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Mercury bioaccumulation and isotopic relation between *Trichiurus lepturus* (Teleostei) and its preferred prey in coastal waters of southeastern Brazil

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ABSTRACT

The trophic transfer of total mercury (THg) and its bioaccumulation from prey species to the predator fish *Trichiurus lepturus* was analysed in coastal waters of southeastern Brazil to evaluate the trace element dynamic in this predator-prey system. The isotopic ($\delta^{13}C$ and $\delta^{15}N$) relation between this predator and its prey allowed inferences on prey assimilation and predator feeding habits. The THg increment varied from 4.5 to 19.5 times between prey and predator, with a biomagnification power of 0.59. The prey species could be divided into three groups regarding $\delta^{15}N$ values: i) 13.6 to 13.2‰ (juvenile conspecifics, *Pellona harroweri*, and *Peprilus paru*); ii) 12.5 to 11.8‰ (*Chirocentrodon bleekerianus*, *Lycengraulis grossidens*, and *Dorytheuthis plei*); and iii) 10.5‰ (*Xiphopenaeus kroyeri*). Based on $\delta^{13}C$ values, the prey groups were: i) -15.3‰ (*X. kroyeri*); ii) -17.6 to -16.8‰ (*C. bleekerianus*, *D. plei*, *P. harroweri*, *P. paru,* and juvenile conspecifics); and iii) -18.7‰ (*L. grossidens*). The values of THg and $\delta^{15}N$ highlighted juvenile conspecifics as the main via of this trace element and the most assimilated prey. The isotopic relation between predator and its prey species showed a feeding activity preferably coastal and pelagic.

Key words: mercury, predator-prey system, southeastern Brazil, stable isotopes.

INTRODUCTION

The species *Trichiurus lepturus* Linnaeus, 1758 is a teleost fish that forms shoals in brackish and marine waters along tropical and subtropical regions worldwide, with importance as fishery resource (Froese and Pauly 2015). During its ontogeny, there is a wide diet shift: juveniles are planktivores, while adults are top predators, feeding on the most abundant prey. In coastal waters of southeastern Brazil (~21-22°S), the feeding habits of adult specimens of *T. lepturus* was detailed by Bittar et al. (2008, 2012). Twelve-eight prey species were recorded for this predator, and seven were the most representative in its diet, in this order: juvenile conspecifics, which represented 33% of the diet*; Pellona harroweri* Fowler, 1917 (17%); *Dorytheuthis plei* Blainville, 1823 (13%); *Chirocentrodon bleekerianus* Poey, 1867 (11%); *Xiphopenaeus kroyeri* Heller, 1862 (11%); *Lycengraulis grossidens* Spix & Agassiz, 1829 (6%); and *Peprilus paru* Linnaeus, 1758 (3%).

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In general, prey identification and quantification, and original size estimates are only possible by stomach content analysis, but it has limitations. Differences in prey digestion rates may lead to under- or overestimation of prey importance in predators' diet (Pierce and Boyle 1991). Many studies have applied multiple trophic markers together to minimize this bias, such as stomach content analysis, trace elements, and stable isotopes (Aubail et al. 2011, Di Beneditto et al. 2011, Kehrig et al. 2013, Connelly et al. 2014). Mercury is a trace element that undergoes biomagnification in animals' tissues through trophic transfer, *i.e.* its concentration increases over consecutive trophic levels (Lavoie et al. 2013). Then, this trace element is suitable as trophic marker. Isotopic ratios of animals' tissues provide records of the prey contribution in the predator diet, indicating prey assimilation after digestion and excretion (Domi et al. 2005, Huckstadt et al. 2007). Furthermore, isotopic composition also indicates the contributions of different sources within a given trophic relation (*e.g.*, aquatic versus terrestrial, coastal versus oceanic, pelagic versus benthic) (Fry 2008).

In this study, the trophic transfer of mercury and its bioaccumulation involving prey species and the fish *T. lepturus* was analysed to evaluate the trace element dynamic in this predator-prey system in southeastern Brazil. Furthermore, the isotopic relation between the predator and its prey allowed inferences on prey assimilation and feeding habits.

MATERIALS AND METHODS

Sampling

The sampling of adult specimens of *T. lepturus* (body size >100 cm) was done between 21º35'S and 22º25'S. These specimens were targets of local commercial fisheries. The preferred prey were collected in the same area during local commercial fisheries (target or by-catch species), taking into account the prey size consumed by the predator (see Bittar et al. 2008, 2012 for details). A sample from the back dorso-lateral muscle (fish), mantle (squid), or abdomen (shrimp) removed from each specimen (predator and prey) was freeze-dried and homogenized with a mortar and pestle for total mercury and stable isotopes analyses.

Total Mercury (THg) Determination

The dried tissue samples (100 mg) were acid digested with 3 mL of H_2SO_4:HNO_3 (1:1v/v) (Merck p.a.) and 1 mL of concentrated H_2O_2 (Merck p.a.) in a 50 mL centrifuge tube at 60 °C in water bath for 45 min. After addition of 5 mL of 5% $KMnO_4$ (Merck p.a.) solution, the digested samples allowed to stand for overnight. The THg concentration in the acid digested solutions was determined by cold vapour atomic absorption spectrometry with a Flow Injection Mercury System (FIMS-400, Perkin Elmer) with auto sampler, using $NaBH_4$ as reducing agent. The limit of detection for THg determination was 0.001 μg g^{-1}. The results were expressed in ng g^{-1} (dry weight).

Quality control involved replicates analysis, strict blank control, and certified reference material.

The samples were analyzed in triplicate, and the coefficients of variation for analytical replicates were below 10%. In each ten triplicates, two blank controls were done to detect reagent impurities or external contamination signals. The accuracy was assessed through certified material DORM-2 (THg: 4.64±0.26 μg g^{-1}) from the National Research Council Canada. The results for THg DORM-2 were 4.54±0.13 μg g^{-1}, demonstrating high precision and accuracy of the analytical method. THg quantified in certified material was within 97% of the mean certified value.

Isotopic Analysis of $\delta^{15}N$ and $\delta^{13}C$

Isotopic analyses of the dried tissue samples (0.5 mg) were completed in a ThermoQuest Finnigan Delta Plus (Finnigan MAT) mass spectrometer

coupled to an elemental analyser. The reference values for $\delta^{15}N$ and $\delta^{13}C$ were atmospheric nitrogen and Pee Dee Belemnite (PDB), respectively, and the results were expressed in parts per thousand (‰). The analytical precision was ±0.3‰ for $\delta^{15}N$ and ±0.2‰ for $\delta^{13}C$, determined by triplicates at each five samplings. The accuracy for elemental and isotopic composition were determined by a certified standard (Protein OAS/Isotope Cert 114859; Elemental Microanalysis), organic carbon and total nitrogen (99%), and $\delta^{13}C$ and $\delta^{15}N$ (100%).

Lipids were not extracted from tissues prior to analysis; however, the C:N ratio in muscle samples from both predator and prey were lower or equal to 3.5, indicating low lipids levels. Therefore, the interpretation of $\delta^{13}C$ was not compromised (Post et al. 2007).

Data Analysis

Differences among species regarding THg concentration and isotopic ratios were analysed by Kruskal-Wallis test with Dunn's multiple comparison test *a posteriori*. A linear regression model was used to test the relation between $\delta^{15}N$ on log-transformed THg concentrations, and the regression slope (*b*) represented the biomagnification power (Kidd et al. 1995). The analyses were performed in GraphPad Prism 5 for Windows, and *p* value <0.05 was chosen to indicate statistical significance.

RESULTS

The concentrations of THg were different among species ($p< 0.0001$). Higher values were registered in the predator *T. lepturus* (Table I). Taking into account the contribution of each prey species in the trophic transfer of THg to the predator, the juvenile conspecifics were the main via (Figure 1). Linear regression testing the relation between $\delta^{15}N$ and THg was significant, showing the biomagnification of this trace element from prey to predator (b= 0.59).

The $\delta^{15}N$ and $\delta^{13}C$ values differed among species ($p< 0.05$, and $p< 0.0001$, respectively). The predator presented the heavier $\delta^{15}N$ values, and the lighter values were found in *X. kroyeri*. The carbon isotopic values were heavier in *X. kroyeri* and lighter in *L. grossidens* (Table I and Figure 2). According to average $\delta^{15}N$ values, the prey species could be divided into three groups: i) 13.6 to 13.2‰ (juvenile conspecifics, *P. harroweri*, and *P. paru*); ii) 12.5 to 11.8‰ (*C. bleekerianus*, *L. grossidens*, and *D. plei*); and iii) 10.5‰ (*X. kroyeri*) (Figure 2). Based on average $\delta^{13}C$ values, the prey species were also divided into three groups: i) -15.3‰ (*X. kroyeri*); ii) -17.6 to -16.8‰ (*C. bleekerianus*, *D. plei*, *P. harroweri*, *P. paru,* and juvenile conspecifics); and iii) -18.7‰ (*L. grossidens*) (Figure 2).

TABLE I
Body dimensions, total mercury concentration (THg), and isotopic ratios ($\delta^{15}N$ and $\delta^{13}C$) of *Trichiurus lepturus* (adult specimens) and its preferred prey.

Species	N	Body size (cm)	Weight (g)	THg ng g^{-1} (dry weight)	$\delta^{15}N$(‰)	$\delta^{13}C$(‰)
Predator *Trichiurus lepturus*	20	142.0±11.0	2,326.0±517.0	1,440±931	14.8±0.4	-16.8±0.3
Prey						
T. lepturus (juvenile)	20	49.1±9.2	66.7±46.9	320±123	13.6±0.8	-17.0±0.4
Pellona harroweri	10	10.0±1.5	13.1±5.0	181±123	13.3±0.5	-17.3±0.8
Dorytheuthis plei	20	4.7±0.7	7.6±3.9	110±39	11.8±0.6	-17.1±0.5
Chirocentrodon bleekerianus	19	10.4±0.8	6.8±0.9	229±79	12.5±0.4	-17.2±0.3
Xiphopenaeus kroyeri	08	9.3±1.8	2.0±0.1	74±25	10.5±0.4	-15.3±0.4
Lycengraulis grossidens	15	11.4±1.1	16.3±4.0	178±45	12.3±0.4	-18.7±1.0
Peprilus paru	10	9.3±1.9	15.7±9.7	89±64	13.2±0.3	-17.6±0.3

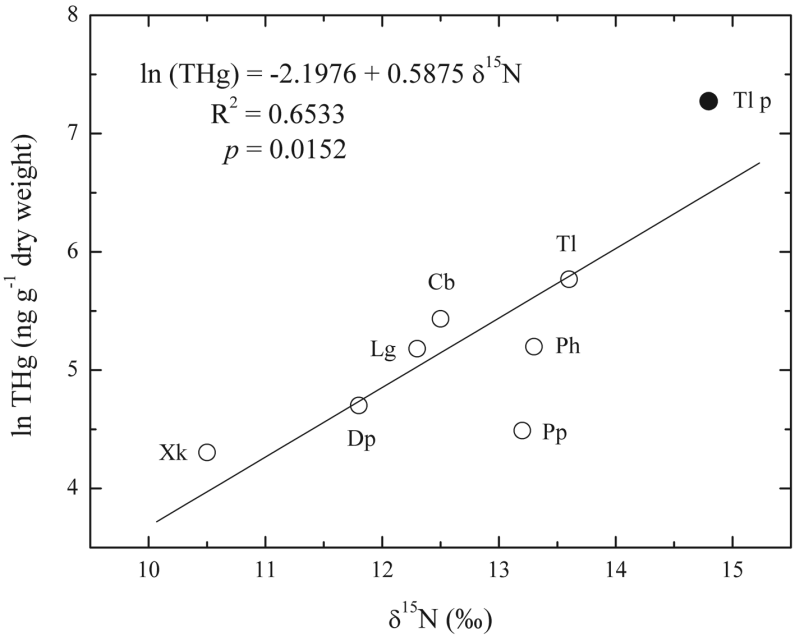

Figure 1- Relationship between $\delta^{15}N$ and THg concentration (log-transformed) for *Trichiurus lepturus* (adult specimens) and its preferred prey. Tl p: *Trichiurus lepturus* predator; Tl: *T. lepturus* prey; Cb: *Chirocentrodon bleekerianus*; Lg: *Lycengraulis grossidens*; Dp: *Dorytheutis plei*; Xk: *Xiphopenaeus kroyeri*; Pp: *Peprilus paru*; and Ph: *Pellona harroweri.*

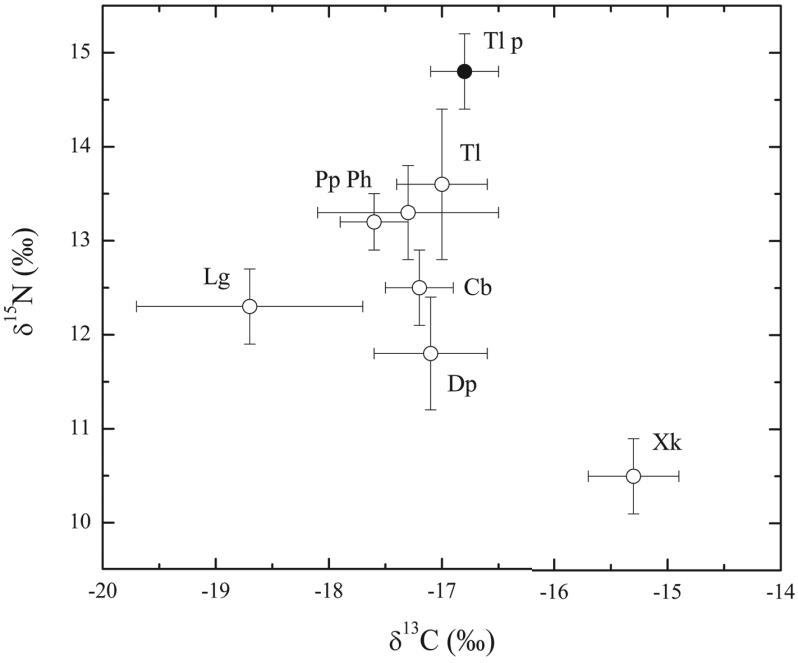

Figure 2 - Relationship between $\delta^{13}C$ and $\delta^{15}N$ in *Trichiurus lepturus* (adult specimens) and its preferred prey. Tl p: *Trichiurus lepturus* predator; Tl: *T. lepturus* prey; Cb: *Chirocentrodon bleekerianus*; Lg: *Lycengraulis grossidens*; Dp: *Dorytheutis plei*; Xk: *Xiphopenaeus kroyeri*; Pp: *Peprilus paru*; and Ph: *Pellona harroweri.* Bars indicate the standard deviations.

DISCUSSION

The values of THg and $\delta^{15}N$, as well the relation between them highlighted juvenile conspecifics as the main via of this trace element to adult specimens of *T. lepturus*, and the most assimilated prey. Thus, cannibalism is confirming as an important feeding strategy to this predator in the study area, as previously recorded through stomach content analysis (Bittar et al. 2008, 2012).

The THg increment varied from 4.5 to 19.5 times between prey and predator, revealing its biomagnification (Table I). Here, the biomagnification power (b= 0.59) considered the increase of THg only from prey to predator, and not along the entire food chain. Jæger et al. (2009) argued that the increase of Hg from specific prey to predator is not an accurate measured of biomagnification. However, previous studies in the same area showed significant Hg transference in many trophic interactions, both predator-prey relations (Carvalho et al. 2008, Kehrig et al. 2009, Di Beneditto et al. 2011, 2013) as entire food chains (Di Beneditto et al. 2012, Kehrig et al. 2013), supporting our results.

Di Beneditto et al. (2012) analysed data on biomagnification power of Hg in marine environments worldwide, and concluded no trend regarding latitude or water temperature. Therefore, the bioavailability of Hg in aquatic ecosystems should be the primary factor driving the magnitude of local biomagnification processes. The main source of Hg to coastal marine species in the study area is a river discharge. This area is permanently influenced by the Paraíba do Sul River (Souza et al. 2010), whose basin was widely impacted with practices of gold-mining and use of mercurial fungicides on plantations until 1980's (Lacerda et al. 1993).

The nitrogen isotope values ($\delta^{15}N$) indicated the high trophic position of the predator relative to its prey, as expected (Fry 2008). The grouping of prey species ($\delta^{15}N$ values) was not conclusive (Figure 2). These prey species are zooplanktivores (*P. harroweri*, *L. grossidens*, and *P. paru*) or carnivores whose prey have small size (juvenile conspecifics, *D. plei*, and *C. bleekerianus*) (Froese and Pauly 2015). Thus, their trophic positions would be similar. However, the prey species of *T. lepturus* varied in length and weight (and probably age) (Table I). Parameters as consumer class, size, and age may influence the isotopic ratios of a given specimen, either alone as combined (Jennings et al. 2002, Caut et al. 2009). For the shrimp *X. kroyeri* the feeding habit may explain the lower $\delta^{15}N$ values, once this species is a benthic feeder whose main feeding resources are small invertebrates and sediment (Branco and Moritz-Júnior 2001).

The isotopic values of $\delta^{13}C$ were similar to those previously registered to marine coastal species in the study area (Di Beneditto et al. 2011, 2012, Kehrig et al. 2013), and showed that most prey species is pelagic. Heavier $\delta^{13}C$ values in the shrimp *X. kroyeri* reflected its benthic habit (Branco and Moritz-Júnior 2001), and lighter values in fish *L. grossidens* indicated its anadromous habit, with seasonal movements between fluvial and marine areas for reproduction (Froese and Pauly 2015). Although $\delta^{13}C$ is not usually applied to distinguish trophic levels, enrichment around ≤1‰ is generally expected from one trophic level to another (Peterson and Fry 1987). Therefore, the enrichment between prey and predator is within the expected interval.

In the study area, the biomagnification process of Hg due to trophic transfer between the prey species and the predator *T. lepturus* is evident. The isotopic relation between them showed a feeding activity preferably coastal and pelagic. In marine tropical waters (our study area) the set of available prey is generally wide, and the model fitted may not represent the real feeding preference of a given predator when only one trophic marker is used. Therefore, the use of multiple trophic markers as complementary approaches tends to reduce bias, providing more reliable results and improving knowledge regarding predator-prey systems.

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RESUMO

A transferência trófica de mercúrio total (THg) e sua bioacumulação das espécies de presas para o peixe predador *Trichiurus lepturus* foi analisada em águas costeiras do sudeste do Brasil para avaliar a dinâmica de elemento traço neste sistema predador-presa. A relação isotópica ($\delta^{15}N$ and $\delta^{13}C$) entre o predador e suas presas permitiu inferências sobre a assimilação das presas e os hábitos alimentares do predador. O incremento THg variou de 4,5 a 19,5 vezes entre a presa e o predador, com uma força de biomagnificação de 0,59. As espécies de presas podem ser divididas em três grupos em relação aos valores de $\delta^{15}N$: i) 13,6 a 13,2‰ (juvenis co-específicos, *Pellona harroweri* e *Peprilus paru*); ii) 12,5 a 11,8‰ (*Chirocentrodon bleekerianus*, *Lycengraulis grossidens* e *Dorytheuthis plei*); e iii) 10,5‰ (*Xiphopenaeus kroyeri*). Com base nos valores $\delta^{13}C$, os grupos de presa foram: i) -15.3‰ (*X. kroyeri*); ii) -17,6 a -16,8‰ (*C. bleekerianus*, *D. plei*, *P. harroweri*, *P. paru*, e juvenis co-específicos); e iii) -18,7‰ (*L. grossidens*). Os valores de THg e $\delta^{15}N$ destacaram os juvenis co-específicos como a principal via deste elemento traço e a presa mais assimilada. A relação isotópica entre o predador e suas espécies de presas demonstrou uma atividade alimentar preferencialmente costeira e pelágica.

Palavras-chave: mercúrio, sistema predador-presa, sudeste do Brasil, isótopos estáveis.

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