Morphological evaluation of the ileum, proximal colon and distal colon of green turtles (*Chelonia mydas*) dead on the slopes in the Environmental Protection Areas of Cananéia-Iguape-Peruíbe and Mosaic Jureia-Itatins Conservation Units, Peruíbe-SP

Avaliação Morfológica do íleo, colo proximal e colo distal das tartarugas-verdes (*Chelonia mydas*) mortas em encalhes nas Áreas de Proteção Ambiental Cananéia-Iguape-Peruíbe e Mosaico de Unidades de Conservação Jureia-Itatins, Peruíbe-SP

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Edris Queiroz Lopes  
Doutor em Ciências pela Universidade de São Paulo  
Instituição: Instituto de Biologia Marinha e Meio Ambiente - IBIMM /Universidade de São Paulo  
Endereço: Fazenda Palmares – Santa Cruz das Palmeiras- SP- Brasil - CEP: 13650-000  
E-mail: edris@ibimm.org.br

Milena Joice Bressan  
Bacharel em Ciências Biológicas pela Universidade Nove de Julho  
Instituição: Instituto de Biologia Marinha e Meio Ambiente-IBIMM  
Endereço: Rua São Joao, 126 – Campo Limpo Paulista-SP –CEP: 13238-212  
E-mail: milena_bressan@hotmail.com

Tatiane Gonçalves de Lima  
Graduanda em Ciências Biológicas pela Universidade Cruzeiro do Sul de São Paulo  
Instituição: Instituto de Biologia Marinha e Meio Ambiente  
Endereço: Fazenda Palmares – Santa Cruz das Palmeiras-SP- CEP: 13650-000  
E-mail: tatiane@ibimmorg.br

Luana Felix de Melo  
Doutoranda em Ciências pela FMVZ-Universidade de São Paulo  
Instituição: Instituto de Biologia Marinha e Meio Ambiente-IBIMM  
Endereço: Av. Prof. Orlando Marques de Paiva, 87–Butantã –São Paulo- SP – CEP: 05508-010  
E-mail: luuh-felix@hotmail.com

Rose Eli Grassi Rici  
Doutora em Ciências Pela Universidade de São Paulo  
Instituição: Universidade de São Paulo  
Endereço: Av. Prof. Orlando Marques de Paiva, 87–Butantã –São Paulo- SP – CEP: 05508-010  
E-mail: roseeli@usp.br
ABSTRACT
Among the consequences of disordered human occupation brings to the environment, we can mention
the changes in the areas used by green turtles for food, which start to present higher amounts of
anthropogenic solid wastes such as plastic debris and other oil derivatives. These materials cause
numerous gastrointestinal disorders in these animals, causing, in most cases, their death. The aim of
this study was to morphological evaluation of the ileum, proximal colon and distal colon of green
turtles (Chelonia mydas) found dead and stranded in Peruibe coastline. Removal of organs from the
digestive system was performed after excision of the ventral part, called plastron. To describe the
intestinal portions, samples were collected from the opening made by the mesenteric border. The
samples were fixed and prepared for microscopic analysis. The results of this study demonstrated the
presence of rectilinear and folded pleats, longitudinal smooth muscle fibers, submucosa formed by
loose connective tissue and blood vessels in the muscular layer; an inner circular layer and a long
longitudinal layer, both with smooth muscle. The presence of folds was found in the proximal colon,
but absent in the distal colon. It was observed alternation of bulging regions (with smooth muscle)
and histologically, pleated mucosa containing glands and lymphocytes, muscular mucosa formed by
smooth muscle and submucosal constituted by loose connective tissue. In scanning electron
microscopy, the smooth muscle layers in the ileum were well evidenced, as well as the presence of
numerous blood vessels in the proximal and distal colon. Therefore, this study can be of great value,
to assist in field work and veterinary medicine in captivity.

Keywords: Chelonia mydas, Gastrointestinal Tract, Anatomy, Food Behavior.
1 INTRODUCTION

Sea turtles are reptiles that are present on Earth for about 150 million years, and have resisted the drastic changes that have taken place over time (BAPTISTOTTE, 1992). Currently, there are seven species, of which five can be found on the Brazil’s coastline (SANCHES, 1999). The green turtle (Chelonia mydas) is considered the most abundant species on the Brazilian coast (FIDELIS et al., 2005), with a greater number of occurrences (strandings, bycatch and sightings), and spawning in the regions of oceanic islands, such as Fernando de Noronha, Trindade and Atol das Rocas (ALMEIDA et al., 2011; MARCOVALDI et al., 2011). It is possible to observe the presence of individuals’ juveniles in the coastal region of Brazil, such as the city of Peruíbe, located on the South Coast of the State of São Paulo (LOPES et al., 2018-2019; MARCOVALDI et al., 2011; SANTOS et al., 2011).

Chelonia mydas is the only species of sea turtle that exhibits variation in feeding behavior during the growth stage. It has two stages of development, divided between pelagic (oceanic) and neritic. The pelagic phase is the one that occurs after birth, with subsequent migration to the neritic environment when they measure between 25 and 35 cm (BJORNDAL, 1997; REICH et al., 2007). In the early stages of life, they have an omnivorous feeding behavior, with tendencies to feed small marine invertebrates (BRAND-GARDNER et al., 1999), and with the habitat change, they modify the food habit, for a totally herbivorous (BJORNDAL, 1997).

Studies by Ferreira (1968) have shown that sea grass are the main food of the green turtle diet. It should be noted that the herbivorous diet plays an important role in the parameters of the history and probability of life of these organisms, since it acts in a significant way in the food chain, mainly in the control of marine algae in the habitat in which they are inserted (BJORNDAL, 1997).

There is still much to be discovered about the morphology of the structures of the gastrointestinal tract of Chelonia mydas. Studies have shown that its morphology is associated with food behavior/preference (SILVA, 2004) and can be observed according to the digestive processes, since this conditionate the specializations of the gastrointestinal tract. According to Magalhães et al. (2007), the length of the organs that are part of the digestive tube can also be correlated with the type of diet of the turtles. Morphometric analysis revealed that the small intestine tends to be longer in carnivores, whereas in herbivores the opposite occurs, the large intestine being larger.

These studies may be used in conjunction with other types of exams, such as endoscopy, tomography, and radiography, to aid in better understanding of normal physiology or pathologies that affect chelonians (MEYER, 1998). According to Holt (1978) and Silva et al. (2007), the studies are
scarce; mainly regarding the evaluations of obstructions of the gastrointestinal tract and fecalomas that causes obstructions of the intestinal transit.

Thus, the objective of this study was to perform the morphological evaluation of the ileum, proximal colon and distal colon of the green turtles (*Chelonia mydas*) death on strandings in the Cananéia-Iguape-Peruíbe Environmental Protection Areas. To know the morphological, physiological and biochemical processes of these animals is extremely important, since it helps to define plans for conservation and management, especially in a rehabilitation center.

2 MATERIAL AND METHODS

To carry out this study, we used 03 individuals of the species *Chelonia mydas*, found dead and collected by the SOS Sea Turtles Project for carrying out monitoring on the beaches of Peruíbe region, metropolitan area of Santos, São Paulo, Brazil. This area of study is part of the Cananéia-Iguape-Peruíbe Area de Proteção Ambiental – Mosaico da Juréia – Itatins, Unidade de Conservação e Estação Ecológica Tupiniquins no Município de Peruíbe (APA-CIP), a federal conservation unit within the Atlantic Forest biome (LOPES et al., 2018; MORAES et al., 2016).

The SOS Turtles Project is developed by the Institute of Biology and Environment (IBIMM) with the purpose of studying and acting on the conservation of these animals in marine ecosystems. It has a license from Tamar/ CMBIO-50132-5, authorization of the CEUA-IBIMM Ethics Committee n°007/18, and the turtles were donated to the School of Veterinary Medicine and Animal Science at University of São Paulo (FMVZ/USP), that was approved by its Ethics committee on the use of animals (CEUA-FMVZ/USP N°. 3829270117).

Among the laboratory procedures performed are the biometry survey, in which the parameters of curvilinear carapace length (CCC) and curvilinear carapace width (LCC) were measured. Necropsy, macro and microscopic analysis were also performed. To obtain CCC measurements, the measuring tape was fixed to the anterior part and extended to the back of the animal's hull, and the LCC measurement, the measuring tape was fixed at the widest horizontal point of the hull on one side and extended to the other. This measure was repeated in three different points of the carapace of each animal, to guarantee greater precision.

Necropsy of the individuals studied was performed according to the procedure described by Wyneken (2001), allowing the macroscopic analysis of the external and internal morphological properties of the ileum, proximal colon and distal colon. To realize the microscopic analysis, segments of the aforementioned parts were collected, being opened by the mesenteric border, washed...
in PBS, placed in wooden rafts with the mucosa facing upwards, and then fixed in 4% paraformaldehyde for 52 hours.

For light microscopy, parts of the samples were dehydrated in an increasing series of ethanols (70 to 100%) and diaphanized in xylol, with subsequent inclusion in histological paraffin, 5 μm thick cuts were performed on the microtome (Leika, German) and stained with hematoxylin-eosin (H&E) (MAIA, 1979). The images were obtained through the Nikon Eclipse E-800 light microscope at Advanced Center in Diagnostic Imaging (CADI - FMVZ/USP), (MELO et al., 2019).

For scanning electron microscopy, part of the samples were fixed in 4% paraformol and dehydrated in 3 increasing sets of alcohols in concentrations of 70%, 80%, 90% and 100% for 5 minutes in each. The samples were dried in a LEICA EM CPD 300 critical point apparatus and, then, glued with carbon glue on aluminum stub bases and subjected to silver plating on the EMITECH K550 device. Finally they were analyzed and photographed in scanning electron microscope LEO 435VP at Advanced Center for Diagnostic Imaging (CADI-FMVZ/USP), (MELO et al., 2019).

3 RESULTS

Macroscopically, after the opening of the plastron, the topography of all organs was documented. They have been in good condition of conservation, being possible to characterize them (figure 1).

![Figure 1](image-url) - Image obtained from the opening after removal of the plastron, showing the positions of the internal organs of the sea turtle (*Chelonia mydas*). Legend: 1 - Esophagus; 2 - Stomach; 3 - Heart; 4 - Liver; 5 - Large Intestine; 6 - Small Intestine; 7 - Pectoral Musculature.
The ileum had longitudinal rectilinear folds and rough regions (Figure 2A). Both the scanning electron microscopy (Figure 2B-D) and the light microscopy (Figure 2E-G) revealed the division of muscle layers and the presence of muscle fibers in the layers and rectilinear folds. The descriptive histology of the ileum was marked by the presence of villi. The results showed that in the juvenile animals, the ileum presented a serous layer, muscular internal and muscular longitudinal, presence of muscular fibers juxtaposed smooth muscle and the lamina propria constituted by loose connective tissue richly vascularized with accumulation of lymphocytes.

Figure 2 - Morphology of the green turtle's ilium (*Chelonia mydas*). In A, the rectilinear folds of the mucosa (juxtaposed) were observed. The position of the ileum stratum was observed in B, C and D by scanning electron microscopy; rectilinear and foliated rectilinear muscle fibers (C); and the longitudinal smooth muscle fibers (cross section) (D). Bar 1μm/Bar 300μm. In E, F, G, longitudinal sections analyzed in photomicrography. Stratum: (IVM) microvilli (SE) - cells that make up ileal serosa tissue; (LM) - longitudinal muscle; (CM) - muscle layer; (LP) - own blade; (MM) - muscular mucosa; (MUC) - mucosa; (VS) blood vessels - Bar 100μm/500μm/Bar 50μm.

Externally, the large intestine (LI) was marked by alternating bulging regions (haustros or sacculations), narrowing and absence of folds (Figure 3A). The proximal colon presented, in its histology, the pleated mucosa containing glands in the lamina propria. In analysis from photomicrography, it is observed that the cellular layers that make up the proximal colon tissue are abundant and vascularized. It was possible to identify the layers of serosa, longitudinal muscle,
muscular layer, lamina propria, muscular mucosa and mucosa (Figure 3B). Through scanning electron microscopy it was possible to observe the cellular layers and blood vessels (Figure 3C and D). The distal colon presents, in its histology, cellular layers, with presence of lymphocytes and great vascularization. The absence of pleated mucosa is observed (Figure 3E). In longitudinal section, it was possible to observe the arrangement of the cellular layers by scanning electron microscopy (figure 3F).

Figure 3 - Large intestine of the green turtle and its rugged regions. In A, macroscopic view of the large intestine. In B, C and D, the proximal colon of the green turtle (Chelonia mydas) observed by light microscopy and scanning electron microscopy. (B) and (C) - The cell layers are identified: (LM) - longitudinal muscle; (SE) - serose; (LM) - longitudinal muscle; (CM) - muscle layer; (LP) - lamina propria; (MM) - muscular mucosa; (MUC) - mucosa. In (D) one has the cellular layers that make up the region of the proximal, rich and vascularized colon, and the blood vessels - microvilli can be visualized. Bar: 100μm. In E, the cellular layers of the distal colon can be seen, with presence of lymphocytes and with high vascularization; stained with HE, 100μm bar. In F, the arrangement of the cellular layers in the longitudinal session is observed. Bar: 300μm.
4 DISCUSSION

There are few studies related to the morphology of sea turtles, consequently the bibliographies on the subject are scarce, which leads us to propose more studies in this area, allowing a better understanding of the feeding and metabolic behavior of these organisms, which was also proposed by Magalhães et al. (2010) and Luz et al. (2003).

Macroscopically, it was possible to visualize the change between the intestinal portions duodenum/jejunum, since the duodenum has reticular folds, while the jejunum/ileum have rectilinear folds. However, it was not possible to identify and delimit the transition of the intestinal portions between jejunum and ileum. Microscopically, the ileum presented rectilinear and foliate folds, corroborating with the results described by Magalhães et al. (2010). According to Queiroz et al. (2009), the chelonians of the family Podocnemididae have a small intestine that is difficult to delimit macroscopically, i.e. visually it is not possible to distinguish the separation between the duodenum, jejunum and ileum. However, this distinction can be realized and identified by means of light microscopy, whose histological sections showed a mucosa formed by reticular and longitudinal zig-zag folds.

The lamina propria present in the ileum is found below the epithelium and serves as a support, fill spaces, nourish, store water / electrolytes and has an important role in defense, since it contains macrophages, mast cells, plasma cells and leukocytes, consisting of an extracellular matrix (HAM, CORMACK, 1983; GARTNER, HIATT, 2007; ROSS, PAWLINA, 2012).

The accumulation of lymphocytes may occur due to the extensive mucosal surface that is exposed to many potentially invasive microorganisms (JUNQUEIRA, CARNEIRO, 2013) and also those that constitute the flora of the gastrointestinal tract, aiding in the digestive process. The presence of these cells in the tissue constitutes an important site of immune responses; in human mammals, also, lymph nodes and dispersed immunocompetent cells can be found (NASCIUTTI, et al., 2016).

In certain regions of the gastrointestinal tract, the mucosa and submucosa form the intestinal folds/projections, known as villi that functionally contribute to increase the absorptive capacity (NASCIUTTI, et al., 2016), as it presents several structures that enlarge its surface, thus increasing the area available for nutrient absorption (JUNQUEIRA, CARNEIRO, 2013) in relation to the greater contact surface / mucosal adhesion (FUGI & HAHN, 1991; MAGALHÃES et al., 2010; PORTER, 1972; WYNEKEN, 2001). It is thus explained that the ileum (small intestine) has folds in the mucosa while the distal colon (portion of the large intestine) does not have, since the first one has the main role of greater absorption of nutrients (MAGALHÃES et al., 2010).
The folds, villi and microvilli have the function of considerably increasing the surface of intestinal lining. According to Junqueira and Carneiro (2003), it is known that the folds can increase the intestinal surface in about 3 times; the villi in 10-fold and the microvilli in about 20-fold. In mammals, these processes are responsible for an approximately 600-fold increase in intestinal surface, resulting in an area of approximately 200m².

These specializations for Chelonia mydas are necessary because is difficulto to digest their feed due to the presence of polysaccharides in the plant cell wall. Among them, cellulose and hemicellulose can be mentioned, as well as the presence of pectic substances, agarans, carrageenans, and others substances that form part of the coating walls and protection of the algae that feed this specie (FERREIRA, 1968; MAGALHÃES et al., 2010; RAVEN, 2014).

The absence of folds in the region of the distal colon was also proposed by Magalhães et al. (2007) who observed that at the beginning of the large intestine it was possible to observe folds and then became devoid of these. The large numbers of cells of the immune system observed should be linked to the variety and abundance of the bacterial population in the large intestine (JUNQUEIRA et al., 2010). Scanning electron microscopy evidenced the great presence of blood vessels and cell layers.

5 CONCLUSION

The morphology of the digestive tube of Chelonia mydas was satisfactory to its food habit, with specific specializations such as differences in intestinal mucosa, folds, large vascularization and a developed defense system. The specializations help in the digestive process and are necessary, because their food is difficult to digest due to the type of food they forage.

The morphological knowledge of structures involved in basic physiological processes in organisms is extremely important in the acquisition of ecological and evolutionary knowledge about species. This work was carried out for the description and morphological characterization of the small intestine (ileum) and large intestine (proximal neck and distal neck) of the green turtle (Chelonia mydas) with the purpose of generating information that would aid comparative analysis with other testudines, as well as assist new studies.
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