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Mercury concentration in larvae of *Eustrongylides* sp. (Nematoda: Dioctophymatoidea) from fish of the Brazilian Amazon

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ABSTRACT

Introduction: Chemical pollution represents a great concern to aquatic organisms, especially fish. Metals enter the aquatic environment from a variety of sources, including natural biogeochemical cycles and anthropogenic sources such as industrial and residential effluents, mining and atmospheric sources.

Objective: To describe the *Eustrongylides* sp. larvae and the interaction with their fish hosts as indicators of mercury (Hg) contamination in the Brazilian Amazon, and the distribution of Hg in the internal organs of fish species *Hoplias malabaricus* and *Pygocentrus nattereri* collected in oxbow lakes on the Tapajós River, in the municipality of Santarém, in the state of Pará.

Methods: Total Hg was analyzed using the Direct Hg Analyzer - DMA-80. Concentrations of Hg in *Eustrongylides* sp. were compared with those found in the tissues/organs of the hosts *H. malabaricus* and *P. nattereri*. Hg concentrations in the host/parasite system were statistically compared using Principal Component Analysis. The bioconcentration factor (BCF) was calculated to assess the bioaccumulation capacity of metals in *Eustrongylides* sp. larvae, comparing the concentration of Hg in the parasite with that accumulated in the musculature of infected hosts.

Results: Hg concentrations in all tissues/organs analyzed were higher in the parasitic species *Eustrongylides* sp. larvae when compared with those found in tissues/organs of *H. malabaricus* and *P. nattereri*. There was an inversely proportional relationship, showing that when *Eustrongylides* sp. larvae are present, the concentration in the parasite is higher than in the musculature of host fish *H. malabaricus* and *P. nattereri*. The BCF of Hg was found by comparing *Eustrongylides* sp. larvae/*H. malabaricus* muscle and was observed during a flood (BCF Hg = 15 364).

Conclusions: The results confirm the greater bioaccumulative capacity of *Eustrongylides* sp. compared to its host. The data indicated the viability of using *Eustrongylides* sp. larvae in biomonitoring programs. It is worth mentioning that fish samples for Hg analysis must be free of parasites since their presence can alter the results.

Key words: fish parasites; Tapajós river; bioaccumulator parasite; heavy metals; pollution.



RESUMEN

Concentración de mercurio en larvas de *Eustrongylides* sp. (Nematoda: Dioctophymatoidea) en peces de la Amazonía brasileña

Introducción: La contaminación química del hábitat acuático representa un gran peligro para organismos acuáticos, especialmente para peces. Los metales ingresan al ambiente acuático desde una variedad de fuentes, incluidos los ciclos biogeoquímicos naturales y fuentes antropogénicas, como efluentes industriales y residenciales, minería y fuentes atmosféricas.

Objetivo: Describir las especies de *Eustrongylides* sp. y la interacción con sus peces hospederos como indicadores de contaminación por mercurio en la Amazonía brasileña, y la distribución en los órganos internos de las especies de peces *Hoplias malabaricus* y *Pygocentrus nattereri* recolectadas en cochas del Río Tapajós, en el municipio de Santarém, del estado de Pará.

Métodos: El Hg total se analizó utilizando el Direct Hg Analyzer - DMA-80. Las concentraciones de *Eustrongylides* sp. se compararon con las encontrados en los tejidos/órganos de los hospederos *H. malabaricus* y *P. nattereri*. Las concentraciones en el sistema hospedero/parásito se compararon estadísticamente utilizando el análisis de componentes principales. Se calculó el factor de bioconcentración (BCF) para evaluar la capacidad de bioacumulación de metales en larvas de *Eustrongylides* sp., comparando la concentración en el parásito con la acumulada en la musculatura de los hospederos infectados.

Resultados: Las concentraciones de Hg en todos los tejidos/órganos analizados fueron mayores en las larvas de la especie parasitaria *Eustrongylides* sp. en comparación con las encontradas en los tejidos/órganos de *H. malabaricus* y *P. nattereri*. Hubo una relación inversamente proporcional, mostrando que cuando las larvas de *Eustrongylides* sp. están presentes, la concentración en el parásito es mayor que en la musculatura de los peces hospederos *H. malabaricus* y *P. nattereri*. El BCF de Hg se encontró comparando *Eustrongylides* sp. larvas/ músculo *H. malabaricus* y se observó durante una inundación (BCF Hg = 15 364).

Conclusiones: Los resultados confirman la mayor capacidad bioacumulativa de *Eustrongylides* sp. en comparación con su hospedero. Los datos indicaron la viabilidad de utilizar larvas de *Eustrongylides* sp. en programas de biomonitoreo. Cabe mencionar que las muestras de pescado para análisis de Hg deben estar libres de parásitos ya que su presencia puede alterar los resultados.

Palabras clave: parásitos de peces; río Tapajós; parásito bioacumulador; metales pesados; contaminación.

INTRODUCTION

The fish species *Hoplias malabaricus*, known in Brazil as “Traíra” belongs to the *Erythrinidae* family with three genera of Characiformes: *Erythrinus* Scopoli, 1777, *Hoplerythrinus* Gill, 1896, and *Hoplias* Gill, 1903 (Nelson, 2006). They are carnivores (piscivores) and are fish widely distributed in the Neotropics, found in all Brazilian states, covering all watersheds in South America. This genus can efficiently adapt to different environmental conditions (Bialecki et al., 2008). The genus *Hoplias* comprises two complexes of species: the *Hoplias lacerdae* group, with nine species: *Hoplias brasiliensis*, *Hoplias aimara*, *Hoplias patana*, *Hoplias teres*, *Hoplias intermedius*, *Hoplias microlepis*, *Hoplias macrophthalmus*, *Hoplias australis*, and *Hoplias curupira*; and the *Hoplias malabaricus* group which, based on cytogenetic and

molecular biology studies, has also been considered a species complex (Jacobina et al., 2011; Marques et al., 2013; Santos et al., 2009; Vitorino et al., 2011).

Another freshwater species, abundant and widely consumed by riverside populations, is the *Pygocentrus nattereri*, known as “Piranha-caju” or “Piranha-vermelha”. It has a carnivorous food preference from the Serrasalminae family, a native, non-endemic, and epicontinental species. They have a reddish color, with a grayish head and back, and some articles even record individuals up to 50 cm. Among the numerous species of Amazonian fish, *P. nattereri* stands out for its economic interest in extractive fishing, handicraft making, aquarium hobby, and the sale of industrialized products, such as dehydrated piranha soup. The species *P. nattereri* has the characteristic of fish from the Amazonian floodplains due to

its abundance in scientific fish samplings, thus being used as an indicator of the stability of these environments (Freitas & Siqueira-Souza, 2009; Murrieta-Morey & Malta, 2016; Silva et al., 2014; Siqueira-Souza & Freitas, 2004).

According to studies by Thatcher and Brites-Neto (1994), adult nematodes that parasitize fish inhabit the digestive tract or body cavities, while in the larval stage, they infect the muscles. There are four larval stages before the adult and, in the case of parasitic forms of fish, the last three are parasitic (Thatcher & Brites-Neto, 1994). Birds, fish, amphibians, and reptiles are hosts for nematodes of the genus *Eustrongylides*, with birds being definitive hosts and the others being intermediate or paratenic hosts (Benigno et al., 2012; Moravec & Kohn, 1993). According to Eiras et al. (2016), these helminths have zoonotic potential in Brazil, but no reports have been described in Brazilian territory (Barros et al., 2006; Benigno et al., 2012; Eiras et al., 2018; Fontenelle et al., 2016). Larvae can be in subcutaneous tissues, liver, mesentery, and between muscle fibers (Melo et al., 2016).

Pollution directly interacts with parasitism in complex ways, making it difficult to describe its effects on disease (Lafferty, 1997). This interaction becomes clearer in reviews of fish parasites (Williams & Mackenzie, 2003). Some pollutants are toxins, which can harm both the host and its parasites' immune systems. Some may even preferentially show higher concentrations in parasite tissues than in hosts (Sures, 2008a). However, observations contrary to this have also been made (Andrews et al., 1988). These possibilities lead to a diverse set of predictions about the effect of toxic pollutants on parasites (Sures et al., 1999).

Specific predictions for some parasites and pollution are possible. Fish parasites provide information about water pollution through both their presence or absence and the parasites' capacity to accumulate heavy metals in tissues, mainly arsenic, copper, lead, zinc, and cadmium (Sures & Taraschewski, 1995). Intestinal parasites like *Proteocephalus percae* and

Acanthocephalus lucii have this ability; their tissues contain 300 times more of these toxins than the muscles and liver of their host fish.

The principal source of mercury, Hg, contamination for humans is the intake of fish and other aquatic organisms. Hg present in fish is approximately 90 % in the form of methylmercury (MeHg). MeHg is the most toxic chemical form of Hg for living beings (Crespo-Lopez et al., 2021). In the Amazon and mainly in the region surrounding the Tapajós River basin, the presence of MeHg in fish that commonly feeds the population has been shown (Lino et al., 2018). Thus, monitoring MeHg in fish is of fundamental importance for understanding the contamination levels in the environment and humans by this metal.

When ingested, fish can be a relevant vehicle for heavy metal transmission. Some studies (Albuquerque et al., 2021; Alcalá-Orozco et al., 2020; da Silva-Costa et al., 2022; Lima et al., 2015; Silva et al., 2019) in fish from Amazonian environments, including in specimens consumed by indigenous communities (Vasconcellos et al., 2021), point to the detection of worrying levels of these metals in species of commercial interest. Human contamination by fish gains importance when considering the preference of Amazonians for this protein source, to the detriment of others (Barreto-Sa et al., 2019; Haddad-Junior et al., 2021; Lino et al., 2018; Lopes et al., 2016). The present study is the pioneer in describing *Eustrongylides* sp. larvae (Nematoda: Dioctophymatoidea) and the interaction with their fish hosts as indicators of the accumulation of contamination by HgT (Total Mercury) in the Brazilian Amazon. It also describes the distribution of HgT in the internal organs of fish of the species *Hoplias malabaricus* and *Pygocentrus nattereri* collected, mainly in oxbow lakes in Rio Tapajós, in the municipality of Santarém, in the state of Pará, which is a place that suffers negative impact of anthropic actions due to gold mining above the Tapajós River, consequently, carrying it to the area where the collections took place.

MATERIALS AND METHODS

Collection Location: Fish were collected by fishing in oxbow lakes of tributaries of the Tapajós River, in the Amazon Basin, near the Alter do Chão district of the municipality of Santarém-Pará, coordinates ($2^{\circ}17'46''$ S; $54^{\circ}53'47.74''$ W - Quantum GIS version 3.14.15, QGIS Development Team, 2020) (Fig. 1). Gillnets measuring 30 m in length and 2.5 m in height, and with a mesh size of 30, 35 and 40 mm between knots were used to capture the fish, along with bait. Collections occurred concurrently to compare data obtained in two fish species (*H. malabaricus* and *P. nattereri*) from the same environment (Fig. 2C, Fig. 2D). The collections took place throughout the year 2022. Sixteen specimens of *H. malabaricus* and 24 specimens of *P. nattereri* were captured. These specimens were obtained in two hydrological periods: in winter (characterized as high waters), collected in the month of April (six specimens of *H. malabaricus* and 14

P. nattereri), and in summer (characterized as dry waters), collected in December (ten specimens of *H. malabaricus* and ten *P. nattereri*). After collection, fishes were euthanized using clove oil (150 mg/L diluted in water), their total length (TL, in centimeters) and whole weight (in grams) were measured using (for the collection of the total length, a measuring tape was used and for the weight a scale was used), then fish were dissected at the collection site, and sex determined according to Vazzoler (1996).

Collection of parasites and organs: After being anesthetized and euthanized, the fish were examined under a stereomicroscope to remove all *Eustrongylides* sp. larvae encapsulated in musculature (Fig. 2A, Fig. 2B), and these were separated for the morphometric study. For morphological identification, we examined ten specimens of parasites, which were clarified according to Santin et al., (2009) and studied by light microscopy, with magnification from 100X to 400X, according to Moravec

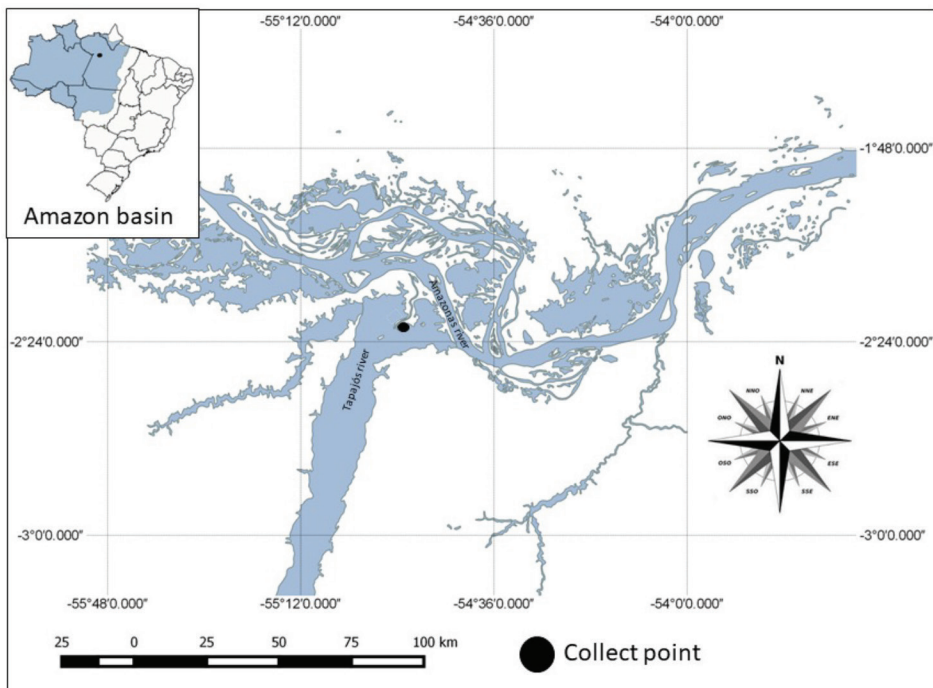


Fig. 1. Map of Brazil, highlighting the Amazon River Basin. Geographic coordinates ($2^{\circ}17'46''$ S; $54^{\circ}53'47.74''$ W - QGIS version 3.14.15, QGIS Development Team, 2020).

and Kohn (1993), after the preparation of temporary slides. The photomicrographs were processed in a Zeiss Axioplan microscope with an Axiocam ERc 5s camera.

The ecological terms used, and the parameters of prevalence, abundance, and mean intensity of infection were calculated according to those proposed by Bush et al. (1997). These parameters were calculated using Quantitative Parasitology 3.0 software (Rozsa et al., 2000), according to Reiczigel et al. (2019).

All *Eustrongylides* sp. larvae and the following fish organs (head, kidney, heart, liver, spleen, small intestine, gonads, and muscles) were frozen at -20 °C for HgT analysis. Muscle samples were free of this type of parasite.

HgT analysis: Aliquots weighing between 0.01-0.05 g (wet weight) were analyzed for HgT concentration. For such analysis, the Direct Mercury Analyzer – DMA – 80 Tri cell (Milestone – Italy) was used. To ensure the reliability

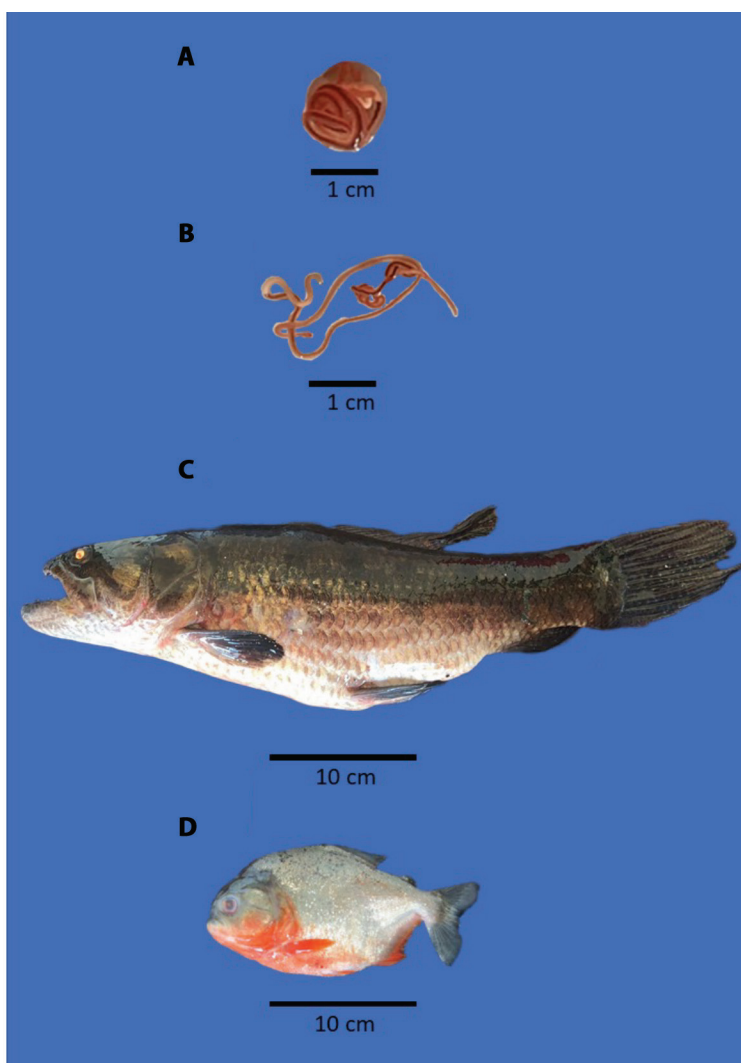


Fig. 2. A. Encapsulated and B. free larvae of *Eustrongylides* sp.; Host species, C. *Hoplias malabaricus* and D. *Pygocentrus nattereri*.



of the results, the certified reference material BCR-463 Tuna Fish (SIGMA) was used, with reference values of 2.85 ± 0.16 mgHg/kg and obtaining values of 2.80 ± 0.07 .

Calculation of the bioconcentration factor (BCF): The Hg bioconcentration factor (BCF) evaluates the capacity of bioaccumulation of the metal in larvae of *Eustrongylides* sp. by comparing the concentration of HgT accumulated in these larvae with that contained in the musculature of infected specimens of *H. malabaricus* and *P. nattereri*. The BCF estimate was calculated and analyzed according to (Schludermann et al., 2003; Sures, 2008b; Sures et al., 1999). BCF was calculated using the expressions: $BCF = (C_{[parasite]}/C_{[host\ tissue]})$.

Statistical analysis: Comparative analyzes referring to HgT concentration in *Eustrongylides* sp. larvae related to that of the fish organs (head kidney, heart, liver, spleen, small intestine, gonads, and muscle), and between oxbow lakes were conducted using Principal Component Analysis (PCA). Spearman's correlation coefficient was used to test for significant associations between HgT concentrations in larvae and host tissues/organs. All statistical tests were performed using the SPSS 8.0 program (Tanaka & Mori, 1997).

RESULTS

Morphological characterization, based on ten specimens of third instar larvae of *Eustrongylides* sp.: body of larvae filiform, yellowish, 60.17-126.20 mm long and 0.65-1.39 mm wide. Cuticles distinctly transversely striated. Three cuticles visible in these stage four larvae: external cuticle, from the second instar, middle cuticle from the third instar, and internal cuticle from the fourth instar. Conical cephalic extremity. Oral opening small, oval, surrounded by 12 cephalic papillae arranged in two circles; each circle formed by two lateral, two subdorsal and two subventral papillae; inner circle papillae smaller, with pointed apices. Pair of small lateral amphids like somatic papillae located

immediately anterior to the lateral cephalic papillae of the outer circle. Four small somatic sublateral field papillae present between the inner and outer circles of the cephalic papillae. Oral cavity 1.77 mm long; esophageal length 12.53-20.15 mm and width 0.27-0.68 mm. Nerve ring encircling esophagus 23.0-38.2 mm from anterior end. Blunt posterior extremity, with slightly elevated region of the anus. Male larvae: developed caudal sucker with a small cuticular border around the entire perimeter. A wide, thick-walled genital tube (ejaculatory duct) with circular muscles extending anteriorly from the rectum, usually with several coils. Tube curving posteriorly near the esophageal-intestinal junction and, after a short distance, expanding into a large hologonic, blunt-ended testis. Seminal vesicle ends just before the genital tube. Spicule incompletely sclerotized. Female larvae: genital primordium consisting of four regions - thick-walled vagina composed of cuboidal epithelium, wide genital tube, and narrow uterus. The genital tube consists of circular muscles and a thin, narrow lumen, curving posteriorly and expanding into a large, conical-tipped hologonic ovary.

Forty fish were collected altogether - 16 *H. malabaricus* (eight males and eight females) and 24 *P. nattereri* (14 males and ten females), with biometric data length/weight of 37.12 ± 9.99 cm / 528 ± 355.90 g, and 17.95 ± 1.64 cm / 167.83 ± 39.72 g, respectively (Fig. 2C, Fig. 2D). The larvae *Eustrongylides* sp. described in this study are all the third stage (L3). By the absolute numerical distribution of larvae in 27 of all the collected fish (67 %), the infection rates obtained were as follows: prevalence of 63 %, abundance of 1.77, and mean intensity of 2.81 larvae/fish. HgT concentrations found in the tissues/organs of *H. malabaricus* and *P. nattereri* and in *Eustrongylides* sp. larvae in hydrological periods (drought and flood) are shown in Fig. 3 and Fig. 4.

HgT concentrations found in the tissues/organs of the hosts *H. malabaricus* and *P. nattereri* and the parasites, *Eustrongylides* sp. larvae, in the hydrological periods (flood and drought) are presented in Fig. 3 and Fig. 4.

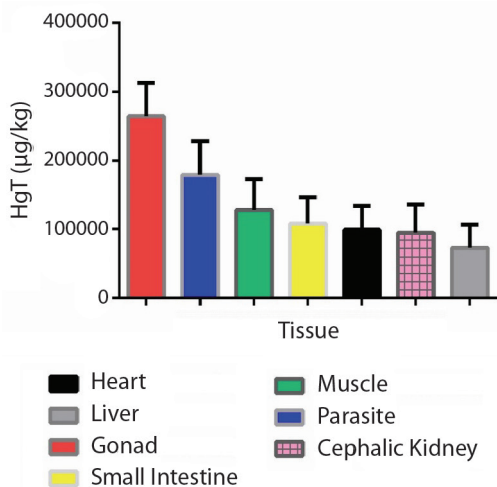


Fig. 3. Total mercury (HgT) concentrations found in the tissues/organs of *Hoplias malabaricus* and *Pygocentrus nattereri* and in *Eustrongylides* sp. larvae during flood and drought.

The concentrations of HgT were significantly higher ($P < 0.01$) in the parasite species when compared with that quantified in the tissues/organs of the hosts. PCA showed that the highest concentration of parasites occurred in *H. malabaricus*, and the highest concentration of

Hg occurred in the gonads of this species. The second highest HgT index was detected in the muscles of both fish species investigated here (Fig. 4).

Spearman's correlation coefficient (r) was crucial to determine the significant relationships between HgT concentrations in *Eustrongylides* sp. larvae and the muscles of *H. malabaricus* and *P. nattereri* parasitized by them. An inversely proportional relationship was observed, demonstrating that the concentration of the metal in these larvae is always higher than that detected in the musculature of these fish ($r = -0.076$; $P \leq 0.001$) (Fig. 5). The HgT BCF in *Eustrongylides* sp. larvae was higher in *H. malabaricus* specimens collected in the flood (BCFHg = 15 364).

DISCUSSION

Several studies have discussed the different roles that parasitic fish species play in the aquatic ecosystem. Previously, these organisms were considered only as a threat to the health of fish, but currently they have shown several other functions, such as environmental bio-indicators or sentinels of pollution of aquatic

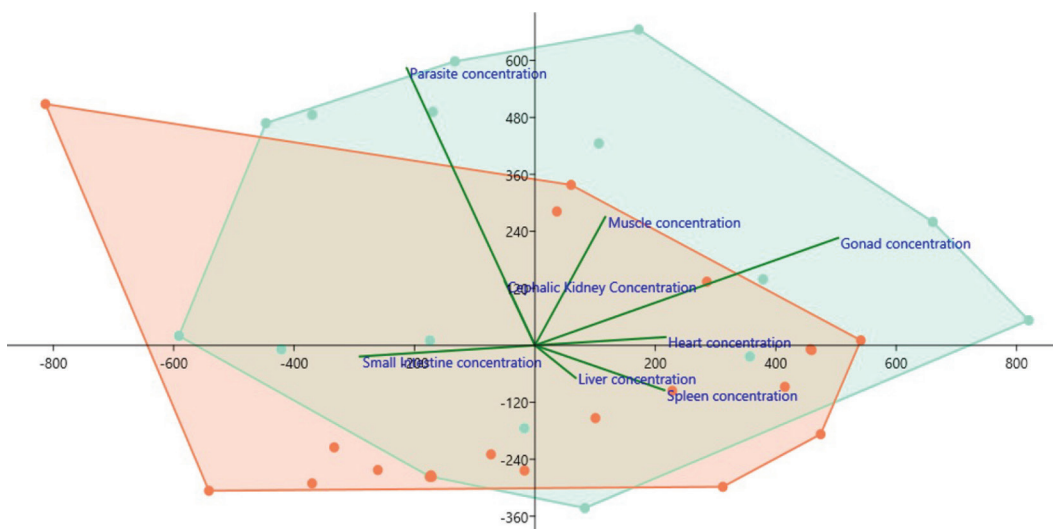


Fig. 4. Total mercury (HgT) concentrations ($\mu\text{g}/\text{kg}$) in the host/parasite system and its characterization by the Principal Component Analysis (PCA). Highlighted in green are the tissue/organ mercury concentrations of the *Hoplias malabaricus* components and in red are the tissue/organ mercury concentrations of the *Pygocentrus nattereri* components.

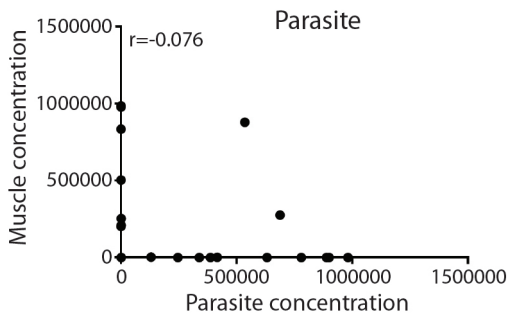


Fig. 5. Spearman's correlation coefficient of total mercury (HgT) concentrations in *Eustrongylides* sp. larvae, when compared with concentration in the musculature of host fish *Hoplias malabaricus* and *Pygocentrus nattereri* ($r = -0.076$; $P \leq 0.001$).

environments (Bush et al., 1997; Heckmann et al., 2015; MacKenzie, 1999; Morsy et al., 2015; Poulin, 1992; Santoro et al., 2020; Sures et al., 1999; Williams and Mackenzie, 2003). Recent studies have recorded the capacity of intestinal fish parasites to accumulate high concentrations of heavy metals in their tissues, mainly species of the phylum Acanthocephala (Sures, 2007; Sures & Siddall, 2003; Vidal-Martínez et al., 2010) and to a much lesser extent, the species of Cestoda (Sures et al., 1999) and Nematoda (Morsy et al., 2015; Sures, 2003; Thielen et al., 2004). Currently, the species of Acanthocephala are considered the most efficient bioaccumulators of heavy metals. According to Sures (2006), the performance of species from other groups, such as Nematoda parasites of fish, needs to be better known.

Studies described by Vidal-Martínez et al., (2010) analyzing the average level of lead (Pb) accumulated in the tissues of the nematode *Anguillicoloides crassus* (Kuwahara, Nimi and Itagaki, 1974), a parasite of the swim bladder of *Anguilla anguilla* (Linnaeus, 1758), recorded that this was lower than what was found in its host and realized that *A. crassus* could excrete heavy metals from a proteinase that degrades hemoglobin. Other studies carried out with nematodes reported that the intestinal parasite *Raphidascaris acus* (Bloch, 1779) accumulated more iron (Fe) and zinc (Zn) (respectively, 68.4

and 86.9 times more) than the liver of its host *Esox Lucius* (Linnaeus, 1758) and that the levels of lead (Pb), chromium (Cr) and cadmium (Cd) in the nematode *Philometra ovata* (Zeder, 1803), an intestinal parasite, were 160, 431 and 119 times higher in the musculature of its host, respectively (MacKenzie, 1999).

Studies focusing on HgT in Amazonian fish have recently been described by Barbosa et al., (2003) and Dórea et al., (2004). Nevertheless, analyses with Hg concentration in parasites, particularly *Eustrongylides* sp. larvae do not exist in the scientific literature. Nematoda larvae of the Anisakidae family (Railliet and Henry, 1912) bioaccumulated lower concentrations of lead (Pb) and copper (Cu) than the host *Dicentrarchus labrax* (Linnaeus, 1758) (da Silva-Costa et al., 2022). The present study showed that *Eustrongylides* sp. larvae were all the third stage (L3) and that their HgT concentrations were higher than in the tissues/organs of the *H. malabaricus* and *P. nattereri* hosts. The study also showed that the ability of *Eustrongylides* sp. to bioconcentrate HgT appears to be related to the site where the host's infection occurred. However, the *Eustrongylides* sp. larvae in *H. malabaricus* have a more evolved spoliation mechanism since they accumulated more HgT when compared to the *Eustrongylides* sp. larvae of *P. nattereri*. However, other factors must be involved, such as the development stage and the feeding aspect of the host species.

The present study detected negative correlation for the concentration of HgT, characterizing it as bioaccumulated in *Eustrongylides* sp. larvae when compared with muscle concentrations in *H. malabaricus* and *P. nattereri* hosts. It seems that the time factor has not decisively influenced the concentration of metals in the *Eustrongylides* sp. larvae in that specific case. This negative correlation observed can be attributed to the kinetics of absorption and the excretion of these metals in this species. Future investigations should reach further elucidation.

Although the *Eustrongylides* sp. larvae analyzed contained high concentrations of HgT, the reproducibility of individuals within the host were not affected. It may suggest that

these parasites have their own detoxification mechanisms. Thus, further investigation and data analysis on free life stages and their exposure to mercury contamination is needed. The results of the present study evidenced HgT concentrations in the muscle and gonads of *H. malabaricus* and *P. nattereri*, especially in the latter organ, which had the highest concentration among all the organs analyzed. Regarding the gonads/roe of Amazonian fish, scientific articles rarely mention anything about consumption habits. When this occurs, however, there are no qualitative or quantitative details such as preferred species or ingested quantities. Eating roe is a common practice of the local population, which means a higher transfer of this heavy metal to consumer individuals.

Using parasites as bioaccumulators of heavy metals is more efficient than using their definitive hosts. Sures et al., (1999) report in their study, that the parasitic species reduce the levels of metals in the tissues of their hosts, masking the actual results. The high BCF HgT values found in the present study were similar in both collection periods - flood and drought. The highest values occurred in the dry season, possibly due to the reduction of the flooded area of the lakes and the consequent higher accumulation of metals in the area. The data obtained in the present study indicate that the *Eustrongylides* sp. larvae have more efficient mechanisms for capturing and accumulating heavy metals than *H. malabaricus* and *P. nattereri*. This HgT uptake and bioaccumulation kinetics by *Eustrongylides* sp. is still not well known, but it was evident that the parasitic species showed higher concentrations of HgT.

Thus, parasitic infections of fish must be considered when using environmental monitoring programs. Otherwise, the levels of pollutants in the hosts may, as investigated by (Sures, 2008b), be erroneously detected and therefore, can invalidate the model. This procedure is essential to use the parasite/host system in ecotoxicological studies. Data in the present work indicate the importance of considering the influence of fish parasite species in biomonitoring programs.

The present work showed the greater bioaccumulative capacity of *Eustrongylides* sp. compared to its host *H. malabaricus* and *P. nattereri*. This feature enabled it to be a potential sentinel organism, which could provide reliable information on pollution or the availability of heavy metals in aquatic environments.

The present study is the first in the Americas that used larvae of a parasitic nematode on fish muscle as bioindicators of HgT accumulation. The results showed the viability of its use, mainly for biomonitoring sensitive aquatic environments in the Brazilian Amazon. Other studies of HgT concentration in fish also recommend verifying that analyzed fish tissue is free of parasites since HgT concentration in the nematodes can be higher than in fish tissue. However, further studies on the kinetics of HgT absorption in *Eustrongylides* sp. and fish are needed.

Ethical statement: the authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

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REFERENCES

- Albuquerque, F. E. A., Herrero-Latorre, C., Miranda, M., Barrêto-Júnior, R. A., Oliveira, F. L. C., Sucupira, M. C. A., Ortolani, E. L., Minervino, A. H. H., & López-Alonso, M. (2021). Fish tissues for biomonitoring



- toxic and essential trace elements in the Lower Amazon. *Environmental Pollution*, 283, 117024.
- Alcala-Orozco, M., Caballero-Gallardo, K., & Olivero-Verbel, J. (2020). Biomonitoring of mercury, cadmium and selenium in fish and the population of Puerto Nariño, at the southern corner of the Colombian Amazon. *Archives of Environmental Contamination and Toxicology*, 79, 354–370.
- Andrews, R. H., Beveridge, I., & Adams, M. (1988). Identification of life cycle stages of the nematode *Echinocephalus overstreeti* by allozyme electrophoresis. *Journal of Helminthology*, 62, 153–157.
- Barbosa, A. C., De Souza, J., Dórea, J. G., Jardim, W. F., & Fadini, P. S. (2003). Mercury biomagnification in a tropical black water, Rio Negro, Brazil. *Archives of Environmental Contamination and Toxicology*, 45, 235–246.
- Barreto-Sa, A., Baltazar De Oliveira, C. S., Lima, A. A. D. S., Sanches-Borges, B. E., Silva-Santos, G. D. F., Mota-Da-Silva, C. I., Vieira-Da-Silva, M. I. C., & Nascimento-Pinheiro, M. D. C. (2019). Fish consumption frequency and lipid peroxidation in the riverside population of Lower Tocantins. *Nutrición Clínica y Dietética Hospitalaria*, 39, 64–68.
- Barros, L. A., Moraes, J., & Oliveira, R. L. (2006). Nematóides com potencial zoonótico em peixes com importância econômica provenientes do rio Cuiabá. *Revista Brasileira de Ciência Veterinária*, 13, 55–57.
- Benigno, R. N. M., Clemente, S. C., Matos, E. R., Pinto, R. M., Gomes, D. C., & Knoff, M. (2012). Nematodes in *Hopleryrtrinus unitaeniatus*, *Hoplias malabaricus* and *Pygocentrus nattereri* (pisces characiformes) in Marajó Island, Brazil. *Revista Brasileira de Parasitologia Veterinária*, 21, 165–170.
- Bialetzki, A., Nakatani, K., Sanches, P. V., Baumgartner, G., Makrakis, M. C., & Taguti, T. L. (2008). Desenvolvimento inicial de *Hoplias aff. malabaricus* (Bloch, 1794) (Osteichthyes, Erythrinidae) da planície alagável do alto rio Paraná, Brasil. *Acta Scientiarum Biological Sciences*, 30, 141–149.
- Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology*, 83, 575.
- Crespo-Lopez, M. E., Augusto-Oliveira, M., Lopes-Araújo, A., Santos-Sacramento, L., Yuki-Takeda, P., Macchi, B. M., do Nascimento, J. L. M., Maia, C. S. F., Lima, R. R., & Arrifano, G. P. (2021). Mercury: What can we learn from the Amazon? *Environment International*, 146, 106223.
- da Silva-Costa, M., Viana, L. F., Lima-Cardoso, C. A., Gonar-Silva-Isacksson, E. D., Silva, J. C., & Florentino, A. C. (2022). Landscape composition and inorganic contaminants in water and muscle tissue of *Plagioscion squamosissimus* in the Araguari River (Amazon, Brazil). *Environmental Research*, 208, 112691.
- Dórea, J. G., Barbosa, A. C., Souza, J., Fadini, P., & Jardim, W. F. (2004). Piranhas (*Serrasalmus* spp.) as markers of mercury bioaccumulation in Amazonian ecosystems. *Ecotoxicology and Environmental Safety*, 59, 57–63.
- Eiras, J. C., Pavanelli, G. C., Takemoto, R. M., & Nawa, Y. (2018). Fish-borne nematodiasis in South America: Neglected emerging diseases. *Journal of Helminthology*, 92, 649–654.
- Eiras, J. C., Pavanelli, G. C., Takemoto, R. M., Yamaguchi, M. U., Karkling, L. C., & Nawa, Y. (2016). Potential risk of fish-borne nematode infections in humans in Brazil-Current status based on a literature review. *Food Waterborne Parasitology*, 5, 1–6.
- Fontenelle, G., Knoff, M., Felizardo, N. N., Torres, E. J. L., Matos, E. R., Gomes, D. C., & São-Clemente, S. C. (2016). Anisakid larva parasitizing *Plagioscion squamosissimus* in Marajó Bay and Tapajós River, state of Pará, Brazil. *Revista Brasileira de Parasitologia Veterinária*, 25, 492–496.
- Freitas, C. E. C., & Siqueira-Souza, F. K. (2009). O uso de peixes como bioindicador ambiental em áreas de várzea da bacia amazônica. *Revista Agrogeoambiental*, 1, 39–45.
- Haddad-Junior, V., de Oliveira, Í. F., Bicudo, N. P., & Marques, M. E. A. (2021). Gnathostomiasis acquired after consumption of raw freshwater fish in the amazon region: A report of two cases in Brazil. *Revista da Sociedade Brasileira Medicina Tropical*, 54, 1–3.
- Heckmann, R. A., Amin, O. M., & Khan, A. (2015). Histopathology of *Centrorhynchus globirostris* (Acanthocephala: Centrorhynchidae) infecting the intestine of the pheasant crow, *Centropus sinensis* (Stephens) in Pakistan. *Scientia Parasitologica*, 16, 151–155.
- Jacobina, U. P., Paiva, E., & Dergam, J. A. (2011). Pleistocene karyotypic divergence in *Hoplias malabaricus* (Bloch, 1794) (Teleostei: Erythrinidae) populations in southeastern Brazil. *Neotropical Ichthyology*, 9, 325–333.
- Lafferty, K. D. (1997). Environmental parasitology: What can parasites tell us about human impacts on the environment? *Parasitology Today*, 13, 251–255.
- Lima, D. P., Santos, C., Silva, R. S., Yoshioka, E. T. O., & Bezerra, R. M. (2015). Contaminação por metais pesados em peixes e água da bacia do rio Cassiporé, Estado do Amapá, Brasil. *Acta Amazonica*, 45, 405–414.
- Lino, A. S., Kasper, D., Guida, Y. S., Thomaz, J. R., & Malm, O. (2018). Mercury and selenium in fishes from the Tapajós River in the Brazilian Amazon: An evaluation

- of human exposure. *Journal of Trace Elements in Medicine and Biology*, 48, 196–201.
- Lopes, I. G., Oliveira, R. G., & Ramos, F. M. (2016). Perfil do consumo de peixes pela população Brasileira. *Biota Amazonia*, 6, 62–65.
- MacKenzie, K. (1999). Parasites as pollution indicators in marine ecosystems: A proposed early warning system. *Marine Pollution Bulletin*, 38, 955–959.
- Marques, D. F., Santos, F. A., Silva, S. S., Sampaio, I., & Rodrigues, L. R. R. (2013). Cytogenetic and DNA barcoding reveals high divergence within the trahira, *Hoplias malabaricus* (Characiformes: Erythrinidae) from the lower Amazon River. *Neotropical Ichthyology*, 11, 459–466.
- Melo, F. T. V., Melo, C. S. B., Nascimento, L. C. S., Giese, E. G., Furtado, A. P., & Santos, J. N. (2016). Morphological characterization of *Eustrongylides* sp. larvae (Nematoda, Dioctophymatoidea) parasite of *Rhinella marina* (Amphibia: Bufonidae) from Eastern Amazonia. *Revista Brasileira de Parasitologia Veterinária*, 25, 235–239.
- Moravec, F., & Kohn, A. B. M. M. F. (1993). Nematode parasites of fishes of the Paraná River, Brazil. Part 3. Camallanoidea and Dracunculoidea. *Folia Parasitologica (Praha)*, 40, 211–2209.
- Morsy, K., Bashtar, A. R., Mostafa, N., El-Deeb, S., & Thabet, S. (2015). New host records of three juvenile nematodes in Egypt: *Anisakis* sp. (Type II), *Hysterothylacium patagonense* (Anisakidae), and *Echinocephalus overstreeti* (Gnathostomatidae) from the greater lizard fish *Saurida undosquamis* of the Red Sea. *Parasitology Research*, 114, 1119–1128.
- Murrieta-Morey, G. A., & Malta, J. C. O. (2016). Parasites with zoonotic potential in *Serrasalmus altispinis* Merckx, Jegue & Santos, 2000 (Characiformes: Serrasalmidae) from floodplain lakes in the Amazon, Brazil. *Neotropical Helminthology*, 10, 249–258.
- Nelson, J. (2006). *Fishes of the world* (4th Ed.). John Wiley and Sons, Inc.
- Poulin, R. (1992). Toxic pollution and parasitism in freshwater fish. *Parasitology Today*, 8, 58–61.
- QGIS Development Team. (2020). *QGIS Geographic Information System*. Open Source Geospatial Foundation Project. <http://qgis.osgeo.org>
- Reiczgel, J., Marozzi, M., Fábíán, I., & Rózsa, L. (2019). Biostatistics for parasitologists – A primer to quantitative parasitology. *Trends in Parasitology*, 35, 277–281.
- Rozsa, L., Reiczgel, J., & Majoros, G. (2000). Quantifying parasites in samples of hosts. *Journal of Parasitology*, 2, 228–232.
- Santin, M., Takemoto, R. M., Pavanelli, G. C., Bialecki, A., & Tavernari, F. C. (2009). Helminths parasitizing larval fish from Pantanal, Brazil. *Journal of Helminthology*, 83(1), 51–55.
- Santoro, M., Iaccarino, D., & Bellisario, B. (2020). Host biological factors and geographic locality influence predictors of parasite communities in sympatric sparid fishes off the southern Italian coast. *Scientific Reports*, 10, 13283.
- Santos, U., Völcker, C. M., Belei, F. A., Cioffi, M. B., Bertollo, L. A. C., Paiva, S. R., & Dergam, J. A. (2009). Molecular and karyotypic phylogeography in the Neotropical *Hoplias malabaricus* (Erythrinidae) fish in eastern Brazil. *Journal Fish Biology*, 75, 2326–2343.
- Schludermann, C., Konecny, R., Laimgruber, S., Lewis, J. W., Schiemer, F., Chovanec, A., & Sures, B. (2003). Fish macroparasites as indicators of heavy metal pollution in river sites in Austria. *Parasitology*, 126, 61–69.
- Silva, M. A., Aride, P. H. R., Santos, S. M., Araujo, R. L., Pantoja-Lima, J., Braga, T. M. P., & Oliveira, A. T. (2014). Preferências e restrições alimentares de moradores do município de Juruá, Amazonas. *Scientia Amazonia*, 3, 106–111.
- Silva, S. F., Oliveira, D. C., Pereira, J. P. G., Castro, S. P., Costa, B. N. S., & Lima, M. O. (2019). Seasonal variation of mercury in commercial fishes of the Amazon Triple Frontier, Western Amazon Basin. *Ecological Indicators*, 106, 105549.
- Siqueira-Souza, F. K., & Freitas, C. E. C. (2004). Fish diversity of floodplain lakes on the lower stretch of the Solimões River. *Brazilian Journal of Biology*, 64, 501–510.
- Sures, B. (2003). Accumulation of heavy metals by intestinal helminths in fish: An overview and perspective. *Parasitology*, 126, 53–60.
- Sures, B. (2006). How parasitism and pollution affect the physiological homeostasis of aquatic hosts. *Journal of Helminthology*, 80(2), 151–157.
- Sures, B. (2007). Host-parasite interactions from an ecotoxicological perspective. *Parasitologia*, 49, 173–176.
- Sures, B. (2008a). Environmental parasitology. Interactions between parasites and pollutants in the aquatic environment. *Parasite*, 15, 434–438.
- Sures, B. (2008b). Host-parasite interactions in polluted environments. *Journal of Fish Biology*, 73, 2133–2142.
- Sures, B., & Siddall, R. (2003). *Pomphorhynchus laevis* (Palaeacanthocephala) in the intestine of chub (*Leuciscus cephalus*) as an indicator of metal pollution. *International Journal for Parasitology*, 33, 65–70.



- Sures, B., Siddall, R., & Taraschewski, H. (1999). Parasites as accumulation indicators of heavy metal pollution. *Parasitology Today*, 15, 16–21.
- Sures, B., & Taraschewski, H. (1995). Cadmium concentrations in two adult acanthocephalans, *Pomphorhynchus laevis* and *Acanthocephalus lucii*, as compared with their fish hosts and cadmium and lead levels in larvae of *A. lucii* as compared with their crustacean host. *Parasitology Research*, 81, 494–497.
- Tanaka, Y., & Mori, Y. (1997). Principal Component Analysis based on a subset of variables: variable selection and sensitivity analysis. *American Journal of Mathematics and Management Science*, 17(1), 61–89.
- Thatcher, V. E., & Brites-Neto, J. (1994). Diagnóstico, prevenção e tratamento das enfermidades de peixes Neotrópicos de água doce. *Revista Brasileira de Medicina Veterinária*, 16, 111–128.
- Thielen, F., Zimmermann, S., Baska, F., Taraschewski, H., & Sures, B. (2004). The intestinal parasite *Pomphorhynchus laevis* (Acanthocephala) from barbel as a bioindicator for metal pollution in the Danube River near Budapest, Hungary. *Environmental Pollution*, 129, 421–429.
- Vasconcellos, A. C. S., Hallwass, G., Bezerra, J. G., Aciole, A. N. S., Meneses, H. N. M., Lima, M. O., Jesus, I. M., Hacon, S. S., & Basta, P. C. (2021). Health risk assessment of mercury exposure from fish consumption in Munduruku indigenous communities in the Brazilian Amazon. *International Journal of Environmental Research and Public Health*, 18, 7940.
- Vazzoler, A. W. M. (1996). *Biología da reprodução de peixes teleósteos, teoria e prática*. Eduem.
- Vidal-Martínez, V. M., Pech, D., Sures, B., Purucker, S. T., & Poulin, R. (2010). Can parasites really reveal environmental impact? *Trends in Parasitology*, 26, 44–51.
- Vitorino, C. A., Souza, I. L., Rosa, J. N., Valente, G. T., Martins, C., & Venere, P. C. (2011). Molecular cytogenetics and its contribution to the understanding of the chromosomal diversification in *Hoplias malabaricus* (Characiformes). *Journal of Fish Biology*, 78, 1239–1248.
- Williams, H. H., & Mackenzie, K. (2003). Marine parasites as pollution indicators: An update. *Parasitology*, 126(S7), S27–41.