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DOI: 10.1016/j.marpolbul.2007.10.030

# Trace element concentrations in blood of free-ranging bottlenose dolphins (*Tursiops truncatus*): Influence of age, sex and location

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Marine mammals serve as good indicators for contaminants accumulation because of their long life span and their place within higher trophic levels (Bossart, 2006; Veinott and Sjare, 2006). A recent review of ecosystem responses to mercury contamination suggested that some marine mammals, such as ringed seals (*Phoca hispida*) and harbor porpoises (*Phocoena phocoena*) would be potentially good indicators of changing mercury loadings in the coastal environments (Wolfe et al., 2007). These species were selected as indicators based on a combination of criteria that include well-characterized life history; capacity for bioconcentrating and accumulating contaminants of concern; common species in the environment; geographically widespread; sensitive and hence indicative of change; easily collected and measured; adequate size to permit resampling of tissue; occurrence in both polluted and unpolluted areas; display correlation with environmental levels of contaminants; and have background data on the natural condition (Jenkins, 1981).

The majority of previous studies on trace elements have documented concentrations in liver, kidney and muscle tissues of marine mammals primarily obtained from stranded carcasses. Accessible samples from free-ranging marine mammals are generally limited to blood, fur/hair, skin biopsy, saliva and feces (Fossi and Marsili, 1997; Kakuschke et al., 2005; Griesel et al., 2006; Andrade et al., 2007). Information on trace element concentrations found in the blood of marine mammals may be useful in comparing contaminant exposure to humans and other mammals (Nielsen et al., 2000). Additionally, monitoring blood levels of trace elements in living marine mammals may also provide data for immunological studies, such as characterization of peripheral blood phagocytes response to mercury; warning signs for adverse health impacts by evaluating the correlation between trace element and mineral elements; and the relationship between exposure to trace elements and infectious disease mortality (Bennett et al., 2001; Lalancette et al., 2003; Kakuschke et al., 2005, 2006; Griesel et al., 2006).

Whole blood is considered the recommended biological fluid for measuring recent exposure to some toxic elements, such as Cd and Pb, which are excreted very slowly in humans (Polkowska et al., 2004). It is difficult to establish the correlations between environmental contaminants and human sickness because of the lack of cause and effect relationships and incomplete exposure mechanisms of contaminants (Sexton et al., 2004). For marine mammals, the correlation between contaminants and possible population declines has also not been established (Barron et al., 2003; Das et al., 2003). Information on baseline trace element levels in the blood of free-ranging marine mammals is limited (Griesel et al., 2006; Brookens et al., 2007; Bryan et al., 2007).

The bottlenose dolphin (*Tursiops truncatus*) was selected in the present study for baseline trace element concentrations because it can serve as a potential sentinel species in coastal areas by reflecting the effects of natural and anthropogenic stressors (Hansen et al., 2004; Bossart, 2006). Age, sex, trophic level, reproductive and health status, season, metabolic rate, tissue type and locations have been documented as factors that may influence trace element concentrations in marine mammals (Das et al., 2003; O'Hara et al., 2003; Brookens et al., 2007). The aims of this study were to determine baseline trace element concentrations in the blood of free-ranging bottlenose dolphin populations and to further determine how concentrations may vary with age, sex and locations. The selection of trace elements in this study was based on both their toxicity (e.g., As and total Hg–THg) and biological function (e.g., Cu and Zn).

Samples were collected from two free-ranging bottlenose dolphin populations, one in Charleston (CHS, $n = 74$), South Carolina (32°46′51″N; 79″°55′33″W) and other in the Indian River Lagoon (IRL, $n = 75$), Florida (27°47′41″N; 80°47′46″W) during the summers of 2003–2005. Biological characteristics of each dolphin sampled including age and length, for each sex are given in Table 1. Dolphins were restrained using established capture-release techniques (Fair et al., 2006). Dolphin ages were determined by the examination of postnatal dentine layers in an extracted tooth (Hohn et al., 1989). Blood samples were drawn into a S-monovette® tube for trace metal analysis (Sarstedt Inc., Newton, NC) from the periarterial venous rete in the flukes as soon as possible after the dolphin was restrained (Bossart et al., 2001; Fair et al., 2006). Samples were immediately frozen in dry ice and then kept at −40 °C until analysis. After thawing, each blood sample was inverted several times to mix well. Aliquots

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Table 1
Biological characteristics of free-ranging bottlenose dolphins sampled in Charleston, SC and Indian River Lagoon, FL in the summers of 2003–2005

| | CHS | | | IRL | | |
|---|---|---|---|---|---|---|
| | *N* | Age (yr) | Length (cm) | *N* | Age (yr) | Length (cm) |
| *Female* | | | | | | |
| Juvenile | 14 | 5 ± 1.2 | 193 ± 9.2 | 9 | 5 ± 0.9 | 203 ± 7.6 |
| | | (3–6) | (176–210) | | (4–6) | (190–217) |
| Adult | 15 | 19 ± 7.6 | 239 ± 10.6 | 13 | 12 ± 5.2 | 230 ± 15.4 |
| | | (8–33) | (217–250) | | (7–26) | (201–253) |
| Total | 29 | 12 ± 9.3 | 217 ± 25.1 | 22 | 9 ± 5.6 | 219 ± 18.6 |
| *Male* | | | | | | |
| Juvenile | 9 | 6 ± 1.9 | 205 ± 19.7 | 19 | 7 ± 1.4 | 215 ± 12.8 |
| | | (4–9) | (183–246) | | (5–9) | (195–236) |
| Adult | 36 | 17 ± 5.8 | 243 ± 16.4 | 34 | 15 ± 4.0 | 244 ± 14.9 |
| | | (10–28) | (212–272) | | (10–27) | (221–274) |
| Total | 45 | 15 ± 6.9 | 236 ± 22.8 | 53 | 12 ± 5.1 | 234 ± 19.8 |
| Total | 74 | 14 ± 8.0 | 228 ± 25.4 | 75 | 11 ± 5.4 | 230 ± 20.4 |

of 1 ml blood were placed into a digestion vessel with ULTREXII nitric acid ($HNO_3$, JT Baker, Phillipsburg, NJ) in a 36-well hotblock (Environmental Express, Mount Pleasant, SC) at approximately 95 °C. Additional ULTREXII 30% hydrogen peroxide ($H_2O_2$, JT Baker) was added to each sample to complete oxidation of organic matter. The blood sample was then evaporated to dryness and redissolved with deionized water and brought up to final volume of 15 ml solution (in 2% $HNO_3$) before analysis.

Total mercury (THg) in each sample was determined using a thermal decomposition (gold) amalgamation atomic absorption spectrophotometer direct mercury analyzer (DMA-80, Milestone Inc., Shelton, CT). Whole blood (200 μl) and 1% $HNO_3$ (100 μl) were added into the quartz sample boat. The method has been validated for solid and liquid matrices using US EPA Method 7473 (http://www.epa.gov/epaoswer/hazwaste/mercury/pdf/7473.pdf). Quality assurance methods included evaluating by measuring empty boats (blank), duplicates and certified reference material (CRM) – Seronorm™ trace element whole blood level III (Ref.: 201705, Lot: OK0337, SERO, Oslo, Norway) with every 10 samples. Recovery of THg in Seronorm whole blood ranged from 96% to 107%, averaging 103 ± 3%. A Perkin Elmer Elan 6100 inductively coupled plasma-mass spectrometry (ICP-MS) connected with an AS-91 AutoSampler (Perkin Elmer, Wellesley, MA) was used to measure the following elements: Al, As, Ba, Cu, Fe, Mn, Se and Zn over the range of 10 μg/L to 2000 mg/L. Multiple-elements ($^{45}$Sc, $^{72}$Ge, $^{103}$Rh, $^{175}$Lu) internal standards (High-Purity Standards, Charleston, SC) were added to each sample and calibration standard solutions. Quality control and quality assurance for ICP-MS included field blanks, method blanks, CRM – Seronorm and matrix spikes. Ba, Cu, Fe and Zn were not certified in this CRM, only information values. The accuracy of these uncertified element measurements were evaluated by recovery tests following sample spiking. Recoveries of all elements in CRM and spiked sample ranged from 88% to 114%, averaging 98 ± 8% and 72% to 112%, averaging 91 ± 11%, respectively. All plasticware was pre-cleaned with reagent grade $HNO_3$ (69.0–70.0%) and hydrochloric acid (36.5–38.0%) to eliminate contamination.

Due to the limited amount of available blood for methylmercury (MeHg) determination, only 25 blood samples (CHS = 17, IRL = 8) in 2003 and 2005 were accessible for the measurement of MeHg by cold-vapor atomic fluorescence spectrometry. These samples were analyzed by the Battelle Marine Sciences Laboratories at the Pacific Northwest National Laboratory (Sequim, WI). The MeHg analysis method followed EPA method 1630 (2001) using standard reference material DOLT-2 (National Research Council Canada) for quality assurance. Recovery of MeHg in DOLT-2 ranged from 101% to 106%, averaging 104 ± 3%. The average percent recovery of matrix spike result was 95 ± 1%, ranged from 94% to 96%.

Descriptive statistics for mean, standard deviation and range were calculated using SAS (Version 9, SAS Institute Inc., Cary, NC) and SPSS (Windows 9.0, SPSS Inc., Chicago, IL) for each concentration; only samples having values greater than those in method detection limit (MDL) were included. Non-parametric statistical methods were applied due to variability of values and relatively small sample sizes. Dolphins <7 and <10 years of age were classified as juvenile female and male, respectively, while those ⩾7 and ⩾10 years of age were categorized as adult female and male, respectively. Sample sizes for each age and sex class varied because some samples had non-detectable values of trace element, while others were below MDL concentrations for some elements in this study. Differences between the means of two age groups were examined by the Mann–Whitney *U* test. Relationships between age and the concentration of each element were evaluated by linear regression. Spearman's rank correlation coefficients were determined among trace elements. Unless otherwise noted, results were considered significant when $p \leqslant 0.05$.

This study consists of the largest existing data set for multiple trace elements in the blood of free-ranging bottlenose dolphins. It is also the first report on concentrations of Al, As, Ba, Mn and MeHg levels in blood of wild dolphins from the southeast Atlantic coast. Concentrations of nine trace elements and MeHg in the blood of CHS and IRL bottlenose dolphins were evaluated by both age and sex classes (Tables 2 and 3). The values found in CHS dolphins ranged from a low of 14 μg/L for Mn < Al < Ba < MeHg < As < THg < Cu < Se < Zn to the highest value of 467 mg/L for Fe (Table 2). A similar order of concentrations was observed in IRL dolphins with the lowest value of 11 μg/L for Mn < Al < As < Ba < MeHg < Se < THg < Cu < Zn to the highest value of 449 mg/L for Fe (Table 3). Measurable levels of all trace elements were found in CHS dolphins except for Al ($n = 62$) and Ba ($n = 72$) and for all IRL dolphin samples except for Al ($n = 40$), Ba ($n = 74$) and Mn ($n = 71$). As expected, the variations in non-essential elements (e.g., As and THg) were higher than

Table 2
Trace element concentrations in blood of free-ranging bottlenose dolphins sampled from the Charleston, SC in the summers of 2003–2005 (mean ± standard deviation and range in μg/L wet weight; mg/L wet weight if noted)

| | Female | | | | Male | | | | Total | *n* |
|---|---|---|---|---|---|---|---|---|---|---|
| | Juvenile | *n* | Adult | *n* | Juvenile | *n* | Adult | *n* | | |
| Al[a] | 34 ± 14<br>(10–65) | 12 | 16 ± 6<br>(9–32) | 14 | 32 ± 10<br>(12–43) | 8 | 27 ± 11<br>(9–53) | 28 | 26 ± 12 | 62 |
| As[a] | 174 ± 91<br>(72–387) | 14 | 128 ± 37<br>(82–227) | 15 | 131 ± 95<br>(30–357) | 9 | 116 ± 59<br>(56–371) | 36 | 131 ± 70 | 74 |
| Ba[a] | 102 ± 103<br>(1–328) | 14 | 47 ± 32<br>(2–94) | 15 | 129 ± 97<br>(29–349) | 8 | 83 ± 31<br>(1–162) | 35 | 84 ± 64 | 72 |
| Cu | 742 ± 110<br>(576–1001) | 14 | 711 ± 147<br>(428–931) | 15 | 779 ± 219<br>(520–1212) | 9 | 736 ± 179<br>(466–1192) | 36 | 737 ± 165 | 74 |
| Fe (mg/L) | 442 ± 101<br>(329–599) | 14 | 455 ± 110<br>(289–673) | 2 | 475 ± 193<br>(250–804) | 9 | 479 ± 124<br>(252–759) | 36 | 467±126 | 74 |
| THg[a] | 100 ± 37<br>(28–151) | 14 | 181 ± 86<br>(47–332) | 15 | 112 ± 51<br>(42–178) | 9 | 159 ± 101<br>(56–573) | 36 | 147 ± 88 | 74 |
| MeHg | 26 | 1 | 91 ± 45<br>(60–123) | 15 | 47 | 1 | 93 ± 27<br>(48–152) | 12 | 86 ± 33 | 16 |
| Mn[a] | 16 ± 4<br>(11–24) | 14 | 14 ± 4<br>(8–19) | 15 | 16 ± 6<br>(10–26) | 9 | 13 ± 4<br>(7–22) | 36 | 14 ± 4 | 74 |
| Se | 842 ± 222<br>(527–1292) | 14 | 767 ± 150<br>(449–953) | 15 | 814 ± 317<br>(385–1349) | 9 | 755 ± 160<br>(488–1097) | 36 | 781 ± 194 | 74 |
| Zn | 2465 ± 330<br>(1827–2937) | 14 | 2270 ± 301<br>(1592–2674) | 15 | 2479 ± 684<br>(1672–3684) | 9 | 2348 ± 358<br>(1708–3153) | 36 | 2370 ± 394 | 74 |

[a] There were significant differences between classes.

Table 3
Trace element concentrations in blood of free-ranging bottlenose dolphins sampled from the Indian River Lagoon, FL in the summers of 2003–2005 (mean ± standard deviation and range in μg/L wet weight; mg/L wet weight if noted)

| | Female | | | | Male | | | | Total | *n* |
|---|---|---|---|---|---|---|---|---|---|---|
| | Juvenile | *n* | Adult | *n* | Juvenile | *n* | Adult male | *n* | | |
| Al | 24 ± 11<br>(10–35) | 5 | 29 ± 9<br>(15–43) | 9 | 22 ± 11<br>(9–40) | 11 | 27 ± 23<br>(8–81) | 15 | 26 ± 16 | 40 |
| As | 47 ± 21<br>(19–78) | 9 | 56 ± 27<br>(22–131) | 13 | 55 ± 23<br>(23–109) | 19 | 56 ± 22<br>(35–149) | 34 | 55 ± 23 | 75 |
| Ba | 99 ± 83<br>(1–264) | 8 | 96 ± 50<br>(1–229) | 13 | 83 ± 104<br>(1–473) | 19 | 85 ± 51<br>(1–198) | 34 | 88 ± 70 | 74 |
| Cu | 749 ± 197<br>(569–1225) | 9 | 779 ± 229<br>(576–1272) | 13 | 832 ± 220<br>(428–1329) | 19 | 813 ± 151<br>(597–1251) | 34 | 804 ± 188 | 75 |
| Fe[a] (mg/L) | 69 ± 126<br>(230–703) | 9 | 405 ± 169<br>(132–872) | 13 | 463 ± 115<br>(296–696) | 19 | 453 ± 103<br>(228–752) | 34 | 449 ± 121 | 75 |
| THg | 832 ± 784<br>(187–2751) | 9 | 719 ± 435<br>(267–1808) | 13 | 655 ± 634<br>(150–2005) | 19 | 591 ± 392<br>(89–1611) | 34 | 658 ± 519 | 75 |
| MeHg | 161 | 1 | | | 342 ± 205<br>(182–573) | 3 | 233 ± 59<br>(156–298) | 4 | 265 ± 135 | 8 |
| Mn[a] | 15 ± 3<br>(10–20) | 8 | 11 ± 2<br>(9–16) | 13 | 12 ± 4<br>(6–20) | 18 | 10 ± 3<br>(5–21) | 32 | 11 ± 4 | 71 |
| Se | 627 ± 129<br>(392–849) | 9 | 562 ± 105<br>(394–784) | 13 | 654 ± 159<br>(430–1005) | 19 | 619 ± 103<br>(446–857) | 34 | 619 ± 124 | 75 |
| Zn[a] | 2985 ± 392<br>(2043–3381) | 9 | 2481 ± 358<br>(1676–2935) | 13 | 2768 ± 420<br>(2271–3504) | 19 | 2728 ± 508<br>(1630–4566) | 34 | 2726 ± 463 | 75 |

[a] There were significant differences between classes.

those for essential elements (e.g., Cu and Zn) which likely reflects the important role these essential elements play in the formation or function of many enzymes (Das et al., 2003). These essential elements are also closely regulated through homeostasis in marine mammals. Levels of Cu, Fe, THg, Se and Zn in blood of bottlenose dolphins are comparable to levels reported in the blood of *Physeter macrocephalus*, *Grampus griseus*, *T. truncatus*, *Phoca vitulina*, *Phoca vitulina richardii* (Nielsen et al., 2000; Shoham-Frider et al., 2002; Griesel et al., 2006; Brookens et al., 2007; Bryan et al., 2007). Mn levels in CHS and IRL dolphins were found to be relatively close to the normal range of 4–14 μg/L reported for human blood (ATSDR, 2000) but considerable lower than that reported in a single whole blood sample of grey seal (*Halichoerus grypus*) (21.6 μg/L, (Kakuschke et al., 2006). Although there were no data available in dolphins for Al, blood Al levels In CHS and IRL dolphins were at least two orders of magnitude lower than those in tissues of cetaceans and pinnipeds (Tilbury et al., 2002; Bustamante et al., 2003). Similar As levels were found in IRL dolphins and in a gray seal (Kakuschke et al., 2006). Concentrations of As in the blood of CHS (mean = 131 μg/L, range = 30–387 μg/L) and IRL (mean = 55 μg/L, range = 19–149 μg/L) dolphins were lower than those in the liver of bottlenose dolphins from South Carolina (range = 100–860 ng/g wet weight) (Beck et al., 1997) and from Australia (range = 200–760 ng/g wet weight) (Law et al., 2003). Fivefold higher Se levels were found in CHS (mean = 781 μg/L, range = 385–1349 μg/L) and IRL (mean = 619 μg/L, range = 392–1005 μg/L) dolphins compared to healthy human blood: 40–200 μg/L (0.5–2.5 μmol/L) (WHO, 1998). Similar Se levels were also observed in blood of harbor seals (Griesel et al., 2006) compared to CHS and IRL dolphins.

The ratio of MeHg to THg has been documented to vary widely in tissues of marine mammals (Wagemann et al., 1998; Joiris et al., 2001; Dehn et al., 2005, 2006; Endo et al., 2005; Brookens et al., 2007). Although in marine mammals, the predominant form of Hg taken up via food is MeHg, limited data are available on the MeHg concentrations in the blood. Blood can serve as an indicator for MeHg exposure since it is a transitory buffer for Hg during its conversion from toxic MeHg to HgSe (Ancora et al., 2002). In this study, MeHg levels in blood of bottlenose dolphins, ranging from 26 to 573 μg/L (ppb), were lower than those reported in liver (261–6960 ppb wet weight), kidney (299–4370 ppb wet weight) and muscle (580–15,400 ppb wet weight) of bottlenose dolphins from other locations (Meador et al., 1999; Endo et al., 2005). MeHg in blood of CHS ($n = 17$) and IRL ($n = 8$) dolphins accounted for approximately 57% and 71%, respectively, of THg in this study. This ratio was lower than those in

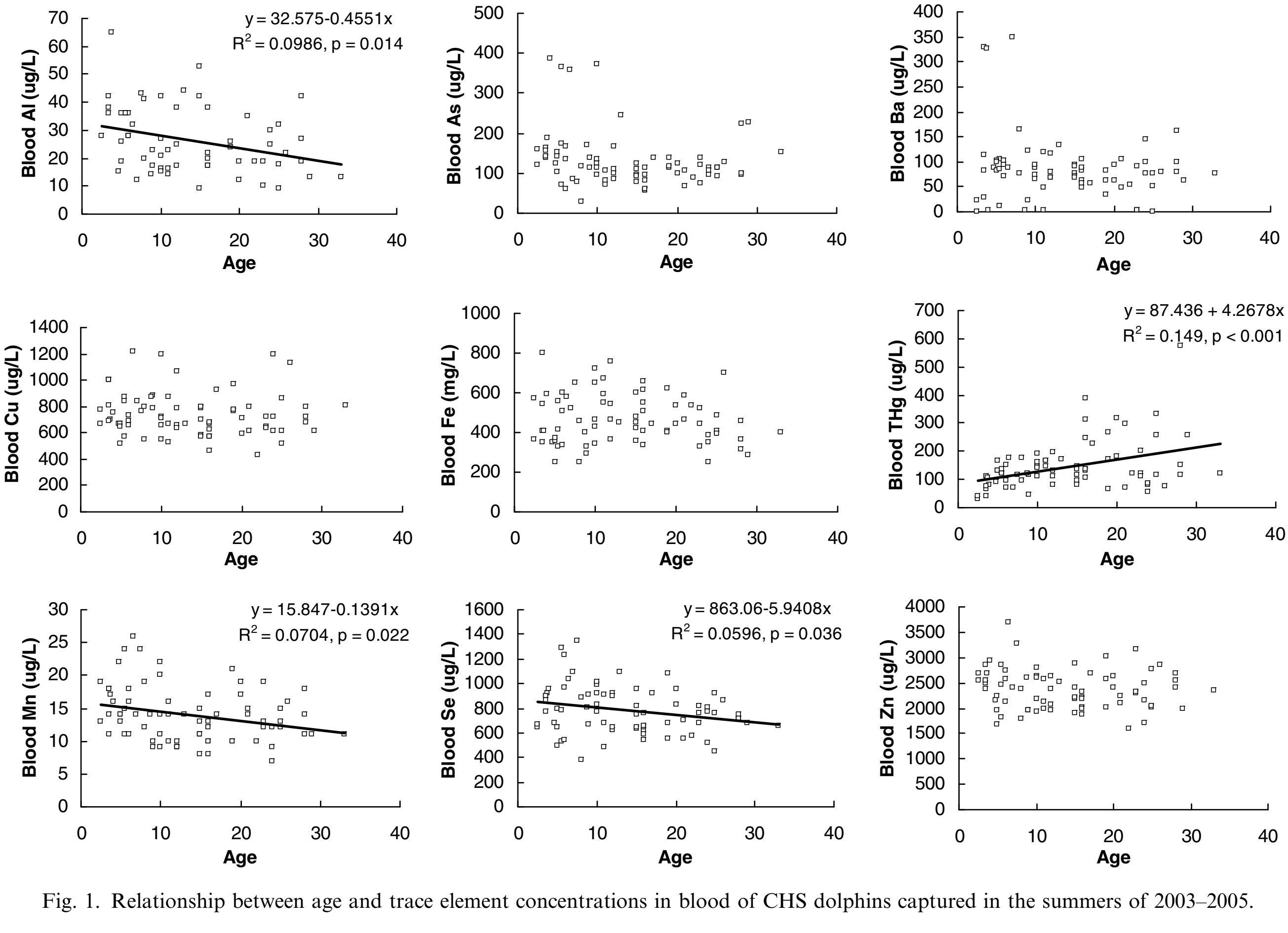

Fig. 1. Relationship between age and trace element concentrations in blood of CHS dolphins captured in the summers of 2003–2005.

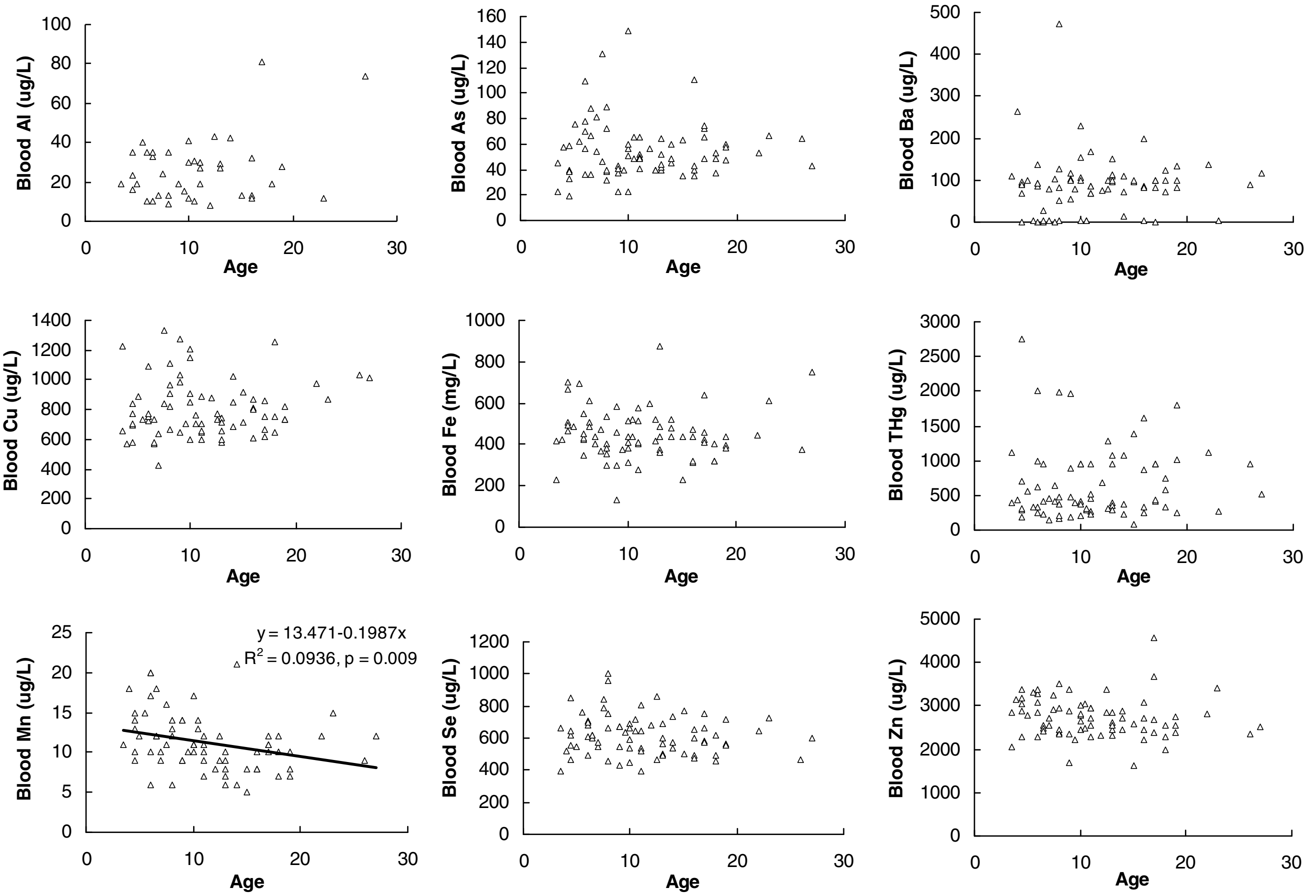

Fig. 2. Relationship between age and trace element concentrations in blood of IRL dolphins captured in the summers of 2003–2005.

blood of Beluga whales (*Delphinapterus leucas*) (Hyatt et al., 1999) but similar to that in muscle of bottlenose dolphins from Japan (Endo et al., 2005) and within the range of 9.7% to 100% in tissues of bottlenose dolphins in the Mediterranean Sea (Storelli and Marcotrigiano, 2000).

Biological and ecological factors affecting trace element concentrations in marine mammals have been reviewed in the past (Das et al., 2003; O'Hara et al., 2003). Samples collected from marine mammals inhabiting different geographic locations are expected to have different trace element concentrations based on dietary exposures (Das et al., 2003; O'Hara et al., 2003; Booth and Zeller, 2005; Brookens et al., 2007). In this study, approximately 1.3–2.3 times significantly higher As, Mn and Se levels in blood were found in CHS dolphins than those in IRL, while the IRL dolphins exhibited significantly higher Cu, THg and Zn levels (Tables 2 and 3). While the mean age of dolphins (Table 1) captured from CHS is greater than those from IRL, three times higher THg levels were found in IRL dolphins suggesting a higher dietary intake of Hg in this population. Atmospheric deposition is a significant source of Hg to the aquatic environment. A consistently higher wet deposition of Hg was reported in Florida, when compared to South Carolina (NADP, 2004; Fulkerson and Nnadi, 2006). Thus, it is likely that dolphins in the IRL and their prey species may be exposed to higher Hg levels. The dietary Hg exposure route from prey species may be the primary factor which contributes to the higher THg levels in IRL dolphins.

Several relationships between the blood concentrations of trace elements and the age of dolphin were observed in this study. The only positive correlation found was between the concentration of THg and age in CHS dolphins (Fig. 1). The accumulation pattern has been reported in blood and various tissues of cetaceans and pinnipeds (Lahaye et al., 2006; Brookens et al., 2007; Bryan et al., 2007). However, this accumulation pattern was not been found in IRL dolphins. The IRL site is an extensive ecosystem which extends 250 km and dolphins were captured in both the southern and northern ranges. It is possible that different dietary exposures may exist within the IRL which could have obscured finding similar correlations between THg and dolphin age. An inverse relationship was found between trace element concentrations of Al, Mn and Se and age in CHS dolphins and Mn and age in the IRL dolphins (Figs. 1 and 2). Another study reported that Al and Mn concentrations in the liver of Baikal seals (*Phoca sibirica*) were strongly influenced by age (Ciesielski et al., 2006). A negative relationship was also found between age and Mn levels in muscle of Baikal seals (Ikemoto et al., 2004b).

Bottlenose dolphins from CHS and IRL exhibited different distribution patterns of several trace elements between age and sex classes that are likely due to different dietary exposure. A significant difference in concentration was detected in CHS dolphins for Al, As, Ba, THg and Mn and in IRL dolphins for Fe, Mn and Zn. Some researchers suggested that there is little gender influence in the accumulation of Hg and Cd in seals and dolphins (Ikemoto et al., 2004a; Lahaye et al., 2006). However, the study by Ikemoto et al. (2004a,b) may be biased due to the relatively small sample sizes for male ($n = 7$) and female ($n = 13$) Baikal seals. In this study, we found significant differences among several trace element concentrations and between males and females. Significantly higher As levels were found in adult females compared to adult males from CHS, while the latter exhibited significantly higher Al and Ba concentrations (Fig. 3). Both adult males and females from CHS showed significantly higher THg levels than those in juveniles (Fig. 4) but not in IRL dolphins. Variability in blood composition and trace element contents may be in part possible explanations to the absence of relationship between THg and IRL dolphins' age. For IRL dolphins, significantly higher Fe levels were found in adult males than that in adult females (Fig. 4). Juvenile males and females showed significantly higher Mn and Zn concentrations than those in adults from IRL (Fig. 5). It is suggested that these higher essential element concentrations in juveniles may either due to high demands for a variety of biochemical processes or limited ability to excrete them (Das et al., 2003).

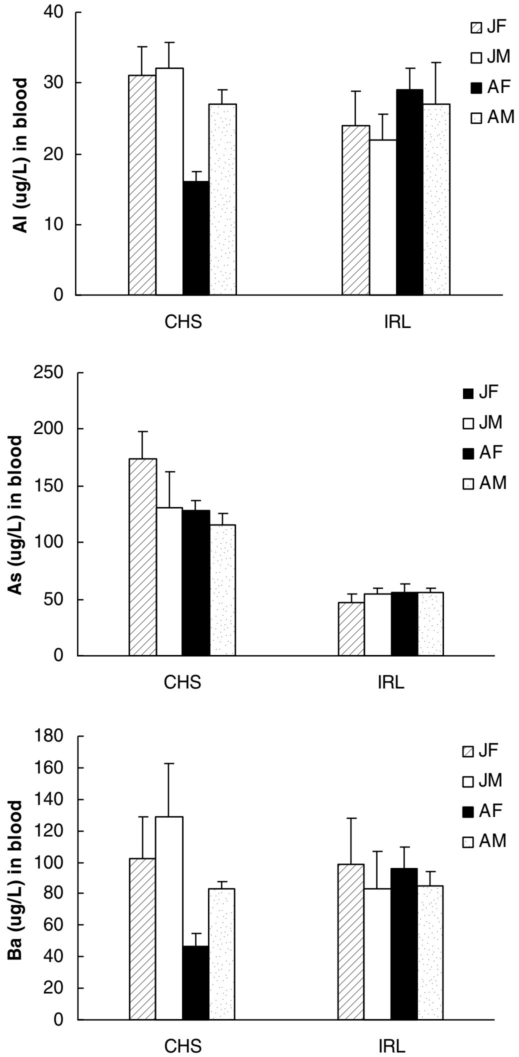

Fig. 3. Concentrations of Al, As and Ba (μg/L) in blood of bottlenose dolphins (JF: juvenile female; JM: juvenile male; AF: adult female; AM: adult male) captured from the CHS and IRL during the summers of 2003–2005.

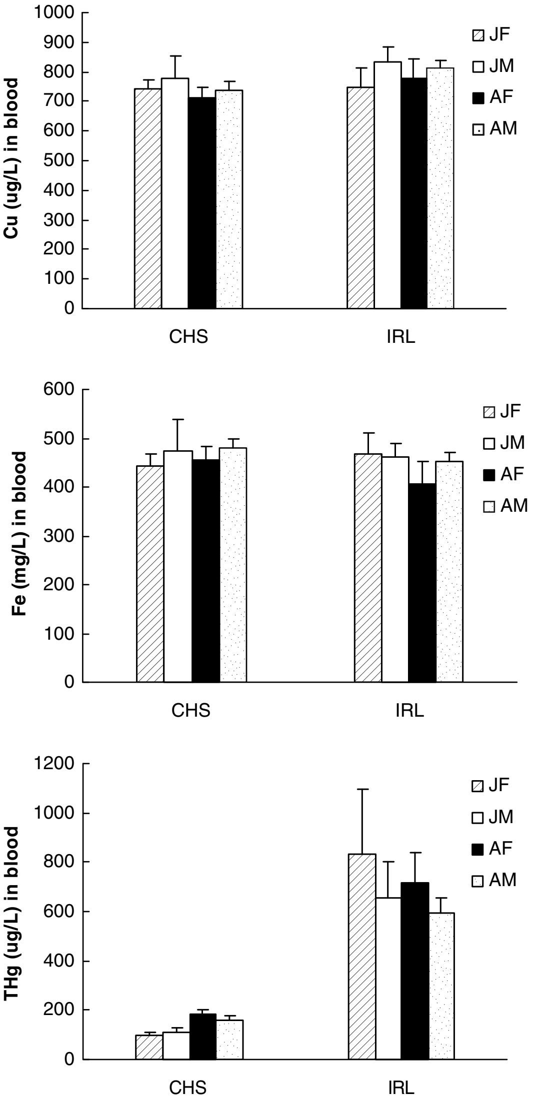

Fig. 4. Concentrations of Cu, THg (μg/L) and Fe (mg/L) in blood of bottlenose dolphins (JF: juvenile female; JM: juvenile male; AF: adult female; AM: adult male) captured from the CHS and IRL during the summers of 2003–2005.

Significant correlations between trace elements concentrations in the blood of CHS and IRL dolphins are shown in Table 4. Correlations found between trace elements in marine mammals have been documented previously. These correlations between trace elements were thought to occur as a result of interaction with both biotic and abiotic factors rather than an indication that the two elements are

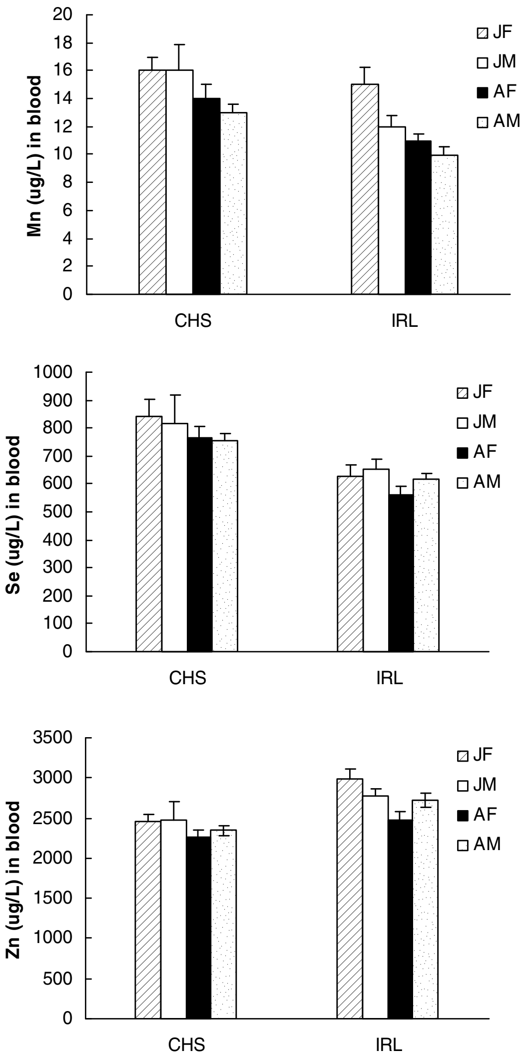

Fig. 5. Concentrations of Mn, Se and Zn (μg/L) in blood of bottlenose dolphins (JF: juvenile Female; JM: juvenile male; AF: adult female; AM: adult male) captured from the CHS and IRL during the summers of 2003–2005.

Table 4
Correlations found between trace elements in blood of free-ranging bottlenose dolphins sampled from Charleston, SC and Indian River Lagoon, FL during the summers of 2003–2005

| Trace element | CHS | IRL |
|---|---|---|
| Al | +Ba | |
| As | +Cu, +Se, +Zn | |
| Ba | –Fe | +Cu, –Fe, +THg, –Se, +V |
| Cu | –THg, +Se, +Zn | |
| Fe | +Se, +Zn | +Mn, +Se, +Zn |
| THg | | +Se |
| Mn | +Se, +Zn | +Se, +Zn |
| Se | +Zn | +Zn |

Underlined: <0.01; other: $p \leqslant 0.05$. Only significant relationships were reported by either + (positive) or – (negative).

directly related (Kunito et al., 2002; Ciesielski et al., 2006; Griesel et al., 2006). In general, significantly positive relationships were found between the following essential elements Cu, Fe, Mn, Se and Zn in the blood of dolphins. A significantly positive correlation involving blood levels of Cu, Se and Zn may possibly result from their functions as major antioxidant enzyme cofactors (Dehn et al., 2006; Griesel et al., 2006). A positive correlation ($p < 0.01$) between Mn and Zn in blood of dolphins has also been reported in liver and kidney of cetaceans and pinnipeds (Kunito et al., 2002; Roditi-Elasar et al., 2003; Ciesielski et al., 2006). Additionally, a positive relationship between Fe and Mn also suggests these essential elements are likely to be regulated in marine mammals as reported by Thompson (1990).

Only IRL dolphins exhibited a positive correlation ($p < 0.01$) between THg and Se. No correlation between blood THg and Se levels has been reported in the sperm whales (*P. macrocephalus*) (Nielsen et al., 2000). Other studies have documented a positive relationship between THg and Se in marine mammals due to the role of Se in protection against THg toxicity (Wagemann et al., 1998; Woshner et al., 2001; Bustamante et al., 2004; Dehn et al., 2005). The molar ratio between Se and THg approached one as the THg concentration exceeds 3 μg/g (~3000 μg/L to blood) in the liver of narwhal (*Monodon monoceros*) (Dietz et al., 2004). However, THg levels in blood of bottlenose dolphins were 5–10 times lower than that in the previous report. The ratio between Se and THg levels in whole blood was close to 1.5 in IRL and 7.2 in CHS dolphins, corresponding to a molar ratio 3.7 in IRL and 18.0 in CHS dolphins. The difference in the Se:THg molar ratio between CHS and IRL dolphins may reflect different Se and THg level in two sets of animals and warrant further investigation on the possibility of Se in protection against Hg toxicity in the blood of dolphins.

In conclusion, the present study provides data on baseline trace element concentrations in free-ranging bottlenose dolphins from the southeast US Atlantic Ocean. Whole blood has proven useful for measuring trace element levels in these dolphins. All trace element levels were within the range of other studies reported for cetaceans, pinnipeds and humans with the exception of Se. Essential elements, which are homeostatically regulated in marine mammals, showed less variation in blood concentrations than the non-essential elements. Our results provided evidence indicating the influence of age, sex and location on several trace elements in these free-ranging bottlenose dolphins. The only positive correlation found between the blood concentrations of trace elements and the dolphins' age was THg in CHS dolphins. Juvenile dolphins showed higher Mn levels than those that in adult dolphins. Higher As, Mn and Se blood levels were found in CHS dolphins than those in IRL, while the IRL dolphins exhibited significantly higher Cu, THg and Zn levels. Additional research determining trace element concentrations in other biological fluid (e.g., urine) and tissues (e.g., teeth) may contribute to further elucidating the role of trace elements and their body burdens in living dolphins and their potential relationship between trace element levels and health status in these dolphin populations. In addition, different chemical speciation of trace elements also warrants further examination to better identify their potential toxicity in marine mammals.

## Acknowledgements

This study was conducted under National Marine Fisheries Permit No. 998-1678, issued to Gregory Bossart, V.M.D., Ph.D., of Harbor Branch Oceanographic Institution (HBOI) in March 2003. The authors would like to thank the numerous researchers and marine mammal specialists representing many institutions including NOAA and HBOI who participated in the field study of dolphins at South Carolina and Florida. We specially thank the NOAA/NCCOS/CCEHBR Marine Ecotoxicology Branch for providing analytical instrumentation. A special thanks to Mr. Wayne McFee for conducting analyses of dental enamel to estimate age of dolphin. This study was supported through NOAA Fisheries Marine Mammal Health and Stranding Response Program, NOAA/NCCOS/CCEHBR and also the Florida Protect Wild Dolphins License Plate Fund. The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the funding agency. This publication does not constitute an endorsement of any commercial product or intend to be an opinion beyond scientific or other results obtained by the National Oceanic and Atmospheric Administration (NOAA). No reference shall be made to NOAA, or this publication furnished by NOAA, to any advertising or sales promotion which would indicate or imply that NOAA recommends or endorses any proprietary product mentioned herein, or which has as its purpose an interest to cause the advertised product to be used or purchased because of this publication. This research was performed while the author held a National Research Council Research Associateship Award at NOAA/NCCOS/CCEHBR.

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doi:10.1016/j.marpolbul.2007.10.030