Occurrence and zoonotic impacts of *Eustrongylides* sp. Jägerskiöld, 1909 in *Serrasalmus gibbus* Castelnau, 1855 from the Upper Tocantins-Araguaia Basin

Ocorrência e impactos zoonóticos de *Eustrongylides* sp. jägerskiöld, 1909 em *Serrasalmus gibbus* Castelnau, 1855 da bacia do alto Tocantins-Araguaia

Ocurrencia e impactos zoonóticos de *Eustrongylides* sp. Jägerskiöld, 1909 en *Serrasalmus gibbus* Castelnau, 1855 de la cuenca alta Tocantins-Araguaia

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Maria Clara Santana Ramos
Undergraduate Student in Biological Sciences
Institution: Universidade Estadual de Goiás (UEG)
Address: Anápolis, Goiás, Brazil
E-mail: mcsr@aluno.ueg.br

Amanda de Oliveira Prates
Undergraduate Student in Ecology and Environmental Analysis
Institution: Universidade Federal de Goiás (UFG)
Address: Goiânia, Goiás, Brazil
E-mail: amandaprates@discente.ufg.br

Thiago Nascimento da Silva
Master in Biodiversity, Ecology, and Conservation
Institution: Universidade Federal do Tocantins (UFT)
Address: Anápolis, Goiás, Brazil
E-mail: thiago_tnsc@hotmail.com

Rafael Braga do Amaral
Doctor in Animal Biodiversity
Institution: Universidade Federal de Goiás (UFG)
Address: Goiânia, Goiás, Brazil
E-mail: rafael.braga70@yahoo.com.br

Gabriela Lidiane Dias Carvalho
Undergraduate Student in Biological Sciences
Institution: Universidade Estadual de Goiás (UEG)
Address: Anápolis, Goiás, Brazil
E-mail: gabrielaldc600@aluno.ueg.br

Emely Ribeiro Votri de Godoy
Undergraduate Student in Biological Sciences
Institution: Universidade Estadual de Goiás (UEG)
Address: Anápolis, Goiás, Brazil
E-mail: emely.godoy@aluno.ueg.br

Guilherme Augustus de Araújo
Master in Animal Biodiversity
Institution: Universidade Federal de Goiás (UFG)
Address: Goiânia, Goiás, Brazil
E-mail: guilhermeaugustus99@hotmail.com

Luciana Damacena-Silva
Doctor of Tropical Medicine and Public Health
Institution: Universidade Estadual de Goiás (UEG)
Address: Goiânia, Goiás, Brazil
E-mail: luciana.silva@ueg.br

ABSTRACT

Larvae of *Eustrongylides* sp., a zoonotic parasite of freshwater fish that is widely reported, can cause harm to humans as well as affect fish welfare. This study aimed to record the occurrence of *Eustrongylides* sp. in *Serrasalmus gibbus* from marginal lakes of the Bonito River (Upper Tocantins–Araguaia basin) and to assess whether parasite abundance is related to abiotic factors (pH, dissolved oxygen, and temperature) and biotic factors (sex and length) of the hosts. Parasitological indices by anatomical site and the impact on the relative condition factor (kn) of the fish were also analyzed. Specimens of *S. gibbus* were collected, transported under refrigeration, and analyzed in the laboratory for biometric parameters and the presence of larvae in different anatomical sites. Parasite identification was based on morphological characteristics, and relationships between environmental and biological variables were tested using Generalized Linear Models. A total of 112 specimens were examined, of which 46 (41.1%) were parasitized, totaling 254 larvae. Water temperature influenced parasite abundance, and larger fish showed higher parasite loads. A kn = 1 suggests that parasitism did not affect fish welfare. Muscle tissue showed the highest prevalence, intensity, and mean abundance of larvae. The results reinforce the importance of parasitological and environmental monitoring in Neotropical ecosystems, highlighting the influence of water temperature and host size on the dynamics of *Eustrongylides* sp. within an integrated One Health perspective.

Keywords: Pirambeba. Carnivorous Fish. Nematoda. Dioctophymatidae. Aquatic Zoonosis.

RESUMO

Larvas de *Eustrongylides* sp., parasito zoonótico de peixes de água doce, amplamente registradas, podem causar danos aos humanos, além de afetar o bem-estar dos peixes. Este estudo teve como objetivo registrar a ocorrência de

Eustrongylides sp. em *Serrasalmus gibbus* de lagos marginais do rio Bonito (bacia do alto Tocantins-Araguaia) e avaliar se a abundância do parasito se relaciona a fatores abióticos (pH, oxigênio dissolvido e temperatura) e bióticos (sexo e comprimento) dos hospedeiros. Também foram analisados os índices parasitários por sítio anatômico e o impacto sobre o fator de condição relativo (kn) dos peixes. Exemplares de S. *gibbus* foram coletados, transportados sob refrigeração e analisados em laboratório quanto aos parâmetros biométricos e à presença de larvas em diferentes sítios anatômicos. A identificação dos parasitos baseou-se em características morfológicas, e as relações entre variáveis ambientais e biológicas foram testadas por Modelos Lineares Generalizados. Foram examinados 112 exemplares, dos quais 46 (41,1%) estavam parasitados, totalizando 254 larvas. A temperatura da água influenciou a abundância de parasitos, e peixes maiores apresentaram maior abundância parasitária. O Kn = 1 sugere que o parasitismo não afetou o bem-estar dos peixes. O músculo apresentou maior prevalência, intensidade e abundância média de larvas. Os resultados reforçam a importância do monitoramento parasitológico e ambiental em ecossistemas neotropicais, evidenciando a influência da temperatura da água e do tamanho do hospedeiro na dinâmica de *Eustrongylides* sp., dentro de uma perspectiva integrada de Saúde Única.

Palavras-chave: Pirambeba. Peixe Carnívoro. Nematoda. Dioctophymatidae. Zoonose Aquática.

RESUMEN

Las larvas de *Eustrongylides* sp., un parásito zoonótico de peces de agua dulce ampliamente registrado, pueden causar daños a los seres humanos, además de afectar el bienestar de los peces. Este estudio tuvo como objetivo registrar la ocurrencia de *Eustrongylides* sp. en *Serrasalmus gibbus* de lagos marginales del río Bonito (cuenca del alto Tocantins-Araguaia) y evaluar si la abundancia del parásito se relaciona con factores abióticos (pH, oxígeno disuelto y temperatura) y bióticos (sexo y longitud) de los hospedadores. También se analizaron los índices parasitológicos por sitio anatómico y el impacto sobre el factor de condición relativo (kn) de los peces. Los ejemplares de *S. gibbus* fueron recolectados, transportados bajo refrigeración y analizados en laboratorio en cuanto a los parámetros biométricos y la presencia de larvas en diferentes sitios anatómicos. La identificación de los parásitos se basó en características morfológicas, y las relaciones entre las variables ambientales y biológicas fueron evaluadas mediante Modelos Lineales Generalizados. Se examinaron 112 ejemplares, de los cuales 46 (41,1%) estaban parasitados, totalizando 254 larvas. La temperatura del agua influyó en la abundancia de parásitos, y los peces de mayor tamaño presentaron una mayor carga parasitaria. Un valor de kn = 1 sugiere que el parasitismo no afectó el bienestar de los peces. El músculo presentó la mayor prevalencia, intensidad y abundancia media de larvas. Los resultados refuerzan la importancia del monitoreo parasitológico y ambiental en los ecosistemas neotropicales, Evidenciando la influencia de la temperatura del agua, de la temperatura y del tamaño del hospedador en la dinámica de *Eustrongylides* sp., dentro de una perspectiva integrada de Una Sola Salud.

Palabras clave: Pirambeba. Pez Carnívoro. Nematoda. Dioctophymatidae. Zoonosis Acuática.

1 INTRODUCTION

Nematodes of the genus *Eustrongylides* Jägerskiöld, 1909, exhibit a cosmopolitan distribution, with records from the Americas, Europe, and Asia (Guardone *et al.,* 2021; Shamsi *et al.,* 2023). This group includes large nematodes, visible to the naked eye, and species such as *Eustrongylides ignotus*, which have been reported in several fish species in Brazil, occurring throughout virtually all of the country's hydrographic basins (Pavanelli; Takemoto; Eiras, 2013). The life cycle of *Eustrongylides* sp. is indirect and involves multiple hosts. Larvae initially develop in aquatic oligochaetes and are subsequently ingested by freshwater fish, where they reach advanced stages and become established in the viscera and musculature. Predatory fish may act as paratenic hosts, accumulating infective larvae (Castiglione *et al.,* 2023). This ability to infect fish makes these hosts potential vehicles for transmission to humans.

The occurrence of *Eustrongylides* sp. larvae in freshwater fish, with zoonotic potential, has been widely reported in different regions of the world. In Italy, the parasite was identified in *Perca fluviatilis*, *Micropterus salmoides*, and *Atherina boyeri* from Lake Trasimeno (Branciari *et al.,* 2016). Similarly, in India, it was detected in 28.7% of *Glossogobius giuris* specimens, with larvae located in various organs and a higher intensity of infection observed during spawning periods (Kaur *et al.,* 2013).

In Brazil, records of infection in *Hoplias malabaricus* and *Pseudoplatystoma fasciatum* indicate prevalence rates ranging from 33% to 93.3%, with larvae predominantly located in the musculature and viscera (Barros; Sigarini; Pinto, 2008; Barros; Moraes Filho; De Oliveira, 2006; Meneguetti *et al.,* 2013; Kuraiem *et al.,* 2019). In addition, *Eustrongylides* spp. have also been found in red piranhas (*Pygocentrus nattereri*) from the Cuiabá River (MT), with 14% of specimens parasitized (Barros *et al.,* 2010).

Among Brazilian fish of the family Serrasalmidae, *Serrasalmus gibbus* stands out as a species with wide distribution, high abundance, and well-developed musculature, frequently used for human consumption (Barros *et al.,* 2010). The consumption of these fish, especially when prepared raw or undercooked, such as in sashimi or marinated dishes, may pose a public health risk due to the possible transmission of *Eustrongylides* sp. (Fábio; Pozza; Lehmann, 2019), which can cause eustrongylidosis — a rare but potentially severe disease characterized by intense abdominal pain, gastrointestinal disorders, and, in extreme cases, intestinal perforation (Eberhard; Ruiz-Tiben, 2014; Cong; Elsheikha, 2021). By consuming raw-prepared fish, humans may act as accidental hosts. Moreover, the disease is believed to be underdiagnosed due to the still limited knowledge about these parasites (Mascarello *et al*., 2017).

In addition to the implications for human health, the presence of parasites can also directly affect the well-being and physiological performance of fish. The physical condition of individuals is influenced by biotic factors, such as sex, size, and parasite interactions, as well as by abiotic environmental conditions, including temperature, dissolved oxygen, and water pH. In the field of fisheries biology, the length-weight relationship is widely used to assess this condition, with the relative condition factor (Kn), defined as the ratio between the observed weight and the expected weight, being one of the main indicators of this balance, whose standard value is equal to 1 (Le Cren, 1951). Changes in Kn may reflect variations in environmental quality, food availability, or the presence of parasitic infections (Guidelli *et al.,* 2011). Complementarily, these physicochemical parameters influence both habitat selection and the behavior of hosts and parasites, directly affecting encounter rates and, consequently, infection levels (Barber; Berkhout; Ismail, 2016).

The hypothesis tested was that the abundance of *Eustrongylides* sp. in *S. gibbus* is influenced by abiotic and biotic factors, and that infection by this parasite reduces the Kn of the fish. Based on this hypothesis, this study aimed to record the occurrence of *Eustrongylides* sp. in *Serrasalmus gibbus* from marginal lakes of the Bonito River (upper Tocantins-Araguaia basin) and to evaluate whether parasite abundance is related to abiotic (pH, dissolved oxygen, and temperature)

and biotic (sex and length) factors of the hosts. Parasitological indices by anatomical site and the impact on the Kn of the fish were also analyzed.

2 METHODOLOGY

2.1 STUDY AREA AND FISH SAMPLING

A total of 112 specimens of *S. gibbus* were obtained over three sampling campaigns conducted in January 2023, July 2023, and April 2024, at two sampling sites: S1 (16°32′32.43″ S, 51°30′41.44″ W; 445422 E / 8171003 N) and S2 (16°32′38.6″ S, 51°29′21.5″ W; 447790 E / 8170812 N), located in marginal lakes of the Bonito River, in the upper Tocantins-Araguaia basin, municipality of Arenópolis, state of Goiás.

The fish used in this study were kindly donated by biologist Thiago Nascimento da Silva Campos. They were kept refrigerated on ice immediately after capture and during transport to the laboratory, ensuring the integrity of the samples. The researcher also provided the physicochemical water data for the sampling sites, including temperature (°C), dissolved oxygen (DO), and pH, obtained during the same periods as the fish collections (Table 1).

Table 1. Mean and standard deviation of the physical and chemical water parameters, by sampling site, in the marginal lakes of the Bonito River, upper Tocantins-Araguaia basin.

Sites	**Parameters**		
	Temp (ºC)	**OD**	**pH**
MPV	---	>5 mg/L	6,0 a 9,0
S1	24,60 ± 3,0	4,62 ± 1,9	7,05 ± 0,4
S2	23,12 ± 3,3	5,02 ± 0,9	7,25 ± 0,4

Abbreviations: MPV - maximum permissible values established by CONAMA No. 357/2005 (Class 2); Temp - Water temperature (°C); DO - Dissolved oxygen (mg/L^{-1}); pH - Hydrogen potential.
Source: Prepared by the authors, 2025.

The sample collections were conducted within the scope of the Aquatic Organism Monitoring Program, in accordance with the authorization issued by the Secretariat of Environment and Sustainable Development of Goiás (SEMAD), under number 64/2023.

In the laboratory, the biometric parameters of the fish were obtained, including total length (TL, cm), standard length (SL, cm), and total weight (TW, g). The length of the fish was measured using a 60-cm stainless steel ruler, and the weight was determined with an Ohaus balance, model LS 2000. Subsequently, the fish were subjected to parasitological analyses.

Kn was determined using the following equation: Kn = (observed total weight / expected total weight) (Le Cren, 1951).

2.2 PARASITOLOGICAL ANALYSES

All fish were necropsied for parasite analysis. The eyes, gills, muscles, and viscera were carefully dissected using tweezers and surgical scissors, and the organs were separated into Petri dishes containing physiological solution (0.8% NaCl). They were then examined under a stereomicroscope (Leica EZ4 W). The parasites were collected with fine tweezers or brushes, fixed in 70% ethanol, and subsequently cleared with Amann's lactophenol between a slide and coverslip (Eiras; Takemoto; Pavanelli, 2006).

The identification and characterization of *Eustrongylides* sp. were based on dichotomous keys (Moravec, 1998; Thatcher, 2006) and articles reporting new records and species descriptions (Pekmezci; Bolukbas, 2021; Correa *et al.,* 2023; Rahmati-Holasoo *et al.,* 2024; Cárdenas *et al.,* 2025). Prevalence data (number of infected hosts divided by the number of examined hosts, multiplied by 100), mean intensity (total number of parasites of a given species found in a sample, divided by the number of hosts infected with that parasite), and mean abundance (total number of individuals of a parasite species in the host sample, divided by the total number of examined hosts) of the nematodes were obtained according to Bush *et al.* (1997).

2.3 STATISTICAL ANALYSIS

To evaluate whether the *S. gibbus* specimens differ in the Kn, compared to the standard value (Kn = 1), a one-sample Student's t-test was used. All analyses were conducted using the Jamovi software (The Jamovi Project, 2024).

A Generalized Linear Model (GLM) with a count distribution (Poisson or Negative Binomial) was employed to evaluate the effect of parasite abundance as a function of fish length and sex, as well as water temperature, dissolved oxygen, and pH. Parasite abundance was considered the response variable, while the biometric, somatic, and limnological variables were used as predictors.

Before fitting the GLM, the presence of overdispersion in the data was assessed (variance greater than the mean, common in count data). When the dispersion ratio was significantly greater than 1, the Negative Binomial distribution was adopted as it is more appropriate. The significance of the effects of explanatory variables on parasite abundance was tested using analysis of variance (ANOVA), and all statistical analyses were performed in the R software (R Core Team, 2024).

3 RESULTS

A total of 254 larvae of *Eustrongylides* sp. were collected from 46 specimens of *S. gibbus* from sites S1 and S2.

Based on the physical and chemical water parameters from sampling sites S1 and S2, previously presented in Table 1 (Methodology section), it was observed that temperature influenced parasite abundance ($p < 0.001$). The mean water temperature was 24.60 ± 3.0 °C, dissolved oxygen avereged 4.62 ± 1.9 mg L^{-1}, and mean pH was 7.05 ± 0.4.

Regarding the host biotic factors (Table 2), the total length of the 112 *S. gibbus* specimens (57 females and 55 males) ranged from 12 to 29 cm (22.50 ± 4.0 cm), while total weight ranged from 26 to 523 g (261.2 ± 121.3 g). The mean value of the was Kn = 1.01 ± 0.1. Among the evaluated factors, only fish length showed a relationship with the abundance of *Eustrongylides* sp. ($p < 0.005$).

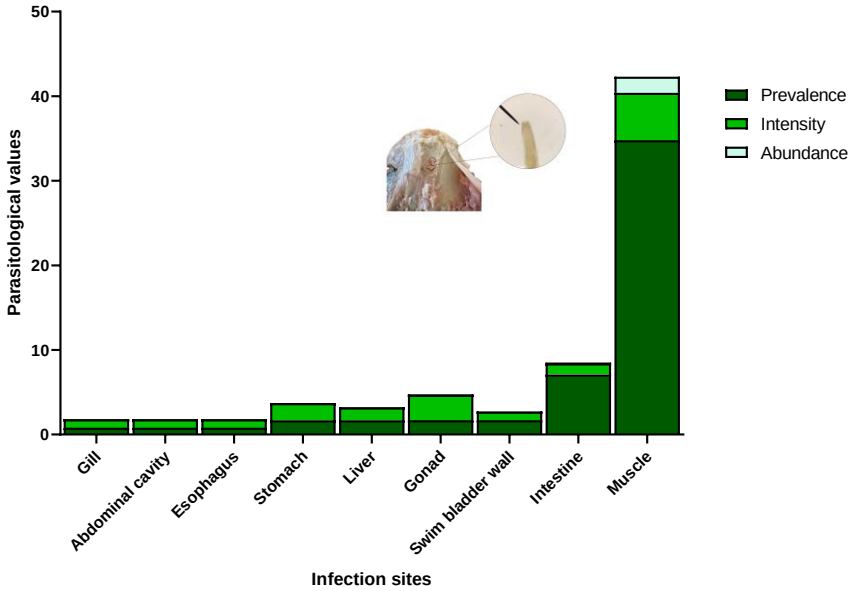
Table 2. Biometric data (means and standard deviations) of *Serrasalmus gibbus* collected at the sampling sites (S1 and S2) in the upper Tocantins-Araguaia basin.

Parameters	Pontos amostrais		
	Sexo	S1	S2
TL (cm)	M	22,06±3,5	21,36±3,4
	F	22,87±4,3	21,05±4,3
SL (cm)	M	18,99±3,3	18,82±3,0
	F	19,80±3,8	18,60±4,9
OW (g)	M	238,05±102,5	207,73±93,9
	F	284,54±136,5	232,25±169,4
Kn	M	1,00±0,1	0,95±0,1
	F	1,02±0,1	0,96±0,1

Abbreviations: TL: Total Length; SL: Standard Length; OW: Observed Weight; Kn: Relative Condition Factor; M: Male; F: Female.
Source: Prepared by the authors, 2025.

Figure 1 presents the data on prevalence, abundance, and mean intensity of infection by anatomical site. Muscle was the most parasitized tissue, showing the highest prevalence (34.8%), followed by the intestine, with 14 larvae (7.1%), and the stomach, liver, gonads, and the wall of the swim bladder, each with 1.7%. In the gills, abdominal cavity, and esophagus, prevalence was 0.8%. The total abundance of 2.26 parasites per anatomical site is illustrated in Figure 1.

Figure 1. Occurrence and distribution by anatomical infection site of *Eustrongylides* sp. found in *Serrasalmus gibbus* at S1 and S2, upper Tocantins River basin, Goiás.



Source: Prepared by the authors, 2025.

4 DISCUSSION

The occurrence of *Eustrongylides* is directly related to environmental conditions and water quality. This relationship reinforces the importance of integrated approaches under the One Health perspective, which considers the interactions among the environment, hosts, and zoonotic parasites (Friend; Franson, 1999; Jiménez Cisneros *et al.,* 2014; Suchona *et al.,* 2025).

In addition to temperature, physicochemical parameters such as pH and the concentration of inorganic ions influence parasite metabolism, affecting larval oxygen consumption and highlighting their sensitivity to water quality (Von Brand, 1943; Calhoun; McDevitt-Galles; Johnson, 2018). Environments with high loads of nutrients and organic matter favor the development and maintenance of *Eustrongylides* spp. (Jiménez Cisneros *et al.,* 2014), as increased nutrient availability enhances primary production and the populations of invertebrates that serve as intermediate hosts (Vidal-Martínez *et al.,* 2010). As this process intensifies, the deterioration of water quality and the emergence of hypoxic conditions (Zhi *et al.,* 2023) may alter aquatic communities and promote the persistence of this zoonotic parasite, which is of environmental and public health relevance (Jiménez Cisneros *et al.,* 2014).

Among the environmental factors analyzed in this study, temperature stands out as one of the main determinants of the growth, development, and survival of aquatic parasites (Kaur; Shrivastav; Qureshi, 2013). Thermal variations may favor the performance of parasites over that of their hosts, resulting in greater abundance when these organisms adapt more effectively to temperature changes (Rohr; Cohen, 2020). *Eustrongylides* spp. shows a preference for warm waters (20–30 °C) and is frequently recorded in eutrophic environments, in which conditions favor its life cycle (Friend; Franson, 1999).

In addition to abiotic factors, variation in parasite occurrence may also be determined by biotic factors, such as the sex, size, weight, and Kn of the hosts (Hoshino; Tavares-Dias, 2014; Amaral *et al.,* 2023). In this context, it was observed that the variable total length of the fish contributed to explaining the abundance of *Eustrongylides* sp. ($p < 0.001$). Larger individuals showed higher

parasite abundance, possibly due to longer exposure time or the greater body surface available for infection (Tavares-Dias *et al.,* 2017; Amaral *et al.,* 2023).

The mean Kn value (1.01 ± 0.1) did not differ from the reference value (Kn = 1) ($p > 0.05$), indicating that infection by *Eustrongylides* sp. did not compromise the well-being of the fish. Studies conducted with *Callichthys callichthys* (Da Silva; Dias, 2012), *Megalechis thoracata* and *Mylossoma duriventre* (Cardoso *et al.,* 2018) also demonstrated no significant variation in Kn in individuals infected by *Eustrongylides* sp.

Regarding host sex, there was no difference ($p > 0.005$) in the prevalence of *Eustrongylides* sp. between males and females, a result that is consistent with what was observed in *Hoplias malabaricus* in the state of Santa Catarina (Cárdenas *et al.,* 2025). However, unlike the present study, research such as that of Ibiwoye *et al.* (2004), Laterça, De Souza Santos and Garcia (2009), and Kaur, Shrivastav and Qureshi (2013) reported higher parasite prevalence in females. These variations may be related to differences in diet, behavior, and the influence of host sex hormones (Pavanelli; Takemoto; Eiras, 2013). Although some studies indicate that females tend to be more parasitized, there is, nevertheless, no consistent scientific evidence that demonstrates a direct relationship between infection rates and sex (Honcharov *et al.,* 2022).

Larvae of this genus have already been reported in 17 orders of fish in different regions of the world (Spalding; Forrester, 1993), which demonstrates their wide distribution. Although records of *Eustrongylides* sp. exist for species congeneric with *Serrasalmus gibbus*, such as *S. rhombeus*, *S. maculatus*, and *S. marginatus* (Vicentin *et al.,* 2011; Pereira, 2016; Morey *et al.,* 2022; Oliveira *et al.,* 2024), this study represents the first record of occurrence for *S. gibbus*.

The detection of 254 larvae of *Eustrongylides* sp. in 46 individuals of *S. gibbus* from sites S1 and S2, resulting in an overall prevalence of 41.07%, indicates a substantial infection level, consistent with that observed in other Neotropical fish species. Similar values were recorded in the Peruvian Amazon in *S. rhombeus*, *Cichla monoculus*, and *Hoplias malabaricus* (around 40%) (Morey *et al.,* 2022; Pasquel Pinedo; Murrieta Morey; Tuesta Rojas, 2022), as well as in populations of *H. malabaricus* in Rio de Janeiro and *Callichthys*

callichthys in Amapá, which showed prevalences close to 44% (Kuraiem *et al.,* 2019; Cardoso *et al.*, 2018). These findings indicate that, regardless of locality or host species, *Eustrongylides* sp. tends to maintain similar infection levels in carnivorous and piscivorous fish.

However, higher prevalence values, exceeding 60%, have been reported in predatory fish such as *Brachyplatystoma tigrinum*, *Hydrolycus scomberoides*, and *Raphiodon vulpinus* in Amazonian aquaculture systems (Morey *et al.,* 2022). These higher values may reflect the presence of all elements involved in the parasite's life cycle. On the other hand, lower prevalences, ranging from 3% to 30%, have been described in species such as *Pseudoplatystoma punctifer*, *Megalechis thoracata*, and *Galaxias maculatus* (Cardoso *et al*., 2018; Torres *et al.,* 2023), indicating that local ecological factors and the feeding characteristics of the species may directly influence infection intensity.

The higher occurrence of larvae in muscle tissue is a relevant finding, as this site has direct implications for public health. Because *Eustrongylides* spp. can remain viable in the muscle of infected fish (Shamsi *et al.,* 2023), the consumption of raw or undercooked fish represents a potential zoonotic risk. In humans, infection can cause gastritis and intestinal perforation, with cases already reported in Sudan and the United States following the consumption of raw fish (Eiras *et al.,* 2018).

In addition to the risk to human health, the presence of this parasite can affect the well-being and physiological performance of fish, reflected in alterations in body condition and in the Kn, as discussed by Le Cren (1951) and Guidelli *et al.* (2011). Thus, infection by *Eustrongylides* sp. may indicate not only a potential imbalance in the host-parasite relationship but also the influence of abiotic factors such as temperature, dissolved oxygen, and pH, variables that modulate both host behavior and parasite transmission dynamics (Barber; Berkhout; Ismail, 2016).

Additionally, the increase in anthropogenic activities, associated with climate change and the eutrophication of freshwater ecosystems, has been linked to a higher incidence of this parasite in aquatic environments (Shamsi *et al.,*

2023), which may favor its maintenance and dispersion in Neotropical ecosystems.

Thus, the results obtained in this study not only expand the knowledge on the occurrence of *Eustrongylides* sp. in *S. gibbus*, but also reinforce the importance of integrating parasitological monitoring, assessment of physiological condition, and sanitary surveillance in order to prevent zoonotic risks and guide sustainable fisheries management strategies.

5 CONCLUSION

The hypothesis that the abundance of *Eustrongylides* sp. in *S. gibbus* is influenced by abiotic and biotic factors was partially confirmed. The results showed that water temperature and host length were the only variables associated with parasite abundance: higher temperatures resulted in greater infection, and larger fish harbored more larvae. The higher occurrence of parasites in the muscle reinforces the sanitary relevance of this finding, as it involves a tissue frequently consumed.

Given this scenario, the results also highlight the importance of preventive measures, such as cooking at high temperatures (>60 °C for at least 10 minutes) or proper freezing (−20 °C for at least 24 hours throughout the entire product), which are effective in preventing viable parasites from reaching the consumer's plate. Prevention also depends on educational actions throughout the production chain, involving fishermen, handlers, cooks, and consumers, in addition to training healthcare professionals to identify and guide suspected cases.

Thus, the results of this study reinforce the need for continuous monitoring, as well as ongoing research aimed at prevention, detection, and understanding of the parasite's ecology in Neotropical ecosystems.

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