Mercury concentrations in lean fish from the Western Mediterranean Sea: Dietary exposure and risk assessment in the population of the Balearic Islands

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Abstract

The present study reports total mercury (THg) and methylmercury (MeHg) concentrations in 32 different lean fish species from the Western Mediterranean Sea, with a special focus on the Balearic Islands. The concentrations of THg ranged between 0.05 mg/kg ww and 3.1 mg/kg ww (mean 0.41 mg/kg ww). A considerable number of the most frequently fish species consumed by the Spanish population exceed the maximum levels proposed by the European legislation when they originate from the Mediterranean Sea, such as dusky grouper (100% of the examined specimens), common dentex (65%), conger (45%), common sole (38%), hake (26%) and angler (15%), among others. The estimated weekly intakes (EWI) in children (7-12 years of age) and adults from the Spanish population (2.7 µg/kg bw and 2.1 µg/kg bw, respectively) for population only consuming Mediterranean fish were below the provisional tolerable weekly intake (PTWI) of THg established by EFSA in 2012, 4 µg/kg bw. However, the equivalent estimations for methylmercury, involving PTWI of 1.3 µg/kg bw, were two times higher in children and above 50% in adults. For hake, sole, angler and dusky grouper, the most frequently consumed fish, the estimated weekly intakes in both children and adults were below the maximum levels accepted. These intakes correspond to maximum potential estimations because fish from non-Mediterranean origin is often consumed by the Spanish population including the one from the Balearic Islands.

Keywords: Estimated weekly intake; Lean fish; Mercury; Methylmercury; Western Mediterranean Sea

1. Introduction

Mercury is widely distributed throughout the environment as consequence of natural and anthropogenic processes. The most common sources of mercury releases include industrial activities, such as artisanal and small scale gold mining, energy production, and chlor-alkali plants, as well as waste sites (UNEP, WHO, 2008). Anthropogenic effects are responsible for the increases of the global inventory of mercury in the oceans. Mercury emissions to the atmosphere due to mining and fossil fuel burning (Hg_0) are deposited into seawaters after oxidation to Hg^{2+}. There, Hg may undergo bioaccumulation and scavenging by organic-rich particles, be eventually transported from surface to deep waters and reduced back to Hg_0 and to methylmercury (Lamborg *et al.*, 2014).

Methylmercury is a more toxic form than the original metal. This compound targets the nervous system, especially during the children developmental stage (Grandjean et al., 1997; UNEP, WHO, 2008). The major source of methylmercury intake in humans is fish and seafood products (Calatayud et al., 2012; Gari *et al.*, 2013; Perello et al., 2014; Cano-Sancho et al., 2015a; Obeid et al., 2017). Food sources other than fishery products may also contribute to mercury body burdens, but mainly in the form of inorganic mercury, which is less toxic than the methylated form (Perello et al., 2014). This compound in fish is bound to tissue protein rather than to fatty deposits. It biomagnifies through the food web and apical predators, which are carnivorous species feeding at the top of the food chain and tend to increase the concentrations.

Until very recently, fish consumption recommendations' to vulnerable population groups, such as infants and pregnant women, have focussed on certain big, migratory and oily fish species (EFSA, 2012; 2015a; AESA, 2006). However, since mercury is associated primarily with muscle tissue rather than to fat deposits, predatory but non-migratory fish species, *e.g.* lean fish, may also accumulate this compound.

Island populations are typically high fish consumers, particularly from local markets. These populations are more prone to accumulate high mercury levels (Grandjean *et al.*, 1997; Myers *et al.*, 2000; Murata *et al.*, 2002). Previous studies on newborns and preschool children from Mediterranean populations have shown high mercury concentrations in blood and hair (Freire *et al.*, 2010; Garí *et al.*, 2013; Llop *et al.*, 2014).

To date, few studies have assessed the potential role of predatory but non-migratory fish species regularly consumed by general and infant populations as mercury sources. The present study is devoted to determine the concentrations of total mercury and methylmercury in a great variety of lean fish species from the Western Mediterranean Sea, with a special focus on the Balearic Islands. This study has been extended to fish specimens from the Portmán Bay, a highly polluted area in Cartagena (Murcia, South Spain), Tunisia and Egypt. Samples from the Atlantic Ocean, in front of Senegal and Mauritania, have also been collected and examined for comparison. The study is also aimed to ascertain whether the concentrations of THg are compliant with the maximum values in fishery products established by the European Legislation (EU, 2011).

Possible human health risks of fish consumption are assesed by examination of the weekly intakes of both children and adults and comparison with the Provisional Tolerable Weekly Intakes (PTWIs) established by the European Food and Safety Authority (EFSA, 2012).

2. Materials and methods

2.1 Sampling

Between February 2014 and August 2016, 407 commercial seafood samples from the Western Mediterranean Sea were collected (Figure 1). Most of them (n = 375) in waters nearby the Balearic Islands (Majorca, n = 174; Menorca, n = 122; Ibiza, n = 79) and the rest of the samples were from Portmán (Múrcia, South of Spain, n = 26), Tunisia (n = 2) and Egypt (n = 4). Additional fish samples from the Atlantic Ocean, in front of the Senegal and Mauritania coasts, were also collected (n = 14).

Thirty-one fish species from the Balearic Islands were selected considering the most consumed by the population (SMAP, 2015). These species were obtained by both commercial and recreational fishing. Samples were collected in action halls and local fish markets. Those from Tunisia, Egypt and the Atlantic Ocean, were obtained from importer facilities in the Balearic Islands.

The specimens from Portmán Bay, *Pagrus pagrus* and *Mullus barbatus*, were collected onboard the R/V Ángeles Alvariño (IEO) in August 2014, in the context of the MIDAS Project.

Information on length, weight, date of sampling and catch location was obtained whenever possible. Some samples were analysed in pools, *e.g.* anchovies (mean specimens in each pool, n = 77), sardines (n = 32), picarel (n = 14), pearly razorfish (n = 6), Atlantic horse mackerel (n = 6), comber and painted comber (n = 5), blackspot seabream (n = 4), black scorpionfish (n = 2) and common sole (n = 2).

2.2 Mercury and methylmercury analyses

Total Mercury (THg) was determined by cold vapor atomic absorption spectrometry, inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7900) and automatic mercury analysis (AMA-254).

Methylmercury was analysed by high performance liquid chromatography coupled to ICP-MS with isotopic dilution (Agilent 7900/Agilent 1200LC).

Mercury and methylmercury concentrations were expressed as Hg (mg/kg) by reference to sample wet weight (ww). Concentration means and ranges were used for data reporting. Statistical analyses and graphs were performed using the R software (R Core Team 2015).

The limits of quantification (LQs) were 0.1 mg/kg ww in both methods. Concentrations below LQs were assumed to be ½ of the LQs, 0.05 mg/kg ww. Similar figures were obtained from the calculation of these values with a reverse Kaplan-Meier estimator (Guillespie et al., 2010).

2.3. Estimated dietary intakes and threshold values

A first estimate of the dietary weekly THg intakes (mg Hg) through fish consumption were calculated by multiplying the median fish concentrations of all analysed samples (mg/kg ww) by the weekly average fish consumptions of the Spanish population, 46.4 g/day and 71.1 g/day for 7-12 year old children and adults older than 17 years, respectively (AESA, 2006). Comparison of these results with those reported by EFSA for Spain give similar figures, e.g. 36.3 g/day and 63.6 g/day for children and adults, respectively (EFSA, 2015b). Estimated Weekly Intakes (EWIs; µg/kg bw) of THg were obtained from the dietary weekly intakes after normalization to the mean body weights of each population group, *e.g*. 34.48 kg for children and 68.48 kg for adults.

The EWIs were compared to the PTWIs of both THg and methylmercury, 4 µg/kg bw and 1.3 µg/kg bw, respectively (EFSA, 2012). The PTWI percentages (%PTWI) were calculated as 100·EWI/PTWI.

In addition to the calculation of EWIs and %PTWIs for total fish consumption, specific calculations for consumption of European hake, angler, common sole and dusky grouper were also performed because these species are the most frequently consumed by the Spanish population (Table 4).

3. Results

The present study reports the concentrations of mercury in 420 fish specimens from 32 species collected at several locations of the Western Mediterranean Sea and the Atlantic coast. Mediterranean morey, red scorpionfish, angler, conger, European hake and dusky grouper were the most studied species, with a total of 27 to 38 specimens for each species examined.

Box plots showing the distribution of THg in the specimens collected in the Balearic Islands are shown in Figure 2. The fish species from the Balearic Islands have been grouped in three trophic levels: those feeding on plankton, those feeding on small fish and crustaceans, and piscivorous species that feed on fish and cephalopods (Riera *et al.* 1995). Five fish species (n = 27 specimens) belonged to the first trophic level, 13 species (n = 112) to the second trophic level, and 13 species (n = 235) to the third (Table 1). The sampling percentages exceeding the maximum mercury levels set forth by the European Commission for human consumption are also indicated. The highest THg concentrations were found in dusky grouper, porbeagle and angler (maximum levels around 3 mg/kg ww), followed by greater amberjack, small-spotted catshark, conger and common dentex (maximum concentrations 1.5 – 2 mg/kg ww). The lowest THg concentrations were found in anchovies, picarel, pearly razorfish and brown meagre. In sardines, transparent goby, anchovy, picarel and round sardinella all of the examined samples had THg levels below detection limit.

The THg concentrations in fish species collected in other sites from the Western Mediterranean Sea (Portmán, Tunisia and Egypt, n = 32) are reported in Table 2. For comparison purposes, THg concentrations in dusky grouper samples collected in the Atlantic Ocean are also shown (n=14; Table 2). The percentages of samples exceeding the EU maximum levels are also indicated. The two fish species collected in Portmán, red mullet (n=19) and red porgy (n=7), show mean mercury concentrations of 0.17 mg/kg ww and 0.24 mg/kg ww, respectively, which are below the EU maximum level. The dusky grouper specimens from Tunisia and Egypt have mercury concentrations above the EU maximum level, 0.71-1.0 mg/kg ww and 0.92 mg/kg ww, respectively. In contrast, only 7% of the dusky grouper specimens from the Atlantic Ocean exceeded the EU maximum level, with mean concentrations of 0.34 mg/kg ww. Two of the three common dentex specimens from Egypt waters (mean concentration 1.1 mg/kg ww) exceeded the EU maximum levels.

Comparisons between THg concentrations found in the present study and other reports from Mediterranean locations are found in Table S1 of the Supporting Information.

Methylmercury was also measured in a subset of specimens in which THg concentrations were close to or above the EU maximum level (Table 3). The percentages of the methylated form with respect to total mercury concentrations involved mean values of 76%, ranging between 48% in dusky groupers from the Atlantic Ocean and 92% in common dentex from the Balearic Islands.

The distributions of mercury concentrations in fish specimens caught nearby each main Balearic Island are shown in Figure 3. Statistical analysis of these data with non-nested multilevel model with varying intercepts (fixed effects) for location (the three islands) and the fish species from which more specimens were available (n = 12) showed no evidence of a differential effect for geographic location.

The EWIs and %PTWI for children and adults for both total fish and individual species, *e.g*. European hake, common sole, angler and dusky grouper, are shown in Table 4. Threshold values for average fish concentrations (µg/g fish) were calculated using mean weekly fish consumption (g fish) and body weight for each population group (kg bw) after accounting for the aforementioned PTWIs (EFSA, 2012). Threshold = PTWI·body weight/mean weekly fish consumption. These values, 0.43 mg/kg ww for children and 0.55 mg/kg ww for adults (Figure 2), provided estimates of the average mercury concentration in fish to fulfil the PTWI according to the average body weight and Mediterranean fish consumption of the population.

4. Discussion

4.1. Mercury and methylmercury fish concentrations

The present study encompasses the 32 lean Mediterranean fish species that are most frequently consumed by the population of the Balearic Islands. The species analysed include those particularly consumed in the early childhood, such as European hake, common sole and angler (AESA, 2006). A significant percentage of these fish specimens exceeded the EU maximum level, 0.5 µg/g ww, except for fish species particularly prone to bioaccumulate this metal, in which it is 1 µg/g ww (Official Journal of the European Union, 2011).

The mecury concentrations in fish from the first trophic level, *e.g.* sardines, anchovies, round sardinella, picarel and tranparent goby, were below this EU maximum level in all samples. The concentrations of this metal exceeded the EU maximum levels in 12% and 28% of the samples examined within the second and third trophic levels, respectively. As expected THg concentration increased at higher trophic level (Storelli *et al.*, 2007), which is consistent with the different feeding habits.

In general terms, the THg concentrations of the present study are in good agreement with other reports from the Western Mediterranean. In the fish species from the first trophic level, the THg concentrations (0.057 mg/kg ww) are in the range of those found in other Mediterranean areas (mean concentrations between 0.030 mg/kg ww and 0.13 mg/kg ww; Yusà *et al.*, 2008; Copat *et al.*, 2012; Bonsignore *et al.*, 2013; Brambilla *et al.*, 2013, Perello et al., 2014; Cano-Sancho *et al.*, 2015b). Only in one study from the Sicily Channel (Copat *et al.*, 2012) higher mean concentrations in sardines, 0.13 mg/kg ww, than in this report were observed.

Hake is the mostly consumed fish by the Spanish population, accounting for about one half and one third of the total fish consumption by children and adults, respectively (AESA, 2006). Nearly one-third of the examined hake samples (26%) exceeded the EU maximum level of 0.5 mg/kg ww (EU, 2006). The hake specimens of the present study have about twice as high mean concentrations as those measured in specimens collected in Catalonia, Valencia and the Italian coast (0.13–0.16 mg/kg ww; Perello et al., 2014; Yusà *et al.*, 2008; Brambilla *et al.*, 2013). However, their THg mean concentration is lower, 0.30 mg/kg ww, than in Roses (Gulf of Lion, Northwestern Mediterranean Sea; Torres *et al.* 2015) and the Central Adriatic Sea (Perugini *et al.* 2014), 0.51 – 0.62 mg/kg ww.

Common sole is the second most frequently consumed fish by the Spanish population, with rates of consumption around 7% in both children and adults (AESA, 2006). THg content in the specimens from the Balearic Islands, 0.45 mg/kg ww, were higher than those found in Catalonia, Italy and from an industrialized area in Tunisia (Martorell et al., 2011; Perello et al., 2014; Brambilla et al., 2013; Zohra and Habib, 2016). Thirty-eight percent of the samples collected around these islands exceeded the EU maximum levels.

The mean THg concentrations of the angler specimens analyzed in the present study (0.74 mg/kg ww) were much higher than those from the Italian coast (mean of 0.13 mg/kg ww; Brambilla *et al.* 2013). The EU maximum level set forth for anglers is 1 mg/kg ww, and around 15% of the examined samples exceeded this limit.

The concentrations of mercury in dusky groupers from the Balearic Islands, 1.6 mg/kg ww (n=10), Tunisia, 0.86 mg/kg ww, and Egypt, 0.92 mg/kg ww, were much higher than the EU maximum levels, 0.5 mg/kg ww (Tables 1 and 2). However, only 7% of the dusky groupers caught in the Atlantic Ocean (mean 0.34 mg/kg ww; n=14) exceeded this maximum level (Table 2). Dusky grouper is also consumed by the Spanish population in both children and adults, although in a minor degree (1.5%).

Conger and common dentex were also found to have mean THg concentrations above the EU maximum level, 0.50 mg/kg ww. Sixty-five percent of common dentex specimens (n=17) exceeded the limit, with a mean concentration of 0.85 mg/kg ww. Three common dentex samples from the Egyptian coast were found to contain even higher concentrations of mercury than in the Balearic Islands (mean 1.1 mg/kg ww; Table 2).

The THg concentrations in horse mackerel from the present study, 0.18 mg/kg ww, were lower than those found in the Italian coast (0.50 mg/kg ww, Brambilla *et al.*, 2013), but higher than those observed in other Mediterranean areas, such as Southern Italy (0.13 mg/kg ww, Bonsignore *et al.*, 2013), Catalonia (0.053-0.099 mg/kg ww, Martorell *et al.*, 2011; Perello et al., 2014) and Valencia (0.030 mg/kg ww, Yusa *et al.*, 2008).

The red porgy samples from the Balearic Islands showed slightly higher THg concentrations than those from Portmán (0.31 mg/kg ww *vs.* 0.24 mg/kg ww, respectively). In contrast, the THg concentrations in red mullets from Portmán and the Balearic Islands exhibited the same concentrations despite being different fish species, red mullets in Portmán (mean 0.17 mg/kg ww; range 0.070 mg/kg ww - 0.44 mg/kg ww) and red stripped mullets (or surmullets) in the Balearic Islands (mean 0.18 mg/kg ww; range 0.050 - 0.49 mg/kg ww). These concentrations were intermediate between those found in studies from red mullets of the Italian coast, including the Adriatic Sea, and Catalonia (average concentrations between 0.23 mg/kg ww and 0.48 mg/kg ww; Storelli and Barone, 2013; Brambilla *et al.*, 2013; Perugini et al., 2014; Perello et al., 2014), Portmán Bay (0.12 mg/kg ww) and other Valencian and Catalan locations (0.074-0.093 mg/kg ww; Martínez-Gómez *et al.*, 2012).

The mean percentage of methylmercury from the subset of specimens (76%, range 52%-92%) was comparable to that reported in other studies (Kuballa *et al.*, 2011). However, a wide range of MeHg/THg ratios was observed in the present study. Whereas dusky grouper specimens from the Atlantic Ocean, the less contaminated, showed percentages of 48%, those from Tunisia and Egypt had percentages of 67% and those from the Balearic Islands (the most contaminated) the highest (81%). Other studies have observed constant MeHg/THg ratios (Brambilla *et al.*, 2013) which is not the case of the present results. The main difference between both studies concerns fish location, whereas the former refers to fish from the FAO Area 37 (Italian coast), those considered in the present study were obtained in a broader area. Most likely, different food chains occurred in the Atlantic Ocean, and the Mediterranean areas of the Balearic Islands, Tunisia and Egypt.

Dietary exposure assessment

The median THg intake due to fish consumption in the Balearic Islands, 0.29 mg/kg ww, involves EWIs of 2.7 µg/kg bw for children aged 7-12 years and 2.1 µg/kg bw for adults when referred to the infant and general Spanish population (Table 4). These values are lower than the PTWI for total mercury intake recommended by FAO/WHO, 4 µg/kg bw (EFSA, 2012) (52% and 67%, respectively). The PTWI for methylmercury are set to 1.3 µg/kg bw (EFSA, 2012) which is lower than the EWIs for children and adults. According to these PTWIs assuming that 90% of total mercury is in the form of methylmercury the observed EWIs for adults and children involve 150% and 190% of the PTWIs, respectively.

An estimation of the differences on fish dietary intakes between adult men and women can be obtained from ENUCAN (2015). It reports separate values for three age groups, 18-44 years (reproductive age in women), 45-64 years and >65 years. The EWIs and PTWIs from these data and these age groups are compared with those of AESA (2006) in Table S2. The results from both studies only differ slightly, 71 g/day and 62 g/day of daily fish consumption rates in AESA (2006) and ENUCAN (2015), respectively. In ENUCAM (2015) the rates between an average adult (> 18 years, 68.5 kg mean body weight) and women of reproductive age (18-44 years, 65 kg mean body weight) are similar, 62 g/day and 57 g/day, respectively. Therefore, the EWIs for these categories are similar 1.8 µg/kg bw as well as the PTWIs, 46% and 45%, respectively. These results suggest that the values calculated in Table 4 for an average adult of the Spanish population are also representative of women of reproductive age.

These values represent the worst case scenarios for total consumption of local fish. The population of the Balearic Island also consumes fish from other Mediterranean areas, as well as from the Atlantic Ocean. The consumption from local sites is less than half of the total.

The EWIs of some fish species such as hake, 0.85 and 0.50 µg/kg bw for children and adults, respectively, are below the THg and MeHg PTWIs. However, their contribution to the mercury burden is not negligible, 21% and 12% in children and adults, respectively, of the PTWI for total mercury and 65% and 38% for total methylmercury.

The present approach in mercury ingestion by fish consumption may be compared with those of other Mediterranean populations. Two studies performed in Italy on specific fish species found higher EWIs than in the present study (Storelli and Barone, 2013; Bonsignore *et al.*, 2013). However, these Italian studies used mean THg values whereas median values were used in the present study, which tend to generate lower estimations as fish mercury distributions tail towards high values.

Studies in Valencia showed that total fish consumption contributed to 43% of PTWI (Yusa *et al.*, 2008) whereas these contributions in Catalonia were 49.3% for adults and 38.5% for children (Llobet *et al.*, 2003; Falcó *et al.*, 2006).

According to their mean body weight and fish consumption habits, the specific threshold values of average THg fish contamination for the Spanish population are 0.55 µg/g ww and 0.42 µg/g ww for children and adults, respectively (Table 4, Figure 2). These values are quite similar to the EU maximum levels, 0.5 µg/g ww (EU, 2011). This agreement suggests that the general EU thresholds are calculated taking as reference high fish consumer populations. Nevertheless, it must be emphasized that an important proportion of the studied fish specimens considered in the present study exceeds this threshold (Figure 2).

5. Conclusions

Fish from the first trophic level, *e.g*. sardines, anchovies, round sardinella, picarel and transparent goby, were below these EU maximum levels recommended for human consumption. Twelve and twenty-eight percent of the fish specimens from the second and third trophic level had mercury concentrations above this threshold. In general, the observed concentrations were in agreement with previous reports on some fish species from the Mediterranean Sea and higher than those observed in the Atlantic Ocean. The rate between methylmercury and mercury in the specimens analysed in the Balearic Islands was also comparable with previous studies but for some species, *e.g*. dusky grouper, was high, 81%, in comparison to specimens from other locations in the Mediterranean Sea or the Atlantic Ocean.

The median THg intake involved EWIs of 2.7 µg/kg bw and 2.1 µg/kg bw for children aged 7-12 years and adults, respectively, which is below the PTWIs recommended by FAO/WHO, 52% and 67% of the threshold values, respectively. However, since 90% of total mercury has been observed to be in the form of methylmercury, the PTWIs of adults and children for this compound were exceeded, 150% and 190%, respectively. These percentages correspond to maximum potential EWI estimates since most of the Spanish population and the population of the Balearic Islands also consume fish from non-Mediterranean origin.

The specific thresholds for the average THg fish contamination calculated for populations of body weights and fish consumption habits such as those considered in the present study show a good agreement with the EU maximum levels. This concordance suggests that the EU thresholds were calculated taking high fish consumer populations as reference.

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Table 1: Total mercury concentrations (mg/kg ww, mean and range) for each fish species in the Balearic Islands (n = 374 but some specimens were analysed in pools as described in the foot note). The percentages of compliance with the EU maximum concentrations for THg are also indicated.

Trophic Level / Fish species	THg					
	N	QF (%)[a]	Mean	Median	Range	% Not compliance
First trophic level (5 fish species)	**27**	**24 (89%)**	**0.058**	**0.050**	**0.050 – 0.15**	**0**
Anchovy (*Engraulis encrasicolus*)[b]	7	6 (86%)	0.057	0.050	0.050 – 0.10	0
Round sardinella (*Sardinella aurita*)	1	0 (0%)	0.11	0.11	–	0
Picarel (*Spicara smaris*)[b]	7	6 (86%)	0.064	0.050	0.050 – 0.15	0
Transparent goby (*Aphia minuta*)	4	4 (100%)	0.050	0.050	0.050 – 0.050	0
Sardine (*Sardina pilchardus*)[b]	8	8 (100%)	0.050	0.050	0.050 – 0.050	0
Second trophic level (13 fish species)	**112**	**23 (21%)**	**0.29**	**0.22**	**0.050 – 1.5**	**12**
Black seabream (*Spandyliosoma cantharus*)	14	3 (21%)	0.19	0.15	0.050 – 0.79	7
Brown meagre (*Sciaena umbra*)	8	5 (63%)	0.094	0.050	0.050 – 0.23	0
Small-spotted catshark (*Scyliorhinus canicula*)	8	0 (0%)	0.78	0.68	0.39 – 1.5	25
Blackspot seabream (*Pagellus bogavareo*)[b]	2	0 (0%)	0.21	0.21	0.11 – 0.30	0
Surmullet (*Mullus surmuletus*) *	9	3 (33%)	0.18	0.15	0.050 – 0.49	0
Red porgy (*Pagrus pagrus*)	12	1 (8 %)	0.31	0.18	0.050 – 1.0	25
Common pandora (*Pagellus erythrinus*) *	10	0 (0%)	0.35	0.24	0.22 – 0.86	0
Common sole (*Solea solea*)[b]	8	2 (25%)	0.45	0.40	0.050 – 1.2	38
Pearly razorfish (*Xyrichtys novacula*)[b]	7	6 (86%)	0.061	0.050	0.050 – 0.13	0
White seabream (*Diplodus sargus*)	9	0 (0%)	0.34	0.31	0.16 – 0.63	11
Comber (*Serranus cabrilla*)[b]	11	1 (9%)	0.30	0.18	0.050 – 0.53	27
Atlantic horse mackerel (*Trachurus trachurus*)[b]	7	2 (29%)	0.18	0.10	0.050 – 0.40	0
Painted comber (*Serranus scriba*)[b]	7	0 (0%)	0.28	0.26	0.20 – 0.46	0
Third trophic level (13 fish species)	**235**	**20 (9%)**	**0.51**	**0.36**	**0.050 – 3.1**	**28**
Dusky Grouper (*Epinephelus marginatus*)	10	0 (0%)	1.6	1.6	0.57 – 3.0	100
Red scorpionfish (*Scorpaena scrofa*)	35	3 (9%)	0.22	0.18	0.050 – 0.58	3
Conger (*Conger conger*)	31	0 (0%)	0.56	0.45	0.17 – 1.8	45
Common dentex (*Dentex dentex*)	17	0 (0%)	0.85	0.78	0.15 – 1.5	65
Black scorpionfish (*Scorpaena porcus*)[b]	2	0 (0%)	0.16	0.16	0.15 – 0.16	0
European barracuda (*Sphyraena sphyraena*)	1	0 (0%)	1.0	1	–	100
Jhon Dory (*Zeus faber*)	16	2 (13%)	0.33	0.21	0.050 – 1.3	19
Common dolphinfish (*Copyphaena hippurus*)	3	3 (100%)	0.050	0.050	0.050 – 0.050	0
European hake (*Merluccius merluccius*)	31	4 (13%)	0.30	0.18	0.050 – 0.99	26
Porbeagle (*Lamna nasus*)	1	0 (0%)	3.0	3.0	–	100
Mediterranean moray (*Muraena helena*)	38	0 (0%)	0.42	0.39	0.24 – 0.68	24
Angler (*Lophius piscatorius*) *	34	0 (0%)	0.74	0.57	0.12 – 3.1	15
Greater amberjack (*Seriola dumerili*)	16	8 (50%)	0.23	0.075	0.050 – 1.9	13

[a]QF: Percentage of samples below the quantification limit. [b]These species were analysed as sample pools. The average numbers of specimens in each individual sample analysed were anchovy (n = 77), picarel (n = 14), sardine (n = 32), black spot seabream (n = 4), common sole (n = 2), pearly razorfish (n = 6), comber (n = 5), Atlantic horse mackerel (n = 6), painted comber (n = 5) and black scorpionfish (n = 2). *The maximum level set forth by the EU is 0.5 mg/kg ww, except for the species labelled with an asterisk (1 mg/kg ww).

Table 2: THg concentrations (mean and range, mg/kg ww) and percentages of compliance for each fish species in Portmán (n = 26 fish specimens analyzed), Tunisia (n = 2), Egypt (n = 4) and the Atlantic Ocean (n = 14).

Location / Fish species	THg			
	n	Mean	Range	% Not compliance
Portmán				
Red mullet (*Mullus barbatus*)	19	0.17	0.070 – 0.44	0
Red porgy (*Pagrus pagrus*)	7	0.24	0.060 – 0.48	0
Tunisia				
Dusky Grouper (*Epinephelus marginatus*)	2	0.86	0.71 – 1.0	100
Egypt				
Dusky Grouper (*Epinephelus marginatus*)	1	0.92	–	100
Common dentex (*Dentex dentex*)	3	1.1	0.90 – 1.2	67
Atlantic Ocean				
Dusky Grouper (*Epinephelus marginatus*)	14	0.34	0.18 – 0.84	7

Table 3: Methylmercury concentrations (mg/kg ww, mean and range) in a subset of samples from the Balearic Islands (n = 79; 16 different fish species), Tunisia (n = 2; dusky grouper), Egypt (n = 4; dusky grouper and common dentex) and the Atlantic Ocean (n = 14; dusky grouper). The percentages of MeHg content among THg are also shown.

Fish specie	MeHg			MeHg/THg
	n	Mean	Range	%
Black seabream (*Spandyliosoma cantharus*)	1	0.88	–	NA
Small-spotted catshark (*Scyliorhinus canicula*)	6	0.70	0.35 – 1.0	79
Red porgy (*Pagrus pagrus*)	3	0.67	0.27 – 1.1	52
Common pandora (*Pagellus erythrinus*)	2	0.66	0.41 – 0.91	79
Common sole (*Solea solea*)	2	0.95	0.69 – 1.2	NA
White seabream (*Diplodus sargus*)	1	0.63	–	NA
Comber (*Serranus cabrilla*)	3	0.44	0.43 – 0.46	86
Dusky Grouper (*Epinephelus marginatus*)	9	1.4	0.57 – 2.3	81
“ “ “ “	2[a]	0.89	0.78 – 1.0	67
“ “ “ “	1[b]	0.62	–	
“ “ “ “	11[c]	0.40	0.040 – 0.94	48
Conger (*Conger conger*)	11	0.68	0.30 – 1.2	83
Common dentex (*Dentex dentex*)	7	1.0	0.24 – 1.5	88
“ “ “ “	3[b]	0.97	0.87 – 1.1	92
European barracuda (*Sphyraena sphyraena*)	1	1.1	–	NA
John Dory (*Zeus faber*)	3	1.1	0.59 – 1.5	NA
European hake (*Merluccius merluccius*)	5	0.46	0.33 – 0.62	75
Mediterranean moray (*Muraena helena*)	12	0.44	0.15 – 0.64	65
Angler (*Lophius piscatorius*)	10	0.93	0.53 – 3.0	87
Greater amberjack (*Seriola dumerili*)	3	0.72	0.05 – 1.5	79

[a] Samples from Tunisia. [b] Samples from Egypt. [c] Samples from the Atlantic Ocean.
NA: Not available. The value could not be calculated due to uncertainties in the applied methodologies.

Table 4. Daily fish consumptions, THg weekly intake through fish consumption, estimated weekly intake (EWI), Provisional Tolerable Weekly Intake percentages (PTWI %) and threshold levels for the maximum acceptable mercury contamination in fish, referred to the infant and general Spanish population.

		Daily fish consumption g/day	Median THg in fish mg/kg ww	THg weekly intakes µg/week	EWI µg/kg bw	PTWI[a] (%)	PTWI[b] (%)	Threshold level[a] mg/kg ww
Total fish	children	46.4 (100%)	0.29	94.2	2.7	68	210	0.42
	adult	71.1 (100%)		144.3	2.1	53	162	0.55
European hake	children	23.27 (50%)	0.18	29.3	0.85	21	65	
	adult	27 (38%)		34.0	0.50	12	38	
Common sole	children	7.56 (16.2%)	0.40	21.2	0.61	15	47	
	adult	7.15 (10%)		20.0	0.29	7	22	
Angler	children	1.76 (3.8%)	0.57	7.0	0.20	5	16	
	adult	1.87 (2.6%)		7.5	0.11	3	8	
Dusky grouper	children	0.83 (1.8%)	1.6	9.3	0.27	7	21	
	adult	0.87 (1.2%)		9.7	0.14	4	11	

Children: 7-12 years of age with a mean body weight of 34.48 kg; Adults: >17 years of age with a mean body weight of 68.48 kg (AESA, 2006).

[a] Based on a THg PTWI of 4 µg/kg bw (EFSA, 2012).

[b] Based on a MeHg PTWI of 1.3 µg/kg bw (EFSA, 2012).

Figure captions

Figure 1. Map of the fish sampling sites in the Mediterranean Sea and Atlantic Ocean considered in the present study.

Figure 2. Boxplots showing total mercury concentrations in each fish species from the Balearic Islands, ordered by trophic level average. The threshold values for total fish consumption of infant (red) and adult (blue) population groups from Spain calculated to fulfil the PTWI of total mercury indicated by FAO/WHO (EFSA, 2012) are also plotted.

Figure 3. Total mercury concentrations in fish from each Balearic Island, grouped by species.