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# Mercury and Arsenic Speciation in the Muscle Tissue of *Scyliorhinus canicula* from the Mediterranean Sea

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Heavy metals have long been recognized as the most deleterious contaminants to biota in the world's marine waters. Within the marine environment metals occur in various forms or species. The different species of trace elements in environmental and biological materials is an important question since the effects or toxicity of an element and its behaviour depend to a great extent on its chemical form. Biochemical and toxicological investigations have, in fact, shown that for living organisms the chemical form of a specific element, or the state oxidation in which the element is introduced into the environment, is crucial, as well as the quantities (Hughes et al., 1995; Jain & Ali, 2000). Therefore, the determination of the total content of a element is certainly not sufficient for the estimation its potential toxic impact on the living organisms.

Arsenic and mercury are nonessential microelements in the marine environment, where they are widely distributed as various inorganic and organic compounds of different toxicity to aquatic life. Usually organometallic compounds are much more toxic than ions of the corresponding inorganic compounds. Mercury obeys this general rule, whereas arsenic represents an exception because most organo-arsenicals are less toxic than inorganic arsenic species.
Despite to the fact that, metal speciation is a critical issue in the evaluation of toxic impact on living organisms most analytical measurements dealt with the total content of a specific element. Very few data are, in fact, available on arsenic speciation in fish (Storelli & Marcotrigiano, 2000; Fattorini et al., 2004), whereas there are comparatively a major quantity of studies on mercury speciation (Joiris et al., 1999; Storelli et al., 2003). Elasmobranch fishes, in particular, have been poorly studied in regard to speciation of both these metals (Storelli et al., 2000; Storelli et al., 2002), thought they constitute an important group of fishes often at the top of food chains in marine ecosystem. Also little is known about the influence of age/size on the accumulation, especially of arsenic, in these marine organisms.

Based on this background the present study provides data about speciation of mercury and arsenic in the muscle of a small scyliorhinid shark, *Scyliorhinus canicula*, representing an important trophic link between invertebrates and larger fish, and discusses the results in relation to age/size of specimens.

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## MATERIALS AND METHODS

During several trawl surveys specimens of *Scyliorhinus canicula* (small spotted shark), were caught in the Adriatic sea along Italian coast from June to September 2003. From total number of specimens were formed pools (n° 12) within which individual fish were collected as a function of their similar size. From the organisms of each pool muscle tissue was removed and preserved at -25 °C until analysis. For total, inorganic and organic As and total Hg and MeHg, the extractive analytical procedures and the instrumental conditions for quantitative analysis have been previously described (Storelli et al., 2000; Storelli & Marcotrigiano, 2000) elsewhere. Briefly, for total As about 3 g of homogenized tissue were digested, under reflux, with 10 ml of the mixture $H_2SO_4$–$HNO_3$-$HClO_4$ for 6 h. After cooling the acid digest was diluted to a final volume with de-ionized water. For inorganic As, samples (about 5 g) were accurately weighed into flat-bottom 100 ml distillation flasks. To each was added 1 ml of hydrobromic acid (48%) and 25 ml of 6.6 M hydrochloric acid and the sample was refluxed and distillate was collected (about 20 ml). A further 20 ml of 6.6 M hydrochloric acid was added to the flask and distillate again and collected to make a total volume of about 40 ml. After cooling, the condenser and receiver were washed with distilled water and the washings added to the distillate and made up to 50 ml. For organic As to the residue was added a mixture of $HNO_3$-$HClO_4$ and $H_2SO_4$. Then samples were slowly evaporated, cooled, and made up to a volume with distilled water. For total Hg, samples (3 g) were digested with a mixture $H_2SO_4$-$HNO_3$ conc. under reflux. The resultant solutions were then diluted to a known volume with deionized water. All atomic absorption measurements were carried out using an atomic absorption spectrophotometer (Analyst 800, P.E.). Hg was determined by the cold vapour technique after reduction by $SnCl_2$, while As were measured as volatile hydrides after reduction by $NaBH_4$ (FIAS-Furnace, P.E.). For MeHg about 1 g of homogenized tissue was pre-washed 3 times with acetone and once with toluene. The pre-washed tissue was acidified with HCl-$H_2O$ (1:1) and extracted with toluene. After centrifugation, the combined toluene extracts were concentrated, diluted to a known volume with toluene, and analyzed by gas chromatography (HRGC-8000 Top C.E.) equipped with an electron capture detector (ECD-400) and with a fused silica capillary SPB-5 Supelco (length = 30 mt, inside diameter = 0.50 mm, 5 μm film). Analytical quality control was achieved using DORM-2 Dogfish Muscle (National Research Council of Canada). Replicate analyses (n=3) (Total Hg 4.51±0.12 mg/kg dry weight; MeHg 4.26± 0.20 mg/kg dry weight; Total As 17.8±1.3 mg/kg d.w.) were in the range of the certified material (Total Hg 4.64±0.26 mg/kg dry weight; MeHg 4.47± 0.32 mg/kg dry weight; Total As 18.0 ±1.1 mg/kg dry weight). Analytical detection limits were for total Hg and total As 10 ng $g^{-1}$ and 5 ng $g^{-1}$ dry weight, respectively. The measurements for each pools were made in triplicate and all data were expressed in mg/kg wet weight.

## RESULTS AND DISCUSSION

Total mercury concentrations in the muscle of small spotted sharks ranged from

**Table 1.** Values of total mercury, methylmercury, and total, organic and inorganic arsenic (mg/kg wet weight) in muscle tissue of small spotted shark.

| Pools | Weight (g) | Total Hg | MeHg | Total As | Org. As | Inorg. As | Sum org.+ inorg. |
|---|---|---|---|---|---|---|---|
| 1 | 33,50 | 0,26 | 0,23 | 6,28 | 5,31 | 0.44 | 5,75 |
| 2 | 66,67 | 0,51 | 0,44 | 6,27 | 6,32 | 0,08 | 6,40 |
| 3 | 97,30 | 0,83 | 0,81 | 5,47 | 5,76 | 0,40 | 6,16 |
| 4 | 106,26 | 0,64 | 0,49 | 4,46 | 5,45 | 0,24 | 5,78 |
| 5 | 145,21 | 0,76 | 0,76 | 7,19 | 7,74 | 0,15 | 7,89 |
| 6 | 154,90 | 0,68 | 0,63 | 7,43 | 8,72 | 0,20 | 8,92 |
| 7 | 202,00 | 1,15 | 1,09 | 6,38 | 6,55 | 0,23 | 6,78 |
| 8 | 207,50 | 0,87 | 0,82 | 14,27 | 13,11 | 0,12 | 13,23 |
| 9 | 242,50 | 1,84 | 1,78 | 8,21 | 8,63 | 0,27 | 8,90 |
| 10 | 280,00 | 2,06 | 1,48 | 7,12 | 9,43 | 0,19 | 9,62 |
| 11 | 282,03 | 1,63 | 1,63 | 8,45 | 9,20 | 0,29 | 9,49 |
| 12 | 319,47 | 1,99 | 1,99 | 13,00 | 14,00 | 0,49 | 14,49 |
| **Min** | 33,50 | 0,26 | 0,23 | 4,46 | 5,31 | 0,08 | 5,75 |
| **Max** | 319,47 | 2,06 | 1,99 | 14,27 | 14,00 | 0,49 | 14,49 |
| **Mean** | 178,11 | 1,10 | 1,01 | 7,88 | 8,35 | 0,26 | 8,62 |
| **St. Dev.** | 92,14 | 0,62 | 0,58 | 2,92 | 2,84 | 0,13 | 2,84 |

0.22 to 1.99 mg/kg (av. 1.08 mg/kg) (Table 1). Comparison with literature data showed that mean mercury levels in organisms here examined were comparable to those detected in specimens either from the same marine environment (Storelli et al., 2002) or from other aquatic systems (Leah et al., 1991). However an appropriate comparison must take account not only the concentration level of metal in the organism, but also the fish size/weight. Animal size is, in fact, recognized to be of importance in determining the rate of physiological processes that influence uptake, distribution and elimination of metals (Phillips & Rainbow, 1993; Canli & Atli, 2003). This certainly appears to be the case of mercury because the levels of this element increase with body size, as larger, older fish have generally higher concentrations than smaller, younger fish. A positive correlation between weight and total mercury concentrations was, in fact, observed in organisms here examined ($r=0.86$, $p<0.0001$) in good agreement with the results reported in the literature for same fish species, for other elasmobranch fish (Storelli et al., 2002), and for teleost fish (Andersen & Depledge 1997; Joiris et al., 1999). As regards methylmercury the concentrations varied from a

minimum of 0.23 to a maximum of 1.99 mg/kg (av. 1.01 mg/kg). To our knowledge no previous data on methylmercury concentrations in these organisms are available, except for one of our recent studies that reported values in line with those found in the present paper (Storelli et al., 2002). Methylmercury is largely responsible for the accumulation of mercury in organisms and the transfer of the element from one trophic level to another. Accumulation of this potent toxicant has been shown to vary in different phyla, due to the distinct routes of uptake of the element (Andersen & Depledge, 1997). It represents 10-30% of the total mercury in marine plants, 20-80% in invertebrates (Claisse et al., 2001), while in fish and higher predators generally accounting for more than 80% of total mercury. Joiris et al., (1999) found that mercury is mostly in the organic form (>85%) in the muscle of sardines, while Storelli et al., (2003) in muscle the tissue of a variety of fish species reported methylmercury percentages between 55 and 100%. Similarly, in the muscle tissue of elasmobranch fish, Walker (1976) reported that 66% of the mercury was in the methylated form, while Storelli et al. (2002) detected methylmercury percentages between 70 and 92%. Also, other cartilaginous fish, such as eagle ray and ghostshark, showed a high percentage of the methylated form, varying from 72% to 83% (Storelli et al., 2002a). The proportions of methylmercury to total mercury on fish presented in our paper fit this general picture well. In fact, the mean percentages ranged from a minimum of 78% to a maximum of 100%. As in the case of total mercury, the methylmercury levels were correlated with fish weight ($r=0.87$, $p<0.0001$) (Fig.1) too.

Results for total, organic and inorganic arsenic in muscle of different species, together with the sum of organic + inorganic are given in Table 1. Between the total arsenic concentrations and the values obtained from the sum of organic + inorganic, no substantial differences have been observed. Hence, in the results total arsenic values will be those of the sum. Total arsenic concentrations vary from 5.75 mg/kg to 14.49 mg/kg (av. 8.62 mg/kg). Available literature data indicate that arsenic concentrations vary greatly amongst different taxa. For example, it is known that crustaceans and molluscs generally contain higher amounts of arsenic than fish. As regards the cartilaginous fish, a recent study shows that total arsenic concentrations vary widely among individuals and species (Storelli & Marcotrigiano, 2004). This large variation in arsenic content is mainly ascribed to diet (Maher & Butler, 1998). In general fish which feed primarily on small fish contain less arsenic than fish whose food consists of bottom living invertebrates, small crustaceans, molluscs and worms. In this respect the relative high arsenic levels in organisms in question might be the resultant of their diet consisting largely of crustaceans and gastropods and in minor extent of little fish. However, independently by factors influencing arsenic content, our values were comparable to those reported for the same species in another study (Storelli & Marcotrigiano, 2004).

The concentrations of organic arsenic between 5.31-14.00 mg/kg (av. 8.35 mg/kg) constituted from 92.3% to 99.1% of the total arsenic. These high percentages showed that arsenic was present prevalently in the organic form in accordance

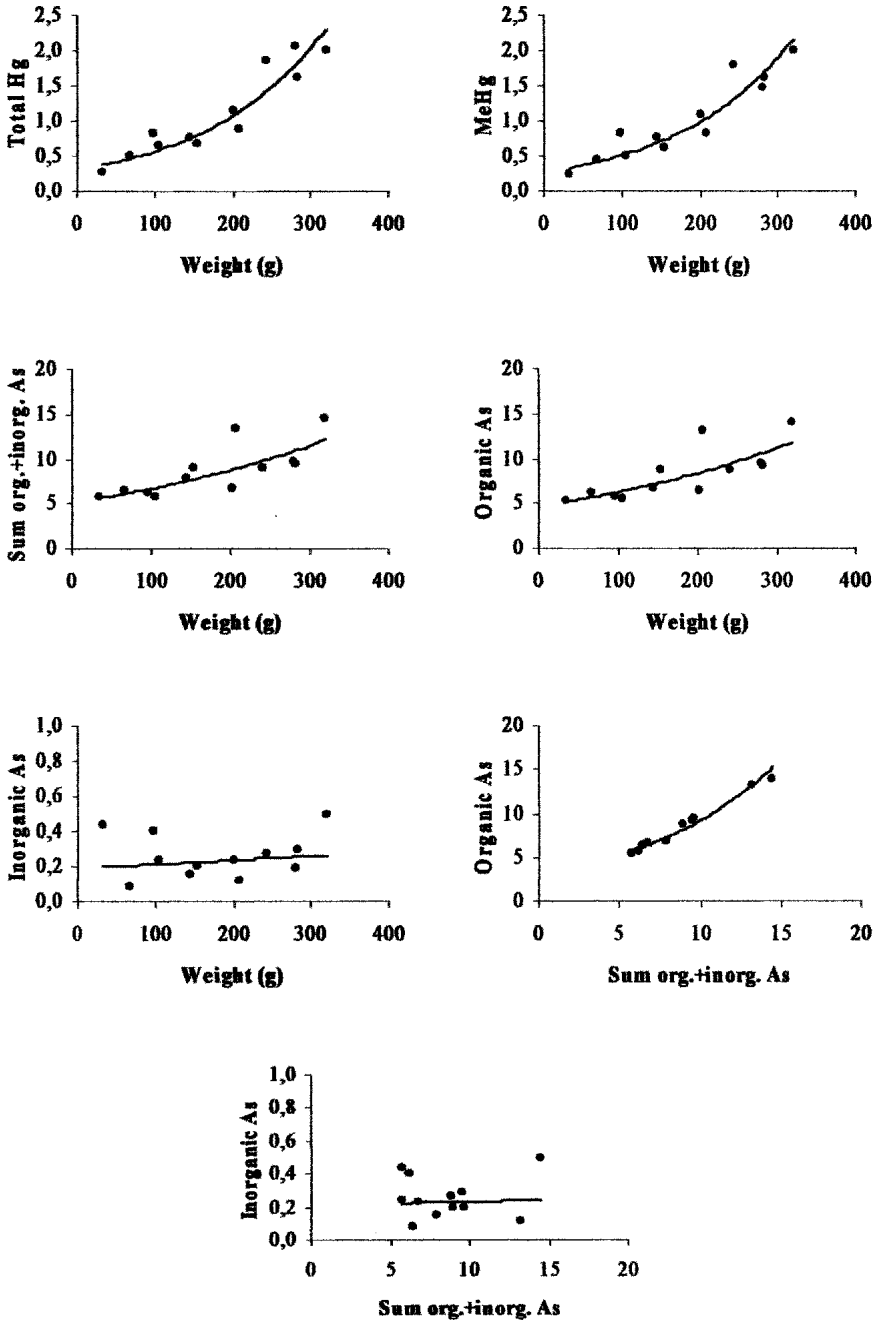


**Figure 1.** Relationship between total mercury, methylmercury, total arsenic (sum organic+inorganic), organic and inorganic arsenic (mg/kg w.w.) and weight and correlation between organic and inorganic arsenic and total arsenic (sum organic + inorganic).

with what detected in fish (Maher et al., 1999; Fattorini et al., 2000) and in other marine organisms, such as reptiles (Storelli & Marcotrigiano, 2000a; Saeki et al., 2000) and mammals (Kubota et al., 2002). Obviously, the levels of inorganic arsenic, between 0.08 and 0.49 mg/kg (av. 0.26 mg/kg), represented only 0.9-7.7% of the whole arsenic. If it is generally accepted that mercury load in fish body is strongly affected by size it is not still clear if the arsenic accumulation is size-dependent. Published literature on the correlation of total arsenic concentrations in tissue muscle with weight have shown that there is a dependency for some species e.g. *Thunnus thynnus* and *Thunnus toggel* (Ashraf & Jaffer, 1988), *Boreogadus saida* (Bohn & McElroy, 1976), *Reinhardtius hippoglossoides, Anarchichas minor* (Bohn, 1975), and *Myoxocephalus scorpius* (Bohn & Fallis, 1978), while for other species, as *S. maculata* (Edmonds & Francesconi, 1981), and *Mugil cephalus* (Maher et al., 1999) no dependency was found. Also for elasmobranch fish an increase in total arsenic concentrations with increasing weight was noted in some species, such as *Galeus melastomus* and *Scyliorhinus canicula*, while other species revealed any dependency of total arsenic concentration on size (Storelli & Marcotrigiano, 2004). The present study revealed a positive correlation between total arsenic concentration and weight of specimens ($r=0.65$, $p<0.003$), indicating that co-accumulation of element was occurring. Generally, the predominant arsenic form in marine organisms is metalloorganic that is known to be efficiently accumulated by tissues of marine animals and not easily excreted as occurs for human and other mammals (Buchet et al., 1994; Neff, 1997). The significant correlation observed between organic arsenic and specimen weight ($r=0.66$, $p<0.003$), as well as the significant increase of organic arsenic fraction with increasing of total arsenic concentration ($r=0.66$, $p<0.0001$) provided convincing evidence of what above mentioned. In contrast, concentrations of inorganic arsenic had no correlation either body weight or with the levels of total arsenic.

An overall analysis of the obtained results confirms that mercury is mainly present as organic form. Likewise, also for arsenic the most common form present in these marine organisms was the organic one. From a toxicological point of view methylmercury concentrations, although not very high, are not to understate because toxic effects of this organic form of mercury are well known. Also high accumulation of organic arsenic in organisms deserves attention because it has recently been shown that an organic compound, dimethylarsinate (DMA), has carcinogenic potential (Yamamoto et al., 1997). It has also found that under specific conditions arsenobetaine present in marine biomass, could be transformed into trimethylarsine, which is a toxic species (Irgolic et al., 1990). What above reported highlights the importance of further studies measuring the different chemical species of organic arsenic to better assess their impact on these organisms.

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