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Fulvio Maffucci, Florence Caurant, Paco Bustamante, Flegra Bentivegna

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1 **Trace element (Cd, Cu, Hg, Se, Zn) accumulation and tissue distribution in**
2 **loggerhead turtles (*Caretta caretta*) from the Western Mediterranean Sea (southern**
3 **Italy)**

4 Maffucci F. 1,*, Caurant F.2, Bustamante P.2, Bentivegna F.

5 1 Stazione Zoologica “Anton Dohrn”, Villa Comunale I, 80121, Napoli, Italy
6 2 Laboratoire de Biologie et Environnement Marins, FRE 2727 du CNRS,
7 Université de La Rochelle, 22, Avenue Michel Crépeau, 17042 La Rochelle Cedex,
8 France

9 * corresponding author. Tel.: +39-081-5323-400; fax +39-081-5833-294

10 E-mail address: maffucci@szn.it (F. Maffucci).

Abstract

Cadmium (Cd), copper (Cu), mercury (Hg), selenium (Se) and zinc (Zn) were determined in the liver, kidney and muscle of 29 loggerhead turtles, *Caretta caretta*, from the South Tyrrhenian Sea (Western Mediterranean). Cd and Hg exhibited a wider range of concentration in the examined tissues than Cu, Se and Zn. For all the elements analysed, no significant differences ($p > 0.05$) were detected between males and females. Trace element concentrations were not influenced by the size of the specimen except Se in the liver, which was negatively correlated with the curved carapace length ($p < 0.001$). The distribution of the analysed elements was comparable to that reported in other marine vertebrates. Thus, the muscles generally displayed the lowest trace element burdens, with the exception of Zn reaching elevated concentrations as high as $176 \mu\text{g g}^{-1}$ dry wt. Kidneys displayed the highest Cd and Se mean concentrations (57.2 ± 34.6 and $15.5 \pm 9.1 \mu\text{g g}^{-1}$ dry weight, respectively), while liver exhibited the highest Cu and Hg values (37.3 ± 8.7 and $1.1 \pm 1.7 \mu\text{g g}^{-1}$ dry weight, respectively). Whatever the tissue considered, toxic elements had elevated coefficients of variation (i.e. from 60% to 177%) compared to the essential ones (i.e. from 14% to 65%), which is a consequence of homeostatic processes for Cu, Se and Zn. Globally, the concentrations of Hg remained low in all the considered tissues, which might be the result of low trophic level in sea turtles. In contrast, the diet of loggerhead turtles would result in a significant exposure to Cd. Highly significant correlations between Cd and Cu and Zn in the liver and kidney suggest that efficient detoxification processes involving MTs occur to prevent Cd toxicity in loggerhead turtles.

Keywords: Bioaccumulation, Trophic transfer, Heavy metals, Marine vertebrates, Sea turtles, Tyrrhenian Sea.

Introduction

Trace elements are natural components of rocks and soil and enter the marine environment as a consequence of weathering and erosion (Garrett, 2000). Many of them are biologically essential but all have the potential to be toxic to biota above a threshold concentration (O'Shea and Geraci, 1999). Following industrialization, unnatural quantities of such elements have been released, and continue to be released into the sea altering the natural biological equilibrium (Haynes and Johnson, 2000). Therefore some concern has arisen regarding their possible adverse effects on marine wildlife, particularly on those long living species such as marine mammals, seabirds and sea turtles, which have the potential to accumulate these contaminants (Caurant et al., 1999; Anan et al., 2001). Although the first two taxa have been thoroughly studied, only a limited number of papers on trace element accumulation in sea turtles have been published to date (see the review of Storelli and Marcotrigliano, 2003). Because of their solitary life style, duration of the pelagic phase and long lasting apnoeas, sea turtles are in fact among the most difficult marine animals to assess (Witherington, 2003).

Two of the seven species of sea turtles are known to reproduce regularly in the Mediterranean Sea, the loggerhead turtle, *Caretta caretta*, and the green turtle, *Chelonia mydas* (Groombridge, 1990). The former is the most abundant and extends its distribution to the whole basin with Western Mediterranean waters providing some of the most important feeding grounds (Margalitoulis et al., 2003). The neritic juvenile stage and adult foraging stage occur in the neritic zone (Bolten, 2003). Turtles feed primarily on sessile or slowly moving benthic preys (Mortimer, 1995;

Bentivegna et al., 2001) although they do capture organisms throughout the water column (Bolten, 2003).

The loggerhead turtle is currently classified as “vulnerable” by IUCN (International Union for the Conservation of Nature and Natural Resources), but it has been suggested to be considered as “endangered” in the Mediterranean Sea (Broderick and Godley, 1996).

Because of the slow renewal of its waters, the large concentrations of human populations along its coasts, the intense maritime activities and the presence of natural sources of pollutants (Bacci, 1989), levels of contaminants in the Mediterranean sea are considered to be relatively high (Turley, 1999). For example several studies on different taxa revealed an enhancement of Hg in cephalopods, fish or dolphins compared to other areas (Renzoni et al. 1973; Bernhard, 1988; André et al., 1991). However the paucity of data regarding trace element burdens in loggerhead turtles from the Mediterranean Sea (Godley et al., 1999, Storelli et al., 1998ab) makes difficult the evaluation of accumulation levels and the comparison among different sites.

The primary objective of this study was therefore to determine the concentrations of five trace elements, Cd, Cu, Hg, Se, and Zn, in the tissues of loggerhead turtles from the Western Mediterranean Sea and to compare these data with those reported from other locations. Secondly, the influence of sex and size on trace element concentrations was tested. Correlations between the elements were determined in order to investigate physiological disorders and the hazard that these pollutants pose to loggerhead turtles survival were discussed.

Materials and methods

Sampling and sample preparation

Tissue samples were taken during necropsies of 29 loggerhead turtles, which stranded dead along the South Tyrrhenian coasts (Western Mediterranean) between March 2000 and May 2001 (Fig. 1). The curved carapace length (CCL), measured to the nearest 1 cm using a flexible meter tape, ranged from 37 to 82 cm. The necropsies were undertaken within 24 hours after the detection of the carcass. Liver, kidney, and pectoral muscle were sampled when possible in a minimum quantity of 20 g and stored in plastic bags to prevent from any contamination. The samples were frozen at -20 °C until the chemical analysis. The sex was determined via visual examination of the gonads. The CCL, sex, sampled tissue and the sampling date of each turtle are shown in Table 1. Tissue samples were freeze dried at - 60 °C and 6 mbar. Fresh and dry weights were determined and the water content, expressed as a percentage of fresh weight was calculated.

Analytical procedures

Approx. 200-300 mg of each homogenized dry sample was heated with supra-pure nitric acid at 60 °C until the solution was clear. Then, the residues were completed to 10 ml with ultrapure Milli-Q quality water. All the elements except Hg were determined by Flame or Graphite Furnace Atomic Absorption Spectrophotometry (AAS) with a Varian spectrophotometer Spectra 250 Plus. Hg analysis was carried out with an Advanced Mercury Analyser spectrophotometer, ALTEC AMA 254, which does not require an acid-digestion of the samples. Aliquots ranging from 10 to 50 mg of dried samples were directly analysed after they had been inserted into the oven of the apparatus. After drying, the samples were heated under oxygen atmosphere for 3 minutes and Hg was subsequently amalgamated on a gold-net. Then the net was heated after a 45 sec. waiting time to liberate the collected Hg, which was measured by AAS.

Standards of dogfish liver DOLT-2 of the NRCC (National Research Council of Canada) were analysed as analytical quality control using the same procedure as the samples. Recoveries of all elements ranged from 93% to 110%. Trace element concentrations are presented as $\mu\text{g g}^{-1}$ of tissue on dry weight (dwt) basis.

Data analyses

Because the data were not normally distributed, statistical analyses of the data were performed by non parametric tests with Minitab 11.2 (Minitab Inc., Pennsylvania). The coefficients of variation (CV) were calculated. The Wilcoxon's test for matched pairs was used to detect differences in the accumulation of trace elements between sex and the Kruskal-Wallis test was used to evaluate the organotropism of each element. Spearman rank coefficients were calculated between pairs of elements in each tissue and between trace element concentrations and CCL. A p value of less than 0.05 was considered to indicate statistical significance (Fowler et al., 1998).

Results

Tissue concentrations

Cd, Cu, Hg, Se and Zn concentrations in the tissues of loggerhead turtles from the Tyrrhenian Sea are reported in Table 2. As no significant differences in trace element concentrations have been found between sexes and size (with the exception of hepatic Se which was negatively correlated with the CCL, Table 3), results in Table 2 were presented together. Trace element concentrations were generally low in muscle, except for Zn which exhibited the highest mean concentration in this tissue. Kidney displayed the highest Cd and Se concentrations, reaching up to 158 and 41.8

$\mu\text{g g}^{-1}$ dwt, respectively. Finally, the liver exhibited the highest Cu and Hg loads, reaching up 41.8 and 8.76 $\mu\text{g g}^{-1}$ dwt, respectively. Toxic elements (i.e. Cd and Hg) were separated from those essential (i.e. Cu, Se, and Zn) according to their coefficients of variation of the concentrations (CV). Indeed, CV range from 60 to 177% for Cd and Hg while they remained lower than 65% for Cu, Se and Zn (Table 2). The mean water content was 68%, 76%, 79% for liver, kidney and muscle, respectively.

Trace element correlations

Table 3 shows the correlations between trace elements within the three tissues. In kidney, Zn was correlated with Cd ($p < 0.001$), Hg ($p < 0.001$) and Se ($p < 0.05$) (Table 3). Renal Hg concentrations were also positively correlated to Cu ($p < 0.05$) and to Cd ($p < 0.05$) (Table 3). In liver, Cd concentrations were correlated to Hg ($p < 0.05$) and Zn ($p < 0.001$), (Table 3). Only hepatic Se was correlated with the CCL ($p < 0.001$) (Table 3).

Discussion

Sea turtles can achieve life span greater than 50 years, and have a potential to bioaccumulate heavy metals and pesticides (Lutcavage et al., 1997). In the costal zone, chronic pollution from industrial, agricultural wastes, and urban runoff constitutes a threat to sea turtles (National Research Council, 1990). Unfortunately, there is little information about this problem. The present results showed that although the Mediterranean Sea is subjected to relative high inputs of pollutants (Kuetting, 1994, Turley, 1999), trace element concentrations in specimens from the Tyrrhenian Sea (South Western Mediterranean) are comparable or even lower than

those determined in the same species from other areas (Table 4). For comparative purposes, data which were originally presented on a wet weight basis were converted to approximate dry weights using the mean water content of each tissue determined in this study (see Results section). In general Cu, Se, Zn concentrations in the tissues of loggerhead turtles did not vary much among sites (Table 4). Consistently, the CV of these elements were the lowest found in all the analysed tissues (Table 1). Therefore it appears that loggerhead turtles control Cu, Se, and Zn concentrations through homeostatic processes in a balance between metabolic requirements and prevention against toxic effects. Consequently, human activity is not likely to be a factor in the accumulation of these elements. On the contrary geographical area influences Cd and Hg concentrations especially in those organs like liver and kidney, which are known to play a main role in their long term accumulation (Table 4). The high CV found (Table 2) imply that Cd and Hg levels are not actively controlled by turtles and should change according to the level of exposure. In this context it is noteworthy that Hg concentrations in all specimens analysed remained very low for an area supposed to have natural mercury enrichment (Renzoni et al., 1973; Bacci, 1989; André et al., 1991; Capelli et al., 2000). More generally, loggerhead turtles do not accumulate Hg to such a great extent (Table 4) as other long living marine vertebrates (Caurant et al., 1994; Cardellicchio et al., 2002). Since this metal is known to biomagnify in the marine food web the absence of long term Hg accumulation suggests that this species, which is an opportunistic predator (Tomas et al., 2002), feeds constantly on preys of low trophic level. This is confirmed by investigations of the stomach and intestine contents of loggerhead turtles in the study area, which revealed that their diet is composed mainly of molluscs and crustaceans (Bentivegna et al., 2001). Such diet involves only few bioaccumulative steps and generally displays low organo-Hg contents compared to a fish based diet (Cappon &

1 Smith, 1982).

2 Cd mean concentrations were comparable within the Mediterranean Sea but
3 lower than those found in other ocean locations (Table 4). However this element
4 which contrary to mercury does not biomagnify (Gray, 2002), accumulates at levels
5 comparable to those reported for other long living marine vertebrates (Caurant et al.,
6 1994). In marine mammals or sea birds, such concentrations (and also higher) are
7 often encountered in animals feeding mainly on squid which are considered to be an
8 important vector of this element to top marine predator (Bustamante et al. 1998).
9 Also loggerhead turtles are most likely to take up Cd via their diet but the paucity of
10 data about the total Cd content and its physico-chemical form within the prey tissues
11 prevents from any final statement.

12 Because no reliable age determination method exists for sea turtle (Bjorndal
13 et al., 1998), we used the curved carapace length (CCL) to evaluate growth related
14 variations in trace element concentrations. Although we analysed the so far largest
15 sample of loggerhead turtles, we did not detect any clear growth related trends in
16 trace element accumulation, the only exception being Se (Table 3). This was
17 surprising, since elements such as Cd and Hg are expected to accumulate with age
18 because of their long biological half life (Gray, 2002). However such trends were
19 previously reported in both green (*Chelonia mydas*) and hawksbill turtles
20 (*Eretmochelys imbricata*) (Gordon et al., 1998; Sakai, 2000b; Anan et al., 2001).
21 Consequently we believe that the size effect in our sample was concealed by the
22 unequal distribution of specimens throughout the size classes, and by the absence of
23 young and very old turtles (CCL < 35 cm and CCL > 82 cm). The early juvenile
24 loggerhead turtles foraging in open ocean pelagic habitats (Bjorndal, 1997) may in
25 fact experience different trace element exposure than older animals which feed on
26 benthic preys.

1 The results of this study show that sex has no significant influence on trace
2 element concentrations in loggerhead turtles. Although no other study has analysed
3 such dependence in this species, no gender differences in trace element
4 concentrations were also observed by Anan et al. (2001) in both green and hawksbill
5 turtles. It might be probable that sexual differences in feeding rates, metabolism or
6 growth rates are reduced in these species.

7 Overall, no particularly high concentrations of trace element in loggerhead
8 turtles have been found during this study. This included Hg, one of the most
9 dangerous marine pollutants, which has been found at very low levels compared to
10 those reported in other long living marine organisms. Although loggerhead turtles
11 show high hepatic Cu concentrations, several authors agreed that these levels are
12 physiologically controlled (Sakai et al., 2000a; Storelli and Marcotrigliano, 2003) as
13 further suggested by the low CV found in the present study (Table 2). Therefore Cu
14 is not likely to affect the health of these endangered animals. The only exception may
15 be the very toxic Cd, which accumulates at relatively high concentrations compared
16 to those reported for other marine vertebrates (Nicholson and Osborn, 1983; Caurant
17 and Amiard-Triquet, 1995; Elinder and Järup, 1996; Storelli and Marcotrigliano,
18 2003). However the significant relationship between Cd and Zn in both liver and
19 kidney (Table 3) suggests the involvement of metallothionein proteins (MTs) in the
20 prevention of Cd toxic effects (WHO, 1992 ; Vogiatzis and Loumbourdis, 1998;
21 ASTDR, 1999a). The presence of these molecules has been recently verified in
22 loggerhead turtle (Anan et al., 2002). Further studies are necessary to evaluate
23 physiological effects of Cd on loggerhead turtles and the potential tolerance to this
24 toxic element via MTs induction because disturbance due to a chronic exposure are
25 expected to happen at a lower concentrations than the toxic limit.

Conclusion

The loggerhead turtles from the Tyrrhenian Sea displayed similar concentrations of trace elements to those reported from other areas. Surprisingly, the Hg concentrations in their tissues remained very low even in the biggest ones (up to 82 cm of length) for an area supposed to be largely contaminated. This result confirms that the loggerhead turtles feed constantly on low trophic level preys, as revealed by stomach content analysis. However, these prey probably accounted for a significant Cd exposure, which is accumulated in relatively high amounts in the kidney and liver. Highly significant correlations between Cd and Cu and Zn in the liver and kidney suggest that efficient detoxification processes involving MTs occur to prevent Cd toxicity in loggerhead turtles.

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1 Table 1. Size (curved carapace length), sex and tissue sampled of 29 loggerhead turtles found dead
 2 on the South Tyrrhenian coast of Italy between 2000 and 2001. (K=kidney, L=liver, M=muscle,
 3 ND=not determined)

Specimen	Sex	Size (cm)	Tissue	Date of sampling
1	♀	75	K, L, M	24-05-00
2	♂	45	K, L, M	26-05-00
3	ND	ND	M	05-06-00
4	ND	71	K, L, M	11-06-00
5	♀	58	K, L, M	12-06-00
6	♂	54	L, M	18-06-00
7	♀	44	K	23-06-00
8	♂	43	L, M	27-07-00
9	ND	63	L, M	07-08-00
10	♀	71	L, M	"
11	♂	64	K, L, M	10-08-00
12	♂	57	K, M	11-08-00
13	♂	62	K, L, M	16-08-00
14	♀	62	K, L	21-08-00
15	♀	71	M	26-08-00
16	♂	53	L, M	01-09-00
17	ND	ND	K, L, M	16-09-00
18	♀	67	K, L, M	22-09-00
19	♀	58	L, M	03-10-00
20	♂	ND	K, L, M	04-10-00
21	♀	37	K, L, M	20-10-00
22	ND	ND	M	03-11-00
23	♂	59	K, L, M	20-11-00
24	♀	70	K, L	"
25	♀	77	K, L, M	29-11-00
26	ND	ND	K, M	03-01-01
27	♂	82	K, L, M	04-01-01
28	♀	51	K, M	16-01-01
29	♀	73	K, L, M	19-03-01

1 Table 2. Trace element concentrations ($\mu\text{g g}^{-1}$ dwt) in the tissues of loggerhead turtles from the
 2 South Tyrrhenean coast of Italy. , n: number of samples, CV: coefficient of variation (%).

Elements	Liver			Kidney			Muscle		
	n	Mean $\pm$ SD	Range (CV)	n	Mean $\pm$ SD	Range (CV)	n	Mean $\pm$ SD	Range (CV)
Cd	14	19.3 ± 34.2	1.6-114 (177)	19	57.2 ± 34.6	10.9-158 (60)	26	0.20 ± 0.20	0.06-0.78 (84)
Cu	14	37.3 ± 8.7	9.4-41.8 (23)	19	2.6 ± 0.7	1.7-4.7 (26)	26	2.7 ± 1.4	0.8-7.0 (50)
Hg	22	1.10 ± 1.70	0.42-8.76 (153)	20	0.90 ± 0.70	0.37-3.41 (71)	26	0.40 ± 0.30	0.14-1.92 (77)
Se	22	9.8 ± 5.3	1.0-24.9 (55)	21	15.5 ± 9.1	4.5-41.8 (59)	26	11.2 ± 4.9	4.0-24.1 (44)
Zn	14	66.0 ± 42.7	23.8-178 (65)	21	97.0 ± 31.7	62.4- 206 (37)	24	$107.0 \pm$	76.4-177 (24)

3

4 Table 3. Spearman correlations between trace elements within the tissues of the loggerhead turtles
 5 *Caretta caretta* from the South Tyrrhenian coast of Italy. Not underlined: $p < 0.05$; underlined: $p <$
 6 0.001. CCL: curve carapace length.

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Elements	Liver	Kidney	Muscle
Cd	<u>+Zn</u> , +Hg	<u>+Zn</u> , +Hg	+ Se
Cu		+ Hg	
Hg	+Cd,	+Cd, +Cu, <u>+Zn</u> ,	
Se	<u>-CCL</u>	+ Zn	+ Cd
Zn	<u>+Cd</u> ,	<u>+Cd</u> , <u>+Hg</u> , +Se	

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Table 4. Reported trace element concentrations ($\mu\text{g g}^{-1}$ dwt) in the tissues of loggerhead turtles from different locations. n : number of samples.
Values reported in wet weight were converted to dry weight using the mean water content as determined in present study

Location	Cd			Cu			Hg			Se			Zn			Reference
	n	Mean ± SD	Range	n	Mean ± SD	Range	n	Mean ± SD	Range	n	Mean ± SD	Range	n	Mean ± SD	Range	
Liver																
Australia	8	51.2 ± 10.3	22.8-110.0	-			6	0.05 ± 0.02	0.0-0.10	6	6.9 ± 0.6	4.44-8.44	5	71.2 ± 9.4	42.8-102	Gordon et al. 1998
Cyprus*	4	8.6	5.1-13.0	-			5	2.41	0.82-7.50	-			-			Godley et al. 1999
Japan	7	29.0 ± 10.3	17.7-45.6	7	55.9 ± 25.5	20.2-105.9	7	4.44 ± 9.15	0.79-25.5	-			7	87.2 ± 13.6	72.5-109.7	Sakai et al. 1995
Japan	7	30.4 ± 10.5		7	55.3 ± 27.9		7	?					7	87.8 ± 14.6		Sakai et al., 2000
West Italy	14	19.3 ± 34.2	1.6-113.0	14	37.3 ± 8.7	9.4-41.8	22	1.1 ± 1.7	0.42-8.76	22	9.8 ± 5.3	1.0-24.9	14	66.0 ± 42.7	23.8-178.0	Present study
East Italy	12	7.60 ± 6.05	3.06-20.23	-			12	1.68 ± 1.04	0.35-3.72	12	15.88 ± 7.40	2.12-27.44	-			Storelli et al. 1998
West France	7	8.1 ± 12.9	0.9-36.9	7	25.8 ± 20.6	7.2-65.3	-						7	78.1 ± 29.7	45.3-120.0	Caurant et al. 1999
Kidney																
Australia	3	117.9 ± 23.7	47.5-164.2	-			3	0.19 ± 0.05	0.14-0.28	3	6.3 ± 0.6	5.32-7.41	5	76.3 ± 3.75	69.6-88.7	Gordon et al. 1998
Cyprus*	2	30.50	18.80-42.20	-			2	0.47	0.13-0.80	-			-			Godley et al. 1999
Japan	7	164.2 ± 67.5	75.4-235.4	4	5.42 ± 0.80	4.12-6.49	7	1.02 ± 0.54	0.16-1.84	-			7	107.5 ± 17.4	80.1-126.7	Sakai et al. 1995
Japan	7	159.6 ± 72.91		7	5.42 ± 0.90		7	?								Sakai et al., 2000
West Italy	19	57.2 ± 34.6	10.9-158	19	2.6 ± 0.7	1.7-4.7	20	0.9 ± 0.7	0.37-3.41	21	15.5 ± 9.1	4.5-41.8	21	97.0 ± 31.7	62.4-206.0	Present study
East Italy	12	24.23 ± 21.407	0.39-64.00	-			12	0.65 ± 0.34	0.30-1.53	12	10.33 ± 3.25	5.73-15.57	-			Storelli et al. 1998
West France	5	55.4 ± 56.6	6.81-148.75	5	9.20 ± 1.92	7.32-11.79	-			-			5	98.3 ± 28.7	68.7-140.8	Caurant et al. 1999
Muscle																
Cyprus*	4	0.57	0.30-1.43	-			7	0.48	Bdl-1.78	-			-			Godley et al. 1999
Japan	7	0.29 ± 0.12	0.19-0.56	7	3.95 ± 1.23	2.52-6.02	7	0.51 ± 0.23	0.26-0.90	-			7	115.2 ± 18.1	92.9-147.6	Sakai et al. 1995
Japan	7	0.31 ± 0.13		7	3.87 ± 1.31		7	?					7	119.0 ± 16.61		Sakai et al., 2000
West Italy	26	0.2 ± 0.2	0.06-0.78	26	2.7 ± 1.4	0.8-7.0	26	0.4 ± 0.3	0.14-1.92	26	11.2 ± 4.9	4.0-24.1	24	107.0 ± 26.1	76.4-177.0	Present study
East Italy	12	0.55 ± 0.63	0.09-2.21	-			12	0.69 ± 0.46	0.17-1.81	12	10.81 ± 3.25	6.51-15.45	-			Storelli et al. 1998
West France	21	0.38 ± 0.23	0.02-0.87	21	3.47 ± 2.14	1.62-10.61	-			-			21	93.3 ± 27.1	58.1-172.8	Caurant et al. 1999

*= Concentrations of metals reported as median.

- 1 Figure legends:
- 2 Fig.1: Sampling location of the 29 loggerhead turtles (*Caretta caretta*) analysed.

1

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Fig 3

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