PLASMA CALCIUM AND PHOSPHORUS LEVELS IN *Chelonia mydas* JUVENILES OBTAINED FROM THREE FEEDING AREAS ON THE BRAZILIAN COAST

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ABSTRACT

The contamination of the marine environment by industrial, agricultural, and domestic effluents is extremely harmful, influencing, in many cases, the abundance and diversity of food resources. In addition to changes in diet, impacted environments provide a favorable scenario for the occurrence of diseases in aquatic organisms. Little is known about the effect of anthropization on the health of *Chelonia mydas* populations in Brazil. This work aimed to evaluate biochemical parameters of juvenile green turtles in three feeding areas in Brazil with distinct degrees of anthropization. From October 2012 to May 2014, 123 specimens of *C. mydas* were captured at three feeding sites along the coast of Brazil, with obvious differences between degrees of anthropization. Of these, 21 specimens were captured at the Vitória Bay, ES, 42 specimens at the Abrolhos Marine National Park, BA, and 60 specimens at the Atol das Rocas Biological Reserve, RN. The biochemical parameters evaluated were calcium (Ca), phosphorus (P) and their ratio (Ca:P). Plasma concentrations of P showed a significant difference between the feeding areas, decreasing according to the distance from the coastal region. The work recommends the study of plasma phosphorus levels as an indicator of the environmental quality of feeding sites in Brazil. For this, studies that correlate the P levels of food components of green turtles with plasma levels should be carried out.

Keywords: Ecotoxicology. Macronutrients. Green turtle.

INTRODUCTION

Sea turtles have complex life patterns as they utilize a variety of ecosystems, including terrestrial habitats, coastal waters, and the open ocean (ANDERSON et al., 2011). Green turtles (*Chelonia mydas*) have a cosmopolitan pelagic distribution in the initial phase, and neritic in the juvenile phase, associated with feeding areas. This species has late sexual maturity (around 20 years) and spawning occurs especially on oceanic islands. They have a complex life cycle and according to the Red Book of Brazilian Fauna Threatened with Extinction (ICMBio, 2018), they are classified as vulnerable to extinction in Brazil.

The diet of *C. mydas* varies throughout its life stages. Newborn green turtles inhabit the open ocean, where they are primarily omnivorous, feeding mainly on invertebrates (BJORNDAL, 1997). After leaving the pelagic habitat, juvenile specimens enter feeding areas near the coast, transitioning their feeding habit to an herbivorous diet (BJORNDAL, 1997; CARDONA et al., 2010). Juvenile and adult green turtles feed mainly on seagrass and algae, although they occasionally consume jellyfish or sponges (BJORNDAL, 1997). In Brazil, their food base is composed mainly of macroalgae and phanerogams, according to the supply of the feeding sites (SANTOS et al., 2011).

The contamination of the marine environment by industrial, agricultural, and domestic effluents is extremely deleterious to aquatic organisms. The eutrophication of the marine environment provides nutrients such as phosphorus (P) and nitrogen (N) in exaggerated amounts to the environment, which can cause imbalances in the ecosystem, such as the dominance of macroalgae (HAMANN et al., 2010). In addition, diets low in calcium (Ca) and high in P stimulate the parathyroid gland to release parathormone (PTH), which mobilizes calcium from bones to help maintain serum calcium levels. This bone demineralization can lead to pathological fractures, tremors, cloacal prolapse, fibrous osteodystrophy, and stunted growth (SNODDY, 2009).

Establishing the relationship between biochemical parameters that are influenced by the degree of anthropization of the environment can contribute to the evaluation of the health of juvenile *C. mydas* populations and the ecosystem in Brazilian feeding sites. Therefore, the objective of this study was to compare the plasma concentration of phosphorus (P), calcium

(Ca) and the Ca:P ratio in three feeding areas on the Brazilian coast with distinct degrees of anthropization.

MATERIAL AND METHODS

A total of 123 clinically healthy *C. mydas* were captured between October 2012 and May 2014. Of these, 21 specimens were captured in the coastal zone at Vitória Bay, Espírito Santo state, 42 specimens at the Abrolhos National Marine Park 60 km off the coast of Bahia state, and 60 specimens at the Atol das Rocas Biological Reserve, an oceanic island 240 km off the coast of Rio Grande do Norte state.

Characterization of the study areas

Vitória Bay, Espírito Santo (20°18'10.08"S/40°17'17.3"W), is an urban cove, with public-use beaches. The area receives effluent from rainwater collection networks and is contaminated with domestic and industrial sewage waste and other pollutants from diffuse sources common to the urban environment. It is located near the mouth of the Santa Maria da Vitória River, which carries sediments and urban and agricultural effluents, besides being a port region. The region of Greater Vitória also has an important steel mill that dumps industrial effluents into the sea (TOREZANI et al., 2010). The beach where the capture took place is considered a degraded environment and has clear signs of eutrophication with an abundance of macrophytic algae (*Ulva* sp.). Algae decomposition increases environmental degradation (SANTOS et al., 2011).

The Abrolhos Marine National Park (-17°57'46.71"S/-38°42'16.01"W), is a federal conservation unit of indirect use. It is located 60 km off the coast of Bahia state. The total area of the Abrolhos bank is 42 km^2 and is under indirect influence of the rivers flowing into the region. The coastal region of the Abrolhos bank has no large urban centers and is characterized by small towns. In the park area, there is a constant traffic of small and medium size boats for the development of activities related to ecotourism. The Abrolhos Marine National Park is an important place for the maintenance of marine biodiversity, where a variety of corals can be found, including endemic species. The island's sewage is treated in septic tanks and the effluent is discharged on Santa Barbara Island. There are no visible signs of eutrophication or direct anthropic impacts related to pollution.

The Atol das Rocas Biological Reserve (33°51'28.06”S/33°49'00.98”W) is an oceanic protected area with low anthropic impact located 240 km off the coast of the state of Rio Grande do Norte. Atol das Rocas is an environment classified as balanced and home to a vast biological diversity (PEREIRA et al., 2010). The transit of watercrafts and the access to the area are prohibited, except for area managers and researchers with prior authorization. In this area there are no visible signs of human impacts and environmental imbalances.

Capture of the animals

The animals were captured at Vitória Bay using net (length: 100 m; mesh size: 10 cm; and height: 6 m). One end of the net was fixed on the shore of the cove, and, with the aid of an inflatable boat, the net was released, arranged in a concave shape toward the beach. The net was then dragged manually. The animals from Abrolhos and Atol das Rocas were captured manually by snorkeling. Animals with fibropapillomatosis were not considered in the sampling.

Collection and storage of blood samples

The blood samples were collected by venipuncture in the cervical venous sinus with disposable 25 x 0.27 cm needles and syringes, obtaining a volume between 3 to 10 mL, according to the size of the animals. The samples were fractionated into tubes containing sodium heparin and stored in an isothermal container at 4 to 8 °C until arrival in the laboratory, where they were centrifuged at 5,000 rpm for 10 minutes to obtain plasma and stored in 2 mL aliquots in plastic tubes at -20 °C until further analysis.

Biochemical analyses

The analyses were performed in an automated system (Beckman Coulter UN2700), using the proper kits and according to the manufacturer's instructions.

Statistical analyses

The comparison of biochemical values between the animals of the three sites was performed using the SPSS 17.0 software, ANOVA test ($p<0.05$), with integrated normality test. Subsequently, the Tukey's test was used to analyze the difference in variance between the parameters of the animals captured at the different feeding sites.

RESULTS AND DISCUSSION

The plasma P concentrations showed a significant difference (Table 1) between the studied areas. The plasma P level decreased with distance from the coastal region. Thus, it was observed that the green turtles from Vitória Bay had higher levels of P, followed by Abrolhos and, finally, Atol das Rocas. The values of the Ca:P ratio of the animals from Abrolhos and Atol das Rocas were similar. However, when compared to the animals captured at Vitória Bay, they were higher (Figure 1).

Table 1 - Biochemical parameters of plasma Ca, P, and the Ca:P ratio of juvenile *Chelonia mydas* specimens obtained from three feeding areas on the Brazilian coast.

	Vitória, ES		Abrolhos, BA		Atol das Rocas, RN	
Parameters	N	Average ± SD	N	Average ± SD	N	Average ± SD
Calcium (mg/dL)	21	6.40 ± 2.29	42	8.04 ± 2.43	60	9.25 ± 12.70
Phosphorus (mg/dL)	21	6.9 ± 2.33 *a*	42	4.76 ± 1.46 *b*	60	3.53 ± 1.28
Ratio Ca:P	21	0.94 ± 0.36 *a*	42	1.88 ± 0.84 *a, b*	60	2.13 ± 1.11 *b*

Note: *a, b*: Different letters indicate a statistically significant difference in the ANOVA test ($p<0.05$).

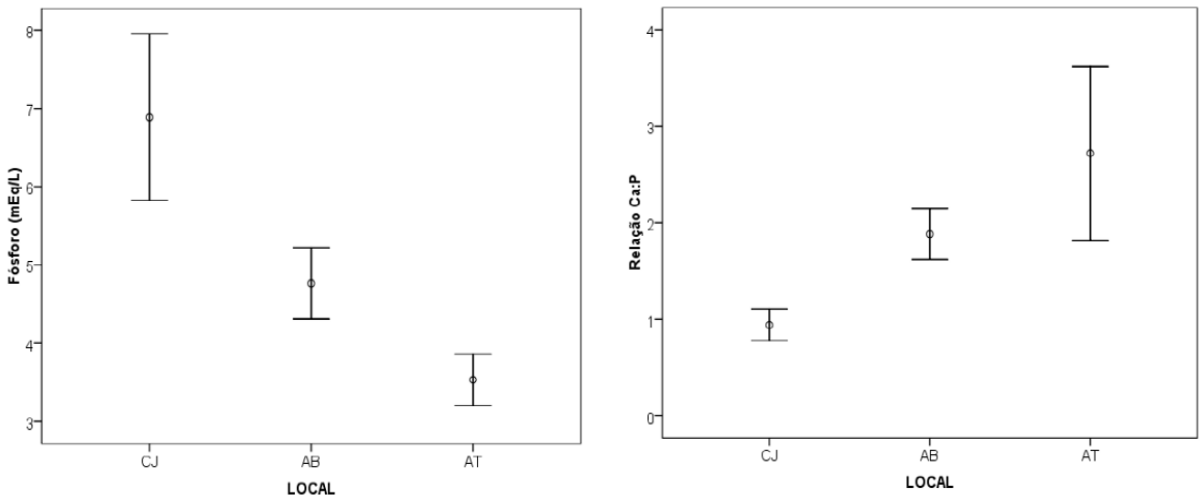

Figure 1 - Biochemical parameters of plasma Ca, P, and the Ca:P ratio of juvenile *Chelonia mydas* specimens, considering the respective feeding site: CJ: Vitória; AB: Abrolhos; AT: Atol das Rocas.
Note: Horizontal bars indicate standard deviation.

Phosphorus and calcium are macronutrients of high importance in the animal organism because they are components of the bone matrix structure, essential for several cellular and extracellular functions (ADKESSON; LANGAN, 2007). The imbalance of one of them leads to the increase or decrease of the other, which may culminate in bone metabolic disease (MOTTA, 2009), a common illness in captivity, where the inversion of the Ca:P ratio was reported as a problem in juvenile *C. mydas* fed with natural dietary items, including fish, shrimp, squid, crabs and scallops (Table 2) during rehabilitation process (HIGGINS, 2003; STRINGER et al., 2010). Moreover, the contamination of the marine environment by excess of P is a serious environmental problem (CARCIOFI; OLIVEIRA, 2006), since it can alter chemical, physical, and microbiological parameters of water bodies, triggering a restructuring of the communities present in the environment, by causing an increase in the biomass of benthic algae and epiphytes, and decline of more selective organisms (LOBBAN; HARRISON, 1994).

Table 2 - Biochemical parameters of plasma Ca, P, and the Ca:P ratio of *Chelonia mydas* specimens undergoing rehabilitation.

Reference	Population	N	Calcium (mg/dL)	Phosphorus (mg/dL)
Aguirre et al., 1995	*Hawaii*	5	8.42 ± 1.02	7.88 ± 0.93
Arthur et al., 2008	Australia	76	6.80 ± 0.84	-
Bolten; Bjorndal, 1992	Bahamas	100	9.10 ± 2.10	6.70 ± 1.20
Flint et al., 2010	Australia	194	6.80 ± 0.84	6.50 ± 1.93
Fong et al., 2010	Taiwan	27	8.87 ± 1.62	-
Snoddy et al., 2009	North Carolina	12	11.20 ± 2.10	9.70 ± 2.60

Studies related to the eutrophication of aquatic environments, such as rivers and lakes, point out that this process causes an increase in the concentration of P and nitrogen (N), and the excess of these nutrients is an important factor in the increasing number of macroalgae (CETESB, 2014).

The plasma P level observed in this study was different from those cited by Mader (1996), Miguel et al. (2022), Santos et al. (2005), and Thrall et al. (2015). The difference in plasma P concentration found in specimens from the three studied areas could be explained by the different degrees of eutrophication related to distance from the shore, resulting in distinct levels of P in food components.

In contrast, the decrease in the Ca:P ratio in specimens captured at Victoria Bay may have occurred due to the high concentration of P found in their blood plasma, since they may be exposed to foods with variable rates of C and P (THRALL et al., 2015). Considering all this, further studies on the level of P in the food components of *C. mydas* and the degree of anthropization of the feeding areas for the species are important not only to corroborate this hypothesis, but also to support it.

CONCLUSION

The serum level of P varied according to the level of anthropization of the evaluated areas, being greater in the bay of Vitória, followed by Abrolhos and, finally, Atol das Rocas. The data obtained in this study is important to understand how green turtles can have their health threatened by feeding in highly anthropized areas, while highlighting the importance of preserving coastal waters for the balance of marine fauna. Furthermore, this study is important as it provides a fundamental knowledge to support proper coastal management decisions and provide information on which future research can be based to optimize the conservation of the species.

DOSAGEM DE CÁLCIO E FÓSFORO EM *Chelonia mydas* JUVENIS OBTIDA EM TRÊS ÁREAS DE ALIMENTAÇÃO NA COSTA BRASILEIRA

RESUMO

A contaminação do ambiente marinho por efluentes industriais, agrícolas e domésticos é extremamente prejudicial, influenciando, em muitos casos, na abundância e diversidade dos recursos alimentares. Além de alterações na dieta, ambientes impactados proporcionam um cenário favorável a ocorrência de enfermidades. Pouco se sabe sobre o efeito da antropização sobre a saúde das populações de *Chelonia mydas* no Brasil. Este trabalho teve como objetivo avaliar parâmetros bioquímicos de tartarugas-verdes juvenis em três áreas de alimentação no Brasil, com graus distintos de antropização. De outubro de 2012 a maio de 2014 foram capturados 123 espécimes de *C. mydas* em três sítios de alimentação ao longo da costa do Brasil, com diferenças óbvias entre graus de antropização. Destes, 21 espécimes foram capturados na baía de Vitória, ES, 42 espécimes no Parque Nacional Marinho de Abrolhos, BA, e 60 espécimes na Reserva Biológica Atol das Rocas, RN. Os parâmetros bioquímicos avaliados foram Cálcio (Ca), fósforo (P) e sua relação (Ca:P). As concentrações plasmáticas de P apresentaram uma diferença significativa entre as áreas de alimentação estudada, diminuindo de acordo com a distância da região costeira. O trabalho recomenda o estudo dos níveis de fósforo plasmático como indicador da qualidade ambiental dos sítios de alimentação no Brasil. Para isso, estudos que correlacionem os níveis de P dos componentes alimentares de tartarugas-verdes com os níveis plasmáticos devem ser realizados.

Palavras-chave: Ecotoxicologia. Macronutrientes. Tartaruga-verde.

CONCENTRACIÓN DE CALCIO Y FÓSFORO EN TORTUGAS JUVENILES *Chelonia mydas* OBTENIDAS EN TRES ÁREAS DE ALIMENTACIÓN EN LA COSTA BRASILEÑA

RESUMEN

La contaminación del medio marino por efluentes industriales, agrícolas y domésticos es extremadamente dañina, influyendo, en muchos casos, en la abundancia y diversidad de los recursos alimentarios. Además de los cambios en la dieta, los entornos afectados proporcionan un escenario favorable para la aparición de enfermedades. Poco se sabe sobre el efecto de la antropización en la salud de las poblaciones de *Chelonia mydas* en Brasil. Este trabajo tuvo como objetivo evaluar los parámetros bioquímicos de tortugas verdes juveniles

en tres áreas de alimentación en Brasil con diferentes grados de antropización. Desde octubre de 2012 hasta mayo de 2014, se capturaron 123 ejemplares de *C. mydas* en tres sitios de alimentación a lo largo de la costa de Brasil, con diferencias evidentes entre los grados de antropización. De estos, 21 ejemplares fueron capturados en Bahía Vitória, ES, 42 ejemplares en el Parque Nacional Marino de Abrolhos, BA, y 60 ejemplares en la Reserva Biológica Atol das Rocas, RN. Los parámetros bioquímicos evaluados fueron calcio (Ca), fósforo (P) y su relación (Ca:P). Las concentraciones plasmáticas de P mostraron una diferencia significativa entre las áreas de alimentación estudiadas, disminuyendo según la distancia a la región costera. El trabajo recomienda el estudio de los niveles de fósforo plasmático como indicador de la calidad ambiental de los sitios de alimentación en Brasil. Para ello, se deben realizar estudios que correlacionen los niveles de P de los componentes del alimento de las tortugas verdes con los niveles plasmáticos.

Palabras clave: Ecotoxicología. Macronutrientes. Tortuga verde.

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REFERENCES

ADKESSON, M. J.; LANGAN, J. N. Metabolic bone disease in juvenile Humboldt penguins (*Spheniscus humboldti*): investigation of ionized calcium, parathyroid hormone, and vitamin D3 as diagnostic parameters. **Journal of Zoo and Wildlife Medicine**, v. 38, n. 1, p. 85-92, 2007.

AGUIRRE, A. A.; BALAZS, G. H.; SPRAKER, T. R.; et al. Adrenal and hematological responses to stress in juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas. **Physiological Zoology**, v. 68, n. 5, p. 831-854, 1995.

ANDERSON, E. T.; HARMS, C. A.; STRINGER, E. M.; et al. Evaluation of hematology and serum biochemistry of cold-stunned green sea turtles (*Chelonia mydas*) in North Carolina, USA. **Journal of Zoo and Wildlife Medicine**, v. 42, n. 2, p. 247-255, 2011.

ARTHUR, K. E.; LIMPUS, C. J.; WHITTIER, J. M. Baseline blood biochemistry of Australian green turtles (*Chelonia mydas*) and effects of exposure to the toxic cyanobacterium *Lyngbya majuscula.* **Australian Journal of Zoology**, v. 56, n. 1, p. 23-32, 2008.

BJORNDAL, K. A. Foraging Ecology and Nutrition of Sea Turtles. In: LUTZ, P. L.; MUSICK, J. A. **The Biology of Sea Turtles**. Boca Raton: CRC Press, 1997. Chapter 8, p. 199-231.

BOLTEN, A. B.; BJORNDAL, K. A. Blood profiles for a wild population of green turtles (*Chelonia mydas*) in the southern Bahamas: size-specific and sex-specific relationships. **Journal of Wildlife Diseases**, v. 28, n. 3, p. 407-413, 1992.

CARCIOFI, A. C.; OLIVEIRA, L. D. Doenças nutricionais. In: CUBAS, Z. F.; SILVA, J. C. R.; CATÃO-DIAS, J. L. **Tratado de Animais Selvagens – Medicina Veterinária**. São Paulo: Roca, 2006. P. 838-860.

CARDONA, L.; CAMPOS, P.; LEVY, P. et al. Asynchrony between dietary and nutritional shifts during the ontogeny of green turtles (*Chelonia mydas*) in the Mediterranean. **Journal of Experimental Marine Biology and Ecology**, v. 393, n. 1-2, p. 83-89, 2010.

CETESB - COMPANHIA AMBIENTAL DO ESTADO DE SÃO PAULO. Norma Técnica L5.306 - Determinação de Clorofila *a* e Feofitina *a*: método espectrofotométrico. **Diário Oficial do Estado de São Paulo**, Caderno Executivo I, v. 124 (71) de 15/04/14, Poder Executivo, Seção I, p. 53-55, 2014.

FLINT, M.; MORTON, J. M.; LIMPUS, C. J.; et al. Development and application of biochemical and haematological reference intervals to identify unhealthy green sea turtles (*Chelonia mydas*). **The Veterinary Journal**, v. 185, n. 3, p. 299-304, 2010.

FONG, C. L.; CHEN, H. C.; CHENG, I. J. Blood profiles from wild populations of green sea turtles in Taiwan. **Journal of Veterinary Medicine and Animal Health**, v. 2, n. 2, p. 8-10, 2010.

HAMANN, M.; GODFREY, M. H.; SEMINOFF, J. A.; et al. Global research priorities for sea turtles: informing management and conservation in the 21st century. **Endangered Species Research**, v. 11, p. 245-269, 2010.

HIGGINS, B. M. Sea Turtle Husbandry. In: LUTZ, P. L.; MUSICK, J. A.; WYNEKEN, J. (Eds.). **The Biology of Sea Turtles**. Vol. II. Boca Raton: CRC Press, 2003. P. 411-440.

ICMBIO - INSTITUTO CHICO MENDES DE CONSERVAÇÃO DA BIODIVERSIDADE. **Livro Vermelho da Fauna Brasileira Ameaçada de Extinção**. Volume IV – Répteis. Brasília: ICMBio/MMA, 2018. 252p.

LOBBAN, C. S.; HARRISON, P. J. **Seaweed Ecology and Physiology**. Cambridge: Cambridge University Press, 1994. 366p.

MADER, D. R. **Reptile Medicine and Surgery**. 4. ed. Philadelphia: W.B. Saunders, 1996. 531p.

MIGUEL, C.; SANTOS, M. R. D.; BIANCHINI, A.; et al. Potential adverse effects of heavy metals on clinical health parameters of *Caretta caretta* from a nesting area affected by mining tailings in Brazil. **Journal of Trace Elements and Minerals**, v. 2, p. 1-12, 2022.

MOTTA, V. T. **Bioquímica Clínica para o Laboratório - Princípios e Interpretações**. 5. ed, Rio de Janeiro: Medbook, 2009. P. 145-166.

PEREIRA, N. S.; MANSO, V. A. V.; SILVA, A. M. C.; et al. Mapeamento Geomorfológico e Morfodinâmica do Atol das Rocas, Atlântico Sul. **Revista da Gestão Costeira Integrada**, v. 10, n. 3, p. 331-345, 2010.

SANTOS, A. L. Q.; MALTA, T. S.; MUNDIM, A. V.; et al. Variação dos constituintes bioquímicos sanguíneos de tartarugas-da-amazônia (*Podocnemis expansa*, Schweigger - 1812) (*Testudinata*) mantidas em criatório comercial. **Archives of Veterinary Science**, v. 10, n. 3, p. 1-8, 2005.

SANTOS, R. G.; MARTINS, A. S.; FARIAS, J. N.; et al. Coastal habitat degradation and green sea turtle diets in Southeastern Brazil. **Marine Pollution Bulletin**, v. 62, n. 6, p. 1297-1302, 2011.

SNODDY, J. E.; LANDON, M.; BLANVILLAIN, G.; et al. Blood Biochemistry of Sea Turtles Captured in Gillnets in the Lower Cape Fear River, North Carolina, USA**. The Journal of Wildlife Management**, v. 73, n. 8, p. 1394-1401, 2009.

STRINGER, E. M.; HARMS, C. A.; BEASLEY, J. F.; et al. Comparison of ionized calcium, parathyroid hormone and 25-hydroxyvitamin D in rehabilitation and healthy wild green sea turtles (*Chelonia mydas*). **Journal of Herpetological Medicine and Surgery**, v. 20, n. 4, p. 122-127, 2010.

THRALL, M. A.; WEISER, G.; ALLISON, R. W.; et al. **Hematologia e Bioquímica Clínica Veterinária**. 2. ed. São Paulo: Roca, 2015. 688p.

TOREZANI, E.; BAPTISTOTTE, C.; MENDES, S. L.; et al. Juvenile green turtles (*Chelonia mydas*) in the effluent discharge channel of a steel plant, Espírito Santo, Brazil, 2000-2006. **Jornal of the Marine Biological Association of the United Kingdom**, v. 90, n. 2, p. 233-246, 2010.

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