Mercury Contamination of Whitecheek Shark (*Carcharhinus dussumieri*): its Relation to the Length and Sex

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Abstract: This study was conducted to assess the total mercury concentration in muscle, liver and fin tissues of whitecheek shark (*Carcharhinus dussumieri*, Carcharhinidae). Approximately 26 percent of all captured Sharks had total mercury concentrations greater than 0.2 mg/kg w.w., 48% contained amounts higher than 0.4 mg/kg w.w. and 22 % had more than 0.6 mg/kg w.w. Only 4 % of all samples had a concentration greater than 0.8 mg/kg w.w. In spite of significant differences in the length distributions of male and female, there were no significant differences in total mercury concentrations of experimental tissues between the genders. In all stations, muscle tissue showed the highest mercury levels (0.73 mg/kg w.w in males and 0.77 mg/kg w.w in famels), followed by Liver (0.28 mg/kg w.w. in males and 0.29 mg/kg w.w. in females) and Fins (0.13 mg/kg and 0.16 mg/kg w.w., respectively). No significant correlation was found between the concentration of mercury in tissues with sex and location, although length had an increasing effect on mercury concentration. In addition, length showed strongly positive correlation with mercury concentration of fins. The current study illustrated that consumption of less than 0.5 kg/week from muscle tissue of the whitecheek shark could result in a daily intake of 80 µg Hg which is more than 45 times the maximum intake concentration established by the World Health Organization.
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1. Introduction

Mercury (Hg) contamination is a great concern which its toxicity in many coastal ecosystems poses some health hazard for human and wildlife (Branco et al., 2004; Tessier et al., 2007; Saniewska et al., 2010). Monomethyl mercury (CH3Hg+) is the most toxic of the mercury compounds that accounts for more than 95% of organic mercury in aquatic muscle tissues (Jewett et al., 2003; Bloom et al., 2004; Branco et al., 2004; Covelli et al., 2009, Liu et al., 2012).The main routes of acute and chronic Hg exposure include inhalation, dermal absorption, and ingestion (Solis et al., 2000; Moreno et al., 2005; Harper and Harris, 2008; Al-Saleh et al., 2009; Burke et al., 2010). Also, toxic responses such problem as malformations, growth reduction, neurological deficiency in levels of certain enzymes, and renal failure are examples of chemically induced effects of Hg exposure (Loumbourdis and Danscher, 2004; Bose-O'Reilly et al., 2010; Rani et al., 2011).

World Health Organization (WHO) had been estimated that around 10×10^6 kg mercury is annually released all over the world (WHO, 1989). About 50 percent of this amount transport from continents to the coastal area by pathways such as surface runoff, atmospheric deposition mainly fluvial transport, and natural weathering processes (Hanten el al., 1998; Babiarz et al., 2003 Carvalho et al., 2008; Stern et al., 2012).

Since degradation of mercury compounds in environment has some complications, the most mercury contaminations in food chain come mainly from existing background mercury pollutants (Merritt and Amirbahman, 2009; Nfon et al., 2009; Lavoie et al., 2010). Recently, public health concerns over mercury toxicity have focused on the potential risk associated by relatively low doses of Hg in the environment (Hsiao et al., 2010; Fang et al., 2011; Huang et al., 2011). Several studies have shown that mercury could bioaccumulate, mainly as methylated forms, in the muscle tissues of aquatic organisms (EPA, 2001; Cai et al., 2007; Negrete et al., 2008). Probably due to some difficulties in sample catching of apices predators like sharks from marine, there are scarce researches on acute and chronic effects of mercury in marine environments (Feng et al., 2004).

Because of predatory behavior, long life, and higher trophic levels, sharks exhibit higher mercury concentrations than other marine fishes (Da Silva et al., 2004; Endo et al., 2008). Whitecheek shark (*C. dussumieri*) is one of the most plentiful sharks of the Persian Gulf which has been inhabited in coastal ecosystem, where point sources of mercury are abundant (Henderson et al., 2007). This ecologically

and economically important species live in continental shelf of south the Indian Ocean with preferable depth of 20 to 60 m and fed from cephalopods, crustaceans, and small fishes (Salini et al., 1992, 1994; Cortés, 1999; Henderson et al., 2007).

Meat and fins of whitecheek shark are used for human consumptions and could consequently represent a model for the study of mercury contaminations in marine environments and the possible intake of mercury by human populations (Ferreira et al., 2004). Accordingly, the objective of the present study was to analysis total mercury concentration in muscle, liver and fin tissues of whitecheek shark, *C. dussumieri*, from the Iranian coastal waters of the Persian Gulf. Besides, correlation between mercury levels and body length, age, and sex of sharks was also considered to clarify risks associated by consumption of the marine resources.

2. Material and Methods

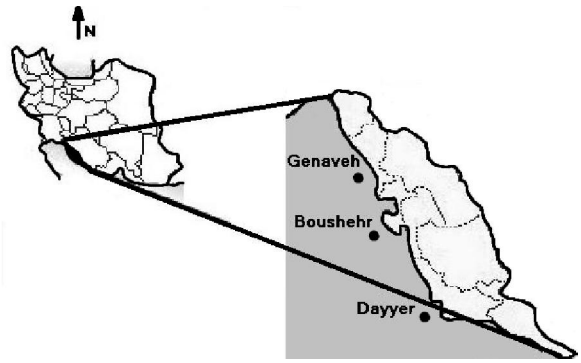
Fish sampling was performed from 3 different stations (Genaveh, Boushehr and Dayer) located in northern coast of the Persian Gulf (50-51° N and 27-29° E), within 20 km of the shoreline (Fig. 1). A total of 149 specimens whitecheek shark (*C. dussumieri*) were captured opportunistically by a local trawl fishing boat from November to December 2011. After capture, all sharks were individually weighted, sized and frozen for preservation before being transported to the laboratory. Besides, the age of each sample was evaluated using the correspondent length of maturity recommended by Compagno (1984). Specimens shorter than the maturity length were considered juveniles, and individuals equal to or larger than the maturity length were considered adults.

Tissue samples were taken in the laboratory by necropsies of each specimen. A portion (approximately 20 g) of muscle, liver and fin were segregated using a clean stainless-steel knife. Care was done to ensure that the samples had no contact with dermal layers or other surrounding surfaces during the dissection. After that, shark tissues washed 3 times with tap water and rinsed by deionized water. To reduce the risk of contamination, each sample were immediately placed in a separate plastic zip-lock bag and frozen at -20°C before analysis. Sex was also determined macroscopically and checked by examination of interior reproductive organs.

Analytical Methods

All samples were processed within two months of being captured. Approximately 2 g of experimental tissues (liver, muscle and fin) were freeze-dried at -50°C and their moisture contents determined by weight loss. Then, the samples were ground, homogenized and sieved (d≤175 μm) for investigation.

The amount of total mercury (THg) in liver, muscle and fin was separately determined by an advanced mercury analyzer (LECO model AMA 254, USA), which did not require pretreatment or acid-digestion of the sample. In brief, aliquots ranging from 20 to 40 mg of freeze-dried sample were placed into oven of the instrument. After drying, each sample was pyrolyzed at 800 °C under oxygen atmosphere for 3 min and elemental mercury vapor was subsequently collected in a gold net (Au-amalgamator). The net was then heated for liberating and measuring of Hg by atomic absorption spectrometry (AAS). Each sample was analyzed in triplicate for assurance of consistent results.



	longitude	latitude
Genaveh	29° 25′ N	50° 25′ E
Boushehr	28° 45′ N	50° 41′ E
Dayer	27° 50′ N	51° 12′ E

Fig. 1. Study area and topology of sampling points.

The accuracy of the procedure was examined by analyzing of certified reference materials including dogfish muscle (DORM-2), dogfish liver (DOLT-3), and lobster hepatopancreas (TORT-2) obtained from the National Research Council of Canada. The standard materials were analyzed according to the procedure described for the tissue samples of whitecheek shark. A suitable conformity was found between certified and obtained values, as recovery ranged from 98.3% to 103.4%. All total mercury results were stated as milligrams per kilogram (mg/kg) of wet weight (w.w.).

3. Results

The total number and body length of the whitecheek shark captured from different stations are shown in Table 1. Of total 149 specimens, the most samples were collected from Dayer station (42.3 %) followed by Genaveh (32.8 %) and Bushehr (24.9 %) stations. The lengths of the sharks varied from 65 to 105 cm. The mean body size of the individuals

showed that all specimens were adult (Based on Compagno, 1984). The total shark samples were composed of 59 (41.6 %) males and 88 (58.4 %) females. Body length of the female samples was significantly higher than males in all sampling station ($p<0.05$), except for Genaveh station which no significant differences was found between males and females. All the shark specimens were showed significant positive correlations between body length and weight ($p<0.05$). Therefore, only relationship between body length and THg levels is reported here.

Overall variations of mercury concentration in different tissues of whitecheek shark were high and concentrations varied, but were usually high in the study area (Table II). Approximately 26 percent of all Sharks had total mercury concentrations greater than 0.2 mg/kg w.w., 48% contained amount higher than 0.4 mg/kg w.w., and 22 % had more than 0.6 mg/kg w.w. Only 4 % of all samples had a concentration greater than 0.8 mg/kg w.w.

Although females had higher length than males, no significant difference was obtained in total mercury concentrations of experimental tissues between the genders ($p>0.05$). In all stations, muscle tissue showed the highest mercury levels (Fig. 1), with an average concentration of 0.73±0.28 mg/kg w.w. in males and 0.77±0.27 mg/kg w.w. in females, followed by the Liver (0.28±0.9 mg/kg and 0.29±0.9 mg/kg w.w., respectively). Fins had the least tissue contamination in all cases, with mercury concentrations varied between 0.13±0.05 to 0.16±0.07 mg/kg w.w.

No significant correlations was found between the concentration of mercury in tissues with sex and location, although length had an increasing effect on mercury concentration ($p>0.05$) (see Table 2). In addition, length showed strongly positive correlation by mercury concentration of fins, whereas other variables like sex and station had not significant effect on fins mercury (Table 3).

Cluster analysis was also applied to provide an outline of similarities between mercury concentrations of different tissues (Fig. 2). It is clear that pattern of mercury level in muscle and fin is closer, whereas kidney concentration was different from the others.

4. Discussions

The current research provides information concerning total mercury levels in muscle, liver, and fin of whitecheek shark, *C. dussumieri*, as a top marine predator caught from northern offshore of the Persian Gulf. Based on the amount of natural mercury in fish (0.15 to 0.2 µg/g wet weight) provided by Johnels et al. (1967), the values found for this species were considered high. Overall concentrations were also high when the results compared to the levels of mercury in the other shark species captured from Florida (Adams and McMichael, 1999), Brazil (Pinho et al., 2002), western equatorial Atlantic Ocean (Ferreira et al., 2004), and western equatorial Pacific Ocean (Endo et al., 2008). The present difference could be related to distinct feeding habits and other species-specific parameters of *C. dussumieri* such as metabolic rate and lifetime.

There are few studies on the segregation of mercury in fish. Coelho et al. (2010) reported mercury content in various tissues of demersal shark and chimaeras from continental shelf and slope waters of southeast Australia. In all specimens, the highest mercury level was recorded in muscle tissue, followed by the heart, gills, liver, and Pancreas. Endo et al. (2008) stated that the average of Hg values in the muscle tissues of tiger and silvertip sharks were higher than that in the corresponding liver tissues. Other shark species have also indicated higher value of total mercury in muscle compared to liver (Taguchi et al., 1979; Lacerda et al., 2000; Adams et al., 2005; Branco et al., 2007; Pethybridge et al., 2010).

Table 1. Number of the fish captured from different station of the Persian Gulf.

	Number			Length (cm)		
	Total	Female	Male	Mean	Female	Male
Genaveh	49	28	21	82.5±7.7	83.8±7.1	80.2±8.1*
Bushehr	37	22	15	84.7±9.2	90.0±9.2	79.6±9.4*
Dayer	63	37	26	87.6±9.6	93.5±10.7	81.3±8.6*

* Shows significant difference at level of p less than 0.05.

Similarly, the present results showed the highest mercury level in the muscle tissue. The liver exhibited comparatively lower mercury level followed by the fin. Current pattern corroborates the hypothesis that mercury is more assimilated from the dietary exposure than the environment, and is easily distributed throughout the body and preferential accumulated in muscle tissue (Lacerda et al., 2000; Wang, 2002; Coelho et al., 2008).

Boening (2000) cleared that dietary behavior has an important effect on mercury accumulation in top marine predators which the results presented in Table

2 support this view. The mercury content in the diet increases as the shark grows larger (Mathers and Johansen, 1985). Whitecheek shark feed on a wide range of crustaceans but preys cephalopods and small benthic as they became greater in size (Henderson et al., 2007; White et al., 2012). Therefore, higher mercury accumulation in muscle is generally associated by dietary uptake (Branco et al., 2007; Pethybridge et al., 2010), while environmental absorption has lower impact on external organs such the skin and fins (Coelho et al., 2010; Pethybridge et al., 2010). Median concentration of mercury in liver could be explained by demethylation mechanism of mercury occurring in liver tissue for elimination and Neutralization of the harmful effect of mercury in sharks (Palmisano et al., 1995; Storelli et al., 2002; Branco et al., 2007).

The current findings support this view that mercury level in muscle tissue has a linear relationship by size/age of the shark (Green and Knutzen, 2003; Branco et al., 2007; Pethybridge et al., 2010). The continuing accumulation may be explained by strong binding of mercury in muscle to trial groups of proteins whose content expands with age (Sfezer et al., 2003; Storelli et al., 2002). Besides, lower growth rates in adult individuals lead to longer exposure and, therefore, more accumulation of mercury (Pinho et al., 2002). On the other hand, the content of Hg in liver was poorly correlated by individuals' lengths which demonstrate that accumulation in this organ does not proceed essentially by age (Branco et al., 2007). The processes of detoxification in liver could cause the elimination of toxicant forms of mercury arrived to this organ (Storelli et al., 2002).

There was no significant difference in total mercury concentration of whitecheek shark between the stations, although Bushehr station showed a slight higher level of contamination. Similar results of mercury accumulation in specimens between stations may be related to their highly migratory nature and identical absorption through the body.

There was no significant difference in total mercury concentration between genders of whitecheek shark, although females exhibited slightly higher level of contamination than males. Differences in mercury levels of males and females may be caused by factors such as energetic requirements, maturation condition, mercury deposition, and transference to eggs and fetuses as previously reported for the other shark species (Taguchi et al., 1979; Walker, 1988; Endo et al., 2008). In the present work, higher mercury levels were found in males, although there were significantly shorter on average than females ($p<0.05$). Differences in mercury concentration could also be clarified by higher growth rate in females, resulting younger female with same length and time for mercury exposure than males (Forrester et al., 1972; Lyle, 1984; Monteiro et al., 1991; Licata et al., 2005). The lower mercury levels in females may be explained by viviparous reproduction of *Carcharhinus* species and nutrients transfer to the fetus through the blood (Lyle, 1984; Compagno et al., 1984; Henderson et al., 2007; Holms et al., 2009). Thus, mercury present in the mother will be easily deposited in the fetus and, therefore, reduced in females compared to that the males.

Table 2. Total mercury concentration (mg/kg wet weight) in tissues of whitecheek shark from different stations of the Persian Gulf.

	Muscle				Liver				Fin			
	Female	Male	Max	Min	Female	Male	Max	Min	Female	Male	Max	Min
Genaveh	0.75 ± 0.20^{ef}	0.72 ± 0.27^{e}	1.20	0.18	0.26 ± 0.10^{c}	0.25 ± 0.09^{c}	0.41	0.02	0.15 ± 0.09^{b}	0.15 ± 0.06^{b}	0.24	0.01
Bushehr	0.76 ± 029^{ef}	0.74 ± 0.33^{e}	1.51	0.10	0.33 ± 0.12^{d}	0.29 ± 0.10^{cd}	0.58	0.08	0.15 ± 0.07^{b}	0.16 ± 0.05^{b}	0.29	0.07
Dayer	0.77 ± 0.32^{ef}	0.71 ± 0.26^{e}	1.35	0.34	0.30 ± 0.10^{cd}	0.30 ± 0.07^{cd}	0.47	0.06	0.18 ± 0.04^{b}	0.10 ± 0.05^{a}	0.30	0.07
Total	0.77 ± 0.27^{ef}	0.73 ± 0.28^{e}	1.51	0.10	0.29 ± 0.10^{cd}	0.28 ± 0.09^{c}	0.41	0.02	0.16 ± 0.07^{b}	0.13 ± 0.05^{ab}	0.24	0.01

Results are present as Mean±SE. means with different superscript are significantly different at level of $p<0.05$.

The whitecheek shark is traditionally consumed by people living in the northern offshore of the Persian Gulf and the eaten of this shark could be one of the major origins of Hg exposure in the human foods. The average of total mercury concentration found in the meat (muscle) of whitecheek shark was 0.73±0.27 g/kg wet weight. The joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended the provisional tolerable weekly intake (PTWI) of 1.6 µg/kg of body weight for organic mercury equivalent to 300 µg of total mercury per person (WHO, 2007). Accordingly, consumption of even less than 0.5 kg muscle tissue of the whitecheek shark per week could result in a daily intake of 75 µg/kg of body weight Hg which is more than 45 times the maximum intake concentration established by the World Health Organization (WHO, 2007; Ferreira et al., 2004). However, lower concentration of THG in the fin let it to be more consumed.

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