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Environmental Pollution 123 (2003) 319–324

ENVIRONMENTAL POLLUTION

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Concentrations of heavy metals in *Sotalia fluviatilis* (Cetacea: Delphinidae) off the coast of Ceará, northeast Brazil

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Received 29 March 2002; accepted 6 September 2002

"Capsule": *As a top predator,* Sotalia fluviatilis *may be at risk from pollutants from outfalls.*

Abstract

Lead (Pb), cadmium (Cd) and mercury (Hg) concentrations on liver and kidney of *Sotalia fluviatilis* (Cetacea: Delphinidae) stranded in the coast of Ceará, Brazil, were studied from 1996 to 1999. Pb levels were usually lower than the detection limit (0.1 μg/g). Concentrations of Cd were significantly higher in kidney than liver, averaging 0.8 μg/g. Mercury accumulation took place mainly in liver with an average concentration of 4.6 μg/g. Both metals were significantly higher in larger mature individuals, but differences between sexes were not significant. The detection of Cd, Hg and Pb in tissue samples of *S. fluviatilis* off the coast of Ceará indicated that heavy metals are locally available in the water, and bioaccumulation may be occurring through the food web. Contamination levels were not considered critical, but could be related to Ceará's growing industrial development. The associated risks of pollution outfalls may pose a threat to marine organisms in a near future, especially for top predators such as *S. fluviatilis*.

Keywords: Cetacean; Heavy metals; Brazil; *Sotalia fluviatilis*; Dolphins

1. Introduction

Marine mammals are long lived and often present high levels of some potentially toxic substances such as heavy metals on body tissues (Becker, 2000). Metals are bioaccumulated with the ingestion of contaminated food items, whereas Hg may also be biomagnified throughout the food web (Aguilar and Borrell, 1995). Yamamoto et al. (1987) observed that Antarctic minke whales (*Balaenoptera acutorostrata*) concentrated on body tissues up to 5.5×10^5 times the concentration of cadmium in the water. Considering the similar ecological roles of marine mammals and men, especially top predators such as dolphins, they may be considered as good models for monitoring metal concentrations to which men may be exposed while consuming marine fish and shelfish.

The accumulation of heavy metals in marine mammals has been studied by Caurant et al. (1994) on *Globicephala melas*, Honda et al. (1983) on *Balaenoptera acutorostrata* and Law et al. (1991) on *Mesoplodon densirostris*, *Physeter macrocephalus* and *Orcinus orca*. Dolphins such as *Stenella coeruleoalba* and *Tursiops truncatus*, may accumulate as much as 44.8 and 9.1 mg/Kg (dry wt.) of mercury (Hg) and cadmium (Cd) in the kidneys, respectively (Leonzio et al., 1992). Marcovecchio et al. (1994) found mercury (Hg) concentrations in the liver of three species of small cetaceans in Argentina: *Tursiops gephyreus* (86.0 μg/g), *Pontoporia blainvillei* (3.8 μg/g) and *Kogia breviceps* (11.7 μg/g).

Sotalia fluviatilis is a small coastal dolphin occurring in coastal and estuarine waters (marine form) and river basins (riverine form) from Central and South America (Simões Lopes,1988; Da Silva and Best, 1996; Carr and Bonde, 2000) The species has been diagnosed as insufficiently known by the World Conservation Union (IUCN, 1991). Threats to the species affecting population stability include pollution by heavy metals and industrial wastes, pesticides, fisheries interactions and habitat degradation (IBAMA, 1997).

Brito-Jr (1994) found Cd concentrations in *Sotalia fluviatilis* between 0.04 and 0.30 μg/g on specimens from Rio de Janeiro and 0.15 μg/g from Pernambuco (NE Brazil). Junin et al. (1998) found higher levels on specimens from

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0269-7491/03/$ - see front matter
PII: S0269-7491(02)00371-8

heavy industrialized areas in Brazil. Concentrations of Cd in the liver of *S. fluviatilis* reached 1.51 μg/g, whereas *Stenella coeruleoalba* and *Delphinus delphis* had respectively 9.83 e 25.0 μg/g. *S. fluviatilis* from Paranaguá Bay (SE Brazil) presented Hg concentrations ranging between 176 and 568 μg/g. These high concentrations were attributed to the presence of a chemical plant in the area.

Sotalia fluviatilis is fairly common, but often occurring in small groups, along the coast of Ceará State, NE Brazil (Oliveira et al., 1995; Alves Jr. et al., 1996; Monteiro-Neto et al., 2000). Current levels of heavy metals in the environment are not known, but appear to be low due to the lack of large industrial areas to date. However, the current economic and industry development in the state may pose a future threat to the species population.

This study investigates the presence of three heavy metals, lead (Pb), cadmium (Cd) and mercury (Hg) on liver and kidney tissues of *Sotalia fluviatilis* (Cetacea: Delphinidae) stranded on the coast of Ceará, Brazil, using the species as a biomonitor of environmental contamination and providing information for the species conservation within areas of increasing human impact and industrial development.

2. Materials and methods

Concentrations of three heavy metals, lead (Pb), cadmium (Cd) and mercury (Hg) were determined on 11 specimens of *Sotalia fluviatilis* (Table 1), incidentally caught on fishing nets or stranded on the beach, from 1996 to 1999, along the coast of Ceará state, northeast Brazil (03°01′ S; 01°15′ W; 04°50′ S; 37°16° W).

Dead animals were submitted to biometry and macroscopic post mortem examination. Animals greater than 160 cm of total length (TL) were considered adults (Bossenecker, 1978).

Necropsy was performed to collect biological material. Approximately 10 g of the posterior part of the liver's left lobe and 10 g of the left kidney were collected with sterile scalps, and cold stored in separate plastic bags at −20 °C for latter analysis, following Geraci and Lounsbury (1993).

Each sample was subdivided into triplicates before the analysis. Samples for Lead (Pb) and Cadmium (Cd) determination were dried at 80 °C for 24 h. After, they were weighted and burned to ashes at 450 °C for 24 h. The ashes were dissolved in a 3:1 HNO_3 and HCl acid solution and evaporated in a hot plate until dryness. The residue was again dissolved in HCL 0.1 N, to a 5.0 ml final volume and filtered. The extract was taken for reading on atomic absorption spectrophotometer AA-1475 Varian (Ishii et al., 1980).

For total mercury (Hg) analysis, 1.0 ml of concentrated H_2O_2, 3.0 ml of H_2SO_4:HNO_3 (1:1) and 5.0 ml of $KMnO_4$ at 5% were added to about 500 mg (wet wt.) of each sample, and then digested in a microwave oven CEM-MDS-2000 (630 ± 50 W) for 30 min. After cooling, NH_4OCl 12% was added to a 10 ml volume. The analysis

Table 1
Results from independent samples *t*-test and Monte Carlo permutation analysis comparing log transformed (log10($x+1$) cadmium (Cd) and Mercury (Hg) concentrations in liver (L) and kidney (K) tissues between male (M) and female (F) immature (Im) and mature (Ma) and *Sotalia fluviatilis* at Ceará[a]

		Independent samples *t*-test						Monte Carlo permutation test Observations GE	
		Mean	SE	DF	*T*	*P*		Test criteria	*P*
Sex							F–M		
Cd-L	M	0.03	0.01	9	−1.32	0.22 NS	0.08	843/10,000	0.08 NS
	F	0.11	0.05						
Cd-K	M	0.09	0.04	9	−1.63	0.14 NS	0.19	543/10,000	0.05 NS
	F	0.28	0.01						
Hg-L	M	0.24	0.12	9	−1.55	0.16 NS	0.41	965/10,000	0.10 NS
	F	0.65	0.22						
Hg-K	M	0.12	0.07	9	−1.69	0.13 NS	0.26	646/10,000	0.07 NS
	F	0.38	0.13						
Maturity							Im–Ma		
Cd-L	Im	0.02	0.01	9	−1.96	0.08 NS	0.10	125/10,000	0.01 **
	Ma	0.13	0.06						
Cd-K	Im	0.08	0.03	9	−2.52	0.03 *	0.25	79/10,000	0.01 **
	Ma	0.33	0.10						
Hg-L	Im	0.11	0.03	9	−4.83	0.00 **	0.76	19/10,000	0.00 **
	Ma	0.88	0.17						
Hg-K	Im	0.05	0.02	9	−5.85	0.00 **	0.46	18/10,000	0.00 **
	Ma	0.52	0.09						

[a] Test criteria: LE, lower than or equal to; GE, greater than or equal to. ** Highly significant. P, Probability level. Number of permutations = 10 000.

was conducted on atomic absorption spectrophotometer with a flow injection system (FIMS-400) and autosampler (AS-90), both from Perkin-Elmer. The system is controlled by a 486DX PC computer, and the Winlab software (Perkin-Elmer) running under Windows (Bastos et al., 1998).

The results for Pb, Cd and Hg concentrations were expressed as the average of three determinations. Values were subjected to a logarithmic transformation ($\log_{10}x$) prior to statistical analysis, in order to reduce contagion and meet model assumptions (Legendre and Legendre, 1998; Zar, 1996).

T-tests (paired and simple) were used to verify the differences on the average metal concentrations between organs, sex and stage of sexual maturity of individuals (Zar, 1996). The simple linear regression was used to further explore the relationship between the metal concentration as a function of total length (TL) of individuals. Statistical analysis was performed using the Windows SPSS (Statistical Package for the Social Science Release 10.0.1) application developed by Norussis/Inc.

Considering the small number of samples, and the possibility of finding biased results using the traditional parametric methods, a permutation approach was taken to test the same hypothesis previously analyzed with *t*-tests. Legendre and Legendre (1998) and Manly (1998) observed that permutation is applicable to small sample sizes, and also to data that does not meet the assumptions of the most common statistical models. Furthermore, Manly (1998) pointed out that such tests are more reliable and less biased at small sample sizes.

Cadmium and Mercury concentration differences between organs were tested using a Monte Carlo permutation model. The difference between the mean concentration of each metal in the Liver (L) and Kidney (K) was calculated (L-K) and used as a benchmark. The concentration values were then submitted to 10 000 permutations constrained within rows, generating 10 000 null differences. Results were compared to the benchmark value, and the proportion of greater than or equal to (positive difference) or lower than or equal to (negative difference) calculated. A result was found significant when less than 5% of the values found through the permutation met the one tail test condition.

A similar model was adopted to test differences in concentrations between sexes and stages of sexual maturity. However, permutations were restricted within columns to prevent mixing of liver and kidney values.

The relationship between animal length and metal concentration (Cd and Hg) in both organs (liver and kidney) was tested using a regression model. Pearson's *R* were calculated and confronted with the 10 000 null values generated by permutation (within column). The proportion of greater than or equal to original *R* values was calculated and used as the probability.

All permutation analysis were performed with Excel's Ressampling (Ressampling Stats), and PopTools v. 2.1 (http://www.dwe.csiro.au/poptools/) macros.

3. Results

Lead concentrations in the liver of *Sotalia fluviatilis* from Ceará, ranged between 0.10 and 0.12 μg/g, with an average of $0.11+0.02$ μg/g. In the kidney, concentrations ranged between 0.11 and 1.28 μg/g, with an average of $0.11+0.51$ μg/g. More than half of the determinations resulted in values below the lower detection limit (0.08 μg/g) of the methodology. Due to these limitations, we did not proceed with any further statistical analysis. Cadmium in the liver was found in concentrations between 0.01 and 1.32 μg/g with an average of $0.22+0.38$ μg/g. In the kidney, Cd concentrations were in the order of 0.01 to 4.09 μg/g, with an average of $0.78+1.18$ μg/g. Mercury was found in concentrations between 0.10 μg/g to 29.51 μg/g and an average of $4.62+8.73$ μg/g in the liver, and 0.06 to 5.63 μg/g with an average of $1.24+1.72$ μg/g in the kidney.

Heavy metal concentrations in the liver and kidney were significantly different for both Cd (paired $t=-3.60$, $P=0.01$; Monte Carlo = 13/10 000, $P=0.00$) and Hg (paired $t=3.21$, $P=0.01$; Monte Carlo = 0/10 000, $P=0.00$), regardless of the statistical methodology used. Average Cd concentrations were higher in the kidney, whereas Hg showed a higher average concentration in the liver.

Significant differences were not detected between males and females for both, Cd and Hg, regardless of the organ. The results were consistent for the *t*-test and Monte Carlo permutation analyses. However, average metal concentration values were consistently higher on females (Table 1).

Mature individuals had significant higher concentrations for all but Cd on liver, when data was analyzed through *t*-test. However, when differences were compared using Monte Carlo, all figures were significant or highly significant (Table 1).

Linear regressions, studying the behavior of Cd concentrations in the liver and kidney of *Sotalia fluviatilis*, in relation to animal body size, resulted in models that explained respectively 35% ($R=0.59$) and 55% ($R=0.74$) of the data variability. Mercury showed similar results with regression lines explaining 71% ($R=0.84$) in the liver and 79% ($R=0.89$) in the kidney (Fig. 1). The correlation pattern of increasing concentration with increasing animal size was highly significant according with the Monte Carlo analysis (TL × Cd-liver = 28/10 000, $P=0.00$; TL × Cd-kidney = 16/10 000, $P=0.00$; TL × Hg-liver = 0/10 000, $P=0.00$; TL × Cd-kidney = 0/10 000, $P=0.00$).

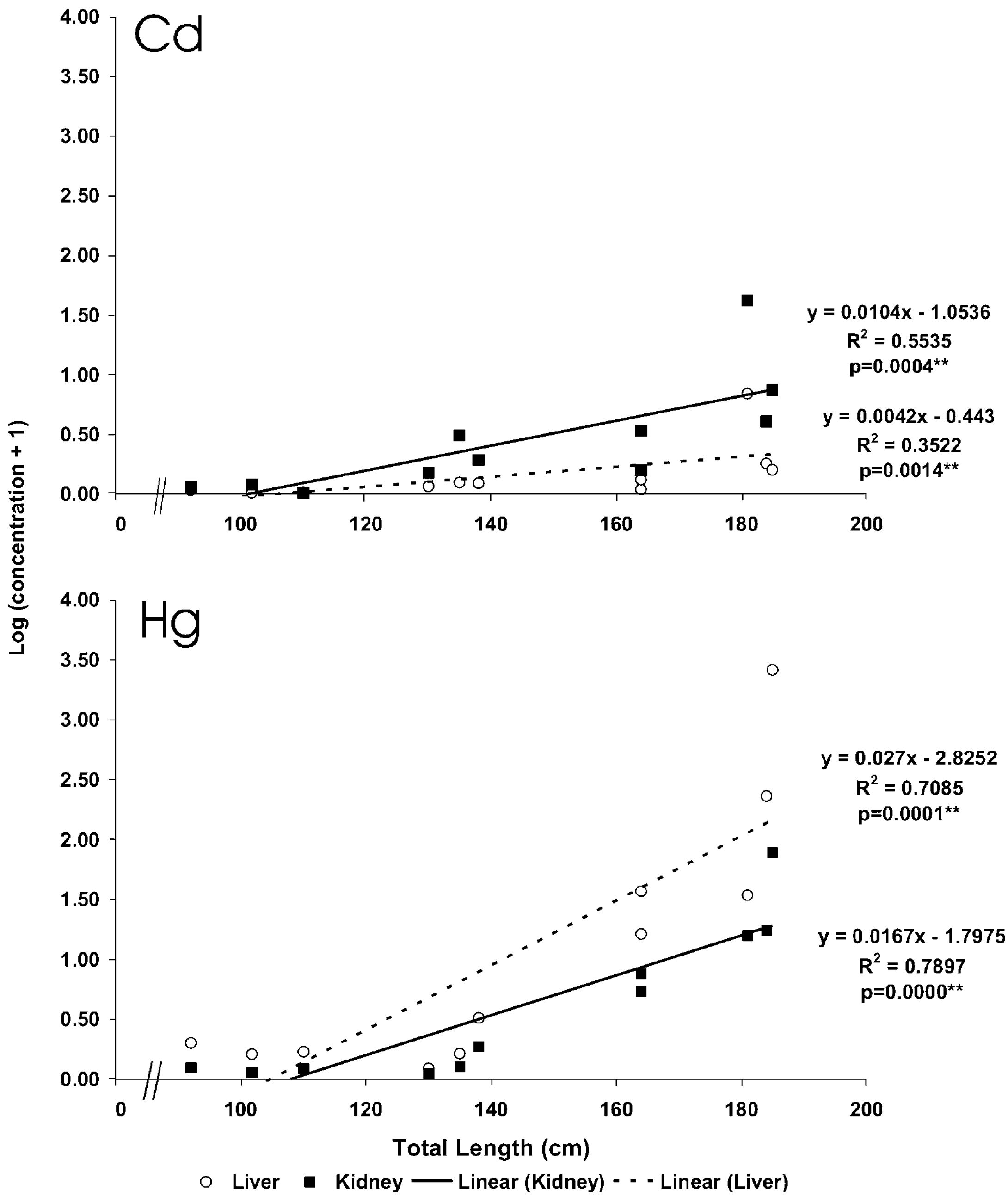

Fig. 1. Linear regressions, studying the behavior of both, Cd and Hg concentrations in the liver and kidneys of *Sotalia fluviatilis*, in relation to body size. Concentration values are transformed by $\log_{10}(x+1)$.

4. Discussion

Lead concentrations in *Sotalia fluviatilis* off Ceará were well below the detection limit of the method in most samples. These results were consistent with the findings of Brito-Jr (1994) and for most of the cetaceans analyzed by Law et al. (1991) and Junin et al. (1998). One possible explanation is that Pb tends to accumulate in bone tissue (Becker, 2000), following a similar metabolic path of calcium. Therefore, skeletal material should be further analyzed to verify possible higher concentrations of Pb in marine mammals.

Cadmium and mercury concentrations stayed within the range of observed values in other studies. Brito-Jr (1994) studying specimens of *S. fluviatilis* from Rio de Janeiro and Pernambuco, observed higher concentrations of Cd in the liver and lower in the kidneys. Similar findings were observed in our study. Reijnders (1988) and Becker (2000) observed that certain organs and tissues may be selective for the accumulation of specific heavy metals. For instance, the filtering function of the kidneys is probably the major cause for Cd to occur at higher levels in the organ. On the other hand, Marcovecchio et al. (1990), observed that the liver was probably the most important organ for the accumulation of Hg for several species of marine mammals. Marine mammals are exposed to methil-mercury present on preyed finfish. (Itano et al., 1984). However, most of the liver accumulated Hg is found at the inorganic form (Wagemann et al., 1988). This might suggest that, the liver functions as a depuration center for toxic methil-mercury coming from a finfish dominated diet. Methil mercury ingested is degraded and stocked under less toxic inorganic forms (Caurant et al, 1996).

Both Cd and Hg concentrations in other small cetacean species were much higher as compared to the present study (e.g. Marcovecchio et al., 1990; Wood and Van Vleet, 1996; Rosas and Lehti, 1996). Differences on heavy metal concentrations may be determined by the predominant food items on the diet of each species, habitat contamination, and metabolic variation among individuals. Squids, a predominant prey item of *Tursiops* spp., *Kogia* spp., and *Pontoporia blainviley*, often accumulate Cd in the liver and gonads (Honda et al., 1983). *Sotalia fluviatilis* however, is a minor squid predator as compared to these species (Borobia and Barros, 1989), which may be responsible for the observed lower values.

The concentrations of Cd and Hg increased with the size of the animals, regardless of the tissue analyzed. In fact, the highest Hg concentration was found on the largest mature female recovered in this study, with 185 cm TL and approximately 19 years of age (Cavallante, 2000). Correlation between heavy metal concentrations and animal size have been reported for *Phocoena phocoena* (Falconer et al., 1983), *Halichoerus grypus* and *Phoca vitulina* (Frank et al., 1992), *Globicephala melas* (Meador et al., 1993), *Delphinapterus leucas* and *Phoca hispida* (Mackey et al., 1996). The positive correlation between metal accumulation and animal size may be a consequence of the fact that larger (and therefore older) animals prey upon larger preys, of higher trophic levels, thus carrying higher metal concentrations in body tissues. Higher concentrations in mature individuals therefore, can be seen as an obvious consequence of animal growth and the bioaccumulation of heavy metals through food chain.

Sex related differences regarding metal concentrations were found to be non significant. Nevertheless, probability values obtained in permutation tests were very close to significance level, specially for Cd on kidney ($P = 0.054$). Differences in heavy metal concentrations in *Stenella coeruleoalba* (Honda et al., 1983) and *Monodon monoceros* (Wagemann et al., 1983) as a function of sex were also non significant. Francis and Bennett (1993), on the other hand, found significant differences in the levels of Hg between sexes of several marine mammal groups. Heavy metal contamination in mature males of many marine mammal species may be higher than in females at the same sexual maturity stage. Rejinders (1988) suggested that females may transfer heavy metals to infants mainly via placenta and, to a lesser extent, by lactation. However, Caurant et al. (1994), found lower Hg concentrations in newborn animals, and attributed that to placentary absorption.

The detection of heavy metals (Cd, Hg and Pb) in tissue samples of *Sotalia fluviatilis* off the coast Ceará, although in small amounts, indicated that those substances are at least locally available in the water, and bioaccumulation may be occurring through the food web. Sources of heavy metals are mostly related to industrial land based activities, and often contaminate coastal instead of oceanic waters (Becker, 2000). Cd has a 10–30 year biological half-life, meaning that even sporadic ingestion of small amounts may result in a significant accumulation in older animals. At the same time, Hg has a longer persistence and high mobility in coastal marine environments, being subjected to strong bioaccumulation process by marine organisms (Rejinders, 1980). Industrial activities at Ceará state involve metallurgical plants, leather processing units and electric components manufacturing. Despite its relatively poor development, their consequent heavy metal input to marine waters may not be neglected.

Due to its top predator position, coastal habits, and small size, *S. fluviatilis* may be placed in a high risk category, subjected to the effects of these environmental pollutants. The contamination of *S. fluviatilis* by heavy metals at Ceará is an indication that industrial outfalls may be reaching rivers, estuaries and eventually reaching coastal marine waters. Even though bioaccumulation levels are not yet critical, further monitoring programs should be conducted.

Acknowledgements

We wish to thank Olaf Malm, Wanderlei Bastos and their staff at the Radio Isotope Laboratory at Universidade Federal do Rio de Janeiro for their help on heavy metal analysis. To the members of Grupo de Estudo de Cetáceos do Ceará for their hard work and friendship. Fernando Rosas contributed with some positive criticism and suggestions. Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq provided fellowships to the authors through the course of this work.

References

Aguilar, A., Borrell, A., 1995. Pollution and harbour porpoises in the eastern north Atlantic: a review. Rep. Int. Whal. Commn. 16, 231–242.

Alves Jr., T.T., Ávila, F.J.C., Oliveira, J.A., Furtado-Neto, M.A.A., Monteiro-Neto, C., 1996. Registros de cetáceos para o litoral do Estado do Ceará, Brasil. Arquivos de Ciências do Mar 30 (1-2), 79–92.

Bastos, W.B., Malm, O., Pfeiffer, W.C., Cleary, D., 1998. Establishment and analytical quality control of laboratories for Hg determination in biological samples in the Amazon, Brazil. Ciência e Cultura 50 (4), 255–260.

Becker, P.R., 2000. Concentration of clorinated hydrocarbons and heavy metals in Alaska Arctic marine mammals. Mar. Pollut. Bull. 40 (10), 819–829.

Borobia, M., Barros, N.B., 1989. Notes on the diet of marine *Sotalia fluviatilis*. Mar. Mamm. Sci. 5, 395–399.

Bossenecker, P.J., 1978. The capture and care of *Sotalia guianensis*. Aquat. Mam. 6 (1), 13–17.

Brito-Jr, J. L., 1994. Concentração de metais traço (Cu, Zn, Fe, Cd, Pb, Cr e Mn) em tecidos de Cetáceos da Costa do Brasil. BS. Monography, Universidade Estadual do Rio de Janeiro.

Carr, T., Bonde, R.K., 2000. Tucuxi (*Sotalia fluviatilis*) ocurrs in Nicaragua 800 Km North of its previously know range. Mar. Mamm. Sci. 16 (2), 447–452.

Caurant, F., Amiard-Triquet, C., Sariau, P.G., 1994. Ecological and biological factors controlling the concentrations of trace elements (As, Cd, Cu, Hg, Se, Zn) in delphinids *Globicephala melas* from the North Atlantic Ocean. Mar. Ecol. Prog. Ser. 103, 207–219.

Cavallante, A.P., 2000. População folicular pré antral em ovários de *Sotalia fluviatilis* no Ceará. 2000. Master Thesis, Programa de Pós-Graduação em Engenharia de Pesca, Universidade Federal do Ceará.

Da Silva, V.M., Best, R.C., 1996. *Sotalia fluviatilis*. Mammalian Species 527, 1–7.

Falconer, C.R., Davies, I.M., Topping, G., 1983. Metals in the common porpoise *Phocoena phocoena*. Mar. Environ. Res. 8, 119–127.

Francis, D.R., Bennett, K.A., 1994. Addition data on mercury accumulation in Northern Michigan River Otters. J. Fresh. Ecol. 9 (1), 1–5.

Frank, A., Golgan, V., Roos, A., Olsson, M., Peterson, L.R., Bignert, A., 1992. Metal concentration in seals from Swedish water. Ambio 8, 529–538.

Geraci, J.R., Lounsbury, V.J., 1993. Marine Mammals Ashore. A Field Guide for Strandings. A & M Sea Grant Publication, Texas.

Honda, K., Tatsukawa, R., Itano, N., Miyazaki, N., Fujiyama, T., 1983. Heavy metal concentrations in muscle, liver and kidney of striped dolphins *Stenella coeruleoalba*, and their variation with body length, weight, age and sex. Agric. Biol. Chem. 47 (6), 1219–1228.

IBAMA, 1997. Mamíferos Aquáticos do Brasil: Plano de ação. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. Imprensa Nacional, Distrito Federal.

Ishii, T., Hirano, S., Matsuba, M., Koyanagi, T., 1980. Determination of trace elements in shellfishes. Bull. Japan. Scient. Soc. 46, 1375–1380.

Itano, K., Kawai, S., Miyakaki, N., Miyazaki, N., Tatsukawa, R., Fujiyama, T., 1984. Mercury and selenium levels in striped dolphins caught off the Pacific Coast of Japan. Agric. Biol. Chem. 48, 1109–1116.

IUCN, 1991. Dolphins, porpoises and whales of the World. In: Klinowska Gland, M. (Ed.), The IUCN Red Data Book. International Union for Conservation of Nature and Natural Resources.

Junin, M., Castello, H.P., Rotman, F., 1998. La contaminacion por plaguicidas organoclorados, PCB's y Metales Pesados en Los Mamíferos Marinos: Un Problema Mundial. Nueva Serie 151, 1–13.

Law, R.J., Fileman, C.F., Hopkins, A.D., Baker, J.R., Harwood, J., Jackson, D.B., Kennedy, S., Martin, A.R., Morris, R.J., 1991. Concentrations of trace metals in livers of marine mammals (seals, porpoises and dolphins) from waters around the British Isles. Mar. Pollut. Bull. 22 (4), 183–191.

Legendre, J., Legendre, P., 1998. Numerical Ecology. Elsevier, New York.

Leonzio, C., Focardi, S., Fossi, C., 1992. Heavy metals and selenium on stranded dolphins of Northern Tyrrhenian (NW Mediterranean). The Sci. Tot. Environ. 119, 77–84.

Mackey, E.A., Becker, P.R., Demiralp, R., Greenberg, R.R., Koster, B.J., Wise, S.A., 1996. Bioaccumulation of vanadium and other trace metals in livers of Alaskan cetaceans and pinipeds. Arch. Environ. Contam. Toxicol. 30, 503–512.

Manly, B.F.J., 1998. Randomization, bootstrap and Monte Carlo methods in biology. Chapman & Hall texts in statistical science. New York.

Marcovecchio, J.E., Moreno, V.J., Bastida, R.O., Gerpe, M.S., Rodriguez, D.H., 1990. Tissue distribution of heavy metal in small cetaceans from the Southwestern Atlantic Ocean. Mar. Pollut. Bull. 21 (6), 299–304.

Marcovecchio, J.E., Gerpe, M.S., Bastida, R.O., Rodriguez, D.H., Moron, S.G., 1994. Environmental contamination and marine mammals in coastal waters from Argentina: an overview. The Sci. Tot. Environ. 154 (2-3), 141–151.

Meador, J.P., Varanasi, V., Robisch, P.A., Chan, S.L., 1993. Toxic metals in pilot whales (*Globicephala meals*) from strandings in 1986 and 1990 on Cape Cod. Can J. Fish Aquat. Sci. 50, 2698–2706.

Monteiro-Neto, C., Alves jr, T.T., Ávila, F.J.C., Campos, A.A., Costa, A.F., Negrão, C.P., Furtado-Neto, M.A.A., 2000. Impact of fisheries on the tucuxi (*Sotalia fluviatilis*) and rough-toothed dolphin (*Steno bredanensis*) populations off Ceará state, Northeastern Brazil. Aquat. Mamm. 26 (1), 49–56.

Oliveira, J.A., Ávila, F.J.C., Alves-Junior, T.T., Furtado-Neto, M.A.A., Monteiro-Neto, C., 1995. Monitoramento do boto cinza, *Sotalia fluviatilis* (Cetacea, Delphinidae) em Fortaleza, Ceará State, Brazil. Arquivos de Ciências do Mar 29 (1-2), 28–35.

Rejinders, P.J.H., 1980. Organochlorine and heavy metal residues in harbour seals from the Wadden Sea and their possible effects on reproduction. Netherlands J. Sea Res. 4 (1), 30–65.

Reijnders, P.J.H., 1988. Ecotoxicological perspectives in marine mammology: research principles and goals for a conservation policy. Mar. Mamm. Sci. 4, 91–102.

Rosas, F.C.W., Lehti, K.K., 1996. Nutritional and mercury content of milk of the Amazon River Dolphin, *Inia geoffrensis*. Comp. Biochem. Physiol. 115^{A} (2), 91–102.

Simões-Lopes, P.C., 1988. Ocorrência de uma população de *Sotalia fluviatilis* Gervais, 1853 (Cetacea:Delphinidae) no litoral sul da sua distribuição, Santa Catarina, Brasil. Biotemas 1, 57–62.

Wagemann, R., Snow, N.B., Lutz, A., Scott, D.P., 1983. Heavy metals in tissues and organs of the narwal (*Monodon monoceros*). Can. J. Fish Aquat. Sci. 40 (2), 206–214.

Wagemann, R., Stewart, R.A., Lockhart, W.L., Stewart, B.E., Povoledo, M., 1988. Trace metals and methyl mercury: associations and transfer in harp seal (*Phoca groenlandica*) mothers and their pups. Mar. Mamm. Sci. 4, 339–355.

Wood, C.M., Van Vleet, E.S., 1996. Copper, cadmium and zinc in liver, kidney and muscle tissue of Bottlenose dolphins (*Tursiops truncatus*) stranded in Florida. Mar. Pollut. Bull. 32 (12), 886–889.

Yamamoto, Y., Honda, K., Hidaka, H., Tatsukawa, R., 1987. Tissue distribution of heavy metals in Weddell seals (*Leptonychotes weddelli*). Mar. Pollut. Bull. 18, 164–169.

Zar, J., 1996. Bioestatistical Analysis, 3rd ed. Prentice Hall, Upper Saddle River, New Jersey.