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Abstract

This research concerns the determination of copper and manganese concentrations in tissues of loggerhead turtles (Caretta caretta) 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 (Caretta caretta) 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 Ca^{2+} 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 HNO_3 65% and 0.2ml $HClO_4$ 70-72% 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 HNO_3 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 (m)	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, Thes/niki	30/9/2001	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	entanglement in fishnet, mutilated LFF, scar around neck	51 days
Erato (m)	Myrsini, Tinos	4/12/2001	8/12/2001	39.1	36.6	7	exhaustion, fishhook swallowing	4 days
Thalassa (m)	Ikaria	12/1/2002	27/1/2002	31	30	3	fishing-line swallowing	15 days

CCL: Curved Carapace Length
CCW:Curved Carapace Width
f: female
m: male
LFF: left front flipper

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

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

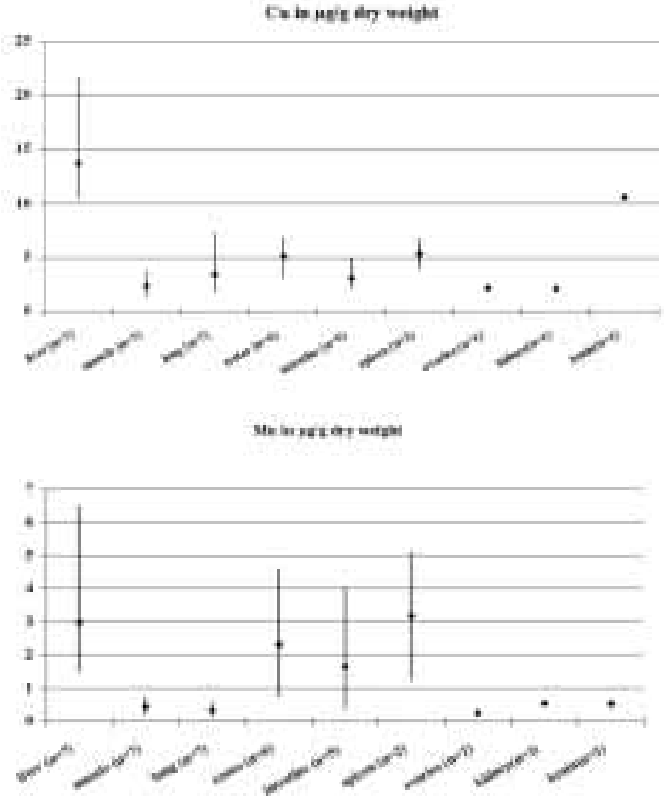

Fig. 1: Distribution of copper and manganese (in μg/g dry weight) in the tissues of the *Caretta caretta* individuals examined (mean and min-max values).

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

Table 4
Copper and manganese concentrations (μg/g wet weight) in tissues of Caretta caretta and Common dolphins from different locations.

Species	Location	Tissue n=samples	Copper			Manganese			Reference
			Mean	Stdev	Range	Mean	Stdev	Range	
Caretta caretta	Japan	Liver (n=6)	17.7	8.93	NA	2.18	0.40	NA	Sakai et al., 2000a
Caretta caretta	Japan	Liver (n=7)	17.9	8.17	6.47-33.9	2.07	0.50	1.44-2.94	Sakai et al., 1995
Caretta caretta	France	Liver (n=7)	8.25	6.59	2.32-20.9	NA	NA	NA	Caurant et al., 1999
Caretta caretta	**Greece**	**Liver (n=7)**	**7.39**	**2.28**	**4.51-11.08**	**1.62**	**1.03**	**0.85-3.96**	**present study**
Common dolphin	Portugal	Liver (n=24)	5.73	0.28	nd-9.01	3.07	0.44	nd-9.12	Zhou et al., 2001
Caretta caretta	Japan	Muscle (n=6)	0.81	0.28	NA	0.28	0.11	NA	Sakai et al., 2000a
Caretta caretta	Japan	Muscle (n=7)	0.83	0.26	053-1.28	0.30	0.12	0.13-0.45	Sakai et al., 1995
Caretta caretta	France	Muscle (n=7)	0.73	0.45	0.34-2.23	NA	NA	NA	Caurant et al., 1999
Caretta caretta	**Greece**	**Muscle (n=7)**	**1.14**	**0.38**	**1.78-0.58**	**0.22**	**0.10**	**0.09-0.44**	**present study**
Common dolphin	Portugal	Muscle (n=24)	2.04	0.48	nd-12.4	nd	nd	nd	Zhou et al., 2001
Caretta caretta	Japan	Lung (n=6)	0.54	0.10	NA	0.12	0.08	NA	Sakai et al., 2000a
Caretta caretta	**Greece**	**Lung (n=7)**	**1.35**	**0.72**	**0.69-2.00**	**0.13**	**0.05**	**0.05-0.22**	**present study**
Caretta caretta	Japan	Intestine (n=6)	0.71	0.23	NA	0.51	0.29	NA	Sakai et al., 2000a
Caretta caretta	**Greece**	**Intestine (n=5)**	**1.40**	**0.60**	**0.98-2.17**	**0.75**	**0.77**	**0.02-1.79**	**present study**
Caretta caretta	Japan	Spleen (n=6)	0.70	0.07	NA	0.37	0.38	NA	Sakai et al., 2000a
Caretta caretta	**Greece**	**Spleen (n=2)**	**-**	**-**	**1.68-2.00**	**-**	**-**	**0.28-1.86**	**present study**

NA: Not Available, nd: not detected

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 μg/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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