



# Do Heavy Metal Concentrations Pose a Threat to Marine Turtles from the Mediterranean Sea?

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Concentrations of heavy metals (Hg, Cd and Pb) were determined in internal organs and nest contents of green turtles *Chelonia mydas* and loggerhead turtles *Caretta caretta* from northern Cyprus, eastern Mediterranean Sea. Concentrations of mercury in liver tissue were higher in loggerhead turtles (median 2.41  $\mu\text{g g}^{-1}$  dry weight) than in green turtles (0.55  $\mu\text{g g}^{-1}$  dry weight). Preliminary data suggest cadmium concentrations to be highest in kidney tissue of loggerhead turtles (median 30.50  $\mu\text{g g}^{-1}$  dry weight) but in liver tissue of green turtles (median 5.89  $\mu\text{g g}^{-1}$  dry weight). Concentrations of lead in internal tissues were often below analytical detection limits in both species, but when measurable, tended to be higher in loggerhead turtles. Concentrations of mercury and cadmium in nest contents from both species were low, often below analytical detection limits, while those of lead were relatively high in loggerhead turtle hatchlings (up to 10.56  $\mu\text{g g}^{-1}$  dry weight). When measurable, concentrations of all three metals tended to be higher in loggerhead turtle nest contents than in green turtle nest contents. Results presented here are consistent with inter-specific differences in diet and trophic status. Heavy metal burdens in loggerhead turtles and green turtles from the Mediterranean are similar or lower than corresponding concentrations in turtles from Japan and Hawaii, but some lead concentrations in Mediterranean loggerhead hatchlings are at levels known to cause subclinical toxic effects in other vertebrates. © 1999 Elsevier Science Ltd. All rights reserved

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Only three species of marine turtle occur regularly in the Mediterranean Sea. These are loggerhead turtle *Caretta caretta*, green turtle *Chelonia mydas* and leatherback

turtle *Dermochelys coriacea* (Groombridge, 1990). Of these, loggerhead and green turtles breed within the region, whilst leatherback turtles are thought to be non-breeding visitors. On a world-wide scale, marine turtle populations are generally in decline (Eckert, 1995; Limpus, 1995). Within the Mediterranean region, estimated annual breeding populations are as few as 2000 female loggerhead turtles and 300–400 female green turtles.

One of the potential threats to the survival of marine turtles is pollution (Hutchinson and Simmonds, 1992; Lutcavage *et al.*, 1997). Of the major categories of potential pollutants, the impacts upon marine turtles of solid debris (Gramentz, 1988; Hobson *et al.*, in press), oil and tar (Gramentz, 1988) and organochlorine residues (McKenzie *et al.*, in press) have been investigated within the Mediterranean region. Kaska (1998) investigated heavy metal contaminants in eggshells, yolk and embryonic livers of loggerhead turtles from Turkey.

Because of the semi-closed nature of the Mediterranean Sea and the relatively large centres of human population that impinge upon its shores, levels of marine contaminants in this ecosystem are considered to be relatively high (Bacci, 1989; Meadows, 1992; Kuetting, 1994; Borrell *et al.*, 1997). Given the endangered status of marine turtles and the potential for heavy metals to have detrimental effects upon marine vertebrates (Bull *et al.*, 1983; Nicholson and Osborn, 1983; Rawson *et al.*, 1993; Work and Smith, 1996), there is a clear need to augment the relatively small amount of data regarding heavy metal burdens in marine turtles from the Mediterranean Sea.

In this paper, we present heavy metal concentrations (mercury, cadmium and lead) in the organs and nest contents of loggerhead and green turtles from northern Cyprus, eastern Mediterranean. We assess to what extent marine turtles in this area exhibit potentially harmful concentrations of heavy metals and we evaluate the usefulness with which turtle nest contents may be

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used to monitor heavy metal contamination in this group.

## Materials and Methods

### Sample collection and preparation

Beaches were patrolled regularly as part of other turtle studies in northern Cyprus. Within an investigation of stranded turtles washed ashore between 1994 and 1996, carcasses were collected for dissection. Curved carapace length (CCL) was measured (using a flexible tape-measure, to the nearest 0.5 cm), and liver, kidney and muscle tissues were dissected from loggerhead turtles ( $n=7$ , mean CCL = 63.5 cm, s.d. = 14.2, range = 56.0–79.0 cm) and green turtles ( $n=6$ , mean CCL = 49.5 cm, s.d. = 16.6, range = 27.5–56.0 cm) within 24 h of stranding. Samples of the contents remaining in previously hatched nests of both species (loggerhead turtles:  $n=48$ ; green turtles:  $n=69$ ) were opportunistically sampled at Alagadi beach, northern Cyprus, following an established protocol (Broderick and Godley, 1996). Only one sample per nest (of a dead hatchling, dead embryo or undeveloped egg) was included in the study. All samples were stored frozen (at ca.  $-20^{\circ}\text{C}$ ) until further treatment. Prior to metal analyses, tissues were thawed at ambient room temperature (ca.  $20^{\circ}\text{C}$ ), then dried to constant mass in an oven at  $50^{\circ}\text{C}$ . Approximate water content, expressed as a percentage of fresh weight, was calculated. However, since samples had been frozen, fresh weights are not presented.

### Metal analyses

Total (organic and inorganic combined) mercury, cadmium and lead concentrations were measured. Total mercury concentrations were determined using a cold vapour atomic absorption spectrophotometry technique following an established methodology (Furness *et al.*, 1986; Thompson and Furness, 1989). Cadmium and lead concentrations were measured using flame atomic absorption spectrophotometry according to the methodology described in Stewart *et al.* (1994). All metal concentrations are presented as  $\mu\text{g g}^{-1}$  (ppm) of tissue

on a dry weight basis. Analytical limits of detection were determined as  $0.01 \mu\text{g g}^{-1}$  dry weight.

## Results

Concentrations of mercury, cadmium and lead in internal tissues of loggerhead and green turtles are presented in Table 1. Loggerhead turtles exhibited higher metal concentrations than green turtles (Table 1). Maximum concentrations in liver tissue of loggerheads were  $7.50$ ,  $12.97$  and  $4.90 \mu\text{g g}^{-1}$  for mercury, cadmium and lead, respectively, compared to  $1.37$ ,  $10.73$  and  $1.84 \mu\text{g g}^{-1}$  in that of green turtles (Table 1). Mercury concentrations were the highest in liver tissue > kidney > muscle for both species, cadmium concentrations in kidney tissue were as high or higher than those in liver tissue, but lowest in muscle tissue, whilst lead concentrations were fairly similar in all three tissues (Table 1). Mean water contents of liver, kidney and muscle tissues were 78%, 72% and 79%, respectively.

Metal concentrations in nest contents of loggerhead and green turtles are presented in Table 2. Mercury concentrations were generally low, with maximum values of  $0.24 \mu\text{g g}^{-1}$  in green turtle hatchlings and  $0.75 \mu\text{g g}^{-1}$  in loggerhead turtle hatchlings (Table 2). Maximum cadmium concentrations were recorded in yolk and albumen of green turtles ( $1.22 \mu\text{g g}^{-1}$ ; Table 2), and in hatchling loggerhead turtles ( $1.45 \mu\text{g g}^{-1}$ ; Table 2). Lead concentrations were higher, with maximum concentrations in hatchlings of  $10.56$  and  $3.86 \mu\text{g g}^{-1}$  in loggerhead and green turtles, respectively. Metal concentrations varied little among the three nest content categories (Table 2). Water content in eggs, embryos and hatchlings ranged from 70% to 75%.

## Discussion

Data presented here, although based on relatively small numbers of turtles, provide convincing evidence that concentrations of heavy metals are likely to reflect marked inter-specific differences in diet. Green turtles are thought to be generally herbivorous, whilst loggerhead turtles are carnivorous (Bjorndal, 1997). Although

TABLE 1

Mercury, cadmium and lead concentrations ( $\mu\text{g g}^{-1}$  dry weight) in internal organs from loggerhead and green turtles found stranded in northern Cyprus. Values are medians, sample size in parentheses and ranges. For analytical limits of detection, see Materials and Methods.

Species	Tissue	Mercury		Cadmium		Lead	
		Median ( <i>n</i> )	Range	Median ( <i>n</i> )	Range	Median ( <i>n</i> )	Range
Loggerhead turtle	Liver	2.41 (5)	0.82–7.50	8.64 (4)	5.14–12.97	BDL (4)	BDL–4.90
	Kidney	0.47 (2)	0.13–0.80	30.50 (2)	18.80–42.20	2.45 (2)	BDL–4.90
	Muscle	0.48 (7)	BDL–1.78	0.57 (4)	0.30–1.43	2.46 (4)	BDL–5.53
Green turtle	Liver	0.55 (6)	0.27–1.37	5.89 (6)	2.53–10.73	BDL (6)	BDL–1.84
	Kidney	BDL (1)	NA	3.46 (1)	NA	1.81 (1)	NA
	Muscle	0.09 (5)	BDL–0.37	0.37 (6)	0.12–0.78	BDL (6)	BDL–2.45

BDL: Below detection limit; NA: Not applicable.

TABLE 2

Mercury, cadmium and lead concentrations ( $\mu\text{g g}^{-1}$  dry weight) in nest contents of loggerhead and green turtles from Alagadi beach, northern Cyprus. Values are medians, sample sizes in parentheses and ranges. For analytical limits of detection, see Materials and Methods.

Species	Sample type	Mercury			Cadmium			Lead		
		Median ( <i>n</i> )	Range		Median ( <i>n</i> )	Range		Median ( <i>n</i> )	Range	
Loggerhead turtle	Hatchling	0.02 (16)	BDL-0.75		0.34 (16)	BDL-1.45		0.13 (16)	BDL-10.56	
	Embryo	0.01 (27)	BDL-0.22		0.21 (29)	BDL-1.09		BDL	BDL-6.48	
	Yolk and albumen	0.19 (3)	0.16-0.57		0.23 (3)	0.23-0.56		0.19 (3)	BDL-3.93	
Green turtle	Hatchling	BDL (24)	BDL-0.24		0.23 (29)	BDL-0.94		BDL (29)	BDL-3.86	
	Embryo	BDL (18)	BDL-0.12		0.33 (16)	BDL-0.93		0.66 (16)	BDL-3.41	
	Yolk and albumen	BDL (17)	BDL-0.19		0.27 (24)	0.05-1.22		BDL (24)	BDL-1.61	

BDL: Below detection limit.

there is a paucity of data regarding diet of these species in the region, these broad dietary differences, leading to corresponding differences in trophic status, were recently confirmed using stable isotope techniques (Godley *et al.*, 1998a). The higher concentrations of metals in loggerhead turtles compared to green turtles reported here (Table 1) are entirely in keeping with these pronounced trophic differences. A similar pattern was also found in a recent study of organochlorine contaminants in tissues from turtles from the same populations (McKenzie *et al.*, in press). Within both species, patterns of metal accumulation followed those described for other marine vertebrates (see Thompson, 1990 for a review), in that mercury concentrations tended to be highest in liver tissue, cadmium concentrations tended to be highest in kidney tissue and lead concentrations tended to be higher in liver and kidney, than in muscle (Table 1).

For comparative purposes, a summary of heavy metal concentrations in internal tissues of marine turtles determined by other studies is presented in Table 3. Data from Aguirre *et al.* (1994) and from Sakai *et al.* (1995), which were originally presented on a wet weight basis, have been converted to approximate dry weight basis using mean water content values determined in this study of liver 78%, kidney 72% and muscle 78% (see Results). Mercury concentrations in loggerhead turtles from Japan (Sakai *et al.*, 1995) were similar to those reported here (Tables 1 and 3). In liver tissue, for example, the median mercury concentration in loggerhead turtles reported by Sakai *et al.* (1995) was *ca.* 1.73  $\mu\text{g g}^{-1}$  (converted dry weight; Table 3), compared to 2.41  $\mu\text{g g}^{-1}$  in this study (Table 1). In contrast, cadmium concentrations in both green turtles from Hawaii (median kidney concentration *ca.* 56.79  $\mu\text{g g}^{-1}$  converted dry weight; Table 3; Aguirre *et al.*, 1994) and from logger-

head turtles from Japan (median kidney concentration *ca.* 162.50  $\mu\text{g g}^{-1}$  converted dry weight; Table 3; Sakai *et al.*, 1995) were considerably higher than those reported here (median kidney concentrations: green turtle 3.46  $\mu\text{g g}^{-1}$ , loggerhead turtle 30.5  $\mu\text{g g}^{-1}$ ; Table 1).

Differences in heavy metal concentrations between populations, may be explained by differences in diet, prevailing environmental contamination in their foraging ranges, by age of individuals sampled, or by a combination of these. Studies in Japan (Sakai *et al.*, 1995) and in Hawaii (Aguirre *et al.*, 1994) incorporated larger individual turtles than those sampled from the Mediterranean here, and were likely, therefore, to have been older. Since cadmium, for example, is known to accumulate in marine vertebrates with age (Stewart *et al.*, 1994; Dietz *et al.*, 1996), this may explain why cadmium concentrations in both conspecific sample sets (Table 3) were higher than those from the Mediterranean (Table 1). Mercury levels were not determined in green turtles from Hawaii (Aguirre *et al.*, 1994), and mercury concentrations in turtles from the Mediterranean (Table 1) were comparable with those from Japan (Sakai *et al.*, 1995; Table 3). The fact that mercury concentrations did not show the same spatial differences as for cadmium (see above) may be explained by the fact that, in general, organisms from the Mediterranean would be expected to have relatively high mercury levels due to the presence of a natural mercury bed in the region (Bacci, 1989).

Concentrations of metals reported in leatherback turtles from the north-east Atlantic (Davenport and Wrench, 1990; Godley *et al.*, 1998b; Table 3) were generally similar to those recorded in green turtles in this study (Table 1), but lower than those reported for loggerhead turtles from Japan (Sakai *et al.*, 1995; Table 3) and in this study (Table 1). Godley *et al.* (1998b) hy-

TABLE 3

Heavy metal concentrations ( $\mu\text{g g}^{-1}$  dry weight) in internal tissues of marine turtles from other locations. Values are medians, samples sizes in parentheses and ranges.

Species	Location	Tissue	Mercury		Cadmium		Lead		Reference
			Median (n)	Range	Median (n)	Range	Median (n)	Range	
Loggerhead turtle	Japan	Liver	1.73 (7)	1.13–37.05	47.73 (7)	25.73–66.36	ND	NA	Sakai <i>et al.</i> (1995)
	Japan	Kidney	1.00 (7)	0.43–1.57	162.50 (7)	64.64–201.79	ND	NA	Sakai <i>et al.</i> (1995)
	Japan	Muscle	0.43 (7)	0.24–0.90	0.29 (7)	0.19–0.56	ND	NA	Sakai <i>et al.</i> (1995)
Green turtle	Hawaii	Liver	ND	NA	23.82 (13)	1.77–118.18	ND	NA	Aguirre <i>et al.</i> (1994)
	Hawaii	Kidney	ND	NA	56.79 (12)	16.86–250.71	ND	NA	Aguirre <i>et al.</i> (1994)
Leatherback turtle	UK	Liver	0.61 (4)	0.29–1.20	8.50 (4)	0.22–88.00	0.08 (4)	0.02–14.00	Godley <i>et al.</i> (1988b)*
	UK	Muscle	0.12 (4)	0.04–0.29	2.10 (4)	0.06–7.50	0.08 (4)	BDL–0.31	Godley <i>et al.</i> (1988b)*

BDL: Below detection limit; ND: Not determined; NA: Not applicable.

\*Also incorporated data from Davenport and Wrench (1990).

pothesised that the highly pelagic nature of this species was likely to contribute to low contaminant burdens, both due to avoidance of the contaminated neritic, and through feeding at a level in the food web which is likely to involve few trophic (and therefore bioaccumulative) steps.

Heavy metal concentrations in nest contents of both species in the present study were generally low, often below quantifiable limits, but lower in green turtles than in loggerhead turtles (Table 2). A preliminary analysis of levels of organochlorines in eggs and hatchlings from the same site showed similar patterns (McKenzie *et al.*, in press). Whilst most egg content samples exhibited low metal concentrations (Table 2), a small number of individual samples exhibited relatively high lead concentrations. Hatchling green turtles exhibited a maximum lead concentration of  $3.86 \mu\text{g g}^{-1}$  and the corresponding value for loggerhead turtle hatchlings was  $10.56 \mu\text{g g}^{-1}$  (Table 2). No toxicological data have been published for marine turtles describing threshold concentrations of heavy metals above which detrimental effects would be likely. Although not directly comparable, a recent review of the toxicity of lead in birds (Franson, 1996) suggested that liver concentrations of lead of as low as *ca.*  $2 \mu\text{g g}^{-1}$  wet weight (approximately  $10 \mu\text{g g}^{-1}$  dry weight) could cause subclinical toxic effects in some species. Similarly, Ma (1996) concluded that in mammals lead concentrations in excess of  $10 \mu\text{g g}^{-1}$  dry weight in liver are consistent with acute poisoning. Lead concentrations in loggerhead turtle hatchlings, in particular, and perhaps in green turtle hatchlings also (Table 2), approach toxic levels reported in other vertebrate groups, and would warrant further study.

Previous work by Stoneburner *et al.* (1980) determined a range of metals (including mercury, cadmium and lead) in egg yolk from loggerhead turtles sampled at four sites in the USA, and noted some relatively high metal concentrations. The concentrations of metals presented by Stoneburner *et al.* (1980) were up to two orders of magnitude higher than those in nest contents reported more recently (Aguirre *et al.*, 1994; Sakai *et al.*, 1995; Kaska, 1998; this study). Such a wide discrepancy between data sets may indeed be due to relatively high levels of contamination in the USA neritic, or alternatively, may reflect inaccuracies in earlier analytical methods.

In conclusion, the preliminary data presented here suggest that metal levels in both green and loggerhead turtles are not likely to be high enough to affect the health of these endangered species. The only exception to this might be relatively high lead concentrations in loggerhead turtle hatchlings, and perhaps also green turtle hatchlings (Table 2). We suggest that for non-invasive monitoring of the potential impact of metal pollution on these species that undeveloped eggs, dead embryos or dead hatchlings are equally useful monitoring units. That nest contents would constitute a non-invasive and meaningful monitor of heavy metal bur-

dens in marine turtles is further supported by the findings of Sakai *et al.* (1995), who demonstrated that egg concentrations correlated with those in the female from which they were sampled. Additionally, whilst metal concentrations tend to be generally low in eggs, given the large number laid by reproductively active females (Broderick and Godley, 1996), excretion of metals via eggs may be a substantial elimination route in this group. Sakai *et al.* (1995) suggested that this might not be important ( $<0.5\%$  of the cadmium burden and  $<5\%$  of the mercury burden per clutch), but appear to have ignored the fact that turtles lay multiple clutches, over many years. At the very least, further monitoring of metal burdens in marine turtles in the Mediterranean Sea region would seem prudent, especially from those ranging into more intensively industrialised regions such as Spain, Italy and Greece.

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