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The "mota" (*Calophysus macropterus*) fishery as the main threat for river dolphins in the Amazon: molecular evidence of commercialization and toxicological analyses

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Abstract

The worldwide problem of overfishing has affected the population abundance trends of many commercial fish species. For this reason, there is a constant need to find new species to replace important commercial species in the market, now scarce and sometimes endangered. This problem can be found in the Amazon, where the fishery of a catfish commonly known as "mota" (Colombia), "simi" or "piracatinga" (Brazil) (*Calophysus macropterus,* Lichtenstein, 1819*)* has become an important economic activity in the region and this species has replaced a number of other overexploited great catfish species from the Amazon and other rivers, including the Magdalena River in Colombia. The widespread distribution of this fish species may be partly the result of its ecological role and its feeding characteristics, as it is considered a scavenger. Due to this high exploitation, ways in which to increase captures have been identified in order to increase the market demand. One of these strategies has been increasing the capture range to include the Peruvian, Brazilian and Colombian Amazon. A second strategy is to use decomposing animal carcasses as bait to attract this fish. Such strategy has now increased the hunting pressure on species such caimans (*Melanosuchus niger* and *Caiman crocodilus*) and river dolphins (*Inia goffrensis y Sotalia fluviatalis*), in order to use its flesh as bait. This hunting pressure increases exploitation of these endangered species. It has been estimated that around 1500 dolphins are killed each year to support this fishing practice. In this study we investigated which catfish species, particularly "mota", are currently commercialized in Colombian fish markets using "DNA barcoding", and to study the concentration of mercury in the tissues of fish molecularly identified as "mota" in the markets. We collected 86 fish samples in markets in four Colombian cities. Sixty-eight of these were identified as "mota" by sequencing of a portion of the mtDNA Cytochrome Oxidase I gene. The mercury concentration of 29 of these samples was analyzed by cold vapor atomic absorption spectroscopy. All samples presented Hg concentration higher than the limit for human consumption established by the WHO (0.5ug/g). These results are worrisome as they suggest that 1) "mota" is a widely used fish species for human consumption in Colombia and 2) "mota" has high concentrations of Hg in their tissues and this needs to be taken into consideration for public health management in the country. Additional studies with increased sample sizes and coverage in the country must be a priority, as well as to establish the level of use of river dolphins and caiman in this fishery in Colombia.

Introduction

The worldwide problem of overfishing has affected the population abundance trends of many commercial fish (Pauly et al. 2002) For this reason, there is a constant need to find new species to replace important commercial species in the market, now scarce and sometimes endangered (Myers and Worm 2003). This problem can be found in the Amazon, where the fishery of a catfish commonly known as “mota” (Colombia), “simi” or “piracatinga” (Brazil) (*Calophysus macropterus,* Lichtenstein, 1819*)* has become an important economic activity in the region and this species has replaced a number of other overexploited great catfish species from the Amazon and other rivers, including the Magdalena River in Colombia (Petrere et al. 2004).

The use of this particular species to fill the market niche previously occupied by the great catfish species from the Amazon and the Magdalena is partly due to the fact that this species is included in the Pimelodidae family, where the great catfish species for human consumption have also been classified, with around 40 species. (Bogotá-Gregory and Maldonado-Ocampo 2006). Also, the widespread distribution of this species in the Amazon region (Salinas and Agudelo 2000) increases the chance for its capture in “black” and “white” water rivers (Saint-Paul et al. 2000).

This widespread distribution may be partly the result of its ecological role and its feeding characteristics, as it is considered a scavenger. For this reason it is frequently found close to human settlement (Salinas and Agudelo 2000). “Mota” has a average size of 20.5 cm and an average weight of 300 gr (Carvalho et al. 2009). This characteristic makes it an “ideal” species to supply consumers demand in big amounts. For this reason, the fisheries effort for this species is around 627,43 tons per month in Southern Amazonia (mostly in Brazil) making it one of the most exploited catfish of the whole Amazonian region (Alonso et al. 2007) .

Due to this high exploitation, ways in which to increase captures have been identified in order to increase the market demand. One of these strategies has been increasing the capture range to include the Peruvian (Ortega et al. 2001), Brazilian (Carvalho et al. 2009) and Colombian Amazon (Zuluaga et al. 2006), including capture in rivers such as the Putumayo (Colombia) (González et al. 2006) and also the low “Llanos” of Venezuela (Castillo 1996). A second strategy, considering low captures in some regions, is to use decomposing animal carcasses as bait to attract this fish (Trujillo et al. 2007).

The latest strategy has now increased the hunting pressure on species such caimans (*Melanosuchus niger* and *Caiman crocodilus*) and river dolphins (*Inia goffrensis y Sotalia fluviatalis*), in order to use its flesh as bait for "mota" (Estupiñan et al. 2003; Silveira and Viana 2003; Trujillo et al. 2007). Hunting this animals is relatively easy and also, it has been documented that a market now exists for this bait in some Amazonian regions (Hernández-Rangel 2010). This hunting pressure increases exploitation of these endangered species. It has been estimated that around 1500 dolphins are killed each year to support this fishing practice (Da Silva et al. 2008).

River dolphin meat has two desirable characteristics for use has bait. Firstly, the meat is very rich in fat, something that appear to attract "mota" and secondly, the strong odor that this meat emits when decomposing attracting "mota" to a particular fishing location in a short time (Estupiñan et al. 2003). Additionally, fishermen that target other species may have a negative interaction with dolphins due to possible competition for similar resources. For this reason, killing a dolphin, perceived as a competitor, may now be an additional source of income, if sold to the "mota" fishermen (Loch et al. 2009).

The commercialization chain of "mota" or "piracatinga" has been identified as follows: the fish is obtained along the Amazon River and then, from particular localities or cities along the river, it is shipped to main cities. For example, in Brazil, Manaos has been identified as the main provider of "piracatinga" to markets in Sao Paulo and Rio de Janeiro. Also, ther is evidence of exports of "piracatinga" filets from Brazil to Haiti (Trujillo et al. 2010). In Colombia, "mota" fishermen bring their catch to a centralized location in Leticia, from where it is shipped and distributed mainly to Bogotá and other cities in the interior (Pérez and Fabré 2009). Commercialization in cities far away from the Amazon may be related to the belief in this region of "mota" being a "taboo" species because it feeds on decomposing meat (Petrere et al. 2004).

In Colombia, there was the suspicion that "mota" was being commercialized as another species, the "capaz" from the Magdalena River (*Pimelodus grosskopfii*) in the big supermarket chains and also in the main central markets in Bogotá and other cities. For this reason, the aim of this study was to indentify via "DNA barcoding", which species of freshwater catfish were commercialized in Colombian markets, particularly in Bogotá.

"DNA barcoding" a molecular technique based on sequencing a portion of the mitochondrial DNA Cytochrome Oxidase I (COI) has been widely used to identify species and part of species that may be legally or illegally traded, including fish species being traded as another one in order to increase its price and availability in the market (Baker 2008). DNA barcodes have allowed identification of species sold as snapper in a number of markets in the USA and also to identify tuna and other fish species used in sushi preparations (Stokstad 2010; Wong et al. 2011). Additionally, the use of DNA barcodes has allowed to understand the trading tendencies of shark fins in Asian markets, as well as provided a tool for species trade monitoring (Abercrombie et al. 2005; Clarke et al. 2006). DNA barcoding may help to establish the geographical origin of a particular sample, and also commercialization or trade routes, being useful to understand how a particular market or fishery works.

An additional aim of this study was to measure mercury concentrations in the fish being commercialized, particularly in the "mota". Considering the increase in mining activities (Uryu, et al 2001), particularly artisanal mining (Baird, 2001), and the change of land use in the Amazonian region (de Lacerda et al 2004), high amounts of mercury have leached into the river. This free inorganic mercury is then converted into organic mercury forms, such as methyl-mercury and then bioaccumulated and biomagnified along Amazonian food webs putting full ecosystems at risk (Dudgeon, et al 2006; Dorea et al, 2006; Porto et al 2005, Lailson-Brito Jr. et al, 2008; Cubillos M, J.C. 2009). Considering its scavenger habits, one would expect this fish to bioaccumulate mercury in high levels, particularly if fed with the remains of top predator such as caimans and river dolphins (Hernández-Rangel, 2010; Silveira and Viana, 2003). For this reason, measuring and monitoring mercury concentrations in this fish is an important need considering the pervasive effects mercury has on human health (Malm et al. 1995; Castilhos et al. 1998).

Methodology

Fish Sampling

Eighty-six fish specimens were obtained from different sources (fishermen, distributors and markets) in seven locations in Colombia (Figure 1), between June and October 2010. Forty-six of these samples where obtained from "source" localities (considering a source places where the fishing activity is done) and these where morphologically identified using identification guides and species identification was confirmed by taxonomic experts in the field. These specimens were also molecularly identified in order to use them for comparison in further analyses. These specimens were obtained in the Colombian and Brazilian Amazon (Puerto Nariño, Leticia and Tabatinga), Colombian Orinoco (Iniria), Meta province (Puerto López) and Putumayo province (Puerto Asís).

Forty specimens were obtained from markets in Bogotá, Girardot and Melgar, where they were sold as "capaz" from the Magdalena River. The latest two cities are located in on the Magdalena River, about 98 Km soutwest from Bogotá. They all were sold at different prices and all were sold under the name "Capaz". A small tissue sample from each specimen was cut and transferred to 70% ethanol and refrigerated at 4°C.

DNA extraction, amplification and phylogenetic analyses

DNA was extracted from tissue samples following a phenol-chlorophorm protocol from (Sambrook et al. 1989) modified for small samples by (Baker et al. 1994). Approximately 558 base pairs (bp) of the mitochondrial COI gene were amplified via PCR, using the primers COXFISH F1-R1 (Ward et al. 2005). Bioline Taq Polymerase was used and the temperature profile was as follow: an initial denaturation for 2 min at 94°C, followed by 35 samples at 94°C for 30s, annealing at 59°C for 45 s, elongation at 72°C for 1 min 20 s, and a final extension 72˚C for 15 min. PCR products were run and visualized in a 1.5% agarose gel stained with ethidium bromide. Successful amplification products were purified using polyethilenglycol and sequenced at Macrogen Korea.

COI sequences were manually edited and aligned with previously published COI sequences from different species from the family Pimelodidae available GenBank (www.ncbi.nlm.nih.gov/) using the software Geneious version 5.3.3 (Biomatters Ltd). This dataset was further used for phylogenetic reconstructions Neighbour-Joining (NJ) in PAUP (Swofford 2011). These reconstructions are consider the standard for DNA barcoding methodology and species ID in a wide variety of taxa (Hebert et al. 2004; Hellberg and Morrissey 2011; Ross et al. 2003).

Mercury analyses

From the total of 86 specimens collected in this study, a subset of 29 samples of antero-dorsal were collected and acid-digested following the protocols described by Sadiq, 1991. Total Hg (Hg_{TOT}) concentrations were determined by cold vapor atomic absorption spectroscopy in a CV-AAS (Perkin – Elmer AAnalyst 300). Analyses were carried out at the spectroscopy laboratory of Universidad de los Andes. Three replicates per each sample were considered to determine the precision and accuracy of Hg determinations. A standard biological reference material (DOLT-2 from NRCC Ottawa, Canada) was measured for calibration purposes and comparisons.

Results

Out of the 86 specimens collected in this study, five species were identified. The morphological indetification from the field was confirmed by sequencing of about 558 bp of the COI gene. These five species were identified as belonging to the family Pimelodidae with high similarity percentages (85%-95) (Figure 2). Only *Calophysus macropterus* showed more than one COI haplotype and clade for this species was supported with a bootstrap value of 100 (Figure 2). COI sequences were also useful to identify the origin of specimens, taking into consideration sequences from specimens collected in field locations in the Colombian Amazon, Brazilian Amazon and Colombian Orinoco. In the neigbour-joining tree (Figure 2) a clade joining specimens from the Amazon was clearly separated from specimens from the Orinoco. *Calophysus macropterus* is the species that is sold in higher proportion in the Colombian markets studied as if it was the "Capaz" from the Magdalena River (*Pimelodus grosskopfii*), but there is evidence of other species also sold as if they were the "capaz". Only three specimens obtained in the markets in Melgar were identified as the real "capaz" (*Pimelodus grosskopfii*).

All samples included in the mercury analyses were indentified as *C. macropterus*. These samples had Hg_{TOT} concentrations higher than the maximum permissible limit of 0,5 ug/g (WHO, 2007) established for human consumption. The concentration values ranged from 1,33 to 2,28 ug/g w.w. Hg_{TOT} concentration compared to fish length for all sampling sites (including locations of origin and one market location) is showed in Figure 3. The bioaccumulation tendency was low according to the correlation coefficients. The variation in Hg_{TOT} concentration among locations is show in Figure 3. For instance, Putumayo shows a higher average of Hg_{TOT} concentration compared to other sampling locations.

Discussion

This study used molecular identification (DNA barcoding) in order determine which species are used in the Colombian markets to replace the traditionally consumed "capaz" from the Magdalena River, as well as to understand the frequency of use of "mota", a scavenger catfish species from the Amazon and Orinoco, to replace "capaz" in the market. We also wanted to determine levels of mercury in these fish, now widely obtained along the Amazon and Orinoco Basins in Colombia and Brazil.

It is very difficult for the final consumer to be able to detect if he is being deceived when buying a particular type of fish, particularly if there is no striking morphological differences allowing clear species differentiation between species. This is the case when the final consumer thinks is buying “capaz” from the Magdalena River but instead he is being sold “mota” or other species, which fishery may be having serious environmental impacts. Additionally, the detection of high concentrations of mercury in the tissue of “mota” is worrisome and may become a serious public health issue. The problem of misleading the public into consumption of particular species based on deceiving their decision making capacity is not unique for this case but has been widely researched in a number of fisheries around the world, Including the case of wild snapper (Carvalho et al. 2011; Cohen et al. 2009; Filonzi et al. 2010; Wong and Hanner 2008; Wong et al. 2011).

Also, the “replacement” species may be presented to the public as filets or other presentations, which may make morphological species identification particularly difficult if not impossible. For this reason, the use of “DNA Barcodes” to investigate what is sold in the market, provides a precise, fast and powerful technique for detecting this kind of fraudulent sale behavior (Galimberti et al. 2013). Its application in market surveys or monitoring to ensure quality of the product being sold can be highly increased (Holmes et al. 2009). Also, it can detect if endangered species are commercialized illegally, as it was the recent case when sushi made with whale meat was detected in a restaurant in the United States (Baker et al. 2010). In our case, it confirmed the suspicions of replacement of *Pimelodus grosskopfii,* “Capaz” from the Magdalena River, being replaced mostly by “Mota” in markets of Colombia. Another three fish species from the Pimelodidae family (*Pinirampus pinirampus, Brachyplatystoma vaillantii* y *Hypophthalmus marginatus*) were identified in this study, all being commercialized in an apparently much smaller proportion than “mota”. The use of species other than “mota” to replace the “capaz” may be due to fluctuations in its capture at different times of the year (Gómez et al. 2008). All these species are distributed in the Amazon and Orinoco basins and none of them in the Magdalena River.

Our results support recently published information by the Colombian government (Ministerio de Agricultura y Desarrollo Rural - MADR 2010), evidencing higher exports from "mota" originating from Colombia and an increase of "mota" in the internal market as lower numbers of "capaz" are been commercialized in the country, evidencing population reductions for this species. The "capaz" from the Magdalena River (*Pimelodus grosskopfii)* is now clasified as a vulnerable species in Colombia (Mojica et al. 2012). The low availability of "capaz" del Magdalena has increased its market price. Currently, 1 pound of "capaz" costs about 6,25 USD, while the cost of "mota" is about 2,5 USD per pound. Such difference in price has also increased the preference for "mota" in the markets.

The final consumer is the only one that is being deceived by this replacement. Based on a reduced number of surveys (data not shown) performed in the three cities included as the "market" in this study, it was clear that the fishermen, restaurant owners and local vendors were aware of the replacement and they identify the mota mostly as "capacete" (see Table 1). Also, it was noticeable that a small number of the people involved in commercialization of "Mota" know or are aware of the way in which they are captured, much less bee aware of the use of river dolphins and caimans as bait (Alves et al. 2012; Gómez et al. 2008; Mintzer et al. 2013; Silva et al. 2011). An "unaware" market may be the cause of approximately 1150 tons of "mota" being obtained in the Brazilian Amazon each year (Trujillo et al. 2010), overexploting this fish and also causing a decrease in numbers in the populations of animals used as bait, similarly to what happened with the crash in population numbers of the Ganges river dolphin (*Platanista gangetica*) in Bangladesh due to hunting pressure to use it has bait (Smith et al. 1998).

The total mercury concentrations found in this study are much higher than other study reports for Piscivorus species at Amazon region (Malm et al. 1995). Also, total mercury concentrations in all the specimens tested were higher than the recommended levels allowed for human consumption by the WHO.

We believe that regulation of this fishery must be implemented in all nations from the Amazon region, particularly Brazil, Colombia and Peru. It is necessary to tract the origin and fishing technique used for capturing “mota”. The alternative of using cattle guts and other organs as bait exists and it is used in some areas of Colombia (Gómez et al. 2008). For this reason, a chain of custody, making clear which bait was used to capture “mota” is necessary in order to allow the final consumer to make an informed decision. Also, increased monitoring and follow up of testing for Mercury content is also needed in order to determine if its consumption should be continued. We suggest that the governmental entities, working in collaboration with NGOs and other institutions should also provide alternatives to the fishing communities regarding sustainable management of aquatic resources and alternatives to the “mota” fishery.

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Figure 1. Sample localities: Into the Putumayo River Region: **1.** Puerto Asís, Putumayo (4 samples). The Magdalena River Region: **2.** Girardot, Cundinamarca (4 samples), **3.** Melgar, Tolima(5 Samples). **4.** Bogota, Cundinamarca (31 samples). The Meta River Region: **5.** Puerto Lopez, Meta. The Amazon River Region: **6.** Puerto Nariño, **7.** Leticia, Amazonas (33 samples), and **8.** The Orinoco River Region: Inirida, Guainía (7 Samples).

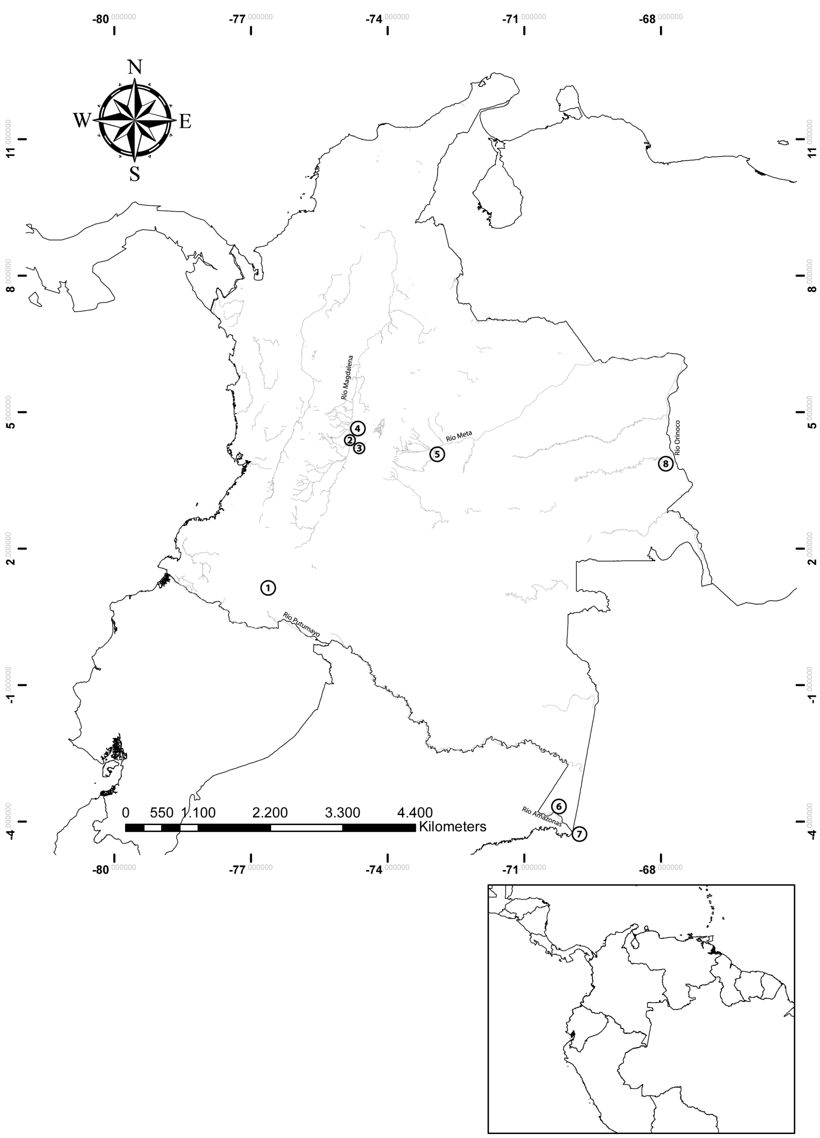

Figure 2. Neighbour- Joining tree constructed based on COI gene. Bootstrap values (in percentage). Branches containing genus *Hypophthalmus, Brachyplatystoma, Pinirampus, Calophysus* and *Pimelodu*s are labelled in purple, yellow, blue, gray and red respectively. Sequences labelled with (*) correspond to previously published sequences available from GenBank.

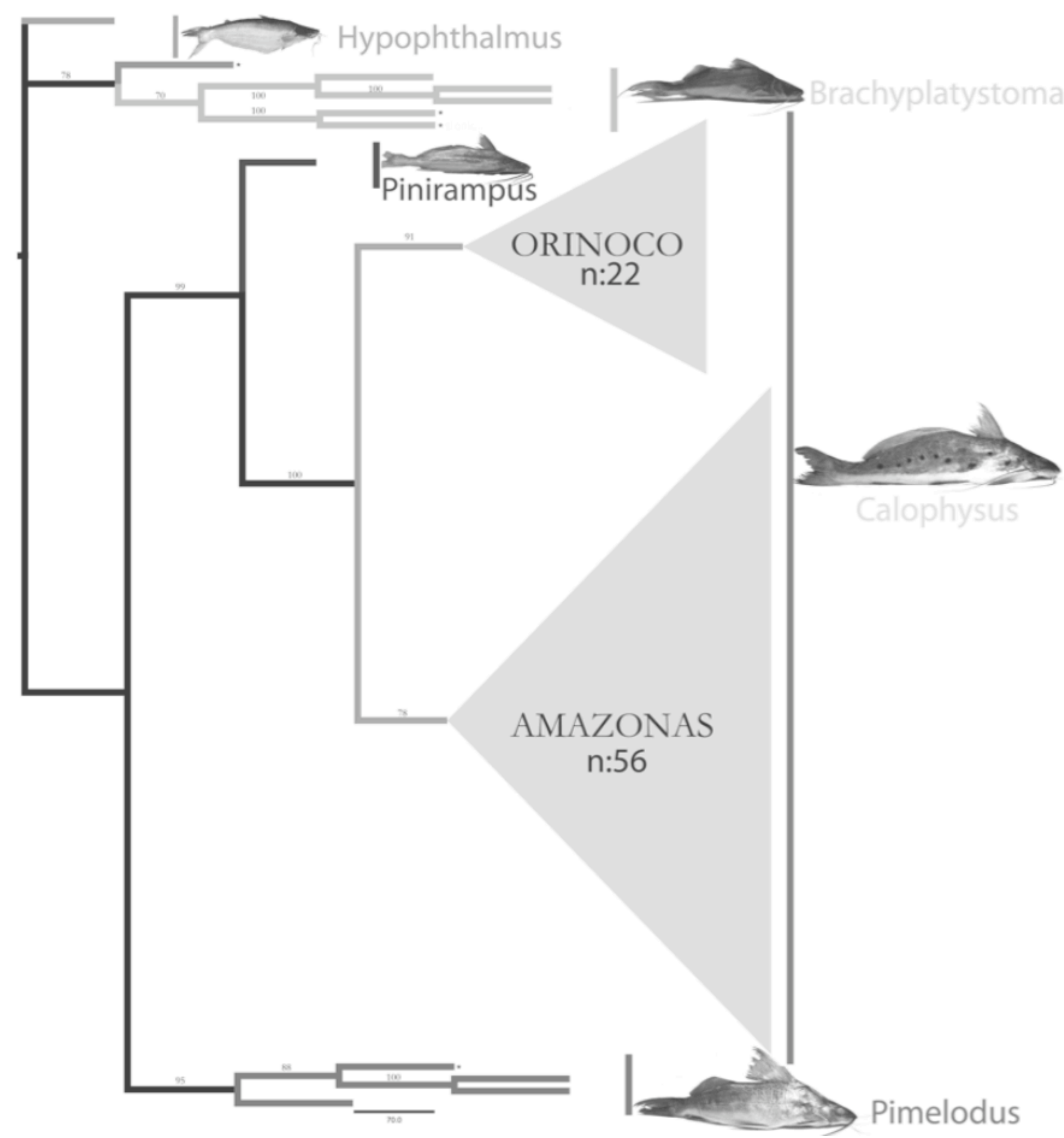

Figure 3. Scatter-plot of muscle Hg concetration as a function of body length

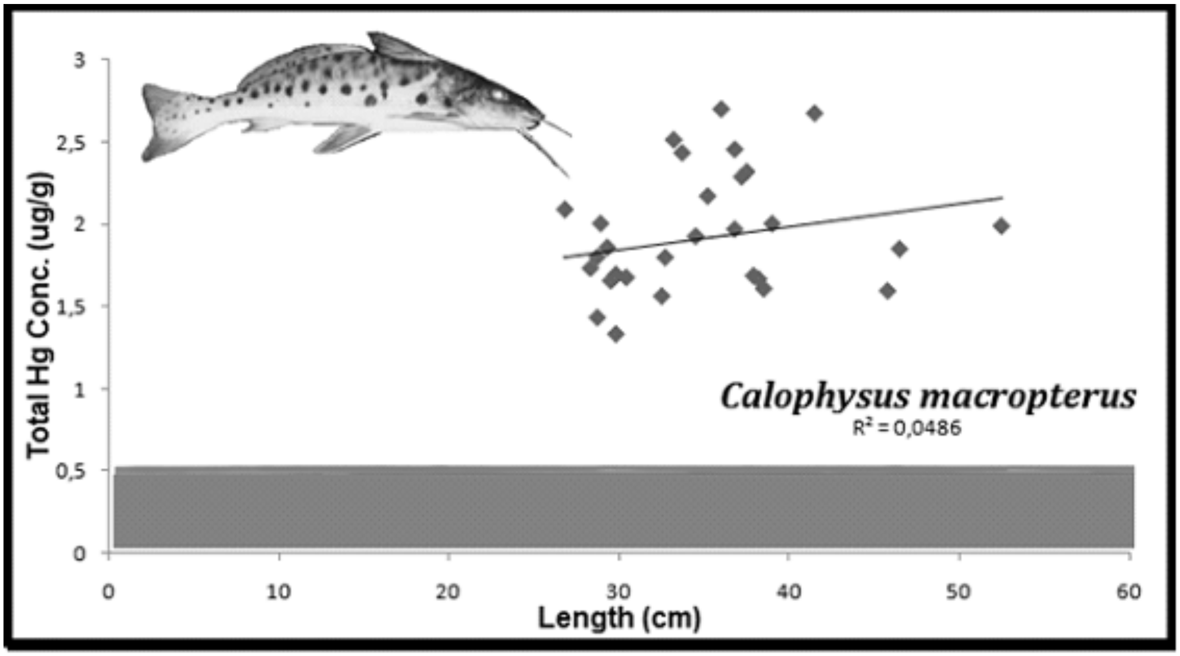

Figure 4. Box-plot of the variation in Hg_{TOT} concetration from specimens from different sampling locations

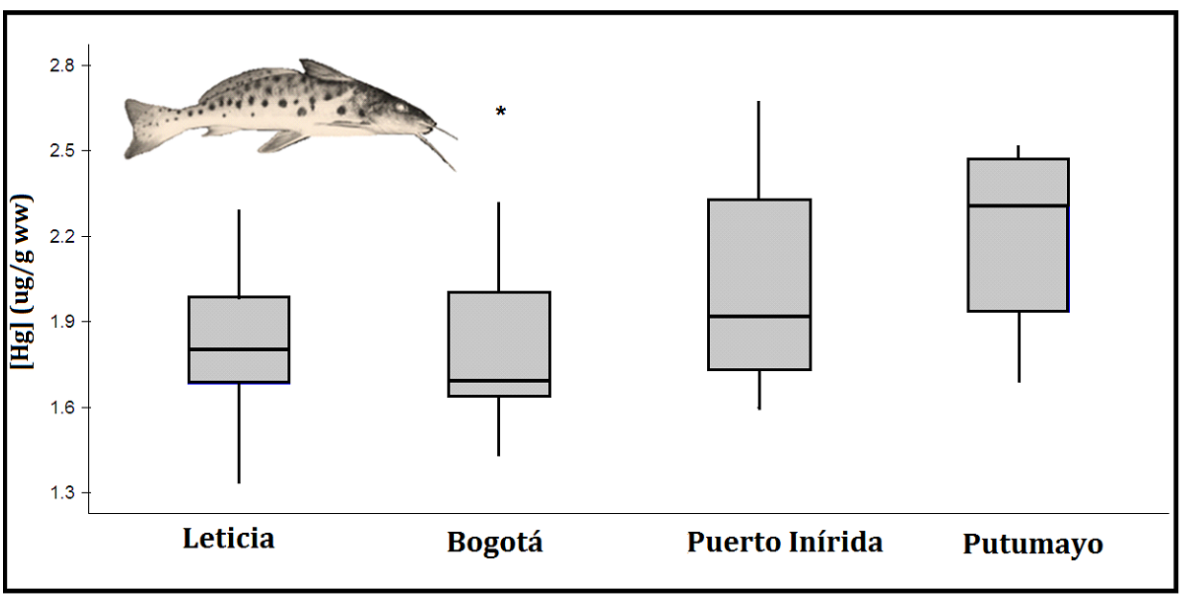

Table 1. Summary of specimens analyzed in this study and the percentage of species representation in the sample of 86 specimens analyzed in total(in bold). Commercial name refers to the name used for a particular species in the markets of Bogotá (B), Girardot (G) and Melgar (M). Local name refers to the name used for a particular species in the Colombian Amazon (Leticia, Puerto Nariño and Puerto Asís), the Brazilian Amazon (Tabatinga), The Colombian Orinoco (Puerto López and Puerto Inírida).

Commercial Name	Local Name	Scientific name	Number of specimens
Capaz (B) Capaceta (B)(M)(G)	Mota, Piracatinga, Zamurito, Simi, Picalon, Capaceta, Barbuchas, mapuro	*Calophysus macropterus*	78 **(90. 7%)**
Capaz (B)	Pirabuton, Blancopobre, Mapuro	*Brachyplatystoma vaillantii*	3 **(3,5%)**
Capaz (B)(M)(G)	Capaz	*Pimelodus grosskopfi*	3**(3,5%)**
Capaz (B)	Barbancho, Barbichato, Piramutaba	*Pinirampus pinirampus*	1 **(1.15%)**
Capaz(B)	Mapará	*Hypophthalmus marginatus*	1**(1.15%)**