) and Martins et al. (2005) (24.6); and in Colombia by Olivero-Verbel (2006) (77.82) in specimens captured in the northern coast and Pardo et al. (2008) (52.6). Nevertheless, the mean intensities of infection reported in Colombia (1.0) by Olivero-Verbel (2006) in Amazonian fish and in Brazil (2.8) by Rodrigues (2010) were lower. Considering specimens of *P. nattereri*, parasitism with *Contracaecum* sp. occurred at higher intensity in the fish examined by Vicentin (2009) and Barros et al. (2010), with mean intensities of 5.75 and 1.04, respectively.

The present data show that the mean intensity of *Eustrongylides* sp. in *H. malabaricus* was closer to 3.93 (MARTINS et al., 2009) and greater than what was previously reported (1.0) by Rodrigues (2010); in *P. nattereri*, it was closer to 2.54 (BARROS et al., 2010), and also greater than what was previously reported (1.0) by Vicentin (2009).

The variables of weight and length were also observed to influence the intensity of infection occurring in specimens of *H. malabaricus*, by Madi and Silva (2005) and Olivero-Verbel et al. (2006). In addition, a positive correlation between the prevalence of parasitism and the weight (600-750 g) of specimens of *H. malabaricus* was detected in the study by Martins et al. (2003). To explain this fact relating to parasitism by *Contracaecum* sp., Madi and Silva (2005) suggested that bigger fish accumulate greater quantities of larvae, since they frequently feed on smaller parasitized specimens and mostly escape from predation by the definitive hosts of these nematodes. The data obtained from this first investigation on specimens of piscivorous fish caught in Arari Lake confirm the role that these fish play in the life cycle of nematodes of public health interest, with high prevalence of the parasites. This may be useful for the sanitary authorities, especially those of the riverfront municipalities on Marajó Island, given that people living in these communities are, historically, the main consumers of the three fish species investigated here.

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