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Trace metals in tissues of gray whale (*Eschrichtius robustus*) carcasses from the Northern Pacific Mexican Coast

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Abstract

Samples of liver, lung, heart, muscle, and blubber tissue from the carcasses of juvenile gray whales (*Eschrichtius robustus*) found stranded on the coast off the Sea of Cortez, México were analyzed for a range of trace metals (Cu, Fe, Zn, Mn, Ni, Pb, and Cd). The highest concentrations of copper, iron, zinc, and manganese were found in liver; nickel and lead in heart, and cadmium in kidney. In all tissues analyzed, iron, zinc and copper were present in the highest concentrations; however, some whales also showed high levels of cadmium in the kidney which could be related to their diet. Elevated concentrations of copper were found only in the liver of one whale. In general, where low levels of iron were found in the liver, they were associated with poor nutrition. Lead, nickel, manganese and zinc levels in liver were within the normal range, indicating that these whales had not been exposed to high levels of these metals.

Keywords: Gray whale; *Eschrichtius robustus*; Heavy metals; Gulf of California; Whale carcasses; Pollution

1. Introduction

Only a few studies have measured trace metal levels in whales (Thompson, 1992; Law et al., 1992; Law et al., 1997; Parsons et al., 1999). Most report metal levels in liver, followed by kidney and muscle, and, to an even lesser extent, other tissues such as stomach, lung, heart, and blubber tissue. It is important to establish the normal range of metal levels in different tissues to diagnose toxic levels as a possible cause of illness or death of whales found stranded on the coast. Although metals are often classified as essential or nonessential, an element can be toxic if abnormally elevated levels are assimilated; such levels can result in metabolic disorders that could be lethal.

Knowing the ranges of metals in different tissues can help in understanding possible routes of assimilation and detoxification in these organisms. The assimilation of metals from water and sediments depends on their chemical association with anionic molecules which in turn depends on many physical and chemical factors as salinity, pH, and temperature (Riley, 1989). In whales, the assimilation of minerals depends on the composition of the diet, the kind and amount of mineral intake, and the rate of mineral excretion (Heath, 1987; Davis and Gatlin, 1991; Tolonen, 1995).

Exposure to heavy metal contaminants is influenced by feeding strategies of organisms (O'Shea et al., 1999). The Gray whale, *Eschrichtius robustus*, is a benthic bottom feeder (O'Shea et al., 1999) that gulfs its prey along with sediments from the sea bottom (Nerini, 1971). Feeding in or near sediments close to the coast may expose whales to many kinds of effluents, including those with elevated concentrations of heavy metals.

2. Materials and methods

Seven samples of lung and heart tissue and five of liver, muscle, and blubber tissue were taken from each of the carcasses of six female and one male juvenile gray whale, *E. robustus*, from the coasts of Sinaloa and Baja California Sur, México, January–June 1999. These were kept in clean bottles on ice for transport to the laboratory where analyses commenced upon arrival.

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Tissues were rinsed with deionized water to remove surface contaminants. From each sample, 1 g of tissue was excised, dried in an oven at 70 °C, and then digested in acid-washed test tubes with a 5:1 mixture of concentrated nitric and perchloric acids (Van Loon, 1985). After slowly boiling to dryness on a hot plate, the cooled, dry samples were redissolved in one ml of concentrated HCl and 24 ml of deionized water in a volumetric flask. Samples were analyzed by Atomic Absorption (BUCK Scientific, East Norwalk, Connecticut, model 200) using an air-acetylene flame. Precision was checked against standard reference material from the National Research Council of Canada (DORM-1 for dogfish) and was within the range of certified values. Recovery of all metals studied was over 95%.

Metal concentrations are reported as per dry weight of tissue. Because some literature results are reported as "per wet weight of tissue", we transformed all literature values to per dry weight values. These were calculated by dividing the wet weight values by a factor of 0.25, the dry/wet weight ratio of most tissues.

3. Results and discussion

Pathological analysis revealed that whales in this study had different degrees and kinds of infections which likely contributed to their deaths. Concentrations of copper, iron, zinc, manganese, nickel, lead, and cadmium in various gray whale tissues are given in Table 1. Greatest concentrations of copper, iron zinc, and manganese were found in liver; nickel and lead in heart; and cadmium in kidney. In all tissues analyzed, iron, zinc, and copper were present in greatest concentration. Manganese, nickel, lead, and cadmium were found in lower concentrations.

In each tissue, the concentration of the elements analyzed in decreasing order are: liver, Fe > Zn > Cu > Mn > Pb > Ni > Cd; lung, Fe > Zn > Cu > Cd > Mn > Ni > Pb; heart, Fe > Zn > Cu > Ni > Pb > Mn > Cd; muscle, Fe > Zn > Cu > Ni > Pb > Cd > Mn; blubber, Fe > Zn > Cu > Ni > Pb> Mn > Cd; and kidney, Fe > Zn > Cu > Cd > Ni ≈ Pb > Mn. Blubber, in general, had the lowest concentrations of all elements studied.

3.1. Copper

In all tissues analyzed, copper was the third most abundant element. The highest concentration of copper was found in liver (57.3 ± 87.7 μg/g) followed by kidney (27.7 ± 18.8 μg/g). According to Tolonen (1995), in vertebrates, the highest concentrations of copper are found in liver, muscle, blood, head, and brain. Metallothioneins, proteins with a high sulphur aminoacid content are also in the liver (Vogt and Quinitio, 1994; Connell and Miller, 1984). In marine mammals, these proteins are charged as a consequence of the homeostatic regulation of essential elements such as copper and zinc (Muir et al., 1988). The regulation of copper levels by this mechanism is irrespective of species and geographical location (Thompson, 1992).

In the present study, only one female specimen of *E. robustus* had concentrations of copper in the liver and

Table 1
Heavy metal concentrations (mean ± S.D. in μg/g dry wt) in tissues of gray whales *E. robustus*

	Cu	Fe	Zn	Mn	Ni	Pb	Cd
Liver (*n* = 5)							
Mean	57.3 ± 87.7	580 ± 334	141 ± 32	7.2 ± 6.57	1.84 ± 0.22	2.06 ± 1.06	1.77 ± 1.11
Range	3.37–228	165–1239	96–173	0.11–16.94	1.53–2.16	0.78–3.62	0.81–3.62
Lung (*n* = 7)							
Mean	5.26 ± 2.59	452 ± 153	83.3 ± 29.3	1.71 ± 1.16	1.59 ± 0.86	1.21 ± 1.37	1.16 ± 2.05
Range	2.34–8.95	262–689	47.4–143	0.36–3.85	0.30–2.37	0.36–4.40	0.10–5.26
Heart (*n* = 7)							
Mean	8.62 ± 6.01	338 ± 94.8	103 ± 41.8	1.08 ± 0.65	2.34 ± 0.67	2.31 ± 0.88	0.68 ± 0.60
Range	2.13–19.54	199–523	60.4–177	0.34–2.20	1.30–2.94	1.28–3.40	0.16–1.81
Muscle (*n* = 5)							
Mean	3.10 ± 1.65	305 ± 217	120 ± 34.4	0.33 ± 0.22	1.39 ± 0.79	1.11 ± 0.69	0.86 ± 1.05
Range	1.12–5.74	151–736	64.4–157	0.11–0.55	0.74–2.93	0.42–1.80	0.05–2.34
Blubber (*n* = 5)							
Mean	1.72 ± 0.90	28.9 ± 14.7	16.0 ± 4.89	0.44 ± 0.13	1.10 ± 0.60	1.06 ± 0.73	0.16
Range	0.30–2.69	9.00–7.8	10.6–25.2	0.31–0.61	0.61–1.95	0.33–1.78	n.d.[a]–0.16
Kidney (*n* = 5)							
Mean	27.7 ± 18.8	342 ± 104	120 ± 32.1	1.76 ± 1.52	2.16 ± 0.88	2.09 ± 2.15	15.4 ± 12.4
Range	16.5–65.0	227–538	91.6–162	0.12–4.08	1.07–3.29	0.34–6.12	1.93–35.1

[a] n.d. = no detectable.

kidney (228 and 65 μg/g, respectively) that were disproportionate to the values recorded for other specimens. These anomalous values were almost 10 and 3 times higher than the second highest concentration found in liver and kidney, 26.52 and 22.2 μg/g, respectively. The unusually high concentration of copper in the liver (228 μg/g) is comparable only with the highest concentration of copper reported in the literature; 285 μg/g in liver of one California sea lion, *Zalophus californianus*, a pinniped (Thompson, 1992). If the anomalous values of copper in liver (228.1 μg/g) and kidney (65.0 μg/g) are not included in the calculation, then the mean for liver and kidney drops to 11.81 and 18.37 μg/g, respectively. These mean values are within the range of values recorded for other cetaceans, for example, in liver (12.8 μg/g) and kidney (15.2 μg/g) of goose-beaked whales, (*Ziphius cavirostris,* Thompson, 1992). Copper values reported in the literature for the liver of bowhead whales are between 11.7 and 35 μg/g (Krone et al., 1999). In *E. robustus*, Varanasi et al. (1994) found concentrations of copper in liver 2.52–100 μg/g, in kidney 1.8–19.6 μg/g. Of 85 marine mammals analyzed by Law et al. (1992), only six had copper concentrations above 120 μg/g. According to Law et al. (1992), copper concentration is a function of age, with greater concentrations found in the young.

Although Krone et al. (1999) did not establish a toxic level of copper for cetaceans, Cardeilhac et al. (1979) considered that toxic concentrations of copper could be obtained through the diet. High copper levels may inhibit Na–K ATPase in the cellular membrane, reducing the capacity to regulate potassium and osmotic balance. High concentrations of copper may also interfere with the absorption of other important metals present in the marine environment (Thompson, 1992).

Because ours is the first study to measure copper levels in other tissues such as lung, heart, and intestine, we were not able to compare our values to other reported values to establish normal copper concentration range in these tissues. More studies are necessary to provide a baseline concentration for copper in these tissues so that we can assess whether a mortality is related to toxic copper levels or some other event.

3.2. Iron

Concentrations of iron in liver of *E. robustus* obtained in this study (between 165 and 1239 μg/g) generally were lower than values previously reported for whale tissues. Varanasi et al. (1994) found concentrations of iron in the liver of *E. robustus* to be between 480 and 16,800 μg/g. Other whales also had higher values of iron in liver: 2228 μg/g in the short-finned pilot whale (*Globicephala macrorhynchus*); 2000 μg/g in the goose-beaked whale (*Ziphius cavirostris*, Thompson, 1992); and 2270 μg/g in the bowhead whale (Krone et al., 1999). The range reported in liver of cetaceans is between 60 and 32,000 μg/g (Krone et al., 1999). Puls (1988) noted that a concentration of iron greater than 3000 μg/g in the liver of terrestrial animals could be considered toxic. Concentrations of iron in liver may be influenced by the amount of blood retained in the tissue and therefore depends, in part, on the length of time between the death of the animal and the collection of the tissues (Thompson, 1992; Krone et al., 1999). We recommended that the liver be as fresh as possible and that it be washed with deionized water to remove excess blood and other contaminants. This will ensure that iron from liver tissues is measured rather than from the blood.

In the kidney of *E. robustus*, we recorded concentrations of iron between 227 and 538 μg/g, within the range reported by Varanasi et al. (1994) for *E. robustus* (between 300 and 600 μg/g). However, in other whales, greater concentrations of iron are recorded for kidney, i.e. in *G.macrorhynchus*(808 μg/g) and *Z. cavirostris* (724 μg/g, Thompson, 1992). In muscle, we found concentrations of iron of 151–736 μg/g. The concentrations recorded for *G. macrorhynchus* and *Z. cavirostris* were 872 and 424 μg/g, respectively (Thompson, 1992). In blubber tissue, in which blood is reduced relative to than in the rest of the tissues, we found the lowest levels of iron.

3.3. Zinc

According to Law et al. (1992), the concentration of zinc in the liver is regulated to between 80 and 400 μg/g. Zinc levels in liver of whales analyzed in this study (96–173 μg/g) are consistent with those of Law et al. (1992), and within the range (6.4 and 640 μg/g liver tissue) reported by Varanasi et al. (1994). Values for zinc are also similar to those reported in other studies: 92 and 174 μg/g for the bottlenose whale, *Hyperoodon ampullatus*, and bowhead whale, *Balaena mysticetus* (Thompson, 1992), and between 88 and 261 μg/g for *B. mysticetus* (Krone et al., 1999). According to Puls (1988), levels higher than 500 μg/g in liver of terrestrial mammals are associated with toxicity.

In kidney *E. robustus*, we found 120 μg/g zinc, in the range previously reported for several cetaceans: *E. robustus*, between 128 and 440 μg/g (Varanasi et al., 1994); *B. mysticetus*, 95.2 μg/g; *Z cavirostris*, 212 μg/g and *S. coeruleoalba*, 120 μg/g (Thompson, 1992). In muscle, *E. robustus* had zinc levels of 120 μg/g, greater than those recorded for *H. ampullatus* (54 μg/g) and *S. coeruleoalba* (between 27.6 and 81.6 μg/g) (Thompson, 1992), but lower than *B. mysticetus* (175 μg/g) (Thompson, 1992). Comparisons of zinc levels in lung, heart and blubber with other reported values could not be made as others have not measured zinc levels in these.

3.4. Manganese and nickel

The most manganese we found in *E. robustus* is 7.2 μg/g in the liver. Varanasi et al. (1994) found 12 μg/g of manganese in the liver. According to Thompson (1992), manganese concentrations in marine mammals are normally found in concentrations lower than 28 μg/g in any tissue. Comparison of manganese levels in other tissues with reported values could not be made because of the lack of such data for other marine mammals.

In *E. robustus* the greatest concentration of nickel was found in the heart (2.34 μg/g), lower amounts were found in the kidney (2.16 μg/g) and liver (1.84 μg/g). These latter values are higher than those found for *E. robustus* by Varanasi et al. (1994): 1.4 μg/g in liver and 0.84 μg/g in kidney. Parsons et al. (1999) reported 0.79 μg/g nickel in the kidney tissue of a pygmy Bryde's whale; this value is also lower than the lowest value obtained by us for *E. robustus.* However, Law et al. (1997) found 3.0 μg/g in the liver tissue of Blainville's beaked whale, that is higher than the values obtained in the present study.

3.5. Lead

In *E. robustus*, the highest concentration of lead was found in the heart (1.28–3.40 μg/g). The kidney contained 0.34–6.12 μg/g of lead, the liver, 0.78–3.62 μg/g. In comparison, Varanasi et al. (1994) found 0.08–1.08 μg/g lead in the liver of *E. robustus*; kidney values were not detectable to 0.4 μg/g. Law et al. (1992) reported 0.6 μg/g in the liver of a female minke whale and considered concentrations of 4.0 μg/g or greater to be indicative of lead pollution. Parsons et al. (1999) recorded 15.88 μg/g in the kidney of a Bryde's whale (*Balaenoptera edeni*). Of all the specimens of *E. robustus* we analyzed, only one had lead values greater than 4.0 and 6.12 μg/g in kidney tissue, 4.40 μg/g in lung. The lead content in the other tissues of this specimen were under 4.0 μg/g.

3.6. Cadmium

In four species of whales, Dietz et al. (1996) found the highest concentrations of cadmium in kidney, followed by liver cadmium levels were lowest in muscle tissue. Dietz et al. (1996) found in kidney mean concentrations of cadmium, 13.9 μg/g in minke whale (*Balaenoptera acutorostrata*) to 217 μg/g in subadult narwhal (*Monodon monoceros*). In comparison, the concentration of cadmium in the kidney of *E. robustus* was between 1.93 and 35.1 μg/g (Table 1). Varanasi et al. (1994) found similar concentrations of cadmium in the liver (0.24–24.8 μg/g) and kidney (0.56–24.4 μg/g) of *E. robustus*. According to Bowless (1999), accumulation of high levels of cadmium in the kidney is mediated by metallothioneins, part of a storage mechanism.

Concentrations of cadmium found in kidney (35.1 μg/g) is much lower than that recorded for the kidney of Ross seals (422 μg/g) and *G. macrorhynchus* (486 μg/g) (Thompson, 1992). Studies involving odontocetes and mysticetes reported cadmium levels in liver between 0.03 and 500 μg/g (Krone et al., 1999). Cadmium values higher than 200 μg/g are associated with toxicity in terrestrial animals; 400 μg/g is indicative of kidney dysfunction (Puls, 1988).

The greatest concentration of cadmium found in liver in the present study (3.62 μg/g) is lower than the maximum concentrations reported by other authors. In livers of minke whale (*B. acutorostrata*), Law et al. (1992) reported 4.4 μg/g while Honda et al. (1987) found concentrations of cadmium of 8.79–133 μg/g. In the later case, the high concentration of cadmium in liver was thought to be caused by a diet primarily of krill which, in some areas, may have very high cadmium content.

In marine mammals, the ratio of cadmium between liver and kidney varies from about 1:2 to 1:5 (Wagemann et al., 1983; Meador et al., 1993). Bowless (1999) noted that the concentration of cadmium in striped dolphins from the western Pacific oceans and in harbor porpoises from Greenlands is four times greater in kidney than in liver. In this study, we calculated a ratio of 1:9 in liver and kidney.

Plankton found off the coast of Baja California in the Sea of Cortez, contain relatively high levels of cadmium (Martin and Broenkow, 1975). Unusually high levels of cadmium were also found in sea-skaters collected in the southern waters of Sea of Cortez (Cheng et al., 1976), as well as in sediment samples from the southern (Baja) Peninsula of California (Méndez et al., 1998), and in water samples collected from a productive squid fishery in the sea of Cortez (Delgadillo-Hinojosa et al., 1999). If high levels of cadmium are present in the environment, and these accumulate mainly in the kidney, it is not surprising that we found elevated concentrations of cadmium in liver and kidney.

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