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Mercury concentration in *Rutilus rutilus* from the Caspian Sea and assessment of health risks

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Abstract

In order to determine the mercury content in muscle of roach (*Rutilus rutilus*) and its potential risks when consumed, samples of the fish were caught in two important fishing areas, Bandar Anzali and Bandar Torkaman. The mean concentration of mercury was 438 and 61 µg Kg^{-1} dw for Bandar Anzali and Bandar Torkaman, respectively. The maximum allowable fish consumption rates were respectively 0.57 Kg per week and 10.86 meals per month for Bandar Anzali and 2.74 Kg per week and 52.47 meals per month for Bandar Torkaman. Hazard quotient (HQ) values for fish samples in both regions were less than one. Correlation results showed no significant relationship between morphological parameters and mercury concentrations in muscle tissue in either region, although there was a significant negative correlation when the samples from both regions were pooled together. The concentrations of mercury in fish in both Bandar Anzali and Bandar Torkaman areas were lower than world standards. Overall, fortunately, the mercury levels in the fish were unlikely to have adverse effects on the health of fish or consumers, although continuous monitoring of contaminant levels is necessary due to the chemical properties of mercury and the frequent introduction of other contaminants into the waters in these areas.

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Introduction

Mercury is a natural element in the environment, but human population growth and, consequently, increased needs, together with industrial development and economic growth have led to an increase in the release of this element into the environment. The release of mercury in the environment increased after the onset of the industrial revolution, and the expansion of human activities has increased the concentration of this element in the atmosphere, soil, water and sediments (Beckers and Rinklebe, 2017; Horowitz et al., 2014).

Mercury is used in various industries and is needed in products such as thermometers, batteries, paints, pesticides, as well as in gold mining and purification, chlorine production, and smelting of lead. About 80 percent of the mercury used in the cited industries is subsequently released into the environment and is capable of being transported over long distances, both in water and atmosphere, due to its persistence and stability (Mondal et al., 2018; Roe, 2003). Mercury that enters seas and oceans, either through mercury-contaminated rivers or by direct industrial and municipal wastewater, eventually converts to methylmercury (MeHg), mercuric mercury (Hg (II)) and cinnabar (HgS). Unfortunately, MeHg becomes a potentially dangerous threat to aquatic and human health once it enters the food chain (Council, 2000).

Human beings are exposed to the dangers of mercury, mainly through the consumption of contaminated fish or when infants are breast-fed. Carnivorous fish accumulate mercury in their tissues and thus old fish with longer exposure time, carry higher concentrations of methylmercury. Smaller fish absorb metals through the skin at a higher rate, due to their high surface to volume ratio (Olivero et al., 2002; Rajamohan et al., 2014). Therefore, various organizations, according to their goals and authority, have been trying to reduce the ways in which mercury enters the environment in order to prevent its adverse effects on societies and organisms. For example, the United States Environmental Protection Agency (EPA) considers the environmental and destructive effects of mercury; the Food and Drug Administration (FDA), considers the safety of foods consumed by humans and possible impact on health and The World Health Organization (WHO) has taken steps to promote the health and well-being of more people in order to improve health worldwide (Malvandi and Alahabadi, 2019).

Environmental mercury has caused irreparable damage to ecosystems and living organisms including humans. Among such problems are the consequences of the release of mercury through industrial wastewater into the water at Minamata, Japan, and in Iraq due to the consumption of bread produced from mercury-contaminated wheat (Saturday, 2018). The presence of mercury in the human body causes severe and irreversible damage to the central nervous system, kidney tissue, and impairs physical and mental development, and brings about balance and genetic disorders. Methylmercury can also impair the function of mitochondria by increasing the production of hydrogen peroxide (Malvandi et al., 2010; Roe, 2003; Stow et al., 2011).

Due to their minerals, vitamins, proteins, and omega-3 fatty acids contents, fish play important role in human nutrition, especially for inhabitants living around aquatic areas. Fish is also recommended for those susceptible to cardiovascular disease, pregnant women, nursing mothers and women of puberty. Omega 3 in the body reduces blood pressure, lowers blood cholesterol, heart disease, stroke, and death following these conditions. Omega 3 also reduces depression and improves the mental health of individuals (Malvandi et al., 2014; Torpy et al., 2006).

One species of fish inhabiting the Caspian Sea is the roach (*Rutilus rutilus*) from Cyprinidae family. The roach is one of the species that is abundantly caught in the southern part of the Caspian Sea every year. This fish is also important in the nutrition and rearing of other fish such as zander, Northern pike, beluga and sturgeon (Mohamadzade Baran et al., 2012). However, factors such as contamination of rivers and wetlands in the spawning areas, decrease in the volume of incoming waters due to dam construction, and overfishing have made *Rutilus rutilus* one of the species in need of protection (Imanpoor et al., 2014).

In this research, the roach species was selected for reasons such as its position at the lower levels of the aquatic food chain, its importance in the diet of strategic sturgeon fish such as beluga (*Huso huso*) and its frequent presence in the human food basket. In this study, mercury concentration in the muscle of roach fish from Bandar Anzali and Bandar Torkaman was investigated. In addition, the potential health risks of mercury for the fish species and for human consumers were estimated by calculating the daily, weekly, and monthly allowable limits. The health risk assessment was also performed by calculating the risk index (HQ) and comparing the mercury levels in the fish with the international standards.

Material and methods

Study area

Roach in the southern basin of the Caspian Sea are mostly caught in two regions, Bandar Anzali and Bandar Torkaman. Therefore, this species was collected from these two regions for the present study. The Anzali international wetland has ecological, economic and social values as a habitat for migratory and non-migratory aquatic species that contribute to human nutrition. Bandar Torkaman, located on the southeast coast of the Caspian Sea, is Iran's largest northern port and the main link between Gorgan gulf and the Caspian Sea, which is important for fisheries and tourism. Today, the health of the unique Caspian ecosystem, surrounding wetlands, aquatic organisms and humans is endangered due to the presence of polluting industries and other sources in the Caspian catchment. Many of these pollutants are derived from discharge of agricultural pesticides and from industrial and municipal wastewater into the lake. Overfishing in these aquatic bodies has reduced their fish populations without giving them time to recover (Baramaki Yazdi et al., 2015; Khodadoust et al., 2015; Mazaheri Kouhanestani et al., 2019), further exacerbating the downgrading condition.

Preparing samples and analyzing data

Twenty specimens of this fish were caught by local fishermen in winter from the two study regions and stored at -24 °C until preparation. Muscle tissue was first separated from each specimen and after weighing, it was completely dried using an oven at 105 °C for 48 h and then powdered. Then, one g of each sample was digested with nitric acid and perchloric acid in a ratio of 3: 1 and subsequently diluted with 10 ml of deionized water. Concentration of mercury were measured by cold vapor atomic absorption spectrometry. The accuracy of the analytical procedures was evaluated using SRM 1633b, SRM 2709 and SRM 2711. The values of recovery and relative standard deviation were in the range of 89–106% and 3% and 6%, respectively. Normality of the data was confirmed by Kolmogorov-Smirnov test. Hence, independent samples t- tests were used to compare the concentration in the samples of the two areas and Pearson's test was applied to analyze the correlation between concentrations of mercury and morphological parameters. The following formula was used to convert dry-weight to wet-weight concentration in each sample (Malvandi et al., 2014). It should be noted that the percent moisture for each sample was obtained separately during preparation of samples.

$$\text{wet weight concentration} = \text{dry weight concentration}\left[1 - \left(\frac{\text{precent moisture}}{100}\right)\right]$$

Assessment of mercury-related health risks for humans

Estimated daily intake (EDI) and estimated weekly intake (EWI) of mercury.

Estimation of daily and weekly intake rates for an adult person were based on the daily and weekly rates of fish consumption and the concentration of the studied element in fish muscle tissue according to the following formula.

$$EWI \text{ or } EDI = \frac{(C \times IR)}{Bw}$$

where EDI is the daily intake rate of the element in the body (µg/kg/day), EWI indicates weekly elemental intake rate in the body (µg/kg/week), C indicates the average concentration of the target element in the tissue (µg g^{-1} in terms of wet weight), IR equals the daily or weekly ingestion of fish (g day^{-1} or g $week^{-1}$), and Bw is the body weight (70 kg for adult human) (Okati and Esmaili Sari, 2018).

Determination of hazard quotient

The hazard quotient (HQ) is the ratio between the exposure time and the reference dose. If this ratio is less than one, there is no threat to the health of the target population, but if the value is one or more than one, the health of the consumer population is considered to be at risk. This index is without a unit and is calculated using the following equation.

$$HQ = \left(\frac{EF \times ED \times FIR \times C}{RFD \times WAB \times TA}\right) \times 10^{-3}$$

where EF is the frequency of exposure (365 days /year); ED indicates the exposure duration (70 years); FIR is the food ingestion rate (36 g /person /day by FAO, 38 g/person/day and 20.5 g/person/ day by the Iranian Fisheries Organization for the coastal provinces of northern Iran and the whole of Iran, respectively); C represents the average concentration of mercury in muscle tissue of fish (µg g^{-1}); RFD represents the oral reference dose defined by USEPA (0.1 µg Hg kg $bw^{-1}d^{-1}$) or the acceptable daily intake of pollutant metal by WHO (0.23 µg Hg kg bw^{-1} d^{-1}); WAB is equal to the mean body weight (70 Kg) and TA is the average exposure time for non-carcinogens (365 days /year ×ED); not taking into account the known methylmercury carcinogenic effect (Mahmoud and Abdel-mohseni, 2015; Malvandi and Alahabadi, 2019).

Determination of allowable fish consumption rate

The following relationship was used to calculate maximum allowable daily consumption rate (CR_{lim}) of fish meat.

$$CR_{lim} = (RfD \times Bw)/MC$$

where, RfD expresses the oral reference dose (mg /kg /day); Bw is equal to the body weight of the consumer (70 kg for adult human) and MC is the concentration of the element in the fish tissue (mg/ kg wet weight). Calculation of maximum allowable fish consumption per month was also conducted using the following equation.

$$CR_{mm} = CR_{lim} \times T/MS$$

where, CR_{lim} represents the maximum allowable fish consumption rate (kg/day); T is the number of days per month (30.44 days); and MS is the amount of fish consumed at each meal (0.227 kg) (Okati and Esmaili Sari, 2018).

Results and Discussion

The mercury concentrations in the muscle tissue of roach fish from the Bandar Anzali and Bandar Torkaman, and some morphological parameters are reported in Table 1.

The mean concentration of mercury in the roach muscle tissue from the Bandar Anzali was 439 µg kg^{-1} dw and from the Bandar Torkaman was 61 µg kg^{-1} dw, which were significantly different ($p<0.05$). High levels of mercury in the edible tissue of fish from the Bandar Anzali could indicate a higher level of mercury contamination of this aquatic environment compared to those from the Bandar Torkaman and could also indicate a high volume of municipal effluents and industrial wastewater inflows into this harbor and the Anzali wetland. Comparison of mercury levels in roach fish in the present study with those reported for other species caught from the Caspian Sea and other water bodies associated with the Caspian Sea is presented in Table 2.

Table 1. The morphological characteristics and mercury concentration in the muscle tissue of roach fish trapped at the Bandar Anzali and Bandar Torkaman

Parameter	Region					
	Bandar Torkaman			Bandar Anzali		
	Max	Min	Average	Max	Min	Average
Total length (Cm)	19.8	17.5	18.93	17.7	16.0	16.72
Standard length (Cm)	17.5	15.6	16.82	15.5	13.80	14.70
Fork length (Cm)	16.4	14.8	15.65	14.4	12.50	13.40
Total weight (g)	85.0	70.0	78.2	53.0	37.0	45.20
Hg (µg kg^{-1} dw)	115.0	28.0	61.4	617.0	231.0	438.70

Table 2. Concentration of Hg in the muscle tissue of the fish caught from the Caspian Sea and other associated aquatic bodies

Species	Value	Study area	References
Rutilus rutilus	439 [a] (86) [c]	Bandar Anzali	This study
Rutilus rutilus	61 [a] (18) [c]	Bandar Torkaman	This study
Rutilus rutilus	180 [b]	Anzali wetland	(Zolfaghari, 2018)
Alosa caspia caspia	2 [b]	Anzali wetland	(Zolfaghari, 2018)
Hemiculter leucisculus	3 [b]	Anzali wetland	(Zolfaghari, 2018)
Esox lucius	136 [a]	Anzali wetland	(Zamani-Ahmadmahmoodi, Bakhtiari, & Rodriguez Martin, 2014)
Cyprinus carpio	990 [a]	Anzali wetland	(Tabatabaie et al., 2011)
Sander lucioperca	300 [a]	Anzali wetland	(Tabatabaie et al., 2011)
Cyprinus carpio	179 [b]	Anzali wetland	(Astani et al., 2016)
Rutilus frisii	200 [b]	Anzali wetland	(Astani et al., 2016)
Carassius auratus	115 [b]	Anzali wetland	(Astani et al., 2016)
Esox lucius	366 [b]	Anzali wetland	(Astani et al., 2016)
Liza aurata	180 [b]	Anzali wetland	(Norouzi et al., 2016)
Rutilus frisii kutum	123 [b]	Anzali wetland	(Nejatkhah Manavi and Mazumder, 2018)
Liza aurata	90 [b]	Anzali wetland	(Nejatkhah Manavi and Mazumder, 2018)
Rutilus frisii kutum	5 [b]	Anzali wetland	(Mousavi Moghadam et al., 2018)
Esox lucius	322 [b]	Anzali wetland	(Molazadeh and Nozari, 2014)
Acipenser stellatus	2180 [a]	Caspian shores (Gilan province)	(Heidary et al., 2012)
Acipenser stellatus	1780 [a]	Caspian shores (Golestan province)	(Heidary et al., 2012)
Acipenser stellatus	150 [b]	Southeast of the Caspian Sea	(Hedayatifard et al., 2017)
Rutilus frisii kutum	4.7 [b]	Bandar Torkaman	(Mousavi Moghadam et al., 2018)
Liza aurata	150 [b]	Bandar Torkaman	(Norouzi et al., 2016)
Liza aurata	75 [b]	Southeast of the Caspian Sea	(Nejatkhah Manavi and Mazumder, 2018)
Rutilus frisii kutum	129 [b]	Southeast of the Caspian Sea	(Nejatkhah Manavi and Mazumder, 2018)
Cyprinus carpio	1000 [a]	Gomishan Wetland	(Tabatabaie et al., 2011)
Sander lucioperca	690 [a]	Gomishan Wetland	(Tabatabaie et al., 2011)
Liza aurata	432 [a]	South coast of the Caspian Sea	(Tabatabaie et al., 2011)

[a] $\mu g\ kg^{-1}$ dw
[b] $\mu g\ kg^{-1}$
[c] $\mu g\ kg^{-1}$ ww

In another study carried out in the Anzali wetland (Zolfaghari, 2018), lower levels of mercury were reported in the roach than those obtained in the present study (Table 2). The results revealed that the reported levels of mercury in *Cyprinus carpio* from Anzali and Gomishan wetlands (Tabatabaie et al., 2011), *Acipenser stellatus* from the Caspian Sea (Heidary et al., 2012) and *Sander lucioperca* from Gomishan wetland (Tabatabaie et al., 2011) were higher than those obtained in the roach from both study areas in the present study. The lowest concentration of mercury from Anzali wetland related to *Alosa caspia caspia*, *Hemiculter leucisculus* (Zolfaghari, 2018) and *Rutilus frisii kutum* (Mousavi Moghadam et al., 2018) and the highest concentration was related to *Acipenser stellatus* (Heidary et al., 2012).

Mercury concentrations in roach species reported from other parts of the world can be seen in Table 3. The comparison of the mercury content in *R. rutilus* showed that mean concentration of this element in samples taken from Bandar Torkaman was lower than those reported in other studies, while the mercury concentration of samples from Bandar Anzali was higher than values reported from the roach caught in the Danube River (Zrnčić et al., 2013), Sanguinet Lake, Aureilhan Lake (Gentès et al., 2013) and less than values reported from the Olt River (Bravo et al., 2014), Boskovice Reservoir, Landstejn Reservoir (Kuklina et al., 2014) and a number of

Swedish lakes (Sonesten, 2001). On the other hand, the mercury concentration of samples from Bandar Anzali was similar to values obtained from samples of Lake Pluszne in Poland (Łuczyńska et al., 2018, 2016) and Neusiedl in Austria (Jirsa et al., 2014).

Table 3. The average mercury concentrations in muscle tissue of *R. rutilus* in the southern Caspian Sea and elsewhere in the world

Regions of study	Country	Concentration	References
Bandar Anzali	Iran	439 [a] (86) [b]	This study
Bandar Torkaman	Iran	61 [a] (18) [b]	This study
Pluszne Lake	Poland	72 [b]	(Łuczyńska et al., 2018)
Pluszne Lake	Poland	95 [b]	(Łuczyńska et al., 2016)
Neusiedl Lake	Austria	84 [b]	(Jirsa et al., 2014)
78 circumneutral lakes in County of Uppsala	Sweden	130 [b]	(Sonesten, 2001)
Danube River	Hungary, Croatia	152 [a]	(Zrnčić et al., 2013)
Olt River	Romania	3855 [a]	(Bravo et al., 2014)
Sanguinet Lake	France	414 [a]	(Gentès et al., 2013)
Aureilhan Lake	France	183 [a]	(Gentès et al., 2013)
Boskovice Reservoir	Czech	990 [a]	(Kuklina et al., 2014)
Landstejn Reservoir	Czech	660 [a]	(Kuklina et al., 2014)
Zitava River (dam Golianovo)	Slovakia	47 [c]	(Toth et al., 2016)
Zitava River (dam Vráble)	Slovakia	40 [c]	(Toth et al., 2016)

[a] μg kg^{-1} dw
[b] μg kg^{-1} ww
[c] μg kg^{-1}

The limitations for mercury concentration have been set by various organizations around the world such as World Health Organization (WHO) and Food and Agriculture Organization (FAO) (500 μg/kg ww) (Majlesi et al., 2017; Sahebi et al., 2018), the US Environmental Protection Agency (USEPA) (300 μg/kg ww) (Sahebi et al., 2018), the Food and Drug Administration (FDA) (500- 1000 μg/kg ww) (Majlesi et al., 2017) and the National Iranian Standards Organization (500 μg/kg) (Koshafar and Velayatzadeh, 2016). According to these standards, the mercury concentrations from the roach in both studied regions were significantly lower than the range proposed by these organizations. The mercury concentrations in fish samples taken from Bandar Torkama were much lower than the standards in comparison with samples taken from Bandar Anzali. As a result, the edible tissue of the specimens from Bandar Torkama were healthier than those of Bandar Anzali in terms of mercury contamination.

The estimated daily and weekly intakes of mercury by humans (EDI and EWI) through the consumption of roach in the two study regions are reported in Table 4. In total, the EDI values varied from 0.0052 to 0.0469 μg g^{-1} for the two regions. The EWI values ranged from 0.1772 to 0.3284 and 0.0367 to 0.0680 μg g^{-1}, for Bandar Anzali and Bandar Torkaman, respectively. Also, the HQ values of roach samples from Bandar Anzali were higher than samples from Bandar Torkaman, although all values of this index were lower than one in the samples (Table 4). As a result, daily exposure of people to this amount of mercury in the roach fish tissues from either study area may not have any adverse effects on their health over their lifetime. Similar results have also been reported in the studies conducted on this species in Bandar Anzali, Iran (Zolfaghari, 2018) and Pluszne Lake, Poland (Łuczyńska et al., 2018). The estimated HQ index for other fish species such as Northern pike from Anzali wetland and two species of kuttum and golden grey mullet caught from the southeast of the Caspian Sea were similar to the results of the present study (Adel et al., 2016; Nejatkhah Manavi and Mazumder, 2018).

Table 4. Estimated daily and weekly intakes (EDI/EWI) and HQ index values for *R. rutilus* from Bandar Anzali and Bandar Torkaman

Index	Region		Index	Region	
	Bandar Torkaman	Bandar Anzali		Bandar Torkaman	Bandar Anzali
EDI_F [a]	0.0092	0.0444	HQ_{FE} [d]	0.000092	0.000444
EDI_{Im} [b]	0.0052	0.0253	HQ_{FW} [e]	0.000040	0.000193
EDI_{In} [c]	0.0097	0.0469	HQ_{ImE} [f]	0.000052	0.000253
EWI_F [a]	0.0644	0.3111	HQ_{ImW} [j]	0.000023	0.000110
EWI_{Im} [b]	0.0367	0.1772	HQ_{InE} [h]	0.000097	0.000469
EWI_{In} [c]	0.0680	0.3284	HQ_{Inwk} [k]	0.000042	0.000204

[a] Based on food ingestion rate by FAO
[b] Based on food ingestion rate in the whole of Iran
[c] Based on food ingestion rate in the coastal provinces of northern Iran
[d] Based on food ingestion rate by FAO and EPA's reference doses
[e] Based on food ingestion rate by FAO and WHO's reference dose
[f] Based on the food ingestion rate in the whole of Iran and EPA's reference dose.
[j] Based on the food ingestion rate in the whole of Iran and WHO's reference dose.
[h] Based on the food ingestion rate in the coastal provinces of northern Iran and EPA's reference dose.
[k] the food ingestion rate in the coastal provinces of northern Iran and WHO's reference dose.

Maximum daily, weekly, and monthly consumption rates of roach samples were 0.08 kg/day, 0.57 kg/week and 10.86 meals per month for an adult from Bandar Anzali, and 0.39 kg/day, 2.74 kg/week and 52.47 meals per month for an adult from Bandar Torkaman. According to the daily, weekly and monthly values above, the health of the consumers is unlikely to be affected.

There were no significant correlations between Hg concentration in muscle tissue and morphological parameters of the roach (total length, standard length, fork length, total weight) in both study areas, separately ($P > 0.05$), whereas for the sum of samples of both study areas significant negative correlations were observed ($-0.792 < r < -0.917$). In other studies, no significant correlations were observed between mercury concentration in muscle tissue with fish length and weight (Asefi and Zamani-Ahmadmahmoodi, 2015; Nasrollahzadeh Saravi et al., 2013). In another study, no correlation was seen between the mercury concentration in different tissues of northern pike and body length (Molazadeh and Nozari, 2014). However, some studies have reported a significant (positive or negative) relationship between morphological parameters such as total length and weight and mercury levels (Nejatkhah Manavi and Mazumder, 2018; Shao et al., 2016; Soares et al., 2018). Therefore, it cannot be generally stated that mercury concentration is correlated with specific morphological parameters.

The mercury accumulated in the fish body can also affect its health. It has been reported by Lepak et al. (2016) that mercury levels below 0.2 ppm will have no or minor effects on the species itself. However, mercury concentration exceeding 0.2 ppm will lead to impaired gene expression and biochemical performance. Concentrations of mercury above 0.3 ppm have adverse effects on behavior, tissues, and reproductive capacity, and if the concentration increases by more than 1.0 ppm, it may impair species growth and cause other adverse effects (Lepak et al., 2016). The results of the present study were less than 0.2 ppm for the roach from Bandar Anzali and Bandar Torkamen, therefore the presence of mercury in the fish indicates no detrimental effects on their health. It should be noted that the values of the above criteria are based on the amount of mercury in the whole body of the fish, whereas in this study, the values were measured only in muscle tissue.

Conclusions

In comparison, the mercury concentrations of roach fish from Bandar Torkaman was lower than Bandar Anzali indicating better water quality in the former. Compared to studies on different fish species at the Bandar Anzali, mercury concentration in *Rutilus rutilus* species was higher than that of carnivorous species such as *Esox Lucius, Sander lucioperca* and *Rutilus rutilus*, while concentration of mercury in *Rutilus rutilus* from Bandar Torkaman was lower than those in the previously studied species (Table 2). There were no significant correlations between the mercury-contamination levels in the fish muscle in the two regions and morphological parameters. Mercury levels in the studied species in both Bandar Anzali and Bandar Torkaman areas were lower than the standards set by the World Health Organization, the US Environmental Protection Agency, Food and Drug Administration, the Food and Drug Administration and the National Iranian Standards Organization. The HQ values were also lower than one, so it is unlikely that the level of mercury in the roach fish samples reported here would have adverse effects on consumer health. However, due to the increasing presence of pollutants in the water bodies, especially in Bandar Anzali (Ghavidel and Moattar, 2014; Jaffari and Hassanzadeh, 2019), continuous monitoring and identification of sources of contamination is necessary to prevent harmful health effects on fish consumers.

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Ethical approval

Approval from the institutional animal care committee was not required because samples of fish caught in local markets and fishing ports were used for this study. In other words, the specimens of the animals studied were not merely captured for the purpose of this study, but were captured by fishermen for human consumption and then the authors purchased them for this study. As a result, the method used in this study was in no way painful or invasive for the animals.

Conflict of Interest/Competing interests

The authors declare that they have no conflict of interest.

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