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Trace metal concentrations in livers and kidneys of sea turtles from south-eastern Queensland, Australia

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Abstract. The concentrations of some or all of arsenic (As), cadmium (Cd), mercury (Hg), selenium (Se) and zinc (Zn) were determined in the livers and kidneys of 50 stranded sea turtles (38 *Chelonia mydas*, eight *Caretta caretta*, three *Eretmochelys imbricata*, one *Lepidochelys olivacea*) from the Moreton Bay region of south-eastern Queensland, Australia. Concentrations of Cd, Se and Zn in the kidney tended to decrease with age, whereas concentrations of Zn in the liver tended to increase. Concentrations of Cd in all sea turtle species (1.7–75.9 $\mu\text{g g}^{-1}$ wet weight) were amongst the highest recorded for marine vertebrates globally. Although there was no obvious association between metal concentrations and particular diseases in *C. mydas*, the high concentrations of Cd found in edible turtle tissues may pose a threat to the health of indigenous people whose diet includes *C. mydas*.

Introduction

Published studies of persistent chemicals (heavy metals and organochlorines) in marine turtles are limited, and mostly confined to serendipitous accessions of stranded animals, or eggs. Worldwide, all species of sea turtles are considered threatened (Baillie and Groombridge 1996), which generally precludes destructive sampling of healthy, wild turtles. Concentrations of 17 trace metals determined in the liver and kidney of 12 Hawaiian green turtles (*Chelonia mydas*) represent the largest sample published to date (Aguirre *et al.* 1994). A survey of trace metals in sediments, indicator organisms and traditional seafoods of the Torres Strait (Gladstone 1996) drew attention to elevated concentrations of Cd, Cu, Hg and Se in tissues obtained from seven *C. mydas*. Concentrations of Cd were considered sufficiently high to pose a threat to the health of indigenous communities for whom *C. mydas* is an important dietary component.

Since late 1990 passive disease surveillance of free-living sea turtles (mostly *C. mydas*) in the Moreton Bay region of south-eastern Queensland (centred on 27°22'S, 153°15'E) has been undertaken by routine necropsy examination of dead or moribund turtles. Tissues obtained at necropsy of 50 sea turtles were analysed for total As, Cd, Hg, Se and Zn, with the primary objective of determining trace metal concentrations in a large sample of *C. mydas* to allow comparisons with other taxa and other regions and to provide a baseline for future comparisons.

As other sea turtle species (including carnivorous loggerhead *Caretta caretta*, olive ridley *Lepidochelys*

olivacea and omnivorous hawksbill *Eretmochelys imbricata*) were sampled in this study, a comparison of trace metal levels in sea turtle species at different trophic levels could be made. This is of particular significance for Hg, which is known to biomagnify (Bryan 1984). The large number of *C. mydas* sampled also allowed analysis of trends in metal accumulation with age, between pairs of metals, and between different tissues (liver *v.* kidney).

Materials and methods

Tissue collection

Details of euthanasia and necropsy procedures are given in Gordon *et al.* (1998). Formalin-fixed tissues were used for metal analysis, since the analytical laboratory had previously demonstrated no loss of metals through leaching into formalin fixative. Samples of liver and kidney were obtained from 50 turtles, comprising 38 *C. mydas*, five *C. caretta*, three *E. imbricata* and one *L. olivacea*. Liver samples only were available from an additional three *C. caretta*. All turtles sampled were obtained from the Moreton Bay region except one *C. mydas* from each of Hervey Bay (25°03'S, 153°05'E) and Shoalwater Bay (22°22'S, 150°23'E). All samples were obtained in the first 14 months of the project (September 1990–November 1991), and were subsequently analysed in three batches. Samples in the first batch ($n = 11$) were analysed for Se, (total) As, Cd and Hg. Samples in the second batch ($n = 20$) were analysed for Zn in addition to these four metals. Preliminary results indicated low levels of Se, As and Hg, so samples in the final batch ($n = 19$) were analysed only for Cd and Zn.

Metal analysis

Reagents. Magos reagents (Magos and Clarkson 1972) for Hg determination were similar to those used by Greenwood *et al.* (1977) except that tri-*n*-butyl-phosphate was used as the antifoam. Intermediate standards for Se and As ($1 \mu\text{g mL}^{-1}$), and for Cd and Zn ($100 \mu\text{g mL}^{-1}$) were prepared freshly from 1 mg mL^{-1} stock atomic absorption solutions (BDH

Chemicals, Australia). Working standards of Cd and Zn were prepared in 1% nitric acid (HNO₃). Working standards of 0, 10, 20, 30, 50 and 100 ng mL⁻¹ of Se were prepared in 50% hydrochloric acid (HCl) (v/v), 5% sulfuric acid (H₂SO₄) (v/v) and 1% potassium iodide solution (KI) (w/v). Working standards of 0, 10, 20, 30 and 50 ng mL⁻¹ of As were prepared in 20% HCl (v/v), 5% H₂SO₄ (v/v) and 1% KI solution (w/v).

Procedures. A Varian model DB1475 atomic absorption spectrophotometer was used for metal determination. A standard procedure which used an acetylene/air flame, described in the Varian operational manual, was adapted for the determination of Cd and Zn following a three-acid digestion process. A cold-vapour atomic absorption spectrophotometric technique was used for Hg determination based on a standard addition method described by Greenwood *et al.* (1977) with modifications (Ng *et al.* 1987). Only the total Hg concentrations were reported here because the inorganic Hg levels in the tested samples were below or near the limit of detection. Levels of As and Se were measured by a hydride-generation atomic absorption spectrophotometric (HGAAS) technique (Ng *et al.* 1987). Briefly, this entailed accurate weighing of liver or kidney tissue (1–2 g) into a 50 mL digestion tube and digestion in a mixture of H₂SO₄ (2.5 mL), HNO₃ (3 mL) and perchloric acid (HClO₄; 2 mL) until only H₂SO₄ remained in the digestion tube. Aliquots of this digest were mixed with appropriate concentrations of HCl and KI (see reagents section) before the HGAAS measurement of As and Se respectively. The limits of detection were defined as twice the signal-to-noise level, which was 0.3, 20, 3, 1 and 20 ng g⁻¹ for As, Cd, Hg, Se and Zn, respectively. Results were expressed as µg g⁻¹ wet tissue.

Wet weight:dry weight ratios for liver and kidney were required for comparisons with other studies. To this end, samples of fresh and formalin-fixed liver and kidney were blotted dry on paper towel and weighed. They were subsequently dried in a 60°C oven until a constant dry weight was reached (4–7 days).

Statistical analysis

When metal concentrations were below the limits of detection, an estimate of half the detection limit was used for the purpose of statistical analysis. For both liver and kidney, scatter plots of metal concentration against curved carapace length (CCL) were examined for each metal. Non-parametric tests were mainly used because much of the data were distributed non-normally. Spearman rank correlation coefficients were calculated between levels of a single metal in liver and kidney, between pairs of metals in either tissue, and between metal levels and CCLs. Body size may have confounded any correlations between metals. Therefore, log-transformed data were used to improve normality, and partial correlation coefficients were determined for pairs of metals with CCL held constant

(Sokal and Rohlf 1981). Mean concentrations of each metal in liver and kidney were compared by use of a paired-sample *t*-test and its non-parametric analogue, the Wilcoxon paired-sample test (Sokal and Rohlf 1981).

Results

A summary of the results of trace metal analysis in 38 *C. mydas* is presented in Table 1, together with published comparative values. A similar summary for other species of sea turtle examined during the present study is presented in Table 2.

The relationship between metal concentrations (in both liver and kidney) and CCL is illustrated for each metal in Figs 1a–e. Values from two *C. mydas* from Hervey Bay and Shoalwater Bay generally fell within the range of values for turtles from Moreton Bay. They were included in analyses so as to present the most complete dataset possible. The scatter plots reveal a roughly linear relationship in the case of Zn, a curvilinear relationship in the case of Se and Cd, and no apparent relationship in the case of As and Hg. For Se and Cd, there appears to be a threshold of approximately 65 cm CCL beyond which there is no appreciable trend in metal concentrations in either liver or kidney.

The results of pairwise correlations between metal concentrations in liver and kidney, and between metal concentrations and CCL, are presented in matrix form in Table 3. Significant positive correlations of metal concentrations with CCL were found for Zn in the liver. Significant negative correlations of metal concentrations with CCL were found for kidney Se, kidney Cd and kidney Zn. Simply put, the older the turtle, the lower the concentrations of kidney Se, Cd and Zn found, but the higher the concentration of liver Zn.

Concentrations of a number of metals were intercorrelated in both liver and kidney (Table 3). Partial correlations, with CCL held constant, were significant between Se and Cd in both liver ($r = 0.535$; $P < 0.05$) and kidney ($r = 0.539$; $P < 0.05$), between Se and Zn in both liver

Table 1. Concentrations of heavy metals (µg g⁻¹ wet weight) in tissues of *C. mydas* in the present study compared with values in the same species from Hawaii (Aguirre *et al.* 1994) and the Torres Strait (Gladstone 1996). BDL, below detection limits

Metal	Tissue	range	Present study			range	Hawaii			Torres Strait ($n = 7$)		
			mean	s.e.m.	n		mean	s.e.m.	n	range	mean	s.e.m.
Se	liver	0.07–2.68	1.18	0.16	23	0.14–2.53	0.79	0.19	12	0.34–3.4	1.06	0.41
	kidney	0.09–1.85	0.59	0.09	23	0.16–1.58	0.46	0.12	12	0.16–1.3	0.45	0.16
As	liver	0.04–0.74	0.26	0.04	23	–	6.40	–	1	0.4–4.3	1.5	0.5
	kidney	0.00–0.69	0.19	0.05	23	–	6.80	–	1	0.07–1.2	0.42	0.16
Cd	liver	2.5–56.9	12.5	2.0	38	0.4–26.0	9.3	2.6	12	6.0–17.0	10.7	1.3
	kidney	1.7–75.9	15.3	2.5	38	4.7–70.2	26.0	6.1	12	12.0–42.0	26.0	4.1
Hg	liver	0.00–0.052	0.021	0.003	23	–	BDL	–	12	0.02–0.17	0.08	0.02
	kidney	0.00–0.049	0.020	0.004	23	–	BDL	–	12	0.01–0.04	0.02	0.004
Zn	liver	16.7–92.7	39.7	3.0	30	18.1–45.8	31.9	2.8	12	24.0–52.0	38.6	3.7
	kidney	15.4–31.8	21.3	0.7	30	12.5–38.1	22.3	2.2	12	19.0–29.0	23.8	1.2

Table 2. Concentrations of heavy metals ($\mu\text{g g}^{-1}$ wet weight) in tissues of loggerhead (*C. caretta*), hawksbill (*E. imbricata*), olive ridley (*L. olivacea*) and leatherback turtles (*D. coriacea*) compiled from the sources indicated. a, Davenport and Wrench (1990); b, Edmonds *et al.* (1994); NA, not analysed

Metal	Tissue	<i>C. caretta</i>				<i>E. imbricata</i>				<i>L. olivacea</i>		<i>D. coriacea</i>		
		present study			<i>n</i>	Sakai <i>et al.</i> (1995)			<i>n</i>	present study	present study	a	b	
range	mean	s.e.m.	range	mean		s.e.m.	range	<i>n</i>						<i>n</i> = 1
Se	liver	1.42–2.70	2.21	0.20	6	NA				2.68–3.65	2	NA	1.41 ^B	NA
	kidney	1.28–1.78	1.52	0.14	3	NA				2.22–2.49	2	NA	NA	NA
As	liver	0.0–1.56	0.46	0.24	6	NA				0.18–1.85	2	NA	0.58 ^B	1.2
	kidney	0.24–1.15	0.71	0.26	3	NA				0.13–0.93	2	NA	NA	NA
Cd	liver	7.3–35.1	16.4	3.3	8	5.7–14.6	9.3	1.3	7	2.4–6.2	3	6.4	0.22 ^B	NA
	kidney	11.4–39.4	28.3	5.7	5	18.1–56.5	39.3	6.1	7	3.6–12.7	3	29.8	NA	NA
Hg	liver	0.0–0.032	0.015	0.006	6	0.25–0.69	0.40	0.06	6 ^A	0.036–0.048	2	NA	0.39 ⁼	NA
	kidney	0.033–0.067	0.045	0.011	3	0.04–0.44	0.25	0.05	7	0.034–0.038	2	NA	NA	NA
Zn	liver	13.7–32.6	22.8	3.0	5	23.2–35.1	27.9	1.7	7	17.7–30.3	3	14.8	2.62 ⁼	NA
	kidney	16.7–21.3	18.4	0.9	5	19.2–30.4	25.8	1.6	7	13.2–20.9	3	18.8	NA	NA

^AOne anomalous value omitted. ^BDry weight basis.

($r = 0.621$; $P < 0.05$) and kidney ($r = 0.571$; $P < 0.05$), and between As and Hg in kidney ($r = 0.635$; $P < 0.05$). Therefore, turtle size or age alone cannot explain the association between metal concentrations in these tissues.

Metal concentrations in liver were correlated with those in the kidney of the same individual for As, Cd, Hg and Se, but not Zn. The Wilcoxon paired-sample test revealed significantly higher mean concentrations of As ($P < 0.01$), Se ($P < 0.001$) and Zn ($P < 0.001$) in liver compared to kidney (Table 1; Figs 1a, d and e). The mean Cd concentration was greater in kidney than in liver. However, this difference was only marginally significant by paired *t*-test ($P = 0.03$) and not significant with the Wilcoxon test ($P = 0.07$). Mean concentrations of Hg were similar in liver and kidney (Table 1; Fig. 1b).

Wet weight:dry weight ratios for liver and kidney of *C. mydas* were 4.9 and 6.8 respectively. Ratios for formalin-fixed tissues were similar to those calculated for fresh tissues, enabling ready comparison between this study and others utilizing fresh tissues. Liver and kidney of *C. mydas* appear to contain more moisture than comparable organs in fish, birds and mammals for which wet weight:dry weight ratios in the order of 3.3–4.0 are quoted (Bryan 1984).

Discussion

The relatively high concentrations of Cd in *C. mydas* tissues are the most notable toxicological finding in this study. Concentrations are one to three orders of magnitude higher than those reported for other marine turtle tissues, including the eggs of loggerhead (Hillestad *et al.* 1974; Stoneburner *et al.* 1980) and olive ridley turtles (Sahoo and Sahoo 1996), as well as leatherback turtle liver (Davenport and Wrench 1990; Table 2), and one to three orders of magnitude higher than concentrations reported in fish and crustaceans (Douglas and Warren 1995; Khan 1996) and

molluscs (Wallace and Moss 1979; White and Beumer 1996) from Moreton Bay. They are higher than values reported globally for most fish and some marine mammals (Bryan 1984). However they are similar to, or slightly lower than values recorded for many seabirds and some cetaceans (Thompson 1990).

The kidney is the organ with the highest concentrations of Cd in marine mammals and seabirds (Bryan 1984; Thompson 1990), and in these groups there is a well-recognized tendency for Cd concentrations to increase with age, largely as a result of sequestration in liver and kidney as Cd–metallothionein complexes (Bryan 1984). The significant decrease in kidney Cd concentrations observed with increasing age of juvenile turtles is counter to the trend in higher vertebrates and cannot be readily explained. Since organ weights were not obtained in the present study, it is unclear whether total Cd in the kidney increases, decreases or remains static as animals grow. It is also uncertain whether *C. mydas* acquire their Cd burden during their initial pelagic lifestage, or after recruiting to coastal areas as benthic feeders. Even as benthic feeders juvenile and adult *C. mydas* appear to segregate within the feeding ground (Limpus *et al.* 1994) which could influence their metal uptake.

The correlation, unconfounded by age effects, between concentrations of Se and Cd in the tissues of *C. mydas* has been observed in seabirds and pinnipeds (Bryan 1984; Thompson 1990). These and other authors have suggested that Se might play a role in the detoxification of Cd.

Gladstone (1996) calculated that weekly consumption of relatively small quantities of green turtle tissues (kidney, liver and intestine) by Torres Strait Islanders would exceed 'provisional tolerable weekly intakes' of Cd, as defined by the World Health Organisation (cited in Anon. 1992), but cautioned that long-term consumption rates needed to be

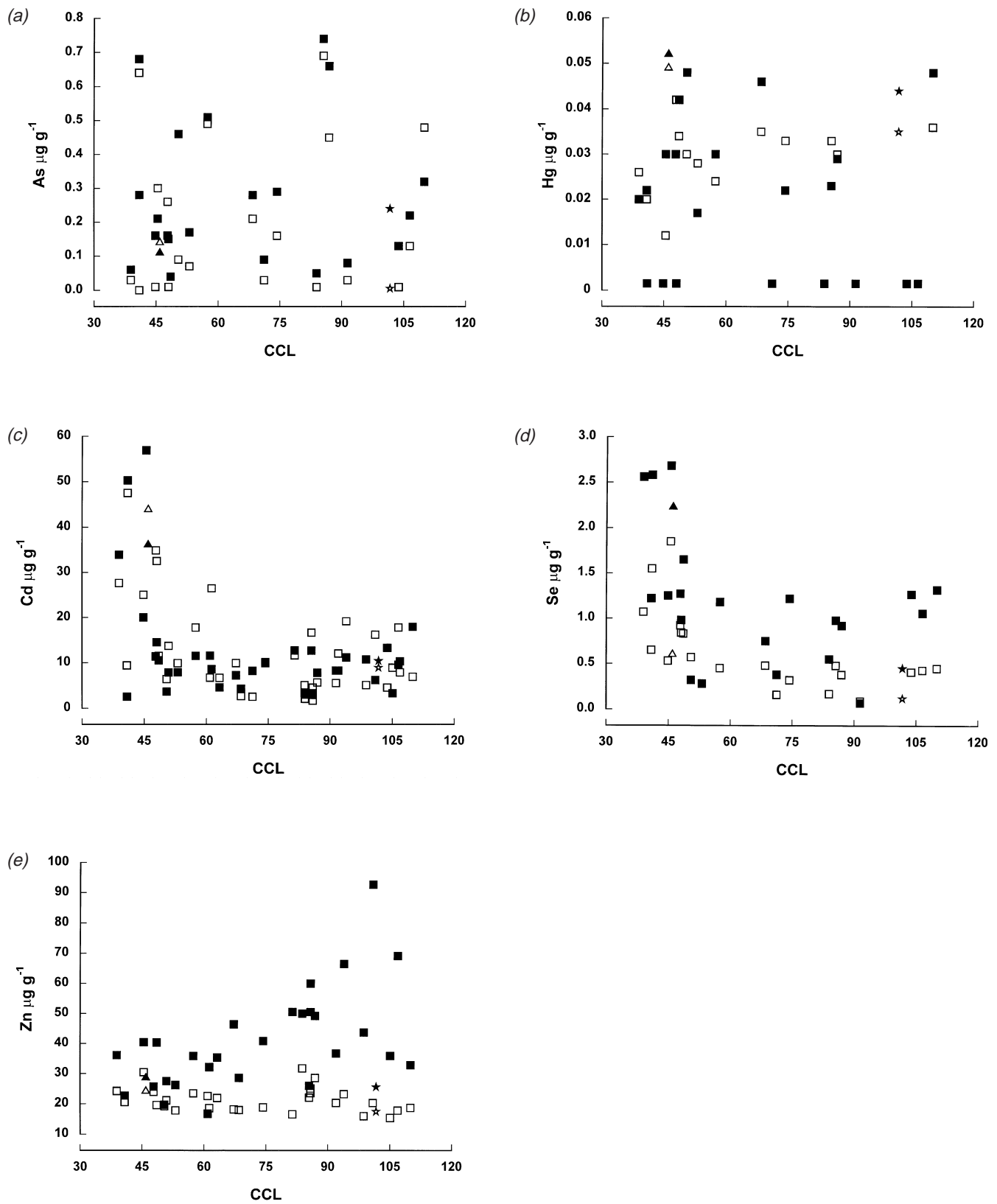


Fig. 1. Concentrations ($\mu\text{g g}^{-1}$ wet weight) of (a) As, (b) Hg, (c) Cd, (d) Se and (e) Zn in livers (closed symbols) and kidneys (open symbols) of *C. mydas* from Moreton Bay (■), Hervey Bay (▲) and Shoalwater Bay (★) plotted against curved carapace length (CCL; cm).

Table 3. Pairwise correlations between metal concentrations in liver (L) and kidney (K) of 38 *C. mydas*. Sign and level of significance (*0.05 > P > 0.01; **0.01 > P > 0.001; ***P < 0.001; ns, not significant) of Spearman rank-correlation coefficients of each metal with all other metals and curved carapace length (CCL), presented in matrix form

	LSe	KSe	LAs	KAs	LCd	KCd	LHg	KHg	LZn	KZn
KSe	+***									
LAs	ns	ns								
KAs	ns	ns	+**							
LCd	+***	+*	ns	ns						
KCd	+**	+***	ns	ns	+***					
LHg	ns	ns	ns	+*	ns	ns				
KHg	ns	ns	ns	+*	ns	ns	+***			
LZn	+*	ns	ns	ns	ns	ns	ns	ns		
KZn	+**	+**	ns	ns	ns	ns	ns	ns	ns	
CCL	ns	-***	ns	ns	ns	-**	ns	ns	+**	-*

determined, as well as analysis of a larger sample size, before conclusions could be drawn regarding the public health implications. The current study lends support to Gladstone's (1996) assertion that high Cd concentrations in green turtle tissues are a naturally-occurring phenomenon, since similar Cd concentrations have now been found in *C. mydas* from three widely separated sites (Table 1).

There was no obvious relationship between high Cd concentrations and particular diseases in *C. mydas* in this study. However, this question could not be adequately addressed since toxicological sampling in the current study was heavily biased towards unhealthy (stranded) turtles. Metal concentrations in five *C. mydas* which were regarded as healthy (deaths were due to misadventure such as drowning) fell within the ranges recorded from overtly diseased turtles.

Concentrations of other trace metals in *C. mydas* in the present study were generally low, tending to fall well below maximum permitted concentrations (MPCs) for foods which are sold, as set out in Standard A12 by the Australia New Zealand Food Authority (Anon. 1998). The MPC for As in seafoods is based on inorganic As; since inorganic As represents only a very small proportion of the total As present in the majority of marine organisms (Edmonds and Francesconi 1993), the total As concentrations reported in this study are likely to be insignificant insofar as public health issues are concerned. Concentrations of Hg in *C. mydas* in the present study are among the lowest recorded for marine animals, being one order of magnitude lower than levels found in leatherback turtles (Davenport and Wrench 1990) and up to six orders of magnitude lower than the very high levels found in some marine mammals (Bryan 1984). Since Hg is the only trace metal for which there is conclusive evidence of biomagnification (Bryan 1984), low levels in *C. mydas* might be expected as a consequence of the low trophic level occupied by the species. However, Hg concentrations in *C. caretta* (which is at a higher trophic

level) were also very low in this study, suggesting minimal Hg contamination in the Moreton Bay region.

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