Trace metal and stable isotope measurements (δ^{13}C and δ^{15}N) in the harbour porpoise *Phocoena phocoena relicta* from the Black Sea

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**“Capsule”:** Trace metal levels in Black Sea harbour porpoises are lower than levels from the North Sea.

**Abstract**

Stable carbon and nitrogen isotopes (δ^{13}C and δ^{15}N) and trace metals (Cd, Zn, Cu, Fe, Se, and Hg) were analysed in the tissues of 46 harbour porpoises (*Phocoena phocoena relicta*) caught in fishing nets along the Ukrainian coasts between 1997 and 1998. Mean δ^{13}C values differed significantly between male and female harbour porpoises suggesting a trophic segregation between sexes with a more coastal distribution for females at least during their gestation and nursing periods. Hepatic Hg was correlated to δ^{13}C measurements, reflecting a different exposure linked to coastal vs offshore feeding habitats. A geographical comparison with existing data from other regions showed general low levels of Hg, Cd, Cu and Zn in the tissues of harbour porpoises from the Black Sea compared to other Atlantic and North Sea areas.

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**Keywords:** Black Sea; Stable isotopes; Trace metals; Marine mammals; *Phocoena phocoena*

1. Introduction

Three cetacean (sub)species inhabit the polluted but highly stratified Black Sea: the harbour porpoise, *Phocoena phocoena relicta*, the common dolphin, *Delphinus delphis ponticus*, and the bottlenose dolphin *Tursiops truncatus ponticus* (Bakan and Büyükgüngör, 2000). During the past century, fisheries have caused a drastic decline in cetacean abundance (Smith, 1982; Buckland et al., 1992; Birkun, 2002). The question of whether pollution has involved in this decline is still unresolved and remains a matter of debate. Since commercial killing of cetaceans was outlawed by all Black Sea nations in the second part of the 20th century, other factors such as by-catch (Birkun, 2002), infectious diseases (Birkun et al., 1999; Müller et al., 2002) and pollution might be involved. Indeed, the Black Sea, the largest enclosed sea in the world, has suffered from an extensive human impact over the past decades due to unmanaged fishing, unrestricted shipping, mineral exploitation and dumping of toxic wastes (Mee, 1992; Bakan and Büyükgüngör, 2000). Though some studies have been performed to assess the environmental quality of the Black Sea, contaminant studies in marine organisms are barely starting to emerge in this area (Tanabe et al., 1997a,b; Tuncer et al., 1998; Joiris et al., 2001; Tuşen, 2003). Furthermore, toxicology and biological data of marine mammals from this area are scarce and sometimes inexistent. Previous studies reported on high organochlorine and butyltin levels in Black Sea harbour porpoises (Madhusree et al., 1997;
Tanabe et al., 1997a,b). In contrast, mercury (Hg) levels in Black Sea harbour porpoises remained low compared to e.g. the southern North Sea (Joiris et al., 2001) while no studies have estimated the levels of zinc (Zn), copper (Cu), cadmium (Cd), selenium (Se) and iron (Fe) in Black Sea cetacean species. Furthermore, trace metal levels in marine mammals depend not only on the contamination of their environment but also on several ecological factors such as diet and trophic position (Das et al., 2002, 2003a). Changes in ratios of stable isotopes of carbon (\(^{13}\)C/\(^{12}\)C) and nitrogen (\(^{15}\)N/\(^{14}\)N) have been used to elucidate trophic relationships within marine food webs (Smith et al., 1996; Hobson et al., 1997; Burns et al., 1998; Lesage et al., 2001) and to investigate the relationship of contaminant uptakes with trophic position (Cabana and Rasmussen, 1994; Nisbet et al., 2002; Das et al., 2000, 2003a; Bearhop et al., 2000, 2002; Gorski et al., 2003). Stable isotope ratios of a consumer are related to those of its prey (De Niro and Epstein, 1978, 1981; Peterson and Fry, 1987). For nitrogen, enrichment in \(^{15}\)N occurs with trophic level (Hobson and Welch, 1992; Michener and Schell, 1994; Thompson et al., 1995; Hobson et al., 1996), whereas \(^{13}\)C enrichment is more closely related to being inshore or benthic feeders relative to offshore or pelagic feeders (Hobson et al., 1995; Smith et al., 1996). Furthermore, stable isotope analysis is often used to provide a continuous variable allowing to assess both trophic level (Michener and Schell, 1994; Hobson et al., 1995) and trophic transfer of contaminants (Kidd et al., 1995; Atwell et al., 1998; Das et al., 2000, 2003a,b; Hobson et al., 2002).

Black Sea cetaceans, including the harbour porpoise, are known to be at great risk of declining and disappearing (Notarbartolo di Sciara and Birkun, 2002). There is obviously an urgent need to collect information on their ecology and contaminant status. By measuring stable nitrogen isotope (\(\delta^{15}\)N) abundance in muscle, the trophic status of the Black Sea harbour porpoise was examined while stable carbon isotope (\(\delta^{13}\)C) analysis was used to investigate potential intra-species segregation according to the source of prey. The present study equally examined Zn, Cu, Cd, Fe, Se and Hg levels in order to compare with trophic data as well as with previous data from the Black Sea and other populations in the North Atlantic. Potential relationships between trace metals and biological data such as sex, age and trophic position have been investigated for evidence of specific bioaccumulation or biomagnification processes.

2. Material and methods

2.1. Sample collection and storage

Liver, kidney and muscle were collected from 46 harbour porpoises by-caught incidentally in fishing nets along the Ukrainian coast between 1997 and 1998. When available, brain tissue was also analysed. Tissue samples were stored at \(-20^\circ\)C until analyses.

2.2. Trace metal analyses

After being weighted and dried for 48 h at 110 \(^\circ\)C, samples were digested in a solution of nitric acid (Merck 456) and slowly heated to 100 \(^\circ\)C until complete digestion. Atomic absorption spectrophotometry (ARL 3510) was used to determine heavy metal concentrations (Cu, Zn, Cd, Fe). Concentrations are expressed as \(\mu\)g g\(^{-1}\) dry weight (dw).

Parallel to the samples, a set of certified material samples (CRM 278 Community Bureau of Reference, Commission of the European Communities) was analysed to ensure the method’s accuracy. Recoveries ranged from 92 to 100% for Cu, Zn, and Fe and 88% for Cd. Limits of detection were 0.01 \(\mu\)g g\(^{-1}\) dw for Cu, 0.33 for Zn, 0.16 for Fe and 0.22 for Cd.

Total Hg was analysed by flameless atomic absorption spectrophotometry (Perkin-Elmer MAS-50A) after sulphuric acid digestion (Joiris et al., 1991).

Selenium was analysed by fluorimetry following complete digestion of the tissue by nitric, perchloric and hydrochloric acids, coupling to EDTA and 2,3-diaminapthalene and extraction by cyclohexane (Mejuto et al., 1987). Quality control measurements for total Hg and Se included replicate analysis resulting in coefficients of variation <10% and analysis of certified material (DORM-1 and DORM-2, NRC, Canada).

2.3. Stable isotope measurements

After drying at 50 \(^\circ\)C (48 h), muscle samples were ground into a homogeneous powder and treated with a 2:1 chloroform:methanol solution to remove lipids. Carbon dioxide and nitrogen gas were analysed on a V.G. Optima (Micromass) IR-MS coupled to an elemental analyser (Carlo Erba). Routine measurements are precise to within 0.3\%\(^{\circ}\) for both \(^{13}\)C and \(^{15}\)N. Stable isotope ratios were expressed in \(\delta\) notation according to the following:

\[
\delta X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000
\]

where \(X\) is \(^{13}\)C or \(^{15}\)N and \(R\) is the corresponding ratio \(^{13}\)C/\(^{12}\)C or \(^{15}\)N/\(^{14}\)N.

Carbon and nitrogen ratios are expressed relative to the v-PDB (Vienna PeeDee Belemnite) standard and to atmospheric nitrogen, respectively. Reference materials were IAEA-N1 (\(\delta^{15}\)N = +0.4 \pm 0.2\%\(^{\circ}\)) and IAEA CH-6 (sucrose) (\(\delta^{13}\)C = \(-10.4 \pm 0.2\%\(^{\circ}\)).
Data are expressed as mean (median) G Brain 68 (62) G Muscle 61 (48) G Kidney 96 (97) G._

Porpoises from Ukrainian coast (J: juveniles; A: adults). Described by Hobson and Welch (1992) and Lesage et al. (2001) were converted to trophic position (TP) using the equation (3):

\[ TP = 2 + \left(D_m - \text{POM - TEF}_{\text{mm}}\right)/\text{TEF} \]

where \( D_m \) = \( \delta^{15}N \) value in marine mammal muscle, POM = \( \delta^{15}N \) value of marine particulate organic matter of the Black Sea (fixed to 4\(^{\circ}{\text{oo}} \), after Fry et al., 1991), and TEF = trophic enrichment factor in \( \delta^{15}N \) for a specific tissue (Hobson and Welch, 1992). This latter value was set to a mean value of 2.4\(^{\circ}{\text{oo}} \), except for marine mammals, for which a TEF value (TEF\(_{\text{mm}}\)) of 2.4\(^{\circ}{\text{oo}} \) was obtained in the muscles of two harbour seals fed on a constant herring diet (Hobson et al., 1996).

### 2.4. Isotopic model

Muscle \( \delta^{15}N \) signatures of harbour porpoise were converted to trophic position (TP) using the equation described by Hobson and Welch (1992) and Lesage et al. (2001):

\[ TP = 2 + (D_m - \text{POM - TEF}_{\text{mm}})/\text{TEF} \]

where \( D_m = \delta^{15}N \) value in marine mammal muscle, POM = \( \delta^{15}N \) value of marine particulate organic matter of the Black Sea (fixed to 4\(^{\circ}{\text{oo}} \) after Fry et al., 1991) and TEF = trophic enrichment factor in \( \delta^{15}N \) for a specific tissue (Hobson and Welch, 1992). This latter value was set to a mean value of 2.4\(^{\circ}{\text{oo}} \), except for marine mammals, for which a TEF value (TEF\(_{\text{mm}}\)) of 2.4\(^{\circ}{\text{oo}} \) was obtained in the muscles of two harbour seals fed on a constant herring diet (Hobson et al., 1996).

### 2.5. Data treatment

Parametric and nonparametric tests were used to compare different groups: Kolmogorov–Smirnov test was used to assume the normality of the data. ANOVA followed by post hoc multiple comparison tests (Tukey test) were used to compare the data between the different sex and age groups (adult males, adult females, juvenile males and juvenile females). Bravais–Pearson coefficient was used to test correlations between the values. Results were accepted as significant when \( p < 0.01 \).

### 3. Results

#### 3.1. Stable isotope analyses

\( \delta^{15}N \) values did not differ significantly between adult males, adult females, juvenile males and juvenile females (\( F_{3,42} = 0.6, p > 0.5 \); Fig. 1, Table 1).

Adult females (\( -20.3 \pm 0.4 \)\(^{\circ}{\text{oo}} \), \( n = 18 \)) were significantly \( 13C \)-enriched as compared to adult males (\( -20.7 \pm 0.3 \)\(^{\circ}{\text{oo}} \), \( n = 11 \); post hoc test, \( p < 0.01 \)). In contrast, \( \delta^{13}C \) values remained similar between juvenile males (\( -20.7 \pm 0.2 \)\(^{\circ}{\text{oo}} \), \( n = 12 \)) and females (\( -20.3 \pm 0.3 \), \( n = 5 \), Fig. 1).

\( \delta^{15}C \) and \( \delta^{15}N \) values did not differ significantly between juveniles and adults (\( p > 0.1 \)).

### Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>( \delta^{15}C )</th>
<th>( \delta^{15}N )</th>
<th>Trophic level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{mean} \text{ (median)} )</td>
<td>( \text{mean} \text{ (median)} )</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>(-20.5 (-20.5) \pm 0.4)</td>
<td>(12.0 (12.0) \pm 0.6)</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>((-21.3 -19.6) n = 46)</td>
<td>((10.4 -13.6) n = 46)</td>
<td></td>
</tr>
</tbody>
</table>

Data are given as average (median) ± standard deviation, (minimum–maximum); \( n \): number of samples.

### Table 2

Trace metal concentrations (\( \mu g / g \text{ dry weight} \)) in the tissues of harbour porpoises (\( Phocoena phocoena \)) from the Ukrainian coast of the Black Sea (nd: non-determined)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Zn</th>
<th>Cd</th>
<th>Cu</th>
<th>Se</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{mean} \text{ (median)} )</td>
<td>( \text{mean} \text{ (median)} )</td>
<td>( \text{mean} \text{ (median)} )</td>
<td>( \text{mean} \text{ (median)} )</td>
<td>( \text{mean} \text{ (median)} )</td>
</tr>
<tr>
<td>Liver</td>
<td>94 (97) \pm 22</td>
<td>1.1 (0.7) \pm 1.5</td>
<td>1135 (1088) \pm 324</td>
<td>20 (20) \pm 7</td>
<td>5.9 (5.2) \pm 3.0</td>
</tr>
<tr>
<td></td>
<td>((47–148))</td>
<td>((&lt;0.1–9.3))</td>
<td>((420–1858))</td>
<td>((6–36))</td>
<td>((1.5–16.3))</td>
</tr>
<tr>
<td></td>
<td>( n = 41 )</td>
<td>( n = 41 )</td>
<td>( n = 41 )</td>
<td>( n = 40 )</td>
<td>( n = 44 )</td>
</tr>
<tr>
<td>Kidney</td>
<td>96 (97) \pm 22</td>
<td>5.8 (5.2) \pm 4.7</td>
<td>906 (738) \pm 479</td>
<td>15 (16) \pm 4.1</td>
<td>8.0 (6.4) \pm 10</td>
</tr>
<tr>
<td></td>
<td>((45–147))</td>
<td>((&lt;0.1–15))</td>
<td>((385–2970))</td>
<td>((5.7–29))</td>
<td>((0.1–57))</td>
</tr>
<tr>
<td></td>
<td>( n = 42 )</td>
<td>( n = 42 )</td>
<td>( n = 42 )</td>
<td>( n = 41 )</td>
<td>( n = 40 )</td>
</tr>
<tr>
<td>Muscle</td>
<td>61 (48) \pm 28</td>
<td>0.3 (&lt;0.1) \± 1</td>
<td>823 (669) \± 508</td>
<td>10 (7) \± 7</td>
<td>2.2 (1.4) \± 1.7</td>
</tr>
<tr>
<td></td>
<td>((30–134))</td>
<td>((&lt;0.1–6.4))</td>
<td>((228–3551))</td>
<td>((3–32))</td>
<td>((0.7–5.5))</td>
</tr>
<tr>
<td></td>
<td>( n = 45 )</td>
<td>( n = 45 )</td>
<td>( n = 45 )</td>
<td>( n = 44 )</td>
<td>( n = 7 )</td>
</tr>
<tr>
<td>Brain</td>
<td>68 (62) \pm 29</td>
<td>0.3 (&lt;0.1) \± 0.8</td>
<td>280 (99) \± 453</td>
<td>23 (22) \± 6</td>
<td>2.1 (1.7) \± 0.7</td>
</tr>
<tr>
<td></td>
<td>((35–119))</td>
<td>((&lt;0.1–3))</td>
<td>((67–1202))</td>
<td>((16–32))</td>
<td>((1.5–3.3))</td>
</tr>
<tr>
<td></td>
<td>( n = 6 )</td>
<td>( n = 6 )</td>
<td>( n = 6 )</td>
<td>( n = 6 )</td>
<td>( n = 7 )</td>
</tr>
</tbody>
</table>

Data are expressed as mean (median) ± SD (min–max) and number of samples analysed.
3.2. Metal levels in the tissues

Metal levels in liver, kidney, muscle and brain are shown in Table 2. Trace metal concentrations did not differ significantly between male and female harbour porpoises; all individuals were therefore pooled.

Zn, Fe and Se were notably higher in the liver and kidney compared to muscle and brain (Table 2). Cu followed a different pattern, with significantly higher concentrations in the porpoises’ liver and brain.

Hg and Se (Pearson correlation, \( r = 0.63 \), \( p < 0.0001 \)) and Zn and Cu (\( r = 0.75 \), \( p < 0.0001 \); Figs. 2 and 3) were both positively correlated in liver tissues.

Hepatic and renal Cd concentrations increased with the age of the individuals (Pearson correlation, \( r = 0.3 \), \( p < 0.05 \) and \( r = 0.5 \), \( p < 0.0001 \) for liver and kidney, respectively). The same significant relationship was observed between the age of the porpoises and Hg concentrations in the liver (Pearson correlation, \( r = 0.65 \), \( p < 0.0001 \)).

3.3. Metal and stable isotope relationship

A significant correlation was observed between \( \delta^{13}C \) values in the muscle and Hg concentrations in harbour porpoise liver (Pearson correlation, \( r = 0.46 \), \( p < 0.003 \), \( n = 41 \); Fig. 4). No significant relationships were observed with Zn, Cd, Cu, Se and Fe or for Hg in other tissues. \( \delta^{15}N \) values were not correlated to metal concentrations in any of the tissues analysed.

4. Discussion

4.1. Pattern of isotopic signatures

Little is known about the life history and ecology of the Black Sea harbour porpoise. One extensive study on harbour porpoise diet was carried out in the 1940s, with 4000 stomachs examined (Tsalkin, 1940 quoted by Tomilin, 1957). The diet consisted mainly of benthic fish while pelagic fish were only taken when they occurred in large and dense schools (Tsalkin, 1940 quoted by Tomilin, 1957). Since this period, the Black Sea has experienced dramatic long-term changes in fish abundance (Daskalov, 2003). More recently, the stomach contents of 94 harbour porpoises collected during the period 1989–1999 were examined (Krivokhizhin et al., 2000). Seven fish species were identified: shad (Alosa pontica), sprat (Sprattus sprattus), European anchovy (Engraulis encrasicolus), whiting (Merlangius merlangus euxinus), pickarel (Spicara smaris), gobies (Gobiidae) and far east haarder (Mugil soiuy), a fish species introduced in the Black Sea during the 1970s (Krivokhizhin et al., 2000).

A mean position of 3.7 was found for the harbour porpoise along the Ukrainian coasts. This estimation is in good agreement with previous data collected from the North Sea with mean trophic position of 3.4 for harbour
Table 3
Trace element concentrations (µg g⁻¹ dry weight) in the liver of harbour porpoises *Phocoena phocoena* from European and Black Sea coasts (selected references). When expressed in wet weight, data are converted to dw assuming a mean water content of 75 %. (na: data not available) Data are expressed as mean ± SD (min-max, when available) and number of samples.

<table>
<thead>
<tr>
<th>Trace Element</th>
<th>Ireland, Law et al., 1992</th>
<th>England and Wales, Bennet et al., 2001</th>
<th>Baltic Sea, Szefer et al., 1994</th>
<th>Norway, Das, 2002; Teigen et al., 1993</th>
<th>Greenland, Paludan et al., 1993</th>
<th>Black Sea, this work; Joiris et al., 2001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>321 ± 184 (176–560)</td>
<td>310 ± 28 (96–144)</td>
<td>120 ± 19 (69–248)</td>
<td>111 ± 47 (145–369)</td>
<td>202 n = 44</td>
<td>94 ± 22 (47–148)</td>
</tr>
<tr>
<td></td>
<td>n = 5</td>
<td></td>
<td>n = 4</td>
<td></td>
<td></td>
<td>n = 41</td>
</tr>
<tr>
<td></td>
<td>173 ± 135 (91–380)</td>
<td></td>
<td>172 ± 10 (infectious diseases)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>n = 8</td>
<td></td>
<td>n = 49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(physical trauma)</td>
<td></td>
<td>(physical trauma)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.6 ± 1 (0.3–1)</td>
<td>0.96 ± 0.2 (0.3–0.4)</td>
<td>0.4 ± 0.5 (0.2–0.7)</td>
<td>13</td>
<td>1.1 ± 1.5 (0.1–9.3)</td>
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<tr>
<td></td>
<td>n = 5</td>
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<td>n = 4</td>
<td></td>
<td></td>
<td>n = 41</td>
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<tr>
<td></td>
<td>0.3 ± 0.2 (0.1–0.5)</td>
<td></td>
<td>0.76 ± 0.12 (n = 49)</td>
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<tr>
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<td>(physical trauma)</td>
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<td>(physical trauma)</td>
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<tr>
<td>Fe</td>
<td>na</td>
<td>na</td>
<td>812 ± 336 (424–1264)</td>
<td>1249 ± 300 (792–1774)</td>
<td>na</td>
<td>1135 ± 324 (420–1858)</td>
</tr>
<tr>
<td></td>
<td>n = 16</td>
<td></td>
<td>n = 16</td>
<td></td>
<td>n = 16</td>
<td>n = 41</td>
</tr>
<tr>
<td>Cu</td>
<td>41 ± 15 (26–64)</td>
<td>47 ± 9 (26–64)</td>
<td>23 ± 8 (18–36)</td>
<td>48 ± 61 (12–217)</td>
<td>48 n = 22</td>
<td>20 (20) ± 7 (6–36)</td>
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<td></td>
<td>n = 5</td>
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<td>n = 4</td>
<td></td>
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<td>n = 40</td>
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<td></td>
<td>98 ± 161 (26–640)</td>
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<td>58 ± 9 (n = 49)</td>
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<td>n = 20</td>
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<td>(physical trauma)</td>
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<tr>
<td>Se</td>
<td>na</td>
<td>40 ± 14 (n = 37)</td>
<td>na</td>
<td>12 ± 9 (n = 92)</td>
<td>11 (n = 44)</td>
<td>5.9 (5.2) ± 3.0 (1.5–16)</td>
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<td></td>
<td>n = 20</td>
<td></td>
<td>(n = 37)</td>
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<td>(infectious diseases)</td>
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<td></td>
<td>29 ± 10 (n = 49)</td>
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<td>13 ± 7 (n = 49)</td>
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<td>(physical trauma)</td>
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<td>(physical trauma)</td>
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<tr>
<td>Hg</td>
<td>56 ± 52 (n = 37)</td>
<td>80 ± 22 (n = 37)</td>
<td>na</td>
<td>12 ± 13 (n = 44)</td>
<td>17 (n = 22)</td>
<td>10.7 (7.2) ± 11 (0.6–35)</td>
</tr>
<tr>
<td></td>
<td>n = 5</td>
<td></td>
<td>(n = 37)</td>
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<tr>
<td></td>
<td>24 ± 42 (n = 49)</td>
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<td>14 ± 10 (n = 44)</td>
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<td></td>
<td>(physical trauma)</td>
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</table>

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porpoise from the southern North Sea (Das et al., 2003b).

$\delta^{13}C$ is preferably used to indicate the origin of carbon sources than as an indicator of the trophic level. The general pattern of inshore, benthos-linked food webs being more enriched in $^{13}C$ compared to offshore, pelagic food webs presents a potentially useful tool. $\delta^{13}C$ values are typically higher in coastal or benthic than in offshore food webs (Hobson, 1999).

The low $\delta^{13}C$ signature of the male porpoises suggests that they obviously fed on more offshore prey with a low $^{13}C$ signature, while females remained in shallow waters. The overall trophic level for both sexes was, however, similar (Fig. 1). Segregation of harbour porpoises in groups of different sex and/or age has been described by few authors (Tomilin, 1957; Kinze, 1994; Santos and Pierce, 2003). In the southern North Sea, adult female porpoises fed at a higher trophic level than adult males while juvenile porpoises displayed no differences between sexes (Das et al., 2003b). The male porpoises were also slightly $^{13}C$-depleted compared to females. The harbour porpoises from this study have been collected mainly during spring and summer. Only two female porpoises (and no males) have been collected in October. The presence during necropsy of near-term foetuses in porpoises during spring indicates this to be the birth period (Birkun et al., 2000; Ozturk and Ozturk, 2003). During the calving and nursing period, females are generally associated with shallow waters (Smith and Gaskin, 1983; Kinze, 1994).

4.2. Trace metal levels

Zn, Cu, Se and Hg in harbour porpoises from the Black Sea are strikingly low compared to other areas such as the North Sea and the Channel, but in the same order of magnitude as in Norway and the Baltic Sea (Teigen et al., 1993; Bennet et al., 2001; Das, 2002; Das et al., 2003a; Table 3). Lower Hg levels were previously described in Black Sea harbour porpoise (Joiris et al., 2001). The special hydrological conditions in the Black Sea, with a limited aerobic surface water mass and an important deeper anoxic zone, might be one reason for an important sink of organic particulate matter with its pollutant load (Joiris et al., 2001). Another reason might be linked to the body condition of the animals. Porpoises used in this study were by-caught porpoises, displaying a good body condition. Hepatic Hg, Se and Zn concentrations may be higher in stranded and emaciated animals (Siebert et al., 1999; Bennet et al., 2001; Anan et al., 2002; Das, 2002).

Zn and Cu show positive correlation (Fig. 3) in the liver. Hepatic Zn and Cu correlations have been reported previously in various marine mammals, as a result of both an antagonistic behavior and high affinities to metallothioneins (Monaci et al., 1998; Anan et al., 2002). The 0.75 slope observed for Black Sea harbour porpoise was similar to the one observed for Norwegian by-caught harbour porpoises (Das, 2002). In harbour porpoise, Hg was correlated to age and Se as a result of a demethylation process (Fig. 2) leading to a long-term tiemannite (HgSe) storage (Nigro and Leoncio, 1996; Das et al., 2002). Strikingly, hepatic Hg was correlated to muscle $\delta^{13}C$ (Fig. 4). This relationship suggests different Hg exposure linked to coastal vs offshore habitat.

No correlations were observed between stable isotope values and other metals. Hepatic and renal Cd concentrations of Black Sea harbour porpoises increased with age but were never associated to $\delta^{13}C$ and $\delta^{15}N$. This differs strongly from the North Atlantic and the North Sea where Cd concentrations were associated to both $\delta^{13}C$ and $\delta^{15}N$ values (Das, 2002; Das et al., 2003a).

High Cd values encountered in some marine mammal species are diet related as a result of ingestion of cephalopods (Bustamante et al., 1998). The low Cd level encountered in the Black Sea porpoises are in agreement with the fact that cephalopods were never recorded in the Black Sea (Mordukhay-Boltovskoy, 1972; Zaitsev and Alexandrov, 1998).

To conclude, trace metal levels of the harbour porpoises from the Black Sea are generally low, at the same order of magnitude as porpoises from Norway and the Baltic Sea, but far lower than levels encountered in porpoises from the North Sea. Stable isotopes were proven useful tools as they evidence a trophic segregation of male and female harbour porpoises, at least in spring and summer. Hg concentrations in harbour porpoises seem to be linked to the coastal vs offshore feeding habits. However, further research on a larger sampling and other prey and cetacean species from the area will allow a better understanding of the transfer of pollutants within the Black Sea.

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