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Comparison of Elements in Bottlenose Dolphins Stranded on the Beaches of Texas and Florida in the Gulf of Mexico over a One-Year Period

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Abstract. We analyzed tissue samples from bottlenose dolphins (*Tursiops truncatus*) that had stranded on beaches in Texas and Florida over a 1-year period starting in September 1991. The concentrations of 10 elements plus methyl mercury (MeHg) were determined in brain, kidney, and liver, and we examined these results for differences based upon age, site, sex, and tissue type. A strong inverse relationship between total mercury (Hg) and the percentage that was MeHg was found in liver, kidney, and brain tissue, presumably due to demethylation of MeHg. A threshold concentration was found for total Hg in brain tissue, indicating that most Hg was present as MeHg up to about 8 years of age. Increases in total Hg after this age were accompanied by an increase in the ratio of total Hg to MeHg, indicating demethylation. Strong relationships were found between total Hg in liver and age and between total Hg and selenium in liver, which have been observed before in many fish- and squid-eating marine mammals. The only difference based on sex of the animals was observed for MeHg, which was higher in females and contrary to the pattern often observed for organic contaminants. Several elements (copper, Hg, lead, zinc) exhibited intersite differences, which were not consistent. Bottlenose dolphin from Florida exhibited the highest levels of MeHg and total Hg, while animals from Texas exhibited the highest levels of lead, copper, and zinc. The essential elements copper and zinc were expected to be the same for the Texas and Florida animals; however, observed differences may indicate population differences in basic physiological levels, dietary intake, or health status.

The Marine Mammal Health and Stranding Response Program was established in 1992 through amendment to the Marine Mammal Protection Act. It gave the National Marine Fisheries Service authority to collect information and study marine mammals that strand on beaches of the United States. Each year approximately 3,000 marine mammal strandings are reported in the United States, of which over 1,000 are cetacean strandings. Bottlenose dolphins (*Tursiops truncatus*) make up the majority of cetacean strandings, which occur frequently on beaches in the Gulf of Mexico. Of all cetacean strandings reported for the years 1990–1995 in the United States, 56.3% (4.7%) (mean and standard deviation of yearly totals) were bottlenose dolphins, and 93% (1.6%) of these occurred in the southeastern region. Bottlenose dolphins are the most common cetacean along the Gulf of Mexico and southwest Atlantic coasts of the United States. Population abundance is estimated to be approximately 17,000 in the coastal areas of the Gulf of Mexico (Mullin *et al.* 1990).

We selected animals that had stranded on the beaches of Texas and Florida in the Gulf of Mexico over a 1-year period and were recovered by the Marine Mammal Stranding Network. Our sample represented about 7–9% of all bottlenose dolphin strandings for this time period from this region. While the causes of such strandings are not well known and will not be solved by analyzing tissue residues, these investigations allow us to characterize the range and occurrence of contaminant chemicals and help us determine if their levels are excessive. Additionally, characterization of essential elements as deficient or excessive may also be beneficial in assessing animal health. Comparison of these values with cause and effect determinations for other mammal species may be useful for gauging the degree of exposure and the potential for adverse effects in marine mammals. In this study we sought to analyze the concentrations of 10 elements in several types of tissue and then compare the results with respect to site, sex, age, and tissue type.

Materials and Methods

Animals

Tissues were taken from 44 bottlenose dolphins (*T. truncatus*) that had stranded on various beaches in Texas and Florida over a 1-year period from September 1991 through September 1992 (Table 1). Animals in this study ranged in age from a few months to over 40 years. Ages were estimated for 29 specimens from growth layers in teeth (Hohn *et al.*

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Table 1. Collection and age data for bottlenose dolphins

Sample Number	Sex	GL-Age	Hg-Age	GC-Age	Length (cm)	Location
						Texas
T1	F		45*	5–> 40	247	Nueces County
T2	F	20			242	Kleberg County
T3	M	3.8			224	Brazoria County
T4	F	11.6			230	Nueces County
T5	F	16			237	Jefferson County
T6	F	1			174	Brazoria County
T7	F	24			255	Galveston County
T8	M	43			247	Brazoria County
T9	F		0.35	0.5–1.5	170	Fulton Beach/Aransas
T10	M		0.06	0	101	Goose Island State Park/Aransas
T11	b		8.5	5–> 40	240	Espiritu Santo Bay/Calhoun
T12	F		0.16	0–0.3	113	Long Island/Calhoun
T13	M		3.1	3–6	211	Matagorda Bay/Calhoun
T14	M		0.25	0	96	CC Marina/Nueces
T15	M	12.5			263	Nueces County
T16	M		0.48	0	98	Rockport/Aransas
T17	F		0.16	0–0.3	115	Rockport/Aransas
T18	M	8			231	Grace Island/Aransas
T19	F		15.1	3–4	202	Espiritu Santo Bay/Calhoun
T20	M	16			250	Espiritu Santo Bay/Calhoun
T21	F		0.22	0–0.2	111	Magnolia Beach/Calhoun
T22	F	3			230	Matagorda Bay/Calhoun
T23	M	1			177	Sand Point Beach/Calhoun
T24	M	16			253	Matagorda Bay/Calhoun
T25	M	8			234	Espiritu Santo Bay/Calhoun
T26	M		8.9	7–> 35	248	Port O'Connor/Calhoun
T27	M	10			235	Espiritu Santo Bay/Calhoun
T28	M		0.97	2– 4	195	Galveston County
T29	F		16.4	5–>40	244	Brazoria County
T30	F		0.04	0	93	Blackberry Island
						Florida
F1	F	11			239	Sarasota
F2	F	3			208	Manasota Beach
F3	M	8			220	Manasota Beach
F4	M	2			195	Anna Maria Island
F5	M	4			210	Manatee County
F6	M	25			226	Manatee County
F7	M	1.3			176	Sarasota County
F8	M	8			226	Manatee County
F9	F	3.3			214	Sarasota County
F10	M	1.3			192	Sarasota County
F11	F	36			246	Longboat key
F12	M	4			233	Sarasota County
F13	F	13			246	Siesta Key
F14	F	9			236	Sarasota County

* Value outside regression, set to 45 years (≈ maximum age). Sex not determined for T11. Sequential numbers used for individuals from Texas (T) Florida (F). GL-Age is age estimated from dental growth layers; Hg-Age is age predicted from Hg in liver; GC-Age is age predicted from growth curves

1989) and are termed GL-age. Teeth were not available for the remaining 15 specimens; however, their ages were predicted with a regression equation (described below) that related age and liver concentration of mercury (Hg-age).

Analytical Procedures

Tissues (liver, kidney, brain, gonad [male], blubber, and stomach contents) were prepared with high-purity nitric acid (SeaStar Chemicals, Sidney, British Columbia) and digested in a microwave system (CEM Corp., Matthews, NC). We analyzed the digests for 10 elements: aluminum (Al), arsenic (As), chromium (Cr), cadmium (Cd), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), and zinc (Zn). Graphite furnace atomic absorption spectroscopy (GFAAS) with Zeeman background correction (Perkin-Elmer Model 5100) was used to analyze As, Pb, and Se. A five-point standard calibration curve was generated using single-element Standard Reference Materials (SRMs) (National Institute of Standards and Technology [NIST]). Inductively coupled plasma spectrophotometry (ICP, Perkin-Elmer Optima 3000) was used to analyze Al, Cr, Ni, and Zn. We analyzed Cd and Cu by both GFAAS and ICP methods.

We measured Hg with cold-vapor atomic absorption spectroscopy (CVAAS) coupled with a flow-injection analysis system (Perkin-Elmer FIAS 200) as described in Meador *et al.* (1993). Methyl Hg (MeHg) was analyzed by Brooks Rand, LTD (Seattle, WA). MeHg samples were digested and ethylated to form volatile methyl ethylmercury. This compound was then chromatographed by cryogenic gas chromatography, pyrolytically broken down to elemental Hg, and measured using a cold vapor atomic fluorescence detector. A certified reference material (CRM) MeHg, DORM (National Research Council of Canada [NRCC]), was analyzed to determine the efficiency of recovery (Table 2).

Lipid content of liver was determined gravimetrically with a methylene chloride extract of the tissue (Meador *et al.* 1993). This method was tested on other marine mammal samples in our laboratory and was shown to produce similar results to the method described by Hanson and Olley (1963). Dry-wet weight ratios were obtained by comparing wet and dry weights before and after drying in a Model 12XL Freeze Dryer (Virtis™, Gardner, NY). Tissue concentrations are based on dry weights unless stated otherwise and are reported in μmol g^{-1} or μg g^{-1} (ppm). To obtain wet weight values, multiply each concentration by the D/W factor (Table 3).

We analyzed five to 10 separate digests for each of five different CRMs (DOLT-2, DORM-1, LUTS-1, and TORT-1 [NRCC] and 1566a [NIST]) concurrently and randomly with our samples to assess analytical error. Including CRMs was a normal part of our protocol to address matrix interference and technique variability; this also helped us better determine recovery rates, accuracy, and precision (Table 2). Raw concentration values were corrected with the results for CRMs (Table 2). To determine precision, a coefficient of variation (CV) was calculated for each CRM that was based on the mean and SD of the five to 10 measured concentrations (Table 2). Only DORM-1 and DOLT-2 were used for mercury (CVAAS) analyses because the concentration of Hg in all other CRMs was close to our limit of detection. The limit of detection (equal to three times the standard deviation of blank samples) for each element is shown in Table 2.

Table 2. Elements analyzed, methods, and quality assurance

Element	Method	LOD (μg/g)	% Cert.		CV	n
Aluminum	ICP	25	95	(4)	4#	5
Arsenic	GFAAS	1.6	104	(24)	17 (8)	38
Cadmium	GFAAS	0.007	109	(17)	12 (6)	21
Cadmium	ICP	1.0	100	(3)	5 (6)	22
Chromium	ICP	2.4	83	(17)	21 (7)	22
Copper	GFAAS	0.1	111	(14)	9 (5)	31
Copper	ICP	5.0	100	(8)	6 (4)	28
Mercury	CVAAS	0.2	120	(5)	15 (10)	18
Methyl mercury	GC/CVAFS	0.005	99.6	(1)	3#	10
Nickel	ICP	1.2	100	(27)	26 (7)	10
Lead	GFAAS	0.1	105	(27)	20 (4)	30
Selenium	GFAAS	0.1	97	(37)	15 (4)	38
Zinc	ICP	4.2	101	(8)	4 (2)	28

Methods were graphic furnace atomic absorption spectrophotometry (GFAAS), inductively coupled plasma spectroscopy (ICP), cold-vapor AAS (CVAAS), gas chromatography/cold vapor atomic fluorescence spectroscopy (GC/CVAFS). % Cert is the mean (SD) for all CRM ratios (measured value over certified value × 100) and was used to correct measured concentrations. CV is the mean (SD) of the coefficients of variation for the CRMs used for each element. Each CV represents up to five CRMs (DOLT-2, DORM-1, LUTS-1, TORT-1, 1566a) with approximately 5–10 analyses per CRM. # indicates only one CRM used for this element. n is the total number of CRM samples. LOD is the limit of detection

Statistical Analyses

Chemical contaminant concentrations are usually lognormally distributed (Gilbert 1987). The Kolmogorov-Smirnov test, a Chi-square test, and probability plotting were used for each element-tissue combination (*e.g.*, Hg in liver) to evaluate the goodness of fit of observed data to the hypothesized distribution. For those data that were lognormally distributed, the usual mean ($\bar{x}$) and variance (δ^2) are biased estimators, hence a correction is required. We used the following equations (Gilbert 1987) for computing the mean and variance for chemical concentrations that were lognormally distributed. This estimator of the mean ($\hat{\mu}$) was used only when the coefficient of variation was greater than 1.2 (Gilbert 1987).

$$\hat{\mu} = \exp\left(\bar{y} + \frac{s_y^2}{2}\right) \quad \text{(Eq. 1)}$$

$$\hat{\delta}^2 = \hat{\mu}^2[\exp(s_y^2) - 1] \quad \text{(Eq. 2)}$$

Because many of the tissue concentrations contained samples that were below the limit of detection, making the data set ''left-censored,'' a correction was necessary for calculation of the mean and standard deviation. We applied Cohen's procedure (a maximum likelihood method) (Cohen 1959, 1961), as described in Gilbert (1987) for the lognormal distribution, to obtain estimates of the mean and variance of these left-censored data sets. Estimating the mean and variance of a left-censored data set with this maximum likelihood method has been shown to reduce the bias in estimating the mean and variance of the data (Gilbert 1987; Newman *et al.* 1989). A comparison of mean and variance values with and without the correction showed substantial differences, especially when the number of below detection values was high.

Linear and nonlinear regression analysis was used to explore relationships in the data. The nonlinear model used for exploring the relationship between MeHg and Hg was an exponential decay function:

$$y = ae^{(nx)} \quad \text{(Eq. 3)}$$

where y was the dependent variable (% MeHg), x was the independent variable (total Hg), and the coefficients a and n were estimated by an iterative nonlinear algorithm. Hockey-stick (also called breakpoint) regression was performed to determine the threshold value when the data displayed a distinct increase above a baseline concentration. The residuals for all regressions (linear and nonlinear) were checked to determine if the assumptions of the model were met.

The inverse prediction method (Zar 1984) was used to predict age for 15 of the 44 animals based on total mercury content in liver. This method is used to predict the value of an independent variable (X = age) with the dependent variable (Y = total mercury). Simply reversing the variables from a standard regression is inappropriate due to the assumption that X is measured without error. Eight of the 15 animals for which age was predicted were under 1 year of age and predictions for animals less than 1 year old were much less variable than for older animals.

Indicator-variable regression analysis was used to detect differences between the Florida and Texas populations for tissue mercury and age. The model was

$$\log_{10} \text{Hg} = \beta_0 + \beta_1 X + \beta_2 I + \beta_3 IX \quad \text{(Eq. 4)}$$

where X = $\log_{10}$ age (covariate); I = indicator variable for location (0 or 1 for Florida or Texas, respectively); IX = interaction term between age and location; βs were coefficients fitted by the regression. If the coefficient of the interaction term (β_3) was significant, it was concluded that the slopes between locations (Florida or Texas) were significantly different. Only age-matched animals were selected for the regression to

Table 3. Mean, standard deviation, and range of concentrations of elements in bottlenose dolphins in 1991 and 1992 strandings

	As					Cd					Cu				
	Mean	SD	Range	BD	n	Mean	SD	Range	BD	n	Mean	SD	Range	BD	n
Texas															
Brain	—	—	—	—	0	0.010	0.014	0.005–0.035	3	10	10	3.0	5.6–12	0	10
Liver	1.3	0.3	1.6–2.0	19	23	0.32	0.18	0.03–0.7	8	14	71	55	7.9–217	0	29
Kidney	1.5	0.6	1.6–2.8	15	23	1.9	1.4	1.1–4.2	11	30	48	40	10–107	0	30
Stomach	—	—	—	9	9	0.46	0.09	0.005–0.11	1	8	26	10	16–33	5	8
Florida															
Brain	—	—	—	—	0	0.004	0.006	0.005–0.019	1	12	12	4.0	7.9–16	1	12
Liver	2.0	0.9	1.7–3.1	1	10	1.6	—	—	10	11	25	6.3	16–36	0	13
Kidney	—	—	—	—	0	4.4	3.1	1.0–5.2	5	13	16	5.2	11–30	0	13
Gonad	—	—	—	4	4	0.10	0.20	0.01–0.3	0	4	6.2	0.4	5.9–6.8	0	4

	meHg					Hg					Pb				
	Mean	SD	Range	BD	n	Mean	SD	Range	BD	n	Mean	SD	Range	BD	n
Texas															
Brain	0.9	0.5	0.4–1.7	0	10	5.4	13.3	0.4–18	0	10	—	—	—	10	10
Liver	6.0	4.9	0.9–23	0	24	212	311	8.3–1404	0	30	0.30	0.50	0.12–2.6	11	30
Kidney	4.5	2.6	1.3–10.4	0	23	33	65	1.0–89	0	29	0.17	0.15	0.1–1.6	11	30
Stomach	2.2	2.6	0.2–4.0	0	9	1.4	1.3	0.3–4.0	0	8	1.70	5.20	0.17–5.7	1	9
Florida															
Brain	2.9	3.3	0.3–5.9	0	11	5.2	9.7	0.23–20	0	10	—	—	—	12	12
Liver	11.0	8.4	2.5–24	0	14	304	547	18–1312	0	13	0.09	0.07	0.14–0.20	10	13
Kidney	9.9	8.1	1.4–19	0	13	68	60	11.2–110	0	12	0.08	0.03	0.13–0.14	11	13
Gonad	2.3	2.1	0.9–3.7	0	4	2.9	2.6	0.9–5.1	0	4	0.16	—	—	3	4
Blubber*	0.6	0.2	0.4–0.7	0	4	—	—	—	—	0	—	—	—	—	0

	Se					Zn					D/W		
	Mean	SD	Range	BD	n	Mean	SD	Range	BD	n	Mean	SD	n
Texas													
Brain	2.8	2.3	1.0–7.8	0	10	57	17	33–73	0	10	0.22	0.04	10
Liver	124	406	2–692	0	30	290	210	80–748	0	12	0.26	0.04	31
Kidney	10.1	6.4	2.4–28	0	29	96	20	65–125	0	10	0.22	0.03	31
Stomach	2.3	1.0	0.6–3.8	0	9	68	31	20–121	0	9	0.23	0.07	8
Florida													
Brain	2.4	2.2	0.8–8.9	0	12	71	11	70–94	0	11	0.20	0.04	10
Liver	65	69	7.7–195	0	13	118	22	97–167	0	12	0.29	0.03	14
Kidney	22	4.6	7.8–31	0	13	78	2.2	62–93	0	13	0.23	0.01	13
Gonad	2.5	0.4	2.1–3.1	0	4	83	5.0	77–89	0	4	0.23	0.05	4

Reported in µg/g dry weight. n = total number of samples analyzed and BD is the number of samples below detection. D/W = ratio of dry to wet weight, blubber not analyzed. Mean with below detection values (BD) determined with maximum likelihood method for censored data. Means with no bd values determined with equations 1 and 2. * blubber in wet weight. Stomach is stomach contents

avoid the overrepresentation of young animals from Texas. All statistical assumptions for this test were met.

Pearson correlation coefficients were determined between elements in tissue and between elements and age. Log_{10}-transformed concentrations were used for all correlations and regressions. Correlations involving total Hg and age were conducted only with animals that were aged from dental growth layers (GL-age). When one or two points had an undue influence on the significance of the correlation, we considered the linear correlation spurious. Coefficients of variation (SD/mean · 100) were calculated for the CRMs. The statistical software packages Statistica (Statsoft, Tulsa, OK) and JMP (SAS, Cary, NC) were used to analyze the data.

Results

As expected, concentrations of metals were variable between tissue types and between individuals (Table 3). While the essential metals (*e.g.*, Cu and Zn) were relatively uniform in concentration, the contaminant metals (Hg, Cd, Pb) were quite variable, usually correlating with age. Values for Al, Cr, and Ni (not reported), which were analyzed with the same frequency as that listed for other tissues, were below detection in most analyses. All concentration data were lognormally distributed, except Cu and Zn in brain tissue, which were normally distributed.

The relationship between mercury in liver and age was very strong (Figure 1a) and less so for Hg in kidney and age (Figure 1b). The regression for all liver data produced a coefficient of determination (r^2) of 0.80. Total Hg in brain also tended to increase with age (Pearson correlation r = 0.65). While a trend of higher total Hg in the Florida animals was present for liver, kidney, and brain as a function of age, indicator-variable regression analysis indicated that only

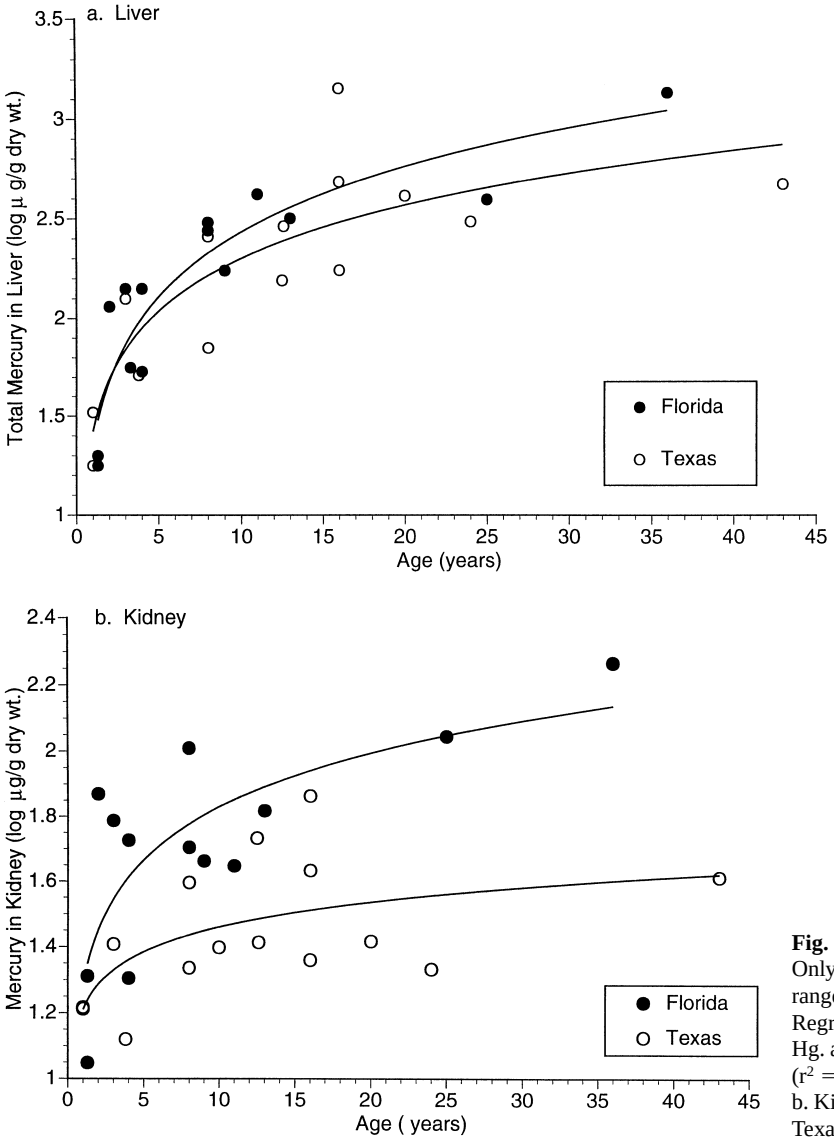

Fig. 1. Total mercury in tissues categorized by location. Only GL-ages (Table 1) that fell within a matched age range for each location (Florida and Texas) were plotted. Regressions were determined with log values, Y is log total Hg. a. Liver. Florida: $Y = 1.36 + 1.08 \cdot \log age$ ($r^2 = 0.83$), Texas: $Y = 1.42 + 0.88 \cdot \log age$ ($r^2 = 0.71$). b. Kidney. Florida: $Y = 1.29 + 0.54 \cdot \log age$ ($r^2 = 0.56$), Texas: $Y = 1.2 + 0.25 \cdot \log age$ ($r^2 = 0.36$)

kidney displayed intersite differences (Figure 1b) that were close to being statistically significant. The following p values were observed for the coefficient of the interaction term from the regression: liver $p = 0.31$; kidney, $p = 0.07$, brain $p = 0.52$.

The results for MeHg supported the trend of higher concentrations in animals stranded on Florida beaches (Figure 2); however the sample sizes were too small for statistical testing. MeHg in liver versus age was the only relationship that displayed differences based on sex of the animal. Normalizing MeHg concentration to lipid content in liver produced no discernible patterns with age or location. Lipid content in liver (data not shown) was not strongly correlated with any other element concentration or with age.

When total Hg concentrations were examined by sex, only brain tissue showed a large difference (mean [SD] Hg: male = 1.8 [1.1] µg/g, female = 6.7 [7.8] µg/g). Mean (SD) Hg in liver was slightly higher in females (263 [342] µg/g) than that for males (203 [300] µg/g). Because the median age for females (10 years) was much higher than the median age observed for males (4 years) and mercury accumulates over time, comparisons of Hg concentrations in tissues must be normalized to age. When total Hg in brain was plotted against age, no differences were observed for males and females.

Because of the strong relationship between age and Hg in liver, ages were predicted with the inverse prediction method described above for animals whose teeth were not examined for growth layers (Table 1). We then compared those predicted ages to a reasonable range of ages for the same specimens using results from growth curves (GC) fitted to length-at-age data (Fernandez and Hohn 1998) (Table 1). Of the 15 predicted ages, nine animals were less than 1 year old, which was easily verified by their length. For individuals older than 5 years, length and age are very poorly correlated (Read *et al.* 1993). Most of the predicted ages from the two methods (Hg-age and GC-age) were consistent; however, notable exceptions include a very small animal with Hg loads suggesting an age closer to 0.5 yr (T16), a specimen with relatively low Hg relative to a reasonable age based on length (T28), and a female with a very high Hg for her length (T19).

There was also a strong correlation between Hg in liver versus kidney (Figure 3a), indicating proportional accumula-

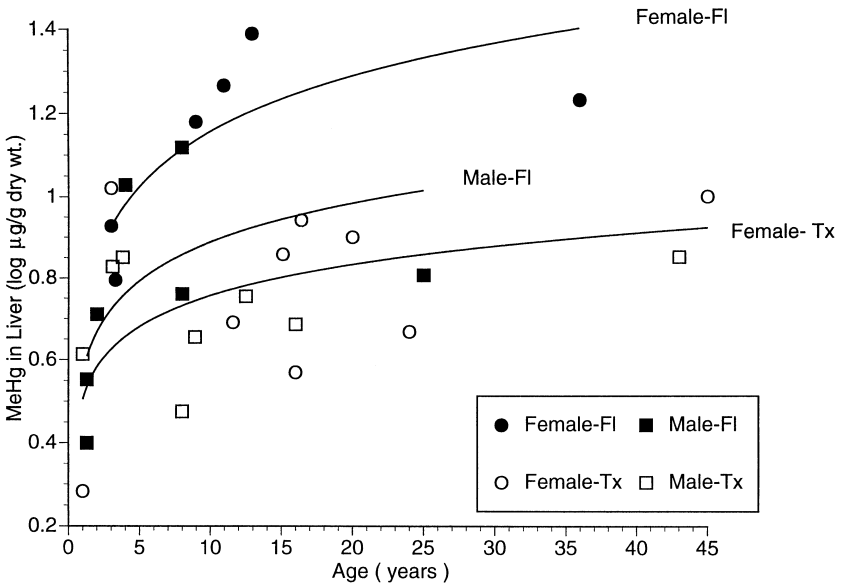

Fig. 2. Methyl mercury in liver versus age. Plot showing site and sex differences in log MeHg as a function of age in years. Only GL- and Hg- ages (Table 1) that fell within a matched age range for each location (Florida and Texas) were plotted

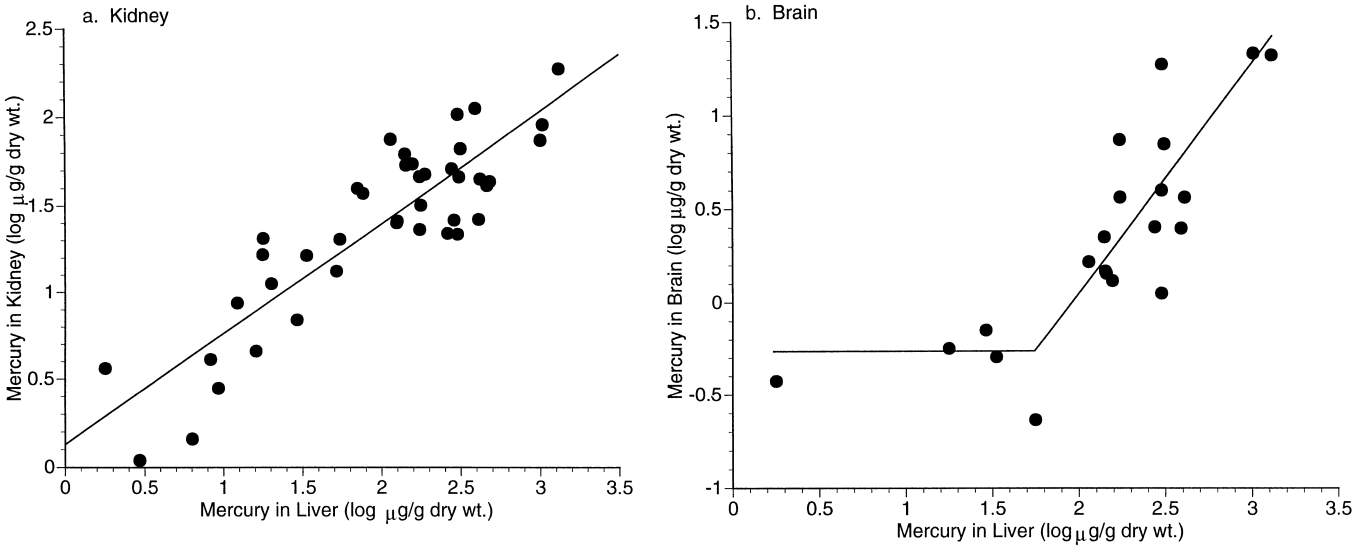

Fig. 3. Total mercury in liver versus kidney and brain. a. Kidney. b. Brain. All data combined. Regression for kidney: $Y = 0.13 + 0.63 \cdot X$ ($r^2 = 0.77$). Hockey-stick regression model for brain: $r^2 = 0.75$, threshold = 56 µg/g

tion. For any given animal, Hg in liver was ≈4.3 times higher than that found in kidney. The relationship between Hg in liver and Hg in brain displayed a threshold indicating that brain Hg did not increase until liver Hg exceeded 56 µg/g dry weight (Figure 3b). Hockey-stick regression determined the threshold and 95% confidence interval (CI) to be 56 (9–89) µg/g and the background concentration to be 0.5 µg/g for this age range. As observed many times before, Hg and Se in liver were very closely associated ($r^2 = 0.92$) in a one to one molar ratio spanning almost three orders of magnitude (Figure 4).

The relationship between MeHg and total mercury in the tissues of bottlenose dolphin is shown in Figure 5. The function used to describe this relationship displayed a high r^2 and indicates that the proportion of MeHg relative to the amount of total Hg declined exponentially, probably due to demethylation. The ratio Hg:MeHg versus age showed that there was a continuous decline in the percentage MeHg as total Hg increased in liver (Figure 6). In brain, a nearly constant ratio was observed for young animals, while there was a distinct threshold for older animals (Figure 6). Hockey-stick regression determined the threshold (95% CI) for brain to be 7.7 (2.3–13) years of age. The pattern for kidney was less strong.

While bottlenose dolphins from Florida tended to have more Hg in their tissues than animals from Texas, the reverse was true for Pb in tissues (Figures 7a–b). Both liver and kidney exhibited substantial intersite differences in Pb content, over all ages, with most of the Florida samples being below the limit of detection.

The trend of decreasing copper in liver with increasing age has been noted before and is confirmed here. Even though there was an insufficient number of young animals from Florida to show the trend of declining liver copper with age, the copper

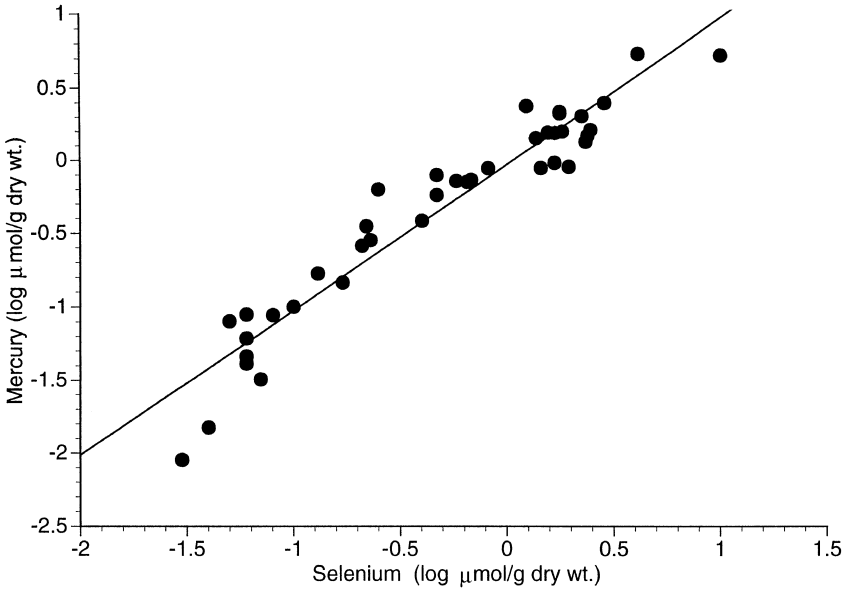

Fig. 4. Total mercury versus selenium in liver of bottlenose dolphin. Texas and Florida data combined. $Y = -0.04 + 0.99 \cdot X$ ($r^2 = 0.92$)

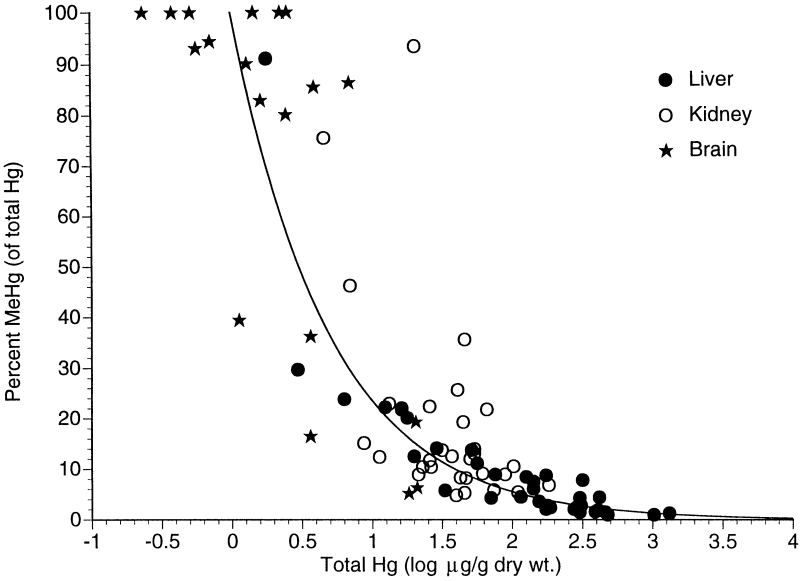

Fig. 5. Total mercury versus percent MeHg of total mercury. Methyl mercury as a percentage of total mercury in liver, kidney, and brain of bottlenose dolphin. Texas and Florida sites combined. $Y = 99.6 \cdot e^{(-1.45 \cdot X)}$ ($r^2 = 0.92$)

content in the liver of animals from Florida was substantially lower for a given age (Figure 8a). Copper in kidney was also lower for the Florida animals and there was no inverse relationship between copper and age for either group (Figure 8b). Similarly, zinc in liver (Figure 9) and kidney (Table 3) were lower in animals from Florida. There was no association between zinc in liver or kidney and age.

Additional correlations for various pair combinations of element concentrations in tissue are shown in Table 4. None of the correlations displayed in the figures are listed in Table 4.

Discussion

Strong associations between elements and between elements and age were observed in this study and many of these patterns (*e.g.*, Hg-Se in liver) have been observed before in other studies of cetaceans. Below we discuss the significance of the observed patterns for mercury, lead, copper, and zinc. Extensive comparisons of mean concentrations between studies are not provided because it was felt that such comparisons are often not informative, especially when the element varies strongly with age.

Mercury

Mercury concentrations in several tissues were highly correlated with age and because the association is linear (based on a log-log plot), a relatively constant input over time can be assumed. The presumed source of mercury to bottlenose dolphin is the diet. Along the coast of Texas bottlenose dolphins feed in about equal proportions on bottom-dwelling fish, shrimp, and squid while along the Gulf coast of Florida the

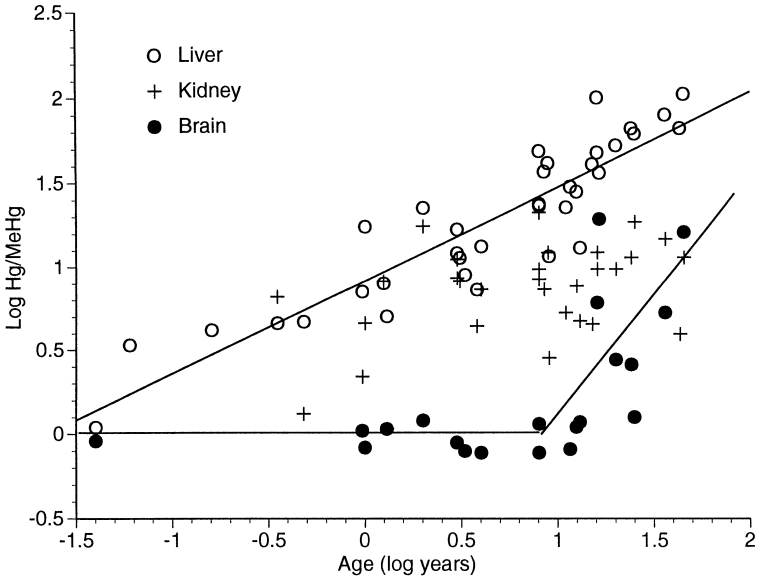

Fig. 6. Ratio of total mercury to methyl mercury versus age for liver, kidney, and brain. Regression for liver: $Y = 0.91 + 0.55 \cdot X$ ($r^2 = 0.82$). Kidney $r^2 = 0.22$. Hockey-stick regression for brain, $r^2 = 0.56$, threshold = 7.7 years

predominant prey types are bottom dwelling fish and mullet (Barros and Odell 1990). Recent studies have shown that Hg in fish and invertebrates (May *et al.* 1987; Bloom 1992) occurs mainly in the methyl form, which is readily assimilated when ingested (Venugopal and Luckey 1978).

The only sex-dependent difference detected was for MeHg in liver. This pattern of higher MeHg concentrations in females versus males is the opposite of that usually described for chlorinated organic contaminants (*e.g.*, PCBs) (Muir *et al.* 1988; Kuehl and Haebler 1995). Sex-based differences observed for organic contaminants are presumably due to differences in lipid cycling (*e.g.*, gestation, lactation) in the female (Gaskin *et al.* 1971; Ridgway and Reddy 1995), which may also control MeHg accumulation due to its hydrophobic nature. However, in our study there was no correlation between the lipid content of liver and MeHg. We found no age-specific difference for total Hg concentrations in liver, kidney, or brain between males and females. Kuehl *et al.* (1994) reported that the mean Hg in the liver of an adult female bottlenose dolphin was more than twice that measured for adult males, although it is not clear if these values were statistically different.

The one-to-one molar association of Hg and Se in liver has been demonstrated in many marine mammal species (Itano *et al.* 1984; Pelletier 1985; Meador *et al.* 1993), and has been proposed to be a result of the demethylation of MeHg (Iwata *et al.* 1982). Iwata *et al.* (1982) suggested that selenium alone was directly involved in demethylation; however only the reduced form (selenide) was effective. Further study by Iwata also determined that selenite was effective in demethylation when reducing agents such as glutathione or L-cysteine were present. A few studies have shown that MeHg is much less toxic when accompanied by an equivalent dose of selenium (Ganther *et al.* 1972; Ohi *et al.* 1980).

For bottlenose dolphin in this study, the Hg-Se association was evident even at very low concentrations of each component (Figure 4), which is contrary to the 100 µg/g threshold reported for Hg by Palmisano *et al.* (1995). The threshold for coaccumulation of Se and Hg in our study appeared to occur at about 0.03 µmol/g. For Se, this is ≈ 2.3 µg Se/g dry weight, which is comparable to Se concentrations in the liver of other marine mammals with low Hg concentrations (Mackey *et al.* 1996). This concentration of Se may be the physiological level for bottlenose dolphin, so it would show no increase until Hg accumulated to this concentration and higher.

Our study supports the previous finding that when total Hg in liver is low, a high percent of that Hg is present as MeHg, and as total Hg increases, the percentage that is MeHg declines exponentially (Falconer *et al.* 1983; Julshamn *et al.* 1987; Meador *et al.* 1993). Even though the percentage of MeHg may have been low in liver, its concentration often reached 10–20 µg/g (dry weight). The results presented here extend this association to kidney and brain tissue, which also show the same pattern of a high percentage MeHg when total Hg is low (Figures 5 and 6) and a reduction in the percentage of MeHg as total Hg increases, indicating demethylation.

It is noteworthy that at concentrations up to 1 µg/g most of the mercury present in brain tissue was in the more toxic methyl form, occurring in the youngest animals up to about 10 years of age. Even though older animals contained up to 20 µg/g total Hg in brain tissue, the percentage that was MeHg was relatively low (Figure 5). Individuals older than 10 years show an abrupt increase in the ratio of Hg/MeHg in the brain (corresponding to a decline in the percentage MeHg of total Hg), which may be the result of a demethylation reaction in this tissue (Figure 6). Both liver and kidney displayed an essentially continuous decline in percentage MeHg over all ages.

Methyl mercury is readily absorbed and is highly toxic to mammals (Venugopal and Luckey 1978). Several studies of small mammals have shown the lethal level of MeHg in brain to be in the range of 12–30 µg/g wet weight (≈60–150 µg/g dry weight) (Wren 1986) and 2–10 µg/g dry weight to be the range for sublethal effects, such as neurological dysfunction (Fehling *et al.* 1975; Arito *et al.* 1982). Concentrations of MeHg in bottlenose dolphin brain were frequently in the 1–3 µg/g (dry weight) range and up to 5.9 µg/g.

The consequences of high Hg concentrations in the liver of these bottlenose dolphin are unknown. One recent study of bottlenose dolphin that had stranded in Florida found excessive

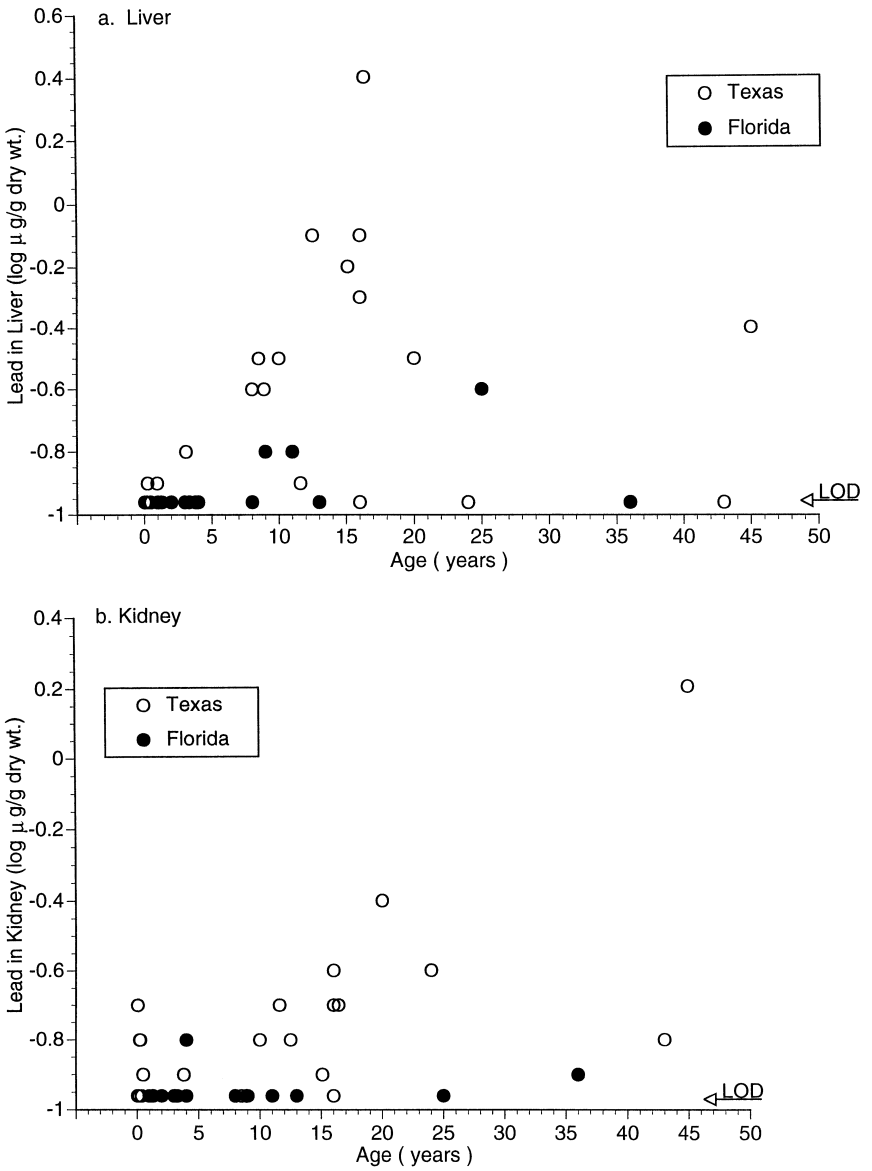

Fig. 7. Lead in bottlenose dolphin tissue versus age. (a) Liver. (b) Kidney. LOD is limit of detection for lead in tissue

lipofuscin pigment (a residue of damaged subcellular membranes in lysosomes) in the liver when Hg was greater than 234 µg/g (dry weight) (Rawson *et al.* 1993). Several of these animals were shown to have active liver disease. In our study, 33% of the bottlenose dolphin sampled had liver Hg levels higher than 234 µg/g. Further research on lipofuscin, which is known to increase with age (Hinton and Grasso 1993), as an indicator of Hg contamination would be valuable for monitoring and assessing effects. Unfortunately, age may be a potentially confounding factor because of the correlation between age and liver Hg.

The trend of higher concentrations of Hg in the Florida animals was likely due to higher exposure concentrations. Geographical differences in environmental concentrations could be due to natural or anthropogenic sources and may result in differential prey concentrations. Another factor is the type of prey consumed by dolphins in each area, which may contain varying levels of contaminants. Testing this hypothesis would require analysis of prey items from each geographic location.

Lead

Our results for lead in liver, which did not vary with sex and were not correlated with age, were more than an order of magnitude lower compared to values observed in bottlenose dolphins from the Atlantic (Kuehl *et al.* 1994), but relatively similar to values for individuals of this species that had stranded on beaches in the Gulf of Mexico (Kuehl and Haebler 1995). Contrary to the pattern observed for mercury, bottlenose dolphins from Texas contained higher concentrations of lead in liver and kidney than dolphins from Florida (Figure 7). While most of the animals from Florida exhibited below-detection concentrations of lead, many of the older individuals from Texas contained detectable levels. As noted above for Hg, the intersite differences observed for Pb in liver and kidney may have been due to higher concentrations in prey species that had been exposed to relatively high environmental concentrations.

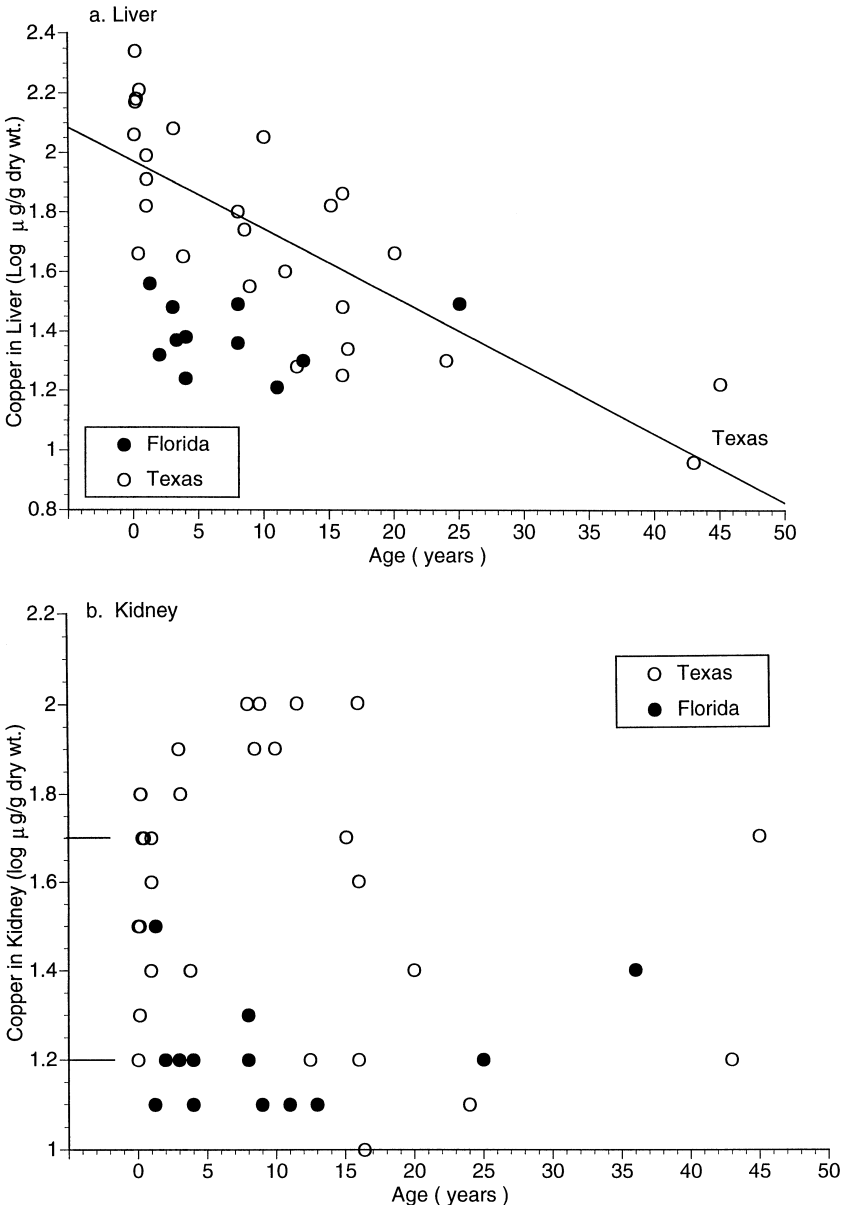

Fig. 8. Copper in bottlenose dolphin tissue versus age. (a) Liver. Regression for liver from Texas animals: $Y = 1.91 - 0.33 \cdot X$ ($r^2 = 0.60$). (b) Kidney. Horizontal lines show mean kidney value for each location

Copper and Zinc

Studies of marine mammals have reported high concentrations of copper in the liver of young marine mammals that declines as animals age (Law *et al.* 1992; Wood and Van Vleet 1996), which is common and normal for vertebrates (Davis and Mertz 1987). In this study, the decreasing trend of copper in liver was evident for the animals from Texas, but not for those from Florida primarily due to the lack of young animals. For both kidney and liver, copper and zinc concentrations were higher in individuals from Texas, compared to those from Florida for a given age (Figures 8 and 9). Normal concentrations of copper and zinc in liver and kidney are not known for this species.

Variable concentrations of copper and zinc in these tissues from the Texas and Florida animals may be due to dietary composition, age, or disease (Davis and Mertz 1987). One explanation for the disparate concentrations is that exposure levels of copper and zinc are higher in Texas than in Florida, and the concentrations observed in bottlenose dolphin liver and kidney reflect those differences. We do not suspect that the intersite differences for Zn and Cu are related to contamination differences because essential metals such as these are highly regulated in the tissues. It is more likely that these differences would reflect deficiencies in the diet rather than excesses from contamination.

Altered concentrations of essential elements, such as copper and zinc, may also be an indication of ill health. Several liver diseases in humans are known to cause elevated copper levels in this organ (Davis and Mertz 1987). Conversely, deficiencies in essential elements in these organs may be an indication of animals in less than optimal health, assuming dietary intake is sufficient.

In pollution monitoring studies, a few authors have noticed that when organic contaminants are elevated in tissues, some elements appear to be depleted (de Goeij *et al.* 1974; Mearns *et al.* 1991). This has lead to the metal-depletion hypothesis that

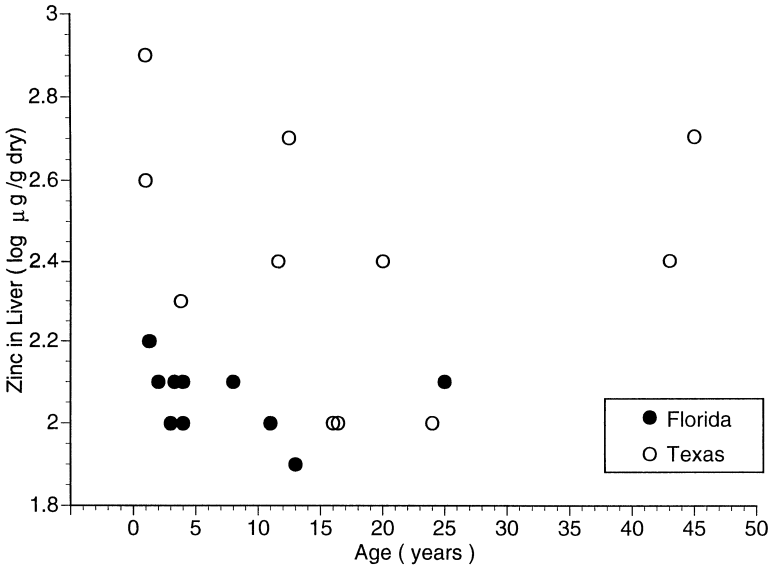

Fig. 9. Zinc in liver. Plot of zinc in liver versus age for each location

Table 4. Product-moment correlations for selected pair combinations

Pair		r	n
Age	Cd-B	0.45	17
Age	Cd-K	0.62	32
Age	Cd-L	0.63	17
Age	Se-L	0.85	42
Age	Ag-L	0.99	6
Age	Se-B	0.56	21
As-L	Cd-L	0.62	11
Cd-B	Se-B	0.53	17
Cd-K	Cd-L	0.69	17
Cd-K	Hg-K	0.62	31
Cd-L	Cd-B	0.57	5
Cd-L	Se-L	0.68	17
Cd-L	As-L	0.62	11
Cu-B	Fe-B	0.49	20
Cu-B	Zn-B	0.68	20
Cu-K	Zn-K	0.68	23
Hg-L	meHg-L	0.64	37
Hg-B	meHg-L	0.72	21
Hg-B	Se-B	0.88	21
Hg-K	Hg-B	0.70	19
Hg-K	meHg-B	0.78	18
Hg-K	Se-K	0.83	41
meHg-B	meHg-L	0.75	20
Se-K	Se-B	0.60	19
Zn-B	Fe-B	0.68	20
Zn-K	Zn-L	0.62	21

Correlations between elements for various tissues (B = brain; L = liver, K = kidney). Age includes known ages and those predicted (see Methods and Table 1). See methods for abbreviations

contamination by organic compounds, such as DDT or polycyclic aromatic hydrocarbons (PAHs), causes elements to be less abundant in those tissues than in tissue from less organically contaminated areas. A mechanism for this phenomenon has been proposed by Brown *et al.* (1987).

Studies that establish the normal range in concentration for the essential elements in different populations may be useful for testing the hypothesis that unhealthy marine mammals have altered concentrations of the essential elements. Such information may be useful in future monitoring studies to assess the health of marine mammal populations, especially as a function of location and diet.

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