Neotropical Ichthyology, 11(1):211-216, 2013
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Mercury and stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) as tracers during the ontogeny of *Trichiurus lepturus*

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This study applies total mercury (THg) concentration and stable isotope signature ($\delta^{15}N$ and $\delta^{13}C$) to evaluate the trophic status and feeding ground of *Trichiurus lepturus* during its ontogeny in northern Rio de Janeiro, south-eastern Brazil. The trophic position of *T. lepturus* is detected well by THg and $\delta^{15}N$ as the sub-adult planktivorous specimens are distinct from the adult carnivorous specimens. The $\delta^{13}C$ signatures suggest a feeding ground associated with marine coastal waters that are shared by fish in different ontogenetic phases. The diet tracers indicated that the fish feeding habits do not vary along seasons of the year, probably reflecting the prey availability in the study area. This fish has economic importance and the concentration of THg was compared to World Health Organization limit, showing that the adult specimens of *T. lepturus* are very close to the tolerable limit for safe regular ingestion.

Este estudo utilizou a concentração de mercúrio total (THg) e a assinatura isotópica ($\delta^{15}N$ e $\delta^{13}C$) para avaliar a posição trófica e a área de alimentação de *Trichiurus lepturus* durante sua ontogenia no norte do Rio de Janeiro, sudeste do Brasil. A posição trófica de *T. lepturus* foi bem detectada pelo THg e $\delta^{15}N$ com os espécimes sub-adultos planctívoros distintos dos espécimes adultos carnívoros. As assinaturas de $\delta^{13}C$ sugerem uma área de alimentação associada a águas marinhas costeiras que são compartilhadas por peixes em diferentes fases ontogenéticas. Os traçadores de dieta indicaram que os hábitos alimentares desse peixe não variam ao longo das estações do ano, refletindo provavelmente a disponibilidade de presas na área de estudo. Esse peixe tem importância econômica e a concentração de THg foi comparada com o limite estabelecido pela Organização Mundial de Saúde, demonstrando que os espécimes adultos de *T. lepturus* estão bem próximos do limite tolerável para uma ingestão regular segura.

Key words: Carbon, Diet tracers, Nitrogen, Trace element, *Trichiurus lepturus*.

Introduction

Mercury (Hg) is a toxic trace element considered an environmental pollutant that bioaccumulates and biomagnifies through all levels of the aquatic food chain. This element has the potential to be employed as a diet tracer and to distinguish the trophic position and/or food preference (Watras *et al.*, 1998; Kehrig *et al.*, 2010; Di Beneditto *et al.*, 2012). Carnivorous fish that show the highest concentrations of Hg are the most sensitive organisms to this element because Hg load assimilates and accumulates in their tissues from feeding (Altindag & Yigit, 2005; Stewart *et al.*, 2008).

Stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope measurements have provided data on fish feeding ecology (*e.g.* Kidd *et al.*, 1995; Corbisier *et al.*, 2006; Al-Reasi *et al.*, 2007; Faye *et al.*, 2011). In general, stable isotope ratios of a predator are related to the isotopic composition of their prey. The isotopic fractionation (enrichment or depletion) allows inference about the processes developed by species in different trophic positions. In general, species with higher or heavier isotope values (enriched) occupy a higher trophic level than those with lower or lighter values (depleted) (Fry, 2006). The enrichment of $\delta^{15}N$ between trophic levels (3-4‰) is more evident than for $\delta^{13}C$ (<1-1‰) (Hobson & Welch, 1992; Hobson *et al.*, 2002). The $\delta^{13}C$ is generally used to indicate different carbon source diets (*e.g.*, inshore *vs*. offshore, pelagic *vs*. benthic, aquatic *vs*. terrestrial) (DeNiro & Epstein, 1978; Peterson & Fry, 1987). The stable isotope enrichment values may be also used to assess the trophic transfer of trace elements along food webs (Bearhop *et al.*, 2000; Silva *et al.*, 2005).

The species *Trichiurus lepturus* L. 1758 is commonly known as ribbonfish; it may be found along tropical and sub-tropical latitudes and is an important fishery resource (FAO, 2005). During fish's development, the specimens can be

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separated into ontogenetic phases according to length and feeding habits: the juveniles (5-30 cm) that feed on planktonic microcrustaceans; the sub-adults (>30-70 cm) and small adults (>70-100 cm) that feed on pelagic macrozooplankton and juvenile fishes, and the adults (>100 cm) that feed on squids and fishes, including co-specifics (Martins *et al.*, 2005). This species moves between estuarine and marine environments (Froese & Pauly, 2012) and adult specimens are considered top predators in marine food chains (Chiou *et al*., 2006; Bittar & Di Beneditto, 2009; Di Beneditto *et al.*, 2012).

The present study evaluates the trophic status and the preferred feeding ground of *Trichiurus lepturus* during its ontogeny by total mercury (THg) concentration and stable isotopes signatures (δ^{15}N and δ^{13}C). The possible implication of this fish consumption for human health, due to mercury concentration in its muscle tissue, is also discussed.

Material and Methods

Study area and sampling. The study area comprises marine coastal waters from northern Rio de Janeiro state (21º35'S 22º15'S) in south-eastern Brazil, from 1 to 56 km away from the coastline, in depths varying from 10 to 50 m (Fig. 1). This area is influenced by the Paraíba do Sul river discharge, whose flux can vary from 180 $m^3 s^{-1}$ in the dry season (April-September) to 4,400 $m^3 s^{-1}$ during the rainy season (October-March) (Carvalho *et al*., 2002). The river plume reaches the ocean waters in velocities of 1.6 and 2.6 km d^{-1} during dry and rainy seasons, respectively (Souza *et al.*, 2010). In this marine coastal area, the surface water temperature ranging from 21 to 24ºC (Laboratório de Ciências Ambientais, unpublished data). The salinity varied from 18 (around 1 km far from the river mouth) to 35-36 (more than 10 km far from the river mouth) (Souza *et al*., 2010).

The Paraíba do Sul river basin covers an area close to 57,000 km^2 in south-eastern Brazil and there are industries, plantations and human communities along its course (Kumlet & Lemos, 2008). Mercury concentration that is exported to the adjacent marine areas is related to the past practices of gold mining and use of mercurial fungicides in sugarcane plantations along this river course, which were banned in 1980 (Lacerda *et al.*, 1993). The annual river cycle also showed an expressive difference of mercury concentration in river water (Almeida *et al*., 2007).

From 2008 to 2010, 40 fish specimens were collected in the study area (Fig. 1). The sampling period was grouped into dry (April-September) and rainy (October-March) seasons. The fish specimens were categorized into ontogenetic phases according to their total length, following the length interval described in Martins *et al*. (2005): sub-adults (>30-70 cm) and adults (>100 cm).

The sub-adult specimens (N= 20) were incidentally captured by trawl nets during commercial fishery of shrimp (*Xiphopenaeus kroyeri*) and Sciaenidae fish conducted between 21º35'S and 21º50'S, around 10-20 m depth. The adult specimens (N= 20), targets of commercial gillnet fisheries practiced in this region, were captured along the whole area indicated in Fig. 1 (between 21º35'S and 22º15'S, from 10 to 50 m depth). After sampling, each individual was measured (total length in cm) and weighed (in grams), and a sub-sample from the back dorso-lateral muscle was removed. Tissue samples were freeze-dried (losing 75±4% of water body) and homogenized with a mortar and pestle for the THg and stable isotope analyses.

Total mercury analysis. Total mercury (THg) was analyzed in dry muscle samples by cold vapor atomic absorption spectrometry with a Flow Injection Mercury System (FIMS) - FIAS 400 (Perkin Elmer) equipped with auto samplers, according to the methodology described in Kehrig *et al.* (2006). The precision and accuracy of the analytical method were controlled by triplicate analysis, blank solutions, and Certified Reference Material (National Research Council-Canada: DORM-2, dogfish muscle sample, and TORT-2, lobster hepatopancreas). The result for DORM-2 was 4.54 ± 0.13 $\mu g\ g^{-1}$ (certified values: 4.64 ± 0.26 $\mu g\ g^{-1}$) and for TORT-2 was 0.28 ± 0.08 $\mu g\ g^{-1}$ (certified values: 0.27 ± 0.06 $\mu g\ g^{-1}$). The method reproducibility was evaluated using the coefficient of variation of the triplicates (less than 15%). Values from adult specimens, which are captured for commercial purposes, were compared with the World Health Organization (WHO) values for mercury intake by humans (WHO, 1976; 1989). The dry weight basis concentration of THg was converted to wet weight considering 75% of water lost during freeze-dry.

Stable isotope analysis. Stable isotope ratios (δ^{13}C and δ^{15}N) were analyzed in dry muscle samples using a Thermo Quest-Finnigan Delta Plus isotope ratio mass spectrometer (Finnigan-MAT) interfaced to an Elemental Analyzer (Carlo Erba). Pee Dee beleminite carbonate and atmospheric nitrogen were used as standard values and the analytical precision was ±0.1‰ for δ^{13}C and ±0.2‰ for δ^{15}N (triplicate samples of every fifth sample). Muscle samples were not previously lipid-extracted, which could interfere with δ^{13}C results. However, Kiljunen *et al.* (2006) and Post *et al.* (2007) stated that a C:N ratio less than 3.0-3.5

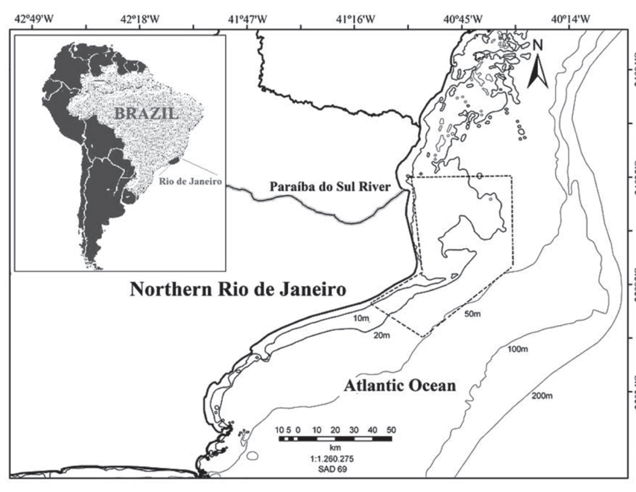

Fig. 1. Northern Rio de Janeiro, in south-eastern Brazil. The sampling area where the *Trichiurus lepturus* specimens were collected is marked with a dashed polygon.

indicates that the muscular tissue contains zero extractable lipid. All C: N ratios calculated were less than 3.5.

Data analysis. The Shapiro-Wilks test was applied to verify the assumptions of normality. Then, the T test was applied to verify differences between sub-adult and adult specimens of *T. lepturus* regarding THg, $\delta^{15}N$ and $\delta^{13}C$. This test was also applied to each ontogenetic phase separately to test differences between dry and rainy seasons. Pearson correlation was used to describe the relation between THg (log-transformed concentrations) and $\delta^{15}N$, considering the fish ontogenetic phases. The statistical analysis was performed using Statistica 7.0 for Windows (StatSoft, Inc 1984-2004, USA) and a p value equal to or less than 0.05 indicated statistical significance (Zar, 2009).

Results

The total length and weight of sub-adult specimens varied from 37.0 to 70.0 cm (mean: 48.5±8.9 cm) and 21.0 to 206.0 g (mean: 53.5±45.7 g), respectively. For adult specimens, the length ranged from 107 to 161 cm (mean: 143.0±11.0 cm) and weight from 880.0 to 3,100.0 (mean: 2,400±504 g) (Fig. 2). Mercury concentrations (dry weight basis) were 159 to 623 µg kg^{-1} (mean: 309±120 µg kg^{-1}) for sub-adults and 248 to 3,594 µg kg^{-1} (mean: 1,290±908 µg kg^{-1}) for adults (Fig. 2). The isotopic signatures of $\delta^{15}N$ in sub-adult and adult specimens were 12.4 to 15.0‰ (mean: 13.6±0.8‰) and 14.0 to 15.5‰ (mean: 15.0±0.8‰), respectively. For $\delta^{13}C$, the sub-adults values were -17.8 to -16.5‰ (mean: -17.1±0.4‰) and the adults values were -17.8 to -16.3‰ (mean: -16.8±0.3‰) (Fig. 2).

Mercury concentrations and $\delta^{15}N$ signatures for sub-adult and adult specimens were significantly different (THg: T test, $t = -5.34$, $N_1 = 20$, $N_2 = 20$, $p < 0.001$ and $\delta^{15}N$: T test, $t = -6.22$, $N_1 = 20$, $N_2 = 20$, $p < 0.001$); with adult specimens showing higher THg concentrations and heavier $\delta^{15}N$ signatures. Meanwhile, $\delta^{13}C$ signatures were similar between both ontogenetic phases ($\delta^{13}C$: T test, $t = -1.69$, $N_1 = 20$, $N_2 = 20$, $p = 0.097$).

Considering the seasonal analysis (dry *vs.* rainy seasons), no significant difference was detected neither for sub-adult specimens (THg: T test, $t = -0.99$, $N_1 = 10$, $N_2 = 10$, $p = 0.335$; $\delta^{15}N$: T test, $t = -0.81$, $N_1 = 10$, $N_2 = 10$, $p = 0.427$ and $\delta^{13}C$: T test, $t = -1.24$, $N_1 = 10$, $N_2 = 10$, $p = 0.229$) nor for adult specimens (THg: T test, $t = 0.33$, $N_1 = 10$, $N_2 = 10$, $p = 0.743$; $\delta^{15}N$: T test, $t = 0.56$, $N_1 = 10$, $N_2 = 10$, $p = 0.581$ and $\delta^{13}C$: T test, $t = 1.05$, $N_1 = 10$, $N_2 = 10$, $p = 0.308$).

During fish ontogeny, the average enrichment for $\delta^{15}N$ and $\delta^{13}C$ was 1.4‰ (from 13.6 to 15.0‰) and 0.3‰ (from -17.1 to -16.8‰), respectively (Fig. 3). Pearson correlation between THg and $\delta^{15}N$ was positive and significant ($r = 0.54$, $p < 0.001$), and this result reveals bioaccumulation and retention of mercury along *T. lepturus* ontogeny (Fig. 4).

Discussion

The trophic status of *T. lepturus* during its ontogeny was well detected by THg concentrations and $\delta^{15}N$ signatures. Both tracers showed that the fish changes its preferential feeding habit, which is an important strategy to minimize intra-specific competition. Previous stomach content analysis of *T. lepturus* had already verified ontogenetic differences in its food preference (Martins *et al*., 2005; Chiou *et al*., 2006; Yan *et al*., 2011). These studies indicate that stomach content analysis is important to the taxonomic recognition of ingested prey. However, this approach requires large sample size and, sometimes, broad research efforts to describe the feeding preference of a given predator. Diet tracers as trace elements and stable isotopes allow the understanding of feeding patterns and trophic interactions among species with smaller sample size (*e.g.*, Gaston & Suthers, 2004; Domi *et al*., 2005; Kasper *et al*., 2009; Kehrig *et al*., 2009; Kehrig *et al*., 2010; Di Beneditto *et al*., 2012). It could be useful in case of endangered species, when sample access is restricted and/or to minimize the research effort.

The $\delta^{13}C$ signatures suggest that in northern Rio de Janeiro the preferred feeding ground of *T. lepturus* is associated with marine coastal areas. Di Beneditto *et al*. (2012) evaluated the trophic chain of this fish, in the same region, and concluded

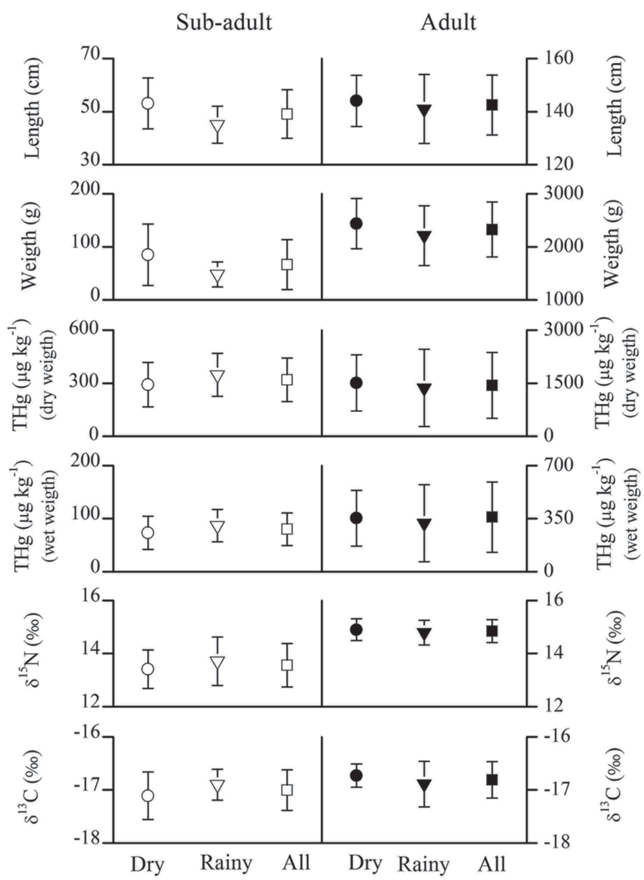

Fig. 2. Length (cm), weight (g), total mercury concentration (THg) in dry and wet weight basis and isotopic signatures ($\delta^{15}N$ and $\delta^{13}C$) of sub-adult and adult specimens of *Trichiurus lepturus*, considering dry season, rainy season, and all sampling periods. Data is shown as a mean and standard deviation. The scale for length, weight, and THg is different for the two ontogenetic phases.

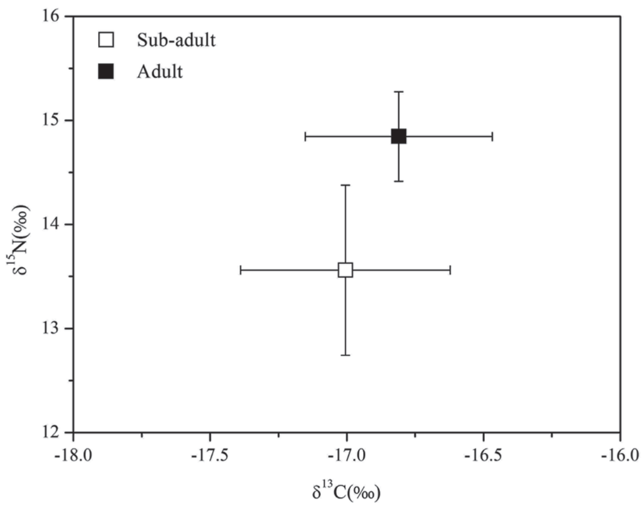

Fig. 3. Relationship between δ15N and δ13C in the muscle of sub-adult and adult specimens of *Trichiurus lepturus*. Bars represent the standard deviation.

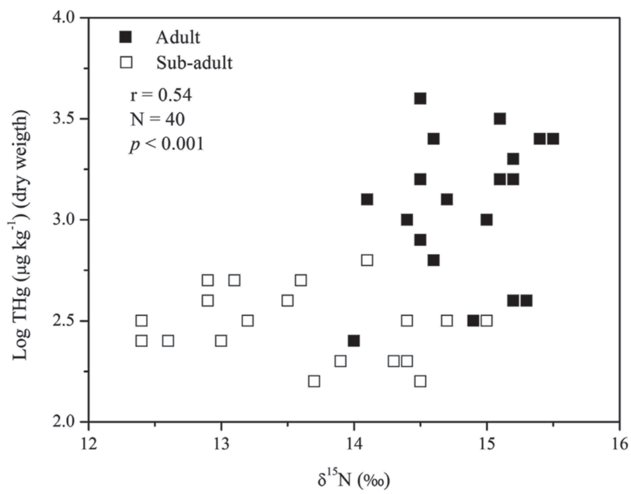

Fig. 4. Correlation between THg (log-transformed) and δ15N in the muscle of sub-adult and adult specimens of *Trichiurus lepturus*.

by THg and δ15N analyses that it is marine plankton based. In this region, the δ13C value of marine plankton is around -19.0 to -20‰ (Laboratório de Ciências Ambientais, unpublished data), which is in accordance with other marine tropical coastal areas from south-eastern Brazil (Rezende *et al*., 2010; Bisi *et al*., 2012). Meanwhile, the δ13C signatures in the mangrove leaves from Paraíba do Sul River estuary ranged from -27.0 to -29‰ (Ribas, 2007). This difference suggests a minimal influence of terrestrial carbon sources to the fish's diet and reinforces the marine plankton role to its trophic chain.

Mercury concentrations indicate that this element has suffered the process of bioaccumulation in the muscular tissue of *T. lepturus*, such as has been observed for other fishes (*e.g.*, Frodello *et al.*, 2000; Adams & Onorato, 2005; Weis & Ashley, 2007; Stewart *et al.*, 2008). The dissolved Hg in the water is easily assimilated and bioaccumulated by organisms with small size and great relative surface area (*e.g*. phytoplankton). Meanwhile, the decrease in dissolved Hg contribution is noted with the increase in organism size. For larger organisms, the element transference via food resources is the main pathway for its assimilation (Reinfelder *et al.*, 1998; Mason *et al.*, 2000; Kehrig *et al*., 2010). The correlation (r = 0.54) between THg and δ15N indicated that change in feeding habits during the fish ontogeny is an important factor in driving the element accumulation. Adult specimens of *T. lepturus* are voracious predators of different sizes of prey that include co-specifics, while juveniles and sub-adults are mainly planktivorous (Martins *et al.*, 2005; Choiu *et al*., 2006; Bittar *et al.*, 2008).

The average enrichment for δ15N was only 1.4‰ between the two ontogenetic phases. In marine ecosystems, δ15N enrichment of 3-4‰ is expected from one trophic level to another (Hobson & Welch, 1992; Hobson *et al.*, 2002). However, the δ15N signatures of *T. lepturus* specimens were significantly different to discriminate the higher trophic position of adult fish. The δ13C signatures for both ontogenetic phases were rather similar, with an average enrichment of 0.3‰. During *T. lepturus* ontogeny, sub-adult and adult specimens are probably sharing the feeding ground. Although the sampling has been done only in marine coastal waters, the δ13C signatures in muscle samples suggest that sub-adult specimens did not enter in the Paraíba do Sul River estuary. The δ13C values of our *T. lepturus* samples are comparable with those obtained by Bisi *et al.* (2012), to the same species, in marine coastal areas from south-eastern Brazil (Sepetiba Bay: -17.2 to -13.8‰ and Ilha Grande Bay: -17.8 to -17.4‰). Moreover, Bizerril (1999) and Bizerril and Primo (2001) conducted extensive surveys on fish species from Paraíba do Sul River basin and did not record this species in the estuarine area.

In the rainy season, a lower concentration of mercury in fish tissues is expected due to dilution of the element in large volumes of water and adsorption onto particulate matter that will be exported to the sea, decreasing its bioavailabitity in the estuary. An opposing pattern is expected during the dry season (Meyer *et al.*, 1998; Barbosa *et al*., 2011). Despite the small sample size for each season (10 specimens of each ontogenetic phase *per* season), the results indicated that THg concentration was not different between seasons for sub-adult and adult specimens. As *T. lepturus* has wide movement along the continental shelf (Cheng *et al*., 2001; Bryan & Gill, 2007; Froese & Pauly, 2012), its mercury levels would reflect the bioaccumulation in different environments and not only the local influence.

The δ15N and δ13C signatures suggest no seasonal changes in the fish feeding habits, which corroborates Bittar *et al.* (2008) and Bittar & Di Beneditto (2009). The same feeding habits all year could reflect the prey availability in the study area. As an adult, *T. lepturus* becomes a carnivorous fish that feeds preferentially on co-specifics, *Pellona harroweri* (teleost), *Chirocentrodon bleekerianus* (teleost), and *Doryteuthis* spp (squids) (Bittar *et al.*, 2008; Bittar *et al.*, 2012). These species are common in northern Rio de Janeiro all year round and are available to the local marine top predators (Di Beneditto & Ramos, 2001; Di Beneditto & Ramos, 2004; Bittar *et al.*, 2008).

This fish species has economic importance locally and around the world, and the evaluation of its quality has become a public health matter. The mercury concentration in fish muscle is in accordance with the limit of 500 THg µg kg^{-1} (wet weight) suggested by the WHO (Fig. 2). However, the WHO-PTWI (provisional tolerable weekly intake) of 3.3 THg µg kg^{-1} per week assumes a consumption of 100 g fish day^{-1} by a 70 kg adult (WHO, 1976, 1989). The adult specimens of *T. lepturus* in northern Rio de Janeiro are very close to the tolerable limit for safe regular ingestion (3.2 THg µg kg^{-1} per week). In Brazil, even in coastal populations, fish is still consumed less than other animal protein sources (*e.g.*, meat and poultry), although marine food ingestion has been increasing in the last ten years (MPA, 2009). If the country maintains this tendency, the public health authorities should monitor mercury levels in this fish species, especially because it has low commercial value and is thus more accessible to the population.

Acknowledgments

We thank fishermen from Atafona Harbour and Silvana Gomes, who provided us with ribbonfish specimens. APM Di Beneditto and CE Rezende were supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (Proc. 300241/09-7 and Proc. 304.615/2010-2, respectively) and Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro - FAPERJ (Proc. E-26/103.038/08 and Proc. E-26/102.697/2008, respectively). This work was partially supported by CNPq INCT Material Transference from the Continent to the Ocean (Proc. 573.601/08-9).

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Submitted January 9, 2012
Accepted January 21, 2013 by Adalberto Luis Val
Published March 31, 2013