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Mercury in the Oriental Sole *(Brachirus orientalis)* Near a Chlor-Alkali Plant in the Persian Gulf, Iran

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Abstract Total mercury in muscle and liver of Oriental sole from the largest inlet in the Persian Gulf was evaluated. Fish were collected from three channels of Moses Inlet near a chlor-alkali plant. Ahamdi and Jafari channels were closest to this plant and Ghanam was farther away. We sampled in August 2007 and February 2008. The overall estimated marginal mean for total mercury in sole tissue was 2.4 ± 0.1 mg/kg wet weight. Mercury in fish was similar in August and February; but muscle from Ahmadi contained higher mercury in August (1 ± 0.2) than in February (0.5 ± 0.01). This trend was reversed in the liver (1.3 ± 0.2 and 3.7 ± 0.3).

Keywords Mercury · Oriental sole (*B. orientalis*) · Muscle · Liver · Persian Gulf · Chlor-alkali plant

Mercury (Hg) pollution is a growing global concern and worldwide. Mercury pollution has increased as much as two–tenfold during the past one and half centuries (Rasmussen et al. 2007; Swain et al. 1992). Universally, it is recognized that the incorporation of Hg into the food chain and its assimilation by humans is a potential health hazard. More recently studies in the Amazonian aquatic ecosystems have clearly linked Hg ingestion through fish consumption to public health issues (Peixoto Boischio and Henshel 2000).

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Mercury pollution by Hg cell chlor-alkali plants has extensively been examined. Human populations living near oceans, lakes, or rivers that accommodate industries, and depend on local catch for food, exhibit high levels of Hg exposure (Pirrone and Mahaffey 2005). Mercury levels in fish depend on fish species and the position of the fish in the trophic chain. Fish that occupy higher trophic levels tend to accumulate more Hg; carnivorous fish accumulate more Hg in their tissues. Classic cases of Hg impact on human health come from studies on the Japanese population which consumed fish from Minamata Bay, Japan (Harada 1978) and on toxic effects of Hg on Iraqis who, not knowingly, consumed seeds "dressed" in Hg (Bakir et al. 1973). More recent studies have found neurobehavioral deficits in fish-consuming Brazilian populations (Dolbec et al. 2000) and along the English-Wabigoon River in Canada (Wheatley 1997).

Oriental sole feeds on bottom-living small crustaceans which bioaccumulate Hg readily. Fish can take up Hg from water column and food directly and bioconcentrate large amounts of Hg in their tissues, methylmercury being the dominant forms of fish Hg. Costal populations in the Persian Gulf heavily depend on Oriental sole for food (Kazemi 2003) and can therefore be at risk for Hg poisoning.

Moses Inlet (30°28′11″ North and 49°12′10″ East, to 30°24′6″ North and 48°55′19″ East) covers an area of 1,350 km^2 (Fig. 1). It houses an active port, an area of oil and gas mining, a petrochemical facility, and a chlor-alkali plant which has been in intermittent use for the past 15 years. This plant is equipped with a treatment facility. According to a government report, dating back to 1998, approximately 31,000 kg of Hg was released in 3 months from this facility. The ecologic significance of the Moses Inlet is attributed to its function as a reservoir that provides adjacent coasts with eggs, larvae and organisms, thereby

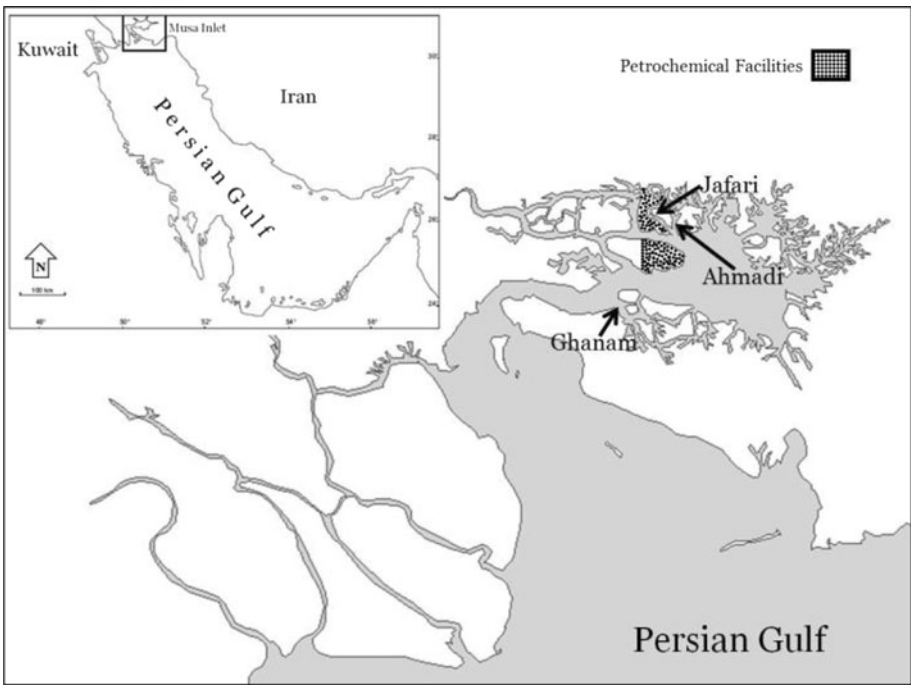

Fig. 1 Khowr-e Musa basin (Moses Inlet) showing sampling sites of Oriental sole; *black shaded areas* indicate petrochemical facilities

maintaining a biological inventory which otherwise would not be so rich.

In Iran, concerns over the public health effects of a chlor-alkali plant located in the Persian Gulf (Khowr-e Musa or Moses Inlet) recently grow to the point where the government called upon public and environmental health agencies to conduct studies, to determine Hg contamination levels in this area; and to investigate its possible adverse health effects. The objective of this study was to measure Hg in Oriental sole, to determine the suitability of this fish for public consumption.

Materials and Methods

We sampled fish in 3 sites within Moses Inlet. Oriental sole ($n = 15$) were collected in August 2007 and in February 2008. Since this fish is protected by law in Iran, we were only able to use a small number of fish. Timing for sample collection was intended to shed light on possible fluctuations in tissue levels of Hg that may occur as a result of changes in environmental factors such as water temperature, nutrients and oxygen levels, and water composition of organic matter. Aquatic methylmercury production varies during different seasons due to changes in such factors and studies have shown that methylmercury production is higher during warmer seasons compared to colder seasons.

Fish were placed in clean polyethylene bags and transported, on ice, to the laboratory where length and weight were recorded and sex was determined. Sex determination was accomplished by microscopic examination of reproductive organ of individual fish. Oriental sole's ovary is granular in appearance while testes appear as white flat structures (Table 1). Dorsal muscle was removed from each fish with a stainless steel knife and liver removed. Liver and muscle tissues were then homogenized. Tissue homogenate was stored in clean-capped glass vials and kept in a freezer (−20°C) until chemical analysis. All glassware were soaked in 10% (v/v) HNO_3 overnight and rinsed with distilled water. Liver or muscle samples (3–5 g) were placed in capped glass flasks using HNO_3/HCl (2:1 v/v) or HNO_3 and potassium permanganate were added. Content of the flasks were allowed to digest for 12 h on a hot plate at 140–150°C until the solution was clear. Flasks were well sealed to prevent sample loss. The digest was then diluted to 25 mL with double distilled water

Mercury levels were determined using a cold vapor atomic absorption spectrometer (model UNICAM 919) with $SnCl_2$ as reducing agent. The spectrometer was equipped with an Hg lamp capable of operation at 253.7 nm. The analyzer had an air circulation pump, a reaction vessel, $SnCl_2$ dispenser, an acidic gas trap and a four-way stopcock with tygon tubes to which a ball valve was attached. Briefly: digests (5 ml sample solutions) were introduced into the reaction vessel using a micropipette (1–5 ml). Mercury vapor was swept into the absorption cell and response was recorded in the form of a sharp peak as described earlier. All samples were analyzed in duplicate and the validity of measurements was checked using blanks and the reference material (CRM: DORM-3). For quality assurance the instrument was calibrated based on a linear six-point calibration curve (0.0, 50, 100, 200, 300 and 400 ppb). Standard calibration curves with an $r^2 = 0.9942$

Table 1 Weight (g), length (cm), sex (*M* Male, *F* Female) of oriental sole from Khowr-e Musa (moses inlet) located in the Persian Gulf

Site	August (2007)						February 2008					
	Weight (g)	Length (cm)	Sex	T-Hg muscle (mg/kg ww)	T-Hg liver (mg/kg ww)	Liver/ muscle Hg ratio	Weight (g)	Length (cm)	Sex	T-Hg muscle (mg/kg ww)	T-Hg liver (mg/kg ww)	Liver/ muscle Hg ratio
Jafari	144	21	M	1.5	3.6	2.3	100	18	M	18	3.4	2.6
	137	23	M	1.6	7.4	4.7	80	17	M	17	3.2	2.5
	154.5	21	M	1.6	4.1	2.6	115	18	M	18	5	3.8
	156	22	F	1.6	5.6	3.6	121	18.40	M	18.40	5.5	4
	135	20	F	1.5	5.5	3.61	124	18	M	18	4.4	3.2
	166	22	F	1.6	4.7	3	134	19.5	M	19.5	5.1	3.7
	123	20	F	1.5	5.5	3.5	83	17	M	17	5.4	4
	158	21	F	1.6	4.2	2.7	107	18	F	18	5.6	4.1
	156	21	F	1.6	4	2.6	100	18	F	18	6.4	4.7
	156	21	M	1.6	4.2	2.6	120	19	F	19	3.5	2.4
	158	22	M	1.6	4.4	2.8	95.5	18	F	18	7.3	5.4
	156	20.3	M	1.3	5.1	3.9	95.5	18	F	18	3.8	2.8
	159	20.5	M	1.3	4.6	3.4	147	21	F	21	3.4	2.2
	160	21.5	M	1.4	6	4.3	88.5	18	F	18	7.9	5.7
	167	23	M	3	5.5	1.9	110	18.4	F	18.4	4.6	3.2
Mean	**152.4**	**21.3**		**1.6**	**5**	**3.2**	**108**	**18.3**		**18.3**	**5**	**3.6**
SEM	**3.2**	**0.24**		**0.1**	**0.24**	**0.2**	**5**			**0.02**	**0.36**	**0.3**
Ahmadi	73.5	19	M	0.53	3.1	5.79	158	21.2	M	0.47	4	9.4
	80	17.5	M	0.5	2.4	5.11	153	21	M	0.35	2.6	7.5
	78.3	17	M	0.5	2.1	4.5	164	21.4	M	0.40	3.4	8.4
	77	17.5	M	0.5	2	4.4	220	23	M	0.48	3	6.2
	73.8	16	M	0.4	2.4	6.5	225	23.5	M	0.49	3	6.1
	191.2	23.5	M	1.2	0.8	0.7	238	24.5	M	0.50	3	6
	208.7	23	F	0.9	0.9	1.04	259	26	F	0.52	2.7	5.2
	165.3	21.5	F	0.86	1.2	1.39	208.5	22	F	0.48	3.6	7.4
	215	24.5	F	1.13	1	0.88	159	21.5	F	0.47	5.7	12
	258	26	F	1.4	0.85	0.62	150	20	F	0.42	5.9	14.1
	875	37	F	2.7	0.5	0.2	158	21.2	F	0.46	5.5	12
	639	34	F	1.1	0.5	0.46	156	21	F	0.46	3.4	7.5
	716	35.5	F	2.03	0.5	0.24	157	21	F	0.5	3.3	7.24
	403	28	F	0.45	0.6	1.33	210	22	F	0.49	3	6.1
	324	27.5	F	0.48	0.8	1.65						
Mean	**292**	**24.5**		**1**	**1.31**	**2.32**	**186.8**	**22.1**		**0.46**	**3.72**	**8.22**
SEM	**69**	**1.8**				**0.6**	**9.8**	**0.43**		**0.01**	**0.30**	**0.7**
Ghanam	88.8	18	M	0.11	2	18.3	243	21	M	0.1	1	7.6
	99.5	18	M	0.11	1.4	12.5	191	23	F	0.1	0.5	4.2
	88.3	17	F	0.11	1.6	14.7	255	21.5	M	0.1	0.6	4.8
	99.6	18	F	0.11	1.5	13.2	183	22	F	0.1	0.5	4.4
	97.5	18	M	0.11	1.3	11.1	220	20	F	0.1	1.5	13.1
	231	24	M	0.12	0.4	3.7	232	20.3	F	0.1	0.8	6.6
	180.5	22	M	0.11	0.6	5.6	250	21	M	0.1	0.78	6
	278	25.5	M	0.13	0.5	4	239	24	F	0.1	0.48	3.3
	186.4	23	M	0.12	0.7	6	294	23	M	0.1	0.5	4
	188.3	24	M	0.12	0.5	4.5	274	26	M	0.1	0.4	2.8
	501.6	32	F	0.16	0.2	1.7	279	25.4	M	0.1	0.4	3.4
	752.2	34	F	0.2	0.2	1.2	311	27	F	0.2	0.4	2.6
	629	33	F	0.2	0.3	1.4	323	27	F	0.2	0.3	2.1
	661.8	33.5	F	0.2	0.2	1.2	239	23	M	0.1	0.5	4
	622.0	32	F	0.2	0.3	1.4	277	25	F	0.1	0.45	3.3

Table 1 continued

Site	August (2007)						February 2008					
	Weight (g)	Length (cm)	Sex	T-Hg muscle (mg/kg ww)	T-Hg liver (mg/kg ww)	Liver/ muscle Hg ratio	Weight (g)	Length (cm)	Sex	T-Hg muscle (mg/kg ww)	T-Hg liver (mg/kg ww)	Liver/ muscle Hg ratio
Mean	**314**	**25**		**0.14**	**0.8**	**7**	**254**	**23.3**		**0.11**	**0.61**	**5**
SEM	**62**	**1.7**		**0.01**	**0.2**	**1.5**	**10.6**	**0.61**		**0.01**	**0.08**	**0.7**

Mercury in liver and muscle as well as liver to muscle rations are presented for August and February

Mean of values and SEM in bold

were run during measurements. To monitor the calibration curve, a continuing calibration verification standard and blank sample was analyzed at intervals and at the end of each analytical batch.

To verify the quality of the analytical results, matrix spike/matrix spike duplicates were analyzed with each analytical batch of about 10 samples. For each run, a triplicate samples and three blanks were carried through the procedure. An analytical recovery was done by adding increasing amounts of mercury to an aliquot of the digested sample. A matrix recovery was also completed for the digestion procedure used by adding a known amount of mercury to the pre-digest sample, which was then digested. To assess the precision of the overall procedure, six replicate analyses of muscle and liver tissues were conducted. All sample analyses were performed in duplicate by the instrument. Precision errors were 4.4%. The limit of detection based on three times the standard deviation ($3S_b$) ($n = 10$) obtained under optimum conditions was 4 ppb.

SPSS statistical software version 17 for Windows was used to analyze data. We used a linear mixed model to analyze our multilevel data (level 1 location within fish and level 2 the fish). The unit of analysis was a fish and two correlated observations were observed on each. Fixed effects in this model were sex, site, time of collection (August or February), fish length, and fish weight. Random effects were location and fish; significance level was put at $p < 0.05$.

Results and Discussion

The overall estimated marginal mean (a term used in SPSS referring to un-weighted means; is important when comparing the means of unequal sample sizes) for T-Hg in our samples was 2.4 ± 0.1 mg/kg wet weight. This value was calculated based on all fish collected from all sites for both collections with length of 22.4 cm, weight of 218 g. There were no differences in fish length across sites and month of collection. Fish length is an important parameter to consider since it is more related to fish age. But, we found no significant correlations between fish length and Hg levels in fish tissue across all three locations in this study. No difference was found in fish Hg between sexes ($p = 0.4$). There was no significant difference between Hg in fish collected in August (2.2 ± 0.1) and February (2.2 ± 0.1). Figure 2 (*top panel*) plots T-Hg in muscle and liver for August and February collections with 95% confidence intervals. The bottom panel indicates that muscle tissue in fish from Ahmadi contained higher Hg levels in August than in February. This trend was reversed in the liver. Fish liver from Ahmadi contained higher Hg in February and lower Hg in the August. Table 1 illustrates liver to muscle Hg ratios.

As a non-essential element, mercury's uptake and elimination is not considered to be well regulated and therefore its tissue concentrations can vary significantly depending on environmental levels of this metal and feeding behavior of the organism. Literature suggests that fish tissues have a large capacity for bioaccumulation of Hg in organic and inorganic forms and the extent of accumulation is species-specific. On the whole, muscle is the major storage site for methylmercury, but other tissues such as liver, which is actively involved in heavy metal metabolism, can also accumulate Hg. Mercury levels in rainbow trout were lower in the muscle than in the liver, but in walleye, carp, and largemouth bass, muscle had the highest Hg concentration. Olson et al. (1978) reported that roughly 95% of Hg in muscle is methylmercury, but a smaller percentage of the T-Hg in liver is methylmercury. Muscle is considered to be a long-term storage site for Hg, but liver receives recently absorbed Hg from the gut via a slow and systemic distribution, and Hg accumulation has been reported for arctic char *(S. alpinus)* (Oliveira-Ribeiro et al. 1999). Recent Hg exposure is reflected in liver and can be a good indicator for environmental exposure to this metal.

Chlor-alkali plants are a major source of aquatic Hg. Mercury in our fish are similar to reports from Cuba, Italy, Portugal, and Argentina; and higher Hg levels have been reported in studies from Colombia and India. High liver/muscle Hg ratio in this study are similar to what has been reported previously where fish were exposed to anthropogenic Hg. Mieiro et al. (2009) reported a high liver/muscle Hg ratio and a reversion of this value to <1. These

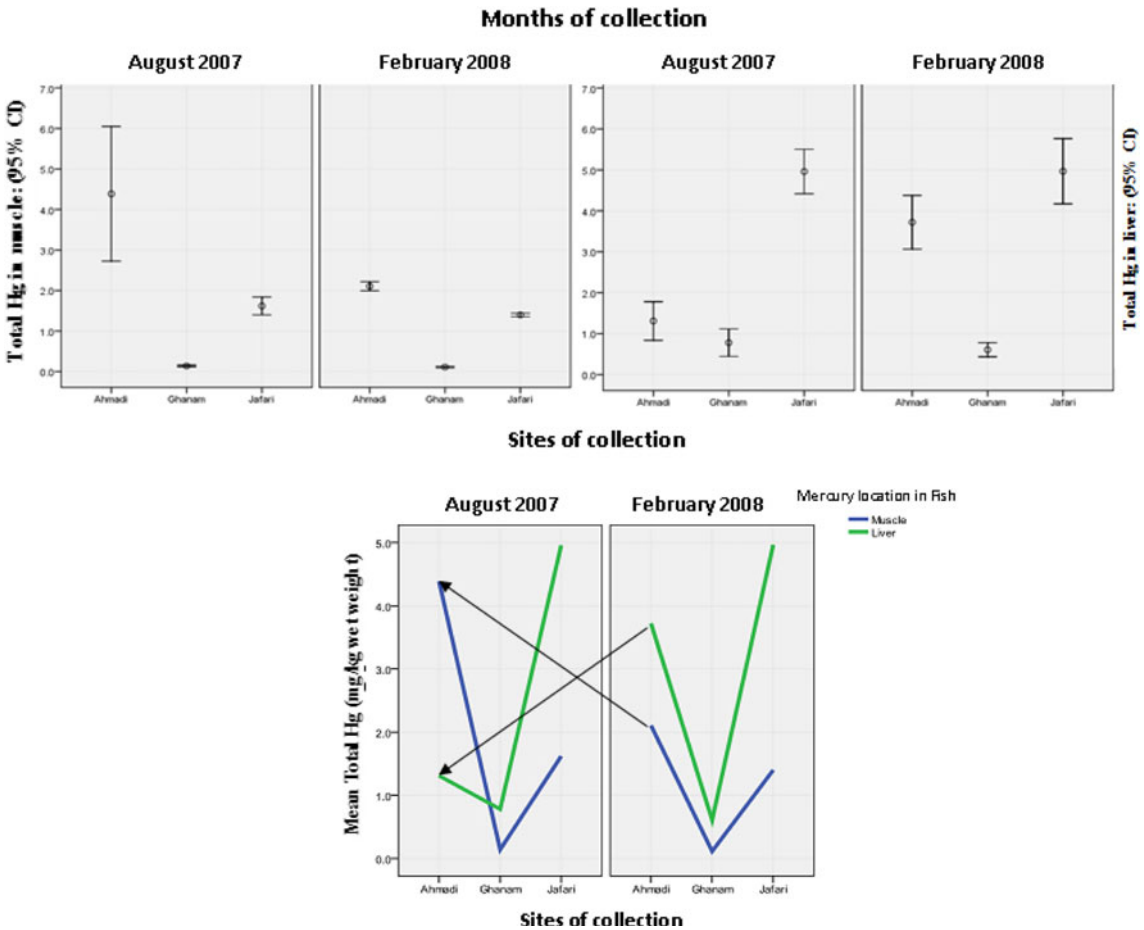

Fig. 2 Mercury in liver and muscle of Oriental sole (mg/kg wet weight) caught in August 2007 and February 2008 near a chlor-alkali plant in the Persian Gulf. *Arrows* point out a change in Hg storage site in fish from Ahmadi

investigators argue that liver has a buffering role in Hg accumulation. After liver capacity for withholding Hg runs out, Hg is able to divert to muscle, at which time its concentration in the muscle starts increasing. Henny et al. (2002) theorizes that as exposure to methylmercury increases, so does the percentage of hepatic inorganic Hg, which indicates greater hepatic demethylation. As a result of metallothioneins' binding and immobilization of inorganic Hg, liver concentrations of Hg relative to muscle may increase. Mercury speciation analysis of our samples could illuminate this issue further.

The World Health Organization has set 5 μg g^{-1} as the tolerable daily intake limit for fish consumption, while the European Union has sets the safety level for Hg in fish at 0.5 μg g^{-1} of fresh weight. Yet other investigators argue that even at levels currently considered "allowable", Hg poisoning can occur in children and young adults who consume polluted fish regularly (Nriagu et al. 1992). We conclude that consumption of fish from Khowr-e Musa (Moses Inlet) can be harmful to the public and measures should be taken by the government to clean up the area and provide the public with clean sources of fish for consumption.

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