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Mineral content in fishes in the lower course of the itapecuru river in the state of Maranhão, Brazil

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Abstract−Concentrations of calcium, iron, potassium, magnesium, phosphorus, zinc, copper, selenium and nickel were determined in the muscle tissue of seven species of fish (*Plagioscion squamosissimus*, *Geophagus surinamensis*, *Prochilodus lacustres*, *Curimata* sp., *Schizodon dissimilis*, *Ageneiosus ucayalensis* and *Hypostomus plecostomus*) collected from the lower course of the Itapecuru River in the state of Maranhão, Brazil. The samples were digested in a nitric-perchloric solution and analyzed using an inductively coupled plasma atomic emission spectrometer, with the construction of specific calibration curves for each element. The highest concentrations of constituent minerals were found for phosphorus, potassium, nickel and magnesium (399.83, 144.60, 90.20 and 29.49 mg $100\,g^{-1}$, respectively) in *G. surinamensi*, *P. lacustres* and *Curimata* sp. The lowest concentrations were found for copper, zinc, iron and selenium (0.12, 0.51, 1.05 and 8.31 mg $100\,g^{-1}$, respectively) in *Curimata* sp., *S. dissimilis*, *A. ucayalensis* and *P. squamosissimus*. The concentrations of all minerals can be considered low and are below the maximum limit established by Brazilian legislation for the human ingestion of fish meat. A comparison of the seven species of fish investigated revealed no statistically significant differences regarding the concentrations of minerals, suggesting that size and different dietary habits do not exert an influence on absorption. The low concentrations of metals, such as Fe, Cu, Zn and Ni, may be related to the environmental conditions of the mouth of the river, which receives ocean inputs that produce particular tide cycles with a strong dispersion capacity, thereby diminishing residence time in the water column and reducing the availability of these metals to species of fish.

Keywords: Fish, Soft Tissue, Minerals, Spectrophotometry

INTRODUCTION

Fish meat is generally considered a valuable source of calcium and phosphorus as well as reasonable amounts of sodium, magnesium, manganese, chloride, sulfur, selenium, chromium, nickel, aluminum, cobalt, zinc, potassium, copper and iron [4]. Some are macronutrients, meaning that the daily requirement reaches 100 mg/day for an adult human. Others are micronutrients or trace elements, the requirements of which are minimal in humans [5]. However, both the deficiency and excess of minerals in food sources can exert harmful effects on humans. Moreover, minerals in excess can be lethal to aquatic organisms and cause biochemical, structural and functional disorders in species of fish. Studies on minerals are essential for understanding the effects associated with the consumption of fish meat by humans. Although the physiological importance is well documented for some animals, many aspects of ingestion, function and bioavailability need to be clarified [6]. Information on the nutritional micronutrient requirements of species of fish is also fragmentary, mainly because many micronutrients are only needed in very small quantities.

Species of fish can be used as biological indicators since they occupy the top of the food chain and accumulate metals, which can be passed on to humans through ingestion, leading to acute or chronic adverse health conditions. Residence time in polluted waters, age and size affect the concentration and bio-magnification of metals in aquatic organisms [7]. Species of fish in the lower course of the Itapecuru River in northeastern Brazil are an extremely important food source for river communities, as these organisms are a source of high quality proteins and minerals to meet nutritional needs as well as provide energy for the body and the maintenance of vital cell processes. Despite the recognized importance of fishing activities for these communities, little is known regarding the availability of minerals in the local diet. Among other species, *Plagioscion squamosissimus*, *Geophagus surinamensis*, *Curimata* sp. and *Prochilodus lacustris* are found in the lower Itapecuru River and have high economic value as well as recognized nutritional quality.

Despite the consensus that the consumption of fish meat is beneficial to humans, scientific records in Brazil on the mineral con-

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stituents found in species of fish are insufficient. Thus, studies that generate knowledge on the quality of fish meat derived from artisanal fishing in Brazil are of considerable importance to assuring an adequate diet and nutritional safety in the low-income populations that reside in tropical river basins.

MATERIALS AND METHODS

Our study is based on data from quarterly collections performed between June 2012 and May 2013 at three sampling sites located in the lower course of the Itapecuru River between the ITALUÍS water catchment system and the mouth of the river in the city of Rosario (Fig. 1). The identification of fish species was based on [8-17]. A taxonomic update was performed by accessing a global information system on fish species denominated *Fishbase* [18].

Among the specimens captured, individuals of six species (*Plagioscion squamosissimus*, *Geophagus surinamensis*, *Curimata* sp., *Schizodon dissimilis*, *Ageneiosus ucayalensis* and *Hypostomus plecostomus*) were selected based on catch volume and marketing potential. Specimens with the best organoleptic characteristics were considered, independently of the size of the species and sex. Total length (cm), total weight (g) and sex were determined. Some individuals of each species were filleted, regardless of gender, anatomical differences and physiological characteristics. A portion of the lateral-medial region (abdominal muscle fillet) was sampled. The samples were skinless, boneless, representative of the edible part of each individual and equivalent to approximately 200 g. The samples subsequently were placed in labeled plastic bags of low-density polyethylene, frozen to –17 °C and transported to the Food and Water Quality Control Laboratory of the Department of Chemical Technology of the Federal University of Maranhão (Brazil), where ash content analyses were performed. The samples were then processed at the Soil Chemistry Laboratory of the Technological Center of Rural Engineering of Maranhão State University (Brazil) for mineral analyses of the chemical elements calcium, iron, potassium, magnesium, phosphorus, zinc, copper, selenium and nickel.

Ash (total mineral) content was determined by heating the organic matrix to a controlled temperature of 550° to 600 °C for four hours. The samples were then placed in a dessicator until reaching room temperature. The inorganic matrix was weighed following the methods described [19]. The heating and cooling procedures were repeated until obtaining a constant weight.

The elemental concentrations were determined through analyses of the ash obtained by dry digestion after complete decomposition of the muscle tissue, as adapted from [20] and [21]. The solutions were analyzed in an inductively coupled plasma atomic emission spectrophotometer (model 720-ES, VARIAN). Specific calibration curves were used for each element and all analyses were conducted in triplicate. A blank reading was performed ten times to calculate the quantification limit (LQ) and the detection limit (LD) of the equipment, which were calculated considering the mean white signal plus ten-times the standard deviation (SD) for LQ and the mean white signal plus three-times standard deviation (SD) to LD, according to definition and criteria established by [22].

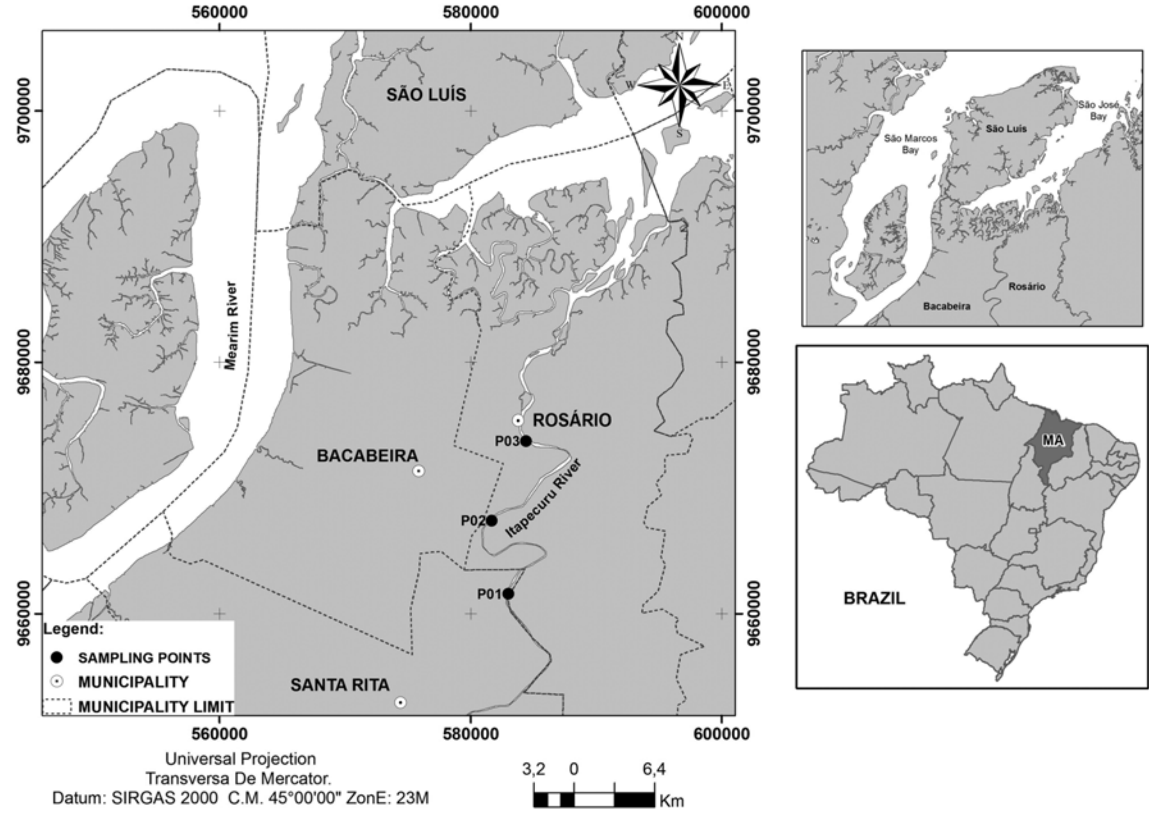

Fig. 1. Location map of sampling sites in lower Itapecuru River.

The entire analytical procedure was tested for both measurement precision and accuracy to assess the degree of reliability which can be allocated to the data generated by this investigation. The precision of the method was established by a calculation of the assay variation coefficients from data of ten independent analyses.

Levene's test was used to determine the homogeneity of assumptions and normality of the data. One-way analysis of variance (ANOVA) was used to compare chemical and nutritional composition among the different species. When the ANOVA results indicated significant differences ($p<0.05$), Tukey's post hoc test was used to identify differences between means, adopting a significance level of $\alpha=0.05$. In cases for which ANOVA assumptions were not met, the non-parametric Kruskal-Wallis test [23] and Mann-Whiney U test were used to compare differences between means. Multivariate analysis employing principal components analysis was used to determine associations between the species sampled and element concentrations based on the variance-covariance matrix. All evaluations were conducted using the Paleontological Statistics (PAST) statistical package, version 2.17 [24].

RESULTS AND DISCUSSION

Table 1 displays the morphometric variables and feeding habits of the species analyzed. Total length and total weight are also presented with standard deviation and range of variation values.

The individuals analyzed were small. The smallest total length (6.7 cm) was recorded for *G. surinamensis* and the largest (38.7 cm) was recorded for *P. lacustris*. Minimum and maximum total weight was 11.3 g and 465.3 g for *P. squamosissimus* and *H. plecostomus*, respectively. Table 2 displays the mineral concentrations in the muscles of the species examined (*P. squamosissimus*, *G. surinamensis*, *Curimata* sp., *P. lacustris*, *S. dissimilis*, *A. ucayalensis* and *H. plecostomus*).

The highest concentrations of constituent minerals (dry weight) were found for phosphorus, potassium, nickel and magnesium (399.83, 144.60, 90.20 and 29.49 mg 100 g^{-1}, respectively) in *G. surinamensi*, *P. lacustres* and *Curimata* sp. Comparatively lower concentrations were found for copper, zinc, iron and selenium (0.12, 0.51, 1.05 and 8.31 mg 100 g^{-1}, respectively) for *Curimata* sp., *S. dissimilis*, *A. ucayalensis* and *P. squamosissimus*. The following results are presented to show the variability in the concentrations of the mineral constituents of the fish species investigated in the present study.

1. Calcium (Ca)

Species of fish absorb calcium directly from the water and do not require additional food-based sources of this element. The level of dissolved Ca in the environment also acts as a stimulus for hormone synthesis and the maintenance of cell membrane integrity. The concentration of Ca in species of fish is mediated by diffusion through the gills and skin as well as by absorption through the intestine. Considering the seven species analyzed herein, the mean Ca concentration was higher in *G. surinamensis* (63.34 mg 100 g^{-1}), *Curimata* sp. (62.81 mg 100 g^{-1}) and *P. lacustris* (60.81 mg 100 g^{-1}). The lowest values of this mineral were detected in *A. ucay-*

Table 1. Taxonomy, morphometric variables and feeding habits of species analyzed

Order	Family	Species	N	Wt (g)	Lt (cm)	Feeding habit
Perciformes	Sciaenidae	*P. squamosissimus*	25	102.5±80.63 (313.7-21.7)	16.2±3.8 (24.6-10.5)	Carnivore
Characiformes	Curimatidae	*Curimata* sp.	12	69.7±21.0 (93.7-35.3)	13.1±1.4 (15.0-10.5)	Detritivore
Perciformes	Cichlidae	*G. surinamensis*	19	34.6±12.4 (61.4-11.3)	9.6±1.1 (11.6-6.7)	Detritivore
Characiformes	Anostomidae	*S. dissimilis*	5	140.8±78.2 (218.7-55.4)	18.9±3.9 (22.5-14.4)	Herbivore
Siluriformes	Auchenipteridae	*A. ucayalensis*	7	47.5±12.2 (61.3-28.6)	16.5±1.5 (18.5-14.2)	Carnivore
Siluriformes	Loricariidae	*H. plecostomus*	7	203.3±155.8 (465.3-32.5)	18.0±5.2 (25.6-10.5)	Detritivore
Characiformes	Prochilodontidae	*P. lacustris*	4	107.9±11.5 (123.2-96.5)	33.2±4.12 (38.7-29.6)	Detritivore

N: sample size; Wt: total length; Lt: total length - Followed by values of mean±standard deviation and amplitude of the variables Wt and Lt

Table 2. Mean and standard deviation (Mean±SD) values of mineral concentrations in seven species of fish from lower Itapecuru River

Minerals mg·100 g^{-1}	Species (Mean±SD)						
	P. squamosissimus	*Curimata* sp.	*P. lacustris*	*G. surinamensis*	*S. dissimilis*	*A. ucayalensis*	*H. plecostomus*
Ca	44.56±12.74	62.81±51.78	60.81*	63.34±1.40	23.32±10.31	21.42±11.73	22.68±13.89
Fe	1.31±0.95	1.34±0.79	1.17*	1.40±0.87	1.73±1.16	1.05±0.07	1.57±0.05
K	85.96±17.29	115.94±89.87	144.6*	85.84±17.88	97.84±5.75	61.09±12.33	75.61±12.97
Mg	22.08±2.41	29.49±14.69	20.46*	25.84±7.92	25.43±1.62	20.55±1.60	21.59±1.51
P	238.76±49.69	298.69±210.92	269.52*	399.83±255.58	220.50±0.01	142.7±12.26	194.75±23.41
Zn	0.58±0.29	1.48±1.99	0.77*	1.39±0.55	0.51±0.04	0.52±0.02	0.62±0.11
Cu	0.47±0.69	0.12±0.15	0.13*	0.28±0.54	-	0.76±0.89	0.18±0.29
Se	8.31±3.72	10.11±3.69	-	9.88±12.01	12.25±0.03	-	0.061*
Ni	61.97±13.03	90.2*	48.24*	38.01±18.66	-	-	63.46*

*Analysis based on only one measurement

alensis (21.42 mg 100 g^{-1}) and *H. plecostomus* (22.68 mg 100 g^{-1}). Mean and standard deviation values ranged from 21.42±11.73 mg 100 g^{-1} to 63.34±1.40 mg 100 g^{-1}. Ca concentrations among the species analyzed were within the FAO-defined range of 19 to 881 mg/100 g [25]. No mean or standard deviation value is presented for *P. lacustris* because only one specimen was analyzed.

2. Iron (Fe)

The concentration of Fe showed little variation among the species studied. Mean and standard deviation values ranged from 1.05±0.07 to 1.73±1.16 mg 100 g^{-1}. The highest concentration for this metal was found in *S. dissimilis* (1.73 mg 100 g^{-1}), whereas *A. ucayalensis* and *P. lacustris* had the lowest values (1.05 and 1.17 mg 100 g^{-1}, respectively). Mean Fe in the seven species studied exhibited the following pattern: *N. dissimilis* (1.73 mg 100 g^{-1})>*H. plecostomus* (1.57 mg 100 g^{-1})>*G. surinamensis* (1.40 mg 100 g^{-1})> *Curimata* sp. (1.34 mg 100 g^{-1})>*P. squamosissimus* (1.31 mg 100 g^{-1})> *P. lacustris* (1.17 mg 100 g^{-1})>*A. ucayalensis* (1.05 mg 100 g^{-1}). Fe concentrations among the species analyzed herein were within the FAO-defined range of 1 to 5.6 mg/100 g [25].

3. Potassium (K)

Potassium is an important mineral for muscle contractions, the transmission of nerve impulses and the metabolism of sugar. K concentrations among the species analyzed herein ranged from 47.65 to 247.90 mg 100 g^{-1} and were within the FAO-defined range of 19 to 502 mg/100 g [25]. Significant differences in K were found among the species, with the lowest mean concentration found in *A. ucayalensis* (61.09 mg 100 g^{-1}) and the highest found in *P. lacustris* (144.6 mg 100 g^{-1}). Mean and standard deviation values ranged from 61.09±12.33 mg 100 g^{-1} to 144.6 mg 100 g^{-1} (the latter figure does not have a standard deviation due to the sampling of only one individual). Ranges were 245 to 443 mg 100 g^{-1} in the Okavango Delta (Africa), 321 to 441 mg 100 g^{-1} in Turkey [26] and 301 to 402 mg 100 g^{-1} in China [28]. Thus, relatively low K values were found in the species caught in the lower course of the Itapecuru River. However, *Curimata* sp. had the highest concentration of this element in the study area (247.90 mg 100 g^{-1}; mean: 115.94 mg 100 g^{-1}). *P. lacustris* had a concentration of 144.6 mg 100 g^{-1}, but the fact that only one specimen of this species was sampled limits the use of descriptive statistics.

4. Magnesium (Mg)

Magnesium is found in muscle tissue and plays a crucial metabolic role in aquatic organisms, particularly in cases involving enzymatic compounds of the electron donor/acceptor system [29]. Magnesium is a macronutrient and an activator of enzymatic systems that control the metabolism of carbohydrates, fats, proteins and electrolytes, acting as a cofactor of oxidative phosphorylation [30]. This metal also exerts an influence on the integrity of the cell membrane and transport within the cell membrane as well as on the transmission of nerve impulses, aiding in muscle contractions and energy metabolism [31].

Magnesium was present in similar concentrations in all species sampled. The highest values were recorded for *Curimata* sp. (29.49 mg 100 g^{-1}) and *G. surinamensis* (25.84 mg 100 g^{-1}). The lowest values were recorded for *P. lacustris* and *A. ucayalensis* (20.46 mg 100 g^{-1} and 20.55 mg 100 g^{-1}, respectively). Mean Magnesium concentrations in the seven species ranged from 20.46 to 29.49 mg 100 g^{-1}, with increasing concentrations in the following order: *P. lacustris* (20.46 mg 100 g^{-1})<*A. ucayalensis* (20.55 mg 100 g^{-1})<*H. plecostomus* (21.59 mg 100 g^{-1})<*P. squamosissimus* (22.08 mg 100 g^{-1})<*S. dissimilis* (25.43 mg 100 g^{-1})<*G. surinamensis* (25.84 mg 100 g^{-1})<*Curimata* sp. (29.49 mg 100 g^{-1}). Magnesium concentrations among the species analyzed were within the FAO-defined range of 4.5 to 452 mg/100 g [25].

5. Phosphorus (P)

Together with calcium and magnesium, phosphorus is one of the main constituents of bones. No significant differences in the concentrations of this mineral were found among the species sampled from the lower course of the Itapecuru River, and the range was 128.27 to 809.82 mg 100 g^{-1}. The highest phosphorus content (809.82 mg 100 g^{-1}) was recorded for *G. surinamensis*, which is detritivorous species and small in comparison to the other taxa. [26] also found higher concentrations of P in a small species (*Barbus poechii*) in comparison to other samples.

The phosphorus range in the present study was higher than the values established by [25] (68 to 550 mg 100 g^{-1}) as well as those recorded for other freshwater fish around the world, such as 232 to 426 mg 100 g^{-1} reported by [27] and 198 to 240 mg 100 g^{-1} reported by [28]. However, the range was lower than that recorded by [32] (1,047 to 1,261 mg 100 g^{-1}). The recommended daily phosphorus intake for adults is 700 mg. Therefore, a serving of only 100 g of fish from the lower course of the Itapecuru River would contribute at least 40% of the daily requirement, which demonstrates the high phosphorous content found in this river. The mean phosphorus concentration among the species studied was between 142.7 mg 100 g^{-1} and 399.83 mg 100 g^{-1}, with the lowest value found in *A. ucayalensis* and the highest in *G. surinamensis*. The following order was found in terms of decreasing concentration: *G. surinamensis* (399.83 mg 100 g^{-1})>*Curimata* sp. (298.69 mg 100 g^{-1})>*P. lacustris* (269.52 mg 100 g^{-1})>*P. squamosissimus* (238.76 mg 100 g^{-1})>*S. dissimilis* (220.50 mg 100 g^{-1})>*H. plecostomus* (194.75 mg 100 g^{-1})> *A. ucayalensis* (142.7 mg 100 g^{-1}).

6. Zinc (Zn)

When accumulated in large quantities in fish, zinc causes histopathological alterations in the gills, such as hyperplasia, lamellar fusion, destruction of the epithelium and the excessive production of mucus [33,34]. It also causes disturbances in the acid-base balance [33] and has immunotoxic effects [35]. [36] evaluated the effect of zinc on the behavior of fish (larvae of *Chironomus* sp.) and found that exposure to this element causes a reduction in food intake. Moreover, zinc can clog interlamellar spaces, blocking the movement of respiration, while also causing delayed growth and maturation [37].

Mean concentrations of zinc varied little among the study sites in the species analyzed herein, with the highest concentration was found in *Curimata* sp. (1.48 mg 100 g^{-1}) and the lowest was found in *S. dissimilis* (0.51 mg 100 g^{-1}). Mean and standard deviation values ranged from 0.51±0.04 to 1.48±1.99 mg 100 g^{-1}. Zinc concentrations among the species analyzed were within the FAO-defined range of 0.23 to 2.1 mg/100 g [25].

7. Copper (Cu)

Numerous studies have examined species of fish that have been exposed to copper [38-40]. Researchers have found that the toxicity of copper can be fostered by multiple stressors that alter the behavior of species of fish through synergistic effects associated with other metals [41]. In general, copper toxicity is highly influenced by the physical and chemical characteristics of the water, such as hardness, alkalinity, pH, temperature and concentration of dissolved oxygen [41]. Therefore, to determine whether a species of fish is more sensitive than another in relation to the copper toxicity, certain physicochemical characteristics of the water should be considered.

Copper concentrations varied little among the species studied. The highest value (0.76 mg 100 g^{-1}) was detected in *A. ucayalensis,* which is a species with carnivorous feeding habits, and the lowest (0.12 mg 100 g^{-1}) was detected in *Curimata* sp., which has detritivorous feeding habits. Copper concentrations among the species analyzed were within the FAO-defined range of 0.001 to 3.7 mg/100 g [25].

Various species of fish from the same body of water may accumulate different amounts of metals. Interspecies differences in metal accumulation may be related to living and feeding habits. [42] found that piscivorous fishes accumulated more mercury, whereas detritivorous species contained more cadmium and zinc. [43] report higher concentrations of mercury in predatory species of fish than non-predatory species. [44] found that lead and zinc concentrations were higher in benthic fish. The results obtained by [45] indicate that carnivorous species accumulate more zinc and nickel than detritivorous species, while the latter contain more cadmium.

8. Selenium (Se)

Selenium is essential for animals and is used to avoid nutritional muscular dystrophy processes when combined with vitamin E. Selenium is an integral component of glutathione peroxidase. The level of this enzyme in the liver or plasma is indicative of the feeding activity of the organism. Selenium deficiency is usually associated with reduced growth. Moreover, Selenium protects species of fish from the spread of the toxicity of heavy metals, such as cadmium and mercury. The analyses in the study area indicate the absence of this element in *P. lacustris* and *A. ucayalensis*. The lowest value (0.061 mg 100 g^{-1}) was found in *H. plecostomus* strain and the highest (10.11 mg 100 g^{-1}) was detected in *Curimata* sp. Se concentrations among the species analyzed were higher the FAO-defined range of 0.018 to 0.068 mg/100 g [25].

9. Nickel (Ni)

Nickel is a transition metal that is relatively abundant in the earth's crust. Brazil has the third largest Nickel reserve in the world [46]. Processes such as mining, smelting, refining, the manufacturing of stainless steel and Ni-CD batteries have resulted in nickel contamination in many aquatic environments [47]. Brazilian law stipulates a maximum permissible limit of 25 μg L^{-1} in freshwater environments, although concentrations higher than 135 mg 100 g^{-1} have been detected in the muscle tissue of several species of fish collected in Brazilian river basins with high levels of heavy metals [48].

Mean concentrations of this metal in the species studied ranged from 38.01 to 90.2 mg 100 g^{-1}. The highest value (90.2 mg 100 g^{-1}) was found in *Curimata* sp. and the lowest (38.01 mg 100 g^{-1}) was in *G. surinamensis*. Nickel range in the present study was higher than the values found by the [49] (8.4 to 10.4 mg 100 g^{-1}). It was not detected in *S. dissimilis* or *A. ucayalensis* during the study period.

ANOVA revealed no significant differences in mean element concentrations ($p>0.05$), demonstrating similarity among species of different sizes and with different eating habits. The first two components of the principal component analysis explained 73.3% of the variability in the data (Fig. 2). *P. lacustris* and *G. surinamensis* correlated positively with component 1, which was associated with concentrations of phosphorus, potassium and calcium, whereas *Curimata* sp. and *S. dissimilis* correlated negatively with component 2, which was associated with concentrations of zinc, magnesium and iron.

The present data are of considerable importance and provide

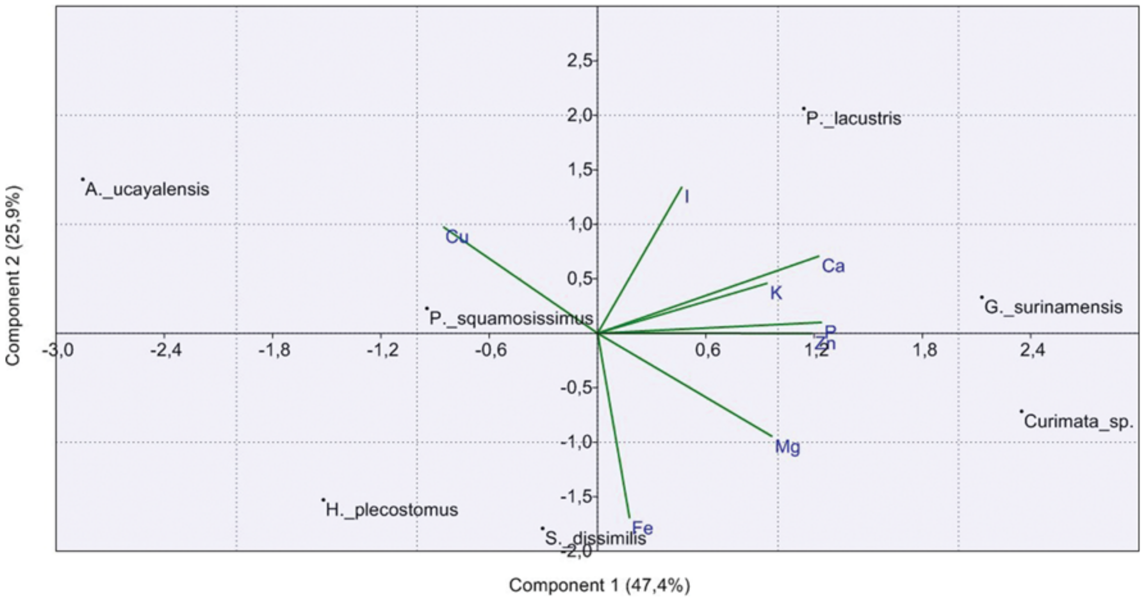

Fig. 2. Factorial plot resulting from principal component analysis of mineral concentrations in species caught in lower Itapecuru River.

essential information for the nutritional management of species in the fish processing industry and intensive aquiculture activities. Moreover, the findings indicate real options that can adequately supply the nutritional needs of individuals and stimulate the consumption of regional species of fish.

CONCLUSION

The mean concentrations of calcium, phosphorus, potassium and magnesium found in species of fish of the Itapecuru River indicate that these organisms constitute an excellent source of nutritional minerals for river communities in the region. The concentrations of copper, iron, zinc and selenium were considered low and were below the maximum limit established by Brazilian legislation for the human ingestion of species of fish. The comparison of the seven species investigated revealed no statistically significant differences in mineral concentrations, which suggests that different sizes and eating habits do not exert an influence on the absorption of these minerals.

Although the lower course of the Itapecuru River is impacted by deforestation and pollution from the release of industrial and domestic sewage, the low concentrations of metals, such as Fe, Cu, Zn and Ni, may be related to the influence of the ocean, which produces tide cycles with a strong dispersion capacity, thereby diminishing residence time in the water column and reducing the availability of these metals to species of fish.

The information produced in the present study is important to the creation of nutritional balance tables with the calculation of nutrient intake and can also contribute as an incentive to aggregate value to fishing activities in the region.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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