NOTE *Toxicology*

Relationships between Carapace Sizes and Plasma Major and Trace Element Status in Captive Hawksbill Sea Turtles (*Eretmochelys imbricata*)

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ABSTRACT. The aim of this study was to evaluate the relationships between carapace parameters as indicators of age and plasma elements in 25 captive hawksbill sea turtles. Particle-induced X-ray emission allowed detection of 23 trace and major elements. There were significant but weak correlations between the virtual carapace surface area and plasma bromide (r= −0.552, P<0.01), phosphorus (r=0.547, P<0.01), lead (r=−0.434, P<0.05) and strontium (r=0.599, P<0.01), while there were no significant correlations with other elements. These results suggest that major and trace plasma elements in captive sea turtles show almost no variation with carapace parameters, suggesting that the increase in plasma elements seen in wild sea turtles might be the result of marine pollution.

KEY WORDS: carapace size, hawksbill sea turtle, major elements, marine ecosystem, trace elements.

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Many chemical pollutants persist and can bioaccumulate in tissues. Because of their long life span of over 50 years and their higher trophic level in the marine food chain, some contaminants could reach toxic levels in sea turtles [2, 3, 19]. In this regard, sea turtles are considered of increasing interest as potential biomonitors for pollution in marine ecosystems [10]. Urban runoff contributes significant amounts of heavy metals into waters in which sea turtles spend a large portion of their life [5]. In consequence, knowledge about heavy metal and element burden in sea turtles is an important focal point to assess their potential impact on these endangered organisms.

In most studies on pollution in marine ecosystems using sea turtles, liver, kidney, muscle, heart and shell tissue samples are obtained during autopsy of animals collected from the beach or caught for commercial purposes [1, 12, 13, 18, 19]. Blood sampling is an excellent, relatively noninvasive method to establish reliable baseline values of element levels in healthy sea turtles [7, 11, 14]. Van de Merwe *et al.* [21] used blood samples to estimate the liver, kidney and muscle accumulation of elements in green sea turtles at found strong correlations between blood and tissue concentrations of As, Cd, Co, Hg and Se. Element levels found in blood samples can be used to study the processes of absorption, accumulation and circulation of metals and metalloids in blood and to correlate them to their bioavailability and potential toxicity [11]. It is necessary to evaluate physiological changes associated with aging before reaching conclusions about the accumulation of elements in the blood with time. To the authors' knowledge, comparative studies on the relationship between age and elements in plasma obtained from sea turtles from the same species and life environment have not yet been published.

It is known that the body weight or carapace parameters such as carapace length (CL) and width (CW) are reliable age determination methods for sea turtles [1–3, 16, 17, 19]. Páez-Osuna *et al.* [17] demonstrated that there was a significant correlation between carapace parameters and Hg in blood. Therefore, the present study was carried out to investigate the possible relationships between carapace parameters as indicators of age and plasma elements in 25 captive hawksbill sea turtles maintained under the same environmental and feeding conditions.

Twenty-five healthy and mature hawksbill sea turtles that were maintained in an outdoor sea pool at ambient temperature at the Okinawa Ocean Expo Research Center (OERC) were included in the study in the time period from March to August 2009. The animals were fed kibinago (*Spratelloides gracilis*), capelin (*Mallotus villosus*), and spear squid (*Loligo bleekeri*) and had free access to cabbage, Chinese cabbage and lettuce. This is a standard sea turtle diet at the OERC, where all animals involved in the study have been living for at least 14 years.

Blood samples (10 m*l*) were taken from the dorsal cervical sinus using a sterile plastic disposable syringe and needle and immediately placed in a heparinized tube. The plasma was separated by centrifugation and stored at −80°C until assay. After blood sampling, the turtles body weight (BW), carapace length (CL) and width (CW) were measured. The virtual carapace surface area (CSA, in cm^2) was estimated

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for an elliptical shape by means of the following equation:

$$CSA\ (cm^2) = \pi/4 \times CL \times CW.$$

The mean concentrations of elements in plasma were measured by particle-induced X-ray emission (PIXE) analysis at the Nishina Memorial Cyclotron Center (Iwate, Japan). The lower limit of detection for this assay is 10^{-5} μg/m*l* of plasma. A detailed description of the preparation, methodology and experimental setup is given elsewhere [4, 20].

Normally distributed data are reported as means ± standard deviations (SD) and non-normally distributed data are expressed as medians and ranges. Statistical analyses were performed using the SPSS Statistics v.20 commercial software package (IBM Corpolation, Somers, NY, U.S.A.). The correlations between BW and carapace parameters and between carapace parameters and each element level in plasma were established by use of the generalized linear model (GLM). When significant correlations were observed between carapace parameters and a given element, the element was also correlated with other elements by using the GLM. Linear regression model analysis was also performed. Plasma element concentrations below the detection limit were statistically taken as 10^{-5} μg/m*l*. The significance level was set at $P<0.05$.

The mean ± SD (and min–max) BW, CL, CW and CSA were 48.3 ± 19.9 kg (27.9–88.0 kg), 73.1 ± 7.3 cm (61.0–88.0 cm), 55.3 ± 7.7 cm (46.4–75.4 cm) and 3205.8 ± 749.9 cm^2 (2221.9–4673.1 cm^2), respectively. The CL, CW and CSA were significantly positively correlated with BW, as evidenced by the following regression equations and correlation coefficients obtained by regression analysis:

$$\begin{aligned} BW\ (kg) &= 2.569 \times CL\ (cm) - 139.480\ (r=0.947, P<0.001) \\ &= 2.267 \times CW\ (cm) - 77.050\ (r=0.878, P<0.001) \\ &= 0.025 \times CSA\ (cm^2) - 32.227\ (r=0.946, P<0.001). \end{aligned}$$

These parameters can be easily measured, and because of their high correlation with BW, they can be useful indicators of marine pollution effects on sea turtles. These results support many field studies [1–3, 12, 13, 16–20] in which the carapace parameters have been used to correlate BW and aging, considering that the BW in sea turtles increases with age.

Table 1 shows the median (min–max) of 23 major and trace elements, Al, As, Br, Ca, Cl, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Ni, P, Pb, S, Se, Si, Sr, Ti, Y and Zn, measured by PIXE. There were significant but weak correlations of BW, CL, CW or CSA with plasma Br, P, Pb or Sr but no other significant correlations with other elements (Table 2).

There is a close relationship between bromide, as well as halogens, and the chloride concentration in plasma ($r=0.741$, $P<0.001$). A structurally and functionally distinct enzyme from neutrophil myeloperoxidase has the unique ability to use halides or pseudohalides (X^-) and H_2O_2 derived from the respiratory burst to generate cytotoxic hypohalous acids, especially hypobromous acid (HOBr) [6, 22]. The eosinophil peroxidase (EPO), through such thing as the EPO–H_2O_2–Br^- system, is also an effective cytotoxin for multiple targets such as multicellular worms or parasites, bacteria, viruses, and host cells [22]. Both HOBr and the EPO–H_2O_2–Br^- system are involved in many of the pathophysiological features of inflammatory disease, which is associated to an increased blood level of Br^- [6]. In this study, the significantly lower Br^- with age suggests that age-related changes in Br^- do not present a clinical problem.

Table 1. Medians (min to max) of major and trace elements in plasma obtained from captive hawksbill sea turtles

(μg/g)	Median	Min	Max
Al	8.33	1.525	31.2
As	0.019	ND	0.144
Br	13.3	9.4	15.9
Ca	72.5	42.1	236.8
Cl	2727.1	508.2	4783.1
Cr	0.039	ND	0.094
Cu	0.515	0.267	0.897
Fe	1.161	0.406	5.089
Hg	0.105	0.009	0.202
K	123.3	28.6	198.9
Mg	24.6	0.008	46.2
Mn	0.015	ND	0.172
Mo	0	ND	0.238
Ni	0.001	ND	0.061
P	107.3	39.1	393.9
Pb	0.132	ND	0.434
S	400	148.5	731
Se	0.256	0.117	0.334
Si	4.117	0.836	22.3
Sr	0.518	ND	3.027
Ti	0.28	ND	0.91
Y	0	ND	0.097
Zn	1.355	0.879	2.2

ND: less than lower limit (<0.00001 μg/m*l*).

Phosphorus and strontium are closely related to calcium metabolism. Indeed, both the plasma P ($r=0.864$, $P<0.001$) and Sr concentrations ($r=0.826$, $P<0.001$) were positively correlated to the Ca plasma levels, and Ca metabolism increases with age in sea turtles. Therefore, it was thought that the homeostasis of Ca in blood takes priority, followed by that of P and Sr.

Sea turtles are well adapted to different marine environments and thrive in tropical, temperate and even subarctic waters [15]. Today, as a result of centuries of worldwide overexploitation for meat, eggs and shells and incidental capture, their numbers have been dramatically reduced to a critical level, especially in the case of hawksbill sea turtles [3, 15]. In consequence, knowledge about the metalloids and heavy metals burden in sea turtles is an important focal point to assess their potential impact on these endangered organisms. Blood is an excellent tissue to measure in a relatively noninvasive way the baseline values of heavy metals and metalloids in sea turtles. For example, blood is the primary means of transportation of Hg throughout the body and target organs [9]. In fact, blood was proved to be an effective predictor of total Hg stored in muscle and the spinal cord [9]. In a previous study, we determined how some elements

Table 2. Person's product-moment correlation coefficient (r) between carapace parameters and plasma major and trace elements in captive hawksbill sea turtles

	CL	CW	CSA	Br	P	Pb	Sr
BW	0.947 c)	0.878 c)	0.946 c)	–0.495 a)	0.510 b)	–0.481 a)	0.523 b)
CL	–	0.841 c)	0.939 c)	–0.487 a)	0.454 a)	–0.463 a)	0.439 a)
CW	–	–	0.974 c)	–0.546 b)	0.592 b)	–0.364	0.676 b)
CSA	–	–	–	–0.552 b)	0.547 b)	–0.434 a)	0.599 b)

BW, body weight (kg); CL, carapace length (cm); CW, carapace width (cm); CSA, virtual carapace surface area (cm^2). a) $P<0.05$, b) $P<0.01$ and c) $P<0.001$.

accumulate in the plasma of wild sea turtles on the coast of Okinawa, Japan [20]. Wild sea turtles have larger plasma concentrations of As and Pb than those in captivity, but there are no significant differences in their Al and Hg levels [20]. However, to the authors' knowledge, comparative studies on the quantitative relationship between the total plasma element levels and natural aging factors, including BW and carapace parameters, have not yet been performed in sea turtles.

Hence, reference basal values obtained from captive sea turtles are useful to understand the extent of metal pollution in these animals. In the present study, no significant positive correlation was seen between aging and elements such as Al, As, Cd, Hg and Pb, which are key to monitoring pollution of oceans. Although it is impossible to determine the feeding patterns of wild turtles, they could be affected by feeding on marine organisms with different degrees of accumulated pollutants.

The PIXE analytical technique selected for the present study is a fast and reliable multi-element qualitative and quantitative method [8]. In this technique, a detector analyzes characteristic X-rays emitted as a result of inner-shell ionization of target atoms. The method works well in small samples and is suitable for determining elements in a solid surface, especially for analyzing medium- and higher atomic weight elements in a matrix consisting of light elements. With this technique, a sample of a few micrograms is sufficient to analyze concentrations in the parts-per-million range [8]. Current analytical technique such as the PIXE used in this study provides reliable, rapid and easy diagnostic methods. In particular, the risk of contamination during the preparation of a sample for the PIXE method is remarkably lower than that for other methods, because the method does not involve a complicated sample preparation process [4, 20].

This study was conducted to assess correlations between carapace parameters and plasma element status using hawksbill sea turtles, excluding young turtles weighing less than 27.9 kg, so future studies need to look for possible correlations between growth and plasma element status in juvenile sea turtles.

In conclusion, the present results suggest that CL, CW and CSA are suitable indicators to estimate the body weight of sea turtles and that plasma trace elements in captive sea turtles show almost no variation with BW and carapace indicators, except for Br, P, Pb and Sr. These findings may be relevant in pollution and marine ecosystem studies where physiological aging is not considered. Therefore, monitoring the increase in the above-mentioned plasma elements in wild sea turtles might be useful in monitoring for possible accumulation caused by marine pollution.

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