Chemical Element Concentrations in the Blood of Green Turtles (Chelonia Mydas) Captured at Fernando De Noronha Marine National Park, Brazil

Fabiola Eloisa Setim Prioste¹, Vanessa Cristina de Oliveira Souza², Mariana Ramos Queiroz², Rosely Gioia-Di Chiacchio¹, Fernando Barbosa Jr³ and Eliana Reiko Matsushima¹

¹Laboratory of Wild Animal Comparative Pathology (Laboratório de Patologia Comparada de Animais Selvagens – LAPCOM), School of Veterinary Medicine and Zootecnhics of the University of São Paulo (Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo – FMVZ-USP), Av. Prof. Dr. Orlando Marques de Paiva, 87, Cidade Universitária, São Paulo, SP, Brazil
²Laboratory of Epidemiology and Biostatistics (Laboratório de Epidemiologia e Bioestatística – LEB), School of Veterinary Medicine and Zootecnhics of the University of São Paulo (Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo – FMVZ-USP), Av. Prof. Dr. Orlando Marques de Paiva, 87, Cidade Universitária, São Paulo, SP, Brazil
³Department of Clinical, Toxicological and Bromatological Analysis (Departamento de Análises Clínicas, Toxicológicas and Bromatológicas), Ribeirão Preto School of Pharmaceutical Sciences (Faculdade de Ciências Farmacêuticas de Ribeirão Preto – FCFRP – USP), Avenida do Café, s/n, Monte Alegre, Ribeirão Preto, SP, Brazil

Abstract

Green turtles may be used as biomonitors of marine environments because of their longevity and feeding habits (omnivorous during the first years of life and herbivorous during the juvenile and adult stages), which can indicate the degree of ocean contamination in the areas where they feed. Studies of metal and chemical element concentrations in the blood of green turtles are still rare; therefore, the results are difficult to interpret, although it appears that the serum levels of certain elements found in green turtles are much higher than the minimal risk levels indicated for human health. The objective of this study was to measure the concentrations of the essential elements Calcium (Ca), Selenium (Se), Zinc (Zn), Manganese (Mn), Cobalt (Co), Copper (Cu) and Molybdenum (Mo) and the metals Aluminum (Al), Arsenic (As), Lead (Pb), Cadmium (Cd), Lithium (Li), Cesium (Cs), Barium (Ba), Rubidium (Rb), Uranium (U), Thorium (Th), Beryllium (Be), Antimony (Sb) and Tellurium (Te) in the blood of 31 juvenile green turtles captured in Fernando de Noronha Marine National Park (Parque Nacional Marinho de Fernando de Noronha), Brazil, and to correlate these concentrations with the curved carapace length to identify possible cumulative effects. Furthermore, because the basal levels for these elements have not yet been established for green turtles, the effects of these chemicals on the health of the species are still unknown. Thus, most of these contaminants should be described as “alarming” until further clarification.

Keywords: Biomonitors; Marine environments; Pollution; Elemental analysis; Mass spectrometry; Sea turtles

Introduction

Chelonia mydas is a sea turtle with a worldwide distribution in tropical and subtropical seas between 40°N and 40°S latitude. This species has the most coastal habit of sea turtles and is capable of entering river and lake estuaries [1]. In these neritic zones (waters close to the shore), green turtles primarily feed on seagrasses and algae [2]. In addition, their feeding habit varies from omnivorous during the first years of life [3] to herbivorous following the post-pelagic phase [4]. This species is considered threatened by the International Union for Conservation of Nature (IUCN) and vulnerable by the List of Endangered Species of the Chico Mendes Biodiversity Institute (Instituto Chico Mendes de Biodiversidade - ICMBio). The decrease in green turtle populations has been attributed to coastal development, accidental capture in fishing gear, human consumption, climate changes, pollution and pathogens [5]. A number of studies conducted on sea turtles in different parts of the world have focused on the detection and measurement of toxic metals and essential elements [6-13], which likely play relevant roles in the decrease of sea turtle populations [14]. Despite the importance of the Brazilian coast for the development of five of the seven sea turtle species, the only study to detect chemical elements in turtles in Brazil was published in 2009 by Barbieri.

Certain chemical elements are essential to life; however, many chemicals can be metabolized or bioaccumulated and become toxic for organisms. Vertebrates exposed to high concentrations of certain chemical elements or small concentrations for long periods of time may exhibit neurological, reproductive, gastrointestinal, respiratory, hepatic, immunological, renal or dermatological symptoms [15].

The objective of this study was to measure the concentrations of the essential elements Calcium (Ca), Selenium (Se), Zinc (Zn), Manganese (Mn), Cobalt (Co), Copper (Cu) and Molybdenum (Mo) and the metals Aluminum (Al), Arsenic (As), Lead (Pb), Cadmium (Cd), Lithium (Li), Cesium (Cs), Barium (Ba), Rubidium (Rb), Uranium (U), Thorium (Th), Beryllium (Be), Antimony (Sb) and Tellurium (Te) in the blood of 31 juvenile green turtles captured in Fernando de Noronha Marine National Park (Parque Nacional Marinho de Fernando de Noronha), Brazil, and to correlate these concentrations with the curved carapace length to identify possible cumulative effects.

*Corresponding author: Fabiola Eloisa Setim Prioste, Laboratory of Wild Animal Comparative Pathology (Laboratório de Patologia Comparada de Animais Selvagens – LAPCOM), School of Veterinary Medicine and Zootechnics of the University of São Paulo (Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo – FMVZ-USP), Av. Prof. Dr. Orlando Marques de Paiva, 87, Cidade Universitária, São Paulo, SP, Brazil, Tel: 55-11-99251-8041; Fax: 55-11-3091-7689; E-mail: fabiolaprioste@gmail.com

Received July 09, 2015; Accepted October 16, 2015; Published October 14, 2015

Citation: Prioste FES, Souza VCO, Queiroz MR, Chiacchio RGD, Barbosa F, et al. (2015) Chemical Element Concentrations in the Blood of Green Turtles (Chelonia Mydas) Captured at Fernando De Noronha Marine National Park, Brazil. J Environ Anal Toxicol 5: 325. doi: 10.4172/2161-0525.1000325

Copyright: © 2015 Prioste FES, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Materials and Methods

Blood collection and specimen identification

Thirty-one juvenile green turtles were intentionally captured at Sueste Beach (Praia do Sueste; 3°86'68"S; 32°42'60"W), Fernando de Noronha Marine National Park, Brazil between 26 April and 4 May 2013 (Figure 1). The specimens were identified using the tag number supplied by the Tamar/ICMBio project. Measurements of the curved carapace length (CCL) [16], a parameter that is used to determine the life stage of the animal, were performed. Because sex cannot be determined in juveniles using phenotypic parameters, sex determination was not performed. One milliliter of blood was collected from the cervical venous sinus using the occipital bone as a reference. The turtles were released immediately following blood collection, and the blood samples were placed in Vacuette® Trace Elements Sodium Heparin (Greiner Bio-one, Americana, SP, Brazil) tubes and kept in ice until they were stored at -20°C for further analysis.

Elemental analysis

The analyses were conducted with an inductively coupled plasma mass spectrometer (ICP-MS) operating with high-purity argon (99.999%, Praxair, Brazil). The sample introduction system was composed of a quartz cyclonic spray chamber and a Meinhard® nebulizer connected by Tygon® tubes to the ICP-MS’s peristaltic pump (set at 20 rpm). The ICP-MS was operated with a Pt sampler and skimmer cones purchased from PerkinElmer (Shelton, CT, USA). High purity de-ionized water (resistivity 18.2 MΩ cm⁻¹) used for preparation of samples and solutions was obtained using a Milli-Q water purification system (Millipore RoIs-DI™, Bedford, MA, USA). All of the reagents were of analytical-reagent grade except for HNO₃, which was previously purified in a quartz sub-boiling still (Kürner Analysetechnik) before use. A clean laboratory and laminar-flow hood capable of producing class 100 air flow were used for preparing the solutions. Rhodium (1000 mg L⁻¹) and multi-element solution (10 mg L⁻¹) were obtained from PerkinElmer. Triton® X-100 was purchased from Sigma–Aldrich (St. Louis, MO, USA). Plastic bottles and cryogenic vials were cleaned by soaking in 15% (v/v) HNO₃ for 24 h, rinsing five times with Milli-Q water and drying in a class 100 laminar flow hood before use. Sample preparation and analysis were performed in a class 1000 clean room. A base blood was derived from sheep (undosed animals) for the matrix-matching calibration. The samples and calibration curves were diluted 1:50 into a 15-mL polypropylene Falcon® tube (Becton Dickinson) with a solution containing 0.01% (v/v) Triton® X-100, 0.5% (v/v) nitric acid and 10 μg L⁻¹ of the internal standards. To verify the accuracy and precision of the blood samples, human blood reference materials (RM) QMEQA507B06 and QME- QA507B03 from the l'Institut National de Santé Publique du Québec (Canada) were analyzed [17].

Data presentation and statistical analysis

Data handling and statistical analyses were performed using the software R (R Development Core Team, 2013).

Correlations between the concentrations of the 20 quantified elements and the CCL were analyzed using the Spearman's rank correlation coefficient because the CCL data were not normally distributed.

Significant correlations between the elements and CCL were tested using Spearman’s correlation test (ρ) or Pearson’s correlation test (r) depending on whether both concentrations exhibited a normal distribution. P values lower than 0.05 (two-tailed) were considered significant.

Results

The median, first quartile and third quartile for the CCL and quantified elements are presented in Table 1. Among the 20 analyzed elements, the Spearman’s correlation test revealed that CCL was significantly negatively correlated with Se (ρ=−0.49, p<0.01) and significantly positively correlated with Zn (ρ=0.42, p=0.02), Pb (ρ=0.41, p=0.02) and Ca (ρ=0.35, p=0.05) (p<0.05).

When both elements exhibited a normal distribution, the correlations were quantified using Pearson’s correlation test, and when both elements exhibited a non-normal distribution, the correlations were quantified using Spearman’s correlation test. The significant correlations observed between the elemental concentrations are presented in Table 2.

Discussion

The concentrations of chemical elements in the blood of the juvenile green turtles appeared to vary greatly from previously reported values for other locations. This result is shown in Table 3, which presents a comparison between our results and the results of studies conducted in different locations worldwide.

The geographical position and urban/industrial development of the areas in the compared studies are listed as follows: Gaus et al. [18] studied green turtles in Gladstone, Australia, an area with large-scale industrial development, varied industries and a seaport; Komoroske et al. [19] measured the element concentrations in green turtles in San Diego Bay, United States, an impacted navigation area; Van de Merwe et al. [13] studied recently deceased green turtles that were under rehabilitation at the Center for Marine Animals in Gold Coast, Australia; and Labrada et al. [20] studied turtles in two coastal lagoons in Baja California, Mexico, an area with urban development and waste discharge. The Fernando de Noronha Marine National Park is considered a clean area because it is an oceanic island without industrial development and with low environmental impacts relative to the other four locations.

We observed a negative correlation between CCL and Se serum levels. This finding is consistent with that of Komoroske et al. [19], who also observed a correlation between CCL and As, Cu, Cd and Hg. The Se levels observed in the present study in the sea turtles from Fernando de Noronha Marine National Park were much lower than those previously reported and almost half the values observed by Komoroske et al. [19]. According to the Agency for Toxic Substances and Disease Registry (ATSDR), exposure to high levels of Se or prolonged exposure.
Blood is considered the most common and accurate compartment for assessing recent exposure to lead [27,28]. The serum Pb concentrations observed in the present study were similar to those reported by Van de Merwe et al. [13]. The positive correlation between the serum Pb concentrations and CCL observed in our study indicates that the studied animals were exposed to Pb, thus increasing their contamination load. This finding is worrisome because minimal risk levels have not been established for this species. Nonetheless, the observed Pb concentrations appeared to be high relative to that of other vertebrates. In humans, serum Pb concentrations higher than 10 µg dL$^{-1}$ result in the decreased biosynthesis of heme enzymes and increased blood pressure, and they promote neurologic effects in children [27].

Cs is found in the environment in its stable form ($^{133}$Cs) combined with other elements in the rocks, soil and dust. Many Cs compounds are dissolved in water. Plants and animals may present Cs in concentrations of 1-300 ppb [29]. The positive correlation between Cs and the CCL indicates that the animals remain in contact with this element.

No other correlations were observed between the quantified elements and CCL. In addition, a comparison of the serum levels of As, Cd, Mn, Co, Cu and Mo observed in the present study with that of previous reports indicated a wide variation. Although Komoroske et al. [19] and Gaus et al. [18] used the same elemental analysis methods (ICP-MS) as in the present study, only the Cu concentrations were similar. Determining baseline data for these elements in green turtles is important for their survival as well as for oceanic health because the concentration of chemical elements in sea turtle tissues are reflective of the concentrations in the environment they inhabit [30].

The highest correlation coefficient among element concentrations was observed between Se and As ($r=0.86$). The correlation between Se and As concentrations has been previously described for several animals, and the simultaneous exposure to these two elements is characterized by an antagonist interaction between the elements [31]. Strong positive linear correlations were observed between Pb and Cs, Pb and Cu, and Cs and Cu ($r>0.60$). Synergies and/or antagonisms

results in increased serum Se concentrations over time [21]. However, decreasing Se levels are observed when the individual has a disease or is at an advanced age. The green turtles at Fernando de Noronha were in good physical conditions, which lead us to hypothesize that this feeding area has low Se concentrations because Se has a relatively short half-life that varies from hours to days. Studies with mammals have shown that Se is usually positively correlated with other metals, especially As, Hg and Cd, although this association was not observed in the present study. The metabolism of Se may be significantly altered by interactions with other metals, chemical products and psychochemical factors [22], which increase the difficulty of interpreting Se concentrations.

Compared with Se, the elements Zn, Pb and Cs were positively correlated with CCL. The mean Zn concentration observed in the present study was slightly higher than the concentration observed by Labrada et al. [20] in two sites in Mexico. Green turtles appear to typically exhibit high Zn serum levels [23-25]. The most important routes of exposure to Zn are feeding and contaminated soil/sediments and serum Zn levels increase rapidly following absorption [26]. Therefore, the archipelago may be the main source of contamination.
between inorganic and organic contaminants are complex and not well understood. Therefore, the data presented here are still preliminary and serve only as a basis for future studies. Further studies are required that include analyses of additional samples.

Conclusions

Green turtles may be used as biomonitor of marine environments because of their longevity and feeding habits (omnivorous during the first years of life and herbivorous during the juvenile and adult stages), which can indicate the degree of ocean contamination in the areas where they feed. Studies of metal and chemical element concentrations in the blood of green turtles are still rare; therefore, the results are difficult to interpret, although it appears that the serum levels of certain elements found in green turtles are much higher than the minimal risk levels indicated for human health.

The exact source of contamination could not be determined, and further studies on the anthropogenic pressure exerted on the marine environment are required because patterns were not observed in the results reported for different locations worldwide. Furthermore, because the basal levels for these elements have not yet been established for green turtles, the effects of these chemicals on the health of the species are still unknown. Thus, most of these contaminants should be described as “alarming” until further clarification.

The methods used here were efficient because blood collection is a minimally invasive and rapid procedure, and the animals were returned to the environment immediately following venipuncture. In addition, the analyses were performed in triplicate, and significant differences were not observed between replicates.

Similar studies using a larger number of animals are currently being conducted in other areas of the Brazilian coast, and the results may further clarify the relationship between inorganic elements and green turtle disease and survival. In addition, similar studies that include the analysis of stomach contents and algae collected from feeding areas will soon be performed to determine the interactions among the elements studied here.

Acknowledgments

The authors wish to thank the Tamar/ICMBio Project and the Experimental and Comparative Pathology Graduate Program of the School of Veterinary Medicine and Zootecnilcs of the University of São Paulo (Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo - FMVZ-USP). This study received financial support from the Foundation for Research Support of the State of São Paulo (FAPESP Process 2012/14319-6) and the Coordination for the Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- CAPES).

References

1. Hirth HF (1997) Synopsis of the biological data on Green Turtle Chelonia mydas (Linnaeus 1758) US Fish and Wildlife Service, Washington, DC.
2. Plotkin P (1997) Adult migrations and habitat use. In: Lutz PL, Musick JA, Wyneken J (eds) The biology of sea turtles. CRC Press, Boca Raton, FL: 225-241.
3. Bjorndal KA (1997) Foraging ecology and nutrition of sea turtles. In: Lutz PL, Musick JA (eds) The biology of sea turtles. CRC Press, Boca Raton, FL: pp 199-231.
4. Bolten AB, Balazs GH (1995) Biology of the early pelagic stage – the "lost year." In: Bjorndal KA (ed) Biology and conservation of sea turtles, revised edition. Smithsonian Institution Press, Washington, DC. pp: 575-581.
5. ICMBio – Instituto Chico Mendes de Conservação da Biodiversidade (2011) Plano de ação nacional para a Conservação das Caus de Marinas [vol. 25] [National plan of action for the conservation of sea turtles]. Serie Espécies Ameaçadas, Brazil.
6. Aguirre AA, Balazs GH, Zimmerman B, Galey FD (1994) Organic contaminants and trace metals in the tissues of green turtles (Chelonia mydas) affected with fibropapillomas in the Hawaiian Islands. Mar Pollut Bull 28: 109-114.
7. Gladstone W (1996) Trace metals in sediments, indicator organisms and traditional seafood of the Torres Strait. Great Barrier Reef Marine Park Authority, Townsville, Australia.
8. Popple AR, Gordon AN, Ng J (1998) Trace metal concentrations in livers and kidneys of sea turtles from south-eastern Queensland, Australia. Mar Freshwater Res 49: 409-414.
9. Saeki K, Sakakibara H, Sakai H, Kunito T, Tanabe S (2000) Arsenic accumulation in three species of sea turtles. Biometals 13: 241-250.
10. Anan Y, Kunito T, Sakai H, Tanabe S (2002) Subcellular distribution of trace elements in the liver of sea turtles. Mar Pollut Bull 45: 224-229.
11. Lam JC, Tanabe S, Chan SK, Yuen EK, Lam MH, et al. (2004) Trace element residues in tissues of green turtles (Chelonia mydas) from South China waters. Mar Pollut Bull 48: 174-182.
12. Agusa T, Takagi K, Iwata H, Tanabe S (2008) Arsenic species and their accumulation features in green turtles (Chelonia mydas). Mar Pollut Bull 57: 782-789.
13. van de Merwe JP, Hodget M, Olszow HA, Whittier JM, Lee SY (2010) Using blood samples to estimate persistent organic pollutants and metals in green sea turtles (Chelonia mydas). Mar Pollut Bull 60: 579-588.
14. Barbari E (2009) Concentration of heavy metals in tissues of green turtles...
(Chelonia mydas) sampled in the Cananéia estuary, Brazil. Braz J Oceanogr 57: 243-248.

15. Moffet DB, El-Masri HA, Fowler BA (2007) General considerations on dose-effect and dose-response relationships. In: Nordberg GF, Fowler BA, Nordberg M, Friberg L (eds) Handbook on the toxicology of metals (3rd edn) Elsevier, Amsterdam. pp: 101-116.

16. Bolten AB (1999) Techniques for measuring sea turtles. In: Eckert KL, Bjorndal KA, Abreu Grobois FA, Donnelly M, editors. Research and management techniques for the conservation of sea turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4, Washington, DC. pp: 126-131.

17. Batista BL, Rodrigues JL, Nunes JA, Souza VCO, Barbosa Jr F (2009) Exploiting dynamic reaction cell inductively coupled plasma mass spectrometry (DRC-ICP-MS) for sequential determination of trace elements in blood using a dilute-and-shoot procedure. Anal Chim Acta 639: 13-18.

18. Gaus C, Grant S, Jin NL, Goot K, Chen L, et al. (2012) Investigation of contaminant levels in green turtles from Gladstone. Final report. National Research Centre for Environmental Toxicology, University of Queensland, Queensland, Australia.

19. Komoroske LM, Lewison RL, Seminoff JA, Deheyn DD, Dutton PH (2011) Pollutants and the health of green sea turtles resident to an urbanized estuary in San Diego, CA. Chemosphere 84: 544-552.

20. Labrador-Martagón V, Rodríguez P, Mendez-Rodriguez L, Zenteno-Savín T (2011) Oxidative stress indicators and chemical contaminants in East Pacific green turtles (Chelonia mydas) inhabiting two foraging coastal lagoon in the Baja California Peninsula. Comp Biochem Physiol C 154: 65-75.

21. ATSDR (2003) Toxicological profile for selenium. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, GA.

22. Eisler R (1985) Selenium hazards to fish, wildlife, and invertebrates: a synaptic review. US Fish and Wildlife Service, Laurel, MD.

23. Ikonomopoulou MP, Olszowy H, Limpus C, Francis R, Whittier J (2011) Trace element concentrations in nesting flatback turtles (Natator depressus) from Curtis Island, Queensland, Australia. Mar Environ Res 71: 10-16.

24. Camacho M, Ortiz J, Boada LD, Zaccaroni A, Silvi M, et al. (2013) Potential adverse effects of inorganic pollutants on clinical parameters of loggerhead sea turtles (Careta caretta): Results from a nesting colony from Cape Verde, West Africa. Mar Environ Res 92: 15-22.

25. Ley-Quíñónez CP, Zavala-Norzagaray AA, Rendón-Maldonado JG, Espinosa-Carreón TL, Canizales-Román A, et al. (2013) Selected heavy metals and selenium in the blood of black sea turtle (Chelonia mydas agasiiizzi) from Sonora, Mexico. Bull Environ Contam Toxicol 91: 645-651.

26. ATSDR (2005) Toxicological profile for zinc. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, GA.

27. ATSDR (2007) Toxicological profile for lead. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, GA.

28. Barbosa F Jr, Tanus-Santos JE, Gerlach RF, Parsons PJ (2005) A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. Environ Health Perspect 113: 1669-1674.

29. ATSDR (2004) Toxicological profile for cesium. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, GA.

30. Grillitsch B, Schiesari L (2010) The ecotoxicology of metals in reptiles. In: Sparling DW, Linder G, Bishop CA, Krest SK (eds) Ecotoxicology of amphibians and reptiles (2nd edn) CRC Press; Boca Raton, FL. pp: 337-348.

31. Zeng H, Uthas EO, Combs GF Jr (2005) Mechanistic aspects of the interaction between selenium and arsenic. J Inorg Biochem 99: 1269-1274.