Total mercury and methylmercury in fish from a tropical estuary

A. P. Baêta[1], H. A. Kehrig[2], O. Malm[2] & I. Moreira[1]
[1]*Chemistry Department,*
Pontificia Universidade Católica do Rio de Janeiro, Brazil
[2]*Biophysics Institute Carlos Chagas Filho,*
Federal University of Rio de Janeiro, Brazil

Abstract

Mercury content, as methylmercury, in aquatic biota varies greatly among species from the same location. Many parameters may affect its accumulation and concentration in fish tissues. The study assessed total mercury (T-Hg) and methylmercury (MeHg) in the muscle, liver and gonad of *Micropogonias furnieri* - carnivorous fish, *Bagre* spp. - omnivorous fish and *Mugil liza* - iliophagous fish from a polluted eutrophic estuary in the Brazilian Southeast coast, Guanabara Bay. Fish were collected during the years 1990, 1999 and 2003. T-Hg was determined by CV-AAS with sodium borohydride as a reducing agent. MeHg was identified and quantified in the toluene layer by GC-ECD. In all cases, the liver appears to be the preferential organ for mercury accumulation. T-Hg in muscle was higher and more variable in carnivorous than in omnivorous and iliophagous fishes. Carnivorous and omnivorous fishes presented a similar percentage of MeHg (99% and 97%) in the muscle. Iliophagous fish, which is at the lower level of this food chain, presented the lowest % MeHg in the muscle and liver, 52% and 9% respectively. However, the percentage of MeHg to T-Hg was around 25% in the liver of carnivorous and omnivorous fishes. In all cases, the gonad presented the lowest T-Hg, and the ratio of MeHg to T-Hg was around 1. In the year 1999, the samples of carnivorous and iliophagous fishes presented the highest T-Hg in the muscle. In this year, the fish specimens of both species showed sexual maturity and the highest total length. The sex of the specimens did not show any influence in the accumulation of mercury by the fish. The total length of the fish specimens presented a significant relationship with T-Hg in the muscle. The different feeding habits of the studied species are important for the accumulation of mercury and methylmercury by the organisms. Mercury, as methylmercury, is biomagnified through this food chain.
Keywords: tropical fish species, different feeding habits, biological parameters, methylmercury, total mercury, Brazilian estuary.

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1 Introduction

Shallow estuarine and near-shore marine waters have become increasingly degraded over recent years. In spite of efforts to improve natural resource management, industrial plants continue to release toxic compounds into the environment, via liquid effluents and atmospheric emissions. Estuaries and coastal zones, particularly near high population density centres, are of special concern, as they receive the largest exposure to chemical contamination due to source proximity. Toxic compounds, such as mercury, can affect productivity, reproduction and survival of coastal and marine organisms, and can eventually be hazardous to human health (IPCS [1]).

The presence and behaviour of mercury in aquatic systems is of great interest and importance since it is the only heavy metal which bioaccumulates and biomagnifies through the aquatic food chain (Lindqvist *et al* [2]). Methylmercury, the most abundant organic form of mercury in the environment, has been recognised as a serious pollutant of aquatic ecosystems. However only limited information about the manner in which it spreads through the tropical estuarine and marine food chains is available. Methylmercury is largely responsible for the accumulation of mercury in organisms (bioaccumulation) and the transfer of mercury from one trophic level to another (biomagnification).

The trophic transfer of trace elements along marine food webs has been increasingly recognized as an important process influencing metal bioaccumulation and geochemical cycling (Fisher and Reinfelder [3]).

In the marine environment, almost all of the mercury in the muscle of fish is methylated (Joiris *et al* [4], Kehrig *et al* [5, 6]). However, the major part of mercury accumulated in the internal organs especially in the liver, exist as inorganic mercury, suggesting that demethylation of methylmercury is possible (Kehrig *et al* [6], Holsbeek *et al* [7]). The literature has proposed that the liver of the aquatic animals may act as an organ for mercury demethylation and/or the sequestration of both organic and inorganic forms of this element from the body (Endo *et al* [8]).

Mercury content in aquatic biota varies greatly among species from the same location. Many parameters may affect the accumulation and concentration of mercury in fish tissues. The concentrations of mercury and methylmercury accumulated by fishes are a function not only the water and sediment quality, but also of seasonal factors, temperature, salinity, diet, spawning and individual variation (Huchabee *et al* [9], Kehrig *et al* [10]).

Guanabara Bay (22°S, 43°W, 400 km^2), in Rio de Janeiro state, can be considered as one of the most important estuaries for fish production on the South-eastern Brazilian coast. The bay receives untreated domestic and industrial sewage from a densely populated area, and from the second largest industrialized region in the country, with around 10,000 industrial plants, two harbours, shipyards and oil terminals (FEEMA [11]). In some areas, the ecosystem is heavily impacted by organic matter, oil and heavy metals, including mercury. Consequently, elevated concentrations of toxic metals and hydrocarbons in sediments and changes in the pelagic and benthic communities can be detected

(FEEMA [11], Carreira *et al* [12]). An important point source of mercury for this estuary is a chlor-alkali plant located at the most polluted region of its watershed, on the North western side. The bay is among the most productive marine ecosystem in Rio de Janeiro State, presenting a high phytoplankton density and also high nutrients concentrations (C, N, P) that result in a high primary production waters, with an average net primary production (NPP) of 0.17 mol C m^{-2} day (Carreira *et al* [12], Rebello *et al* [13]). This estuary has been the object of numerous studies, but very few of them dealing with mercury and methylmercury in the aquatic biota (Kehrig *et al* [5, 6, 10], Costa *et al* [14]).

The present study assesses the accumulation of total mercury (THg) and methylmercury (MeHg) concentrations and the ratios of MeHg to THg in the muscle, liver and gonad of three species of fish, *Micropogonias furnieri* - carnivorous fish, *Bagre* spp. - omnivorous fish and *Mugil liza* - iliophagous fish from a polluted eutrophic estuary in the Brazilian Southeast coast, Guanabara Bay.

These fish species, which are present in high abundance and are widely distributed on the Southeast Brazilian coast, are those most frequently consumed by human populations. These organisms are characteristic of tropical areas in the Southern Atlantic.

2 Materials and methods

A total of 110 fish specimens with different feeding habits, *Micropogonias furnieri* – carnivorous fish (N=65), *Bagre* spp. – omnivorous fish (N=14), *Mugil liza* – iliophagous fish (N=31), were collected in different periods between 1990 and 2003 at Guanabara Bay. Fish samples were obtained from local fishermen who use a variety of fishing techniques. Following determination of the weight and total length of the specimens, a skinless cube of dorso-lateral muscle tissue was extracted. Cubes of tissue were stored in airtight plastic bags at below –10°C until analysis. Muscle, liver and gonad samples were analysed for total mercury (T-Hg) and methylmercury (MeHg) at Federal University of Rio de Janeiro laboratory.

For determination of total mercury, wet tissue samples were acid-digested and subjected to cold vapour atomic absorption spectrometry (FIMS system, Perkin-Elmer) with sodium borohydride as a reducing agent (Bastos *et al* [15]). For methylmercury, an analytical procedure developed at the National Institute for Minamata Disease (NIMD-Japan) laboratory and subsequently adapted at the UFRJ laboratory was used. The methylmercury analysis in the wet fish tissues was performed by digesting samples with an alcoholic potassium hydroxide solution followed by dithizone-toluene extraction and analysis by Gas Chromatography with Electron Capture Detector (GC-ECD) (Akagi and Nishimura [16]; Kehrig and Malm [17]). Precision and accuracy of the analytical methods were determined using certified standard materials from International Atomic Energy Agency (IAEA-350) and National Research Council Canada (DORM-2). Certified reference material IAEA-350 and DORM-2 was analysed in all sample batches. The overall reproducibility for the analysis period was determined from the results obtained using certified samples. On the basis of triplicate analyses, the coefficient of variation (a measure of random error in determination of mercury concentration) was less than 10%.

The results for total mercury DORM-2 (N=15) were 4.54 ± 0.13 $\mu g.g^{-1}$. The CRM has a certified T-Hg value of 4.64 ± 0.26 $\mu g.g^{-1}$. Our routine methylmercury results for the reference sample IAEA 350 (N=39) were 3.59 ± 0.38 $\mu g.g^{-1}$; the CRM MeHg value is 3.65 ± 0.35 $\mu g.g^{-1}$.

3 Results and discussion

In this study, the concentrations of total mercury (T-Hg) and methylmercury (MeHg) (on a fresh weight basis), and the percentage of methylmercury (% MeHg) in the tissues, muscle and liver, of the three fish species presented data that did not differ greatly to the measurements reported in the literature for fishes with different feeding habits from the South America coastal regions: Argentina (Marcovecchio [18]), Brazil (Niencheski *et al* [19], Sant'Anna *et al* [20], Kehrig *et al* [5, 6, 10], Pinho *et al* [21]), Suriname (Mol *et al* [22]) and Uruguay (Viana *et al* [23]).

The average total mercury concentration (T-Hg) and the percentage of methylmercury as T-Hg (% MeHg) in the tissues of the fish (muscle, liver, and gonad) from Guanabara Bay, and also the total length and weight of the fish specimens are summarised in table 1.

In all cases, T-Hg in the liver of the three fish species (*Micropogonias furnieri*, *Bagre* spp., *Mugil liza*) were higher than those found in their muscle tissue and gonad, presenting statistically significant difference ($p \ll 0.05$). These high hepatic mercury concentrations are probably related to the role played by the liver in terms of pollutant bio-transformation (Frodello *et al* [24]).

The average ratio of liver T-Hg: muscle T-Hg was 2.4 (range 1.2-3.9, n=14) for the carnivorous, 4.2 (range 2.9-5.9, n=14) for the omnivorous and 94.5 (range 1.1-485.5, n=13) for iliophagous fishes.

Carnivorous and omnivorous fishes presented the average ratio of liver MeHg: muscle MeHg close to 1:1, 0.82 for carnivorous and 0.99 for omnivorous. However, the muscle and liver of the carnivorous and omnivorous fishes, both predators' species, presented similar MeHg concentrations ($p \gg 0.15$). Meanwhile, the concentrations of methylmercury in the muscle and liver of the iliophagous fish presented a significant difference ($p=7 \times 10^{-6}$) and the average ratio liver MeHg: muscle MeHg was 26.9. Nevertheless, iliophagous fish showed lower % MeHg in the liver (9%) than carnivorous (26%) and omnivorous (22%) fishes, as shown in table 1.

In all cases, the gonad presented the lowest T-Hg, and the percentage of MeHg to T-Hg was around 100.

Micropogonias furnieri, a carnivorous benthic fish, which foods on bottom fauna mainly macro and microcrustacea (copepod), polychaeta, mollusc and fish (Vazzoller [25]), showed a higher capacity to accumulate total mercury and methylmercury than the other studied fish species with different feeding habits and lower trophic levels, not considering total length and weight differences.

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Fish feeding on copepods ingested more methylmercury than inorganic mercury owing to the larger fraction of methylmercury found in the soft tissues of the copepods Lawson and Mason [26].

Carnivorous species, which are on top end of the aquatic food chain, are a good indicator of mercury in fish (Malm *et al* [27]). Of these, all analysed specimens presented the highest total mercury concentration in their muscle tissue, always showing the values below the maximum limit of 1.0 μg Hg.g^{-1} wet wt. established for human intake of predatory fish by Brazilian legislation (Brasil [28]).

Table 1: Average total mercury concentration (T-Hg) and the percentage of methylmercury as T-Hg (% MeHg) in the tissues of the fish (muscle, liver, and gonad) collected at Guanabara Bay.

Fish species	Total length (mm)	Weight (g)	Tissues	T-Hg (μg.g^{-1} wet wt.)	% MeHg
Micropogonias furnieri (carnivorous fish) – N=65	401	720	Muscle	0.14 ± 0.10	99
			Liver	0.21 ± 0.13	26
			Gonad	0.03 ± 0.02	100
Bagre spp. (omnivorous fish) – N=14	390	660	Muscle	0.07 ± 0.03	97
			Liver	0.29 ± 0.10	22
			Gonad	*	*
Mugil liza (iliophagous fish) – N=31	315	584	Muscle	0.01 ± 0.008	52
			Liver	0.12 ± 0.08	9
			Gonad	0.01 ± 0.007	100

* not analysed.

Total mercury concentrations in carnivorous fish muscle ranged from 0.043 to 0.27 μg.g^{-1} wet wt (N=30) in 1990 (Kehrig *et al* [10]), from 0.063 to 0.56 μg.g^{-1} wet wt. (N=20) in 1999 (Kehrig *et al* [5]) and from 0.040 to 0.28 μg.g^{-1} wet wt. (N= 15) in 2003 respectively. The average of the methylmercury to total mercury ratios was 99%, indicating that MeHg was the predominant form of Hg in carnivorous fish muscles (table 1). The methylmercury concentration at higher trophic levels reflects uptake at low trophic levels and other factors, such as diet and growth (Watras *et al* [29]).

Bagre spp., an omnivorous benthic fish, which feeds mainly on organic detritus, small fishes and invertebrates (Blaber [30]), showed lower T-Hg and MeHg in its muscle than carnivorous fish. The concentrations in *Bagre* spp. muscle (N=14) ranged from 0.046 to 0.18 μg T-Hg.g^{-1} and from 0.049 to 0.16 μg MeHg.g^{-1} and the average of MeHg was 97%.

In this study, *Mugil liza*, an iliophagous fish, which feeds mainly on benthic diatoms (Blaber [30]), showed the lowest concentrations of T-Hg and MeHg and also the lowest % MeHg in the muscle. Total mercury concentrations in iliophagous fish muscle ranged from 0.002 to 0.027 μg.g^{-1} wet wt (N=16) in

1999 (Kehrig *et al* [5]) and from 0.014 to 0.12 $\mu g.g^{-1}$ wet wt. (N= 15) in 2003 respectively. The average of the percentage of MeHg was 52.

Highly significant differences were observed between the average of MeHg concentrations in the muscle of the carnivorous and omnivorous fishes ($p=1 \times 10^{-8}$) and also between the ones in the same tissue of the carnivorous and iliophagous fishes ($p=1 \times 10^{-8}$).

In the year 1999, the samples of carnivorous fish presented the highest T-Hg concentrations in the muscle, 0.17 ± 0.09 $\mu g.g^{-1}$ wet wt. (fig. 1a). In this year, all carnivorous specimens showed sexual maturity and also the highest total length (350 mm - 577 mm). The sexual maturity in *Micropogonias furnieri* occurred at around 450 mm or 4 years old (Vazzoler *et al* [31]). However, the specimens of carnivorous fish colleted in the years 1990 and 2003 presented similar T-Hg in their muscle, 0.11 ± 0.046 $\mu g.g^{-1}$ wet wt and 0.096 ± 0.082 $\mu g.g^{-1}$ wet wt respectively (fig 1a). In theses years, the fish specimens were smaller and younger than the ones sampled in 1999, and also did not present sexual maturity. The total length of the specimens sampled in 1990 and in 2003 ranged from 190 mm to 525 mm and 340 mm to 430 mm respectively. A significant difference ($p << 0.05$) was observed between T-Hg in the muscle of the organisms sampled in the years 1999 and 2003.

Iliophagous fish presented higher T-Hg concentrations in the muscle tissue, 0.019 ± 0.004 μg $THg.g^{-1}$ wet wt., in the samples collected in the year 1999 than in the ones from 2003, 0.004 ± 0.002 μg $THg.g^{-1}$ wet wt (fig 1b). In the year 2003, all the specimens of iliophagous fish presented lower total length (290 mm – 355 mm) than the ones from the year 1999 (370 mm – 500 mm). The sexual maturity of *Mugil liza* occurred at around 400 mm.

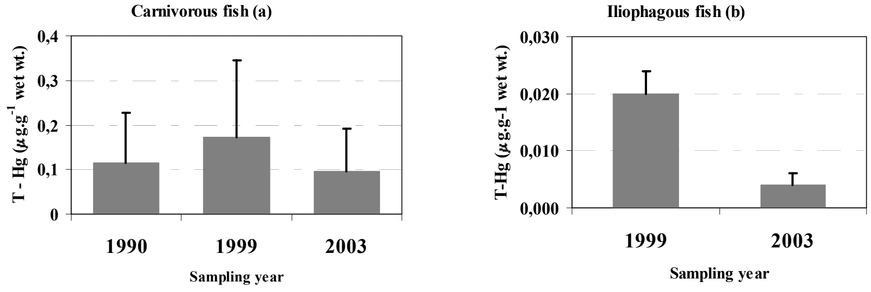

Figure 1: Average total mercury in the muscle tissue of the carnivorous fish (a) and iliophagous fish (b) collected at Guanabara Bay in different years.

In all sampling years, carnivorous fish always presented higher T-Hg in the muscle than iliophagous fish in the same tissue, due to they occupy different trophic levels in the food chain. Carnivorous fish are at the top level and the iliophagous fish at the bottom level of the Guanabara Bay food chain. So, it is important to note that regarding mercury dietary intake, which can influence mercury uptake in marine organisms; there is a marked difference between the concentrations of total mercury and methylmercury in fishes with different feeding habits, carnivorous, omnivorous and iliophagous, and also occupying different trophic levels in the food chain.

In this study, the amplification of the total mercury and methylmercury concentrations and also the percentage of methylmercury in the muscle tissue of the fishes probably are related to the increase of the trophic level position in the food chain. This could be indicating that biomagnification might be occurring throughout the Guanabara Bay food chain.

However, mercury in aquatic environments is not simply transferred from prey to predator tissues; it is accumulated by complex mechanisms. Methylmercury is largely responsible for the bioaccumulation of mercury in organisms and the transfer of mercury from one trophic level to another, causing biomagnification.

A variety of biotic and abiotic factors have been identified which can affect the efficiency that marine animals accumulate metals and metalloids in their tissues (Reinfelder *et al* [32]). Many parameters may affect the mercury accumulation: such as specimen size, sexual maturity, sensitivity to seasonal, feeding habit, trophic position, water quality and environmental contamination (Huchabee *et al* [9], Kehrig *et al* [10]).

A significant positive relationship was observed between T-Hg in the muscle and liver of carnivorous fish ($R=0.91$; $p << 0.05$). However, mercury content in the tissues, muscle and liver, of the omnivorous and iliophagous fishes did not present any relationship ($p>>0.15$).

A significant positive relationship was found between T-Hg concentrations in the muscle of carnivorous ($R = 0.56$; $p << 0.05$) and iliophagous ($R = 0.82$; $p <<0.05$) fishes and their total length (L). The concentration of mercury in the muscle of fish showed a linear increase with the total length, presenting the following relations as eqn (1) for carnivorous and eqn (2) for iliophagous.

$$[T - Hg] = 0.042L - 0.98 \tag{1}$$

$$[T - Hg] = 0.0064L - 0.17 \tag{2}$$

However, the T-Hg concentrations in the muscle of omnivorous fish did not show any relationship with its total length ($p>>0.05$).

No significant difference ($p >> 0.05$) was observed between the average of T-Hg concentration in the female fish specimens and in males of all analysed fish species.

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