Ecological and biological determinants of trace elements accumulation in liver and kidney of *Pontoporia blainvillei*

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Abstract

The present work tested whether ecological and biological variables have an influence on the assimilation of trace elements by the tissues of a cetacean from the Western South Atlantic Ocean. No significant differences were observed in the concentrations for both sexes. As individuals from the two sampling areas belong to distinct genetic and morphological populations, animals of similar body length were older on the southeastern than on the southern coast. The liver showed the highest concentrations of mercury, whereas the highest levels of cadmium were found in the kidney. Hepatic mercury, cadmium and selenium in individuals from the south coast were about four times as high as those from the southeast coast. However, arsenic in the liver and kidney were similar in both coastal areas. Hepatic mercury, cadmium and selenium concentrations increased with body length in individuals from the southeastern coast, although no significant correlations (P > 0.05) were observed between body length from either area and the renal and hepatic As concentrations. A significant positive linear relationship was observed between molar concentrations of Hg and Se in the liver of all individuals from both areas (r² = 0.93; P < 0.001), presenting Se:Hg ratios close to 4. Differences found among the concentrations of Hg, Cd and Se in dolphins from both areas were probably due to the preferred prey, bioavailability of elements in each marine environment, and environment variables (water temperature, net primary production). As a consequence, concentrations of trace elements in the tissues of this species can be considered to be a result of the surrounding environment.

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1. Introduction

Marine mammals are very sensitive to environmental changes and have been considered good bioindicators of environmental contamination (Capelli et al.,

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These organisms have high potential for accumulating some trace elements, such as mercury (Hg), selenium (Se), arsenic (As) and cadmium (Cd), since they have relatively long life spans, and generally occupy a high trophic level in the marine food chain (Caurant et al., 1994; Kubota et al., 2001; Kunito et al., 2004). The analysis of tissues from different species of whales and dolphins has been used as a tool for the assessment of marine pollution by trace elements (Leonzio et al., 1992; Caurant et al., 1994; Hobson et al., 2004).

Trophic transfer of trace elements along marine food chains has been recognized as an important process influencing metal and metalloid bioaccumulation and geochemical cycling (Fisher and Reinfelder, 1995). It has been shown that the food chain is the major pathway for selenium, arsenic, cadmium and mercury bioaccumulation in aquatic animals (Shibata et al., 1992; Bustamante et al., 1998; Kubota et al., 2001).

Selenium and arsenic are recognized as micronutrients for animals, acting in the activities of enzymes (Shibata et al., 1992). Conversely, cadmium and mercury are exogenous and harmful elements, which accumulate during growth (Feroci et al., 2005). Some experiments have investigated the reduction in bioavailability of some trace elements, such as arsenic, mercury and cadmium, by selenium (Sasakura and Suzuki, 1998; Feroci et al., 2005). Selenium and arsenic can affect the physiological role of other elements as well as some metabolic pathways, inducing other effects on aquatic organisms (Shibata et al., 1992).

It has also been reported that the liver of aquatic organisms may act as an organ for demethylation and/or the sequestration of both organic and inorganic forms of mercury (Wagemann et al., 2000; Endo et al., 2002; Kehrig et al., 2006a), and that selenium is involved in both of these mechanisms (Palmisano et al., 1995; Caurant et al., 1996; Ikemoto et al., 2004).

It is known that not only the longer life of cetaceans is responsible for the mechanism of metal bioaccumulation, but also a number of physiological factors such as the diet of cetaceans seem to be responsible for enhancing this process (Monaci et al., 1998). Marine mammals assimilate contaminants mainly by ingestion, which may vary according to the species consumed. Squids are the main source of cadmium for cetaceans as they are naturally rich in this metal (Bustamante et al., 1999). Fish prey is generally the main source of selenium and mercury, including methylmercury, for cetaceans, whereas it is known that crustaceans are an important source of arsenic for them (Kubota et al., 2001; Monteiro-Neto et al., 2003).

These mechanisms are better studied and understood in marine mammals from the Northern Hemisphere (Leonzio et al., 1992; Caurant et al., 1994; Dietz et al., 1996; Wagemann et al., 1996; Capelli et al., 2000; Anan et al., 2002; Szefér et al., 2002; Watanabe et al., 2002; Roditi-Elasar et al., 2003). However, it is also essential to understand these patterns for species from the Southern Hemisphere, where studies are limited (e.g. Kemper et al., 1994; Marcovechio et al., 1994; Junin et al., 1998; Gerpe et al., 2002; Lailson-Brito et al., 2002a,b; Bustamante et al., 2003; Monteiro-Neto et al., 2003; Kunito et al., 2004; Kehrig et al., 2004).

Pontoporia blainvillei (Gervais and D’ Orbigny, 1844) is a small coastal cetacean endemic to the Western South Atlantic, distributed from the Gulf San Matias (42°10’S), Peninsula Valdés, Argentina (Crespo et al., 1998) up to Itaúnas (18°25’S), southeastern Brazil (Siciliano, 1994). This dolphin is at the upper level of the marine food chain and inhabits mainly shallow waters (up to around 30 m or a little further) (Secchi et al., 2003b), therefore it is particularly vulnerable to anthropogenic activities, specially fisheries (Secchi et al., 2003a). It has a restricted home range and feeds mainly on teleost fish (up to 10 cm) and cephalopods (Di Beneditto and Ramos, 2001; Danilewicz et al., 2002).

Genetic studies have identified at least two different populations of franciscana, one of smaller individuals ca. 18°–27°S and another of larger organisms ca. 28°–42°S (Secchi et al., 1998; Ott, 2002; Lázaro et al., 2004). In the southeastern coast individuals attain sexual maturity between 2 and 3 years old, and between 115 cm (male) and 130 cm (female) in length (Di Beneditto and Ramos, 2001), while in the southern area sexual maturity is reached at about 3.5 years of age and at a much larger size for both sexes (Danilewicz, 2003; Danilewicz et al., 2004).

The present study aimed at answering the following question: do different environmental conditions and biological characteristics influence the assimilation of trace elements by the liver and kidney of cetaceans? Thus the hypothesis tested here is that for P. blainvillei, different environmental conditions (tropical and sub-tropical waters) and biological characteristics (sex, age and population) lead to different accumulations of trace elements in their organs.

2. Materials and methods

2.1. Samples

Liver and kidney samples of 31 individuals of franciscana incidentally caught in fishing nets were freeze...
dried and analyzed for mercury (Hg), selenium (Se), arsenic (As) and cadmium (Cd). Eighteen individuals were caught off the Brazilian southeastern coast (SE) (21°18'S to 25°25'S) from 1998 to 2005; and thirteen off the Brazilian southern coast (S) (29°30'S to 33°30'S) between 2003 and 2004 (Fig. 1). The samples were supplied by the
Biological Specimen Banking from the Bioscience and Biotechnology Center (UENF) and the Department of Oceanography (FURG).

Samples covered mature and immature individuals with body length varying from 68 to 147 cm (southeast) and 99.5 to 153.5 cm (south). Immature individuals (71%) and males (65%) predominated (Table 1).

### 2.2. Trace element analysis

Samples were digested in a sulphuric–nitric acid mixture and mercury was determined by cold vapor atomic absorption spectrometry with a Flow Injection Mercury System (FIMS)–FIAS 400 (Perkin Elmer, USA) equipped with auto sampler AS90 (Perkin Elmer, USA) and using sodium borohydride as a reducing agent (Kehrig et al., 2006b).

Samples were also digested in nitric acid (Deaker and Maher, 1997) and selenium, arsenic and cadmium were determined by graphite furnace atomic absorption spectrometry, using an Analytic Jena Model ZEEnit 60 spectrometer (Analytik Jena, Germany) with Zeeman Effect background correction equipped with MPE-52 auto sampler. Palladium nitrate was used as a chemical modifier (Seixas et al., 2007).

Precision and accuracy of the analytical methods were determined and monitored using certified reference materials obtained from the International Atomic Energy Agency (IAEA 407 and IAEA 436 — Tuna fish sample) and the National Research Council of Canada (DORM 2 — Dogfish muscle sample). Results demonstrated a good precision and accuracy of the analytical method. Trace elements quantified in the certified reference materials (Hg, Se, As, Cd) were within 89 and 107% of the mean certified values and the coefficients of variation (SD/mean) were lower than 15%.

### 2.3. Statistical analysis

Statistical analyses were performed using STATISTICA® 6.0 for Windows (StatSoft, Inc. 1984–2001, USA). The analysis of variance was done by factorial ANOVA followed by a Post-Hoc test (Duncan test). A multiple regression statistic ($r^2$) was performed to verify the existing relationship between body length and concentration of individual trace elements and inter-element relationship (on a molar basis) for both tissues. The relationship of individual trace element concentrations between liver and kidney was also verified. Values

<table>
<thead>
<tr>
<th>Sampling area</th>
<th>Trace element</th>
<th>Tissues</th>
<th>Equation regression</th>
<th>$r^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Southeast coast</strong></td>
<td>Hg</td>
<td>Liver</td>
<td>$Hg = 0.06 L - 3.82$</td>
<td>0.25</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kidney</td>
<td>$Hg = 0.02 L - 0.77$</td>
<td>0.13</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Se</td>
<td>Liver</td>
<td>$Se = 0.06 L - 3.09$</td>
<td>0.33</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kidney</td>
<td>$Se = 0.10 L - 3.45$</td>
<td>0.31</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>Liver</td>
<td>$Cd = 0.03 L - 2.77$</td>
<td>0.62</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kidney</td>
<td>$Cd = 0.21 L - 18.56$</td>
<td>0.65</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>As</td>
<td>Liver</td>
<td>$As = 0.01 L - 0.03$</td>
<td>0.07</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kidney</td>
<td>$As = 0.01 L - 0.02$</td>
<td>0.16</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>South coast</strong></td>
<td>Hg</td>
<td>Liver</td>
<td>$Hg = 0.07 L - 6.71$</td>
<td>0.53</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Se</td>
<td>Liver</td>
<td>$Se = 0.38 L - 34.51$</td>
<td>0.17</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>Liver</td>
<td>$Cd = 0.03 L - 1.93$</td>
<td>0.11</td>
<td>&gt;0.05</td>
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<td></td>
<td></td>
<td>Kidney</td>
<td>$Cd = 0.10 L - 6.14$</td>
<td>0.07</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>As</td>
<td>Liver</td>
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<td>0.17</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kidney</td>
<td>$As = 0.01 L + 0.46$</td>
<td>0.01</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Significant ($P<0.05$) — in bold.
are presented as mean ± standard deviation (SD) based on a dry weight basis.

3. Results and discussion

3.1. Effect of body length (or age) and sex on the accumulation of trace elements

No significant difference between body length and sex for individuals from both populations was found (ANOVA, \( P > 0.05 \)), suggesting that both groups are equally represented in the sample and making it possible to compare them (Monaci et al., 1998).

Despite the fact that animals collected at both areas presented similar body length, they are not necessarily of the same age. Individuals from southeastern coast were older than those from the southern coast of similar size. Franciscanas from Rio de Janeiro (southeast) reach asymptotic length when they are approximately 4 years old and 117 cm (male) or 145 cm long (female) (Ramos et al., 2000). On the other hand, individuals from Rio Grande do Sul (south) attain asymptotic length when they are 4 years old and about 130 cm long (male) or 6 years old and 146–152 cm long (female) (Barreto and Rosas, 2006). These differences are not only clinal since individuals from intermediate areas (i.e. Santa Catarina, Paraná and São Paulo states) are smaller than those from both Rio Grande do Sul and Rio de Janeiro (e.g. Secchi et al., 2003b). Genetic data indicate strong differences between individuals from these two areas (Secchi et al., 1998; Ott, 2002; Lázaro et al., 2004).

Data analysis from the two sampling populations showed that hepatic mercury, cadmium and selenium concentrations increased with body length for the southeastern individuals (Table 2), whereas no significant correlations \( (P > 0.05) \) were found between body length from either area and renal and hepatic As concentrations. No significant correlations \( (P > 0.05) \) were found between body length of individuals from the southern coast and hepatic concentrations of all trace elements (Hg, Se and Cd).

Previous studies with this species also presented a significant relationship between hepatic trace element concentrations (Hg, Cd and Se) and body length (Lailson-Brito et al., 2002a; Gerpe et al., 2002; Kunito et al., 2004). However, in the literature, the correlation between hepatic arsenic concentration and body length is still unclear for marine mammals (Kubota et al., 2001), and limited information is available for this particular species (Kunito et al., 2004). There is no previous information in the literature about the relationship between As concentration in the kidney and body length.

The relationship between age and Hg, Se and Cd concentration in marine mammals has been extensively examined (Leonzio et al., 1992; Wagemann et al., 1995; Caurant et al., 1994; Dietz et al., 1996; Monaci et al., 1998; Szefer et al., 2002; Monteiro-Neto et al., 2003; Ikemoto et al., 2004; Kunito et al., 2004). In general in long-lived marine mammals, the hepatic concentration increases with age (Ikemoto et al., 2004), since the biological half-life is rather long in these animals, due to age-related increase in concentration, and the strong affinity of some trace elements (e.g. Cd and Hg) to the SH group in cysteine.

However, some species of cetaceans (such as harbor porpoise, Phocoena phocoena) and pinnipeds (such as baikal seal, Phoca sibirica; ribbon seal, Phoca fasciata; and Steller sea lion, Eumetopias jubatus) have shown a significant positive correlation between hepatic As concentration and age (Kubota et al., 2001). This fact could be related to a higher uptake than excretory rate of arsenic that leads to an increase in arsenic concentration with body length (or age) (Kubota et al., 2001).

Even though not observed for franciscana, an increase on renal concentrations of Hg and Cd with body length has been reported for Sotalia guianensis from the northeastern coast of Brazil (Monteiro-Neto et al., 2003) and with Hg and Se for Stenella coeruleoalba from the Mediterranean Sea (Monaci et al., 1998). In the literature, no information is available concerning the relationship between As concentration in the kidney and body length of P. blainvillei.

ANOVA showed no differences in the analyzed trace elements (Hg, Se, As and Cd) between sexes within the same sampling population. It must be considered, however, that juveniles predominated (22 out of 31). Marine mammals normally present no sex-related differences in the accumulation of trace elements (O’Shea, 1999), as seen for the franciscana from the southeastern (Lailson-Brito et al., 2002a) and southern–southeastern coast of Brazil (Kunito et al., 2004) and also the Buenos Aires Province coast, Argentina (Gerpe et al., 2002), for S. guianensis from different Brazilian coastal areas (Lailson-Brito et al., 2002b; Monteiro-Neto et al., 2003), and for striped dolphins from the Mediterranean Sea (Monaci et al., 1998; Cardellicchio et al., 2002).

3.2. Concentrations of trace elements in the tissues

The mean concentration of mercury (Hg) in liver (5.98 ± 2.31 μg g⁻¹) was about four times as high as those found in the kidney (1.51 ± 1.27 μg g⁻¹). However, the mean concentrations of Se in the liver (6.52 ± 3.99 μg g⁻¹) and kidney (7.83 ± 8.07 μg g⁻¹) were similar. It is well known that Hg accumulates preferentially in the liver (Thompson,
which is probably related to the role played by the liver in terms of pollutants bio-transformation, metabolizing nutrients and essential elements as well as removing some non-essential elements and toxins from the bloodstream (Frodello et al., 2000). This pattern was also observed in previous studies with franciscana from Argentina (Gerpe et al., 2002) and Brazil (Lailson-Brito et al., 2002a).

The mean concentration of cadmium (Cd) in kidney (4.40±2.40 μg g⁻¹) was about five times as high as those found in their liver (0.96±0.95 μg g⁻¹). Cadmium accumulates preferentially in the kidney of marine mammals (Thompson, 1990; Law, 1996). Previous studies with franciscana have shown that the kidney is the main target organ for cadmium (Gerpe et al., 2002; Lailson-Brito et al., 2002a), which is probably related to the filtering and elimination function of this organ, and also due to the Cd hard bond with metallothionein present in the kidney (Monaci et al., 1998; Das et al., 2000).

Metallothioneins (MTs) are important in the detoxification of non-essential elements (such Cd and Hg) in organs of different species of animals, such as mollusks and mammals (Kägi, 1991; Dalling et al., 1997; Das et al., 2002, 2006). However, MTs are proteins induced by various physiological and toxicological stimuli, including heavy metals. Dynamic interactions have suggested that MTs play a major role in detoxification of Cd in internal organs (i.e. liver and kidney) of marine mammals, since the percentage of the cytosolic Cd bound to MTs can reach almost 100% in the liver and kidney (Dalling et al., 1997; Das et al., 2000, 2002). However, MTs appear to play a minor role in the binding and detoxification of Hg by marine mammals, since the percentage of hepatic and renal Hg bound to MTs is very low (generally less than 10%) in the tissues. In general, Hg is mainly associated with selenium (HgSe) under a detoxified form in the insoluble fraction of the tissues (Das et al., 2000, 2002, 2006).

Although the liver of marine mammals at high trophic positions plays an important role in arsenic metabolism (Kubota et al., 2002), the mean arsenic concentration in the liver (1.36±1.23 μg g⁻¹) and kidney (1.12±0.93 μg g⁻¹) of franciscana found here were similar, suggesting no differences in accumulation patterns. However, previous studies found small differences in arsenic concentrations between the liver and kidney of harp seal and hooded seals (Brunborg et al., 2006).

Information on arsenic concentration in tissues of franciscana is still very limited in the literature, and only data about hepatic arsenic concentrations have been reported (Table 3) (Kunito et al., 2004).

### 3.3. Regional differences on accumulation of trace elements

No significant differences were found between mean concentrations of Hg, Cd, Se and As in the kidney and As in the liver from both studied populations (ANOVA, P>0.05). Mean concentrations of Hg, Cd and Se were

### Table 3

<table>
<thead>
<tr>
<th>Cetacean species (N)</th>
<th>Hg (μg g⁻¹)</th>
<th>Se (μg g⁻¹)</th>
<th>As (μg g⁻¹)</th>
<th>Cd (μg g⁻¹)</th>
<th>Marine environment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (18)</td>
<td>2.6</td>
<td>3.2</td>
<td>1.1</td>
<td>0.6</td>
<td>Tropical (Brazil)</td>
<td>This study</td>
</tr>
<tr>
<td>A (13)</td>
<td>10.7</td>
<td>11.1</td>
<td>1.7</td>
<td>1.5</td>
<td>Sub-tropical (Brazil)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>12.7</td>
<td>–</td>
<td>–</td>
<td>11.0</td>
<td>Temperate (Argentina)</td>
<td>Marcovecchio et al. (1994)</td>
</tr>
<tr>
<td>C</td>
<td>286.0</td>
<td>–</td>
<td>–</td>
<td>2.7</td>
<td>Sub-tropical (Australia)</td>
<td>Kemper et al. (1994)</td>
</tr>
<tr>
<td>C</td>
<td>(0.5–33.9)</td>
<td>–</td>
<td>–</td>
<td>(≈0.0–33.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>(19.2–102.0)</td>
<td>–</td>
<td>–</td>
<td>(9.0–11.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>52.9</td>
<td>–</td>
<td>–</td>
<td>47.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (18)</td>
<td>29.0</td>
<td>–</td>
<td>7.5</td>
<td></td>
<td>Temperate (Argentina)</td>
<td>Gerpe et al. (2002)</td>
</tr>
<tr>
<td>A (17)</td>
<td>(3.0–24.4)</td>
<td>–</td>
<td>–</td>
<td>(≈0.0–2.1)</td>
<td>Tropical (Brazil)</td>
<td>Lailson-Brito et al. (2002a)</td>
</tr>
<tr>
<td>B (15)</td>
<td>11.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Tropical (Brazil)</td>
<td>Lailson-Brito et al. (2002b)</td>
</tr>
<tr>
<td>E (2)</td>
<td>42.6</td>
<td>23.0</td>
<td>–</td>
<td>38.2</td>
<td>Temperate (New Caledonia)</td>
<td>Bustamante et al. (2003)</td>
</tr>
<tr>
<td>C (1)</td>
<td>50.0</td>
<td>20.1</td>
<td>1.4</td>
<td>5.6</td>
<td>Sub-tropical (Australia)</td>
<td>Law et al. (2003)</td>
</tr>
<tr>
<td>B (11)</td>
<td>(0.3–4.4)</td>
<td>–</td>
<td>–</td>
<td>(0.03–4.4)</td>
<td>Equatorial (Brazil)</td>
<td>Monteiro-Neto et al. (2003)</td>
</tr>
<tr>
<td>B (15)</td>
<td>25.4</td>
<td>34.0</td>
<td>–</td>
<td>–</td>
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<td>Kehrig et al. (2004)</td>
</tr>
<tr>
<td>A (23)</td>
<td>3.5</td>
<td>9.1</td>
<td>1.2</td>
<td>0.4</td>
<td>Sub-tropical (Brazil)</td>
<td>Kunito et al. (2004)</td>
</tr>
<tr>
<td>B (20)</td>
<td>77.0</td>
<td>38.0</td>
<td>0.8</td>
<td>0.7</td>
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</tr>
<tr>
<td>D (2)</td>
<td>140.0</td>
<td>79.0</td>
<td>0.7</td>
<td>30.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(A=Pontoporia blainvillei; B=S. guianensis; C=Tursiops truncatus; D=Stenella frontalis; E=Kogia breviceps).

Wet weight basis concentration was converted to dry weight basis concentration assuming that moisture content was 69.7% (Yang and Miyazaki, 2003).

aN — number of samples; b(min–max).
significantly higher \((P<0.05)\) in the liver of individuals from the southern coast (Fig. 2). Individuals from the south also presented the highest variation in the concentration of Hg, Cd and Se in the liver. Although a number of physiological and environmental factors may also have some influence, the feeding behavior (based on fish and cephalopods rich in Hg, Se, As and Cd) and the complexity of the marine food chain are the major factors responsible for the process of bioaccumulation of trace elements in long-lived marine animals (Shibata et al., 1992; Bustamante et al., 1998; Monaci et al., 1998; Kubota et al., 2001).

On the basis of the results presented here, the regional differences in trace concentrations found in franciscana may be due to the fact that they represent distinct genetic and demographic populations (e.g. Secchi et al., 2003b), which have different nutritional, developmental and reproductive characteristics (e.g. Ramos et al., 2000; Barreto and Rosas, 2006; Danilewicz, 2003; Secchi et al., 2003b). Individuals of similar size from the southeastern coast were older than the southern coast. This may be the reason that animals from the southeastern coast presented the lowest concentrations of trace elements.

The lower concentrations of trace elements in franciscana tissues from the southeastern Brazilian coast might be a result of their preferential dietary habits, based on *Stellifer* sp., *Anchoa filifera*, *Pellona harroweri* and *Isopisthus parvipinnis* (Di Benedetto and Ramos, 2001) whereas the southern franciscanas feed mainly on *Cynoscion guatucupa*, *Trichiurus lepturus*, *Macrodon ancyloodon*, *Umbrina canosai*, *Micropogonias furnieri*, *Loligo sanpaulensis* and *Urophycis brasiliensis* (Danilewicz et al., 2002; Secchi et al., 2003a).

### 3.4. Comparison with others regions worldwide

In this study, concentrations of mercury, selenium, arsenic and cadmium found in the liver of *P. blainvillei* from the two populations studied, southeastern (SE) and southern (S), were of the same order of magnitude as those reported in earlier studies with samples from Brazil and Argentina. However, they were generally lower than those found in the liver of other cetaceans (*S. guianensis*; *Tursiops truncatus*; *Stenella frontalis*; *Kogia breviceps*) from coastal regions in the Southern Hemisphere (Table 3).

Although Hg, Se and Cd measurements in the kidney are available for toothed cetaceans from coastal regions worldwide (Leonzi et al., 1992; Augier et al., 1993; Caurant et al., 1994; Kemper et al., 1994; Wagemann...
et al., 1996; Monaci et al., 1998; Meador et al., 1999; Capelli et al., 2000; Frodello et al., 2000; Cardellicchio et al., 2002; Endo et al., 2002; Lailson-Brito et al., 2002b; Szefer et al., 2002; Watanabe et al., 2002; Monteiro-Neto et al., 2003; Roditi-Elasar et al., 2003; Ikemoto et al., 2004), little information is reported for arsenic (Caurant et al., 1994; Meador et al., 1999; Law et al., 2003) and other trace elements in P. blainvillei (Gerpe et al., 2002; Lailson-Brito et al., 2002a). Despite this limitation, concentrations of trace elements in the kidney of franciscana could be considered lower than data reported for other dolphins (S. guianensis; T. truncatus; S. coeruleoalba) from coastal regions of the Southern Hemisphere (Table 4).

However, it is difficult to compare data for trace elements in species with different feeding habits, life span, and from various locations worldwide. In this case, it is better to compare the trace element concentrations between individuals of the same species that present a similar life span. Life span seems to be a significant factor influencing on trace element accumulation in internal organs of cetacean species.

### 3.5. Inter-element relationships in the liver and kidney

#### 3.5.1. Selenium to mercury relationship

In the present study, a significant positive linear relationship was found between the molar concentrations of Se and Hg in the liver of organisms from both populations (Fig. 3a, b). The high correlation between Hg and Se in the organs of marine mammals is well known (Wagemann et al., 1998; Meador et al., 1999; Capelli et al., 2000; Das et al., 2000; Dietz et al., 2000; Endo et al., 2002) and could be reflecting a direct association between these two elements in the liver, which could be explained by the existence of a detoxification mechanism involving both elements (i.e. Se detoxifies Hg and other metals such as Ag) (Arai et al., 2004).

Some studies have reported a 1:1 molar ratio of Se and Hg in the liver of marine mammals (Leonzio et al., 1992; Palmisano et al., 1995; Meador et al., 1999; Capelli et al.,

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**Table 4**

<table>
<thead>
<tr>
<th>Cetacean species (N)a</th>
<th>Hg (μg g⁻¹ dry wt.)</th>
<th>Se (μg g⁻¹ dry wt.)</th>
<th>As (μg g⁻¹ dry wt.)</th>
<th>Cd (μg g⁻¹ dry wt.)</th>
<th>Marine environment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (18)</td>
<td>1.4</td>
<td>7.0</td>
<td>0.7</td>
<td>3.4</td>
<td>Tropical (Brazil)</td>
<td>This study</td>
</tr>
<tr>
<td>A (13)</td>
<td>1.7</td>
<td>8.8</td>
<td>1.4</td>
<td>5.5</td>
<td>Sub-tropical (Brazil)</td>
<td></td>
</tr>
<tr>
<td>A (18)</td>
<td>7.6</td>
<td>–</td>
<td>–</td>
<td>28.2</td>
<td>Temperate (Argentina)</td>
<td>Gerpe et al. (2002)</td>
</tr>
<tr>
<td>A (15)</td>
<td>(1.9–18.1)b</td>
<td>–</td>
<td>–</td>
<td>(≈ 0.0–5.3)b</td>
<td>Tropical (Brazil)</td>
<td>Lailson-Brito et al. (2002a)</td>
</tr>
<tr>
<td>C (1)</td>
<td>1.2</td>
<td>8.4</td>
<td>0.6</td>
<td>&lt;0.01</td>
<td>Sub-tropical (Australia)</td>
<td>Law et al. (2003)</td>
</tr>
<tr>
<td>B (11)</td>
<td>(0.3–24.8)b</td>
<td>–</td>
<td>–</td>
<td>(0.04–18.0)b</td>
<td>Equatorial (Brazil)</td>
<td>Monteiro-Neto et al. (2003)</td>
</tr>
</tbody>
</table>

(A=Pontoporia blainvillei; B=S. guianensis; C=Tursiops truncatus).

Wet weight basis concentration was converted to dry weight basis concentration assuming that moisture content was 77.3% (Yang and Miyazaki, 2003).

aN — number of samples; b(min–max).

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**Fig. 3.** Relationship between molar concentrations of trace elements in the liver of Pontoporia blainvillei from south and southeast Brazilian coast areas.
2000; Dietz et al., 2000; Wagemann et al., 2000; Cardellicchio et al., 2002; Endo et al., 2002; Bustamante et al., 2003; Law et al., 2003; Kehrig et al., 2004), as was also seen for other dolphin species (S. guianensis) from the Brazilian coast (Kehrig et al., 2004; Kunito et al., 2004). However, in this study, Se concentrations were higher than those of Hg in the liver, on a molar basis, (Se/ Hg molar ratio = 4:1). In a previous study with franciscana from the southern Brazilian coast, Se concentrations were even higher than those of Hg (Se/Hg molar ratio = 8:1) (Kunito et al., 2004). The difference in the metabolism of the cetacean species could be influencing the accumulation of mercury and selenium, as reflected in the Se/Hg molar ratio.

It is important to observe that S. guianensis feeds preferentially upon different species of fish (Di Beneditto and Ramos, 2004), whereas franciscana feeds mainly on small fish and cephalopods (Di Beneditto and Ramos, 2001). Marine mammals feeding preferentially on fish rather than cephalopods normally accumulate higher concentrations of Hg in the liver (Watanabe et al., 2002).

In the marine environment, almost all Hg present in fish and cephalopods is methylated (Caurant et al., 1996; Das et al., 2000). Since the major part of Hg accumulated in marine mammal internal organs, especially in the liver, occurs as inorganic mercury (I-Hg), it is suggested that demethylation of methylmercury (MeHg) may occur in the liver (Caurant et al., 1996; Wagemann et al., 1998). A number of reported observations, such as the long half-life of mercury in the liver (≥ 10 years), the dependence of Hg on age, and the often-observed one to one relationship between Hg and Se (on a molar basis), are readily explained by the temporal accumulation of mercuric selenide (HgSe) in the liver (Wagemann et al., 2000; Arai et al., 2004). Mercuric selenide is considered to be a stable end-product of the demethylation process of methylmercury. Although not readily eliminated from the liver, it is inert and apparently non-toxic (Nigro and Leonzio, 1996; Wagemann et al., 2000; Kehrig et al., 2006a).

In the kidney of franciscana, on the other hand, Se concentrations were much higher than those of Hg on a molar basis (Se/Hg molar ratio = 16:1). Although Se/Hg molar ratios are close to one, as generally found for other marine mammals (Caurant et al., 1994; Meador et al., 1999; Endo et al., 2002; Brunborg et al., 2006), no significant correlation was found between their molar concentrations in franciscana (P > 0.05) (Fig. 4a, b). Some studies have also reported the presence of HgSe granules in the kidney of cetaceans (Das et al., 2000; Arai et al., 2004). However, no previous study reported the selenium to mercury relationship in the kidney of P. blainvillei.

3.5.2. Selenium to cadmium relationship

A significant positive relationship was observed between the molar concentrations of Se and Cd ($r^2 = 0.67; P < 0.001$) in the liver of individuals from the southeastern area (Fig. 3a, b). This association has often been observed in the liver of pilot whales (Globicephala melas) (Caurant et al., 1994), striped dolphins (S. coeruleoalba) (Monaci et al., 1998) and bottlenose dolphins (T. truncatus) (Meador et al., 1999). However, in this study, molar Se concentrations were much higher than molar Cd concentrations (mean Se/Cd ratio = 107:1), as reported in previous studies (Monaci et al., 1998; Meador et al., 1999).

Se protects mammals from the toxic effects of Cd and these elements may form equimolar Cd–Se complexes in the liver (Caurant et al., 1994). It is also known that metallothioneins (MTs) play the main role in the binding...
and detoxification of non-essential elements such as Cd in marine mammals (Das et al., 2000).

A non-significant correlation was found between the molar concentrations of Se and Cd \( (P \geq 0.05) \) in the kidney (Fig. 4a, b). Similar results were found by Monaci et al. (1998) and Meador et al. (1999) \( (\text{Se/Cd ratio} = 13:1) \).

In the literature, little information about the selenium to cadmium relationship in the tissues of marine mammals is available, and no previous study reported this inter-element relationship, either in the liver or in the kidney of franciscana.

### 3.5.3. Selenium to arsenic relationship

Although hepatic concentrations of Se were higher than As concentrations \( (\text{Se/As molar ratio} \approx 5) \), a non-significant correlation \( (P > 0.05) \) between these elements was found (Fig. 3a, b). Conversely, a significant positive linear relationship was found between the molar concentrations of Se and As \( (r^2 = 0.46; P < 0.05) \) in the kidney of individuals from the southern population, while renal concentrations of Se were also higher than those of As \( (\text{Se/As molar ratio} = 9.03) \) (Fig. 4a, b). A significant relationship between the concentrations of Se and As was also seen in the kidney of pilot whales from the North Atlantic Ocean (Caurant et al., 1994). However, there is little information on the relationship between Se and As in tissues of marine mammals (Caurant et al., 1994) and no previous study reported their inter-element relationship, either in the liver or in the kidney of franciscana.

Studies have shown that selenium reduces the toxicity of As compounds (EPA, 1998; Eisler, 2000). In addition, As can also antagonize Se toxicity (EPA, 1998; Eisler, 2000). Although the interaction of Se and As compounds is complex and not well understood, it has been suggested that formation of an arsenic–selenium compound occurs (Gailer et al., 2000). This compound was isolated from the bile of rabbits inoculated with these elements and identified by X-ray spectroscopy (EPA, 1998).

### 3.5.4. Mercury to cadmium relationship

A significant positive relationship was observed between the molar concentrations of Hg and Cd in the liver of franciscana from the southeastern population \( (r^2 = 0.77; P < 0.001) \) and also in the liver of individuals from the southern population \( (r^2 = 0.41; P < 0.05) \).
However, non-significant correlations were found between the molar concentrations of Hg and Cd \((P > 0.05)\) in the kidney of animals from either area.

There is a lack of information in the literature concerning the relationship between mercury and cadmium, both in the liver and in the kidney of toothed cetaceans from coastal regions worldwide (Caurant et al., 1994; Monaci et al., 1998; Roditi-Elasar et al., 2003). And, no previous study reported this inter-element relationship, either in the liver or in kidney of \(P.\) blainvillei.

Some studies have reported significant positive correlations between concentrations of Hg and Cd in the liver and in the kidney of marine mammals (Caurant et al., 1994; Monaci et al., 1998). In contrast to these results, a negative relationship between Hg and Cd concentrations was reported for beluga whales (Wagemann et al., 1990) and striped and bottlenose dolphins (Roditi-Elasar et al., 2003). Wagemann et al. (1990) suggested a competition mechanism to explain the negative correlation between Hg and Cd concentrations found. A feasible hypothesis to explain the positive correlation between the molar concentrations of Hg and Cd in the liver of franciscana can be the cumulative effects of Cd and Hg with increasing age (Caurant et al., 1994).

### 3.6. Relationship of trace element concentration between tissues

The relationship between the trace element concentrations in the liver and kidney of franciscana within the same sampling population was tested by multiple regression statistics (Fig. 5).

The concentrations of As, Hg and Cd showed a significant relationship between liver and kidney, indicating proportional accumulation (Fig. 5); whereas no correlation was observed between hepatic and renal Se concentrations. Other studies have also reported significant inter-tissues relationships for the concentrations of non-essential elements, such as mercury and cadmium, in marine mammals (Meador et al., 1999; Roditi-Elasar et al., 2003; Ikemoto et al., 2004). Very few studies reported As accumulation in several marine mammals species (such as whales, seals and dolphins) and most of them only in the liver (Kubota et al., 2001; Kubota et al., 2002). Ikemoto et al. (2004) reported non-significant inter-tissues relationship (liver and kidney) for selenium in different species of pinnipeds. According to Ikemoto et al. (2004), this is mostly due to the homeostatic control of the concentration of essential elements in tissues, but not for non-essential elements. Among the elements with significant inter-tissue correlation, Cd and Hg are classified as soft acids or chalcophilic elements (Haraguchi, 1999). Because elements belonging to this group have a high affinity to the SH group in cysteine, they show high accumulation in tissues as the liver and kidney (Haraguchi, 1999).

In the literature, no previous information about inter-tissues relationship for trace element concentrations (Hg, Se, As, Cd) in franciscana was reported.

### 4. Conclusions

The present study provides new information on trace element concentrations in the internal organs, mainly in the kidney, of a small dolphin species from the Southwestern Atlantic. Relatively low concentrations of trace elements were found in the liver and kidney of franciscana when compared with other marine mammal species worldwide, mainly those from the South Hemisphere.

Based on the results presented here, we can conclude that the life span presented a significant influence on the hepatic accumulation of trace elements, whereas this pattern was not observed for renal trace element accumulation.

The significant differences found in the hepatic concentrations of Hg, Se and Cd in individuals from the southern and southeastern Brazilian coast can be attributed partly to differences in the distinct populations, the prevalent environmental conditions (water temperature and primary production), and also to other factors, such as the level of food contamination. The principal intake of heavy metals in \(P.\) blainvillei is via food. Probably, the preys available for individuals from the southern Brazilian coast are richer in Hg, Cd and Se than those available for individuals from the southeastern coast. The low trace element concentrations observed in individuals from the southeastern coast may also be related to the low bioavailability of trace elements in the southeastern environment.

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