

Concentration of Mercury in Selected Tissues of the Caspian Lamprey (*Caspiomyzon wagneri*) Migrants in Spawning Season

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Received: 12.3.2011

Accept: 16.4.2011

ABSTRACT

Background: Mercury (Hg) is considered a global pollutant because Hg⁰ which is the predominant form of atmospheric Hg resides in the atmosphere for as long as 0.5 to 2 years. Mercury has many negative effects on the reproductive, respiratory, and immune systems.

Methods: In this study, 24 Caspian lampreys (*Caspiomyzon wagneri*) were transported to the university laboratory and then stored in -20 °C until they were dissected. The liver, muscle, skin, ovaries, and testes were all dissected out. All samples were freeze-dried and ground by a mortar and pestle into powder. The specimens were analyzed by a Leco AMA254 mercury analyzer.

Results: The order of mercury concentration in the lamprey tissues was as follows: Muscle > ovaries > liver > skin > testes. The mean values of mercury in muscle and testes were 192.25 ± 7.10 and 21.42 ± 1.48 Hg ng/g dry weight, respectively. There were no significant differences (N = 24) between the sexes in the Hg level of most tissues except for gonads.

Discussion: A comparison with some ammocoetes of jawless fishes shows a 10 times less concentration than other records. This difference probably is due to non-parasitic behavior and use of various sources of nutrition in other species.

Conclusion: In comparison of other kind of sea lamprey, due to detritivore habits of Caspian lamprey on a very specific part of food web (non-live food only in the sea floor), the supposed species can introduce as a special indicator of mercury and heavy metal levels in the aquatic ecosystem.

Keywords: Biomonitor, Caspian Lamprey, Mercury level, Shirud River, Iran.

INTRODUCTION

Heavy metals are naturally occurring trace elements of the aquatic environment, but their background levels in the environment have increased, especially in areas where industrial, agricultural, and mining activities are widespread. As a result, aquatic animals are exposed to elevated levels of heavy metals (1). Some are necessary in trace amounts for normal growth, like zinc, copper, and cobalt; others, however, such as mercury, cadmium, and lead have no biological significance (2). In the environment, mercury (Hg) comes both from anthropogenic activities and natural

cycling in the biosphere. This includes Hg segregation from rocks and minerals, evaporation from water, and atmospheric transport and deposition back to land and water, with oceans playing an important function in this global cycling (3). When mercury enters the environment, it circulates in the atmosphere and finally deposits in lakes, seas, and oceans (4). Metals that are deposited in the aquatic environment may accumulate in the food chain and cause ecological damage, posing a threat to human health due to biomagnification over time as well (5). Mercury (Hg) is a highly toxic non-essential

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metal of major concern due to its harmful environmental effects, as elevated exposure in humans and wildlife has become an extensive phenomenon. This derives from the particular biogeochemical properties of mercury and its behavior in aquatic ecosystems (6). Today, the most Hg pollution exists in aquatic environments where Hg is converted to methyl mercury (MeHg) by aquatic biota. Because of the high affinity of MeHg to sulfhydryl-groups of proteins, this heavy metal is rapidly incorporated into the food chain, bioaccumulating in aquatic organisms, and biomagnifying from one trophic level to the next (7).

In their distribution, lampreys (Petromyzontiformes) are of significant ecological, cultural, and economic importance (8). *Caspiomyzon wagneri* is endemic to the Caspian Sea and rivers in its northern, western, and southern watersheds (9), and it migrates to the Volga, Ural, Terek, and Kura rivers (9, 10). The Caspian lamprey in the southern Caspian basin (Iran) migrates to such rivers as Shirud, Talar, Babolrud, Gorganrud, Tajan, Haraz, Sardabrud, Aras, Tonekabon, Polrud, Sefidrud, and Anzali Lagoon (11, 8). For *Caspiomyzon wagneri*, the migrations used to be in the order of 1500 km; now they are less than 450 km (12). Over half of all the lamprey species are considered to be endangered, vulnerable, or extinct in at least a portion of their range (12). The Caspian lamprey is vulnerable generally in Europe (12), sharply declining in Russia (14, 12), extirpated from the Sefid River, and rare in Anzali Lagoon and tributaries, Iran. The *Caspiomyzon wagneri* is unique among lampreys, as indirect evidence suggests that it feeds exclusively as a scavenger in the adult stage (12). Based on the Renaud et al's study (13), lampreys are suitable biomonitors for environmental mercury pollution. The main reasons for conducting this study were:

- 1) The target fauna is a near threatened (NT) species that has already been included in IUCN red list category, little information is available on this.
- 2) This is an endemic species of the Caspian Sea fauna and the only living

members of the primitive group, jawless fish class in this region.

3) Determining the mercury concentration in different organs of the specimens of lamprey studied and the comparison of the results with international human consumption advisory limits because this fish is consumed by humans in some countries.

MATERIALS AND METHODS

Study area

The study was carried out in the Shirud River (34° 44'–36° 51' N, 50° 48'–50° 49' E; Figure 1). The length of this river is about 36 km, the width in the estuary is 50–80 m, and the depth is 1.5–2.5 m. The upper substrate is composed of pebbles mixed with gravel and sand and beneath it is mostly sand and mud. The river has a high water flow as well as clearness.

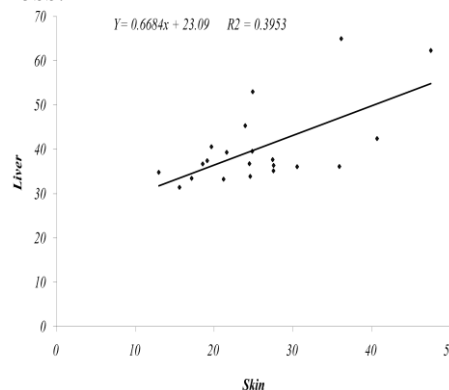


Figure 1. Correlation of mercury concentration (ng/g) between skin and liver of Caspian lamprey

Collection of samples

We bought 24 specimens of Caspian lamprey (*Caspiomyzon wagneri*) from local fishermen from March to April, 2010. The fish were first transported to the university laboratory for biometric measurements (Table 1). The fish were then stored in polyethylene nylons, and kept in -20°C until they were dissected and their sex was determined (16 males and 8 females).

Table 1. Descriptive statistics of mean for body length, body weight for Caspian lamprey

Variable	Mean	SE	Min	Max
Body length (cm)	36.36	0.66	30	43.50
Body weight (g)	94.38	5.12	47.50	130.49

Preparation and analysis of the samples

The liver, muscle, skin, ovaries, and testes were all dissected out. All samples were freeze-dried, then ground by a mortar and pestle into powder (15). The moisture contents of the tissues were determined according to weight loss before and after freeze-drying (16).

Mercury was measured using a LECO AMA 254 (advanced mercury analyzer, USA) in accordance with ASTM standard No. D-6722. The mercury content was measured without mineralization using the single-purpose atomic absorption spectrometer AMA 254. In a stream of oxygen, on a catalytic column, 50 to 100 mg of each sample was placed into the boat of the AMA 254 analyzer, dried, combusted, and decomposed. The main advantage of using the thermal decomposition technique for mercury determination is that it does not require complex manipulation of the sample.

In order to assess the analytical capacity of our method, the accuracy of the total Hg analysis was checked by running the three samples of the Standard Reference Materials (SRM), namely, the National Institute of Standards and Technology, SRM 1633b, SRM 2709, and SRM 2711, in seven replicates (17, 18). The recovery was between 93.5% and 106%. The detection limit of the instrument used was 1 ng/g of dry weight.

Statistical analysis

The statistical analysis was performed using Excel (version 2003) and SPSS software (version 15.0). Data were tested for goodness of fit to a normal distribution using Kolmogorov–Smirnov test. The data were not normally distributed.

Mann-Whitney test was used for detecting significant differences in the mercury concentration among the four tissues and between different tissues of male and

female as well. For correlating the length and weight of the specimens with the mercury concentrations in different tissues and determining the correlation between mercury levels in the different tissues, Spearman test was used.

RESULTS

Mercury concentration in the tested tissues of lamprey decreased in the following order: muscle > ovaries > liver > skin > testes (Table 2). The average concentrations of mercury found in muscle, ovaries, liver, skin, and testes were 192.25, 47.77, 47.67, 28.17, and 21.42 ng/g, respectively. The difference between the tissues was significant ($P < 0.05$) except for the difference between liver and ovaries, and skin and testes. The difference in Hg concentration between sexes was significant ($P = 0.000$). The Mann-Whitney test results showed that the difference in the mercury concentration between the sexes in the skin was significant ($P = 0.038$), but no significant differences were seen between muscle ($P = 0.528$) and liver ($P = 0.528$). The correlation between the mercury concentrations for the biometric variables (body length and weight) was measured; no significant difference was observed between all the tissues and these two variables, except a weak positive correlation between the length and liver ($P = 0.096$, $r = 0.36$).

On the other hand, when the correlations between all the tissues were analyzed, a weak correlation was seen between skin and liver ($P = 0.165$, $r = 0.29$).

Table 2. Mercury concentration (ng/g dry weight) in tissues of lamprey from Shirud River in Iran

Species		Muscle	ovaries	Liver	Skin	Testes
Lamprey	Mean±SE	192.2±7.10	47.77±11.68	47.67±5.47	28.17±3.34	21.42±1.48
	Range	79.92-259.08	23.88-128.06	31.42-145.96	12.96-94.64	12.18-36.58

DISCUSSION

Saei-Dehkordi (19) reported that the mercury concentrations ($\mu\text{g/g}$) in species from the Persian Gulf range between 0.120 ± 0.042 and 0.527 ± 0.190 . The highest concentration was detected in *Thunus thunggol*. The order of mercury concentrations in the rest of the species was *Psettodes erumei* > *Epinephelus coioides* > *Acanthopagrus latus* > *Scomberomorus commerson* > *Pomadasys argenteus* > *Caranx sem* > *Rachycentron conadum* > *Sphyræna jello* > *Platycephalus indicus* > *Parastromateus niger* > *Nemipterus japonicus* > *Chirocentrus dorab* > *Pampus argenteus* > *Trichiurus lepturus*.

The mercury concentrations in muscles of haemulidae (*Pomadasys* sp.), platycephalidae (*Platycephalus* sp.), serranidae (*Epinephelus tauvina*), and *Pampus argenteus* were 0.05- 0.11, 0.14–0.68, 0.1–0.26, and 0.02–0.11 $\mu\text{g/g}$, respectively as reported for these four Persian Gulf fish species that were sampled in January 2004 (19, 20). Simonin et al (21) measured mercury levels in four fish species collected from New York State lakes during 2003-2005. They focused on fish species known to accumulate higher levels of mercury, due primarily to the fact that these species are piscivorous and that they may live longer than other fish.

Burger and Gochfeld (22) examined total mercury levels in liver and muscle of Pacific cod (*Gadus macrocephalus*), collected from the northern Pacific and Bering Sea waters in the Aleutian Chain around Nikolski, Amchitka, and Kiska Islands (Alaska). The mercury levels ranged from 0.008 to 0.86 ppm in muscle but up to 1.25 ppm in the liver. Although the average mercury levels were higher in the muscle than liver, the differences were relatively small. The mercury levels in muscle were more strongly correlated with age

than weight, although the differences were not great. Alonso (23) reported significant differences in Hg content between *Mugil incilis* (detritivorous) and *Eugerres plumieri* (omnivorous), observed in both Cartagena Bay ($P < 0.001$) and the Cienaga Grande de Santa Marta ($P = 0.008$), namely, in Cartagena Bay the median values were 7.3 times higher in *E. plumieri* than in *M. incilis*. The great differences may reflect the role of trophic transfer of Hg through the food chain, where the predatory fish species contained higher Hg levels than the non-predatory fish.

In this study, only a weak correlation was seen between liver and body length, but no significant correlation was observed between muscle and body length. The absence of correlation between muscle Hg and length has been reported for *Mugil incilis* (23) and silvertip sharks (24).

This lack of correlation which was observed in our study may have resulted from a limited range in the body length. In contrast, other studies have shown only statistical relationships between these two parameters (25, 26, 27).

In the present study, a significant difference in mercury concentration was seen between the gonads of different sexes. A further investigation of sea lamprey (*Petromyzon marinus*) and ammocoetes in relation to the four different streams reflected the level of contamination in their nursery streams (28). We suggest that a similar situation also occurs for the Caspian lampreys when the females arrive in the egg development stage and are exposed to a contaminated river. These differences could be due to a combination of several factors:

- 1) The nature of more ovarian than testicular fat because methyl-mercury is lipophilic (29; 30; 31). On the average, more than 70 percent

of the total mercury in animal tissue is organic mercury (32; 33; 34, 35). This ratio reaches 90 to 100 percent, especially in aquatic fish (36). Therefore, mercury is accumulated in eggs during gonad development.

2) Between the development and evolution of the ovaries and testes (stages 1, 2, and... 5) there exists a time difference. The ovarian evolution is slower than that of the male sexual gonad. So we can suppose that the opportunity for exposure to pollution of female sexual gonads is more than that of male gonads, and this is a factor that has a direct impact on the significant differences. As a result, ovaries will be better indicators than testes (8).

3) Criterion of biological element accumulation in the organism's body is age, but there is very little information about age of lampreys that migrate to rivers.

Mirlean (37) evaluated mercury levels at three lakes in southern Brazil and assessed the relationships between mercury in fish tissues at sites close to (industrial and suburban areas) and distant from (protected conservation area) sources of mercury emissions. In each lake, the planktivore *Geophagus brasiliensis* had the lowest mean mercury concentration. The piscivorous *Oligosarcus jenynsii* had the highest mercury concentrations in the suburban and natural lakes, but this did not occur in the industrial-area lake. The highest concentrations measured (> 400 ng/g) in the

study were for *O. jenynsii* from the suburban lake.

This is predictable because muscle is fatter than the other organs, so it is there that the maximum accumulation of mercury is seen.

In this study, the mercury concentration in muscle was higher than in liver, corresponding to other similar studies conducted on *Oncorhynchus Keta* (38), *Galeocerdo cuvier* (25), *Abramis brama L.* (39).

The average concentration of mercury in the lamprey muscle was similar to that in *Plagioscion auratus* and *P. surinamensis* (40) (Table 3).

The mercury concentration in the muscle of the Caspian lamprey was compared with the lamprey ammocoetes occurring in different locations (Table 4). The mercury levels in lamprey ammocoetes may be more than in mature ones and decontamination occurs in this species. It is recommended to have the amount of mercury in lamprey ammocoetes measured. Also, differences in mercury levels between Caspian lamprey and other species may occur due to nonparasitic and detritivorous food habits.

Table 3. Mercury concentrations detected in lamprey (*Caspiomyzon wagneri*) from Shirud River, Iran, in comparison to mercury concentrations reported in fish in others studies

Class	Species	Tissue	Mean	SD or SE	Location	References
Jawless fish	<i>Caspiomyzon wagneri</i>	Muscle	0.19 ^b	7.10 ^c	Shirud River, Iran	This study
	<i>Caspiomyzon wagneri</i>	Ovaries	0.04 ^b	11.68 ^c	Shirud River, Iran	This study
	<i>Caspiomyzon wagneri</i>	Liver	0.04 ^b	5.47 ^c	Shirud River, Iran	This study
	<i>Caspiomyzon wagneri</i>	Skin	0.02 ^b	3.34 ^c	Shirud River, Iran	This study
	<i>Caspiomyzon wagneri</i>	Testes	0.02 ^b	1.48 ^c	Shirud River, Iran	This study
	<i>Petromyzon marinus</i>	Muscle	1.35 ^a	0.19 ^c	Lake Ontario, North America	41
	<i>Myxine formosana</i>	Muscle	12.29 ^b	2.88 ^d	southwestern Taiwanese waters	42
	<i>Myxine formosana</i>	Liver	10.61 ^b	5.22 ^d	southwestern Taiwanese waters	42
	<i>Paramyxine nelson</i>	Muscle	7.78 ^b	1.29 ^d	southwestern Taiwanese waters	42
	<i>Paramyxine nelson</i>	Liver	3.29 ^b	1.98 ^d	southwestern Taiwanese waters	42
chondrichthyes	<i>larhinus albimargius</i>	Muscle	1.80 ^a	0.45 ^d	Japan	25
	<i>Carcharhinus albimarginatus</i>	Liver	0.70 ^a	0.42 ^d	Japan	25
Osteichthys	<i>Acipenser persicus</i>	Muscle	0.33 ^b	—	Caspian Sea	43
	<i>Huso huso</i>	Muscle	1.4 ^b	—	Caspian Sea	43

Acipenser gueldenstaedtii	Muscle	0.32 ^b	–	Caspian Sea	43
Acipenser stellatus	Muscle	0.06 ^b	–	Caspian Sea	43
Acipenser nudiiventris	Muscle	0.67 ^b	–	Caspian Sea	43
Anguilla Anguilla	Muscle	0.11 ^a	98.89 ^d	Flanders, Belgium	44
Thunus tonggol	Muscle	0.53 ^a	0.190 ^d	Persian Gulf, Iran	19
Pomadasys argenteus	Muscle	0.23 ^a	0.059 ^d	Persian Gulf, Iran	19
Acanthopagrus latus	Muscle	0.40 ^a	0.128 ^d	Persian Gulf, Iran	19
Nemipterus japonicas	Muscle	0.17 ^a	0.078 ^d	Persian Gulf, Iran	19
Epinephelus coioides	Muscle	0.40 ^a	0.129 ^d	Persian Gulf, Iran	19
Oncorhynchus Keta	Muscle	0.08 ^a	0.011 ^d	Alaska	38
Abramis brama L.	Muscle	0.08 ^b	0.03 ^d	Lake Balaton	39
Abramis brama L.	Liver	0.08 ^b	0.03 ^d	Lake Balaton	39
Sander vitreus	Muscle	0.66 ^a	–	New York	21
Micropterus dolomieu	Muscle	0.62 ^a	–	New York	21
Gadus macrocephalus	Muscle	0.17 ^a	0.01 ^c	Aleutians	22
Gadus macrocephalus	Liver	0.11 ^a	0.01 ^c	Aleutians	22
Esox lucius	Muscle	1.51 ^a	0.296 ^d	Alaska	45
Thymallus arcticus	Muscle	0.15 ^a	0.032 ^d	Innoko National Wildlife Refuge, Alaska	46
Clarias mossambicus	Muscle	0.04 ^a	–	Tanzania	47
Staghorn sculpin	muscle	0.05 ^a	9 ^d	San Francisco Bay	48
Engraulis mordax	Muscle	0.04 ^a	21 ^d	San Francisco Bay	48
Parasilurus asotus	Muscle	0.11 ^a	0.109 ^d	Guizhou Province, China	49
Dorosoma cepedianum	Muscle	0.027 ^a	–	Caddo Lake, Texas, USA	50

^a μg/g ww ^b μg/gdw^c SE ^d SD**Table 4.** Mercury concentration (ng/g dry weight) in lamprey ammocoetes muscle from other locations for comparison with the present study

Species	N	Range	Locality	References
Caspiomyzon wagneri	24	80-260	Shirud River	This study
Ichthyomyzon fossor	6	3189-5489	Chateauguay River	13
Lampetra appendix	7	1748-6793	Chateauguay River	13
Lampetra appendix	3	1055-1862	Sainte-Anne River (west bank)	13
Petromyzon marinus	4	1000-2085	Sainte-Anne River (west bank)	13
Petromyzon marinus	3	854-1981	Sainte-Anne River (east bank)	13
Petromyzon marinus	3	3705-4834	Saint-Maurice River	13

ACKNOWLEDGEMENT

This work was funded by Tarbiat Modares University of Iran. The authors wish Ellen Vuosalo Tavakoli for the final English editing and Claude Renaud, staff of canadian museum of nature for scientific comments.

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