ECOTOX – Brazil

J. Braz. Soc. Ecotoxicol., v. 5, n. 1, 2010, 9-18
doi: 10.5132/jbse.2010.01.003

JBSE

Biomarkers for Mercury Exposure in Tropical Estuarine Fish

A. P. C. Rodrigues[1,2], P. O. Maciel[3], L. C. C. Pereira da Silva[1], C. Albuquerque[4], A. F. Inácio[4], M. Freire[4], A. R. Linde[4], N. R. P. Almosny[3], J. V. Andreata[5], E. D. Bidone[2] & Z. C. Castilhos[1]*

[1]Centre for Mineral Technology, Av. Ipê, 900, CEP 21941-590, Rio de Janeiro – RJ, Brazil
[2]Department of Geochemistry, Fluminense Federal University, Outeiro São João Batista, s/n, CEP 24020-150, Niterói – RJ, Brazil
[3]School of Veterinary, Fluminense Federal University, Rua Vital Brazil Filho, 66, Niterói – RJ, Brazil
[4]Brazilian Ministry of Health, Av. Brasil, 4365, CEP 21040-360, Manguinhos, Rio de Janeiro – RJ, Brazil
[5]Laboratory of Fish Ecology, Santa Úrsula University, Rua Fernando Ferrari, 75, Botafogo, Rio de Janeiro – RJ, Brazil

(Received May 30, 2008; Accepted August 7, 2009)

ABSTRACT

Several studies have evaluated human risks due mercury (Hg) exposure through fish consumption. However, relatively few studies have explored effects of environmental Hg concentrations in biota, especially tropical fish species. The aim of this work was to assess in situ hematological, biochemical and genotoxic effects in tropical fish due to environmental exposure to mercury in estuarine ecosystems. A total of 282 fishes were collected from September 2003 to October 2005 in two estuarine areas: Ribeira Bay (reference area - 22° 55' to 23° 02' S and 44° 18' to 44° 26' W) and Guanabara Bay (highly impacted area by human activities - 22° 40' to 23° 00' S and 43° 00' to 43° 20' E). Total mercury levels in fish from Guanabara were twice higher than in Ribeira bay for the catfish species *Genidens genidens* (Ariidae), with significant differences among areas after standardization using length intervals (exposure time indicator). The species *Haemulon steindachneri* (Haemulidae) showed the highest mercury concentration, reflecting its position in trophic chain. Among effect biomarkers, only haematocrit, global leucometry and micronucleus assays seemed to reflect the differences on mercury exposure among areas, what may support their use for evaluations of fish exposure to mercury compounds. However, it's necessary both laboratory experiments to establish cause-effect relationship and a continuous in situ study to obtain more information, involving more trophic levels, searching for sensible species to mercury exposure.

Keywords: biochemical, Guanabara Bay, hematology, micronuclei, Ribeira Bay.

RESUMO

Biomarcadores para Avaliação da Exposição Mercurial de Peixes Tropicais Estuarinos

Muitos estudos avaliam os riscos à saúde humana associados à exposição por mercúrio (Hg) através da ingestão de peixes. Entretanto, relativamente poucos estudos exploram os efeitos deste na biota, especialmente em espécies de peixes tropicais. O objetivo deste trabalho foi avaliar efeitos hematológicos, bioquímicos e genotóxicos in situ em espécies de peixes tropicais de ecossistemas estuarinos devido a exposição ambiental ao mercúrio. 282 peixes foram coletados entre Setembro/2003 e Outubro/2005 em áreas estuarinas no Rio de Janeiro: Baía da Ribeira (área de referência - 22° 55' a 23° 02' S e 44° 18' a 44° 26' O) e Baía de Guanabara (área altamente impactada pela ação antropogênica - 22° 40' a 23° 00' S e 43° 00' a 43° 20' L). As concentrações de Hg no músculo dos peixes na Baía de Guanabara foram quase o dobro das encontradas na Baía da Ribeira para a espécie de bagre *Genidens genidens* (Ariidae) onde houve diferença significativa após padronização por tamanho (indicador de tempo de exposição). A espécie *Haemulon steindachneri* (Haemulidae) apresentou as concentrações mais altas, refletindo sua posição na cadeia trófica. Dentre os biomarcadores, o hematócrito, a leucometria global e as frequências de

* Corresponding author: Zuleica Carmen Castilhos, e-mail: zcastilhos@cetem.gov.br

micronúcleo e núcleo bilobado pareceram refletir melhor as diferenças na exposição por mercúrio nas áreas estudadas, o que daria suporte a escolha destes para avaliação de exposição de peixes a mercúrio. Todavia, faz-se necessário tanto ensaios em laboratório para estabelecimento da relação causa-efeito quanto a continuação de estudos in situ para maiores esclarecimentos, envolvendo mais níveis tróficos, buscando possíveis espécies mais sensíveis.

Palavras-chaves: bioquímica, Baía de Guanabara, hematologia, micronúcleo, Baía da Ribeira.

INTRODUCTION

Mercury (Hg) is considered as one of the most toxic metals by the World Health Organization (WHO, 1990) due to carcinogenic characteristics of methylmercury (MeHg), which is its most common organic form. It is believed that inorganic mercury is transformed by anaerobic bacteria into MeHg, especially in aquatic ecosystems, where this process seems to be more efficient (Ullrich *et al.*, 2001). Most of the studies indicate that this process occurs preferentially in anaerobic organic superficial sediments. In the other hand, in aerobic environmental conditions, mercury associated to sulfur may be oxidized, forming soluble Hg II, which is assimilated by bacteria and soon methylated (WHO, 1990).

When methylmercury is formed, mercury can reach top predators through its bioaccumulation and biomagnification in food chain. According to organism's trophic level and presence or absence of migratory behavior, fish can show higher mercury concentrations, being largely used in order to assess mercury contamination in aquatic ecosystems. Bruggeman (1982) demonstrated that mercury bioaccumulation factor between non-carnivorous and carnivorous fish species is about 10 times higher in the last one and this relation has been also reported in in situ studies in Brazil (Castilhos, 1999).

Mercury bioavailability depends on biologic variables, e.g. differences on absorption and excretion mechanisms according to species and/or life stage, being important to evaluate mercury assimilation rates by juveniles and adults fish specimens. Some physical-chemical processes may interfere in mercury bioavailability as adsorption by clays, sulfides or organic matter, precipitation, sedimentation, changes in pH, salinity or dissolved oxygen. These factors will determine which mercury species will be available in the ecosystem, consequently will determine which one the organism is going to be exposed to.

Several studies have evaluated human health risks due mercury exposure through fish consumption (Hacon *et al.*, 1997; Malm, 1998; Castilhos, 1999). However, relatively few studies have explored effects of environmental Hg concentrations in fish, especially tropical species (Lopez-Carillo; Lopez-Cervantes, 1993; Castilhos *et al.*, 2004; Silva, 2004). The effects on biota could be expressed by biomarkers responses.

Conceptually, biomarkers are defined as any alteration induced by xenobiotics in cellular or biochemical components or processes, structures or functions that is measurable in a biological system or sample (ATSDR, 1994), in order to identify effects at a tissue/organ before they are apparent at a clinical/pathological level. Effect biomarkers of benthic organism or fish are largely used to assess different contamination degrees of aquatic ecosystems (Lopez-Carillo; Lopez-Cervantes, 1993; Castilhos *et al.*, 2004; Silva, 2004), using as reference results from non-contaminated areas.

Fish exposed to different Hg concentrations in laboratory experiments have showed several effects, such as: hormonal and reproduction alterations due effects during the larval stage (WHO, 1990); alterations on hematological parameters (Olson *et al.*, 1973; Gill & Pant, 1985; Berntssen *et al.*, 2004); histopathological alterations in liver and kidney (Who, 1990); decreasing of enzymatic activities (Gill *et al.*, 1990); problems during gonad development (Wiener; Spry, 1996); reduction of eggs incubation success and survival during embryo-larval stages (Mckim *et al.*, 1976; Friedmann *et al.*, 1996; Latif *et al.*, 2001; Hammerschmitd *et al.*, 2002); decreased locomotor activity, reduction of escape capacity, brain lesions and death (Takeuchi, 1968 apud Wiener *et al.*, 2003); genotoxic effects (Nepomuceno, 1997).

Some of those effects were also found in field studies such as hematological alterations (Castilhos *et al.*, 2004); decrease of enzymatic activities (acetylcholinesterase) (Lopez-Carillo; Lopez-Cervantes, 1993); and genotoxic effects (Rodrigues; Castilhos, 2003).

Hematological parameters as red blood cells counting, hemoglobin concentration, hematocrit, leukocytes, neutrophils and mononuclear cells counting and Mean Corpuscular Volume (MCV) are largely applied to diseases diagnostics, including effects due exposure to toxic substances. Increase of the leukocytes number and neutrophils counting in fish exposed to MeHg were observed in laboratory studies (Oliveira Ribeiro *et al.*, 2006), related to tissue damages such as necrosis in different organs. MeHg exposure could also affect the mechanism of red blood cell turnover, inducing an anemic state (Lohner *et al.*, 2001; Souto, 2004; Silva, 2004).

Acetylcholinesterase is an enzyme that hydrolyzes acetylcholine molecules and it is an important regulatory enzyme that controls the transmission of nerve impulses across cholinergic synapses. Its inhibition has been linked to organophosforades, carbamates and other pesticides exposure (Fonseca *et al.*, 2008; Guimarães *et al.*, 2007; Lavado *et al.*, 2006; Moretto *et al.*, 2004), resulting in an excessive stimulation of cholinergic nerves, consequently causing alterations in swimming behavior, tremors and convulsions (Fernández-Vega *et al.*, 2002 and Miron *et al.*, 2005 *apud* Fonseca *et al.*, 2008). Few information is available concerning its inhibition due methylmercury exposure. Previous studies of our group demonstrated that *Geophagus brasiliensis* sampled in Guandu River (MUNIZ *et al.*, 2005) and catfishes (*Genidens genidens*) from Guanabara Bay (Rodrigues, 2006), presented negative

correlations among acetylcholinesterase activity and mercury levels. On the other hand, for tucunarés (*Cichla* sp.) from Tapajós River (gold mining area) a positive correlation was found, suggesting the increase of acetylcholinesterase activity due mercury exposure (Souto, 2004).

Micronuclei assays (MN) is considered to be one of the most useful methods for evaluating genotoxicity and clastogenic effects due to inorganic and/or organic compounds exposure in aquatic systems (Cavalcante *et al.*, 2008; Monserrat *et al.*, 2007). Micronuclei are formed by chromosome fragments or whole chromosomes that lag at cell division due to the lack of centromere, damage, or a defect in cytokinesis (Heddle *et al.*, 1991 apud Cavas, 2008). Other morphological nuclear alterations (NAs) are also described, including bilobed nuclei, although the mechanisms responsible for NAs have not been fully explained (Cavas, 2008). Thus, the aim of this work was to assess in situ hematological, biochemical and genotoxic effects in tropical fish due to environmental exposure of mercury in estuarine ecosystems.

MATERIAL AND METHODS

Study areas

Guanabara Bay (Figure 1) (22° 40' to 23° 00' S and 43° 00' to 43° 20' E) has a surface area of 380 Km^2, being the second largest bay in Brazil. The time for water renewal is about 10 to 20 days (Wasserman *et al.*, 2000). It has high salinity (29.4 ± 4.8 S), decreasing to internal part of the bay (Kjerfve *et al.*, 1997). It has been impacted by domestic and hospital waste and industries effluent with high concentrations of toxic metals since 50's. Nowadays, about 14,000 industries are located to its circuit, discharging 4,800 Kg of metals daily and domestic wastes are responsible for the releasing of 465 t.day^{-1} of sewage without pre-treatment (Pereira & Gomes, 2002). Besides the large input, metals are rapidly adsorbed by organic matter, showing low bioavailability, being storage in sediment surface (Kehrig *et al.*, 1998; Kehrig *et al.*, 2001; Wasserman et al, 2000). However, Campos (2000) showed that

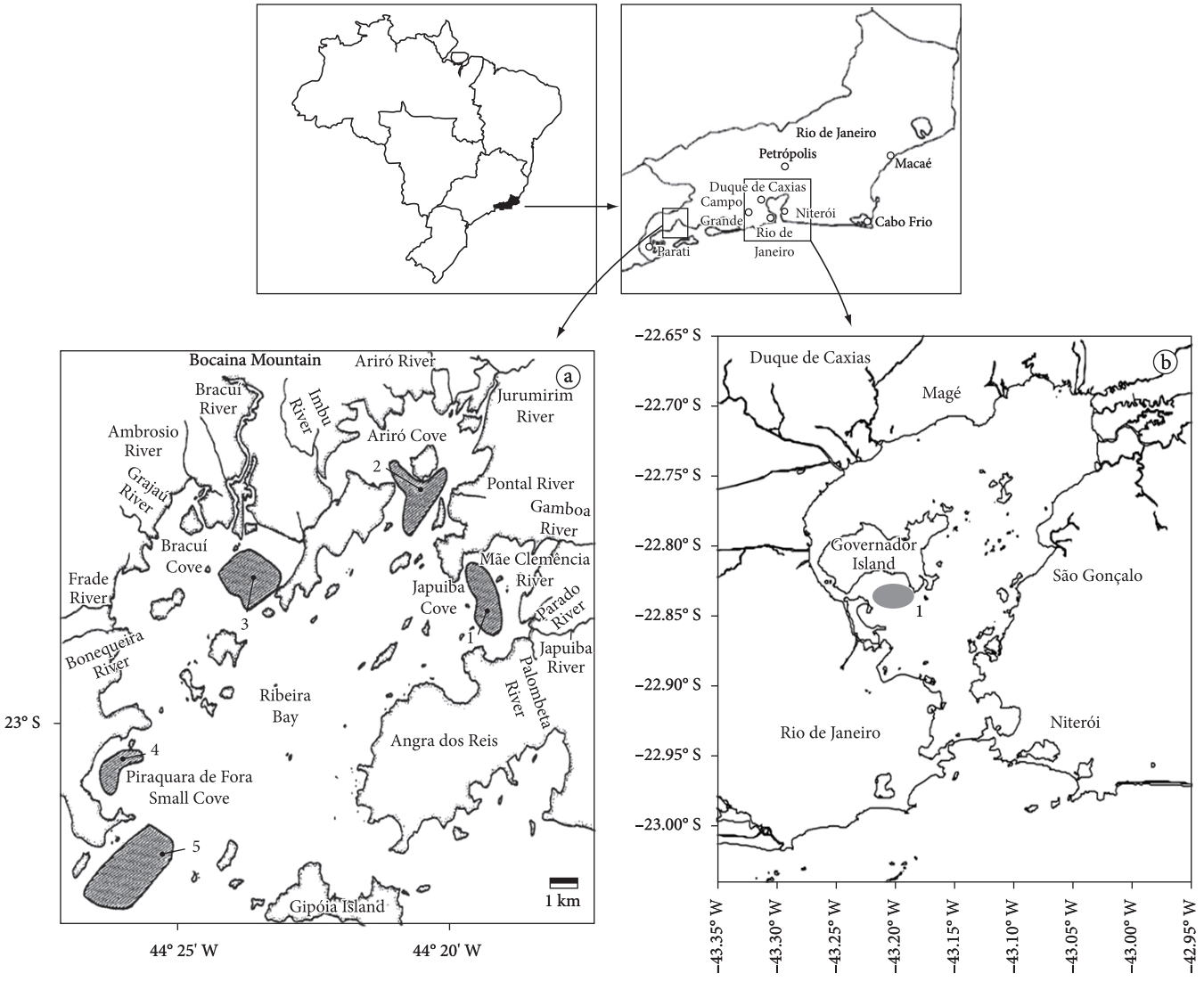

Figure 1 – Map of Ribeira (a) and Guanabara (b) Bays, located at Rio de Janeiro State, Brazil.

the major potential ecological risks in this bay are due mercury and cadmium exposure.

Ribeira Bay (22° 55' to 23° 02' S and 44° 18' to 44° 26' W) has a surface area of 172 km^2 (Figure 1) (Lima, 1985). Despite the increase of tourism activities in the last 10 years, there are no punctual sources of metals or organic matter (Cardoso *et al.*, 2001). The most important anthropogenic activity is the thermonuclear industries (Angra I and II) that use Ribeira Bay's water to cool their reactors systems. Mercury concentrations are very low in surface sediment (28 to 53 $ng.g^{-1}$) and in fish (<200 $ng.g^{-1}$) (Cardoso *et al.*, 2001). There is no record for disturbances on fish assembly during the last years and up today were registered 52 fish families, like Ariidae, Haemulidae e Sciaenidae (Andreata *et al.*, 2002).

Fish species

In order to verify mercury effects, it's very important the choice of fish species. A good biomonitor may be sedentary, belong to high trophic level and well distributed among continental coast, what makes possible comparisons among areas. In this study four fish species were chosen: two species of catfish, Ariidae family (*Genidens genidens* and *Aspistor luniscutis*), which are well distributed, with high frequencies, feeding on benthic organisms (Chaves & Vendel, 1996); one species from Haemulidae family (*Haemulon steindachneri*), which is a carnivorous species, feeding on benthic invertebrates and small fish (Nelson, 1994); and, one species from Scianidae family (*Micropogonias furnieri*), which is a carnivorous species feeding on zooplankton, with high commercial value and migratory characteristics in adult stage (Carvalho-Filho, 1999). In this study were used only juvenile specimens to infer local influences.

Sampling

Fish were collected every two months, between September 2003 and September 2005 at Ribeira Bay and only one sampling was made at Guanabara Bay on October 2005. It was used a bottom doors trawl with 10.5 m of length, being scattered during 30 minutes by a boat, once a time in five stations at Ribeira Bay and at two stations at Guanabara Bay. Unfortunately there was no possibility of continue the sampling campaigns at Guanabara Bay, being chosen as sampling stations, the most contaminated area of this bay, as suggested by Campos (2000).

At Ribeira Bay, a total of 198 fish were collected, 96 specimens of *G. genidens*, 31 specimens of *A. luniscutis*, 33 specimens of *H. steindachneri* and 38 specimens of *M. furnieri*. At Guanabara Bay, a total of 84 fish specimens were colleted, 70 specimens of *G. genidens* and 14 specimens of *M. furnieri*.

Blood sampling

Soon after collection, blood samples from captured fishes were obtained by caudal puncture with an EDTA-containing syringe. Three blood smears for each specimen were made as soon as possible. After blood collection, fishes were brought to the laboratory, where each specimen was weighed (Wt), lengthed (Lt) and individual axial muscle (fillet) was removed, placed in eppendorf tubes and frozen.

Mercury determination

Mercury determination was performed by using Lumex, a portable atomic absorption spectrometer, specific for total mercury determination (HgT), coupled with pyrolysis reactor, which function is the thermal destruction of the sample. 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). For mercury determinations in erythrocytes and plasma, blood samples were centrifuged (3000 rpm; 5 minutes). Blood samples were analyzed until 10 days after sampling. The maximum error accepted was 10%.

Biomarkers

This study assessed the following effect biomarkers: micronucleus, hemogram and acetylcholinesterase (AChE) activity in muscles. Although they are not specific biomarkers for mercury, previous works of our group (Rodrigues & Castilhos, 2003; Souto, 2004; Silva, 2004) demonstrated linear relationship between mercury concentrations and these biomarkers.

Micronucleus assay

For micronucleus (MN) and bilobed nuclei analysis (Bombail *et al.*, 2001), blood samples were smeared on clean glass slides, dried, fixed with methanol, and stained with Giemsa. Three smears per sample were observed under a light microscope with immersion oil (1000×). One thousand blood erythrocytes per smear were scored. Only non-refractory particles with the same color of erythrocyte nuclei were interpreted as micronuclei.

Hemogram

Hemograms were performed at the same day of the sampling, using a method described in Almosny & Santos (2001), where all cellular types are in the Newbauer$^{(Improved)}$ cell counting chamber, being proceeded erythrocytes, trombocytes and leukocytes counting together. Haematocrit was obtained through the microhematocrit technique with centrifugation (14,000 G; 5 minutes). The mean corpuscular volume (MCV) was calculated as follows: MCV = [(Hematocrit) × 100]/ Total erythrocyte counting (Almosny & Santos, 2001). For total plasmatic protein (TPP) determination, blood samples were placed in capillary tubes, centrifuged (5 minutes) and then TPP was measured at clinical refratometer (Almosny & Santos, 2001).

Acetylcholinesterase activities

The determinations of the AChE activity in fish muscles followed the method described by Oliveira Silva *et al.* (2000), with modifications, where they are quantified based in the reaction

described by Ellman *et al.* (1961). Small portions of muscle samples were weighted and homogenized in sodium phosphate 0.12 M, pH 7.6 (6:1). The samples were centrifuged (9,000 G; 20 minutes; 8 °C). While the samples were centrifuging, test tubes were prepared containing 2 mL of sodium phosphate and 0.5 mL of DTNB 2 mM. At the time of lecture, 500 µL of acetylcholine and 25 µL of sample were added at those previously prepared tubes. Enzymatic activities were determined in spectrophotometer in kinetic form ($\lambda = 412$ nm), being obtained after two minutes of reaction, the absorbance per minute. For protein determination, muscles samples were diluted (1:10) in sodium phosphate solution 0.12 M pH 7.6. In test tubes were added 4.3 mL of H_2O distilled, 200 µL of NaOH 25% and 200 µL of diluted sample. The blank contained 4.5 mL of H_2O distilled and 200 µL of NaOH 25%. In the first tube (blank) was added 300 µL of Folin reagent, being homogenized on a vortex during 30 seconds. The same process of homogenization was made to all tubes. After 5 minutes, the absorbance was measured in photometric model, in a spectrophotometer Shimadzu UV 1601 ($\lambda = 660$ nm). The absorbance were converted to protein concentration ($mg.mL^{-1}$) using an albumin curve. The specific activity of AChE ($\mu moles.min^{-1}.mg^{-1}$ protein) was obtained by ratio of the enzyme activity and the protein concentration (Cunha *et al.*, 1991).

Statistical analysis

Statistical differences on Hg concentrations, hematological and biochemical parameters between different species from distinct areas were tested using parametric (Student's T-test) and nonparametric tests of significance (Mann-Whitney U-test). The significant level (p) considered was $p < 0.05$. Correlations were determined using Pearson correlation coefficient and Spearman rank correlation coefficient. In order to verify differences between areas for genotoxic responses (MN and BN), it was used a statistical test for rare events in cell genetics described in Pereira (1991).

RESULTS

Results for biometry and mean mercury concentrations in muscles, blood, erythrocytes and plasma for all species at both areas are shown in Table 1. For some specimens was not possible blood sampling and for others the blood samples were not sufficient for erythrocyte/plasma separation and posterior mercury determination. *H. steindachneri* and *A. luniscutis* were not observed during the sampling at Guanabara Bay.

Almost all species showed total mercury levels in muscle below of 200 $ng.g^{-1}$, except *H. steindachneri* (mean of 310 $ng.g^{-1}$), which presented the highest mercury concentration (maximum value of 817.0 $ng.g^{-1}$). At the first moment, catfish species (*G. genidens*) showed no difference for mercury levels in muscles between areas, but specimens collected in Ribeira Bay were bigger in size than in Guanabara Bay (T-test, $p < 0.001$; t-value: 4.63), what may means different ages, consequently different exposure times. So, in order to compare mercury accumulation in both areas, fish were separated in length range groups. This separation was made only for *G. genidens* (<200 mm specimens), since this species was the only one with a reasonable number of specimens within similar length at both areas. After this, the difference between areas was very clear (t-test; $p < 0.001$; t-value: –7.04), showing almost two times higher mercury concentrations in muscle at Guanabara Bay (101.9 ± 43.2; $n = 69$) than from Ribeira Bay (58.6 ± 26.8; $n = 67$). These results reinforce the necessity of fish length normalization when muscle is the chosen matrix for mercury exposure assessment in order to indicate the same exposure time.

Considering general data, mercury levels in blood of *G. genidens* were higher at Ribeira Bay (t-student test; $p < 0.001$; t-value: 2.63), in the other hand erythrocytes levels were not different among areas. With standardized data, mercury levels in fish erythrocytes from Guanabara Bay were higher than in Ribeira Bay (Mann-Whitney U test; $p < 0.01$; W-value: 10.0), suggesting a higher availability of organic mercury in Guanabara Bay.

The results found for effect biomarkers are shown in Table 2. Among hematological parameters, analyzing the reference values obtained at Ribeira Bay, the mean corpuscular volume was lower in *M. furnieri* than in *A. luniscutis* and *G. genidens* (ANOVA; $p < 0.05$), what seems to be a characteristic of these species. Among areas, haematocrit of *G. genidens* is higher at Ribeira Bay (t-test; $p < 0.05$; t-value: 2.11) and no difference was found for mean corpuscular volume, ie., erythrocytes of catfishes from both areas have the same volume, but in Guanabara

Table 1 – Mean values ± standard deviation found for biometric parameters and total mercury concentrations (wet weight) in muscle (HgM), blood (HgB), erythrocytes (HgEr) and plasma (HgPl) of the studied tropical fish species, Rio de Janeiro State, Brazil. (n) = number of sampled specimens. * The number of samples for HgPl determinations was 10, however in some cases, Hg levels were below the equipment detection limit (5 $ng.g^{-1}$).

Species	Length (mm)	Weight (g)	HgM ($ng.g^{-1}$)	HgB ($ng.g^{-1}$)	HgEr ($ng.g^{-1}$)	HgPl ($ng.g^{-1}$)
			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)		

bay they are in lower number (lower haematocrit), suggesting a non-regenerative anemia scenario for *Genidens genidens*.

G. genidens MN and BN frequencies were higher and lower, respectively, at Guanabara Bay ($p < 0.001$) than in Ribeira Bay. *M. furnieri* erythrocytes showed no difference on MN frequency (both were nulls), however BN frequency was 10 times higher at Guanabara Bay. Comparing the present results for MN and BN frequencies with a previous work in 2003 (Figure 2) at Guanabara Bay (Rodrigues; Castilhos, 2003), there is no significant difference, indicating that in the last two years, there was no changes in genotoxic scenarios for catfish from this bay.

AChE activity mean of *G. genidens*, after length normalization, showed a tendency to be lower in Guanabara Bay (0.92 ± 0.41 µmoles.min^{-1}.mg^{-1} protein; n = 38) than in Ribeira Bay (1.14 ± 0.66 µmoles.min^{-1}.mg^{-1} protein; n = 13), although they are non-significant statistically ($p > 0.05$).

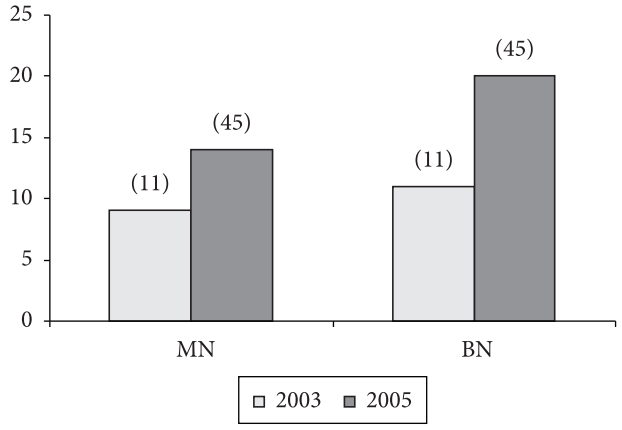

Figure 2 – Micronuclei (MN) and Bilobed Nuclei (BN) frequencies found in catfish of Ariidae family at Guanabara Bay in 2003 and in 2005. (n) = number of specimens.

DISCUSSION

Although mercury levels in muscles of *G. genidens* after standardization at Guanabara Bay were almost twice higher than at Ribeira Bay (Table 3), the observed values at Guanabara Bay are not proportional to mercury concentrations in sediment, which are many times higher than in Ribeira Bay. Campos (2000) found values up to 2,000 ng.g^{-1} of total mercury in sediments from the most contaminated sites at Guanabara Bay. On the other hand, at Ribeira Bay the maximum value found was 53 ng.g^{-1}. This may be related to high organic matter concentration due to domestic wastes that allows the formation of a more stable mercury-organic complex, being mercury unavailable to biota incorporation.

Observing the results for linear equations, the ratio between Hg in erythrocytes/blood and Hg in muscle is close to 1:10 in fish from reference site and to 1:100 in fish from contaminated site. This may indicate a higher rate of mercury accumulation in the contaminated area, even considering that most part of mercury that reaches this bay would be adsorbed by organic matter. These ratios could be used in order to predict mercury levels in fish muscles through mercury determination in blood samples, being a good strategy to avoid the sacrifice of animals during biomonitoring.(Figures 3 and 4)

Concerning the results for biomarkers, micronuclei and bilobed nuclei incidence showed positive (Pearson; +0.39; $p < 0.01$; n = 45) and negative (Pearson; –0.35; $p < 0.05$; n = 45) correlations with Hg in muscle, respectively. In *M. furnieri* the bilobed nuclei frequency seems to be related to fish growth, since it presented negative correlations with length and weight (Spearman; –0.82; $p < 0.005$; and –0.69; $p < 0.05$, respectively, n = 10).

Sanchez-Galan *et al.* (2001) demonstrated that both cadmium and mercury can induce micronuclei expression in eels when injected (tested concentration: 1.7 mg metal per Kg body weight), being the induction of 2.64 and 2.35 micronuclei per 1,000 cells for cadmium and mercury respectively.

Table 2 – Mean values ± standard deviation found for erythrocyte counting (E), haematocrit (Ht), mean corpuscular volume (MCV), total plasmatic protein (TPP), acetylcholinesterase activities (AChE) and absolute frequencies of micronucleus (MN) and bilobed nuclei (BN) of the studied tropical fish species, Rio de Janeiro State, Brazil. (n) = number of sampled specimens.

Parameters	*G. genidens*	*M. furnieri*	*H. steindachneri*	*A. luniscutis*
		Ribeira Bay		
E (10^6/mm^3)	1.27 ± 0.49(16)	2.57 ± 0.20(5)	1.93 ± 0.69(14)	1.55 ± 0.32(10)
Ht (%)	28.1 ± 6.4 (19)	26.5 ± 7.1(6)	29.7 ± 6.6 (17)	34.0 ± 5.2 (13)
MCV (fL)	259.2 ± 90.3 (16)	100.8 ± 24.7 (5)	184.9 ± 125.9 (14)	241.5 ± 68.3 (10)
TPP (g.dL^{-1})	4.3 ± 0.5 (17)	4.4 ± 0.5 (6)	4.7 ± 0.8 (17)	4.6 ± 1.0 (11)
MN (frequency/counted cells)	5/19,000	0/4,000	0/2,000	15/25,000
BN (frequency/counted cells)	12/19,000	9/4,000	0/2,000	3/25,000
AChE (µmoles.min^{-1}.mg^{-1} protein)	0.79 ± 0.54 (33)	0.19 ± 0.04 (19)	0.76 ± 0.25 (10)	0.22 ± 0.15 (23)
		Guanabara Bay		
E (10^6/mm^3)	1.11 ± 0.32 (39)	2.65 ± 0.61 (2)	–	–
Ht (%)	24.1 ± 7.9 (42)	18.0 ± 2.8 (2)	–	–
MCV (fL)	218.2 ± 60.3 (39)	68.5 ± 5.0 (2)	–	–
TPP (g.dL^{-1})	3.9 ± 0.7 (42)	4.0 ± 0.57 (2)	–	–
MN (frequency/counted cells)	14/45,000	20/45,000	–	–
BN (frequency/counted cells)	0/10,000	108/10,000	–	–
AChE (µmoles.min^{-1}.mg^{-1} protein)	0.91 ± 0.41 (39)	0.61 ± 0.10 (11)	–	–

Porto *et al.* (2005) found significantly higher mean frequencies of MN in *Prochilodus nigricans* (detritivorous), *Mylossoma duriventris* (omnivorous) and *Hoplias malabaricus* (piscivorous) from the Madeira River when compared to frequencies from the same species from the Solimões River. Madeira River is highly impacted by artisanal gold mining areas that use mercury during amalgamation process, being mercury the most important toxic metal present in this environment quantitatively and qualitatively. In addition, mean frequencies of MN from piscivorous species were almost fivefold higher than the detritivorous and/or omnivorous species (Porto *et al.*, 2005). These results reinforce the evidences of mercury genotoxic action.

MN frequencies of the present study are close to those frequencies found in Porto *et al.* (2005) for Amazonian fish (Madeira river: *P. nigricans* 19 MN in 50,000 erythrocytes counted; *M. duriventris* 24/60,000; *H. malabaricus* 44/25,000; and for Solimões river: *P. nigricans* 5/50,000; *M. duriventris* 6/60,000; *H. malabaricus* 2/30,000), at least at the same magnitude.

Other field studies that determined MN frequencies in fish erythrocytes demonstrated the same tendency of higher frequencies in polluted areas (Lemos *et al.*, 2007; Porto *et al.*, 2005), where there are more physical and chemical stressors, such as toxic metals. At Rio de Janeiro State, Linde-Arias *et al.* (2008) studied MN frequencies in *Oreochromis niloticus* from Paraíba do Sul River basin and the highest frequency was found in fish from Guandu River, which is responsible for water supply to metropolitan region of Rio de Janeiro State.

About hemogram results, haematocrit (Pearson, 0.37 $p < 0.05$; $n = 42$) and mean corpuscular volume (Pearson, 0.50 $p < 0.001$; $n = 39$) showed correlations with Hg in muscles for *Genidens genidens* from Guanabara bay. These correlations demonstrate the increasing of erythrocytes proportion in blood, and their corpuscular volume due to mercury exposure, supporting the non-regenerative scenario previously described for this fish species at the polluted area. Souto (2004) and Silva (2004) found similar results in Amazonian fish species (*Cichla* sp), where mercury would be directly involved in ionic imbalance, which may induce haematocrit decreasing and mean corpuscular volume increasing.

For *Aspistor luniscutis* from Ribeira bay, positive correlation between Hg levels in blood/muscles and total plasmatic protein (Spearman, 0.72; $p < 0.05$ and 0.81; $p < 0.01$, respectively,

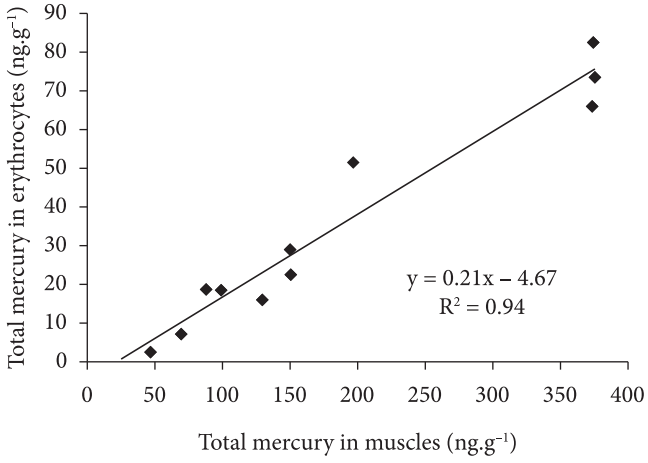

Figure 3 – Linear correlation among total mercury concentrations in muscles and in erythrocytes of catfish *Genidens genidens* sampled at Ribeira Bay, reference area – RJ, Brazil.

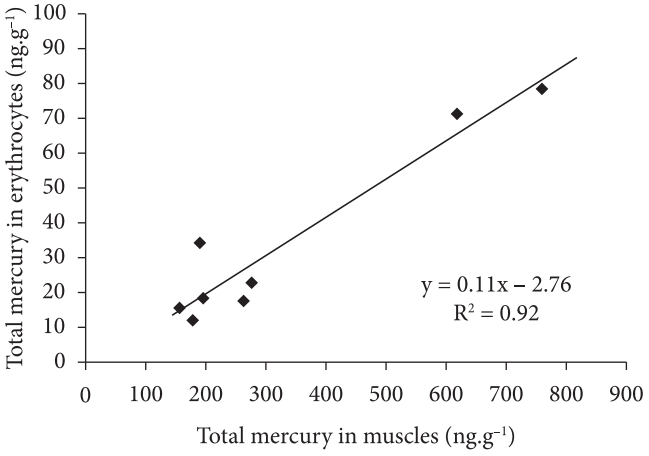

Figure 4 – Linear correlation among total mercury concentrations in muscles and in total blood of *Haemulon steindachneri* sampled at Ribeira Bay, reference area – RJ, Brazil.

Table 3 – Results found for *Genidens genidens* from Guanabara and Ribeira Bays (Rio de Janeiro-Brazil), before and after standardization by length intervals (<200 mm), for all assessed biomarkers. (n) = number of sampled specimens. *t-student test; $p < 0.05$; **t-student test; $p < 0.01$; ***t-student test; $p < 0.001$; + Mann-Whitney, U test; $p < 0.05$; # test for rare events in cell genetics; $p < 0.05$; ## test for rare events in cell genetics; $p < 0.001$.

Biomarkers	Non-Standardized			Standardized		
	Ribeira Bay	Guanabara Bay		Ribeira Bay	Guanabara Bay	
Hg in muscle ($ng.g^{-1}$)	99.9 ± 86.9 (93)	102.3 ± 43.0 (70)	n.s.	58.6 ± 26.9 (67)	101.9 ± 43.3 (69)	***
Hg in blood ($ng.g^{-1}$)	21.6 ± 27.8 (23)	10.4 ± 5.7 (46)	***	7.2 ± 1.7 (7)	10.1 ± 5.2 (45)	n.s.
Hg in erythrocytes ($ng.g^{-1}$)	35.3 ± 28.1 (11)	28.2 ± 7.3 (10)	n.s.	11.0 ± 7.5 (4)	27.7 ± 7.6 (9)	**
Hg in plasma ($ng.g^{-1}$)	12.9 ± 9.3 (9)	4.2 (1)	n.s.	10.1 (1)	–	–
Erythrocytes counting ($10^6/mm^3$)	1.27 ± 0.49 (16)	1.11 ± 0.32 (39)	n.s.	1.38 ± 0.52 (6)	1.11 ± 0.33 (38)	n.s.
Haematocrit (%)	28.1 ± 6.4 (19)	24.1 ± 7.9 (42)	*	25.1 ± 7.7 (9)	23.5 ± 6.9 (41)	n.s.
Mean corpuscular volume (fL)	259.2 ± 90.3(16)	218.2 ± 60.3 (39)	n.s.	215.4 ± 86.6 (6)	212.7 ± 50.5 (38)	n.s.
Total plasmatic proteins ($g\ dL^{-1}$)	4.3 ± 0.5 (17)	3.9 ± 0.7 (42)	*	4.3 ± 0.6 (8)	3.8 ± 0.7 (41)	+
MN (frequency/counted cells)	5/19,000	14/45,000	#	1/4,000	14/40,000	n.s.
BN (frequency/counted cells)	12/19,000	0/10,000	##	4/4,000	20/40,000	#
AChE ($\mu moles.min^{-1}.mg^{-1}$ protein)	0.79 ± 0.54 (33)	0.91 ± 0.41 (39)	n.s.	1.14 ± 0.76 (13)	0.92 ± 0.41 (38)	n.s.

n = 9) were found and may indicate that TPP would increase with mercury exposure. Besides TPP levels in *Genidens genidens* from Ribeira bay are higher than in contaminated area (Mann-Whitney; $p < 0.05$), suggesting a possible mechanism of mercury binding by blood and muscle proteins.

These results were also found by Berntssenn *et al.* (2004), in laboratory experiments with *Salmo salar*, where TPP had a significant increase with the exposure to high mercury levels (5 ppm). Besides, in the same study, Berntssenn *et al.* (2004) also demonstrated that as mercury levels in fish muscles increased, haematocrit decreased, similar to the results of this work, as observed for *G. genidens* from Guanabara Bay. Although this effect could not be attributed specifically to mercury exposure, since this reduction can be induced by many other factors, another field studies at mining areas at Tapajós River (Brazilian Amazon), where mercury is the main chemical stressor, but the only one, demonstrated that mercury would induce anemia in carnivorous species from genus *Cichla* (Castilhos *et al.*, 2004).

AChE activities were different among species (ANOVA; $p < 0.05$), but no significant among areas. At Ribeira Bay, *Micropogonias furnieri* showed the lowest activity (0.19 ± 0.04 μmoles.min^{-1}.mg^{-1} protein) and *Genidens genidens* the highest one (0.79 ± 0.25 μmoles.min^{-1}.mg^{-1} protein). At Guanabara Bay, it was observed the same tendency, being AChE activities lower in *Micropogonias furnieri* and higher in *Genidens genidens*. This variation among species was pointed out by Magnotti *et al.* (1994 apud Oliveira *et al.*, 2007), that suggested the existence of two big groups, one of high enzymatic activity and the other of low enzymatic activity.

Although no differences on acetylcholinesterase activity were found among areas, ie there was not evidences of inhibition of this enzyme in this study, the negative correlation among its activity and mercury levels in muscles and in blood (Pearson; –0.57; $p < 0.001$; n = 39) of *G. genidens* from Guanabara Bay suggest a possible inhibition relationship, as found in other studies of our group at this bay (Rodrigues & Castilhos, 2003).

Differences in sensitivity against anticholinesterase compounds have been reported between several fish and invertebrate species (Monserrat *et al.*, 2007) and these differences may be due to several factors, such as the ability of the active site of the enzyme to fit the alkyl chain of substrates such as acetylthiocholine iodide (AcSCh) and butyrylthiocholine iodide (BSCh). Monserrat *et al.* (2007) showed that, generally, fish species cholinesterases are more sensible to anticholinesterase compounds when compared to invertebrate. However, the authors considered as an exception the estuarine white mouth croaker *Micropogonias furnieri*. In the present study, this species has not shown inhibition evidences.

Briefly, our results indicate that (i) both in Ribeira and Guanabara Bays, fish muscles and blood showed low mercury levels; (ii) in order to compare areas using mercury levels in muscles as matrix is extremely important to standardize the data, through length, age or other variable that expresses time; (iii) although mercury input in Ribeira Bay is lower, mercury is more bioavailable than in Guanabara Bay, where mercury seems to be rapidly removed by organic matter to sediment; (iv) mercury is accumulated predominantly in erythrocytes of fish in comparison with plasma, suggesting a MeHg exposure; (v) although mercury is bioavailable, the levels found in fish from Ribeira bay could be considered natural, except for *Haemulon steindachneri*, then the results found for biomarkers at this bay may be used as reference values for these tropical fish species; (vi) haematocrit was lower, with no changes on MCV, in *G. genidens* from Guanabara Bay, suggesting a non-regenerative anemia; (vii) micronuclei and bilobed nuclei frequencies were different among areas, especially for *Genidens genidens*.

It is important emphasize the necessity of more sampling stations at Guanabara Bay to analyze if low mercury availability also occurs in lower oxygenated areas and near some important streams, where punctual sources of mercury are located. Guanabara Bay may be considered as a time bomb. In the case of organic material decreasing associated with an increase in oxygen saturation, mercury and other metals may become available to local biota and their effects could be aggravated.

Further studies are necessary in order to a better understanding of methylmercury accumulation on erythrocytes, assessing methylmercury and inorganic mercury biochemical effects in fish. Although sampling frequency was not the same at both areas, these results are extremely important since they may be used for guidelines and reference for further studies with tropical fish species or bioavailability of mercury at other aquatic ecosystems.

Acknowledgements – The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support; Laboratory of Fish Ecology from Santa Úrsula University for fish species identification and sampling; and, all people involved in this work direct or indirectly, making it possible.

REFERENCES

ALMOSNY, N. R. P. & SANTOS, L., 2001, Laboratory support in wild animal medicine. *In:* M. Fowler & Z. Cubas (eds), *Biology, Medicine and Surgery of South American Wild Animals*. Iowa State University Press,, p. 500-505. doi: 10.1002/9780470376980.

ANDREATA, J. V., MEURER, B. C., BAPTISTA, M. G. S., MANZANO, F. V., TEIXEIRA, D. E., LONGO, M. M. & FRERET, N. V., 2002, Composição da assembléia de peixes da Baía da Ribeira, Angra dos Reis, Rio de Janeiro, Brasil. *Rev. Bras. Zool.*, 19: 1139-1146. doi: 10.1590/S0101-81752002000400019.

ATSDR, Agency for Toxic Substances and Disease Registry, 1994, *Toxicological Profile*. US. Dept. of Health and Human Services, Atlanta. TP-93/05, CAS# 7439-97-6.

BERNTSSEN, M. H. G., HYLLAND, K., JULSHAMN, K., LUNDEBYE, A. K. & WAAGBO, R., 2004, Maximum limits of organic and inorganic mercury in fish feed. *Aquacult. Nutr.*, 10: 83-97. doi: 10.1046/j.1365-2095.2003.00282.x.

BOMBAIL, V., AW, D., GORDON, E. & BATTY, J., 2001, Application of the comet and micronucleus assays to butterfish (*Pholis gunnelus*) erythrocytes from the Firth of Forth, Scotland. *Chemosphere*, 44: 383-392. doi:10.1016/S0045-6535(00)00300-3.

BRUGGEMAN, W. A., 1982, Hydrophobic interactions in the aquatic environment. *In:* O. Hutzinger (ed), *The handbook of environmental chemistry*. Spring-Verlag, Germany.

CAMPOS, A. N., 2000, *Ensaio de adaptação e utilização de um 'índice de risco ecológico potencial' para controle da contaminação por metais pesados em ambiente costeiro tropical*: Baía de Guanabara, RJ – Brasil. Dissertação (Mestrado em Geociências-Geoquímica Ambiental) - Universidade Federal Fluminense, Niterói.

CARDOSO, A. G. A., BOAVENTURA, G. R., SILVA FILHO, E. V. & BROAD, J., 2001, Metal distribution in sediments from the Ribeira Bay, Rio de Janeiro-Brazil. *J.Braz.Chem. Soc.*, 12: 767-774. doi:10.1590/S0103-50532001000600013.

CARVALHO-FILHO, A., 1999, *Peixes*: Costa Brasileira. São Paulo: Melro. 320p.

CASTILHOS, Z. C., 1999, *Gestão em poluição ambiental: análise da contribuição dos garimpos de ouro na contaminação por mercúrio da ictiofauna e das águas fluviais na região do rio Tapajós, estado do Pará, Brasil*. Tese (Doutorado em Geociências-Geoquímica Ambiental) - Universidade Federal Fluminense, Niterói.

CASTILHOS, Z. C., ALMOSNY, N. R. P., SOUTO, P. S., PEREIRA DA SILVA, L. C. C., LINDE, A. R. & BIDONE, E. D., 2004, Bioassessment of ecological risk of amazonian ichthyofauna to mercury. *Bull. Environ. Contam. Toxicol.*, 72: 671-679. doi: 10.1007/s00128-004-0297-0.

CAVALCANTE, D. G. S. M., MARTINEZ, C. B. R. & SOFIA, S. H., 2008, Genotoxic effects of Roundup® on the fish *Prochilodus lineatus*. *Mutat Res.,* 655: 41-46. doi:10.1016/j.mrgentox.2008.06.010.

CAVAS, T., 2008, In vivo genotoxicity of mercury chloride and lead acetate: Micronucleus test on acridine orange stained fish cells. *Food Chem. Toxicol.*, 46: 352-358. doi: 10.1016/j.fct.2007.08.015.

CHAVES, P. T. C. & VENDEL, A. L., 1996, Aspectos da alimentação de *Genidens genidens* (Valenciennes) (Siluriformes, Ariidae) na Baía de Guaratuba, Paraná. *Rev. Bras. Zool.*, 13: 669-675. doi: 10.1590/S0101-81751996000300016.

CUNHA, J. B., LIMA, J. S. & FARIA, M. V. C., 1991, Brain acetylcholinesterase as an in vitro detector of organophosphorus and carbamate insecticides in water. *Water Res.*, 25: 835-840. doi: 10.1016/0043-1354(91)90164-L.

ELMAN, G. L., COURTNEY, K., ANDRES, J. R. A. & FEATHERSTONE, R., 1961, A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, 7: 89-95. doi: 10.1016/0006-2952(61)90145-9.

FONSECA, M. B., GLUSCZAK, L., MORAES, B. S., MENEZES, C. C., PRETTO, A., TIERNO, M. A., ZANELLA, R., GONÇALVES, F. F. & LORO, V. L., 2008, The 2,4-D herbicide effects on acetylcholinesterase activity and metabolic parameters of piava freshwater fish (*Leporinus obtusidens*). *Ecotoxicol. Environ. Saf.,* 69: 416-420. doi: 10.1016/j.ecoenv.2007.08.006.

FRIEDMANN, A. S., WATZIN, M. C., BRINCK-JOHNSEN, T. & LEITER, J. C., 1996, Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). *Aquat. Toxicol.*, 35: 265-278. doi: 10.1016/0166-445X(96)00796-5.

GILL, T. S. & PANT, J., 1985, Mercury-induced blood anomalies in the freshwater teleost *Barbus conchonius* Ham. *Water, Air Soil Pollut.*, 24: 165-171. doi: 10.1007/BF00285441.

GILL, T. S., TEWARI, H. & PANDE, J., 1990, Use of the fish enzyme system in monitoring water quality: effects of mercury on tissue enzymes. *Comp. Biochem. Physiol*., 97: 287-292. doi: 10.1016/0742-8413(90)90143-W.

GUIMARÃES, A. T. B., SILVA DE ASSIS, H. C. & BOEGER, W., 2007, The effect of trichlorfon on acetylcholinesterase activity and histopathology of cultivated fish *Oreochromis niloticus*. *Ecotoxicol. Environ. Saf.*, 68: 57-62. doi: 10.1016/j.ecoenv.2006.08.005.

HACON, S., ROCHEDO, E. R. R., CAMPOS, R. R. R. & LACERDA, L. D., 1997, Mercury exposure through fish consumption in the urban area of Alta Floresta in the Amazon basin. *J. Geochem. Explor.,* 58: 209-216. doi: 10.1016/S0375-6742(96)00066-0.

HAMMERSCHMITD, C. R., SANDHEINRICH, M. B., WIENER, J. G. & RADA, R. G., 2002, Effects of dietary MeHg on reproduction of fathed minnows. *Environ. Sci. Technol.*, 36: 877-883. doi: 10.1021/es011120p.

KEHRIG, H. A., MALM, O. & MOREIRA, I., 1998, Mercury in a widely consumed fish *Micropogonias furnieri* (Demarest.1823) from four main Brazilian estuaries. *Sci. Total Environ.*, 213: 263-271. doi: 10.1016/S0048-9697(98)00099-0.

KEHRIG, H. A., MOREIRA, I., MALM, O. & PFEIFFER, W. C., 2001, Especiação e acumulação de mercúrio pela biota da Baía de Guanabara – RJ. *In:* R. Moraes, M. Crapez, W. Pfeiffer, M. Farina, A. Bainy & V. Teixeira, *Efeitos de poluentes em organismos marinhos*. Arte Ciência Villipress.

KJERFVE, B., RIBEIRO, C. H. A., DIAS, G. T. M., FILIPPO, A. M. & QUARESMA, V. S., 1997, Oceanographic characteristics of an impacted coastal bay: Baia de Guanabara, Rio de Janeiro, Brazil. *Cont. Shelf Res.*, 17: 1609-1643. doi: 10.1016/S0278-4343(97)00028-9.

LATIF, M. A., BODALY, R. A., JOHNSTON, T. A. & FUDGE, R. J. P., 2001, Effects of environmental and maternally derived methylmercury on the embryonic and larval stages of walley (*Stizostedion vitreum*). *Environ. Pollut.*, 111: 139-148. doi: 10.1016/S0269-7491(99)00330-9.

LAVADO, R., UREÑA, R., MARTIN-SKILTON, R., TORREBLANCA, A., DEL RAMO, J., RALDÚA, D. & PORTE, C., 2006, The combined use of chemical and biochemical markers to assess water quality along the Ebro River Ramo. *Environ. Pollut.*, 139: 330-339. doi: 10.1016/j.envpol.2005.05.003.

LEMOS, C. T., RÖDEL, P. M., TERRA, N. R., D'AVILA DE OLIVEIRA, N. C. & ERDTMANN, B., 2007, River water genotoxicity evaluation using micronucleus assay in fish erythrocytes. *Ecotoxicol. Environ. Saf.*, 66: 391-401. doi: 10.1016/j.ecoenv.2006.01.004.

LIMA, S. C., 1985, *Estudo sedimentológico, textural, mineralógico e geoquímico dos sedimentos superficiais e do material em suspensão na Baía da Ribeira – Angra dos Reis – RJ*. Dissertação (Mestrado em Geociências-Geoquímica Ambiental) - Universidade Federal Fluminense, Niterói.

LINDE-ARIAS, A. R., INÁCIO, A. F., DE ALBURQUERQUE, C., FREIRE, M. M. & MOREIRA, J. C., 2008, Biomarkers in an invasive fish species, *Oreochromis niloticus*, to assess the effects of pollution in a highly degraded Brazilian river. *Sci. Total Environ.*, 399: 186-192. doi: 10.1016/j.scitotenv.2008.03.028.

LOHNER, T. W., REASH, R. J., WILLET, V. E. & ROSE, L. A., 2001. Assessment of tolerant sunfish populations (*Lepomis* sp.) inhabiting selenium-laden coal ash effluents. *Ecotoxicol. Environ. Saf.*, 50: 203-216. doi: 10.1006/eesa.2001.2097.

LOPEZ-CARILLO, L. & LOPEZ-CERVANTES, M., 1993, Effect of exposure to organophosphate pesticides on serum cholinesterase levels. *Arch. Environ. Health*, 48: 359-363.

MALM, O., 1998, Gold mining as a source of mercury exposure in the Brazilian Amazon. *Environ Res.,* 77: 73-78. doi: 10.1006/enrs.1998.3828.

MCKIM, J. M., OLSON, G. F., HOLCOMBE, G. W. & HUNT, E. P., 1976, Long-term effects of methylmercury chloride on three generations of brook trout (*Salvelinus fontinalis*): Toxicity, accumulation, distribution, and elimination. *J. Fish. Res. Board Can.*, 33: 2726-2739.

MONSERRAT, J. M., MARTÍNEZ, P. E., GERACITANO, L. A., AMADO, L. L., MARTINS, C. M. G., PINHO, G. L. L., CHAVES, I. S., FERREIRA-CRAVO, M., VENTURA-LIMA, J. & BIANCHINI, A., 2007, Pollution biomarkers in estuarine animals: Critical review and new perspectives. *Comp. Biochem. Physiol.*, 146: 221-234. doi: 10.1016/j.cbpc.2006.08.012.

MORETTO, M. B., LERMEN, C. L., MORSCH, V. M., BOHRER, D., INEU, R. F., DA SILVA, A. C., BALZ, D. & SCHETINGER, M. R. C., 2004, Effect of subchronic treatment with mercury chloride on NTPDase, 5'-nucleotidase and acetylcholinesterase from cerebral cortex of rats. *J. Trace Elem. Med. Biol*., 17: 255-260. doi: 10.1016/S0946-672X(04)80027-0.

NELSON, J. S., 1994, *Fishes of the World*. 3 ed. John Wiley & Sons, New York, 600p.

NEPOMUCENO, J. C., 1997, Detection of micronuclei in peripheral erythrocytes of *Cyprinus carpio* exposed to metallic mercury. *Environ. Mol. Mutagen.*, 30: 293-297. doi: 10.1002/(SICI)1098-2280(1997)30:3<293::AID-EM7>3.0.CO;2-M.

OLIVEIRA RIBEIRO, C. A., FILIPAK NETO, F., MELA, M., SILVA, P. H., RANDI, M. A. F., RABITTO, I. S., ALVES COSTA, J. R. M. & PELLETIER, E., 2006, Hematological findings in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methylmercury, inorganic lead, and tributyltin chloride. *Environ. Res.*, 101: 74-80. doi: 10.1016/j.envres.2005.11.005.

OLIVEIRA SILVA, J. J., ALVES, S. R., INÁCIO, A. F., MEYER, A., SARCINELLI, P. N., FERREIRA, M. F., CUNHA, J. C. & MOREIRA, J. C., 2000, Cholinesterase activities determination in frozen blood samples: An improvement to the occupational monitoring in developing countries. *Hum. Exp. Toxicol*., 19: 173-177. doi: 10.1191/096032700678827762.

OLIVEIRA, M. M., SILVA FILHO, M. V., CUNHA BASTOS, V. L. F., FERNANDES, F. C. & BASTOS, J. C., 2007, Brain acetylcholinesterase as a marine pesticide biomarker using Brazilian fishes. *Mar. Environ. Res.*, 63: 303-312. doi: 10.1016/j.marenvres.2006.10.002.

OLSON, K. R., BERGMAN, H. L. & FROMM, P. O., 1973, Uptake of methyl mercury chloride and mercuric chloride by trout: A study of uptake pathways into the whole animal and uptake by erythrocytes in vitro. *J. Fish. Res. Board Can.*, 30: 1293-1299.

PEREIRA, C. A. B., 1991, Teste estatístico para comparar proporções em problemas de citogenética. *In:* N. Rabello-Gay, M. A. Rodrigues & R. Monteleone-Neto (eds.), *Mutagênese, Teratogênese e Carcinogênese*: métodos e critérios para avaliação. Sociedade Brasileira de Genética, p. 113-121.

PEREIRA, R. C. & GOMES, A. S., 2002, *Biologia Marinha*. Interciência, Rio de Janeiro, 382p.

PORTO, J. I. R., ARAUJO, C. S. O. & FELDBERG, E., 2005, Mutagenic effects of mercury pollution as revealed by micronucleus test on three Amazonian fish species. *Environ. Res.*, 97: 287-292. doi: 10.1016/j.envres.2004.04.006.

RODRIGUES, A. P. C. & CASTILHOS, Z. C., 2003, Avaliação de risco ecológico em ecossistemas aquáticos contaminados por mercúrio. Estudo de caso: Ilha das Enxadas, Baía de Guanabara, RJ. *In: Jornada de Iniciação Científica*, 11., CETEM, Rio de Janeiro.

RODRIGUES, A. P. C., 2006, *Avaliação de risco ecológico associado à contaminação mercurial em dois estuários do estado do Rio de Janeiro: Baía de Guanabara e Baía da Ribeira.* Dissertação (Mestrado em Geoquímica) - Universidade Federal Fluminense, Niterói, 97p.

SANCHEZ-GALAN, S., LINDE, A. N., AYLLON, F. & GARCIA-VAZQUEZ, E., 2001, Induction of micronuclei in Eel (*Anguilla anguilla* L.) by heavy metals. *Ecotoxicol. Environ. Saf.*, 49: 139-143. doi: 10.1006/eesa.2001.2048.

SILVA, L. C. C. P., 2004, *Avaliação da contaminação mercurial sobre parâmetros hematológicos e bioquímicos plasmáticos de tucunarés (Cichla spp.) oriundos da região do Rio Tapajós, Pará, Brasil.* Dissertação (Mestrado em Medicina Veterinária) - Universidade Federal Fluminense.

SOUTO, P. S. S., 2004, *Risco ecológico associado a contaminação mercurial em ecossistemas aquáticos da Amazônia: Região do rio Tapajós, estado do Pará, Brasil. Caracterização através de biomarcadores no gênero Cichla (tucunarés).* Tese (Doutorado em Geociências-Geoquímica Ambiental) - Universidade Federal Fluminense, Niterói.

ULLRICH, S. M., TANTON, T. W. & ABDRACHITOVA, S. A., 2001, Mercury in the aquatic environment: a review of factors affecting methylation. *Crit. Rev. Env. Sci. Technol.*, 31: 241-293. doi:10.1080/20016491089226.

WASSERMAN, J. C., FREITAS-PINTO, A. A. P. & AMOUROUX, D., 2000, Mercury concentrations in sediment profiles of a degraded tropical coastal environment. *Environ. Technol.*, 21: 297-305. doi: 10.1080/09593332108618117.

WHO, World Health Organization, Environmental Health Criteria (EHC 101), 1990, *Methylmercury*. Geneva, 143p.

WIENER, J. G. & SPRY, D. J., 1996, Toxicological significance of mercury in freshwater fish. *In:* W. N. Beyer, G. H. Heinz & A. W. Redmon-Norwood, *Environmental Contaminants in Wildlife*: Interpreting Tissue Concentrations. Lewis Publishers, Boca Raton, Florida, p. 297-339.

WIENER, J. G., KRABBENHOFT, D. P., HEINZ, G. H. & SCHEUHAMMER, A. M., 2003, Ecotoxicology of mercury. *In:* D. J. Hoffman, B. A. Rattner, G. A. Burton Jr. & J. Cairns Jr. *Handbook of Ecotoxicology*. 2ª ed. Lewis Publishers, Boca Raton, Florida.