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Copper and manganese in loggerhead turtles (Caretta caretta) tissues in the Mediterranean

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Abstract

This research concerns the determination of copper and manganese concentrations in tissues of loggerhead turtles <u>(Caretta caretta)</u> found injured in various locations of Greek waters. The specimens were transported through the National Stranding Network run by ARCHELON to the Sea Turtle Rescue Centre, and were treated there. Measurement of copper and manganese form the beginning of a further research that includes measurement of Cd, Pb, Fe and Ni. Our intention was to obtain initial data values in several tissues of <u>(Caretta caretta)</u> in Greek waters and detect any bioaccumulation trend. In general the highest concentrations for both Cu and Mn were measured in the liver. The lowest concentrations for Cu were found in the muscle samples and for Mn in the lung samples. There was no indication of bioaccumulation for either of the two metals or the examined tissues. However, a negative trend was observed between curved carapace length (CCL) and (a) intestine copper concentration and (b) liver and lung manganese concentration. The comparison between specimens from various territories showed that the results were in agreement, taking into account the size of the specimens.

Keywords: Caretta caretta; Copper; Manganese.

Introduction

Sea turtles are widely distributed in the world's oceans, found in tropical, sub-tropical and temperate waters. From the seven remaining sea turtle species (LUTZ & MUSICK, 1997), only three are present in the Mediterranean Sea: Loggerhead (Caretta caretta), Green turtle (Chelonia mydas) and Leatherback (Dermochelys coriacea) (ARNOLD & BURTON, 1985). Chelonia mydas and Caretta caretta are the only species that nest in the Mediterranean basin (GROOMBRIDGE B., 1990). The most important nesting sites for Caretta caretta are

located in Greece, Turkey, Cyprus and Libya, whereas fewer rookeries exist in Tunisia, Israel and Lebanon (MARGARITOULIS *et al.*, 2003). According to TOMAS *et al.* (2001) the western Mediterranean loggerhead's early to late juvenile population (34.0-69.0cm CCL) feed upon fish, pelagic tunicates, crustaceans, mollusks and other invertebrates.

In the Mediterranean the loggerhead is considered to be threatened and is protected by international conventions, European Commission directives and national legislation (MARGARITOULIS D., 2000). The level of pollution in the Mediterranean is higher than in the open ocean, due to its semi-closed shape and to the fact that many large cities and industries are located along its coastline (GODLEY et al., 1995, WWF, 1989, HOBSON et al., 1997). There is also intense shipping activity (UNEP, 1996, GROOMBRIDGE B., 1990). Therefore, marine turtles are exposed to increased metal concentrations, mainly through the food chain, which could potentially affect their health (GODLEY et al., 1999). As SAKAI et al. (1995) point out there is a need for monitoring chemical pollutants towards an effort to conserve the loggerhead population as a whole.

Some metals like copper and manganese are essential for marine organisms in adequate concentrations (CLARK, 1999). Vertebrates require copper as a catalytic cofactor for biological processes such as respiration, iron transport, oxidative stress protection, peptide hormone production, pigmentation, blood clotting and normal cell growth and development (PUIG & THIELE, 2002). Manganese also plays many roles in biological systems ranging from acting as a simple Lewis acid catalyst to being an element that can transverse several oxidation states to carry out water oxidation (YOCOM & PECORARO, 1999). However, exposure to these metals is known to cause toxic effects. Copper participates in redox reactions that generate the hydroxyl radical, which causes catastrophic damage to lipids, proteins and DNA (PUIG & THIELE, 2002) and manganese blocks Ca²⁺ channels, and thus affects neuromuscular transmissions in both vertebrates and in benthic marine invertebrates (BADEN & NEIL, 2003).

The purpose of this research was to determine the levels of copper and manganese in several tissues of specimens of *Caretta caretta* that live in the eastern Mediterranean. An attempt was made to detect the bioaccumulation trend for these two metals.

Materials and Methods

The studied specimens were transported injured to the ARCHELON Sea Turtle Rescue Centre through the National Stranding Network run by ARCHELON during the period December 2000-January 2002. The animals died at the Rescue Centre and, during routine necropsies, various tissues were extracted and stored in polyethylene bags at -8°C until further analysis. The tissues were liver, muscle, lung and intestine (two spleen samples and one kidney, one ovary and one brain tissue were also taken). Information about the location where the individuals were found, their sex, their biometry, the cause of death and the storing period is shown in Table 1. The samples were lyophilized and five aliquots of approximately 200-300 mg of each sample were digested in PTFE vessels with a mixture of 5ml HNO3 65% and 0.2ml HClO₄70-72% at 80°C. After the mixture was diluted the vessels were tightly closed and left for 2h on the plate. The residue was collected using HNO₃ 0.3N solution, to 25ml plastic bottles. Copper and manganese were determined by Graphite Furnace Atomic Absorption Spectrometer Varian SpectrAA 640Z with Zeeman background correction.

Two reference materials: Bovine BCR No 184 and Tuna fish IAEA-350 (1989-1991), were analyzed using the same procedure for analytical quality control. The results were in good agreement with the certified values (Table 2).

Results and Discussion

Table 1
Information about the location the individuals were found, their sex, their biometry,
the cause of death and the keeping period.

Name	location found	date found	date of death	CCL, cm	CCW, cm	weight kg	, cause of death	keeping time
Xanthie (n	n)Kefalonia	10/12/2000	20/10/2001	66	61.7	32	wound on the scull	8 months
Iota (m)	Glyfada, Attiki	21/9/2001	21/9/2001	61.5	59.3	-	multiple extensive wounds on the carapace	immediate death
Kiriaki (m)) Vonitsa, Ait/nia	24/9/2001	31/10/2001	56.8	54	21	wound on the scull	37 days
Xenia (f)	Ag.Triada,	30/9/2001 Thes/niki	30/10/2001	70	62	37	wound on the scull, fishhook swallowing	30 days
David (m)	Matala, Crete	1/12/2001	21/1/2002	82.5	71	52.1 ir	entanglement fishnet, mutilate LFF, scar around neck	51 days d
Erato (m)	Myrsini, Tinos	4/12/2001	8/12/2001	39.1	36.6	7	exhaustion, fishhook swallowing	4 days
Thalassa (1	n) Ikaria	12/1/2002	27/1/2002	31	30	3	fishing-line swallowing	15 days
CCL: Curv CCW:Curv f: female m: male LFF: left fr	ed Carapace ed Carapace ont flipper	e Length e Width						

Table 2

Copper and manganese concentrations in reference materials for 95% confidential interval.

		Cu µg/g	Mn ng/g
Bovine	certified values	2.36±0.06	334±28
BCR No 184	determined values	2.16 ± 0.25	308 ± 52
n=5	range	2.00-2.50	253-368
	recovery	91.5%	92.2%
Tuna fish	certified value	2.83	NA
IAEA-350,	range	2.55-3.10	NA
1989-1991	determined value	2.21 ± 0.14	NA
n=5	range	2.07-2.42	NA
	recovery	78.1%	NA
NA: Not Available			

copper and manganese in the various tissues sample is a result that requires further of the Caretta caretta examined are presented investigation, although SAKAI et al. (2000a) in Figure 1 (in μ g/g dry tissue weight). The liver samples gave the highest values for both Cu concentrations in the brain tissues of adult

The range and mean concentrations of and Mn. The high Cu value of the single brain also found comparatively high Cu



Fig. 1: Distribution of copper and manganese (in $\mu g/g$ dry weight) in the tissues of the *Caretta caretta* individuals examined (mean and min-max values).

 Table 3

 Correlation coefficients between the two metals and the CCL for 95% confidential interval. (n: number of samples)

	liver	muscle	lung	testes	intestine	
Cu	0.175	0.120	-0.393	-0.180	-0.855	
Mn	-0.807	-0.027	-0.740	0.061	-0.053	

loggerheads (n=7). Elevated Mn values were obtained for testes and intestine. The muscle samples (along with the ovary and kidney samples) gave the lowest copper values while the lung samples (along with the ovary) gave the lowest manganese values. An increased range was observed for copper in liver samples and for manganese in liver, testes and intestine samples. Values for Cu in spleen tissues (n=2) were higher than the mean for the Japanese individuals (SAKAI *et al.*, 2000a). One of the

two spleen Mn values was six times higher than the average for the Japanese individuals (SAKAI *et al.*, 2000a).

Table 3 presents the correlation between the metal concentrations and the CCL. The CCL is used because there is a relation between CCL and the age of the individuals, although there is no standard method of age determination for sea turtles (BJORNDAL *et al.*, 1998). It is obvious that there is not any positive correlation and thus no

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4	Manganese Reference	ge Mean Stdev Range	v 2.18 0.40 NA Sakai et al., 2000a	13.9 2.07 0.50 1.44-2.94 Sakai et al., 1995	20.9 NA NA NA Caurant et al., 1999	1.08 1.62 1.03 0.85-3.96 present study	01 3.07 0.44 nd-9.12 Zhou et al., 2001	v 0.28 0.11 NA Sakai et al., 2000a	.28 0.30 0.12 0.13-0.45 Sakai et al., 1995	2.23 NA NA NA Caurant et al., 1999	1.58 0.22 0.10 0.09-0.44 present study	2.4 nd nd nd Zhou et al., 2001	v 0.12 0.08 NA Sakai et al., 2000a	2.00 0.13 0.05 0.05-0.22 present study	v 0.51 0.29 NA Sakai et al., 2000a	2.17 0.75 0.77 0.02-1.79 present study	v 0.37 0.38 NA Sakai et al., 2000a	2.00 0.28-1.86 present study	
Tissue n=samples Copper	pper	dev Range	93 NA	.17 6.47-33.9	59 2.32-20.9	28 4.51-11.08	28 nd-9.01	28 NA	26 053-1.28	.45 0.34-2.23	38 1.78-0.58	.48 nd-12.4	.10 NA	72 0.69-2.00	23 NA	60 0.98-2.17	.07 NA	- 1.68-2.00	
	s Coj	Mean St	17.7 8.	17.9 8.	8.25 6.	7.39 2.	5.73 0.	0.81 0.	0.83 0.	0.73 0.	1.14 0.	2.04 0.	0.54 0.	1.35 0.	0.71 0.	1.40 0.	0.70 0.		
	Tissue n=samples		Liver (n=6)	Liver $(n=7)$	Liver $(n=7)$	Liver (n=7)	Liver (n=24)	Muscle $(n=6)$	Muscle $(n=7)$	Muscle $(n=7)$	Muscle $(n=7)$	Muscle $(n=24)$	Lung (n=6)	Lung (n=7)	Intestine (n=6)	Intestine (n=5)	Spleen (n=6)	Spleen (n=2)	
2	Location		Japan	Japan	France	Greece	Portugal	Japan	Japan	France	Greece	Portugal	Japan	Greece	Japan	Greece	Japan	Greece	
	Species		Caretta caretta	Caretta caretta	Caretta caretta	Caretta caretta	Common dolphin	Caretta caretta	Caretta caretta	Caretta caretta	Caretta caretta	Common dolphin	Caretta caretta	Caretta caretta	Caretta caretta	Caretta caretta	Caretta caretta	Caretta caretta	

bioaccumulation trend for the two metals. On the other hand there is a negative correlation between intestine copper concentration and the curved carapace length and between liver and lung manganese concentration and the CCL. This negative correlation could be attributed to detoxification processes involving metallothionein, a metal binding protein (SAKAI *et al.*, 2000b).

Table 4 shows results of studies that concern the same species in Japan (SAKAI et al., 2000a) and on the Atlantic coasts of France (CAURANT et al., 1999) as well as common dolphins in Portugal (ZHOU et al., 2001) The values of the present study were transported to $\mu g/g$ wet tissue weight so that they could be compared to the other research values. Copper values in Mediterranean liver samples were significantly lower than those found in Japan and somewhat lower than those measured in France. As for Mn, the values lie slightly lower than those obtained in Japan. The twofold values of Cu that were obtained in the measured intestine and lung samples are noteworthy, while the concentration in muscle samples is only slightly higher. The Mn concentrations for muscle and lung are similar, while intestine samples gave slightly higher values compared to Japanese specimens. Common dolphins from Portuguese waters gave lower Cu concentrations in liver, almost twofold values for Cu in muscle samples and for Mn in liver samples while no Mn was detected in muscle samples.

The results of the present research indicate that, despite a few variations in the studied metal concentrations between Mediterranean turtles and sea turtles from Japanese waters, there is no significant danger to them by trace metal bioaccumulation. In all cases the trace metal concentrations do not seem to pose toxic health danger to the sea turtles as they fall into the ranges reported by other researchers and as STORELLI and MARCOTRIGIANO (2003) underline that such values are not likely to be high enough to affect the health of these endangered species. However, relative research has to be continued along with research on physiological effects of chemical pollutants on sea turtles, since there is inadequate information on these fields.

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