

Total mercury in the fish *Trichiurus lepturus* from a tropical estuary in relation to length, weight, and season

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The Goiana River Estuary (7°30'S 34°47'W) is a typical estuary of the semi-arid tropical regions. This estuary shelters a rich fauna of fish, crustaceans and mollusks which play an important role in the life of traditional populations. It is also the main recipient of the effluents from the sugarcane agro-industry and sewage from settlements and villages. *Trichiurus lepturus* (n = 104), from the Goiana Estuary were examined for total mercury contents during ten months (2005 to 2007) spanning two dry seasons and part of a rainy season. The studied individuals showed weight (W) (204.1±97.9 g) and total length (TL) (63.1±10.1 cm, range 29.5-89.0 cm) with a significant (p<0.05) correlation. Correlation between TL and Hg-T (r = 0.37286) and between W and Hg-T (r = 0.38212) were positive and significant (p<0.05). Two-way ANOVA (n = 81) showed that TL and W had significant difference (p<0.05) among seasons. The Hg-T showed differences in relation to the factor season (p<0.05). The correlation between Hg-T and rainfall showed a negative and significant relation (r = -0.56; p<0.05). Rainfall strongly influenced the bioaccumulation of mercury in this species. Drier months showed relatively higher mercury concentrations than the end of the rainy season. Less rainfall, and consequently less particulate matter and less primary production in the estuary, make mercury more bioavailable. Fish from this estuary are fit for human consumption at all times of the year.

O estuário do rio Goiana (7°30'S 34°47'W) é um típico estuário de região tropical semi-árida. Esse estuário abriga uma fauna rica de peixes, crustáceos e moluscos que tem um importante papel na vida da população tradicional. Ele também é o principal receptor de efluentes da agroindústria da cana-de-açúcar e esgoto das comunidades e vilas. *Trichiurus lepturus* (n = 104), do estuário do rio Goiana foram examinados para o conteúdo de mercúrio total durante dez meses (2005 a 2007) ao longo de duas estações secas e parte de uma estação chuvosa. Os indivíduos estudados apresentaram peso (P) (204,1±97,9 g) e comprimento total (CT) (63,1±10,1 cm, variação 29,5-89,0 cm) com uma correlação significativa (p<0,05). As correlações entre CT e Hg-T (r = 0,37286) e entre P e Hg-T (r = 0,38212) foram positivas e significativas. A ANOVA (2 fatores) (n = 81) mostrou que o CT e P apresentaram diferenças significativas (p<0,05) entre as estações. O Hg-T apresentou diferença em relação ao fator estação (p<0,05). A correlação entre Hg-T e a chuva apresentou uma relação negativa e significativa (r = -0,56; p<0,05). A precipitação pluviométrica influenciou fortemente a bioacumulação de mercúrio nessa espécie. Os meses secos apresentaram relativamente concentrações de mercúrio maiores do que o final da estação chuvosa. Isso sugere que a menor precipitação pluviométrica, e consequentemente a menor quantidade de material particulado e menor produtividade primária no estuário, torna o mercúrio mais biodisponível. Peixes desse estuário podem ser consumidos pela população humana ao longo de todo ano.

Key words: Estuarine fish, Mercury bioavailability, Risk assessment, Seasonal changes, Trichiuridae.

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Introduction

Due to its high toxicity and association with historic events of contamination in aquatic environments, mercury occupies a place of prominence among heavy metals (Kasper *et al.*, 2007). It is known to be a persistent environmental pollutant (McAloon & Mason, 2003) and to suffer a number of transformations in the aquatic environment once directly or indirectly introduced. It is an element that has both natural and anthropogenic sources (OSPAR, 2004) to coastal environments, usually gaining access to it through estuaries.

The presence and behaviour of mercury in aquatic systems is then of great interest and importance. Also, it is the only heavy metal which bioaccumulates and biomagnifies through all levels of the aquatic food web (Lindqvist *et al.*, 1991; Kehrig *et al.*, 1998). Mercury levels in marine organisms tend to increase with growth and trophic level (Cronin *et al.*, 1998; Power *et al.*, 2002; Kehrig *et al.*, 2009; Seixas *et al.*, 2009).

Fish are considered good indicators for the evaluation of mercury pollution (Kasper *et al.*, 2007) and, are also an important fisheries resource (Hylander *et al.*, 2000). The presence of mercury in fish allows for the assessment of the metal's bioavailability in aquatic systems. Fish of different species have been frequently used as bioindicators of environmental contamination by mercury in estuaries (Kehrig *et al.*, 2002; Sarger, 2002; Kehrig *et al.*, 2004; Weis & Ashley, 2007). Top predators can be used as bioindicator of mercury contamination and biomagnification. Fish consumed by human populations, can be used to monitor aspects of mercury contamination of aquatic environments related to human health (Malm *et al.*, 1995; Al-Majed & Preston, 1999) and resource sustainability. Several species of fish use tropical estuaries as nursery, feeding grounds, and shelter at different stages of their life cycles (Beck *et al.*, 2001; Blaber, 2007).

Variations in the concentrations of mercury in fish may also be linked to the migration of fish to areas where food is more, or less, contaminated, and will also depend on the residence time in each area (Francesconi & Lenanton, 1992; Al-Majed & Preston, 1999). Seasonal differences in fish mercury levels (Weis *et al.*, 1986; Kehrig *et al.*, 1998; Weis & Ashley, 2007) due to different environmental factors were attributed to differences in temperature, dissolved oxygen levels and aquatic primary production (Huchabee *et al.*, 1979).

Trichiurus lepturus Linnaeus, 1758 is a cosmopolitan species that occurs along the whole tropical and sub-tropical latitude belt, including the Western Atlantic and the Brazilian coast (Figueiredo & Menezes, 2000; Martins & Haimovici, 2000; Di Benedetto & Ramos, 2004; Barletta *et al.*, 2005; Martins *et al.*, 2005; Di Benedetto & Siciliano, 2007; Barletta *et al.*, 2008). *Trichiurus lepturus* is near the top of the marine food web. It is preyed by many species of cetaceans, seals, sharks, and consumed by human coastal populations (Di Benedetto & Ramos, 2004; Garcia-Rodriguez & Auriolles-Gamboa, 2004; Di Benedetto & Siciliano, 2007). It is fished using a number of different techniques from shallow estuaries, where it is fished with artisanal tools, to deep waters where it

can be accidentally captured in tuna long lines (by catch). This species is a marine straggler (Barletta & Blaber, 2007; Elliot *et al.*, 2007), switching between estuaries and the adjacent seas as life cycle and food supplies demand.

The present study aimed at determining the concentration of total mercury (Hg-T) in the muscle of *Trichiurus lepturus* and its relations with biological variables (weight and length), as well as to detect seasonal variations related to the tropical rainfall regime. It was also verified if the level of mercury in fish was within the limit allowed by the World Health Organization (WHO) for safe human consumption. This study was the first in tropical South America to systematically use *T. lepturus* as bioindicator of mercury pollution and, to our knowledge, the first in the world comparing levels of mercury in this species along seasons.

Material and Methods

Study area

The Goiana Estuary (7°30'S 34°47'W) has 17 km of main channel from Goiana City to its mouth, and spreads across an area of ~475 000 m², including the floodplain (Fig. 1). The area is encircled by mangrove lined shores and islands. The estuary shelters a rich fauna of fish, crustaceans and mollusks which play an important role in the lives of traditional populations, determining quality and quantity of their subsistence catch and artisanal fisheries activities (Barletta & Costa, 2009). In this estuary, *T. lepturus* is frequently consumed and sold by the local population. It is a typical estuary of the semi-arid tropical regions, and the Brazilian East coast has dozens of small estuaries with similar natural and anthropic characteristics. The mangrove forests surrounding the estuary are rapidly losing area to sugarcane plantations and urban areas. This estuary is the main recipient of the effluents from the sugarcane agroindustry and sewage from settlements and villages. However, it is generally considered to be well preserved, due to the remaining extents of mangrove forests.

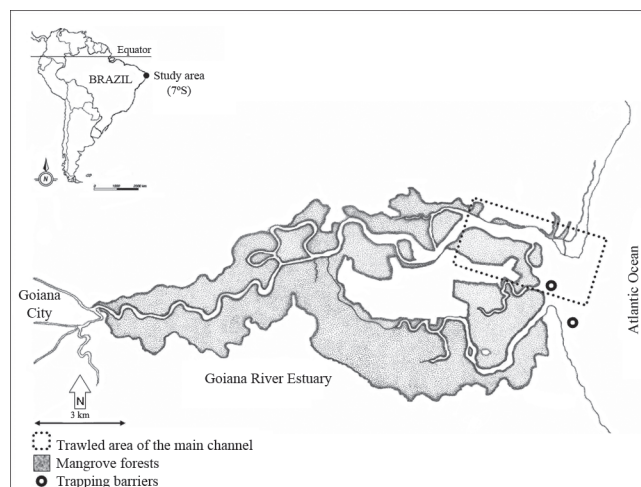


Fig. 1. Location and details of the study area, Goiana Estuary at the Brazilian Northeast.

It supports traditional communities with fisheries (mollusks, fish and crustacean), wood from mangroves and some small tourist business and its associated services (ferry boat, bars and restaurants).

Fish samples

Fish samples (n = 104) were taken from the low estuary from November 2005 to February 2006 and August 2006 to January 2007, at a trapping barrier (Barletta *et al.*, 1998) used by the traditional community, which works as a fish trap or fish weir. Samples were also taken with an otter trawl net along the main channel of the low estuary (Barletta *et al.*, 2005).

Individuals were measured (total length in cm) and weighed (g) immediately after sampling. A sample from the back dorso-lateral muscle was removed from each individual (FAO/SIDA, 1983). Tissue samples were placed in disposable plastic centrifuge tubes, labeled and frozen (-18°C). All tissues were freeze-dried and lost 75% water, on average.

Mercury analysis

A muscle sample of 0.05 g (dry weight) was acid digested with 3.0 mL of a H₂SO₄:HNO₃ (1:1 v/v) mixture and 1.0 mL of H₂O₂ in an open system at 65°C for 1 hour, or until a complete digestion. Potassium permanganate (KMnO₄) was added and after cooling for 24 h, and just before mercury determination, samples were clarified with hydroxylamine, and completed with Milli-Q water. Mercury was determined by cold vapor atomic absorption spectrometry (FIMS - Perkin Elmer AS 90), using sodium borohydride (NaBH₄) as the reducing agent (Kehrig *et al.*, 2006; Kehrig *et al.*, 2008).

Precision and accuracy of the analytical methods were determined and monitored using certified reference materials obtained from the International Atomic Energy Agency (IAEA 436 - tuna fish muscle). The analytical results showed good precision and accuracy (Table 1).

Except when related to human intake (based on wet weight), all other total mercury concentration results presented here are on a dry weight base.

Statistical analysis

Statistical analyses were performed using STATISTICA® 6.0 for Windows (StatSoft, Inc. 1984-2001, USA). Linear regressions were used to test the existence of relationships between the biological variables (weight and total length), the concentration of total mercury and rainfall. Total mercury concentration and weight data were log-transformed.

Two-way ANOVA was used to test differences in concentration of total mercury among seasons and months (dry season 1: November 2005, December 2005, January 2006; end rainy season: August 2006, September 2006, October 2006; dry season 2: November 2006, December 2006 and January 2007) and total length and weight among seasons and months. The Cochran's test was used to check the homogeneity of variances. Tukey's test (*post hoc*; p<0.05) was used whenever significant differences were detected (Quinn and Keough, 2003).

Table 1. Certified reference values and analyzed values for total mercury (µgHg.g⁻¹) in certified standard materials TORT-2 (lobster hepatopancreas) and IAEA 436 (tuna fish).

	Certified Reference Value	Present study (April/2007) (n = 8)	(January/2007) (n = 10)
	Mean±S.D.	Mean±S.D.	
TORT-2 (NRCC)	0.27±0.06	0.24±0.1	0.29±0.03
IAEA 436 (IAEA)	4.64±0.26	4.32±0.56	-

Results

Rainfall

The total monthly precipitation in the area can be divided in four main hydrological seasons (Barletta *et al.*, 2005). The months during the present work spanned across two dry seasons and one rainy season. During D1, dry season 1 (November 2005 to January 2006) mean (±SD) rainfall was 12.7±4.2 mm. During R, the end of the rainy season (August 2006 to October 2006) mean (±SD) rainfall was 91.2±89.2 mm. Finally in D2, dry season 2 (November 2006 to January 2007) mean (±SD) rainfall was 91.5±12.9 mm (Fig. 2). There was no *T. lepturus* capture during the early rainy season of 2006.

Biological variables and Total mercury concentration in *Trichiurus lepturus* - Seasonal variations.

Mean (±SD) weight (204.1±97.9 g; total biomass = 222.7 g) and total length (63.1±10.1 cm; range 29.5-89.0 cm) showed that the studied group (n = 104) was composed of sub-adult (30-70 cm; n = 71) and adult fish (>70 cm; n = 33) according to the size intervals for the Western Atlantic (Martins & Haimovici, 1997). The analysis of all samples (n = 104) of *Trichiurus lepturus* presented a positive and significant (p<0.05) correlation between weight and total length (r = 0.8273) (Fig. 3a). The correlation between total length and log total mercury concentrations (r = 0.37286) was also positive and significant (p<0.05) (Fig. 3b). The correlation (r = 0.38212) between weight and log total mercury was similarly positive and significant (p<0.05) (Fig. 3c).

ANOVA (n = 81) showed that total length had a significant difference (p<0.05) among seasons. Total length in D1 was greater than in D2 and R, respectively (Fig. 4a). There was a significant interaction between factors season and month. Weight showed a significant difference (p<0.05) for the factors season and month. In D1 weight was greater than in D2 and R respectively (Fig. 4b). Also, a significant interaction between the factors season and month was found. Log total mercury concentrations in *T. lepturus* differed significantly among seasons (p<0.05). Total mercury concentrations in D1 were greater than in D2 and R respectively (Fig. 4c). Significant differences among the months comprising each season were not detected for log total mercury concentrations. Significant interactions between the factors season and month were not observed in relation to log total mercury concentrations.

Table 2. Biological parameters and respective mean total mercury concentrations ($\mu\text{gHg}\cdot\text{T}\cdot\text{kg}^{-1}$ in the muscle) of *Trichiurus lepturus* from Goiana Estuary in the study period.

Seasons	Year	N	Length (cm) Mean \pm SD (Range)	Weight (g) Mean \pm SD (Range)	$\mu\text{gHg}\cdot\text{T}\cdot\text{kg}^{-1}$ Mean \pm SD (Range)
D1	2005/2006	27	67.0 \pm 6.8 (56.0-79.6)	256.0 \pm 73.7 (107.5-387.0)	773.4 \pm 207.5 (408.7-1283.4)
R	2006	27	54.2 \pm 9.8 (26.0-69.0)	104.6 \pm 55.9 (18.6-252.0)	331.2 \pm 138.5 (154.3-654.1)
D2	2006/2007	27	66.8 \pm 7.4 (53.0-78.0)	220.9 \pm 73.0 (113.0-354.1)	370.1 \pm 78.8 (241.5-551.6)

When comparing D1, R and D2, it was observed that in D1, individuals showed a slightly larger length, more weight and higher concentrations of mercury (Table 2). The correlation ($r = -0.56$) between rainfall and log total mercury was negative and significant ($p < 0.05$) (Fig. 5).

Mercury concentration in *Trichiurus lepturus*: is Human consumption possible?

Average total mercury in fish muscle during the present work was $501.0 \pm 247.5 \mu\text{gHg}\cdot\text{T}\cdot\text{kg}^{-1}$ dry wt., corresponding to $125.3 \pm 61.9 \mu\text{gHg}\cdot\text{T}\cdot\text{kg}^{-1}$ wet wt. (Table 3). It follows that D1 had an average total mercury content of $193.4 \pm 51.9 \mu\text{g}\cdot\text{kg}^{-1}$ wet wt.; R $82.8 \pm 34.6 \mu\text{g}\cdot\text{kg}^{-1}$ wet wt. and; D2 showed a mean mercury concentration of $92.5 \pm 19.7 \mu\text{g}\cdot\text{kg}^{-1}$ wet wt (Table 2). The World Health Organization Provisional Tolerable Weekly Intake (PTWI) allows the ingestion of $3.3 \mu\text{gHg}\cdot\text{T}\cdot\text{kg}^{-1}$ per week (wet wt.) for the general population. A 70 kg person,

eating 100g of *T. lepturus* from the Goiana Estuary daily, would ingest, on average, $1.26 \mu\text{g}$ of total mercury per week. In D1 this intake would be of $1.96 \mu\text{g}$ per week; R $0.84 \mu\text{g}$ per week and, D2 $0.91 \mu\text{g}$ per week (Table 2).

Discussion

Trichiurus lepturus was not captured during the early and high rainy seasons at the Goiana Estuary using the available techniques. Probably during this time it seldom penetrates the estuary since shoals of its main prey (sardines and anchovies) are not present either (Bittar *et al.*, 2008). Both, prey and predator, probably remain along the coast where salinity is more stable at this time of the year (Barletta *et al.*, 2005), which confirms its status as a marine straggler reported in the literature (Barletta & Blaber, 2007; Elliot *et al.*, 2007).

Correlations between size and concentration of total mercury and weight and concentration of total mercury confirm that this metal is undergoing bioaccumulation in piscivorous fish of this species at the Goiana Estuary. The correlation between mercury concentration and biological parameters in fish can be influenced by age/growth rate, season and food availability (quantity and quality) (Huchabee *et al.*, 1979; Kehrig *et al.*, 1998). The present results suggest a process of bioaccumulation of mercury at the Goiana Estuary, which may be noted by the increase in the concentration of total mercury in fish with the increase of size and weight. This same correlation of increased concentration

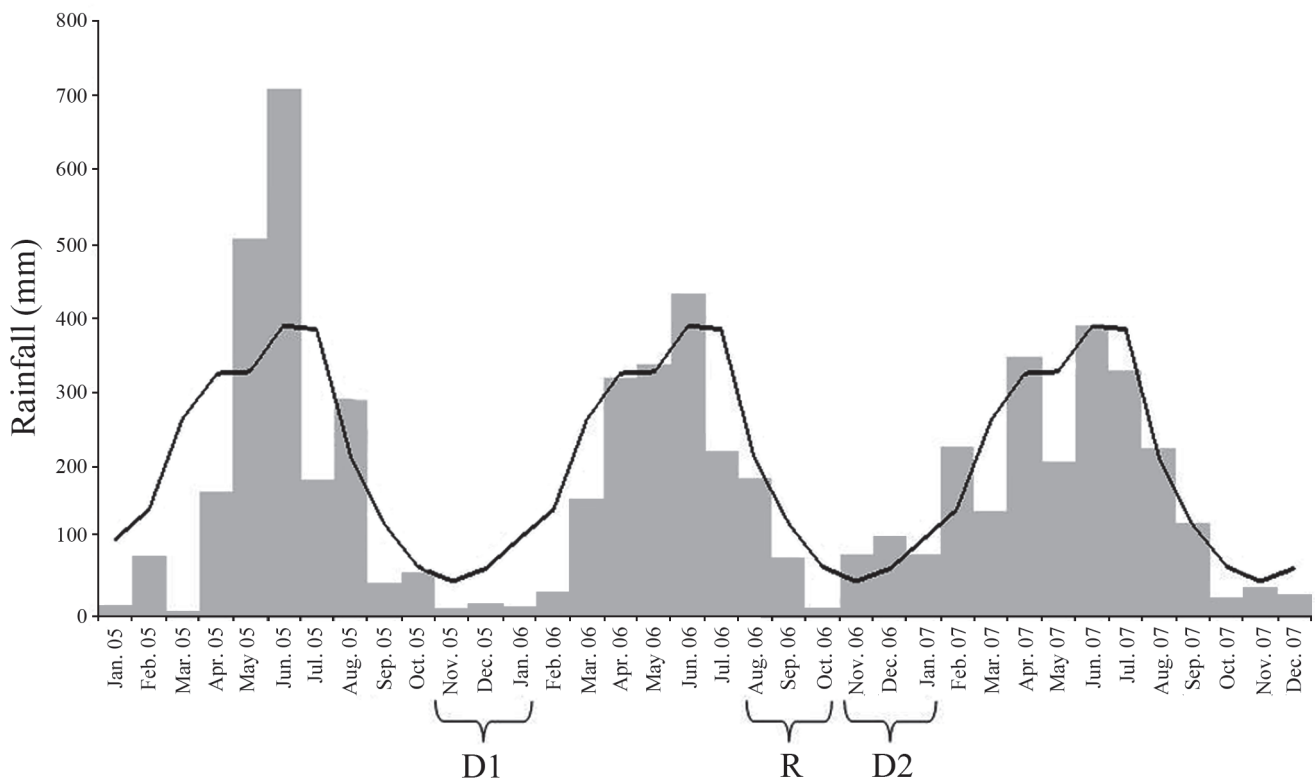


Fig. 2. Rainfall in the study area from a historic (1961 to 1990) data set (solid line) and from 2005-2007 (bars). Source: <http://www.inmet.gov.br> - meteorological station Recife-82.898).

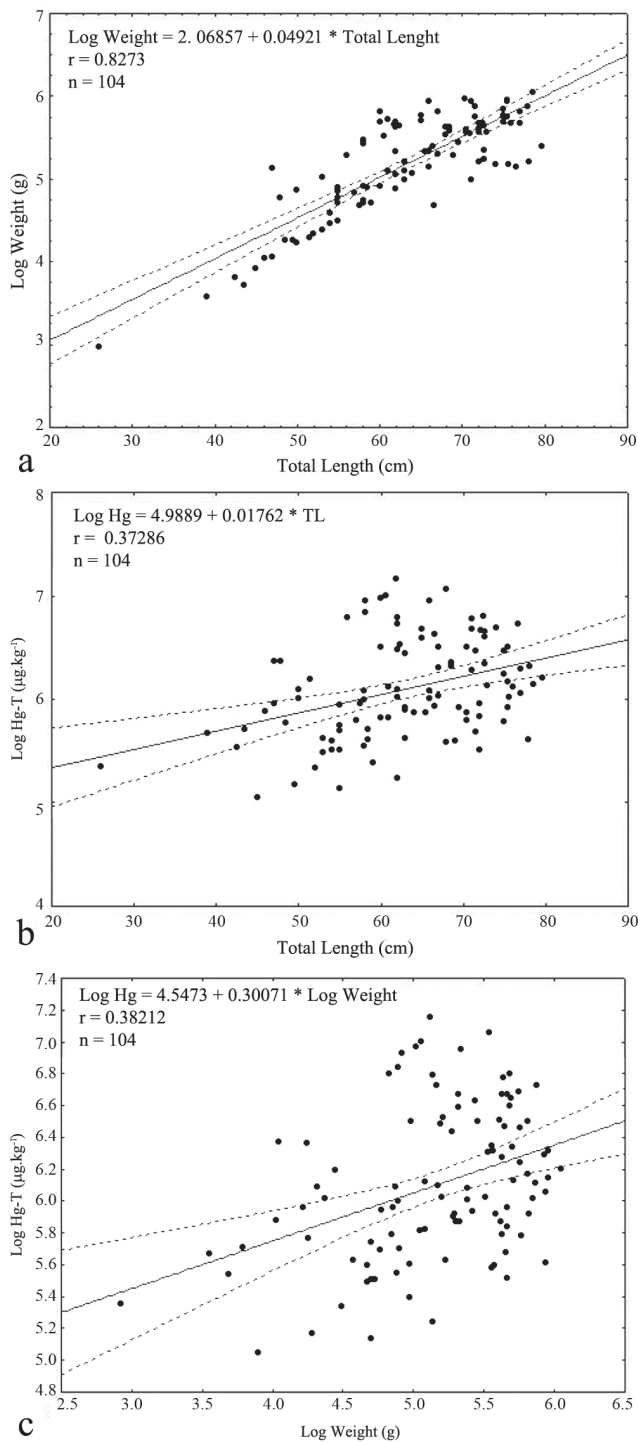


Fig. 3. Correlations ($n = 104$). **a.** Log weight (g) in respect to total length (cm); **b.** Log (mgHg-T.kg⁻¹ in the muscle) in relation to total length (cm); **c.** Log (mgHg-T.kg⁻¹) in relation to log weight (g) of *Trichiurus lepturus* from the Goiana Estuary from November 2005 to January 2007. All plots show a 95% confidence interval.

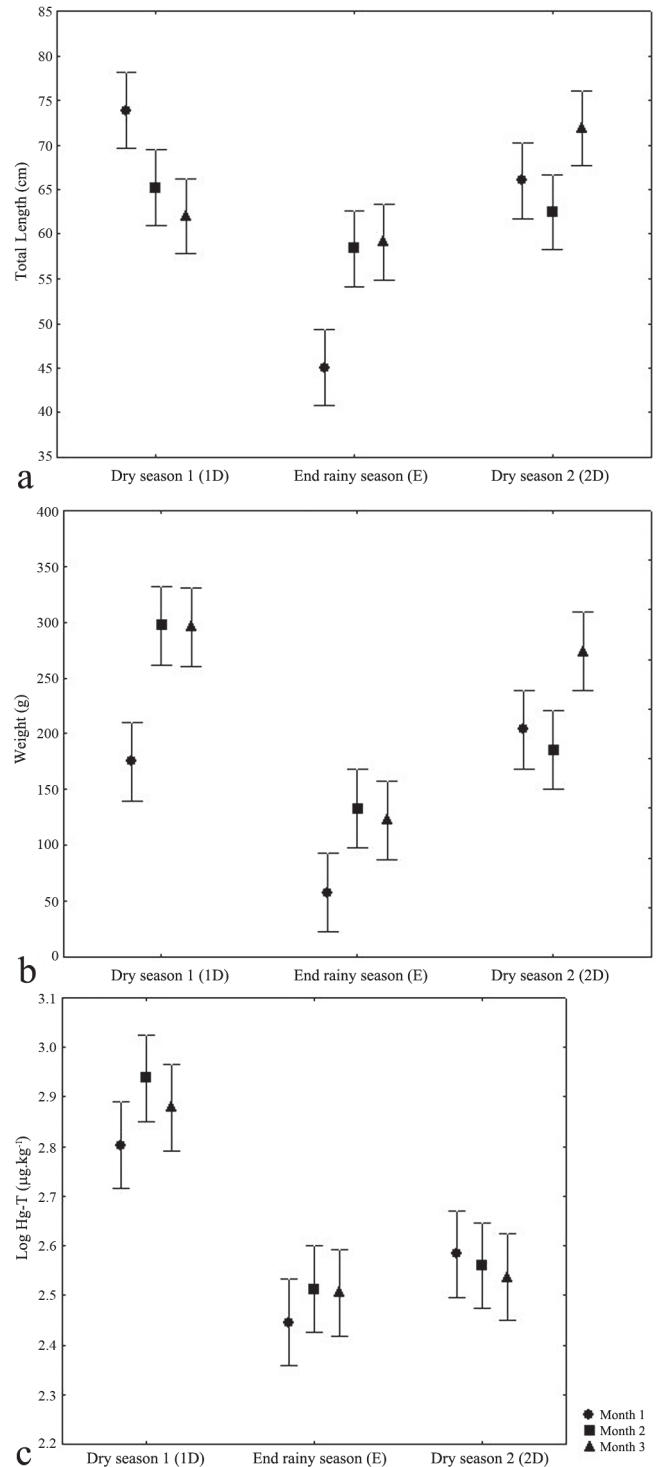


Fig. 4. Two-way ANOVA. **a.** Total length (cm); **b.** Weight (g); **c.** Log (mgHg-T.kg⁻¹ in the muscle) considering seasons and months (1 = dry season 1 - November and December 2005, January 2006; 2 = end of the rainy season - August to October 2006; 3 = dry season 2 - November and December 2006, January 2007).

Table 3. Comparisons of (Mean±S.D.) length (cm), weight (g), total mercury concentration in the muscle (mgHg-T.kg⁻¹) and mean estimates of weekly intake (assuming a regular consumption of 100 g fish.day⁻¹ wet wt.) for *Trichiurus lepturus* from four different countries/regions based on WHO (1976, 1989). **a.** Present study; **b.** Prudente *et al.*, 1997; **c.** Mol *et al.*, 2000; **d.** Kehrig *et al.*, 2004; **e.** Kehrig *et al.*, 2009.

Country	N	Length (cm) (Range)	Weight (g) (Range)	µgHg-T.kg ⁻¹ (dry wt.) (Range)	µgHg-T.kg ⁻¹ (wet wt.)	µgHg-T.kg ⁻¹ (wet wt.) per week
Brazil ^a	104	63.1±10.1 (26.0-79.6)	204.1±97.9 (18.6-424.0)	501.0±247.5 (154.3-1283.4)	125.3	1.26
Philippines ^b	1	51.0		123.0	30.8	0.31
Suriname ^c	2	114.8±5.9 (110.6-119.0)	1190.0±490.0 (843.0-1536.0)	620.0±283 (600.0-640.0)	155.0	1.55
Brazil ^d	28	87.5±19.3 (62.0-138.0)	515.2±350.7 (180.0-1360.0)	312.6±156.8 (152.0-840.0)	78.2	0.78
Brazil ^e	17			1070±1060	267.5	2.68

of total mercury with length and weight of fishes, was also observed for other species in other situations (Kehrig *et al.*, 1998; Pinho *et al.*, 2002; Kehrig *et al.*, 2004; Adams & Onorato, 2005; Weis & Ashley, 2007).

A lower concentration of mercury in fish will be expected with higher precipitation. This can be explained by the dilution of mercury in the larger volumes of water and adsorption onto particulate matter that will be directly exported to the sea without a long residence time within the estuary. With more rainfall in dry season 2, the estuary presented a predominantly eutrophic condition with a lot of suspended matter entering the system. Both these factors dilute mercury inputs, with a consequent decrease of its bioavailability (Kehrig *et al.*, 1998; Kehrig *et al.*, 2001), resulting in lower mercury concentrations in fish.

The main likely source of mercury to this estuary is diffuse land runoff. The lower basin of the Goiana River is almost totally taken by sugarcane plantations and urban areas,

leaving only a very narrow mangrove fringe around the estuary. Urban effluents contribution is another probable source, since the area has no sewage treatment systems.

When *Trichiurus lepturus* preys inside estuaries, probably it is contaminated with mercury due to the trophic transfer of this element along the food web, which has been acknowledged as an important process influencing bioaccumulation (Fisher & Reinfelder, 1995). When leaves to coastal waters *T. lepturus* acts as a transporting agent of mercury from the estuary to the sea. When consumed by its own predators such as *Sotalia guianensis* (Cetacea: Delphinidae) (Di Benedetto & Ramos, 2004; Di Benedetto & Siciliano, 2007), *T. lepturus* will transfer its mercury burden to other levels of the marine trophic web.

There are only few studies considering mercury contamination of this socially and economically important species at estuarine and coastal regions around the world. Most of them have limited number of individuals and time span. Comparing total mercury (wet wt.) in *Trichiurus lepturus* from the Goiana Estuary - Brazilian Northeast: 125.3 µgHg-T.kg⁻¹ wet wt., with results from Guanabara Bay - Brazilian Southeast: 78.2 µgHg-T.kg⁻¹ wet wt. (Kehrig *et al.*, 2004), Suriname: 155.0 µgHg-T.kg⁻¹ wet wt. (Mol *et al.*, 2000), Philippines: 30.8 µgHg-T.kg⁻¹ wet wt. (Prudente *et al.*, 1997), and the Northern Coast of Rio de Janeiro - Brazilian Southeast: 267.5 µgHg-T.kg⁻¹ wet wt. (Kehrig *et al.*, 2009), it is seen that at all sites fish are below the limit suggested by the WHO (500 µgHg-T.kg⁻¹ wet wt.), above which regular human consumption should be controlled.

When present in shallow coastal and estuarine waters *T. lepturus* is frequently captured and consumed by traditional populations as those living around the Goiana Estuary. In the present results indicate that *T. lepturus* did not exceed the WHO-PTWI of maximum 3.3 µgHg-T.kg⁻¹ per week, and can continue to be part of the daily diet of the local population. However, pregnant women and small children should avoid frequent consumption of this species since, for these groups, *T. lepturus* in this estuary are near the tolerable limit for safe regular ingestion, in this cases, a maximum of 1.6 µgHg-T.kg⁻¹ per week.

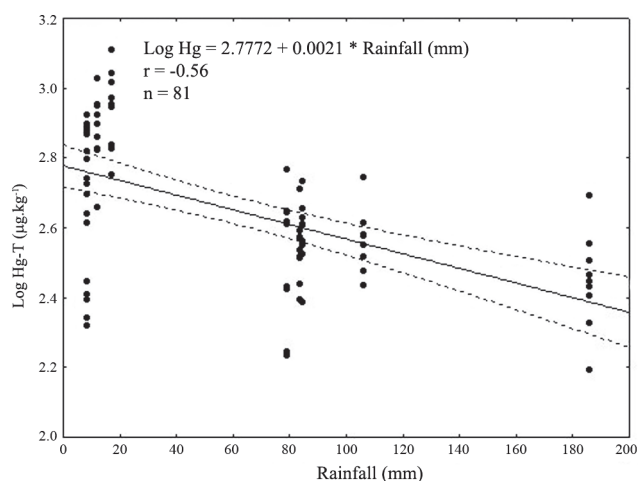


Fig. 5. Correlation (n = 81) between rainfall (mm) and log (µgHg-T.kg⁻¹ in the muscle) of *Trichiurus lepturus* from the Goiana Estuary (1 = dry season 1 - November and December 2005, January 2006; 2 = end of the rainy season - August to October 2006; 3 = dry season 2 - November and December 2006, January 2007).

Conclusions

The total mercury concentration in *T. lepturus* at Goiana Estuary suggested bioaccumulation, because the increasing concentration of total mercury presented direct relation to length and weight of individuals observed.

Mercury concentrations were inversely correlated to rainfall. Differences in mercury concentrations were also detected for the two subsequent dry seasons.

Fish from the Goiana Estuary can still be safely consumed by the local population. The proposed heavy dependency of total mercury levels in fish from water quality indicates that land use practices must be closely watched in order to guarantee the best possible options are put in place to prevent water contamination and food web bioaccumulation and biomagnification.

Trichiurus lepturus is largely consumed by coastal populations of tropical and sub-tropical countries all over the world. It is also consumed by a number of marine mammals over which there is a strong conservation interest. Therefore knowledge of its degree of contamination might contribute to public health issues as well as marine conservation actions. Studies on mercury and other contaminants including this species as bioindicator could help elucidate mechanisms through which pollutants are being transferred not only through the food web, but also from estuarine to coastal and finally to open waters, since this species is a marine straggler and promotes a link among different ecosystems along this ecocline. In addition, using the same species in marine pollution studies, especially if part of a mosaic of species, allows for wide range comparisons of marine food web contamination.

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