Total-Hg and organic-Hg in *Cephalopholis fulva* (Linnaeus, 1758) from inshore and offshore waters of NE Brazil

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(With 3 figures)

Abstract

To investigate whether source proximity or bioavailability is the major factor controlling both Hg concentration and Hg speciation in marine fishes, total- and organic-Hg content in muscle and liver tissues from different populations of *Cephalopholis fulva* (piraúna) from inshore and offshore waters of the Brazilian northeastern coast were analyzed. Average total-Hg in muscle (104 ng.g^{-1} w.w.) and liver (2,865 ng.g^{-1} w.w.) tissues, as well as organic-Hg concentrations in muscle (169 ng.g^{-1} w.w.) and liver (1,038 ng.g^{-1} w.w.), were much higher in the offshore population of *C. fulva* than in the inshore ones. In the inshore population total-Hg and organic-Hg average concentrations in muscle tissue were similar and reached only 49 ng.g^{-1} w.w., while in liver they reached 412 ng.g^{-1} w.w. for total-Hg and 180 ng.g^{-1} w.w., for organic-Hg. Concentrations of both Hg species in the two populations were higher in liver than in muscle. The average percentage contribution of organic-Hg to the total Hg content was higher in muscle (98-100%) than in liver (42-53%), but similar between the two populations. Total-Hg and organic-Hg concentrations in muscle and liver from the offshore population showed significant ($P < 0.05$) positive correlation with fish length. However, in the inshore population only the total-Hg and organic-Hg in muscle tissues correlate significantly with fish size. Although the coastal environments are enriched in total Hg relative to open waters, the significant higher Hg concentrations in the offshore population of *C. fulva* and the significant correlation found between organic-Hg in liver with fish size suggest higher bioavailability of Hg in offshore waters relative to inshore ones.

Keywords: total mercury, organic mercury, fish populations, *Cephalopholis fulva*, NE Brazil.

Mercúrio total e mercúrio orgânico em *Cephalopholis fulva* (Linnaeus, 1758) em águas costeiras e mar aberto no nordeste do Brasil

Resumo

As concentrações de Hg-total e Hg-orgânico foram determinadas em diferentes populações de *Cephalopholis fulva* (piraúna) capturadas em águas costeiras e em bancos oceânicos do litoral nordeste do Brasil. A comparação entre as duas populações permitiu investigar o efeito da proximidade de fontes sobre as concentrações, e a especiação de Hg em músculo e fígado desta espécie. As concentrações médias de Hg-total em músculo (104 ng.g^{-1} w.w.) e fígado (2,865 ng.g^{-1} w.w.), assim como as concentrações de Hg-orgânico em músculo (169 ng.g^{-1} w.w.) e fígado (1,038 ng.g^{-1} w.w.) foram muito maiores na população capturada nos bancos oceânicos do que na população costeira. Nesta, as concentrações médias de Hg-total e Hg-orgânico na musculatura de *C. fulva* foram similares e baixas (49 ng.g^{-1} w.w.), enquanto que atingiram 412 ng.g^{-1} w.w. de Hg-total e 180 ng.g^{-1} w.w. de Hg-orgânico no fígado destes animais. As concentrações das duas espécies de Hg foram significativamente maiores no fígado do que na musculatura. A contribuição percentual média de Hg-orgânico para a concentração total de Hg nos peixes foi maior para músculo (98-100 %) que para fígado (42-53 %), mas semelhante entre as duas populações. As concentrações de Hg-total e Hg-orgânico na musculatura e no fígado de *C. fulva* mostraram-se positivamente correlacionadas com o tamanho do animal ($P < 0,05$). Entretanto, na população costeira somente as concentrações destas espécies de Hg na musculatura apresentaram correlações significativas com o tamanho do animal. Embora o ambiente costeiro seja relativamente enriquecido em Hg, em relação aos bancos oceânicos, as maiores concentrações de Hg foram verificadas na população oceânica de *C. fulva*. A correlação significativa entre Hg-orgânico no fígado e tamanho do animal no fígado sugerem uma maior biodisponibilidade do Hg em águas oceânicas quando comparada às águas costeiras.

Palavras-chave: mercúrio total, mercúrio orgânico, populações de peixes, *Cephalopholis fulva*, litoral nordeste.

1. Introduction

Mercury levels in the aquatic biota respond to local bioavailability and vary among species of a given site and within a same species from different places. Also, Hg concentrations are different among different organs of a given species. For example, Storelli et al. (2005) measuring concentrations of total Hg and organic-Hg in the edible fish tissue from the Ionian and Adriatic seas, detected higher concentrations in striped mullet (*Mullus barbatus*), a benthic species, than in hake (*Merluccius merluccius*), a pelagic species. Romeo et al. (1999) evaluating Hg levels in muscle, gills and liver tissues in different fish species from the Mauritania coast, observed that Hg concentrations in the gills and muscle of the pelagic species are very low, whereas higher concentrations were found in the liver of benthic species. Joiris et al. (1999) determining total-Hg and organic-Hg in sardines from Tunisia found similar concentrations in the northern and eastern areas and significantly lower concentrations in the southern zone. Higher Hg concentrations occurred in the liver compared to muscle tissues regardless of the area sampled. In general, most previous studies have also shown a positive correlation between Hg concentration and fish length, suggesting biomagnification (Adams, 2004; Pinho et al., 2002; Joiris et al., 2000; Phillips et al., 1997).

Analyzing Hg speciation in muscle tissue of fish, numerous studies have demonstrated that most of the Hg present is bioaccumulated as metyl-Hg (Storelli et al., 2005), the most toxic form due to its neurotoxic properties (WHO, 1990). Anderson and Depledge (1997) reported percentages of organic-Hg varying from 63% and 86% of different fish species from Azorean waters. Joiris et al. (1999), analyzing muscle tissue of sardines from Tunisia, found that organic-Hg represents about 85% of the total Hg present in the fish muscle. Storelli et al. (2002; 2003) and Kehrig et al. (2005) found organic-Hg percentages varying from 75 to 100% and 94 to 100%, in muscle tissue of tuna and other carnivorous fish species, respectively.

The spatial variability of Hg concentrations in populations of a single species has mostly been evaluated when comparing polluted and unpolluted sites. Less attention is given on the variability of Hg concentration and distribution in populations of a single species inhabiting different sites in a similar area, such as inshore and offshore waters of a given coast where, although under the influence of similar Hg sources, Hg may be present with different bioavailability to fish uptake.

The aim of this study was to analyze the total Hg and organic-Hg concentrations in the muscle and liver tissues of *Cephalopholis fulva*, a benthic grouper of significant economic importance, collected from inshore and offshore waters, free of any significant anthropogenic Hg source (Marins et al., 2004), on the Ceará coast in northeastern Brazil.

2. Materials and Methods

The red grouper *Cephalopholis fulva* (Linnaeus, 1758) occurs along the Western Atlantic from southern North Carolina and Bermuda to southeast Brazil, including the Bahamas and the continental and insular coasts of the Caribbean Sea, Fernando de Noronha Islands and Atol das Rocas atoll (Figueiredo and Menezes, 1980; Cervigón, 1991, Heemstra and Randall, 1993). The species is exclusively marine and inhabits coastal areas from up to 150 m deep, living close to coral reefs and rocky bottoms, in rifts and caves where it hides during the day. According to Cervigón (1991), it is found in up to 50 m depth, being more common in up to 25 m. The alimentary diet of *C. fulva* consists of small fish and benthic invertebrate (Randall, 1967). This species doesn't present migratory habits, i.e. once a population settles it does not mix with others from different areas (Heemstra and Randall, 1993).

Specimens of *C. fulva* were caught in inshore waters in the Ceará continental shelf, NE Brazil and adjacent offshore oceanic waters (Figure 1). Both stations are located in front of the central portion of the Ceará coast receiving effluents from the most urbanized part of the coast and where major rivers are also located. The inshore population (11 individuals) was sampled at between 20 to 60 m deep by trawling the sandy coralline bottom of the continental shelf at about 20 nautical miles from the shore, whereas the offshore population (14 individuals) was sampled at the Mundau Oceanic Bank at 60 m deep, about 140 nautical miles from the shore. The Ceará Abyssal Plain of 3,500 m in depth extends between the two sites (MMA, 2004), representing an immense environmental barrier for demersal fish, guaranteeing that the two populations are specifically linked to their own substrate and habitat.

Fishes were brought to the laboratory where length, weight and sex were recorded. Soon after, samples were dissected to separate liver and muscle tissues and preserved at –25 °C until analysis. Total Hg and organic-Hg were determined by selective reduction after Magos (1971) and Limaverde Filho and Campos (1999). Samples of

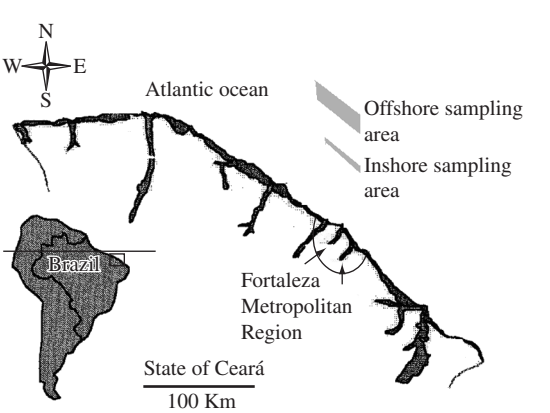

Figure 1. Map of the NE Brazilian coast where *C. fulva* were collected for Hg analysis.

liver and muscle tissues (0.5 g wet weight) were digested with 4 mL 40% NaOH and 1 mL of a 1% cystein solution in a heating plate at 70 °C. Extracts, at room temperature, were taken to 10 mL with 1% NaCl. A 10 mL aliquot of the digested extract was treated with 1 mL 1% SnCl/CdCl solution for the reduction of total Hg present in the sample. A second aliquot was treated with 1% SnCl solution (1 mL), reducing only the inorganic Hg present. The organic-Hg fraction was estimated by the difference of the two reduction steps. Determination of Hg concentrations was performed with Cold Vapor Atomic Absorption Spectrophotometry (CVAAS) in a Varian model AA-6, equipped with a cold vapor generator.

The accuracy of total-Hg and organic-Hg determinations were tested by simultaneous analysis of Dogfish Muscle Certified Reference Material for Trace Metals – DORM-2, with average recovery of 96.1% for total-Hg and 98.0% for organic-Hg (Table 1). These results demonstrated accuracy and precision of the analytical method.

3. Results and Discussion

Table 2 gives fish length, total Hg, organic-Hg and % organic-Hg of the total Hg content in *C. fulva*. Average offshore fishes were larger (313 ± 43 mm) than inshore fishes (263 ± 49 mm). Total-Hg and organic-Hg concentrations were much higher in liver than in the muscular tissue for both populations studied. In the offshore population, liver total-Hg concentrations reached 2,865 ± 3,293 ng.g^{-1} whereas in muscle, average concentrations reached 174 ± 104 ng.g^{-1}. Organic-Hg in this population reached 1,038 ± 1,031 ng.g^{-1} in liver and 169 ± 97 ng.g^{-1} in muscle. In the inshore population total-Hg concentrations in liver reached 412 ± 406 ng.g^{-1} whereas in muscle, average concentrations reached 49 ± 28 ng.g^{-1}. Organic-Hg in the inshore population reached 180 ± 154 ng.g^{-1} in liver and 49 ± 28 ng.g^{-1} in muscle. The ratios between total Hg in liver to that of in muscle were 8.4 for the inshore population and 16.5 for offshore waters, much higher than most ratios reported in literature. For example, Joiris et al. (1999) found a ratio of 1.4 in *Sardinella aurita* and 1.7 for *Sardina pilchardus* sampled in Bangladeshi waters. In *Cephalopholis nigri* from Mauritania coast the ratio was 1.9 (Romeo et al., 1999). In carnivorous species sampled in SE Brazil, however, Kherig et al. (2005) found liver/muscle ratios of Hg concentrations of 1.3 and of 12 for herbivorous species.

Total-Hg concentrations in the offshore population were significantly higher (174 ± 104 ng.g^{-1} and 2,865 ± 3,293 ng.g^{-1}) in muscle and liver tissues respectively, than in inshore fishes (49 ± 28 ng.g^{-1} and 412 ± 406 ng.g^{-1}, in muscle and liver tissues respectively). Similarly, organic-Hg concentrations in the offshore population were also significantly higher (169 ± 97 ng.g^{-1} and 1,038 ± 1,031 ng.g^{-1}) in muscle and liver tissues respectively, than in inshore fishes (49 ± 28 ng.g^{-1} and 180 ± 154 ng.g^{-1} in muscle and liver tissues respectively). The percentage of organic-Hg to the total content was higher and constant in muscle, being 100% in the population of inshore waters and between 80 and 90% in the population of offshore waters. The organic-Hg fraction in liver of both populations was lower ranging from 42 to 53% in offshore and inshore fishes respectively. Similar distribution was found for *Sardinella*

Table 1. Expected and measured Hg concentrations in the certified reference material DORM-2 (National Research Council of Canada), (n = 5).

Hg (μg.g^{-1})	Certified concentration	Measured concentration
Total-Hg	4.64 ± 0.26	4.46 ± 0.03
Organic-Hg (Methyl-Hg)	4.47 ± 0.32	4.38 ± 0.03

Table 2. Fish length, total Hg, organic-Hg and % of organic-Hg of the total Hg content in *Cephalopholis fulva* (Linnaeus, 1758), from two different populations sampled in inshore and offshore waters of the Ceará continental shelf, NE Brazil.

Fish population	Length (mm)	Total Hg in muscle (ng.g^{-1} wet wt)	Total Hg in liver (ng.g^{-1} wet wt)	Organic-Hg in muscle (ng.g^{-1} wet wt)	Organic-Hg in liver (ng.g^{-1} wet wt)	Organic-Hg in muscle (%)	Organic-Hg in liver (%)
Inshore							
Mean	263	49	412	49	180	100	53
SD	49	28	406	28	154	0	20
Minimum	195	23	90	23	54	100	33
Maximum	333	94	1,104	94	522	100	93
n	11	11	11	11	11	11	11
Offshore							
Mean	313	174	2,865	169	1,038	98	42
SD	43	104	3,293	97	1,031	5	12
Minimum	260	31	238	31	146	80	29
Maximum	380	425	10,190	425	3,048	100	62
n	14	14	13	14	13	14	13

aurita and *Sardina pilchardus*, with the organic-Hg fraction constant and high, from 85 to 97% in muscle tissue and only 20 to 50% in liver (Joiris et al., 1999) and for *Micropogonias furnieri* from SE Brazil, with 98 and 31% of organic-Hg in muscle and liver tissue respectively.

Total-Hg and organic-Hg concentrations in muscle and liver from the offshore population showed significant ($P < 0.05$) positive correlation with fish length (Figures 2 and 3). However, in the inshore population only the total-Hg and organic-Hg in muscle tissues

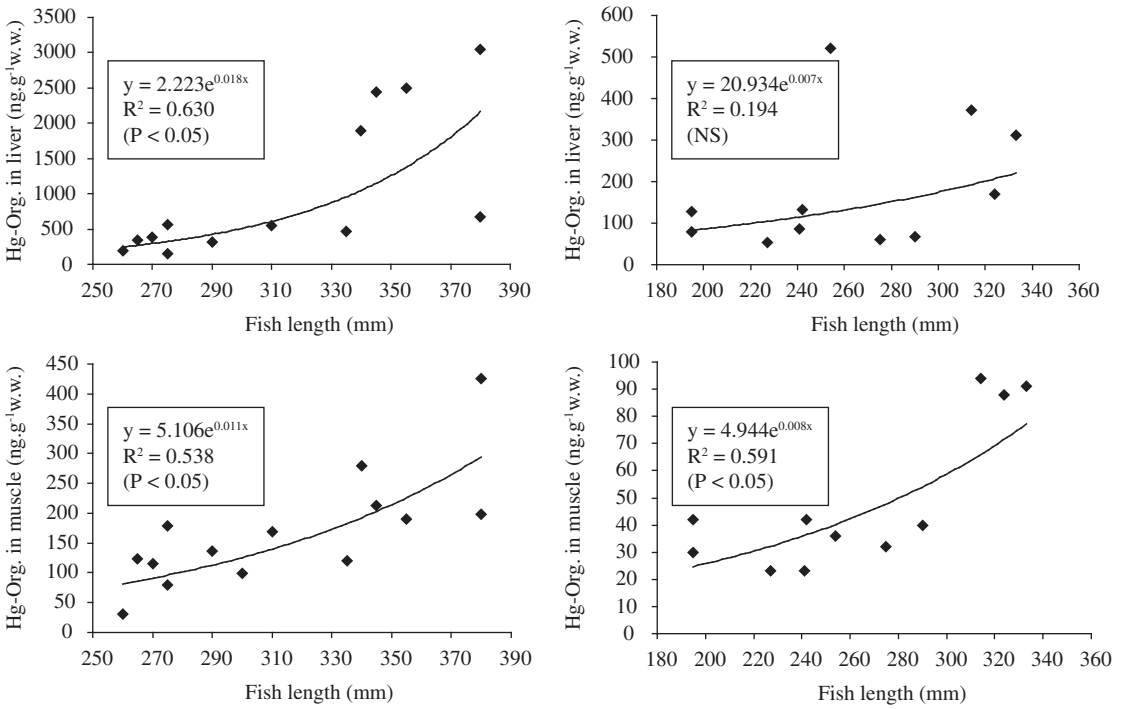

Figure 2. Correlation between organic-Hg concentrations and fish length in *C. fulva* from inshore (right) and offshore (left) waters of NE Brazil.

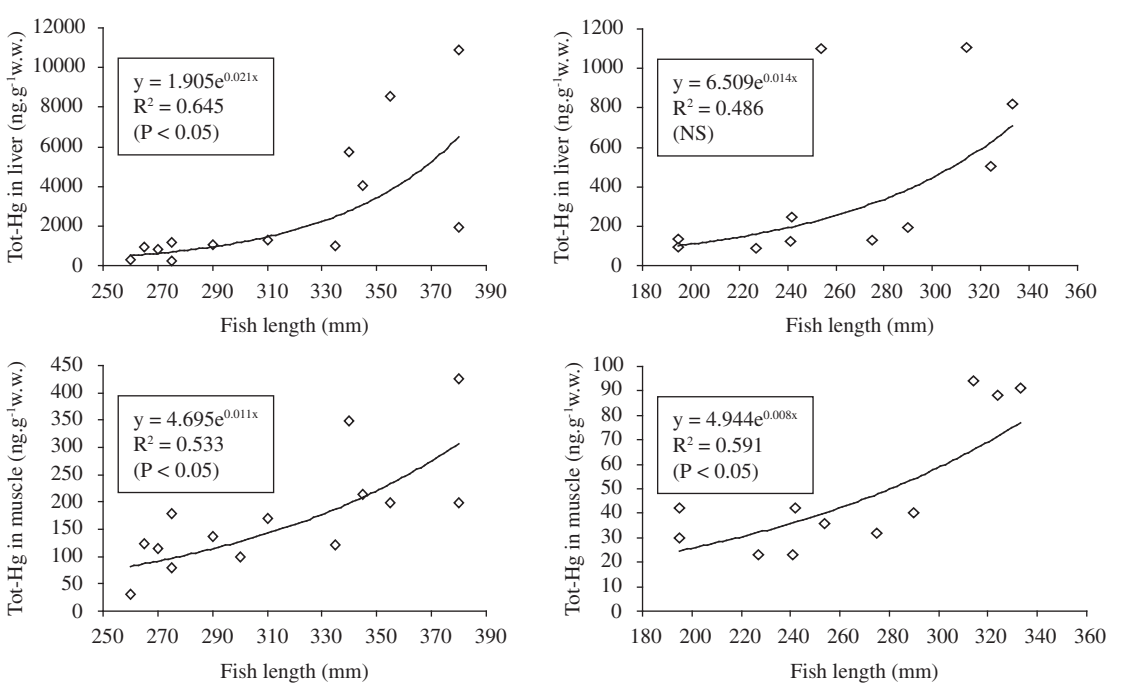

Figure 3. Correlation between total-Hg concentrations and fish length in *C. fulva* from inshore (right) and offshore (left) waters of NE Brazil.

correlate significantly with fish size. The significantly higher Hg concentrations in the offshore population of *C. fulva* and the significant correlation found between organic-Hg in liver with fish size suggest intake of Hg from biological sources through the food web and therefore, is mostly organic Hg (Storelli et al., 2005; Adams, 2004; Pinho et al., 2002; Joiris et al., 1995). Also these different concentrations suggest higher bioavailability of Hg in offshore waters relative to inshore waters.

The difference in Hg concentrations between offshore and inshore populations can be explained by differences in size. From Figures 2 and 3 one can deduce that fish length could explain a twofold increase in concentrations at maximum and would hardly explain differences in liver concentrations. Difference in Hg availability between the two areas may contribute to a great extent to the difference. Various studies (Leermakers et al., 1995; Lacerda et al., 2001; Marins et al., 2002; Paraquetti et al., 2004) showed an increase in Hg reactivity in offshore waters in comparison with waters closer to the continent. In waters under the influence of continental inputs, Hg is mostly associated with clastic suspended matter (Lacerda and Gonçalves, 2001; Paraquetti et al., 2004). Ocean wards, Hg associated with dissolved organic matter increases, resulting in higher bioavailability (Paraqueti et al., 2004). On the Ceará coast, Marins et al. (2002) showed increasing dissolved Hg concentrations towards the ocean along estuarine and coastal waters. Similarly, on the SE Brazil continental platform, Lacerda et al. (1993; 2004) showed that Hg could be transported from continental sources to deep offshore waters, being correlated to clastic material close to the shore and to organic complexes offshore. Therefore, offshore transport followed by increasing bioavailability could explain the differences in Hg concentrations between the two fish populations.

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