Environmental Research 144 (2016) 73–80

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Sex-related mercury bioaccumulation in fish from the Madeira River, Amazon

Wanderley R. Bastos [a,*], José G. Dórea [b], José Vicente E. Bernardi [c], Angelo G. Manzatto [a], Marilia H. Mussy [a,e], Leidiane C. Lauthartte [a], Luiz D. Lacerda [d], Olaf Malm [e]

[a] Laboratório de Biogeoquímica Ambiental, Universidade Federal de Rondônia, Brazil
[b] Faculdade de Ciências da Saúde, Universidade de Brasília, Brazil
[c] Instituto de Química, Universidade de Brasília, Brasília, Brazil
[d] Laboratório de Biogeoquímica Costeira, Instituto de Ciências do Mar, Universidade Federal do Ceará, Brazil
[e] Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 29 August 2015
Received in revised form
1 November 2015
Accepted 2 November 2015
Available online 14 November 2015

Keywords:
Madeira River
Amazon
Fish
Mercury
Muscle
Sex

ABSTRACT

Sex plays an important role in the kinetics and dynamics of methylmercury in some animals. Although fish is the main source of mercury exposure to consumers, the role of sex in fish-Hg bioaccumulation is less known. We studied total Hg (THg) concentrations in 2538 samples (males = 1052, females = 1486) of fish from different trophic levels (herbivorous, planctivorous, detritivorous, omnivorous, carnivorous, piscivorous); for each species we made a post hoc estimation of the minimum number of samples required to detect variance-based differences between sexes. Only five of the 41 studied species showed significant difference between sexes; but, no consistent dominant pattern of THg concentrations favored either sex. When grouped by trophic levels, overall mean difference in THg concentrations between males and females were not statistically significant. Correlation analysis showed sex-dependent THg bioaccumulation as a function of condition factor was statistically significant and negative for all trophic levels (detritivorous, herviborous, omnivorous, planctivorous, carnivorous, and piscivorous). *Conclusions*: Sex is not the main driver of Hg bioaccumulation in most Amazonian fish species; however, studies have to consider the minimum number of samples required to ascertain sex effects on THg bioaccumulation. Therefore, neither the surveillance of environmental pollution nor the current food advisories based on muscle THg need to change because of fish sex.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Sex plays an important role in the kinetics and dynamics of Hg metabolism in animals where it has been extensively studied (Thomas et al., 1986; Hirayama and Yasutake, 1986). Although fish are the main source of mercury (Hg) exposure to consumers, the role of fish sex in Hg bioaccumulation is less known. There are far fewer studies relating sex differences in pollutant levels (including Hg) in fish than in other wildlife (Burger, 2007). Nevertheless, the effects of Hg on fish biology (Alvarez et al., 2006) are gaining attention and laboratory studies have demonstrated adverse effects of methylmercury (MeHg) on biochemical processes and behavior (Rhea et al., 2013).

Skeletal muscles in fish account for over 60% of body weight (Li et al., 2014) and constitute the main storage compartment for MeHg. Studies have suggested that the rate of feeding and rate of Hg elimination may be species specific and influenced by sex. In species like salmon, during spawning season physiological starvation bring changes in rates of catabolism between juvenile and adult fish (Baker et al., 2009). Males displaced lower total Hg (THg) concentrations than females in European catfish (Squadrone et al., 2015), and in threespine stickleback fish (Willacker et al., 2013). Nevertheless, the elimination rate of Hg in northern pikes was higher in males than in females (Madenjian et al., 2014a). Such differences in mercury metabolism may lead to sex differences in muscle Hg concentrations. Recently, our own studies have suggested that differences in sexes may influence Hg metabolism in Amazonian catfish (Bastos et al., 2015b).

Burger (2007) has observed that there is size related sexual dimorphism in some species; a predominance of females in larger size classes is considered a reproductive tactic to increase fertility (Nikolsky, 1963; Lowe-McConnell, 1999). Studies reported sex difference in the size of lake trout showing that males with larger body weight also showed higher Hg concentrations than females (Madenjian et al., 2011). However, there are innumerous studies reporting non-statistically significant sex differences in fish-Hg

* Corresponding author.
E-mail address: bastoswr@unir.br (W.R. Bastos).

http://dx.doi.org/10.1016/j.envres.2015.11.001
0013-9351/© 2015 Elsevier Inc. All rights reserved.

concentrations (Magalhães et al., 2007; Weis and Ashley, 2007; Burger et al., 2007; Endo et al., 2008; Harmelin-Vivien et al., 2009; Adams, 2009; Martinez-Gomez et al., 2012; Mela et al., 2014).

Inconsistencies in sex-related differences in THg concentrations may relate to species, fish size (sexual dimorphism included), and trophic levels. An abundant collection of specimens and fish species have been collected from the Madeira River in the Amazon (Bastos et al., 2015a). Such data bank is a valuable source of information and unique opportunity to compare a large number of species. In this study we explore sex dimorphism in relation to environmental mercury in fish muscle focusing on body weight and length (condition factor). Therefore, we took into consideration size and trophic level to study Hg concentrations in females and males in a large variety of freshwater fish from the Amazon.

2. Materials and methods

A larger study exploring THg bioaccumulation in relation to spatial differences and temporal changes has appeared elsewhere (Bastos et al., 2015a). This study represents fish collected along the Madeira River between the cities of Guajará-Mirim-RO (Long 243219.152 – Lat 8805970.844-UTM datum SAD 69) and Humaitá-AM (Long 497790.381 – Lat 9170102.251-UTM datum SAD 69) over the period between 2009 and 2013. Of the sampled species only 41 had a sufficient number of specimens of both sexes to be compared.

Fish species and respective feeding habit classifications were established according to Santos et al., (2006); based on secondary sexual characteristics and gonad inspection, sex was determined macroscopically (Vazzoler, 1996). Besides the conventional classification we also used the trophic level classification proposed by Fishbase (Boettiger et al., 2012; Fishbase, 2015). For each specimen, besides sex, total or fork length (L_t) (mm) and total weight (W_T) (g) were recorded; the condition factor (CF_k) for each specimen was calculated as $CF_k=(W_T)/(L_t)^3$. In our large sample (41 species and 2538 specimens) differences in shape and size can be normalized (accounting for length and weight) to better represent Hg concentrations. Furthermore, within a species, CF of 1 or higher indicates relatively healthy fish, while CF <0.8 indicate underweight. We also removed a sample of muscle (approximately 20 g, wet weight) for THg determination.

THg concentrations in muscle were determined by an in-house method developed and validated as described elsewhere (Bastos et al., 2015a); approximately 500 mg (wet weight) of muscle tissue was analyzed after treatment with solutions of H_2SO_4:HNO_3 (1:1, Tédia, Brazil) and $KMnO_4$ (5% w/v-Merck). After sample digestion, 4.0 mL of $KMnO_4$ solution (5% w/v) was added and left for 30 min more in the digestion block (Tecnal-Mod.007A, Piracicaba, São Paulo, Brazil) at 60 °C for 1 h; this was followed by cooling to room temperature ($\pm$ 25 °C), and addition of hydroxylamine hydrochloride (12% solution, w/v-Merck) drops. The digested samples were diluted with 10.0 mL of ultra-pure H_2O (Milli-Q Plus, Millipore, Bedford, MA, USA). THg readings were done by cold vapor atomic absorption spectrophotometry (Flow Injection Mercury System- FIMS -400- Perkin Elmer, Ueberlingen, Germany) (Malm et al., 1989; Bastos et al., 1998).

All glassware followed our routine protocol of washing in 10% HNO_3 and was rinsed with ultra-pure H_2O. In our routine analysis we used triplicate analyses of samples, and certified reference materials (BCR®, Sigma-Aldrich RTC Inc., USA). Method accuracy of THg determinations were ensured by the use of certified material (Tuna Fish, BCR-463 – THg= 2.850 ± 0.160 mg/kg) which was run with each batch of samples; mean recovery of 96% (2.750 ± 0.500 mg/kg). Limits of detection (LOD) for THg were 0.0007 mg/kg.

Table 1
Observed and estimated minimum number of specimens necessary to estimate sex differences (minimum n=significant for 80%) in mercury (THg) concentrations of male (M) and female (F) fish.

Scientific name	N Obs M	N Obs F	*t* Test	*p*	Minimum (*n*)
Acestrorhynchus falcirostris	22	22	−1.467	0.1497	28 M; 28 F
Acestrorhynchus heterolepis	11	21	−0.2714	0.7879	12 M; 6 F
Ageneiosus inermis	14	10	0.4850	0.6325	149 M; 78 F
Anodus elongatus	43	58	0.2007	0.8413	36 M; 27 F
Auchenipterichthys thoracatus	70	52	0.9574	0.3403	7 M; 6 F
Auchenipterus ambyiacus	17	30	0.1779	0.8596	150 M; 11 F
Auchenipterus nuchalis	6	9	1.6997	0.1130	24 M; 17 F
Calophysus macropterus	21	25	2.9645	0.0122	13 M; 10 F
Cichla pleiozona	21	22	−2.0849	0.0433	9 M; 6 F
Curimata knerii	10	5	−1.6681	0.1192	172 M; 345 F
Hemiodus microlepis[a]	9	32	0.7717	0.4403	2,857 M; 800 F
Hemiodus unimaculatus	10	55	1.4454	0.1483	190 M; 34 F[a]
Hoplias malabaricus[a]	29	51	−0.8549	0.3952	53 M; 30 F
Hydrolycus scomberoides	22	33	−0.7106	0.4804	29 M; 19 F
Hypophthalmus marginatus	5	28	−1.0050	0.3227	237 M; 42 F
Jurengraulis juruensis	5	17	1.219	0.2369	77 M; 23 F
Leporinus friderici	15	21	0.0636	0.9497	30 M; 22 F
Mylossoma duriventre	91	43	0.6615	0.5094	253 M; 533 F
Nemadoras humeralis	18	25	3.4749	0.0012	166 M; 119 F
Oxydoras niger	7	6	0.4248	0.6792	6 M; 8 F
Pellona castelnaeana	56	86	0.2803	0.7796	2357 M; 1535 F
Pellona flavipinnis	8	7	−0.3633	0.7222	28 M; 32 F
Pimelodus aff. Blochii	39	42	−1.1239	0.2645	41 M; 39 F
Pinirampus pirinampu	36	56	2.3428	0.0213	76 M; 48 F
Plagioscion squamosissimus	99	153	1.2625	0.2079	2 M; 2 F
Potamorhina altamazonica	62	53	−0.6886	0.4925	12 M; 14 F
Potamorhina latior	26	51	−1.047	0.2983	8 M; 4 F
Prochilodus nigricans	22	22	1.219	0.2369	28 M; 28 F
Psectrogaster rutiloides	11	21	−0.2714	0.7879	12 M; 6 F
Pterodoras granulosus	10	18	0.3933	0.6973	8 M; 4 F
Pygocentrus nattereri	18	29	−0.2207	0.8263	164 M; 91 F
Rhaphiodon vulpinus	43	72	0.4672	0.6412	22 M; 13 F
Satanoperca sp.	6	6	−1.2703	0.2327	25 M; 25 F
Schizodon fasciatus	32	53	0.2801	0.7801	28 M; 17 F
Serrasalmus elongatus	9	12	−0.9243	0.3669	11 M; 6 F
Serrasalmus rhombeus	39	57	0.3178	0.7513	31 M; 22 F
Serrasalmus sp.n. robertsoni	8	16	−0.8816	0.3875	209 M; 104 F
Sorubim elongatus	10	12	0.0165	0.9870	8 M; 7 F
Triportheus albus	21	39	0.9822	0.3301	2953 M; 1588 F
Triportheus angulatus	47	99	−0.5040	0.6151	1427 M; 677 F
Triportheus auritus	15	17	−0.4598	0.6490	58 M; 52 F

n=number of observations.
[a] Mann–Whitney *U* test.

2.1. Statistical analysis

With this dataset (n=2,538; 1052 males and 1486 females) we analyzed differences between male and female within species and within trophic levels. We used the Kolmogorov–Smirnov one-sample test (to analyze the normality of all study data) and applied appropriate statistical analysis. Group means of THg according to the fish sex were compared using parametric (Student *t* test) and nonparametric (Mann–Whitney *U* test as an alternative to

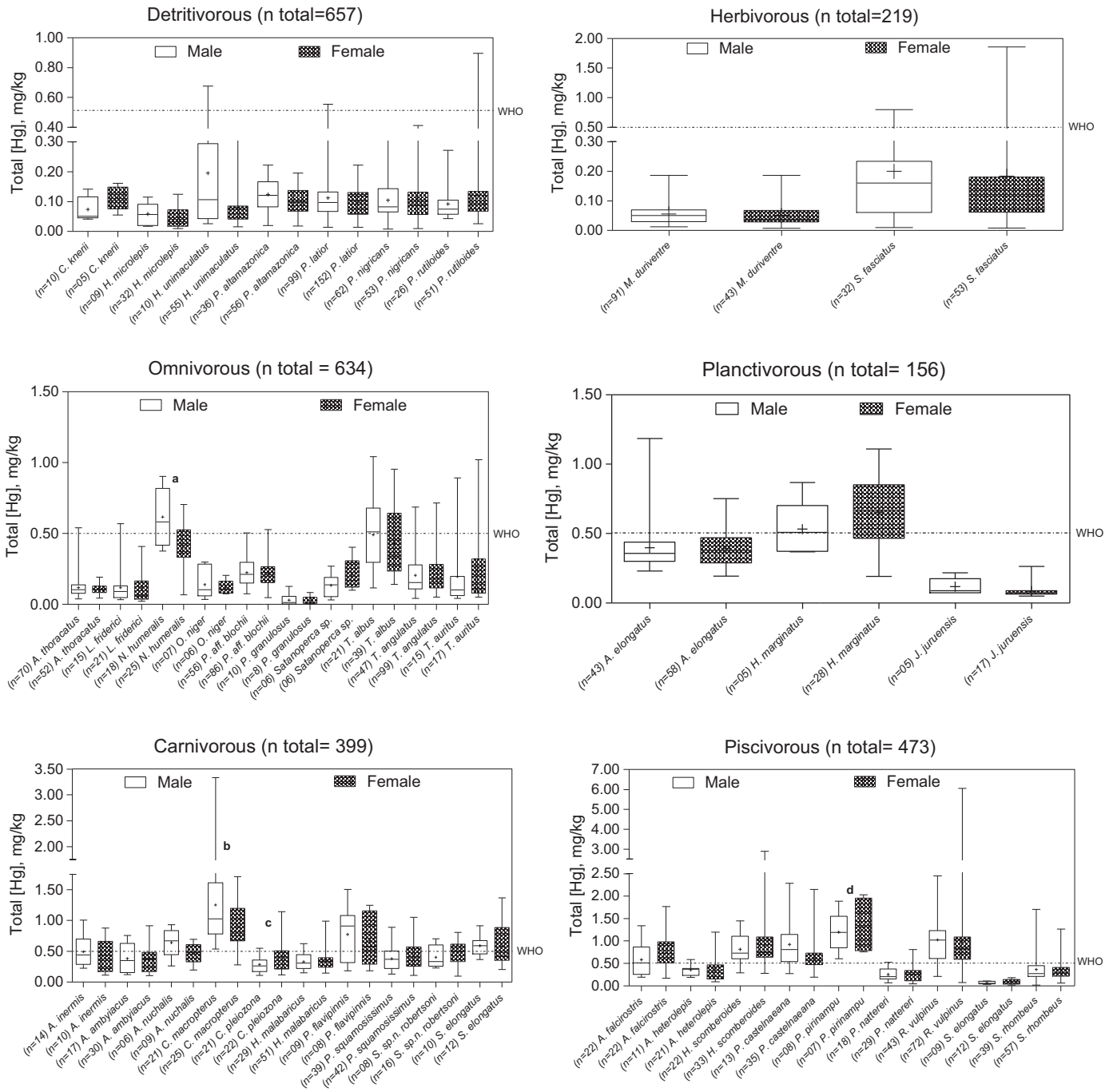

Fig. 1. Whisker-box plot representing medians and quartiles of THg in muscle of males and females by species according to trophic levels. N total= 2538, the mean is identified by +. Letter superscript represents level of statistical significance: $a=0.0012$; $b=0.0122$; $c=0.0433$; $d=0.0213$. The dotted line is the limit of WHO (1990).

independent samples) tests as required. The assumption variance equality was verified by Levene test; if conditions were not met, then we evaluated the mean differences between groups using alternatives to the *t*-test from heteroscesdastic variance (Zar, 2010). We used correlation analysis to study the association between THg fish concentrations and CF condition factors (within conventional trophic levels).

We followed the rationale of Gagnon and Hodson (2012) to estimate the approximate numbers of fish needed to identify statistical significance between sexes in order to maximize the chances of correctly identifying the number of specimens that were collected to evaluate Hg bioaccumulation in males and females. According to Gagnon and Hodson (2012), we assumed that sample size is a function of the degree of inter-sex differences between species and the variability of THg concentrations. Using THg concentrations from fish species of the Madeira River (Table 1), the number of fish required to detect an inter- male and female difference at $\alpha=0.05$ was calculated using the publicly available program G/Power3.1.3 (http://www.psycho.uni duesseldorf.de/abteilungen/aap/gpower3/). We used the following criteria: *t*-tests, mean difference between two independent groups, a priori compute required sample size – given a, power and effect size as suggested by Gagnon and Hodson (2012). Statistical methods used are outlined in Zar (2010).

To compare THg concentrations in males and females we used the means and SD of each species from the population statistics in

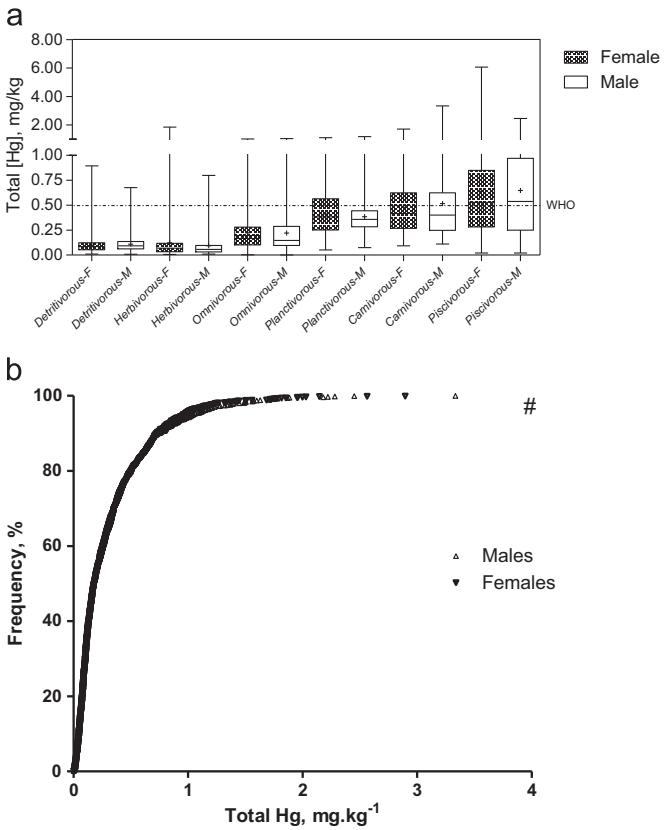

Fig. 2. Total Hg concentrations in male and female fish; (a) represents whisker-box plot (medians and quartiles; means are identified by +) according to trophic levels. (b) Shows frequency distribution of total Hg in all male and female studied fish; #=Female outlier, 6.0.

our data bank. In our case we needed to compare the means from the two groups, along with the average of each group to be compared, and we also needed to determine the effect size "*d*" of male and female fish. Calculations of minimum number of males and females were performed for powers of 0.80, corresponding respectively to 80% chance of obtaining a significant difference among groups at $1-\alpha=0.2$; this test is a factor influencing the minimum number of males and females, in the present case, in the collected sample. As discussed in Fairweather (1991), it is assumed, as in many environmental science studies, that the power should be at least $\alpha=0.80$, i.e., an 80% chance of detecting a difference in THg concentration between males and females.

All statistical analyses were performed using the software package XLStat version 2.03 (Addinsoft, 2010) and PRISM (version 4.0; San Diego, CA) software, which was also used for data summarization (in figures) and correlation analysis. We accepted a value of <0.05 as statistically significant.

3. Results

We studied THg concentrations as a function of sex in 2538 samples (males=1052, females=1486) of 41 species of fish of different trophic levels (detritivorous, herbivorous, omnivorous, planctivorous, carnivorous, and piscivorous). THg for both males and females of all species are illustrated in Fig. 1 by conventional classification. There was no clear pattern of sex differences in THg concentrations for 90% of the studied species; females showed higher THg than males only for *P. pirinampu* (fishbase trophic level 4.5 ± 0.80), and the opposite for the *C. macropterus* and *C. pleiozona* (fishbase trophic level 3.2 ± 0.47 and 4.2 ± 0.5, respectively), and *N. humeralis* (fishbase trophic level 2.8 ± 0.4) species.

Table 1 shows details of statistical analysis with actual and estimated minimum number of observations for species. The magnitudes of the inter-sex differences within each species were estimated; 11 of the 41 species studied, due to THg variability, showed sufficient numbers for relevant sex effects of the studied fish populations. Indeed, only two of the statistically significant species showed had a large enough number of specimens for a robust statistical comparison.

When analyzed by fish trophic levels, THg concentrations showed the expected hierarchical pattern of THg concentrations (Fig. 2a); detritivorous and herbivorous species showed the lowest while piscivorous showed the highest THg concentrations. However, there were no statistically significant differences between males and females within trophic levels. To further illustrate similarity between sexes in relation to muscle THg concentrations, Fig. 2b shows a frequency distribution for all fish samples; trends were indistinguishable between males and females. Overall, THg > 0.50 mg/kg (limit recommended by the FAO/WHO, 2007 for non-predatory fish) were mostly seen in carnivorous and piscivorous species.

Due to differences in shape and sizes of the 41 species, we analyzed THg as a function of the condition factor for all species. Fig. 3 illustrates the correlation analysis between fish CF and THg concentration by sex; in all cases the relationship was statistically significant and negative.

4. Discussion

Based on *a priori* understanding of THg bioaccumulation, sex can influence Hg metabolism in some species of fish but other unmeasured factors are stronger determinants of muscle-THg bioaccumulation. Overall, distribution frequency of THg concentration is indistinguishable between males and females. For the 88% of the studied species there was no difference between sexes, and for the few that showed significant difference there was no systematic dominance of male or female. Our results for sex related THg in Amazonian fish are in agreement with the collective tendency showed by published studies (Table 2). Recent isotope studies in walleye (*Sander vitreus*), confirm that sex-based differences in Hg concentrations are weak (Ofukany et al., 2015); which also concur with our recent work with Amazonian catfish (Bastos et al., 2015b). In the present work, we only had two species (*P. pirinampu and S. elongatus*) but only one (*P. pirinampu*) showed statistically higher Hg in female.

Sexual dimorphism may be linked to differences in muscle-Hg concentrations for some species (Lepak et al., 2012). Additionally, sex-related metabolic differences in growth and behavior may also affect Hg metabolism and bioaccumulation (Henderson et al., 2003; McClain et al., 2006; Rennie et al., 2008). The rate of THg bioaccumulation and condition factor indicated a significant relationship for some groups (Piscivorous, Carnivorous, and Omnivorous) of fish. Indeed, studying the slopes of the mercury and total length relationships in six species (*Sander vitreus; Pylodictis olivaris; Morone chrysops; Ictalurus punctatus; Cyprinus carpio; Carpiodes carpio; Dorosoma cepedianum*) of fish, McClain et al. (2006) showed a significant difference only for male and female walleye. However, for Canadian walleye populations, no such differences were reported (Ofukany et al., 2015).

Stacy and Lepak (2012) discussed the role of sexual dimorphism and behavior as determinant traits driving differences in THg bioaccumulation between males and females. In some apex species (like walleye), sexual size dimorphism can influence resource partitioning, resulting in male and female prey difference and accompanying diet composition. Dietary characteristics of prey

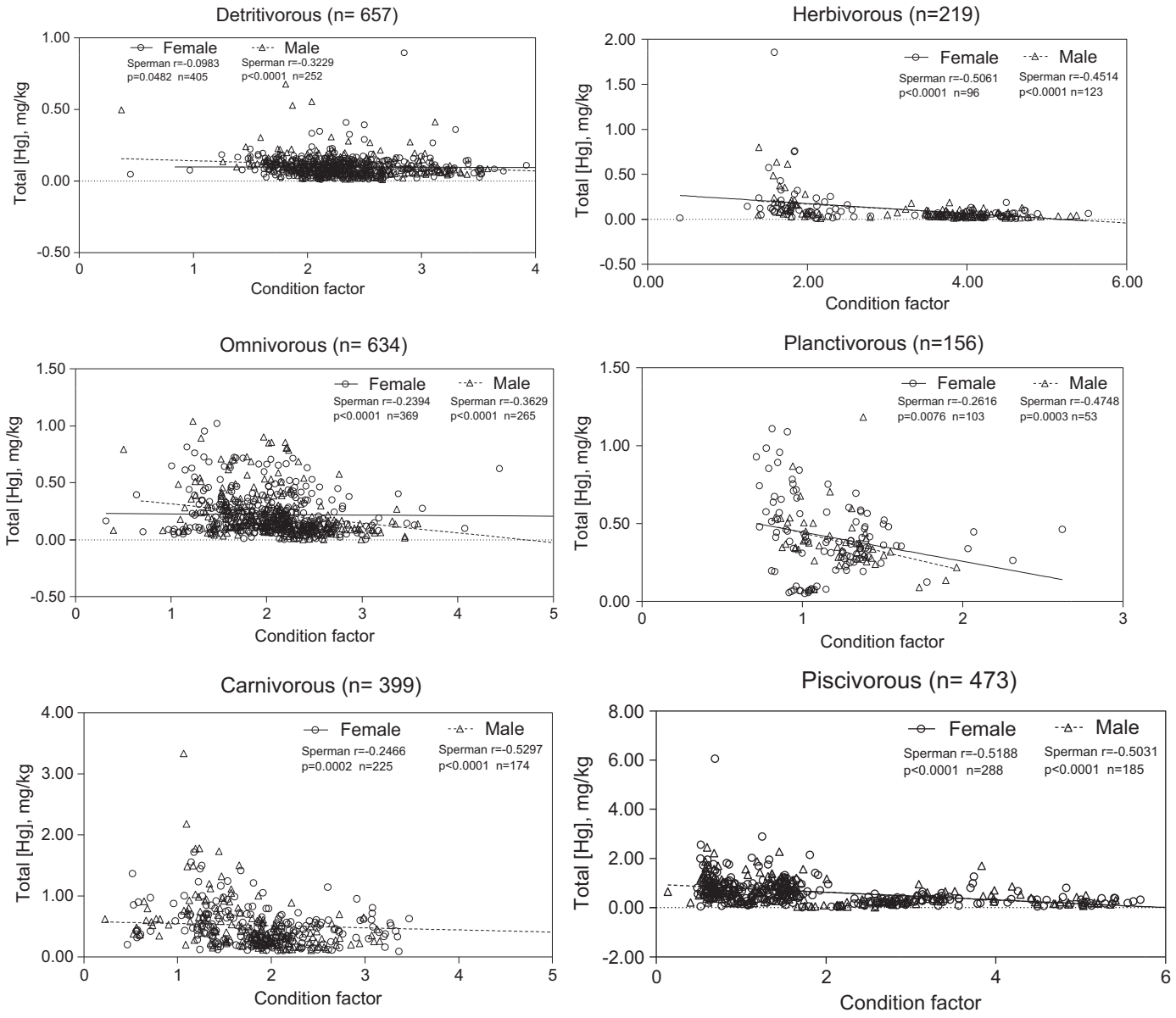

Fig. 3. Correlation analysis between total Hg concentration in muscle and condition factors (calculated as $CF_K=(W_{eight})/(L_{ength}^3)$) by trophic levels (solid lines integrate trends).

could explain the difference in THg between male and female walleyes (Stacy and Lepak, 2012). However, in different studies summarized in Table 2, walleye (*Sander vitreus*) showed opposite trends, i.e, males with higher (McClain et al., 2006) and lower (Visha et al., 2015) THg concentrations than females. Collectively, the studies summarized in Table 2 (39) done with few (1–5) species indicated that in most of them (29) there were no significant differences between males and females. In our study and in those summarized in Table 2, the few statistically significant sex-related differences favored either males or females depending on the species.

It is worth noting that we estimated the minimum number of samples (for each studied species) based on the variance of THg in males and in females (Table 1). Among the 41 species, the variance within each species was high for both males and females. This resulted in an insufficient sample size to detect mean difference between sexes. Such variability could be associated with biotic and abiotic factors (Wren, 1986), as well as random errors related to sampling, and analysis (Brower et al., 1997; Krebs, 1999). In wild fish, sampling variability is uncontrollable, and the variance may be such that the measured parameter may not be comparable between males and females. The post hoc evaluation indicated that we attained satisfactory sampling only for 11 of the 41 species (Table 1).

After applying the g-power test suggested by Gagnon and Hodson (2012), only two of the species (Table 1) achieved the minimum number of specimens for a meaningful statistical outcome. The use of the g-power test is not without criticism (Hoenig and Heisey, 2001). Therefore, to avoid overuse, future studies should judiciously establish its assumptions and interpret results with the necessary caution and within the limits of its application. The limited number of species (12%) showing sex differences in Hg concentrations, so far carries little biological significance. Nevertheless, neither the surveillance of environmental pollution nor the current food advisories based on muscle THg need to change because of fish sex.

Our study addressed condition factor and sex in a large number of specimens from a wide range of species. Additionally, the inherent difficulty it adequately assessed fish trophic levels (crucial

Table 2
Total mercury (THg) concentrations in muscle of several species of fish as a function of sex (M=males; F=females) in different studies indicating statistically significant (M > F; M < F) and non-significant (M/F) difference.

Scientific Name	*n*	Fishbase Trophic Level	M > F	M/F	M < F	References
Amazonian fish (41 species) [a]	2538	2.0 to 4.5	+	+	+	Current study
Sphyrna lewini	40	4.1 ± 0.5			+	Bergés-Tiznado et al. (2015)
B. filamentosum; B. platynemum; B. rousseauxii; B. vaillantii; P. hemiliopterus; P. punctifer; P. tigrinum; Z. zungaro	165	4.2 to 4.6			+	Bastos et al. (2015b)
Sander vitreus, Salvelinus namaycush	1023; 745	4.5; 4.3		+	+	Visha et al. (2015)
Lota lota	50	3.8 ± 0.2			+	Madenjian et al. (2015)
Esox lucius	6	4.1 ± 0.4				Madenjian et al. (2014a)
Petromyzon marinus	78	4.4 ± 0.8	+			Madenjian et al. (2014b)
Epinephelus diacanthus;Parastromateus niger; Alectis indicus	64; 63; 68	3.6; 3.8; 2.9		+		Abdolvand et al. (2014)
Hoplias malabaricus	43	4.5 ± 0.0		+		Mela et al. (2014)
Myoxocephalus scorpius	33	3.9 ± 0.0		+		Sonne et al. (2014)
Sander vitreus	26	4.5 ± 0.0	+			Stacy and Lepak (2012)
Platichtys flesus;	74	3.3 ± 0.2	+			Polak-Juszczak (2012)
Mullus barbatus	8	3.1 ± 0.1		+		Martinez-Gomez et al. (2012)
Aphanopus carbo Lowe, 1839	NG	4.5 ± 0.8		+		Maulvault et al. (2012)
Salvelinus namaycush	119	4.3 ± 0.5	+			Madenjian et al. (2011)
S. pilchardus; S. japonicus; T. trachurus	19;25;25	3.4 to 3.7		+		Vieira et al. (2011)
S. vitreus; O. tshawytscha; S. namaycush; E. lucius; M. dolomieu; C. clupeaformis; P. flavescens	887	3.2 to 4.5	+	+		Gewurtz et al. (2011)
Thunnus albacares	68	4.4 ± 0.4		+		Ordiano-Flores et al. (2011)
Prionace glauca	38	4.4 ± 0.2		+		Escobar Sanchez et al. (2011)
C. monoculus	27	3.9 ± 0.7		+		Rabitto et al. (2011)
E. baxteri, C. crepidater, D. calcea; S. mitsukurii	227	4.2 to 4.4		+		Pethybridge et al. (2010)
Scyliorhinus canicula	48	3.8 ± 0.3		+		Coelho et al. (2010)
Sphyrna zygaena	10	4.9 ± 0.5			+	Escobar Sanchez et al. (2010)
Coryphaena hippurus	385	4.4 ± 0.0		+		Adams (2009)
C. carpio; Capoeta sp	225	3.1; NG		+		Ebrahimi and Taherianfard (2010)
M. barbatus, M. ponticus, M. surmuletus	208	3.1 to 3.5		+		Harmelin-Vivien et al. (2009)
Galeocerdo cuvier	41	4.5 ± 0.0		+		Endo et al. (2008)
Micropterus dolomieu	45	3.6 ± 0.2			+	Murphy et al. (2007)
Prionace glauca, L. 1758; Xiphias gladius, L. 1758	64	4.4 ± 0.2		+		Branco et al. (2007)
	52	4.5 ± 0.2		+		
Morone americana	168	3.1 ± 0.3		+		Weis and Ashley (2007)
P. pychis, P. blennoides, P. americanus, C. conger, P. acarne, T. picturatus, L. caudatus M. Moro	295	3.3 to 4.3		+		Magalhães et al. (2007)
Micropterus salmoides	53	3.8 ± 0.4		+		Foster et al. (2000)
X. gladius; T. albacares; K pelamis;C. hippurus; A. solandri	69	4.3 to 4.5		+		Kojadinovic et al. (2006)
Sander vitreus	79	4.5 ± 0.0	+			McClain et al. (2006)
Acipenser transmontanus	57	3.3 ± 0.1		+		Webb et al. (2006)
Thunnus thynnus	14	4.5 ± 0.8		+		Licata et al. (2005)
Stizostedion vitreum vitreum	567	4.5 ± 0.0	+			Henderson et al. (2003)
M. canis	79	3.6 ± 0.2		+		Penedo de Pinho et al. (2002)
Salmo trutta L.	20	3.4 ± 0.1		+		Gutenmann and Lisk (1990)
Xiphias gladius L.	136	4.5 ± 0.2	+			Monteiro and Lopes (1990)
A rupestris; L. auritus; L. gibbosus; L. macrochirus	NG	3.2 to 3.4			+	Nicoletto and Hendricks (1988)
Mustelus antarcticus Guenther	153	4.5 ± 0.6	+			Walker (1976)

n=number of observations.
NG= Not given.
[a] M > F *n*=2; M < F *n*=3; M/F *n*=36.

factor for Hg bioaccumulation) was partly overcome by using fishbase classification. These are strength of the study. Here, we demonstrate the limited influence of sex on fish-Hg bioaccumulation. It was clear from our analysis that we may have had an insufficient number of samples. Furthermore, we were not able to assess stomach contents of the studied species, which constitutes another limitation of the study.

5. Conclusions

Sex is not the main determinant of muscle-Hg bioaccumulation, and does not seem to be more important for fish advisories than fish trophic level and size. However, because it occurs in some specific species, it remains a potential research topic in fish biology.

Acknowledgments

This work was partly supported by the National Research Council of Brazil-CNPq (Grants 552331/2011-2 and 458977/2014-4). The authors also gratefully acknowledge the support of the Laboratory of Ichthyology and Fishing Federal University of Rondônia and Santo Antônio Energia.

References

Abdolvand, S., Esfahani, S.K., Dmirchi., S., 2014. Mercury (Hg) and Methyl Mercury (MMHg) bioaccumulation in three species (sea food) from Persian Gulf. Toxicol. Environ. Health Sci. 6 (3), 192–198.

Adams, D.H., 2009. Consistently low mercury concentrations in dolphinfish, *Coryphaena hippurus*, an oceanic pelagic predator. Environ. Res. 109 (6), 697–701. http://dx.doi.org/10.1016/j.envres.2009.05.004.

ADDINSOFT, 2010. Xlstat for excel, version 2010.2.03. Addinsoft, New York.
Alvarez, M., del., C., Murphy, C.A., Rose, K.A., McCarthy, I.D., Fuiman, L.A., 2006. Maternal body burdens of methylmercury impair survival skills of offspring in Atlantic croaker (*Micropogonias undulatus*). Aquat. Toxicol. 80, 329–337. http://dx.doi.org/10.1016/j.aquatox.2006.09.010.
Baker, M.R., Schindler, D.E., Holtgrieve, G.W., St Louis, V.L., 2009. Bioaccumulation and transport of contaminants: migrating sockeye salmon as vectors of mercury. Environ. Sci. Technol. 43 (23), 8840–8846. http://dx.doi.org/10.1021/es901798f.
Bastos, W.R., Malm, O., Pfeiffer, W.C., Cleary, D., 1998. Establishment and analytical quality control of laboratories for Hg determination in biological and geological samples in the Amazon. Brasil. Ciênc. Cult. 50, 255–260.
Bastos, W.R., Dórea, J.G., Bernardi, J.V.E., Lautharte, L.C., Mussy, M.H., Lacerda, L.D., Malm, O., 2015a. Mercury in fish of the Madeira River (temporal and spatial assessment), Brazilian Amazon. Environ. Res. 140, 191–197. http://dx.doi.org/10.1016/j.envres.2015.03.029.
Bastos, W.R., Dórea, J.G., Bernardi, J.V.E., Lautharte, L.C., Mussy, M.H., Hauser, M., Dória, C.R.C., Malm, O., 2015b. Mercury in muscle and brain of catfish from the Madeira river, Amazon, Brazil. Ecotoxicol. Environ. Saf. 118, 90–97. http://dx.doi.org/10.1016/j.ecoenv.2015.04.015.
Bergés-Tiznado, M.E., Márquez-Farías, F., Lara-Mendoza, R.E., Torres-Rojas, Y.E., Galván-Magaña, F., Bojórquez-Leyva, H., Páez-Osuna, F., 2015. Mercury and selenium in muscle and target organs of Scalloped Hammerhead Sharks *Sphyrna lewini* of the SE Gulf of California: dietary intake, molar ratios, loads, and human health risks. Arch. Environ. Contam. Toxicol. . http://dx.doi.org/10.1007/s00244-015-0226-8, Sep 14. [Epub ahead of print]
Boettiger, C., Lang, D.T., Wainwright, P.C., 2012. Fishbase: exploring, manipulating and visualizing fishbase data from R. J. Fish. Biol. 81, 2030–2039.
Branco, V., Vale, C., Canário, J., Santos, M.N., 2007. Mercury and selenium in blue shark (Prionace glauca, L. 1758) and swordfish (Xiphias gladius, L. 1758) from two areas of the Atlantic Ocean. Environ. Pollut. 150, 373–380.
Brower, J.E., Zar, J.H., von Ende, C.N., 1997. Field and laboratory methods for general ecology. In: McGraw-Hill (Ed.), Field and Laboratory Methods for General Ecology, 4th Ed. , p. 273.
Burger, J., Gochfeld, M., Shukla, T., Jeitner, C., Burke, S., Donio, M., Shukla, S., Snigaroff, R., Snigaroff, D., Stamm, T., Volz, C., 2007. Heavy metals in Pacific cod (*Gadus macrocephalus*) from the Aleutians: location, age, size, and risk. J. Toxicol. Environ. Health A 70, 1897–1911. http://dx.doi.org/10.1080/15287390701551159.
Burger, J., 2007. A framework and methods for incorporating gender-related issues in wildlife risk assessment: gender-related differences in metal levels and other contaminants as a case study. Environ. Res. 104, 153–162. http://dx.doi.org/10.1016/j.envres.2006.08.001.
Coelho, J.P., Santos, H., Reis, A.T., Falcão, J., Rodrigues, E.T., Pereira, M.E., Duarte, A.C., Pardal, M.A., 2010. Mercury bioaccumulation in the spotted dogfish (*Scyliorhinus canicula*) from the Atlantic Ocean. Mar. Pollut. Bull. 60 (8), 1372–1375. http://dx.doi.org/10.1016/j.marpolbul.2010.05.008.
Ebrahimi, M., Taherianfard, M., 2010. Concentration of four heavy metals (cadmium, lead, mercury, and arsenic) in organs of two cyprinid fish (Cyprinus carpio and Capoeta sp.) from the Kor River (Iran). Environ. Monit. Assess. 168 (1–4), 575–585. http://dx.doi.org/10.1007/s10661-009-1135-y.
Endo, T., Hisamichi, Y., Haraguchi, K., Kato, Y., Ohta, C., Koga, N., 2008. Hg, Zn and Cu levels in the muscle and liver of tiger sharks (*Galeocerdo cuvier*) from the coast of Ishigaki Island, Japan: relationship between metal concentrations and body length. Mar. Pollut. Bull. 56, 1774–1780. http://dx.doi.org/10.1016/j.marpolbul.2008.06.003.
Escobar-Sánchez, O., Galván-Magaña, F., Rosíles-Martínez, R., 2010. Mercury and selenium bioaccumulation in the smooth hammerhead shark, *Sphyrna zygaena* Linnaeus, from the Mexican Pacific Ocean. Bull. Environ. Contam. Toxicol. 84 (4), 488–491. http://dx.doi.org/10.1007/s00128-010-9966-3.
Escobar-Sánchez, O., Galván-Magaña, F., Rosíles-Martínez, R., 2011. Biomagnification of mercury and selenium in blue shark *Prionace glauca* from the Pacific Ocean off Mexico. Biol. Trace Elem. Res. 144 (1–3), 550–559. http://dx.doi.org/10.1007/s12011-011-9040-y.
Fairweather, P.G., 1991. Statistical power and design requirements for environmental monitoring. Aust. J. Mar. Freshw. Res. 42, 555–567. http://dx.doi.org/10.1071/MF9910555.
FAO/WHO, 2007. Evaluation of certain food additives and contaminants. Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series 940. Available at: ⟨http://whqlibdoc.who.int/trs/WHO_TRS_940_eng.pdf⟩ Fishbase Information 2015. Accessed Sep 23 2015.
Foster, E.P., Drake, D.L., DiDomenico, G., 2000. Seasonal changes and tissue distribution of mercury in largemouth bass (*Micropterus salmoides*) from Dorena Reservoir, Oregon. Arch. Environ. Contam. Toxicol. 38, 78–82.
Gagnon, M.M., Hodson, P.V., 2012. Field studies using fish biomarker–how many fish are enough? Mar. Pollut. Bull. 64, 2871–2876. http://dx.doi.org/10.1016/j.marpolbul.2012.08.016.
Gewurtz, S.B., Bhavsar, S.P., Fletcher, R., 2011. Influence of fish size and sex on mercury/PCB concentration: importance for fish consumption advisories. Environ. Int. 37, 425–434. http://dx.doi.org/10.1016/j.envint.2010.11.005.
Gutenmann, W.H., Lisk, D.J., 1990. Higher average mercury concentration in fish fillets after skinning and fat removal. J. Food Saf. 11 (2), 99–103. http://dx.doi.org/10.1111/j.1745-4565.1990.tb00043.x.
Harmelin-Vivien, M., Cossa, D., Crochet, S., Banaru, D., Letourneur, Y., Mellon-Duval, C., 2009. Difference of mercury bioaccumulation in red mullets from the northwestern Mediterranean and Black seas. Mar. Pollut. Bull. 58, 679–685. http://dx.doi.org/10.1016/j.marpolbul.2009.01.004.
Henderson, B.A., Collins, N., Morgan, G.E., Vaillancourt, A., 2003. Sexual size dimorphism of walleye (*Stizostedion vitreum vitreum*). Can. J. Fish. Aquat. Sci. 60, 1345–1352. http://dx.doi.org/10.1139/f03-115.
Hirayama, K, Yasutake, A., 1986. Sex and age differences in mercury distribution and excretion in methylmercury-administered mice. J. Toxicol. Environ. Health 18, 49-60.
Hoenig, J.M., Heisey, D.M., 2001. The abuse of power: the pervasive fallacy of power calculations for data analysis. Am. Stat. 55, 19–24. http://dx.doi.org/10.1198/000313001300339897.
Kojadinovic, J., Potier, M., Le Corre, M., Cosson, R.P., Bustamante, P., 2006. Mercury content in commercial pelagic fish and its risk assessment in the Western Indian Ocean. Sci. Total Environ. 366 (2–3), 688–700. http://dx.doi.org/10.1016/j.scitotenv.2006.02.006.
Krebs, C.J., 1999. Ecology Methodology, 2nd Ed. Benjamin/Cummings, California, USA, p. 620.
Lepak, J.M., Hooten, M.B., Johnson, B.M., 2012. The influence of external subsidies on diet, growth and Hg concentrations of freshwater sport fish: implications for management and fish consumption advisories. Ecotoxicology 21, 1878–1888. http://dx.doi.org/10.1007/s10646-012-0921-4.
Li, D., Lou, Q., Zhai, G., Peng, X., Cheng, X., Dai, X., Zhuo, Z., Shang, G., Jin, X., Chen, X., Han, D., He, J., Yin, Z., 2014. Hyperplasia and cellularity changes in IGF-1-overexpressing skeletal muscle of crucian carp. Endocrinology 155 (6), 2199–2212. http://dx.doi.org/10.1210/en.2013-1938.
Licata, P., Trombetta, D., Cristani, M., Naccari, C., Martino, D., Calò, M., Naccari, F., 2005. Heavy metals in liver and muscle of bluefin tunA (*Thunnus thynnus*) caught in the Straits of Messina (Sicily, Italy). Environ. Monit. Assess. 107 (1–3), 239–248. http://dx.doi.org/10.1007/s10661-005-2382-1.
Lowe-McConnell, R.H., 1999. Estudos e cológicos de comunidades de peixes tropicais, São Paulo, EDUSP. 535p.
Madenjian, C.P., Keir, M.J., Whittle, D.M., 2011. Sexual difference in mercury concentrations of lake trout (Salvelinus namaycush) from Lake Ontario. Chemosphere 83 (7), 903–908. http://dx.doi.org/10.1016/j.chemosphere.2011.02.053.
Madenjian, C.P., Blanchfield, P.J., Hrenchuk, L.E., Van Walleghem, J.L., 2014a. Mercury elimination rates for adult northern pike Esox lucius: evidence for a sex effect. Bull. Environ. Contam. Toxicol. 93 (2), 144–148. http://dx.doi.org/10.1007/s00128-014-1256-z.
Madenjian, C.P., Johnson, N.S., Siefkes, M.J., Dettmers, J.M., Blum, J.D., Johnson, M. W., 2014b. Mercury accumulation in sea lamprey (Petromyzon marinus) from Lake Huron. Sci. Total Environ. 470–471, 1313–1319. http://dx.doi.org/10.1016/j.scitotenv.2013.10.081.
Madenjian, C.P., Stapanian, M.A., Cott, P.A., Krabbenhoft, D.P., Edwards, W.H., Ogilvie, L.M., Mychek-Londer, J.G., DeWild, J.F., 2015. Females exceed males in mercury concentrations of Burbot Lota lota. Arch. Environ. Contam. Toxicol. 68 (4), 678–688. http://dx.doi.org/10.1007/s00244-015-0131-1.
Magalhães, M.C., Costa, V., Menezes, G.M., Pinho, M.R., Santos, R.S., Monteiro, L.R., 2007. Intra- and inter-specific variability in total and methylmercury bioaccumulation by eight marine fish species from the Azores. Mar. Pollut. Bull. 54, 1654–1662. http://dx.doi.org/10.1016/j.marpolbul.2007.07.006.
Malm, O., Pfeiffer, W.C., Bastos, W.R., Souza, C.M.M., 1989. Utilização do Acessório de Geração de Vapor Frio para Análise de Mercúrio em Investigações Ambientais por Espectrofotometria de Absorção Atômica. Ciên e Cult. 41, 88–92.
Martinez-Gomez, C., Fernandez, B., Benedicto, J., Valdes, J., Campillo, J.A., Leon, V.M., Vethaak, A.D., 2012. Health status of red mullets from polluted areas of the Spanish Mediterranean coast, with special reference to Portman (SE Spain). Mar. Environ. Res. 77, 50–59. http://dx.doi.org/10.1016/j.marenvres.2012.02.002.
Maulvault, A.L., Anacleto, P., Machado, R., Amaral, A., Carvalho, M.L., Lourenco, H.M., Nunesa, M.L., Marques, A., 2012. Effect of sex, maturation stage and cooking methods on the nutritional quality and safety of black scabbard fish (*Aphanopus carbo* Lowe, 1839). J. Sci. Food Agric. 92, 1545–1553. http://dx.doi.org/10.1002/jsfa.4741.
McClain, W.C., Chumchal, M.M., Drenner, R.W., Newland, L.W., 2006. Mercury concentrations in fish from Lake Meredith, Texas: implications for the issuance of fish consumption advisories. Environ. Monit. Assess. 123 (1–3), 249–258. http://dx.doi.org/10.1007/s10661-006-9194-9.
Mela, M., Neto, F.F., Yamamoto, F.Y., Almeida, R., Grötzner, S.R., Ventura, D.F., de Oliveira Ribeiro, C.A., 2014. Mercury distribution in target organs and biochemical responses after subchronic and trophic exposure to neotropical fish *Hoplias malabaricus*. Fish. Physiol. Biochem. 40, 245–256. http://dx.doi.org/10.1007/s10695-1013.
Monteiro, L.R., Lopes, H.D., 1990. Mercury content of swordfish, *Xiphias gladius*, in relation to length, weight, age, and sex. Mar. Pollut. Bull. 21 (6), 293–296. http://dx.doi.org/10.1016/0025-326X(90)90593-W.
Murphy, G.W., Newcomb, T.J., Orth, D.J., 2007. Sexual and seasonal variations of mercury in small mouth bass. J. Freshw. Ecol. 22, 135–143. http://dx.doi.org/10.1080/02705060.2007.9664153.
Nicoletto, P.F., Hendricks, A.C., 1988. Sexual differences in accumulation of mercury in four species of centrarchid fishes. Can. J. Zool. 66 (4), 944–949. http://dx.doi.org/10.1139/z88-140.
Nikolsky, G.V., 1963. The Ecology of Fishes. Academic, Nova York, p. 352.
Ofukany, A.F., Hobson, K.A., Wassenaar, L.I., Bond, A.L., 2015. The efficacy of scale sampling for monitoring trace element concentrations and stable isotopes in commercially harvested walleye (*Sander vitreus*). Isot. Environ. Health Stud. 26, 1–13. http://dx.doi.org/10.1080/10256016.2015.1023797.

Ordiano-Flores, A., Galván-Magaña, F., Rosiles-Martínez, R., 2011. Bioaccumulation of mercury in muscle tissue of yellowfin tuna, *Thunnus albacares*, of the eastern Pacific Ocean. Biol. Trace Elem. Res. 144 (1–3), 606–620. http://dx.doi.org/10.1007/s12011-011-9136-4.

Penedo de Pinho, A., Guimarães, J.R.D., Martins, A.S., Costa, P.A., Olavo, G., Valentin, J., 2002. Total mercury in muscle tissue of five shark species from Brazilian offshore waters: effects of feeding habit, sex, and length. Environ. Res. 89 (3), 250–258. http://dx.doi.org/10.1006/enrs.2002.4365.

Pethybridge, H., Cossa, D., Butler, E.C., 2010. Mercury in 16 demersal sharks from southeast Australia: biotic and abiotic sources of variation and consumer health implications. Mar. Environ. Res. 69 (1), 18–26. http://dx.doi.org/10.1016/j.marenvres.2009.07.006.

Polak-Juszczak, L., 2012. Bioaccumulation of mercury in the trophic chain of flatfish from the Baltic Sea. Chemosphere 89, 585–591. http://dx.doi.org/10.1016/j.chemosphere.2012.05.057.

Rabitto, I.S., Bastos, W.R., Almeida, R., Anjos, A., de Holanda, I.B.B., Galvão, R.C.F., Neto, F.F., de Menezes, M.L., Dos Santos, C.A., de Oliveira Ribeiro, C.A., 2011. Mercury and DDT exposure risk to fish-eating human populations in Amazon. Environ. Int. 37 (1), 56–65. http://dx.doi.org/10.1016/j.envint.2010.07.001.

Rennie, M.D., Purchase, C.F., Lester, N., Collins, N.C., Shuter, B.J., Abrams, P.A., 2008. Lazy males? Bioenergetic differences in energy acquisition and metabolism help to explain sexual size dimorphism in percids. J. Anim. Ecol. 77 (5), 916–926. http://dx.doi.org/10.1111/j.1365-2656.2008.01412.x.

Rhea, T.D., Farag, A.M., Harper, D.D., McConnell, E., Brumbaugh, W.G., 2013. Mercury and selenium concentrations in biofilm, macroinvertebrates, and fish collected in the Zankee Fork of the Salmon River, Idaho, USA, and their potential effects on fish health. Arch. Environ. Contam. Toxicol. 64 (1), 130–139. http://dx.doi.org/10.1007/s00244-012-9816-x.

Santos, G.M., Ferreira, E., Zuanon, J., 2006. Peixes Comerciais de Manaus. Manaus: Ibama-AM.

Sonne, C., Bach, L., Søndergaard, J., Rigét, F.F., Dietz, R., Mosbech, A., Leifsson, P.S., Gustavson, K., 2014. Evaluation of the use of common sculpin (*Myoxocephalus scorpius*) organ histology as bioindicator for element exposure in the fjord of the mining area Maarmorilik, West Greenland. Environ. Res. 133, 304–311. http://dx.doi.org/10.1016/j.envres.2014.05.031.

Squadrone, S., Benedetto, A., Brizio, P., Prearo, M., Abete, M.C., 2015. Mercury and selenium in European catfish (*Silurus glanis*) from Northern Italian Rivers: Can molar ratio be a predictive factor for mercury toxicity in a top predator? Chemosphere 119, 24–30. http://dx.doi.org/10.1016/j.chemosphere.2014.05.052.

Thomas, D.J., Fisher, H.L., Sumler, M.R., Marcus, A.H., Mushak, P., Hall, L.L., 1986. Sexual differences in the distribution and retention of organic and inorganic mercury in methyl mercury-treated rats. Environ. Res. 41, 219–234.

Stacy, W.L., Lepak, J.M., 2012. Relative influence of prey mercury concentration, prey energy density and predator sex on sport fish mercury concentrations. Sci. Total Environ. 437, 104–109. http://dx.doi.org/10.1016/j.scitotenv.2012.07.064.

Vazzoler, A.E.A.M., 1996. Biologia da reprodução de peixes teleósteos: teoria e prática. Maringá: EDUEM, p. 169.

Vieira, C., Morais, S., Ramos, S., Delerue-Matos, C., Oliveira, M.B., 2011. Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: intra- and inter-specific variability and human health risks for consumption. Food Chem. Toxicol. 49, 923–932. http://dx.doi.org/10.1016/j.fct.2010.12.016.

Visha, A., Gandhi, N., Bhavsar, S.P., Arhonditsis, G.B., 2015. A Bayesian assessment of the mercury and PCB temporal trends in lake trout (*Salvelinus namaycush*) and walleye (*Sander vitreus*) from lake Ontario, Ontario, Canada. Ecotoxicol. Environ. Saf. 117, 174–186. http://dx.doi.org/10.1016/j.ecoenv.2015.03.022.

Walker, T.I., 1976. Effects of species, sex, length and locality on the mercury content of school shark *Mustelus antarticus* (Guenther) from south-eastern Australian waters. Aust. J. Mar. Freshw. Res. 27, 603–616.

Webb, M.A., Feist, G.W., Fitzpatrick, M.S., Foster, E.P., Schreck, C.B., Plumlee, M., Wong, C., Gundersen, D.T., 2006. Mercury concentrations in gonad, liver, and muscle of white sturgeon *Acipenser transmontanus* in the lower Columbia River. Arch. Environ. Contam. Toxicol. 50 (3), 443–451. http://dx.doi.org/10.1007/s00244-004-0159-0.

Weis, P., Ashley, J.T., 2007. Contaminants in fish of the Hackensack Meadowlands, New Jersey: size, sex, and seasonal relationships as related to health risks. Arch. Environ. Contam. Toxicol. 52 (1), 80–89. http://dx.doi.org/10.1007/s00244-006-0093-4.

WHO (World Health Organization), 1990. Environmental Health Criteria. Methylmercury vol. 101. WHO/IPCS, Geneva, Switzerland.

Willacker, J.J., von Hippel, F.A., Ackerly, K.L., O'Hara, T.M., 2013. Habitat-specific foraging and sex determine mercury concentrations in sympatric benthic and limnetic ecotypes of threespine stickleback. Environ. Toxicol. Chem. 32 (7), 1623–1630. http://dx.doi.org/10.1002/etc.2213.

Wren, C.D., 1986. A review of metal accumulation and toxicity in wild mammals I. Mercury. Environ. Res. 40, 210–244.

Zar, J.H., 2010. Biostatistical Analysis. Northern Illinois University, p. 960p. 5/E.