Total mercury concentrations in muscles of little tunny species (*Euthynnus alletteratus*) sold in the city of Cabo Frio, State of Rio de Janeiro, Brazil

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Mercury (Hg) is a trace metal that is considered a major contaminant of fresh and marine waters due to natural sources and anthropogenic sources such as gold mining and industrial processes. From a toxicological point of view, Hg is one of the most widely studied elements, especially in the area of food, due to its high toxicity, high absorption levels, and low excretion rates, which lead to an increase in its concentrations in the food chain. It enters the human body through the consumption of fish, posing a risk to human health. Owing to these facts, this study aimed to determine total mercury (THg) concentrations in the tuna species, little tunny (*Euthynnus alletteratus*), to compare the concentrations between males and females, and to analyze whether these concentrations fall within the maximum limit established by Brazilian and international legislations for predatory (carnivorous) fish, which is 1.0 $mg.kg^{-1}$. In our study, the Hg concentration found in males ranged from 0.034 $mg.kg^{-1}$ to 1.930 $mg.kg^{-1}$, while in females it ranged from 0.036 $mg.kg^{-1}$ to 1.980 $mg.kg^{-1}$. The maximum values discovered were nearly twice as high as the recommendations of Brazilian and international legislations for carnivorous fish. Therefore, it is concluded that regular consumption of this species may endanger human health.

Keywords: Contaminant, Predatory fish, Tuna.

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O mercúrio (Hg) é um metal traço considerado um importante contaminante de águas doces e marinhas devido a fontes naturais e antrópicas, como garimpo de ouro e processos industriais. Sob o ponto de vista toxicológico, o Hg é um dos elementos mais estudados, com destaque na área de alimentos, em função de sua alta toxicidade, seus altos níveis de absorção e baixas taxas de excreção, que levam ao aumento de suas concentrações ao longo da cadeia alimentar. O Hg entra no corpo humano através do consumo de peixe, representando um risco para a saúde humana. Diante desses fatos, este estudo teve como objetivo determinar as concentrações de mercúrio total (HgT) na espécie de atum bonito-pintado (*Euthynnus alletteratus*), comparar as concentrações entre machos e fêmeas e analisar se as mesmas estão de acordo com os limites máximos estabelecidos pelas legislações internacional e brasileira para peixes predadores (carnívoros), que é de 1.0 $mg.kg^{-1}$. Em nosso estudo, a concentração de Hg encontrada em machos variou de 0.034 $mg.kg^{-1}$ a 1.930 $mg.kg^{-1}$, enquanto que nas fêmeas variou de 0.036 $mg.kg^{-1}$ a 1.980 $mg.kg^{-1}$. Os valores máximos encontrados eram quase duas vezes mais elevados que o recomendado pelas legislações internacional e brasileira para peixes carnívoros. Portanto, conclui-se que o consumo regular dessa espécie pode colocar em risco a saúde humana.

Palavras-chave: Atum, Contaminante, Peixes predadores.

INTRODUCTION

Mercury (Hg) is a metal found in the atmosphere, earth, and water. Hg can enter the environment and, consequently, the ecosystems in two ways: naturally and anthropically (Pires *et al*., 1988; Mason, 2009; Pirrone *et al*., 2010; Guynup, 2012; Sisinno, Oliveira-Filho, 2013; Gworek *et al*., 2016; Maria *et al*., 2017; Sundseth *et al*., 2017; EEA, 2018). Hg occurs naturally in the environment as a result of degassing of the Earth's surface (Mason, 2009; Pirrone *et al*., 2010; Guynup, 2012), volcanic activities (Pires *et al*., 1988; Ferrara *et al*., 2000; Nriagu, Becker, 2003; Pyle, Mather, 2003; Mason, 2009; Guynup, 2012; Sisinno, Oliveira-Filho, 2013; Gworek *et al*., 2016; Maria *et al*., 2017; Sundseth *et al*., 2017; EEA, 2018), forest fires, mineral rock dissolution, and erosion (Pires *et al*., 1988; Sisinno, Oliveira-Filho, 2013; EEA, 2018). Anthropically, Hg occurs in the environment as a result of environmental pollution caused by human activities, such as the disposal of industrial waste in the environment, primarily from electrochemical industries that produce chloralkali (Lacerda, Marins, 1997), and the production of paper, paint (Canela, 1995), and fluorescent lamps (Durão Júnior, Windmöller, 2008). Hg is also released into the environment as a result of gold mining emissions (Lacerda, Marins, 1997; Martiniano *et al*., 2008; Chen, Driscoll, 2018; EEA, 2018), mining, agricultural activities (Pires *et al*., 1988; WHO, 2008; Sisinno, Oliveira-Filho, 2013), coal burning (Lacerda, Marins, 1997; Pacyna *et al*., 2006; Guynup, 2012; Sisinno, Oliveira-Filho, 2013), oil products (petroleum derivatives) (Lacerda, Marins, 1997; Wilhelm, 2001), and burning of waste, particularly hospital waste (USEPA, 1993; Sisinno, Oliveira-Filho, 2013).

Once released into the aquatic ecosystem in its inorganic form, Hg binds to dissolved organic matter present in the environment (WHO, 1990) and may undergo changes in its chemical form, solubility, mobility, viability, and toxicity (Ravichandran, 2004) via processes mediated primarily by microorganisms in water bodies associated with bottom sediment, suspended particulate matter, and high decomposition rates (Bisinoti, Jardim, 2004; Lacerda, Malm, 2008) before being transformed into its organic form, methylmercury (MeHg). This form is the most toxic to humans because of its teratogenic effect and strong affinity for nerve cells (WHO, 1990). The water quality of an ecosystem also interferes with the biogeochemical cycle of Hg as environmental pollution caused by this metal is mainly associated with the possibility of MeHg formation, a process known as methylation (Bisinoti, Jardim, 2004; Lacerda, Malm, 2008).

Marine organisms play a direct role in Hg dynamics owing to their bioaccumulation and biomagnification capacity, thus increasing the concentration of this metal along the food chain (Ferreira *et al*., 2012). Several studies have shown that predatory fish at higher trophic levels have high Hg concentrations due to the element's ability to biomagnify in these animals via the marine food chain (Bargagli *et al*., 1998; Storelli *et al*., 1998, 2007). Studies have also shown that high exposure to Hg in humans is associated with the consumption of contaminated predatory fish (Storelli *et al*., 2002, 2005a, b, 2007), making this the group with the highest potential for contamination in humans (Ferreira *et al*., 2012).

Tunas are preferably oceanic fish (epipelagic and mesopelagic), large migratory predators that occupy high trophic positions in the marine ecosystems, and an important fishing resource for many nations (Collette, Nauen, 1983; WHO, 2008; FDA, 2020). Due to its exceptional capacity to move across large distances, the high Hg contamination in tuna species is an indicator of the widespread and high levels of global pollution of this element in the oceans. When it comes to food safety, Hg contamination in local and imported tuna products has been a constant concern for many nations (WHO, 2008; FDA, 2020). Although many publications have reported concentrations of Hg in the muscles of some species of tuna, such as skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*), albacore (*Thunnus alalunga*), bigeye tuna (*Thunnus obesus*), and Atlantic bluefin tuna (*Thunnus thynnus*) (Sun, Chang, 1972; Menasveta, Siriyong, 1977; Boush, Thieleke, 1983; Storelli, Marcotrigiano, 2001; Storelli *et al*., 2002, 2005b; Yamashita *et al*., 2005; Besada *et al*., 2006; Medeiros *et al*., 2008; Silva *et al*., 2011; Ferreira *et al*., 2012; Chen *et al*., 2014; Manhães *et al*., 2020; Vieira *et al*., 2024), only two studies were found on Hg concentrations in little tunny (*Euthynnus alletteratus*) (Manhães *et al*., 2020; Vieira *et al*., 2024).

The main source of Hg in people without occupational exposure is via ingestion of food, and the highest levels of MeHg in the diet come from fish and its derivatives (WHO, 1976; Wheeler, 1996; Storelli *et al*., 2005a). When Hg, mostly in the form of MeHg, is consumed by eating fish, it binds to sulfhydryl radicals of proteins, forming a fairly stable chemical bond that makes it difficult to eliminate by the organism. There is no type of treatment, including cooking, that can eliminate Hg in fish (Mariño, Martín, 1976; Lacerda, Meneses, 1995; Guenka *et al*., 2003).

Worldwide, there have been several instances of MeHg contamination of the population due to the consumption of food, especially contaminated fish. The most serious incident occurred in 1953 in Minamata, Japan, which resulted in neurological symptoms and teratogenic effects in an entire generation. This disorder was named Minamata disease (Fujiki, Tajima, 1992; Weiss, 1996; Harada *et al*., 1998). After the incident, which became known as the Minamata disaster, MeHg persisted in the environment for over 40 years due to its dissolution in particulate matter in the sediment. According to Harada (1995) and Harada *et al*. (1998), Hg concentrations in bay sediments reached levels of up to 2.010 $mg.kg^{-1}$ (dry weight). During and after the incident, several neurobehavioral effects associated with Hg contamination were observed in humans, including tremors, sensory disturbances, lack of motor coordination, and reduction of the visual field (Tsubaki, Irukayama, 1976). Other studies have shown that even at low concentrations, Hg can accumulate in the body, causing degenerative diseases, such as Alzheimer's disease, Parkinson's disease, and lupus (Zahir *et al*., 2005; Karagas *et al*., 2012). Fishing could be authorized again in the contaminated area only after several decontamination actions had been carried out, including removing sediment containing concentrations of the element above 25 parts per thousand from most of the bay and isolating the most contaminated areas (Hosokawa, 1993). Even after decontamination was completed and fishing was permitted again in the bay, high Hg concentrations were reported in samples of water, sediment, and mussels at various points throughout the bay, demonstrating the need for frequent monitoring of Hg-contaminated areas (Haraguchi *et al*., 2000).

According to Mendez *et al*. (2001), the concentration of Hg in several fish species has been intensively monitored since the Hg poisoning incident that occurred in Minamata, and intake values are well established. The Food and Drug Administration established an Hg limit of 1 $\mu g.kg^{-1}$ (wet weight – WW) to regulate the concentration of this metal in commercial fish. However, according to the author, Hg is considered a potential risk to human health when it comes to swordfish *Xiphias gladius* and tuna consumption, among other species.

Arraial do Cabo region has an area of special bioconservacionist value, the Arraial do Cabo Marine Extractive Reserve (ACMER / RESEXMAR Arraial do Cabo), created by presidential decree on January 31, 1997, at the request of the local community. Fishing in this area has been carried out for centuries, and the upwelling phenomenon contributes to increasing local fish stocks. Arraial do Cabo MER covers an area of 56,769 hectares, stretching from Massambaba to "Pontal Beach" (the end of Forte Beach), on the border with the city of Cabo Frio. Only fishing vessels from Arraial do Cabo are permitted to fish in the reserve (Silva, 2006). One of the most common fish in the region is the little tunny species (A. S. Ribeiro, pers. comm; M. F. Lima, pers. comm). This species is migratory, predator, and occupy high trophic positions in the marine ecosystems (Collette, Nauen, 1983; WHO, 2008; FDA, 2020), throughout their areas of occurrence. The little tunny species occur almost year-round on the coast of Arraial do Cabo and have commercial value, being highly caught, sold, and consumed by the local population. Although the region has no history of environmental contamination, some mercury studies have been conducted in this upwelling area. One of them, conducted by Silva (2006), aimed to determine Hg speciation in the first links of the food chain (phytoplankton and zooplankton), as well as to quantify the Hg content in a group of planktophagous and carnivorous fish belonging to different trophic levels and the pelagic food chain. According to the author, Hg speciation in marine plankton demonstrated that the inorganic form Hg^{2+} was predominant in phytoplankton and zooplankton, while Hg speciation in fish demonstrated that the highest percentage of methylmercury relative to total mercury was observed in the muscle tissue of skipjack tuna (*Katsuwonus pelamis*) and the lowest percentage in the muscle tissue of brazilian sardinella (*Sardinella brasiliensis*). Still according to the author, methylmercury biomagnified throughout the pelagic food chain analyzed in the study, while inorganic mercury bioconcentrated at the base of the food chain. The author concludes that constant monitoring of this metal is necessary in upwelling areas, as these locations, together with coastal marine areas, play an important economic role, being responsible for 98% of global fishing production.

This study aimed to determine total mercury (THg) concentrations in the muscle of specimens of little tunny captured off the coast of Arraial do Cabo and sold in the city of Cabo Frio, the east coast of the State of Rio de Janeiro; compare THg concentrations between males and females; and analyze whether these concentrations fall within the maximum limits established by the WHO for human consumption, which is 1.0 mg.kg^{-1} for carnivorous fish (FAO, 2011). The Brazilian legislation, in accordance with WHO, has currently determined a maximum Hg limit of 0.5 mg.kg^{-1} in fish for non-predator/non-carnivorous species and 1.0 mg.kg^{-1} for predator/carnivorous species (ANVISA, 1998; MAPA, 1999).

MATERIAL AND METHODS

Study area (sampling site). The coast of the Arraial do Cabo region (22°57’58”S 42°01’40”W) on the east coast of the State of Rio de Janeiro in southeastern Brazil has a unique geomorphology, making it one of the most projected points towards the sea on the Brazilian coast with an important dynamic of coastal and ocean currents in its waters and the presence of the upwelling phenomenon, which has led to the high primary productivity of its waters (Valentin, Coutinho, 1990; Valentin, 1994; Calado *et al*., 2010, 2020; Silva *et al*., 2023).

Time and place of sample collection. A total of 90 fish samples of *E. alletteratus* (N = 30) were acquired and used during the study. A 300 g piece of muscle from the left anterior dorsal region (due to the large muscle mass in this area) was removed from each individual and divided into three smaller pieces of 10–50 g for analysis in triplicate. The sex ratio (M:F) found was 2:1. The specimens were carefully chosen for their integrity and physical condition. The samples were collected during fish landings in the months of January (summer) and July (winter), 2018 (15 specimens in each period), mainly at the Cabo Frio Municipal Fish Market.

Analysis of stomach contents. Analysis of the stomach content was performed to identify and quantify the food items found in the stomachs of the analyzed little tunny samples. This was used to determine the most consumed prey by *E. alletteratus* while passing through or staying back on the Brazilian coast and in the study area in summer (January: peak period of upwelling on the coast of Cabo Frio) and winter (July: period in which the upwelling is nonexistent or sporadic in the study area). To identify the food items consumed, the literature on the subject was referred to, such as identification guides of fish of the Brazilian coast (Figueiredo, Menezes, 1980; Carvalho-Filho, 1994, 1999; Szpilman, 2000; Figueiredo *et al*., 2002; Bernardes *et al*., 2005).

Extraction and pretreatment of samples. The specimens were acquired as a whole and packed in cool boxes (ice boxes) and then transported to the laboratory, where they were measured, weighed, and analyzed to obtain biological samples. From each sample (individual), 300 g of muscle was extracted from the anterior dorsal region of the body for analysis. The samples were divided into triplicate, with three smaller aliquots, duly homogenized into pieces weighing 10–50 g each, and washed with ultra-pure distilled water. They were then immediately stored in ziplock polyethylene bags, which were previously washed with 65% nitric acid, and stored in a freezer at -20°C until the analysis.

Determination of THg. For this technique, the process requires a prior acid digestion of the samples to decompose the organic matter. Microwave digestion in a closed vessel was conducted using the ETHOS UP microwave sample digester (Milestone, Sorisole, Italy). To determine the THg in the biological samples, Cold Vapor Atomic Fluorescence Spectrometry (CV-AFS) technique was used, according to the methodology described by Sánchez-Rodas *et al*. (2010). This technique has low detection limits (DL) (below µg L^{-1}), a limit of quantification (LQ) of 0.05 µg/L (5 µg.kg^{-1} or 0.005 mg.kg^{-1}), and a wide linear calibration range (from µg L^{-1} to mg L^{-1}) (Sánchez-Rodas *et al*., 2010). The PSA 10.025 Millennium Merlin Mercury Analyzer (spectrometer) (PS Analytical), with an LD of 0.0001 mg.kg^{-1} and an LQ of 0.05 µg/L (under these conditions, the LD is 0.02 µg/L), was used. All samples were analyzed in triplicate in order to increase the reliability of the analytical results.

Validation of the analytical method for determining THg. The accuracy of the analytical method was determined by analyzing the following certified reference materials the NRCC-DORM-2 (dogfish muscle) and the NRCC-DORM-4 (fish protein), both produced and certified by the National Research Council of Canada (NRCC) (Bustamante *et al*., 2003; Branco *et al*., 2004; NRCC, 2013; Morgano *et al*., 2015). The same methodology was used to analyze the samples. The mean percentage of THg recovery in the certified samples was 90%, indicating that there was no significant difference between the certified values and those evaluated for THg.

Statistical analysis of the results. For the statistical analysis of the results, simple descriptive statistics were used. These were obtained using the statistical functions of the software program Microsoft Excel 2007 and Shapiro-Wilk, Bartlett, Student's t (independent), Kruskal-Wallis, and Multiple Comparisons (Nemeny) tests using the software program Python (version 3.7). The Monte Carlo method was used to repeat successive simulations (10,000 times) in relation to the random selection of 10 male samples out of the 20 acquired in order to perform comparative analyses between males (N = 10) and females (N = 10). The statistical tests mentioned were repeated for each random simulation.

Calculation of human health risk assessment. The human health risk assessment methodology proposed by the United States Environmental Protection Agency (USEPA) (USEPA, 1989) was used to compare a potential exposure level over a specific period of time with the reference dose (RfD) for a similar exposure period. The RfD is the estimated daily intake value of a substance, which in the case of Hg is 0.3 µg (0.3

μg.kg^{-1} or 0.0003 mg.kg^{-1}) THg/kg/day. According to the USEPA (1989), the toxicity exposure ratio is known as the hazard quotient (HQ), which is the ratio (comparison) between the estimated dose (D) (the quantity assessed) and the RfD. The D is calculated according to the concentration of Hg in the fish (to be ingested) and the rate of fish intake. Thus, the D can be calculated using the formula D = C × I / W × 1000, where

D = estimated dose (assessed dose)
C = concentration of Hg in the fish (μg/g = μg.g^{-1} WW)
I = rate of intake of the fish (g/day)
W = average body weight of an adult person (70 kg)

To perform the calculation using the formula in question, the values obtained in this study were converted from mg.kg^{-1} → μg.kg^{-1} → μg.g^{-1}, as C = Hg concentration in the fish (μg/g = μg.g^{-1} WW).

RESULTS

Specimens analyzed. The total length (TL) of the males ranged from 0.76 to 1.13 m and the total weight (TW) ranged from 3.135 to 13.835 kg. For the females, the TL ranged from 0.75 to 1.08 m and the TW ranged from 3.387 to 8.356 kg (Tab. 1). The gonads were macroscopically analyzed to determine the sex and the stage of gonadal maturation of the individual samples. In terms of sex, 20 males and 10 females were identified (Tab. 1). According to the methodology described by Vazzoler (1981), all of the specimens were classified as mature adults (C = mature, N = 17 and D = spawned, N = 13) (Tab. 1) in terms of gonadal maturation stage.

Stomach content of the analyzed specimens. Of the 30 fish analyzed in this study, 27 (90%) had food items in their stomachs, of which 162 were identified. The most abundant food item consumed by the species was Brazilian sardinella (*Sardinella brasiliensis* – according to Figueiredo *et al*., 2010) (N = 86, 53%), followed by broadband anchovy (*Anchoviella lepidentostole*) (N = 66, 41%), tropical two-wing flyingfish (*Exocoetus volitans*) (N = 7, 4%), and the round scad (*Decapterus punctatus*) (N = 3, 2%) (Tab. 1). In 10 (37%) of the 27 fish, it was not possible to identify and quantify the stomach contents as they had been heavily digested. Only 3 (10%) fish had empty stomachs (Tab. 1).

THg concentrations in the analyzed specimens. Regarding the THg concentrations in the specimens of little tunny analyzed in this study, the Hg concentration in males ranged from 0.034 to 1.930 mg.kg^{-1} (WW), whereas in females it ranged from 0.036 to 1.980 mg.kg^{-1} (WW) (Tab. 2). It is important to highlight that the maximum levels found (male = 1.930 and female = 1.980) (Tab. 3) were almost twice as high as those recommended by the international and Brazilian legislations for carnivorous fish, which is 1.0 μg.g^{-1} / 1.0 mg.kg^{-1}. Regarding the mean and standard deviation, the minimum mean observed in males was 0.065 mg.kg^{-1} and the maximum mean was 1.747 mg.kg^{-1} (overall mean and standard deviation = 0.819 ± 0.439 mg.kg^{-1}), while the minimum

TABLE 1 | Information regarding the specimens of little tunny analyzed in this study. # / N = Number; TL = Total Length; TW = Total Weight; Sex (M = Male, F = Female); GMS = Gonadal Maturation Stage (C = Mature, D = Spawned); FI = Food Items (A = Absent, U = Unidentified) / N = Number (U = Unidentified).

#	TL (m)	TW (kg)	Sex	GMS	FI/N
1	0.83	3.135	M	C	Broadband anchovy (*Anchoviella lepidentostole*)/N = 13
2	0.86	3.386	M	C	Broadband anchovy (*Anchoviella lepidentostole*)/N = 8
3	1.05	6.826	M	D	Brazilian sardinella (*Sardinella brasiliensis*)/N = 35
4	0.85	3.863	F	C	Brazilian sardinella (*Sardinella brasiliensis*)/N = 12
5	0.83	5.835	M	C	Brazilian sardinella (*Sardinella brasiliensis*)/N = 18
6	0.78	4.823	F	C	U / U
7	0.75	3.742	F	C	Tropical two-wing flyingfish (*Exocoetus volitans*)/N = 5
8	0.93	5.835	M	D	U / U
9	1.12	13.356	M	D	U / U
10	1.13	13.835	M	D	Brazilian sardinella (*Sardinella brasiliensis*)/N = 3
11	1.03	5.878	M	D	Brazilian sardinella (*Sardinella brasiliensis*)/N = 6
12	0.97	5.586	M	D	Brazilian sardinella (*Sardinella brasiliensis*)/N = 3
13	0.86	4.742	M	C	Brazilian sardinella (*Sardinella brasiliensis*)/N = 6
14	0.76	3.535	M	C	Brazilian sardinella (*Sardinella brasiliensis*)/N = 3
15	0.93	5.853	M	D	Round scad (*Decapterus punctatus*)/N = 3
16	1.05	6.958	F	D	Broadband anchovy (*Anchoviella lepidentostole*)/N = 15
17	0.85	4.974	M	C	Broadband anchovy (*Anchoviella lepidentostole*)/N = 12
18	1.05	7.583	F	D	Broadband anchovy (*Anchoviella lepidentostole*)/N = 18
19	1.08	8.356	F	D	U / U
20	1.02	8.823	M	D	U / U
21	0.78	3.387	F	C	U / U
22	0.87	4.295	M	C	U / U
23	0.90	4.843	M	D	A / 0
24	0.93	4.386	F	D	U / U
25	0.83	3.758	F	C	A / 0
26	0.88	5.674	M	C	Tropical two-wing flyingfish (*Exocoetus volitans*)/N = 1
27	0.76	3.157	M	C	U / U
28	0.82	4.362	F	C	Tropical two-wing flyingfish (*Exocoetus volitans*)/N = 1
29	0.78	3.528	M	C	A / 0
30	0.80	3.485	M	C	U / U

mean observed in females was 0.051 $mg.kg^{-1}$ and the maximum mean was 1.421 $mg.kg^{-1}$ (overall mean and standard deviation = 0.861 ± 0.473 $mg.kg^{-1}$) (Tab. 2). The overall mean and standard deviation for the species were 0.833 ± 0.443 $mg.kg^{-1}$ (Tab. 3).

In relation to Hg concentrations between males and females, the results were as follows: Shapiro-Wilk test showed that the data are normal / parametric; Bartlett test showed that the variances are homogeneous; and Student t test (independent) showed no statistically significant difference ($p>0.05$) between males and females. In the

TABLE 2 | Total mercury concentrations in the muscle of *Euthynnus alletteratus* samples analyzed in this study. THg = Total Mercury; # / N = Number; Sex (M = Male, F = Female); SBS 1 (Sub-Sample 1), SBS 2 (Sub-Sample 2), and SBS 3 (Sub-Sample 3); SD = Standard Deviation; CV = Coefficient of Variation. Note: The samples were analyzed in triplicate (sub-samples).

THg $mg.kg^{-1}$							
#	Sex	SBS 1	SBS 2	SBS 3	Mean (±)	SD	CV
1	M	0.037	0.169	0.134	0.113	0.068	60.339
2	M	0.044	0.095	0.172	0.104	0.064	62.159
3	M	0.050	0.037	0.108	0.065	0.038	58.157
4	F	0.036	0.064	0.054	0.051	0.014	27.641
5	M	0.034	0.091	0.173	0.099	0.070	70.343
6	F	0.604	0.490	1.040	0.711	0.290	40.809
7	F	0.074	0.212	0.063	0.116	0.083	71.374
8	M	0.955	0.611	0.922	0.829	0.190	22.886
9	M	1.440	1.560	1.360	1.453	0.101	6.926
10	M	1.930	1.490	1.820	1.747	0.229	13.110
11	M	0.829	1.040	1.170	1.013	0.172	16.989
12	M	0.911	0.866	0.855	0.877	0.030	3.382
13	M	0.591	0.713	0.920	0.741	0.166	22.435
14	M	0.911	0.786	0.871	0.856	0.064	7.457
15	M	0.793	0.769	0.951	0.838	0.099	11.804
16	F	0.901	0.831	0.786	0.839	0.058	6.904
17	M	0.949	0.705	0.938	0.864	0.138	15.950
18	F	1.980	0.933	1.350	1.421	0.527	37.094
19	F	1.010	1.480	1.490	1.327	0.274	20.675
20	M	1.490	1.300	0.954	1.248	0.272	21.775
21	F	1.420	1.440	1.170	1.343	0.150	11.199
22	M	1.010	0.985	0.861	0.952	0.080	8.382
23	M	0.918	1.050	0.916	0.961	0.077	7.988
24	F	0.873	0.824	1.030	0.909	0.108	11.839
25	F	0.932	1.070	0.789	0.930	0.141	15.103
26	M	0.862	1.020	0.824	0.902	0.104	11.524
27	M	0.877	0.933	0.983	0.931	0.053	5.696
28	F	0.991	1.070	0.834	0.965	0.120	12.499
29	M	1.000	0.939	0.744	0.894	0.134	14.952
30	M	0.845	1.040	0.794	0.893	0.130	14.539

TABLE 3 | Conversion of the results found in this study to be used in the formula determined.

Maximum content found in this study	Average content found in this study	Maximum content found in this study	Average content found in this study
Conversion $mg.kg^{-1} \rightarrow \mu g.kg^{-1}$	Conversion $mg.kg^{-1} \rightarrow \mu g.kg^{-1}$	Conversion $\mu g.kg^{-1} \rightarrow \mu g.g^{-1}$	Conversion $\mu g.kg^{-1} \rightarrow \mu g.g^{-1}$
1.980 → 1980	0.833 → 833	1980 → 1.98	833 → 0.833

comparison of Hg concentrations between length classes (P = 0.70–0.85 m; M = 0.85–1.0 m; and G = 1.0–1.15 m, with an interval of 15 cm between each class) based on the TLs of the analyzed specimens, the following results were obtained: Shapiro-Wilk test showed that the data are non-normal / non-parametric for the length class P and normal / parametric for the length classes M and G; Bartlett test showed that the variances are homogeneous; and Kruskal-Wallis and Multiple Comparisons (Nemeny) tests showed no statistically significant difference ($p>0.05$) in Hg concentrations between the length classes of the analyzed specimens. Another important observation in this study was that 15 (50%) of the 30 samples of *E. alletteratus* analyzed exceeded the maximum limit for Hg consumption established by the international and Brazilian legislation for carnivorous fish (1.0 $\mu g.g^{-1}$ / 1.0 $mg.kg^{-1}$).

According to the human health risk assessment calculation proposed by the USEPA (1989) and described below, the HQ for the species analyzed in this study was 2.4 (maximum value) and 1 (mean value). In addition, according to the USEPA (1989), HQ < 1 indicates the potential nonexistence of effects that are harmful to health (antagonistic), while HQ > 1 indicates the potential existence of effects that are harmful to health, with possible contrary (antagonistic) effects.

Calculation of the maximum value:

$$D = C \times I / W \times 1000$$

$$D = 1.98\ \mu g.g^{-1} \times 25\ g.day^{-1} / 70\ kg \times 1000 = \mathbf{0.71\ \mu g\ Hg/kg.day}$$

$$HQ = D / RfD \rightarrow HQ = 0.71 / 0.3 = \mathbf{2.4}$$

Calculation of the average:

$$D = C \times I / W \times 1000$$

$$D = 0.833\ \mu g.g^{-1} \times 25\ g.day^{-1} / 70\ kg \times 1000 = \mathbf{0.30\ \mu g\ Hg/kg.day}$$

$$HQ = D / RfD \rightarrow HQ = 0.30 / 0.3 = \mathbf{1}$$

Considering the average Brazilian consumption of fish of 25g/day, the HQ for the specimens of *E. alletteratus* analyzed in this study was high, reaching values above and equal to 1 (1.000). These results confirm that THg concentrations in the fish were higher than the maximum permissible level of 1.0 $mg.kg^{-1}$ / 1.0 $\mu g.g^{-1}$ for predator/carnivorous species (ANVISA, 1998; MAPA, 1999). However, it is important to note that determining daily fish consumption may not accurately reflect reality, as an individual may not consume fish on a daily basis but may consume large quantities in a short period of time (Ferreira *et al*., 2012).

DISCUSSION

The main Hg absorption route in marine vertebrates is via food, which together with the low rate of excretion leads to increased concentrations of the element in the food chain (Legat, Lailson-Brito, 2010). Therefore, understanding the eating habits and prey of marine vertebrates is important because it can provide insight into the levels of Hg contamination in these animals.

Although the objective of this study was not to analyze the Hg concentrations in the food items found in the stomachs of the analyzed fish, it is important to emphasize that fish that consume large quantities of other fish tend to accumulate more Hg (Ferreira *et al*., 2012). This fact is confirmed in a study by Storelli *et al*. (2005b) who found a statistically significant difference ($p<0.0001$), with the highest Hg concentrations observed in Atlantic bluefin tuna (*Thunnus thynnus*, 0.20 $\mu g.g^{-1}$) samples when compared to swordfish (*Xiphias gladius*, 0.07 $\mu g.g^{-1}$) samples. Gorni (2010) studied trophic interactions between pelagic species from the Southwest Atlantic Ocean, including analysis of stomach contents of swordfish (*Xiphias gladius*), yellowfin tuna (*Thunnus albacares*), bigeye tuna (*Thunnus obesus*), and albacore (*Thunnus alalunga*). In general, swordfish stomachs contained mostly cephalopod mollusks, whereas tuna stomachs contained more fish, confirming the piscivorous diet of these animals. In this study, the stomachs of the little tunny samples analyzed contained only fish species native to the Brazilian coast. Given that tuna are transient animals, the specimens used in this study may have migrated and fed in different areas of the Brazilian coast, making it impossible to determine the source of the ingested food. *E. alletteratus* is an opportunistic predator that feeds on almost everything that appears in its area of occurrence, including small pelagic fish, primarily those of the Clupeidae family (sardines), cephalopod mollusks such as squid, crustaceans, and other marine invertebrates (Collette, Nauen, 1983; Carvalho-Filho, 1994, 1999; Szpilman, 2000; FishBase, 2013; García, Posada, 2013).

According to Beckvar *et al*. (1996), differences in Hg bioaccumulation may also be related to the different nutritional needs of organisms at different life stages. According to Svobodová *et al*. (1999), the age of the fish is an important factor because of the possible changes in feeding and the time (duration) of Hg exposure of the animal. Several studies have linked the age, length, and weight of the fish to their Hg concentrations (Bache *et al*., 1971; Monteiro *et al*., 1991; Morrison, Thérien, 1995; Kehrig *et al*., 2001; Ikingura, Akagi, 2003). According to Ikingura, Akagi (2003), the relationships between length and weight can be used to predict THg concentrations in fish. According to Svobodová *et al*. (1999), in addition to the relationship between Hg concentration and muscle tissue, there is also a relationship between Hg concentration, length and weight of fish, and the liver. Hg is mostly concentrated in the liver due to the presence of the metallothionein protein, which is rich in sulfhydryl groups, with which Hg has a high affinity (Olson *et al*., 1978; Roméo *et al*., 1999; Berntssen *et al*., 2004). In this study, the age of the little tunny specimens was not determined. However, through macroscopic analysis of the gonads, it was possible to identify the sex and gonadal maturation stage. In terms of the stage of maturation, all the fish samples were classified as mature adults. In terms of size, the specimens with the highest length and weight had the highest Hg concentrations.

Studies conducted by Capelli *et al*. (1983) and Beckvar *et al*. (1996) demonstrate that differences in Hg accumulation between male and female fish can be attributed to differences in diet. However, the authors point out that the physiological differences between the two sexes are important in relation to Hg concentrations. Furthermore, understanding these physiological differences is important because the gonads play a role in eliminating organic mercury. In this study, no statistically significant differences were observed in diet and Hg concentrations ($p>0.05$) between males and females of *E. alletteratus*.

The high biomagnification of Hg in large pelagic fish, such as tuna, swordfish, and shark, is normally attributed to their trophic level in the food chain (Ferreira *et al*., 2012). However, Branco *et al*. (2007) found that the level of Hg accumulation varies according to the region where the fish live, although they belong to the same species and trophic level. The authors found that THg and MeHg concentrations were significantly higher in swordfish caught near Ecuador than in swordfish caught in areas close to the Archipelago of the Azores, most likely because of the type and quantity of food available to the fish in those regions. All the fish used in this study were caught from the coast of Arraial do Cabo, east coast of the State of Rio de Janeiro, southeastern Brazil, an area known for its natural beauty and the absence of environmental contamination. One of the main characteristics of this region is the presence of the upwelling phenomenon, which is responsible for the high primary productivity of its waters (Valentin, Coutinho, 1990; Valentin, 1994; Calado *et al*., 2010). Of the 30 specimens of little tunny analyzed in this study, 27 (90%) had food items in their stomachs. *Sardinella brasiliensis* was the prey most consumed by the species, followed by *A. lepidentostole*, *E. volitans*, and *D. punctatus*.

In addition to diet and trophic level, other specific factors must also be taken into account when comparing Hg concentrations in different species, such as the different metabolism of predatory fish. The high Hg concentration normally found in fish such as tuna and swordfish, which occupy high trophic levels, is not attributed to the diet of these species alone. As they are predators, these fish have high metabolic activity and a high feeding rate as they require a continuous supply of energy. As a consequence, the intensity of predation and food consumption are extremely high. Associated with a higher rate of absorption and a lower rate of excretion, this contributes significantly to Hg accumulation in the tissues and biomagnification along the food chain (Storelli, 2005b; Damiano *et al*., 2011).

Variations in Hg concentrations may also be related to the migration of fish to more contaminated areas or the time spent in these areas (Francesconi, Lenanton, 1992; Al-Majed, Preston, 2000). According to Kim (1995), Beckvar *et al*. (1996), and Goldstein *et al*. (1996), other factors that may influence Hg concentrations are the physiological characteristics of the species, amount of food eaten, and size of prey ingested.

The geographical distribution of *E. alletteratus* is limited almost exclusively to the Atlantic Ocean (Collette, Nauen, 1983; Vaske Jr. *et al*., 2020; Vieira *et al*., 2021). The species do not tolerate low oxygen concentrations and low temperatures, spending most of their time in shallow waters above the thermocline (Collette, Nauen, 1983; Szpilman, 2000). Mercury pollution can be a threat to tuna and tuna-like species (Ueno *et al*., 2003) since a mercury increase in oceans has been shown (Drevnick *et al*., 2015). Compared to other tuna species, little tunny feed on smaller prey, such as small pelagic fish, squid, crustaceans, and other invertebrates (Collette, Nauen, 1983; Szpilman, 2000). Smaller prey, such as sardines and anchovies (García, Posada, 2013), tend to show low THg concentrations (Storelli *et al*., 2005b; Voegborlo *et al*., 2006). According to Trudel and Rasmussen (2006), mercury bioaccumulation in fish tends to increase with age, especially when older fish assess (consume) larger and more contaminated prey.

Zheng *et al*. (2019) conducted a study in which they showed that the bioaccumulation of mercury in marine fish was highly variable, and its concentration was affected by the specific physiological and ecological characteristics of different fish species. According to the authors, Hg exposure can produce teratogenic, neurotoxic effects, and reproductive toxicity. These effects can cause damage to cells, tissues, proteins, genes, and consequently, to the survival, growth, and behavior of marine fish. According to Huang *et al*. (2011) and O'Bryhim *et al*. (2017), in marine fish, exposure to Hg can lead to reduced liver function and metabolism, altered behavior, impaired reproduction, deformity, damage to the gills and olfaction organs, and mortality.

Regarding the teratogenic effects of mercury on marine fish, malformations due to mercury exposure exert a devastating effect on fish, since they not only affect the external morphology, survival mode, and growth rate of fish, but can also affect the behavioral characteristics of these animals, such as long-distance migration, hunting, and avoiding predators (Webber, Haines, 2003; Mora-Zamorano *et al*., 2016, 2017). The most common malformations observed in fish occur in the spine, but these animals can also have malformations in the head region, fins, and bladder. The most obvious spinal deformities include lordosis (sacral doris), kyphosis (sacral curvature), and scoliosis (lateral curvature) (Sfakianakis *et al*., 2015; Morcillo *et al*., 2016). Deformities, especially skeletal deformities, can interfere with the fish's ability to interact with the environment, reducing their chances of survival (Huang *et al*., 2011). Bone deformities can also result from poor nutrition (Liu *et al*., 2016).

Regarding the neurotoxic effects of mercury on marine fish, Hg can accumulate in the brain and thereafter can cause significant damage (Wang *et al*., 2015). A study conducted by Keyvanshokooh *et al*. (2009) observed changes of the proteome in the brain of juvenile beluga (*Huso huso*), confirming that methylmercury induces toxicity through oxidative stress and apoptosis, which suggested that chronic methylmercury exposure can cause an important metabolic defect in the brain. According to Pereira *et al*. (2016), mercury may also induce morphological changes in the brain, such as changes in the total number and volume of neurons and glial cells in specific areas of the brain, accompanied by changes in swimming behavior. Senger *et al*. (2010) demonstrated in a study of white seabream (*Diplodus sargus*) that neurobehavioral deficits induced by mercury exposure can be passed down steadily to the next generation. These deficits were transmitted through sperm and persisted in each generation. According to the authors, this discovery showed that such neurological diseases can persist in a fish population for generations, even after the source of pollution has been removed. Regarding the reproductive toxicity of mercury on marine fish, Hg can accumulate in the gonads of fish and may affect the reproductive system, in addition to inhibit the growth and development of fish gonads (Liao *et al*., 2006).

The upwelling phenomenon is characterized by the surfacing of deep waters, generally cold and rich in mineral salts. In the deep layers, nutrients are not consumed by primary producers due to the absence of photosynthesis in these areas, and they tend to accumulate, potentially reaching the surface layers during upwelling events. A natural ecological effect resulting from upwelling phenomenon is the increase in local biological production (Valentin *et al*., 1975). Upwelling areas, along with coastal areas, play an important economic role, accounting for 98% of the world's fish production. The number of fish in areas where upwelling occurs increases, as many species are attracted by the greater availability of food. Upwelling not only increases available fish stocks but also facilitates the predictability (prospects) of fishing activities (Silva, 2006).

Upwelling also plays a significant role in Hg bioavailability. Organic forms of mercury produced in deep waters of minimal oxygen content below the thermocline (Mason, Sullivan, 1999) can reach the surface. Thus, Hg can be incorporated by primary producers through diffusion and gradually accumulate in subsequent levels of the food chain. In top-levels consumers, such as carnivorous fish, Hg reaches higher concentrations than those found in their prey, characterizing the process of biomagnification (Silva, 2006).

Organisms that occupy high trophic levels, such as tuna and tuna-like species, may exhibit high concentrations of mercury (Peterson *et al*., 1973). These fish are fast-swimming opportunistic top predators (FAO, 1997; Amorim *et al*., 2011), and they have a diverse diet (Collette, Nauen, 1983; Amorim *et al*., 2011). Due to the fact that the diet is the main route of trace element accumulation in marine organisms (Wang, 2002; Silva *et al*., 2011), these fish can present elevated Hg concentrations in their tissues (Voegborlo *et al*., 2006). Being highly migratory (Collette, Nauen, 1983), tuna and tuna-like species can be sentinels of Hg global pollution and other pollutants, thus providing information about the contamination status of offshore waters and open seas (Ueno *et al*., 2003; Endo *et al*., 2016). In view of these facts, *E. alletteratus* can be used as an indicator or a bioindicator of environmental contamination by micropollutants, as well as a sentinel of the environmental health of certain aquatic systems in relation to Hg contamination. The results found in the samples analyzed in this study are significant both in terms of ecotoxicology and public health. In terms of ecotoxicology, little tunny is a potential bioindicator of marine pollution in coastal and ocean ecosystems. However, pollution does not appear to be the primary cause of the high Hg levels found in this species. Miller *et al*. (1972) found values ranging from 0.26 to 0.64 $\mu g.g^{-1}$ in specimens of tuna and swordfish from museums dating from 1946 to 1978, a time period preceding the contributions of pollutants from industrial activities.

Because of the cumulative effect of this metal on organisms, it is critical to consider the maximum THg values in the analysis of the results and the frequency of fish consumption of a given population when studying fish contamination by Hg. The mean THg values should also be considered, as regular consumption of these animals may pose a risk to human health. Furthermore, the limits established by the legislation for consumption should be revised and should be specific to the culture and dietary habits of each population. These facts have alarmed scientists and public health and fisheries agencies, prompting them to take more effective measures when deciding whether to authorize the consumption of certain fish species, especially large predatory fish. Programs to monitor this metal in fish, which may be carried out at the initiative of a specific sector or institutional partnerships, are valuable tools for reducing the risks associated with the consumption of Hg-contaminated fish.

When it comes to public health, the findings are concerning, as half of the samples exceeded the maximum Hg limit recommended by the international and Brazilian legislations for human consumption in carnivorous fish (1.0 $\mu g.g^{-1}$ / 1.0 $mg.kg^{-1}$). Another concerning factor is the frequency with which these fish are consumed in areas where they are caught and sold along the Brazilian coast. *E. alletteratus* can be found all year on the east coast of the State of Rio de Janeiro, in the regions of Cabo Frio and Arraial do Cabo, but it is most abundant in spring and summer, when it is caught and sold. The species has commercial value in this region and is widely consumed by the local population. Little tunny may pose a risk to human health depending on how frequently it is consumed. Thus, the frequency with which these fish are consumed and the potential risks to human health must be considered, especially for groups that are more vulnerable to the effects of Hg poisoning, such as children and pregnant women.

The information presented in this paper serves as a warning to avoid consuming these fish and emphasizing the importance of continuous monitoring of Hg concentrations in tuna sold in the State of Rio de Janeiro. It will raise awareness among public health sector and fisheries agencies about the need for more effective actions to control *E. alletteratus* consumption. Programs to monitor this metal in tuna and other fish species should be encouraged because they are valuable tools for reducing the risks associated with eating Hg-contaminated fish. It is also important to encourage further research on the subject, particularly regarding the contamination routes of these animals.

Few studies have been conducted on Hg in organisms from upwelling environments. The available literature is insufficient to establish the risks to consumers of consuming fish containing Hg caught in upwelling areas. Despite the uncertainty regarding the origin of the little tunny specimens used in this study (capture area of the individuals), although the species is considered common on the coast of Arraial do Cabo, and the small sample size (20 males and only 10 females – 30 muscle pieces, each divided into three smaller aliquots, to be analyzed in triplicate), due to the high cost of analysis (R$ 108.00 Brazilian reais x 90 sub-samples = R$ 9,720.00 total cost), the results show that constant monitoring of *E. alletteratus* with regard to Hg concentrations is necessary, and that this species should be consumed with caution.

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Marcelo Tardelli Rodrigues: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.
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