Infection and susceptibility of three fish species from the Paraná River, Presidente Epitácio, State of São Paulo, Brazil, to *Contracaecum* sp. larvae (Nematoda: Anisakidae)

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ABSTRACT. Infection by *Contracaecum* sp. (Nematoda: Anisakidae) in tucunaré (*Cichla ocellaris*), corvina (*Plagioscion squamosissimus*) and traíra (*Hoplias malabaricus*) collected from the Paraná River near the city of *Presidente Epitácio*, a region not yet studied in the São Paulo State, Brazil, was evaluated. The fishes were captured monthly with net and hook from August/2000 to August/2001 for parasitological analysis. The highest water temperature (26.7ºC to 28.8ºC) and the highest pluviosity rate (291.6mm) were recorded, respectively, between October/2000 and June/2001 and from November/2000 through May/2001. It was observed a prevalence up to 70% and a mean intensity of 9.5 parasites in *H. malabaricus* collected in September/2000. Prevalence, mean intensity and mean abundance of *Contracaecum* sp. larvae in *H. malabaricus* were significantly ($p<0.01$) higher than those observed in *C. ocellaris* and *P. squamosissimus*. No difference was found between *C. ocellaris* and *P. squamosissimus*. In *H. malabaricus* the highest prevalence ($p<0.05$) occurred in October, November/2000 and in June, July and August/2001. Positive correlation between parasitism and fish weight was also observed. The high susceptibility of *H. malabaricus* to *Contracaecum* sp. larvae was confirmed in the studied region.

Key words: *Contracaecum, Cichla ocellaris*, *Plagioscion squamosissimus, Hoplias malabaricus*, prevalence, mean intensity.

RESUMO. Infecção e susceptibilidade de três espécies de peixe do rio Paraná, Presidente Epitácio, Estado de São Paulo, Brasil, a larvas de *Contracaecum* sp. (Nematoda: Anisakidae). Estudou-se a infecção por *Contracaecum* sp. (Nematoda: Anisakidae) em tucunarés (*Cichla ocellaris*), corvinas (*Plagioscion squamosissimus*) e traíras (*Hoplias malabaricus*) provenientes do rio Paraná próximo à cidade de Presidente Epitácio, região ainda não estudada do Estado de São Paulo, Brasil. Os peixes foram capturados mensalmente pelo período de agosto de 2000 a agosto de 2001 para análise parasitológica. O período de outubro/2000 a junho/2001 foi o de maiores temperaturas (26,7ºC a 28,8ºC) e entre novembro/2000 e maio/2001 de maiores índices de pluviosidade (291,6mm). Observou-se prevalência de até 70% e intensidade média de até 9,5 parasitos em setembro/2000 em *H. malabaricus*. A prevalência, intensidade média e abundância média de *Contracaecum* sp. em *H. malabaricus* foram significativamente ($p<0,01$) maiores do que as observadas em *C. ocellaris* e *P. squamosissimus*. Não houve diferença entre o parasitismo nesses dois últimos hospedeiros. Em *H. malabaricus,* as maiores prevalências ($p<0,05$) ocorreram em outubro, novembro/2000 e junho, julho, agosto/2001. Observou-se correlação positiva do parasitismo com o peso dos peixes. Ficou comprovada a maior susceptibilidade de *H. malabaricus* a larvas de *Contracaecum* sp. na região estudada.

Palavras-chave: *Contracaecum, Cichla ocellaris, Plagioscion squamosissimus, Hoplias malabaricus*, prevalência, intensidade média.

Introduction

Larvae of anisakid nematodes can be observed in the muscle or adhered to the internal organs of marine and freshwater fish. The majority of papers are limited to occurrence or description reports (Wooten, 1978; Rego *et al*., 1985; Moravec *et al*.,

1985, 1995, 1997; Valtonen *et al*., 1988; Rokicki *et al*., 1993; Timi *et al*., 2001; Zdzitowiecki, 2001).

In Brazil, nematodes of the *Contracaecum* genus were observed in *Pimelodella lateristriga* and *Acestrorhamphus* sp. (Travassos *et al*., 1928); in *Astyanax bimaculatus*, *A. fasciatus* and *A. schubarti* from the Mogi Guaçu River, São Paulo (Kloss, 1966); in *Scomberomorus cavalla* and *S. maculatus* from Ceará (Klein, 1973); in *Harengula clupeola* from Bahia (Guimarães and Cristofaro, 1974); in *Macrodon ancylodon* from Maranhão (Vicente and Fernandes, 1978); in *Hoplias malabaricus* from Rio de Janeiro (Fabio, 1982); in *Pomatomus saltatrix* from Rio de Janeiro (Rego *et al*., 1983); in *Acestrorhamphus macrolepis*, *Bergiaria* sp., *Crenicichla lepidota*, *Geophagus brasiliensis*, *H. malabaricus* and *Pimelodus ortmanni* from the Basin of the Iguaçu River (Kohn *et al*., 1988); in *Pseudoplatystoma fasciatum* from Mato Grosso (Eiras and Rego, 1989); in *Galeocharax humeralis*, *G. knerii*, *Rhaphiodon vulpinus*, *H. malabaricus*, *Plagioscion squamosissimus* and *C. lepidota* from the Paraná River (Moravec *et al*., 1993); in *P. corruscans* and *Cichla monoculus* from the Paraná River, Porto Rico (Machado *et al*., 1994, 1996, 2000); in *Pagrus pagrus* and *Caranx latus* from Rio de Janeiro (Barros, 1994; Luque and Alves, 2001); in *Lutjanus purpureos* and *Coryphaena hippurus* from the Northeast of Brazil (Barros and Cavalcanti, 1998).

This work evaluated prevalence, seasonality and intensity of infection by *Contracaecum* sp. larvae in tucunaré (*Cichla ocellaris*), corvina (*Plagioscion squamosissimus*) and traíra (*Hoplias malabaricus*) collected from the Paraná River, Presidente Epitácio, São Paulo State, Brazil.

Material and methods

This work was developed in the base of the Unoeste Aquaculture Center, situated in the edges of the Paraná River, near the city of Presidente Epitácio, São Paulo state, and in the Aquatic Organisms Pathology Laboratory of the Unesp Aquaculture Center, Jaboticabal, São Paulo state. Parasitic fauna of the fishes in this region had not been studied until now. A sample of 128 tucunarés, *C. ocellaris* Bloch and Schneider, 1801 (Cichlidae), 111 corvinas, *P. squamosissimus* Heckel, 1840 (Sciaenidae) and 119 traíras, *H. malabaricus* Bloch, 1794 (Erythrinidae) were collected with net and hook from August/2000 to August/2001. The fishes were divided in size classes for analysis of total prevalence during the period.

When the samples were taken, aquatic parameters such as dissolved oxygen, temperature, electric conductivity, pH, alkalinity, transparency and pluviosity indexes were analysed. After sacrificing the fishes, the nematodes were carefully dissected from their cysts in the muscles or peritoneum, maintained in 0.65% saline solution, fixed in AFA 65ºC and preserved in alcohol 70% with 10% glycerine. Then, after dehydration and clarification with glycerine or Faia creosote, 20 specimens of each host were drawn in camera lucida. Identification was made according to Travassos *et al.* (1928), Hartwich (1974) and Vicente and Pinto (1999). The prevalence, the mean intensity and the mean abundance were calculated according to Bush *et al*. (1997). The variance analysis was performed from transformed data in arc sin SQRT (X+0.50) and average comparison by the Tukey test. Correlation analysis was employed to verify the significance among pluviosity, temperature and dissolved oxygen related to the parasite.

Results

No changes in the electric conductivity, alkalinity and transparency of water were observed. Although a slight decrease in the pH (between February and August/2001) and in the dissolved oxygen (between November/2000 and July/2001) have been related. The period of October/2000 through June/2001 showed the highest temperature variation, from 26.7ºC to 28.8ºC, and from November/2000 to May/2001, the highest pluviosity index was reported, reaching 291.6mm (Table 1).

Table 1. Pluviosity and aquatic parameters in the Paraná River, Presidente Epitácio, state of São Paulo, Brazil from August/2000 through August/ 2001

Months	Pluviosity (mm)	Oxygen (mg/l)	Temperature (ºC)	Electric conductivity (µS/cm)	pH	Alcalinity (mg/l)	Transparency (meters)
Aug/2000	64.8	9.1	23.6	50.1	7.1	27.4	2.4
Sep/2000	195.4	8.0	22.6	81.8	7.3	27.4	2.4
Oct/2000	67.0	7.3	26.7	56.4	7.1	27.4	2.4
Nov/2000	142.4	6.4	28.7	49.0	7.3	27.4	2.4
Dec/2000	215.1	6.2	27.7	49.8	7.3	27.4	2.4
Feb/2001	291.6	6.3	28.4	55.1	6.4	27.4	2.4
Mar/2001	130.6	3.7	28.2	57.0	6.4	27.4	2.4
Apr/2001	57.2	6.5	27.1	56.5	6.4	27.4	2.4
May/2001	140.8	6.5	28.8	56.6	6.4	27.4	2.4
Jun/2001	52.9	6.7	27.6	56.5	6.4	27.4	2.4
Jul/2001	31.8	6.0	23.6	57.0	6.7	29.5	2.4
Aug/2001	50.0	3.8	22.0	56.7	6.4	41.0	2.4

Table 2. Parasitological evaluation of fish infected by *Contracaecum* sp. in the Paraná River, Presidente Epitácio, State of São Paulo, Brazil from August/2000 through August/2001. IF/EF: infected fish/examined fish; P: prevalence; MI: mean intensity (variation); MA: mean abundance

Months	*Cichla ocellaris*				*Plagioscion squamosissimus*				*Hoplias malabaricus*			
	IF/EF	P (%)	MI	MA	IF/EF	P (%)	MI	MA	IF/EF	P (%)	MI	MA
Aug/00	1/8	13	2.0 (0-2)	0.1	0/10	0	0	0	2/10	20	5.0 (2-8)	1.0
Sep/00	0/10	0	0	0	1/10	10	1.0 (0-1)	0.1	2/10	20	9.5 (3-16)	1.9
Oct/00	0/10	0	0	0	0/2	0	0	0	6/10	60	1.8 (1-4)	1.1
Nov/00	0/10	0	0	0	0/10	0	0	0	5/9	56	2.4 (3-4)	1.2
Dec/00	0/10	0	0	0	0/9	0	0	0	2/10	20	3.5 (3-4)	0.7
Jan/01	0/10	0	0	0	1/7	14	2.0 (0-2)	0.2	0/0	0	0	0
Feb/01	0/10	0	0	0	0/3	0	0	0	0/10	0	0	0
Mar/01	0/10	0	0	0	0/10	0	0	0	0/10	0	0	0
Apr/01	1/10	10	3.0 (0-3)	0.3	3/10	30	3.0 (1-5)	0.9	5/10	50	4.4 (2-6)	2.2
May/01	1/10	10	1.0 (0-1)	0.1	1/10	10	1.0 (1-3)	0.1	2/10	20	3.0 (2-4)	0.6
Jun/01	0/10	0	0	0	0/10	0	0	0	5/10	50	8.2 (1-25)	4.1
Jul/01	1/10	10	1.0 (0-1)	0.1	0/10	0	0	0	7/10	70	6.8 (2-17)	4.8
Aug/01	0/10	0	0	0	1/10	10	1.0 (0-1)	0.1	5/10	50	6.6 (1-16)	3.3

The nematode identified as larvae of *Contracaecum* sp. Railliet and Henry, 1912 (Nematoda: Anisakidae) had the same characters in each host and was found encysted into the muscles or adhered to the internal organs. When fish were analysed in weight classes, a positive correlation of parasitism in *C. ocellaris* weighing 900g to 1,150g and *P. squamosissimus* weighing 800g to 1,510 g was observed, as shown in the equation in Figure 1. On the other hand, the strongest positive correlation has been verified in *H. malabaricus* weighing 600g to 750g, as also shown in Figure 1.

During this period, *C. ocellaris* showed 12% prevalence in August/2000, April, May, July/2001, while *P. squamosissimus* showed 10, 14, 30, 10 and 10% respectively in September/2000, January, April, May, August/2001. On the other hand, *H. malabaricus* presented the highest prevalence (70%) in September/2000. The mean intensity of infection (3.0) and mean abundance (0.9) in *C. ocellaris* and *P. squamosissimus* were similar in both cases (3 parasites per host), being present in four out of the 13 months contemplated in the research. However, high mean intensities (9.5 and 8.2) in *H. malabaricus* occurred in September/2000 and June/2001 respectively; high mean abundance (4.1 and 4.8) in June and July/2001, respectively (Tables 2 and 3). Statistical analysis demonstrated high prevalence; mean intensity and mean abundance of *Contracaecum* in *H. malabaricus* significantly different ($p<0.01$) of those observed in *C. ocellaris* and *P. squamosissimus*. No difference was observed between the last ones. Positive correlation ($p<0.05$) between prevalence and pluviosity in October, November/2000 and June, July, August/2001 was related in *H. malabaricus*. The correlation between prevalence and dissolved oxygen in *C. ocellaris* showed $r=0.74$. In the other hosts, the correlation was lower than $r=0.46$.

Discussion

Special consideration must be taken to the elevated indexes of pluviosity and water temperature observed in November/2000 and May/2001. The other analysed parameters did not vary in the studied period. However, this fact is to be expected when an environment of this size is being considered.

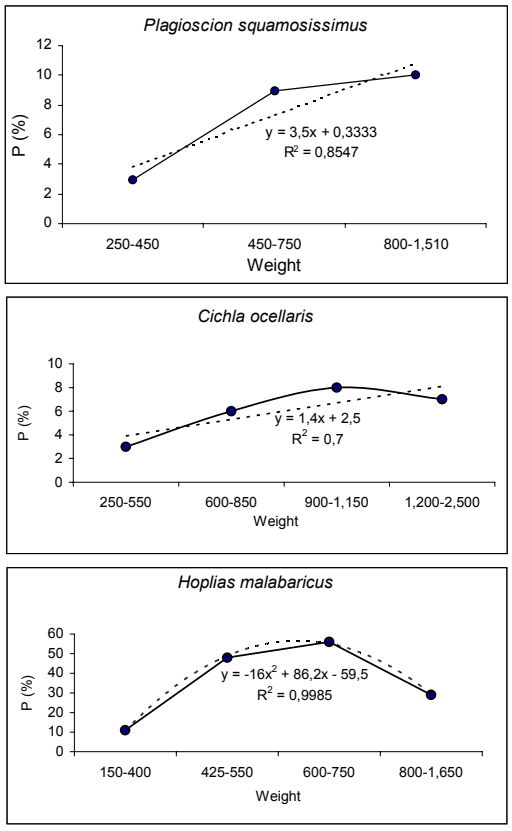

Figure. 1. Correlation between classes of weight and prevalence (P) of *Contracaecum* sp. in three fish species from the Paraná river, Presidente Epitácio, state of São Paulo, Brazil

Table 3. Statistical analysis of prevalence (P), mean intensity (MI) and mean abundance (MA) of *Contracaecum* sp. in fish from the Paraná River, Presidente Epitácio, state of São Paulo, Brazil. Different letters indicate significant difference between the hosts

Hosts	Variables		
	P	MI	MA
Cichla ocellaris	9.18 b	5.42 b	4.23 b
Plagioscion squamosissimus	6.56 b	5.65 b	4.41 b
Hoplias malabaricus	31.69 a	11.24 a	7.81 a
V.C.	76.19	46.17	33.81
F	17.10**	11.98**	15.42**
S.D.	12.05	3.43	1.85

V.C.: variation coefficient; S.D.: standard deviation; ns: not significant; **p<0.01

In this work, *H. malabaricus* showed high prevalence, mean intensity and mean abundance of *Contracaecum* sp. larvae when compared to *C. ocellaris* and *P. squamosissimus*. It was not possible to correlate parasitism with pluviosity and water temperature. This fact was due to the presence of the nematode in rainy months (October, November/2000), as well as in the drought period (June, July, August/2001). The prevalence in *H. malabaricus* was very high when compared with those observed in the literature: *S. scombrus* (8.7%) (Rego *et al*., 1985); fish from the Baltic Sea (20%) (Valtonen *et al*., 1988); fish from the Penisula of Yucatan, México (46%) (Moravec *et al*., 1995); several fish species from Venezuela (9 to 100%) (Moravec *et al*., 1997) and *Engraulis anchoita* from the Argentina and Uruguai Seas (39.7%) (Timi *et al*., 2001). High prevalence of *Contracaecum* sp. larvae was also related in *Parachaenichthys charcoti* collected from the Shetland Islands, Antarctica (Zdzitowiecki, 2001). In the present work, the distribution of parasite in *H. malabaricus* was regular during the period. Because of this fact, it is difficult to affirm any seasonality in their occurrence, although the prevalence related to the pluviosity in October, November/2000 and June, July, August/2001 was higher than the number observed in the other months. The mean intensity (9.5) and mean abundance (4.8) in *H. malabaricus* were higher than the ones related by Wootten (1978), Valtonen *et al*. (1988), Moravec *et al*. (1995, 1997) and Timi *et al*. (2001), and lower than the results obtained by Zdzitowiecki (2001) in Antarctic fish.

In Brazil, studies on the occurrence of *Contracaecum* larvae in fish during a regular sampling period are scarce. Fabio (1982) reported 64.4% in *H. malabaricus* from Rio de Janeiro; 40% in anchovy from Rio de Janeiro (Rego *et al*., 1983); 100.0% in several species of fish including *H. malabaricus* from the Basin of Iguaçu River (Kohn *et al*., 1988); in *P. squamosissimus* and *H. malabaricus* from the Paraná River (Moravec *et al*., 1993); 8.2% in *P. corruscans* from the Paraná River (Machado *et al*., 1994); up to 46.3% in porgy from the cost of Rio de Janeiro and Northeast Brazil (Barros, 1994; Barros and Cavalcanti, 1998); 30% in fishes from the Sepetiba Bay, Rio de Janeiro (Takemoto *et al*., 1996); up to 100% in *C. monoculus* from the Paraná River (Machado *et al*, 2000); 18.2% in *C. latus* from Rio de Janeiro (Luque and Alves, 2001). The variation in prevalence rates of *Contracaecum* larvae in fish from the different regions studied can be observed. In the present work, *C. ocellaris* and *P. squamosissimus* showed significantly low infection rates when compared to *H. malabaricus*. In this case *H. malabaricus* showed higher mean intensity than the one observed in other fish species from the Paraná River (Machado *et al*., 1994; 1996). On the other hand, Machado *et al*. (2000) have reported 49.1 mean intensity, compared to 9.5 in this work. However, similar results in mean intensity and mean abundance were related by Luque and Alves (2001) and the same mean intensity (9.5) by Kohn *et al*. (1988) from the same host in the Iguaçu River. Ecological studies have been made on the relationship between parasite/host depending on habits and involved fish species (Luque *et al*. 1996; Takemoto *et al*. 1996; Luque and Alves, 2001). Anisakid nematode larvae of the genus *Thynnascaris* sp. in the mesentery of *P. squamosissimus* from Volta Grande Reservoir, State of Minas Gerais, presented significant increase in the prevalence and mean intensity values during the rainy months (Martins *et al*., 2000). The same was not observed in three fish species infected by *Contracaecum* sp. from the region of Presidente Epitácio. Although Luque and Chaves (1999), Takemoto *et al*. (1996), Luque and Alvez (2001) and Machado *et al.* (2000) have not reported significant difference between length and fish parasitized by *Contracaecum* sp., this work agrees with Machado *et al*. (1994), who have observed positive correlation of the length of *P. corruscans* with *Contracaecum* sp. infection.

The results show the importance of parasite fauna evaluation involving fish collected from different regions in the same river course. It may vary according to the host, climatic conditions and parasite species. In conclusion, such research must be put in evidence when analysing mean intensity and mean abundance of fish from the other localities. Finally, not only the high susceptibility of *H. malabaricus* to *Contracaecum* larvae and the absence of relation between infection and pluviosity, but also the positive correlation of parasitized fish with age are important data to be compared with future studies in Brazil.

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