# Total Mercury Content in the Tissues of Freshwater Chelonium (Podocnemis Expansa) and a Human Health Risk Assessment for the Amazon Population in Brazil

**Fábio Targino Júnior** (fabiotargino@id.uff.br)
Universidade Federal Fluminense
**Joanna Damazio de Nunes Ribeiro**
Universidade Federal Fluminense
**Julia Siqueira Simões**
Universidade Federal Fluminense
**Carla Silva Carneiro**
Universidade Federal do Rio de Janeiro
**Stella Maris Lazzarini**
Centro de Pesquisa e Preservação de Mamíferos Aquáticos
**Aline Ramos Souza**
Universidade Federal do Amazonas
**Micheli Silva Ferreira**
Universidade Federal Fluminense
**Sérgio Borges Mano**
Universidade Federal Fluminense
**Erick Almeida Esmerino**
Universidade Federal Fluminense
**Eliane Teixeira Társico**
Universidade Federal Fluminense

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**Research Article**

**Keywords:** Public Health, Food safety, Environmental pollution, Trace elements, Biomonitoring, Freshwater toxicology

**Posted Date:** November 23rd, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-2266817/v1

# Abstract

Researchers are highly aware of the silent, negative effects caused by mercury pollution in gold mining areas. Freshwater turtles are culturally part of the diet of riverside populations in the Amazon region and this area presents mercury (Hg) pollution issues mainly due to gold mining activities. Thus, this research aimed to evaluate the total mercury (THg) content in the different organs of river turtles (P. expansa) and carry out a human health risk assessment associated with the consumption of these animals. Skin (n=28), muscle (n=19) and brain (n=2) samples were analyzed by Atomic Absorption Spectrometry (TDA-AAS) and a DMA-80™ mercury analyzer used for the total mercury determinations. The average values found for THg in the skin, muscle and brain samples were, respectively, 0.1045 mg.kg-1, 0.1092 mg.kg-1 and 0.0601mg.kg-1. Thus, THg was observed even though the P. expansa were kept in captivity, possibly due to previous contamination by air, water and food. The Hazard Quotient (HQ) was calculated considering a 9.07g.day-1 intake dose of P. expansa and the consumption of turtles once a week showed a HQ=2.45, which may cause long-term injuries to human health. Although the muscle concentrations were below the maximum limit established by the World Health Organization (WHO) and Brazilian regulatory agencies, it is important to evaluate consumption factors such as amount ingested, frequency and animal gender, which may cause a potential risk to regular consumers due to mercury bioaccumulation. The WHO may consider various aspects in order to warn the amazon population about the severity and silent hazard of this metal, especially due to the importance of this matrix in the region. This region urgently needs government actions to inhibit clandestine mining and to prevent future serious, chronic health problems of the entire population.


# 1 Introduction

Mercury (Hg) is an essential toxic trace element found in the Amazonian ecosystem due to its use in the amalgamation stage of gold extraction (Castilhos et al, 2015, Lino et al, 2019, Miserendino et al, 2017, Pinto et al, 2019). This toxic contaminant requires public health attention due to its toxicity, high absorption and low excretion after ingestion (Okpala et al, 2017). The toxic element is present in the aquatic environment where it is methylated, bioaccumulates and biomagnifies, providing the possibility for incorporation by human beings, who are at the top of the food chain (Kasper et al, 2014). Metal pollution is less visible than others but may cause intense and extensive effects on ecosystems and humans (Vardhan et al, 2019).

Mercury can have toxic effects on tissues and systems such as the nervous, digestive and immune systems, and organs such as the lungs, kidneys, skin and eyes (WHO, 2017). The organic form, methyl mercury (MeHg), is a potent neurotoxin inducing oxidative stress activity, neuroinflammatory action, and changes in the heart rate (Alexander et al., 2011). A recent study demonstrated the toxicity of high concentrations of MeHg on thyroid cell oncogenesis by acting on the ERK-mediated pro-oncogenic signal transduction pathway (Maggisano et al., 2020). Mercury is eliminated into the environment in the inorganic form and may undergo sediment methylation by physical, chemical, and biological mechanisms, turning into methylmercury (MeHg), the most toxic form of this metal (Bonotto et al., 2018, Vieira et al., 2018). Due to its geochemical cycle, mercury can be found in areas far away from its mobilized source (Ma, Du, and Wang, 2019). Inorganic Hg reaches the atmosphere, and, by precipitation, is deposited on the bottom of rivers, lakes, and other watercourses (Beckers and Rinklebe, 2017). Also, humans may demonstrate pathognomonic effects (Kim, Kabir, and Jahan, 2016).

To protect human health and limit dietary exposure to toxic metals, international agencies and Brazilian regulations have provided guidelines for the human intake of trace elements (WHO/FAO, 1996, Brasil, 2019). According to the World Health Organization (WHO), to estimate the amount of mercury per unit of body weight that can be ingested over a lifetime without health risks, the joint FAO/WHO Expert Committee recommended Provisional Tolerable Weekly Intakes (PTWIs) for MeHg of 1.6mg.$Kg^{-1}$ body weight (FAO, WHO, 2017).

The Amazon population's diet varies according to the season, but aquatic animals are the primary protein source for most riverside people, especially children. The consumption of turtles by natives and settlers is historically and culturally developed by Amazon communities (Silva and Begossi, 2009), and the amazon turtle (*P. expansa*) is the most consumed species. After colonization, chelonian commerce improved and the local population could explore the meat, eggs, oil, and carapace as an additional financial source. Due to their long life-span, aquatic turtles can accumulate Hg over extended periods and therefore it could be of interest to monitor environmental contamination (Rebelo and Pezzuti, 2000, Silva and Begossi, 2009). The history of gold mining and a hydroelectric power plant has influenced Hg availability in the environment and living organisms.

Therefore, it is essential to develop research and environmental biomonitoring programs to measure the Hg concentration in Amazonian turtles and assess the risk of exposure to this population. Thus, this research aimed to evaluate the presence of total mercury (THg) in different organs of the *P. expansa* chelonian species and carry out a human health risk assessment associated with the consumption of these animals.

# 2 Materials And Methods

## 2.1 Experimental area

The location studied was Vila Balbina, in the municipality of Presidente Figueiredo, located in the North of Amazonas State (latitude 1º55'07.0 S and longitude 59º28'05.9 W), Brazil. This location was considered for study due to the extensive mining activity that has occurred there for decades. The chelonian samples were obtained from the *Centro de Preservação e Pesquisa de Quelônios Aquáticos* (CPPQA), located near the River Uatumã mouth, a tributary of the black waters inserted in an area with anthropogenic actions, such as metal extractions (niobium-Nb and tin-Sn).

## 2.2 Chelonian sampling

Twenty-eight samples of Giant South American freshwater turtles (*P. expansa)* were collected in June 2018 from the CPPQA captivity area, and the carapace length of each animal measured. Skin and muscle samples (~2cm³) were obtained by biopsy punch (3mm) from the pelvic member using a previous local anesthesia. Considering the difficulty of animal handling, 28 skin samples and 19 muscle samples were obtained, but only two brain samples were collected by the CPPQA when the animals died. The samples were frozen (-18ºC ± 2°C) stored in polypropylene packing and flown to a chemical laboratory for analysis at the Federal Fluminense University. The animals were gender identified by observation of the reproductive organ dimorphism (Ceballos et al., 2012).

### 2.3 Chelonian consumption data

Ninety one Balbina residents answered a questionnaire about the consumption of chelonians in the area. The questionnaire involved social-economic questions and the consumption of chelonian meat and eggs with respect to frequency, occasion, amount, and preferences.

### 2.5 Instrumentation

The Direct mercury analyzer DMA80™ (Milestone Inc., Shelton, USA) involves thermal decomposition, catalytic conversion, amalgamation, and atomic absorption spectrophotometry. The heating stages are implemented to first dry and then thermally decompose the sample in a quartz cuvette, and a flow of oxygen carries the decomposition products through a hot catalyst bed. The mercury content is reduced to Hg(0) and then carried along with the reaction gases to an amalgamator, where the mercury is selectively trapped. Subsequently, the amalgamator is heated and releases the trapped mercury to the single beam, fixed wavelength atomic absorption spectrophotometer. The absorbance is measured at 253.7 nm as a function of the mercury content. All non-mercury vapors and decomposition products are flushed from the system by the continuous flow of gas (Milestone Inc., Shelton, USA).

### 2.6 Analytical Procedures

The analyses were carried out according to the analytical procedures of the US Environmental Protection Agency - EPA 7473 (2007). An aliquot of 0.210 g of the chelonian sample was directly and accurately weighed into a quartz cuvette. The DMA80™ used does not require pretreated samples. According to the WHO/FAO recommendations, the samples were weighed on a wet weight basis (wwb).

The samples were submitted to the following heating stages: starting temperature of 200±10ºC, drying stage for 60 seconds at 250±10ºC, thermal decomposition at 650±10ºC for 120 seconds, and finally 100 seconds for the analytical reading and cooling. An amalgamator selectively trapped the Hg, and the oxygen flow removed any remaining gases or decomposition products (24 seconds), the amalgamator was rapidly heated (12 seconds), releasing the Hg vapor. The atomic absorbance was read at 253.7 nm.

The accuracy of the analytical procedure was evaluated by analyzing the Certified Reference Material (CRM) of DORM-2 (dogfish muscle tissue) obtained from the National Research Council Canada (NRCC). The CRM control samples were analyzed without any treatment, and non-analyzed aliquots were used to determine the moisture content according to the certificate instructions for analysis. The certified material concentration was determined in triplicate and reached 4.470 $\mu g.g^{-1}$.

### 2.7 Limits of detection and quantification

The limits of detection (LOD) and quantification (LOQ) were calculated from the mean concentration and relative standard deviation of ten blanks and the slope of the analytical curve ($1.5x10^{-6}$ $mg.kg^{-1}$ of THg). These limits were calculated as 3 and 10 times the standard deviation of 10 blanks and multiplied by the dilution factor used for sample preparation (1g of sample/25 mL), using the following equations:

$LOD = 3 \times s$ and $LOQ = 10 \times s$

where: s = the average standard deviation in units of instrumental response, of at least ten independent determinations of analytical blanks.

The limits of detection (LOD) and quantification (LOQ) were 94$\mu g.kg^{-1}$ and 314$\mu g.kg^{-1}$, respectively. The recovery value mean was 84.93%. The value of the LOQ was confirmed using ten independent replicates.

### 2.8 Human health risk assessment

The human health risk was assessed by the EPA/WHO method (1989), assessing the exposure and characterizing the risk associated with the consumption of *P. expansa*. The exposure assessment was represented by I (Intake dose), which involves the description of the nature and size of the populations studied exposed to the chemical agent (Hg in this study), and the magnitude and duration of the exposure, calculated according to the following equation:

$$I = (CF \times IR \times FI \times EF \times ED) / (BW \times AT)$$

The Intake dose (I) was defined by the ratio between the sample and the estimated weight of the exposed population. The following factors were taken into account: Chemical concentration (CF) in *P. expansa* muscle (male, female and total mean), the intake rate (IR) used was the average daily consumption of turtle of 9.07 $g.person.day^{-1}$ as estimated by Isaac et al. (2015), fraction of food ingested (FI) obtained from a contaminated source (considered as the total food fraction in this study), exposure frequency (EF) consumption considered annually, monthly and weekly, exposure duration (ED) was established from the average life expectancy for Brazilian people of 75.8 years (IBGE, 2016), body weight (BW) was considered as 70 kg (US EPA, 1989), and average time (AT) as the annual exposure frequency by individuals during their life expectancy (AT=ED×365 days).

For the risk characterization, the intake dose (I) was compared with the RfD (Reference dose of Hg), which, according to WHO (2010), is $3.0 \times 10^{-4}$ $mg.kg.day^{-1}$, calculated using the following equation.

$$HQ = I / RfD$$

HQ greater than 1 (HQ>1) order of magnitude indicating health risks.

### 2.9 Statistical analysis

The one-way ANOVA and Tukey's test were applied using XLSTAT™ 2014.1 (Addinsoft Inc., New York, NY, USA) with a significance level of 95% ($P<0.05$) to establish any significant differences between the means. A correlation analysis was applied and Pearson's linear coefficient calculated amongst the muscle and skin tissues. All analyses were carried out in triplicate.

# 3 Results And Discussion

## 3.1 Results

### 3.1.1 THg content

The average carapace length for females was 74.42 cm (n=14) and 47.18 cm (n=16) for males. The present results show that although the female carapaces were larger than those of the males, higher THg concentrations were not observed in the female animals. The muscle tissue samples showed the highest concentrations of THg (0.6929mg.kg$^{-1}$) as compared to maximum values in the skin of (0.5457mg.kg$^{-1}$) and in the brain of (0.0610mg.kg$^{-1}$). The mean THg concentrations found in the skin (n=28), muscle (n=19) and brain (n=2) were, respectively, 0.1045 mg.kg$^{-1}$, 0.1092 mg.kg$^{-1}$, and 0.0601mg.kg$^{-1}$. The results are shown in Table 1.

Regarding the tissues, the muscle should be considered the most important, because of its higher accumulation of THg in *P. expansa*as compared to the skin, but the present research found no significant difference ($P<0.05$). However, a notable difference was detected in the tissue concentrations, considering a minimum of "not detected" in the muscle and skin and a maximum of 0.6929mg.kg$^{-1}$ in the muscle. The average values found in the male samples demonstrated greater THg bioaccumulation in the male muscle than in the female muscle, and a significant difference ($P<0.05$) was observed between the female muscle samples and the samples of the other tissues.

A positive, gender independent correlation was observed ($R^2$=0.87) between the skin and muscle concentrations. Also, the correlation was positive ($R^2$ = 0.50) between tissues (muscle and skin) for males, but no such correlation was observed for females ($R^2$ = 0.43). The present research found no correlation between carapace length and THg concentrations in the muscle or skin for males ($R^2$ = 0.011) or females ($R^2$ = 0.15), possibly due to the high variation between the tissue concentrations in the animal diet and the diversity of origin.

### 3.1.2 Human health risk assessment

Amongst those interviewed, 18% (15/91) consumed chelonian meat, especially tracajá (*P. unifilis*) and the Giant South American river turtles (*P. expansa*). However, only 8% (7/91) reported consuming the eggs.

Regarding the consumption data used to calculate the HQ, 8/91 ate chelonian meat at least once a year, 5/91 ate it once a month, and 2/91 once a week during through the egg-laying months (September to December). The people interviewed confirmed that these animals and eggs were caught in Vila Balbina, Presidente Figueiredo, Amazon.

The Hazard Quotient (HQ) was calculated for the different consumption intervals (annually, monthly, and weekly) with respect to the Thigh concentrations in male plus female *P. expansa*, and the mean THg values for both genders separately. Table 2 shows the HQ results obtained for the Human risk assessment of *P. expansa* muscle.

### 3.2 Discussion

#### 3.2.1 THg content

The total mercury content in the different turtle tissues is related to Hg kinetics. Researchers have indicated that the total mercury content in freshwater fish is related to the species and habitat evaluated (Yoshimoto et al., 2016, Zang et al., 2020). Brazilian studies have reported a THg content in Amazon fish muscle of over 0.500 mg $kg^{-1}$, above the limit recommended by World Health Organization (Custódio et al., 2020, Queiroz et al., 2018, WHO, 2003). Although there are few studies with Amazonian freshwater chelonians, it is essential to consider the influence of the environment on the contents of trace elements.

The *P. Unifilis* caught in the lower River Xingu accumulated lower concentrations of THg throughout its life when compared to other species of Amazon turtles. Thus, it appears that the Amazon basin rivers have mercury in a bioavailable form that can be accumulated by organisms. Recent studies have shown that *P. unifilis* and *Podocnemis expansa* are predominantly herbivorous, but may consume insects, crustaceans, and molluscs (Balensiefer and Vogt, 2006, Vogt, 2008). The present authors observed that some studies indicate a difference in feeding preference between genders, with the females consuming more fruits and seeds and the males more stalks and shoots (Teran et al., 1995). Thus, the authors postulated that the difference in Hg concentrations between males and females could be explained by the absence of difference in trophic levels between species gender (Lara et al., 2012). Frossard et al. (2021) identified a maternal transfer of metals (essential or not) to the eggs in tests with udines nesting eggs, triggering genotoxic effects in the hatchlings. Research carried out by Schneider et al. (2011) evaluated non-lethal and non-invasive ways of collecting samples from Amazonian chelonian species for Hg monitoring. They found no significant differences between the carapace and internal organs of P. *erythrocephala* and P. *sextuberculata*. In the present study, the THg concentrations in the turtle skin and muscle indicated that anthropic actions might impact animals in different environments.

Different THg concentrations may be observed between males and females due to changes in eating habits (Schneider et al., 2009) and the elimination of Hg via the eggs during the reproductive period (Jagoe et al., 2003, Frossard et al., 2021). However, in contrast, the present study diverged from this observation and the THG concentration for males did not significantly differ from that of the females. Schneider et al. (2010) found 0.0624 $mg.kg^{-1}$ THg in *P. expansa* muscle samples, 0.432 $mg.kg^{-1}$ THg in *C.*

*fimbriatus* muscle and Schneider et al. (2009) found 0.033 $mg.kg^{-1}$ THg in *P. erythrocephala* muscle. These animals present distinct eating behaviors between the genders and life periods. Small amounts of fish and crab were observed in studies that analyzed the chelonian gastrointestinal content, but their primary food source is seeds and fruits, especially those from riverside trees and flooded forests. However, the literature also reported that juvenile turtles prefer to eat fish and other meat.

### 3.2.2 THg bioaccumulation

Toxic metals entering the river body would be absorbed in sediments and migrate to the sediment and biota through a biological and chemical process (Eagles-Smith et al., 2016, Windsor et al., 2019), so researchers began to pay more attention to pollution by toxic metals (Kronberg et al., 2016, Pignati et al., 2018, Yi et al., 2008).

The bioaccumulation of toxic metals by reptiles results from surface contact with the water and food chain (Schneider et al., 2011, Zupo et al., 2019), and the concentrations depend on the environmental levels of trace metals in the habitat, since they are absorbed and stored in the tissues (Yi, Yang, & Zhang, 2011). Researchers have shown that the mercury concentrations in the muscles of freshwater animals varied widely depending on the capture location (Eagles-Smith et al., 2016, Zang et al., 2020).

Captive breeding possibly contributed to reduced mercury concentrations when compared to the data reported by Schneider et al. (2014). The researchers evaluated the THg concentrations in the muscles and keratin samples from the carapaces of free-living P. *expansa* in the Amazonas region and found THg concentrations in the muscle of 0.1 $mg.kg^{-1}$ and in the carapaces of 2.8 $mg.kg^{-1}$. However, the diet and food consumption were human-controlled, thus interfering with the Hg kinetics.

In addition, the muscle THg concentration is related to eating habits, which interferes with the biomagnification of this trace element. On carrying out research on the THg concentrations in Amazon chelonian muscles from different species, Scheneider et al. (2010) found concentrations of 0.106 $mg.kg^{-1}$ in P. *expansa,* corroborating the results of the present research.

*C. fimbriatus*, a predatory carnivorous animal, is adapted for collecting fish and other animals in the aquatic environment (Lemell et al., 2019) via a well-developed suction system and head movements in the direction of the prey (Lemell et al., 2010). In contrast, when raised in captivity, young P. *expansa* prefer meat and fish (Ojast et al., 1971), which may explain the higher average THg concentrations found in C. *fimbriatus* specimens as compared to P. *expansa*.

Different Amazonian reptiles have been used to monitor THg bioaccumulation. Eggins et al. (2015) evaluated this trace metal in various *C. crocodilus* tissues, this being an alligator from the River Purus (Amazonas state), and found a THg concentration of 0.389 $mg.kg^{-1}$. Correia et al. (2014) evaluated THg in alligators from the Mamirauá reserve (Tefé, Amazonas State), and found THg concentrations of 0.407

$mg.kg^{-1}$, these animals showing predatory eating behavior. Thus, the THg concentrations represent the biomagnification principle, which is associated with the consumption of fish, chelonians, small mammals, and birds.

### 3.2.3 Human Health Risk Assessment

Although the established PTWI limits (WHO 1990, WHO 2017) suggest secure mercury intake values, researchers and global health organizations highlight that this metal is dangerous at any concentration due to its bioaccumulation characteristics, and thus there is no healthy mercury level for humans (Manavi and Mazumder, 2018, US EPA, 1997). Bioavailability can be affected by controlling the biotic and abiotic factors. The most critical tool that can be used to reduce mercury in the environment and in animals is the prevention of pollution, which is considered a long-term goal (Burger and Gochfeld, 2011). Balbina village (Vila Balbina), in Amazon State, is highly influenced by mining and damming, both anthropogenic activities on the River Uatumã causing environmental impact and interfering with the Hg biological cycle (Kehrig et al, 1998).

The maximum tolerable limit of 0.5 mg $MeHg.kg^{-1}$ for fish (except for predatory fish) (FAO, 2016) is established for seafood based on a weekly consumption. The Brazilian regulatory agencies also set the acceptable THg level for non-predatory fish, crustaceans, mollusks, cephalopods, and bivalve mollusks at 0.5 $mg.kg^{-1}$, and at 1.0 $mg.kg^{-1}$ for predatory fish (Brasil, 2019). Although the agencies did not determine a THg limit for chelonians, considering the limit established for fish, the THg concentration found in *P. expansa* muscle (0.1045 $mg.kg^{-1}$) did not exceed the legislation values, although two turtles presented THg concentrations above 0.5 $mg.kg^{-1}$.

A Hazard Quotient (HQ) above 1 (HQ> 1) represents a potential health risk to consumers (US EPA, 2009). To determine the HQ for the consumption of P. expansa, it is crucial to consider the weekly consumption of the male categories. When considering the mean THg concentration in males and females for monthly or annual consumption, the HQ was <1 in all categories. Research carried out by Correia et al. (2014) evaluated the HQ for alligator consumption by riverside populations and determined a value of 7.19, considering this quotient due to the higher Hg concentrations in these animals as compared to *P. expansa*, since they show predatory feeding behavior and are piscivores.

One of the characteristics of the consumption of turtles in the Amazon area is its seasonality, with the hunting of turtles and other animals for consumption being more intense in preserved and isolated areas (Isaac et al., 2015). In the present study, 18% (15/91) of the chelonian meat consumers interviewed considered it rare and occasional, only occurring in months with greater availability. Turtle consumption is taboo among the Amazonian population due to preservation practices of these species and constant supervision by environmental agencies (Silva, 2007). Although in this research, only a small percentage of individuals reported consuming turtle meat and eggs, the true consumption might be higher. Female specimens are more consumed since they are easier to capture, their meat is more appreciated, and the yield is higher (Miorando et al, 2015).

There is little information available on the risk evaluation associated with chelonian consumption. The authors highlighted that total mercury concentrations in river fish from Malaysia and Ghana were influenced by the habitat, feeding habits, species, and body length, and the increased HQ was due to the nearby gold mining area (Doke and Gohlke, 2018). Brazilian researchers have reported HQ values ranging from 1.5 to 28.5 in the River Amazon and concluded that the elevated risk was associated with artisanal gold mining areas, where people also catch fish (Castilhos et al, 2015). They suggested population risks at almost the sampling points due to consumption. It appears there is considerable contamination of living beings that share the same environment with turtles due to their consumption without considering food safety (Manavi and Mazumder, 2018). Hence, determining the allowable limit for the consumption of these chelonians (daily, weekly or monthly) is crucial to preserve public health.

# 4 Conclusions

*P. expansa* chelonians, kept in captivity in Vila Balbina (Balbina village) at the mouth of the River Uatumã, accumulated THg in the muscles, skin and brain, with higher concentrations in the skin and muscle. Regarding gender differences, the males showed higher THg concentrations than the females. In general, the concentrations found in the Amazonian giant river turtle muscles were lower than the limits established for fish consumption.

The mercury content is mainly associated to animal's diet and is considered to accumulate in organisms. It is essential to notice that depending on the consumption characteristics, the frequency and animals' gender may be a risk to public health. Although the THg concentrations are below the acute metal toxicity level, the bioaccumulation characteristic deserves attention because of its long-term effects on human health.

A comprehensive study on this trace element in the Amazonian area is recommended to determine allowable consumption limits and identify the location most involved with contaminants. There are clear benefits and risks from the consumption of fishery products, so the population should be provided with enough information to maximize the positive health benefits while minimizing the risks from contaminants. General and scientific knowledge is scarce, reinforcing the importance of developing communication tools involving scientists, health professionals, regulators and the general public.

More extensive studies on Hg contamination using turtles as bioindicators, with different approaches such as physiology, histopathology and the analysis of biomarkers, should be carried out in the Amazon region. This is important for a better understanding of similar phenomena reported in previous studies and because such approaches are incredibly rare in the Amazon basin, especially in River Xingu area.

# Declarations

## 5.1 Eticals approval and consent to participate

This work was evaluated and accepted by the UFF Human Ethics Committee (number 2899092), SisBio (number 63587-3), and the UFF Animal Ethics Committee (number 1012300319).

### 5.2 Consent for publication

The authors accept the publication of this article in the jornal database.

### 5.3 Availability of data and materials

All data generated during analyzed this study are included in this article.

### 5.4 Competing interests

The authors declare no conflicts of interest.

### 5.5 Authors contribuitions

Fábio José Targino Moreira da Silva Júnior: Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Visualization. Joanna Damazio de Nunes Ribeiro: Methodology, Validation, Formal Analysis, Investigation, Data Curation, Visualization. Julia Siqueira Simões: Writing – Original Draft. Stella Maris Lazzarini: Collect samples, Resources. Aline Ramos Souza: Collect samples, Resources. Micheli Silva Ferreira: Conceptualization, Resources, Supervision, Project Administration. Sérgio Borges Mano: Writing – Original Draft. Erick Almeida Esmerino: Writing – Original Draft. Eliane Mársico: Resources, Writing – Original Draft, Visualization, Supervision, Project Administration.

### 5.6 Funding

This research was financed in part by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* – Brazil (CAPES). (financing code 001), Brazil and by the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPQ), Brazil (Grant number 311974/2019–8).

### 5.7 Acknowledgements

The authors are also grateful to *Eletrobras Amazonas Geração e Transmissão de Energia S/A* – (ELETRONORTE) and to the *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio), namely to Gilmar Nicolau Klein.

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# Tables

Tables 1 and 2 are available in the Supplementary Files section.

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