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Parasitic fauna of *Mugil curema* in a estuarine ecosystem in the northern coast of the Santa Catarina State, Brazil

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ABSTRACT

Mugilids hold a characteristic parasitic fauna that varies according to the environment they live in. Epidemiological studies of this fauna are important for prophylaxis and treatment of zoonotic diseases. The aim of this study was to investigate the parasitic fauna of *Mugil curema* for 2 years, from November 2015 to October 2017, in estuarine waters of the Northshore of Santa Catarina. A total of 282 specimens were captured with a gillnet in the Parati River, Araquari, SC, Brazil. The fish were necropsied, and its parasitic fauna studied. Tha parasite prevalence rate was 96.81% (273/282), being 97.30% (72/74) females, 94.55% (52/55) males and 97.39% (149/153) nonidentified gender. The prevalence of *Neoechinorhynchus curemai* was 43.97% (124/282), *Ergasilus lizae* 77.66% (219/282), *Lernaeenicus longiventris* 73.05% (206/282), *Ascocotyle* (*Phagicola*) *longa* in the liver 24.04% (96/282) and in the muscle 36.88% (104/282), and Haloporidae was found in 26.13% (29/111). The Parati River has peculiar characteristics on its dynamics, which interferes in the parasitological rates of the fish living there, a relation that still needs better investigation.

1. Introduction

Fish, like other animals, have their own parasitic fauna. This includes several parasite species organized in taxonomic groups. Parasite pathogenesis is multifactorial, affected by factors such as tissue or organ type, parasite type, and parasitic stress or intensity (Eiras, 2004; Luque, 2004).

Mullets, also known as mugilids, are part of the Mugilidae family of grey mullets. Culturally, they are a part of the gastronomic tradition of the Rio Parati region in Araquari, SC, Brazil, since they can be obtained easily. In this region, several preparation methods are used for the consumption of these fish; its roe is also greatly appreciated by the region's population. Traditionally, a salty, dry, and smoked fish called "cambira" is produced with mugilids. However, there is the possibility of raw fish consumption in oriental food preparations; thus, increasing zoonotic disease potential.

Parasitic fauna are directly connected to the general environmental characteristics it habituates. The Parati river and its fauna are under anthropic influence as a result of conurbation, with a population of more than 500 000 inhabitants, agroforestry, and harbor and metal mechanic industrial exploration.

The accomplishment of this study aimed to investigate the parasitic fauna of *Mugil curema* Valenciennes, 1836, along two years, from November 2015 to October 2017, in estuary waters, Parati River, Araquari SC, an affluent of the Babitonga bay complex Northshore of Santa Catarina, Brazil.

2. Material and Methods

This study aimed to investigate the parasitic fauna of a fish called *Mugil curema* (Valenciennes, 1836) for two years (November 2015 to October 2017) in Parati River, Araquari, SC, Brazil of the Babitonga Bay complex, in the north shore of Santa Catarina, Brazil.

This study was approved by the Federal Institute of Santa Catarina *Campus* Araquari Ethical Committee on the Animal Use (CEUA 107/2015) and by the Chico Mendes Institute for Biodiversity Conservation (ICMBio, SISBIO 48661-1, 48661-2 e 48661-3).

The Parati river watershed (coordinates: 26°23’07.0” S 48°45’07.0” W and 26°21’31.8” S 48°42’51.7” W) is in the north shore of the Santa Catarina State, South of Brazil, and covers 72km². The height of the shed area varies from 14 m at the source to 0 m at the river mouth, at Canal do Linguado, Babitonga Bay.

M. curema were identified according to Harrison (2003) and Menezes et al. (2015). An average of 12 fish per month were collected using a gillnet with a 7 cm spacing between knots that is 2 m high and 150 m long. The fish were carefully removed from the gillnet and transported to the IFC Campus Araquari Veterinary Diagnosis Laboratory, where they were euthanized according to the CFMV n.1000/2012 of the May 11, 2012, and the CONCEA n.13 of September 26, 2013, resolution precepts. Fish necropsy was performed, following the protocols described by Eiras et al. (2006), Jerônimo et al. (2012), and Eiras et al. (2013).

The oral, nasal, and opercular cavities of the fish were inspected to search for ectoparasites, and cutaneous mucus was scraped using a microscope slide. The scraped content was analyzed using a stereoscope. The operculum was opened, and the gills were carefully removed and inspected under a stereoscope to search for copepods. The liver was separated from the stomach and intestines, and a muscular tissue sample of approximately 5 g was taken to search for cysts holding *Ascocotyle* (*Phagicola*) *longa* metacercariae. Additionally, a muscular tissue fragment approximately 10 cm long, 3 cm wide, and 1 cm thick was obtained to search for potential nematode maggots encrusted within the muscular tissue. The intestinal contents were deposited in a petri dish and inspected under a stereoscope.

3. **Results**

The *M. curema* captured in the Parati River between November 2015 and October 2017 showed a prevalence of parasitic infestations approximating 96.81% (273/282) of the samples obtained. The parasite prevalence in female, male, and sexually unidentified *M. curema* was 97.30% (72/74), 94.55% (52/55) and 97.39% (149/153), respectively (Tables 1–3).

	Neoechinorhynchus curemai	*Ergasilus lizae*	*Lernaeenicus longiventris*	*Ascocotyle (P.) longa*		Haloporidae
				Liver	Muscle	
Females	35/74^{a} (47.30%)	63/74^{a} (85.14%)	55/74^{a} (74.43%)	22/74^{ab} (29.73%)	21/74^{a} (28.38%)	7/31^{ab} (22.58%)
Males	20/55^{a} (36.36%)	44/55^{a} (80.00%)	30/55^{b} (54.55%)	15/55^{a} (27.27%)	21/55^{a} (38.18%)	7/32^{a} (21.88%)
Non identified gender	69/153^{a} (45.10%)	112/153^{a} (73.20%)	117/153^{a} (76.47%)	59/153^{b} (38.56%)	62/153^{a} (40.52%)	15/48^{b} (31.25%)
Total	124/282 (43.97%)	219/282 (77.66%)	206/282 (73.05%)	96/282 (34.04%)	104/282 (36.88%)	29/111 (26.13%

Distinct letters indicate significant difference (p<0,05).

Table 1 *– Parasite prevalence ± standard deviation of Mugil curema captured in the Parati River, Araquari SC, from November 2015 to October 2017.*

	Neoechinorhynchus curemai	*Ergasilus lizae*	*Lernaeenicus longiventris*	*Ascocotyle (P.) longa*		Haloporidae
				Liver[(1)]	Muscle[(1)]	
Females	2.66±2.69^{a} (35)[(2)]	12.59±10.69^{a} (63)	3.61±2.89^{ab} (54)	2.68±4.07^{a} (22)	1.86±1.31^{a} (21)	10.29±6.75^{a} (7)
Males	5.85±7.65^{b} (20)	13.43±11.69^{a} (44)	2.63±1.42^{a} (35)	2.73±1.81^{ab} (15)	3.88±5.58^{b} (21)	12.00±11.30^{a} (7)
Non identified gender	3.04±2.16^{ab} (69)	10.38±16.16^{b} (112)	4.09±3.27^{b} (117)	5.07±8.47^{b} (58)	2.85±2.72^{ab} (62)	8.13±7.28^{a} (15)
Total	3.39±3.85 (124)	11.63±13.93 (219)	3.71±2.97 (206)	4.15±7.00 (96)	2.86±3.34 (104)	9.59±8.14 (29)

[1]standardized to 5 g; [2]parasitized fish; distinct letters indicate significant difference (p<0,05).

Table 2 *– Average parasite intensity ± standard deviation of Mugil curema captured in the Parati River, Araquari SC, from November 2015 to October 2017.*

	Neoechinorhynchus curemai	*Ergasilus lizae*	*Lernaeenicus longiventris*	*Ascocotyle (P.) longa*		Haloporidae
				Liver(2)	Muscle(2)	
Females (74)(1)	1.26±2.27[a]	10.72±10.83[a]	2.64±2.95[ab]	0.84±2.57[a]	0.56±1.11[a]	2.32±5.31[a]
Males (55)	2.13±5.35[a]	10.94±11.78[a]	1.97±1.70[a]	0.79±1.57[a]	1.54±3.95[a]	2.63±7.08[a]
Non identified gender (153)	1.37±2.10[a]	7.64±14.59[b]	3.12±3.34[b]	1.97±5.81[a]	1.17±2.24[a]	2.54±5.50[a]
Total (282)	1.49±3.05	9.09± 13.22	2.71±3.03	1.45±4.58	1.09± 2.48	2.50±5.90

[1]number of samples; [2]standardized to 5 g; distinct letters indicate significant difference (p<0,05).

Table 3 *– Parasite abundance ± standard deviation of Mugil curema captured in the Parati River, Araquari SC, from November 2015 to October 2017.*

From the 282 fish that were examined, 163 were parasitized by *A*. (*P*.) *longa* metacercariae in the liver and in the muscular tissue sample; in 157 fish, cysts in the liver or in the muscular tissue samples were found; in 43 fish, cysts in the liver and in the muscular tissue sample were found; in 53 fish, the cysts were found only in the liver; and in 61 fish, the cysts were found only in the muscular tissue samples.

4. Discussion

The life cycle of a parasite depends on geographic and climatic conditions, with varying temporal and spatial effects on parasite epidemiology, yielding seasonal and regional differences (Eiras, 2004; Luque, 2004). Such information supports the parasite prevalence data found in the *M. curema* captures from the Parati river between November 2015 and October 2017. The Parati river, and its fish and parasite fauna, are under the influence of the Babitonga Bay waters, rainfall rates, temperature, and relative air humidity of the region located between the Atlantic shore and the "Serra do Mar", in addition to the anthropic action previously described. Owing to this, the Parati River has its own characteristics and dynamics, which possibly interfere in the parasite prevalence rates of the fish that habituate there. However, this relationship needs to be further explored.

Previously, Alves (2014) studied metazoan parasites in *M. curema*, in the north shore of Rio de Janeiro State, Brazil and concluded that *M. curema* had low richness in parasite species compared to richness in studies of parasite fauna in mugilids from the Brazilian shore. This study partially agrees with the conclusion drawn by Alves (2014), where a relatively low richness of parasite fauna was also observed, mostly when compared to other mugilid species such as *M. liza*. However, when making these comparisons, we must consider that biota of the Parati river and the influence of Babitonga Bay in this river are unique, resulting in variation.

Campelo et al. (2013) studied how seasonal changes in the aquatic environment influence parasite rates in mugilids. Of the 180 captured mugilids in their study, 166 (92.22%) were *M. curema*. Seasonal effects in the intensity and abundance of *Ligophorus* sp. in the dry period and in *Myxobolus* sp. in the rainy season were observed; thus, there are environmental impacts in the region of Pina Bay, Pernambuco State, Brazil. In this study, the prevalence distribution pattern, parasite intensity, and abundance of *M. curema* did not reveal a pattern that suggests seasonality throughout the two years of study in the Parati River. However, in Northeast Brazil, there is a clear division in the dry period and rainy season throughout the year, which does not occur in South Brazil. However, there are strong temperature fluctuations throughout the year, which are not observed in Northeast Brazil.

4.1. Acanthocephala

A study by Cavalcanti et al. (2012) investigated the prevalence of *N. curemai* in *M. curema* from coastal waters in the Rio Grande do Norte State, Brazil and observed a 22.37% (11/54) prevalence, with varying levels of infection between males and females. Additionally, a 43.97% (124/282) prevalence was observed at Araquari, SC, in the estuarine waters of the Parati River. However, in this study, the difference in prevalence was not significant ($p < 0.05$), between the female, male and sexually unidentified fish. It was discussed that acanthocephalus presence is affected by fish migratory behavior for reproduction in the sea. Considering the methodology and results of this study, it was not possible to confirm if acanthocephalus presence is correlated to its migratory behavior.

According to Santos et al. (2013), acanthocephalus seem to regulate their population density in space through intraspecific competition. In fish examined in this study, the average intensity of acanthocephalus infection was 3.55 specimen per fish, with a standard deviation of 3.99 and a range of 30 (1-31) models. Notably, of the 282 fish examined and the 124 fish parasitized by *N. curemai*, 91 (73.39%) had a parasite intensity of five or more and only five (4.03%) fish had a parasite intensity of 10 or more. In other species such as *P. lineatus*, an average parasite intensity of 20.9 was found, with a range of 1–250 specimens per fish (Martins et al., 2001).

4.2. Crustacea

Cavalcanti et al. (2011) investigated the presence of crustacean ectoparasites in *M. curema* in the north shore of Rio Grande do Norte State, Brazil, and found that 66.66% of *Caligus bonito* and 33.33% of *Caligus* sp. *C. bonito* were found only in the males of *M. curema* during the dry season, and *Caligus* sp. were found only in the females during the rainy season; the prevalence of caligids was 3.23%. Of the detected copepods from the Ergasilidae family, *Ergasilus versicolor* (91.67%) occurred during both dry and rainy seasons while *E. lizae* (8.33%) occurred only during the rainy season. The prevalence of *E. versicolor* and *E. lizae* were 35.48% and 3.23%, respectively. The favorite mounting location of copepods and isopods was the gill chamber of *M. curema*. In this study, the ergasilid found was *E. lizae*, with a 77.66% (219/282) prevalence. There was no significant difference ($p < 0.05$) in prevalence between females, males and sexually unidentified fish. However, the prevalence was not lower than 73.20% for any of the aforementioned categories. Additionally, the parasites exclusively mounted the gill arches, supporting the study by Calvacanti et al. (2011).

The gills have a very organized anatomical and histological structure used for gas exchange, osmoregulation, and nitrogen compound excretion. They are delicate, easily irritable, and responsive to stimuli. Any environmental substance or mechanical action, such as parasites, trigger a response that may produce gill hypertrophy, edema, necrosis, and epithelial peeling, usually observed in acute lesions. Hyperplasia, secondary lamella fusion, and necrosis are observed in chronic lesions (Saraiva, 2006). In the captured fish, intense bloody mucous production was observed macroscopically when parasitized by ergasilids, proportional to the parasite intensity; this supports the aforementioned responses. Moreover, fish with fused gill lamellae were found. However, this finding was not proportional to the prevalence nor the parasite intensity of the ergasilids in *M. curema* from the Parati River. The secondary gill was always the one with the highest parasite intensity, most likely because of its morphology, which provides a kind of shelter in its recesses for the parasites.

Cavalcanti et al. (2005) studied the parasitic mechanism of the copepod ergasilid in *M. curema* from Ponta Negra, Rio Grande do Norte State, Brazil and found differences in parasite occurrence between fish gender and time of year. Cavalcanti et al. (2011) also studied the parasitic mechanism of crustaceans in *M. curema* in the Rio Grande do Norte State shore, Brazil, and found differences between season and gender. Furthermore, Campelo et al. (2013), found seasonal effects in the intensity and abundance rates of ectoparasites in *M. curema* in Pina Bacia, Pernambuco State, Brazil. In this study, the temporospatial effect in the prevalence and intensity of ergasilids in *M. curema* was not observed. Of note, it must be considered that in South Brazil, there is no division between the dry and rainy periods.

Carvalho (1953; 1957) described the parasitism of *L. longiventris* in *Xenomelaniris brasiliensis*. In this study, having more than one parasite model per fish was rarely observed. Additionally, the parasite was distributed throughout the whole body, from the peduncle to the head. We also confirmed that it is not possible to generalize the parasitism pattern of *L. longiventris* parasitism pattern to one specimen per fish since in a study by Cananéia (1952), 70 exemplars of this copepod were observed in only one blind mullet. Fonsêca et al. (2000) studied mugilids in Itamaracá PE, Brazil and observed a relative parasite abundance of 1% for *L. longiventris.* Furthermore, Masood et al. (2015) studied copepod parasitism in *M. cephalus* in Pakistan and observed that in *L. longiventris* infestation, the parasites were generally in the head, near the eyes, mouth, operculum and in the tail peduncle. Partially supporting the aforementioned descriptions, the *M. curema* specimens in this study parasitized by *L. longiventris* showed a clear preference for the fin region as a mounting location after observation from head to caudal fin; the incidence was 3.71 exemplars per fish.

4.3. Trematoda

Other than a study published by Knoff et al. (1997) at Rio de Janeiro, all other studies from 2002 onwards indicate a 99-100% prevalence of cysts holding *A*. (*P*.) *longa* metacercariae in the Brazilian shore. In this study, a 57.45% (162/282) prevalence of cysts holding *A*. (*P*.) *longa* in *M. curema* from the Parati river was found. A hypothesis that might explain the difference between the prevalence rates of the mentioned cysts in *M. curema* captured in the Parati river is the low prevalence of the first intermediate host of *A*. (*P*.) *longa*, *Heleobia australis*; this still needs to be elucidated. On the other side of the trematode epidemiological chain in the Parati river, potential predators of mugilids were observed throughout the studied period, such as potential definitive hosts of *A*. (*P*.) *longa* and mammals such as *Lontra longicaudis* and *Phocoena phocoena*.

Antunes and Almeida Dias (1994) found that 100% of *M. liza* and *M. curema* were contaminated by *A*. (*P*.) *longa* in the liver and heart, and 5.25% in the muscular tissue at Baixada Santista, Coast of São Paulo State, Brazil. It was also observed that the encysted metacercariae could be kept alive for up to three days in the fish and seven days in the prepared microscope slides, even after refrigeration. In this research, the observed prevalence of *A*. (*P*.) *longa* in *M. curema* was 34.04% (96/282) in the liver and 36.88% (104/282) in the muscular tissue samples. There is a discrepancy in the results that might be explained by the use of a different type of cyst research methodology, but what raises awareness in the 1994 study is the survival time of the cysts, even under refrigeration. This is a clear indication of the potential zoonotic risk of fish, if consumed in an inappropriate way.

Namba et al. (2012) justified the significantly higher density of *Ascocotyle* sp. metacercariae in *M. curema* viscera captured in the river region by the fact that the fish have access to the river, which substantiates the potential proximity between intermediate hosts and infectious parasitic forms. The opposite was observed in the Parati River, where the parasitic prevalence and density of *A*. (*P*.) *longa* in *M. curema* was relatively low compared to other sites of the Brazilian shore. This could have possibly occurred because of the low prevalence of the trematode first intermediate host.

Namba et al. (2012) found *Ascocotyle* sp. metacercariae in 100% of mugilids, *M. curema*, and *M. liza*, obtained from the fish trade of the city of Iguape, São Paulo, Brazil, between January 2009 and February 2010. Based on the results, these authors advised about inappropriate consumption of *M. curema* and *M. liza* and the generation of a potential zoonotic risk for the consumer population owing to the high prevalence and intensity of metacercariae in their tissues. The number of cysts holding *A*. (*P*.) *longa* metacercariae in *M. curema* from the Parati river was below the one found by Namba et al. (2012). However, it is still recommended that the fish must undergo through a thermal process before consumption.

The Haloporidae specimen found in this study were morphologically similar to the descriptions of Andres et al. (2015) for *Xiha fastigata* (Thatcher et Sparks, 1958) comb. n. Syn. *Dicrogaster fastigatus* Thatcher and Sparks (1958), and to the descriptions of Thatcher and Sparks (1958), Bargiela (1987), Knoff et al. (1997), Overstreet (1971), Lado et al. (2013), and Montes et al. (2013) for *Dicrogaster fastigatus*. Bargiela (1987) found a high correlation between parasitic intensity and fish length of *M. cephalus*, which was not observed in this study. Additionally, Knoff et al. (1997) described *D. fastigatus* parasitizing *M. liza* in the coast of Rio de Janeiro. However, there is no report about this parasite in *M. curema* in Brazil. Therefore, this is the first description of *X. fastigata*, proving and identifying the trematode parasitizing this fish species.

5. CONCLUSIONS

The local fish population studied has a characteristic behavior in epidemiological descriptors in terms of its parasitic fauna, which differentiates of other studies, in the Brazilian coast, of the same fish species.

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