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Human Health Risk of Methylmercury from Fish Consumption at the largest floodplain in Colombia

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4 **Abstract**
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7 Colombia is one of the countries with the highest emissions of mercury (Hg) to the
8 environment, due to its use in gold mining. This study evaluated the human health risk
9 from exposure to Hg through fish consumption in 11 municipalities located in the Mojana
10 region, northern Colombia. The study participants (n = 839) were categorized into three
11 population groups: children (CHD), women of childbearing age (WCHA) and the rest of
12 population (RP). Total Hg (THg) and methylmercury (MeHg) concentrations were
13 evaluated in the top ten most consumed fish species (n = 292). Median concentrations
14 (range: 0.22 – 0.58 mg/kg ww) of the five carnivorous fish species exceeded the reference
15 limit established by FAO/WHO (0.2 µg/g ww) for vulnerable populations. For 6 of the 10
16 studied fish species, the estimated weekly intake (EWI) in children was above the
17 provisional tolerable weekly intake (PTWI, 1.6 µg/kg bw/week) of MeHg established by
18 JECFA. EWI values for WCHA were above PTWI in 4 fish species, whereas in general, for
19 RP group values were below PTWI (3.2 µg/kg bw/week). Our assessment of potential risks
20 to MeHg exposure indicated that most of the consumed fish could generate negative
21 effects in vulnerable groups, because according to ingestion rate, MeHg permissible is, in
22 some cases, up to 4 times higher than reference limits. Consumption advisories should be
23 a guidance to avoid risk, gain nutritional benefits, and sustain fish populations. Because its
24 high MeHg levels as well as high ingestion, it is recommended that inhabitants of this
25 region should stop eating certain kinds of fish and the whole fish as a single meal. To
26 reduce its harmful intake, we have proposed a didactic strategy based on marbles that
27 control the portion of fish they are eating.
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54 **Key words:** gold mining; contaminated fish; estimated weekly intake; public health;
55 vulnerable populations; Mojana region; Colombia.
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4 **1. Introduction**
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7 Food security is a major global concern, given the demographic expansion and the
8 decreasing availability of food resources (FAO, 2017). Fish plays an essential role, since it
9 can help to reduce the indices of malnutrition, by their content of essential amino acids,
10 protein of high quality, essential fatty acids, vitamins, and minerals (FAO, 2016; Kris-
11 Etherton et al., 2002). In addition, the consumption of fish reduces the risk of coronary
12 heart disease, diabetes and hypertension and contributes to the development and fetal
13 growth (Fuentes-Gandara et al., 2018a; Swanson et al., 2012).
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23 According to the United Nations Development Programmed (UNDP), in the coastal
24 populations of Colombia, a 90% of the animal protein consumption comes from fish,
25 mainly as the result of very low access to other types of proteins. Nevertheless, the high
26 levels of metals currently reported in rivers such as the San Jorge, Cauca, and
27 Magdalena, have limited the consumption of this food source to the coastal population in
28 northern areas where there is no mining exploitation (Fuentes-Gandara et al, 2018b). In
29 the Mojana region, located in northern Colombia, it is estimated that in the 11
30 municipalities that make up the region, there are about 35,000 people dependent on
31 fishing, making fish the most important source of protein supply for the inhabitants of the
32 shores of wetlands, marshes and streams (DNP, 2012; Marrugo-Negrete et al., 2008).
33 However, this zone is under strong anthropic pressure, including the discharge of large
34 quantities of wastewater, both domestic and mining wastes containing mercury (Hg) from
35 artisanal gold mining processes in the mining areas of southern Bolívar, northern
36 Antioquia, and the upper part of the San Jorge River (Marrugo-Negrete et al., 2008; 2015,
37 2018). The deposition in sediments of toxic metals such as Hg and their transfer to the
38 water column, leads to their bioaccumulation and biomagnification in the aquatic food web,
39 representing a great risk to environmental sustainability and the health of fish ultimate
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4 consumers (Nawab et al., 2018). The risk assessment associated to the exposure of the
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6 Mojana population to Hg and in particular to MeHg through fish consumption is interesting
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8 considering their proven adverse effects in humans, such as cognitive deficit in children
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10 even at trace levels (Freire et al, 2010, Díez, 2009). The formation of MeHg from inorganic
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12 Hg is of special concern because, this organic form of Hg, is easily bioaccumulated and
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14 biomagnified in aquatic food webs (Driscoll et al., 2013). Moreover, the greatest source of
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16 protein intake of the population, could also be one of the inputs of this neurotoxic into the
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18 population, due to bioaccumulation processes (Wang et al., 2019; Salazar-Camacho et al.,
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20 2017).

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25 Consequently, the aim of this work was to assess the risk to human health posed by Hg
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27 exposure due to the consumption by riverine populations of the most common fish in the
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29 Hg-impacted Mojana region of Colombia. Likewise, this study intends to provide practical
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31 tools for the inhabitants of the Mojana region to initiate their own consumption strategies,
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33 taking into account that the suppression of fish from the diet is often not an option for these
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35 inhabitants because fish consumption is their major source of protein.
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42 **2. Materials and methods**

43 44 *2.1 Study area, collection and processing of samples*

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47 The Mojana region is located in the Northwest of Colombia (8°00'- 9° 30' N, 75° 15'- 73°
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49 45' W). It has an area of 5.545 km² and a population of 413.604 inhabitants (DANE, 2017),
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51 it includes the municipalities of Achí, Ayapel, Caimito, Guaranda, Majagual, San Benito
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53 Abad, San Marcos, Magangué, San Jacinto del Cauca, Nechí and Sucre (Fig. 1). The
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55 region is surrounded by three rivers: Magdalena, Cauca and San Jorge. During the rainy
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57 season (May - December), this area receives discharges of Hg and other heavy metals
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4 transported by the Cauca River from gold mining residues in the mines located in the
5 departments of Bolívar and Antioquia, connected to the Mojana region (Calao & Marrugo-
6 Negrete, 2015).
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11 From April to June 2018, 292 samples of the ten most consumed fish species were
12 obtained with the inhabitants living in the above-described municipalities. The samples
13 were individually packed in plastic bags, labeled and refrigerated at 4 °C for later transport
14 to the laboratory. In the laboratory, the dorsal muscle was subsampled for each caught fish
15 (3 cm and 10 g) was stored at -20 °C until analysis. The determination of total Hg (THg)
16 and MeHg was performed individually for each dorsal muscle.
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25 *2.2 Analysis of Hg and MeHg*

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28 For THg analysis, 0.02 g of lyophilized fish sample was weighed, then introduced into the
29 direct mercury analyzer DMA 80 Tricell Milestone, according to the EPA method 7473
30 (EPA, 1998). The accuracy of the method was determined by the analysis of certified
31 reference material (DORM-2, dogfish-muscle) samples from the National Research
32 Council of Canada (NRCC). The analyzed samples were determined with a calibration
33 curve whose working range was 0.05-800 ng Hg. The estimated correlation coefficient was
34 0.9993. The recovery percentage was determined doping samples of fish with known
35 concentrations of THg and MeHg. The THg concentration determined in DORM-2 was
36 $4.62 \pm 0.12 \mu\text{g/g}$ (certified value $4.64 \pm 0.26 \mu\text{g/g}$) with an average recovery of 99.6% (n =
37 3). A detection limit (LOD) of $0.003 \mu\text{g/g}$ was calculated as 3 times the standard deviation
38 of a series of blank samples (n = 3). The limit of quantification (LOQ) was calculated by
39 multiplying the standard deviation by ten ($0.01 \mu\text{g/g}$).
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56 MeHg was extracted from fish samples with hydrobromic acid and toluene. The extract
57 was then mixed with a solution of L-cysteine. An aliquot of 100 μL was taken from the
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4 aqueous phase for analysis in DMA 80 TriCell Milestone (EUR-25830 EN-2013) (Cordeiro
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6 et al., 2013). The concentration of MeHg determined in DORM-2 was $4.43 \pm 0.07 \mu\text{g/g}$,
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8 with an average recovery of 99% ($n = 3$). The LOD was $0.007 \mu\text{g/g}$, whereas LOQ was
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10 $0.023 \mu\text{g/g}$.

11 12 13 14 *2.3 Data analysis*

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16 Kolmogorov–Smirnov and Shapiro-Wilk were used to evaluate the normality of the data.
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18 The centre and dispersion of the samples did not follow a normal distribution and thus the
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20 median and the 75% confidence intervals (inter-quartile range) were used for descriptive
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22 analysis. A p value of 0.05 was chosen to indicate the statistical significance. The
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24 statistical analysis were performed using the IBM SPSS statistics, version 23.
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28 29 *2.4 Human health risk assessment*

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31 The Codex Alimentarius risk assessment for food safety (UNEP/WHO, 2008) was used to
32
33 determine the risks of dietary habits and food consumption of the region's population,
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35 including enquiries of the National Institute for Drug and Food Surveillance (INVIMA) and
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37 the Food Safety Risk Assessment Unit (ERIA) (INS/ERIA, 2015). In the assessment of
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39 potential health risk, 839 volunteers were studied. The volunteers replied to a
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41 questionnaire in which they indicated their gender, educational status, average body
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43 weight and dietary habits. In particular, the questionnaire included questions about the
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45 habitual intake of fish, focusing on the frequency they consumed fish, including the number
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47 of fish meals consumed per week and the type of fish. Subjects were categorized into
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49 three population groups: 264 children (CHD) (2 - 15 years old), 267 women of childbearing
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51 age (WCHA) (16 - 49 years old) and 308 respondents called the rest of the population
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53 (RP) (men >15 years and women > 49 years). The general characteristics of each
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4 population group are presented in Table 1. Details for all the municipalities are provided in
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6 Supplementary Material, Tables S1-S2.
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9 Potential risk of human exposure to Hg was assessed with the estimated weekly intake
10 (EWI) of Hg per kg of bodyweight of the studied individual ($\mu\text{g}/\text{kg}$ body weight (bw)/week)
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12 using the equation described by UNEP (2010):
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$$15 \quad 16 \quad 17 \quad 18 \quad 19 \quad 20 \quad 21 \quad 22 \quad 23 \quad 24 \quad 25 \quad 26 \quad 27 \quad 28 \quad 29 \quad 30 \quad 31 \quad 32 \quad 33 \quad 34 \quad 35 \quad 36 \quad 37 \quad 38 \quad 39 \quad 40 \quad 41 \quad 42 \quad 43 \quad 44 \quad 45 \quad 46 \quad 47 \quad 48 \quad 49 \quad 50 \quad 51 \quad 52 \quad 53 \quad 54 \quad 55 \quad 56 \quad 57 \quad 58 \quad 59 \quad 60 \quad 61 \quad 62 \quad 63 \quad 64 \quad 65$$
$$EWI = \frac{IR \times C}{BW} \quad (1)$$

21 where C is the median concentration of MeHg ($\mu\text{g}/\text{kg}$) in fish, IR is the weekly intake
22 (g/week) of fish, and BW is the bodyweight of the person (kg). The IRs were calculated
23 taking into account the consumed portion of fish (g), the number of portions (day), and the
24 frequency of consumption (days/week) in each of the different municipalities of the Mojana
25 region.
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33 The concentration of MeHg that the consumed fish species should contain to avoid
34 exceeding the EWI concentration established by FAO/WHO (2007) (e.g. the PTWI) was
35 estimated by considering the EWI and the amount of fish eaten per week. Thus, MeHg
36 permissible was calculated by the following equation:
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$$[\text{MeHg}]_{\text{permissible}} = \frac{C \times \text{PTWI}}{\text{EWI}} \quad (2)$$

49 where C is the median concentration of MeHg ($\mu\text{g}/\text{kg}$) in fish and PTWI is the provisional
50 tolerable weekly intake, a reference value established by the Joint FAO/World Health
51 Organization (WHO) Expert Committee on Food Additives (FAO/WHO, 2017). For women
52 of childbearing age and children, its value is $1.6 \mu\text{g}/\text{kg}$ bw/week and for the rest of the
53 population is $3.2 \mu\text{g}/\text{kg}$ bw/week (JECFA, 2006).
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4 Considering the MeHg concentrations of the fish consumed by the inhabitants of the study
5 area, we estimated the maximum amount of fish (in g) that people should eat weekly to
6 avoid Hg exposure (MFW).
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$$MFW = \frac{PTWI \times IR}{EWI} \quad (3)$$

17 Finally, the formula proposed by Zhang et al. (2019) was used to reflect the degree of Hg
18 contamination in the most consumed fish species:
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$$21 \quad 22 \quad 23 \quad 24 \quad 25 \quad 26 \quad 27 \quad 28 \quad 29 \quad 30 \quad 31 \quad 32 \quad 33 \quad 34 \quad 35 \quad 36 \quad 37 \quad 38 \quad 39 \quad 40 \quad 41 \quad 42 \quad 43 \quad 44 \quad 45 \quad 46 \quad 47 \quad 48 \quad 49 \quad 50 \quad 51 \quad 52 \quad 53 \quad 54 \quad 55 \quad 56 \quad 57 \quad 58 \quad 59 \quad 60 \quad 61 \quad 62 \quad 63 \quad 64 \quad 65$$
$$P_i = \frac{C_i}{S_i} \quad (4)$$

26 where P_i is the pollution index, C_i and S_i are the median concentration of the metal in the
27 fish muscle and the value of the evaluation criteria, respectively. Two reference limits were
28 used: 0.2 $\mu\text{g/g}$ ww for vulnerable populations such as children under 15 years of age and
29 women of childbearing according to FAO/WHO; and a threshold of 0.5 $\mu\text{g/g}$ ww according
30 to the JEFCA and the Colombian norm, Resolution 122 of 2012 (Minsalud, 2012) for the
31 rest of population.
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42 **3. Results and discussion**

46 *3.1 Most consumed fish species in the Mojana region*

48 Results of the survey show which fish species are most commonly eaten by the
49 inhabitants of the Mojana region. Although 22 fish species are consumed in the region, for
50 this study we considered the ten fish species most commonly consumed in the Mojana
51 (Fig.2). Among these, five were non-carnivorous species (NC) stand out: *Prochilodus*
52 *magdalenae*, *Oreochromis niloticus*, *Cyphocharax magdalenae*, together with *Leporinus*
53 *muyscorum* and *Pimelodus clarias*. It should be highlighted that 3 of them are within the
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4 most consumed by people in the Mojana, *P. magdalenae* about a 90%, *O. niloticus* a 35%
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6 and finally *C. magdalenae* a 33%.
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10 Globally, a per capita apparent fish consumption 19.7 of kg/year has been reported, with
11 estimates pointing to an increase in the coming years (FAO, 2016). Fish consumption is
12 very important as it might represent about 20% of the animal protein consumed by
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14 approximately 3100 million people. In Colombia, the per capita apparent fish consumption
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16 is 4.73 kg/year, which is close to 4 times lower than the average in Latin America (18
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18 kg/year) (MADR/FAO, 2015). According to the National Survey of Nutritional Status
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20 (ENSIN, 2010), only 27% of Colombians consume fish or seafood weekly, implying that
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22 the risk to health by consumption of Hg-contaminated fish is low. However, the Ministry of
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24 Agriculture and Rural Development and the FAO (2015) is highly concerned because fish
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26 consumption in Colombia is regionalized, with high consumption in areas where the
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28 purchasing power or the supply of other protein sources are low, e.g. in coastal areas. The
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30 Mojana region is of special due to the high per capita apparent fish consumption, with an
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32 average of 28 kg/year and values of 22, 30 and 32 kg/year for CHD, WCHA and RP
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34 groups, respectively (Table 1).
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41 *3.2 Concentration of THg and MeHg in most consumed fish*

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44 Median concentrations of THg and MeHg ($\mu\text{g/g ww}$) and the MeHg percentages (MeHg%)
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46 of the carnivorous (C) and non-carnivorous (NC) species reported as having the highest
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48 consumption among the inhabitants of La Mojana are described in Figure 3. Results
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50 showed that two carnivorous species (*Sorubim cuspicaudus* and *Ageneiosus pardalis*)
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52 exceeded the permissible Hg values established by the JECFA/WHO ($0.5 \mu\text{g/g ww}$), and
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54 *Caquetaia kraussii* is very close; moreover all of them exceeded the 75th percentile.
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4 THg concentration in fish were higher Hg in carnivores species than in non-carnivores; this
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6 would indicate the occurrence of biomagnification processes.
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10 There are many factors that can determine THg concentration in fish such as dietary habit,
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12 contaminant concentration, water temperature, salinity, pH, seasonal change, atmospheric
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14 conditions (El-Moselhy et al., 2014), physiology of organisms, toxicokinetics (absorption,
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16 distribution, biotransformation and excretion), toxic dynamics, species ecology (Wagner &
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18 Boman, 2003; Weber et al., 2013), exposure times (Kojadinovic et al., 2007) and
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20 intramuscular fat content (Farkas et al., 2003). The variability of the THg and MeHg
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22 concentrations in the muscle of the studied fish could be explained by one or several ones
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24 of these factors.
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28 As mentioned in the introduction, the most abundant form of Hg accumulated in fish is
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30 MeHg (Wu et al., 2019). In this study, all the fish species except one (e.g. *P. clarias*)
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32 presented a percent of MeHg as THg (MeHg%) higher than 80%. However, significant
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34 differences in MeHg% ($p < 0.05$) were found between fish species (Fig. 3). The species *P.*
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36 *magdalenae*, which is the most consumed fish in the Mojana, presented the largest
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38 MeHg%. Although the average concentration of THg and MeHg did not exceed the
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40 permissible limit established by the WHO (i.e. $0.5 \mu\text{g/g ww}$). Bradley et al. (2017),
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42 concluded that, for humans, bioaccessibility of Hg might vary from 2 to 100% for MeHg
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44 and from 0.2 to 94% for Hg. The total average absorption might range from 12-79% for
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46 MeHg to 49-69% for Hg. Hg present in fish is a toxic contaminant to humans, regardless of
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48 its chemical speciation. Therefore the high THg and MeHg concentrations measured in the
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50 studied fish combined with the high fish consumption of the region is of concern in La
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52 Mojana, specially due to long-term low dose exposure to neurotoxic MeHg.
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3.3 Risk assessment

The intake rate (IR) is the amount of fish that an individual consume per unit of time, and serves to consider the consumption preference of each person surveyed. According to WHO criteria (2008), IR values over 100 g of fish per day are considered as a high consumption, especially when evaluating vulnerable groups. Hence, in this study the consumption of more than 700 g/week of fish could be considered a high IR. As can be seen in Table 2, *P. magdalenae* is the only species that exceeded this consumption value in the three population groups, *C. magdalenae* and *C. kraussii* in the RP group, although intake for *O. niloticus* in the WCH group was very close (678 g/week). The food meal size for children in the Mojana region has an average value of 131 ± 73 g, whereas for WCHA and RP are 144 ± 80 g and 168 ± 87 g, respectively. In all the cases, these values are higher than the portion size of 114 g, agreed by the USEPA (2002). In addition, Health Canada (2008) defines as an individual with high frequency of fish consumption, those people whose consumption is equal to or greater than three times a week. In this sense, frequency of consumption in the three population groups in the Mojana was 3 days/week for the species *P. magdalenae*.

Table 2 shows the estimated weekly intake (EWI) obtained in this study, with the highest values for carnivorous fish species in CHD group (ranging from 2.2 to 5.5 $\mu\text{g}/\text{kg}$ bw/week), following the RP and WCHA groups. Values for non-carnivorous fish ranged from 0.1 to 1.9 $\mu\text{g}/\text{kg}$ bw/week in all the groups. The maximum EWI value for MeHg in the CHD group was 5.5 $\mu\text{g}/\text{kg}$ bw/week, about 3 to 4 times the PTWI. In the vulnerable groups, the highest EWIs values were recorded in *C. kraussii* for CHD, and *S. cuspidus* for WCHA; while the lowest value was recorded in non-carnivorous *O. niloticus*, about 16-fold lower than PTWI. Most of the EWI values calculated for the RP group were below the PTWI, except

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4 for *C. kraussii*, with values close to the PTWI for *P. magdaleniatum* and *S. cuspidus*,
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6 that may pose a threat to the general population.
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10 On the other hand, according to the surveys on food consumption, in all the villages of the
11 Mojana region, the amount of fish consumed weekly ranged from 1329 g/week of *P.*
12 *magdalenae* for RP group to 194 g/week of *P. clarias* for CHD group. The calculated
13 amount of allowed fish weekly (MFW) (Table 2) for *C. kraussii*, *P. magdaleniatum* and *S.*
14 *cuspidus* is much lower than fish consumed by any group. These results suggest that
15 young consumers should eat 72 g per week of *S. cuspidus*, and the amount they really
16 consume is 4 times higher. For the other two groups, RP and WCHA groups eat 1.5 and 4
17 times more amount than maximum permitted.
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28 Considering the surveys, the CHD group should eat only *C. magdalenae* and *O. niloticus*
29 since the ingestion rate is higher than MFW for all the rest of fish species. The WCHA
30 could only eat non-carnivorous *P. magdalenae*, *C. magdalenae* and *O. niloticus*. Finally,
31 the RP should eat more fish species (7 over 10) since their IR is higher than MFW.
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37 38 3.4 Diagnosis of the population 39

40 The pollution index was used to reflect the contamination degree with Hg of each of the
41 fish species (Zhang et al., 2019), using the permissible limits established by the FAO/WHO
42 (0.2 µg/g ww) and the JEFCA (0.5 µg/g ww).
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47 Table 2 shows the Pi values calculated according to FAO/WHO, where species *S.*
48 *cuspidus* and *A. pardalis* presented a “moderate” pollution degree (see Supplemental
49 material, Table S3), whereas for *S. kraussii* was between grade 1 and 2, which
50 corresponds to a slight pollution. On the other hand, when the pollution index was
51 calculated based on the JEFCA and the Colombian norm, all species showed a “clean”
52 pollution degree, except for *S. cuspidus* and *A. pardalis* that show grade 2. This
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4 situation leads to considering whether the establishment of a general permissible limit that
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6 does not distinguish the level of vulnerability of populations such as children, women of
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8 childbearing age and frequent consumers, is consistent when attempting to provide safe
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10 consumption conditions for the inhabitants of any population.
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14 In the US, the Environmental Protection Agency and the Food and Drug Administration
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16 jointly issued consumption advice for commercial fish that was intended to protect young
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18 children and women of childbearing age against the harmful effects of MeHg. In Colombia,
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20 there are no specific fish consumption advisories issued from any public policy that could
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22 warn vulnerable groups. In fact, advice about what type of and how much fish to consume
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24 is often confusing or contradictory, because of both risks and benefits. Advisory
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26 consumption strategies should be adequate to the social, cultural and environmental
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28 situation of a very complex region such as the Mojana floodplain. Inhabitants of this region
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30 should be advised to eat fish as a source of low-fat protein and omega-3 fatty acids but to
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32 limit consumption of some fish species such as *Sorubim cuspicaudus* and *Ageneiosus*
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34 *pardalis*, because of their Hg high levels. Also, traditional fishing practices and wild fish-
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36 based diet is a cultural value that communities will preserve, no matter if fish populations
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38 decline. Then, it is important to provide a fish consumption guidance highlighting clear
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40 messages avoiding risk, gaining nutritional benefits, and sustaining fish populations.
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45 According to our survey, fish consumers from any population group usually eat a whole
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47 fish, and therefore we have to assume that the recommended amount for consumption
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49 (MFW) is equal to the total weight of the fish. Based on the Fishbase database (Fishbase,
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51 2018) it is possible to estimate the length of a fish for a given weight to be aware about the
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53 exactly size they should catch to not exceed the MFW. The estimated length (EL) was
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55 calculated as the length of fish for the given maximum amount of fish that a person should
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57 be consumed weekly (i.e. MFW). Table 3 compares EL with the minimum catch size
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4 (MSC) established by AUNAP for a sustainable fishing in Colombia (SEPEC et al., 2013).
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6 MSC values are higher than the related EL for all the carnivorous fish species (Table 3).
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8 For example, the size of *P. magdaleniatum* that AUNAP recommend is 3 times higher than
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10 EL related to MFW, whereas for *S. cuspidatus* and *A. pardalis*, MSC is 2-fold higher.
11
12 Results suggest that people should catch fish sizes as recommend AUNAP, however they
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14 should not eat the whole fish. Accordingly, besides the guidance on the fish species that
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16 can be consumed, it becomes necessary to propose more didactic consumption strategies
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18 for the inhabitants of this region, seeking its implementation in the short term.
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22 It is unrealistic to think that in each house there is a balance with capacity to weigh such
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24 small portions (e.g. 56 g). Therefore, a didactic strategy based on common object and that
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26 allows to calculate the size of the maximum portion that an inhabitant of the region could
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28 consume according to their population group has been proposed. The selected object was
29
30 the popular crystal ball or marble, a small spherical toy often made from glass or agate.
31
32 They are very popular for children games and are used for a variety of games called
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34 marbles. These balls vary in size, but most commonly they are about 13 mm (1/2 in) in
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36 diameter and an average weight of 10 g. Thus, the sum of the weight of each marble can
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38 be identified as the equivalence to the weight of those small portions of carnivorous fish
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40 (Table 4). For the carnivorous species, the equivalent weight (EW) that corresponds to the
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42 MFW of different fish species are represented by small blue balls. As example, the
43
44 maximum number of balls of fish that children should eat is 9 per week, in the case of *C.*
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46 *kraussii* or 13 in case of *H. malabaricus* and *P. magdaleniatum*.
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51 **Conclusions**

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54 The highest concentrations of both THg and MeHg were measured in carnivorous fish
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56 species, particularly in *C. kraussii*, *S. cuspidatus* and *A. pardalis*. Six of the ten most
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58 consumed fish species in the Mojana region exceeded the permissible Hg values
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4 established by the FAO/WHO for vulnerable groups. The EWI indicates some risks for
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6 children and women of childbearing age groups with the highest values for carnivorous fish
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8 species in children group (ranging from 2.2 to 5.5 µg/kg bw/week). Results suggest that
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10 the consumption of carnivorous *C. kraussii* *A. pardalis* *H. malabaricus*, *S. cuspicaudus*
11
12 should be reduced by any of the inhabitants of the region. Except for *C. magdalenae*, *O.*
13
14 *niloticus*, *L. myscorum*, and *P. clarias* the rest of fish consumed by children, provide
15
16 quantities of MeHg that will undoubtedly trigger consequences on their health, exceeding 3
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18 and 4 times the maximum intake of MeHg established for *A. pardalis* and *C. kraussii*,
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20 respectively.
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25 In order to prevent the toxicological risks associated to Hg exposure through fish
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27 consumption, we provided a didactic strategy based on marbles, an object that most of the
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29 population has access. Our advisory consumption strategy is therefore adequate with the
30
31 social, cultural and environmental situation of the region and provides clear guidance on
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33 how to avoid risk of Hg exposure, maintain gain nutritional benefits, and sustain fish
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35 populations.
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39 40 **Acknowledgements**

41
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54 Córdoba.
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58 59 **Ethical considerations:**

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For the research development, its objective was submitted to the Ethics Committee of the Faculty of Health Sciences of the University of Córdoba. The process of inclusion and voluntary participation of the subjects was carried out in conformity with the national (Resolution N° 8430 of October 4 of 1993, Republic of Colombia, Ministry of Health) and international regulations (Declaration of Helsinki and its Amendments, World Medical Association (WMA), Edinburgh, Scotland, October 2000). It was essential to obtain the signature of the informed consent of all the participants.

Conflict of interest

The authors declare there are no conflicts of interest.

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Table 1. General characteristics of studied population in the Mojana region, Colombia.

Characteristics		Population			
		Children (CHD)	Women of childbearing age (WCHA)	Rest of population (RP)	Total
Number of participants (n)		264	267	308	839
Gender	Female	127	267	39	433
	Male	137	0	269	406
Studies	Yes	259	246	278	783
	No	5	21	30	56
Body weight (kg)		35±3	67±6	70±3	57±16
Consumed portion of fish (g)		131±73	144±80	168±87	148±15
Fish consumption (g/day)		59±26	82±29	88±44	76±35

Table 2. Estimate of the potential risk in the population, by consumption of fish in The Mojana region. The order of fish species is from higher to lower consumption. EWI ($\mu\text{g}/\text{kg}$ bw/week) is the Estimated Weekly Intake of MeHg; MeHg permissible is the permissible safety level ($\mu\text{g}/\text{g}$), and MFW (g) is the estimated maximum amount of fish that can be weekly consumed per person. CHD: children, WCHA: women of childbearing age, RP: rest of population. Pi: pollution index; C: carnivorous; NC: non-carnivorous. Values in bold means $\text{IR} > \text{MFW}$.

Species Common name	IR (g/week)			EWI ^a ($\mu\text{g}/\text{kg}$ bw/week)			MeHg permissible ($\mu\text{g}/\text{g}$)			MFW (g/week)			Pi ^b
	CHD	WCHA	RP	CHD	WCHA	RP	CHD	WCHA	RP	CHD	WCHA	RP	
<i>P. magdalenae</i> (NC)	838	1048	1329	1.9	1.3	1.5	0.08	0.13	0.18	706	1331	2803	0.4
<i>C. magdalenae</i> (NC)	529	591	818	0.5	0.3	0.4	0.11	0.12	0.33	1719	2530	6775	0.2
<i>C. kraussii</i> (C)	525	542	737	5.5	3.1	3.9	0.10	0.15	0.36	139	231	553	2.3
<i>O. niloticus</i> (NC)	390	678	545	0.1	0.1	0.1	0.09	0.13	0.48	4025	9721	18376	0.1
<i>H. malabaricus</i> (C)	399	454	681	2.2	1.3	1.9	0.11	0.17	0.33	241	448	1070	1.1
<i>P. magdaleniatum</i> (C)	319	579	613	3.2	3.0	3.0	0.24	0.22	0.63	132	313	600	2.0
<i>A. pardalis</i> (C)	307	449	349	4.8	3.7	2.4	0.19	0.06	0.28	75	108	374	2.9
<i>S. cuspidatus</i> (C)	316	583	355	4.4	4.2	2.8	0.08	0.12	1.05	72	136	227	2.9
<i>L. muyscorum</i> (NC)	292	250	481	1.4	0.6	1.1	0.04	0.09	0.55	151	237	982	1.0
<i>P. clarias</i> (NC)	194	552	251	0.9	1.3	0.5	0.05	0.12	0.25	133	380	686	1.1






^aPTWI: 1.6 $\mu\text{g}/\text{kg}$ bw/week for children (CHD) and women of childbearing age (WCA); PTWI: 3.2 $\mu\text{g}/\text{kg}$ bw/week for rest of population.

^bFor Pi calculation the threshold value of 0.2 $\mu\text{g}/\text{g}$ ww for vulnerable populations was used

Table 3. Comparison of the estimated length (EL) values obtained by Fishbase that are related to the maximum amount of fish consumed weekly (MFW) with in comparison Minimum size of catch (MSC) suggested by the AUNAP. It should be noted that only carnivorous fishes were considered.

Species	MFW (g)	EL (cm)	MSC (cm)
<i>C. kraussi</i>	88	13.7	20
<i>H. malabaricus</i>	131	20.1	25
<i>P. magdaleniatum</i>	131	27.2	80
<i>S. cuspicaudus</i>	56	21.2	45
<i>A. pardalis</i>	57	18.1	35

Table 4. Didactic strategy for the calculation of small portions of carnivorous fish consumed by inhabitants of the Mojana region, Colombia. CHD: children 2–15 years old, WCHA: women of childbearing age 16-49 years old, and RP: rest of population, men > 16 years old and women > 49 years old.

Species	CHD 2–15 years old	WCHA 16-49 years old	RP Men > 16 years old and Women > 49 years old
 <i>C. kraussi</i> (Mojarra amarilla)	MFW : 88 g EW* : 9 marbles	MFW : 146 g EW* : 15 marbles	MFW** : 175 g EW* : 17 marbles
 <i>H. malabaricus</i> (Moncholo)	MFW : 131 g EW : 13 marbles	MFW** : 121 g EW* : 12 marbles	
 <i>P. magdaleniatum</i> (Bagre rayado)	MFW : 131 g EW : 13 marbles	MFW** : 154 g EW* : 15 marbles	
 <i>S. cuspicaudus</i> (Blanquillo)	MFW : 56 g EW : 6 marbles	MFW : 105 g EW* : 10 marbles	MFW** : 144 g EW* : 14 marbles
 <i>A. pardalis</i> (Doncella)	MFW : 57 g EW : 6 marbles	MFW : 83 g EW* : 8 marbles	MFW : 175 g EW* : 17 marbles

Ew: Equivalent weight; ** The equivalent value of half a portion is presented.

Figure 1. Map of the Mojana floodplain with the location of the 11 municipalities included in this study

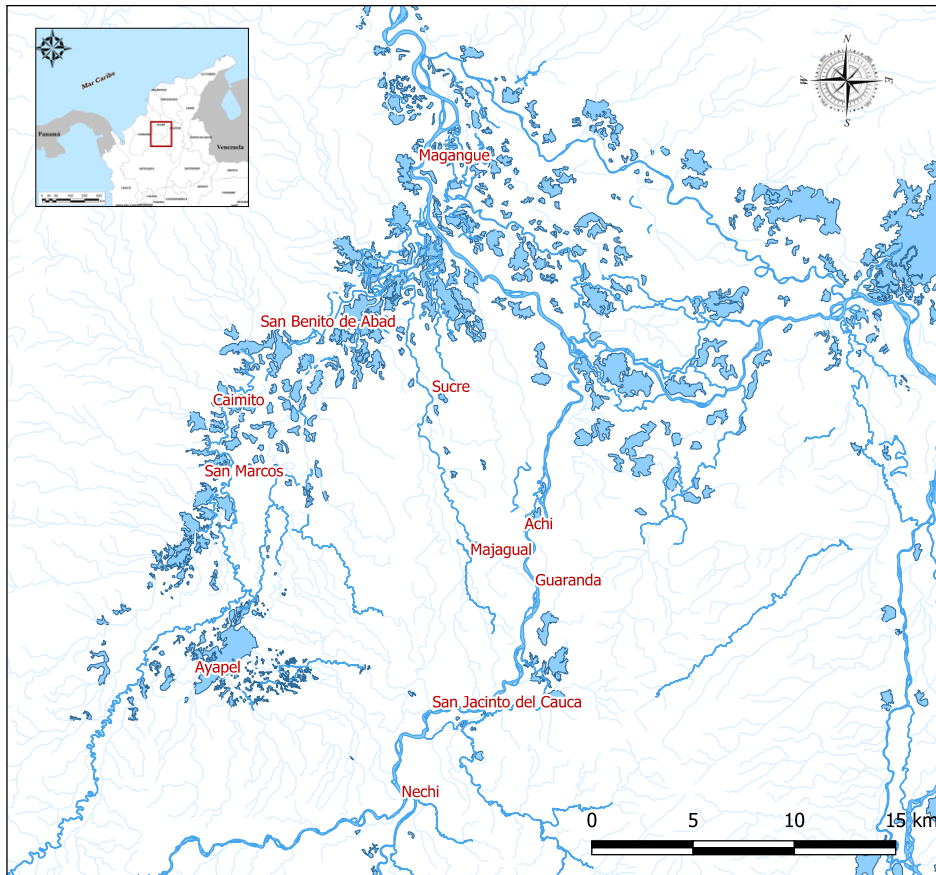


Figure 2. Fish species of higher consumption among the inhabitants of the Mojana region, Colombia. The number of individuals consuming one of the species (n) and its equivalence in percentage (%) compared to the total number of people surveyed are presented. Total number of participants (n = 839). C: carnivorous; NC: non-carnivorous

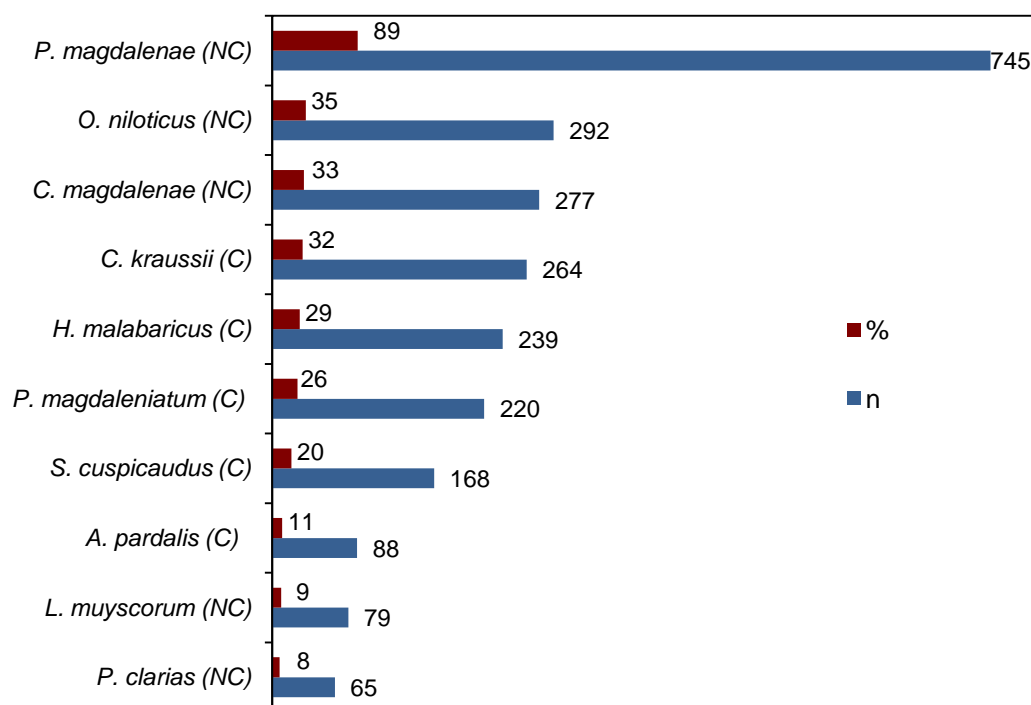
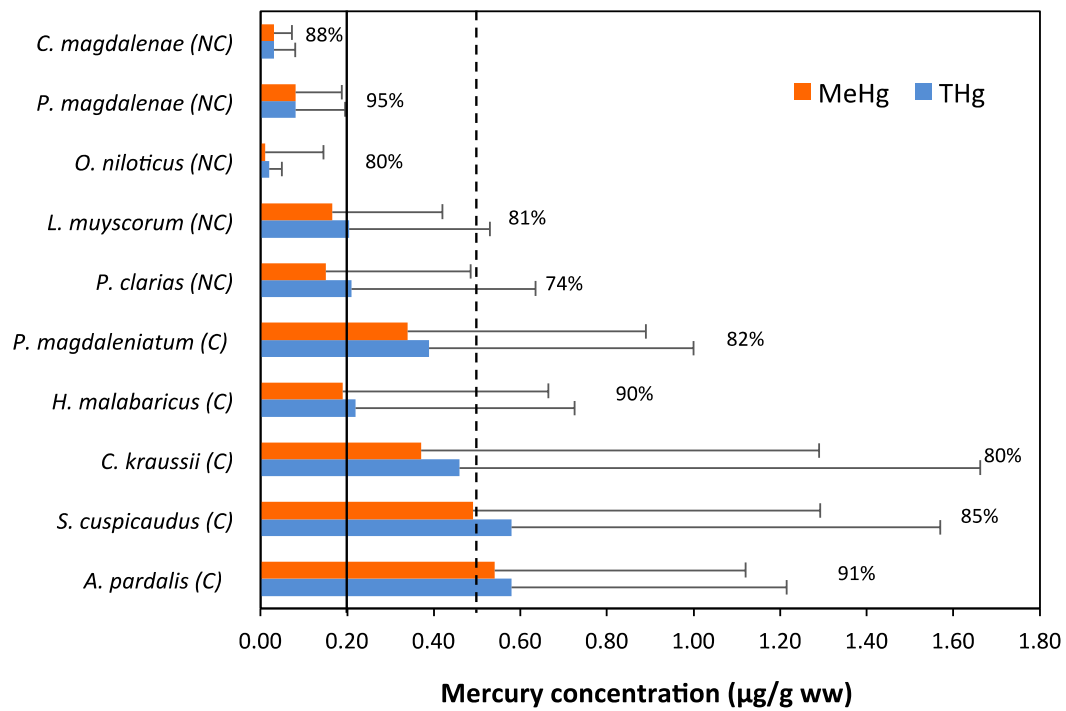


Figure 3. Concentrations of THg and MeHg ($\mu\text{g/g ww}$) and MeHg% in fish mostly consumed by inhabitants of the Mojana region. Boxes depict median values (P50) and whiskers (P75). Continuous line refers the WHO recommended threshold level for vulnerable people (THg=0.2 $\mu\text{g/g ww}$). Dashed line refers the maximum level recommended by the WHO recommended for human consumption (THg=0.5 $\mu\text{g/g ww}$).



Supplementary Material

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Graphical Abstract

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