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Comparative Study on Trace Metal Accumulation in Liver of Mediterranean Deep-Sea Fish and Their Selenium/Mercury Molar Ratios

**Grazia Barone · Arianna Storelli ·
Rosanna Mallamaci · Maria Maddalena Storelli**

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Abstract The objectives of this study were to determine and compare the concentrations of Hg, Cd, Pb, Zn, Cu, Ni and Se in the liver of macrourid fish as *Trachyrinchus scabrus*, *Nezumia sclerorhynchus* and *Coelorhynchus coelorhynchus* from the Mediterranean Sea, Italy. It was also carried out to evaluate the relationship between metal concentration and fish size and to explore selenium/mercury molar ratio. The highest concentrations were in *T. scabrus*, followed by *N. sclerorhynchus* and *C. coelorhynchus*. In all species, any element displayed significant correlation between metal body burden and fish size, except Hg. The mean selenium/mercury ratios were greater than one in all fish species indicating that Se antidotal effect in counteracting Hg occurred. This report represents one of the few surveys providing information on trace metal in deep-sea fish from Mediterranean Sea constituting, thus, an essential baseline work with which future levels may be compared.

Keywords Trace metals · Deep-sea fish · Concentration-size relationship · Se/Hg molar ratio · Mediterranean Sea

1 Introduction

One of the most important forms of aquatic pollution is represented by heavy metals, which are continuously introduced into the environment via natural and anthropogenic processes, including urban and industrial discharges, agriculture, mining and combustion. Once released in the marine environment, metals are readily adsorbed onto particulate matter and precipitate to deep-sea floor creating a potential source of pollution. Nevertheless, the deep sea is a vast area which remains largely unexplored in terms of its chemical contamination. At the present time, if it is possible to have a general view of the littoral pollution of most seas and oceans in the world, only few results have been reported concerning the contamination of the deeper part of the oceans. Monitoring environmental impact through the accumulation of metals by marine biota is, in fact, applied to a limited number of studies on deep sea (Adachi et al. 2012; Arima et al. 1979; Asante et al. 2010; Company et al. 2010; Cronin et al. 1998; Koenig et al. 2013; Mormede and Davies 2001a, 2001b; Oehlenschlager 2009; Siscar et al. 2014; Yamada et al. 2001), probably due to the technical difficulty of sample collection. Scarce and often outdated is also the knowledge of metal levels in Mediterranean deep-sea fauna. Kress et al. (1998) working with five deep-sea fish species from the South Eastern Mediterranean Sea, report the concentrations of essential and non-essential metals as well as other studies account for the metal content in deep-sea fauna captured in North Eastern Mediterranean Sea (Siscar et al. 2014) and in North Western Mediterranean Sea along Catalan coast

G. Barone · A. Storelli · R. Mallamaci · M. M. Storelli (✉)
Biosciences, Biotechnologies and Biopharmaceutical Department, University of Bari, Strada Prov. le per Casamassima Km 3, 70010 Valenzano (Ba), Italy
e-mail: mariamaddalena.storelli@uniba.it

(Koenig et al. 2013). However, these few studies are usually addressed to the edible muscle, while even more scarce is the information available for liver (Company et al. 2010; Martins et al. 2006; Mormede and Davies 2001a, 2001b; Siscar et al. 2014), an organ with a high pollutant accumulating capacity and recommended as an environmental indicator of water pollution (Licata et al. 2005). Marine environmental pollution is a worldwide problem, but the Mediterranean Sea presents a more dramatic situation due to its particular conformation of semi-enclosed water body, entirely landlocked and subject to an intense anthropogenic impact (Angelidis et al. 2011; Damiano et al. 2011). Our group has already reported on organochlorine compound concentrations (Storelli et al. 2004, Storelli et al. 2007; Storelli and Perrone 2010) as well as polybrominated diphenyl ethers (Covaci et al. 2008) in the liver of different deep-sea fish species from Adriatic Sea (South-Eastern Mediterranean Sea), revealing the presence of an extensive contamination of the marine environment with hot spots of concern (Storelli et al. 2009). On completion of this picture, the present study, adding up an investigation on the status of trace metals in deep fish species from the same marine area, aims to (1) compare the metal concentrations among different deep-sea fish, (2) investigate the relationship between metal concentration and fish size, (3) determine the selenium/mercury (Se/Hg) molar ratio and (4) provide information about the severity of pollution through a comparison with literature data. To this end, we determined the levels of three non-essential metals (Hg, Cd and Pb) and four essential metals (Zn, Cu, Se and Ni) in the liver of different deep-sea fish belonging to the family Macrouridae, namely *Trachyrinchus scabrus*, *Nezumia sclerorhynchus* and *Coelorhynchus coelorhynchus*. This issue is particularly relevant, not only to fill a knowledge gap on the state of chemical contamination in Mediterranean deep-sea fauna but also in consideration of the increasing interest in deep-sea fisheries due to depleted fish stocks of the world's oceans (Ramirez-Llodra et al. 2011).

2 Materials and Methods

2.1 Sample Collection

Specimens of *T. scabrus* (roughsnout rat-tail) (specimen number 260, total length 32.8–49.6 cm, average 39.9 ± 5.2), *N. sclerorhynchus* (roughtip grenadier) (specimen number 307, total length 15.2–29.3 cm, average 20.7 ± 4.7) and *C. coelorhynchus* (hollowsnout grenadier) (specimen number 246, total length 15.5–19.3 cm, average 17.7 ± 1.4) were caught along the Apulian coast (about 200 km) in the Southern Adriatic Sea (Mediterranean Sea, Italy) during two cruises conducted between May and September 2009. The sampling sites (Fig. 1) were selected based on the geographical features of the Adriatic Sea, which generally presents shallow waters except for a deep depression along the Apulian coast (300–1200 m). Species choice was based on their availability at the sampling location. From the total number of specimens, pools were formed (*T. scabrus*: no. 10; *N. sclerorhynchus*: no. 9; *C. coelorhynchus*: no. 6) within which individual fish were gathered as a function of their similar size (Table 1). From fish of each pool, liver was taken, homogenized and kept in a deep freeze at −20 °C until chemical analysis.

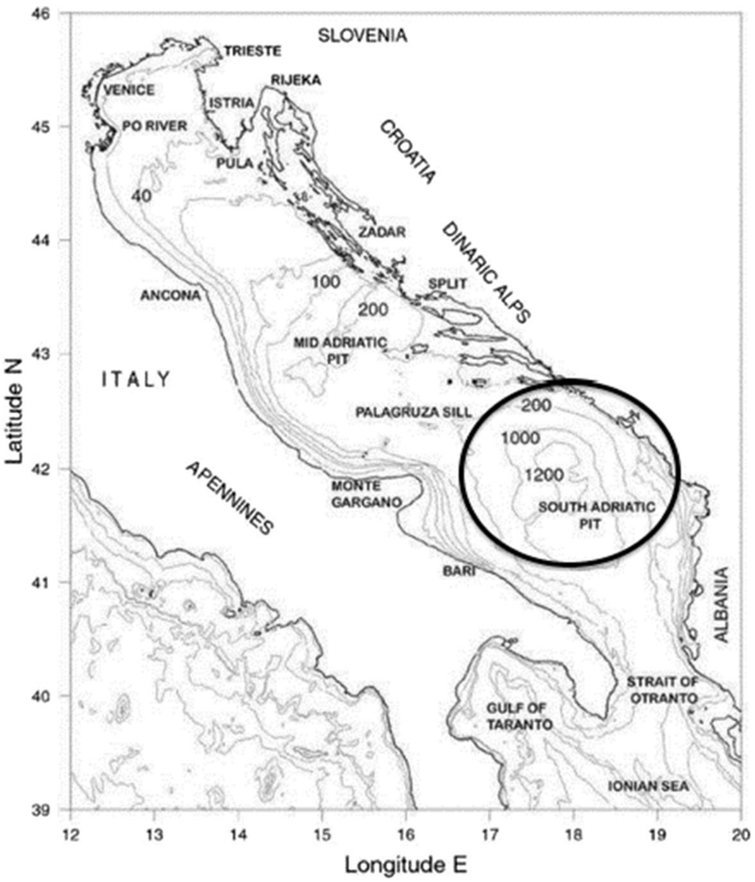
2.2 Chemical Analyses

The extractive analytical procedure and the instrumental conditions for determining metal concentrations have been described in detail elsewhere (Barone et al. 2013). Briefly, aliquots (about 1.0–2.0 g) of the samples were digested to a transparent solution with a mixture of HNO_3–$HClO_4$ (8:3) for Cd, Pb, Ni, Zn and Cu determination and with a mixture of H_2SO_4–HNO_3 (1:1) for Hg and Se. The completely digested samples were allowed to cool temperature and diluted with deionized water according to the method recommended by Official Italian Agencies (GURI 1994). The content of metals was determined by atomic absorption spectrophotometer (Shimadzu AA 7000). Zn and Ni were analysed by flame; Cd, Pb and Cu by using graphite furnace (high-density tube) (GFA-7000); Hg and Se were measured by using a hydride vapour generator (HVG-1) after reduction by $NaBH_4$. The selenium/mercury molar ratio was obtained by using the molecular weight (200.59 for Hg and 78.9 for Se).

2.3 Quality Control and Assurance

- Reference tissue (Tort-2 Lobster Hepatopancreas, National Research Council of Canada, Ottawa, Ontario, Canada) was treated and analysed in the same way as the samples. Results (Hg 0.28 ± 0.03; Cd 26.2 ± 2.4; Pb 0.32 ± 0.18; Se 5.71 ± 0.56; Cu

Fig. 1 Sampling site



101 ± 13; Zn 188 ± 12; Ni 2.3 ± 0.23 μg g^{-1} dry weight) were in good agreement with the certified values (Hg 0.27 ± 0.06; Cd 26.7 ± 0.60; Pb 0.35 ± 0.13; Se 5.63 ± 0.67; Cu 106 ± 10; Zn 180 ± 6; Ni 2.5 ± 0.19 μg g^{-1} dry weight) and the standard deviation were low, proving good repeatability of the methods. The results for standard reference material displayed recoveries of the

Table 1 Number of pools and individuals and mean size ranges of deep-sea fish species selected for the study

T. scabrus			*N. sclerorhynchus*			*C. coelorhynchus*		
No pools	No individuals	Mean size ranges	No pools	No individuals	Mean size ranges	No pools	No individuals	Mean size ranges
1	35	32.8 ± 0.1	1	32	15.2 ± 0.1	1	41	15.6 ± 0.2
2	29	35.2 ± 0.1	2	33	15.8 ± 0.2	2	43	16.7 ± 0.1
3	31	35.8 ± 0.1	3	34	17.4 ± 0.1	3	38	17.7 ± 0.2
4	30	37.4 ± 0.1	4	35	18.1 ± 0.1	4	40	18.1 ± 0.1
5	30	38.1 ± 0.1	5	32	20.3 ± 0.2	5	44	18.6 ± 0.1
6	27	40.3 ± 0.2	6	36	20.7 ± 0.1	6	40	19.3 ± 0.1
7	25	40.7 ± 0.1	7	35	23.6 ± 0.1			
8	22	43.6 ± 0.1	8	34	25.6 ± 0.1			
9	18	45.6 ± 0.1	9	36	29.3 ± 0.2			
10	13	49.6 ± 0.1						

elements ranging from 91 to 104% ($n = 3$). The limit of detection (LOD) (Hg 5; Cd 0.12; Pb 10; Se 1; Cu 26; Zn 24; Ni 26 ng g^{-1} wet weight) is defined as the concentration corresponding to three times the standard deviation of blanks, and the standard of quantification (LOQ_s) are the following: Hg 13; Cd 0.40; Pb 38; Se 3.6; Cu 81; Zn 87; Ni 79 ng g^{-1} wet weight. Two blank samples were analysed together with each sample batch. Metal concentrations in blanks were below the detection limits in all the analyses. Blanks and calibration standard solutions were similarly analysed as the digested sample solution, and calibration curves were constructed. Analyses were duplicated to check the reproducibility of the results. Relative standard deviations among replicates were always less than 10%. Recovery tests were performed for the investigated metals in selected samples by spiking analysed samples with aliquots of the metal standards and then carrying out digestion. The recovery percentages ranged from 96 to 99%. Throughout the manuscript, metal concentrations are presented as μg g^{-1} wet weight basis.

2.4 Statistical Analysis

Kruskal-Wallis test was conducted to determine whether there were metal concentration differences as a function of species. Simple linear regression coefficient was used to examine the correlations between the metal load and the length of fish and between Se/Hg ratio and fish length. There were significant positive correlations between total body length and weight for all species (*T. scabrus*: $R = 0.91$, $P < 0.001$; *N. sclerorhynchus*: $R = 0.87$, $P < 0.002$; *C. coelorhynchus*: $R = 0.83$, $P < 0.04$). However, to investigate the influence of size on metal accumulation, the length was chosen because less subject to fluctuation than body weight (Diaz et al. 1994). The level of significance was set at $P \leq 0.05$.

3 Results and Discussion

3.1 Between-Species Differences in Metal Concentrations

Mean and range values for trace metals measured in the three macrourids analysed are illustrated in Table 2. Within the different fish species, mean Hg concentrations varied from 3.45 to 16.01 μg g^{-1} wet weight, with the highest levels in *T. scabrus*, including a surprisingly high value of 26.18 μg g^{-1} wet weight in one of the livers analysed. Mean Cd concentrations were between 0.60 and 1.78 μg g^{-1} wet weight, with the maximum value in *T. scabrus* at 4.72 μg g^{-1} wet weight, while Pb recorded mean concentrations from 0.64 to 0.81 μg g^{-1} wet weight, with the highest levels of 2.20 μg g^{-1} wet weight in *T. scabrus*. Statistical analyses data showed some significant concentration differences among the various species tested but no systematic trend. *T. scabrus* exhibited the highest concentrations of Hg ($P < 0.003$) and Cd ($P = 0.05$), followed by *N. sclerorhynchus* and *C. coelorhynchus*, containing similar levels of these two elements ($P > 0.05$), while for Pb contamination, image was essentially comparable among the three species ($P > 0.05$). Concerning essential metals, mean Zn and Se concentrations varied from 18.22 to 119.38 μg g^{-1} wet weight and from 8.76 to 12.26 μg g^{-1} wet weight, with the highest levels associated to *T. scabrus*, with values of 154.15 and 26.63 μg g^{-1} wet weight, respectively. Mean Cu concentrations varied from 9.50 to 12.33 μg g^{-1} wet weight, the maximum being in *N. sclerorhynchus* at 16.79 μg g^{-1} wet weight, while Ni with mean concentrations ranging from 4.51 to 12.55 μg g^{-1} wet weight exhibited the highest levels of 16.95 in one sample of *T. scabrus*. According to statistical analysis, there were significant differences between Ni concentrations ($P < 0.03$). On the contrary, Zn and Cu displayed comparable levels in *T. scabrus* and *N. sclerorhynchus* ($P > 0.05$) but higher than those in the other macrourid fish ($P = 0.05$), while any difference in terms of Se burden was observed among the species tested ($P > 0.05$). An assortment of synergistic factors can be at the origin of the metal concentration differences between fish species collected in the same marine area. Among them, it is unquestionable the importance of the diet: animal feeding on crustaceans and molluscs appears to retain higher Cd concentrations (Bustamante et al. 1998) than piscivorous biota that accumulate preferentially Hg (Havelková et al. 2008; Roméo et al. 1999; Storelli et al. 1998), as well as a crustacean-based diet determines an enrichment of Cu (Vas 1991). In our case, the three species tested show, in general, similar feeding habits having a benthopelagic diet (Carrassón and Matallanas 2002). In this framework, it would appear that feeding habits are not central in the metal load variation among the species

Table 2 Range and mean ± standard deviation of metal concentrations (μg g^{-1} wet weight) and Se/Hg molar ratios

	T. scabrus	*N. sclerorhynchus*	*C. coelorhynchus*
Hg	16.01 ± 7.07 (7.19–26.18)	6.04 ± 5.14 (1.87–16.27)	3.45 ± 1.45 (1.18–5.15)
Cd	1.78 ± 1.31 (0.67–4.72)	0.92 ± 0.50 (0.38–2.04)	0.60 ± 0.14 (0.40–0.81)
Pb	0.81 ± 0.69 (0.26–2.20)	0.64 ± 0.31 (0.27–1.34)	0.75 ± 0.22 (0.45–1.02)
Cu	9.89 ± 2.42 (6.32–14.35)	12.33 ± 3.26 (6.15–16.79)	9.50 ± 0.21 (7.99–10.50)
Ni	12.55 ± 2.90 (6.71–16.95)	6.80 ± 2.12 (4.37–10.36)	4.51 ± 0.57 (3.71–5.30)
Zn	119.38 ± 26.31 (80.75–154.15)	105.84 ± 29.48 (75.41–150.55)	18.22 ± 1.79 (15.85–20.73)
Se	12.26 ± 5.71 (6.21–26.63)	11.80 ± 3.94 (5.78–19.55)	8.76 ± 1.46 (6.90–11.00)
Se:Hg	1.95 ± 1.06 (1.28–4.92)	4.97 ± 3.19 (2.35–12.11)	6.51 ± 4.65 (3.41–16.37)

considered. Stergiou and Karpouzi (2002) indicate, instead, that for most Mediterranean species including *T. scabrus* and *C. coelorhynchus*, trophic level increases with increasing fish body size. *T. scabrus*, in particular, seems to exhibit changes in its diet in relation to length, with larger-sized individuals including also fish in their feeding pattern. The greatest size range of *T. scabrus* and the lowest of *C. coelorhynchus* coupled with ontogenetic diet shifts offer a plausible explanation about the interspecific metal variations here recorded. This seem to be particularly true in regard to Hg which exhibits a differential load in relation to size/mass; a tendency also firmed up by the significant effect of length on Hg levels encountered in the present study and discussed below. An important increase in fish size linked with depth has been also noted in various deep-sea organisms of Mediterranean Sea, including *T. trachyrinchus* and *C. coelorhynchus* supporting the general "bigger-deeper" phenomenon (Massuti et al. 1995; Rotllant et al. 2002). This direct proportionality is of crucial importance in consideration of the vertical Hg distribution with a pronounced increase in concentrations with increasing depth (Choy et al. 2009; Koenig et al. 2013). This mercury-depth-size inclination could be a supplementary possible justification for the lower concentrations of Hg encountered in *C. coelorhynchus* smaller size samples than in the other two macrourid fish. However, the interspecies variability can be linked to numerous others factors as food behaviour, i.e. a high uptake rate as a consequence of higher feeding rate for some species with respect to others, differences in fish growth cycle, sex, physiological state and/or species-specific metabolic activity. In this latter regard, the accumulation of essential metals in liver is likely correlated to its function in metabolism. Zn and Cu play a pivotal role in the enzymatic processes and relatively high levels are necessary to maintain these biological functions. Additionally, it is generally believed that marine organisms are actively capable of regulating internal concentrations up to thresholds above which regulations break down and net accumulation of the metals occur. In our case, the prominent Zn content and the relatively higher Cu values in *T. scabrus* and *N. sclerorhynchus* than *C. coelorhynchus* could indicate an elevated uptake consequent to major physiological needs, a disrupted metal metabolism, or might be a result of the activity of metallothioneins, proteins that can be binded to Cu and Zn, thus reducing their toxicity and allowing the hepatic tissue to concentrate them at elevated levels. Although metallothioneins were not investigated in the present study, the significant correlations between Cu and Zn observed in *T. scabrus* ($R = 0.64$, $P < 0.05$) and *N. sclerorhynchus* ($R = 0.79$, $P = 0.01$), but not in *C. coelorhynchus*, provide clues for the presence of metallothionein-mediated Cu and Zn detoxification processes. On the other hand, Siscar et al. (2014) in deep-sea fish found significant correlations between metallothioneins and Cu and Zn confirming the key role of these proteins in handling essential metals. However, further studies are needed to understand the accumulation and detoxification strategies in these deep-sea fish.

3.2 Relationship between Metal Content and Fish Body Length

As mentioned above, fish size is an important parameter when discussing metals' accumulation (Canli and Atli 2003; Farkas et al. 2003; Noël et al. 2013; Storelli et al. 2006). In the present study, statistically reliable and robust correlations were encountered between length and Hg concentrations in all the three macrourid species (*T. scabrus* $R = 0.75$, $P < 0.02$; *N. sclerorhynchus* $R = 0.69$, $P < 0.04$; *C. coelorhynchus* $R = 0.81$, $P = 0.05$) (Fig. 2), with the lowest statistical significance in *C. coelorhynchus* probably due to the narrow number of pools tested, while no body length-dependent accumulation was observed for Cd and Pb. Concerning essential metals, heterogeneous slopes were noted. In particular, some relationships between fish length and Zn and Cu content, although did not reach significance, have negative trends as observed in *N. sclerorhynchus* (Zn $R = -0.47$, Cu $R = -0.46$, $P > 0.05$) and *C. coelorhynchus* (Zn $R = -0.52$, Cu $R = -0.24$ $P > 0.05$), but not in *T. scabrus* for which no relationships was observed. Se showed a slight but not significant tendency to increase with increased body length in all the three species, while Ni did not display any body length-dependent accumulation. To the best of our knowledge, there are no reports of changes in metal hepatic concentrations correlated with size in deep-sea fauna, except for a study reporting a strong positive correlation between body length and hepatic Hg and Cd concentrations in *N. aequalis* and *Lepidion eques* from Atlantic Ocean (Mormede and Davies 2001a). Understanding the changes in metal hepatic concentrations correlated with size ultimately requires data from several different deep-sea fish species before patterns can be established. However, in general terms, size-dependent concentrations here described are, generally, supported in literature. Positive correlation between Hg hepatic content and marine organism size has been well demonstrated (Barone et al. 2013; Canli and Atli 2003), while the results for Cd and Pb are often contradictory, as some observe a length-related accumulation (Agah et al. 2009), others absence of any correlation (Stange et al. 1996) and still others a metal tendency to decrease with increased body length (Khezri et al. 2014). Contrasting findings are also reported for Se, with positive correlation reported for bluefish (Burger et al. 2013) and no consistent size pattern for large predators as skipjack tuna (Kojadinovic et al. 2007) and marine mammals (Ikemoto et al. 2004; Seixas et al. 2007). Conversely, there is a general consensus about the tendency for Zn and Cu concentrations to decrease with increases in body size. This trend, commonly encountered in hepatic tissue of various marine organisms including elasmobranch fish, such as torpedinid (Barone et al. 2013) and sharks (Cornish et al. 2007; Endo et al. 2008), teleost fish (Canli and Atli 2003) and marine mammals (Endo et al. 2007), is generally ascribed to different metabolic rates of accumulation and depuration of essential metals between smallest, rapidly growing animals, with respect to the oldest, showing a decline of metabolic activity. Concerning Ni, the very few published data reported negative correlations between hepatic levels and fish length (Mohammadnabizadeh et al. 2014).

3.3 Se/Hg Molar Ratios

One of the most important detoxification strategies observed in marine and terrestrial organisms (Decataldo et al. 2004; Martoja and Berry 1980; Nigro et al. 2002) relies on the insolubilization of metals as mineral concretions. An example is represented by Hg and Se, which form a complex, known as tiemannite, which is stored as inert concretions in organism hepatocytes. The interactions between these two elements, leading to ameliorate the toxic effects of Hg, have become one of the strongest and most general examples of interactions between a heavy metal and a micronutrient. The mechanisms contributing to this protective effect are still not well understood because generally, Se is thought to sequester Hg and reduce the bioavailability in organisms

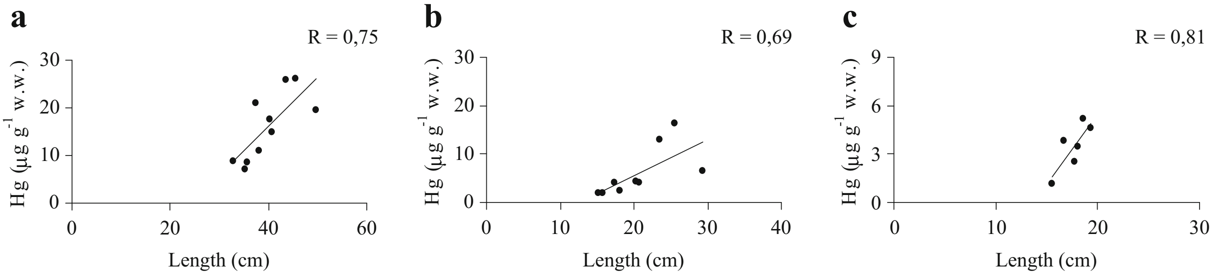


Fig. 2 Relationship between length (cm) and Hg levels (µg g^{-1} wet weight) for *T. scabrus* (**a**), *N. sclerorhynchus* (**b**) and *C. coelorhynchus* (**c**)

(Sormo et al. 2011), but the results from other studies are often contradictory and suggest that supplemental Se performs a protective action against Hg toxicity by balancing the loss and sequestration of Se by Hg (Dang and Wang 2011). However, regardless of the elaborate mechanism ruling the interactions between these two elements, it has been asserted that an excess of Se protects against Hg toxicity, and that Se/Hg molar ratios exceeding 1 are largely protective for adverse Hg effects (Peterson et al. 2009a, b; Ralston 2008). In the fish in question, clear and significant intra- and interspecific differences in Se/Hg ratio were displayed and with *C. coelorhynchus* having the highest values (Se/Hg = 3.41–16.37, average = 6.51), followed by *N. sclerorhynchus* (Se/Hg = 2.35–12.11, average = 4.97) and *T. scabrus* (Se/Hg = 1.28–4.92, average = 1.95) ($P < 0.001$) (Table 1). As might be expected *C. coelorhynchus*, a smaller species, having the lower levels of Hg showed higher Se/Hg ratios than did larger species such as *T. scabrus* containing higher levels of Hg. This trend is the result of the fact that selenium is homeostatically regulated in the body, while Hg levels are not regulated but increase proportionally with fish size. Se/Hg molar ratio was, in fact, significantly negatively correlated with length in all the three species (*N. sclerorhynchus* $R = -0.76$, $P < 0.002$; *C. coelorhynchus* $R = -0.87$, $P < 0.003$), although in *T. scabrus* ($R = -0.37$, $P > 0.05$), such a negative correlation did not appear of statistical significance. This negative trend means that as the fish get bigger and Hg levels increase, the ratio decreases and the potential protective effect of Se decreases. However, in the fish in question, the values were all above 1, indicating that an antidotal effect of Se in counteracting Hg occurred, although it is interesting to note that in *T. scabrus*, almost equimolar ratios were observed in many cases, suggesting that they had less Se available to protect against Hg toxicity.

3.4 Comparison with Literature Data

A geographic comparison about trace element content in the examined species is difficult because few data are available in deep-sea fish liver. However, as can be seen in Table 3, Hg levels detected here are extremely higher than those published for deep-sea fauna from Atlantic Ocean (Company et al. 2010; Martins et al. 2006; Mormede and Davies 2001a, 2001b), but they are in good agreement with concentrations reported for fish of the deep waters of the North-western Mediterranean Sea (Siscar et al. 2014). With regard to Cd, the detected levels in our study are generally comparable with those reported for other deep-sea fish from Mediterranean Sea (Siscar et al. 2014), higher than those encountered in *N. aequalis* and *L. eques* from Atlantic Ocean (Mormede and Davies 2001b) but lower with respect to concentrations registered in different deep-fish sampled in Rockall Trough area–Atlantic Ocean (Mormede and Davies 2001b) and near the Atlantic hydrothermal vents, areas with particularly metal-enriched waters (Company et al. 2010). For Pb, values encountered in the present study are lower than those noted in *C. mediterraneus* from North-western Mediterranean Sea (Siscar et al. 2014) but higher than those registered in species sampled in the Rockall Trough off Scotland (Mormede and Davies 2001a, 2001b). As regards Se, it is impossible to compare the results obtained in this study due to the lack of literature data, while for Ni, our concentrations are much higher than those detected in fish from NW Mediterranean Sea (Siscar et al. 2014) and in deep fauna living near hydrothermal vents in the Mid-Atlantic Ridge off Azores (Company et al. 2010). Concerning Cu and Zn, a pronounced interspecific heterogeneity can be discerned which is an expected finding, because these metals are homeostatically regulated in a species-dependent manner. However, in samples of *T. scabrus* and *N. sclerorhynchus* analysed, Zn reaches values extremely high when compared with those reported for deep fauna from Mediterranean and non-Mediterranean regions, except for a case (see *Antimora rostratus*). Also for Cu, although in a more moderate measure with respect to Zn, the concentrations were variable among species, with our levels generally higher than those previously reported for other deep-sea fish from different marine areas. From the little data available for comparison emerges that metal concentrations in our study are generally higher than the levels encountered in fish from Atlantic Ocean, especially for Hg, confirming the strong presence of this element in Mediterranean deep-sea biota (Koenig et al. 2013; Siscar et al. 2014). Concerning Ni, the presence of high levels observed in fish examined, especially where data from Eastern and Western Mediterranean Sea are compared, seems to be more delicate to understand. The wide fluvial system composed by Po river, its tributaries and a series of secondary rivers can partly contribute to the high levels of some metals, including Ni, observed in this sub-basin of the Mediterranean sea (Tankere et al.

Table 3 Comparison of metal content (μg g^{-1} wet weight) in liver of different deep-sea fish from other marine regions

Species	Location	Hg	Cd	Pb	Cu	Ni	Zn	Se	References
Macrouridae									
T. scabrus	Adriatic Sea	7.19–26.18 16.01	0.67–4.72 1.78	0.26–2.20 0.81	6.32–14.35 9.89	6.71–16.95 12.55	80.75–154.15119.38	6.21–26.63 12.26	This study
N. sclerorhynchus	Adriatic Sea	1.87–16.27 6.04	0.38–2.04 0.92	0.27–1.34 0.64	6.15–16.79 12.33	4.37–10.36 6.80	75.41–150.55105.84	5.78–19.55 11.80	This study
C. coelorhynchus	Adriatic Sea	1.18–5.15 3.45	0.40–0.81 0.60	0.45–1.02 0.75	7.99–10.50 9.50	3.71–5.30 4.51	15.85–20.73 18.22	6.90–11.00 8.76	This study
T. scabrus	Mediterranean Sea	7.02	1.76	0.10	12.29	ND	56.13	14.00	Siscar et al. (2014)
C. mediterraneus	Mediterranean Sea	27.61	1.72	2.00	3.11	0.96	10.83	23.00	Siscar et al. (2014)
N. aequalis	Mediterranean Sea	4.35	0.76	0.30	4.39	0.65	19.77	22.00	Siscar et al. (2014)
N. aequalis	Rockall Trough Atlantic Ocean	0.02–0.22 0.08	0.14–0.84 0.29	0.01–0.13 0.05	0.90–7.49 1.92	–	0.90–7.49 16.18	–	Mormede and Davies (2001a)
Moridae									
M. moro	Mediterranean Sea	4.68	0.38	0.15	2.97	ND	14.18	2.50	Siscar et al. (2014)
M. moro	Atlantic Ocean	0.40–2.20	–	–	–	–	–	–	Martins et al. (2006)
M. moro	Atlantic Ocean	–	1.48–4.58 3.03	–	2.37–5.73 4.05	0.93–2.65 3.58	NA	–	Company et al. (2010)
L. lepidion	Mediterranean Sea	4.82	1.13	0.30	8.04	ND	37.21	11.50	Siscar et al. (2014)
L. eques	Rockall Trough Atlantic Ocean	0.02–0.28 0.05	0.15–0.86 0.27	0.001–0.08 0.03	1.53–8.54 3.07	–	9.29–47.08 15.05	–	Mormede and Davies (2001a)
A. rostrata	Atlantic Ocean	–	0.64–1.03 0.83	–	6.89–9.86 8.38	0.48–0.76 0.62	NA	–	Company et al. (2010)
Aphanopodinae									
A. carbo	Rockall Trough Atlantic Ocean	–	2.06–18.24 6.98	<0.05–0.47 < 0.05	<1.00–39.05 11.87	–	29.42–108.70 62.35	–	Mormede and Davies (2001b)
Alepocephalidae									
A. rostratus	Mediterranean Sea	0.89	2.18	0.38	2.41	ND	233.03	4.00	Siscar et al. (2014)
Ipnopidae									
B. mediterraneus		0.87	0.09	0.20	0.83	ND	8.09	2.00	

Table 3 (continued)

Species	Location	Hg	Cd	Pb	Cu	Ni	Zn	Se	References
	Mediterranean Sea								Siscar et al. (2014)
Synaphobranchidae									
Synaphobranchus spp.	Atlantic Ocean	–	1.30–9.58	–	2.76–26.46	1.08–4.01	–	–	Company et al. (2010)

NA not available, *ND* not detected

2000). Consistent amounts of this element, on the other hand, have also been reported in different studies examining water and sediment quality as well as biota of the Adriatic Sea (Barone et al. 2013; Dinelli et al. 1996; Franzellitti et al. 2004), supporting the idea of a regional background value. This issue should be deepened considering the several physicochemical factors of water which are known to modify nickel toxicity to fish. For example, acute lethality of Ni increases with decreasing water pH and decreases as hardness, alkalinity and total suspended solids increase (Hoang et al. 2004). However, further studies are also needed due to the marked toxicity of this element to fishes, including surfacing, rapid mouth and opercular movements and, prior to death, convulsions and loss of equilibrium (Svecevičius 2010).

4 Conclusion

This study fills a gap by providing information on trace metal concentrations in the liver of three macrourid fish from the Adriatic Sea (Mediterranean Sea, Italy). In addition, it brings results on relationship of metals with fish length and explores Se/Hg molar ratio. Overall, the bioaccumulation of the studied trace metals differs among fish species in relation to various variables as size, diet and depth habitat. With regard to concentration vs. fish size, the correlation patterns here described generally reflect what was encountered in literature. Concerning Se/Hg molar ratio, the values obtained, all greater than one, suggest that Se is capable to counter the Hg toxicity, although *T. scabrus* shows almost equimolar ratios indicating a potential hazard for this species. From the comparison with literature data emerges that metal levels in the present study are high, especially for Hg, adding weight to the idea that the Mediterranean region is a hot spot of concern for this element. Intriguing are also Ni data, and a further characterization of its concentration in a wider range of deep-sea species would be the next step for future biomonitoring assessments. As a final consideration, research on this topic remains a necessary basic work in order to provide a better understanding either of the possible metal impact on the health of these deep-sea fish or the contamination of the Mediterranean deep-sea environment.

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