Mercury concentrations in dusky grouper *Epinephelus marginatus* in littoral and neritic habitats along the Southern Brazilian coast

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A R T I C L E  I N F O

Article history:
Received 4 July 2016
Received in revised form 28 November 2016
Accepted 5 December 2016
Available online 13 December 2016

Keywords:
Total mercury
Trophic position
Epinephelidae
Human health risk

A B S T R A C T

Our study incorporated a comprehensive suite of parameters (i.e., body size, age, diet and trophic position) to investigate mercury concentration in dusky groupers *Epinephelus marginatus*. This study was carried out in rocky bottoms in littoral and neritic habitats along the Southern Brazilian coast. We also determined spatial variation in mercury concentrations in individuals inhabiting both zones, which may provide insights into how dietary differences or potential pollution sources affect bioaccumulation. A total of 244 dusky groupers was analyzed to determine total mercury concentrations. Our study revealed that when considering similar body sizes, individuals inhabiting littoral rocky habitats had higher concentrations of mercury probably due to proximity to pollution sources associated with human activities in the estuary and its drainage basin. Furthermore, large individuals (>650 mm and ~8 years old) showed mercury contamination levels that are potentially harmful for this endangered fish species and above the acceptable limits for human consumption.

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1. Introduction

Mercury is a non-essential toxic element commonly found in aquatic ecosystems. It is released through a variety of natural and anthropogenic processes including geologic weathering, gold mining, and burning of coal (Pacyna et al., 2010). In aquatic systems, inorganic mercury can be transformed by microbial activity into organic methylmercury (MeHg) which biomagnifies in the food web and results in high concentrations of methylmercury in piscivorous species (including humans). Previous studies have shown deleterious effects of mercury on health at concentrations >0.3 mg kg⁻¹ wet weight (Sandheinrich et al., 2011; Depew et al., 2012). These concentrations are not uncommon to aquatic food webs suggesting that health and reproduction of wild fish populations may be impacted at environmentally relevant concentrations of MeHg (Beckvar et al., 2005; Webb et al., 2006; Sandheinrich and Wiener, 2011). A literature review by Depew et al. (2012) of mostly freshwater fishes found that concentrations of mercury within this range results in decreases in gonadosomatic index (GSI), sex steroid production, spawning success, fertilization, fecundity, and increases in ovary apoptosis.

Large-bodied and long-lived fish species such as the dusky grouper (*Epinephelus marginatus*) are well recognized for their ability to bioaccumulate mercury (Evers et al., 2009; Tremain and Adams, 2012). Dusky grouper are exploited by fishermen in rocky bottoms along the coasts of South America (Figueiredo and Menezes, 1980; Condini et al., 2007), Europe (Heemstra and Randall, 1993) and Africa (Fennessy, 2006), and are widely consumed by human populations (Heemstra and Randall, 1993). Mercury bioaccumulation in other epinephelids has been investigated mainly in the northern hemisphere (Hassan et al., 2007; Evers et al., 2009; Tremain and Adams, 2012; Harris et al., 2012, but see Lacerda et al., 2007 and Kutter et al., 2009), and prior studies on mercury contamination of large-bodied fishes in the south-western Atlantic are restricted to other fish families such as sciaenids, trichiurids, and scombrids (Viana et al., 2005; Medeiros et al., 2008; Di Benedetto et al., 2013). These studies focused mainly on levels of mercury in fish tissues, typically muscle, and usually related contamination levels with body size (Adams and Onorato, 2005; Lacerda et al., 2007) and/or trophic position of the individual estimated by stable isotopes (Bank et al., 2007; Hassan et al., 2007; Tremain and Adams, 2012; Di Benedetto et al., 2013; Sluis et al., 2013). In several cases, these studies report that mercury concentrations in large-bodied fishes are above the proposed threshold (0.3 mg kg⁻¹ wet weight) required to safeguard these wild populations (Adams and Onorato, 2005; Lacerda et al., 2007; Evers et al., 2009). In some instances, they are also above the safety levels (0.5 mg kg⁻¹ wet weight, World Health Organization - WHO) for human consumption (Cai et al., 2007; Hassan et al., 2007; Harris et al., 2012; Sluis et al., 2013). Taken together, these studies provide evidence that mercury concentrations in dusky grouper are likely to occur at levels that may negatively affect fish health and pose a risk for human consumption.

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Dusky grouper is an important fisheries species of conservation concern (classified by IUCN as “endangered”; Cornish and Harmelin-Vivien, 2004), thus, additional information is needed on factors (e.g. body size, age, trophic position, habitat use) that affect mercury concentrations in order to support conservation and socio-ecological aspects of fisheries management (e.g. size limits, consumption advisories). Dusky grouper also provide a unique ecological model for the study of effects of mercury on fish populations due to a complex life-history strategy that includes slow growth rates, late maturation, aggregate spawning, and sexual reversal (Heemstra and Randall, 1993; Craig et al., 2011). The first step in the study of potential mercury effects on population dynamics is an understanding of ecological factors that mediate mercury concentrations in the individuals.

Our study provides the first account of total mercury bioaccumulation for an Epinephelus species in the Southwestern Atlantic, and has direct relevance for conservation of this threatened species and human health of coastal communities that rely on this fishery. In contrast with most prior studies on mercury contamination in groupers, our study incorporated a more comprehensive suite of parameters (i.e., body size, age, stage of reproductive maturation, sex, diet and trophic position) that may be expected to affect variation in mercury concentration. We also determined spatial variation in mercury concentrations in individuals inhabiting littoral and neritic zones which may provide insights into how dietary differences or potential pollution sources affect bioaccumulation. Our previous research at these sites (Condini et al., 2015) found that even though there was no relationship between body size and trophic position, dusky grouper at the littoral site had a diet dominated by fishes. Individuals at the neritic site consumed similar amounts of shrimp and fishes except for the largest size class which consumed a greater proportion of fishes. Based on this, we expected a strong positive relationship between mercury concentration and body size (length, weight) and age due to bioaccumulation, but not with trophic position (i.e. biomagnification). Furthermore, the greater proportion of fish in the diets of dusky grouper from the littoral zone and the largest size class from the neritic zone should correspond with higher Hg accumulation rates due to longer lifespans and greater proportion of Hg associated with assimilated biomass of prey fishes versus crabs and shrimp.

2. Materials and methods

2.1. Study area and sample collection

This study was carried out in rocky habitats located adjacent to Patos Lagoon Estuary. Patos Lagoon Estuary constitutes one of the largest and most well-studied estuarine regions in South America (see Seeliger et al., 1997 and Odebrecht et al., 2010) and has been exploited by fishermen since the nineteenth century (Von Ihering, 1896). This estuary also contains the third most important Brazilian harbor that engages mainly in transport of chemical products, grains, and agricultural machines, among other potential pollutant sources (Odebrecht et al., 2010; SPRG, 2016). Mirlean et al. (2003) report mercury concentrations up to 21 mg kg⁻¹ in suspended particulate matter of the shallow waters of Patos Lagoon Estuary. The authors link this contamination to domestic sewage and stormwater runoff sources. Nienchiesi et al. (2001) and Kutter et al. (2009) quantified mercury contamination in fishes in Patos Lagoon Estuary and adjacent marine area and found mercury concentrations <0.3 mg kg⁻¹ for nearly all of species. These studies sampled primarily smaller-bodied species that feed at lower trophic positions, but also included some larger predatory species (e.g. Cynoscion guatucupa, Pomatomus saltatrix, Macrodon ancyloplus) and one specimen of E. marginatus.

Dusky grouper were sampled from two marine sites adjacent to Patos Lagoon Estuary. The first is in the littoral zone along a pair of rocky jetties (locally known as ‘Molhes da Barra de Rio Grande’) located at the mouth of Patos Lagoon Estuary (Fig. 1; 32°09’S, 52°05’W). The pair of rocky jetties is approximately 4.5 km long and extends from the littoral zone to the sea. They were built in the beginning of the 20th century to maintain a navigation channel to allow regular maritime access to the harbor of Rio Grande. Over time, the area was colonized by diverse marine benthic fauna (Capitoli, 1996) and by dusky grouper, mainly juveniles and immature females (Condini et al., 2007). The second study area (Carpinteiro Bank) is located in the neritic zone approximately 16 nautical miles offshore and between 20 and 30 m isobaths (Fig. 1; 32°16’S, 51°47’W) and was composed of beach rocks with a high level of cementation by recrystallized calcium carbonate (Buchmann et al., 2001; Abreu and Calliari, 2005). Both study locations represent rocky habitats within a coastal landscape that is otherwise dominated by homogeneous sandy substrates. Individuals were collected in the littoral and neritic zones between January 2008 and April 2009 and between January 2010 and May 2011, respectively.

Collected specimens were stored directly on ice and taken to the laboratory for processing. Each individual was measured for total length (TL, mm) and weighed (g). The new Hg contamination dataset presented in this study builds on previously published research conducted by the authors on maturity stages, age and trophic ecology of E. marginatus in the same study area (i.e. Condini et al., 2014a, 2014b, 2015). Maturity stage and sexes of the dusky grouper were determined by histological analysis of gonads (Condini et al., 2014a) and age estimates were determined by readings of sagittal otoliths (Condini et al., 2014b). Condini et al. (2015) previously described the trophic ecology of dusky grouper at these sites based on stomach contents and stable isotope data (δ¹³C and δ¹⁵N). The same anterior–dorsal muscle tissue samples from each individual that were used to determine stable isotopes composition are analyzed in this study for mercury concentration. The current study builds on the aforementioned previous findings for sex stage, age and trophic ecology by testing for relationships between each of these factors and muscle mercury concentration. Importantly, muscle tissue collected for these analyses is representative of the fish fillet usually consumed by humans (Adams and Onorato, 2005).

2.2. Mercury analysis

Subsamples of dried and homogenized muscle tissue (n = 68 littoral, n = 176 neritic) were weighed to the nearest 0.3 mg using an analytical balance. Total mercury concentration was determined on a direct mercury analyzer (DMA-80; Milestone Inc., Monroe, Connecticut) using thermal decomposition, gold amalgamation, and atomic absorption spectrometry (USEPA, 1998). Calibration curves were generated using three reference materials from the National Research Council of Canada: MESS-3 (marine sediment: certified value = 91 ± 9 ng of total Hg g⁻¹ of dry weight), TORT-2 (lobster hepatopancreas: certified value = 270 ± 60 ng g⁻¹), and DOLT-4 (dogfish liver tissue: certified value = 2580 ± 220 ng g⁻¹). Quality assurance included blanks, duplicate samples, and reference samples. Blank (empty boats) were analyzed every 20 samples with a mean Hg content of 0.0004 ± 0.0005 mg kg⁻¹ (range = 0–0.0015, n = 22). Duplicate samples were analyzed every 20 samples with a mean relative percent difference of 3.69 ± 2.85% (range = 0.41–9.36%, n = 15). Reference samples (MESS-3, TORT-2 and DOLT-4) were analyzed every 10 samples with a mean percent recovery for each of: MESS-3:3101.35 ± 3.54% (n = 8), TORT-2110.01 ± 13.63% (n = 8), and DOLT-4:98.90 ± 2.44% (n = 8). Results are presented as wet weight mercury concentrations, assuming a standard 80% moisture content of muscle. This conversion was done in order to compare our dusky grouper muscle mercury concentrations to other reported literature values as well as toxicity benchmarks which are generally determined as wet weights.

2.3. Data analyses

Individuals were pooled into four size classes (<351 mm TL, 351–500 mm TL, 501–650 mm TL, and >650 mm TL) in order to evaluate
mercury contamination along the size increment of the species. These size classes were chosen based on prior studies of dusky grouper reproduction, age structure and feeding habits for the study region (Condini et al., 2014a, 2014b, 2015, respectively). However, all statistical analyses were conducted using continuous data and ecological factors of interest, with and without the inclusion of individuals >650 mm TL from the neritic zone (no individuals of this size were present in the littoral zone). Conducting complementary analyses with and without the largest size
class is relevant for a couple reasons. First, excluding individuals >650 mm TL allows for a more direct comparison between sites with completely overlapping size distributions. Second, sexual reversal occurs at approximately the 650 mm TL threshold (Condini et al., 2014a), and this important physiological and ecological change may affect mercury accumulation differently in this largest size class compared with smaller size classes.

Relationships between factors expected to affect mercury concentration ([Hg]) (i.e. total length, weight, age and δ15N) were tested at the ‘global’ scale, i.e. all individuals from both sites, using linear regression, and relationships were compared between sites using analysis of covariance (ANCOVA). Relationships between independent variables are depicted in Fig. 2, and ANCOVAs with site as the factor and the independent variables from the global regressions as the covariate are depicted in Fig. 3. As noted above, all of the aforementioned analyses were conducted with and without individuals >650 mm TL. An additional series of ANCOVAs tested for differences in the relationship between each factor and [Hg] for individuals <650 mm TL vs. >650 mm TL from the neritic site (Hoeinghaus et al., 2006). All analyses were conducted using R version 3.1.2.

3. Results and discussion

All individuals captured from the littoral zone were females, and few males were caught from the neritic zone (94% females, 6% males). Total lengths (TL) of individuals from the littoral zone ranged from 260 to 640 mm and total weights (TW) ranged from 0.34 to 5.23 kg (Table 1). In the neritic zone, TL ranged from 290 to 1160 mm and TW ranged from 0.31 to 25.00 kg. Approximately 53% of all individuals were below the TL of first maturation (496 mm TL), thus our sample population is approximately equally split between sexually immature and mature individuals. Importantly, the lack of large males from the littoral site is not due to sampling bias, but rather is a natural aspect of this system. Large males only occur at the neritic site, likely due to the absence of suitable habitat (i.e. large crevices) in the rocky jetties of the littoral zone and occurrence of spawning aggregations in the neritic zone (Condini et al., 2014a, 2014b).

Total mercury concentrations across both study sites ranged from 0.01 to 0.90 mg kg$^{-1}$ wet weight, with higher concentrations in larger, older fishes (TL, weight and age, respectively: $R^2 = 0.77$ and $R = 0.88$, both $P < 0.001$; $R^2 = 0.84$ and $R = 0.92$, both $P < 0.001$; and $R^2 = 0.81$ and $R = 0.90$, both $P < 0.001$; Fig. 3a–c). These positive relationships between mercury contamination and fish size and age reflect a well-established pattern, which occurs due to the greater assimilation rate of mercury versus the slow rate at which mercury is eliminated, i.e. bioaccumulation (McKim et al., 1976; Adams and Onorato, 2005; Evers et al., 2009; Tremain and Adams, 2012). In contrast, δ15N was not correlated with [Hg] ($R^2 = 0.02$ and $R = 0.14$, both $P > 0.05$). Mercury concentrations typically correlate with δ15N within and among species (Wang, 2002; Hussey et al., 2014) because nitrogen isotope ratios are useful tracers of trophic position which is also frequently correlated with body size and age in fishes (i.e. reflecting both bioaccumulation and biomagnification). Dusky grouper in this study, although representing a broad range in body sizes and ages (Fig. 2), have similar trophic levels throughout their ontogeny (Condini et al., 2015), as evidenced by the limited difference in δ15N among individuals (Table 1, Figs. 2d and 3d). That being said, dusky grouper exhibit differences in prey ingested and assimilated among sites and size classes (Condini et al., 2015). Bayesian mixing models based on carbon and nitrogen stable isotope data of dusky grouper and potential prey taxa (Condini et al., 2015) indicate greater assimilation of fishes by grouper in the littoral zone (approximately 80% contribution across size classes) compared with

![Fig. 2.](image-url) Relationship between total length (mm) and weight (g), age (years) and δ15N (‰) (A, C and D) and between log total length and log weight (B) of dusky grouper from littoral and neritic habitats along the Southern Brazilian coast.
Fig. 3. Relationships between total mercury concentration (mg kg$^{-1}$ wet weight) and total length (mm), weight (g), age (years) and $\delta^{15}$N (‰) (A through D, respectively) of dusky grouper from littoral and neritic habitats along the Southern Brazilian coast. Significant regressions are plotted for both sites for each factor. Shading indicates toxicity thresholds at 0.3 mg kg$^{-1}$ and 0.5 mg kg$^{-1}$.

Table 1
Comparison of mercury concentration (mg kg$^{-1}$ wet weight), total length (mm), total weight (kg), age (years) and $\delta^{15}$N (‰) of the dusky grouper (Epinephelus marginatus) from littoral and neritic habitats along the Southern Brazilian coast.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;351 mm</td>
<td>Littoral zone (n = 10)</td>
<td>0.09 ± 0.03</td>
<td>0.06–0.12</td>
<td>Neritic zone (n = 26)</td>
</tr>
<tr>
<td>Hg (mg kg$^{-1}$)</td>
<td>318.4 ± 24.2</td>
<td>260–349</td>
<td>318.7 ± 17.8</td>
<td>290–350</td>
</tr>
<tr>
<td>Total length (mm)</td>
<td>0.63 ± 0.12</td>
<td>0.34–0.79</td>
<td>0.56 ± 0.13</td>
<td>0.31–0.87</td>
</tr>
<tr>
<td>Total weight (Kg)</td>
<td>3.9 ± 0.8</td>
<td>3–5</td>
<td>2.1 ± 0.4</td>
<td>2–3</td>
</tr>
<tr>
<td>Age (years)$^{*}$</td>
<td>17.55 ± 0.45</td>
<td>16.82–18.29</td>
<td>17.66 ± 0.39</td>
<td>17.09–18.32</td>
</tr>
<tr>
<td>351–500 mm</td>
<td>Littoral zone (n = 40)</td>
<td>0.14 ± 0.03</td>
<td>0.07–0.24</td>
<td>Neritic zone (n = 57)</td>
</tr>
<tr>
<td>Hg (mg kg$^{-1}$)</td>
<td>433.9 ± 37.1</td>
<td>368–500</td>
<td>419.5 ± 39.5</td>
<td>353–409</td>
</tr>
<tr>
<td>Total length (mm)</td>
<td>1.54 ± 0.41</td>
<td>0.86–2.34</td>
<td>1.38 ± 0.41</td>
<td>0.70–2.27</td>
</tr>
<tr>
<td>Total weight (Kg)</td>
<td>4.7 ± 0.8</td>
<td>3–6</td>
<td>3.6 ± 1.0</td>
<td>2–7</td>
</tr>
<tr>
<td>Age (years)$^{**}$</td>
<td>17.89 ± 0.35</td>
<td>16.78–18.58</td>
<td>17.74 ± 0.48</td>
<td>16.34–18.47</td>
</tr>
<tr>
<td>$\delta^{15}$N (%)</td>
<td>Littoral zone (n = 18)</td>
<td>501–650</td>
<td>501–650</td>
<td>Neritic zone (n = 57)</td>
</tr>
<tr>
<td>&lt;650 mm</td>
<td>0.21 ± 0.03</td>
<td>0.15–0.28</td>
<td>0.18 ± 0.03</td>
<td>0.13–0.26</td>
</tr>
<tr>
<td>Hg (mg kg$^{-1}$)</td>
<td>555.8 ± 42.7</td>
<td>501–640</td>
<td>567.9 ± 43.2</td>
<td>501–650</td>
</tr>
<tr>
<td>Total length (mm)</td>
<td>3.31 ± 0.92</td>
<td>2.04–5.23</td>
<td>3.51 ± 0.93</td>
<td>2.11–5.90</td>
</tr>
<tr>
<td>Total weight (Kg)</td>
<td>6.7 ± 1.4</td>
<td>5–11</td>
<td>6.2 ± 1.3</td>
<td>4–10</td>
</tr>
<tr>
<td>Age (years)$^{***}$</td>
<td>17.90 ± 0.31</td>
<td>17.44–18.77</td>
<td>17.94 ± 0.37</td>
<td>17.09–18.82</td>
</tr>
<tr>
<td>&gt;650 mm</td>
<td>Littoral zone (n = 0)</td>
<td>17.98 ± 0.61</td>
<td>16.47–19.14</td>
<td>Neritic zone (n = 36)</td>
</tr>
</tbody>
</table>

$^{*}$ Age in the littoral zone n = 8 and neritic zone n = 26;
$^{**}$ Age in the littoral zone n = 33 and neritic zone n = 51;
$^{***}$ Age in the littoral zone n = 16 and neritic zone n = 55.
the neritic zone (approximately 40% for all size classes <650 mm TL but approaching 60% for individuals >650 mm TL; shrimp were the other primary prey type, contributing 30–50% and decreasing with body size).

The aforementioned differences in diet between sites likely contribute to the significant differences in mercury accumulation rate between individuals from the littoral and neritic zones (Fig. 3a–c). Regression slopes in ANCOVA analyses for total length, weight and age were significantly steeper for the littoral site ($F = 7.746, df = 1, P < 0.01; F = 10.045, df = 1, P < 0.01; F = 5.993, df = 1, P = 0.05$, respectively), indicating greater rate of mercury accumulation compared to the neritic site (for individuals <650 mm TL). This may be due to the greater proportion of fish in the assimilated diet of littoral zone grouper (see Condini et al., 2015), even though trophic position did not differ between sites or size classes, because the fish species primarily consumed (mainly sciaenids, e.g. whitemouth croaker Microgobias furnieri, argentine croaker Umbrina canosa, southern kingcroaker Menticirrhus americanus, barbel drum Ctenoscaena gracilicornis, and striped weakfish Cynoscion guatucupa) are longer lived than alternative prey (e.g. blue crabs, shrimp) and thus are expected to carry greater total mercury in their tissues due to bioaccumulation. Similarly, Tremaín and Adams (2012) investigated relationships between total mercury contamination and feeding ecology in some epinephelids and found that species feeding mainly on fish exhibited higher mean mercury concentrations than those species preying upon invertebrates.

Dusky grouper >650 mm TL from the neritic zone had a significantly higher slope in our ANCOVA analysis than smaller size classes (individuals <650 mm) ($F = 111.066, DF = 1, P < 0.001$, representing an Hg accumulation rate ∼3 × faster than the smaller size classes (Fig. 3a). The slopes between smaller size classes (<650 mm) from the littoral and neritic zones were not significantly different (Fig. 3a), suggesting that part of the observed increase in accumulation rate with length is due simply to bioaccumulation. This largest size class in the neritic zone exhibits a significant increase in the proportion of fish in the assimilated diet and larger prey in general (Condini et al., 2015), but fish still contribute less to the assimilated diet than for individuals of all size classes from the littoral zone. Site specific differences in the species composition of prey may play a role (e.g. cephalopods such as Octopus spp. consumed only in the neritic zone; Condini et al., 2015), though our data are insufficient to assess the importance of specific prey taxa.

Another key ecological factor that occurs only in the largest size class is sexual reversal. Several Epinephelidae species, including dusky grouper, are protogynous hermaphrodites, undergoing sex change from female to male at larger sizes (Heemstra and Randall, 1993). We are unaware of any physiological studies that have examined potential influences of hermaphroditism (e.g. due to hormone changes) on mercury bioaccumulation. Although our sample size for comparing sexes in the Gulf of Mexico and found mean concentrations similar to those we observed in the present study. Many of the fish species consumed by dusky grouper (e.g. whitemouth croaker) use the estuarine regions as a feeding and growing site during their early stages of life and then migrate to the ocean to spawn. It seems plausible that the mercury concentrations observed in this dusky grouper population occur mainly due to the consumption of fish prey that spends the majority of their life in the estuarine region of Patos Lagoon. That is, fish moving from the estuary to the coastal zone are serving as a vector for mercury transport and accumulation in coastal fisheries. This is potentially significant due to the expected greater potential for mercury methylation in shallow wetland habitats of the estuary and concentrated point sources in that region. Further investigation on mercury contamination of marine estuarine-dependent fishes integrated with natural markers (e.g., otolith chemistry, stable isotopes) to reveal fish movement patterns between estuary and adjacent sea would be necessary to evaluate this hypothesis.

Total mercury concentrations in individuals from the littoral zone were below suggested threshold values (0.3 mg kg$^{-1}$) to prevent fish health impacts (Beckvar et al., 2005; Sandheinrich et al., 2011; Depew et al., 2012). A similar pattern was observed for dusky groupers with total length <650 mm TL caught at the neritic zone. However, two-thirds of the individuals >650 mm TL at the neritic zone ($n = 36$) had mercury concentrations above the 0.3 mg kg$^{-1}$ toxicity threshold (Fig. 3a). Although most individuals of dusky grouper showed mercury concentration below safety levels, the majority of larger, reproductively mature individuals (>650 mm TL) had higher concentrations (>0.3 mg kg$^{-1}$) indicating potential harmful consequences for fish health and human consumption. Thus, consumption of dusky grouper larger than 650 mm TL by humans should be avoided or limited in this region of the Southwestern Atlantic to prevent exposure to mercury.

Mercury contamination values found in the present work were considerably higher than prior results reported for this lagoon by Niencheski et al. (2001) and Kutter et al. (2009). Niencheski et al. (2001) investigated only two estuarine species (Odontesthes bonariensis and Microgobias furnieri) and all analyzed individuals had mercury concentrations below 0.3 mg kg$^{-1}$. In contrast, Kutter et al. (2009) analyzed several freshwater, estuarine and marine species, but only two freshwater species (Oligosarcus jenynsii and O. robustus) showed mercury contamination values above 0.3 mg kg$^{-1}$. Kutter et al. (2009) analyzed a single large (900 mm TL) dusky grouper specimen, which showed a lower value (215.8 mg g$^{-1}$ wet weight, equivalent 0.22 mg kg$^{-1}$) than the average value observed in this study for individuals of similar body size (e.g. 0.53 mg kg$^{-1}$ wet weight). Unfortunately, they did not report the site location where this specimen was caught, but considering its large body size and expected size distribution of this species in this region (Condini et al., 2014a), it most likely came from offshore waters and not from the rocky jetties in the littoral zone. In contrast, Tremaín and Adams (2012) determined mercury levels in several Epinephelus grouper species (E. adscensionis, E. drummondhayi, E. flavolimbatus, E. latgara, E. morio and E. nigritus) in the Gulf of Mexico and found mean concentrations similar to those we observed in the present study.

4. Conclusions

Our study revealed that large individuals of dusky grouper occurring in the southernmost limit of its distribution in the Southwestern Atlantic have mercury contamination levels that are potentially harmful for this endangered fish species and also above the acceptable limits for human consumption. Mercury contamination in this species was correlated both with site locations and body sizes. Mature larger-body individuals (>650 mm and >8 years old) exhibited the highest mercury concentrations. When considering similar body sizes, individuals inhabiting littoral rocky habitats adjacent to a large coastal lagoon had higher concentrations of mercury probably due to proximity to pollution sources associated with human activities in the estuary and its
diagnostic features. Based on prior diet studies with this population, we hypothesized that fish consumption (mainly of estuarine-dependent fish) was the most likely trophic link leading to mercury contamination, especially in the littoral zone. Further studies are needed to identify which human activities are the primary sources of mercury contamination and also to test our proposed hypothesis on the prevalence of estuarine-dependent fish prey as the main trophic link leading to mercury contamination in both littoral and offshore subpopulations of this endangered species.

**Acknowledgements**

This study received financial support from the Ministry of Science and Technology (MCT/CNPq) through the Brazilian Long Term Ecological Research Programme (BR-LTER) at Site 6: Patos Lagoon estuary and Fundação O Boticário de Proteção a Natureza (9562.20122). MVC thanks a fellowship provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). AMG acknowledges fellowship support from CNPq (310141/2015-0).

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