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Using carbon and nitrogen stable isotope modelling to assess dietary mercury exposure for pregnant women in Baja California Sur, Mexico

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Abstract

Introduction: Previous studies of mercury (Hg) in pregnant women in the area of La Paz, Baja California Sur (BCS), Mexico found a proportion of individuals had concentrations of total Hg ([THg]) above some thresholds of concern set by health agencies. The [THg] were associated with fish and seafood consumption as well as other factors; although it was unclear which marine diet items could potentially be contributing to the concentrations observed.

Method: We examined [THg] and monomethylmercury concentration ([$MeHg^+$]) in the archived hair of 70 pregnant women from BCS as well as in diet items including fish, shellfish, and staple items (rice, beans, corn, and flour). We measured stable isotopes of carbon and nitrogen and employed a Bayesian stable isotope mixing model to investigate the proportion of fish and seafood in the isotopic profiles of archived hair samples.

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Ethics approval and consent to participate

Hair samples and survey information were collected under the approval of the Comisión Nacional de Investigación Cienti fica del Instituto Mexicano del Seguro Social (permit number 2016–785-013) and Consejo Nacional de Bioetica (CONBIOETICA-09-CEI-009–2016060).

Availability of data and material

The datasets generated and analyzed during the current study are not publicly available due to concurrent parallel studies but are available from the corresponding author on reasonable request.

Conflicts of interest

The authors declare that they have no competing interests.

Results: Concentrations of Hg species were low in staple foods and ranged from below detection limit to 5.71 parts per billion (ppb) wet weight. In hair, geometric mean [THg] was 658 ppb and [$MeHg^+$] was 395 ppb, which were lower than previous reports. Percent $MeHg^+$ was positively correlated with higher $\delta^{15}N$ values.

Conclusions: The largest carbon contributors to the diet of the study participants were corn and rice, and our analysis of fish contribution to diet varyingly agreed with the self-reported fish consumption. This report highlights the ability to discriminate potential sources of Hg from a diverse diet and the limitations of dietary recall studies.

Keywords

Fish consumption; Mercury; Methylmercury; Mixing models; Stable isotopes

1. Introduction

Mercury (Hg) is a global pollutant. Human exposure to monomethylmercury ($MeHg^+$) is generally through the consumption of freshwater and marine fish (vertebrates) and seafood[1] (invertebrates) (Clarkson and Magos, 2006). Inorganic mercury (Hg^{2+}) is poorly absorbed by mammals compared to $MeHg^+$ which tends to effectively bioaccumulate and biomagnify through trophic levels (Clarkson and Magos, 2006; Eagles-Smith et al., 2016). Aquatic environments typically have higher concentrations of $MeHg^+$ than terrestrial environments due to the methylation of inorganic Hg species which occurs via sulfate and iron-reducing bacteria in hypoxic or anaerobic environments such as those found in marine or freshwater sediment (Hu et al., 2013).

Inorganic Hg species are known to cause nephrotoxicity (Clarkson and Magos, 2006) while $MeHg^+$ is especially neurotoxic to the developing fetus and neonate as it crosses the placental barrier (Ceccatelli et al., 2010). Although the neurotoxicity of $MeHg^+$ in animal models and humans is well documented (Clarkson and Magos, 2006; Bernhoft, 2012), there is considerable disagreement and conflicting data regarding long term effects of fish consumption during pregnancy (Sheehan et al., 2014; Myers et al., 2015). In some populations with high proportions of marine diet items (i.e. Seychelles) there have been only a few reports of negative associations between developmental outcomes and maternal [$MeHg^+$] (Myers et al., 2003; van Wijngaarden et al., 2013), and the most recent examination of the original study cohort (at 22–24 years) found no convincing evidence for adverse effects of fish consumption during pregnancy (van Wijngaarden et al., 2017). In contrast, a similar longitudinal study found evidence of neurological deficits in children of mothers who had concentrations of 10–20 ppm [THg] in hair (Grandjean et al., 1998) with numerous caveats to consider but are not reviewed here.

Although there is some evidence that low-dose $MeHg^+$ exposure via fish consumption is correlated with developmental deficits in children, the lack of observed toxicity in longitudinal studies may be due, at least in part, to nutritional components of fish which may

[1]In Baja California the term *marisco*, which translates to seafood, describes various invertebrate species such as shrimp, crabs, clams, and scallops.

counteract $MeHg^+$ induced toxicity and may support proper fetal development (Egeland and Middaugh, 1997; Gribble et al., 2016; Oken et al., 2016). Fish contain high concentrations of antioxidants including selenium (Se) and long chain omega-3 polyunsaturated fatty acids (PUFAs), which are essential for normal brain development and function. In an animal model, pups from pregnant mice concurrently exposed to both $MeHg^+$ and Se did not show the same developmental deficits as mice exposed solely to $MeHg^+$. Several studies have found a positive correlation between fish consumption and developmental endpoints (Sakamoto et al., 2004; Oken et al., 2008; Mahaffey et al., 2011; Strain et al., 2012), although some have noted that selecting fish with high concentrations of omega-3 PUFAs and low [$MeHg^+$] and other toxicants (i.e. organohalogens, biotoxins) would be optimal (Egeland and Middaugh, 1997; Gribble et al., 2016). In humans, benefits in birth and developmental outcomes with the consumption of fish oil supplements during pregnancy have been reported (Sakamoto et al., 2004; Olsen et al., 1992; Dunstan et al., 2008).

The two states of Baja California peninsula in Mexico (Baja California and Baja California Sur (BCS), Fig. 1) are bordered by the Pacific Ocean and the Gulf of California (also known as Sea of Cortez), which together constitute the majority of fisheries production for the country (Erisman et al., 2010). Fish and seafood are important dietary components for many residents of BCS, although the frequency and identity of species consumed are not well known (Gaxiola-Robles et al., 2013, 2014). Initial findings examining total mercury concentrations ([THg]) in the hair of a cohort of pregnant women found that a sizeable proportion (54 out of 75 women) had hair [THg] above the EPA advisory guideline of 1000 parts per billion (ppb) and some (6 of 75 women) had concentrations above 5000 ppb (Gaxiola-Robles et al., 2014). In a companion study, nitrogen isotope composition ($\delta^{15}N$) was used as an indicator of fish consumption that was associated with higher [THg], although interestingly lower mean [THg] was seen in the women who reported consuming fish and shellfish most frequently (Bentzen et al., 2014). Other factors significantly associated with [THg] playing a potential role were passive tobacco exposure and body mass index.

Reports of Hg concentrations in marine fish landed and consumed from the Pacific Ocean waters in Mexico have been mainly focused on top predatory fish, some of which had concentrations higher than the U.S. Food and Drug Administration (FDA) action level of 1000 ppb wet weight (García-Hernández et al., 2007; Ruelas-Inzunza et al., 2008; Barrera-García et al., 2012). Mexico ranks as the 6th largest producer and 6th largest importer of shark products, with a large portion of the meat destined for domestic consumption (Dent and Clarke, 2015). Per capita consumption estimates of shark are relatively low compared to other categories of seafood; however, $MeHg^+$ concentrations in large predatory taxonomic groups, such as sharks and tunas, can be several orders of magnitude higher than more commonly consumed items, such as shrimp and tilapia (Murillo-Cisneros et al., 2018). It is important to note that not all fish with high Hg concentrations are large predatory species some fish appear to store $MeHg^+$ in muscle over liver (based on concentration) and in long-lived species this can lead to relatively high [$MeHg^+$] (Harley et al., 2015). At the time of writing, we could find no comprehensive surveys of frequency of species consumption for fish consumers in this region, although landing and per capita consumption data for the country are compiled by the Comisión Nacional de Acuacultura y Pesca (CONAPESCA).

Stable isotopes of carbon ($\delta^{13}C$) and nitrogen are used to reconstruct diet through multidimensional mixing models (Moore and Semmens, 2008). These models are based on the principles that lighter isotopes of C and N are more easily incorporated into biochemical processes (heavier isotopes preferentially excreted), resulting in tissular enrichment of heavier isotopes. Across trophic levels this results in significant enrichment of N^{15} (usually 3—4‰ per trophic level in most mammalian tissues, known as the trophic enrichment factor, TEF) and smaller but measurable enrichment of C^{13} (1—2‰ TEF) with increasing trophic levels (Kelly, 2000). Food web movement of stable isotopes in marine systems is somewhat complicated by the fact that different environments (i.e. benthic versus pelagic) display different $\delta^{13}C$ values in primary producers (France, 1995).

In wildlife, stable isotope mixing models (SIMMs) are increasingly utilized as tools for assessing the diet and ecology of difficult to observe species and habitats (Parnell et al., 2013; Phillips et al., 2014a). In human populations, C and N stable isotopes have been validated for use as an assessment of dietary intake of sugars (Nash et al., 2014) and marine items (O'Brien et al., 2017). In omnivorous species SIMMs are often complicated by the fact that dietary items can have highly variable concentrations of C and N, however several models can account for difference in nutrient content across diet items (Phillips et al., 2014b). It might seem imprudent to conduct chemical diet assessments in situations where dietary recall and communication with participants is possible, self-reported consumption and diet recalls may be of limited use and are occasionally conflicting with chemical analyses (O'Brien, 2015). In addition, Hg in hair represents several weeks or months of accumulation based on the length analyzed (growth rate ~1 cm $month^{-1}$ (LeBeau et al., 2011)), and dietary assessments aimed at assessing consumption over long time periods (i.e. food frequency questionnaires, FFQs) have multiple shortcomings and inaccuracies and their merit is highly debated (Shim et al., 2014). Thus, a more detailed chemical feeding ecology based on assessment of Hg exposure in conjunction with dietary recall data is warranted (Bentzen et al., 2014).

Fish consumption is almost always considered the primary route of Hg exposure for non-occupational sources and pathways (Clarkson and Magos, 2006), although some reports have indicated that other dietary sources of Hg should be considered. While fish may be commonly consumed for some individuals, it is likely that consumption of staple foods derived from corn, rice, and beans are higher in both frequency of consumption and portion size. $MeHg^{+}$ has been found in appreciable concentrations in some sources of rice (Qiu et al., 2008; Zhang et al., 2010) as well as high-fructose corn syrup (HFCS), which the authors suggest considering in dietary assessments (Dufault et al., 2009).

In the present study, our objective was to evaluate potential sources of dietary Hg exposure by measuring [THg] and [$MeHg^{+}$] in hair and diet items and stable isotope compositions of C and N. We report [THg] and [$MeHg^{+}$] in pregnant women from La Paz and in several species of fish, some of which have not been previously assessed for Hg in this region. Using stable isotopes we examined the relative contributions of diet items using Bayesian SIMMs. We also evaluate whether major dietary sources of Hg can be evaluated using chemical analysis by comparing the results of our SIMMs to data from self-reported dietary surveys.

2. Methods

2.1. Sample collection

Fish and seafood samples were purchased (generally as whole eviscerated fish) from fish markets in La Paz between March 2013 and May 2015. The full list of species examined is presented in Table 1. Samples of corn flour, wheat flour, and tortillas were purchased from supermarkets in La Paz.

Deidentified hair samples were provided from archived samples by Dr. Gaxiola-Robles (CIBNOR, IMSS) collected under the approval of the Comisión Nacional de Investigación Científica (permit number 2016–785-013). Occipital hair samples were obtained according to the protocol established in McDowell et al. (2004). Rice and bean samples donated by each study participant were collected to reflect the type of staple items likely to be served in their own home.

A questionnaire was provided to each participant containing questions about the frequency of consumption and portion sizes of various foods including finfish, seafood (invertebrates), pork, beef, beans, rice and other staples (a questionnaire is provided as supplemental material). The form also contained questions regarding the participant's occupation, smoking habits, dental amalgams, potential pesticide exposure, and other questions potentially relevant to Hg exposure. Archived questionnaire data was matched to the hair samples using deidentified numerical designations.

2.2. Sample processing

Hair samples were washed in order to remove external surface contamination using Triton X-100 detergent and Milli-Q water (Millipore, Bedford, Maryland, USA). Purchased whole fish remained frozen in a cooler until arrival at the University of Alaska Fairbanks (UAF), at which point they were stored at −20 °C until processing. Once thawed, morphometric measurements were obtained for each fish/invertebrate including length and mass. For invertebrates, total mass was recorded (including exoskeleton) and length was measured as carapace width at the widest point. Species identification was done via morphologic examination and consultation with local fish biologists. For the green crab (*Callinectes bellicosus*) samples, the frozen specimens were disarticulated upon their arrival at UAF and claws could not be matched to the main body. However, since muscle from both parts are consumed, these were analyzed independently. Sex was not determined since most of the fish purchased from the markets were without internal organs, making visual sex identification impossible. A small portion of the cranial fillet was obtained from fish samples (1—6 g) with the skin removed. From the chocolate clam (*Megapitaria squalida*) samples of the mantle, foot, and abductor muscle were obtained, and from the crabs muscle samples were obtained from the carapace (peropodal) and claw (cheliped). All samples were placed into Whirl-Paks (Nasco, Fort Atkinson, Wisconsin, USA) prior to analysis.

2.3. Freeze drying and homogenization

Samples including finfish, seafood, tortillas, and hair were freeze dried in order to facilitate Hg and stable isotopes of C and N measurements. Samples in Whirl-Paks were placed

into glass vacuum jars and were freeze-dried using a Labconco FreeZone 6 L lyophilizer (Labconco, Kansas City, Missouri, USA). Samples were freeze-dried for 36–78 h depending on the water content. In the case of finfish, seafood, and tortilla samples percent moisture was calculated as the proportion of sample mass lost during freeze drying. Rice, beans, and flour were analyzed in their dried form (without cooking). In order to increase reproducibility and precision, all samples were homogenized using a steel-ball Cryomill (Retsch Inc, Newton, Pennsylvania, USA) for 1–2 min at 25 Hz.

2.4. Total mercury analysis

[THg] was determined using a Direct Mercury Analyzer (DMA-80, Milestone Inc, Milestone, Shelton, Connecticut, USA) which combines sample decomposition, catalysis, and atomic absorption spectrophotometry (Harley et al., 2015). The detection level was set to the lowest point on the calibration curve as 0.5 ng. Standard reference materials (SRMs) were selected in order to approximate both expected [THg] as well as simulate the wide variety of matrices analyzed in this study. SRMs used were IAEA 085 and IAEA 086 (hair matrix, International Atomic Energy Agency, Vienna, Austria), DORM-4 (fish protein, National Research Council (NRC) Canada, Ottawa, Canada), NIST-1570a (spinach, National Institute of Standards and Technology), Gaithersburg, Maryland, USA) and TORT-3 (lobster hepatopancreas, NRC Canada). An internal laboratory standard (Hg in 3.7% HCl) was also used. Recovery of SRMs for THg were between 87 and 103%. Samples were run in triplicate, except in the event of greater than 15% relative standard deviation (RSD) in which case they were run in quintuplicate. Samples for which two replicates were below the detection level of the machine (0.5 ng) were only run in duplicate. Replicates were averaged using arithmetic mean prior to use in reporting and statistics. Samples that were below detection level (BDL) were factored in to mean calculations (unless otherwise noted) by dividing the minimum detection limit (MDL) by the square root of 2. MDLs were calculated for each sample as the detection level of the machine divided by the mass of the sample ran (approximately 0.03 g–0.15 g). Different matrices have different recommended masses (i.e. corn versus fish tissue) in laboratory and manufacturer protocols which resulted in slightly different MDLs for various tissues (~2ppb–16 ppb).

2.5. Methylmercury analysis

Approximately 0.001–0.05 g of sample was digested using two different methods. Nitric acid (30% v/v) was used in hair digestion as recommended by Brooks Rand Inc. Briefly, subsamples were weighed out into 40 mL TraceClean vials (VWR, Radnor PA) and 10 mL of 30% HNO_3 was added. Samples were digested in a hot water bath at 65 °C for 36−48 h. Samples were removed from the water bath and 20 mL of milliQ water was added to bring the total digestion volume to 30 mL.

For all other sample digestions we followed the established method outlined in Bloom (1989) using potassium hydroxide (KOH) dissolved in methanol (25%). Samples were weighed in TraceClean vials and 10 mL of KOH in methanol was added. Samples were digested in the dark at room temperature for approximately 48 h, at which point 20 mL of methanol was added to bring the total digestion volume to 30 mL. All digestions were analyzed within two weeks.

Aliquots of samples and SRMs (liquid standard, IAEA-085 and IAEA-086) were run on the Brooks Rand methylmercury cold vapor atomic fluorescence spectroscopy (CVAFS) detection system (including the MERX autosampler) using a 7 point calibration curve (Taylor et al., 2011). Methylmercury was analyzed using the Brooks Rand Model III detector (Brooks Rand, Seattle, Washington, USA) using a modified method of EPA Method 1630 in order to incorporate the MERX autosampler. Briefly, 40 mL glass vials were filled with milliQ water, 300 μL of acetate buffer, and a 30–100 μL aliquot of digestion. An ethylating reagent consisting of 2% sodium tetraethylborate ($NaBEt_4$) in 2% KOH/water was added and the septa caps were screwed on tightly to the vials. Each vial was then sequentially pulled into a bubbler, after which point we followed EPA Method 1630 as outlined in Harley et al. (2015). Recovery of SRMs for $MeHg^+$ was 95.8 ± 7.6%. Percent $MeHg^+$ was calculated as the slope of a robust linear regression between [THg] and [$MeHg^+$] as described in Wagemann et al. where [$MeHg^+$] = $a \bullet [THg]^{+} b$ (Wagemann et al., 1997). This method is a robust estimator to calculate average ratios of highly correlated variables.

Similar to THg, samples were run in triplicate, except in the event of greater than 15% RSD. Samples where two replicates were below the detection level of the machine (0.5 pg) were only run in duplicate. Replicates were averaged using arithmetic mean prior to use in reporting and statistics. Concentrations are reported in wet (fresh) weight unless otherwise noted. For fish tissues and tortilla samples this represents the concentration in dry weight (dw) which was back-calculated to reflect the concentration of the tissue as to represent the fish purchased by the consumer (without freeze drying). However, for staple foods the wet weight will refer to the uncooked food (e.g. dried beans, rice). This concentration of cooked staple foods at the “end of the fork” (i.e. as it reaches the consumer) is likely to be lower than what is reported here since the foods can take on water during cooking and are often mixed with other ingredients.

2.6. C and N stable isotopes analysis

Stable isotopes of carbon and nitrogen were analyzed following the methods reported by Bentzen et al. (2014). Approximately 0.2–0.5 mg of dry sample was folded into pressed tin capsules (Elemental Microanalysis, Cambridge, UK) and analyzed using a DeltaVPlus continuous-flow isotopic ratio mass spectrometer (CFIRMS, Thermo Scientific, Bremen, Germany) and elemental analyzer (Costech Scientific, Valencia, CA, USA) at the Alaska Stable Isotope Facility (ASIF) at UAF. Standard reference materials (meat based protein peptone, Sigma Chemical, St. Louis, Missouri, USA) were analyzed approximately every 10 samples to ensure ongoing precision, and blank samples were analyzed approximately every 20 samples to detect drifting values or contamination. Recovery of the peptone stable isotope SRM was 101 ± 2.1%.

Stable isotope values are presented in δ notation; that is, expressed as a part per thousand (‰) deviation from internationally recognized standards (carbon, Vienna PeeDee belemnite; nitrogen, atmospheric air). Thus.

Equations (1) and (2)

$$\delta N^{15}(‰) = \left\{ \frac{\left(\frac{sample\ N^{15}}{sample\ N^{14}}\right) - \left(\frac{standard\ N^{15}}{standard\ N^{14}}\right)}{\left(\frac{standard\ N^{15}}{standard\ N^{14}}\right)} \right\} \cdot (1000) \quad (1)$$

and

$$\delta C^{13}(‰) = \left\{ \frac{\left(\frac{sample\ C^{13}}{sample\ C^{12}}\right) - \left(\frac{standard\ C^{13}}{standard\ C^{12}}\right)}{\left(\frac{standard\ C^{13}}{standard\ C^{12}}\right)} \right\} \cdot (1000) \quad (2)$$

Samples were run in two or more replicates. In cases where the RSD for values exceeded 20%, samples were reanalyzed in triplicate or higher. The arithmetic mean of replicates was obtained for each sample and used in further analyses.

2.7. Stable isotope mixing model (SIMM)

In order to conduct a non-biased quantitative assessment of the potential contributions of different items in the participants' diet, we employed a mixing model based on a Bayesian framework (*simmr* in R) (Parnell et al., 2010). We followed the best practices for SIMM as outlined in Phillips et al. (2014a). In addition to the traditional Bayesian two-dimensional mixing model approach, this method allows corrections for the relative concentrations of C and N in our source data. Although not always recommended, this correction has been suggested in instances where there are large differences in elemental concentrations, as is sometimes found in omnivorous diets (Phillips et al., 2014a; Koch and Phillips, 2002). Since we sampled both staple foods (relatively low N concentrations) as well as seafood (relatively high N concentrations), we had large variation in elemental concentrations of our sources; we therefore employed the concentration dependent correction to our model. We also employed a k-neighbor joining algorithm to group sources according to similarity in $\delta^{15}N$ and $\delta^{13}C$ values (Rosing et al., 1998). Mixing models are dependent on a trophic enrichment factor (TEF) defined as the difference between the stable isotope ratios between the diet items and the tissue of interest in the consumer. These are defined using Δ notation as.

Equations (3) and (4)

$$\Delta N^{15} = \delta N^{15}_{tissue} - \delta N^{15}_{diet} \quad (3)$$

$$\Delta N^{13} = \delta N^{13}_{tissue} - \delta N^{13}_{diet} \quad (4)$$

The stable isotope values for the diet items need to be adjusted based on the TEF in order to accurately assess the contribution of each item to the consumer's diet. It has been suggested that the TEF is the most important factor in SIMMs, and small variations in TEF values can

lead to large differences in assessments (Martínez del Rio et al., 2009; Caut et al., 2009). Values based on experimentally controlled feeding studies have indicated that TEFs are species, tissue, sex, and diet specific (Roth and Hobson, 2000). Unfortunately, there are few experimental studies examining TEFs for human hair, and even fewer that have examined females specifically. One study examined $\Delta^{15}N$ and $\Delta^{13}C$ in women (n = 20) from Fiji with a similar diet to the women in our study (fish, staples, meat, shellfish) (Hedges et al., 2009). TEFs for nitrogen in the literature are somewhat variable, but the convention has been a 3–4‰ increase per trophic level in $\delta^{15}N$, although most studies examining hair or keratin have found this value to be slightly higher (Hedges et al., 2009; Hola et al., 2015). In this study we use TEFs as provided in Caut et al. for mammalian hair, where $\Delta^{15}N = 2.59$ and $\Delta^{13}C = -0.417\left(\delta^{13}C_{source}\right) - 7.878$ (Caut et al., 2009).

We utilized the *simmr* package developed for the R programming language (R Core Team. R, 2017) using 100,000 iterations and 1000 burn-ins (Parnell et al., 2010). In order to determine if our chemical analysis correlated with self-reported consumption habits, we repeated these mixing models separating groups according to their frequency of seafood consumption. To simplify this analysis, we refined seafood consumption frequency groups into three categories (1) once per month or less, (2) once every other week, and (3) once per week or more. Using these values, we were able to achieve good convergence based on the Gelmen diagnostics. All further statistical analysis was done in R (R Core Team. R, 2017). All graphical representations were generated using the ggplot2 package (Wickham, 2009).

3. Results

Of the bean and rice samples that were donated from our study participants, no bean samples (0/70, 0%) had concentrations above minimum detection level for $MeHg^+$, while nearly all rice samples (65/70, 93%) had detectable [$MeHg^+$]. The geometric mean (±standard error (SE)) of [$MeHg^+$] in rice was 2.16 ± 0.09 ppb ww, with the minimum concentration BDL and the highest concentration of 5.17 ppb. No bean or rice samples had [THg] above the detection limit for this assay, approximately 16 ppb. We also tested store-bought items including 10 flour samples (5 corn and 5 wheat) and 27 tortillas (15 flour and 12 flour). None of the tortilla or flour samples had consistently detectable [$MeHg^+$]. Percent $MeHg^+$ was not calculated for staple foods due to many samples being below the detection limit for [THg] and the disparate detection levels of the respective assays. Our MDL for [THg] was approximately 16 ppb for most matrices, while the MDL for [$MeHg^+$] was at least an order of magnitude less (approximately 0.5 ppb). Thus, some samples had detectable concentrations of [$MeHg^+$] but concentrations of [THg] that were BDL.

THg and $MeHg^+$ concentrations in green crabs and chocolate clams were typically low, as presented in Table 1. Mean [THg] was lowest in invertebrate species (<3 ppb in chocolate clam, < 30 ppb in green crab) and highest in mojarra (*Diapterus peruvianus*, 202 ppb). Methylmercury concentrations were highly correlated to [THg] and the average $\%MeHg^+$ of THg was 94% (95% confidence interval 93.1%–94.3%), indicating average $\%MeHg^+$ of [THg] was approximately 94% for all marine samples. Standard errors for aggregated crab samples were elevated by one carapace and one claw sample that had concentrations

>100 ppb for both THg and $MeHg^+$. The $\%MeHg^+$ for these samples were 94% and 89%, respectively.

Ages of participants ranged from 18 to 56 years (median = 27) and body mass index (BMI) ranged from 18 to 63 (median = 28). Concentrations of Hg in hair samples are presented in Table 2. Geometric mean (±SE) of [THg] was 658 ± 74 and $[MeHg^+]$ was 395 ± 56 ppb ww. There were 17 individuals with [THg] above the EPA reference concentration of 1.0 ppm (Rice et al., 2003; Trasande et al., 2016). Average percent $MeHg^+$, derived using robust linear regression as described above, indicated that on average 76% of the [THg] in hair was $MeHg^+$ (R^2= 0.74), although 17 individuals had % $MeHg^+$ less than 50% and 3 individuals had $\%MeHg^+$ less than 25%. The [THg] was significantly lower in this study than in Gaxiola-Robles et al. when compared using a student's t-test (p = 0.001) (Gaxiola-Robles et al., 2014). Carbon isotope compositions ($\delta^{13}C$) ranged from −15.2‰ to −18.3‰ (median = −17.0‰, standard error = 0.1) and nitrogen ($\delta^{15}N$) ranged from 8.4‰ to 10.8‰ (median 9.2‰, SE = 0.1). The $\delta^{13}C$ and $\delta^{15}N$ values were not well correlated (r = 0.18). There was a significant positive association between [THg] and $\delta^{15}N\left(p \ll 0.01, R^2 = 0.23\right)$as well as $[MeHg^+]$ and$\delta^{15}N\left(p \ll 0.01,\ R^2 = 0.37\right)$, although considerably more variability in $[MeHg^+]$ was explained by $\delta^{15}N$. Percent $MeHg^+$ was also positively associated with $\delta^{15}N$ values ($p \ll 0.01, R^2 = 0.14$, Fig. 2) although not with $\delta^{13}C$ values.

Neither $[MeHg^+]$ nor [THg] varied significantly between groups of self-reported fish consumption (Fig. 3a). We also noted no significant differences between fish consumption groups and $\delta^{15}N$ values (Fig. 3b). This was the case for groups based on the frequency of consumption of seafood (not shown). There was a significant difference in $\%MeHg^+$ between participants who had zero dental amalgams and participants who had one or more (students t-test, p = 0.02, Fig. 4). Due to homogeneity of responses, we did not make comparisons of Hg species related to smoking.

We determined $\delta^{15}N$ and $\delta^{13}C$ values from each finfish, seafood, and staple food sample analyzed and executed a mixing model to attempt to account for the relative contribution of each diet item in the source. Our initial model contained each fish species (i.e. Table 1) and staple food (rice, corn, beans, etc.) as a separate source. However, this model was inhibited by too many sources due to overlapping stable isotope ranges of many food items. We employed a k-nearest neighbor algorithm (Rosing et al., 1998) to group sources according to similarity in $\delta^{15}N$ and $\delta^{13}C$ values (Fig. 5) and we identified six discrete groups which were fed back into the SIMM. We restricted our model to six sources rather than further grouping because smaller groups, while potentially mathematically sound, would differ largely from groupings based on ecological and dietary considerations (for instance, mojarra would be inappropriately grouped with crabs and clams). The $\delta^{15}N$ and $\delta^{13}C$ values (±standard deviation (SD)) for each diet group are presented in Fig. 6. Literature stable isotope values for beef and chicken (from (Jahren and Kraft, 2008)) are included in this figure to display the potential for them to exhibit a corn-based composition, however these values were not generated from our analysis.

The results of the SIMM output are presented in Table 3. The percent of diet attributed to mojarra (mean proportion = 0.01, SD = 0.01) and fish (mean proportion = 0.02, SD = 0.01) were quite low, while beans + flour and rice make up significant, although somewhat variable, proportions of diet. Corn made up a significant proportion of all participants diet (mean proportion = 0.71, SD = 0.01).

In order to compare our chemical analysis with self-reported consumption habits, we repeated these mixing models separating groups according to their frequency of seafood consumption. We found only small differences in the estimated portions of fish in the diet of each of the three groups (Fig. 7a). Using the results of each model iteration (n = 100,000) each self-reported consumption group was ranked in terms of proportion of fish consumption according to the SIMM (i.e. *at least once a week* > *once every other week* > *once a month or less*) and found that the most frequent consumption group (at least once per week) was selected as the highest fish consumers in 39% of iterations, as compared to 31% and 30% for the other two groups.

We also ran our SIMM to compare fish consumption across groups generated according to their [$MeHg^+$] and %$MeHg^+$ in hair (Fig. 7b). Using the three approximately equal sized groupings of participants, we found that the model selected the high [$MeHg^+$] group as the highest fish consumers in approximately 50% of iterations. The model selected the high %$MeHg^+$ group in 43% of iterations (Fig. 7c).

4. Discussion

Hair mercury concentrations in this study were lower than previously reported in Gaxiola-Robles et al. (2014), from the same region and an order of magnitude lower than concentrations in pregnant women from known high fish consumption communities (i.e. the Seychelles (van Wijngaarden et al., 2017)). Concentrations of THg reported here are less than the 95 percentile of frequent fish consumers reported in the National Health and Nutrition Examination Survey (NHANES) study (McDowell et al., 2004), although nearly all (65 out of 70, 96%) of the individuals in this study had hair [THg] higher than the median concentration of all participants in that study.

There are several potential reasons for the different average [THg] hair concentrations between the two BCS-bases studies. Although these were not the same women examined in the previous study (Gaxiola-Robles et al., 2014), they were from a similar area, age, and socio-economic class. One explanation could be a general decrease in fish consumption, although our $\delta^{15}N$ values did not differ significantly from those of Bentzen et al. (2014). However, a switch in preferred species from one with higher [THg] (i.e. mojarra, elasmobranchs) to one with lower [THg] (i.e. cochito) could explain differences in [THg] that were not associated with changes in $\delta^{15}N$ values. Although species-specific consumption was not assessed in the previous studies, in our study a large portion of respondents reported consuming pierna (*Caulolatilus princeps*) (41%) and cochito (*Balister polyepis*) (30%), two species that we found to have relatively low [THg].

Hair [$MeHg^+$] is not commonly measured, and is generally assumed that the percent $MeHg^+$ of total Hg in hair is at least 80% (Cernichiari et al., 1995). However, to our knowledge there is no standardized method of calculating average %$MeHg^+$, with some reports using simple arithmetic means (Harada et al., 1998), while others have advocated for a robust linear regression (Wagemann et al., 1997) making it difficult to compare results across studies. The median %$MeHg^+$ in our study was 67%, although 17 individuals had %$MeHg^+$ less than 50%. While we support the calculation of the average ratio of $MeHg^+$:THg using methods other than arithmetic mean, we suggest here that there were individuals whose %$MeHg^+$ values differ greatly from the estimate of average %$MeHg^+$ using the slope parameter. Different tissues can have different %$MeHg^+$ related to the protein (cysteine) content, lipid type and content, or *in situ* demethylation; thus, it is conceivable that hair %$MeHg^+$ could vary from blood or urine (Squadrone et al., 2015). An alternative explanation is that these individuals have exposure to sources of inorganic Hg in addition to $MeHg^+$ through diet or other routes of exposure (e.g., amalgams, dentistry occupation, hair and skin products).

Shallow hydrothermal activity in the Gulf of California discharges water enriched with a number of minerals including Hg, which may contribute to marine inorganic or organic (following methylation) Hg concentrations (Prol-Ledesma et al., 2004). Although other sources of inorganic mercury exposure have been implicated for Mexican citizens including cosmetic and beauty supplies, some of which contain mercury concentrations between 20,000 and 36,000 ppm (Dickenson et al., 2013). Imported cosmetic products were identified as a potential source of inorganic mercury exposure in adults from New York City (McKelvey et al., 2011). It has been suggested that inorganic mercury is absorbed through skin across the epidermis via sweat glands and sebaceous glands (Chan, 2011; Park and Zheng, 2012), which would explain high concentrations of Hg found in urine of patients displaying symptoms of Hg toxicity after self-reported use of a Mexican skin lightning cream (Weldon et al., 2000). Indeed, a study in 2011 found that 6 of 15 creams produced in Mexico had detectable concentrations of Hg, some as high as 36,000 ppm (Peregrino et al., 2011), well above the FDA's allowable limit of 1 ppm. Although the dermal absorption rate of the Hg (unknown form) from lightning creams is not well described, there is strong evidence that there is at least partial absorption (Clarkson and Magos, 2006; Peregrino et al., 2011) and even poor absorption efficiencies could lead to potentially toxic exposures.

Concentrations of THg and $MeHg^+$ in the chocolate clam were quite low (2−5 ppb), which is consistent with the current concentration reported by the FDA and Atwell et al. (1998). To our knowledge, this is the first report of Hg concentrations in this species from this region. Concentrations of THg and $MeHg^+$ were slightly higher in the crab samples, and although Hg concentrations are not commonly measured in crab species, our values were more or less consistent with previous reports (Burger et al., 2007). One crab sample of both carapace and claw muscle tissue had [THg] and [$MeHg^+$] 5–10 times higher than the other samples (135 [$MeHg^+$] and 112 [THg] ppb). Interestingly, this individual had the lowest $\delta^{15}N$ and $\delta^{13}C$ values of the crabs, nearly a full trophic level (3‰) lower than the mean $\delta^{15}N$. Crabs and other detritivores are likely exposed to variable concentrations and forms of Hg due to opportunistic feeding on detritus, so variation in [Hg] and C and N stable isotopes are perhaps not unexpected. While it is surprising to see significantly higher concentrations in

an individual feeding on a lower trophic level, we will not draw any conclusions from one sample on a poorly studied species. Further analysis of [Hg] in detritivores from the region are warranted, especially given reports of hydrothermal activity in the region (Prol-Ledesma et al., 2004).

Concentrations of Hg in finfish were generally low and based on their C and N stable isotope profiles most appeared to be low trophic level feeders. However, even the higher trophic level species (cabrilla (*Mycteroperca rosacea*) and sierra (*Scomberomorus sierra*), mean $\delta^{15}N > 18$) had geometric mean concentrations less than 100 ppb. We caution that despite the low concentrations reported here, there could be seasonal or spatial variation in Hg concentrations within a species. More robust sample sizes over larger temporal and spatial settings should be assessed prior to considering consumption recommendations. Of the species mentioned as most frequently consumed in self-reporting, we analyzed 5 of 6 (83%) of finfish species and 2 of 4 (50%) shellfish species. There were a few species that were reported to be consumed by the study participants that were not measured for Hg including shrimp and scallops; however, these items are generally not known to have high [THg]. Some studies from this region have shown high [THg] in sharks and elasmobranchs (Barrera-García et al., 2012; Murillo-Cisneros et al., 2018). These species were not asked about specifically but were not mentioned in self-reporting.

The highest concentrations of THg and $MeHg^+$ in this study were in mojarra (Table 1). This species has been noted to have mean concentrations of 580 ppb ww from the Sinaloa coast of Mexico, a value which is higher than the general guidance level for fish mercury from both the World Health Organization (WHO) and the European Commission (EC) (Ruelas-Inzunza et al., 2008). Another study found mean [THg] in *D. peruvianus* to be the highest (2556 ppb dry weight) of 19 species analyzed from the Gulf of California (García-Hernández et al., 2007). The [THg] found in *D. peruvianus* are slightly unusual considering the size and ecology of the species. The mean length of *D. peruvianus* in this study (16.7 cm, n = 12) was in agreement with previous reports on the species (16.8 cm) (Spanopoulos-Zarco et al., 2015), yet it was notably smaller than other fish species assessed in this study. There are only a few resources regarding dietary assessments for the species; however, most indicate that they are benthic carnivorous or omnivorous fish whose prey include mainly bivalves and marine worms (Phyla Mollusca and Echinodermata), although Lopez-Peralta and Arcila (2002) also note detritus as a dietary component in their stomach content analysis. Further investigations into the feeding ecology, life history, and metabolism of this species is warranted to assess their role in human Hg exposure.

Stable isotopes of C and N from hair samples were analyzed in order to assess potential sources of Hg through diet (Bentzen et al., 2014). Overall, our isotope values were very similar to Bentzen et al. although in this study we did not observe nearly as broad a range in [THg] in human hair (Bentzen et al., 2014). Interestingly, $\%MeHg^+$ appeared to be positively associated with $\delta^{15}N$ values. Bentzen et al. found that $\delta^{15}N$ values were associated with [THg] that was likely driven by finfish and seafood consumption (Bentzen et al., 2014). We also found a positive association between $\delta^{15}N$ values and [THg], and a stronger association between $\delta^{15}N$ and [$MeHg^+$] and % $MeHg^+$. This supports the notion that the seafood and fish are sources of $MeHg^+$ exposure; however, the low $\%MeHg^+$

among infrequent fish consumers ($\delta^{15}N < 9$) could also potentially be explained by these individuals having exposure to Hg (potentially inorganic) via routes other than seafood. The 47% of infrequent fish consumers had one or more dental amalgams, while 26% of moderate consumers and 43% of frequent consumers had amalgams. Dental amalgams have also been known to increase tissular inorganic Hg concentrations, and other studies have found that some of the variation in $\%MeHg^+$ can be explained by the presence of dental amalgams (Vieira et al., 2013). In our study, $\%MeHg^+$ was indeed significantly lower in individuals with one or more dental amalgams, although there were no significant differences in [THg] or [$MeHg^+$] between those with amalgams and those without. There are numerous factors to consider regarding Hg exposure via dental amalgams including the timing of the procedure as well as the type of amalgam used. It would be worthwhile to design a specific study examining Hg exposure via dental amalgams and those working in the dentistry profession in this population that would need to strategically account for Hg exposure from fish.

Corn has a fairly unique C isotope composition among human staple foods due to its C_4 carbon fixation which results in lower $\delta^{13}C$ compared to other terrestrial plants (O'Brien, 2015). We note that, while all respondents had significant contribution of corn in their diets, this does not imply that only corn products (i.e. flour) are contributing. Many livestock species including cattle and chicken are fed diets with large proportion of corn, thus their $\delta^{13}C$ values are quite similar to corn (Jahren and Kraft, 2008). We did not measure stable isotopes in these sources, although many of the study participants reported frequent consumption of beef, chicken, and pork, which would contribute to corn-like $\delta^{13}C$ values we observe here (see Fig. 6).

There are a number of assumptions and sources of error built into SIMMs, and while we attempted to control for most of these, there are a few confounding factors that should be addressed. First, there is evidence that individual variation in uptake and metabolism can lead to variation in C and N stable isotope values in consumers. For instance, Sponheimer et al. found large variation (up to 3‰) in $\delta^{15}N$ values in hair of mammals that were fed identical diets (Sponheimer et al., 2003). It is also possible that individuals in this study could have exogenously altered the chemical composition of their hair, i.e. via bleaching or washing. Indeed, a number of samples had evidence of artificial coloration. However, it appears that stable isotope values of human hair are unaffected by shampoo and only slightly affected by aggressive bleaching (O'Connell and Hedges, 1999), thus, we do not see this as a large source of error.

Overall, our SIMM agreed with self-reported fish consumption. We did not see significantly different $\delta^{15}N$ values across fish consumption groups; however, using a stable isotope mixing model we found that frequent fish consumers indeed had slightly higher proportions of fish in their diet. Our model also indicated a relatively high proportion of rice in the diet (mean proportion = 0.15) which supports self-reporting in that 80% of respondents reported eating rice more than once a week. The ubiquity of these staple foods, similarity of stable isotope values, and the lack of data concerning portion sizes inhibited a more thorough examination of these staple foods in terms of daily Hg intake. Our model comparing groups based on $MeHg^+$ concentrations and $\%MeHg^+$ in hair was more successful at distinguishing

the chemical signature of fish consumption, which provides further evidence that chemical analysis of diet is warranted even in circumstances where dietary recall is possible.

5. Conclusion

We have presented [THg] and [$MeHg^+$] in commonly consumed fish species from La Paz, Baja California, Mexico. To our knowledge, this is the first report of Hg in some of these species from this region. We found lower Hg concentrations in hair samples from pregnant women than previous reports (Gaxiola-Robles et al., 2014), which could indicate a change in dietary preference. We show here that chemical analyses and stable isotope modelling are a valuable tool in evaluating human diet and can be used to determine potential sources of contaminants. Further monitoring of human Hg exposure and Hg dynamics in the local environment and biota could provide a more complete picture of Hg exposure for humans in this area.

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HIGHLIGHTS

- [THg] in hair (pregnant women) was lower than previous reports, and [THg] in fish were low compared to thresholds.
- Stable isotope mixing models reveal limitations in dietary recall studies and the value of chemical analyses.
- Percent methylmercury was positively associated with δ15 N values.

Fig. 1.

A map of the southern part of the Baja California peninsula, Mexico. La Paz, Baja California Sur (BCS) is situated on the Gulf of California, although it lies close to the Pacific Ocean near the fishing villages of Todos Santos and El Pescadero. Commercial fish are harvested from both coasts of the peninsula. The continuous line approximately denotes the 12-mile territorial zone. Map is from OpenStreetMaps.

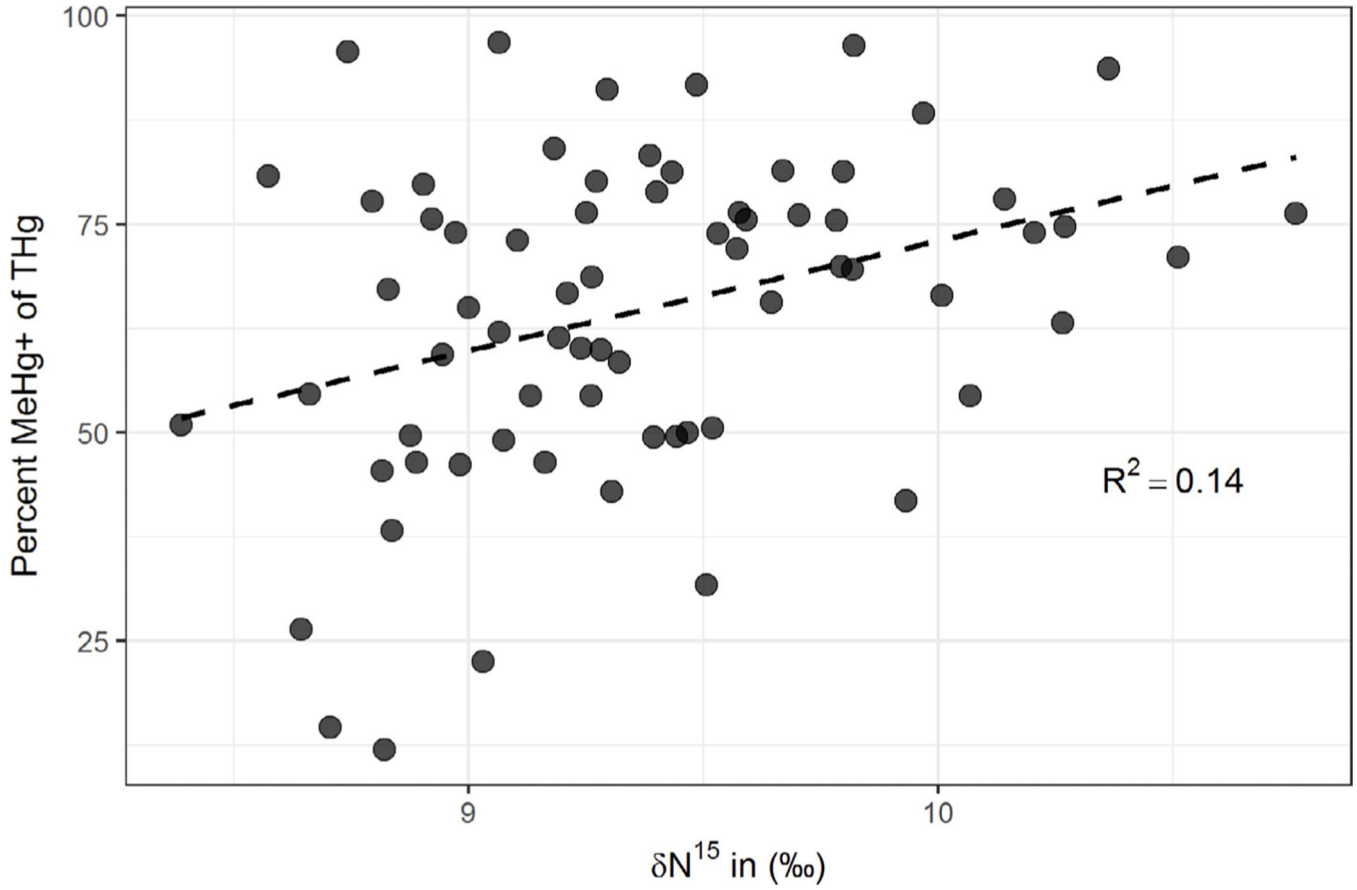

Fig. 2.

Linear relationship between %$MeHg^+$ of [THg] and $\delta^{15}N$, an estimator of fish consumption, in human hair samples (n = 70). Seventeen individuals had %$MeHg^+$ less than 50%. $MeHg^+$ - monomethylmercury, THg – total mercury, $\delta^{15}N$ - stable isotope ratio of N.

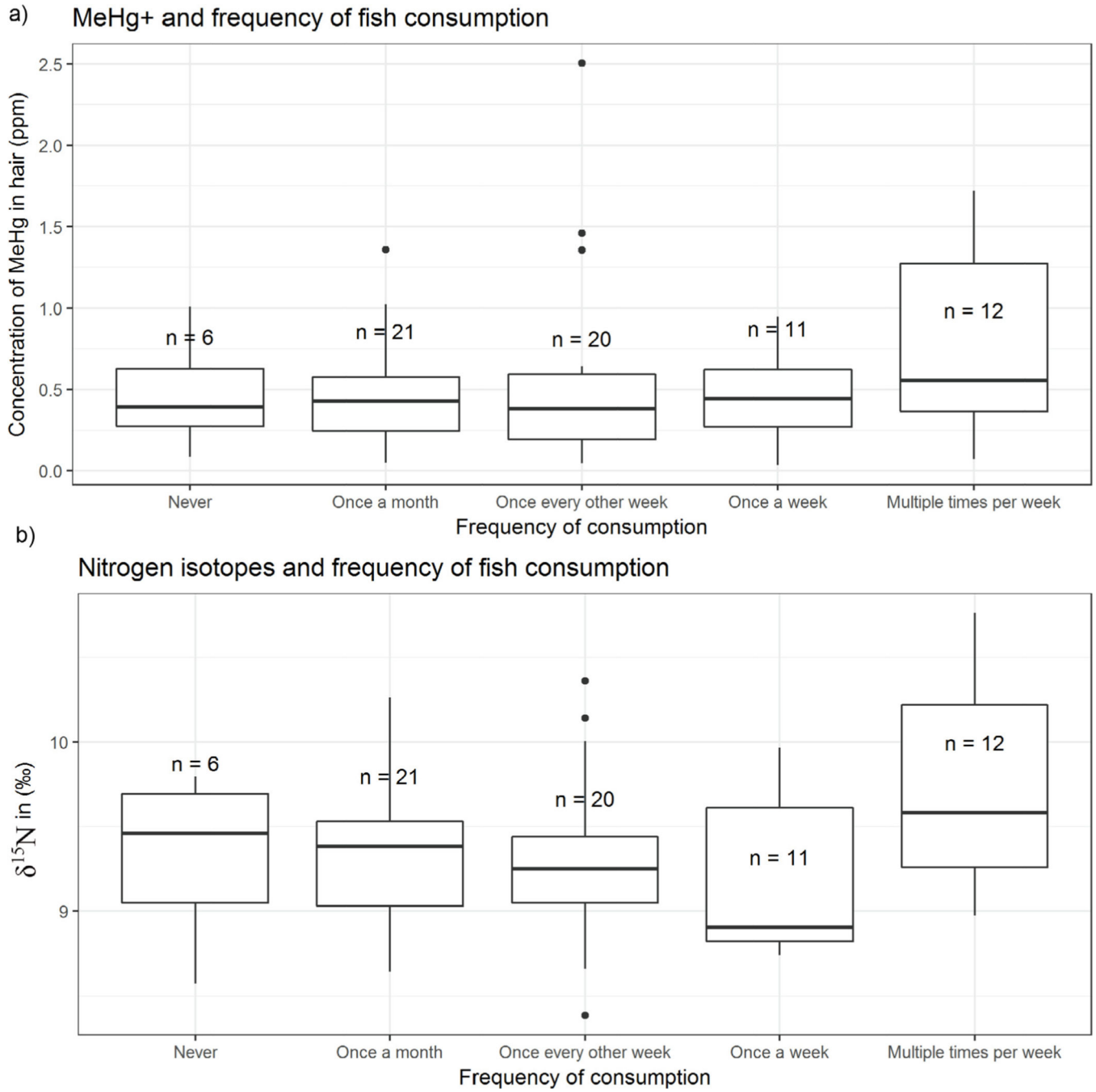

Fig. 3.

(a) Concentrations of $MeHg^{+}$ in hair samples grouped by self-reported fish consumption, and (b) $\delta^{15}N$ in hair based on self-reported consumption of fish. Boxplots represent first quartile, median, and third quartile while whiskers represent the highest and lowest datum within 1.5 interquartile range (IQR). Individual points are data outside 1.5*IQR. $MeHg^{+}$ - monomethylmercury, $\delta^{15}N$ - stable isotope ratio of N.

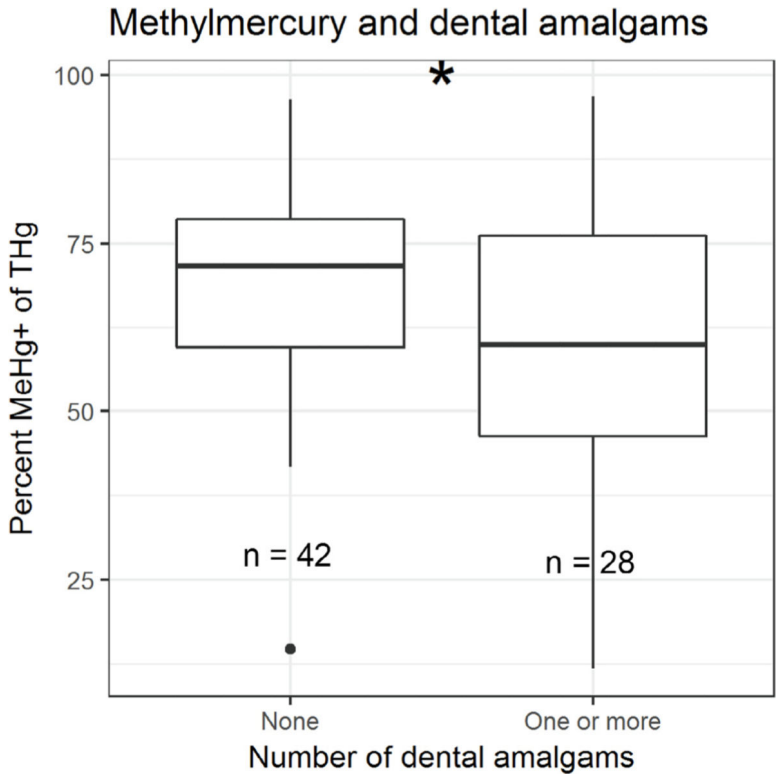

Fig. 4.

Differences in the percent $MeHg^+$ of [THg] between study participants with and without dental amalgams. Asterisk indicates significance at $\alpha = 0.05$ level (student's t-test). %.? $MeHg^+$ - monomethylmercury, THg – total mercury.

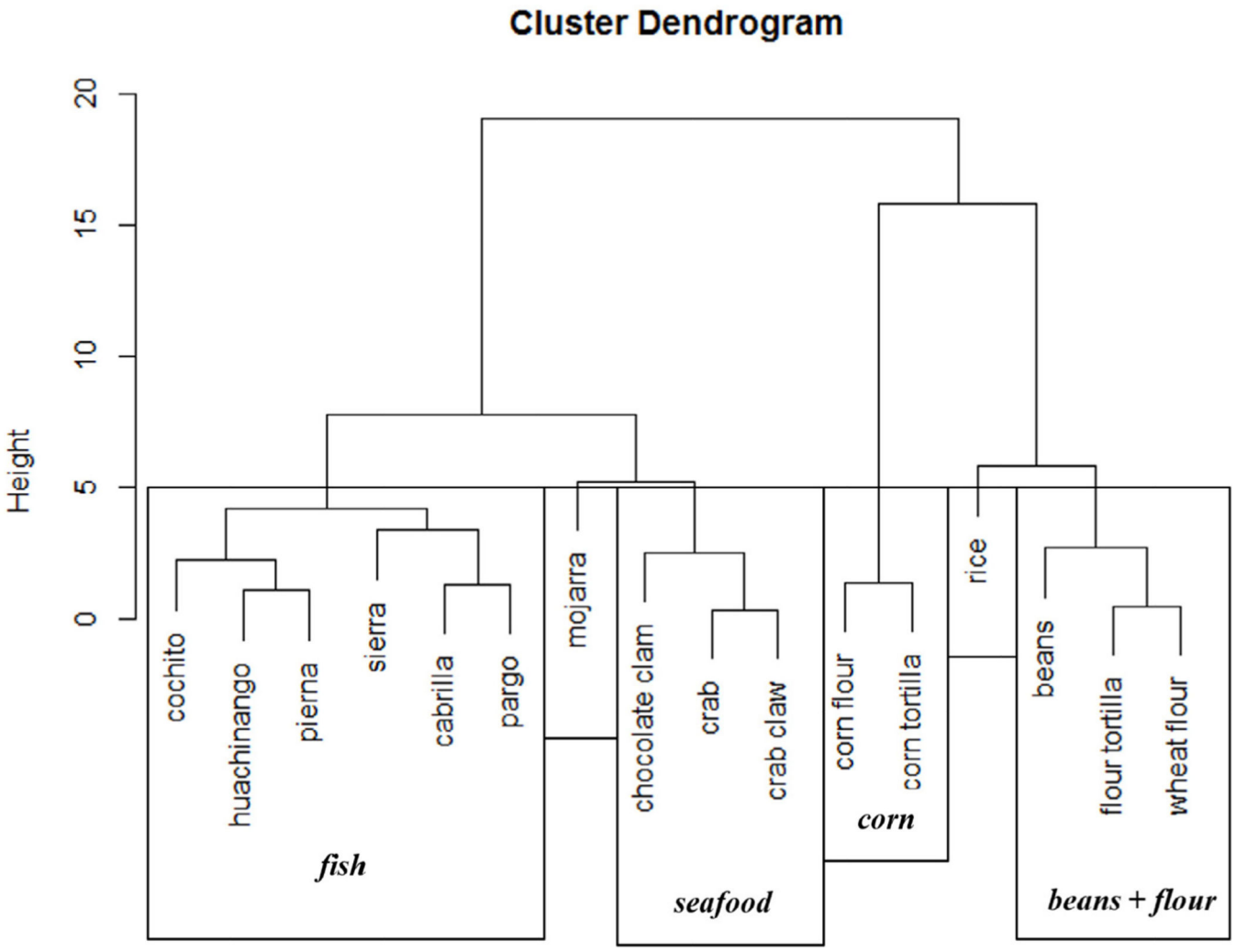

Fig. 5.

Results of clustering k-neighbor joining clustering algorithm based on $\delta^{15}N$ and $\delta^{13}C$ values. Groupings (n = 6) used in further analysis are shown in boxes. $\delta^{15}N$ - stable isotope ratio of N, $\delta^{13}C$ - stable isotope ratio of C.

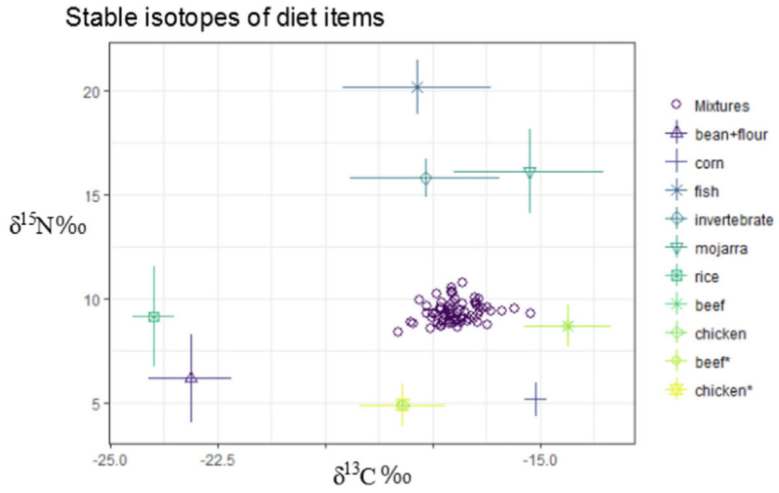

Fig. 6.

Average stable isotope values of grouped dietary items (grouped according to k-neighbor clusters presented in Fig. 5). Values for beef and chicken are provided from Jarhen and Kraft (Jahren and Kraft, 2008) to show the corn signature present in these protein sources, however they were not included in the mixing model. Mixtures provided are individual hair samples (n = 70) from pregnant women in La Paz, Baja California, Mexico.

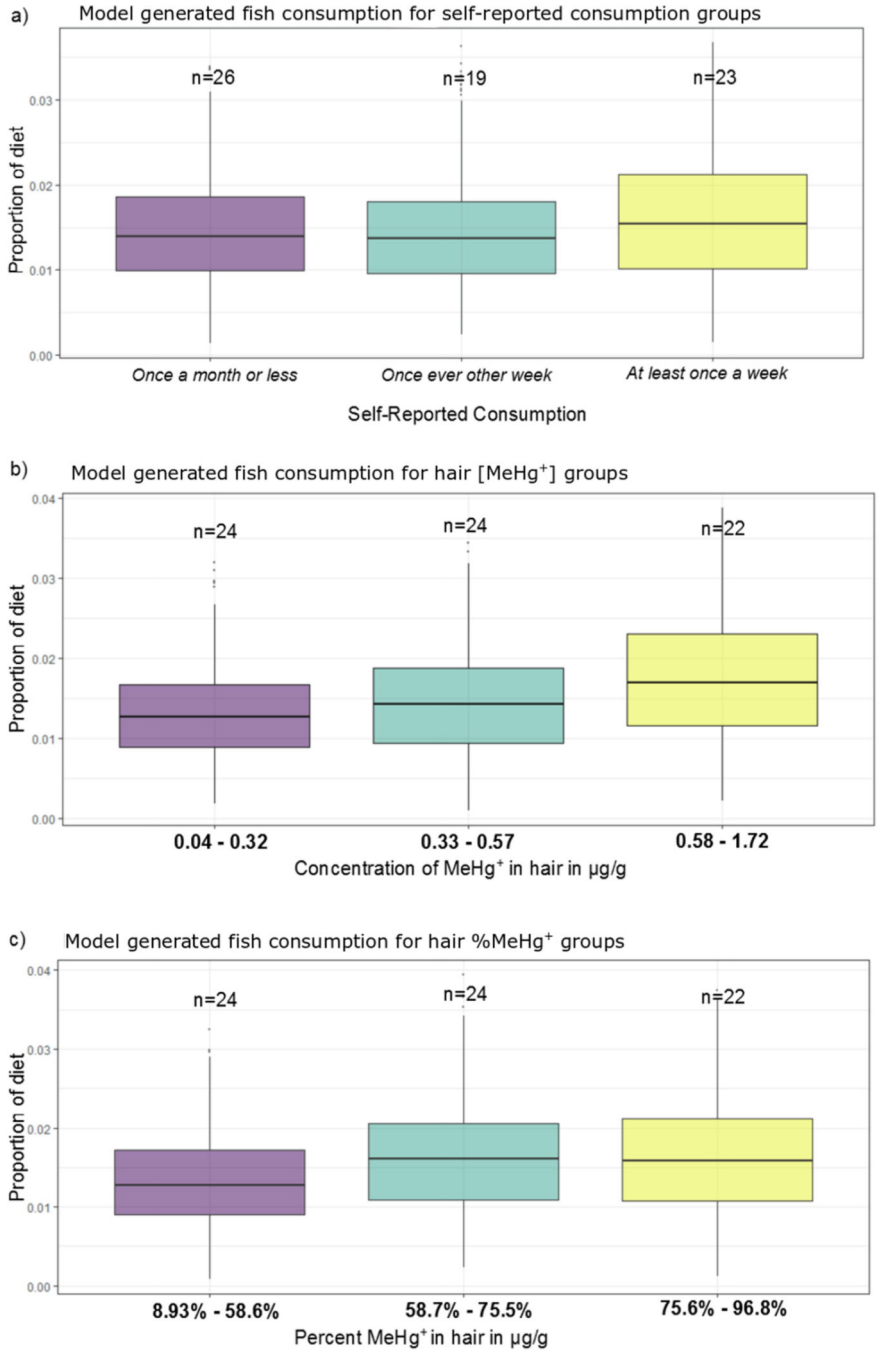

Figure 7.

a) *simmr* model output proportions of fish in the diet of each self-reported fish consumption group, b) model output proportions of fish consumption based on groups according to [$MeHg^+$] in hair, and c) model output proportions for groups based on %$MeHg^+$ in hair. $MeHg^+$ - monomethylmercury.

Table 1

Mercury concentrations for fish and seafood. Data presented as geometric mean ± standard error. Percent $MeHg^+$ for each species is calculated using geometric mean of individually calculated $[MeHg^+]/[THg]*100$.

Common name		Species	Tissue	Number	[THg] in ppb ww	[$MeHg^+$] in ppb ww	%MeHg
Spanish	**English**						
Almeja chocolata	chocolate clam	*Megapitaria squalida*	mantle	5	2.37 ± 0.05	1.40 ± 0.04	59%
			abductor	5	2.91 ± 0.21	2.37 ± 0.22	82%
			foot	5	2.96 ± 0.17	2.24 ± 0.15	75%
Cangrejo verde	green crab	*Callinectes bellicosus*	carapace	6	27.50 ± 21.00	25.63 ± 20.30	93%
			claw	8	20.62 ± 12.11	19.30 ± 10.75	94%
Cabrilla	leopard grouper	*Mycteroperca rosacea*	muscle	4	86.80 ± 18.80	78.90 ± 19.22	91%
Cochito	finescale triggerfish	*Balister polylepis*	muscle	4	23.00 ± 20.56	21.90 ± 20.87	95%
Huachinanago	red snapper	*Lutjanus peru*	muscle	4	30.09 ± 2.89	29.44 ± 2.98	98%
Mojarra	Peruvian mojarra	*Diapterus peruvianus*	muscle	8	196.68 ± 36.04	178.54 ± 30.74	91%
Pargo	yellow snapper	*Lutjanus argentiventris*	muscle	5	77.64 ± 13.91	71.48 ± 14.16	92%
Pierna	ocean whitefish	*Caulolatilus princeps*	muscle	6	51.57 ± 26.76	46.76 ± 30.57	91%
Sierra	Pacific sierra	*Scomberomorus sierra*	muscle	6	73.42 ± 2.57	70.21 ± 4.01	96%

[THg] – concentration of total mercury.

[$MeHg^+$] – concentration of methylmercury.

ppb – parts per billion (μg/kg).

ww – wet weight.

%MeHg – $[MeHg^+]/[THg]*100$.

Table 2

Mercury concentrations in human hair. Values expressed as geometric mean in parts per billion (μg/kg) wet weight. Data are compared to thresholds (1 ppm and 5 ppm) similar to Gaxiola-Robles et al. (2014).

Study	Hg Species	Median	Geometric mean	Standard error	n > 1 ppm	n > 5 ppm
This study	[THg]	688	658 ±	74	17/70 (24%)	0 (0%)
	[$MeHg^+$]	432	395 ±	56	10/70 (14%)	0 (0%)
	%$MeHg^+$[a]	76.3%	60.4%	2.3%		
Gaxiola-Robles et al. (Harley et al., 2015)	[THg]	1520	1389	463	54/75 (72%)	6/75(8%)

[THg] – concentration of total mercury.

[MeHg+] – concentration of methylmercury.

[a]Percent $MeHg^+$ calculated as the slope of the regression (a) where [MeHg] = a • [THg] + b. For further explanation see text and Wagemann et al. (1997).

Table 3

Mean proportions and standard deviations (SD) of items in the diet of all study individuals generated by the mixing model (*simmr*).

Food Item	Proportion	SD
corn	0.71	0.01
rice	0.15	0.04
bean + flour	0.08	0.04
invertebrates	0.03	0.01
fish	0.02	0.01
mojarra	0.01	0.01