New insights into mercury bioaccumulation in deep-sea organisms from the NW Mediterranean and their human health implications

Samuel Koenig [a,b]*, Montserrat Solé [a], Cristal Fernández-Gómez [b] and Sergi Díez [b]

[a] Institut de Ciències del Mar (ICM-CSIC). Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Catalunya, Spain.

[b] Institut de Diagnosi Ambiental i Estudis de l'Aigua (IDAEA-CSIC). C/ Jordi Girona 18, 08034 Barcelona, Catalunya, Spain.

* Corresponding author. Tel.: +34 93 230 95 00; fax: +34 93 230 95 55. E-mail address: koenig@icm.csic.es

Abstract

A number of studies have found high levels of mercury (Hg) in deep-sea organisms throughout the world's oceans, but the underlying causes are not clear as there is no consensus on the origin and cycling of Hg in the ocean. Recent findings suggested that Hg accumulation may increase with increasing forage depth and pointed to the deep-water column as the origin of most Hg in marine biota, especially its organic methylmercury (MeHg) form. In the present study, we determined total mercury (THg) levels in 12 deep-sea fish species and a decapod crustacean and investigated their relationship with the species' nitrogen stable isotope ratio ($\delta^{15}N$) as an indicator of their trophic level, average weight and habitat depth. THg levels ranged from 0.27 to 4.42 µg/g w.w. and exceeded in all, except one species, the recommended 0.5 µg/g w.w. guideline value. While THg levels exhibited a strong relationship with $\delta^{15}N$ values and to a lesser extent with weight, the habitat depth, characterized as the species' depth of maximum abundance (DMA), had also a significant effect on Hg accumulation. The fish species with a shallower depth range exhibited lower THg values than predicted by their trophic level ($\delta^{15}N$) and body mass, while measured THg values were higher than predicted in deeper-dwelling fish. Overall, the present results point out a potential risk for human health from the consumption of deep-sea fish. In particular, for both, the red shrimp *A. antennatus*, which is one of the most valuable fishing resources of the Mediterranean, as well as the commercially exploited fish *M. moro*, THg levels considerably exceeded the recommended 0.5 µg/g w.w. limit and should be consumed with caution.

Keywords: mercury bioaccumulation, trophic level, habitat depth, deep-sea fish, red shrimp *Aristeus antennatus,* Mediterranean

1. Introduction

Mercury (Hg) is a trace element of natural and anthropogenic origin that can be found throughout the atmosphere, biosphere and geosphere. In aquatic environments, mercury is readily transformed by chemical and biological (*i.e.* bacterially mediated) pathways into organomercury compounds such as methylmercury (MeHg), greatly affecting its solubility, volatility, bioavailability and toxicity (Díez, 2009). Methylmercury, the most toxic mercury species, is known to have numerous adverse effects, including neurotoxicity, genotoxicity and endocrine disruption on a wide range of vertebrates, including fish (Scheuhammer et al., 2007; Depew et al., 2012), as well as invertebrate species (Carrasco et al., 2008; Azevedo-Pereira and Soares, 2010; Faria et al., 2010).

The origin and cycling of MeHg in the marine environment is still not fully understood. Some studies have suggested that MeHg could originate from deep-sea sediments (Kraepiel et al., 2003; Ogrinc et al., 2007), while a recent study conducted in open Mediterranean waters concluded that most of the MeHg found in the water column had been generated in situ by planktonic organisms (Cossa et al., 2009). After entering the aquatic food web, MeHg tends to bind to sulfhydryl groups of proteins and biomagnifies in higher trophic level organisms (Mason et al., 2006). The presence of mercury in commercially important seafood, especially in large predatory pelagic fish is of major concern with regard to human consumption. Increasing anthropogenic emissions and growing public awareness of the potential health impacts of mercury have lead to the establishment of advisories and consumption limits for the general population and particularly for sensitive subgroups (e.g. pregnant women and young children). The concentration limit for total Hg (THg) in fish for human consumption was set at 1 mg/kg w.w. for predatory fish and 0.5 mg/kg w.w. for non-predatory species (FAO/WHO, 1991; EC, 2001). In general, more attention has been devoted to fish such as tuna, shark, king mackerel, swordfish and tilefish due their high position in the trophic chain; however, less attention has been paid to deep-sea fish and especially deep-sea crustaceans (e.g. shrimp).

Deep-sea species are thought to accumulate higher levels of heavy metals than more shallow-water species, possibly as a result of their higher longevity and trophic levels (Mormede and Davies, 2001). In this context, relatively high levels of mercury have been found in a number of deep-sea fish (Monteiro et al., 1996; Cronin et al., 1998;

Mormede and Davies, 2001; Storelli et al., 2002; Chiu and Mok, 2011). However, a previous study conducted in the North Pacific Ocean has also highlighted that foraging depth may also influence mercury accumulation in fish and that mercury levels are higher in deeper-feeding pelagic predators (Choy et al., 2009).

The objectives of the present study were: (1) to determine mercury levels in twelve deep-sea fish species and a deep-sea crustacean and investigate their relationship with the trophic level, size and depth of occurrence of the species (2) to give recommendations regarding the safe consumption of the selected fish species and the red shrimp *Aristeus antennatus*, a very popular seafood and one of the most valuable fishing resources of the Mediterranean.

2. Materials and methods

2.1. Sampling

Sampling cruises were conducted off the coast of Blanes, Catalan Sea (CS) onboard the R/V *Garcia del Cid* (CSIC) in February 2009 and in the southern Balearic Sea in the western basin (WM), the western Ionean Sea in the central basin (CM) and the eastern basin (EM) onboard the R/V *Sarmiento de Gamboa* (CSIC) in May 2009 (Fig. 1). Animals were caught using a OTMS otter trawl (Sardà et al., 1998) at depths ranging from 900 m to 2000 m. The selected species include the most abundant and thus ecologically the most relevant deep-sea species within the Mediterranean deep-sea (Tecchio et al., 2011). Onboard, muscle tissue was dissected and stored at -20 °C until further analysis.

2.2. Mercury analysis

THg was determined in pooled samples, consisting of 10 individuals per pool for all fish species and 5 individuals per pool for the crustacean *A. antennatus*, as male and female shrimp were analyzed separately. An equal portion of 0.2 g or 0.4 g were taken from the dorsal muscle tissue of each individual and subsequently homogenized. The measurements were performed using an advanced mercury analyser AMA-254, manufactured by Altec (Prague, Czech Republic) and distributed by Leco (St. Joseph,

MI, USA). This instrument is based on catalytic combustion of the sample, preconcentration by gold amalgamation, thermal desorption and atomic absorption spectrometry (AAS). Samples were taken directly from the freezer, cut into 50-150 mg pieces, precisely weighed in a nickel boat and automatically introduced into the AMA. Replicate analyses were conducted for each pooled sample and the measured values accepted if the relative standard deviation was lower than 10%, otherwise analysis were run again. The entire analytical procedure was validated by analysing blanks and CRM DORM-2 and DORM-3 samples at the beginning and end of each set of tissue samples (usually 10), ensuring that the instrument remained calibrated during the course of the study. Blanks consisted of an empty boat. Detection and quantification limits were calculated from blank measurements with THg values of 0.2 and 0.7 ng/g w.w., respectively (Díez et al., 2007).

2.3. Statistical analysis

For comparisons of THg levels among species, a linear regression analysis on Log THg (ng/g w.w.) data was performed using the species' habitat depth, trophic level and body mass as continuous variables. The habitat depth was chosen as the depth of maximum abundance (DMA) of each species, which was extracted from the *DeepMed Research Group Database* (ICM-CSIC). This parameter represents the depth at which the population of a given species exhibits its highest abundance within the species' depth range, thus representing the optimal habitat depth of the species across its depth range (Company and Sardà, 1998). It is noteworthy that DMA values do not necessarily coincide with sampling depths as the database extends beyond the depth range sampled in the present study (900-2000 m). However, in contrast to the DMA, the actual sampling depth is not necessarily representative of the species' ecological depth, similarly to the approach adopted in the studies by Choy et al. (2009) and Monteiro et al. (1996), where the variation of Hg accumulation with depth was also investigated. The trophic level was characterized as the nitrogen stable isotope ($\delta^{15}N$) values determined in a study by Tecchio et al. (2012) on the same samples as analyzed in this work, except for *Coelorinchus mediterraneus* and *Nezumia aequalis* data, taken from Polunin et al. (2001). It is noteworthy that the $\delta^{15}N$ determinations by Tecchio et al. (2012) were performed on the same samples The average weight (g) of the species was used instead of body size because for some fish species (i.e. macrouridae) the standard

length was recorded as the anal length instead of total body length and the comparison of size among species was therefore not possible. All interaction levels between factors (*i.e.* δ^{15}N*DMA; δ^{15}N*weight; DMA*weight) were not significant and were therefore omitted in the final analysis.

3. Results and discussion

3.1 Interspecies comparisons

In the present study, THg levels were used as proxy for organic mercury content because MeHg is the major chemical form of mercury stored in fish muscle tissues (80-90 % of the total mercury) (Harris et al., 2003). A similar relationship between THg and MeHg has been demonstrated for the crustacean *A. antennatus*, with an average 85 % MeHg content in muscle tissue (Minganti et al., 1996).

Values for total mercury (THg) concentrations in muscle tissue ranged from 0.27 to 4.42 µg/g w.w. and only one fish species, namely *Lampanyctus crocodilus*, exhibited total mercury (THg) levels below the European limit for safe consumption of 0.5 µg/g w.w. (Table 1). Furthermore, for six species, THg content was determined in samples from different sites within the Mediterranean (Fig. 2, Table S1). However, due to the fact that only one pooled sample was analyzed for some sites and species the statistical difference of these site differences could not be determined. The measured values for all species at all sites exceeded the recommended consumption limit (Fig. 2) and a relatively uniform THg contamination pattern was observed across all sites. However, it is noteworthy that only a limited number of samples were included in this study and that previous studies showed that THg and MeHg levels significantly vary both vertically and horizontally within Mediterranean waters (Kotnik et al., 2007).

The high level of mercury contamination of the Mediterranean Sea has been the subject of extensive studies for the last decades and has resulted in international campaigns such as the UNEP MED POL program, to assess the impact of mercury contamination on the Mediterranean marine environment (Aston and Fowler, 1985; UNEP/FAO/WHO, 1987; Cossa and Coquery, 2005). Moreover, our results are concordant with previous results of mercury concentrations in Mediterranean deep-sea

organisms from the central (Storelli et al., 2002; Drava et al., 2004) and the eastern Mediterranean basin (Hornung et al., 1993). In contrast, mercury levels in deep-sea fish from the Atlantic and Pacific Ocean, including species also analyzed in the present study such as *Nezumia aequalis* (Mormede and Davies, 2001) and *Coryphaenoides guentheri* (Cronin et al., 1998), generally exhibited Hg levels of one order of magnitude lower than those recorded within this work. Several potential explanations for these high mercury concentrations in Mediterranean biota have been proposed, including volcanic activity and higher anthropogenic emission rates (*e.g.* mining), although more recent findings suggest a biological rather than geochemical origin (Cossa and Coquery, 2005). In particular, the oligotrophic nature of the Mediterranean Sea could enhance the methylation of mercury within the water column thus resulting in higher bioaccumulation of MeHg at the base of the food chain, which is subsequently transferred to larger predators (Cossa et al., 2009; Heimbürger et al., 2010; MerMex Group, 2011).

In addition, linear regression analyses of data from all species exhibited a significant effect of the species' nitrogen stable isotope ratio ($\delta^{15}N$), depth of maximum abundance (DMA) and body weight on THg content in muscle tissue ($R^2 = 0.85$, $P < 0.0001$, Fig. 3). However, the crustacean *Aristeus antennatus* was an outlier (Fig. 3d) and the fit of the model improved when only the 12 fish species were included in the analysis, with DMA, $\delta^{15}N$ and weight accounting for 96 % of the variability in THg among fish species (Table 2a). Of the three factors included in the analysis, variations in $\delta^{15}N$ explained most of the variability of THg among fish species, while the effect of the DMA was second most important followed by the average weight of the species (Table 2a, Fig. 3a,b,c). Furthermore, a stepwise regression analysis was performed to determine whether the introduction of each factor improves the fit of the respective model. According to the Mallows' Cp values, the inclusion of the factors DMA and weight both improved the fit of the model and the best fit was obtained when all three factors were included (Table 3). The strong relationship between THg and $\delta^{15}N$ observed in the present study (Fig. 3a) is consistent with the general understanding that mercury, in particular MeHg, tends to bioaccumulate in higher tropic level organisms (Senn et al., 2010). Interspecific mercury variation in fish has been attributed to size/mass in numerous studies (Choy et al., 2009), a trend which is also confirmed by the significant effect of weight on THg levels observed in the present study.

While the variation of mercury burden among species according to the trophic level and body mass is well understood, the influence of habitat depth on mercury accumulation is less clear. To illustrate the effect of DMA on THg, a linear regression model with $\delta^{15}N$ and weight was fitted (Table 2b) and predicted values were contrasted against measured THg levels in biota across depth ranges (Fig. 4). THg levels encountered in fish were lower than predicted based on $\delta^{15}N$ and weight at 500-1300 m depth and higher than predicted at 1500-2800 m depth (Fig. 4). The present results thus clearly indicate that mercury levels increase with increasing habitat depth in fish, with a particularly pronounced difference between the shallower depth range (500 - 900 m) and the highest depth of 2800 m. This increasing THg trend with depth is in accordance with former studies that reported elevated mercury levels in deep-sea fish from the Mediterranean Sea (Hornung et al., 1993; Storelli et al., 2002), as well the Atlantic (Monteiro et al., 1996; Mormede and Davies, 2001; Bebianno et al., 2007) and Pacific Ocean (Chiu and Mok, 2011). Furthermore, this increasing trend in Hg contamination with depth has been previously described in a study on large pelagic predators such as tuna in the North Pacific Ocean, where species that forage at greater depths accumulated higher mercury loads than species that feed on epipelagic prey (Choy et al., 2009).

In contrast to the deep-sea fish, the crustacean *A. antennatus* did not follow the same trend and the predicted THg value (based on $\delta^{15}N$ and weight, see equation in Table 2b) was lower than the actual observed level, despite a relatively shallow DMA of 700 m (Fig. 4). This could be due to distinct reasons. For instance, the crustacean might present higher bioaccumulation kinetics for mercury than fish as a result of higher uptake through diet and/or lower metabolism. However, to our knowledge no information exists regarding differential Hg accumulation mechanisms between fish and crustaceans and further research is needed to corroborate this hypothesis. Another potential explanation arises from the difference in the depth profile of juvenile recruitment between the deep-sea fish species and the crustacean *A. antennatus*. Deep-sea fish recruitment takes place at shallower depths and juveniles progressively migrate to greater depths during their lifetime ("bigger-deeper") (e.g. Massutí et al., 1995; Morales-Nin et al., 1996; Rotllant et al., 2002). In contrast, the recruitment of *A. antennatus* occurs at greater depths and juveniles become more abundant with increasing depth ("smaller-deeper") (Sardà et al., 2004). Hence, it is possible that although adult *A. antennatus* are most abundant at 700 m, juveniles feed at greater

depths, which could result in higher bioaccumulated mercury levels in adult shrimp than predicted by their trophic level.

3.2. Public health issues

For the decapod crustacean *Aristeus antennatus* (red shrimp), a species of high commercial value, only one out of thirteen analyzed samples did not exceed the recommended value of 0.5 μg/g w.w (Table S1). Although previous studies already detected relatively high levels of mercury in this species from the Ligurian Sea (Minganti et al., 1996; Drava et al., 2004), the present findings further stress the need to investigate the mercury content in this species and potentially issue advisories on the human consumption of red shrimp. This issue is particularly important considering the fact that shrimp are reported to generally contain lower mercury levels than most fish species and thus to be safe for human consumption, as recommended for instance by the US Food and Drug Administration (USFDA, 2002) or the Catalan Food Safety Agency (ACSA, 2008). However, these recommendations are based on values detected in shallow-water shrimp species (e.g. 0.12 μg/g w.w. in *Penaeus setiferus*; Domingo, 2008), which appear to have lower Hg content than *A. antennatus*.

The US Environmental Protection Agency (USEPA) adopted a revised reference dose (RfD) for MeHg of 0.1 μg mercury per kg body weight per day (USEPA, 1997). In 2006, a provisional tolerable weekly intake (PTWI) of 1.6 μg MeHg/kg body weight/week was established in the 67st meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006). We assumed that the concentration of total Hg is equal to that of MeHg (Bloom, 1992) and a body weight of 70 kg for adult males was also assumed. Therefore, based on these assumptions, the guideline value calculated from RfD of US EPA and PTWI of JECFA was 7.0 μg Hg/day and 16.1 μg Hg/day, respectively. Based on the mean Hg concentration in red shrimp in Table 1 (1.04 μg/g w.w.) and the shrimp consumption rate of 3.5 g/day in Catalonia, Spain (Domingo, 2008), dietary Hg exposure from shrimp consumption was estimated at 3.64 μg Hg/day, which was significantly lower than the US EPA and JECFA guidelines. However, the estimated exposure value represents 52 % of the RfD and 23 % of the PTWI for adult males, in contrast to the reported 2 % of PTWI deduced from Hg levels detected in shallow-water shrimp species (Domingo, 2008).

Moreover, the deep-sea fish species *Mora moro* (commom mora) is also commercially exploited and mercury levels in samples from the NW Mediterranean and the Central Mediterranean exceeded by far the 0.5 µg/g w.w. limit (Fig. 2). Although the other fish species included in the present study are currently not relevant for human consumption, the fact that all except one were found to have higher mercury levels than the recommended value indicates that mercury contamination might be of particular concern for deep-sea fisheries. This issue is particularly relevant considering the fact that deep-water fisheries, which are of relatively modern origin, are continuously expanding and deep-sea fish are becoming increasingly important as a human food resource (Morales-Nin and Panfili, 2005; Ramirez-Llodra et al., 2011).

4. Conclusions

The present study has shown that apart from the species' trophic position and body mass, the habitat depth also significantly influences the THg accumulation in deep-sea fish. Fish species with a shallower depth distribution exhibited lower than expected THg concentrations while the contrary was observed for deeper-dwelling species. Thus, when taking into account the trophic level and weight of each species, THg contamination appears to increase with habitat depth. This trend is concordant with observations from other studies on Hg accumulation in deep-sea fish throughout the world's oceans, and appears to be a general phenomenon rather than an exception. These findings are particularly relevant considering the fact that the exploitation of deep-sea waters is becoming increasingly important as a fisheries resource and the consumption of deep-sea organisms might be of particular concern with regard to human health. As shown in the present study, THg exceeded in all except one species the recommended 0.5 µg/g w.w. guideline value, including the highly valuable red shrimp *A. antennatus* and the commercially exploited fish *M. moro*.

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References

ACSA, 2008. Chemical contaminants in fish and shellfish consumed in Catalonia, Spain. http://www.gencat.cat/salut/acsa/html/es/dir1599/doc16977.html.

Aston, S.R., Fowler, S.W., 1985. Mercury in the open mediterranean: Evidence of contamination? Sci. Total Environ. 43, 13-26.

Azevedo-Pereira, H., Soares, A., 2010. Effects of Mercury on Growth, Emergence, and Behavior of Chironomus riparius Meigen (Diptera: Chironomidae). Arch. Environ. Contam. Toxicol. 59, 216-224.

Bebianno, M.J., Santos, C., Canário, J., Gouveia, N., Sena-Carvalho, D., Vale, C., 2007. Hg and metallothionein-like proteins in the black scabbardfish Aphanopus carbo. Food Chem. Toxicol. 45, 1443-1452.

Bloom, N.S., 1992. On the Chemical Form of Mercury in Edible Fish and Marine Invertebrate Tissue. Can. J. Fish. Aquat. Sci. 49, 1010-1017.

Carrasco, L., Díez, S., Soto, D.X., Catalan, J., Bayona, J.M., 2008. Assessment of mercury and methylmercury pollution with zebra mussel (Dreissena polymorpha) in the Ebro River (NE Spain) impacted by industrial hazardous dumps. Sci. Total Environ. 407, 178-184.

Chiu, K.-H., Mok, H.-K., 2011. Study on the Accumulation of Heavy Metals in Shallow-Water and Deep-Sea Hagfishes. Arch. Environ. Contam. Toxicol. 60, 643-653.

Choy, C.A., Popp, B.N., Kaneko, J.J., Drazen, J.C., 2009. The influence of depth on mercury levels in pelagic fishes and their prey. Proc. Natl. Acad. Sci. 106, 13865-13869.

Company, J.B., Sardà, F., 1998. Metabolic rates and energy content of deep-sea benthic decapod crustaceans in the western Mediterranean Sea. Deep Sea Research Part I: Oceanographic Research Papers 45, 1861-1880.

Cossa, D., Averty, B., Pirrone, N., 2009. The origin of methylmercury in open mediterranean waters. Limnol. Oceanogr. 54, 837-844.

Cossa, D., Coquery, M., 2005. The Mediterranean Mercury Anomaly, a Geochemical or a Biologocal Issue, in: Saliot, A. (Ed.), The Mediterranean Sea. Handbook of environmental chemistry. Springer, Heidelberg, pp. 121-130.

Cronin, M., Davies, I.M., Newton, A., Pirie, J.M., Topping, G., Swan, S., 1998. Trace metal concentrations in deep sea fish from the North Atlantic. Mar. Environ. Res. 45, 225-238.

Depew, D.C., Basu, N., Burgess, N.M., Campbell, L.M., Devlin, E.W., Drevnick, P.E., Hammerschmidt, C.R., Murphy, C.A., Sandheinrich, M.B., Wiener, J.G., 2012. Toxicity of dietary methylmercury to fish: Derivation of ecologically meaningful threshold concentrations. Environ. Toxicol. Chem. 31, 1536-1547.

Díez, S., 2009. Human Health Effects of Methylmercury Exposure. Rev. Environ. Contam. Toxicol. 198, 111-132.

Díez, S., Montuori, P., Querol, X., Bayona, J.M., 2007. Total Mercury in the Hair of Children by Combustion Atomic Absorption Spectrometry (Comb-AAS). J. Anal. Toxicol. 31, 144-149.

Drava, G., Capelli, R., Minganti, V., De Pellegrini, R., Orsi Relini, L., Ivaldi, M., 2004. Trace elements in the muscle of red shrimp Aristeus antennatus (Risso, 1816) (Crustacea, Decapoda) from Ligurian sea (NW Mediterranean): variations related to the reproductive cycle. Sci. Total Environ. 321, 87-92.

EC, 2001 Commission regulation (EC) No. 466/2001. Official Journal of the European Communities. European Economic Community, Brussels, Belgium.

FAO/WHO, 1991. Codex Alimentarius Guideline Levels for Methylmercury in Fish. CAC/GL 7-1991.

FAO/WHO, 2006. Summary and Conclusions of the Sixty-Seventh Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Rome.

Faria, M., López, M.A., Díez, S., Barata, C., 2010. Are native naiads more tolerant to pollution than exotic freshwater bivalve species? An hypothesis tested using physiological responses of three species transplanted to mercury contaminated sites in the Ebro River (NE, Spain). Chemosphere 81, 1218-1226.

Harris, H.H., Pickering, I.J., George, G.N., 2003. The Chemical Form of Mercury in Fish. Science 301, 1203.

Heimbürger, L.-E., Cossa, D., Marty, J.-C., Migon, C., Averty, B., Dufour, A., Ras, J., 2010. Methyl mercury distributions in relation to the presence of nano- and picophytoplankton in an oceanic water column (Ligurian Sea, North-western Mediterranean). Geochim. Cosmochim. Acta 74, 5549-5559.

Hornung, H., Krom, M.D., Cohen, Y., Bernhard, M., 1993. Trace metal content in deep-water sharks from the eastern Mediterranean Sea. Mar. Biol. 115, 331-338.

JECFA, 2006. Joint FAO/WHO Expert Committee on Food Additives http://www.chem.unep.ch/mercury/Report/JECFA-PTWI.htm.

Kotnik, J., Horvat, M., Tessier, E., Ogrinc, N., Monperrus, M., Amouroux, D., Fajon, V., Gibičar, D., Žižek, S., Sprovieri, F., Pirrone, N., 2007. Mercury speciation in surface and deep waters of the Mediterranean Sea. Mar. Chem. 107, 13-30.

Kraepiel, A.M.L., Keller, K., Chin, H.B., Malcolm, E.G., Morel, F.M.M., 2003. Sources and Variations of Mercury in Tuna. Environ. Sci. Technol. 37, 5551-5558.

Mason, R., Heyes, D., Sveinsdottir, A., 2006. Methylmercury Concentrations in Fish from Tidal Waters of The Chesapeake Bay. Arch. Environ. Contam. Toxicol. 51, 425-437.

Massutí, E., Morales-Nin, B., Stefanescu, C., 1995. Distribution and biology of five grenadier fish (Pisces: Macrouridae) from the upper and middle slope of the northwestern Mediterranean. Deep-Sea Res. Part I Oceanogr. Res. Pap. 42, 307-330.

MerMex Group, 2011. Marine ecosystems' responses to climatic and anthropogenic forcings in the Mediterranean. Prog. Oceanogr. 91, 97-166.

Minganti, V., Capelli, R., De Pellegrini, R., Orsi Relini, L., Relini, G., 1996. Total and organic mercury concentrations in offshore crustaceans of the Ligurian Sea and their relations to the trophic levels. Sci. Total Environ. 184, 149-162.

Monteiro, L.R., Costa, V., Furness, R.W., Santos, R.S., 1996. Mercury concentrations in prey fish indicate enhanced bioaccumulation in mesopelagic environments. Mar. Ecol. Prog. Ser. 141, 21-25.

Morales-Nin, B., Massutí, E., Stefanescu, C., 1996. Distribution and biology of *Alepocephalus rostratus* from the Mediterranean Sea. J. Fish Biol. 48, 1097-1112.

Morales-Nin, B., Panfili, J., 2005. Seasonality in the deep sea and tropics revisited: what can otoliths tell us? Mar. Freshw. Res. 56, 585-598.

Mormede, S., Davies, I.M., 2001. Trace elements in deep-water fish species from the Rockall Trough. Fish. Res. 51, 197-206.

Ogrinc, N., Monperrus, M., Kotnik, J., Fajon, V., Vidimova, K., Amouroux, D., Kocman, D., Tessier, E., Zizek, S., Horvat, M., 2007. Distribution of mercury and methylmercury in deep-sea surficial sediments of the Mediterranean Sea. Mar. Chem. 107, 31-48.

Polunin, N.V.C., Morales-Nin, B., Pawsey, W.E., Cartes, J.E., Pinnegar, J.K., Moranta, J., 2001. Feeding relationships in Mediterranean bathyal assemblages elucidated by stable nitrogen and carbon isotope data. Mar. Ecol. Prog. Ser. 220, 13-23.

Ramirez-Llodra, E., Tyler, P.A., Baker, M.C., Bergstad, O.A., Clark, M.R., Escobar, E., Levin, L.A., Menot, L., Rowden, A.A., Smith, C.R., Van Dover, C.L., 2011. Man and the Last Great Wilderness: Human Impact on the Deep Sea. PLoS One 6, e22588.

Rotllant, G., Moranta, J., Massutí, E., Sardà, F., Morales-Nin, B., 2002. Reproductive biology of three gadiform fish species through the Mediterranean deep-sea range (147-1850 m). Sci. Mar. 66, 157-166.

Sardà, F., Cartes, J.E., Company, J.B., Albiol, A., 1998. A Modified Commercial Trawl Used to Sample Deep-Sea Megabenthos. Fish. Sci. 64, 492-493.

Sardà, F., D'Onghia, G., Politou, C.Y., Company, J.B., Maiorano, P., Kapiris, K., 2004. Deep-sea distribution, biological and ecological aspects of Aristeus antennatus (Risso, 1816) in the western and central Mediterranean Sea. Sci. Mar. 68, 117-127.

Scheuhammer, A.M., Meyer, M.W., Sandheinrich, M.B., Murray, M.W., 2007. Effects of Environmental Methylmercury on the Health of Wild Birds, Mammals, and Fish. Ambio 36, 12-19.

Senn, D.B., Chesney, E.J., Blum, J.D., Bank, M.S., Maage, A., Shine, J.P., 2010. Stable Isotope (N, C, Hg) Study of Methylmercury Sources and Trophic Transfer in the Northern Gulf of Mexico. Environ. Sci. Technol. 44, 1630-1637.

Storelli, M.M., Giacominelli-Stuffler, R., Marcotrigiano, G.O., 2002. Total and methylmercury residues in cartilaginous fish from Mediterranean Sea. Mar. Pollut. Bull. 44, 1354-1358.

Tecchio, S., Ramírez-Llodra, E., Sardà, F., Company, J.B., 2011. Biodiversity of deep-sea demersal megafauna in western and central Mediterranean basins. Sci. Mar. 75, 341-350.

Tecchio, S., van Oevelen, D., Soetaert, K., Moodley, L., Ramírez-Llodra, E., Sardà, S., 2012. Increasing community niche width with depth and oligotrophy in deep-sea megabenthos. In preparation.

UNEP/FAO/WHO, 1987. Assessment of the state of pollution of the Mediterranean Sea by mercury and mercury compounds and proposed measures. MAP Tech. Rep. Ser. 18. UNEP/MAP, Athens.

USEPA, 1997. Mercury Study Report to Congress. Office of Air Quality Planning and Standards and Office of Research and Development, EPA 452/R-97-0003, Washington, DC.

USEPA, 2005. Water quality criterion for the protection of human health. Methylmercury. US Environmental Protection Agency, Office of Science and Technology, Office of Water, Washington DC.

USFDA, 2002. Consumer advisory: an important message for pregnant women and women of childbearing age who may become pregnant about the risks of

mercury in fish. US Federal Drug Administration.
http://w.w.w.fda.gov/oc/opacom/hottopics/mercury/backgrounder.html

Table

Table 1 Biological characteristics and mean total mercury (THg) levels (min.-max-) of 12 deep-sea fish species and the crustacean *A. antennatus.*

Species	**N**	**DMA**	**$\delta^{15}N$**	**Weight (g) ± S.E.**	**Diet**	**THg (µg/g ww)**
Alepocephalus rostratus (*Ar*)	8	1300 m	9.86	370 ± 49	Macroplankton (non-migratory)[a]	0.64 (0.30-1.32)
Bathtypterois mediterraneus (*Bm*)	1	1750 m	11.39	17 ± 1	Benthopelagic plankton (non-migratory)[b]	1.23
Cataetyx laticeps (*Cl*)	1	2800 m	12.75	430 ± 60	Epibenthic prey[c]	4.42
Coelorinchus mediterraneus (*Cm*)	3	1500 m	12.60*	23 ± 2	Infauna[a]	1.46 (1.40-1.56)
Coryphaenoides guentheri (*Cg*)	1	1750 m	11.00	16 ± 1	Benthic feeder[d]	0.92
Coryphaenoides mediterraneus (*Cm**)	1	2700 m	10.97	88 ± 17	Benthopelagic and benthic feeder[d]	1.96
Lampanyctus crocodiles (*Lc*)	1	500 m	8.09	43 ± 4	Macroplankton (migratory)[a]	0.27
Lepidion lepidion (*Ll*)	10	1200 m	11.20	64 ± 12	Benthopelagic and benthic feeder[e]	0.94 (0.21-1.71)
Mora moro (*Mm*)	2	1100 m	11.78	584 ± 48	Nekto-suprabenthos (Active predator)[e]	2.40 (2.38-2.43)
Nezumia aequalis (*Na*)	1	600 m	13.78*	55 ± 4	Nekto-suprabenthos[a,f]	1.79
Nezumia sclerorhynchus (*Ns*)	5	1200 m	12.22	25 ± 3	Nekto-suprabenthos[f]	1.44 (0.97-1.86)
Trachyrhynchus scabrus (*Ts*)	1	900 m	10.25	190 ± 12	Infauna[a]	0.65
Aristeus antennatus (*Aa*)	13	700 m	9.61	17 ± 5	Infauna[a]	1.04 (0.48-2.24)

N: number of pools used to calculate THg (each pool consists of n = 10 individuals for all fish species and n = 5 for *Aa*)
DMA: depth of maximum abundance
$\delta^{15}N$: nitrogen stable isotope values from Tecchio et al. (In prep.) , except values indicated by * from Polunin et al. (2001)

[a] (Cartes et al., 2002); [b] (Carrassón and Matallanas, 2001); [c](Mauchline and Gordon, 1984); [d] (Carrassón and Matallanas, 2002); [e] (Carrassón et al., 1997); [f] (Marques and Almeida, 1998)

Table 2 Linear regression results and Type III sum of squares of Log THg (ng/g w.w.) values in deep-sea fish (n = 12) as a function of depth of maximum abundance (DMA), nitrogen stable isotope ratio ($\delta^{15}N$) and average weight of the species.

Factors	**Source**	**df**	**Sum of squares**	**Mean squares**	**F**	**Pr > F**
a) DMA, $\delta^{15}N$, Weight	R^2 = 0.96, Log THg = 1.75E-04*DMA + 0.15*$\delta^{15}N$ + 4.75E-04*Weight + 1.04					
	Model	3	1.058	0.353	65.33	<.0001
	Error	8	0.043	0.005		
	Corrected Total	11	1.101			
	DMA	1	0.162	0.162	30.05	0.001
	$\delta^{15}N$	1	0.533	0.533	98.75	<.0001
	Weight	1	0.091	0.091	16.89	0.003
b) $\delta^{15}N$, Weight	R^2 = 0.81, Log THg = 0.17*$\delta^{15}N$ + 5.72E-04*Weight + 1.04					
	Model	2	0.896	0.448	19.62	0.001
	Error	9	0.205	0.023		
	Corrected Total	11	1.101			
	$\delta^{15}N$	1	0.731	0.731	32.02	0.000
	Weight	1	0.136	0.136	5.95	0.037

Table 3 Summary of the factor selection using stepwise regression analysis of Log THg (ng/g w.w.) values in deep-sea fish (n = 12) as a function of depth of maximum abundance (DMA), nitrogen stable isotope ratio ($\delta^{15}N$) and average weight of the species.

No. of variables	Variables	R^2	Mallows' Cp
1	$\delta^{15}N$	0.69	55.212
2	$\delta^{15}N$ / DMA	0.88	18.887
3	$\delta^{15}N$ / DMA / Weight	0.96	4.000

Fig. 1 Map of sampling sites across Mediterranean Sea

Fig. 2 Mean total mercury (THg) levels ± S.D. (µg/g wet weight) in six deep-sea species from various sampling sites across the Mediterranean Sea (Blanes and theWestern, Central and Eastern Mediterranean areas). For species abbreviation names see Table 1. Dashed line indicates the 0.5 µg/g w.w. recommended value for human consumption.

Fig. 3 Relationship between Log THg levels (ng/g wet weight) and (a) nitrogen stable isotope values ($\delta^{15}N$), (b) depth of maximum abundance (DMA) and (c) weight. (d) Measured versus predicted Log THg levels based on the equation for the linear regression for for 12 deep-sea fish species and the red shrimp *Aristeus antennatus*. For species abbreviation names see Table 1.

Fig. 4 Difference between measured and predicted Log THg levels (based on $\delta^{15}N$ and weight, see Table 3b) in fish from different depth ranges and the crustacean *A. antennatus.* The abbreviation of the species names included in each depth strata are provided below the bars. Full species names and their respective depth of maximum abundance (DMA) are listed in Table 1. Values shown are mean ± S.E.M.

Figure 1

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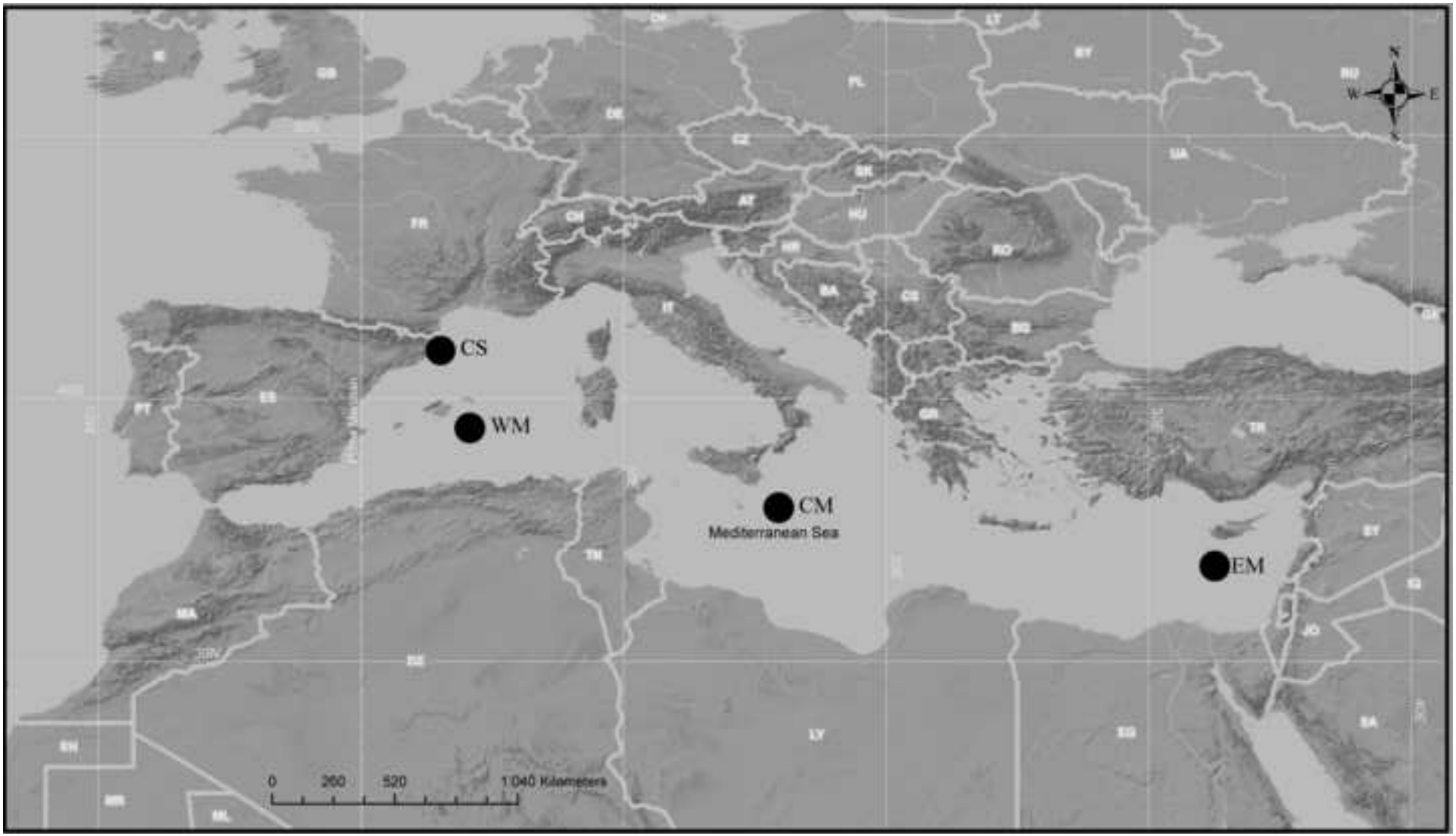

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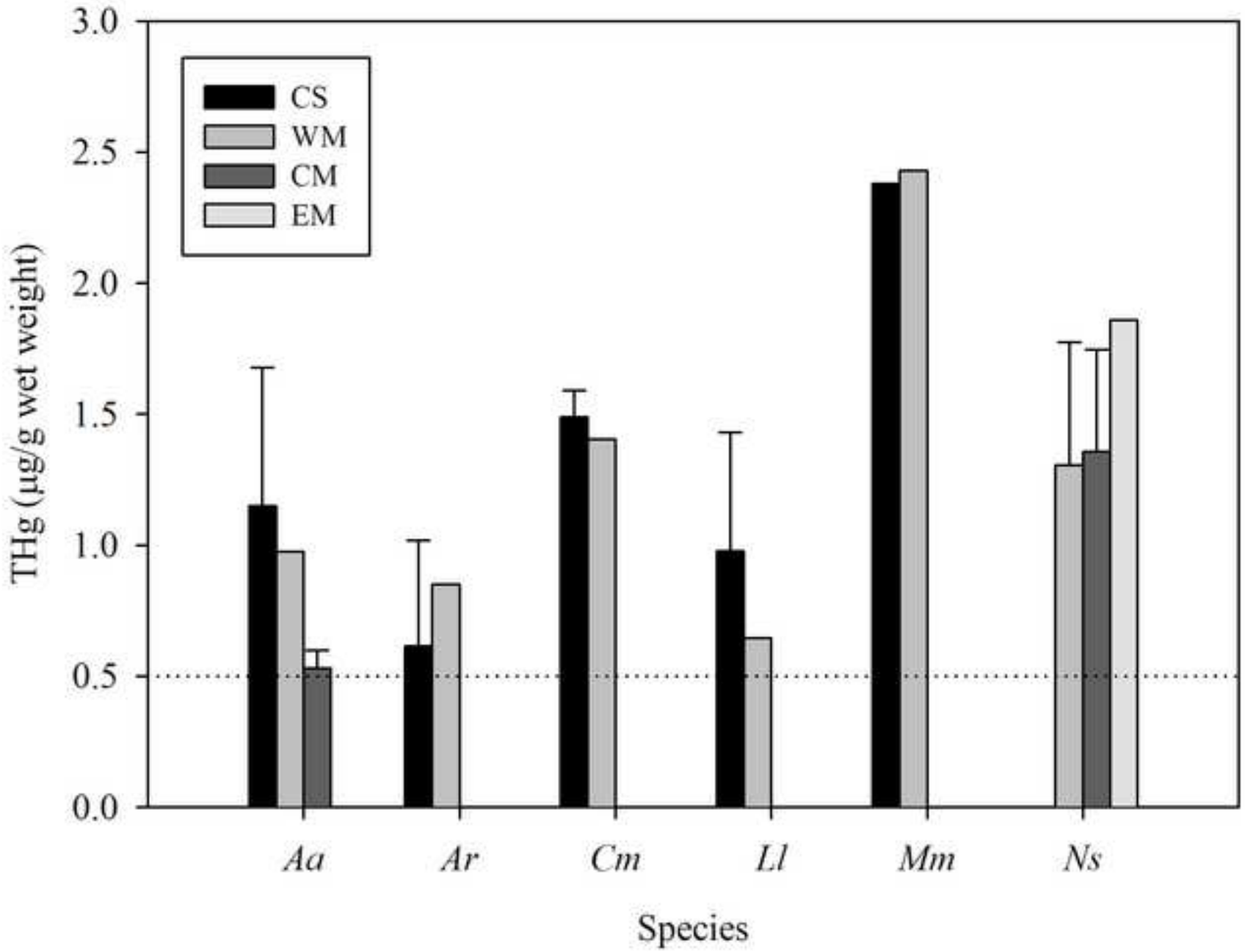

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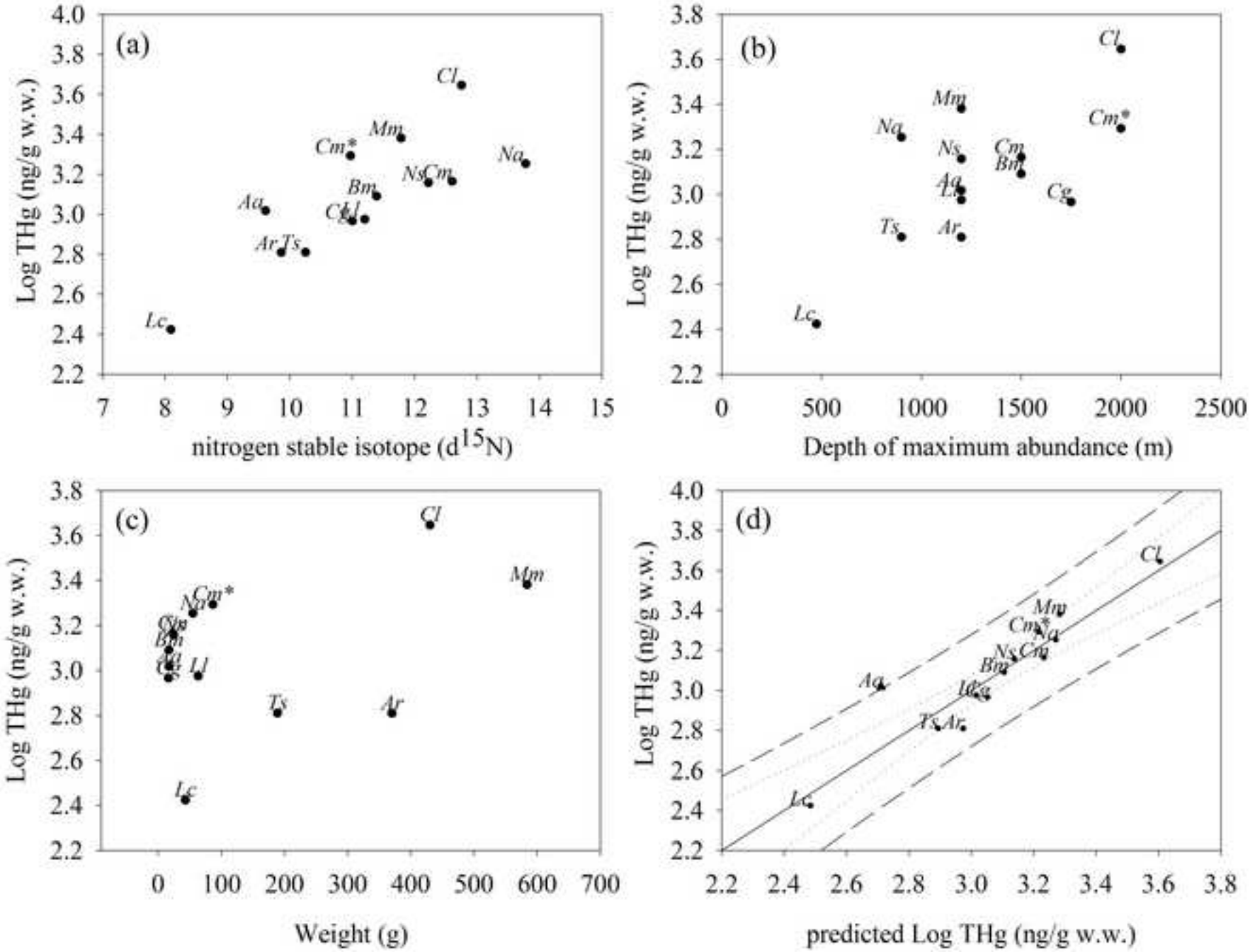

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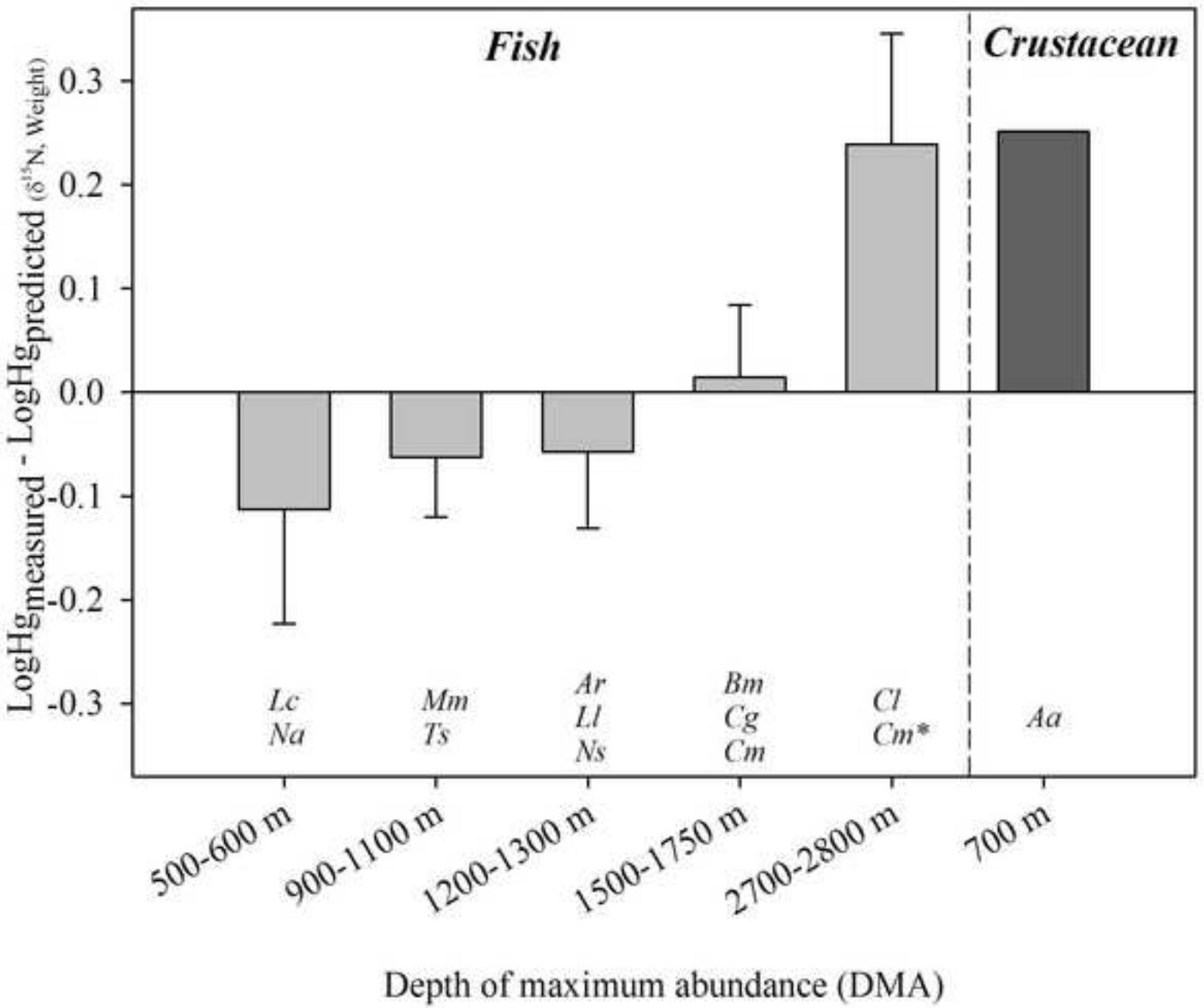

Supplementary Material

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