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Original Contribution

Mercury in Populations of River Dolphins of the Amazon and Orinoco Basins

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Abstract: In the Amazon and Orinoco basins, mercury has been released from artisanal and industrial gold mining since the Colonial time, as well as a result of deforestation and burning of primary forest, that release natural deposits of methyl mercury, affecting the local aquatic vertebrate fauna. This study reports the presence of mercury in river dolphins' genera *Inia* and *Sotalia*. Mercury concentrations were analysed in muscle tissue samples collected from 46 individuals at the Arauca and Orinoco Rivers (Colombia), the Amazon River (Colombia), a tributary of the Itenez River (Bolivia) and from the Tapajos River (Brazil). Ranges of total mercury (Hg) concentration in muscle tissue of the four different taxa sampled were: *I. geoffrensis humboldtiana* 0.003–3.99 mg kg^{-1} ww (n = 21, M_e = 0.4), *I. g. geoffrensis* 0.1–2.6 mg kg^{-1} ww (n = 15, M_e = 0.55), *I. boliviensis* 0.03–0.4 mg kg^{-1} ww (n = 8, M_e = 0.1) and *S. fluviatilis* 0.1–0.87 mg kg^{-1} ww (n = 2, M_e = 0.5). The highest Hg concentration in our study was obtained at the Orinoco basin, recorded from a juvenile male of *I. g. humboldtiana* (3.99 mg kg^{-1} ww). At the Amazon basin, higher concentrations of mercury were recorded in the Tapajos River (Brazil) from an adult male of *I. g. geoffrensis* (2.6 mg kg^{-1} ww) and the Amazon River from an adult female of *S. fluviatilis* (0.87 mg kg^{-1} ww). Our data support the presence of total Hg in river dolphins distributed across the evaluated basins, evidencing the role of these cetaceans as sentinel species and bioindicators of the presence of this heavy metal in natural aquatic environments.

Keywords: Amazon, Bioindication, Gold mining, Mercury contamination, Orinoco, River dolphins

INTRODUCTION

The Amazon and Orinoco basins are home to the highest diversity of river dolphins on the planet (Mosquera-Guerra

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et al. 2018a, b, c), represented by the genera *Inia* (*I. geoffrensis*: *I. g. geoffrensis*, Amazon basin; *I. g. humboldtiana*, Orinoco basin; *Inia boliviensis* Mamore, Itenez, Grande and the upstream of the Madeira River, in Bolivia and Brazil; and *Inia araguaiaensis*, Tocantins and Araguaia basins) and *Sotalia* (*S. fluviatilis*, Amazon) (Caballero et al. 2007; Shostell and Ruiz-García 2010; Hrbek et al. 2014; Gravena et al. 2015; Mosquera-Guerra et al. 2018a). Species within the genus *Inia* are the largest river dolphins known, with more than 2 m (150–207 kg) of body size, and showing sexual dimorphism (da Silva 1994, 2009), while *S. fluviatilis* is the smallest species in the family Delphinidae, with an average body size of 1.5 m (35–55 kg) and no sexual dimorphism (da Silva 1994; Da Silva and Best 1994; Caballero et al. 2007).

Amazon River dolphins are considered top predators in freshwater ecosystems with a wide trophic spectrum (at least 43 species in 30 fish families); in average, adult river dolphins in the genus *Inia* consume 2.5–3.0 kg day^{-1} (Best and da Silva 1989). These predators are intrinsically dependent on the flood pulse dynamics of the basin and its effect on the temporal and spatial distribution of their prey (Mosquera-Guerra et al. 2018b). Species in these two genera perform longitudinal migrations, with differential spatial ecology between males and females. Males can have displacements of more than 400 km (Mosquera-Guerra et al. 2018b), whereas females are philopatric, remaining in wetlands where they reproduce and take care of their offspring (Trujillo 2000; Mosquera-Guerra et al. 2018b). River dolphin pregnancy lasts between 12.3 and 13 months, with 3.62 and 4.56 years between births. They have only one offspring that reaches its maturity between 8 and 10 years, and with an average life span of 40 years (Martin and da Silva 2018).

As active top predators characterized by: (1) high feeding requirements; (2) a wide trophic spectrum of prey; (3) active behaviour; (4) extensive migrations; (5) high biomass; and (6) extended longevity, South American river dolphins are susceptible to exposure and ultimately accumulation of contaminants such as mercury. River dolphins are among the most threatened cetaceans on the planet (Reeves et al. 2003), and their natural habitats have been intensively affected by anthropogenic activities, such as gold mining, timber deforestation, agricultural expansion and, more recently (2000's), the construction of hydropower dams, primarily in Bolivia, Brazil and Peru (Anderson et al. 2018). To date, there are 140 dams operating or under construction, as well as at least 428 planned dams only for the Amazon basin (Forsberg et al. 2017; Latrubesse et al. 2017; Anderson et al. 2018; Mosquera-Guerra et al. 2018a). This has resulted in the presence of large amounts of chemical contaminants, including organochlorines and mercury (Barbosa et al. 1997; Boas-Villas 1997; Amorim et al. 2000; Bahía-Oliveira et al. 2004; Passos and Mergler 2008; Hacon et al. 2009). Changes in the biogeochemical cycles, associated with the transformation of these environments, have resulted in changes in the processes of bioaccumulation, biotransference and biomagnification of mercury in the food web of aquatic ecosystems (Porcella 1994; Morel et al. 1998; Ullrich et al. 2001; Sarica et al. 2005).

In this study, we document the presence of total Hg in wild populations of river dolphins through the analysis of this pollutant in muscle tissue samples of 46 individuals, including dead, stranded and captured and released animals. Samples were obtained as part of a remote-monitoring study at the Orinoco River (border between Colombia and Venezuela), the Amazon River in Colombia and Peru, the Itenez River in Bolivia and Tapajos River in Brazil. Samples from the Arauca River, in Colombia, were obtained from stranded individuals.

MATERIALS AND METHODS

Individuals, Collecting Localities and Tissues Collection

A total of 46 individuals were analysed, including: stranded and released individuals ($n = 8$, 17.39%); and captured and released animals ($n = 33$, 71.74%); as well as carcasses (dead individuals) found floating in the river ($n = 5$, 10.87%). Captures were associated with the deployment of transmitters as part of the Program of Satellite Monitoring of River Dolphins (Annex I); fieldwork spanned between 2015 and 2018. Sampling localities included the following sub-basins of the Amazon: Amazon River (Colombia, $n = 7$), Caballococha Lake (Peru, $n = 2$), San Martin River (Bolivia, $n = 8$) and Tapajos River (Brazil, $n = 8$); as well as at the Orinoco basin, in the Arauca River (Colombia, $n = 8$) and the Orinoco River (Colombia, $n = 13$) (Fig. 1).

Each individual was measured, its weight was recorded, and its stage of age was estimated based on body length following: Martin and da Silva (2018), da Silva (2009) and Da Silva and Best (1994) ("Appendix"), also including dead animals, since they were detected in general good

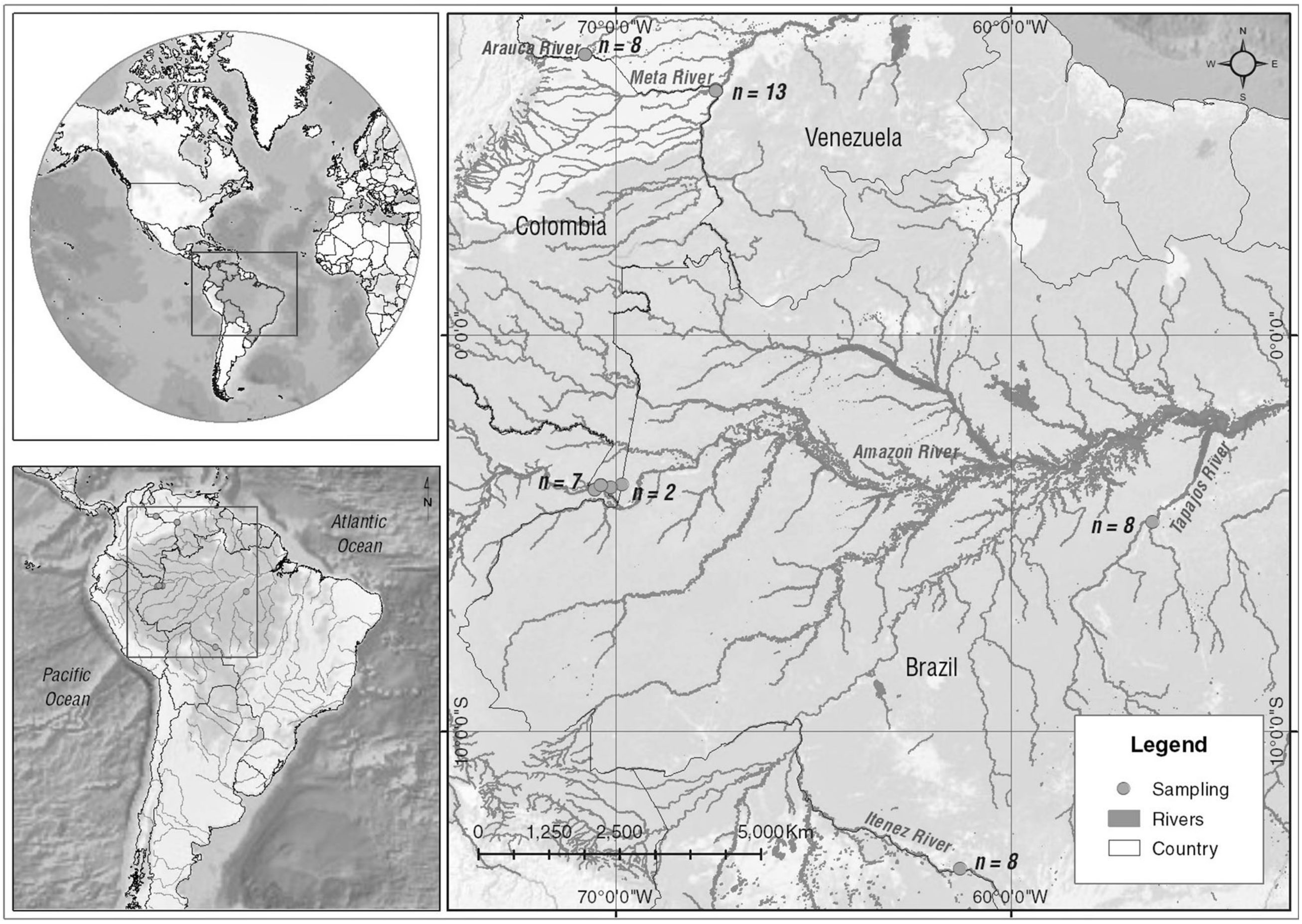

Figure 1. Locations where tissue samples were collected from river dolphins in the Amazon and Orinoco basins for mercury assessment.

conditions with a very low degree of decomposition of no more than 24 h after death. In addition, necropsies were performed in dead animals, according to protocols described in Geraci and Lounsbury (2003).

For each individual, we extracted between 1 and 2 g of muscle tissue, taken from the dorsal region under standard conditions; samples were divided into small pieces and stored at − 4°C in plastic containers, previously washed in acid and taken for mercury assay, following protocols by Kuiken and Hartmann (1993).

Mercury Analysis

Once on the laboratory, samples were acidified and stored it in a refrigerator at approximately − 4°C to prevent changes in volume due to evaporation (Eaton et al. 1998). To prevent contamination, sample containers and equipment were cleaned following the protocols of the Chemical Methods Manual for Fish and Seafoods (Canadian Food Inspection Agency. Amend 4. 1999-16.2) (Eaton et al. 1998).

Dolphin tissue samples were pre-treated, through microwave-assisted acid digestion, following the methods proposed in the section 3030 K of the Chemical Methods Manual for Fish and Seafoods, procedure also implemented in Wei-Wei et al. (2006). Readings of contamination with Hg in dolphin tissue samples were performed following the spectrometric cold vapour atomic absorption standard method in the Chemical Methods Manual for Fish and Seafoods (Canadian Food Inspection Agency. Amend 4. 1999-16.2) (Eaton et al. 1998), on an iCE 3000 Series Atomic Absorption Spectrometer—Thermo Fisher Scientific. Approximately 1 g wet weight of tissue was dried in an oven to constant weight, as suggested by Siebert et al. (1999). The mercury detection limit was 0.003 mg kg^{-1}, and the mercury quantification limit was 0.1 mg kg^{-1} of dolphin muscle tissue; all concentrations were reported in grams per kilogram wet weight. The statistical analyses were also performed with values expressed as wet weight (ww).

No appreciable Hg contamination was detected in blanks. Tuna tissue was used as certified referenced material. Laboratory analyses for Hg did not include replicates, since we were only able to extract small amounts of tissue (< 2 g) from alive individuals that constitutes 89% ($n = 41$) of our samples ($n = 46$).

Statistical Analyses

The statistical analyses were carried out in R version 3.3 (R Core Team 2013). A Shapiro–Wilks test was conducted to account for data normality in data set (Hg concentration, dolphins' size and weight). According to this test, the Hg data were not adjusted to a normal distribution (P value: 2.6×10^{-16}). Hence, we performed a Mann–Whitney U test for comparing Hg concentration between females and males. Additionally, a Kruskal–Wallis test for multiple comparisons was performed to determined Hg level differences in dolphin tissues among basins (Amazonas, Orinoco and Itenez).

In order to determine the relationship between size and weight, we performed a linear correlation between these two variables for all analysed individuals (42 adults, two subadults and two newborns). Afterwards, we performed a linear correlation between: weight/length index (calculated for all individuals) and total Hg concentration for each taxon. Variables were log transformed before performing the statistical analyses.

Finally, we did a box plot of the variation in total Hg concentration by sex; and a second box plot, in order to compare Hg concentrations for *Inia* and *Sotalia fluviatilis* in our study and those reported for *S. guianensis* from the Brazilian Atlantic coast. A significance level of alpha = 0.05 was adopted for all analyses, and the data are presented as median (Me), maximum and minimum values.

RESULTS

We report total Hg concentration from 46 dolphin individuals, discriminated as follows: *I. g. geoffrensis* ($n = 15$; 10 Males; 5 Females) from the Amazon basin in Colombia, Brazil and Peru; *I. g. humboldtiana* ($n = 21$; 14 Males; 7 Females), from the Orinoco basin in Colombia; *I. g. boliviensis* ($n = 8$; 7 Males; 1 Female), from the Itenez and San Martin Rivers in Bolivia; and *S. fluviatilis* ($n = 2$; 1 Male; 1 Female), from the Amazon River in Colombia (Table 1; "Appendix").

We also documented a high variability in total Hg concentration values among analysed samples (taxonomic and geographic: basin and sub-basin). Mercury was detected in 100% of the analysed dolphins, with maximum values recorded for the genus *Inia*. *Inia. g. humboldtiana* was the subspecies with the highest individual total Hg concentration values documented in this study, recovered from a juvenile male (3.99 mg kg^{-1} ww) and an adult female (3.50 mg kg^{-1} ww), both from the Arauca River in the Colombian Orinoco. *Inia g. geoffrensis* had a maximum total Hg concentration value of 2.60 mg kg^{-1} ww, in an adult male from the Tapajos River, Brazil. Interestingly, concentration values for *I. g. geoffrensis* from the Amazon basin in Colombia, and *I. boliviensis* from the San Martin River in the Bolivian Amazon, reported the lowest total Hg concentrations with values under 0.5 mg kg^{-1} ww. Finally, *S. fluviatilis* presented a maximum total Hg concentration value of 0.87 mg kg^{-1} ww, in a female from the Amacayacu River, in the Colombian Amazon (Table 1).

We report a weak linear correlation ($R^2 = 0.21$; P value = 0.00135), between size and weight among analysed individuals (42 adults, 91.30%, two subadults, 4.34%; and two newborns, 4.34%) (Fig. 2); as well as weak statistical support for a series of linear correlations between the weight/length index and total Hg concentration (F-statistic: 1.134; P value = 0.2927), for: (1) all the analysed taxa; (2) *I. g. geoffrensis*; (3) *I. g. humboldtiana*; and (4) *I. boliviensis*; (Fig. 3a–d, respectively). Since *S. fluviatilis* was represented by two individuals, we only report their position on the Cartesian plane (Fig. 3e).

The data for body size and weight were not normally distributed. We did not find statistically significant differences in body size and weight between males and females (Kruskal–Wallis Chi-squared = 0.18473, $df = 1$, P value = 0.6673; ANOVA: F value: 0.0103; P value = 0.749) (Fig. 4). Finally, we found no significant differences in total Hg levels among basins (Amazon, Orinoco and Itenez) (Kruskal–Wallis Chi-squared = 5.366, $df = 2$, P value = 0.06836; ANOVA P value = 0.749, respectively).

Although our results showed no relationship between total Hg concentration and body size (F-statistics = 2.122; P value = 0.1523), we found a significant (F-statistics 4.194; P value = 0.04656), but weak correlation ($R^2 = 0.087$) between Hg concentration and weight.

Table 1. Concentration of Total Hg in Muscle Tissue of River Dolphins (*Inia* and *Sotalia*) in the Amazon and Orinoco River Basins.

Taxa	Hg concentration (mg kg^{-1} wet weight)				Body length (cm)		
	Sex	Males	Females	All	Males	Females	All
I. g. geoffrensis	Median	0.55	0.5	0.55	179	162	178
	Maximum	2.6	1.6	2.6	205	199	205
	Minimum	0.1	0.1	0.1	60	120	60
	No. individuals	10	5	15	10	5	15
I. g. humboldtiana	Median	0.4	0.1	0.4	173.5	190	188
	Maximum	3.99	3.5	3.99	227	214	227
	Minimum	0.004	0.1	0.004	159	138	138
	No. individuals	14	7	21	14	6	20
I. boliviensis	Median	0.1	0.2	0.1	2.08	208.5	
	Maximum	0.4	0.2	0.4	248	226	248
	Minimum	0.03	0.2	0.03	169		
	No. individuals	7	1	8			
Sotalia fluviatilis	Value	0.87	0.1	0.67	142	148	148
							148
	No. individuals	1	1	2	1	1	2

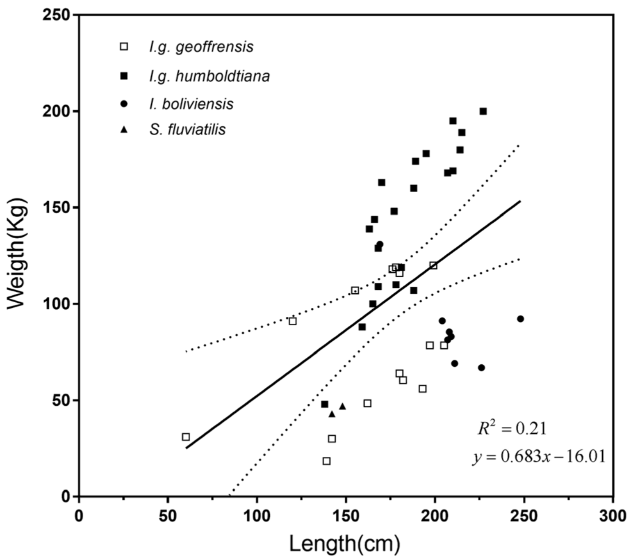

Figure 2. Linear correlation between size and weight among river dolphin individuals included in our samples.

DISCUSSION

Evidence of mercury in the aquatic ecosystem in the Amazon and Orinoco basins has been well documented since the 1980s (Martinelli et al. 1988; Lacerda 1997; Lacerda and Salomens 1998). The main source of this pollutant is gold extraction, mechanized, artisanal and industrial, with an estimate of more than 200,000 tn deposited into aquatic ecosystems since Colonial time (Pfeiffer et al. 1989; Villas Bôas 1997; Bahía-Oliveira et al. 2004). Currently, the artisanal small-scale gold mining sector is considered the major consumer of Hg and also the main source of mercury emissions in Latin America and the Caribbean (UNEP/ROLAC 2014). Estimates for 2010 show that mercury released into the atmosphere worldwide by this sector accounted for 71% of the overall emissions, reaching 77% in South America (UNEP/ROLAC 2014). In the Amazon, 63% of the mercury was found to be released by activities related to gold mining (Roulet et al. 1998a, b, 2000; Artaxo et al. 2000; Guimaraes et al. 2000). For the Amazon, the amount of Hg released to the ecosystem by the gold mining sector was estimated at 3000 tn between 1987 and 1994, with an approximate average range between 100 and 200 tn year^{-1} (Cid de Souza and Bidone 1994; Aula et al. 1994; Guimaraes et al. 1995; Palheta and Andrew 1995; Villas Bôas 1997; Kehrig et al. 1997; Lacerda 1997; Barbosa and Dorea 1998; Veiga et al. 1994, 1999; Veiga 1997).

Illegal gold mining is widespread in South America, and it is present in Brazil, in the: Tapajos (Roulet et al. 1998a, b; Dos Santos et al. 2000), Paraiba, Tocantins, Madeira, Xingu, Negro, Amapari, Solimões and Amazon

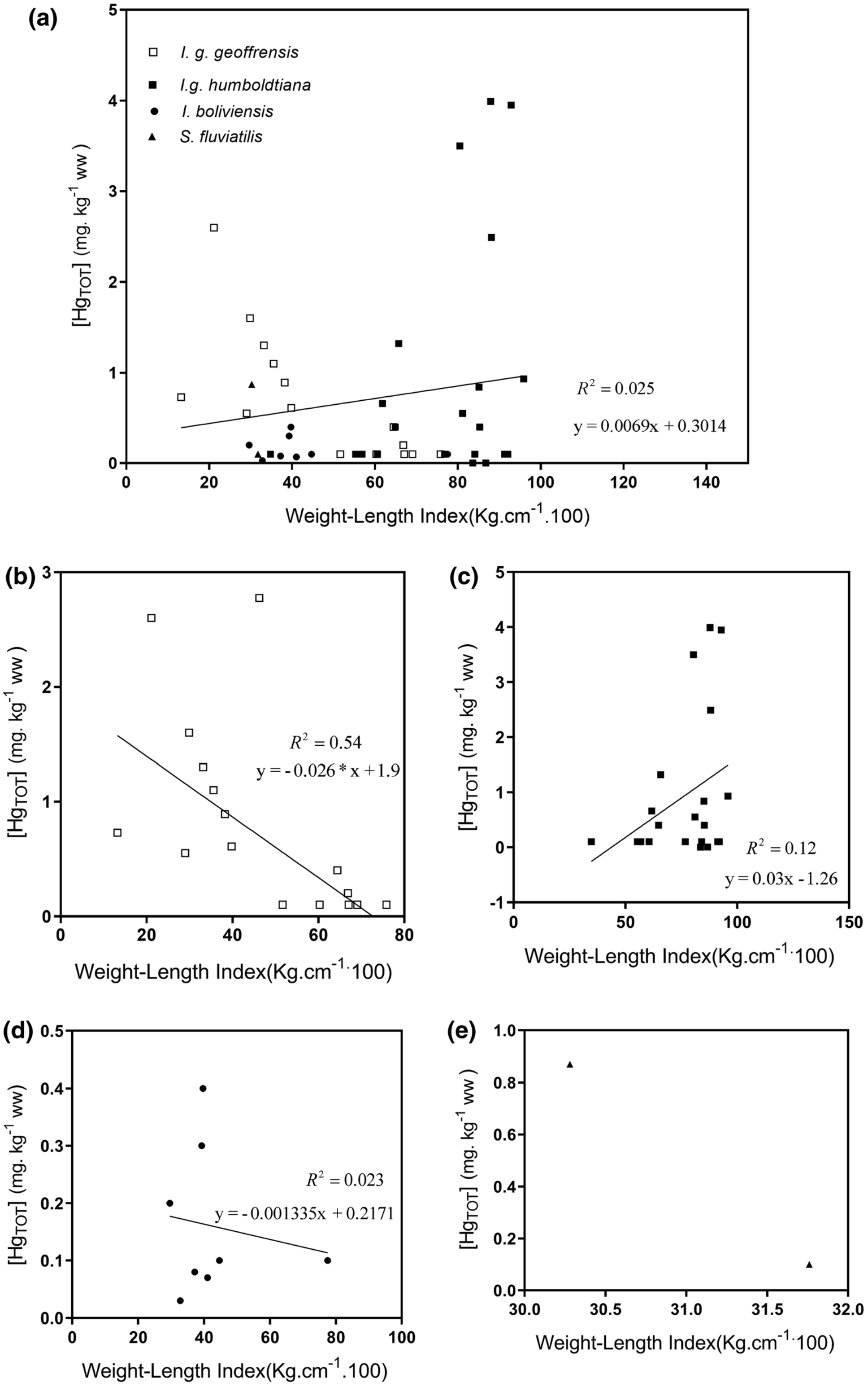

Figure 3. Linear correlation between weight/length index and total Hg concentration for: **a** all the analysed taxa; **b** *I. g. geoffrensis*; **c** *I. g. humboldtiana*; **d** *I. boliviensis*; and **e** the position of the two sampled individuals of *S. fluviatilis* on the Cartesian plane.

Rivers; in Bolivia, verified at the: Madeira, Beni and Itenez Rivers (Pouilly et al. 2013); in Colombia, at the Putumayo and Caqueta Rivers (Nuñez-Avellaneda et al. 2014); in Ecuador, at Nambija River; and in French Guiana, along tributaries of the Negro River Basin (Barbosa and Dorea 1998) (Figs. 5, 6).

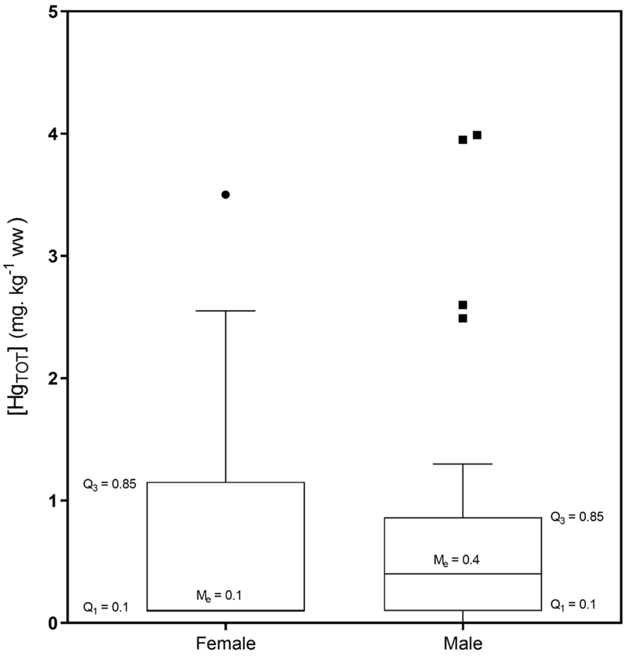

Figure 4. Box plot of the variation in total Hg concentration by sex and sampling locations.

Additionally, mercury also exists in the natural soil deposits in the Amazon Basin, and it is released into the aquatic environment through deforestation and burning, whereby the methylmercury bioaccumulates up the food chain (Souza Araujo et al. 2016). As a summary, 63% of the mercury entering the aquatic ecosystem is thought to be related to gold mining (Roulet et al. 1998a, b, 2000; Artaxo et al. 2000; Guimaraes et al. 2000), 31% from runoff from deforestation and 3% from atmospheric emissions from burning (Roulet et al. 1998a, b).

The presence of mercury in aquatic environments constitutes one of the major problems globally, affecting marine and continental ecosystems, such as the Amazon and Orinoco basins, with records of this contaminant in fish, otters and recently in river and coastal dolphins (Rosas and Lethi 1996; Dias Fonseca et al. 2005; Siciliano et al. 2008; Panebianco et al. 2011; Salinas et al. 2013; Nuñez-Avellaneda et al. 2014; Mosquera-Guerra et al. 2015a, b, c; Venturieri et al. 2017). Currently, high levels of mercury concentrations are a public health problem, with indigenous people and fishermen of the Tapajos and Teles Pires Rivers in the Brazilian Amazon having high levels of this contaminant, with values that exceeded the WHO safe limit (0.5 mg kg^{-1}), based on hair analyses (Dorea et al. 2005).

Wildlife is exposed primarily to methylmercury (MeHg) through their diet, rather than to other chemical forms of Hg, due to its persistence and high mobility within the food web of aquatic ecosystems (Porcella 1994; Morel et al. 1998; O'Shea 1999; Ullrich et al. 2001; Sarica et al. 2005; Moura et al. 2012), primarily in the omnivorous and carnivorous levels, mostly affecting top predator species (Aula et al. 1994; Malm et al. 1995, 1997; Lebel et al. 1997; Evans et al.,1998; Basu et al. 2005; Sarica et al. 2005; Markert 2007; Molina et al. 2010; Bossart 2011).

Despite dolphins being considered the most effective top predators in the aquatic ecosystems of the Amazon and Orinoco basins (Gomez-Salazar et al. 2012), a role that they share with other mammalian species such as otters, as well as carnivorous reptiles and fish (Trujillo 2000), we only statistically test for biomagnification of total Hg in our data on dolphin's weight. We failed in finding a correlation between total Hg concentration and body size, due to differences in adult individual's body size. Among river dolphins, differences in body size can be found at: (1) the genus level (*Inia* and *Sotalia*); (2) among species and subspecies of *Inia* (*I. g. geoffrensis I. g. humboldtiana* and *I. boliviensis*); and even (3) at the population level, such as *I. g. geoffrensis* which proved to be smaller in the Tapajos River than in the Amazon River (da Silva 2009). However, all the analysed individuals in this work presented high concentrations of total Hg in their tissues, in agreement with data reported in Rosas and Lethi (1996), who showed evidence of the presence of this heavy metal in maternal milk of *I. g. geoffrensis* from Manaus (Brazil); as well as data in Mosquera-Guerra et al. (2015b) that confirmed the presence of this pollutant in muscle tissues in stranded individuals of *I. g. humboldtiana* (Arauca, Orinoco) and *I. g. geoffrensis* (Amazon).

Sotalia guianensis distributed along the Brazilian Atlantic coast are also affected by the presence of mercury. High concentrations of this heavy metal have also been evidenced in populations from Rio de Janeiro State, with values ranging between 1.07 ± 0.35 (0.2–1.66 μg g^{-1} ww) in the muscle tissue of 20 individuals (Moura et al. 2011); Amazon coast: 0.4 ± 0.16 (0.07–0.79 μg g^{-1} ww, $n = 27$ (Moura et al. 2012); Northern Rio de Janeiro State: 0.98 μg g^{-1} ww, $n = 21$ (Kehrig et al. 2009) and 0.73 (0.34–1.42 μg g^{-1} ww), $n = 20$ (Carvalho et al. 2008); Espirito Santo State: 1.8 ± 0.46 μg g^{-1} ww, $n = 5$ (Lopes et al. 2008) and Guanabara Bay: 0.7 (0.2–2.5 μg g^{-1} ww), $n = 15$ (Kehrig et al. 2004), evidencing the wide distribution of this pollutant throughout the basin and its high mobility in the aquatic trophic networks (Sarica et al. 2005) (Fig. 7).

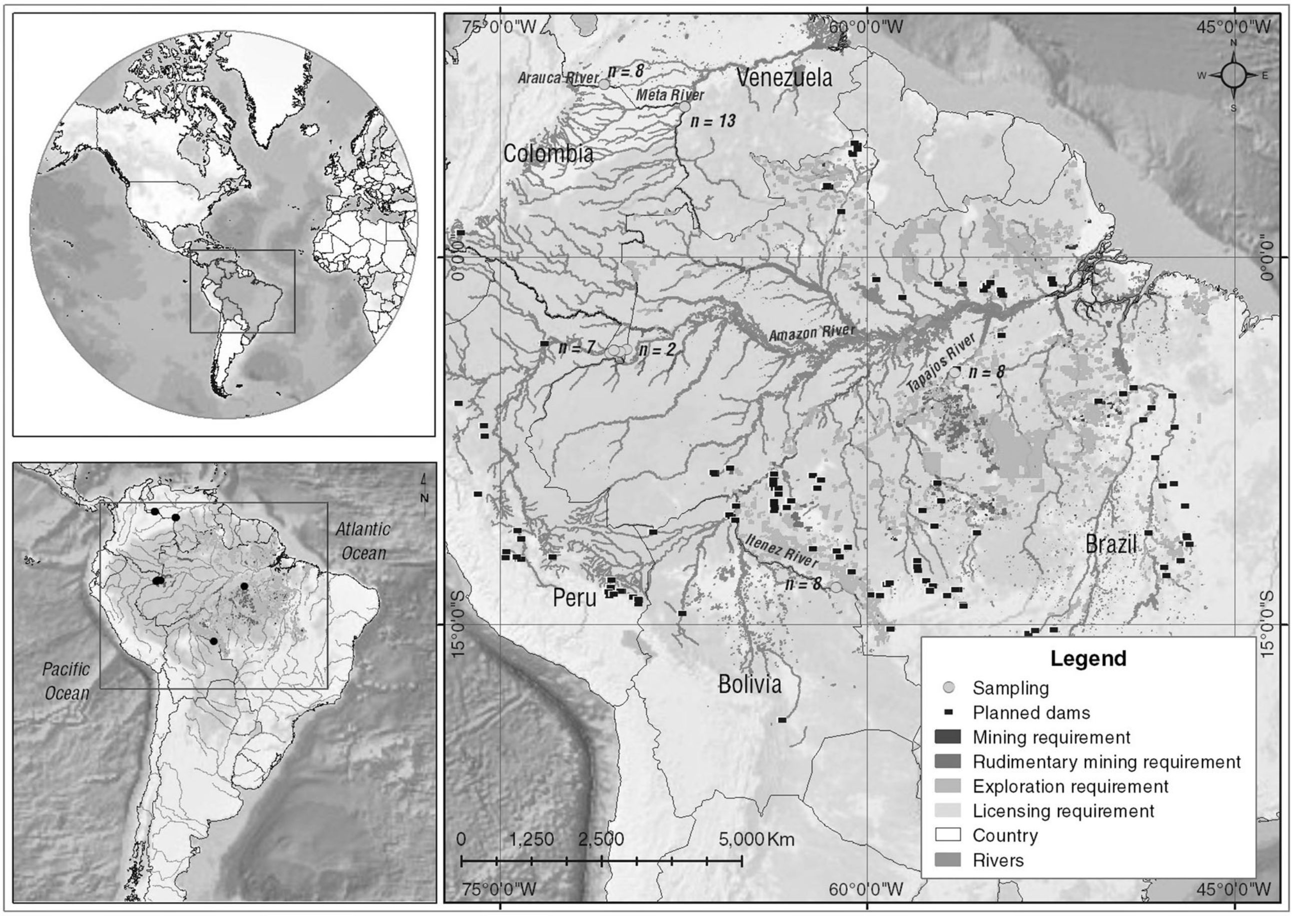

Figure 5. Current gold mining operations in the Amazon and Orinoco basins and the locations where river dolphins were sampled. Adapted from WCS (2017).

As mentioned, the presence of mercury is not only restricted to dolphin populations. Data on mercury concentration from muscle tissue samples among other Amazon aquatic vertebrates are presented in Nuñez-Avellaneda et al. (2014), in which the presence of total mercury was evaluated in muscle tissue samples of eleven fish species at four locations in the Colombian Amazon, reporting values that ranged between 0.0116 and 2.0123, $M_e = 0.3549$ mg kg^{-1} Hg. Additionally, Mosquera-Guerra et al. (2015a) reported that 54% ($n = 103$) of the total tissue samples, of the catfish species *Calophysus macropterus* (omnivore) from the Amazon, presented ranges between: 0.11 and 1.66 mg kg^{-1}, coinciding with that in Salinas et al. (2013) for the same cat fish species. Data on Hg concentrations from muscle tissue samples in other Amazon aquatic top mammals predator include: the giant otter (*Pteronura brasilinsis*), from Rio Negro in the Pantanal, Brazil, with a mean mercury concentration 0.17 mg g^{-1} in muscle tissue samples in the Amazon (Dias Fonseca et al. 2005).

In the southern Atlantic Ocean, Marcovecchio et al. (1990) reported values of mercury concentration of 3.8 µg g^{-1} ww in liver tissues for the same region, in one female of the Franciscan dolphin. Similar results are reported in Asian coastal and riverine dolphins by Wei–Wei et al. (2006) in some key tissues: liver 87.94 (1.4–181 µg g^{-1} ww), kidney 21.8 (43 µg g^{-1} ww), small intestine 17.04 (2.4–66 µg g^{-1} ww) and stomach 2.65 (0.65–5.2 µg g^{-1} ww) of five Yangtze finless porpoises (*Neophocaena phocoenoides asiaeorientalis*) in Eastern Dongting Lake, China; Zhou et al. (1993) document concentrations of total Hg for *N. p. sunameri* in: liver 10.24 (0.31–34.7 µg g^{-1} ww) and kidney 1.735(0.756–3.01 µg g^{-1} ww) for populations distributed in the Yellow Sea (China); Zhang et al. (1996) in the same species reported total Hg concentrations for: liver 76.05 (0.23–34.93 µg g^{-1} ww), kidney 8.23 (0.06–29.93 µg g^{-1} ww), small intestine 0.36 (0.06–1.46 µg g^{-1} ww) and stomach 0.54 (0.00–1.96 µg g^{-1} ww) in the Bohai Sea (China); Fujise et al. (1988) reported concen-

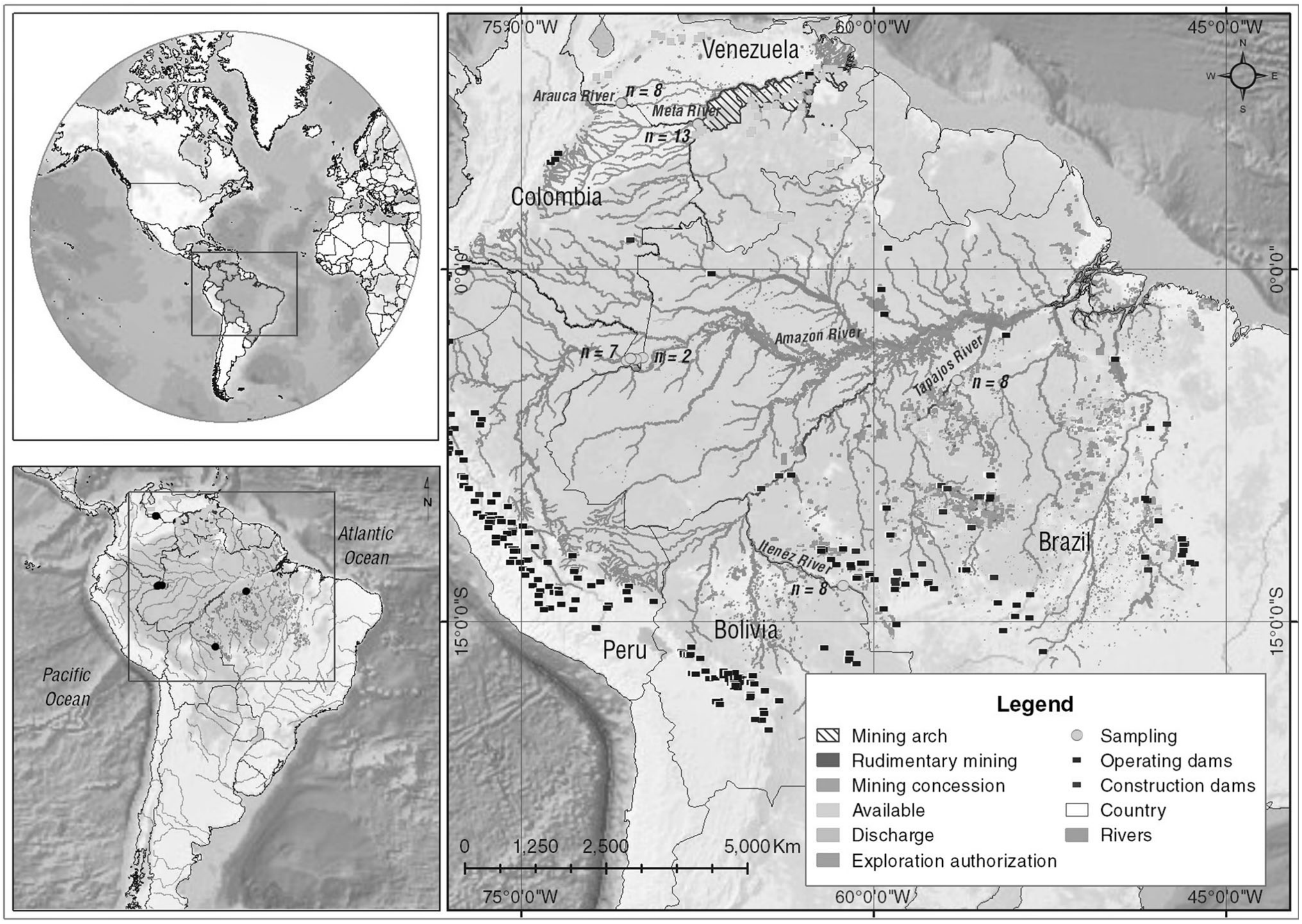

Figure 6. Projected gold mining operations in the Amazon and Orinoco basins and the locations where river dolphins were sampled. Adapted from WCS (2017).

trations values in liver (3.38 μg g^{-1} ww) and kidney (1.8 μg g^{-1} ww), in populations of *Phocoenoides dalli* for in the Pacific Northwest, and Yang (2001) reported concentration values of 7.8 (2.31–28.8 μg g^{-1} ww) in liver, for the same species, obtained from populations of the Japan Sea. Additionally, in North Eastern Europe, Siebert et al. (1999) reported concentrations of total Hg in: liver 12.1 (0.2–13.0 μg g^{-1} ww) and kidney 2.3 (0.1–33.5 μg g^{-1} ww) for 60 individuals of *Phocoena phocoena*, distributed in the North and Baltic Seas.

The presence of mercury in aquatic environments affects mammalian species at several levels (Scheuhammer et al. 2007). Mercury is known to be at least partially responsible for the decline of North American otters (*Lutra canadensis*) and the European otter (*Lutra lutra*) (Evans et al. 1998; Gutleb et al. 1998; Wren 1985). Although incidents of Hg poisoning in wild mammals are rare, this is perhaps a result of the practitioner's inability to observe and demonstrate the impacts, rather than an absence of the disease (Wren 1986).

Effects of the mercury on dolphins are documented by Krishna et al. (2003), with Atlantic bottlenose dolphins (*Tursiops truncatus*) having liver abnormalities associated with chronic accumulation of Hg. Cardellicchio et al. (2002) reported that a synergy between Hg with other pollutants could result in the death of striped dolphins (*Stenella coeruleoalba*) found in the Mediterranean coasts; primary damage was caused to the central nervous system, including a motor and sensory deficit and behavioural deficiency, anorexia, lethargy, reproductive disorders and death of foetuses as well as deficiencies of the immune system, facilitating the appearance of infectious diseases and pneumonia. High concentrations of mercury also generated serious disorders in liver, kidney and brain tis-

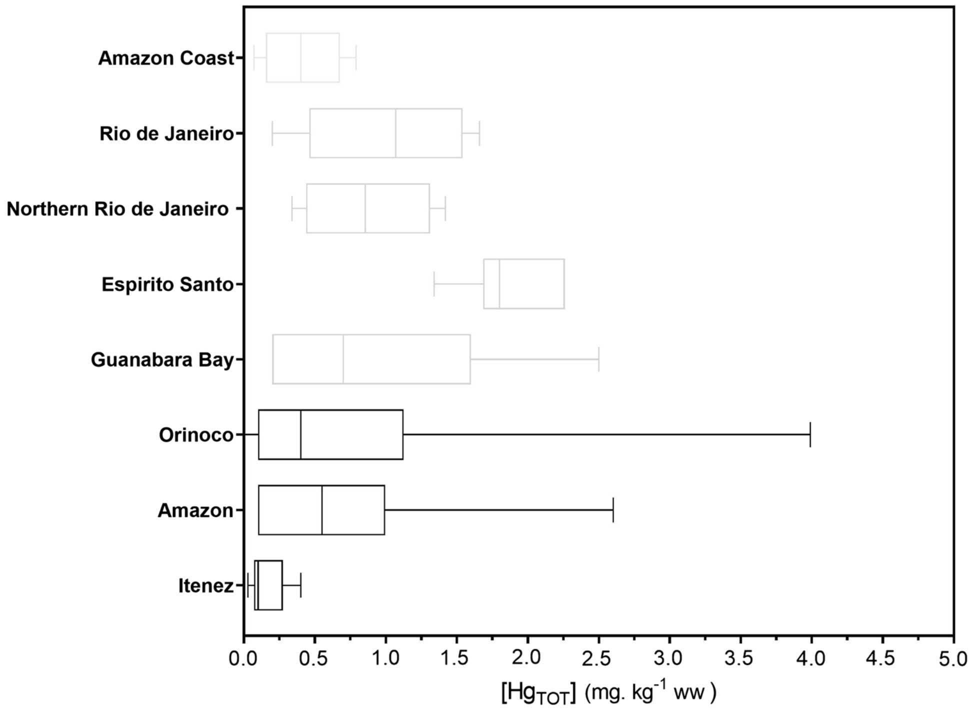

Figure 7. Box plot of total Hg concentrations (μg g^{-1} ww) in muscle tissues of *S. guianensis* from the Brazilian Atlantic coast: Guanabara Bay (Kehrig et al. 2004); Espirito Santo (Lopes et al. 2008); Northern Rio de Janeiro State (Carvalho et al. 2008); Rio de Janeiro (Moura et al. 2011); Amazon coast (Moura et al. 2012); *Inia* and *Sotalia* from Amazon, Orinoco and Itenez Rivers in the present study.

sues of striped dolphins (Augier et al. 1993). Although effects caused by high concentrations of Hg have not been studied yet, mercury is present in river dolphins since very early stages of development. Mosquera-Guerra et al. (2015b) reported the presence of Hg in a river dolphin foetus (0.16 mg kg^{-1} wet weight) collected in the Amazon.

Freshwater dolphins are sensitive to environmental perturbation, evidencing specific responses to changes in their habitats and rendering them useful bioindicators for monitoring the health of riverine ecosystems (Ichihashi and Tatsukawa 1993; Aguilar et al. 1999; O'Shea 1999; Gomez-Salazar et al. 2012). It is urgent to generate information on the effects of mercury bioaccumulation on populations of river dolphins. To date, no studies have been conducted on the effects of this pollutant on *Inia* and *Sotalia*, raising the concern for the persistence of species in these two genera. This is particularly true, if we take into consideration the increment in intensity and frequency of other types of threats affecting these cetaceans, a situation that elevated *Inia's* conservation status from Data Deficient up to Endangered (Da Silva et al. 2018).

CONCLUSIONS

Our data support the presence of total Hg in river dolphin tissues in the Amazon, Itenez and Orinoco basins, evidencing the role of these cetaceans as bioindicators of the presence of this heavy metal in natural aquatic environments.

These results indirectly point towards the complexity of the Hg biogeochemical cycle in the analysed environments and call our attention on the need to incorporate other factors, such as mercury measurements at different levels of the trophic web, as well as elements of movement ecology of these cetaceans in future analyses. River dolphins are keystone species in the South American largest river basins, making it critical to address this growing threat through transboundary cooperation among countries towards reducing the use of mercury, as stated in the Minamata Convention. Mercury contamination further exacerbates the conservation status of these aquatic mammals, populations of which are currently experiencing the negative effects of habitat degradation and fragmentation, due to the construction of hydropower dams, conflicts with fisheries and the effects of climate change.

Additional studies to evaluate *Inia* and *Sotalia* conservation status under these emergent scenarios are urgent, particularly for the middle and lower Amazon basin. In this context, it is necessary to extend this type of analysis along the main tributary rivers such as Caqueta/Japura, Putumayo/Içа, Madeira, the lower Amazon basin and the Araguaia–Tocantins complex.

The dolphins' position as top predators makes these organisms sentinel species of water resources. Therefore, changes in their health should be interpreted as an early warning on ecosystem degradation and even human health and wellbeing. The two analysed basins are homeland to some vulnerable sectors of the human population, which constitute ancestral fish-eating societies that face an imminent risk. This last statement should be interpreted under the philosophy of the sustainable development goals in which "no one is left behind".

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APPENDIX

See Table 2.

Table 2. Concentration of Total Hg in River Dolphin (*Inia* and *Sotalia*) in the Amazon and Orinoco River Basins Analysed in Muscle Tissue (mg kg^{-1} ww).

Taxon	Localities	Basin	Hg (mg kg^{-1} wet weight)	Sex	Sample origin	LT (cm^{-1})	Weight (kg^{-1})	Collection date
I. g. geoffrensis	Puerto Nariño (Colombia)	Amazon	0.2	M	T/A	178	119	07 July 2015
I. g. geoffrensis	Puerto Nariño (Colombia)	Amazon	0.1	M	T/N	60	31	13 January 2016
I. g. geoffrensis	Puerto Nariño (Colombia)	Amazon	0.1	F	D/A	120	91	04 March 2017
I. g. geoffrensis	Puerto Nariño (Colombia)	Amazon	0.1	M	T/A	176	118	04 March 2017
I. g. geoffrensis	Puerto Nariño (Colombia)	Amazon	0.1	F	T/A	199	120	10 November 2017
I. g. geoffrensis	Caballococha (Perú)	Amazon	0.1	F	D/A	155	107	10 November 2017
I. g. geoffrensis	Caballococha (Perú)	Amazon	0.4	M	D/A	180	116	04 March 2017
I. g. geoffrensis	Tapajos River (Brasil)	Amazon	1.1	F	T/A	180	64	07 October 2017
I. g. geoffrensis	Tapajos River (Brasil)	Amazon	0.73	M	T/SA	139	18.4	07 October 2017
I. g. geoffrensis	Tapajos River (Brasil)	Amazon	2.6	M	T/SA	142	30	08 October 2017
I. g. geoffrensis	Tapajos River (Brasil)	Amazon	0.61	M	T/A	197	78.4	08 October 2017
I. g. geoffrensis	Tapajos River (Brasil)	Amazon	1.6	F	T/A	162	48.4	10 October 2017
I. g. geoffrensis	Tapajos River (Brasil)	Amazon	1.3	M	T/A	182	60.4	10 October 2017
I. g. geoffrensis	Tapajos River (Brasil)	Amazon	0.89	M	T/A	205	78.4	11 October 2017

Table 2. continued

Taxon	Localities	Basin	Hg (mg kg^{-1} wet weight)	Sex	Sample origin	LT (cm^{-1})	Weight (kg^{-1})	Collection date
I. g. geoffrensis	Tapajos River (Brasil)	Amazon	0.55	M	T/A	193	56	11 October 2017
I. g. humboldtiana	Arauca (Colombia)	Orinoco	1.32	F	S/A	181	119	13 January 2017
I. g. humboldtiana	Arauca (Colombia)	Orinoco	0.66	F	S/A	178	110	04 March 2017
I. g. humboldtiana	Arauca (Colombia)	Orinoco	0.4	M	S/A	1.68	109	04 March 2017
I. g. humboldtiana	Arauca (Colombia)	Orinoco	0.1	M	S/A	165	100	04 March 2017
I. g. humboldtiana	Arauca (Colombia)	Orinoco	3.99	M	S/A	215	189	04 March 2017
I. g. humboldtiana	Arauca (Colombia)	Orinoco	0.93	M	S/A	170	163	04 March 2017
I. g. humboldtiana	Arauca (Colombia)	Orinoco	3.5	F	S/A	210	169	04 April 2015
I. g. humboldtiana	Arauca (Colombia)	Orinoco	0.1	M	S/A	168	129	04 March 2017
I. g. humboldtiana	Puerto Carreño (Colombia)	Orinoco	0.1	M	T/A	159	88	04 March 2017
I. g. humboldtiana	Puerto Carreño (Colombia)	Orinoco	2.49	M	T/A	227	200	04 March 2017
I. g. humboldtiana	Puerto Carreño (Colombia)	Orinoco	0.4	M	T/A	163	139	04 March 2017
I. g. humboldtiana	Puerto Carreño (Colombia)	Orinoco	0.004	M	T/A	166	144	06 January 2016
I. g. humboldtiana	Puerto Carreño (Colombia)	Orinoco	0.003	M	T/A	177	148	06 January 2016
I. g. humboldtiana	Puerto Carreño (Colombia)	Orinoco	0,84	M	T/A	188	160	06 January 2016
I. g. humboldtiana	Puerto Carreño (Colombia)	Orinoco	3.95	M	T/A	2.1	195	28 January 2018
I. g. humboldtiana	Puerto Carreño (Colombia)	Orinoco	0.1	F	T/A	195	178	09 November 2017
I. g. humboldtiana	Puerto Carreño (Colombia)	Orinoco	0.1	M	T/A	189	174	28 January 2018
I. g. humboldtiana	Puerto Carreño (Colombia)	Orinoco	0.1	F	T/A	214	180	28 January 2018
I. g. humboldtiana	Puerto Carreño (Colombia)	Orinoco	0.55	M	T/A	207	168	28 January 2018
I. g. humboldtiana	Puerto Carreño (Colombia)	Orinoco	0.1	F	T/A	188	107	28 January 2018
I. g. humboldtiana	Puerto Carreño (Colombia)	Orinoco	0.1	F	T/N	98	48	28 January 2018
I. boliviensis	Itenez River (Bolivia)	Itenez	0.1	M	T/A	169	131	1 November 2017
I. boliviensis	San Martin River (Bolivia)	Itenez	0.1	M	T/A	204	91.2	1 November 2017
I. boliviensis	San Martin River (Bolivia)	Itenez	0.08	M	T/A	248	92.25	1 November 2017
I. boliviensis	San Martin River (Bolivia)	Itenez	0.4	M	T/A	209	83	1 November 2017
I. boliviensis	San Martin River (Bolivia)	Itenez	0.07	M	T/A	208	85.4	1 November 2017
I. boliviensis	San Martin River (Bolivia)	Itenez	0.03	M	T/A	211	69.2	1 November 2017
I. boliviensis	San Martin River (Bolivia)	Itenez	0.2	F	T/A	226	67	2 November 2017
I. boliviensis	San Martin River (Bolivia)	Itenez	0.3	M	T/A	207	81.3	3 November 2017
Sotalia fluviatilis	Puerto Nariño (Colombia)	Amazon	0.1	F	D/A	148	47	13 January 2017
Sotalia fluviatilis	Amacayacú (Colombia)	Amazon	0.87	M	D/A	142	43	04 March 2017

Nomenclature for the origin of samples: captures for transmitter deployment (T); stranded animals (S); dead animals (D). Age of the individuals: adult (A) and newborns (N).

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