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Bioaccumulation of Cadmium, Copper and Zinc in some Tissues of Three Species of Marine Turtles Stranded Along the French Atlantic Coasts

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Cadmium, copper and zinc have been analysed in some tissues and organs of Loggerhead, Kemp's Ridley (only muscle for this species) and Leatherback turtles stranded along the Atlantic coasts of France. The pancreas analysed only in Leatherback turtles exhibited the highest metal concentrations, which is very surprising for an organ which does not play a role in the detoxification processes. The distribution of these elements in kidney, liver and muscle were quite similar to that found in marine mammals or seabirds. Nevertheless, mean cadmium concentrations in the kidney were as high as 13.3 μg g^{-1} wet weight in the Loggerhead turtles and 30.3 μg g^{-1} wet weight in the Leatherback turtles. Such high concentrations in the Leatherback turtles have never been recorded before. The main source of cadmium for marine turtles is probably the food. The Leatherback turtles are known to feed mainly on jellyfish in this area. Ten times higher cadmium concentrations have been determined in jellyfish compared to fish. This would imply a greater exposure to cadmium for Leatherback turtles, which probably need to eat great quantities of jellyfish to cover their needs.

Keywords: cadmium; copper; zinc; bioaccumulation; sea turtles; Atlantic coasts of France.

Introduction

Sea turtles like marine mammals and seabirds have a long lifespan and occupy high trophic levels in the marine food web. Nevertheless, if numerous studies have been carried out on both last species showing their utility as biological indicators of chemical pollution (reviewed in Wagemann and Muir, 1984; Walsh, 1990; Furness, 1993; Aguilar and Borrell, 1997), studies on heavy metals or organic compounds bioaccumulation in sea turtles are very limited and use eggs more often than tissues and organs (Stoneburner *et al.*, 1980; Sakai *et al.*, 1995; Vazquez *et al.*, 1997). However, besides all the human activities involving mortality in sea turtles, that is consumption of meat, degradation of nesting beaches and feeding habitats, impact of fishing and shellfishing industries..., sea turtles are also probably affected by marine pollutants and there is a need for monitoring chemical pollutants in an effort to conserve their populations (Sakai *et al.*, 1995).

Five species of marine turtles have been recorded in the French Atlantic waters: the leatherback turtle *Dermochelys coriacea*, the green turtle *Chelonia mydas*, the loggerhead turtle *Caretta caretta*, the hawksbill turtle *Eretmochelys imbricata* and the Kemp's Ridley turtle *Lepidochelys kempii*. The leatherback turtle is frequently observed along the French Atlantic coasts especially in summer, with a maximum of records in the "*Pertuis charentais*" (Fig. 1). Leatherback turtles are known to travel thousands of miles annually around the ocean basins of the world and the origin of these individuals could be the French Guiana where is located the most important stock of the species in the world (Duguy, 1983). In 1995, a leatherback turtle ringed in French Guiana has been caught accidentally in the French waters and has confirmed this migration.

The individuals of loggerhead and Kemp's Ridley turtles found along the French Atlantic coasts are all juveniles as most of the specimens found in northern Europe. Brongersma (1972) suggested that these juvenile turtles are most probably carried from the Western Atlantic on the Gulf Stream and North Atlantic Current.

The purpose of this study was to assess the presence of cadmium, a toxic metal relatively abundant in the Gironde estuary (south limit of the "*Pertuis charentais*", see Fig. 1) (Boutier *et al.*, 1989), copper and zinc in different tissues of turtles stranded along these coasts.

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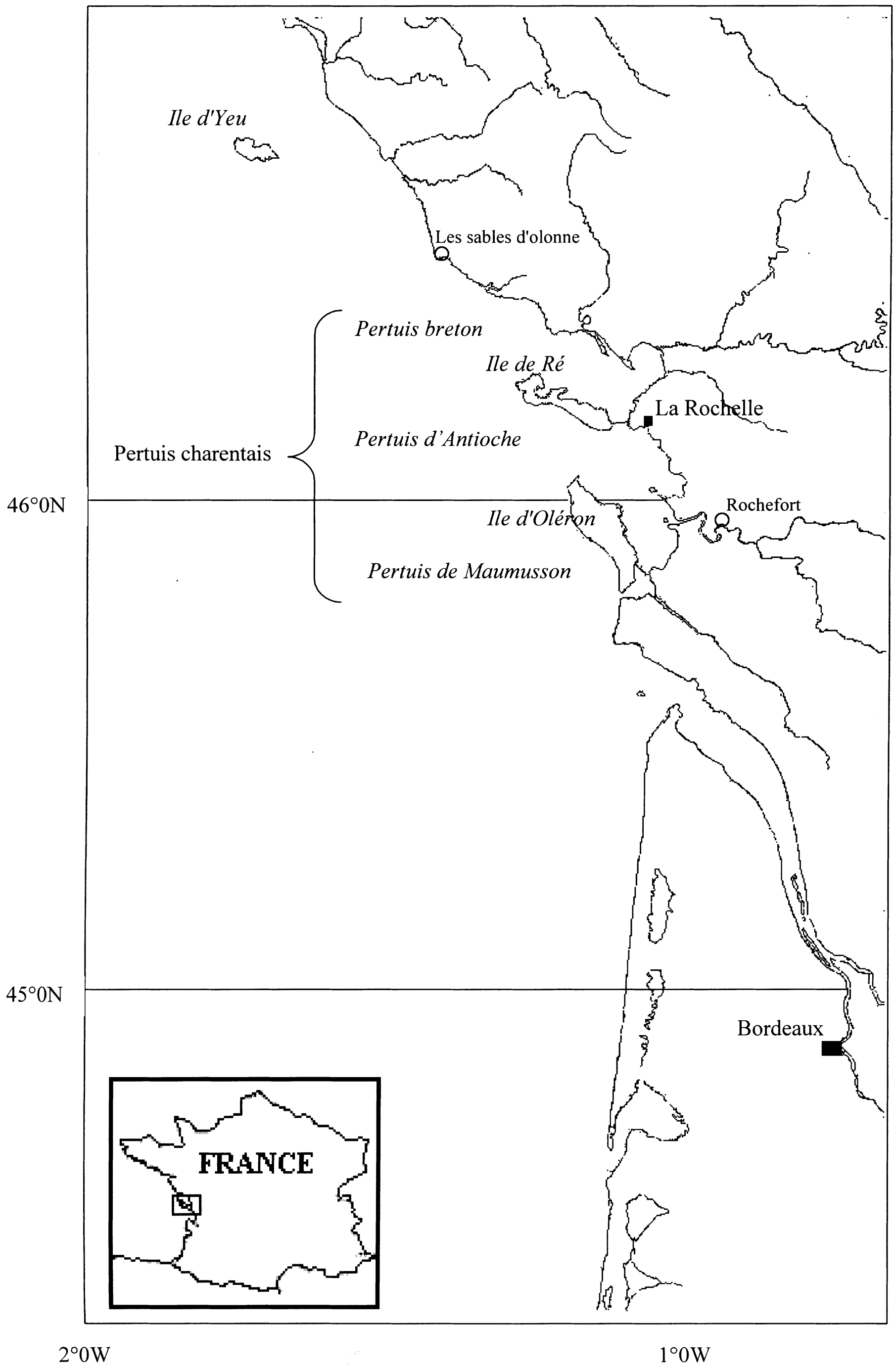

Fig. 1 Localization of all the "*Pertuis charentais*": the area of the stranded sea turtles.

Materials and Methods

Samples

Tissues samples (pectoral muscle, kidney and liver) from leatherback, loggerhead and Kemp's Ridley turtles have been collected from stranded specimens for this study. For two specimens of loggerhead and two specimens of leatherback turtles, numerous organs and tissues including heart, spleen, intestine, lungs and bladder have been sampled in addition to muscle, kidney and liver. The biometry of the individuals and the details of the sampling are shown in Table 1. All samples were stored at −20°C until chemical analysis.

Analytical procedure

Tissue samples were dried for several days at 60°C to constant weight. Tissues and organs were analysed for cadmium (Cd), copper (Cu) and zinc (Zn). Two aliquots of approx. 200 or 300 mg of each homogenized dry sample were heated with a mixture of 5 ml of supra-pure 65% nitric acid and 200 μl of supra-pure 70%

TABLE 1

Size (cm) and weight (kg) of loggerhead, leatherback and kemp's Ridley turtles, stranded on the Atlantic coasts of France (SCL: Standard Carapace Length).

	SCL	Weight
Caretta caretta		
(Loggerhead turtle, $n = 21$)		
Mean	29.4 ± 15.3	2.8 ± 2.2
Min–max	21.3 – 34.5	0.3 – 7.5
Lepidochelys kempii		
(Kemp's Ridley turtle, $n = 6$)		
Mean	25.8 ± 5.2	2.5 ± 1.5
Min–max	21.3 – 34.5	1.4 – 5.2
Dermochelys coriacea		
(Leatherback turtle, $n = 16$)		
Mean	145.7 ± 22.6	–
Min–max	115 – 188	–

perchloric acid at 80°C until the solution was clear. After evaporation, the residues were dissolved in 10 ml of 0.3 N supra-pure nitric acid. Cd, Cu and Zn were determined by Flame Atomic Absorption Spectrophotometry (AAS) with a Varian spectrophotometer Spectra 250 Plus with deuteriun background correction. Concentrations are expressed in μg g^{-1} wet weight. As analytical quality control, standards of the NRC Canada (dogfish liver DOLT-2) were analysed using the same procedure. Our results were in good agreement with the certified values. Measurements were also validated by the IAEA Intercalibration Exercise (Coquery *et al.*, 1997).

Results

Organotropism

Cadmium, copper and zinc concentrations in different organs of two loggerhead and two leatherback turtles are shown in Figs. 2 and 3.

Pancreas has been analysed only in the leatherback turtles and exhibited the highest concentrations in the case of the three elements. Concentrations were always

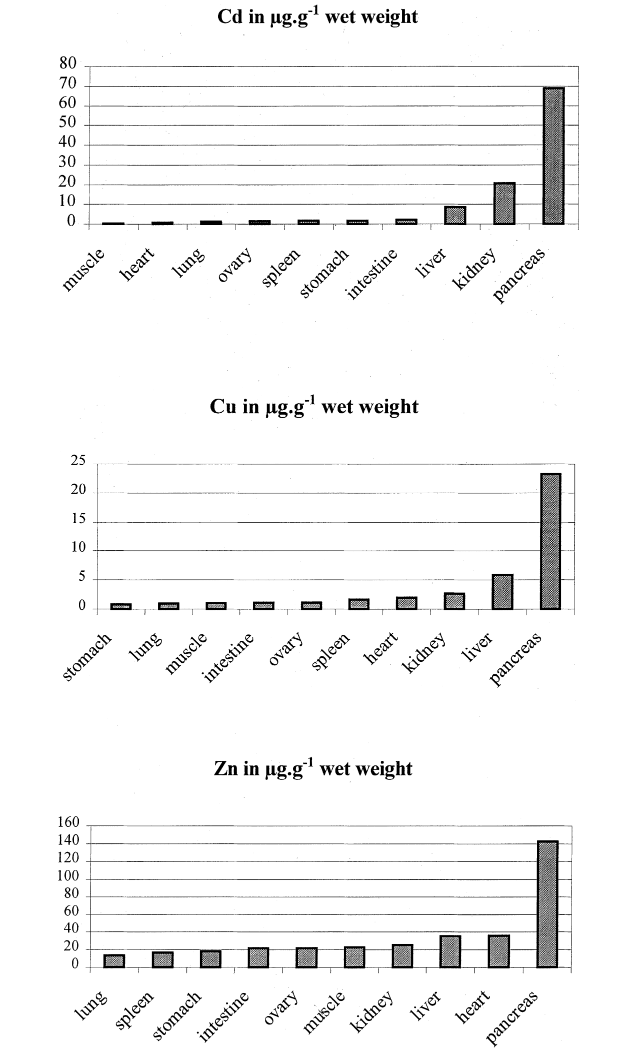

Fig. 2 Distribution of cadmium, copper and zinc concentrations (in μg g^{-1} wet weight) in some organs and tissues of two individuals of leatherback turtle stranded along the French Atlantic coasts.

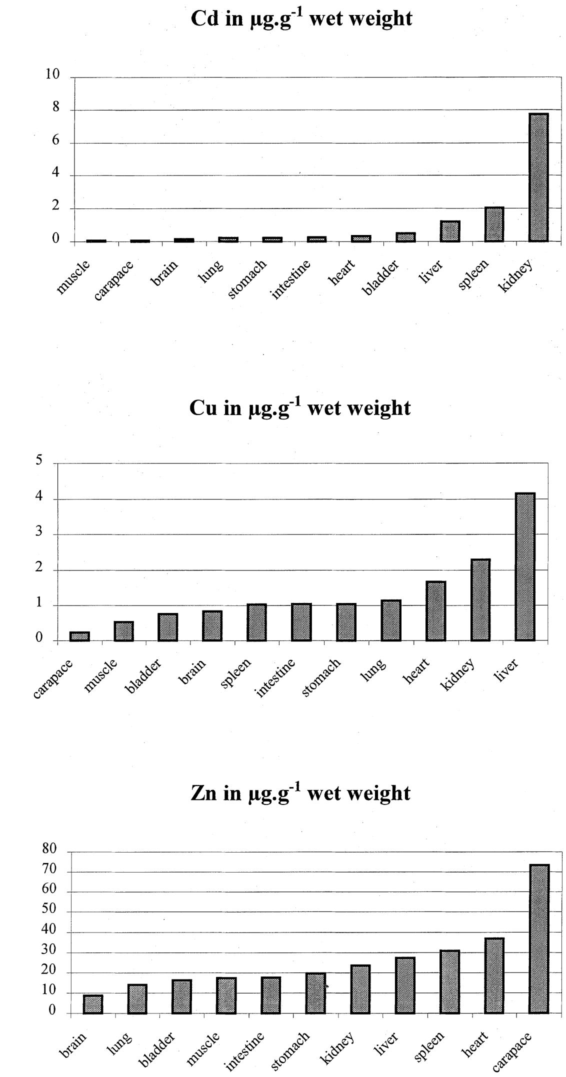

Fig. 3 Distribution of cadmium, copper and zinc concentrations (in μg g^{-1} wet weight) in some organs and tissues of two individuals of loggerhed turtle, stranded along the French Atlantic coasts.

much higher than in the other organs with 68.8 µg Cd g^{-1}, 23.2 µg Cu g^{-1} and 142.3 µg Zn g^{-1}.

In both species, kidney and liver exhibited high cadmium and copper concentrations: 20.5 and 7.72 µg g^{-1} for cadmium in the kidney in leatherback and loggerhead turtles, respectively ; 2.65 and 2.29 µg g^{-1} for copper in the kidney in leatherback and loggerhead turtles, respectively; 8.4 and 1.22 µg g^{-1} for cadmium in the liver in leatherback and loggerhead turtles, respectively; 5.84 and 4.14 µg g^{-1} for copper in the liver in leatherback and loggerhead turtles, respectively. On the other hand, the spleen accumulated cadmium in a different proportion in both species. It was the second organ for cadmium accumulation in the loggerhead turtle after the kidney and only the fifth one in the leatherback turtles with concentration much lower than in the kidney and the liver.

Concerning zinc, except the cases of the pancreas in the leatherback turtles and the carapace in the loggerhead turtles, the concentrations were relatively homogeneous in the organs. In both species, heart exhibited the highest concentrations.

Bioaccumulation in all individuals

Muscle, liver and kidney are the tissues which have been analysed in nearly all the stranded individuals and metal concentrations are compared with other studies. Heavy metal concentrations in the muscle, liver and kidney of turtles are shown in Table 2.

The variability of the coefficients of variation reflected the individual variations typical of each element. Zn exhibited the lowest coefficients of variation (contained between 14% and 38%). It is an essential element for which the individual variations are known to be limited as a consequence of homeostasis processes. Nevertheless Cu, which is also an essential element, exhibited higher coefficients of variation than Zn, contained between 12.3% and 79.6%. The highest coefficients of variation were observed for Cd (contained between 57.1% and 159.7%), which is a toxic element. In loggerhead and leatherback turtles, kidney tissue exhibited the highest Cd concentrations whereas liver tissue exhibited the highest Cu concentrations. The most streaking feature of this study was the high Cd concentrations encountered in the leatherback turtles. In the three tissues including muscle, Cd concentrations were much higher in this species than in the loggerhead and Kemp's Ridley turtles.

Discussion

Similar distribution patterns of Cd and Cu have been reported in marine mammals or seabirds (Honda *et al.*, 1983; Wagemann and Muir, 1984; Wagemann *et al.*, 1990; Furness, 1993; Caurant *et al.*, 1994; Wenzel *et al.*, 1996; Debacker *et al.*, 1997), but the ratio between Cd in kidney and Cd in liver was much higher in turtles than in marine mammals or seabirds. Zinc concentrations in turtles were quite homogenous in the different tissues, compared to mammals. This would suggest a slight different organotropism of these trace elements in turtles compared to seabirds or marine mammals.

The pancreas exhibited high metal concentrations which have never been previously reported in marine vertebrates. This organ has been analysed in a study carried out by André *et al.* (1990) about cadmium con-

TABLE 2

Heavy metal concentrations (µg g^{-1} wet weight, mean ± standard deviation, min–max) in tissues of Loggerhead, Leatherback and Kemp's Ridley turtles from the Atlantic coasts of France.

	Cd	Cu	Zn
Liver			
Loggerhead turtle ($n = 7$)			
Mean	2.58 ± 4.12	8.25 ± 6.59	25.0 ± 9.5
Min–max	0.30 – 11.8	2.32 – 20.9	14.5 – 38.4
Leatherback turtle ($n = 18$)			
Mean	6.84 ± 3.66	8.61 ± 4.40	29.2 ± 4.1
Min-max	0.60 – 14.7	1.05 – 19.7	21.9 – 36.5
Kidney			
Loggerhead turtle ($n = 5$)			
Mean	13.3 ± 13.6	2.21 ± 0.46	23.6 ± 6.9
Min–max	1.68 – 35.7	1.76 – 2.83	16.5 – 33.8
Leatherback turtle ($n = 5$)			
Mean	30.3 ± 28.1	2.68 ± 0.33	25.7 ± 7.7
Min–max	8.47 – 62.0	2.36 – 3.02	18.5 – 33.8
Muscle			
Loggerhead turtle ($n = 21$)			
Mean	0.08 ± 0.05	0.73 ± 0.45	19.6 ± 5.7
Min–max	0.004 – 0.18	0.34 – 2.23	12.2 – 36.3
Leatherback turtle ($n = 16$)			
Mean	0.35 ± 0.20	0.95 ± 0.49	25.9 ± 5.9
Min–max	0.16 – 1.00	0.40 – 2.56	18.3 – 37.3
Kemp's Ridley turtle ($n = 6$)			
Mean	0.09 ± 0.09	0.98 ± 0.50	16.4 ± 3.3
Min–max	0.01 – 0.26	0.44 – 1.85	13.3 – 20.5

tamination of tissues of a delphinid species *Stenella attenuata*. Cd average concentration was 5.64 μg g^{-1}, much lower than in kidney (48.7 μg g^{-1}) or liver (8.7 μg g^{-1}). Nevertheless, in humans, cadmium is known to accumulate in the pancreas which can exhibit higher concentrations than in the liver because of a faster elimination in this last organ (Lauwerys, 1990).

Our data have been compared with loggerhead turtles from Japan (Sakai *et al*, 1995) and from the Adriatic Sea (Storelli *et al*., 1998), and green turtles from the Hawaiian Islands (Aguirre *et al*., 1994). Cu concentrations in the liver of green turtles were much higher than those of loggerhead and leatherback turtles, whereas they were comparable between the species in muscle and kidney. Again zinc concentrations were quite homogenous between species in the three organs (Tables 2 and 3). Although Cd concentrations exhibited a great individual variability, the mean concentrations were high in all the species, especially in the kidney (Tables 2 and 3), which appears to contain the highest levels. Nevertheless, concerning the leatherback turtle, the concentrations reported in a single individual killed in a net in Cardigan Bay (UK) were considerably lower than the concentrations found in our study (Table 3). Cd concentrations were outstandingly higher in the three organs of loggerhead and especially leatherback turtles of our study than in mammals of the same area. The mean Cd concentrations in 19 common dolphins (*Delphinus delphis*), stranded along the French Atlantic coasts were respectively 1.2 ± 1.58 μg g^{-1} wet weight in the liver, 4.18 ± 4.31 μg g^{-1} wet weight in the kidney and 0.02 ± 0.02 μg g^{-1} wet weight in the muscle (Caurant, unpublished data). Except in the case of Davenport and Wrench's study, the high Cd concentrations seem to be a common point in the three species of turtles studied whatever their origin was (Table 3).

Different ecological and biological factors control concentrations of trace elements in vertebrates. The age is known to be one of them, especially for toxic elements such as Cd whose concentrations are generally low at birth and accumulate with age. Skeletochronology – the use of incremental growth marks in the humerus to estimate age-has been used in several studies to assign ages to sea turtles (Zug *et al*., 1995; Parham and Zug, 1997). Nevertheless, this technique has recently been criticized by Bjorndal *et al*. (1998), who underline that the two assumptions upon with this technique is based: (1) growth marks in the humerus of sea turtles are laid annually; (2) there is a constant proportional allometry between radial growth of the humerus and longitudinal growth of the carapace-must be validated before being applied. Moreover, growth rates of turtles are quite variable within the same cohort and populations (Zug *et al*., 1995) and depend on the temperature, the food and are also probably different between wild or captive animals. Thus, there is a great difficulty in comparing

TABLE 3

Heavy metal concentrations (μg g^{-1} wet weight, mean ± standard deviation, min-max) in tissues of different turtles species from different areas (NA: Not Analysed)[a].

	Cd	Cu	Zn	Origin	Reference
Liver					
Loggerhead turtle ($n = 7$)					
Mean	9.29 ± 3.3	17.9 ± 8.17	27.9 ± 10.4	Japan	Sakai *et al*. (1995)
Min–max	5.66 – 14.6	6.47 – 33.9	24.2 – 35.1		
Loggerhead turtle ($n = 12$)					
Mean	2.24 ± 1.78	NA	NA	Adriatic Sea	Storelli *et al*. (1998)
Min–max	0.78 – 5.97				
Green turtle ($n = 13$)					
Mean	9.30 ± 8.58	87.6 ± 61.7	30.6 ± 10.4	Hawaiian Islands	Aguirre *et al*. (1994)
Min–max	0.39 – 26.0	1.30 – 189	15.1 – 45.8		
Leatherback turtle ($n = 1$)	0.22[a]	0.15 ± 0.04[a]	2.62 ± 0.15[a]	British Island	Davenport and Wrench (1990)
Kidney					
Loggerhead turtle ($n = 7$)					
Mean	39.4 ± 16.2	1.30 ± 0.20	25.8 ± 4.17	Japan	Sakai *et al*. (1995)
Min–max	18.1 – 56.5	0.99 – 1.56	19.2 – 30.4		
Loggerhead turtle ($n = 12$)					
Mean	7.52 ± 6.65	NA	NA	Adriatic Sea	Storelli *et al*. (1998)
Min–max	0.12 – 19.9				
Green turtle ($n = 12$)					
Mean	26.0 ± 20.2	3.60 ± 2.60	22.3 ± 7.2	Hawaiian Islands	Aguirre *et al*. (1994)
Min–max	4.77 – 70.2	1.10 – 10.5	12.5 – 38.1		
Muscle					
Loggerhead turtle ($n = 7$)					
Mean	0.062 ± 0.026	0.83 ± 0.26	24.2 ± 3.80	Japan	Sakai *et al*. (1995)
Min–max	0.041 – 0.117	0.531 – 1.28	19.5 – 31.0		
Loggerhead turtle ($n = 12$)					
Mean	0.14 ± 0.16	NA	NA	Adriatic Sea	Storelli *et al*. (1998)
Min–max	0.023 – 0.55				
Leatherback turtle ($n = 1$)	0.06[a]	0.26[a]	1.89[a]	British Island	Davenport and Wrench (1990)

[a] μg g^{-1} dry weight.

bioaccumulation of trace elements in turtles from different areas. Nevertheless Cd concentrations in this study seem all the more high since the loggerhead and Kemp's Ridley turtles are small juvenile individuals and loggerhead turtles for example can take 20–63 years to reach sexual maturity and live for 50 to more than 100 years (Parham and Zug, 1997). Concerning the leatherback turtles, a study about the nesting of this species in French Guiana has allowed to measure 834 females (Fretey, 1979). The mean straight-line carapace length was 1.67 m with a minimum of 1.35 m and a maximum of 1.89 m and the sexual maturity would be reached at 1.35 m. According this study, the specimens of our samples are mature individuals (Table 1) but no data allow to determine their age.

Food is probably the main source of exposure to heavy metals and other trace elements for marine vertebrates. In marine mammals or seabirds, such concentrations (and also much higher) are often encountered in animals feeding mainly on cephalopods which are known to accumulate Cd in higher levels than fish and are considered to be an important vector of this element to top marine predators (Bustamante *et al.*, 1998). Hatchling appears to be nearly omnivorous but the diets of adults are more specialized and differ among species-leatherback turtles feed on jellyfish and loggerhead turtles are mostly carnivorous (Bowen and Avise, 1995). A study carried out by Godley *et al.* (1997) suggests that loggerhead turtles in the eastern Mediterranean Sea do indeed feed upon benthic molluscs and crustacea, at shallow to moderate depths, from both rocky and sedimentary habitats. For both post-pelagic juveniles and adults of these species, study of prey items in other regions of the world have found the diet to be dominated by benthic molluscs, crustaceans and coelenterates (Godley *et al.*, 1997). Although such a study has not been done on the stranded individuals of this study, the post-mortem examinations very often revealed plastic fragments mixed with benthic preys in the stomach (Duguy *et al.*, 1998). These food items are not very specific and the high Cd concentrations found in sea turtles could also be the consequence of the metabolism or physiology of these species which would lead to a great accumulation of this element.

In temperate latitudes, the leatherback turtle is associated with swarms or jellyfish such as *Rhizostoma* or *Cyanea* (Davenport and Wrench, 1990). In the "*Pertuis charentais*" *Rhizostoma pulmo* is present in great quantities and the leatherback turtles have often been observed feeding on this species, and ingesting up to 10 individuals per day (Duron-Dufrenne, 1978; Duguy, 1983). This species is probably the unique air breathing vertebrate feeding mainly upon medusea. This constitutes a food chain which is very poorly studied and that difficulty explains such high Cd concentrations found in the liver and the kidney tissues of the species. Cd has been analysed in one jellyfish caught in the "*Pertuis charentais*". The concentration was 0.27 $\mu g\ g^{-1}$ dry weight, which is of the same order as the concentrations found in the plankton in this area (1.14 μg/g dry weight in the microzooplankton, 0.2 in the Mysidacae and 0.5 in the copepods, unpublished data), but 10 times higher than in fish. Moreover the energy metabolism of the leatherback turtles is unknown, but the energetic value of the jellyfish is probably very low. Thus the turtles would have to ingest a great quantity of jellyfish to cover their needs and this could imply a high exposure to Cd for the leatherback turtles, at least higher than for fish-eating vertebrates like some coastal dolphins.

Turtles are certainly very long-lived animals and would therefore appear to be very good indicators of the degree of contamination of the oceanic food web. However the data on concentrations of heavy metals in these species appear to be very difficult to interpret because the lack of information on the age of the individuals, especially in the case of leatherback turtles, and because generally too few individuals have been analysed to evaluate the bioaccumulation with age. In the future, the analysis of individuals coming from the French Guiana could allow to evaluate and discriminate the importance of age and food in the bioaccumulation of metals.

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