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Total mercury and fatty acids content in selected fish marketed in Quito – Ecuador. A benefit-risk assessment

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ABSTRACT

Total mercury and fatty acids contents were determined in muscles of croaker, snapper, dolphinfish, blue marlin, and shark, from different markets in the Metropolitan District of Quito, Ecuador. Fifty-five samples were collected and analyzed for total mercury using cold vapor atomic fluorescence spectrometry, and the fatty acids were analyzed using gas chromatography equipped with a flame ionization detector. The lowest total mercury levels were found in snapper [0.041 $\mu g \cdot g^{-1}$ wet weight (ww)] while blue marlin showed the highest (5.883 $\mu g \cdot g^{-1}$ ww). EPA + DHA ranged from 1.0 $mg \cdot g^{-1}$ in snapper to 2.4 $mg \cdot g^{-1}$ in shark. A high omega-3/omega-6 ratio was found for all fish types; however, the HQ_{EFA} for the benefit–risk ratio was above 1, suggesting an evident risk to human health. Based on our results, consumption of croaker and dolphinfish is recommended up to one serving per week, considering the importance of EFAs intake and avoiding fish with elevated MeHg content. Therefore, Ecuadorian authorities could enhance public standards for seafood safety and develop consumer advice for pregnant women and young children to determine good fish choices or those to avoid.

1. Introduction

Pelagic and demersal fish constitute important industrial fisheries in Ecuador, providing different products such as canned fish, frozen fish, and other marine ingredients (fish meal and fish oil) [11].

Fish is an important protein source, providing beneficial nutrients in the human diet, like essential fatty acids (EFA), lipid-soluble vitamins (A and D), and minerals (sodium, potassium, calcium, magnesium, and phosphorus) [26,41,76]. Even though the consumption of marine foods provides numerous health benefits, it is also the main pathway for human exposure to mercury (Hg) ([70]; World Health Organization [[104]).

Coastal ecosystems are the interface between mercury (Hg) sources—atmosphere, rivers, and ocean—where trace metals like Hg can be transported and deposited [52]. Waste from anthropogenic activities, including domestic sewage, ship trash, and industrial waste, among others, increase the level of contaminants in coastal environments [45], which in turn increases the bioavailability of inorganic Hg (iHg) and methylmercury (MeHg) for uptake by aquatic biota [97]. Mercury enters the aquatic environment in various forms, mainly as its inorganic form (Hg^{+2}) (C.-B. [54]), which is converted to MeHg by bacterial activity [30,35]. MeHg is a toxic, stable organic compound that is consumed by marine microorganisms, and its concentration increases through the food chain up to higher trophic levels, affecting not only aquatic species but also reaching humans through the food web [25,56]. According to several studies, MeHg represents around 64–100% of total mercury (THg) content, depending on the size and age of the fish [105]. Also, it has been reported that 0.67–1.60% of the inorganic form ingested can be

Abbreviations: ALA, α-linolenic acid; ARA, arachidonic acid; BW, body weight; CHD, coronary heart disease; CRM, certified reference material; CV-AFS, cold vapor atomic fluorescence spectrometry; DHA, docosahexaenoic acid; DMQ, Metropolitan District of Quito; dw, dry weight; EFAs, essential fatty acids; EPA, eicosapentaenoic acid; EWI, estimated weekly intake; FAO, Food and Agriculture Organization; GC-FID, gas chromatograph equipped with a flame ionization detector; HQ_{EFA}, hazard quotient for fish consumption when a person obtains from the fish the recommended dose of EFA; JECFA, Joint FAO/WHO Expert Committee on Food Additives; LA, linoleic acid; LC-PUFAs, long-chain polyunsaturated fatty acids; MeHg, methylmercury; MoS, Margin of Safety; MUFAs, monounsaturated fatty acids; PTWI, provisional tolerable weekly intake; RfD, oral reference dose; SFAs, saturated fatty acids; THg, total mercury; THQ, target hazard quotient; US-EPA, US Environmental Protection Agency; WHO, World Health Organization; ww, wet weight.

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methylated into MeHg in fish intestines [40]. For people who consume fish and fish predators, MeHg is rapidly transported and distributed to various tissues and organs through the bloodstream [48,49,6]. MeHg is a neurotoxin that causes damage to the central nervous system; it also has far-ranging gonadotoxic and carcinogenic properties [10,6,65,69]. To reduce potential exposure to THg and MeHg through fish consumption, maximum acceptable concentration limits in fish species (0.5 $\mu g \cdot g^{-1}$) and predatory fish (1.0 $\mu g \cdot g^{-1}$), have been established by the Food and Agricultural Organization [31] and the Ecuadorian Institute for Standardization NTE INEN 183 (Instituto Ecuatoriano de Normalización ([43]. Moreover, the Joint FAO/WHO Expert Committee on Food Additives [JECFA]) reviewed consumption advisories as provisional tolerable weekly intake (PTWI), establishing 4 $\mu g \cdot kg^{-1}$ week·BW^{-1} (body weight) and 1.6 $\mu g \cdot kg^{-1}$ week·BW^{-1} for iHg and MeHg, respectively [103].

On the other hand, within the health-beneficial substances associated with marine foods are the two groups of long-chain polyunsaturated fatty acids (LC-PUFAs): the 'omega-3-fatty acids' (ω-3) and 'omega-6-fatty acids' (ω-6) [19,107].

The ω-3 fatty acids are the α-linolenic acid (C18:3n3; ALA), the eicosapentaenoic acid (C20:5n3; EPA), and the docosahexaenoic acid (C22:6n3; DHA), whereas the main ω-6 fatty acids are the linoleic acid (C18:2n6; LA) and the arachidonic acid (C20:4n6; ARA) [5,107]. ALA and LA are mainly found in high proportions in foods derived from plant sources such as seeds, nuts, and plant oils, including African palm, corn, sunflower, soybean, olives, pumpkin seeds, safflower, walnuts, canola, flax seeds, and flaxseed oil [19,5,98]. Meanwhile, EPA and DHA are primarily found in seafood, which is the richest source of ω-3 [21,5,64].

ALA and LA can be converted into EPA and further to DHA through various biochemical pathways. However this endogenous conversion is limited in humans with low percentages, 8% and 4% for EPA and DHA, respectively, therefore sources of ω-3 LC-PUFAs should be included in the human diet for general health [107,19,5,64].

It is well known that the consumption of EPA and DHA can prevent several cardiovascular disorders, such as decreasing the risk of myocardial infarction, heart arrhythmias, and the risk of sudden cardiac death; lower blood pressure, improving triglyceride concentrations and platelet aggregation, heart-rate variability, and enhance the immune system. Additionally, LC-PUFAs have beneficial effects in the prevention of cognitive disorders and brain development for children and aged people; and protect against depression and cancer [21,38,64,76]. It has been reported that ω-3 fatty acids (EPA and DHA) can antagonize some of the adverse effects of Hg in human body regarding to heart diseases and childrens brain development [36,83].

In developing countries, the Food and Agriculture Organization (FAO) of the United Nations reported that fish consumption increased from 5.2 kg in 1961 to 19.4 kg in 2017 [32].

Considering that fish consumption is the main source of LC-PUFAs, EPA and DHA in the human diet, and also an exposure pathway to MeHg [91,102], the present study was conducted as a first approach in samples of commonly consumed fishes at the Metropolitan District of Quito, Ecuador. To the best of our knowledge, there are no studies related to the risk-benefit of essential fatty acids against mercury in fish species consumed in Ecuador. Therefore, this work aimed to: a) quantify THg levels in muscles of various fish types commonly consumed in Quito-Ecuador; b) evaluate compliance with food safety regulations; c) assess the related mercury health risk due to fish ingestion; and d) estimate the lipids content and profile of fatty acids in the studied fish types to evaluate their hazard quotient of benefit-risk ratio.

2. Materials and methods

2.1. Sampling

The Metropolitan District of Quito (DMQ for its name in Spanish) is the capital of Ecuador, characterized by a high population density, with approximately 2.8 million habitants [42]. Due to the DMQ location in the Andean region, in Ecuador, marine species are mainly consumed compared to freshwater species [2,23], thus fish and seafood provided from the Coastal region are delivered in supermarkets and open markets for local consumption.

In order to provide a baseline of risk–benefit assessment of fish consumption for residents of the DMQ, five representative fish types commonly consumed were selected. Fifty-five fish samples were purchased from 14 different main city supermarkets, and 2 of the biggest wholesale seafood markets, considering the availability of the fish at each vendor. The collected fish types were: croaker locally known as "corvina" (*Cynoscion* spp.; n = 15), snapper "pargo" (*Lutjanus peru*; n = 8), dolphinfish "dorado" (*Coryphaena hippurus*; n = 9), blue marlin "picudo" (*Makaira nigricans*; n = 8), and shark "tollo" (*Mustelus mento*; n = 15).

Muscle tissue samples were transported to the Centro de Estudios Aplicados en Química (CESAQ-PUCE) laboratory. Each sample was washed with high-quality reagent water (resistivity of 18.2 MΩ·cm at 25 ºC) and then placed in plastic bags, which were then immediately frozen and stored at − 25 ºC until analysis.

2.2. Total mercury content and analyses

Fish samples were freeze-dried (Labogene, Bjarkesvej 5, Denmark) for 48 h at − 50 ºC and 0.150 hPa, and weighed to determine their water content, to report the results as wet weight (ww). Each sample was homogenized grinding with a mortar and pestle, and then samples were then transferred to a plastic bag. These were stored in a desiccator until the acid digestion procedure.

For the acid digestion procedure, the Yánez-Jácome et al. [105] method was used. Approximately 0.15 g portion of each dried sample was weighed directly in each high-pressure polytetrafluoroethylene vessel (MARSEasyPrep) where the digestion took place. One mL of HNO_3 (Fisher Chemical, Ottawa, Canada, Certified ACS, CAS# CAS 7697–37–2, PubChem CID: 944), 1 mL of H_2O_2 (Fisher Chemical, Ottawa, Canada, Certified ACS, CAS# 7722–84–1, PubChem CID: 784) and 1 mL of $HClO_4$ (Fisher Chemical, Ottawa, Canada, Certified Optima, CAS# 7601–90–3, PubChem CID:24247) were added to each vessel. Samples were gently mixed, and vials were left open for 10 min before closing them. Acid digestion was performed in a MARS 6 microwave (CEM, Matthews, NC, USA) meanwhile THg analyses were performed using Mercur Plus equipment that uses cold vapor atomic fluorescence spectroscopy (CV-AFS) (Analytik Jena, Jena, Germany) [105].

For THg analyses, one random sample from each fish type was selected, digested and analyzed by sextupled. All measurements were performed in duplicate for each sample, and the mean concentration was reported. Linear regression coefficients higher than 0.99 were obtained for calibration curves. The instrumental limits of detection and quantitation were 0.055 μg·L and 0.183 μg·L, respectively. The mean recovery (mean ± SD) of THg in the DORM-5 (fish protein) certified reference material (CRM) was 93% ± 2%, within the certified range (0.316 ± 0.017 $\mu g \cdot g^{-1}$).

The THg concentrations in the samples were expressed as μgs per g ww ($\mu g \cdot g^{-1}$).

2.3. Total lipid extraction

Total lipid extraction was performed according to the modified Folch method [51]. Approximate 500 mg of each dried muscle sample was homogenized for 10 s in 10 mL of a mixture 2:1 of methylene chloride (analytical grade, Merck, Darmstadt, Germany, CAS# 75–09–2, PubChem CID: 6344) and methanol (analytical grade, Merck, Darmstadt, Germany, CAS# 67–56–1, PubChem CID: 887) using an IKA T10 Ultra Turrax homogenizer (Staufen, Germany), repeating this extraction twice. The residue of extraction was filtrated through a glass filter cup (pore size 4) with a 1 mm of Celite® (analytical grade, Sigma Aldrich, USA) layer. The filtrate was transferred to a 100 mL glass separatory funnel, 5 mL of 0.73% sodium chloride (NaCl, analytical grade, Merck, Darmstadt, Germany, CAS# 7647–14–5, PubChem CID: 5234) solution was added, and then vigorously hand shaken for 30 s. After an overnight separation, the lower phase was collected in a 15 mL brown glass flask, and the solvent was evaporated to dryness at 40 °C using a nitrogen flow (Glas-Col, Terre Haute, IN, USA). The free-solvent oil was weighted to calculate the total oil content (% dry weight, dw).

2.4. Fatty Acid Methyl Ester analyses

The fatty acids profile was determined as methyl esters (FAME) according to the method described by [51]. To the total oil extracted into the 15 mL brown glass flask, 4 mL of 0.5 M sodium hydroxide (NaOH, analytical grade, Merck, Darmstadt, Germany, CAS# 1310–73–2, PubChem CID: 14798) in methanol solution was added, and then the tube was heated at 90 °C using a water bath for 10 min. Once the tube cooled down to room temperature, 5 mL of 20% Boron trifluoride (BF3) in methanol (Sigma Aldrich, MO, USA) solution was added to the tube and heated for 5 min at 90 °C. After cooling down to approximately 30 – 40 °C, 2 mL of hexane (Chromatography grade, Merck, Darmstadt, Germany, CAS# 110–54–3, Pub Chem CID: 8058) and 3 mL of a supersaturated NaCl solution were added. The tube was vigorously hand-shacked for 15 s. The hexane phase containing FAMEs was carefully collected after phase separation, filtered through a PTFE 0.5 μm syringe filter, and injected in a Perkin Elmer Clarus 500 gas chromatograph equipped with a flame ionization detection (GC-FID) (Perkin Elmer, Waltham, MA, USA). A FAMEwax capillary column (Restek, Lisses, France, 30 m in length, 0.25 mm internal diameter, 0.20 μm film thickness) was used. The carrier gas was helium at a flow rate of 1.2 mL min^{-1}. The injector temperature was set at 230 °C and the detector temperature was 260 °C. Separation was conducted using an initial temperature of 100 °C maintained for 2 min. Then column was temperature programmed at 10 °C min^{-1} to 140 °C, 3 °C min^{-1} to 190 °C, and 30 °C min^{-1} until 260 °C, keeping this temperature for 5 min. FAME identification was done using a commercial mixture standard (37 components FAME Mix C4-C24, Sigma Aldrich, Leramie, WY, USA). All fatty acid analyses were performed in duplicate. Fatty acids were reported in g·100 g^{-1} of free-solvent oil.

2.5. Statistical analyses

Analytical data were treated to calculate the arithmetic mean, standard deviation, hazard indexes, and CRM recovery using Microsoft Office Excel (Microsoft office, 2016). After a logarithmic transformation of THg concentrations, a one-way ANOVA was applied to compare the THg content in the five fish types from the different markets, followed by Post-Hoc Tukey test.

PUFAs content between the five fish types was assessed using non-parametric methods (Kruskal Wallis) followed by a pairwise Wilcox test.

A PCA test was applied to visualize the differences between fish types regarding their THg and EFAs (EPA + DHA) content. The R software environment for statistical computing and graphics (https://w.w.w.r-project.org/) was used to plot and analyze the data.

2.6. Provisional weekly intake and target hazard quotient

The Environmental Protection Agency of the United States (US-EPA) recommends the assumption that all Hg in fish is present as MeHg to be most protective of human health [95], therefore for the present study, the potential health risk was assessed using the THg concentrations as MeHg in muscle tissue of the five fish types.

First, Eq. (1) was used to calculate the estimated weekly intake (EWI). An average mean body weight of 14.5 kg, 60 kg, and 70 kg was assumed for children, women, and men, respectively.

$$EWI = (Cx \quad IR)/BW \tag{1}$$

where EWI is reported as μg·kg^{-1} week·BW^{-1}; C is the mean or maximum concentration of MeHg found in fish (mg·kg^{-1} ww); and IR is the weekly ingestion rate (g·$week^{-1}$).

The calculated EWI was compared to the PTWI of 1.6 μg·kg^{-1} week·BW^{-1} for MeHg suggested by the JECFA [41,70,79], to obtain the Margin of Safety (MoS).

The MoS is a ratio, derived from the scientific laws of toxicology, and from the mathematical laws of statistical analysis, between (a) that dose of a food additive, agricultural residue, or nutritional factor on a body weight basis, that causes a measurable undesirable effect on an appropriate laboratory animal or upon man, and (b) that dose which man eats on a daily basis [62]. A value of MoS > 1 indicates there could be adverse effects derived from fish consumption [33].

The target hazard quotient (THQ) considers the oral reference dose (RfD) to estimate consumers' non-carcinogenic health risk from the intake of trace metals through contaminated fish [57]. RfD estimates the level of a consumer's daily exposure without significant health risk over a lifetime [104]. A THQ > 1 implies a high adverse non-carcinogenic health risk from fish consumption, while a THQ < 1 suggests no adverse health effects [3].

THQ was calculated by Eq. (2) [28,3,78,88]:

$$THQ = \frac{C \quad x \quad FIR \quad x \quad EFr \quad x \quad ED}{RfD \quad x \quad BW \quad x \quad ATn} x \quad 10^{-3} \tag{2}$$

where C is the mean concentration of THg in fish (mg·kg^{-1} ww); FIR is the fish ingestion rate for children and both gender adults (0.057 kg·$week^{-1}$ and 0.113 kg·$week^{-1}$, respectively); EFr is the exposure frequency, or the number of exposure events per year (from 365 days·$year^{-1}$ for people who eat fish seven times a week to 52 days·$year^{-1}$ for people who eat fish once per week); ED is exposure duration (70 years for adults and 6 years for children) [28,3,88], equivalent to the average lifetime; RfD is the oral reference dose specific for MeHg (0.0001 μg·g^{-1}·day^{-1}) [103]; BW is body weight; and ATn is the average exposure time for non-carcinogens (EFr x ED) (days).

Latin America as a region has one of the lowest per capita fish consumption rates—10.5 kg·$year^{-1}$—[32], and Ecuador has the lowest rate of Latin American countries—7 kg·$year^{-1}$ per capita—[13,22], even though fisheries are one of the country's most important economic industries. Therefore, 52 days·$year^{-1}$ was used for the number of exposure events per year for THQ calculations.

2.7. Hazard quotient for benefit-risk ratio

The fatty acids EPA y DHA content were expressed in g·100 g^{-1} of edible fish muscles according to Eq. (3) [56,75]:

$$FA = \left[\frac{(P * FC)}{100}\right] * C, \tag{3}$$

where FA is the fatty acid (g·100 g^{-1} muscle of fish); P is the fatty acid percentage (% of total lipids); FC is the free-solvent oil content expressed in fresh weight (g·100 g^{-1} of fish muscle); C is the conversion factor, calculated according to Weihrauch et al. [101]; Yilmaz [106], considering the total lipid (TL) content (Eq. (4)).

$$C = 0.933 - 0.143/TL \tag{4}$$

The hazard quotient for the benefit-risk ratio for fish consumption of both MeHg and the content of EFAs (EPA + DHA) was assessed according to [37] and [56], using Eq. (5):

$$HQ_{EFA} = \left(\frac{R_{EFA} \times c}{EFA \times RfD \times AW}\right) \tag{5}$$

where: R_{EFA} is 250 mg^{-1} [96]; c is the content of metal in fish (μg·g^{-1});

Table 1
Mercury levels in muscle tissue of fish species under study, estimated weekly intake, Margin of Safety (MoS) and recommended weekly intake of fish meat for children, women, and men, based on mean, minimum and maximum values found in samples.

Fish species *n*	THg ($\mu g \cdot g^{-1}$ ww)	Children[a]			Women[b]			Men[c]		
		MeHg EWI ($\mu g \cdot kg^{-1}$ week·BW^{-1})	MoS	Recommended weekly intake (g fish/week)	MeHg EWI ($\mu g \cdot kg^{-1}$ week·BW^{-1})	MoS	Recommended weekly intake (g fish/week)	MeHg EWI ($\mu g \cdot kg^{-1}$ week·BW^{-1})	MoS	Recommended weekly intake (g fish/week)
Croaker (*Cynoscion spp.*)* *n* = 15	0.176 ± 0.106 (0.072 – 0.491)	0.69 (0.28 – 1.92)	0.43 (0.18 – 1.20)	132	0.33 (0.14 – 0.93)	0.21 (0.09 – 0.58)	545	0.29 (0.12 – 0.80)	0.18 (0.07 – 0.50)	636
Snapper (*Lutjanus peru*)* *n* = 8	0.173 ± 0.126 (0.041 – 0.447)	0.68 (0.16 – 1.75)	0.42 (0.10 – 1.09)	134	0.33 (0.08 – 0.84)	0.20 (0.05 – 0.53)	555	0.28 (0.07 – 0.72)	0.18 (0.04 – 0.45)	647
Dolphinfish (*Coryphaena hippurus*)** *n* = 9	0.760 ± 0.500 (0.138 – 1.622)	2.97 (0.54 – 6.34)	**1.86** (0.34 – 3.96)	31	1.44 (0.26 – 3.06)	0.90 (0.16 – 1.92)	126	1.23 (0.22 – 2.63)	0.77 (0.14 – 1.64)	147
Blue marlin (*Makaira nigricans*)** *n* = 8	2.569 ± 1.575 (1.056 – 5.883)	10.05 (4.13 – 23.00)	**6.28** (2.58 –14.38)	9	4.86 (2.00 – 11.12)	**3.03** (1.25 – 6.95)	37	4.16 (1.71 – 9.53)	**2.60** (1.07 – 5.96)	44
Shark (*Mustelus mento*)** *n* = 15	0.803 ± 0.394 (0.314 – 1.792)	3.14 (1.23 – 7.01)	**1.96** (0.77 – 4.38)	29	1.52 (0.59 – 3.39)	0.95 (0.37 – 2.12)	120	1.30 (0.51 – 2.90)	0.81 (0.32 – 1.81)	140

MeHg maximum level (Food and Agricultural Organization [FAO], 2019)
* Fish: 0.5 $\mu g \cdot g^{-1}$; ** Predatory fish: 1.0 $\mu g \cdot g^{-1}$
[a] Body weight of 14.5 kg for children; [b] Body weight of 60 kg for women; [c] Body weight of 70 kg for men
In Bold MoS > 1

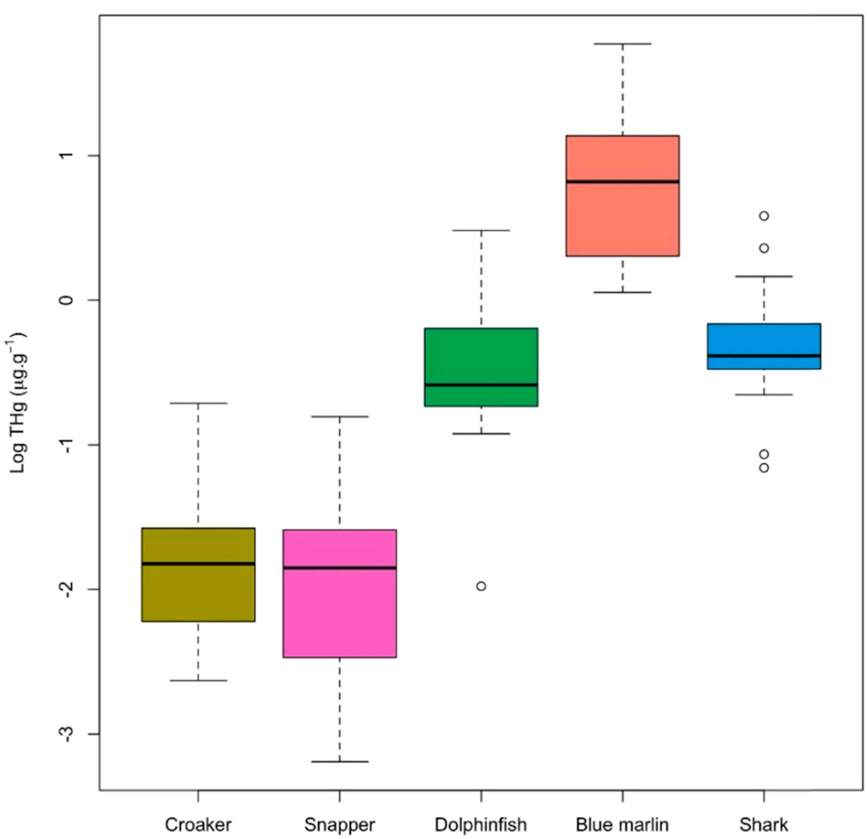

Fig. 1. Boxplot of total mercury content ($\mu g \cdot g^{-1}$) in log scale, for the five fish types under study.

EFA is the sum of the content of EPA + DHA ($mg \cdot g^{-1}$); RfD is the reference dose of metal (0.0001 $ug \cdot g^{-1} \cdot day^{-1}$); AW is an average body weight (14.5, 60, and 70 kg for children, women, and men respectively).

If $HQ_{EFA} < 1$, there is a health benefit from fish consumption and the consumers are safe, whereas an $HQ_{EFA} > 1$ suggests a high probability of adverse risk to human health.

3. Results and discussion

3.1. Content of mercury in fish tissue, estimated weekly intake, and target hazard quotient

The highest THg levels were found in the muscles of blue marlin, shark, and dolphinfish (Table 1). The 22.2% of dolphinfish samples exceeded the threshold limits established by the [31] and [43], meanwhile, 100% of blue marlin and 20% of shark samples presented values over the corresponding threshold limits. Snapper and croaker tissues had the lowest THg contents, however, one sample of croaker was near the maximum limit with 0.491 $\mu g \cdot g^{-1}$ (Fig. 1). Significant differences were found between THg concentrations in fish tissues of the five types under study ($p < 0.01$). Tukey test showed THg differences between blue marlin, which had the highest THg content, followed by dolphinfish and shark, and croaker and snapper with the lowest concentrations.

In a study performed in dolphinfish from the Eastern Pacific Ocean in Manta city, Ecuador, the reported THg levels were higher than the permissible limit (1 $\mu g \cdot g^{-1}$) in 55% of the analyzed samples [4]. Another study performed by [87] on dolphinfish consumed in Machala city, Ecuador, showed similar results, with THg concentrations exceeding the maximum threshold established (0.5 $\mu g \cdot g^{-1}$) with a mean value of 1.970 ± 0.85 $\mu g \cdot g^{-1}$. Regarding croaker, [87] also found a mean THg content of 1.421 ± 0.998 $\mu g \cdot g^{-1}$, a value higher than the results in our study.

For estimation intake calculations, 57 g fish·week^{-1} and 113 g fish·week^{-1} were considered for children (between 2 and 11 years old), and adults, respectively. The [29] recommends that no more than three meals per week include fish for adults, with a serving size of 113 g of animal protein before cooking per eating occasion. For children, it recommends serving fish one to two times per week, with serving sizes smaller than adult portions depending on the child's age [29].

Values obtained were compared to MeHg PTWI, considering that most of the mercury in fish is MeHg, and most (greater than 95%) of the MeHg in fish ingested is readily absorbed into the body through the gastrointestinal tract [104]. PTWI is expressed on a weekly basis, showing a long-term exposure risk for contaminants that may accumulate in the human body [70]. The highest MoS values were found for children in blue marlin, shark, and dolphinfish. However, for children and adults, blue marlin is a fish species with MoS values that cause concern for human health (1.07 – 14.38).

In an study performed by Franco-Fuentes et al., [33] in dolphinfish from the Galapagos Islands, Ecuador, the MoS was below 1, nevertheless, according to the results from this study, Hg content found in the species might come from natural volcanic activity of the Galapagos Islands, differently to our results in which fish species come from the Ecuadorian coast which is principally affected by sources of anthropogenic contamination.

According to the [29], fish with mercury content above 0.46 $\mu g \cdot g^{-1}$, as marlin and shark, are fish choices to avoid.

A United Kingdom review published in 2004, reported that one portion of shark or marlin (140 g) would result in a dietary MeHg exposure close to or above 3.3 $\mu g \cdot g^{-1}$ BW per week limit. Consumption of fish at this level could harm the fetus of women who are pregnant or become pregnant within a year, given the half-life of MeHg in humans of about 70 days [8]. Blue marlin is a pelagic billfish that show the highest mercury levels of all fish species [20,61]. Variations of THg concentrations in marlin species from different sites could be attributed to different factors such as body length, age, food habits, elemental bioavailability, and contaminant sources [73].

Quantities of safe ingestion of fish meat were calculated (Table 1). Dolphinfish, croaker, and snapper are considered good choices [29], however, according to the results obtained in this study, consumption of dolphinfish is recommended to be limited to one serving per week. Considering the highest THg content found in dolphinfish, its maximum quantities suggested for consumption per week are 14, 59, and 69 g for children, women, and men respectively. In the study performed by Araújo & Cedeño-Macias [4], 178 g per week for common dolphinfish was assessed as the maximum quantity of consumption for adults. These values were obtained considering a PTWI for inorganic mercury of 4 $\mu g \cdot kg^{-1}$ (BW). Considering a scenario of a PTWI of 1.6 $\mu g \cdot kg^{-1}$ (BW) and according to the THg concentration of 1.6 $\mu g \cdot g^{-1}$ [4], 70 g per week would be the suggested ingestion portion, which is in good agreement with the present study.

[15] and [66] assessed the human health risks associated with different shark species for consumption in Ecuador. The former showed that an adult person (considering 60 kg of BW) should consume only up to 30 g per week of blue shark (*Prionace glauca*) meat to reduce possible risks, while children (considering 30 kg) can consume up to 14.5 g per week. The latest indicated that none of the analyzed species is edible, except for pelagic thresher (*Alopias pelagicus*), limited to one portion per week. However, it is well known that the consumption of shark meat represents a serious human health risk for the populations [46,60,90], as this fish species has a slow-growing and tends to accumulate a large amount of MeHg [34].

All the THQ values calculated for the five fish types under study were lower than one for children, women, and men, even considering the samples with the highest THg concentration in edible tissue (0.230, 0.111, and 0.095 in blue marlin for children, women, and men, respectively). These results suggest that there is no significant potential health risk for the exposed population over a lifetime, considering a fish intake once per week. Also, the WHO [104] reported that intakes of fish up to about two times higher than the PTWI would not pose risks of neurotoxicity in adults, although, in the case of women of childbearing age, the intake should not exceed the PTWI, to protect the embryo and fetus.

For the present study, samples came from Esmeraldas, located in Ecuador's coastal zone. In this province, illegal, artisanal, and small-scale mining is carried out, where Hg is widely used for gold amalgamation, and impacts on the rivers in the north of the province due contamination by gold mining have been reported [68,74]. Hg water pollution is also attributed to current systems that can move metals from industrialized countries such as Japan and China towards the central Pacific [4]. Moreover, untreated domestic and industrial water discharges into the coastal zone, as well as runoff from crop areas, and mining tailings released into rivers [87], contribute to the Hg burden in the world̓s oceans. Approximately, 300 tonnes per year of Hg are transported from rivers, representing 130% of the estimated global Hg budget ([86]; United Nations Environment Programme [UNEP], Chemicals and Health Branch Geneva, 2019).

Volcanic activity has also been considered a contributor to high Hg levels in fish from the Eastern Equatorial Pacific [4]. According to [6], high Hg concentrations are particularly found in volcanic regions. Five hundred tonnes per year of Hg are released directly into the atmosphere from volcanoes [94], contributing to the total atmospheric Hg input. On the other hand, in Ecuador, the presence of non-essential metals such as Hg is mainly attributed to geological environments and geological processes of volcanic-origin soils which are the most abundant in the country [80]. The Andean region and the Andean Amazon have been considered active mercury belts due to the constant volcanic and tectonic activity [59]. It has been reported that rivers drain Andean volcanic soils [99], affecting the lower parts of the Amazonian river network with metals such as Hg [12]. In the Ecuadorian Amazon, a link between deforestation, soil erosion, and the leaching of naturally occurring Hg has been demonstrated [59], additionally, in the Brazilian Amazon, erosion increased the superficial sediment mercury concentrations in the different aquatic systems [81].

3.2. Lipid content and fatty acids profiles

In the present study, lipid content was determined gravimetrically as the free-solvent oil extracted from the dry muscle, expressed in fresh

Table 2
Fatty acids profile (%) of selected fish species marketed in Quito – Ecuador.

Fatty acid (%) $\bar{x} \pm s$ (range)	Croaker (*Cynoscion spp.*)	Snapper (*Lutjanus peru*)	Dolphinfish (*Coryphaena hippurus*)	Blue marlin (*Makaira nigricans*)	Shark (*Mustelus mento*)
n	15	8	9	8	15
C14:0	0.44 ± 0.19 (0.16 – 0.84)	1.65 ± 0.62 (0.83 – 2.67)	0.57 ± 0.42 (0.20 – 1.66)	0.52 ± 0.19 (0.36 – 0.96)	0.25 ± 0.17 (0.08 – 0.74)
C15:0	0.42 ± 0.16 (0.21 – 0.79)	0.53 ± 0.11 (0.39 – 0.71)	0.40 ± 0.20 (0.10 – 0.69) [a]	0.47 ± 0.10 (0.36 – 0.63)	0.10 ± 0.08 (0.03 – 0.37)
C16:0	29.01 ± 3.44 (23.84 – 35.58)	32.16 ± 1.73 (28.71 – 34.13)	19.75 ± 2.90 (16.00 – 24.26)	24.07 ± 2.01 (20.75 – 26.61)	19.92 ± 4.61 (13.60 – 30.45)
C17:0	0.73 ± 0.21 (0.38 – 1.16)	1.12 ± 0.14 (0.92 – 1.37)	0.90 ± 0.30 (0.48 – 1.22)	1.58 ± 0.28 (1.21 – 1.99)	0.44 ± 0.21 (0.21 – 0.96)
C18:0	10.15 ± 1.33 (8.60 – 13.66)	12.20 ± 0.85 (11.05 – 13.94)	12.71 ± 3.32 (8.94 – 19.74)	12.85 ± 0.86 (12.01 – 14.52)	19.48 ± 4.32 (14.33 – 25.55)
C20:0	0.22 ± 0.01 (0.21 – 0.23)[a]	0.39 ± 0.09 (0.28 – 0.55)	0.20 ± 0.04 (0.14 – 0.25) [a]	0.25 ± 0.03 (0.23 – 0.28)[a]	–
C21:0	0.24 ± 0.07 (0.19 – 0.29)[a]	0.09 ± 0.02 (–)[a]	–	–	0.32 ± 0.22 (0.10 – 0.71)[a]
C22:0	–	0.17 ± 0.05 (0.12 – 0.24)[a]	0.06 ± 0.02 (–)[a]	–	–
C23:0	–	–	0.11 ± 0.04 (0.08 – 0.16) [a]	–	0.29 ± 0.01 (–)[a]
C24:0	–	0.29 ± 0.09 (0.18 – 0.47)	0.24 ± 0.11 (0.13 – 0.35) [a]	0.25 ± 0.21 (–)[a]	–
Σ SFA	**40.78 ± 3.75 (35.44 – 47.81)**	**48.47 ± 2.61 (43.37 – 51.88)**	**34.49 ± 4.06 (28.70 – 41.27)**	**39.61 ± 1.56 (37.74 – 41.99)**	**40.63 ± 2.99 (34.06 – 48.12)**
C14:1 n5	–	–	0.14 ± 0.06 (0.07 – 0.21) [a]	–	0.08 ± 0.04 (0.03 – 0.15)[a]
C15:1n5	0.18 ± 0.06 (0.11 – 0.29)[a]	0.49 ± 0.14 (0.24 – 0.73)	6.83 ± 2.09 (4.04 – 9.75)	4.97 ± 0.70 (3.86 – 6.05)	5.82 ± 2.20 (1.76 – 9.86)
C16:1n7	2.17 ± 0.45 (1.54 – 3.05)	2.56 ± 0.68 (1.49 – 3.61)	1.27 ± 0.52 (0.59 – 2.55)	1.02 ± 0.24 (0.80 – 1.52)	1.07 ± 0.53 (0.43 – 2.00)
C17:1n7	0.56 ± 0.16 (0.24 – 0.74)[a]	0.47 ± 0.04 (0.40 – 0.51)	0.35 ± 0.17 (0.12 – 0.61)	0.36 ± 0.06 (0.30 – 0.46)	0.21 ± 0.09 (0.12 – 0.43)[a]
C18:1t9	–	–	0.26 ± 0.14 (0.14 – 0.49) [a]	0.30 ± 0.12 (0.22 – 0.53)[a]	0.20 ± 0.05 (0.12 – 0.24)[a]
C18:1n9	19.45 ± 4.77 (11.26 – 25.23)	20.74 ± 3.00 (16.36 – 24.55)	11.64 ± 3.39 (7.51 – 18.50)	12.23 ± 1.31 (9.29 – 13.85)	11.82 ± 3.10 (6.50 – 16.92)
C20:1n9	0.44 ± 0.18 (0.19 – 0.70)	0.51 ± 0.10 (0.37 – 0.67)	0.71 ± 0.34 (0.25 – 1.25)	0.41 ± 0.08 (0.34 – 0.59)	1.56 ± 0.60 (0.61 – 2.82)
C22:1n9	–	0.26 ± 0.09 (0.17 – 0.40)	0.31 ± 0.16 (0.19 – 0.60)	0.29 ± 0.18 (0.05 – 0.51)[a]	0.16 ± 0.00 (–)
C24:1n9	–	0.28 ± 0.06 (0.21 – 0.38)	0.27 ± 0.12 (0.17 – 0.40) [a]	0.39 ± 0.11 (0.32 – 0.59)[a]	–
Σ MUFA	**22.67 ± 4.88 (14.57 – 29.01)**	**25.29 ± 3.57 (20.13 – 30.19)**	**21.36 ± 4.41 (14.41 – 27.81)**	**19.63 ± 0.95 (18.05 – 20.42)**	**20.31 ± 3.17 (13.27 – 25.72)**
C18:2n6	0.91 ± 0.20 (0.68 – 1.25)	0.67 ± 0.10 (0.57 – 0.90)	0.62 ± 0.14 (0.46 – 0.83)	0.99 ± 0.06 (0.91 – 1.07)	0.64 ± 0.29 (0.35 – 1.35)
C18:3n3	–	0.14 ± 0.03 (0.08 – 0.19)	0.18 ± 0.08 (0.10 – 0.33) [a]	0.20 ± 0.01 (–)[a]	–
C20:2n6	0.30 ± 0.07 (0.19 – 0.41)	0.35 ± 0.09 (0.25 – 0.54)	0.41 ± 0.09 (0.29 – 0.56)	0.42 ± 0.07 (0.32 – 0.57)	0.49 ± 0.19 (0.29 – 0.96)
C20:3n6	0.17 ± 0.04 (0.12 – 0.24)[a]	0.13 ± 0.03 (0.10 – 0.18)[a]	0.20 ± 0.12 (0.07 – 0.33) [a]	0.21 ± 0.02 (0.19 – 0.23)[a]	0.26 ± 0.07 (0.16 – 0.38)[a]
C20:4n6	5.47 ± 0.94 (3.98 – 7.67)	3.48 ± 0.87 (2.45 – 5.30)	7.32 ± 3.26 (3.71 – 14.16)	7.23 ± 0.65 (6.03 – 8.31)	7.23 ± 1.35 (4.31 – 9.32)
C20:5n3 (EPA)	4.22 ± 1.11 (2.63 – 6.28)	2.96 ± 0.39 (2.33 – 3.54)	3.46 ± 1.15 (1.99 – 5.27)	3.27 ± 0.43 (2.63 – 4.10)	2.86 ± 1.16 (1.08 – 4.56)
C22:6n3 (DHA)	25.58 ± 4.59 (17.75 – 32.37)	18.57 ± 4.21 (11.54 – 25.94)	32.03 ± 3.44 (26.65 – 35.63)	28.72 ± 1.82 (26.42 – 31.20)	27.58 ± 2.95 (22.07 – 33.38)
Σ PUFA	**36.52 ± 6.26 (25.61 – 46.74)**	**26.24 ± 5.38 (17.95 – 36.50)**	**44.08 ± 2.68 (37.66 – 47.39)**	**40.76 ± 2.24 (37.61 – 44.21)**	**39.02 ± 4.25 (30.31 – 46.34)**
Σ ω-3	**29.79**	**21.67**	**35.66**	**32.18**	**30.44**
Σ ω-6	**6.85**	**4.63**	**8.55**	**8.85**	**8.61**
Ratio ω-3/ω-6	**4.35**	**4.68**	**4.17**	**3.64**	**3.54**

[a] Not found in all samples; "—": Not detected; "(–)": FA detected in only one sample

tissue of each sample. Results were 0.72 ± 0.12, 0.64 ± 0.15, 0.81 ± 0.08, 0.83 ± 0.47, 1.00 ± 0.47 g·100 g^{-1} of fresh muscle for croaker, snapper, dolphinfish, blue marlin, and shark, respectively. In previous studies in croaker, the lipid content determined was about double than our findings (1.30 g·100 g^{-1} meet) [67]. Similar higher results were found for red snapper (1.4 g·100 g^{-1} meet) [16], dolphinfish (2.05 g·100 g^{-1} meet) [92], and blue marlin 2.60 g·100 g^{-1} meet [14]. For other shark species, results had lower lipid content (0.6 – 0.8 g·100 g^{-1} meet) [16].

Fish have been historically considered the principal source of EFAs in the human diet. In addition, fish and other seafood have also a well-balanced amino acid composition and contain taurine and choline, vitamins and minerals [50]. Even though the samples in our study presented a low percentage of total lipids in the fresh muscle, considering their valuable content of unsaturated fatty acids (EPA and DHA), they

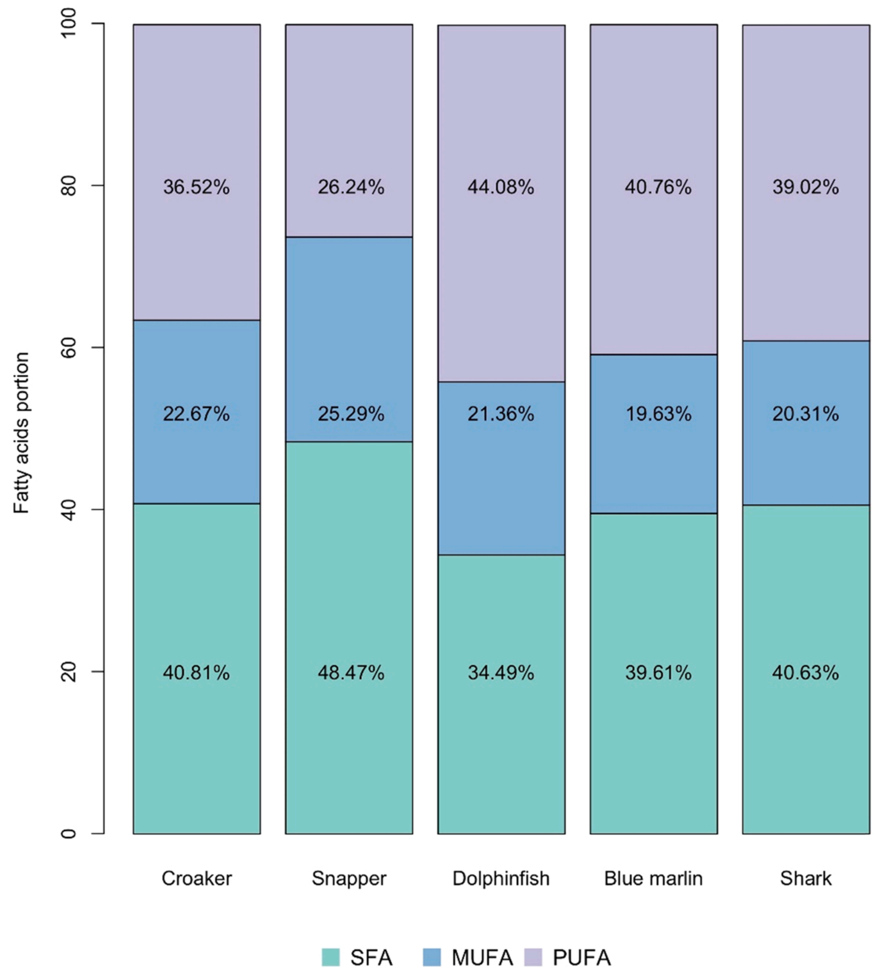

Fig. 2. Portions (%) of total saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in the free-solvent lipid content of the five fish types studied.

can represent one of the nutritional sources for the human diet.

Regarding fatty acids content, the five fish types showed a wide variability of compounds and portions (Table 2). The major saturated fatty acids (SFA) were palmitic acid (C16:0; between 13.60% for shark and 35.58% for croaker) and stearic acid (C18:0; 8.60% in croaker – 25.55% in shark). For monounsaturated fatty acids (MUFA), the major were palmitoleic acid (C16:1 n7; ranging between 0.43% in shark to 3.61% in snapper), and oleic acid (C18:1 n9; 6.50% in shark and 25.23% in croaker). Finally, the major polyunsaturated fatty acids, arachidonic acid (ARA, C20:4n6; between 2.45% in snapper and 14.16% in dolphinfish), eicosapentaenoic acid (EPA; 20:5n3; 1.08% in shark to 6.28% in croaker), and docosahexaenoic acid (DHA; 22:6n3; 11.54% for snapper and 35.63% for dolphinfish).

Moreover, in some cases, species had a particular amount of specific fatty acids —i.e. in the case of snapper, a higher portion of miristic acid (C14:0) was found compared with the other fish types under study. Also dolphinfish, blue marlin, and shark presented high portions (3.86 – 9.86%) of 5-pentadecenoic acid (C15:1n5) compared to croaker and snapper (0.11 – 0.73%) — (Fig. 2).

Other MUFAs were not found in all fish types. Miristoleic acid (C14:1n5) was present only in dolphinfish and shark; meanwhile, elaidic acid (18:1t9) was only found in dolphinfish, blue marlin, and shark. Snapper, dolphinfish, and blue marlin presented low values of α-linolenic acid (C18:3n3).

Mehta & Nayak, [67] reported a higher portion of SFA in croaker (70.07 ± 1.45%) than our study. In addition, the portions of EPA and DHA were not similar to our results, 3.55 ± 0.08%, and 11.36 ± 1.98%, respectively. Similar SFA content (41.72%) in snapper was found by Santamaría-Miranda et al., [84], however, C20:4n6 (8.28 ± 0.59%) and EPA (6.92 ± 0.18%) were about the double of the reported values in the present study. For dolphinfish, all the values reported by [9] were similar to ours, for both SFA (31.50%) and individual proportions of C16:0, C18:0, C18:1n9, C20:4n6, EPA, and DHA. Also for blue marlin, both SFA (32.10%) and individual proportions of C16:0, C18:0, C18:1n9, EPA, DHA were similar, except for C20:4n6 (4.70%) which was lower than our findings. Finally, for shark, no fatty acids information was found regarding *Mustelus mento* specie. However, in other shark species such as *Carcharhinus leucas*, *Glyphis garricki,* and *Glyphis glyphis*, Every et al., [27] found low proportions of SFA, MUFA, and PUFA. Significant lower proportions of EPA and DHA were detected. For EPA, values ranged between 0.52% and 0.94%, while DHA showed proportions of 4.25 – 7.97%, for the three shark species.

It has been reported that marine predators as sharks, generally have very low carbohydrate diets, and amino acids from protein intake can be broken down, producing fewer and simpler FA, restricted to 14:0, 16:0, and 18:0 saturated FA and their monounsaturated isomers 14:1n-5, 16:1n-7, and 18:1n-9, respectively [44]. Nevertheless, different shark tissues have been found to store higher saturated fats and polyunsaturated fats in structural tissues (e.g., muscle), whereas higher monounsaturated fats are often found in tissues used for energy storage (e.g., liver) [27].

The wide inter- and intra-specific variation of lipids have been strongly associated with several factors as diet [53,85] mainly because fatty acids content is attributed to the different trophic levels [39],

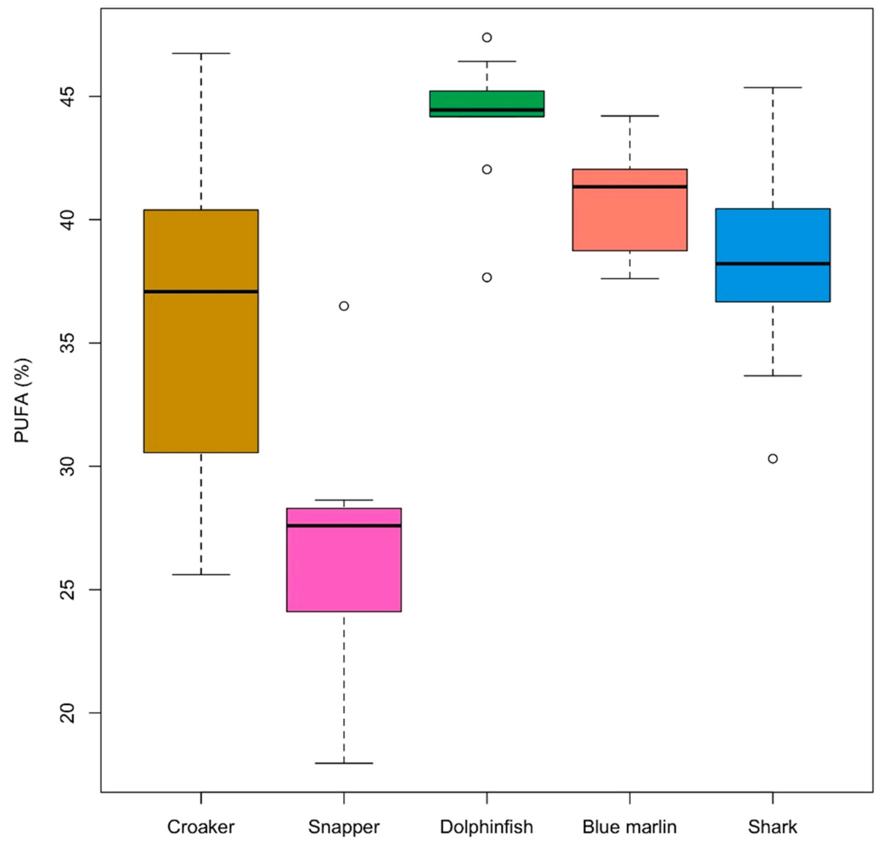

Fig. 3. Boxplot of polyunsaturated fatty acids (PUFA) (%) for the five fish types under study.

Table 3
Hazard quotient for the benefit-risk ratio of total mercury and essential fatty acids content.

HQ_{EFA}	Croaker (*Cynoscion spp.*)	Snapper (*Lutjanus peru*)	Dolphinfish (*Coryphaena hippurus*)	Blue marlin (*Makaira nigricans*)	Shark (*Mustelus mento*)
Children	19,2	30,8	60,2	219,5	57,6
Women	4,6	7,4	14,5	53,0	13,9
Men	4,0	6,4	12,5	45,5	11,9

oceanographic conditions [1], and seasonal variability [108,18,89].

Kruskall Wallis test showed differences between PUFAs concentration among the five fish types, and Wilcox test confirmed that PUFAs content in dolphinfish differs from other fish types, showing the highest levels (Fig. 3).

Essential fatty acids, EPA and DHA, have shown significant benefits in cardiovascular, cognitive, and eye health, lowering the risk of coronary heart disease (CHD) death and sudden death [5,71]. Before the introduction of agriculture, the diet of hunter-gatherer societies was mainly based on the consumption of animals, including meat, fish, and nuts, which provided greater amounts of ω-3 LC PUFA [50]. After the agricultural revolution until now, the human diet has drastically changed, increasing the consumption of cereals and vegetable oils rich in ω-6 PUFA and low in ω-3 PUFA. However, human genetics have not been designed for drastic changes in the human diet, so the human organism has remained adapted to a higher ω-3 PUFA intake than the actual consumption [50].

Due to the high consumption of ω-6 fatty acids, the ratio of ω-3/ω-6 may have decreased [17]. The Food and Agriculture Organization recommends the consumption of EFAs in a ratio of 1:5 or 1:10 [67,72]. Therefore, a modest consumption of 250–500 $mg{\cdot}d^{-1}$ of EPA and DHA has been suggested to lower CHD risk by 25% or more [71,96]. For the present study, a recommended daily intake of 250 mg of EFAs has been considered.

The ratios ω-3/ω-6 from all the species studied are much higher than the recommendations, suggesting a benefit due to the consumption of the lipids in these species. Regarding previous studies related to the ω-3/ω-6 ratio, Mehta & Nayak, [67] reported 3.11 for croaker, fairly lower than our findings (Table 2).

Although the five species presented a high ω-3/ω-6 ratio, the low level of total fatty acids found in the fresh muscle to the risk of MeHg content present in the samples, showed a benefit-risk ratio HQ_{EFA} greater than 4.0 (Table 3). In addition, based on the MoS values, dolphinfish, blue marlin, and shark consumption should be avoided by children and pregnant women, since MeHg may cause major risk to these critical subpopulations [62,104].

To reach the amount of EFAs that would provide health benefits from consuming any of the five fish types, populations would need to eat high amounts of fish per serving, however, this would increase the amount of MeHg ingested and human risk. A study performed by [56] reported a hazard quotient for the benefit–risk ratio HQ_{EFA} below 1, ranging from 0.07 in mackerel to 0.90 in tench. These findings suggest that the intake of EPA + DHA and the concentration of THg in fish poses no evident risk to human health. On the other side, authors stated that the differences in the chemical composition of fish, including fatty acids, are related to the fish species, but are also conditioned by other individual and environmental factors [56].

The first axis (46.96%) of the PCA (Fig. 4) explains the variation mainly from EPA and DHA variables, while the second PCA axis (37.61%) was based on the THg and EPA content. The graph shows that croaker and dolphinfish differentiate from the other fish groups by their EFAs levels while having, at the same time, less concentrations of THg making them good dietary options. Blue marlin presents high THg concentrations and low EFAs levels. This fish group should be avoided

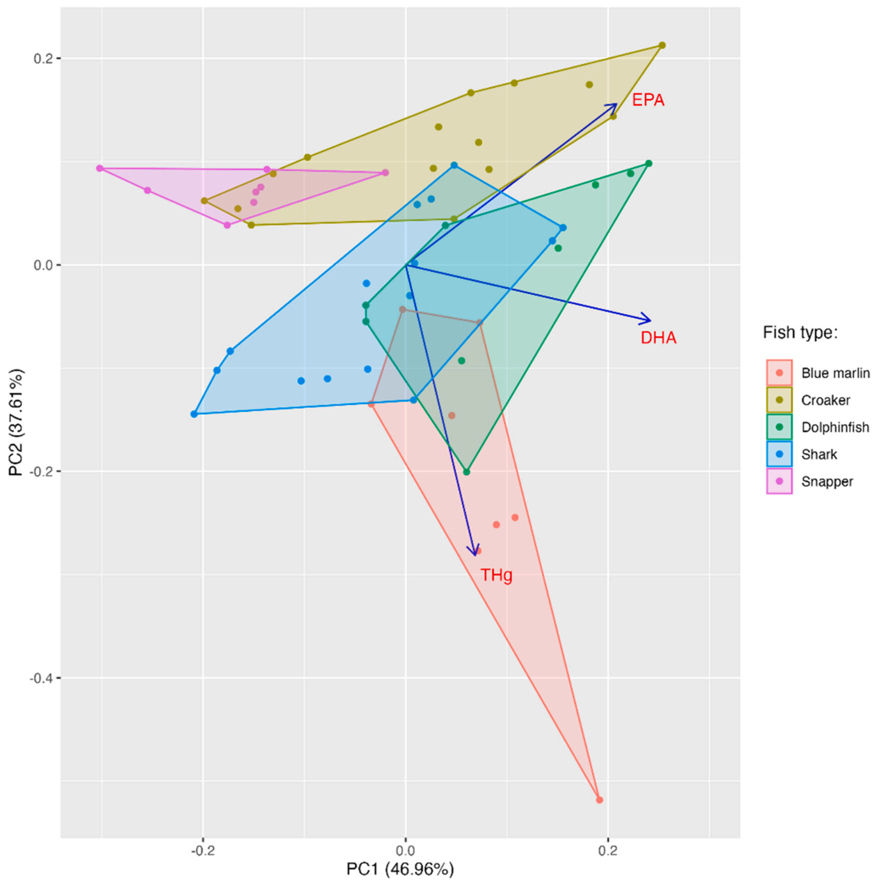

Fig. 4. Principal Component Analysis (PCA) correlation plot showing THg concentrations and EFAs content in the five fish types under study.

for human consumption. Even though studies have demonstrated not significant relation between mercury and omega-3 PUFAs [77,102], it has also been reported that the protective effects of fish consumption and PUFAs may be attenuated by Hg content found in fish; and reciprocally, the harmful effects of MeHg may be attenuated by PUFAs and fish consumption [24]. [82] reported the positive correlation between THg and DHA, which exert opposing effects on growth and function of the developing brain, and are regulated by the amount and species of fish consumed by pregnant women.

Despite the fact that fish is a source of proteins and PUFAs, MeHg can pose a serious health threat to pregnant women, fetuses, and young children. MeHg can cross the placenta, penetrate the blood-brain barrier and concentrate in the fetus, resulting in neurodevelopmental delays, behavioral changes, and reduced cognitive and motor ability [7,47,55,63].

The FDA & US-EPA have provided consumption advice related to the best seafood choices higher in the EPA and DHA and lower in MeHg [93]. Thereby Ecuador's national authorities should establish mechanisms to assess and identify the MeHg content in fish and shellfish, which are locally sold, to protect the human health of consumers, increasing omega-3 PUFAs intake by eating fish species with low MeHg content, and reducing MeHg exposure from large and carnivorous fish [58,82].

Given the country's poor economic conditions in rural communities located mainly in the Amazon and Coastal zones, rural populations consume more seafood. These populations are not able to consume various types of food products, and Hg exposure in rural communities could pose a health threat, as it has been shown that socio-cultural factors are important in determining mercury exposure [100]. Additionally, national authorities could develop strategies to determine good fish choices or those to avoid considering the natural and anthropogenic pollution background of Ecuador.

4. Conclusions

Even though THg content in all fish species under study was variable, blue marlin, shark, and dolphinfish had the highest concentrations. Omega-3-fatty acids' levels in shark were higher compared to the other fishery products, followed by dolphinfish with an assessed value of EPA + DHA of 248.6 mg, which reaches the recommended daily intake for an adult. In a daily diet based on the fish types under study, the hazard quotient for the benefit-risk ratio is above one, suggesting an evident risk to human health. On the other hand, the calculated THQ for MeHg showed that there is no significant potential health risk for the exposed population over a lifetime for all the species analyzed, considering one weekly intake.

Based on our results, consumption of croaker and dolphinfish is recommended up to one serving per week, in view of the importance of EFAs intake and avoiding fish with elevated MeHg levels, such as blue marlin and shark. Regarding to snapper, neither benefits nor risks were found in terms of EFAs and MeHg, respectively.

Further investigations should be conducted for other frequently consumed species in Ecuador, including more markets along the country, to evaluate their compliance with THg permissible limits; provide information to enhance Ecuadorian public standards for seafood safety, especially for pregnant women and young children; and incorporate information related to EFAs, which can counteract some of the adverse effects of Hg.

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CRediT authorship contribution statement

Gabriela S. Yánez-Jácome: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **David Romero-Estévez:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Pamela Y. Vélez-Terreros**: Writing – review & editing. **Hugo Navarrete:** Conceptualization, Project administration, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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