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Hematological and histopathological changes in *Hoplias malabaricus* from the São Francisco River, Brazil caused by larvae of *Contracaecum* sp. (Nematoda, Anisakidae)

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Summary

Until now studies in Brazil of the pathological action of *Contracaecum* sp. larvae in *Hoplias malabaricus* have been rare. The aim of the present study was to analyze and describe histopathological changes in the stomach of *H. malabaricus* caused by different intensities of infection by larvae of *Contracaecum* sp., and the hematological effects of such infection. The mean intensity of infection by L3 larvae of *Contracaecum* sp. correlated positively with Hematocrit percentage (Hct%) and correlated negatively with mean corpuscular volume (MCV). These findings are possibly related to the passage of larvae from the stomach to the mesentery, potentially due to hemorrhaging t. Fish with high infection intensity had larvae of *Contracaecum* sp in the submucosal of the stomach, which caused destruction of Type I and Type III collagen (finer and more delicate collagen fibers which do not form beams and are permeated by fundamental substances) around the parasite. Microscopic analysis showed that crypts were arranged regularly throughout the stomach mucosal, varying in number, size and depth, depending on the region of the stomach.
Keywords: fish parasites; helminth*;* histology

Introduction

The fish species *Hoplias malabaricus* (Bloch 1794) (PISCES: ERYTHRINIDAE), popularly known in Brazil as *traíra*, or wolf fish, is widely distributed in South and Central America, occurring most commonly in hydrographic basins from Costa Rica to Argentina (Oyakawa, 2003). It is a carnivorous species, uses ambush strategies, and is essentially piscivorous in its adult phase (Hahn *et al*., 1997; Shibatta *et al*., 2002).
Studies of the hematological parameters of fish are important as they provide relevant information about the physiological capacity of such animals (Viljoen & Vuren, 1991; Ballarin *et al*., 2004; Wells *et al*., 2005), serving as a useful tool for evaluating the immunologic system (Ballarin *et al*., 2004; Tavares-Dias & Moraes, 2004; Tavares-Dias & Moraes, 2007). Hematocrit, hemoglobin concentration and red blood cell size values are important indicators of the oxygen transport capacity of water, oxygen removal rate, and oxygen availability in tissues (Graham *et al*., 1985; Tavares-Dias & Moraes, 2004; Wells *et al*., 2005).
Hematological parameters can also provide information about the health of a given fish population, and are important indicators of changes in the environment or to the physiology of these animals (Paiva *et al*., 2000a, b).
Hematological alterations in fish cultivated in Brazil have been characterized in *Cyprinus carpio* (Paiva *et al*., 1997) and *Piaractus mesopotamicus* (Tavares-Dias *et al*., 1999a) infested by *Argulus* sp.; in *Mugil platanus* infected by *Trypanosoma* sp. *Haemogregarina, Trichodina*, Monogenea, Copepods and Hirudinea (Paiva *et al*., 1997); in *Piaractus mesopotamicus* and *Leporinus macrocephalus* infected by Monogenea; in *Ichthyophthirius multifiliis*, *Trichodina* sp, *Piscinoodinium pillulare* and *Lernaea cyprinacea* (Tavares-Dias *et al*., 1999b); in *Oreochromis niloticus* infected by *Ichthyophthirius multifiliis* (Tavares-Dias *et al*., 2002); and in *Hoplias malabaricus* infected by L3 larvae of *Contracaecum* sp. (Corrêa *et al*., 2013).
Larvae of *Contracaecum* sp. have been found in various fish species, with specificity uncharacterized (Kohn *et al*.,. 1985; Vicente *et al*., 1985; Kohn & Fernandes, 1987; Moravec, 1998). These larvae pass through the wall of the digestive tract, frequently settling

in the mesentery. Specimens of *H. malabaricus* act as intermediary and/or paratenic hosts, acquiring the parasite through the ingestion of aquatic invertebrates, mainly micro crustaceans and/or through preying on other, smaller fish (Madi & Silva, 2005).

Until now studies in Brazil of the pathological action of *Contracaecum* sp. larvae in *H. malabaricus* have been rare (Martins *et al*., 2005). The aim of the present study was to analyze and describe the histopathological changes in the stomach of *H. malabaricus* caused by different intensities of infection by larvae of *Contracaecum* sp., and to analyze the hematological parameters of *H. malabaricus*, to determine the epidemiological indices of *H. malabaricus* in the São Francisco River Basin.

Material and Methods

Specimens of *H. malabaricus* (n = 54) were collected from Inhuma Lake in the municipal district of Puín, Minas Gerais, near the Serra da Canastra mountain range (20°10'53"S and 45°50'32"W) in the São Francisco River Basin. The fish were collected with gillnet fishing nets with different meshes, and the captured fish were transported to the research laboratory of CEPTA/ICMBio in the town of Pirassununga, SP (21°55'55"S and 47°22'37"W), where they were kept in a tank with running, filtered water and were observed for two weeks. The fish were transported in a 1000 liter box and artificial aerated with liquid oxygen. They were monitored every hour, with 5 – 7 milligrams held per liter and the temperature maintained between 23 to 26 °C.

The sites were georeferenced (GPS) with the authorization of ICMBio-N°. 27447-2/2010-2012. The procedures for fish collection and euthanasia were approved by the CEPTA/ICMBio Ethics Committee under protocol N° 2090-1.

Fish were collected every month from February 2010 to March 2012, alternating between the four lagoons, making a total of seven collections from each lagoon. Blood collection and hematological analysis was performed at the Continental Fish Hematology Laboratory (CEPTA/ICMBio).

After biometry, 3 ml of blood was collected from the caudal vein of each fish, using a syringe containing 25,000 UI/5mL of heparin. Blood samples were used to determine hematocrit and hemoglobin concentration levels, using the methods developed by Collier (1944) and Goldenfarb *et al*., (1971), respectively. The erythrocyte (Er) count was performed by the indirect method (Dacie & Lewis 2007). Hemoglobin concentration (Hb) was determined by the cyanomethemoglobin method and microhematocrit (Hct) by blood centrifugation of the total volume of blood (5 min/12,000 rpm) in capillary tubes. Erythrocyte indices such as mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated in accordance with Dacie and Lewis (2007).

Leucocyte and white blood cell counts were performed using blood smears stained by May-Grunwald-Giemsa, in accordance with the methods described by Dacie and Lewis (2007). Blood extensions were photographed using a Zeiss Axioplan2 photomicroscope equipped with Leica IM50 software.

Using such quantification methodology the number of leucocytes, thrombocytes, and approximately 2,000 erythrocytes were counted for each extension. The total number of leucocytes and thrombocytes was estimated from the proportion of total erythrocytes (using a Neubauer chamber), according to Hrubec and Smith (1998). Following biometry (total length and weight), the fish were necropsied, sex was determined (Vazzoler, 1996) and helminth collection was performed.

Fish were euthanized by cervical cord transection, weighed, measured (total length) and had all their organs examined. The parasites found were collected and fixed in accordance with Eiras *et al*., (2006). Following this procedure, the fish were necropsied to analyze parasites in their stomachs. A voucher specimen was catalogued in a scientific collection at the Zoology Museum of the State University of Campinas – UNICAMP- N° Zuec-nma-03.

Larvae of *Contracaecum* sp., were collected in 0.9 % saline solution, heat killed, and conserved in glycerin alcohol (Eiras *et al*., 2006). Subsequently, they were clarified with lactophenol, identified, counted, measured and photographed using the IM50 software (Leica). Identification of nematode parasites was performed in accordance with Moravec (1998).

The histological sections of the stomach were divided into three groups: Group I (control), uninfected fish; Group II, fish with low infection intensity (7 – 20 larvae) chosen randomly; Group III, fish with a high parasite load (more than 700 larvae) chosen randomly. The samples were fixed in Bouin and processed in accordance with standard histological practice (Culling *et al*., 1985) for inclusion in paraffin. Slides were produced from the fundic region of the stomach, using sections with a thickness of 5µm, stained with Hematoxylin and Eosin (H&E) and Masson`s Trichome stain. The slides were observed using a light microscope and photographed using IM50 software (Leica).

Student's *t*-test (P<0.05) was used to compare the blood parameters of parasitized and non-parasitized fish.

Nonparametric Spearman rank correlation was used to determine the infestation intensity and the relative condition factor (Kn) of fish. This procedure was performed using PROC CORR (SAS Inc. 1996). The same procedure was used to correlate the biometry of the fish, and the frequency of *Contracaecum* sp. Kn was calculated for each host, according to Gomiero and Braga (2003), using the Gnumeric 1.1 statistical program. Spearman's rank correlation was used to correlate mean infection intensity, hematological parameters and Kn.

Prevalence, mean infection intensity and mean abundance of *Contracaecum* sp was calculated in accordance with Bush *et al*., (1997). Statistical analysis was performed using the SAS (SAS Inc. 1996) program. The comparison of the mean infection intensity and biometry of *Contracaecum* sp. was analyzed using ANOVA (one criteria) with Tukey's Test (p<0.05) a posteriori. The Chi-Squared Test (X^2) was used to evaluate possible differences between the infection intensity and the sex of the fish, with a significance level of $p \leq 0.05$.

Results

The total length of fish varied from 15.0 to 38.5 cm ($\bar{x}$ = 25.7; s = ±5.26 cm) and weight varied from 45.0 to 640.0 g ($\bar{x}$ = 228.1; s = ±134.4 g).

Table 1. Variation amplitude (Ax), mean ($\bar{x}$) and Standard Deviation (s) of the blood parameters of *H. malabaricus* collected from lagoons São Francisco River, Brazil, from February 2010 to March 2012

	Parasitized fishes (n = 52)			**Non parasitized fishes (n = 2)**		
Parameters	Ax	$\bar{x} \pm S$	*p*	Ax	$\bar{x} \pm S$	*p*
Hct (%)	8 – 74	28.41 ± 13.44	<0.05	25 – 64	32.60 ± 18.31	<0.05
Er (10^6µL)	1.45 – 3.79	2.70 ± 0.40	0.028	2.3 – 3.59	2.69 ± 0.40	0.017
Lc (t) (µL)	6315 – 73474	32953 ± 16894	0.123	15150 – 59400	32192.5 ±19332.6	0.355
Tb (µL)	2,965 – 56737	15,633 ± 10,961	0.982	5800 – 27300	15155 ±9113.82	0.005
Hb (g/dL)	2.15 – 2.70	2.32 ± 0.09	0.074	2.21 – 2.43	2.32 ± 0.11	0.087
MCV (fl)	28.73 – 395.49	116.78 ± 64.94	<0.05	95.23 – 286.36	147.68 ± 91.40	<0.05
MCH (pg)	7.77 – 15.68	8.99 ± 1.67	0.173	7.62 – 10.16	8.45 ± 2.01	0.005
MCCH (%)	2.85 – 32.95	9.33 ± 4.16	0.287	3.55 – 8.78	6.80 ± 2.25	0.037

Hct = Hematocrit; Er = Erithrocity; $Lc_{(t)}$ = Total leucocytes; Tb = Trombocity; Hb = Hemoglobin; Mean Corpuscular Volume (MCV), Mean Cellular Hemoglobin (MCH); Mean Concentration of Cellular Hemoglobin (MCCH); Statistically differ between parasitized and non parasitized by *t* test (P<0.05)

Of the total examined fish, 28 were male and 26 were female. Of these, 51.36 % of males and 48.64 % of females were infected with parasites. Prevalence was 96.2 %, mean infection intensity was 196.3 parasites/fish, mean abundance was 192.1, and there were 35,926 larvae.

Erythrocytes, thrombocytes, and leucocytes were observed in the blood extensions of parasitized and non-parasitized *H. malabaricus*. The erythrocytes of the fish were elongated and elliptical, with a centralized nucleus. Thrombocytes were stick cells, with the nucleus occupying practically all of the cytoplasm. The size, shape and color of Leucocytes differed from other cell types.

The mean values of the blood parameters of parasitized and non-parasitized *H. malabaricus* are shown in Table 1.

The mean Kn of the *H. malabaricus* specimens was 1.01, indicating that conditions in the studied lagoons were healthy. Only one positive correlation was found between Kn and thrombocytes (Table 2).

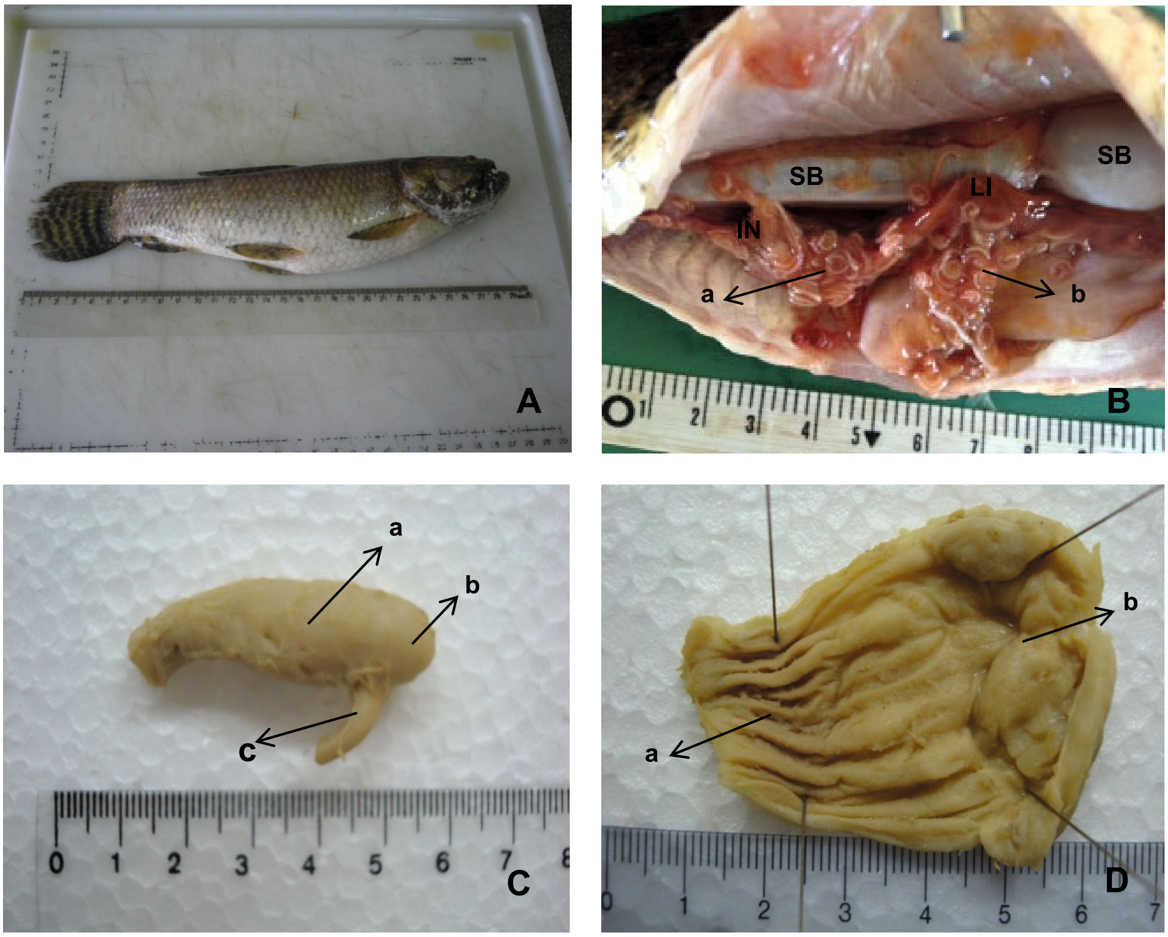

Fig. 1. **A** – *Hoplias malabaricus*. **B** – Open general cavity of *H. malabaricus* with *Contracaecum* sp.; (a) larvae in mesentery region; (b) Larvae adhered to stomach; SB – Swim Bladder; LI – Liver; IN – Intestine. **C** – Stomach of *H. malabaricus* with cardial region highlighted (a); fundic region highlighted (b) and pyloric region highlighted (c). **D** – Mucosal folds of cardial region (a) and fundic region (b)

Table 2. Spearman rank correlation (r_s) between the Relative Condition Factor (Kn) and the hematological parameters of *H. malabaricus* parasitized collected from lagoons in São Francisco River, Brazil, from February 2010 to March 2012

Eritrogram	$\bar{x} \pm S$	r_s	P
Hct (%)	28.41 ± 13.44	0.01	0.89
Er (10^6µL)	2.70 ± 0.40	-0.15	≤0.01
Tb (µL)	15,633 ± 10,961	0.25	≤0.01
MCV (fl)	116.78 ± 64.94	0.09	0.40
MCCH (%)	9.33 ± 4.16	0.01	0.92

Hct = Hematocrit; Er = Erythrocyte count; Tb = Thrombocyte count; Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCCH)

The results of the Chi-Squared Test revealed that mean infection intensity was significantly greater in male fish (χ^2 = 33.72; $p < 0.05$).

It was found that larger fish had a greater quantity of larvae of *Contracaecum* (ANOVA $F_{1,496}$= 6.93 $p < 0.0001$).

Larvae of *Contracaecum* sp. were present in in the mesentery (Fig. 1A) or adhered to the stomach, intestine, liver and/or swim bladder (Fig. 1B) of *H. malabaricus*. According to Peretti & Andrian (2008) and Morrison & Wright (1999) based on the presence, orientation and thickness of the mucosal crypts or distribution of the gastric glands, the stomach of a fish can be divided into three regions: cardial, fundic and pyloric. As such, the stomach is of a siphonal type, with cardial and fundic regions aligned on the same plane, while the pyloric region is perpendicular (Fig. 1C). The mucosal crypts of the cardiac region are parallel, extending to the fundic region (Fig. 1D). In the pyloric region, the mucosal folds are narrower and more numerous (Fig. 1D).

Lesions arising from a high intensity of *Contracaecum* sp were frequently observed in the mucosal of the fundic region (Fig. 2A). Lesions arising from *Contracaecum* sp in the mucosal of the pyloric region were not observed (Fig. 2B).

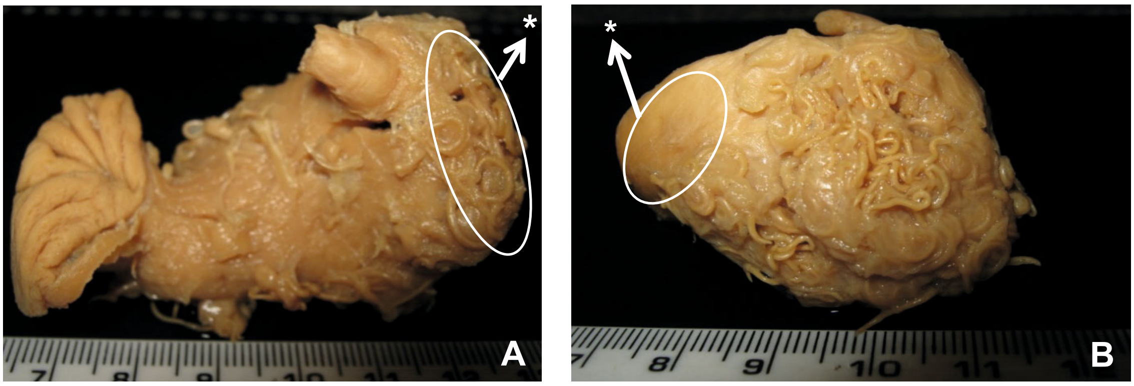

Fig. 2. **A (*)**. Fundic region of stomach of *Hoplias malabaricus* with high intensity of *Contracaecum* sp.; **B (*)**. Pyloric region of the stomach, where lesions caused by larvae of *Contracaecum* sp were not observed

An analysis of the control histological sections (Fig. 3) showed a well-developed, highly vascularized mucosal, with high, rounded folds. The slide is comprised of loose connective tissue of the tubular glands with a high number of acidophilus cells and few basophilic cells opening within the crypts. The mucosal musculature is evident, and is formed by smooth muscle fibers. The submucosal layer, which is non-glandular, is well developed and irrigated, comprised of loose connective tissue and has smooth muscle fibers. The muscular layer is formed of smooth muscle fibers and has a thinner outer longitudinal beam than the internal circular beam. The serosa has loose connective tissue with collagen fibers and nerves, and is irrigated with small vessels.

Macroscopic comparison of superimposed histological slides, following the sequence Group I, Group II and Group III, displayed gastric hypertrophy. Macroscopic analysis of the histological sections allowed comparison between the size difference of the control stomach and stomachs infected with parasites.

Microscopy revealed epithelial destruction with a reduction of glandular volume (Fig. 4). A large increase of the submucosal where the larvae of *Contracaecum* sp were found was observed. Collagen next to the mucosal was in the form of beams parallel to the same, and laterally closer to the mucosal layer. The fibers were more delicate and were not organized into characteristic beams.

Fish with a high infection intensity had larvae of *Contracaecum* sp in the submucosal of the stomach, causing destruction of Type I and Type III collagen (finer and more delicate collagen which do not form beams and are permeated by fundamental substances) around the parasite.

Microscopic analysis showed crypts regularly arranged throughout the stomach mucosal, varying in number, size and depth, depending on the region of the stomach.

Discussion

Variation in the quantity of erythrocytes, hematocrits and hemoglobins is related to the physiological aspects of the breathing and feeding habits of fish (Ranzani-Paiva *et al*., 2000). These researchers, analyzing *H. malabaricus,* found Hb values of 6.6 g.dL^{-1}. In the present study Hb values in lakes were extremely low (2.32). The mean values of infection intensity of L3 larvae of *Contracaecum* sp. correlated significantly with Hct (%) and MCV parameters, which is possibly related to the passage of larvae from the stomach to the mesentery in the form of a hemorrhage.

According to Ueda *et al*., (1997), the mean number of total thrombocytes in freshwater teleosts may vary from 2,000 to 68,400 µL^{-1} of blood. Camargo *et al*., (1986) found a variance of 28,200 to 76,000 µL^{-1} of blood in *H.malabaricus*. In the present study, the number of thrombocytes of fish in the lakes was lower, most probably due to the defense mechanism of the parasite in relation to the immune system of the host.

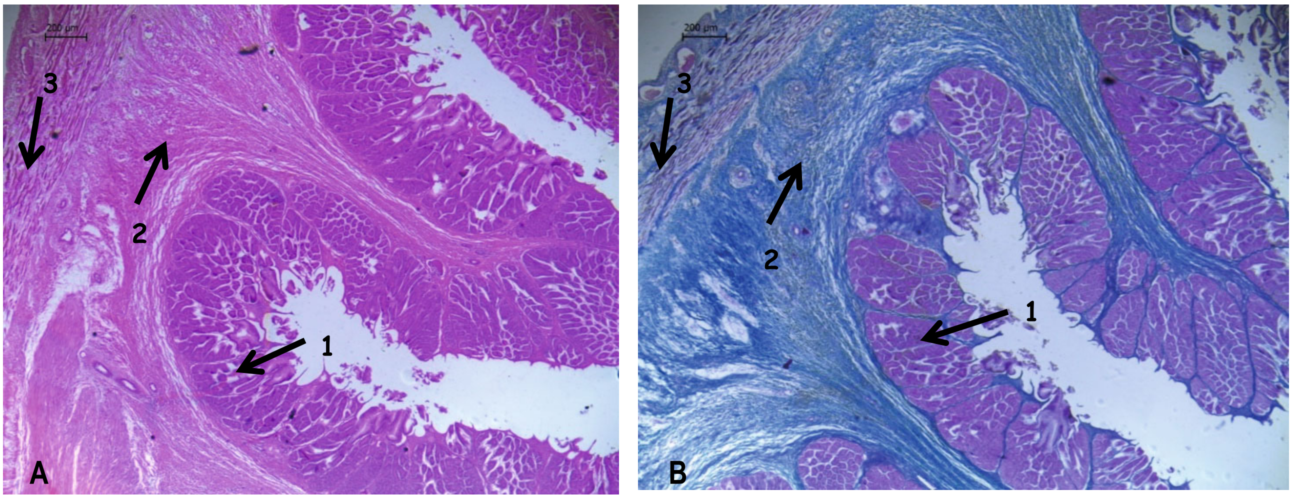

Fig. 3. Photomicrographs of histological sections of stomach *H. malabaricus*. Fixed in Bouin, stained with H&E. Increase.
A – Photomicrographs of gastric wall: 1 → mucosal; 2 → submucosal; e, 3 → muscular layer. **B** – coloring included: Masson`s Trichrome. Same arrangement as Figure 3A – with submucosal highlighted in blue. Bar= 200µm

However fish with the relative condition factor of anemia, an indicator of the degree of healthiness of fish, remained unaltered. There was no significant alteration of Kn, due to these larvae being acquired through digestion, and their accumulation may interfere significantly with the weight of fish. The aggregation of parasites in the host population may increase with the age of the host (Lizama *et al*.,. 2006).

According to these results, the highest relative condition factors were found in the more infected fish, showing that these fish are capable of withstanding a greater number of parasites, corroborating the findings of Moore (1987); Cone (1995) and Lizama *et al*., (2006).

The gastric glandular cells were not classified in the present study. Microscopic analysis revealed cells with granular content in the cytoplasm. Stoskopf (1993) found that the gastric glandules are formed of oxintopeptic cells.

A high infection intensity of *Contracaecum* sp. resulted in lesions to the tissues of the mucosal of the fundic region of the stomach of *H. malabaricus,* caused by the erosive and mechanical action of the larvae and tissue destruction, with likely digestive consequences. The large number of larvae (Group III) did not alter the pattern of the lesions, but resulted in more extensive lesions.

In relation to the presence of larvae of *Contracaecum* sp., previous studies have also found high levels of infection intensity in *H. malabaricus* (Machado *et al*., 1996; Guidelli *et al*., 2003; Martins *et al*., 2003; Madi & Silva 2005; Martins *et al*., 2005). The high prevalence of *Contracaecum* sp. observed in the present study may be due to piscivorous birds frequenting the lakes. Such birds include White crane (*Casmerodius alba*), Brazilian teal (*Amazonetta brasiliensis*), Cocoi heron (*Ardea cocoi*), Green kingfisher (*Chloroceryle americana*), Ringed kingfisher (*Ceryle torquatus*), Neotropic cormorant (*Phalacrocorax brasilianus*), American darter (*Anhinga anhinga*) and Snowy egret (*Egretta thula*).

Paraguassu *et al*., (2005) attributed a high infection intensity by larvae of *Contracaecum* sp. in *H. malabaricus* to possible preying on smaller fish, resulting in a cumulative infection. However, this study found a prevalence of 14 % of *Contracaecum* sp, which differed greatly from the values of the present study, which reported a

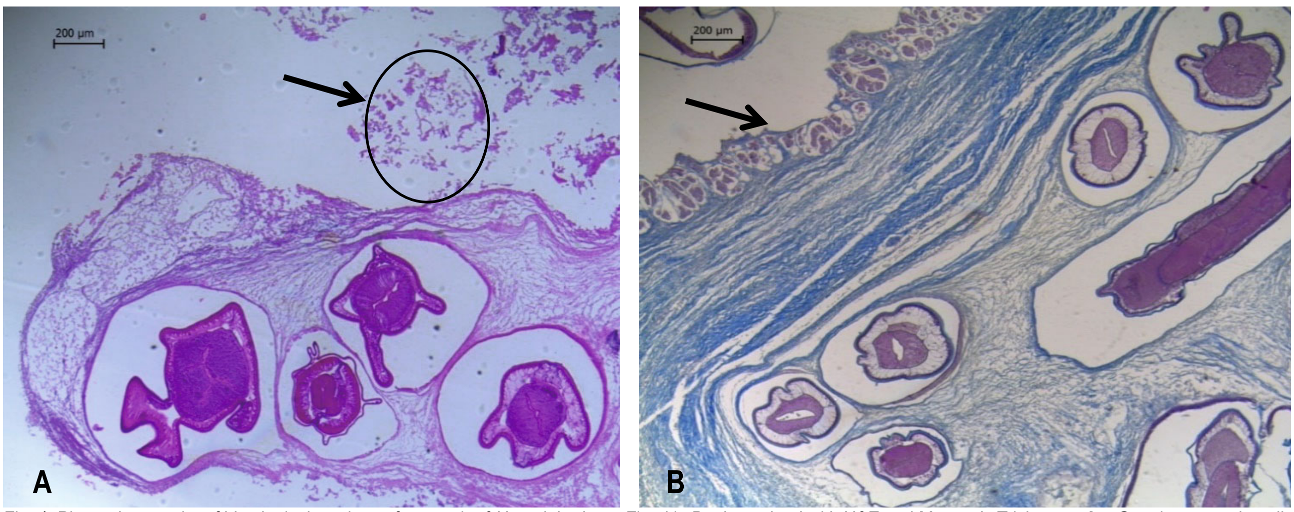

Fig. 4. Photomicrographs of histological sections of stomach of *H. malabaricus*. Fixed in Bouin, stained with H&E and Masson's Trichrome. **A** – Gastric mucosal totally and/or partially destroyed →; presence of larvae of *Contracaecum* sp., in the submucosal (hypertrophied) encapsulated larvae near loose connective tissue.
B – Coloring included: Masson`s Trichrome. Same arrangement as Figure 4A- Submucosal highlighted in blue. Bar= 200µm

prevalence of 96.2 %, mean infection intensity of 196.3 parasites/fish, mean abundance of 192.1, and 35,926 larvae.

It may be suggested that infection intensity was influenced by the sex of *H. malabaricus,* with a greater intensity among males. This finding differs from the results of Machado *et al*., (2002), who reported that a higher number of larvae among females. This divergence may be due to behavioral and dietary similarities between males and females in the study environment. Recently the studies of Corrêa *et al*., (2013) in 61 *H. malabaricus* in oxbow lakes of the Mogi-Guaçu River, showed a high abundance of 90.4 % for *Contracaecum* sp., with greater infection among males than females. In the present study, however, the abundance of the group of *Contracaecum* sp. nematodes were significantly higher among females of *H. malabaricus* than in the study by Corrêa *et al*., (2013). Other authors relate the intensity of parasites to the size and sex of the host. In a study by Loureiro *et al*., (2012) of *Cetengraulis edentulus* with the isopod parasite *Livoneca desterroensis*, from estuaries in the north of Brazil, it was found that this parasite causes more stress, resulting in a change in the growth and development of the host, although the type of parasitism was different to *Contracaecum* sp. Takemoto and Pavanelli (2000), describing aspects of the ecology of proteocephalid cestode parasites of Surubim fish (Pimelodidae), which are endoparasites. Changes were found as a result of the sex of the host, serving to corroborate the data found this role.

The results of the present study corroborate those of Von Zuben (1997), who found that the aggregation of parasites in the host population may increase with the age of the host, as larger fish possessed a greater quantity of larvae of *Contracaecum* sp.

Considering the results of the present study, it may be suggested that a high intensity of infection by larvae of *Contracaecum* sp. altered the hematological variables of *H. malabaricus,* as well as creating an imbalance in the host/parasite relationship.

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References

Ballarin, L., Dall'oro, M., Bertotto, D., Libertini, A., Francescon, A., Barbaro, A. (2004): Haematological parameters in *Umbrina cirrosa* (Teleostei, Sciaenidae): a comparison between diploid and triploid specimens. *Comp. Biochem. Physiol. A*, 138: 45 – 51. PMid:15165570. DOI: 10.1016/j.cbpb.2004.02.019

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W. (1997): Parasitology meet ecology on its own terms: Margolis *et al*.,. revisited. *J. Parasitol*., 83 (4): 575 – 583. PMid:9267395. DOI: 10.2307/3284227

Camargo, F.T., Costa, J.R.V., Souza-Cruz, A.C., Rocha, W., Vignoli, V.V. (1986): Estudo das séries hematológicas da traíra *Hoplias malabaricus* (Pisces-Teleostea). *Esc. Fam. Odont.*, 9:63 – 69

Collier, H.B. (1944): The standardization of blood haemoglobin determinations. *Can. Med. Assoc. J.*, 50: 550 – 552. PMid:20323122 PMCid:PMC1581573

Cone, D.K. (1995): Monogenea (Phylum Platyhelminthes). In: Woo P.T.K. (Ed). *Fish diseases and disorders. Protozoan and Metazoan Infections.* Wallingford: CABI: 289 – 328

Corrêa, L.L., Karling, L.C., Takemoto, R.M., Ceccarelli, P.S., Ueta, M.T. (2013): Hematological alterations caused by high intensity of L_3 larvae of *Contracaecum* sp Railliet & Henry, 1912 (Nematoda, Anisakidae) in the stomach of *Hoplias malabaricus* in lakes in Pirassununga, São Paulo. *Parasitol. Res*., 112 (8): 2783 – 2789. DOI 10.1007/s00436-013-3446-8

Culling, C.F.A., Allison, R.T., Barr, W.T. (1985): *Cellular pathology technique.* 4.ed. London: Butterworths: 642

Dacie, J.V., Lewis, S.M. (2007): *Practical haematology*. 7th ed. London: Churchill Livingstone: 556

Eiras, J.C., Takemoto, R.M., Pavanelli, G.C. (2006): *Métodos de estudo e técnicas laboratoriais em parasitologia de peixes*. Editora Universidade Estadual de Maringá, 173

Goldenfarb, P.B., Bowyer, F.P., Hall, E., Brosious, E. (1971): Reproducibility in the haematology laboratory: the microhematocrit determination. *Amer. J. Clin. Path.*, 56: 35 – 39. PMid:5556212

Gomiero, L.M., Braga, F.M.S. (2003): Relação peso-comprimento e fator de condição para *Cichla* cf. *ocellaris* e *Cichla monoculus* (Perciformes, Cichlidae) no reservatório de Volta Grande, rio Grande - MG/SP. *Acta Sci. Biol. Sci*., 25(1): 79 – 86. DOI: 10.4025/actascibiolsci.v25i1.2119

Graham, M.S., Haedrich, R.L., Fletcher, G.L. (1985): Hematology of three deep-sea fishes: A reflection of low metabolic rates. *Comp. Biochem. Physiol. A Comp. Physiol.*, 80 (1): 79 – 84. DPI: 10.1016/0300- 9629(85)90682-6

Guidelli, G.M., Isaac, A., Takemoto, R.M.; Pavanelli, G.C. (2003): Endoparasite infracommunities of *Hemisorubim platyrhynchos* (Valenciennes, 1840) (Pisces: Pimelodidae) of the Baía River, upper Paraná river floodplain, Brazil: specific composition and ecological aspects. *Braz. J. Biol.*, 63 (2): 261 – 268

Hahn, N.S., Adrian, I.F., Fugi, R., Almeida, V.L.L. (1997): Ecologia trófica. In: Vazzoler, A.E.M., Agostinho, A. A., Hahn, N. S. (Ed.) *A planície de inundação do alto rio Paraná: Aspectos físicos, biológicos e socioeconômicos*. Maringá: EDUEM, a. 209 – 228

Hrubec, T.C., Smith, S.A. (1998): Hematology of fish. In: Feldman B.F., Zinkl, J.G., Jain, N.C. (Eds) *Schalm's*. *Vet. Hematol.*, 5: 1120 – 1125

Kohn, A., Fernandes, B.M.M., Macedo, B., Abramson, B. (1985): Helminths parasites of freshwater fishes from Pirassununga, SP, Brazil. *Mem. Inst. Oswaldo Cruz*., 80 (3): 327 – 336. DOI: 10.1590/S0074-02761985000300009

Kohn, A., Fernandes, B.M.M. (1987): Estudo comparativo dos helmintos parasitos de peixes do rio Mogi Guassu, coletados nas excursões realizadas entre 1927 e 1985. *Mem. Inst. Oswaldo Cruz*., 82 (4): 483 – 500

Lizama, de Los M.A.P., Takemoto, R.M., Pavanelli, G.C. (2006): Parasitism influence on the hepatosplenosomatic and weight/length relation and relative condition factor of *Prochilodus lineatus* (VALENCIENNES, 1836) (PROCHILODONTIDAE) of the upper Paraná River floodplain, Brazil. *Rev. Braz. Parasitol. Vet.*, 15 (3): 116 – 122

Loureiro, S. N., Videira, M. N., Giarrizzo, T. (2012). Host-parasite interaction between sardines *Cetengraulis edentulus* (Cuvier, 1829) and the isopod parasite *Livoneca desterroensis* (Isopoda, Cymothoidae) in an estuary in Northern Brazil. *Rev. Pan-Amaz Saude*, 3 (2): 25 – 31

Machado, M.H., Pavanelli, G.C., Takemoto, R.M. (1996): Structure and diversity of endoparasitic infracommunities and the trophic level of *Psedoplatystoma corruscans* and *Schizodon borelli* (Osteichthyes) of the high Paraná river. *Mem. Inst. Oswaldo Cruz*., 91 (4): 441 – 448

Machado, M.H., Pagotto, J.P.A., Yamada F.H. (2002): Ocorrência de larvas de *Contracaecum* sp. (Nematoda: Anisakidae) em *H. malabaricus* (Bloch, 1794) (Pisces, Erythrinidae) do reservatório da Usina Hidrelétrica de Segredo (Bacia do Iguaçu), Paraná, Brasil. *Resumos do VII Encontro Brasileiro de Patologistas de Organismos Aquáticos*, Foz do Iguaçu, 134

Madi, R.R., Silva, M.S.R. (2005): *Contracaecum* Railliet & Henry, 1912 (Nematoda, Anisakidae): o parasitismo relacionado à biologia de três espécies de peixes piscívoros no reservatório do Jaguari, SP. *Rev. Bras. Zoociências,* 7 (1): 7 – 14

Martins, M.L., Santos, R.S., Kazuyuki, T., Marengoni, N.G., Fujimoto, R.Y. (2003): Infection and susceptibility of three fish species from the Paraná River, Presidente Epitácio, State of São Paulo, Brazil, to *Contracaecum* sp. Larvae (Nematoda: Anisakidae). *Acta Sci. Anim. Sci*., 25 (1): 73 – 78

Martins, M.L., Onaka, E.M., Fenerick Jr., J. (2005): Larval *Contracaecum* sp. (Nematoda: Anisakidae) in *Hoplias malabaricus* and *Hoplerythrinus unitaeniatus* (Osteichthyes: Erythrinidae) of economic importance in occidental marshlands of Maranhão, Brazil. *Vet. Parasitol.*, 127(1): 51-59. DOI: 10.1016/j.vetpar.2004.09.026

Moore, J. (1987): Some roles of parasitic helminths in trophic interactions. A view from North America. *Rev. Chil. Hist. Nat.*, 60 (2): 159 – 179

Moravec, F. (1998): *Nematodes of freshwater fishes of the Neotropical region*. Praga, Academia, 464

Morrison, C.M., Wright Jr., J.R. (1999): A study of the histology of the digestive tract of the Nile tilapia. *J. Fish Biol.,54:* 597 – 606

Oyakawa, O.T. (2003): Family Erythrinidae. In: Reis, R.E., Kullander, S.O., Ferraris Jr, C.J. (Ed) *Checklist of the Freshwater Fishes of South and Central America*. Porto Alegre: EDIPUCRS. 238 – 240

Paiva, M.J.T.R., Ishikawa, C.M., Campos, B.E.S., Eiras, A.C. (1997): Haematological characteristics associated with parasitism in mullets, *Mugil platanus* Günther, from the estuarine region of Cananéia, São Paulo, Brazil. *Rev. Bras. Zool*., 14 (2): 329 – 339. DOI: 10.1590/S0101- 81751997000200007

Paiva, M.J.T.R., Ishikawa, C.M., Eiras, A.A., Felizardo, N.N. (2000a): Haemotological analysis of *Chara, Pseudoplatystoma fasciatum* in captivity. Aqua 2000. Responsible aquaculture in the new millennium. Nice, France. May 2 – 6 2000*. Eur Aquacult Soc Special Publication.*, 28:590

Paiva, M.J.T.R., Souza, A.T.S., Pavanelli, G.C., Takemoto, R.M., Eiras, A.C. (2000b): Hematological evaluation in commercial fish species from the floodplin of the upper Paraná River, Brazil*. Acta Sci Biol Sci*., 22 (2): 507 – 513

Paraguassú, A.R., Luque, J.L., Alves, D.R. (2005): Metazoários parasitos do acará, *Geophagus brasiliensis* (Quoy & Gaimard, 1824), (Osteichthyes: Cichlidae) do Reservatório de Lajes, Estado do Rio de Janeiro, Brasil. *Rev. Bras. Parasitol.Vet.*, 14 (1): 35 – 39

Peretti, D., Andrian, I.F. (2008): Feeding and morphological analysis of the digestive tract of four species of fish (*Astyanax altiparanae*, *Parauchenipterus galeatus*, *Serrasalmus marginatus* and *Hoplias* aff. *malabaricus*) from the upper Paraná river floodplain, Brazil. *Braz. J. Biol*., 68 (3): 671 – 679

Ranzani-Paiva, M.J.T., Ishikawa, C.M., Eiras, A.A., Felizardo, N.N. (2000): Hematological analysis of *Chara, Pseudoplatystoma fasciatum* in captivity. Aqua 2000. Responsible aquaculture in the new millennium. Nice, France. May 2 – 6 2000. Europ Aquac Soc Special Pub., 28:590

SAS Institute Incorporation (1996): *SAS User´s Guide: Statistics*. Release 6.12. North Caroline, Cory, 1098

Shibatta, O.A., Orsi, M.L., Bennemann, S.T., Silvasouza, A.T. (2002): Diversidade e distribuição de peixes na bacia do rio Tibagi. In: Medri, M.E., Bianchini, E., Shibatta, O. A., Pimenta, J.A. (Eds) *A bacia do rio Tibagi.* Londrina: EDUEL, 403 – 423

Stoskopf, M.K. (1993): *Fish medicine. Philadelphia*: W.B. Sauders Company. 882

Takemoto, R.M., Pavanelli, G.C. (2000); Aspects of the ecology of proteocephalid cestodes parasites of *Sorubim lima* (Pimelodidae) of the upper Paraná River, Brazil: I. Structure and influence of host's size and sex.*Rev. Bras. Biol*60 (4): 577 – 584

Tavares-Dias, M., Martins, M.L., Kronka, S.N. (1999a): Evaluation of the haematological parameters in *Piaractus mesopotamicus* Holmberg (Osteichthyes: Characidae) with *Argulus* sp. (Crustacea, Branchiura) infestation and treatment with organophosphate. *Rev Bras Zool.*, 16 (2): 553 – 555. DOI: 10.1590/S0101-81751999000200019

Tavares-Dias, M., Schalch, S.H.C., Martins, M.L. (1999b): Hematologia de teleósteos brasileiros com infecção parasitária. I. Variáveis do *Leporinus macrocephalus* Garavello & Britski, 1988 (Anostomidae) e *Piaractus mesopotamicus* Holmberg, 1887 (Characidae). *Acta Sci Biol Sci*., 21 (2): 337 – 342

Tavares-Dias, M., Moraes, F.R., Martins, M.L., Santana, A.E. (2002): Haematological changes in *Oreochromis niloticus* (Osteichthyes: Cichlidae) with gill ichthyophthiriasis and saprolegniosis. *B Inst Pesca*., 28 (1): 1 – 9

Tavares-Dias, M., Moraes, F.R. (2004): *Hematologia de peixes teleósteos*. Ribeirão Preto, São Paulo: Villimpress 144

Tavares-Dias, M., Moraes, F.R. (2007): Leukocyte and thrombocyte reference values for channel catfish (*Ictalurus punctatus* Raf.), with an assessment of morphological, cytochemical, and ultrastructural features. *Vet Clin Pathol* 36:49 – 54. PMid:17311194. DOI: 10.1111/ j.1939-165X.2007.tb00181.x

Ueda, I.K., Egami, M.I., Matushima, E.R. (1997): Estudos hematológicos em *Oreochromis niloticus* (Linn.; Sassoaeus, 1758) (Cichilidae, Teleostei)- Parte I. *Braz. J. Vet. Res. Anim. Sci.*, 34: 270 – 275

Vazzoler, A.E.A.M. (1996): Biologia da reprodução de peixes teleósteos: teoria e prática. Maringá: *EDUEM.*196

Vicente, J.J., Rodrigues, H.O., Gomes, D.C. (1985): Nematóides do Brasil 1ª parte: Nematóides de peixes. *Atas Soc. Biol. Rio de Janeiro*, 25: 1 – 79

Viljoen, B.C.S., Vuren, J.H.J. (1991): Physical characteristics of the blood plasma of blood *Labeo ruddi* and *Labeo rosae* (Pisces: Cyprinidae). *Comp. Biochem. Physiol. A*, 100: 873 – 875. DOI: 10.1016/0300-9629(91)90306-W

Von Zuben, Claudio J. (1997): Implicações da agregação espacial de parasitas para a dinâmica populacional na interação hospedeiro-parasita. *Rev. Saúde Pública*, 31 (5): 523 – 530

Wells, R.M.G., Baldwins, J., Seymour, R.S., Christian, K., Brittain, T. (2005): Red blood cell function and haematology in two tropical freshwater fishes from Australia. *Comp. Biochem. Physiol. A*, 141: 87 – 93. PMid:15893948. DOI: 10.1016/j.cbpb