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Mercury Accumulation and Speciation in Muscle Tissue of Different Species of Sharks from Mediterranean Sea, Italy

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Domestic sewage, industrial effluents, combustion emission, mining operations and metallurgical activities introduce significant amounts of trace metals into the marine environment. Although heavy metals in trace concentrations are normal constituents of marine organisms, at high levels they are potentially toxic and may disrupt the biological activities of aquatic ecosystems. Among the toxic trace metals, mercury is one of the most hazardous environmental pollutants in the marine environment and this hazard is augmented by its persistence. The toxicology and environmental behaviour of mercury is quite complex, since the toxicity, mobility, and bioaccumulation of mercury depend on its chemical form (D'Itri, 1990). The majority of the mercury released into the marine environment is inorganic but can be converted to the methylmercury form, that is most harmful, by bacteria living in the sediment. As organometallic compound it passes easily across cell membranes and the high affinity for sulfhydryl groups of proteins causes its rapid absorption in living organisms. As a result, an accumulation of mercury, mainly as methylmercury, in marine organisms occurs with concurrent bioamplification phenomena through the trophic chains.

Sharks as predators at the top of many food chains may represent sites for biological accumulation of pollutants and, therefore, may be considered good indicators of the extend of mercury contamination in the marine ecosystems (Vas, 1991). The objectives of this study are to provide data about total and organic mercury concentrations in the muscle of different species of sharks from various areas of the Mediterranean Sea, to examine the relationship between specimen weight and mercury and methylmercury concentrations, and, finally, to compare Hg concentrations in sharks of the same species but from different areas of the Mediterranean Sea, in order to provide information about the severity of mercury pollution in these regions.

MATERIALS AND METHODS

From June to September 1999, 1081 sharks belonging to different species were caught in the eastern Mediterranean Sea. Table 1 shows the different species, the number of specimens, the range of length and weight and for few species the number of pools constituted by specimens of similar size. Muscle tissue was taken

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from the samples and preserved at -25 °C until their analysis. For the analyses of total Hg, homogenized samples of the tissue (1-3 g wet weight) were digested to a transparent solution with 10 mL of the mixture H_2SO_4-HNO_3 (1:1) under reflux. The resultant solutions were then diluted to a known volume with deionized water (G.U. 1990), and the total Hg concentrations were measured in atomic absorption spectrophotometry (Perkin Elmer 5000) by the cold vapour technique after reduction by $SnCl_2$ (A.V.A. Thermo Jarrel Ash Corp.). Methylmercury was determined following the method described by Hight and Corcoran (1987). Homogenized samples of the tissue (1 g) were prewashed 3 times with 10 mL of acetone and once with 10 mL of benzene. The prewashed tissue was acidified with 5 mL HCl-H_2O (1+1) and extracted 3 times with 10 mL of benzene. After centrifugation, the combined benzene extracts were concentrated in Kuderna-Danish glassware. The extracts were diluted to 25 mL with benzenemixed with 5 g Na_2SO_4, and analyzed by gas cromatography (Carlo Erba model HRGC-5300) equipped with a ^{63}Ni electron capture detector (ECD-400), and splitless injection tecnique was used. The column consisted of a fused silica capillary SPB-5 Supelco (length = 30 mt, inside diameter = 0.50 mm, 5 μm film). Acid washed glassware, analytical grade reagents and double distilled deionized water were used in the tissue analysis. In order to check on the purity of the chemical used, a number of chemicals blanks were run; there was no evidence of any contamination in these blanks. Analytical quality control was achieved using TORT-1 Lobster Hepatopancreas (National Research Council of Canada). The values found (Hg: 0.32 ± 0.02 mg/kg d.w.; MeHg: 0.123 ± 0.014 mg/kg d.w.) agreed with the certified values (Hg: 0.33 ± 0.06 mg/kg d.w.; MeHg: 0.128 ± 0.014 mg/kg d.w.).

RESULTS AND DISCUSSION

Table 2 shows range and mean values of total Hg and MeHg concentrations, expressed in mg/kg wet wt., in muscle tissue of different shark species from various areas of the Mediterranean Sea. Among the different species of sharks the highest levels of total mercury were detected in hammerhead shark (18.29 mg/kg). High mean concentrations were found in gulper shark (9.66 mg/kg), longnose spurdog (4.53 mg/kg), and in kitefin shark (4.38 mg/kg) too. Mean values of 1.27 mg/kg and 1.49 mg/kg were found in sharpnose sevengill and in small spotted shark respectively, while in the black-mouthed dogfish the mean total mercury concentrations ranged from 1.01 to 2.66 mg/kg. Low mean levels were found in velvet belly (0.63 mg/kg) and in smoothhound (0.31 mg/kg).

There was a considerable variation in total mercury concentrations among the different species. Biotic (species, sex, age, season, tissue sampled, diet, metabolism) and abiotic (temperature, location specific metal contamination and geological differences) factors may affect Hg accumulation in marine organisms. Among these different ecological and physiological factors, the diet and the position in the trophic web are determining elements (Cabana and Rasmussen, 1994; Kidd et al., 1995). Hammerhead shark is an efficient predator, its diet

Table 1. Biometric data and specimen number of different species of sharks.

Species	Location	n	n° pools	Range length (cm)	Range weight (g)
Blackmouth dogfish *(Galeus melastomus)*	Adriatic Sea (Italy)	164	13	24.9-55.3 45.6±8.6	41.58-440.3 272.4±119.5
Blackmouth dogfish (Galeus *melastomus)*	Adriatic Sea (Albania)	164	12	19.3-50.5 39.2±9.8	33.5-319.5 179±92.2
Blackmouth dogfish (Galeus *melastomus)*	Ionian Sea	273	27	12.6-52.1 33.3±12.7	6.0-395.7 140±117.2
Blackmouth dogfish (Galeus *melastomus)*	Aegean sea	218	18	18.8-63.0 39.8±12.1	16.18-547.3 178.5±149.1
Small spotted shark *(Scyliorhinus canicula)*	Adriatic Sea (Italy)	70	7	36.5-49.0 43.7±4.6	140-408 282.4±98.9
Kitefin shark *(Dalatias licha)*	Ionian Sea	3	---	82-104 96.7±12.7	2044-7200 5481±2977
Gulper shark *(Centrophorus granulosus)*	Adriatic Sea (Albania)	25	---	89.3-92.2 91±12.8	4472-4872 4752±167.8
Longnose spurdog *(Squalus blainvillei)*	Adriatic Sea (Albania)	20	---	60.0-74.1 67.6±4.2	778-2375 1881±565.0
Velvet belly *(Etmopterus spinax)*	Ionian Sea	120	7	17.4-29.1 23.2±4.4	14.3-94.5 60.5±29.6
Smoothhound *(Mustelus mustelus)*	Adriatic Sea (Italy)	15	---	89.3-91.0 91.2±0.8	2790-2842 2817±23.7
Sharpnose sevengill *(Heptranchias perlo)*	Ionian Sea	8	---	73.0-78.6 74.5±1.8	975-1050 994.5±24.5
Hammerhead *(Sphyrna zygaena)*	Ionian Sea	1	---	277	97000

n = number of individual sharks.

consists mainly of benthic fish as small sharks, rays, and to a lesser extent crustaceans and cephalopods, and it is one of the upper links in the food chain. Therefore its high mercury concentrations may be a product of mercury biomagnification through the trophic web and may also be related to its feeding behaviour. In this respect, it has been emphasized in several study that animals, which have close relationship with sediment, shows metal levels comparatively higher than those in pelagic fish (Storelli et al., 1998a; Kalay et al., 1999). Consequently, animals which feed mainly on benthic organisms tend to concentratecontaminant to a higher degree than other species. Also the high mercury values found in kitefin shark, gulper shark and in longnose spurdog may be related to their carnivorous diet. Significant differences between total mercury levels in gulper shark respect to longnose spurdog ($p < 0.05$) were found. Because both species are from the same sea zone (Aegean Sea), have similar feeding

Table 2. Total mercury (T-Hg), methylmercury (MeHg) concentrations (mg/kg w.w.) and % MeHg in muscle tissue samples of sharks from different regions.

Species	Provenience	Muscle Tissue		
		T-Hg	MeHg	% MeHg
G. melastomus	Adriatic Sea (Italy)	0.68-5.03 2.66±1.24	0.47-3.70 2.11±0.96	57-100 79.8±12.84
G. melastomus	Adriatic Sea (Albania)	0.25-2.06 1.01±0.62	0.23-1.99 1.01±0.58	72-100 92.3±9.91
G. melastomus	Ionian Sea	0.25-2.84 0.82±0.62	0.25-2.20 0.74±0.52	72-100 91.5±8.32
G. melastomus	Aegean sea	0.85-5.47 2.14±1.44	0.58-4.32 1.55±1.23	43-100 70.3±16.52
S. canicula	Adriatic Sea (Italy)	0.79-2.56 1.49±0.61	0.68-2.00 1.23±0.49	77-89.5 82.6±5.10
D. licha	Ionian Sea	3.58-6.00 4.38±1.07	3.24-5.0 3.81±0.69	78-95 88±0.06
C. granulosus	Adriatic Sea (Albania)	8.75-10.51 9.66±0.69	7.90-10.0 9.09±0.83	89.4-96.9 92.9±2.98
S. blainvillei	Adriatic Sea (Albania)	3.90-7.44 4.53±1.19	3.22-7.24 4.05±1.29	81-98 91.8±8.01
E. spinax	Ionian Sea	0.17-1.07 0.63±0.29	0.17-0.97 0.58±0.26	86.3-100 90.8±4.70
H. perlo	Ionian Sea	1.13-1.41 1.27±1.70	1.00-1.41 1.20±0.17	86.3-100 91.3±5.04
M. mustelus	Adriatic Sea (Italy)	0.23-0.37 0.31±0.06	0.18-0.28 0.23±0.05	69-80 75±0.05
S. zygaena	Ionian Sea	*18.29±0.03	*16.06±0.04	87.7 ±0.05

*mean of 3 replicate samples

behaviour and habitat, the reason for the differences observed may be due to size. For both species the maximum size is of the same order 110 cm *ca*, (Fao Fisheries, 1987); therefore, the gulper shark samples having a length very close to the maximum size, are older and have accumulated mercury for a longer time, respect to longnose spurdog ones. The relatively low total mercury levels in kitefin shark, voracious predator that feeds of different types of fish, could be explained with the small samples size examined that reflects a short period of exposure (age) to pollutants uptake. In black-mouthed dogfish the highest mean Hg concentrations were found in the samples from Adriatic Sea along the Italian coast (2.66 mg/kg) and in specimens caught in the Aegean Sea (2.14mg/kg), while in the samples from the Ionian Sea (0.84 mg/kg) and from the Adriatic Sea along the Albanian coast (1.01 mg/kg) the mean Hg levels were significantly lower ($p < 0.001$). Several hypothesis may explain the variability of Hg levels detected in black-mouthed dogfish from different marine areas. First of all there may be differences in the relative abundances of the various species in the black-mouthed

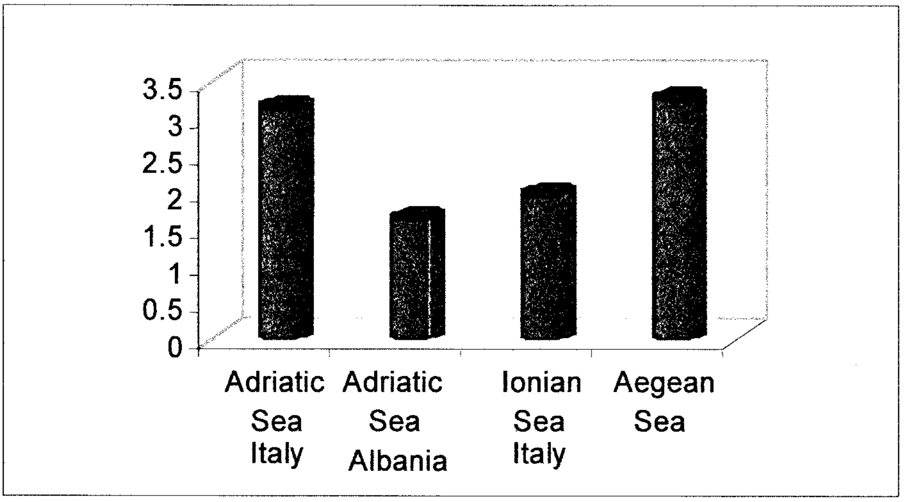

Figure 1. Comparison of total mercury levels in blackmouth dogfish similar sized specimens from different marine areas.

dogfish diet (i.e. crabs, molluscs and little fish) at various localities and/or that these species of prey vary in their ability to absorb metal. Secondarily, the abiotic component of the environment (water and sediments) at the different localities contains different metal levels which result in different quantities of the metal being absorbed either via the food chain or directly through the respiratory integuments of these sharks, or both (Walker, 1976). In this regard, the comparison between mercury concentrations in similar sized specimens (ca 280 g) of black-mouthed dogfish from different areas (Fig. 1) can provide valuable information on the level of this metal in the different environments. In fact, it was put in evidence that, in eastern Mediterranean seawater, the areas subjected to a higher contamination are Aegean Sea and Adriatic Sea along the Italian coast. Several previous studies labelled certain coastal areas and especially several closed gulfs in the Aegean Sea as polluted by heavy metals (Catsiki et al., 1991) because of a very rapid industrialization which took place without the adequate provisions for protective measures to maintain the quality of the marine environment (Fytianos et al., 1999). During the last decades, also along the Adriatic coast of Italy an increasing industrial activity developed, affecting the quality of the environment. It is not by chance that a comparison with a previous study (Storelli et al., 1998b) carried out on the same species collected in the Adriatic Sea showed lower total mercury levels than those found in the present one (e.g. Table 3).

The small spotted shark is a small scyliorhinid shark, common in the mid to deep-waters of the eastern Mediterranean, that prey primarily on invertebrates such as crustaceans and molluscs. Compared with the benthic blackmouth dogfish with

Table 3. Comparison of shark total Hg concentrations (mg/kg w.w.) measured in this study with those from other surveys.

Species	Provenience	Muscle	Reference
		Hg	
G. melastomus	Adriatic Sea (Italy)	0.68-5.03	Present study
G. melastomus	Adriatic Sea (Albania)	0.25-2.06	Present study
G. melastomus	Ionian Sea	0.25-2.84	Present study
G. melastomus	Aegean Sea	0.85-5.47	Present study
G. melastomus	Eastern Mediterranean Sea (Israel)	0.99-8.76	Hornung et al., 1993
G. melastomus	Adriatic Sea (Italy)	0.14-3.39	Storelli et al., 1998
G. melastomus	Adriatic Sea (Albania)	0.20-1.21	Storelli et al., 1998
C. granulosus	Adriatic Sea (Albania)	8.75-10.51	Present study
C. granulosus	Eastern Mediterranean Sea (Israel)	0.48-8.37	Hornung et al., 1993
E. spinax	Ionian Sea	0.17-1.07	Present study
E. spinax	Eastern Mediterranean Sea (Israel)	1.83-4.58	Hornung et al., 1993

which it shares some similarities (i.e. size and diet), the small spotted shark exhibited lower mercury concentrations. The reasons for this difference may be related to the physiological differences between two species, as different growth rates resulting in specimens of equal size being of a different age, or a differentrate of the uptake of pollutants, or the quantity of food taken by different species.

Among the species analyzed the lowest values, undoubtedly related to diet, were found in smoothhound and velvet belly samples. Velvet belly has a pelagic feeding habits. Juveniles feed on cephalopods while the adults feed mainly on crustaceans. Also smoothhound, a bentho-demersal shark, shows a diet similar to those of velvet belly. Studies carried on the stomach content of smoothhound from Adriatic sea (Costantini et al., 2000) revealed that the predominant preys were crustaceans (41%) molluscs (30%) and fish (27%). Animals feeding mainly on cephalopods or crustaceans, as opposed to those that are piscivorous, tend to concentrate mainly cadmium or arsenic than mercury, because it is known that cephalopods have high levels of cadmium (Bustamante et al., 1998; Storelli and Marcotrigiano, 1999) and crustaceans shows high arsenic concentrations (Cullen and Reimer, 1989; Storelli and Marcotrigiano 2000a).

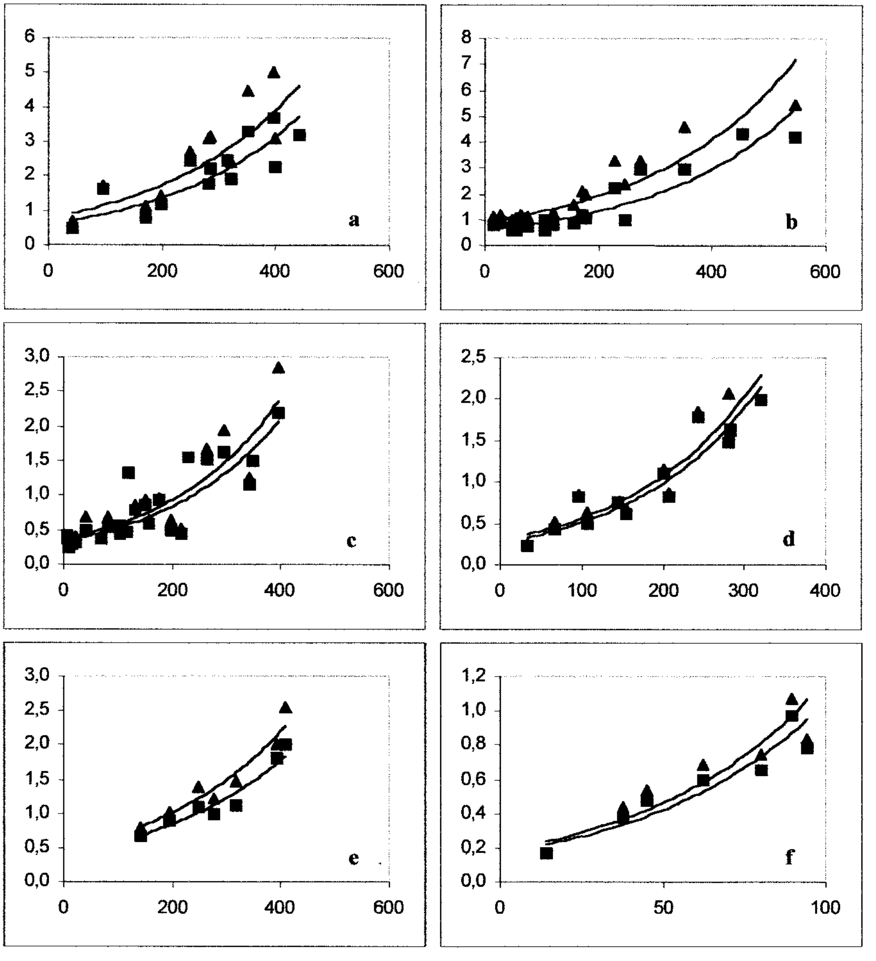

Figure 2. Correlation between total mercury (▲) and methylmercury (■) concentrations (mg/kg w.w.) and weight (g) in muscle of sharks. **a** = Blackmouth dogshark (Adriatic Sea Italy); **b** = Blackmouth dogshark (Aegean Sea); **c** = Blackmouth dogshark (Ionian sea); **d** = Blackmouth dogshark (Adriatic Sea Albania); **e** = small spotted shark; **f** = Velvet belly

Although there is a wealth of information on the concentrations of mercury in marine organisms from Mediterranean Sea there are comparatively few data for mercury levels in sharks, especially for the eastern Mediterranean. Particularly focused on this matter is the study by Hornung et al., (1993) whose values were compared with ours (table 3). The comparison revealed similar mercury levels in the muscle of gulper shark and blackmouth dogfish, while in kitefin shark the data obtained in the present study were very low.

Methylmercury is the predominant form in fish muscle tissue, generally, and the data on sharks presented in our paper fit this general picture well. In fact, mean methylmercury percentages with respect to total mercury were between 70 and 93%. These results are in agreement with those reported in literature for different marine organisms. Kamps and Miller (1972) detected methylmercury percentages from 67% to 100% of the total mercury in swordfish and canned tuna. Joris et al., (1999, 2000) found that mercury is mostly in the organic form in the muscle of different fish, while Walker (1976) and Storelli et al., (2001) reported in shark muscle mean methylmercury percentage of 66% and 91% respectively.

Specimens of few species were divided into different size groups to investigate the influence of growth on bioaccumulation. Animal size is recognized to be of importance in determining the rate of physiological processes that influence uptake, distribution and elimination of pollutants (Barron, 1990; Gutenmann et al., 1992). Significant positive correlations between total mercury, methylmercury concentrations in muscle and weight were observed for black-mouthed dogfish from different areas (Adriatic Sea-Italy Hg: r=0.74, MeHg: r=0.72; Aegean Sea Hg: r=0.86, MeHg: r=0.80; Ionian Sea Hg: r=0.76, MeHg: r=0.75; Adriatic Sea-Albania Hg: r=0.87, MeHg:r=0.87) small spotted shark (Hg: r= 0.94, MeHg: r= 0.93) and velvet belly (Hg: r=0.86, MeHg: r=0.89) (Fig. 2) confirming the results reported for several shark species (Forrester et al., 1972; Lyle 1986; Hornung et al., 1993; Storelli et al., 1998) and for marine teleosts (Storelli et al., 1998, 2000b; Joris et al., 2000).

The results showed high levels of mercury in all the species examined, except in velvet belly and smoothhound. The presence of such high concentrations in muscle tissue is not surprising given the bioaccumulative properties of Hg and the high trophic levels of these long-lived species. Characteristically, sharks are long-lived with comparatively slow rates of growth. Their longevity and slow growth rates considered in conjunction with their high trophic position probably contribute significantly to the accumulation of high concentrations of mercury (Lyle, 1984). Besides, our data suggest once more the importance of the dietary input to body mercury loading in marine organisms. Moreover, this study showed that mercury concentrations in the tissues of sharks, were considerably high in the marine areas of Aegean sea and Adriatic sea along the Italian coast.

These data provide a useful baseline against which to measure any future changes in local pollution and suggest that measures should be taken now to prevent the contamination of these marine environments.

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