



Mercury bioaccumulation patterns in deep-sea fishes as indicators of pollution scenarios in the northern Pacific of Mexico

Edgar Cruz-Acevedo^a, Miguel Betancourt-Lozano^a, Dana Isela Arizmendi-Rodríguez^b, Hugo Aguirre-Villaseñor^c, Daniela Aguilera-Márquez^d, Jaqueline García-Hernández^{d,*}

^a Centro de Investigación en Alimentación y Desarrollo A.C., CIAD-Mazatlán Unit, Avenida Sábalo-Cerritos S/N, Mazatlán 82112, Sinaloa, Mexico

^b Instituto Nacional de Pesca y Acuicultura, Centro Regional de Investigación Pesquera-Guaymas, Miguel Alemán Sur 608, Col. La Cantera 85400, Guaymas, Sonora, Mexico

^c Instituto Nacional de Pesca y Acuicultura, Centro Regional de Investigación Pesquera-Mazatlán, Calzada Sábalo-Cerritos S/N, Mazatlán 1177, Sinaloa, Mexico

^d Centro de Investigación en Alimentación y Desarrollo A.C., CIAD-Guaymas Unit, Carretera al Varadero Nacional km 6.6, Col. Las Playitas, Guaymas, Sonora 85480, Mexico

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ABSTRACT

Mercury (Hg) bioaccumulation in deep-sea fauna (> 200 m depth) of the northern Mexican Pacific is unknown. We measured the total Hg concentration (THg $\mu\text{g/g}$ wet weight) in 18 fish species, caught between 90 and 1100 m depth, in two ecoregions of the Mexican Pacific: The Gulf of California (GC) and the western coast of Baja California (BC), in the Southern Californian Pacific. We assessed spatial (ecoregion and depth) and biological (species, tissues, trophic position, sex and size) patterns of bioaccumulation. The highest THg concentrations were observed in liver (geometric mean: $1.28 \pm 2.31 \mu\text{g/g}$, range: < Method Detection Limit (MDL)– $16.17 \mu\text{g/g}$), followed by muscle (geometric mean: $0.34 \pm 0.53 \mu\text{g/g}$, range: < MDL– $6.12 \mu\text{g/g}$) and gonad (geometric mean: $0.29 \pm 0.49 \mu\text{g/g}$, range: < MDL– $2.18 \mu\text{g/g}$). The maximum limit established for human consumption ($1 \mu\text{g/g}$ wet weight) was exceeded by 7.8% of the muscle samples and 31% exceeded the protection threshold for possible harmful effects in fish ($0.2 \mu\text{g/g}$ wet weight). We observed that THg concentrations tended to increase at higher trophic levels, total lengths and condition factors. Our results indicate a clear differentiation of the mercury bioaccumulation patterns between GC and BC, where GC species (200–500 m) had consistently higher liver/muscle concentration ratios than those from BC (600–1100 m). Altogether, the findings presented in this study are possible evidence of differential scenarios of environmental contamination.

1. Introduction

Due to its toxicity and high availability in the environment, mercury (Hg) is the most relevant pollutant in the oceans (Boening, 2000; Batrakova et al., 2014). Its distribution in the sea is governed by different biogeochemical processes, adsorption by particles and oceanographic processes at different scales, meanwhile factors such as trophic position, sex, age (size) and reproductive stage may influence the bioaccumulation of this metal in organisms (Burger et al., 2007; Hinck et al., 2007). These factors can determine temporal and spatial patterns in the concentrations of Hg in both, environment and biota (Burger et al., 2007; Batrakova et al., 2014).

More than 75% of Hg that enters the marine environment comes from the atmosphere, in inorganic form: Elemental (Hg^0), divalent (Hg^{2+}) and particulate (Hg^p), and to a lesser extent as methylmercury,

MeHg (CH_3Hg^+), its most toxic and bioaccumulative form (Sunderland and Mason, 2007; Batrakova et al., 2014). MeHg constitutes up to more than 90% of the total Hg in individuals (Harris, 2003; Alpers et al., 2005).

Up to 70% of the world total Hg is deposited in the deep-sea (> 200 m depth) (Booth and Zeller, 2005; Batrakova et al., 2014). Most of the inorganic Hg transformation to MeHg occurs in environments with low concentrations of oxygen, such as sediment and the mesopelagic zone, driven by sulfate-reducing bacteria (Boening, 2000; Sunderland and Mason, 2007; Sunderland et al., 2009). Due to this biotransformation, MeHg concentrations in water, sediments and deep-sea biota are generally higher than in shallow waters (Boening, 2000; Chiu and Mok, 2011; Batrakova et al., 2014).

Most contaminants are bioaccumulated in large quantities in the liver, the main detoxification organ; however, due to its affinity to thiol

* Corresponding author.

E-mail address: jaqueline@ciad.mx (J. García-Hernández).

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(-SH), a structural muscle compound, Hg tends to bioaccumulate in muscle too (Boening, 2000; Booth and Zeller, 2005). In fishes, concentrations above 5 µg/g wet weight in muscle are considered very high and an indication of polluted sites (Scheuhammer et al., 2012). However, concentrations below this limit have been related to adverse effects in growth, behavior and reproduction in different fish species (0.25–1.2 µg/g wet weight of whole body); a concentration of 0.2 µg/g whole body concentration has been suggested as a protection limit for juvenile and adult fish (Beckvar et al., 2005 and references therein). For human consumption, the maximum allowed limits in fish muscle established in the international law vary, although, they are generally established between 0.3 and 1 µg/g wet weight (UNEP, 2002; DOF, 2009a; Health Canada, 2017).

The monitoring of Hg contamination in deep-sea species has focused mainly on commercially important fisheries (Mormede and Davies, 2001; Pethybridge et al., 2010; Chiu and Mok, 2011). In some of these species, Hg has been observed at higher concentrations than in shallow water species of similar habits, exceeding permissible limits for human consumption (Mormede and Davies, 2001; Martins et al., 2006; Chiu and Mok, 2011).

Different studies report relatively high concentrations of Hg in shallow water fishes of coastal and pelagic environments from the Mexican Pacific, mainly in the northwest region (Ruelas-Inzunza and Páez-Osuna, 2005; García-Hernández et al., 2007, 2018; Ruelas-Inzunza et al., 2011, 2013; Zamora-Arellano et al., 2017). However, the deep-sea of the Mexican Pacific is a poorly explored environment until recent times (Hendrickx, 2012; Cruz-Acevedo et al., 2018), and Hg bioaccumulation patterns in its fauna are unknown. In the context of the Minamata agreement, the identification of contaminated sites (Article 12) and the monitoring of pollution trends in the different environmental and biotic compartments (Article 19; UN, 2017) are relevant objectives. The objective of this work is to present the first evaluation of contamination by Hg in deep-sea fishes in the northwest Mexican Pacific, exploring possible patterns of bioaccumulation between species, tissue, trophic levels and sizes and assessing the relation between total Hg concentrations, depth gradient and geographic regions.

2. Material and methods

2.1. Sampling of specimens

Eighteen fish species were caught in two marine ecoregions (Wilkinson et al., 2009) of the northwest Pacific of Mexico (Table 1, Fig. 1): 14 species from the lower continental shelf and upper slope of the northern-central Gulf of California (GC: 90–850 m depth) and four species from the upper/middle continental slope of the northern-central portion of the western coast of Baja California (BC: 600–1100 m depth), in the Southern Californian Pacific.

The species from BC were caught on board the oceanographic vessel “El Puma”, during TALUD (Hendrickx, 2012; Cruz-Acevedo et al., 2018), a research project focused on exploring the biodiversity of the continental slope of the Mexican Pacific (cruise: TALUD XVI-B, extreme latitudes: 28°47'N–31°46'N, depth interval: 610–1100 m). TALUD used a benthic sledge (2.35 m wide, 0.90 m high) equipped with a collecting net of about 5.5 cm stretched mesh size (Cruz-Acevedo et al., 2017, 2018).

The species from GC were caught during the TALUD XIV cruise (extreme latitudes: 28°11'N–29°04'N, depth interval: 181–847 m) and during four consecutive years of sampling (commercial fishing and fisheries prospections from the Instituto Nacional de Pesca y Acuicultura) in the Pacific hake (*Merluccius productus*) fishing zone (2014, 2015, 2016 and 2017; extreme latitudes: 28°03'N–30°28'N, depth interval: 92–462 m). The fishes were identified to species level and frozen at –20 °C until dissected. For the comparison of the Pacific hake Hg concentrations, we incorporated 14 specimens of this species

from the Montereyan Pacific Transition (MPT: extreme latitudes: 34°26'N–37°25'N, depth interval: 248–483 m).

In the laboratory, total length (TL) and total weight (TW) of the specimens were measured. Later, their organs were extracted from the abdominal cavity to obtain the eviscerated weight (EW), with the exception of some specimens from the hake fishery that were dissected on board (30% of the individuals) and were not weighed without guts. The liver, gonad and part from the fish's muscle were also individually weighed, homogenized and frozen to –20 °C until the Hg analysis.

2.2. Determination of THg concentrations

Samples were weighed (precision 0.01 g), lyophilized during 72 h (LABCONCO Lyophilizer 79340–00, pressure: 133×10^{-3} mBar, temperature: –40 °C), weighed again and grounded in a ceramic mortar. Percent humidity was obtained for each sample using the difference in the sample weights. Of each type of lyophilized tissue -gonad, liver and muscle- aliquots of 0.01 g, 0.01 g and 0.25 g were taken, respectively. Samples were acid digested in two stages, the first, with 50% nitric acid, and the second with 30% hydrogen peroxide in a Microwave digestion system CEM Corp. Mod. MARS_X with three pressure and temperature ramps (65 PSI–100 °C, 100 PSI–120 °C, 140 PSI–140 °C), following the EPA method 3052 (Tobergte and Curtis, 2013).

Total mercury concentrations (THg) were obtained using anodic stripping voltammetry (ASV) at the rotating gold electrode, with a 797 VA Computrace instrument (Metrohm AG • Ionenstrasse 9100 Herisau, Switzerland) using the Application Bulletin 96/5 e method (Metrohm, 2014). The ASV is an EPA approved methodology (EPA, 1996) comparable with spectrophotometric methods for the analysis of mercury in fish tissues (Augelli et al., 2007; Giacomino et al., 2017). Voltammetric parameters used are presented in Table A.1.

For quality control/quality assurance (QA/QC) purposes, a blank, a duplicate and a certified reference material (DOLT-4, Dogfish Liver Certified Reference Material for Trace Metals, NRCC) were included in the digestion of samples. Using the percent humidity for each sample, concentrations were transformed from dry weight to wet weight and are expressed in µg/g. The method performance variables obtained from the analyses of fish samples are presented in Table A.2. The Method Detection Limit (MDL = 0.002 µg/g) was calculated according to Helsel (2012).

2.3. Data analysis

The condition factor (K), which relates the weight and size of each individual to estimate indirectly its health condition (Froese, 2006), was calculated using the following formula: $K = EW/TL^b$, where “K” is the condition factor, “EW” is the eviscerated weight, TL is the total length and “b” is the slope of the length-weight relation of the species. As the information regarding the trophic position of deep-sea fishes is scarce, the average trophic level of some species was assigned from the data of the closest species available in the literature (see Livingston and Bailey, 1985; Hoff et al., 2000; Nelson, 2006; FishBase, 2017).

Due to the incidence of censored data (14% of the total samples), the nonparametric Kaplan-Meier estimator was used (function “cenfit” from “NADA” package in R 3.4.2) to calculate the basic statistic of THg concentrations in the species. This method is recommended for databases with less than 50% of observations below the MDL (Helsel, 2012; R Core Team, 2017). Statistical comparisons of THg concentrations were performed only for the species with at least five measurements per tissue. All statistical analysis were made using the R 3.4.2 software, considering the significance of $\alpha = 0.05$ (R Core Team, 2017).

The comparisons of THg concentrations between species and between trophic levels were made using the “Censored Regressions using Maximum Likelihood Estimation” (CENMLE; function “cenmle” from “NADA” package; Helsel, 2012). The CENMLE method allows testing hypotheses similar to the analysis of variance when the database has

Table 1

Total mercury concentrations (THg µg/g wet weight) in tissues of deep-sea fishes of northern Mexico and hake of Central California, in the Eastern Pacific. Data showed: geometric mean ± standard deviation, parenthesis: total samples/censored samples (Helsel, 2012).

Species	Acronym	Depth (m) Min-max	n	Trophic level	Gonad (THg)	Liver (THg)	Muscle(THg)
Gulf of California							
<i>Hyporthodus acanthistius</i>	Hpa	223 – 256	3	3.9	–	–	0.772, 1.033 (3/1)
<i>Brotula clarkae</i>	Brc	216 – 251	3	4.1	–	–	0.570 ± 0.721 (3/0)
<i>Lepophidium negropinna</i>	Len	216 – 256	14	3.8	–	0.985 (4/3)	0.490 ± 0.531 (14/3)
<i>Merluccius productus</i>	Mep	92 – 462	165	4.4	0.239 ± 0.439 (26/2)	1.641 ± 2.632 (143/3)	0.135 ± 0.243 (165/48)
<i>Mustelus henlei</i>	Mus	204 – 296	7	3.6	–	1.194 ± 0.451 (7/2)	ND (7)
<i>Hydrolagus colliei</i>	Hyc	243 – 462	30	3.7	1.136 ± 0.755 (3/0)	0.786 ± 1.959 (26/3)	0.749 ± 0.708 (30/4)
<i>Symphurus oligomerus</i>	Syo	181 – 414	20	3.3	–	–	0.305 ± 0.130 (20/0)
<i>Galeus piperatus</i>	Gap	234 – 462	6	3.8	–	1.004 ± 0.059 (3/0)	0.511 ± 0.374 (6/1)
<i>Coelorinchus scaphopsis</i>	Coe	260 – 414	26	3.6	–	7.439 (1/0)	0.487 ± 0.708 (26/1)
<i>Cherublemma emmelas</i>	Che	327 – 443	7	3.6	–	–	0.314 ± 0.083 (7/0)
<i>Physiculus rastrelliger</i>	Phn	181 – 443	7	3.4	0.149 ± 0.049 (3/0)	–	0.274 ± 0.065 (7/0)
<i>Sebastes sinensis</i>	Ses	385 – 443	9	3.5	–	–	0.642 ± 0.375 (9/1)
<i>Eptatretus stoutii</i>	Eps	414	2	4.2	–	–	1.516, 1.097 (2/0)
<i>Cephalurus cephalus</i>	Cec	847	12	3.7	–	–	0.434 ± 0.254 (12/0)
Western coast off Baja California							
<i>Nezumia stelgidolepis</i>	Nes	760	14	4.4	0.163 ± 0.072 (4/1)	0.332 ± 0.255 (10/0)	1.072 ± 1.489 (14/0)
<i>Nezumia liolepis</i>	Nel	611 – 1101	29	3.3	–	0.166 ± 0.222 (9/1)	0.423 ± 0.263 (29/0)
<i>Sebastobolus altivelis</i>	Seb	611 – 1101	19	3.3	–	0.071 ± 0.051 (14/2)	0.466 ± 0.482 (19/0)
<i>Microstomus pacificus</i>	Mic	705 – 1101	7	3.2	–	0.574, 0.531 (2)	0.454 ± 0.473 (7/0)
Central California							
<i>Merluccius productus</i>	Mep	249–483	14	4.4	–	1.398 ± 1.281 (9/1)	0.428, 0.947 (14/12)
Number of samples					36	228	394

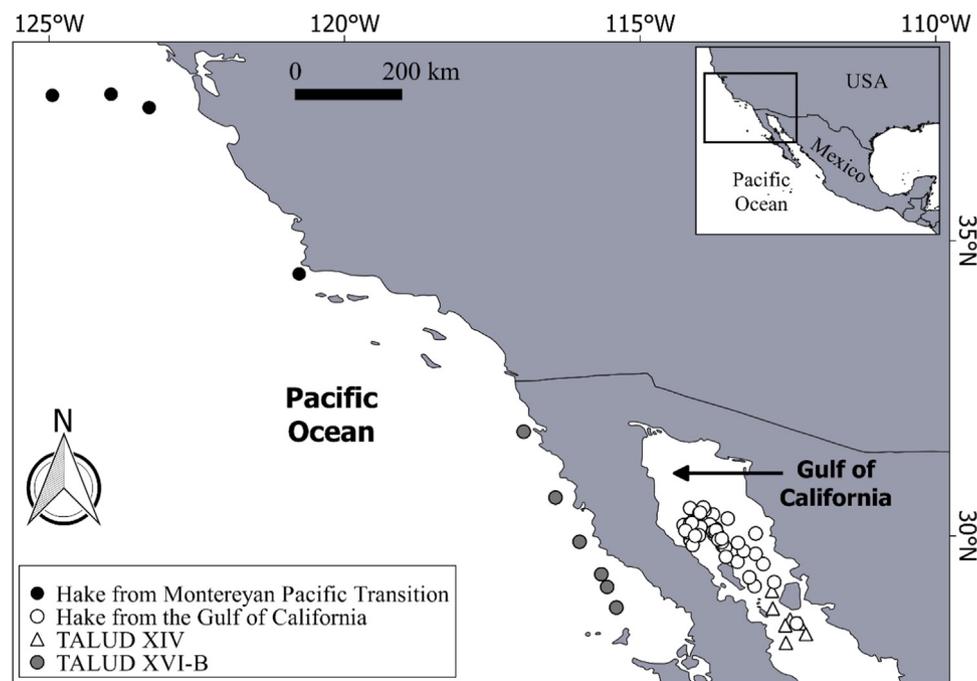


Fig. 1. Sampling sites for the quantification of total mercury (THg) in deep-sea fishes of northern Mexico and Central California, in the Eastern Pacific.

censored data, reporting a Chi-square analysis (Helsel, 2012). The CENMLE analysis was performed for liver and muscle, respectively, excluding the gonad from the analysis, due to the small number of samples of this tissue (Table 1). The species with significant differences were identified by pair-wise comparisons, using the CENMLE estimator. To reduce the probability of reporting false positives, *p-values* were adjusted by the “False Discovery Rate” (FDR), using the “fdrtool” package (Benjamini and Hochberg, 1995; Strimmer, 2008).

The comparisons within species were made for those with THg concentrations measured in two or more tissues (*M. productus*, *Hydrolagus colliei*, *Nezumia liolepis*, *Nezumia stelgidolepis* and *Sebastobolus altivelis*). Due to the presence of censored data, THg concentrations in *M. productus*, *H. colliei*, *N. liolepis* and *S. altivelis*, were compared by

CENMLE analysis (Helsel, 2012). For *M. productus* in GC, the inter-annual variation of THg concentrations between sexes and tissues was compared using two independent comparisons (CENMLE does not evaluate more than one explanatory variable at a time), grouping the factors as follows: year-tissue and year-sex. Later, within each year of capture, THg concentrations were compared using the combination of sex-tissue as an explanatory variable. To compare THg concentrations in hake from different ecoregions (GC and MPT in 2017) CENMLE analyses were performed, evaluating the effect of the site and the combination site-tissue. The sex was not considered in the analysis because of the reduced number of females and immature organisms from MPT (Table 1). The comparisons to *H. colliei* were performed between the sex-tissue groups. Because *N. liolepis* and *S. altivelis* were

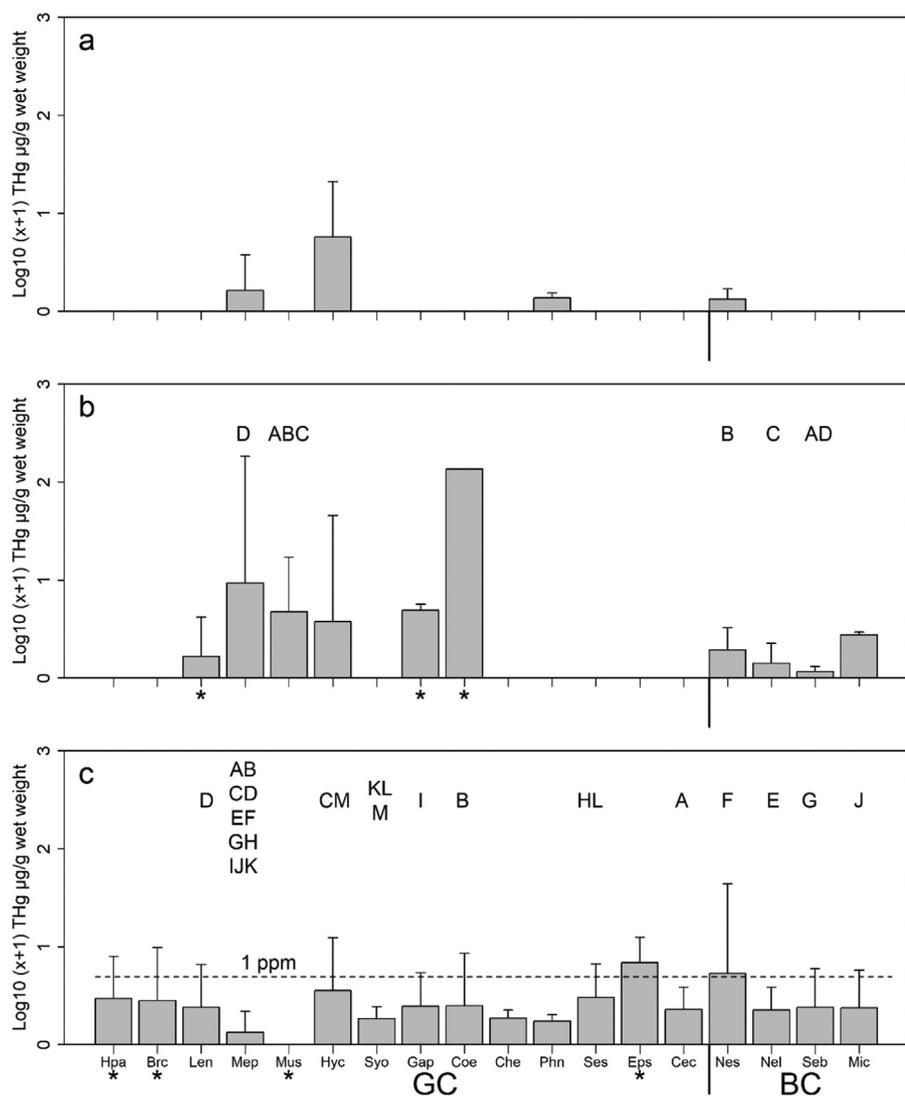


Fig. 2. Concentration of total mercury (THg) in gonad (a), liver (b) and muscle (c) of deep-sea fishes from northern Mexico, in the Eastern Pacific. Each pair of equal capital letters indicate species with significant differences. GC: Gulf of California, BC, western coast off Baja California, Brc: *Brotula clarkae*, Cec: *Cephalurus cephalus*, Che: *Cherublemma emmelas*, Coe: *Coelorinchus scaphopsis*, Eps: *Eptatretus stoutii*, Gap: *Galeus piperatus*, Hyc: *Hydrolagus collicie*, Hpa: *Hyporthodus acanthistius*, Len: *Lepophidium negropinna*, Mep: *Merluccius productus*, Mic: *Microstomus pacificus*, Mus: *Mustelus henlei*, Nel: *Nezumia liolepis*, Nes: *Nezumia stelgidolepis*, Phn: *Physiculus rastrelliger*, Ses: *Sebastes sinensis*, Seb: *Sebastolobus altivelis*, Syo: *Symphurus oligomerus*. Dashed line: maximum allowed concentration of mercury in fish muscle for human consumption (UNEP, 2002; DOF, 2009a), asterisks: species not included in statistical analysis.

not sexed, their comparisons were only made between tissues (liver and muscle). For species subjected to more than two factors and compared by overall CENMLE analyzes, pair-wise CENMLE comparisons were performed and p values were adjusted through FDR of the "fdrtool" package (Benjamini and Hochberg, 1995; Strimmer, 2008; Helsel, 2012). As *Nezumia stelgidolepis* did not have censored data, THg concentrations in this species were evaluated through a two way ANOVA with interactions, using as independent factors sex and type of tissue. Tukey HSD tests were used to identify the groups with different THg concentrations (Chambers and Hastie, 1993; R Core Team, 2017).

The liver/muscle index (THg concentration in liver/THg concentration in muscle) was calculated for individuals with THg data in both tissues (n = 177). According to Kružíková et al. (2013), an index higher than one suggests a Hg-contaminated environment, whereas values below one could indicate negligible Hg pollution (Hg mainly of natural origin). Species with more than five individuals were selected and their liver/muscle indices were compared through a one-way ANOVA. Tukey HSD tests were used to identify groups with different liver/muscle indices (Chambers and Hastie, 1993; R Core Team, 2017). For *M. productus*, the liver/muscle index was compared between the sampling years, using a one-way ANOVA.

To evaluate possible patterns of bioaccumulation of Hg throughout the depth and trophic levels, Spearman correlation (r_s) analysis were performed regarding the liver and muscle THg concentrations (including < MDL observations), as well for the liver/muscle indices

(Di Giulio and Hinton, 2008; Helsel, 2012). The variations on THg concentrations regarding size (THg versus TL) and the body condition (THg versus K) were evaluated for all the specimens of each species (for both tissues when possible). For *M. productus* correlations were calculated for each ecoregion and sex. When censored data were absent on THg measurements, the Pearson correlation coefficient (r) was used to establish associations; otherwise, Spearman correlations were used (Helsel, 2012). All correlations were calculated using the "agricolae" package (De Mendiburu, 2016).

3. Results

A total of 648 THg measurements were obtained in 18 deep-sea fish species. Table 1 summarizes the Hg concentrations by species and tissue. In general, the highest concentrations were recorded in the liver (geometric mean: 1.28 ± 2.31 µg/g, range: < MDL–16.17 µg/g), followed by muscle (geometric mean: 0.34 ± 0.53 µg/g, range: < MDL–6.12 µg/g) and gonad (geometric mean: 0.29 ± 0.49 µg/g, range: < MDL–2.18 µg/g). The five highest individual concentrations were found in liver, four of them in *M. productus* (16.2, 12.7, 11.6 and 11.2 µg/g) and one in *H. collicie* (9.6 µg/g). Regarding muscle, the highest individual concentrations were found in four different species: *N. stelgidolepis* (6.12 µg/g), *H. collicie* (3.25 µg/g), *Coelorinchus scaphopsis* (3.45 and 1.98 µg/g) and *Lepophidium negropinna* (1.98 µg/g). Regarding suitability for human consumption, 21.5% of muscle

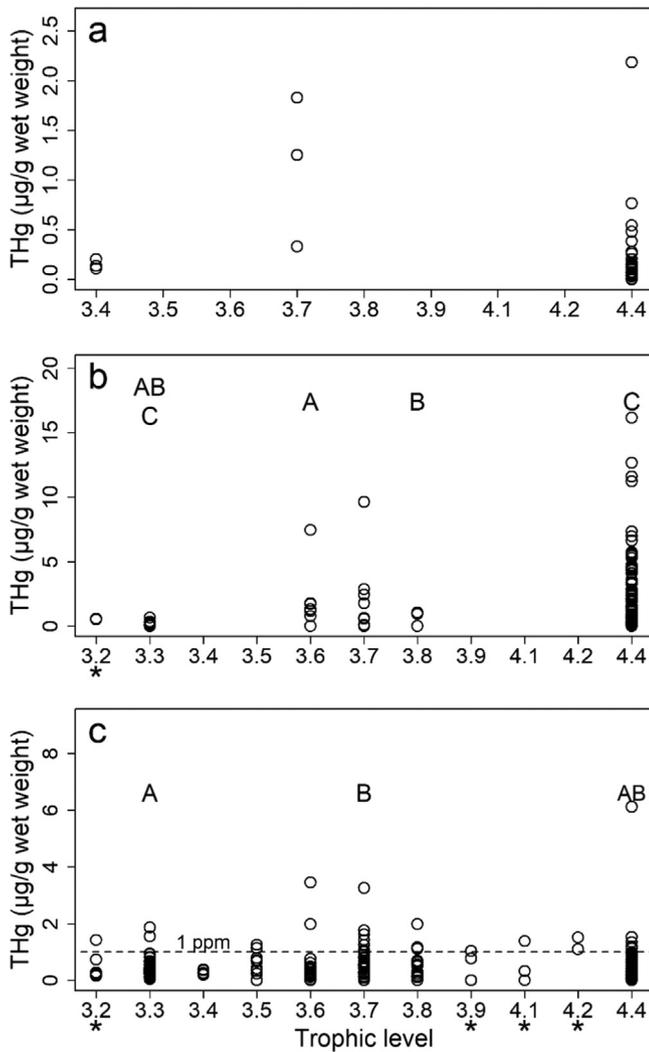


Fig. 3. Concentration of total mercury (THg) in gonad (a), liver (b) and muscle (c) of deep-sea fishes from northern Mexico, in the Eastern Pacific, ordered by trophic levels. Each pair of equal capital letters indicate species with significant differences. Asterisk: species not included in comparisons.

measurements (85 samples) exceeded 0.5 µg/g and 7.8% (31 samples) exceeded 1 µg/g, the more commonly used maximum permissible limit of THg concentration (UNEP, 2002; DOF, 2009a; Health Canada, 2017).

3.1. THg concentrations between species

THg concentrations were different between species (Fig. 2), in both liver (X^2 df: 5, n: 218 = 12.78, $p < 0.001$) and muscle (X^2 df: 13, n: 379 = 92.29, $p < 0.001$). In liver, paired comparisons showed that *M. productus* had higher THg concentrations than *S. altivelis*, whereas *Mustelus henlei* had higher concentrations than *N. liolepis*, *N. stelgidolepis* and *S. altivelis* (Fig. 2b). Respect to muscle, *M. productus* had lower THg concentrations than several species and *Symphurus oligomerus* had lower THg concentrations than *H. colliei* and *Sebastes sinensis* (Fig. 2c). Regarding trophic level (Fig. 3), liver THg concentration in species of levels 3.6, 3.8 and 4.4 were higher than in level 3.3 (X^2 df: 4, n: 226 = 10.61, $p = 0.031$; Fig. 3b), whereas, in muscle, levels 3.3 and 3.7 had higher THg concentrations than level 4.4 (X^2 df: 7, n: 386 = 35.98, $p < 0.001$; Fig. 3c). In general, THg concentrations in liver were higher at higher trophic levels (r_s n: 228 = 0.31, $p < 0.001$), whereas muscle (r_s n: 394 = -0.43, $p < 0.001$) showed the opposite trend. Liver THg concentrations were not related to depth (r_s n: 228 = -0.08, $p = 0.25$; Fig. 4b). However, a decreasing trend of the liver/muscle

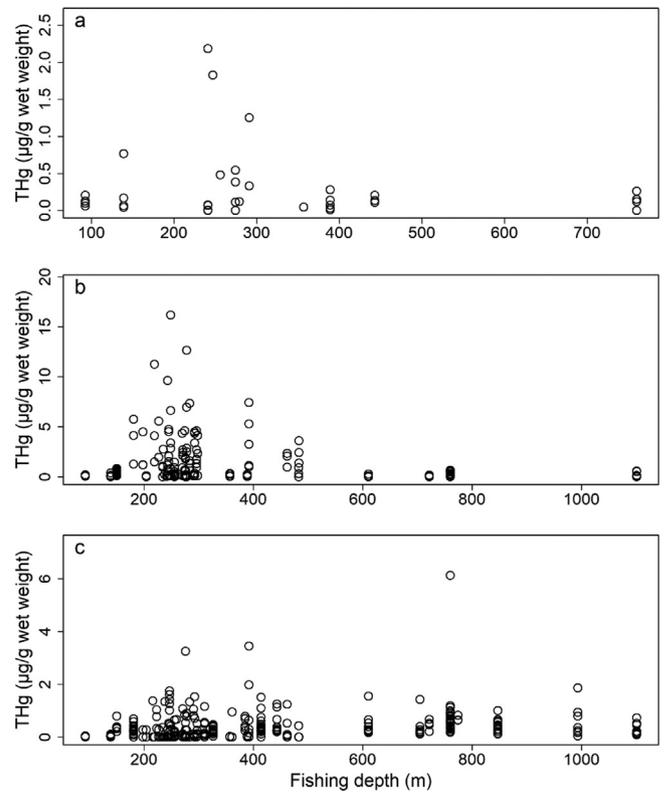


Fig. 4. Concentration of total mercury (THg) in gonad (a), liver (b) and muscle (c) of deep-sea fishes from northern Mexico, in the Eastern Pacific, ordered by fishing depth.

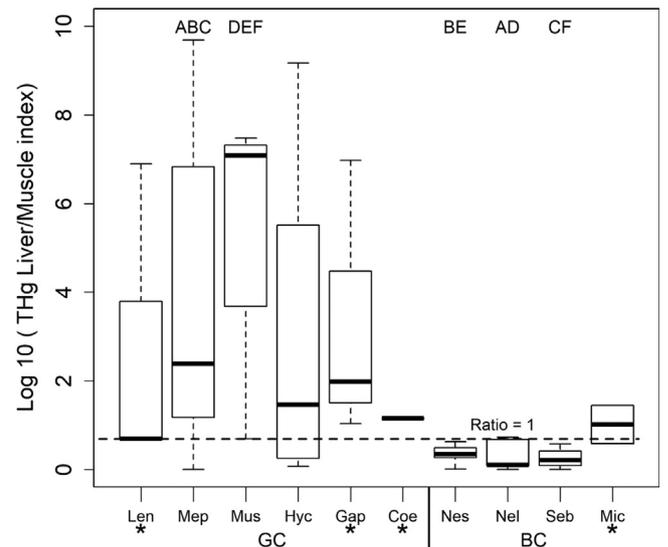


Fig. 5. Liver/muscle index of the concentration of total mercury (THg) in deep-sea fishes from northern Mexico, in the Eastern Pacific. Each pair of equal capital letters indicate species with significant differences. GC: Gulf of California, BC, western coast off Baja California, Coe: *Coelorinchus scaphopsis*, Gap: *Galeus piperatus*, Hyc: *Hydrolagus colliei*, Len: *Lepophidium negropinna*, Mep: *Merluccius productus*, Mic: *Microstomus pacificus*, Mus: *Mustelus henlei*, Nel: *Nezumia liolepis*, Nes: *Nezumia stelgidolepis*, Seb: *Sebastes altivelis*. Dashed line: liver/muscle index = 1, asterisks: species not included in statistical analysis.

indices according to depth was influenced by high liver THg concentrations between the 200 and 500 m depth and lower muscle THg concentrations at higher depths (r_s n: 394 = 0.34, $p < 0.001$; Fig. 4c). The species from GC had higher liver/muscle indices than BC species (F

Table 2

Correlation indexes for the following comparisons: Concentration of total mercury (THg $\mu\text{g/g}$ wet weight) versus total length (TL), and THg concentration versus condition factor (K), in tissues of deep-sea fishes from northern Mexico and Central California, in the Eastern Pacific. Asterisks: significant correlations, plus symbol: Spearman correlation.

Species/correlation	Total samples	Gonad	Liver	Muscle
<i>Cephalurus cephalus</i>				
TL	–	–	–	–0.08
K				0.49
<i>Cherublemma emmelas</i>				
TL	–	–	–	–0.32
K				–0.65
<i>Coelorrhinus scaphopsis</i> ⁺				
TL	–	–	–	0.39
K				0.19
<i>Galeus piperatus</i> ⁺				
TL	0.20	–	–	0.77
K	0.03			0.14
<i>Hydrolagus colliei</i> ⁺				
TL	0.12	–	–0.05	0.11
K	0.06		0.11	0.01
<i>Merluccius productus</i> GC ⁺				
TL	0.15	0.06	0.06	0.17
Males		–0.50	–0.23	0.08
Females		0.22	0.10	0.14
K	0.09	0.18	0.38*	–0.15
Males		–0.50	0.42	–0.61*
Females		0.18	0.37*	0.09
<i>Merluccius productus</i> CAL ⁺				
TL	0.15	–	–0.04	–0.27
K	–0.11		–0.10	–0.28
<i>Microstomus pacificus</i>				
TL	–0.03	–	–	–0.05
K	0.64			0.74*
<i>Mustelus henlei</i> ⁺				
TL	–0.29	–	–0.41	–
K	0.09		0.19	
<i>Nezumia liolepis</i> ⁺				
TL	0.14	–	0.36	0.20
K	0.33 *		–0.05	0.30
<i>Nezumia stelgidolepis</i> ⁺				
TL	0.09	–	–0.16	0.04
K	0.41 *		0.81*	0.01
<i>Physiculus rastrelliger</i>				
TL	0.10	0.05	–	0.77*
K	0.21	–0.13		0.12
<i>Sebastes sinensis</i> ⁺				
TL	–	–	–	0.80*
K				–0.5
<i>Sebastolobus altivelis</i> ⁺				
TL	0.14	–	–0.31	0.67*
K	–0.01		0.21	–0.28
<i>Symphurus oligomerus</i>				
TL	–	–	–	–0.03
K				–0.13

df: 9, 167; n: 177 = 4.82, $p < 0.001$; Fig. 5), with significant higher concentrations found in *M. productus* and *M. henlei* from GC compared to three species from BC: *N. liolepis*, *N. stelgidolepis* and *S. altivelis* (Tukey HSD, $p < 0.05$).

3.2. THg concentrations within species

Physiculus rastrelliger, *S. sinensis* and *S. altivelis* had higher muscle THg concentrations at larger sizes. The health condition (K) was positively correlated with liver THg concentration of *M. productus*, *N. liolepis* and *N. stelgidolepis*, and muscle of *M. pacificus* (Table 2, Fig. B.1).

THg concentrations in *H. colliei* were not different between sex-tissue groups (X^2 df: 4, n: 59 = 2.04, $p = 0.73$, Fig. 6a), *S. altivelis* had higher THg concentrations in muscle than in liver (X^2 df: 1, n: 33 = 8.71, $p = 0.003$, Fig. 6b), as well as *N. liolepis* (X^2 df: 1, n: 38 = 6.92, $p = 0.01$, Fig. 6c). For *N. stelgidolepis*, differences were found between tissues (F

df: 2, 23; n: 28 = 4.26, $p = 0.02$; muscle > liver and gonad, Tukey HSD), but sex was not a determinant factor (F df: 1, 23; n: 28 = 0.93, $p = 0.34$ Fig. 6d), neither the sex-tissue interaction (F df: 1, 23; n: 28 = 1.31, $p = 0.26$).

Hake specimens from GC had significant differences in the THg concentrations between years (X^2 df: 3, n: 287 = 32.48, $p < 0.001$; Fig. 7), year-sex (X^2 df: 8, n: 287 = 43.41, $p < 0.001$) and year-tissue (X^2 df: 8, n: 287 = 132.12, $p < 0.001$) combinations. Hake specimens caught in 2014 and the muscle of females from 2015 had lower THg concentrations compared to the rest of the combinations. When comparing individual years, CENMLE analyses showed differences in THg concentrations between tissues and sexes ($p < 0.05$), showing, mostly higher concentrations in liver than in muscle. For 2014, female livers had the highest THg concentrations (Fig. 7a), whereas for 2015 the livers of both sexes had higher THg concentrations than muscle of females (Fig. 7b). Liver of females in 2016 had higher THg concentrations than the rest of the groups (Fig. 7c) and liver of females in 2017 had higher concentrations than in muscle (Fig. 7d). No THg differences were observed between hake specimens from GC and MPT caught in 2017 (X^2 df: 1, n: 41 = 3.11, $p = 0.07$; Figs. 7d and 7e); however, in both ecoregions hake had higher THg concentrations in liver than in muscle (X^2 df: 3, n: 41 = 21.18, $p < 0.001$). The liver/muscle index was lower in 2014 than in the other sampling years (F df: 4, 121; n: 126 = 11.90, $p < 0.001$; Fig. 7f).

4. Discussion

The inter and intraspecific differences observed in the THg concentrations of deep-sea fishes from the northern Pacific of Mexico are a reflection of differences in life history (trophic position), biological factors (type of tissue, size, condition factor, and, to a lesser extent, sex) and exposure (possibly due to both natural and anthropogenic scenarios), likely determined by geographic region and depth (Cutshall et al., 1978; Kružíková et al., 2013).

4.1. Inter and intraspecific comparisons

The capture site (ecoregion and depth) and trophic position were determinant factors influencing the inter-specific differences of liver THg concentration, whereas muscle showed more homogeneous values, being *M. productus* the GC species with lower THg concentrations in muscle, but also the one with higher concentrations in liver. Although the trophic interval among the species in this study seems short (from 3.2 to 4.4), this factor elicited the greatest influence on the THg distribution among the species, particularly in liver, where the level 4.4 (*M. productus* and *N. stelgidolepis*) presented the significantly highest concentrations. Predatory species, such as tertiary consumers (trophic level 4) and top predators (trophic level 5), tend to have higher concentrations of pollutants due to biomagnification, regardless of the type of environment in which they live (EPA, 1997; Cossa et al., 2012; Pethybridge et al., 2012).

In environmental toxicology, longer life means greater exposure time; therefore, there is an expected relationship between pollutant concentrations and size of organisms, which is normally used as an indirect measure of age (Cossa et al., 2012; Walker et al., 2012). This was true only for *P. rastrelliger*, *S. sinensis* and *S. altivelis*, where larger individuals showed higher THg concentrations in muscle. However, this trend was not observed in liver, indicating that other environmental factors are influencing bioaccumulation in these species (Kružíková et al., 2013).

Despite its limitations, the condition factor (K) is commonly used to estimate biological condition, where organisms with higher K values are those that have a greater weight than expected for their size, which could be linked to energy reserves and fitness (Froese, 2006; Ramos-Santiago et al., 2010; Cifuentes et al., 2012). When an organism is affected by pollutants, a decreasing trend in its condition could be

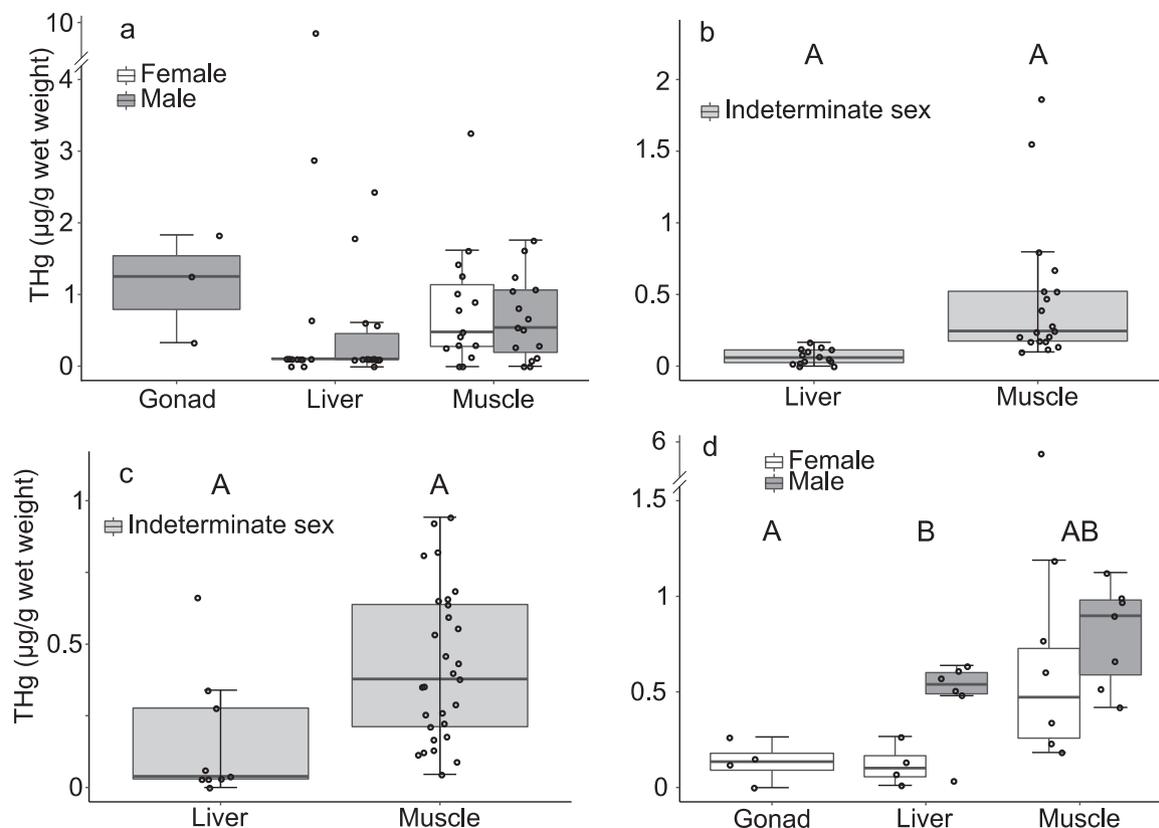


Fig. 6. Comparison of the total mercury (THg) concentration between tissues and/or sexes of some fish species from northern Mexico, in the Eastern Pacific. Each pair of equal capital letters indicate comparisons with significant differences. a: *Hydrolagus colliei*, b: *Sebastolobus altivelis*, c: *Nezumia liolepis*, d: *Nezumia stelgidolepis*.

expected (Lundebye et al., 1997; Bervoets and Blust, 2003). However, individuals of *M. productus*, *N. stelgidolepis*, *M. pacificus* and *N. liolepis* with higher body mass tended to present higher THg concentrations. Possibly, their THg concentrations were not too unfavorable to the organism's well-being or survival and K was determined by other factors (e.g., habitat quality and food availability) (Bervoets and Blust, 2003).

Eighty-three percent of the liver/muscle index values from GC exceeded the threshold of one, in contrast with BC that showed only 5% of its values above one. According to Abreu et al. (2000) and Havelková et al. (2008), when Hg environmental concentrations are minimal, the muscle shows the greatest bioaccumulation, however, when Hg environmental contamination is high, the liver tends to present higher concentrations than the muscle. This seems to agree with the fact that GC presented the highest THg concentrations in liver, in depths between 200 and 500 m, a zone known with the highest biotransformation rate of inorganic Hg to MeHg due to a greater amount of particulate organic matter and low concentrations of dissolved oxygen (Sunderland and Mason, 2007; Di Giulio and Hinton, 2008; Batrakova et al., 2014).

The analysis within species showed contrasting results. *Sebastolobus altivelis*, *N. liolepis* and *N. stelgidolepis*, species caught mainly in the middle slope of BC, had the highest THg concentrations in muscle, whereas *M. productus*, from the lower continental shelf and upper slope of GC, consistently had the highest values of THg in the liver between the different sampling years. Regarding sex, females usually eliminate higher concentrations of pollutants than males during reproduction, which transfer to their offspring or gonads (Hoffman et al., 2002; Burger et al., 2007). However, no clear effect of sex was observed in the THg concentrations, possibly due to the dispersion of the data and the variation in the availability of individuals of each sex for the comparisons. Regarding the organs, different bioaccumulation patterns of THg in liver and muscle were observed between the ecoregions, indicating

greater Hg contamination in the lower continental shelf and upper slope of GC (90–500 m depth) than in the upper and middle slope of BC (600–1100 m depth).

Merluccius productus was the only species that could be spatially and temporarily compared. This mesopelagic species is one of the most abundant fishes between 100 and 500 m depth in the California Current and the Upper Gulf of California and is the only fish species commercially caught in the continental slope of the Mexican Pacific (Cutshall et al., 1978; Ressler et al., 1991; Mazorra-Manzano et al., 2008). The highest individual THg concentrations in this species were found in liver, mainly in 2016. Sex was not determinant for most of the comparisons for this species, possibly due to the variability of the number of specimens for each sex and the detoxification of Hg during the release of the gonads by the females, since most of the samples were taken during the spawning season (December–March; Smith, 1995). On the other hand, the differences observed in THg concentrations between sampling years could be related to variations in oceanographic conditions, since vertical and horizontal migrations, as well as feeding habits of *M. productus* are affected by environmental factors, mainly temperature (Ressler et al., 1991). Between 2015 and 2016, a very strong “El Niño-ENSO” event occurred, increasing the average water temperature in the sea in the East Pacific and affecting the behavior of populations of different species (McClatchie et al., 2016; Iskandar et al., 2018; NOAA, 2018). A relationship between latitude and Hg concentration in *M. productus* was previously observed, possibly influenced by the presence of sub-populations with different horizontal migration intervals (Cutshall et al., 1978). However, the THg concentrations of hake from GC and MTP in 2017 were not different, but in both ecoregions, liver had higher THg concentrations than muscle. This finding and the differences observed between GC and BC species may be evidence that Hg contamination in the study area corresponds to a vertical pattern instead of a latitudinal one. Further studies considering a greater geographical range of sampling sites are desirable to better

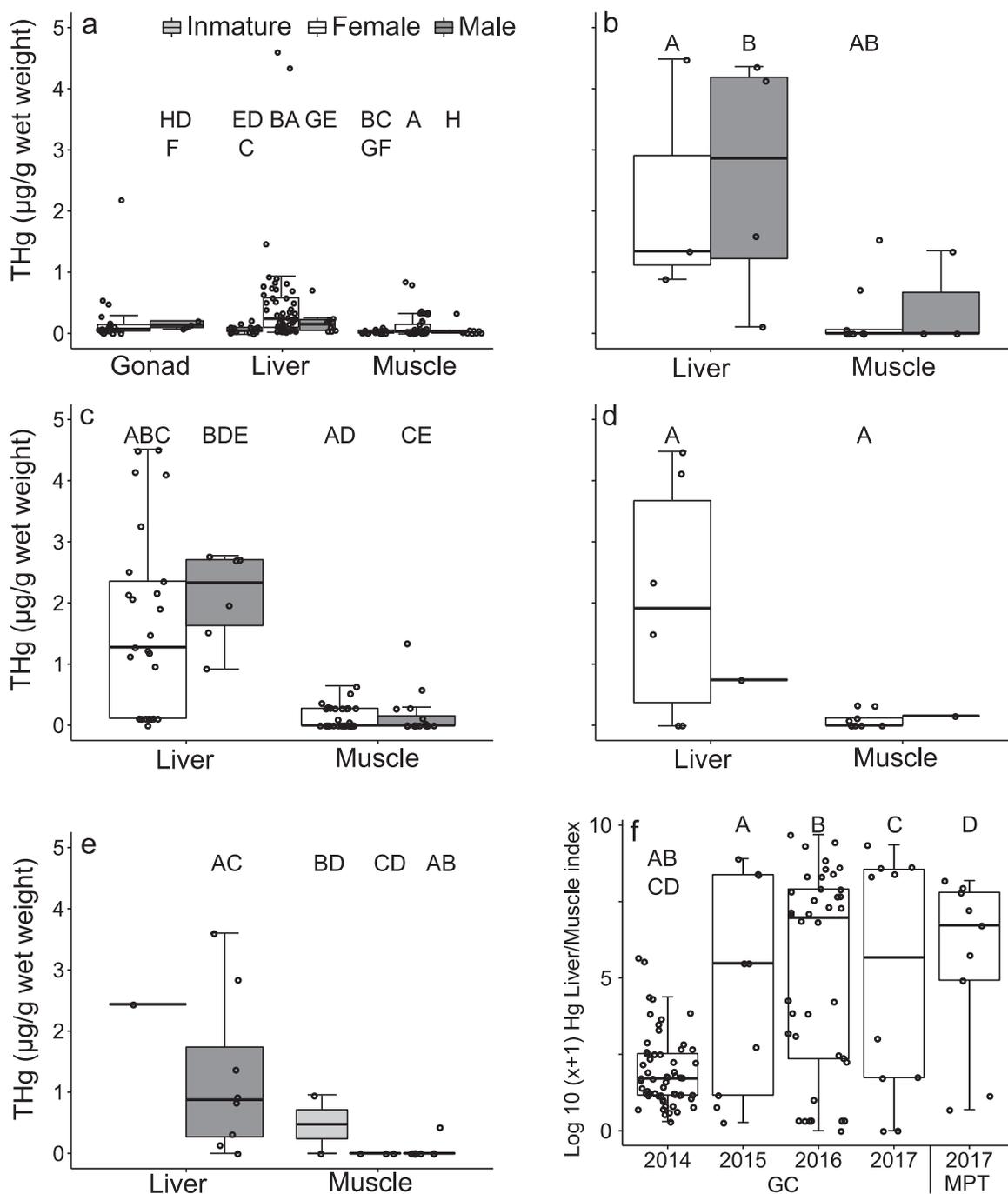


Fig. 7. Comparison of the total mercury concentration (THg) between years, tissues and sexes of the Pacific Hake (*Merluccius productus*) from the Gulf of California (GC; a: 2014, b: 2015, c: 2016, d: 2017) and the Montereyan Pacific Transition (MPT; e: 2017), f: Liver/muscle index. Each pair of equal capital letters indicate comparisons with significant differences.

understand the regional patterns of Hg contamination.

4.2. Regional variations in THg concentrations

The THg concentrations in muscle were similar to those reported for shallow water fishes from northwestern Mexican Pacific (García-Hernández et al., 2007, 2018; Ruelas-Inzunza and Páez-Osuna, 2005; Ruelas-Inzunza et al., 2011, 2013; Zamora-Arellano et al., 2017). In contrast, some liver THg concentrations of organisms from GC were higher than previously recorded from shallow water fishes (Ruelas-Inzunza et al., 2013). Our results, therefore, might indicate a higher contamination by Hg in the upper slope of this ecoregion, compared

with its continental shelf (Kružíková et al., 2013) and it is possibly related to a greater bioavailability of organic Hg (Sunderland and Mason, 2007; Di Giulio and Hinton, 2008; Batrakova et al., 2014).

The differential bioaccumulation of THg between BC and GC species may indicate different amounts of Hg entering the environment and/or differences in their local transport and deposition processes (Batrakova et al., 2014). The hydrothermal vents located in GC and BC (around 27°N and 32°N, respectively; Prol-Ledesma et al., 2004, DOF, 2009b), could indeed be a natural source of Hg for marine biota (García-Hernández et al., 2015), by releasing heavy metals from the earth's crust, as has been demonstrated in other regions of the world (Pruski and Dixon, 2003; Dixon et al., 2004; Martins et al., 2006). Regarding

anthropogenic factors, the Colorado River transports heavy metals and Persistent Organic Compounds (OCs) from the USA and the agricultural valleys of Mexicali and San Luis Río Colorado to GC (Hinck et al., 2007; García-Hernández et al., 2013). Urban settlements (Di Giulio and Hinton, 2008) and mining located around the Peninsula of Baja California (Alpers et al., 2005; Servicio Geológico Mexicano, 2016a, 2016b; García-Hernández et al., 2015) can also be important sources of Hg for both ecoregions.

Marine currents move large quantities of Hg in the ocean (Sunderland and Mason, 2007; Batrakova et al., 2014). The California Current brings water from the North Pacific to BC (Hickey, 1979; Durazo and Baumgartner, 2002) and, considering that central California is one of the most contaminated areas by Hg in the USA (Fairey et al., 1998; Choe and Gill, 2003; Alpers et al., 2005), the marine circulation is a possible source of Hg into the Mexican Pacific. The northern GC circulation is different from the rest of the Eastern Pacific, dominated by strong horizontal and vertical circulation processes and has a limited water exchange with the adjacent regions (Álvarez-Borrego and Schwartzlose, 1979; Álvarez-Borrego, 2008). Besides depth, these particularities in the oceanographic conditions of GC could determine a greater deposition of Hg in comparison with BC, which is a more dynamic region with seasonal variations in circulation (Lynn and Simpson, 1987; Durazo and Baumgartner, 2002; Wilkinson et al., 2009).

5. Conclusions

The presence of THg concentrations that can affect the health of the organisms, the recording of values above different thresholds of permissible limits for human consumption and the regional differences in the Hg bioaccumulation patterns, point out that Hg contamination can be relevant in the health of deep-sea fish communities of the northern Pacific of Mexico. In this regard, the higher concentrations found in the liver of GC species could represent recent exposures to Hg, so it is necessary to continue with the evaluation of Hg contamination in this ecoregion.

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Author contributions

ECA, JGH, MBL and HAV contributed to the conception and design of the study. DIAR, JGH and HAV managed the donation of the samples. DIAR led the hake prospecting cruises in the Gulf of California. HAV coordinated the fish sampling during the TALUD project. ECA, JGH and DAM performed the analytical procedures and organized the database. ECA performed the statistical analysis. ECA, JGH and MBL wrote the first draft of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

Declarations of interest

None.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dsr.2019.01.002.

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