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Short Communication

Centesimal composition and meat yield of *Hoplias malabaricus*: association with intestinal parasites

Composição centesimal e rendimento da carne de *Hoplias malabaricus*: associação com parasitos intestinais

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Abstract

Hoplias malabaricus is a non-migratory fish commonly found in the Mogi Guaçu River basin, mainly feeding on fish, small crustaceans and insects. It forms part of the diet for humans, birds and some mammals. This fish has great nutritional value, with both good quality and good quantities of essential vitamins and amino acids. Regarding parasitic fauna, this fish can host different species of helminths in its gastrointestinal tract. The aim of the present study was to investigate the possible interference of parasitism in the meat yield from *H. malabaricus* and the centesimal composition. For this purpose, fish specimens were collected from marginal lagoons of the Mogi Guaçu River (Pirassununga, state of São Paulo, Brazil) using hooks and fishing nets. We found that all specimens of *H. malabaricus* were parasitized by at least one species, including larvae of *Contracaecum* sp. (Nematoda: Anisakidae). Parasitism did not have any significant influence on centesimal composition, but meat yield was negatively correlated with the abundance of larvae.

Keywords: Mogi Guaçu river fish, *Hoplias malabaricus*, intestinal helminths, musculature centesimal composition, meat yield.

Resumo

Hoplias malabaricus é um peixe comumente encontrado na bacia do rio Mogi Guaçu. Não realiza migração e alimenta-se de peixes, pequenos crustáceos e insetos. Faz parte da dieta de seres humanos, aves e outros mamíferos piscívoros. Apresenta grande valor nutricional em relação à quantidade e à qualidade de vitaminas e aminoácidos essenciais. Em relação à fauna parasitária, este peixe pode albergar diferentes espécies de helmintos em seu trato gastrointestinal. O presente estudo teve como objetivo verificar a eventual interferência do parasitismo no rendimento e na composição centesimal da carne do pescado de *H. malabaricus*. Para isso, foram realizadas coletas por um ano, em lagoas marginais do rio Mogi Guaçu, Pirassununga, estado de São Paulo, Brasil, utilizando-se anzol e rede de espera. Como resultado, todos os exemplares de *H. malabaricus* estavam parasitados por, pelo menos, uma espécie de parasita, prevalecendo larvas de *Contracaecum* sp. (Nematoda: Anisakidae). O parasitismo não afetou a composição centesimal da carne do peixe, mas foi encontrada uma correlação negativa forte entre o rendimento da carne e o número de larvas de parasitos.

Palavras-chave: Peixes do rio Mogi Guaçu, *Hoplias malabaricus,* helmintos intestinais, composição centesimal da musculatura, rendimento da carne.

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Introduction

Fish meat is used as food by both humans and other animals. It is rich in proteins and has a reserve of polyunsaturated fatty acids, such as eicosapentaenoic and docosahexaenoic omega 3 series, thus providing numerous benefits for its consumers (Durmuş, 2019).

Hoplias malabaricus (Bloch, 1794) (Characiformes: Erythrinidae), commonly known in Brazil as *traíra* (trahira in English), is a neotropical predatory fish with importance for both commercial and subsistence fishing (Oliveira et al., 2018). It serves as food for birds and mammals (Meneguetti et al., 2013). It has lentic and lotic habits and its diet consists of other fish and insects (when young) and their fry feeds on microcrustaceans (copepods and cladocerans) (Oliveira et al., 2018).

Hoplias malabaricus is considered to be a lean fish with low fat content (about 0.84%) and has high nutritional value due to its high protein content (Santos et al., 2001). About 800,000 kg of this fish are produced through Brazilian aquaculture annually (IBGE, 2016).

Hoplias malabaricus can be parasitized by different helminths, predominantly nematodes, such as *Contracaecum* larvae (Nematoda: Anisakidae) (Oliveira et al., 2018). The metabolic requirements of these parasites, especially regarding proteins, carbohydrates and lipids, may be reflected not only in terms of fish health, such that the consequent nutrient deficits may be detrimental for fish growth (Frantz et al., 2018), but also in terms of reduction of the nutritional value and/or meat yield from the fish (Hajji et al., 2015).

We found no available studies showing the effect of nematode infection on fish yield, therefore, the aim of the present study was to identify the gastrointestinal helminthological fauna of *H. malabaricus* from the marginal lagoons of the Mogi Guaçu River (Pirassununga, state of São Paulo, Brazil) and its influence on meat yield and centesimal composition.

Material and Methods

Specimens collection and identification

Five specimens of *H. malabaricus* were collected every month (February 2017 to January 2018) from marginal lagoons of the Mogi Guaçu River, in Pirassununga, state of São Paulo, Brazil (21° 59′ 46″ S; 47° 25′ 33″ W), using fishing nets and hooks. The fish were weighed and measured (standard length), and gender and sexual maturity (Stage A – immature; Stage B - in maturation; Stage C – mature) were determined (Vazzoler, 1996). The fish were then euthanized through pithing, and the abdominal cavity was exposed through longitudinal sectioning from the anus to the mouth. The stomach, intestine, liver and gallbladder were removed and searched for helminths. The nematodes found were collected, counted and fixed. Fish fillets were removed (always by the same handler), using a sharp knife, and fish yield and centesimal composition were compared between parasitized and non-parasitized specimens (Britto et al., 2014).

Helminth prevalence and average intensity were calculated as described by Bush et al. (1997). The helminths were screened under a stereoscopic microscope in a Petri dish containing saline solution, counted, killed by heating and placed in glycerinated alcohol or fixed in Railliet-Henry solution. They were clarified with glycerinated alcohol, lactophenol, lactic acid or methyl salicylate to view internal structures and for identification. Slides were mounted using Canadian balsam (May, 1922) for identification (Luque et al., 2011).

Relative condition factor (Kn)

The relative condition factor (Kn) (a quantitative measurement that is used to ascertain fish wellbeing) was determined through the relationship between the standard length (Ls) and the total weight (Wt) of each specimen, using the equation $W = a.L^b$ (a and b are constants, estimated through linear regression on the transformed equation $\log W = \log a + b \times \log L$). Under normal conditions, the expected value is Kn = 1. If any episode, such as parasitism, causes interference to fish health, the Kn value will vary (Tavares-Dias et al., 2010).

Analysis on centesimal composition

Centesimal composition was analyzed through measurement of humidity, total proteins, total lipids and total mineral salts. For this, groups of fishes were formed according to their intensity of infection: low (less than

100 parasites; group 1) and high (more than 100 parasites; group 2), and without parasites (control group). All analyses were done in triplicate. Since all specimens collected from the Mogi Guaçu River were parasitized, for the control group, non-parasitized fish raised in tanks at the National Center for Continental Fish Research and Conservation (CEPTA) were used.

Total humidity was calculated through the oven drying method, in which the fillets were cut and homogenized while they were frozen, dried in an oven, ground up and weighed. The samples resulting from this drying were used for total protein and total lipid analyses. Total protein content was determined following the Kjeldahl method (Horwitz, 1980). Total lipid content was determined following the methodology described by Bligh & Dyer (1959). Ash analysis was used to assess the mineral salt content, through the difference in relation to the sum of other components (% total humidity + % total protein + % total lipids + % mineral salts = 100%).

Meat yield assessment

Fish fillets, without scales or fishbones, were removed using a thin knife. Fish yield was compared between parasitized and non-parasitized specimens, as described by Britto et al. (2014).

To assess meat yield, specimens were selected based on the quantity of parasites: three specimens with less than 100 larvae (group I); three specimens with more than 100 parasites and less than 300 parasites (group II); and three specimens with more than 300 parasites (group III). Six specimens (three males and three females) without parasites were used as control groups (male control group and female control group). Yield was calculated proportionally to the fillet weight and the fish weight value.

Statistical analysis

All the data were analyzed for normality using the Shapiro-Wilk test. Parametric tests were used when the data showed normal distribution and nonparametric tests were used when the data did not have normal distribution.

Correlations between the number of *H. malabaricus* parasites and their weight, length, Kn, gender, sexual maturity and collection period were investigated through Spearman's correlation and their significances and statistical differences within each maturity stage was analyzed by Mann-Whitney test.

Analysis of variance (ANOVA) (p-value<0.05) was also used to compare the centesimal compositions of muscles with and without *H. malabaricus* infection. For statistical analysis on meat yield, analysis of variance (ANOVA) was applied. Pearson's correlation coefficient (*r)* was also used to assess whether there was any correlation between fish meat yield and the number of parasites. The statistical tests were performed using the GraphPad Prism software, v7.0.

Ethics statement

This project was approved by SISBIO, under number 57102-1 and by the Ethics Committee for Animal Use of the National Center for Continental Fish Research and Conservation (CEUA/CEPTA number 02031.000003/2017-68), as validated by the Ethics Committee for Animal Use of the State University of Campinas (UNICAMP).

Results

Specimens collected

Between February 2017 and January 2018, 59 specimens (39 females and 20 males) of *H. malabaricus* were collected and examined.

All specimens were parasitized with *Contracaecum* sp. larvae (type 2), and five specimens also carried other nematodes, one was infected with *Goezia* sp. (2 larvae) and four with *Eustrongylides* sp. (one fish with two larvae and the remaining with one larva each). Because of the small number of coinfected specimens, these were not included in the analysis. No platyhelminths or acanthocephalans were found.

Regarding *H. malabaricus* control specimens (non-parasitized), 29 specimens (17 females and 12 males) were collected and examined (Table 1).

Table 1. Biological data from specimens collected, based on their infection status gender and maturity stage (A – immature; B - in maturation; C – mature). N – Number of specimens.

	Gender	Maturity stage	N	Number of parasites	Standard length (cm)	Weight (g)
				Average	Average	Average
Infected	Male	A	1	188 ± 0	26.2 ± 0	343.0 ± 0
		B	20	155.9 ± 26.9	**26.9 ± 0.72[a]**	**391.4 ± 31.2[b.c.d]**
		Total (average)	21	157.4 ± 25.6	**26.9 ± 0.68[g]**	**389.1 ± 29.7[h]**
	Female	B	13	146.3 ± 27.4	24.3 ± 0.77	**274.3 ± 29.0[c.d]**
		C	26	207.2 ± 29.0	**26.3 ± 0.65[f]**	**383.8 ± 31.6[e]**
		Total (average)	39	186.9 ± 21.6	**25.6 ± 0.52[j]**	**347.3 ± 24.4[i]**
Uninfected control	Male	A	1	0 ± 0	22.0 ± 0	192.0 ± 0
		B	11	0 ± 0	**22.8 ± 0.92[a]**	**253.6 ± 30.8[b]**
		Total (average)	12	0 ± 0	**22.4 ± 0.62[g]**	**222.8 ± 20.9[h]**
	Female	A	3	0 ± 0	23.0 ± 0.47	233.8 ± 21.0
		B	5	0 ± 0	22.0 ± 0.85	207.0 ± 32.7
		C	9	0 ± 0	**21.9 ± 0.67[f]**	**216.7 ± 20.9[e]**
		Total (average)	17	0 ± 0	**22.3 ± 0.16[j]**	**219.1 ± 4.6[i]**

Numbers with the same letter show statistical differences between each other (p-values: a = 0.032; b = 0.0139; c = 0.0268; d = 0.038; e = 0.0005; f = 0.006; g = 0.0034; h = 0.0137; i = 0.0015; j = 0.0002).

Apart from the Kn, all other variables from Table 1 did not pass the Shapiro-Wilk test, therefore a non-parametric approach was used.

Based on gender or sexual maturity, no significant differences in the numbers of parasites infecting *H. malabaricus* were found. Regarding length and weight, both the infected males and the infected females showed significant differences, compared with uninfected fish (Table 1).

Using the Spearman correlation test, we found a positive correlation between the number of parasites and their length (r = 0.6450; p-value < 0.0001) and between the number of parasites and their weight (r = 0.6130; p-value < 0.0001).

No significant correlations were found between Kn and the number of parasites, gender, length, weight or season.

Centesimal composition

Centesimal composition analysis was done to compare the control fish (non-parasitized) (Humidity%: 78.91±0.14; Lipids%: 0.49±0.028; Protein%: 19.48±0.49; Mineral salts%: 1.12) with fish with low numbers of parasites (less than 100 parasites; group 1) (Humidity%: 79.49±1.45; Lipids%: 0.57±0.026; Protein%: 19.02± 1.62; Mineral salts%: 1.02) and with fish that were highly parasitized (more than 100 parasites; group 2) (Humidity%: 78.86±0.93; Lipids%: 0.50±0.038; Protein%: 19.55±0.66; Mineral salts%: 0.82). No statistical differences were found between the groups (Two-Way ANOVA, p > 0.05).

Fish yield

Overall, there was a significant reduction in fish yield with increasing numbers of parasites. Table 2 and Figure 1 shows the average biological values for the fish (standard length, weight, number of *Contracaecum* sp. larvae and yield from *H. malabaricus* fillets). Pearson's correlation coefficient (r) was -0.900, with a significance level (p-value) of 0.037. This indicated that there was a statistically strong, negative correlation between fish yield and the number of *Contracaecum* sp. larvae, i.e. meaning that the higher the number of parasites was, the lower the yield would be.

Table 2. Lengths of *H. malabaricus* specimens, meat weight, intensity of infection by *Contracaecum* sp. larvae. (mean ± standard error) and meat yield.

Group	Length (cm)	Weight (g)	Number of parasites	Yield (%)
Group I	20.5 ± 0.76	163 ± 18	74.3 ± 12.7	34.9 ± 2.74[a]
Group II	24.5 ± 1.6	306 ± 86	154 ± 20	26.9 ± 4.63 [b]
Group III	27.8 ± 0.92	430 ± 39	345 ± 23	23.9 ± 1.54[a.b.c]
Female control group	22.3 ± 0.88	220 ± 33	0 ± 0	32.5 ± 0.75[c]
Male control group	21 ± 0.76	184 ± 15	0 ± 0	34.7 ± 1.4[c]

Group I – less than 100 *Contracaecum* sp. larvae, Group II – 100 to 300 *Contracaecum* sp. larvae; Group III – more than 300 *Contracaecum* sp. larvae. [abc]Yield values with the same letters show statistical differences between each other (One-way ANOVA (*Fisher's LSD*); $p < 0.05$).

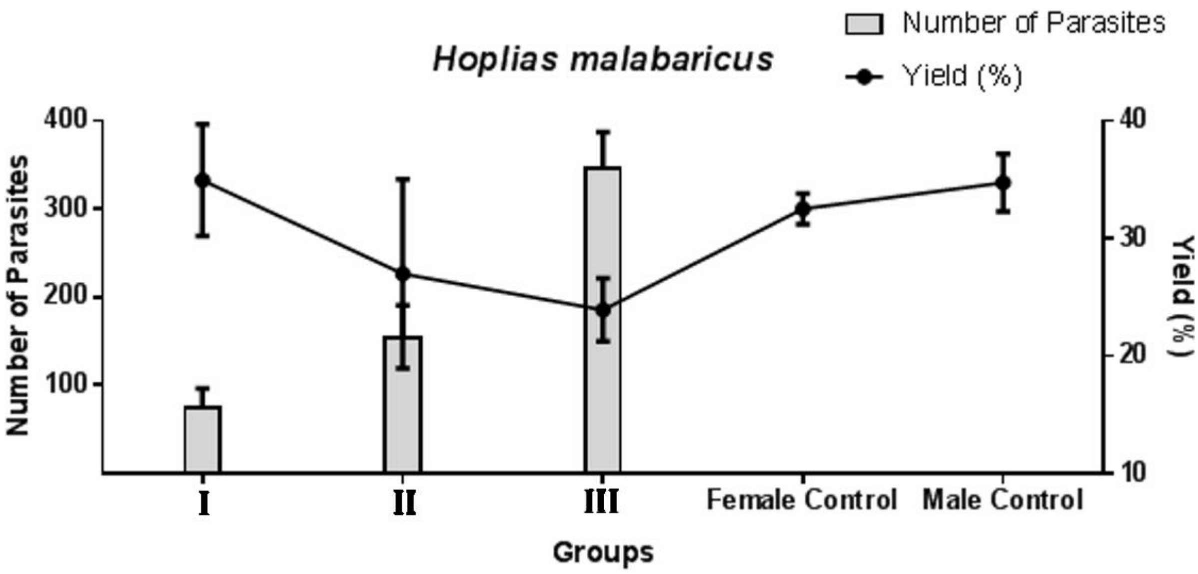

Figure 1. Graphical representation between the number of parasites and meat yield of *H. malabaricus* (Group I - less than 100 *Contracaecum* sp. larvae, Group II - 100 to 300 *Contracaecum* sp. larvae; Group III - more than 300 *Contracaecum* sp. larvae).

Discussion and Conclusion

All the specimens of *H. malabaricus* that were collected were parasitized, with predominance of *Contracaecum* sp. larvae. This was similar to the results found by Madi & Silva (2005) in the Jaguarí reservoir (São Paulo, Brazil) and by Oliveira et al. (2018) in the Eastern Amazon region (Brazil). Since all collected fish (100%) were parasitized, tank raised fish had to be used as non-parasitized control. Although using tank raised fishes might bring some limitations when comparing to specimens collected from the Mogi Guaçu marginal lagoons, the absence of statistical difference in centesimal composition between low parasitized fish and the control group suggests, at least for the analyzed parameters, this limitations might not be significant.

In this study, we also found *Eustrongylides* sp. larvae encysted in the cavity and muscles of 1% of the fish collected, while Rodrigues et al. (2017) found that 7.1% of *H. malabaricus* were positive for this nematode in flooded regions of the Maranhão lowlands (Baixada Maranhense, Maranhão, Brazil).

Polyansky & Bychowsky (1963) suggested that there might be a correlation between the number of parasites and their weight, length and Kn, in which the host body mass would have an influence on the intensity of infection, such that the higher the body mass was, the greater the number of parasites might be. Poulin (2000) also stated that older fish accumulated higher numbers of parasites and that bigger fish offered more space for parasites to become established. These authors also believed that higher infection rates would occur through ingestion of higher quantities of parasitized prey.

Guidelli et al. (2011) reported that fish parasitized by different genera of endoparasites showed lower condition factor (Kn) values than those parasitized with larval nematode stages, but without any significant difference. This finding seems to be in accordance with our results, considering that no significant difference was found between Kn nor any correlation with the number of *Contracaecum* sp. larvae.

Regarding the centesimal composition, we obtained values similar to those obtained by Santos et al. (2001) and Araújo et al. (2018), who analyzed the centesimal composition of uninfected *H. malabaricus*. In our comparison between infected and uninfected fish, we did not find any significant differences between the groups. This suggested that *Contracaecum* sp. larvae do not interfere with the centesimal composition of the meat and that, despite infection, *H. malabaricus* can still be considered to be a lean and high-protein fish. However, it is important to notice that there was a correlation between the number of *Contracaecum* sp. larvae and reduction of meat yield, which was in line with the association between the number of parasites and the fish yield, such that fish with higher numbers of parasites had lower meat yields.

There are several factors that may interfere with meat centesimal composition and/or meat yield. In this study, we assessed the effect of parasite infection, specifically by *Contracaecum* larvae, since this parasite has high prevalence and usually has high intensity of infection. Although we did not find any significant differences in the centesimal composition of infected fish meat, we did, however, find a strong negative correlation between the number of parasites and the meat yield. It is therefore important to track *Contracaecum* infection, since reduction in fish yield results in commercial losses.

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