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Relationship between Mercury Concentrations in the Blood with that in the Muscle of Four Estuarine Tropical Fish Species, Rio de Janeiro State, Brazil

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Abstract The aim of this work was to assess the relationship between mercury concentrations in the blood with that in muscle for non-invasive mercury contamination assessment in fish. At Ribeira Bay were collected 198 fishes of 4 species (*Genidens genidens*, *Arius luniscutis*, *Haemulon steindachneri, Micropogonias furnieri*). At Guanabara Bay were collected 84 fishes of 2 species (*Genidens genidens*, *Micropogonias furnieri*). Means of mercury concentrations in fish muscles in both areas were below 500 ng/g. The mean ratio, including all specimens of all species, for mercury in muscle-to-whole blood was 13.4:1, for muscle-to-erythrocytes, 6.5:1 and for erythrocytes-to-plasma, 6.5:1. Further studies are necessary to insure that blood could be used as an exposure biomarker, in order to assess mercury availability in aquatic ecosystems.

Keywords Mercury · Blood · Fish · Fish fillet

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Mercury (Hg) levels in erythrocytes have been used as indicator for human exposure to methylmercury (MeHg), due to its high affinity with erythrocytes (WHO 1990). On the other hand, for diagnosis of inorganic mercury exposure the most common indicator is mercury concentrations in human plasma (Schütz et al. 1994). Concerning aquatic ecosystems, fishes are largely applied as bioindicators for Hg bioavailability in the system due to mercury bioaccumulation characteristics. Olson et al. (1973) showed that in fish's erythrocytes about 90% of mercury is in the organic form, what is similar to MeHg percentile in fish muscles. Inorganic Hg interacts with plasma's proteins of fish, mainly with albumin and globulin (Cember et al. 1968). Erythrocytes and plasma ratio seems to depend on each species. In humans, this ratio is of 9–10:1. In rodents, is ten times higher: 100–200:1 (WHO 1991; Suzuki et al. 1971 *apud* Carrier et al. 2001). However, up to now, this relation is not well described to fish species and it is not known if there are changes in these ratios associated with trophic levels or areas.

This simple and practical methodology for mercury exposure evaluation would be applied for environmental assessments, whatever aquatic or terrestrial ecosystems. However, there are few studies involving blood as an exposure biomarker, especially in tropical countries. Most of the available literature involves species from temperate areas, with high influence of seasonality (Wayland 2001; Ribeyre and Boudou 1984; Olson et al. 1973; Cember et al. 1968). Furthermore, erythrocytes have biological half-life of days, varying according to fish species. Then, blood is a renew character that would permit to compare mercury bioavailability in different areas at an actual scenario, reflecting the exposure of those animals in the last few days. Also, as blood sampling does not sacrifice the captured specimens, Hg in blood or in its elements could be

used to infer Hg concentrations in muscles to evaluate Hg accumulation in fish. The aim of this work was to assess the relationship between Hg concentrations in the blood with that in the muscle of four tropical fish species of different trophic levels from two estuaries of Rio de Janeiro State, Brazil, in order to apply a noninvasive mercury contamination assessment.

Materials and Methods

Guanabara Bay is located in Rio de Janeiro State, Brazil (Fig. 1) (22° 40′–23° 00′ S and 43° 00′–43° 20′ E). It has a surface area of 380 Km^2, being one of the largest bays of Brazil. The time for water renew is about 10–20 days (Wasserman et al. 2000). It has high salinity (29.4 ± 4.8 S), decreasing in the internal part of the bay, reflecting the influence of rivers water (Kjerfve et al. 1997 *apud* Campos 2000). It has been impacted by domestic and hospital wastes and industries' effluent with high concentrations of toxic metals since 50s, when industrial development increased in Rio de Janeiro city and other municipalities of Rio de Janeiro State. Nowadays, about 10 000 industries are located to its circuit, discharging 4,8 kg of metals daily and domestic wastes are responsible for the releasing of 465 *t* of organic matter per day (Pereira and Gomes 2002). Despite the metals loads to bay's waters, organic matter seems to complex these substances at the water column and consequently low metals concentrations are found on general biota, suggesting a low bioavailability of these metals for aquatic organisms (Wasserman et al. 2000; Kehrig et al. 1998). Then sediments seem to be the environmental compartment where metals have been stocked and stabilized. However, Campos (2000) showed that the major potential ecological risks are for mercury and cadmium levels presented in this bay.

Ribeira Bay (22°55′–23°02′S and 44°18′–44°26′W) has a surface area of 172 km^2 (Fig. 1). Although tourism activities had increased in the last 10 years, there are no point sources of metals or organic matter (Cardoso et al. 2001). The most important anthropogenic activity is the thermonuclear Angra I and II that use Ribeira Bay's water to cool their reactors systems, besides an increasing activity at Angra dos Reis' Port. Mercury concentrations are very low in surface sediment (28–53 ng/g) (Cardoso et al. 2001). There is no record of disturbances on fish assembly during the last years and up to now 52 fish families were registered, such as Ariidae, Haemulidae and Sciaenidae (Andreata et al. 2002).

For this study were chosen four fish species: two species of catfish (*Genidens genidens* and *Aspistor luniscutis*), which are well distributed, with high frequencies, feeding on benthic organisms (Chaves and Vendel 1996); one species from Haemulidae family (*Haemulon steindachneri*), which is a carnivorous species, feeding on benthic invertebrates and small fishes (Nelson 1994); and, one species from Scianidae family (*Micropogonias furnieri*), which is a carnivorous species, feeding on zooplankton and fish, with high commercial value and migratory characteristics in adult stage (Carvalho-Filho 1999), but in this study were used only juvenile specimens, to infer local influences.

Fish were collected bimonthly between September 2003 and September 2005 at Ribeira Bay and only one sampling was made at Guanabara Bay on October 2005. Sampling was carried out using bottom doors trawl with 10.5 of

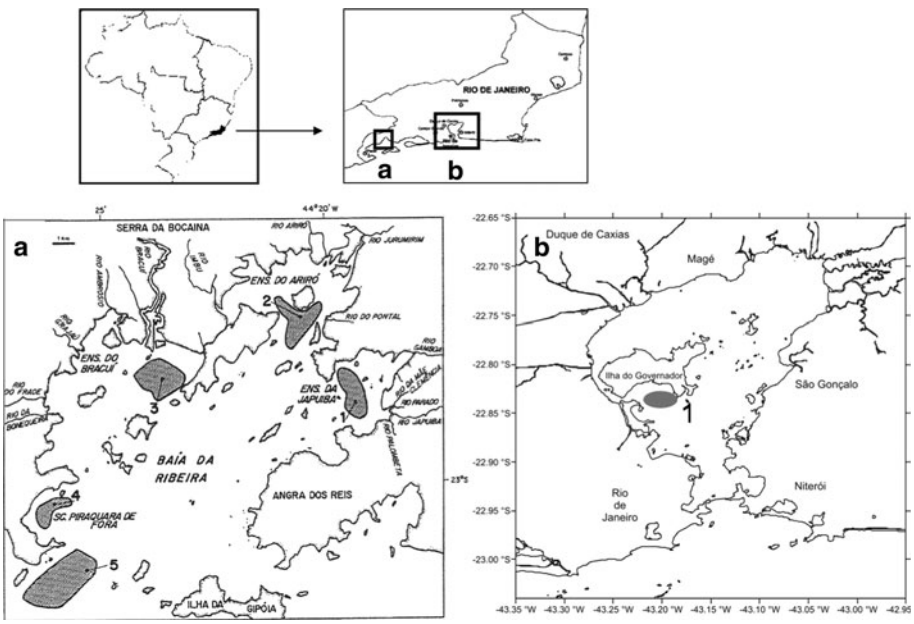

Fig. 1 Map of Ribeira (a) and Guanabara (b) Bays, located at Rio de Janeiro State, Brazil

length, being scattered during 30 min by a boat, once a time in five stations at Ribeira Bay and at one station at Guanabara Bay. This station at Guanabara Bay was chosen since it is known as the most contaminated site of this bay, being representative of the worst scenario of mercury exposure.

At Ribeira Bay, a total of 198 fish were collected, 96 specimens of *Genidens genidens*, 31 specimens of *Aspistor luniscutis*, 33 specimens of *Haemulon steindachneri* and 38 specimens of *Micropogonias furnieri*. At Guanabara Bay, a total of 84 fish specimens were colleted, 70 specimens of *Genidens genidens* and 14 specimens of *Micropogonias furnieri*. Unfortunately, no specimens of *A. luniscutis* and *Haemulon steindachneri* were collect at Guanabara Bay.

Soon after collection, blood samples from captured fishes were collected by caudal puncture with an EDTA-containing syringe. In some cases, blood collection was not successful. Then it was not possible to obtain blood samples from all collected fish specimens. Moreover, the volume of blood that was collected ranged from 0.1 to 1 mL. Thus, for some samples (with lower volume of blood collected) were not possible to perform total mercury determinations in plasma and erythrocytes, only in whole blood. Blood samples were placed in eppendorf tubes and refrigerated. After blood collection, fishes were frozen and brought to the laboratory. Weight (Wt) and total length (Lt) of each specimen were measured and individual axial muscle (fillet) were removed, placed in eppendorf tubes and frozen.

Total mercury was determined using a Lumex, which is a portable atomic absorption spectrometer, specific for total mercury determination (HgT), coupled with pyrolysis reactor. The equipment was calibrated with certified samples (NIST Buffalo River Sediment 2704 and Draw Elements in Coal Fly Ash 1633b). Triplicates were made using small muscles and blood masses for each replicate (20–40 mg). The minimum analytical precision accepted was 90%. Mercury determinations in blood were performed within the first 10 days after sampling. First, all blood samples were used for total mercury determinations as whole blood. If after this determination in whole blood there was a sufficient blood volume left to perform mercury determinations in erythrocyte and in plasma, the sample was centrifuged (500G/15 min) to separate these blood fractions. Hg concentration is presented as ng/g wet weight.

Muscle-to-whole blood, muscle-to-erythrocyte and erythrocyte-to-plasma ratios were calculated based on Hg concentrations found for matched samples of the same fish specimen. The ratio was calculated individually and the mean value for each species will be shown as results. Statistical differences on Hg concentrations among areas were tested using parametric (Student's *T* test) or non-parametric tests of significance (Mann–Whitney *U* test). One-way ANOVA followed by Duncan pos-hoc were performed when appropriate. The significant level considered was ≤ 0.05. Correlations were determined using Pearson and Spearman rank correlation coefficients.

Results and Discussion

Results are shown in Table 1. The difference on the number of analyzed specimens found for length and weight is due to lost material. For some specimens was not possible blood sampling and for others the blood samples were not sufficient for mercury determination in erythrocyte and plasma.

Generally, total mercury levels (Table 1) in muscle were below 500 ng/g (WHO established limit for human consumption), being the maximum value found 817.0 ng/g for *H. steindachneri* at Ribeira Bay. At the first moment, catfish species (*G. genidens*) showed no difference for

Table 1 Mean values ± standard deviation of biometric parameters and total mercury concentrations (wet weight) in muscle, blood, erythrocytes and plasma in four fish species

Fish Species	Length (mm)	Weight (g)	HgM (ng/g)	HgB (ng/g)	HgEr (ng/g)	HgPl (ng/g)
Ribeira Bay						
G. genidens	156.9 ± 54.9 (96)	38.0 ± 45.1 (92)	99.9 ± 86.9 (93)	21.6 ± 27.8 (23)	35.3 ± 28.1 (11)	12.9 ± 9.3 (9)
A. luniscutis	246.2 ± 48.2 (30)	176.3 ± 90.3 (30)	177.6 ± 78.0 (31)	38.7 ± 20.0 (25)	91.9 ± 62.0 (10)	10.7 ± 6.0 (10)
M. furnieri	223.2 ± 66.3 (38)	133.0 ± 145.4 (38)	78.6 ± 88.7 (37)	12.9 ± 5.0 (4)	38.0 (1)	6.9 (1)
H. steindachneri	195.9 ± 26.1 (33)	106.0 ± 30.3 (33)	310.1 ± 206.1 (33)	30.7 ± 18.4 (18)	38.0 ± 2.8 (2)	5.0 (1)
Guanabara Bay						
G. genidens	125.9 ± 30.8 (70)	20.8 ± 19.9 (70)	102.3 ± 43.0 (70)	10.4 ± 5.7 (46)	28.2 ± 7.3 (10)	4.2 (1)*
M. furnieri	105.9 ± 9.8 (14)	12.0 ± 3.5 (13)	56.8 ± 13.0 (14)	5.4 ± 3.9 (10)		

* The number of HgPl determinations was 10, however all the other values were below the equipment detection limit (5 ng/g)

n number of sampled specimens, *HgM* total mercury in fish muscles, *HgB* total mercury in fish blood, *HgEr* total mercury in fish erythrocytes, *HgPl* total mercury in fish plasma

mercury levels in muscles between areas, what was not expected, but it was easily explained by the differences on fish length, where specimens collected at Ribeira Bay were bigger than at Guanabara Bay (T test, $p < 0.001$). Then, for a real comparison of those areas, fishes were grouped by length. This separation was possible only for *G. genidens* (<200 mm specimens), since this species was the only one with a reasonable number of specimens with the same length at both areas. After this, the difference between areas was very clear (T test; $p < 0.001$), showing almost two times higher mercury concentration in muscle of fish from Guanabara Bay (101.9 ± 43.2; n = 69) than from Ribeira Bay (58.6 ± 26.8; n = 67). These results reinforce the importance of fish length normalization, since it will indicate the same exposure time when you analyze muscles matrix.

Mercury levels in blood of *Genidens genidens* were not significantly different among areas using general data, what indicates a similar bioavailability of mercury in both areas. However, for specimens of the same length, mercury levels in Guanabara Bay were higher than in Ribeira Bay (ANOVA; $p < 0.001$). In the same way, at the first moment there was no difference between mercury concentrations at both areas, suggesting a similar rate of accumulation (assimilation). However, when data was compared using fish with the same length, mercury concentrations in erythrocytes were also higher at Guanabara Bay than at Ribeira Bay. This result could suggest a higher methylmercury exposure for catfishes from Guanabara Bay. Although these specimens of same length could not have the same age stage or metabolic rates, the length standardization seemed to be more effective for discriminate differences among areas.

Significant linear correlations (Pearson; $p < 0\ 001$) between Hg levels in blood or erythrocytes and Hg levels in muscle for all studied species, except *M. furnieri*, were found. The resulted equations for each species were: *G. genidens* from Ribeira Bay: [HgT Erythrocytes] = 0.21[HgT Muscle]—4.7 ($R^2 = 0.94$); *A. luniscutis*: [HgT Erythrocytes] = 0.49 [HgT Muscle]—22.5 ($R^2 = 0.74$); *H. steindachneri*: [HgT blood] = 0.11[HgT Muscle]—2.8 ($R^2 = 0.92$); and *G. genidens* from Guanabara Bay: [HgT blood] = 0.05[HgT Muscle] + 3.6 ($R^2 = 0.37$). Observing the results for linear equations, the Hg erythrocytes or blood-to-muscle relationship is close to 1:10. However, *Genidens genidens* from Guanabara Bay showed a relation of 1:100, indicating a higher rate of mercury accumulation in this area.

These relationships between mercury in blood and in muscles were assessed also using the muscle-to-whole blood, the muscle-to-erythrocyte and the erythrocyte-to-plasma ratios (Table 2). The mean ratio, including all specimens of all species, for Hg in muscle-to-whole blood was 13.4:1 (n = 118), for muscle-to-erythrocytes, 6.5:1 (n = 32) and for erythrocytes-to-plasma, 6.5:1 (n = 18).

In general, the mean ratios by fish species for mercury in muscle-to-whole blood were above 5:1, indicating mercury accumulation on fish muscles. Comparing areas, for the catfish *G. genidens* the ratios were quite similar. On the other hand, for white mouth croaker *M. furnieri* the highest mean value was found for specimens from Guanabara Bay. These results may indicate differences on accumulation and exposure to mercury of these species, being linked to their behavior and food habits.

Regarding the ratios for Hg in muscle-to-erythrocyte, in general, considering all specimens of all species, the mean value found was 6.5 ± 8.6 (n = 32), being the maximum ratio found 41.9 for one specimen of *G. Genidens* from Ribeira Bay. The maximum mean values found were 10:1 and 14:1 for *G. genidens* and *H. steindachneri*, respectively, both from Ribeira Bay. Comparing the results for *G. genidens* by areas, these could suggest a similar accumulation by muscles of the available mercury on bloodstream at both areas.

Since the number of samples of total mercury determinations in both erythrocyte and plasma from the same specimen is low, the results for erythrocytes-to-plasma ratio will be presented as an indication. The erythrocytes and plasma mercury levels followed the tendency pointed in the literature, where mercury would be concentrated at erythrocytes (Ribeyre and Boudou 1984). The ratio values found were around 7:1, except for *G. genidens* from Ribeira Bay (5:1), indicating higher concentrations of

Table 2 Mean values ± standard deviation found for muscle-to-whole blood, muscle-to-erythrocyte and erythrocyte-to-plasma ratios of four fish species

Fish Species	HgM:HgB	HgM:HgEr	HgEr:HgPl
Ribeira Bay			
G. genidens	17.8 ± 19.1 (23)	10.9 ± 13.4 (10)	4.8 ± 2.6 (7)
A. luniscutis	6.9 ± 7.9 (25)	4.1 ± 3.9 (9)	7.6 ± 3.7 (9)
M. furnieri	5.2 ± 2.0 (2)	3.1 (1)	–
H. steindachneri	17.9 ± 17.2 (18)	14.1 ± 5.9 (2)	7.2 (1)
Guanabara Bay			
G. genidens	12.4 ± 10.4 (41)	3.0 ± 0.9 (10)	7.6 (1)
M. furnieri	17.5 ± 10.3 (9)	–	–

n number of specimens, *HgM:HgB* muscle-to-whole blood ratio, *HgM:HgEr* muscle-to-erythrocyte ratio, *HgEr:HgPl* erythrocytes-to-plasma ratio

mercury on erythrocytes than in plasma. So these ratios could suggest a higher accumulation of methylmercury among other Hg species in both areas. In previous work (unpublished data), a ratio of 3.5:1 was found for another catfish species *Netuma barba* from Guanabara Bay, with linear relationship of [HgT Muscle] = ([HgT Erythrocytes]/0.1020)—0.9821 ($R^2 = 0.49$) (see mean values at Table 3).

Considering the erythrocyte-to-plasma ratio of *G. genidens* from Ribeira Bay (5:1) and the ratio from Guanabara Bay (7:1), one could suggest a higher bioavailability of methylmercury at Guanabara Bay, considering that methylmercury has high affinity for erythrocytes and inorganic mercury for plasma proteins (Olson et al. 1973; Cember et al. 1968). However there is only one specimen for this ratio at Guanabara Bay, being necessary to increase the number of specimens in order to confirm these previous results. It's important also to point out that there is no standard for mercury and methylmercury levels in tropical fish blood, being necessary further studies.

These results may express differences on mercury forms which fish have been exposed to. The ratio found at Ribeira Bay would indicate a higher inorganic mercury exposure, although no differences were observed for mercury levels in plasma. It may be because of low number of analyzed specimens and high number of mercury determinations that were below the equipment detection limit.

As correlations between mercury concentrations in the tissues and fish length were found, correlations involving the evaluated ratios and length and weight were investigated. Strong and significant correlations were found only for *G. genidens*. In Ribeira Bay, negative correlations between muscles-to-whole blood ratio and length (−0.69; $p < 0.05$) and this ratio and weight (−0.58; $p < 0.05$) were found, suggesting a decreasing on muscles accumulation or increasing on mercury contents in blood in adults specimens. In the same way, for *G. genidens* from Guanabara Bay this correlation was also observed (−0.36; $p < 0.05$).

Table 3 Summary of biometric and mercury levels (wet weight) in a catfish *Netuma barba*, from Guanabara Bay estuary, Rio de Janeiro-Brazil (March 2003—previous study, unpublished data)

Parameters	Unit	N	Mean ± SD	Min	Max
Biometrics					
Weigth	g	14	673 ± 962	225	3,950
Length	mm	14	328 ± 92	225	590
Total mercury					
Muscles	μg/kg	14	126.1 ± 40.2	66.95	203.03
Blood	μg/kg	14	34.4 ± 30.2	9.29	107.74
Erythrocytes	μg/kg	10	20.2 ± 16.0	9.04	63.56
Plasma	μg/kg	10	10.5 ± 10.9	1.58	30.17

As mercury in muscles and mercury in blood/erythrocytes indicate different times of exposure (long and short times, respectively), ratios were also calculated using Hg concentrations in muscles normalized by fish length (Table 4). Considering the catfish *G. genidens*¸ which has the highest number of specimens, the HgM:HgB ratios still similar among areas. The HgM:HgEr ratios vary between species and area, however the number of specimens is very low, increasing the data variability.

Another important point is if juveniles and adults present similar ratios. In this work gonad stage and sex were not evaluated. Regarding information about maturity length for some of the studied species, all specimens included on the analysis were mature, which unfortunately could not provide results concerning possible differences among life stages, what will be studied further.

Although the assimilation/accumulation of mercury in blood seems to be more efficient at Guanabara Bay, considering the proportion, it was expected higher concentrations in fish from this area. Mercury levels in sediment at Guanabara Bay are many times higher than in Ribeira Bay. Campos (2000) found values up to 2 000 ng/g of total mercury in sediments at the sampling point of the present work. On the other hand, at Ribeira Bay the maximum value found was 53.0 ng/g (Cardoso et al. 2001). These results may be related to high organic matter concentration due to domestic wastes that allows the formation of a more stable mercury-organic complex, being most part of mercury that reaches Guanabara Bay waters unavailable to biota incorporation, besides complex formation with sulfides and chlorides ions. Nevertheless it is important to note that mercury levels available to biota at Guanabara Bay, even with those biogeochemical mechanisms for metals complexation, are twice higher than in Ribeira Bay. Then, if these processes diminish their action, mercury that now is stored in sediment would be transformed to methylmercury and further assimilated by biota, being Guanabara Bay considered as a time bomb.

Table 4 Mean values found for muscle-to-whole blood and muscle-to-erythrocyte ratios after Hg in muscles normalization

Fish Species	HgM:HgB	HgM:HgEr
Ribeira Bay		
G. genidens	0.10 (23)	0.06 (10)
A. luniscutis	0.03 (25)	0.02 (9)
M. furnieri	0.02 (2)	0.01 (1)
H. steindachneri	0.10 (18)	0.07 (2)
Guanabara Bay		
G. genidens	0.11 (41)	0.02 (10)
M. furnieri	0.16 (9)	–

n number of specimens, *HgM:HgB* muscle-to-whole blood ratio, *HgM:HgEr* muscle-to-erythrocyte ratio

It's important to explain that Brazilian Government in partnership with private companies developed a rehabilitation program in order to restore water quality at Guanabara Bay, in particular, concerning about the eutrophication process. The Guanabara Bay Depollution Program has as one of its objectives to decrease the biochemical oxygen demand (BOD) in waters until 90%, what will change the trophic conditions of this bay. The concept of "time bomb" considers that after the actions of this program, the improvements in water will reflect in sediments characteristics such as Eh and consequently pollutants, including mercury, could be released to water column, being more bioavailable then before. Our group at Environmental Geochemistry Department is developing models in order to predict different contaminations scenarios according to the objectives of this depollution program.

Concluding, the ratio of mercury in erythrocytes and in plasma in fish seems a practical proxy to assess the bioavailable mercury form in the system. Although deeper investigations on relationships of mercury in muscles and in blood/erythrocytes are necessary, for now, these ratios could be used for qualitative evaluations of mercury contamination, during a screening phase, indicating an interval of possible mercury concentrations in muscles. Additionally, it's extremely important to continue the development of more robust ratios, using higher number of specimens of different life stage and increasing the study areas, in order to investigate deeply if there are no variations of these ratios, enabling to infer blood samples as a good exposure biomarker, avoiding the sacrifice of specimens during contamination assessments.

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