
Are red mullet efficient as bio-indicators of mercury contamination? A case study from the French Mediterranean

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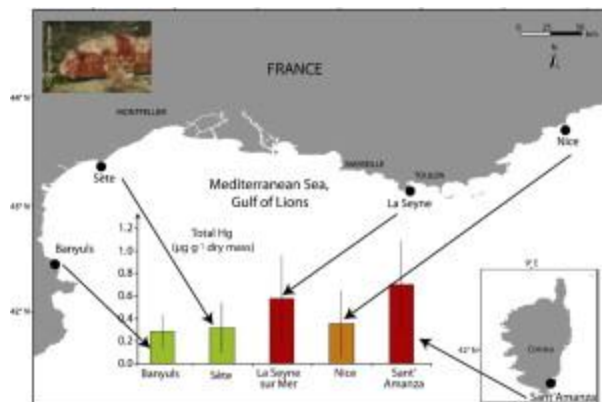
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Abstract :

Mercury (Hg) is one of the main chemicals currently altering Mediterranean ecosystems. Red mullet (*Mullus barbatus* and *M. surmuletus*) have been widely used as quantitative bio-indicators of chemical contamination. In this study, we reassess the ability of these species to be used as efficient bio-indicators of Hg contamination by monitoring during 18 months Hg concentrations in muscle tissue of mullet sampled from 5 French Mediterranean coastal areas. Mean concentrations ranged between 0.23 and 0.78 $\mu\text{g g}^{-1}$ dry mass for both species. Values were consistent with expected contamination patterns of all sites except Corsica. Results confirmed that red mullets are efficient bio-indicators of Hg contamination. Nevertheless, the observed variability in Hg concentrations calls for caution regarding the period and the sample size. Attention should be paid to environmental and biologic specificities of each studied site, as they can alter the bioaccumulation of Hg, and lead to inferences about environmental Hg concentrations.

Graphical abstract



Highlights

► Hg was monitored in *Mullus* spp. from 5 French Mediterranean zones during 18 months. ► All concentrations were below recommended European health safety levels. ► Hg trends were consistent with environmental contamination except in Corsica. ► Oligotrophy could explain the high and unexpected values in Corsica. ► Biotic and abiotic parameters must be considered when using bioindicators.

Keywords : Mercury, Bio-indicator, Fish, *Mullus* spp., Mediterranean

Introduction

Chemical contamination is one of the main threats currently causing alteration to marine ecosystems (Halpern et al., 2008), with observed impact on individuals, populations, species and ecosystems (Fleeger et al., 2003). Chemical pressure is high in the Mediterranean Sea, a semi-enclosed sea surrounded by highly industrialized and densely populated countries (Durrieu de Madron et al., 2011). Among all chemicals, mercury (Hg) is of great concern, as it has neurotoxic, immunotoxic and teratogenic effects on vertebrates (Scheuhammer et al., 2007; Tartu et al., 2013). Metallic mercury (Hg^0) is emitted in the environment from natural (mainly volcanic emissions) and anthropogenic sources, with an increase in the anthropogenic sources since the end of the 18th century (Streets et al., 2011). Hg^0 is highly volatile and can be transported during long periods of time and over long distances. After deposition as Hg^{II} , Hg is methylated by heterophilic bacteria as methylmercury (MeHg). In marine environments, this process occurs in low oxygen zones in the water column, and at the water/sediment interface (Blum et al., 2013; Cossa et al., 2009; Heimbürger et al., 2010). MeHg is the chemical form of Hg of greatest concern, as it can enter the trophic networks after being uptaken by the phytoplankton. High MeHg concentrations are observed in high trophic level species, due to the high level of biomagnification of MeHg throughout the trophic networks. To cope with such high Hg concentrations, physiological mechanisms were developed against the adverse effect of metallic contaminants. The synthesis of metallothionein is commonly proposed as an important detoxifying mechanism in fishes. Metallothionein is a class of low molecular-weight proteins able to bind to metals and to play a role in the detoxifying process as high metallothionein concentration are measured in fish sampled in contaminated zones (Filipović and Raspor, 2003; Zorita et al. 2009). Others detoxifying mechanisms can involve antioxidant synthesis or metabolisation of contaminants in the liver (Company et al. 2010).

Monitoring Hg contamination can be performed by assessing environmental concentrations in water or sediment. This approach produces an immediate measure of contamination in a specific place but is complex to use, mainly due to low concentrations and the high temporal and spatial variability observed. In addition, high contaminant concentrations in the environment do not always lead to high values and adverse effects on organisms and vice-versa (Beyer et al., 1996; Martínez-Gómez et al., 2012). Consequently, in most cases, contamination has been assessed by bio-monitoring, *i.e.* inferring the contamination in the environment from values measured in organisms. This approach can provide time and space integrated information about the actual Hg contamination, and also about the bio-available

fraction of the contaminant, which actually enters the trophic networks. Determining Hg concentrations in marine organisms is also required for sanitary purpose, as consumption of contaminated products is the main pathway leading to human intoxication (Endo et al., 2013; Martí-Cid et al., 2007). Nevertheless, this approach is rendered complex because of the influence of various biotic and abiotic factors on the bioaccumulation (Phillips and Rainbow, 1998).

Red mullet (*Mullus* spp.) exhibit several features allowing them to be considered as appropriate bio-indicators of mercury contamination. These species are observed off all Mediterranean shores, providing a basis for wide-ranging geographical comparison of the results (Kucuksezgin et al., 2001; Labropoulou et al., 1997; Lombarte et al., 2000; Morat et al., 2012; Reñones et al., 1995; Tserpes et al., 2002). The biological and ecological features of this species, such as diet, growth, spatial patterns and reproduction have been extensively studied and may provide a basis for explaining the contamination patterns observed (Bautista-Vega et al., 2008; Cresson et al., 2014 a,b; Labropoulou et al., 1997; Lombarte et al., 2000; Machias et al., 1998; Machias and Labropoulou, 2002; Morat et al., 2012; Reñones et al., 1995). Since food is considered to be the main source of Hg contamination in fish (Hall et al., 1997), a good knowledge of dietary habits is crucial to the understanding of contamination patterns. Both *M. barbatus* and *M. surmuletus* have similar mesocarnivorous diets based on benthic invertebrates, such as crustaceans and annelids (Bautista-Vega et al., 2008; Cresson et al., 2014b; Labropoulou et al., 1997; Machias and Labropoulou, 2002). With increasing size, *M. barbatus* changes its diet, consuming larger shrimps and annelids and avoiding small crustaceans. In contrast, *M. surmuletus* has the same diet whatever the size (Bautista-Vega et al., 2008; Labropoulou et al., 1997). *Mullus* species are considered to be rather territorial (Morat et al., 2012), even if spatial and depth segregation between large and small individuals has been reported (Lombarte et al., 2000). Due to their strong benthic affinity, their burrowing activity and the low range of movement of these species, the level of contamination in *Mullus* spp can be considered representative of the local contamination level in the environment. These species are also expected to accumulate pollutants at a higher rate than other fish species (Zorita et al., 2008) and were recommended as suitable species for the monitoring of environmental contamination (UNEP/RAMOGE 1999). Finally, these species have a commercial value and have been fished mainly by small-scale coastal fisheries since Roman and Greek antiquity (FAO, 2012; Leleu et al., 2014; Tipton, 2008; Tserpes et al., 2002). For the French Mediterranean, red mullet annual catches ranged between 280 and 500 metric tons

before 1990 and decreased to nearly 200 t in recent years (FishStatJ, FAO). A high Hg concentrations in muscle tissue of fish could thus represent a concern for human health. As a consequence, Hg concentrations have been extensively surveyed in *Mullus surmuletus* and *M. barbatus* throughout the Mediterranean Sea (Capelli et al. 2004; Conti et al., 2012; Copat et al., 2012; Corsi et al., 2002; Falcó et al., 2006; Harmelin-Vivien et al., 2009; Kucuksezgin et al., 2001; Martínez-Gómez et al., 2012). But as previously observed by Dierking et al (2009), most of the studies on contamination in fish were carried out over a single area, where contamination was expected to be high. Similarly, papers comparing Hg concentration in *M. barbatus* and *M. surmuletus* are scarce (e.g., Harmelin-Vivien et al., 2009) and no data are available regarding temporal variations of Hg content of the fish tissue. Finally, no study has investigated Hg concentration in fishes from Corsica. Consequently, on the basis of sampling of *M. barbatus* and *M. surmuletus* from five French Mediterranean coastal zones during 18 months, we assessed temporal and spatial patterns of Hg concentrations in Mediterranean red mullet muscle, and determined to what extent these species can be considered as good quantitative bio-indicators of Hg contamination of their surrounding environment.

Materials and methods

Sample collection and preparation

Individuals of the two main Mediterranean mullid species (*Mullus barbatus barbatus* Linnaeus, 1758, hereafter *M. barbatus* and *Mullus surmuletus* Linnaeus, 1758) were collected every two months from June 2012 to December 2013 by fishermen operating trammel nets in shallow (~ 20 - 50 m depth) coastal waters of the French Mediterranean (Fig. 1), at two sites in the Gulf of Lions (Banyuls-sur-Mer, hereafter Banyuls, and Sète), two in the Provence area (La Seyne-sur-Mer, hereafter La Seyne, and Nice) and one in south-eastern Corsica (Sant' Amanza).

These sites were chosen as they were thought to reflect different levels of contamination: Corsica is considered to be lightly impacted by contamination pressure, and is commonly used as the reference site in contamination studies (e. g. Andral et al., 2004; Lafabrie et al., 2007; Pergent-Martini, 1998). The pattern is similar at Banyuls and Sète, situated away from large cities and harbors. Low Hg concentrations measured in sediment (Ifremer Chemical Contamination Monitoring Network, Table S1) and in fish (Dierking et al., 2009) at these sites are consistent with a low chemical contamination in these sites. On the other hand, fishes

from La Seyne were caught in the bay of Toulon, one of the major Hg hotspots in the French Mediterranean (Andral et al., 2004; Tessier et al., 2011). Similarly, Hg concentrations are high in Nice harbor sediments and mussels (Andral et al., 2004) but fishes were collected outside this harbor and could thus be considered as less impacted by this contamination.

At each location, fishermen were asked to collect at least 2 kg of fish. In order to ensure that the sampling was representative of the actual catches, they received no further instruction. Mainly, they were asked to fish in the zone where they usually work, and not to balance the sampling if one species appeared predominant. If necessary, fishes were caught in several fishing operations performed throughout the month and were stored frozen until the total quantity reached ~ 2 kg.

In the lab, species were separated. Individuals were measured (standard length, to the nearest mm), weighed (total mass, to the nearest 0.1 g) and dissected. A sample of muscle located on the caudal peduncle was collected for total Hg analysis, as required by the international standards (Nakhlé et al., 2007). Muscle samples were stored frozen, before freeze-drying and grinding. Sagittal otoliths were removed from the cranial cavity to determine fish age.

Mercury determination

Total Hg concentration was determined by the semi-automated atomic absorption spectrophotometer (AMA-254, Altec Ltd., Praha, Czech Republic) in three steps. First, the muscle sample is burnt and mercury is volatilized. Then the evaporated elemental mercury is captured by a gold trap. Finally, the trap is heated (800°C), Hg swept into the flow cell, and Hg content is determined by spectrophotometric atomic absorption. The accuracy of the analysis is assessed with a standard certified material (DORM-4, National Research Council of Canada) chosen in accordance with the assumed Hg content of the sample. Certified material is tested for every ten fish samples. Measured values were always within the confidence limits of the certified materials (certified values: $0.410 \pm 0.055 \mu\text{g g}^{-1}$; found values: $0.386 \pm 0.009 \mu\text{g g}^{-1}$). This technique allows the determination of the total Hg but not the methylmercury (MeHg), the organic mercury compound of major concern. However, total Hg is a good proxy of MeHg, as MeHg represents 80 to 100 % of the total Hg in *Mullus* spp. (Storelli et al., 2005; Tolga Gonul and Kucuksezgin, 2007). Concentrations are expressed relative to the dry mass (dm) of individuals. One determination of Hg concentration was performed for each individual.

Age determination

As recommended by international expert groups (ICES, 2012) and described in Mahé et al. (2013), two techniques were used in order to achieve the most precise estimation of the fish age: observation under transmitted light, as well as observation under reflected light before and after burning the whole otolith. After preparation, otoliths were immersed in 5 % alcohol water. Otoliths were observed with a binocular microscope connected to a computer with TNPC software (digital processing of calcified structures, www.tnpc.fr). Each otolith was analyzed by three different experts. Age was determined for 917 individuals.

Numerical analyses

Statistical analyses were run to test (1) spatial differences of length for each species, (2) if a correlation was apparent between length and Hg concentration, and (3) to test the effect of species, fish age, sampling site and time on Hg concentration. Finally, as age could not be determined for all individuals, effect of age on Hg concentration was tested separately.

Comparison of mean length between sites and considering both species separately were performed with Kruskal-Wallis non-parametric test (KW), as lengths were not normally distributed. The effect of length on Hg concentration was determined for each species separately by linear Pearson correlation. The general pattern for each species (*ie* without considering spatial variation) was tested first. Then, spatial differences were tested by considering all sites separately.

The effect of species, sites and sampling dates on Hg concentration was assessed using analysis of variance (ANOVA), as no effect of length on Hg concentration was detected. The effect of age on Hg concentration was tested first on all individuals together, then considering species and sites separately. Individuals aged 0 and 4 were poorly represented (always less than 3 individuals for each species at each site) and were consequently not included when species and sites were separated.

All ANOVAs were run on log-transformed Hg concentration, as Hg was not normally distributed. When significant differences were observed, Student's Least Square Distance (LSD) post hoc test was performed. Numerical analyses were run with R statistical software (R Core Team, 2014).

Results

Sample size, biometry and age

A total of 1042 fishes were captured, including 556 *Mullus surmuletus* and 486 *M. barbatus*. *M. surmuletus* was the predominant species at all sites except at Nice (Table 1). The actual number of fishes by species, site and date is detailed in Table S2. Individuals aged 1 and 2 years represent 90% of the sampling (496 and 328 respectively), followed by individuals aged 3 (69). Individuals aged 0 and 4 years were under-represented (22 and 2 respectively). Significant differences of fish length between sites were observed for *M. barbatus* (KW $H_{(4,486)} = 168.78$, $p < 0.0001$) and *M. surmuletus* (KW $H_{(4,556)} = 56.65$, $p < 0.0001$). At similar ages, *M. barbatus* individuals from Sant'Amanza were always larger than counterparts from all other sites (Fig. S1). This trend was not observed for *M. surmuletus*.

Mercury concentrations

All measured Hg concentrations were lower than the $2.5 \mu\text{g g}^{-1}$ (dm) threshold defined by the European Commission as a safety value for mercury concentration in foodstuffs (EC, 2006). Three individuals exhibited concentrations above $2 \mu\text{g g}^{-1}$, and 15 between 1.5 and $2 \mu\text{g g}^{-1}$ (Fig. 2). Most of these Hg-rich fishes were *M. surmuletus* captured at Sant'Amanza (8 individuals) and individuals of both species from La Seyne (5 individuals).

Average Hg concentrations ranged between $0.23 \pm 0.19 \mu\text{g g}^{-1}$ dm and $0.78 \pm 0.36 \mu\text{g g}^{-1}$ dm with significant differences between sites (Table S3). Higher Hg concentrations were measured at Sant'Amanza and at La Seyne (ANOVA $F_{(4,989)} = 82.26$, $p < 0.0001$). A significant variation of Hg content was observed between *M. surmuletus* ($0.46 \pm 0.33 \mu\text{g g}^{-1}$, dm) and *M. barbatus* ($0.34 \pm 0.28 \mu\text{g g}^{-1}$ dm; ANOVA $F_{(1,986)} = 12.03$, $p < 0.001$). This trend appeared to be largely driven by the discrepancy in Hg concentration occurring at Sant'Amanza between *M. barbatus* ($0.33 \pm 0.27 \mu\text{g g}^{-1}$ dm) and *M. surmuletus* ($0.76 \pm 0.36 \mu\text{g g}^{-1}$ dm), since no significant difference was detected when excluding fishes from Corsica (ANOVA $F_{(1,986)} = 0.20$, $p = 0.66$).

Temporal variations of Hg concentration were detected (Table S3). Considering both species together, the highest values were measured in April 2013. High values were also observed during spring or summer when separating species and sites (Fig. 3). The highest values were observed in April for *M. barbatus* at Nice, in June for both species at Sète, in June and August for *M. surmuletus* at La Seyne and in August for *M. surmuletus* at Sant'Amanza. In addition, this trend is repeated from one year to another when data are available on both years. For example, at La Seyne, concentrations measured for *M. surmuletus* in June 2012 (0.99

$\pm 0.58\mu\text{g g}^{-1}$) and in August 2013 ($0.81 \pm 0.33 \mu\text{g g}^{-1}$) were higher than all other concentrations measured for this species at this site.

Correlations between Hg, length and age

Length and Hg concentration were poorly correlated (Fig. 4; Table S4). Regarding age, a significant (ANOVA $F_{(4,910)} = 18.67$, $p < 0.0001$) increase of Hg concentration with ageing was detected when considering all individuals together, regardless of their species or origin (Fig. 6). When separating species and sites (Table 2), significant differences in Hg concentration between ages were observed for *M. barbatus* at Banyuls, Sète and Nice, and for *M. surmuletus* at La Seyne, Nice and Sant'Amanza. Concentrations were always higher for older individuals, whether differences were significant or not.

Discussion

Albeit Hg content has been largely investigated in Mediterranean red mullet (*M. barbatus* and *M. surmuletus*), few studies have compared results obtained from different areas. Similarly, seasonal variations of Hg concentration have rarely been investigated in marine teleosts whereas seasonal cycles can potentially affect Hg concentrations. This study has made good this lack, and identified certain features that may be the cause of the patterns observed.

Inter- and intra-specific variability of Hg concentrations

Mean mercury concentrations ranged between 0.23 and $0.78 \mu\text{g g}^{-1}$, consistently with previously reported results for mullids from the Mediterranean Sea (Table. 3). Most of the values measured were below the $2.5 \mu\text{g g}^{-1}$ dm safety value defined by the European Commission (EC, 2006). The similar Hg burden within the two species, when individuals from Corsica are excluded, is consistent with previous results (Capelli et al. 2004; Harmelin-Vivien et al., 2009). At the size considered here (10 to 22 cm), *M. barbatus* and *M. surmuletus* have a similar diet, based on small benthic invertebrates (Bautista-Vega et al., 2008; Labropoulou et al., 1997; Machias and Labropoulou, 2002). As diet is the main source of Hg contamination in fishes (Hall et al., 1997), this similar diet may explain the absence of difference in Hg concentration. The high inter-specific discrepancy observed only at Sant'Amanza, with lower values in *M. barbatus*, could be linked with the faster growth of *M. barbatus* and justifies an extensive assessment of the Corsican situation (see below).

It is generally considered that Hg bioaccumulation occurs all along the fish life. In other words, larger and older fishes display higher mercury concentrations, meaning that the rate of Hg uptake in fish tissue are higher than their rate of excretion. Several biological processes have been invoked to explain this process, such as accumulation due to chronic exposure to contaminant, lower biodilution due to lower somatic growth and investment in reproduction for mature individuals or ontogenetic change of diet (Cossa et al., 2012; Harmelin-Vivien et al., 2009; Magalhães et al., 2007). Here, length was not a good descriptor of Hg contamination, what is comparable with the results of Harmelin-Vivien et al. (2009) who observed few correlations and only for *M. barbatus*. Age appeared to be a better descriptor of Hg bioaccumulation, as age provides absolute information about exposure time whereas length is dependent upon the life history parameter of each individual. Considering this discrepancy is crucial when comparing Hg concentrations of individuals living in different environments, where abiotic parameters can affect metabolism and growth. The low correlation between length and Hg concentration could be caused by the high inter-individual variability. This trend is clearly illustrated by the dispersion of the points in Figure 5. Due to individual life history, two individuals can be exposed to different Hg contamination. In addition, individuals exposed to the same Hg contamination may exhibit different Hg concentrations as they potentially have a different metabolism. This idea is also supported by the trend observed for *M. barbatus* at La Seyne and for *M. surmuletus* at Banyuls and Sant'Amanza. The largest individuals exhibited lower Hg concentrations than the smallest individuals, inconsistently with the expected bioaccumulation of Hg. It could be hypothesized that the spatial segregation between small and large individuals previously described (Machias et al., 1998; Lombarte et al., 2000) causes this unexpected trend. In those papers, authors described a migration of larger individuals towards deeper waters and the predominance of smaller individuals in shallower zones. Here, if the small individuals are living and feeding in the bay, they would be under the influence of the highly contaminated sediments of the bay of Toulon (Tessier et al., 2011). On the contrary, due to ontogenetic migration, larger individuals would live and feed outside the bay, in environments with lower Hg contamination. This result is similar with those of Greenfield et al (2013) who observed different Hg concentration for two fish species caught at the same site, as one is sedentary, whereas the other has an extensive range of movement. The different Hg concentration in fish reflects the exposition to different Hg sources. Further study, focused on a larger number of individuals and on a large range of sizes is required to reach a definitive conclusion on that point. It should also include complementary analyses, such as the determination of stable

isotope ratios of C and N, to verify whether or not large individuals belonged to a different trophic network, with lower Hg concentrations.

Temporal variation of Hg concentration

Seasonal variation of Hg content in marine fishes is poorly documented (Greenfield et al. 2013; Kucuksezgin et al., 2001; Mathieson et al., 1996). As Hg has a higher affinity for proteins, muscle displays a higher Hg concentration than gonads (Webb et al., 2006). Thus, Hg losses due to spawning are negligible (Trudel and Rasmussen, 2006). As a consequence, most studies documented no sex variation of Hg content in fishes (Harmelin-Vivien et al., 2009; Magalhães et al., 2007; Martínez-Gómez et al., 2012). The seasonal variation observed in the present study could be linked with a seasonal variation of feeding intensity, as diet is the main source of Hg in fishes (Hall et al., 1997) and less than 0.1 % of the Hg results from a direct uptake of Hg from water (Trudel and Rasmussen, 2006). Higher Hg concentrations were measured between April and August, concomitantly with the reproductive season of red mullet in the Mediterranean (Machias and Labropoulou, 2002; Reñones et al., 1995; Vassilopoulou and Georgakopoulos-Gregoriades, 1993). In contrast to other fish species which store reserves prior to the reproductive season to supply reproductive needs, red mullet exhibit intensive feeding activity during the spawning season (Lloret et al., 2007). Increasing the feeding activity would consequently increase the Hg contamination at this time. In addition, a seasonal variation of Hg bioavailability was previously documented. A higher Hg concentration in summer for several benthic invertebrates such as crustaceans, annelids or mollusks was recently documented (Cardoso et al. 2014). Such invertebrates are the main prey of red mullet, so a similar trend for Mediterranean invertebrates could explain the seasonal variation observed. In addition, a decrease in Hg methylation in winter previously measured in the Mediterranean could cause the lower Hg values measured in red mullet sampled in winter (Heimbürger et al. 2010).

Geographical comparison of Hg concentration in red mullet

In this work, *Mullus* spp. individuals were sampled at five Mediterranean sites exhibiting different environmental Hg contamination. Results segregated three groups of locations depending on their Hg concentration: Banyuls and Sète with low Hg concentrations, Nice where intermediate values were measured, and finally La Seyne and Sant'Amanza where Hg concentrations were the highest. Results measured in red mullet were consistent with environmental values and contamination pressure at all sites except Sant'Amanza.

Low Hg concentration at Sète and Banyuls are consistent with previous values obtained in the same zone in sediment, mussels (Table S1) and in the muscle of common sole *Solea solea* (Dierking et al., 2009). These authors explained the low values they recorded by the absence of a major Hg source in the immediate vicinity, compared to other sites in the Gulf of Lions. At Nice, the harbor is a major hotspot of Hg contamination in sediment but fishes were sampled away from the harbor. A weak influence of the harbor and the city may explain the intermediate Hg concentrations observed at Nice. Similarly, the high mercury concentrations measured for both species in La Seyne were consistent with previous knowledge of high Hg content locally measured in sediment and caged mussels (Andral et al., 2004; Tessier et al., 2011). Values in red mullet were thus representative of the environmental Hg contamination at these four sites.

The high mercury concentrations in *M. surmuletus* from Sant'Amanza might appear surprising. Corsica is commonly considered as a weakly impacted zone, subjected to limited anthropogenic pressures. Several early papers compared Hg concentration in sediment, mussels or the seagrass *Posidonia oceanica* and observed lower mercury concentrations in Corsica than in other parts of the Western Mediterranean (Andral et al., 2004; Galgani et al., 2006; Lafabrie et al., 2007; Pergent-Martini, 1998; Pergent et al., 2011; Serrano et al., 2013). The oligotrophy of Corsican waters (in comparison with the waters of the Gulf of Lions and Provence area, Bosc et al., 2004) may be a major factor causing the discrepancy between low environmental Hg concentrations and high concentrations in red mullet.

First, phytoplanktonic communities in Corsica are dominated by nano- and picophytoplankton, just as in all oligotrophic zones (Garrido et al., 2014). Heimbürger et al. (2010) showed that Hg methylation is enhanced in the presence of nano- and picophytoplankton. Thus, a higher amount of organic mercury is available for bio-amplification in oligotrophic zones. In addition, due to the lower amount of biogenic particles, mercury is less bio-diluted by biogenic particles and is more easily integrated at the basis of trophic networks (Cossa et al., 2012). Higher methylation and lower biodilution at the base of the trophic network were proposed as explanations of the different Hg concentrations in red mullet sampled in the Gulf of Lions and in the Black Sea. *M. barbatus ponticus*, sampled in the eutrophic Black Sea, exhibit markedly lower Hg concentrations than *M. barbatus barbatus* and *M. surmuletus* in the mesotrophic Gulf of Lions, whereas Hg concentrations in water are similar in the two geographic zones and these fish occupy the same trophic position (Harmelin-Vivien et al., 2009). Whilst *Mullus* spp. are benthic burrowing feeders, the latter

and other recent results based on carbon and nitrogen stable isotopes ratios demonstrated that the trophic network they belong to is dependent upon the falling pelagic primary production (Cresson et al., 2014 a,b). If Hg is less biodiluted, integration of this matter in the trophic network would result in a higher Hg contamination. Secondly, due to the lower amount of particles, the growth of organisms could be slower in oligotrophic zones. With regard to contamination, a slow-growing organism synthesizes less tissue than a fast-growing one and bio-dilutes less the Hg contamination in tissues. Slow growing organisms thus display a higher Hg content. Oligotrophy explained the lower condition indices and Hg burden measured for caged mussels deployed in Corsica (Andral et al., 2004) and also the lower growth rate and the higher Hg for hake *Merluccius merluccius* sampled in the Gulf of Lions, in comparison with fish sampled in the eutrophic Bay of Biscay in the Atlantic (Cossa et al. 2012). Here it could be the cause of the lower Hg values in fast growing *M. barbatus* individuals (Fig. S1).

It is of interest to note that several authors also observed a discrepancy between environmental values and *Mullus* spp. body burden (Martínez Gómez et al., 2012; Storelli and Marcotrigiano, 2005). Authors attributed this discrepancy to the input of Hg from distant zones (Storelli and Marcotrigiano, 2005) or to a dietary switch and the consumption of more contaminated prey for individuals displaying high Hg concentration (Martínez-Gómez et al., 2012). Even if these hypotheses should not be rejected *per se*, no consideration was given to biological and abiotic factors. As demonstrated earlier, oligotrophy can increase the amount of Hg available for bio-accumulation in the trophic networks. In addition, previous results demonstrated that, under eutrophic conditions, fish grow faster and dilute more Hg contamination in newly synthesized tissues, resulting in a lower Hg concentration in faster-growing fishes (Cossa et al., 2012). In the work of Martínez-Gómez et al (2012), lipid content and condition factor (ratio between eviscerated mass and cubic length) were presented. Fishes sampled at the site with higher Hg concentrations in sediment exhibited higher lipid content and condition factors. Thus, the faster growth of these fishes may have diluted their Hg burden and this might explain the lower and unexpected Hg concentrations at a more contaminated site. Considering the abiotic features of the environment and the resulting biological status of the organisms is thus crucial to correctly interpret contamination patterns.

Conclusions

The results obtained here confirmed that *Mullus* spp. caught along the French Mediterranean coast are efficient bio-indicators of mercury contamination. This is not surprising as those species have been identified as such and already used in several studies. The geographical trend observed is consistent with knowledge regarding different levels of chemical contamination, mainly at La Seyne on the one hand and at Sète and Banyuls on the other hand. In addition, as *M. barbatus* and *M. surmuletus* are widely consumed, it is crucial to assess their Hg contamination, to detect and prevent possible human contamination while consuming these fishes. The quite low average values, comprised between 0.23 ± 0.19 and $0.78 \pm 0.36 \mu\text{g g}^{-1} \text{dm}$, make this rather unlikely, even if several high values were observed. Nevertheless, observations from Corsica coasts underline again that caution must be taken when bio-indicators are used to infer environmental contaminant impregnation. Biological factors of the bio-indicator species and specificities of the studied zone must be kept in mind to interpret Hg concentrations in biota. The high inter-individual variability of Hg concentrations, potentially linked with individual life history and metabolism, calls for extensive sampling. Seasonal variation should also be considered, as the annual cycle of the species could impact Hg content. Finally, the Corsican case also calls for caution, as the level of productivity and fish growth could blur the data. Raw values observed in biota are not always a direct indicator of environmental Hg concentrations. A good knowledge of these biological processes (*e.g.* diet or growth) and of the abiotic parameters they are ruled by (*e.g.* temperature or productivity of the zone etc.) is required for an accurate interpretation of Hg concentrations, and for the comparison of contamination between studies investigating different zones.

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Figures

Fig. 1: Map of the sampling sites.

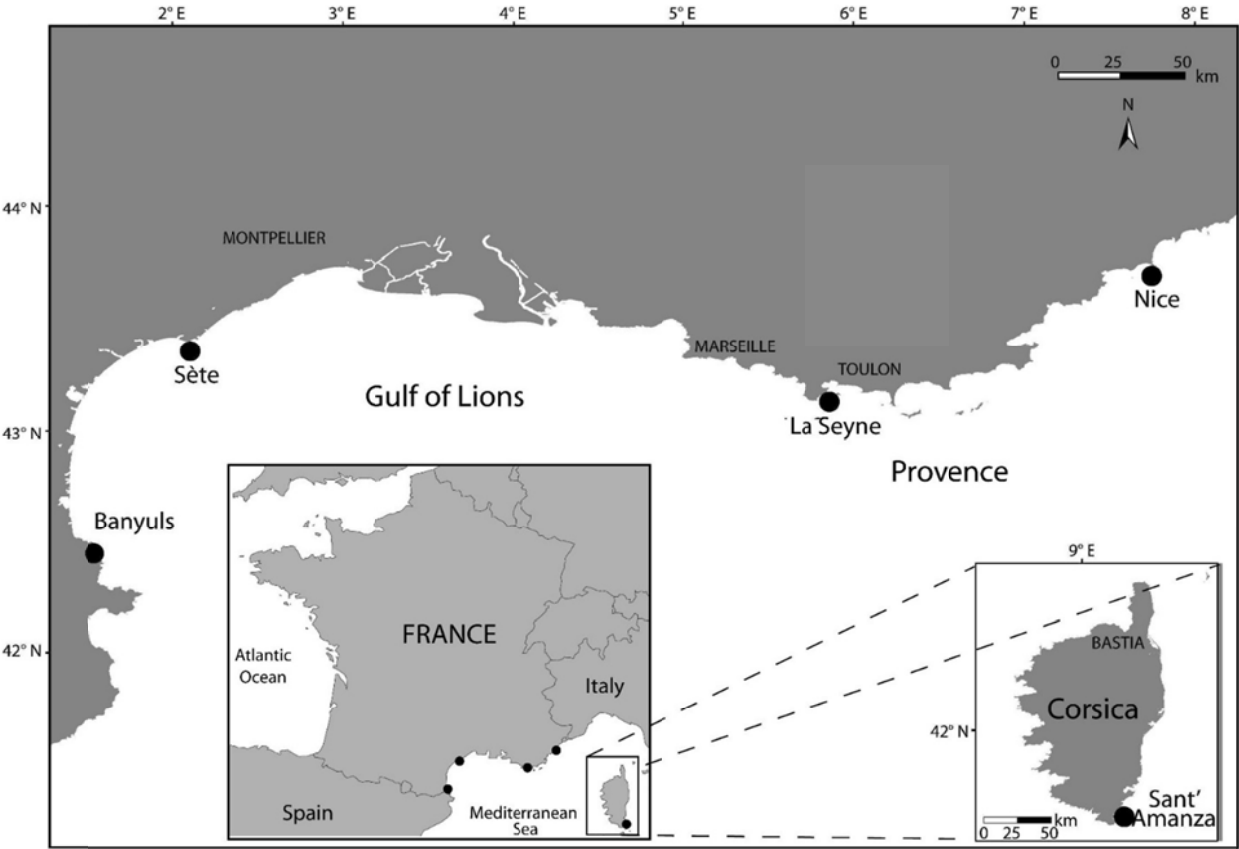


Fig. 2: Boxplots of Hg concentration ($\mu\text{g g}^{-1}$ dm). Box hinges are the 1st and 3rd quartiles, line is the median value, whisker extends from the hinge to the highest or lowest value that is within 1.5 x interquartile range. Points are outlier values. Horizontal hatched lines represent the maximum levels in food stuffs established by European Commission (EC, 2006) at $2.5 \mu\text{g g}^{-1}$ dm. Numbers below each site are the mean \pm sd values. n: number of individuals. LS: La Seyne, SA: Sant'Amanza

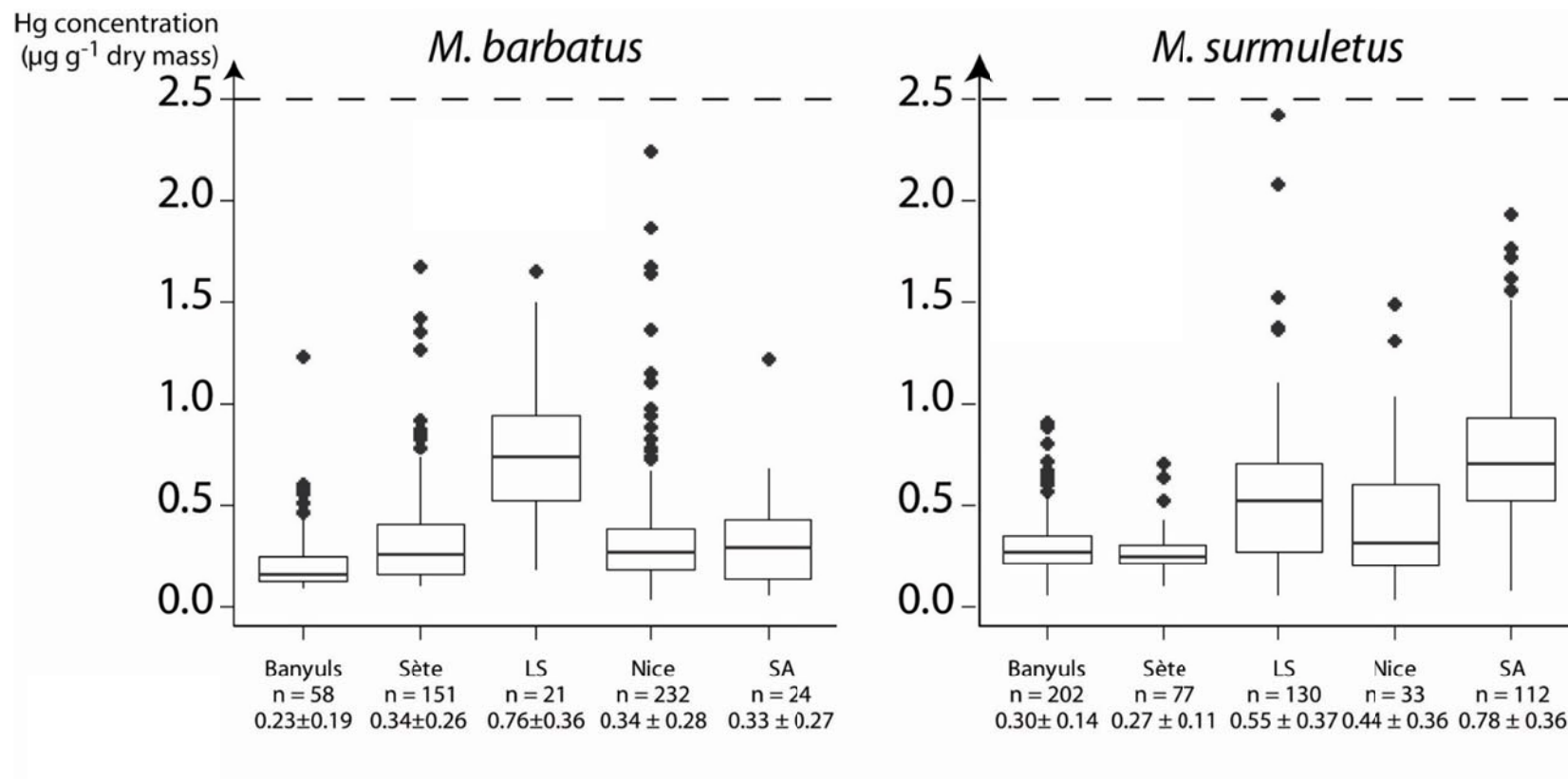


Fig. 3: Temporal variation of mean Hg concentration in each site. Months are represented by their first letter (J: June; Ap: April; Au: August). Box hinges are the 1st and 3rd quartiles, line is the median value, whiskers extend from the hinge to the highest or lowest value that is within 1.5 x interquartile range. Points are outlier values.

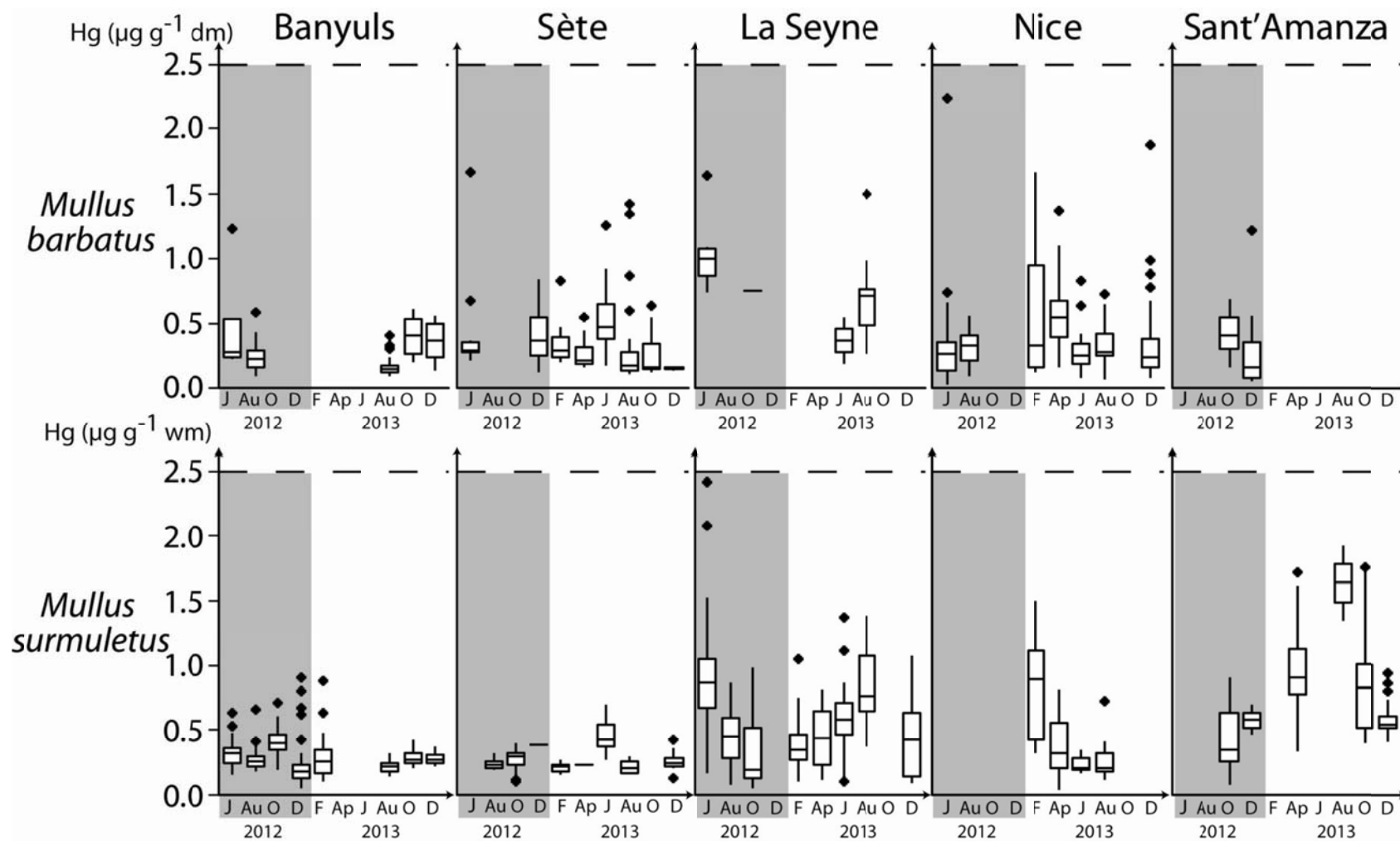


Fig. 4: Linear correlations between length (in cm) and Hg concentration ($\mu\text{g g}^{-1}$ dm). ***: p-value <0.001; ** p-value <0.01, *: p-value < 0.05, NS, non-significant, p-value ≥ 0.05

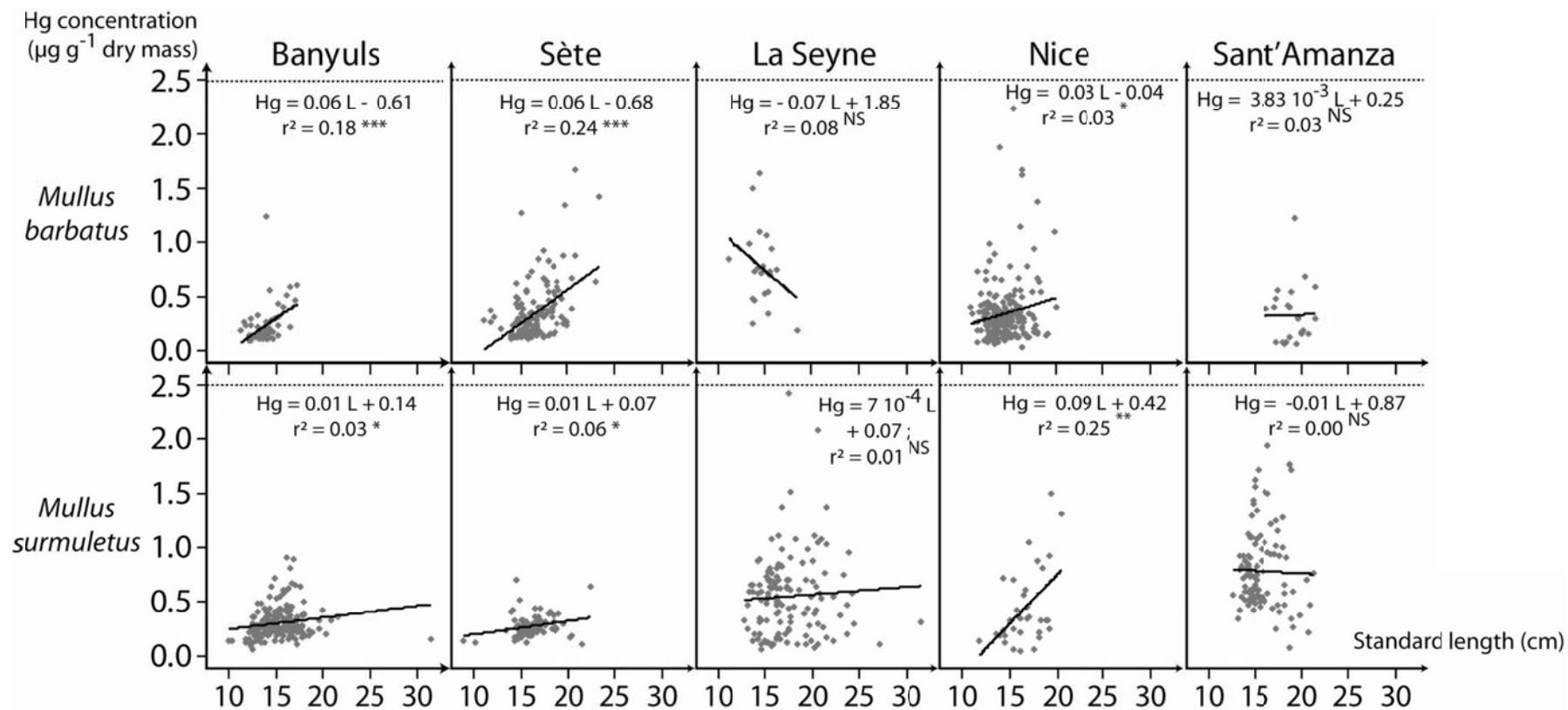


Fig. 5: Increase of the mean Hg concentration ($\mu\text{g g}^{-1}$, dm) with increasing age in both species and all sites. Ages with similar letter have similar Hg concentration. Individuals aged 0 and 4 were not included in the analysis.

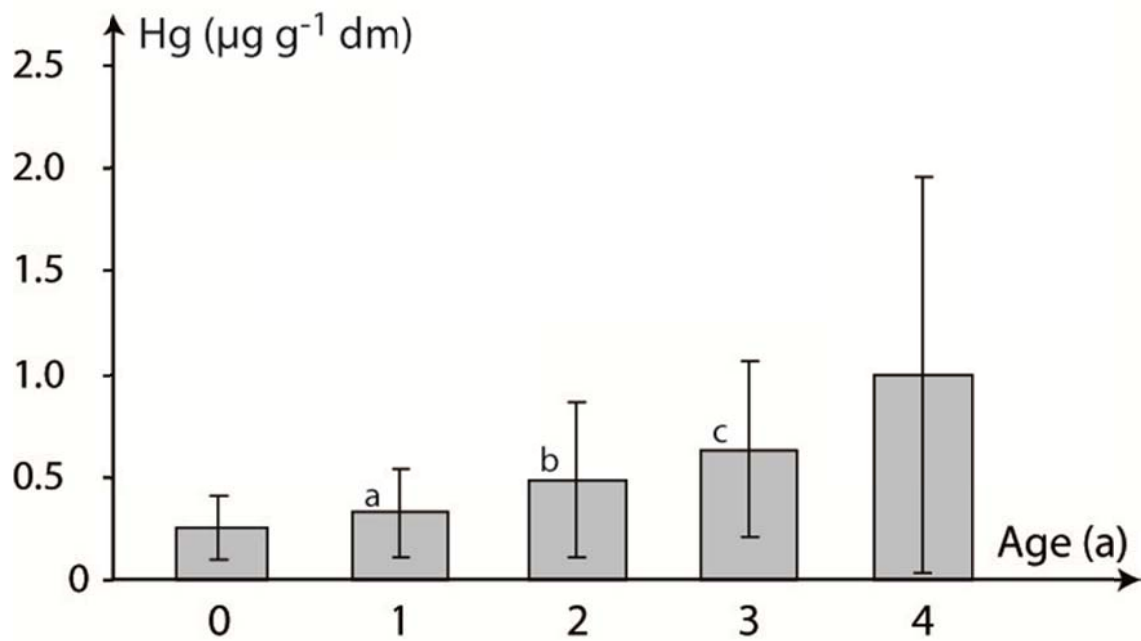


Table 1: Actual number of individuals sampled by species, sites and age classes. nd: individuals whose age was not determined

Species	Sites	Ages (a)						Total
		0	1	2	3	4	nd	
<i>M. barbatus</i>	Banyuls	3	39	9	1		6	58
	Sète		63	52	25	1	10	151
	La Seyne		2	6				13
	Nice	6	132	66	2	26		232
	Sant' Amanza		9	12	3			24
<i>M. surmuletus</i>	Banyuls	10	118	53	2		19	202
	Sète	1	37	24	3		12	77
	La Seyne	2	34	43	21	1	31	132
	Nice		6	19	6		2	33
	Sant' Amanza		56	44	6		6	112

Table 2: Comparison of mean Hg concentration by age at all sites. Comparison were performed by ANOVAs, on log-transformed Hg concentration, with length as a covariate. Individuals aged 0 and 4 years were present in low number and were not included. In the post hoc column, numbers refers to the age classes compared

Species	Site	Stats	P value	Post Hoc (age)
<i>M. barbatus</i>	Banyuls	$F_{(2,46)} = 5.85$	0.004	1 < 2 < 3
	Sète	$F_{(2,137)} = 32.60$	<0.0001	1 < 2 < 3
	La Seyne	$F_{(1,6)} = 0.19$	0.67	
	Nice	$F_{(2,197)} = 8.94$	<0.0001	1 < 2 < 3
	Sant'Amanza	$F_{(2,21)} = 1.68$	0.21	
<i>M. surmuletus</i>	Banyuls	$F_{(2,170)} = 1.29$	0.30	
	Sète	$F_{(2,61)} = 0.56$	0.57	
	La Seyne	$F_{(2,95)} = 4.81$	0.010	1 < 2 = 3
	Nice	$F_{(2,28)} = 3.23$	0.004	1 < 2 < 3
	Sant'Amanza	$F_{(2,103)} = 9.09$	< 0.0001	1 < 3 = 2

Table 3: Mean Hg concentrations recorded for *M. barbatus* or *M. surmuletus* in bibliographical references in the Mediterranean Sea. When available, sampling size (n) and length parameters (mean, standard deviation, minimal and maximal lengths) were collected from the paper. If needed, wet mass was converted to dry mass using the equation dry mass = 5 wet mass. na: data not available in the paper

Species	Site	Country	n	Length (cm)		Hg concentration ($\mu\text{g g}^{-1}$ dm)		Reference
				Mean \pm sd	Min - Max	Mean \pm sd	Min - Max	
<i>M. barbatus</i>	Ligurian Sea	Italy	30	na	14-16	0.122 ± 0.01	na	Conti et al 2012
	Ligurian Sea	Italy	30	na	na	0.40 ± 0.15	na	Copat et al 2012
	Catalan Sea	Spain	20	na	na	na	0.7 - 1.8	Falcó et al. 2006
	Aegean Sea	Turkey	na	na	10.6 - 18.6	0.45 ± 0.23	0.07 - 2.5	Kucuksezgin et al. 2011
	Adriatic Sea	Italy	14	na	na	0.48 ± 0.09	0.05 - 1.07	Perugini et al. 2009
	Gulf of Lions	France	132	14.8 ± 3.6	8.5 - 24.5	1.11 ± 1.15	0.02 - 8.92	Harmelin-Vivien et al. 2009
	Balearic Sea	Spain	43	na	11.7 - 14.2	2.28 ± 1.00	1.07 -3.57	Hernández Hernández et al 1990
<i>M. surmuletus</i>	Gulf of Lions	France	85	16.6 ± 4.6	9.6 - 31	0.92 ± 1.06	0.001 - 6.06	Harmelin-Vivien et al. 2009
	Balearic Sea	Spain	38	na	9.1 - 14.2	0.71 ± 0.34	0.18 - 1.09	Hernández Hernández et al 1990
	Ionian Sea	Italy	78	15.3	9.5 - 21.2	1.05 ± 0.33	0.18 - 4.23	Capelli et al. 2004

Supplementary material

Fig. S1: Comparison of fish standard lengths for each age class (in years) and in each site. Box hinges are the 1st and 3rd quartiles, line is the median value, whiskers extend from the hinge to the highest or lowest value that is within 1.5 x interquartile range.

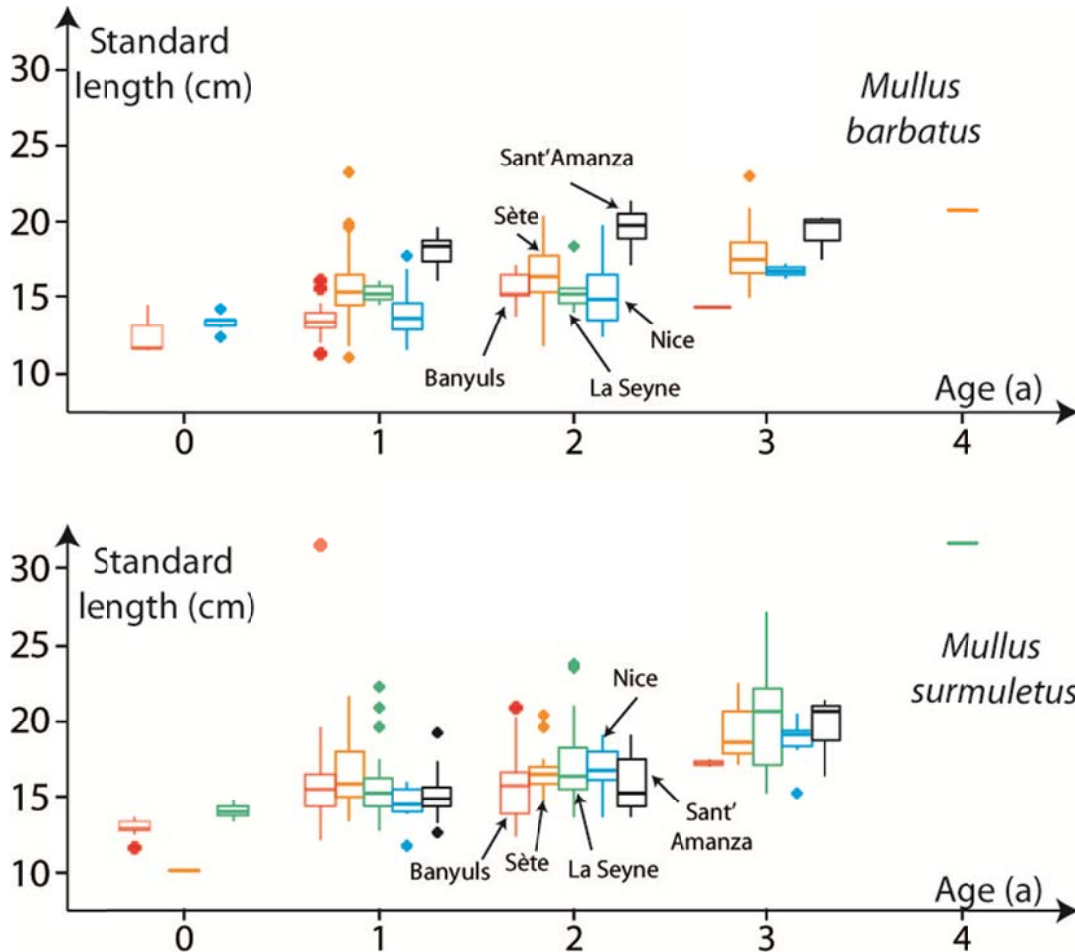


Table S1: Comparison of Hg concentrations in sediment and caged mussels. Concentrations in the sediment were measured within the Ifremer contamination survey network (ROCCHSED) during 2011 campaign (one measurement at each site) or came from bibliographical references. Concentration in mussels are the means \pm standard deviation of crude values measured during 2000, 2003, 2006, 2009 and 2012 RINBIO campaigns, and uncorrected with condition indices (calculated as the ratio between flesh mass and shell mass for mussels collected in 2012; refer to Andral et al. 2004 for details of the methodology used)

	Banyuls	Sète	La Seyne	Nice	Sant' Amanza
Sediment ($\mu\text{g g}^{-1}$)	0.06 ^a	0.06 ^a	1.86 ^a 2.26 – 3.24 ^b	nd	0.03 ^{a,c}
Mussels condition index	0.13	0.15	0.10	0.09	0.09
Mussel raw Hg concentration ($\mu\text{g g}^{-1}$)	0.07 \pm 0.02	0.04 \pm 0.01	0.25 \pm 0.01	0.09 \pm 0.03	0.12 \pm 0.06

a: Ifremer chemical contamination survey network, JF Chiffolleau pers. comm.; b: range of values in the zone of fish sampling, data read on the map of Teissier et al. (2011) ; c: Galgani et al. (2006)

Table S2: Number of fishes sampled, by species, site and date

Month	<i>Mullus barbatus</i>					<i>Mullus surmuletus</i>					Total
	Banyuls	Sant' Amanza	La Seyne	Nice	Sète	Banyuls	Sant' Amanza	La Seyne	Nice	Sète	
June 2012	4		6	43	10	29		16			108
August 2012	9			30		27		13		10	89
October 2012		9	1			29	10	14		21	84
December 2012		15				33	2			1	73
February 2013				13	17	22		10	8	12	82
April 2013				11	20		38	28	13	1	111
June 2013			2	44	20			25	3	8	102
August 2013	37		12	41	32	16	2	9	9	6	164
October 2013	4					24	25				79
December 2013	4			50	4	22	35	17		18	150
Total	58	24	21	232	151	202	112	132	33	77	1042

Table S3: Results of the comparison of Hg concentrations, with ANOVA. Post-hoc column present the results of LSD post-hoc comparison tests, with letters referring to the sampling sites (Ba: Banyuls; Se: Sète, Ni: Nice, LS: La Seyne; SA: Sant'Amanza), months (F: February, Ap: April, Ju: June, Au: August, O: October, D: December) and years (12: 2012, 13: 2013)

Parameter	Stat	p-value	Post-hoc
Site	$F_{(4,989)} = 82.26$	<0.0001	Ba = Se < Ni < LS < SA
Date	$F_{(9,986)} = 6.08$	<0.0001	D12 = Au12 = Au13 < O12 = D13 = Ju13 = F13 = O13 = J12 < Ap13
Species	$F_{(1,986)} = 12.03$	< 0.0001	<i>M. barbatus</i> < <i>M. surmuletus</i>
Site x Date	$F_{(25,986)} = 12.01$	<0.0001	
Site x Species	$F_{(4,986)} = 1.42$	0.225	
Date x Species	$F_{(9,986)} = 2.72$	0.006	

Table S4: Parameters of linear regressions between Hg concentration and length (L). Linear relationships were considered to be significant when $r^2 \geq 0.25$ and $p \leq 0.05$

	All sites	Banyuls	Sète	La Seyne	Nice	Sant'Amanza
<i>M. barbatus</i>	Hg = 0.03 L - 0.06 $r^2 = 0.04$; $p < 0.0001$ ***	Hg = 0.06 L - 0.61 $r^2 = 0.18$; $p < 0.0001$ ***	Hg = 0.06 L - 0.68 $r^2 = 0.24$; $p < 0.0001$ ***	Hg = -0.07 L + 1.85 $r^2 = 0.03$; $p = 0.21$	Hg = 0.03 L - 0.04 $r^2 = 0.03$; $p = 0.01$	Hg = $3.83 \cdot 10^{-3}$ L + 0.25 $r^2 = 0.03$; $p = 0.93$
<i>M. surmuletus</i>	Hg = 0.02 L + 0.18 $r^2 = 0.02$; $p = 0.003$ **	Hg = 0.01 L + 0.13 $r^2 = 0.14$; $p = 0.014$ *	Hg = 0.01 L + 0.08 $r^2 = 0.06$; $p = 0.03$ *	Hg = $7.04 \cdot 10^{-3}$ L + 0.42 $r^2 = 0.01$; $p = 0.50$	Hg = 0.09L - 1.06 $r^2 = 0.25$; $p = 0.003$ **	Hg = -0.01 L + 0.87 $r^2 = 0.00$; $p = 0.78$