An Acad Bras Cienc (2023) 95(Suppl. 2): e20220978 DOI 10.1590/0001-3765202320220978
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ANIMAL SCIENCE

Abundance of *Procamallanus (Spirocamallanus) inopinatus* (Nematoda: Camallanidae) in Characiformes fish and associated factors in Midwest Brazil

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Abstract: The success of *Procamallanus* (*Spirocamallanus*) *inopinatus* infection in fish involves a complexity of variables. This study aims to evaluate the relationship between abundance of *P.* (*S.*) *inopinatus* with biometric and somatic parameters, sex, relative condition factor (Kn) and hosts diet, as well as to evaluate length relationship of the parasites and the hosts. The fishes were collected by the mesh method and data, length, weight, sex, gonad and liver weight, Gonadosomatic index (GSI) and hepatosomatic index (HSI), Kn and stomach content were recorded. Twenty-seven specimens of *P.* (*S.*) *inopinatus* were collected in the intestine from *Serrasalmus rhombeus* and 52 from *Leporinus friderici*. In general, the prevalence, mean intensity and mean abundance of infection was higher in *L. friderici*. The total abundance was explained by the variables GSI, HSI total length, gonad and liver weight. Fish relative condition factor (kn) and sex were not influenced by the infection, being that the parasite infection did not impair the body condition of the hosts. There is no relationship between host length and parasite length in any of the evaluated fish species. On average, *S. rhombeus* parasites are 0.69 cm larger than *L. friderici* parasites.

Key words: Fish parasite, food guild, lotic environment, nematoda, parasitological descriptors.

INTRODUCTION

Nematoda of the genus *Procamallanus* Baylis, 1923 (Camallanidae, Procamallaninae) are predominantly parasites of freshwater fish (Moravec 1998). In South America, these parasites occur in Argentina, Paraguay, Peru, Venezuela and Brazil (Moravec et al. 1997, Kohn et al. 2011, Tavares-Dias et al. 2017, Oliveira et al. 2018, Morais et al. 2019, Ramallo et al. 2020, Rivadeneyra et al. 2020). Camallanid nematodes, such as *Procamallanus* (*Spirocamallanus*) *inopinatus* Travassos, Artigas & Pereira, 1928 infects several South American freshwater fish, including the ones living in Brazil (Neves et al. 2020). Infection by *P.* (*S.*) *inopinatus* can induce pathological lesions and reduce host fitness. *Brycon cephalus* (Characiformes) parasitized by *P.* (*S.*) *inopinatus* showed ulcer and necrosis in the intestine and decreased weight (Rivadeneyra et al. 2020). However, no harm was observed in infected *Serrasalmus rhombeus* (Linneaus, 1776) (Lima 2010) and *Leporinus friderici* Bloch, 1794 (Oliveira et al. 2017). In general, nematodes do not cause great damage to wild fish. Nevertheless, the fish health can be compromised by environmental

stress increasing their susceptibility to parasitic harm (Cohen et al. 2020).

Host fish size, considered an expression of its age, has continuous growth throughout life that, in a way, influences the size of parasitic infrapopulation, as well as the abundance of parasites, showing that this density can increase with the size and age of the host, which will allow a larger body surface area available for attachment (Poulin & Leung 2011, Tavares-Dias et al. 2014). Besides, several abiotic factors influence the parasites abundance, such as physical and chemical properties of the water, depth of the habitat, seasons (Tavares-Dias et al. 2017, Oliveira et al. 2018, Morais et al. 2019) and geographic factors (Cantatore & Timi 2015) and other factors can be influence the parasite infrapopulation size such as environmental changes, period of exposure of the fish to the parasites and type of food ingested by the host (Moreira et al. 2005, Poulin & Leung 2011, Takemoto et al. 2009, Bellay et al. 2013, Tavares-Dias et al. 2014).

Prevalence, intensity, and abundance of *P.* (*S.*) *inopinatus* are dependent on population densities of intermediate and definitive hosts present in aquatic ecosystems (Blasco-Costa et al. 2015), as well as on predator-prey interaction associated to the parasitic infection (Neves et al. 2020). Thus, acquisition and maintenance of parasites in host fish are explained by heterogeneous behavior patterns, which are considered the main factors that explain the non-uniformity of parasitic abundance (Amarante et al. 2016).

In Brazil, *P.* (*S.*) *inopinatus* was found in five orders as Characiformes, Cichliformes, Osteoglossiformes, Pleuronectiformes and Siluriformes with 71.6% of freshwater teleost species parasitized by this nematode species belong to the order Characiformes (Neves et al. 2020). The wide distribution of *P.* (*S.*) *inopinatus* in freshwater ecosystems in Brazil was associated with its low specificity for host fish (Yamada & Takemoto 2013, 2017, Morais et al. 2019, Neves et al. 2020). Nonetheless, only two records were reported in the Tocantins River basin, at the UHE Lajeado reservoirs and the São Salvador UHE reservoir (Yamada & Takemoto 2013), and the knowledge concerning parasitic fauna and host-parasite interactions of these Characiformes species remain scarce in this basin.

The knowledge of parasitic fauna of freshwater fish has made it possible to draw up lists of the biodiversity of helminths from different basins. Such information can further be used to examine the ecosystem function and evaluate their state of conservation in the neotropical zone (Salgado-Maldonado 2006, Salgado-Maldonado & Rubio-Godoy 2014). Thus, the current study aimed to evaluate the relationship between abundance of *P.* (*S.*) *inopinatus* with biometric and somatic parameters, sex, relative condition factor (Kn) and total diet of the two host fish species *S. rhombeus* and *L. friderici* from the upper Tocantins river basin, Midwest Brazil, as well as to evaluate the length relationship of the parasites and the length of the hosts. The association of organs such as liver and gonads, total length, weight, and relative body condition factor (Kn) to parasite abundance is an important method to understand the ecological relation between host and parasite (Pavanelli et al. 2013). Furthermore, the abundance of *P.* (*S.*) *inopinatus* are dependent on population densities of intermediate and definitive hosts present in aquatic ecosystems (Blasco-Costa et al. 2015), as well as on predator-prey interaction associated to the parasitic infection (Neves et al. 2020).

MATERIALS AND METHODS

The study was carried out on the Traíras River (Figure 1a-c), one of the main tributaries of the Maranhão River, both belonging to the Hydrographic Basin of the Tocantins-Araguaia River, located in the municipality of Niquelândia, State of Goiás, Brazil (Segplan 2019). Four sampling sites (S1, S2, S3 and S4) are located in the Private Reserve Legado Verdes do Cerrado (Figure 1c). This study was approved by the Chico Mendes Institute for Biodiversity Conservation - ICMBio (process n. 02010.000260/01-73) and by the Biodiversity Authorization and Information System - SISBIO (process n. 71279-1) and was developed in accordance with the principles adopted by the National Council for the Control of Animal Experimentation (CONCEA) and with approval from the Ethics Committee in the Use of Animals of State University of Goiás (N° 003 - CEUA/ UEG).

A total of 17 specimens of *S. rhombeus* and 23 specimens of *L. friderici* were collected during October 2019 and January 2020 (Tables

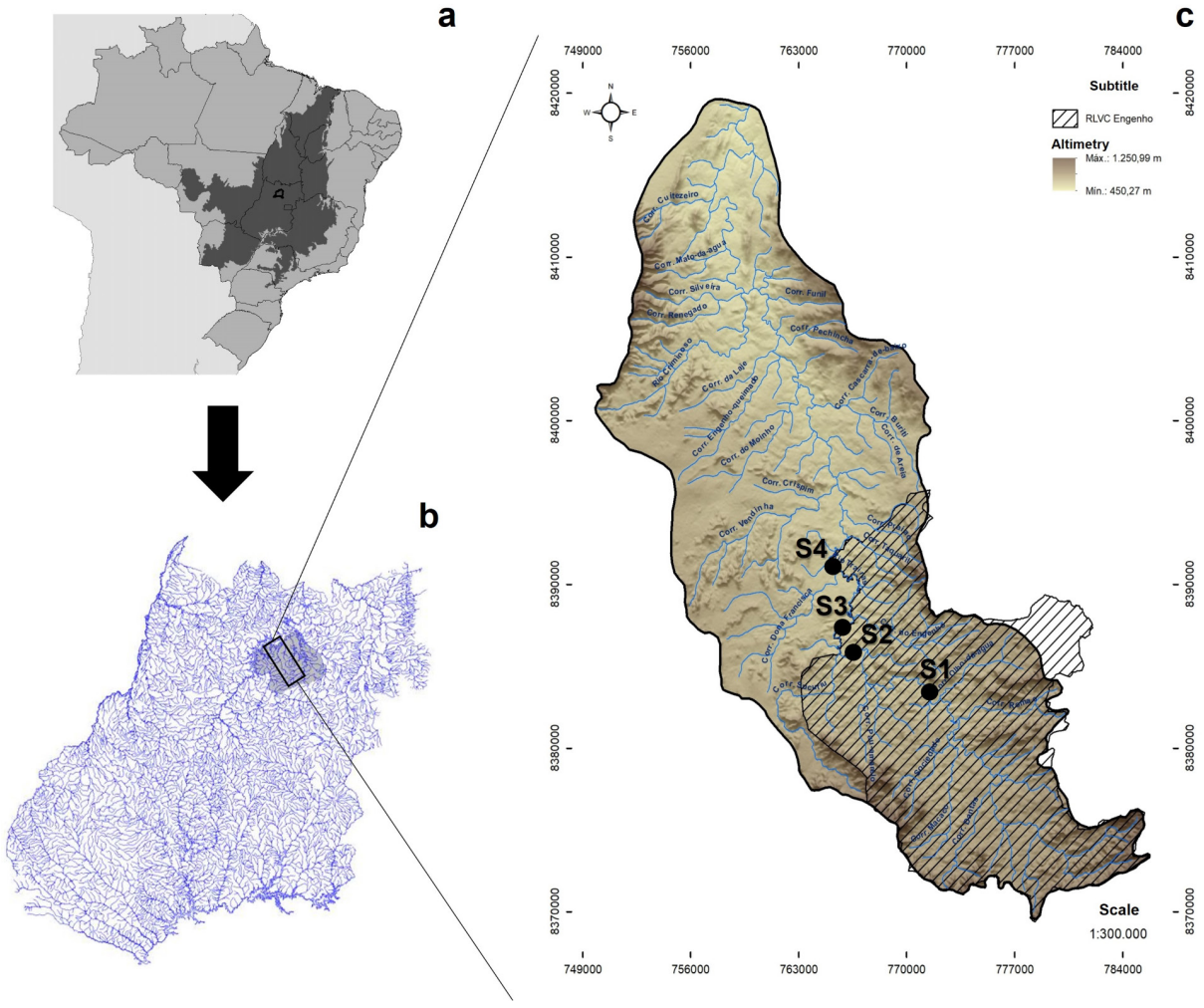

Figure 1. Sampling sites along the Traíras River basin, municipality of Niquelândia, State of Goiás, Brazil. (a) map of Brazil with delimitation of the Cerrado Biome; (b) map of the State of Goiás, with emphasis on the municipality of Niquelândia; (c) map of the Traíras River basin and sampling sites (S1 to S4) in the Private Reserve Legado Verde do Cerrado (dashed area), Niquelândia, Goiás, Brazil.

SI and SII in Suplementary Material) by the mesh method (waiting nets) using meshes with openings between 12, 15, 25, 40, 50 and 80 mm between opposite nodes accordingly to Oliveira & Tejerina-Garro (2010) with modifications. Fish species were identified according to Melo et al. (2005). Afterwards, the fish were anesthetized in clove-oil-derived eugenol (250 mg L^{-1}) in a 13-L box (45.5 x 30 x 10 cm) and euthanized by hypothermia (Aydin & Barbas 2020). Next, the biometric parameters of fish, such as total length (TL) (cm), standard length (SL) (cm), total weight (TW) (g), were measured. Fish were stored in a polystyrene box with ice and transported to the laboratory and kept in a freezer (-20 °C) until further analysis. All the fishes were defrosted and necropsied for parasites analysis. The eyes, gills, muscle and visceras were carefully dissected and individualized in a Petri dish with physiological solution (0.8% NaCl) (Eiras et al. 2006) and examined under a stereoscopic microscope (STEMI 508; Zeiss, UK).

Relative body condition factor was determined by the following equation: Kn = (observed total weight / expected total weight) (Le Cren 1951). Gonadosomatic index (GSI) and hepatosomatic index (HSI) were determined by the following equations: GSI = (gonad weight / total weight) x 100 and HSI = (liver weight / total weight) x 100 (Muniz et al. 2016), respectively. Furthermore, four gonadal maturation stages (immature, maturing, mature, and spawned) were established for females and males according to color, transparency, superficial vascularity, flaccidity, size and position in the abdominal cavity and, specifically in the case of ovaries, the degree of oocytes visualization (Vazzoler 1996).

Nematodes were collected under a stereomicroscope (STEMI 508; Zeiss), fixed by immersion in Alcohol-Formaldehyde-Acetic Acid - AFA (Ethanol 70º GL; Formalin 40%; Glacial Acetic Acid P.A. 100%) solution for 24 h, then preserved in 70% ethanol, followed by clarification with four drops of Amann's Lactophenol solution (Eiras et al. 2006). Identification and characterization of nematodes were based on dichotomous keys (Moravec 1998, Thatcher 2006), updated by articles from new records (Oliveira et al. 2017, Tavares-Dias et al. 2017, Morais et al. 2019). Nematodes were identified, and recorded using a STEMI 508 stereomicroscope (Zeiss) associated with the AxioCam 105 color camera and the ZEN Blue 2.6 software. Life stage, morphological structures, and sex were determined on a light microscope (Olympus, US).

For scanning electron microscopy (SEM) analysis, the nematodes were fixed by immersion in 10% paraformaldehyde for 4 h, washed in 0.2 M PBS buffer at pH 7.2, dehydrated in an increasing series of ethanol (70% and 100%), dried with liquid carbon dioxide (CO_2) in a critical point dryer (Autosamdri® 815 A). Then, the dry parasites were coated with gold in a Denton Vacuum Sputter Coater (Denton Vacuum, LLC, Moorestown, NJ, USA) and analyzed by a scanning electron microscope (SEM) (Jeol JSM-6610).

Prevalence, mean intensity and mean abundance of parasites were determined according to Bush et al. (1997). Thus, the prevalence was estimated by dividing the number of fish parasitized by the number of fish analyzed multiplied by 100; the mean intensity was obtained by dividing the number of parasites found by the number of parasitized fish; and the mean abundance was calculated by dividing the number of parasites found by the number of fish analyzed. Additionally, the parasites total length (TL) (mm) was measured using a microscope with an eyepiece micrometer (Olympus, US).

After dissecting the fish, stomachs were fixed by immersion in 10% formalin for 30 days

(Magnoni 2009) and subsequently kept in 70% ethanol. Stomach content analysis was based on the method of volumetric or gravimetric frequency (volume of the item in relation to total volume of food in the stomachs) (Kawakami & Vazoller 1980). For diet composition, the degree of gastric repletion (0-3) was recorded (Hyslop 1980). Food items consumed by the fish were classified into six categories: *i*) plant material - flowers and leaves (new or disintegrating); *ii*) fish - whole fish or pieces, scales, pieces of fins and skeleton; *iii*) insect - immature stages and adult insects (aquatic and terrestrial); *iv*) sediment - various granulometries and with different amounts of algae, organic matter and debris; *v*) crustacean - crab and shrimp legs, shrimp, crab, copepods; *vi*) seeds.

Student t-test was used to evaluate whether the two fish species and sex differ in relation to the (Kn) (Siegel 1975). Also, Pearson's correlation was used to assess the relationship between parasite length and host length. Monte Carlo test with 1000 randomizations was used to determine the accuracy of Student t-test. All analyses were conducted in the WPerm package in R. Furthermore, Fisher's non-parametric test was used to assess whether the abundance of parasites has any effect on the host sex. This analysis was used based on the fact that normality and homoscedasticity assumptions were not achieved according to the Shapiro-Wilk and Levene tests, respectively. The Fisher's non-parametric test was conducted in GraphPad Prism 6.

To investigate the influence of biometric parameters and total diet of the hosts on the abundance of parasites, model selections were made using the Akaike's Information Criterion (AIC) (Burhman & Anderson 2002). This analysis consists of the construction of several linear regression models with the selection of those that best explain the variable of interest. The AIC values were corrected considering the sample size (AICc), thus models with delta value AICc <2 were considered as *good models* (Burhman & Anderson 2002). Before conducting the models selection, the existence of collinearity between the biometric variables of the hosts was evaluated using the Variance Inflation Factor. Variables with VIF> 10 were considered collinear (Alin 2010). Thus, the variables used to predict abundance of parasites in *L. friderici* were length, Kn, gonad weight, GSI, liver weight, HSI and total diet. For the models of *S. rhombeus*, length, Kn, gonad weight, HSI and total diet were used. Variance Inflation Factor was calculated using the vifstep function of the usdm package (Naimi 2017) in RStudio software (R Core Team 2020). Linear regressions and model selection were performed using the Sam program (Rangel et al. 2010). The significance level of $p<0.05$ was assumed for all analyses. Graphics were made using GraphPad Prism 6 software.

RESULTS

A total of 79 specimens of *P. (S.) inopinatus* was collected in the hosts intestinal lumen, of which 27 was found in *S. rhombeus* and 52 in *L. friderici* from S1, S2, S3 and S4 (Figure 1). The total infection prevalence in *S. rhombeus* and *L. friderici* was 53% (9 parasitized/17 analyzed) and 74% (17/23), respectively. The mean intensity and mean abundance were 3.0 ± 1.7 (ranging from 1–5 parasites per infected host) and 1.59 ± 0.9 (1–2 parasites per analyzed host) in *S. rhombeus*, and 3.06 ± 1.0 (2–4 parasites per infected host) and 2.26 ± 0.6 (1–3 parasites per analyzed host) in *L. friderici,* respectively. Biometric parameters and somatic indexes of both host fish (*S. rhombeus* and *L. friderici*) are recorded (Table SI, SII).

To assess whether infection by *P. (S.) inopinatus* influenced the biometric parameters and somatic indexes of the hosts, several

models were generated by linear regression to explain the abundance of parasites in *L. friderici* (n = 127 models) and in *S. rhombeus* (n = 31 models). The best model to explain the parasitic abundance in *L. friderici* includes the variables TL, gonad weight, GSI, liver weight and HSI (R^2 = 0.65; delta AIC = 0). The length proved to be the most important variable among the linear regression models for *L. friderici* (Importance = 0.91). However, when this variable was analyzed independently, it was not considered ideal to explain the abundance of parasites (R^2 = 0.14; delta AIC = 6.67), as it was not significant (rs = -0.07; p = 0.73). In opposition, the best model for *S. rhombeus* included only the gonadal weight (R^2 = 0.08; delta AIC = 0) (Importance = 0.38). Nevertheless, another four models with unique variables were also considered to be relevant for *S. rhombeus* (delta AIC <2), and include total diet (R^2 = 0.06; delta AIC = 0.48) (Importance = 0.30), HSI (R^2 = 0.03; delta AIC = 1.01) (Importance = 0.25), total length (R^2 = 0.007; delta AIC = 1.46) (Importance = 0.24) and Kn (R^2 = 0.004; delta AIC = 1.51) (Importance = 0.21).

Parasitic abundance did not influence the IGS of *L. friderici* (R^2 = 0.009; delta AIC = 10,055), while the IGS of *S. rhombeus* was not selected for analysis with the linear regression models because it was excluded by the Variance Inflation Factor (VIF> 10). Regarding *L. friderici* gonadal maturation stages, 41.18% were in immature stage, 5.88% in maturing stage, 17.65% mature stage and 35.29% in spawned stage.

Results showed high percentage of *S. rhombeus* (58.8%) and *L. friderici* (73.9%) infected by *P.* (*S.*) *inopinatus*. Among the infected *S. rhombeus,* 55.6% were male and 44.4% female, while 41.2% of infected *L. friderici* were male and 58.8% female. However, total parasite abundance did not differ significantly between sexes of both fish hosts (Fisher's exact test, p = 0.26).

The relative condition factor (Kn) of *L. friderici* (1.00 ± 0.15) and *S. rhombeus* (1.01 ± 0.11) showed no significant difference between infected and non-infected fish (t = 0.028; p = 0.97) (Figure 3).

Serrasalmus rhombeus presented a carnivore feeding behavior ingesting mainly small fish and insects, while *L. friderici* ingested a wide variety of food items, such as plant material, terrestrial insect, crustacean, sediment, fish and seeds (Table II). Food items and their volumes varied between fish species from the same site and among each fish species from different sampling sites. The total diet of *S. rhombeus* influenced the abundance of parasite (R^2 = 0.063; delta AIC = 0.485) (Importance = 0.30). However, total diet did not change the parasitic abundance in *L. friderici* (R^2 = 0.002; delta AIC = 10.231).

There are significant differences in the length of the infrapopulations of *P.* (*S.*) *inopinatus* between the two host species (t = -0.69; p = 0.0012). *Serrasalmus rhombeus* parasites are 0.69 cm larger than *L. friderici* parasites. However, there were no relationship between the length of the parasites and the length of the hosts *S. rhombeus* (r = 0.07; p = 0.84) and *L. friderici* (r = 0.18; p = 0.51).

Morphological characteristics of *P.* (*S.*) *inopinatus* in *S. rhombeus* and *L. friderici* (Figure 2) agreed with the descriptions given by Moravec (1998). Larger sized nematodes with almost smooth cuticle. Oral openning circular, surrounded by eight submedian cephalic papillae arranged in two circlets and small lateral amphids, and an orange-brown and thick-walled buccal capsule approximately as long as wide. The inner oral surface was provided with numerous thin spiral thickenings, which occupied no more than 2/3 of the buccal capsule. Muscular oesophagus expanded at their posterior part was observed. Gravid

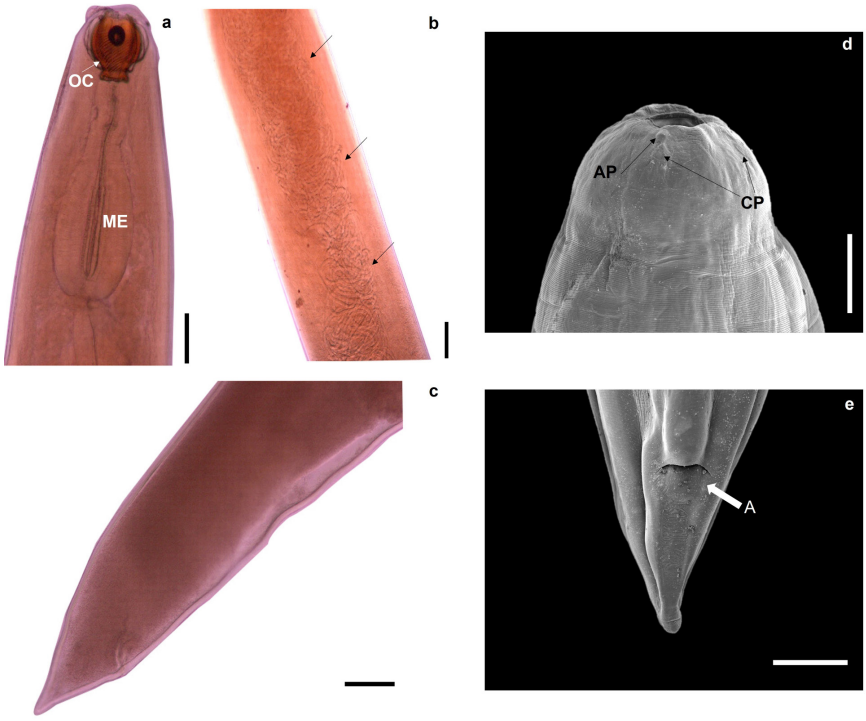

Figure 2. ***Procamallanus* (*Spirocamallanus*) *inopinatus* in *Serrasalmus rhombeus*, micrographs at light microscope (a-c) (lateral view of a gravid female) and *Leporinus friderici* scanning electron microscopy (SEM) (d) (subapical view) and (e) (ventral view). a) Anterior region; b) Uterus with numerous larvae (black arrows); c) Posterior region. d) Cephalic papillae (CP), amphids (AP) of a female. e) tail of the female, in detail the disposition of the anus (A). Abbreviations: OC, Oral Capsule; ME, Muscle Esophagus. Bar scale: a, c = 50 µm; b = 100 µm; d-e = 50 µm.**

females showed uterus containing eggs and larvae located in the anteromedial region. Non-gravid females showed uterus not containing eggs and larvae. Adult male (Table I) presented small conical tail, spicules well sclerotized and gubernaculum absent.

DISCUSSION

Studies have demonstrated the infection and distribution patterns of *P.* (*S.*) *inopinatus* in freshwater teleost fish in Brazil (Oliveira et al. 2017, Morais et al. 2019, Ailán-Choke et al. 2020, Neves et al. 2020). Our findings contribute to the first record of *P. (S.) inopinatus* from the upper Tocantins river basin, Midwest Brazil. Prevalence of *P. (S.) inopinatus* in *S. rhombeus* (53%) and in *L. friderici* (74%) observed in this study was similar to that reported in *L. friderici* (76.6%) from the Amazon River basin (Oliveira et al. 2017), while higher prevalence (90%) was observed in *L. friderici* from the Paraná River basin (Feltran et al. 2004) and in *Astyanax altiparanae* (73.3%) from the Paraná River basin (Doro Abdallah et al. 2012). In addition, high prevalence (100%) also was observed in *Pygocentrus nattereri* Kner, 1858 in delta lakes in Central Amazon (Morais et al. 2019). However, was reported low prevalence of *P. (S.) inopinatus* in *Serrasalmus marginatus* Valenciennes, 1837 (5.49%) and *Pygocentrus nattereri* (22.37%) in the "Rio Negro", Central Pantanal (Vicentin et al. 2011), as well as in *Colossoma brachypomum* (Cuvier, 1818) (1.7% to 25.7%) from fish farms in the State of Amapá (Dias et al. 2015) and in *Cichlasoma bimaculatum* (5.4%) in the Igarapé Fortaleza, State of Amapá (Tavares-Dias et al. 2017). This data show heterogeneity of results regarding the prevalence of *P. (S.) inopinatus* in the Brazil Characiform fish. Parameters related to trophic levels, life histories, and geographic distributions of fish in Brazilian watersheds are important for understanding infection prevalence levels (Neves et al. 2020).

Our data showed similar mean intensity between *S. rhombeus* from site S1 and in *L.*

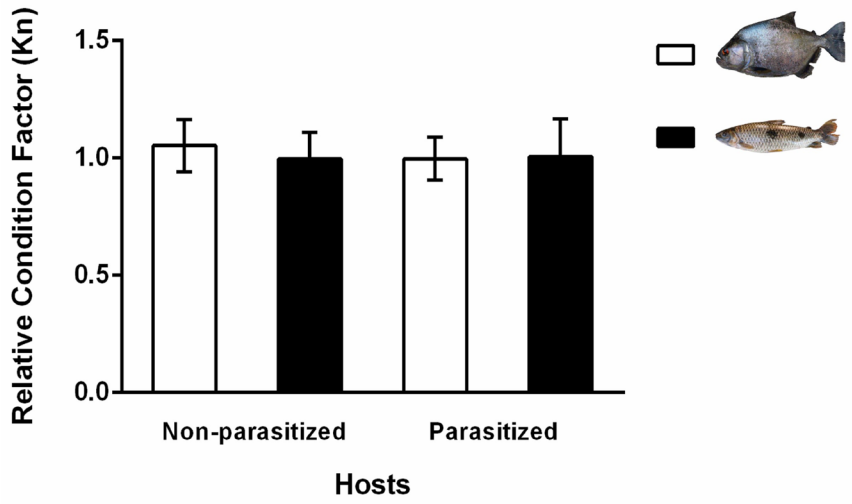

Figure 3. Relative condition factor (Kn) of *Leporinus friderici* (1.00 ± 0.15) and *Serrasalmus rhombeus* (1.01 ± 0.11) not infected and infected by *Procamallanus* (*Spirocamallanus*) *inopinatus*. Fish were collected in the Traíras river, upper Tocantins river basin, Goiás. Results are expressed as mean values ± 95% confidence intervals.

friderici from site S4 with value 4. The mean abundance was higher in *L. friderici* from site S1. The difference between sampling sites was associated with the variability in the food items consumed by the fish resulting in greater or lower ingestion of intermediate hosts infected by *P. (S.) inopinatus*. Both parasitological descriptors were higher than those reported for *S. rhombeus* (Lima 2010) and *L. friderici* (Oliveira et al. 2017). We can infer that the presence of food itens at as insects (Moreira et al. 2005, Costa Silva et al. 2019), microcrustaceans and molluscs (Camargo et al. 2016, Oliveira et al. 2017) that they are certainly a probable hosts intermediate for *P.* (*S.*) *inopinatus*, and these endoparasite metazoans are usually acquired by ingestion due to their indirect and long life cycle. Thus, larger hosts support a higher degree of infection by these parasites because such parasites are not pathogenic and cause little damage to the host (Hoshino & Tavares-Dias 2014).

To understand the parasite-host-environment relationship, data from host fish such as body length has been considered an important variable. Fish with longer lengths provides a larger surface area for attachment and are considered a more stable habitat for the parasites that, often display a higher longevity compared to the ones living in small fish (Pavanelli et al. 2013). Additionally, small fish offer insufficient habitat spaces for the parasites (Hoshino & Tavares-Dias 2014). On the other hand, there is agreement in the literature on the relationship that the parasites can have a short life cycle and therefore are constantly infected and eliminated by the hosts (Hoshino 2013, Tavares-Dias et al. 2017, Morais et al. 2019). For example, *L. friderici* from Igarapé Fortaleza, a tributary of the Jari River, showed a positive correlation between total length and abundance of *P. (S.) inopinatus* (Oliveira et al. 2017). On the other hand, studies conducted with *Metynnis lippincottianus* (Cope, 1870) from Igarapé Fortaleza, tributary of the Amazon River, Amapá, Brazil, showed that the abundance of *P. (S.) inopinatus* parasites was higher in small fish (Hoshino 2013), and no effect interaction of fish length and parasitic abundance was observed (Hoshino & Tavares-Dias 2014). This pattern was also observed in fish from the Paraná River (Franceschini et al. 2013, Camargo et al. 2016) and another a tributary of the Amazon River (Tavares-Dias et al. 2017), in which helminth abundance was associated with behavioral factors of the parasite such as a short life cycle or rapid elimination during your passage

Table I. The total length mean (mm) (mean ± SD) in 26 (XXVI) infrapopulations of *Procamallanus (Spirocamallanus) inopinatus* of *Leporinus friderici* (I-XVII) and *Serrasalmus rhombeus* (I-IX) from the Traíras river, upper Tocantins river basin, Goiás. N: Number of parasitized hosts, nº: number gravid females, n.o.: no observation, (n): number of parasites collected from each hosts.

Hosts Characicformes and infrapopulations (n)		Mean length		Gravid females	
	N	Non-gravid Female	Male	n°	Mean length
Leporinus friderici	17				
I (2)		-	-	2	12.99±8.5
II (2)		n.o.	-	2	n.o.
III (3)		-	-	3	25.73±4.6
IV (2)		-	-	2	23.85±0.4
V (1)		-	-	1	18.40±0
VI (1)		-	-	1	20.32±0
VII (1)		-	-	1	17.95±0
VIII (1)		-	-	1	20.77±0
IX (1)		-	-	1	19.25±0
X (4)		-	-	4	20.89±1.6
XI (1)		-	-	1	21.32±0
XII (1)		13.67±0	-	0	-
XIII (1)		-	-	1	17.32±0
XIV (21)		n.o.	n.o.	0	-
XV (3)		-	15.87±0	-	-
XVI (2)		-	-	2	19.86±1.4
XVII (5)		11.36±3.9	-	0	-
Serrasalmus rhombeus	9				
I (5)		-	-	5	30.95±2.8
II (7)		-	-	7	27.35±5.9
III (2)		n.o.	-	2	n.o.
IV (1)		-	-	1	20.21±0
V (3)		27.91±0.9	-	0	-
VI (2)		-	-	2	25.68±6.0
VII (3)		-	-	3	26.27±6.0
VIII (2)		-	-	2	20.84±0.5
IX (2)		n.o.	-	2	-

through the host's digestive tract. Furthermore, as observed by Franceschini (2013) and Tavares-Dias et al (2017), helminths abundance did not interfere with fish Kn. Even in the case of *S. rhombeus* in which parasites were 69% larger than parasites from *L. friderici*, the fish health conditions remained the same regardless of infection.

The host fish sex did not influence the mean abundance and mean intensity of *P.* (*S.*) *inopinatus* in this study. However, the abundance of this parasite in *S. rhombeus*, from floodplain lakes in Central Amazonia was higher in male fish (Lima 2010). Meanwhile, *P.* (*S.*) *inopinatus* found in marine and freshwater fish hosts, showed higher mean abundance in female hosts (Amarante et al. 2016).

Although *P.* (*S*) *inopinatus* infection did not influence the GSI of *S. rhombeus* and *L. friderici*, higher abundance of parasite was observed in fish with immature and maturing gonadal stages compared to other gonadal stages. Alterations on GSI related to parasite abundance has been described in *Eustrongylides* sp. larvae (Kaur et al. 2013) and *Philometra* sp. (Selvakumar et al. 2014). The positive correlation could be associated to the fact of the reproductive period is a stressful phase for the fish, as they become more susceptible to infections (Pavanelli et al. 2013). On the other hand, the current study demonstrated that *P. (S.) inopinatus* infection has no effects on GSI of both host fish.

Food contain observed in *S. rhombeus* and *L. friderici* (i.e., plant material, fish, insect, sediment, crustacean, seeds) were similar to

Table II. Volume (cm³) of food items obtained from the stomachs of *Leporinus friderici* and *Serrasalmus rhombeus* from the Traíras River, upper Tocantins River Basin, Goiás. N: Number of stomachs analyzed, n.o.: no observation. Results are express as mean ± SD.

Hosts Characicformes and food items (cm³)	N	Sites			
		S1	S2	S3	S4
Leporinus friderici	23				
Plant material		6.1 (0.9 ± 0.7)	n.o.	0.5 (0.5 ± 0)	4.45 (2.2 ± 1.4)
Fish		0.9 (0.5 ± 0.1)	n.o.	n.o.	n.o.
Terrestrial insect		3.7 (0.5 ± 0.5)	n.o.	n.o.	0.3 (0.3 ± 0)
Sediment		0.8 (0.2 ± 0.2)	n.o.	n.o.	0.2 (0.2 ± 0)
Crustacean		1.6 (0.8 ± 1.0)	n.o.	n.o.	n.o.
Seeds		0.2 (0.2 ± 0)	n.o.	n.o.	n.o.
Total		13.3	n.o.	0.5	4.95
Serrasalmus rhombeus	17				
Plant material		n.o.	0.2 (0.2 ± 0)	n.o.	n.o.
Fish		8.4 (2.8 ± 2.8)	20.8 (5.2 ± 4.4)	4.1 (4.1 ± 0)	1.7 (1.7 ± 0)
Terrestrial insect		n.o.	n.o.	1.3 (1.3 ± 0)	2.1 (2.1 ± 0)
Total		8.4	21.0	5.4	3.8

those reported for *S. rhombeus* in "Solimões" floodplain lakes (Lima 2010) and in *L. friderici* in the Amazon Basin, Brazil (Costa Silva et al. 2019). Also, microcrustaceans and molluscs were identified as food contain for *L. friderici* from lotic environments (Oliveira et al. 2017), which can reflect a smaller number of these intermediate hosts and, consequently, of endoparasites these environments (Camargo et al. 2016). In *Pygocentrus nattereri* collected in floodplain lakes in Central Amazonia, Brazil, microcrustacean food item was associated with a higher prevalence of infection by *P. (S.) inopinatus* (Morais et al. 2019). Therefore, eating habits of host fishes can influence the presence of *P.* (*S*). *inopinatus* (Shamsi 2013). A study on feeding habits and trophic ecology of Lutjanids reported the fish preference for macrocrustaceans living in areas of submerged vegetation, a diet associated to benthic fish (Guevara et al. 2007). This could explain the presence of nematodes in *L. friderici*, as some parasite groups have an indirect life cycle and their first intermediate host is generally a crustacean among other invertebrates.

Body length is a good predictor of parasite abundance. Larger fishes ingest a higher amount of potentially infected prey. Besides, they are considered a more stable habitat for the parasites that, often display a higher longevity compared to the ones living in smaller fish on the relationship that the parasites can have a short life cycle and therefore are constantly eliminated by the hosts smaller (Pavanelli et al. 2013, Amarante et al. 2016).

Carnivorous fish would be susceptible to obtain larger parasitic abundance because they are at the top of the food chain (Machado et al. 1996). However, in this study, *S. rhombeus* (carnivore) showed lower abundance of *P. (S.) inopinatus* in comparison to *L. friderici* (omnivore). Similar data were reported for *S. marginatus* from Rio Negro, Pantanal, Mato Grosso do Sul (Vicentin et al. 2011), as well as in *Cichlasoma bimaculatum* from the Igarapé Fortaleza basin, tributary of the Amazon River, Amapá, Amazon Region (Tavares-Dias et al. 2017). The abundance of *P.* (*S.*) *inopinatus* were similar for detritivorous, omnivorous, carnivorous and piscivorous hosts. These patterns correlated with differences in the transmission strategies of these parasite taxa (Neves et al. 2020). Additionally, carnivorous fish with diets based on invertebrates and fish; and omnivorous fish with diets containing only invertebrates, had higher richness of endohelminth communities than herbivorous and planktivorous fish (Simková et al. 2001, Baia et al. 2018). Thus, omnivorous diet was a predictor that these hosts present diversified consumption of food items, at least when they are still young, which may have caused a higher eating of intermediate hosts, and what the knowledge of the relationship between *P.* (*S.*) *inopinatus* and its intermediate hosts will only be possible when its biological cycle is completely studied in the laboratory (Neves et al. 2020).

We report the first record of nematode *P.* (*S.*) *inopinatus* in two species of Characiformes fish (*S. rhombeus* and *L. friderici*) in a river in Central-West Brazil. *Procamallanus* (*Spirocamallanus*) *inopinatus* showed higher mean abundance in *L. friderici*. Our data showed that the difference between sampling sites was associated with the variability in the food items consumed by the fish resulting in greater or lower ingestion of intermediate hosts infected by *P.* (*S.*) *inopinatus*. The total length was considered the most important variable related to parasitic abundance in *L. friderici*, although it was not considered ideal to explain the parasitic abundance. The host fish sex did not influence the mean abundance and mean intensity of *P.* (*S.*) *inopinatus*. There are differences in the

length of the individuals that make up the infrapopulations of parasites between the two fish species. On average, *S. rhombeus* parasites are larger than *L. friderici* parasites.

Thus, we conclude that the success of the infection involves a complexity of variables, with greater importance for the prey-predator relationship. Nonetheless, it will be necessary to perform further studies about the life cycle of *P.* (*S.*) *inopinatus* to clarify which species could act as intermediate and definitive host.

Acknowledgments

This work was funded by Fundação de Amparo à Pesquisa do Estado de Goiás – FAPEG (proc. n. 201810267001535), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil – CAPES (funding code 001). The authors thank the Reserva Legado Verdes do Cerrado (LVC), Companhia Brasileira de Alumínio (CBA)/Grupo Votorantim and Universidade Estadual de Goiás, Campus Central Sede: Anápolis-CET for supporting the development of the research.

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SUPPLEMENTARY MATERIAL

Tables SI-SII

How to cite
AMARAL RB, LEÃO GR, CAMPOS TNS, BORGES KM, GRANO-MALDONADO MI, LINO CNR, TAKEMOTO RM, ROCHA TL & DAMACENA-SILVA L. 2023. Abundance of *Procamallanus* (*Spirocamallanus*) *inopinatus* (Nematoda: Camallanidae) in Characiformes fish and associated factors in Midwest Brazil. An Acad Bras Cienc 95: e20220978. DOI: 10.1590/0001-3765202320220978.

Manuscript received on November 5, 2022;
accepted for publication on March 12, 2023

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