Mercury and stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) as tracers during the ontogeny of *Trichiurus lepturus*

Ana Paula Madeira Di Beneditto¹, Vanessa Trindade Bittar¹, Carlos Eduardo de Rezende¹, Plínio Barbosa Camargo² and Helena Amaral Kehrig³

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 subtropical latitudes and is an important fishery resource (FAO, 2005). During fish’s development, the specimens can be

---

¹Universidade Estadual do Norte Fluminense, Centro de Biociências e Biotecnologia, Laboratório de Ciências Ambientais, Campos dos Goytacazes, 28013-620 RJ, Brazil. anapaula@uenf.br
²Universidade de São Paulo, Centro de Energia Nuclear na Agricultura, Laboratório de Ecologia Isotópica, Piracicaba, 13416-100 SP, Brazil.
³Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho, Laboratório de Radioisótopos Eduardo Penna Franca, Rio de Janeiro, 21941-902 RJ, Brazil.
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 (δ15N and δ13C). 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 (21°35'S 22°15'S) in south-eastern Brazil, from 1 to 56 km away from the coastline, in depths varying from 50 to 500 m (Fig. 1). This area is influenced by the Paraiba do Sul river discharge, whose flux can vary from 180 m³ s⁻¹ in the dry season (April-September) to 4,400 m³ 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 24°C (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 Paraiba do Sul river basin covers an area close to 57,000 km² 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 (*Xiphopecten kroyeri*) and Sciaenidae fish conducted between 21°35'S and 21°50'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 21°35'S and 22°15'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 µg g⁻¹ (certified values: 4.64 ± 0.26 µg g⁻¹) and for TORT-2 was 0.28 ± 0.08 µg g⁻¹ (certified values: 0.27 ± 0.06 µg 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 (δ13C and δ15N) 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 belemnite carbonate and atmospheric nitrogen were used as standard values and the analytical precision was ±0.1‰ for δ13C and ±0.2‰ for δ15N (triplicate samples of every fifth sample). However, Kiljunen et al. (2006) and Post et al. (2007) stated that a C:N ratio less than 3.0-3.5 could interfere with δ13C results. Stable isotope ratios (δ13C and δ15N) were analyzed in dry muscle samples by standards.

**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.
well detected by THg concentrations and T. lepturus. This result reveals bioaccumulation and retention of mercury along 10, N2 = 10,

\(10, \text{ N}2 = 10,\)

\(N1 = 10, N2 = 10,\) respectively. For \(N1 = 10, N2 = 10,\) were 12.4 to 15.0‰ (mean: 13.6±0.8‰) and 14.0 to 15.5‰ (mean: 15.0±0.8‰), respectively (Fig. 3). Pearson correlation between THg 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...
by THg and δ^{15}N analyses that it is marine plankton based. In this region, the δ^{13}C 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 δ^{13}C 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 *Trichiurus 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 δ^{15}N indicated that change in feeding habits during the fish ontogeny is an important factor in driving the element accumulation. Adult specimens of *Trichiurus 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 δ^{15}N was only 1.4‰ between the two ontogenetic phases. In marine ecosystems, δ^{15}N enrichment of 3-4‰ is expected from one trophic level to another (Hobson & Welch, 1992; Hobson et al., 2002). However, the δ^{15}N signatures of *Trichiurus lepturus* specimens were significantly different to discriminate the higher trophic position of adult fish. The δ^{13}C 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 δ^{13}C signatures in muscle samples suggest that sub-adult specimens did not enter in the Paraíba do Sul River estuary. The δ^{13}C 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 bioavailability 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 δ^{15}N and δ^{13}C signatures suggest no seasonal changes in the fish feeding habits, which corroborates Bittar et al. (2008) and Bittar & Di Benedetto (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 bleekeri anus* (teleost), and *Doryteuthis* spp.
is in accordance with the limit of 500 THg kg⁻¹ (wet weight) suggested by the WHO (Fig. 2). However, the WHO-PTWI (provisional tolerable weekly intake) of 3.3 THg 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 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 Benedetto 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).

**Literature Cited**


Submitted January 9, 2012

Accepted January 21, 2013 by Adalberto Luis Val

Published March 31, 2013