Mercury content and consumption risk of the invasive *Arapaima gigas* (paiche) in Bolivia

Contenido de mercurio y riesgo de consumo de la especie invasora paiche *Arapaima gigas* en Bolivia

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ABSTRACT

Mercury is a global pollutant present in the environment. The most frequent non-occupational human exposure pathway is fish consumption. Generally, fish species of large size and with carnivorous or omnivorous feeding habits have higher mercury concentrations. The invasive fish *Arapaima* (popularly known as paiche), an omnivorous large-sized species introduced in northern Bolivian Amazon about 50 years ago after escape from aquaculture in Peru, has successfully colonized this region and is now the main commercial fish species. Consumption of this species in Bolivia is increasing, and an evaluation of the risk of mercury exposure for human health is warranted. Muscle samples from 86 fish were taken from four different sub-basins (Orthon, Madre de Dios, Beni and Yata). 8.1% of the samples showed mercury content above the safe consumption recommendation of the World Health Organization (0.5 mg kg^{-1}). Samples from the Madidi and Yata rivers scored the highest mean concentrations (0.367 and 0.306 mg kg^{-1}, respectively), whereas individuals from the lower Beni sub-basin showed the lowest (0.105 mg kg^{-1}). According to our results, the maximum recommended paiche meat consumption is 316 g per week, divided into two meals, which is in agreement with international recommendations for fish consumption (227 g to 340 g per week), although this can vary according the place of origin of the meat. It is concluded that, following these recommendations, mercury exposure through paiche consumption does not represent a risk for human health.

Keywords: Bioaccumulation, fish consumption, contamination, chemical toxic trace, methylmercury, Amazonian artisanal fisheries, Upper Madeira drainage.

RESUMEN

El mercurio es un contaminante cosmopolita presente en el medio ambiente. La exposición humana no ocupacional más frecuente es el consumo de pescado. Generalmente, las especies de peces de gran tamaño y con hábitos de alimentación carnívoros u omnívoros tienen concentraciones más altas de mercurio. El pez invasor *Arapaima gigas* (popularmente conocido como paiche) del drenaje del Alto Madera, una especie omnívora de gran tamaño introducida en el norte de la Amazonía boliviana hace unos 50 años a través de la incipiente acuicultura en Perú, ha colonizado con éxito esta región y ahora es la principal especie pesquera comercial. El consumo de esta especie está aumentando en Bolivia, por lo que se realizó una evaluación para conocer el riesgo de la exposición al mercurio para la salud humana. Se tomaron muestras de músculo de 86 peces de cuatro subcuencas diferentes (Orthon, Madre de Dios, Beni y Yata). El 8.1% de las muestras presentaron contenido de mercurio por encima de la recomendación de consumo seguro propuesto por la Organización Mundial de la Salud (0.5 mg kg^{-1}). Las muestras de los ríos Madidi y Yata mostraron las concentraciones medias más altas (0.367 y 0.306 mg kg^{-1}), mientras que los individuos de la porción baja de la subcuenca Beni mostraron las más bajas (0.105 mg kg^{-1}). De acuerdo a nuestros resultados, el consumo máximo recomendado de carne de paiche es de 316 g por semana, repartidos en dos comidas, lo cual está de acuerdo con las recomendaciones internacionales para el consumo de pescado (227 g a 340 g por semana), aunque esto puede variar de acuerdo al lugar de origen de la carne. Se concluye que, tomando estas recomendaciones, la exposición al mercurio a través del consumo de paiche no representa un riesgo para la salud humana.

Keywords: Bioacumulación, consumo de pescado, contaminación, traza de químico tóxico, metilmercurio, pesca artesanal amazónica, drenaje del Alto Madera.

INTRODUCTION

Mercury exposure of humans is a major health concern worldwide, in particular for fetuses and young children (WHO 2010). In fetus it inhibits brain development and in adults it affects the sensory-motor activity (tremor, gait and balance disorders, headaches, muscle and articular aches, among others) (Guimaraes *et al*. 2000). Toxic effects of mercury and its compounds are related to the affinity for the sulfide group, present in several proteins and compounds in the body (Bidone *et al.* 1997, Boischo & Henshel 2000), reducing the capacity to eliminate it.

Elemental mercury is common in soil, particularly that of ancient geological origin (Gworek *et al*. 2020). It is also present in the upper atmosphere, primarily from human industrial activity, from where it is precipitated to the ocean and freshwater through rain. Additionally, unregulated gold mining, deforestation, and formation of reservoirs often contribute mercury to aquatic systems (Gworek *et al.* 2020). This elemental mercury is converted to a more soluble and biologically active methylmercury by bacteria (Compeau & Bartha 1985, Pfiffer *et al*. 1989, Guimaraes *et al*. 2000). Methylmercury progressively accumulates in the food chain through biomagnification, as absorption at the different levels generally is higher than elimination rates (Hylander *et al.* 2000, DeForest *et al.* 2005, Lopez 2005, Pouilly *et al.* 2013). Additionally, as mercury can bioaccumulate, it is expected that older fish have higher mercury content than younger ones, and the same pattern can be expected with size (Farkas *et al*. 2003, Donald *et al.* 2015, Barocas *et al.* 2023).

The main source of mercury exposure in the human population is through fish consumption. Almost all mercury in fish is present as methylmercury, an organic toxic compound that affects the neurological system. Nevertheless, fish are considered a high-value food (Balami *et al*. 2019), and the FAO/WHO (2007) has set a provisional tolerable weekly intake (PTWI) of 1.6 µg methylmercury kg^{-1} body weight, which takes into account elimination rates for normal adults. Several reviews have proposed fish consumption levels and species selection that balance the health benefits of eating fish, and the risk of mercury contamination (Abelsohn *et al*. 2011, Maulu *et al*. 2021).

The paiche, or *Arapaima gigas*, is a large-sized Amazonian fish, introduced to the Bolivian Amazon in the 50s and 60s (Carvajal-Vallejos *et al.* 2017). It is now inhabiting the Abuná, Madre de Dios, Beni, Yata, Iténez and Mamoré sub-basins and has become the principal commercial fish species in this region (Van Damme *et al*. 2024). More than 50% of the current fishery landings consist of paiche (Rico Lopez *et al*. 2023), making it one of the most consumed Amazonian species in Bolivia.

Amazonian aquatic ecosystems have remarkably high mercury concentrations, from seasonally mobilized naturally high levels in ancient soils exasperated by deforestation and placer gold mining (Crespo-López *et al*. 2021), as well as from further mobilization by new hydroelectric reservoirs (Arrifano *et al*. 2018, Crespo-López *et al*. 2021). Paiche is at the top of the food chain, with a carnivorous diet tending to omnivory (Villafán *et al*. 2020, Rejas *et al*. 2023), likely biomagnifying mercury, potentially putting consumers at risk. The fish may be an indicator species for overall mercury contamination in the environment, and characteristics such as age and size may also be good indicators of the mercury content in fishes.

The objective of the current study is to evaluate mercury content in paiche muscle from different localities of the northern Bolivian Amazon and explore if its mercury content has any relationship with paiche size. The final objective of this study is to assess the risk of its consumption by local communities and urban people.

MATERIAL AND METHODS

Sampling area

The study was carried out in the Bolivian Amazon basin, Upper Madeira drainage. This basin covers 72.7% of the territory, stretching from the Andean mountains over 6 000 m to 210 m of elevation (Guyot *et al.* 1995). The area is impacted by strong seasonal rains of 1 300 mm yr^{-1}, 75% of which fall during the four summer months of December to March (Espinoza *et al.* 2008). The main sub-basins are the Beni and Mamoré, which are tributaries and form the Madera (Bolivia) or Madeira (Brazil) River, itself a major tributary of the Amazon. The Mamoré sub-basin covers 45% (494 851 km^2) of Bolivian territory, extended over the central Andean Mountains, Brazilian Precambrian shield and Chaco-Beni lowlands. The Beni sub-basin represents 15% of the area (164 651 km^2), and spans from the north Andean Mountains in Bolivia, also collecting Peruvian waters through the Madre de Dios River (Guyot 1993, Molina 2007).

In general, these rivers have a large sediment load. Half of the sediment in the Amazon River comes from the Madeira River (306 $x10^6$ t $year^{-1}$), 72% is contributed by the Beni River (192 $x10^6$ t $year^{-1}$) and 18% by the Mamoré (65 x 10^6 t $year^{-1}$). Ten percent comes from other minor tributaries in Bolivia and Brazil. The water quality is variable between sub-basins, depending on the river origin and substrate of the riverbed (Guyot *et al*. 1995, McClain & Naiman 2008).

Sample collection

The fish were collected by local professional fishers using artisanal fishing gear. Tissue samples were collected in the field at central landing sites. A total of 86 samples were taken from seven different locations, including both rivers and oxbow lakes, during the low water season of 2015 (Table 1).

Standard length (cm) of sampled fish was measured, whereas trunk length was measured in fish collected in markets, as they arrived without skin and head. To estimate the standard length of these fish, a regression analysis derived from 38 individuals was used to convert trunk length (TrL) to standard length (SL) resulting in the equation SL= 0.0082 + 1.32 (TrL) ($R^2 > 0.9$). Fish from the Negro River were not measured, hence these samples were not used in the analysis of the relationship between mercury content and size. Muscle samples of approximately 5 cm^3 were taken from the dorsal body portion, using ultraclean methodology. Samples were put on ice (4 °C) and subsequently frozen (-15 to -20 °C) for shipment to the Laboratorio de Calidad Ambiental – Universidad Mayor de San Andrés, La Paz, Bolivia.

TABLE 1. Locations of the paiche *Arapaima gigas* samples obtained in the Northern Bolivian Amazon during the low water season of 2015. The column "Locality" shows the identity assigned to each sample for analysis throughout this study (note that individuals from Mentiroso lagoon were included for the analyses within Miraflores lagoon, as there were only three individuals, and the lagoons are less than 3 km apart).

Sub-basin	n	Origin	Locality	Coordinates
Orthon	9	Manuripi River	R. Orthon	-11.464715 S / -67.992247 W
Madre de Dios	10	Miraflores lake	L. Miraflores	-11.081822 S / -66.404036 W
	3	Mentiroso lake	L. Miraflores	-11.095048 S / -66.575031 W
Beni	8	Florida lake	L. Florida	-11.786801 S / -66.834062 W
	13	Garcero lake	L. Garcero	-11.803373 S / -66.844563 W
	20	Madidi River	R. Madidi	-12.594990 S / -67.015028 W
	10	Negro River	R. Negro	-13.031893 S / -67.030304 W
Yata	13	Tucunaré lake	L. Tucunare	-11.240573 S / -65.680307 W

Mercury content was measured using digestion and atomic fluorescence (Ruelas-Inzunza *et al*. 2011). Concentrations were reported by the laboratory in dry weight (dw), as is common in analytical work. Where needed, concentrations in wet weight were calculated from the percentage moisture of the sample (Hg ww = Hg dw H). Concentrations by wet weight (ww) is a conventional measure to report concentrations in fish for consumption (RAMP 2009).

Statistical analysis

To assess the effect of sampling locality on mercury content in the samples, a linear model analysis with "Locality" as factor was performed. Subsequently, a pairwise comparison between localities was performed, however, given that sample sizes were unequal, and the assumption of variance homogeneity was not fulfilled (see Results), a Fisher-Welch approximation (Best & Rayner 1987) and a Games-Howell post-hoc test (Games & Howell 1976) were used. To correct the problem of multiple testing, a Holm's adjustment was performed on the pairwise comparisons (Chen *et al*. 2017).

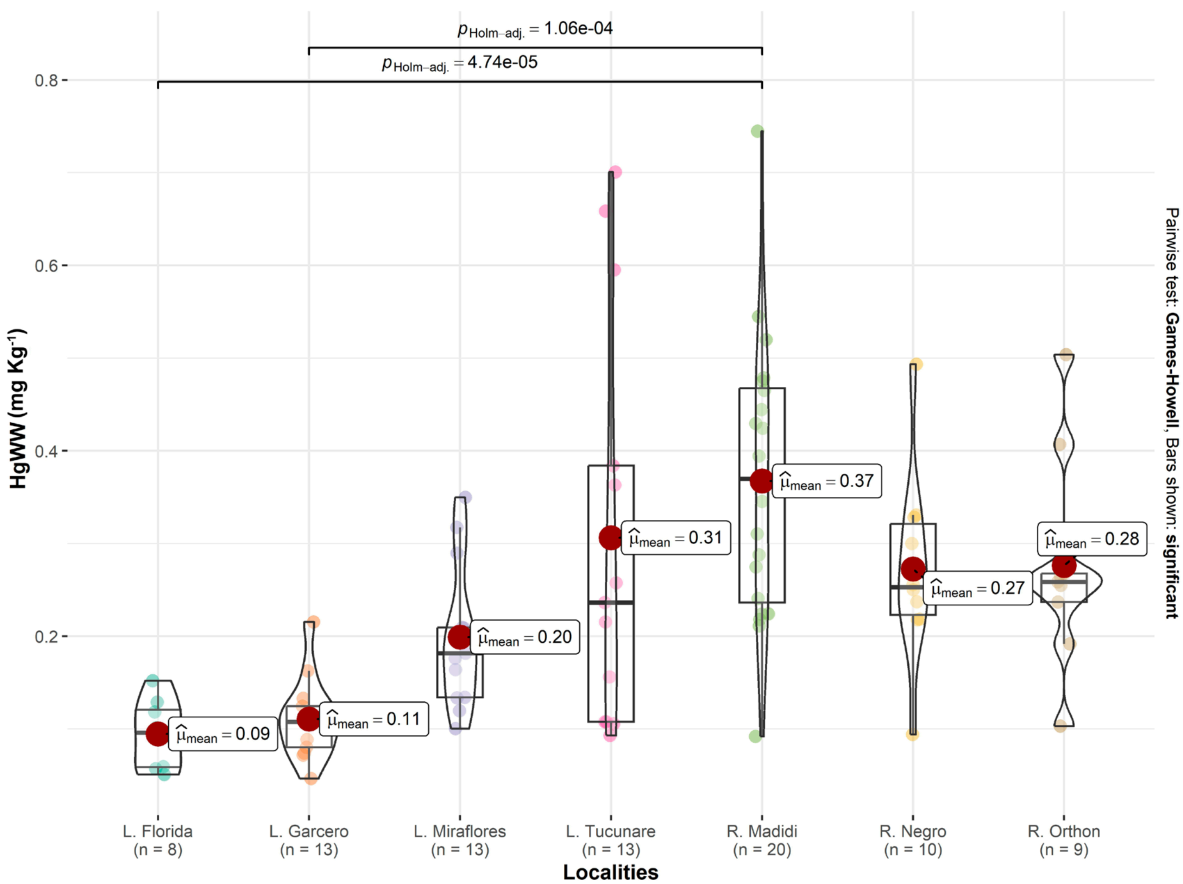
To explore the relationship between size (measured as standard length) and mercury concentration (measured as mg kg^{-1} of wet weight) in the fish, a linear model with "locality" and "size" as independent variables, was used. As the effect of locality proved to be significant (see Results), the size effect was explored for each locality separately. Finally, in order to address the general tendency of mercury concentration and its relationship with size in paiche, a regression with the residuals of locality vs. mercury concentration (i.e. after eliminating the effect of locality) was performed.

RESULTS

We found significant differences in the mercury content in paiche from different localities ($F_{Welch(6,\ 31.83)}$ = 15.88, $p < 0.001$). The Games-Howell post hoc comparison showed that R. Madidi (mean = 0.37 Hg ww) had higher mercury concentration than L. Florida and L. Garcero (mean = 0.09 and 0.11 Hg ww, respectively). Given the high variance of the data and after using Holm's correction for multiple testing, no other comparisons were found to be significant (Fig. 1).

A significant positive relationship between size and mercury content was only found In L. Garcero, L. Miraflores and L. Tucunare (Fig. 2). Few individuals (seven in total, unrelated with size) surpassed the WHO's recommendation of mercury content in fish (Fig.2). However, in general (after accounting for differences between localities), a significant positive relationship between size and mercury content was found ($F_{1,\ 74}$ = 15.7, $p < 0.001$, Fig. 3).

FIGURE 1 . Differences in mercury content (expressed as mg kg-1 wet weight) between localities where the paiche ***Arapaima gigas*** was collected in Bolivia. Boxplot and violin graphs show data variance, the red dot indicates the mean. Upper bars show significant differences between localities (Games-Howell comparison, Holm's adjustment for multiple testing).



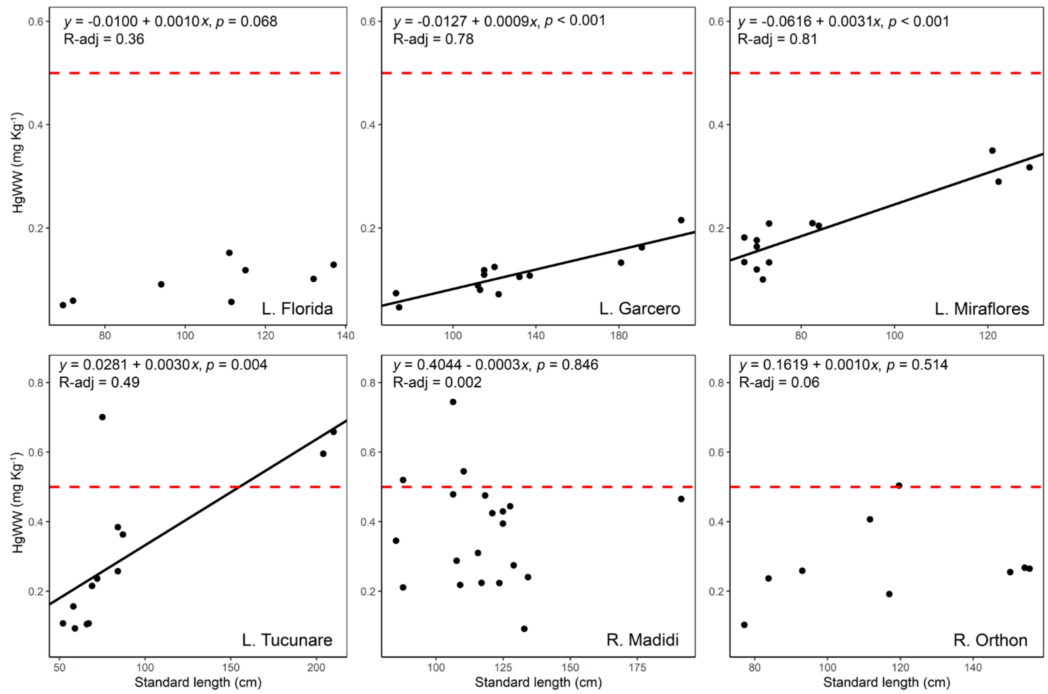


FIGURE 2. Relationship between size (as standard length in cm) and mercury content (as mg kg^{-1} of wet weight) in the paiche *Arapaima gigas* for each sampling locality from Bolivia. Only significant relationships show the regression line (black, solid line). Red dashed line shows WHO limit of mercury content in fish.

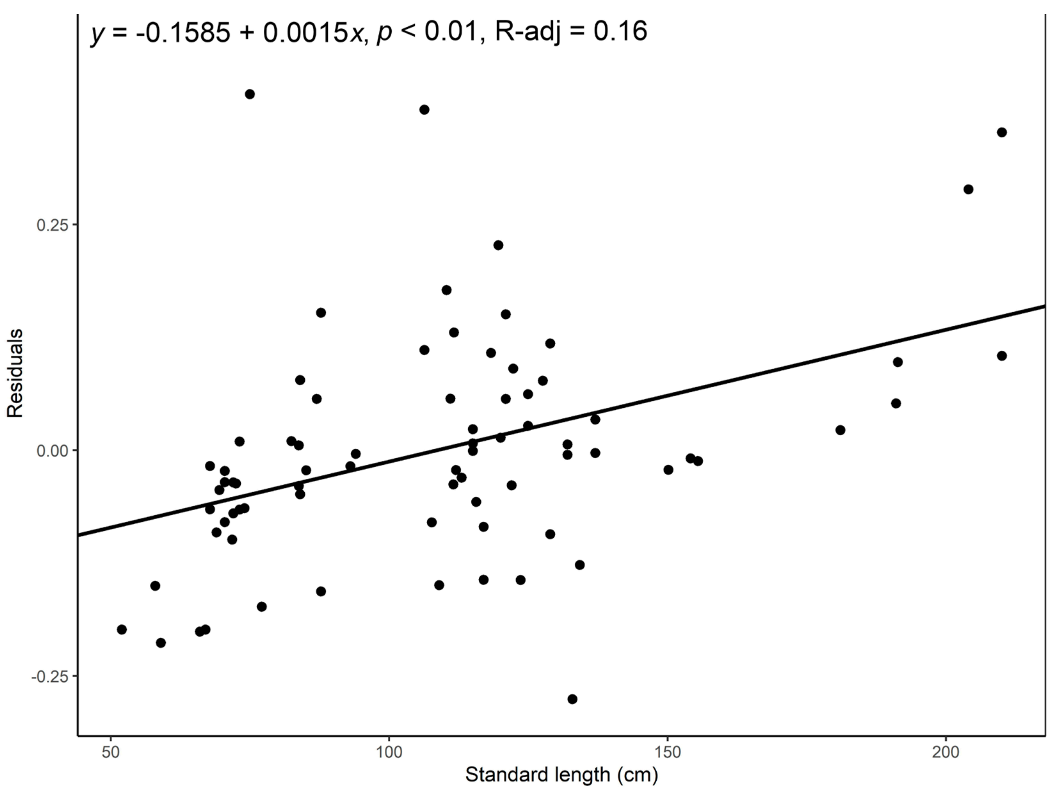


FIGURE 3. Relationship between size (as standard length in cm) and mercury content in the paiche *Arapaima gigas* from Bolivia after accounting for differences between localities (residuals).

DISCUSSION

This study presents the first analysis and description of the mercury content and its relationship with size in the invasive paiche from Bolivia. Our results showed contents below the general average, in comparison with other commercial fish species from the Bolivian Amazon (Supplementary material Table S1). Excluding the effect of locality, the carnivorous *Brachyplatystoma rousseauxii, Cichla pleiozona, Plagioscion squamosissimus, Pseudoplastystoma fasciatum, P. tigrinum,* and *Pygocentrus* nattereri showed higher mercury content than the paiche (Supplementary Material 1), probably because paiche is omnivorous (Villafan *et al.* 2020).

Mercury content observed in the paiche from the Brazilian Amazon is higher than values obtained for Bolivian samples, denoting intermediate mercury content for this species (Porvari 1995, Boischio & Henshel 2000, Gali *et al*. 2005, Bastos *et al*. 2008, Machado 2012) (Supplementary Material Table S2). The Bolivian Amazon drainage runs to the Madeira River Basin, which represents the natural border between Bolivia and Brazil in its upper portion. Most Brazilian studies on mercury content in the paiche were carried out in this basin, and the reported contents are similar to those obtained for the Bolivian paiche. Studies from the Porto Velho area, 300 km downstream from the Bolivia-Brazil border, showed that the mercury content in paiche was similar to the one obtained in the present study, despite anthropogenic alterations as fluvial gold mining and the (mega)hydroelectric dams of Jirau and Santo Antônio (Porvari 1995, Bastos *et al*. 2008, Machado 2012). In contrast, Boischio & Henshel (2000) analyzed paiche samples from locations along the border Bolivia-Brazil and found 15.5 times greater mercury content than other places within the same basin.

In the Bolivian Amazon, fluvial gold mining, changes in soil use to agriculture and forest fires are sources of external mercury discharge to the aquatic ecosystems. Besides environmental variability, the season during the sampling and analysis procedure could potentially explain part of the observed difference, as was suggested by some authors (Hylander *et al*. 2000, Bastos *et al*. 2008).

The wide variation of mercury content observed in the paiche samples (range of 0.698 mg kg^{-1} between the lowest and highest observed values), could be related to the large and heterogeneous area considered in the study area. This variation was also observed in Brazil (Supplementary material Table S2). The mercury content in fish is related to the natural concentrations in sediments or water, increased by other perturbations such as deforestation or gold mining, and conditions for methylation (Campeau & Bartha 1985, Mauro *et al*. 1999, Guimaraes *et al*. 2000, Roulet 2001, Pouilly *et al.* 2013). Future research should focus on the interaction of these factors with accumulation in different fish species in the Bolivian Amazon.

The percentage of fishes with mercury concentrations over the limit recommended for consumption by the WHO (0.50 mg kg^{-1} for carnivorous fish; IPCS 1990) is used as an indicator of system contamination levels (Bastos *et al*. 2007, Bastos *et al*. 2008, Pouilly *et al*. 2012). Mercury contents in 28% of fishes (816 total individuals) passed the limit in the Brazilian portion of the Madeira River basin (Bastos *et al*. 2007), while 36% were over the limit (1 100 total individuals) in the Jamari and Madeira rivers (Bastos *et al*. 2008). In 2016, Pouilly & Molina (2014) reported that 31% of fish (229 total specimens) from the Bolivian drainage of Madeira River surpassed the limit. Samples from the Beni sub-basin had 70% (of 26 individuals), Mamoré sub-basin had 0% (of 308 individuals), and Iténez sub-basin had 1% (of 416 individuals).

The present study found that 8.1% of the paiche samples analyzed were above the WHO limit. Only three of six locations had some fish with mercury content above the WHO limit: three in Yata, three in Madidi, and one in Manuripi (R. Orthon). Interestingly, the paiche from the Yata sub-basin were the most affected, though this system is the least affected by disturbances as gold mining or other anthropogenic activities releasing mercury into the aquatic systems. The low portion of the Yata sub-basin runs on the western end of the Brazilian Shield craton (SNHN 1998). It is known that atmospheric mercury deposition is higher in ancient soils and their associated waters flowing over them (Lin *et al.* 2010). This could explain the high mercury content observed in some paiche samples obtained in this locality.

Large omnivorous or carnivorous fish at the top of the food chain could be expected to bioaccumulate mercury significantly with age, showing a clear positive relationship between size and mercury content. However, mercury levels are a net result of accumulation and excretion, which results in actual relationships that are neither linear nor often in equilibrium (Singh *et al.* 2011). In particular, the mercury balance in paiche could be affected by changes in diet over time, as the fish moves around in a complex, seasonally changing linked ecosystem of flooded forest, oxbow lakes, and rivers. In this study, the relationship between size and mercury content is dependent on the locality, although once this effect is removed, there is a clear, positive relationship between mercury content and size. Some factors are likely to influence these results, including seasonal variation in diet availability, regional variability in mercury content in the soil and water, individual diet selectivity, and differential elimination rates of mercury according to the locality.

Without doubt, availability of prey species changes seasonally for the paiche, entering the forest during flooding. However, sampling during this study was in the same season, so such variation would not contribute directly to the departure from the expected relationship between mercury content and fish size.

Geographical variation in mercury concentration in the Amazon has been demonstrated (Siqueira *et al.* 2018, Crespo-Lopez *et al.* 2021). In the present study, collection sites are a significant source of variation in the data, but a good correlation between mercury content and size is absent within fish from half of the collection sites. This suggests that either this regionality is not the main reason for the bioaccumulation discrepancy, or the scale of mercury patchiness is smaller than the area sampled, while the individual fish ranges are not.

Recent data indicate that the diet of paiche in Bolivia is quite varied, including, in some cases, aquatic plants that may or may not be ingested incidentally (Villafan *et al.* 2020). There is also evidence that diets change with age (Rodrigues & Cargnin-Ferreira 2017, Villafan *et al.* 2020) and, in captive paiche, that individuals may have different dietary preferences (Lima *et al.* 2018). As the majority of the fish sampled were of relatively small size, they may be at a stage where diet selection habits are being developed or enforced by social interactions with other paiche or fish species. The prolonged parental care period of this species, suggests that learning is an important part of the paiche development. Studies with closer evaluation of diets in comparison with available prey are needed, in the wild or captivity, to address this question.

Mercury elimination mechanisms and rates are unknown for paiche, as they are for most fish (Bataglioli *et al.* 2019). Osteoglossomorph fish, such as the paiche, are additionally part of an ancient offshoot from other teleosts (Hilton & Lavoué 2018), with unknown physiological idiosyncrasies. Depending on the physicochemical water characteristics of each locality, it is possible that these may affect the elimination rate of mercury in paiche.

Paiche is increasingly important to commercial fisheries and fish consumption in Bolivia (Navia *et al.* 2017). While originally sold primarily as surubí or pintado (local name for the high value catfish species of genus), it is increasingly recognized under its own name (Carvajal-Vallejos *et al.* 2017), with a growing market – including amongst consumers that are eating fish for the first time. Our study suggests that, in general, the paiche is relatively safe to consume when recommended fish consumption levels are respected. Nevertheless, there are gaps in our understanding of mercury exposure pathways in this fish species, and why some individuals have high levels. Until more knowledge is generated, it is useful to use the reference table provided by the World Health Organization (WHO) and Food and Agriculture Organization (FAO) for provisional tolerable weekly intake (TWI) for inorganic mercury. This table suggests a maximum of 4 µg kg^{-1} body weight (WHO/FAO 2007), meaning a person of 70 kg can eat 316 g of paiche meat per week, or 16.5 kg each year. The Environmental Protection Agency (US-EPA 2009) advises to limit weekly fish intake to between 227 g and 340 g, divided into two meals. Considering these suggestions, paiche consumption does not seem to represent a high risk to local communities, within these consumption levels.

As a conclusion, the mercury concentrations in Bolivian paiche are within the reported mercury content for the species in the Amazon basin (Brazil) and commercial fish species in Bolivia. This does not represent a serious risk to the human health of consumers, if consumers follow international guidelines for fish consumption. According to our results, it is probable that larger fish present higher risk of mercury contamination, although additional studies must be carried out, including on the variables controlling bioaccumulation in paiche, the spatial and temporal dietary variability, and the environmental bioavailability of mercury.

SUPPLEMENTARY MATERIAL

Table S1. Mercury content in different commercial fish from the Bolivian Amazon.

Table S2. Comparison of mercury content reported for the paiche *Arapaima gigas* in different studies and areas from the Amazon basin.

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