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Dietary exposure to methylmercury from fish consumption by Indigenous communities along the Napo River, Peru

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Abstract

Recently in the Peruvian Amazon, there has been increased concern regarding exposure to the neurotoxin methylmercury (MeHg) through fish consumption.

Objective: To measure local Indigenous communities' methylmercury exposure secondary to consumption of the most commonly eaten fish species, and assess if weekly methylmercury exposure by women of reproductive age (15 – 49 years) surpassed established tolerable weekly intake values for this vulnerable population.

Methods: The team surveyed households, by means of a convenience sample, to establish the five most commonly consumed fish, the fishes' average household feeding capacity, as well as the average total fish length. We sampled fish in the region shared by local fishermen, and analyzed these tissue samples for methylmercury levels to calculate risk exposure. A limitation was using a convenience sample for the dietary survey, which could introduce the risk of low generalizability to the population. Such a risk is low in this situation, however, as household characteristics are homogenous.

Results: A total of 205 fish representing 19 different species were sampled. The median mercury concentration in the 9 most commonly–consumed fish species was < 0.073 mg/kg ww. The highly carnivorous chambira (*H. scomberoides)* was the only exception, with a median mercury concentration of 0.30 mg/kg ww. Total weekly methylmercury exposure weighted by species–specific consumption frequencies for local women of reproductive age (15 – 49 years) was 0.635 µg/kg bw/week. This is less than the most conservative tolerable weekly intake value of 0.70 µg/kg bw/week, as established by international health agencies.

Conclusions: Given low exposure to MeHg and the reliance of fish as a primary protein source, it was concluded that it is reasonably plausible the benefits of fish consumption at the observed rates outweigh the risks of methylmercury poisoning in this population. We do caution however, that young children and women of child–bearing age might benefit from choosing fish other than chambira wherever possible.

Key Words methylmercury, Peruvian Amazon, nutrition, child and maternal health

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INTRODUCTION

Recently, there has been increased concern regarding human exposure to methylmercury from fish consumption, especially among Indigenous inhabitants of the Amazon River Basin where fish is their primary protein source.[1–7] Over the last few decades, greater amounts of inorganic mercury have been released into the environment due to various naturally–occurring and anthropogenic sources, including but not limited to: burning of fossil fuel;[8] forest fires;[9] deforestation;[10–13] major oil spill events;[14,15] seasonal flooding of organic soils, including during reservoir creation;[16–18] and mercury released as a result of artisanal gold mining.[19–22]

It is known that a portion of inorganic mercury (Hg^{2+}) deposited to the environment is converted to methylmercury (MeHg), principally by sulphate–reducing bacteria in aquatic sediments.[23,24] Once incorporated into bacterial and phytoplankton tissue, this new source of methylmercury is readily accumulated in animal tissues at progressively higher concentrations up the food web to higher trophic levels.[25,26] Higher concentrations of mercury are observed in fish, particularly in large, long–lived carnivorous species,[27,28] where the majority (> 90%) of mercury in tissue is in the form of methylmercury.[29]

Consumption of fish is the primary mode of methylmercury exposure in humans.[27] Given the dependence of remote communities of the Amazon Basin on fish as their primary protein source, there is concern regarding exposure to methylmercury. Boischio and Hanshel,[30] as well as Fabre and Alonso,[31] have estimated a mean daily fish consumption of between 243 g and 500 g of fish for some Amazon riverside populations. Depending on the methylmercury concentration in fish, this dose may have the potential to exceed guidelines intended to be protective of health.

While there is a positive correlation between hair methylmercury concentration and exposure,[1,2,7] dietary factors such as selenium from tropical fruits and nuts have been reported to act as moderators in the toxic effects of methylmercury exposure to humans.[32–34] Effects of excessive exposure to methylmercury vary in presentation and severity, but may include neurodevelopmental, neuro–behavioral, visual, and neuromotor deficits, as well as, cytogenetic damage, immune alterations and cardiovascular toxicity.[19, 35–40] Fetuses and young children are more susceptible than adults to the risk of neurologic impairment due to greater sensitivity during early stages of brain development. For these reasons, the World Health Organization considers mercury as one of the top ten chemicals of public health concern.[41] However, while fish may contain methylmercury, fish is also an excellent source of high quality protein and is one of the best food sources of omega–3 fatty acids, vitamin D and essential elements including selenium, iodine, magnesium, iron and copper.[42] There is also evidence that regular fish consumption benefits cardiovascular health and child development. Thus, risks of exposure to methylmercury must be carefully balanced with direct health benefits, in addition to the cultural and community benefits that accrue from the pursuit of fish.[42-44]

Objectives

The impetus for this study arose as a result of participatory research conducted in 2012 by our group, the University of British Columbia (UBC) Global Health Initiative, a part of UBC's Division of Global Health (Dept. of Family Practice), and conducted in partnership with the Centro de Salud de Santa Clotilde (CSSC). The CSSC is a healthcare centre responsible for the Micro Red Napo health catchment area, which lies along the Napo River in the Napo and Torres Causanas Districts, Province of Maynas, Department of Loreto, Peru (Figures 1 & 2). The CSSC serves a catchment area of at least 28 communities along the Napo River.

Participatory research conducted in the area identified that local communities along the Napo River expressed concern over methylmercury in water and fish following the results of a national government study; consequently, mercury in fish became a perceived health risk by community members.[45] These results caused our group and the medical staff of the CSSC to investigate whether local fish had elevated levels of mercury and further estimate dietary exposure to methylmercury via fish consumption.[27]

To address community concerns, the research team completed a three–pronged approach. Firstly, a household dietary diversity survey was completed. Secondly, using the results of the survey to inform the sampling method, fish tissue samples of the most commonly consumed fish species were undertaken to measure MeHg tissue concentrations of the reported fish. Thirdly, combining the results of the first and second study components, weekly MeHg exposure was calculated and compared with international health organization recommended threshold limits, to assess potential risk of continued fish consumption in the region by the most vulnerable populations: women of reproductive age, children and youth 17 years old and younger.

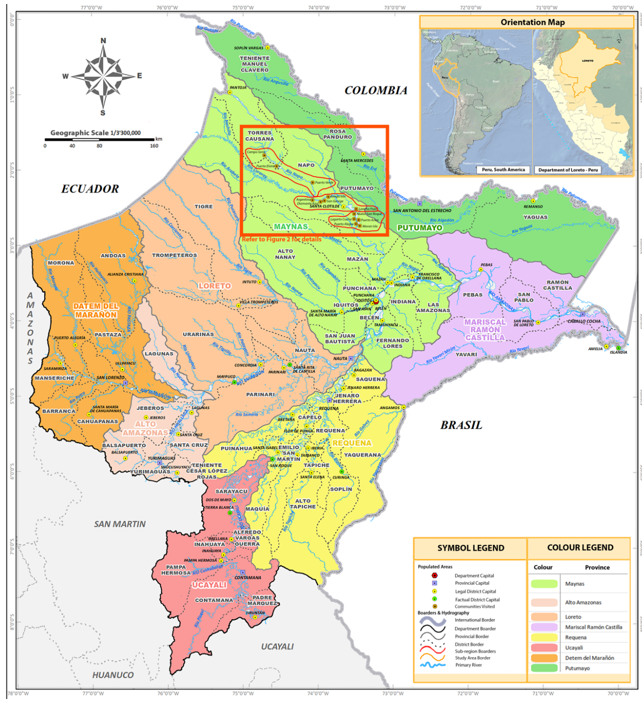

Figure 1 Map of the Department of Loreto, with the study area indicated, and an insert of both, where Peru is in relation to South America, and where the Department of Loreto is in relation to Peru. Original map from Gobierno Regional Loreto.[63]

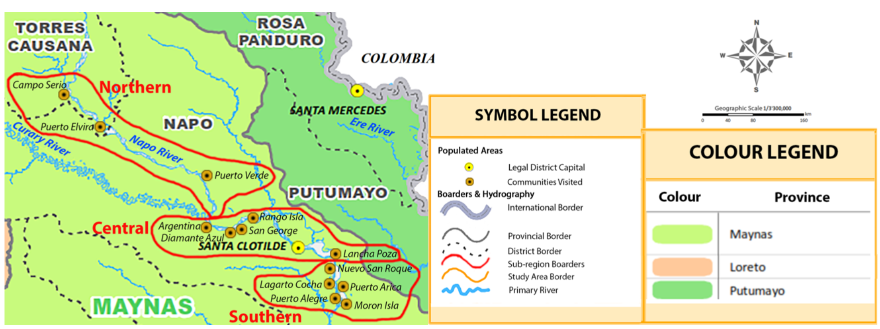

Figure 2 Enlarged map of the study area with both the stratified study sub-areas outlined and labeled, and the communities visited indicated. Original map from Gobierno Regional Loreto.[63]

MATERIALS AND METHODS

Ethics

Ethical approval for the survey component of the study was obtained from the University of British Columbia's Behavioral Research Ethics board (H13–00303). The research team also obtained local support for the research proposal from the Director of Dirección de Atención Integral de Salud and the Director of Centro Nacional de Alimentación y Nutrición through the Director of the Centro de Salud de Santa Clotilde (CSSC). It was confirmed by the same University's ethic board that no ethic approvals were required for the fish sampling component of the study.

Geography

The Napo River is a large upstream tributary of the Amazon with its headwaters in eastern Ecuador, flowing eastwards through north–western Peru.[46] The study area stretches 178 km upstream, and 72 km downstream of the CSSC (Figures 1 & 2). All inhabitants of this Napo River region are indigenous to the area, with the exception of some healthcare professionals.

Household Dietary Survey

The survey used a convenience sampling method, sampling 10% of households in each community visited.[48] The survey asked participants to self–report the five most commonly consumed fish in the household and provide an estimate of both the feeding capacity and total fish length. Validity of the survey tool was assessed and verified using a sample population from the Santa Clotilde community prior to undertaking this study. The inclusion criterion to participate in the surveys were for participants to be the primary meal preparer and/or cook for their household. Only one survey was completed per household.

Fish Tissue Sampling

The fish sampling protocol for this study was based on a frequency analysis of the fish identified as the five most commonly consumed by at least 32 (17%) of the 190 participating families who completed the dietary survey (Table 1).

The CSSC served as the central location for archival and storage of all tissues collected. Reasonable efforts were made to acquire a sufficiently large sample size of each fish taxon from each of the three sub–regions to determine if spatial patterns existed, as having had a spatial difference exist could result in potentially altered health communication information for communities in those zones (Table 2).

Fish were collected in the fishing waters of 13 communities along the Napo River over a 250 km length of the Napo River, Peru. These communities were among those surveyed during the household survey component of the study. Given the large size of the study area and geographically distant communities included in the study, one further objective was to determine if there were spatial differences in the fishes' tissue mercury concentration; and thus, exposure. To determine this, we divided the study area into three sub–areas: the northern, central and southern study sub–areas (Figures 1 & 2). Fish were collected from three, five and five communities in each study sub–area, respectively. Twelve communities were visited directly. Fish from a thirteenth community, Lancha Poza, from the central sub–area, were harvested directly by community's residents and brought to Santa Clotilde's morning market, where a research team member bought and sampled the fish.

Fishing Methods

All fishing and sampling was conducted early in August, 2014. All fishing locations were accessed from a local community's dugout fishing vessel while on dedicated fishing expeditions from Santa Clotilde with the support of local community fishers. Fish were caught using gill nets, angling and weighted throw nets by locals. Gill nets were set in tributaries that fed into the Napo River and were situated away from the community's main living area and populace. Nets were set overnight, or for an extended period of time during the day. Weighted fishnets were used in the Napo River itself and occasionally in a tributary; and in both cases, thrown off of a dugout fishing vessel.

Fishing locations were situated where locals of each community fish daily, by using the same methods typically employed to catch their fish. One or two days were spent at each community to collect a representative sample. Additional fish were provided to the sampling team by community members who agreed to have the team sample fish from their catch–of–the–day.

Tissue Sampling Technique

Fish tissue samples were harvested from the caudal musculature using a stainless steel knife. Scales and skin were excised, with a minimum 10 g of muscle retained. For small size fish, tissue from both flanks was composited. Samples taken from *Loricaria sp.* (a catfish species) were an exception, which had its samples taken bilaterally from epaxial muscle tissue on the dorsolateral aspect of its body. Tissue samples were placed into either a Whirl–Pac® or a Ziploc® bag and coded with an indelible marker to indicate local fish name, sample number and sampling location. Immediately following the labelling process, samples were placed into an insulated cooler with large ice planks. Fish were kept on ice for a maximum of 3 days until samples could be frozen. Samples were maintained in a frozen state until transport to the ALS Global laboratory in Lima, Peru. There, tissue samples were analyzed for total mercury concentration (mg/kg wet weight) using Cold Vapour Atomic Fluorescence Spectroscopy (CVAFS) with a detection limit of 0.002 mg/kg wet weight (ww). No permits were required to undergo any of the fish sampling methodology outlined above.

Fish Taxa Identification

Digital photographs of representative specimens of each species were taken in the field. To determine the scientific name for the sampled fish species, we initially referred to identification sources such as Fishbase and Ortega *et al.* that contained photographs and descriptions that could be compared with our photographs.[49,50] Following use of these initial sources, a literature search was completed to find additional taxonomic details.[51–53] Finally, assistance with identifications for six more difficult taxa was provided by several experts in the fish fauna of South America (see Acknowledgments).

Methylmercury Exposure Assessment

All mercury measured in the fish samples were assumed to be methylated mercury as a conservative health measure when assessing subsequent methylmercury exposure.[29] Equations 1 – 4 were used by the study team to calculate weighted weekly mercury exposure from fish consumption by females of reproductive age (15 – 49 years of age), measured as micrograms of methylmercury per kilogram of consumer's body weight per week (μg MeHg/kg bw/week) (Appendix I).[54] Dietary survey results provided inputs for the variables in equations 1–3 that relate to fish consumption patterns (Appendix I). The average weight of local women of fertile age (61.1 kg) was retrieved from a national demographic report.[54] Fish lengths were converted to weight as follows:

$$W = a \times L^b$$

The species–specific terms 'a' and 'b' were taken from Fishbase.[55] Finally, the median fish tissue mercury concentrations by species were informed by laboratory results of this study (Table 3).

Equation 1 solves for the amount of methylmercury local women of reproductive age are exposed to through consumption of an entire fish, exclusive of bones or head (Appendix I). Equation 2 calculates the exposure to methylmercury by the average local woman of fertile age per meal, based on fish mercury concentration and meal size (μg MeHg /meal) (Appendix I). Equation 3 calculates the weekly exposure to methylmercury by the average local woman of fertile age, weighted by consumption frequency of dominant taxa (Appendix I). The last equation, equation 4, calculates the total weighted weekly methylmercury exposure (μg MeHg/kg bw/week) to local women of fertile age, weighted by the frequency of meals per week that include each fish taxon analyzed according to fish consumption patterns (Appendix I).[45]

The local fish named "zungaro" represented a species complex of various fish from the taxonomic order *Siluriformes* (catfishes), rather than a single species. To best assess methylmercury exposure to locals from its consumption, a representative species from the taxonomic order *Siluriformes* was chosen as a proxy for the weight–length relationship factors 'a' and 'b'. Based on field observations, *Pseudoplatystoma punctifer* was chosen to be representative of "zungaro."

Statistical Methods

Normality of all data was evaluated using a Shapiro–Wilk Test, where a significant finding constituted a non–normal distribution. Statistically significant, non–normally distributed, descriptive statistics are presented as their median and interquartile range. Among the data from fish that were caught in all three sub–areas, a one–way ANOVA was used on normally distributed data, and a Kruskal–Wallis Test was performed on statistically significant, non–normally distributed data. All fish that were caught in only two sub–areas, which resulted in statistically significant non-normal distributions, had a Wilcoxon rank–sum test performed on the data. The aforementioned tests were used to determine if there were significant differences in median mercury concentrations within species across the different regions in the study area, which might prompt different management decisions. The statistical analyses were performed using JMP® Version 9.0.1 for Mac.

RESULTS

Dietary Survey

Households included in the survey hosted a median (interquartile range) of 6 (4–7) individuals. A total of 190 adult women were interviewed, representing 1,129 inhabitants of the study region (Figures 1 & 2). The variety of fish taxa consumed was diverse, with 100 identified in total. Of these, fish taxa were selected for further study based on being reported as the top five most consumed by at least ten percent of households sampled. Results are shown in Table 1, inclusive with median reported total fish length and the feeding capacity of each taxa. The fish taxa cited as one of the top five most consumed by more than half of respondents were boquichico (n = 151, 76%) and palomenta (n = 133, 67%).

Table 1 Descriptive characteristics of fish reported as among the five most commonly consumed by at least 10% of the participating households (n = 190).

Local Fish Name	Incidence[1]	Feeding Capacity[1,2]	Total Fish Length (cm)[1]
Boquichico	151 (75.5)	2 (1.5 – 3.0)	23 (17.8 – 27.9)
Palometa	133 (66.5)	2 (1.0 – 2.0)	18 (15.2 – 22.9)
Lisa	71 (35.5)	2 (2 – 3)	25 (22.9 – 30.5)
Fasaco	71 (35.5)	3 (2.0 – 3.3)	28 (25.4 – 33.0)
Sardina	58 (29.0)	1 (1.0 – 2.0)	15.2 (12.7 – 20.3)
Bujurqui	52 (26.0)	1 (1.0 – 1.0)	12.7 (10.8 – 15.2)
Zungaro	42 (21.0)	6.5 (5 – 10)	61 (47 – 89.5)
Carachama	39 (19.5)	1 (1.0 – 2.0)	23 (20.3 – 25.4)
Chambira	32 (16.0)	4 (2.2 – 4)	36 (24.1 – 45.7)
Shuyo	32 (16.0)	2 (1.8 – 3.0)	25 (22.9 – 31.1)
Tucunare	31 (15.5)	4 (3.0 – 7.5)	35.6 (28.6 – 44.4)
Sabalo	31 (15.0)	3 (2.0 – 5.5)	37 (29.2 – 50.8)
Cunchi	28 (14.0)	1 (1.0 – 1.0)	18 (15.2 – 20.8)
Acarahuasu	20 (10.0)	2 (2.0 – 2.0)	23 (20.3 – 25.4)

[1]median (interquartile range) or n (%)
[2] usual number of household members fed by one fish of this species

Fish Tissue Sampling

A total of 205 fish tissue samples, representing 19 different taxa, were analysed for total mercury concentration (mg/kg wet weight) (Table 2). The consensus on the identification to the lowest possible taxonomic level of detail is provided in Table 2.

Table 2 Results from the fish survey that informed the fish tissue sampling methodology.

Local Name	Scientific Name[a]	Frequency of Most Commonly Consumed Species[b]	Fish Diet	Target Sample Size
Boquichico	*Prochilodus nigricans*	151 (79)	Planktivorous	20
Palometa	*Mylossoma aureum*	133 (70)	Varied Diet	20
Fasaco	*Hoplias malabaricus*	71 (37)	Piscivorous	20
Lisa	*Schizodon fasciatus*	71 (37)	Herbivorous	15
Sardina	*Triportheus rotundatus*	58 (30)	Planktivorous	15
Bujurqui	*Chaetobranchus flavescens*	52 (27)	Detritus, Insects, Plankton	15
Zungaro	*Siluriformes*[a]	42 (22)	Benthivore	15
Carachama	*Loricaria sp.*	39 (20)	Insect larvae, detritus	15
Chambira	*Hydrolycus scomberoides*	32 (17)	Piscivorous	15

[a]Order, taxonomic level
[b] n (%)

The five most sampled fish by local name, corresponding taxa and respective proportion, were all representatives of the taxonomic order *Characiformes* (characins) including boquichico (*Prochilodus nigricans*, 19.5%), sardina (*Triportheus rotundatus*, 15.6%), palometa (*Mylossoma sp.*, 11.2%), fasaco (*Hoplias malabaricus*, 10.7%) and chambira (*Hydrolycus scomberoides*, 9.8%).

More fish were captured in the southern sub–area, while the greatest diversity of fish were caught from the central sub–area (Table 3). Despite differences in sample sizes among the areas fished, a statistical comparison of mercury concentrations across areas did not reveal a significant geographic difference for any species (Table 3).

Table 3 Summary of fish mercury data by taxa and sample size from the total (T) study area, and the northern (N), central (C), and southern (S), sub-regions (SR) of the study area, taken in August, 2014. A P-value of < 0.05 indicates a statistically significant geographic difference in the median mercury concentrations within species.

Local Fish Name	Scientific Name	SR	Sample Size[a,b]	Median Fish Tissue [Hg] (mg/kg ww)[b]	*P*–value
Boquichico	*Prochilodus nigricans*	T	40 (19.5)	0.046 (0.031–0.055)	0.06
		N	20 (9.8)	0.038 (0.026–0.050)	
		C	13 (6.3)	0.052 (0.036–0.071)	
		S	7 (3.4)	0.048 (0.040–0.065)	
Sardina	*Triportheus rotundatus*	T	32 (15.6)	0.060 (0.046–0.082)	0.26
		N	17 (8.3)	0.052 (0.034–0.079)	
		C	4 (1.9)	0.073 (0.052–0.128)	
		S	11 (5.4)	0.067 (0.052–0.078)	
Fasaco	*Hoplias malabaricus*	T	22 (10.7)	0.069 (0.056–0.140)	0.09
		N	4 (1.9)	0.051 (0.045–0.060)	
		C	3 (1.5)	0.093 (0.070–0.129)	
		S	17 (8.3)	0.075 (0.063–0.123)	
Palometa	*Mylossoma aureum*	T	21 (10.2)	0.016 (0.014–0.029)	0.79
		N	12 (5.6)	0.017 (0.013–0.027)	
		C	2 (1.0)	0.018 (0.015–0.022)	
		S	7 (3.4)	0.016 (0.015–0.090)	
Chambira	*Hydrolycus scomberoides*	T	20 (9.8)	0.299 (0.10–0.478)	0.63
		N	3 (1.5)	0.498 (0.277–0.568)	
		C	6 (2.9)	0.342 (0.286–0.416)	
		S	7 (3.4)	0.243 (0.094–0.554)	
Lisa	*Schizodon fasciatus*	T	14 (6.8)	0.028 (0.02–0.051)	0.16
		N	9 (4.4)	0.027 (0.017–0.036)	
		C	4 (1.9)	0.035 (0.029–0.065)	
Bujurqui	*Chaetobranchus flavescens*	T	15 (7.3)	0.044 (0.034–0.06)	0.15
		N	3 (1.5)	0.038 (0.031–0.292)	
		C	5 (2.4)	0.034 (0.031–0.049)	
		S	7 (3.4)	0.051 (0.044–0.173)	
Carachama	*Loricaria sp.*	T	7 (3.4)	0.035 (0.011–0.049)	0.06
		C	2 (1.0)	0.014 (0.002–0.029)	
		S	9 (4.4)	0.049 (0.037–0.061)	
Sabalo	*Brycon stolzmanni*	C	5 (2.4)	0.078 (0.039–0.092)	
Ractacara	*Psectrogaster sp.*	C	5 (2.4)	0.092 (0.064–0.11)	
Bacalow	*Pellona casteinaeana*	C	1 (0.4)	0.548 (—)	
Zungaro	[Species Complex]	T	21 (10.2)	0.072 (0.047–0.158)	0.83
		N	4 (1.9)	0.033 (0.030–0.290)	
		C	6 (2.9)	0.095 (0.058–0.161)	
		S	11 (5.4)	0.073 (0.056–0.196)	
Doncella	*Pseudoplatystoma sp.*	T	7 (3.4)	0.118 (0.35–0.257)	NA[c]
		N	2 (1.0)	0.202 (0.029–0.376)	

		C	1 (0.4)	0.118 (—)	
		S	4 (1.9)	0.135 (0.044–0.242)	
Tigre	*Pseudoplatystoma sp.*	T	6 (2.9)	0.058 (0.053–0.065)	NA[c]
		N	1 (0.4)	0.061 (—)	
		S	5 (2.4)	0.057 (0.049–0.067)	
Achara	*Siluriformes*[d]	T	3 (1.5)	0.033 (0.033–0.73)	NA[c]
		N	2 (1.0)	0.033 (0.033–0.33)	
		S	1 (0.4)	0.073 (—)	
Bocon	*Siluriformes*[d]	T	2 (1.0)	0.086 (0.051–0.121)	
Camotillo	*Siluriformes*[d]	C	1 (0.4)	0.071 (—)	
Chiripira	*Loricariidae sp.*	S	1 (0.4)	0.416 (—)	
Mota	*Siluriformes*[d]	C	1 (0.4)	0.283 (—)	

[a] numbers and proportions are not mutually exclusive
[b] n (%) or median (interquartile range)
[c] NA = Not applicable
[d] Order, taxonomic level

Mercury Exposure Assessment

Median mercury concentration of fish muscle was < 0.07 mg/kg ww for almost all commonly consumed fish taxa (Tables 2 & 3). The only exception to this pattern was for *Hydrolycus scomberoides*, a large carnivorous species (36 cm) (Table 4), locally known as chambira (Figure 3). Median mercury concentration of this species was 0.30 mg/kg ww (n = 20), several times higher than the next highest concentration found in a species. Besides chambira, only a small number of infrequently captured fish also had elevated tissue mercury concentrations relative to other species captured in this survey. These included single individuals from only three species: mota (Order *Pimelodidae*) at 0.28 mg/kg ww, chiripira (*Loricariidae sp.*) at 0.42 mg/kg ww and bacalow (*Pellona casteinaeana*) at 0.55 mg/kg ww (Table 3).

Table 4 Weekly methylmercury exposure (mg/kg bw/wk) by females of average weight (61.1 kg), who are between 15 and 49 years of age, and are from the study region, based on fish consumption data resulting from the household surveys completed earlier in the study.

Local Fish Name	Total Length (cm)[a]	Total Weight (g)	Grams/Meal	Median Fish Tissue [Hg] (mg/kg ww)[a]	Weekly Feeding Frequency (%/week)	Weighted Exposure (µg/kg bw/week)
Boquichico	23 (17.8 – 27.9)	196	65	0.046 (0.031–0.055)	23	0.082
Palometa	18 (15.2 – 22.8)	75	25	0.016 (0.014–0.029)	20	0.009
Fasaco	33 (25.4 – 33.0)	228	51	0.069 (0.056–0.140)	11	0.044
Lisa	25 (22.9 – 30.5)	338	113	0.028 (0.02–0.051)	11	0.040
Sardina	15 (12.7 – 20.3)	73	48	0.060 (0.046–0.082)	9	0.030
Bujurqui	13 (10.8 – 15.2)	23	15	0.044 (0.034–0.06)	8	0.006
Zungaro	61 (47.0 – 89.5)	2537	260	0.072 (0.047–0.158)	6	0.140
Carachama	23 (20.3 – 25.4)	135	90	0.035 (0.011–0.049)	6	0.022
Chambira	36 (24.1 – 45.7)	932	155	0.299 (0.100–0.478)	5	0.262
Total Average Weekly Exposure						0.635

[a]median (interquartile range)

Figure 2 Profile photograph of a representative *Hydrolycus scomberoides*, which is locally known as chambira, and abroad as vampire fish, that was sampled in this study.

Based on a combination of the dietary survey results (Table 1) and findings of mercury concentrations in the most commonly consumed fish species (Table 3), the weighted total weekly exposure of methylmercury by females of reproductive age in the region was 0.635 μg/kg bw/week (Table 4).

DISCUSSION

Dietary Survey

The Amazon Basin holds the greatest diversity of freshwater fish in the world and this is reflected in the diet of locals in this region.[56] The diversity of local diet is evidenced by the results of the dietary survey, which indicated that 100 fish taxa were consumed. However, the dietary survey results also indicated that about 14 species comprised the majority of the diet, with other species being consumed rarely (Table 1). There is a possibility one of the more rarely consumed fish species could provide a disproportionately large exposure of methylmercury to the regional population. In such a circumstance, however, the health effects are mitigated by the low risk associated with a single or infrequent, relatively high dose exposure to methylmercury from fish consumption. Such considerations are out of the scope of the present study, but also relevant and important for potential pursuit by future studies.

Edible mass of fish ranged from 15 g – 260 g across fish taxa (Table 3), feeding 1 – 7 people, depending on fish size (Table 3). The lightest fish meals (15 g) came from bujurqui (*Chaetobranchus flavescens*), which had the smallest feeding capacity (1 people/fish) due to a combination of its small median total length (23 cm), and its flat body shape that reduces the amount of meat available to eat per unit length of the fish (Table 3). The heaviest fish meals (260 g) came from the catfish species complex zungaro (Order *Siluriformes*), which had the largest feeding capacity (6.5 people/fish), due to its large size (64 cm) and cylindrical body shape, both of which contributed to increasing available meat per unit length of the fish (Tables 3).

A primary limitation of the dietary survey was our sampling technique. A convenience sample method was selected as there was insufficient phone, internet or formal mail service in the study area (Figures 1 & 2), thus limiting sampling methodology options.[47] Convenience sampling introduces the possibility of a lack of generalizability to the entire population, however this is less likely in a population such as this one, with minimal disparity in household characteristics such as occupation, resources, or revenue.

Geography

Napo River width averaged 1.5 km, with an average annual discharge rate of 5,700 m^3/sec, and large variations between wet (7,294 m^3/s) and dry (1,234 m^3/s) seasons.[47] The large variation in the Napo River's volumetric flow rate introduces the potential for seasonal variation in the fish muscle mercury concentration during the various hydrological cycles in this region of the Amazon. The hydrological cycles include the following seasons: flood, demonstrated by an increase in water level; wet, when the river has peak water levels; ebb, demonstrated by a decrease in water level; dry, when the river has valley water levels. Research published after this study was conducted showed there was a measurable, yet statistically insignificant, difference in the mercury concentration of fish muscle tissue from the Solimões River, Brazil, between the ebb and the flow seasons there.[57] The Napo River is an indirect upstream tributary of the Solimões River. These geographic differences limit the generalizability of Ferreira de Silva *et al.*'s study results to this study's results. However, there was one species that was common between this study and the one conducted by Ferreira de Silva *et al.*, *Hoplias malabaricus* (Tables 1 & 2). When comparing exclusively the ebb and flood seasons, Ferreira de Silva *et al.* demonstrated that *H. malabaricus* contained higher muscle tissue mercury concentration during the flood season, and contained a lower muscle tissue mercury concentration during the ebb season. The measured fish muscle mercury concentration differences between hydrologic cycles in the western Amazon basin, although not statistically significant, show there is a possibility that the exposure to methylmercury by the regional population in this study may be different among hydrologic seasons, and is a limitation of our study design. Assessing methylmercury exposure through fish consumption across at different points in the hydrological cycle could be investigated in future research.

Fish Tissue Sampling

The frequency that a fish taxa was reported as amongst those commonly consumed by a household in the dietary survey had a cut–off of being reported by a minimum of 17% (n = 32) of the total 190 respondents. This cut–off was chosen to ensure a sufficient number of samples was collected among the fish species reported as most commonly consumed.

Mercury Concentrations in Fish Tissue

Although low sample size for some sub–areas may have reduced our ability to detect possible spatial differences (Table 2), the relative difference in mercury concentration between areas within species was small. Thus, we are confident that median mercury concentrations integrated across the entire study area (Figures 1 & 2), within species, accurately represents the concentration that an individual is exposed to when consuming a particular species. Fortunately, for the vast majority of fish species consumed, median mercury concentrations averaged less than 0.07 mg/kg (Tables 3 & 4), suggesting that exposure would be correspondingly low, especially considering the small average fish size per meal (< 92 g of fish/meal/person) consumed by most people (Table 4).

As noted above, chambira was the only species with elevated mercury identified, consumed by about 16% of survey respondents as one of the five most commonly consumed fish species (Table 1). However, only 4 (2%) survey respondents in the dietary survey reported their households having eaten chambira as a part of their last meal. Thus, while chambira has relatively elevated mercury, it does not appear to be consumed regularly; and thus, would not be a significant source of methylmercury exposure for a majority of people. Importantly, should methylmercury become a concern again at a future date, it would be possible to utilize chambira as a sentinel taxon. If levels remain low in chambira, other fish are also likely to be within safe methylmercury exposure levels.

Of the nine most commonly consumed fish, the median mercury concentration was < 0.072 mg/kg ww, with the exception of *H. scomberoides* (Table 3).

Methylmercury Exposure

The most vulnerable populations to methylmercury are fetuses, breastfeeding infants and children younger than 13 years.[58] Adolescents up to 18 years of age may also be potentially more sensitive to methylmercury toxicity when compared to adults because of continued myelination and remodelling of the brain during this age.

There is considerable debate regarding the tolerable intake value or reference dose (RfD in μg/kg bw/d or week) for methylmercury as advocated by various health agencies. For example, the US Environmental Protection Agency (EPA) is the most conservative, using a RfD of 0.1 μg/kg bw/day,[59] which is equivalent to a provisional tolerable weekly intake (PTWI) of 0.70 μg/kg Hg bw/week. The EPA employs a benchmark analysis using Seychelles, New Zealand and Faroe Islands studies to calculate their RfD, with an uncertainty factor (UF) of 10. As of 2001, the US Food & Drug Administration (FDA) also adopted the same RfD of 0.1 μg/kg bw/day as the EPA.[60] In the USA, the Agency of Toxic Substances and Disease Registry (ATSDR) has set a minimal risk level (MRL) of 0.3 μg/kg bw/day of methylmercury,[61] equivalent to a PTWI of 2.1 μg/kg bw/week, which is three times higher than the EPA and FDA PTWI. The MRL derived by the ATSDR is based on the study by Davidson, *et al.*,[62] and includes a UF of 4.5.

Health Canada's provisional tolerable daily intake (pTDI) for adults is 0.47 μg/kg, and 0.20 μg/kg/day for pregnant and breastfeeding mothers, as well as children 12 years and younger .[42] A pTDI of 0.20 μg/kg/day is equivalent to a PTWI of 1.4 μg/kg bw/week, which is double that of the EPA and FDA for the same population.

A global reference point comes from the Joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives' (JECFA's), which most recently recommended a PTWI of 1.6 μg/kg bw/week for pregnant and breastfeeding women, as well as children 17 years and younger.[58] Note that none of these guideline values account for the holistic health benefits of consuming fish, so risk communication is extremely important as it is critical that Indigenous people are not dissuaded from consuming fish. Fortunately, despite the variability in RfDs for methylmercury, the weighted total weekly methylmercury exposure in this study of 0.64 μg/kg bw/week was below even the most conservative RfD guideline value of 0.70 μg/kg bw/week (Table 4).

Recommendation

The weighted total methylmercury exposure rate generated by this study is 0.64 μg/kg bw/week, which is lower than the most conservative provisional tolerable weekly intake values proposed by the EPA and the FDA,[59,60] and much less than that proposed by Health Canada and JECFA.[42,58] Based on this finding, it is reasonably plausible that current fish consumption practices pose no risk to local community members. However, despite chambira's apparent infrequent weekly consumption rate by locals, the research team acknowledges its elevated mercury concentration (0.30 mg/kg ww) relative to other fish species in this study (Table 2). For this reason, to be protective of the fetus and young children, we would be cautious to allow women of child–bearing age to consume it in anything beyond small quantities, and defer to other fish species when possible. Fortunately, chambira is a highly unique looking fish species (Figure 3), making it straightforward to establish an outreach program to help consumers identify and avoid this potentially problematic species.

CONCLUSION

Results of this study reveal that mercury concentrations for the majority of commonly–consumed fish species from the Napo River of Peru are low. There are no regional within–species differences in fish mercury concentration across the study sub–areas. Given the overall low concentrations and low exposure to riparian locals due to infrequent and small meal size, it is highly unlikely that there are any adverse health risks due to mercury exposure, even to the most sensitive population. In fact, because fish appear to be the main protein source, we would become concerned if too few fish are eaten. Nevertheless, because there remains an unmeasured heightened risk to women of child–bearing age that consume chambira, even in small quantities, it is advisable for this demographic to defer chambira consumption where possible. Otherwise, it is reasonable to assume that residents can continue with their fish consumption practices given the nutritional benefit of fish and their low mercury concentration relative to health guidelines in this region of the Peruvian Amazon.

APPENDIX I

$$\frac{\left(a(M.Total\ Fish\ L.\ (cm)^{b}) \times \frac{2}{3} \times Potential\ Hg\ Dose\ \left(\frac{\mu g\ of\ Hg}{g\ of\ wet\ weight}\right)\right)}{National\ Average\ Weight\ of\ Female\ of\ Fertile\ Age\ (kg)} = \frac{\mu g\ of\ M.Hg}{Dressed\ Fish_{Avg.F.F.}}$$

Equation 1 – Calculates the amount of methylmercury local women between the ages of 15 and 49 years–old (women of fertile age) are exposed to per dressed fish eaten, not including the bones. M. = median; L. = length; a = length–weight relationship factor a ; b = length–weight relationship factor b; Hg = mercury; A.F.F. = average fertile female.

$$\frac{\mu g\ of\ M.Hg}{Dressed\ Fish_{Avg.F.F.}} \times \frac{M.\ proportion\ of\ fish\ eaten\ (\%)}{1\ meal} = \frac{M.Hg\ Intake\ (\mu g)}{Fish\ Meal}_{Avg.F.F.}$$

Equation 2 – Calculates the exposure to methylmercury per meal (µg/kg bw/meal) by the average local woman of fertile age from the consumption of a single prepared fish meal.; M. = median; No. = number; Avg.F.F. = average fertile female; M.Hg methylmercury.

$$\frac{M.Hg\ Intake\ (\mu g)}{Fish\ Meal}_{Avg.F.F.} \times \frac{M.\ No.\ of\ meals\ eaten\ weekly}{Fish\ Taxon} = \frac{Weekly\ M.Hg\ Intake}{Fish\ Taxon}_{Avg.F.F.}$$

Equation 3 – Calculates the weekly exposure to methylmercury (µg/kg of bw/week) by the average local woman of fertile age from the weighted number of meals eaten per week for a specific fish taxon. M. = median; No. = number; Avg.F.F. = average fertile female; M.Hg = methylmercury.

$$\sum_{All\ fish\ taxa} \frac{Weekly\ M.Hg\ Intake}{Fish\ taxon}_{Avg.F.F.} = Weighted\ Total\ Weekly\ M.Hg\ Intake_{Avg.F.F.}$$

Equation 4 – Calculates the total weighted weekly exposure to methylmercury by an average local woman of fertile age from current fish consumption habits. Avg. F.F. = average fertile female; M.Hg = methylmercury.

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AUTHOR CONTRIBUTIONS

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