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Characterization of the feeding behavior of the green turtle (*Chelonia mydas*) in the Juréia-Itatins Mosaic's Conservation Units, South Coast of the São Paulo state

Caracterização do comportamento alimentar da tartaruga-verde (*Chelonia mydas*) nas unidades de conservação do Mosaico Juréia-Itatins, Litoral Sul do estado de São Paulo

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ABSTRACT
The *Chelonia mydas* occurs most frequently in the Cananéia-Iguape-Peruíbe Environmental Protection Area and Conservation Units Jureia-Itatins, using coastal regions of the continent and islands of the region as a feeding area. It presents a habitat change during its ontogenetic development. The individuals migrate from the pelagic environment, in this stage they had an omnivorous diet, when they change this feeding area, to the neritic environment, they can have predominantly

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herbivorous´ diet. However, recent studies have suggested that both habitats change and, consequently, the diet changes may be optional. The objective of this study was to characterize the feeding behavior of green turtles in the municipality of Peruíbe, south coast of São Paulo. The data were collected from October/2018 to October/2019, with the monitoring of the beaches and the study of the specimens found. This study was approved by the Ethics Committee on The Use of Animals (CEUA/IBIMM) n°. 008/18 and by the Biodiversity Authorization and Information System (SISBIO/ICMBio/MMA) n°. 50132. In the period, 7 individuals were collected who were submitted to biometrics, necropsy and food content collection to verify their composition. Organic and inorganic constituents were separated and the macroalgae found in the esophagus and stomach samples were submitted to a second screening, using morphology and staining as criteria. The mass frequency of each phylum of macroalgae was calculated. The results showed that the individuals were juveniles with CCL 36.33 cm and presented predominantly herbivore feeding behavior, because there was no food from animal origin, consisting only of shell fragments of molluscs and exoskeletons of crustaceans, whose intake may have been accidental. The total survey of macroalgae present in the esophagus and stomach were Chlorophyta (42.76%), Rhodophyta (33.19%) and Heterokontophyta (24.05%), the presence of diatoms was observed complementing the diet of the animals. In addition, it was found that all had anthropogenic residues in their gastrointestinal tracts, and 73.33% of total constituents found are associated with plastic and plastic bags from fishing activities. It was concluded that the Region of Peruíbe is an important feeding and development habitat for the species. A problem related to solid waste pollution was observed which consequently shows the need to develop environmental education work with fishers, population and tourists oriented to recycling and the reduction of anthropogenic actions on site.

Keywords: Testudines, Food, Environmental Protection Area, Herbivory, Aquatic chelonians.

RESUMO

A tartaruga-verde (*Chelonia mydas*) a espécie de tartaruga marinha que ocorre com maior frequência na Área de Proteção Ambiental Cananéia-Iguape-Peruíbe e Unidades de Conservação do Mosaico Jureia-Itatins, utilizando regiões costeiras do continente e ilhas da região como área de alimentação. Apresenta uma alteração de habitat durante o seu desenvolvimento ontogenético. Os indivíduos migram do ambiente pelágico, estágio que apresentam uma dieta onívora, para o ambiente nerítico, cuja dieta tende a ser preferencialmente herbívora. Contudo, estudos recentes têm sugerido que tanto a mudança de hábitat e, consequentemente, a alteração da dieta pode ser facultativos. Este trabalho teve o objetivo de caracterizar o comportamento alimentar das tartarugas-verdes no município de Peruíbe, litoral Sul de São Paulo. A coleta de dados foi realizada no período de outubro/2018 a outubro/2019, com o monitoramento das praias e o estudo dos exemplares encontrados. Este trabalho foi aprovado pela Comissão de Ética sobre Uso de Animais (CEUA/IBIMM) nº 008/18 e pelo Sistema de Autorização e Informação em Biodiversidade (SISBIO/ICMBio/MMA) nº 50132. No período, foram coletados 7 indivíduos que foram submetidos aos procedimentos de biometria, necropsia e coleta do conteúdo alimentar para verificar sua composição. Foram separados os constituintes orgânicos e inorgânicos e os exemplares de macroalgas encontrados nas amostras de esôfago e estômago foram submetidos a uma segunda triagem, utilizando como critério a morfologia e a coloração. Procedeu-se ao cálculo da frequência em massa de cada filo de macroalgas. Os resultados mostraram que os indivíduos eram juvenis com CCC médio 36,33 cm e apresentaram comportamento alimentar predominantemente herbívoro, pois não havia itens alimentares provenientes de origem animal, consistindo apenas em fragmentos de concha de moluscos e exoesqueletos de crustáceos, cuja ingestão pode ter sido acidental. O levantamento total de macroalgas presentes no esôfago e estômago foram Chlorophyta (42,76%), Rhodophyta (33,19%) e Heterokontophyta (24,05%), foi observada a presença de diatomáceas complementando a dieta dos animais. Além disso, foi constatado que todos possuíam resíduos antropogênicos em seus tratos gastrointestinais, sendo que 73,33% na frequência

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dos constituintes totais encontrados estão associados a sacolas plásticas e plásticos proveniente das atividades de pesca. Conclui-se que a região de Peruíbe é um habitat de alimentação e desenvolvimento importante para a espécie. Foi evidenciado um problema relacionado a poluição por resíduos sólidos, o que consequentemente evidencia a necessidade de desenvolver um trabalho de educação ambiental com os pescadores, moradores e turistas direcionado para reciclagem e a redução das ações antropogênicas no local.

Palavras-chave: Testudines, Alimentação, Área de Proteção Ambiental, Herbivoria, Quelônios aquáticos.

1 INTRODUCTION

The green turtle (*Chelonia mydas*) is one of five species of sea turtles that occur on the Brazilian coast and has cosmopolitan distribution, being found in coastal environments (foraging or feeding areas), in tropical and subtropical seas, usually between latitudes 40ºS and 40ºN (ALMEIDA et al., 2011; HIRT, 1997, LOPES *et al.*, 2018 e 2019).

There is a difficulty in the study of species eating behavior due to its migratory behavior and "lost years", a period between the birth of specimens and return to the coast, when they present pelagic habits (BJORNDAL, 1996). The change in eating habit occurs when individuals migrate from the pelagic environment; stage that presents an omnivorous diet, with a strong tendency to carnivory (BJORNDAL, 1985); for feeding areas, in a neritic environment, where they switch to a basically herbivore diet (BJORNDAL, 1996). Recent studies have suggested that this change of habitat is optional and, consequently, the same occurs with diet change (HATASE *et al*., 2006).

According to the standards of the Tamar Project, the growth stage of sea turtles can be estimated according to carapace curvilinear length measurements (CCL), being considered as hatchlings, individuals with CCL up to 29.9 cm; between 30 cm and 95.9 cm are juveniles and above 96 cm are adults (PRIOSTE, 2016).

However, to characterize the behavior of turtles it should also be considered that the feeding habitats vary greatly in their attributes to abiotics (the depth of the sea, the type of sediment characteristics and the presence/absence of tidal flow) and biotics (the availability of food, the selectivity and digestibility of the individual) (EHRHART; OGREN, 1999; SAZIMA; SAZIMA, 1983). According to Hirt (1997), the diet varies according to the habitat, because the algae present in the diet of *C. mydas* vary depending on the maritime region and the populations are present. This variation can be observed in studies conducted in the Rio de Janeiro city, in Cananéia (south coast of São Paulo state) and on the south coast of Rio Grande do Sul.

In the stomach contents of *C. mydas,* with CCL medium size of 42.5cm, collected on the coast of the Rio de Janeiro city, between 2007 and 2009, thirteen types of marine algae were identified

(PEDRINI *et al.*, 2010). The researchers observed that in the studied region, the specimens have preference for shallow water marine plants. In a study developed by Nagaoka and collaborators in 2012 at the Estuarine-lagoon complex of Cananéia, green turtles presented an omnivorous diet and it is important to emphasize that intake of invertebrates may have been both incidental and selective. The same result was obtained in a survey conducted in Rio Grande do Sul, whose diet of the specimens analyzed, with an average CCL of 37.7 cm, can be considered opportunistic and generalist, because they did not tend to herbivorous PEDRINI *et al.*, 2010). A factor that impacts the life of green turtles is the presence of anthropogenic residues, mainly flexible plastics and nylon, and are probably related to eating habits and accidental ingestion, since these residues may be entangled to macroalgae (SECCO *et al.* 2010; AWABDI *et al.*, 2013).

The Ceará, Rio de Janeiro, Bahia, Paraná, Rio Grande do Sul and São Paulo states coasts are considered "monitored feeding areas" for the species *Chelonia mydas,* in these states there are great availability and abundance of algae along the entire coast, mainly Chlorophyta and Rhodophyta types (SFORZA *et al.*, 2017). Juveniles individuals can occupy these feeding areas for several months when food resources are available (HART; FUJUSAKI, 2010; MAKOWSKI *et al.*, 2006). According to Avanzo Neto and Fujii (2016) the diversity of macroalgae belonging to the ficoflora of the São Paulo State presents 218 (65.14%) Rhodophyta species; 71 (21,2%) Chlorophyta and 46 (13.7%) Heterokontophyta.

A continuous data collection that characterizes the diet of *Chelonia mydas* in the south coastal region of the São Paulo State is fundamental for the knowledge and maintenance of this species, in addition provides information that assists in the conservation and protection of feeding areas, a determining factor for the existence and permanence of sea turtles both on the Brazilian coast and in all oceans.

The objective of this study was to characterize the feeding behavior of green turtles, from the gastrointestinal content analysis of dead specimens found in Peruíbe´s beaches, South Coast, São Paulo State.

2 MATERIAL AND METHODS

The project was developed by the Institute of Marine Biology and Environment (IBIMM), which executes preserving programs of the sea turtles, based on the “Projeto SOS Tartarugas Marinhas”. This study was approved by the Ethics Committee on The Use of Animals (CEUA/IBIMM) n°. 008/18 and by the Biodiversity Authorization and Information System

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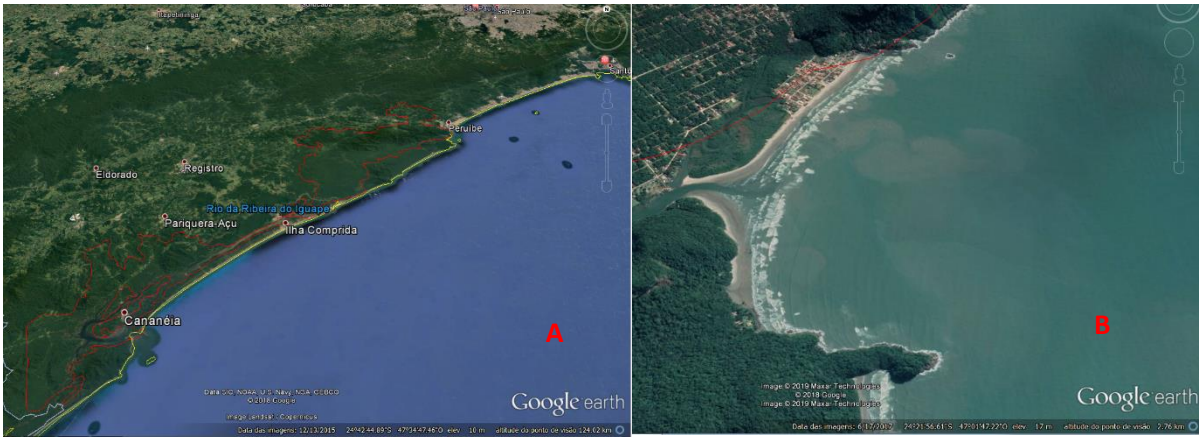
(SISBIO/ICMBio/MMA) n°. 50132. The research was conducted with the monitoring of the beaches and study of the collected animals.

Monitoring phase

The monitoring phase was developed on the Guaraú Beach (S 24° 19' 2", W 46° 59' 44"), in the Peruíbe city, South Coast of São Paulo State, that is situated in the "The Cananéia-Iguape-Peruíbe Environmental Protection Area (APA) - Juréia-Itatins Mosaic of Conservation Units and Tupiniquins Ecological Station" – APA-CIP, a federal conservation unit of sustainable use (figure 1) that it is part of the “Atlantic Forest South-East Reserves” World Heritage site and UNESCO Biosphere Reserve.

Figure 1 - **(A)** Location of the Environmental Protection Area of Cananéia-Iguape-Peruíbe (APA-CIP) e Conservation Units Mosaic Jureia-Itatins, on the south coast of the State of São Paulo, Brazil; **(B)** - Guaraú Beach Monitored Area, Peruíbe-SP.



Source: Google Earth, 2020.

Data collection

The data collection was performed from October/2018 to October/2019 and 7 (seven) dead specimens of the *species Chelonia mydas* were found. As soon as found, the individuals were referred to the Institute of Marine Biology and Environment Research Center (IBIMM) and submitted to the biometrics procedure according to the method proposed by Wyneken (2001). The sizes of the animals were measured with measuring tape by obtaining the *Over the Curve Carapace Length* (CCL) and *Over the Curve Carapace Width* (CCW) and were measured the body mass using a digital scale (LOPES *et al.*, 2018).

The necropsy was performed, starting with the opening of the plastron and removal of the pectoral muscles. The organs of the gastrointestinal tract were found from the mouth, delimited with a string and separated, being cut with the surgical scissors. Then, with a measuring tape, the length measurements of each organ were obtained: esophagus, stomach and total intestine (without

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delimiting it in the small and large intestine). The organs compose the digestive system were cut with the aid of a scalpel in a longitudinal cutting to remove the food content.

The samples obtained from the inside of the esophagus, stomach and intestine were subjected to weighing of the total content, with the aid of the analytical balance Denver Instrument Company TR-4102 model (accuracy of 0,01 g). Organic and inorganic constituents were separated. The macroalgae specimens found in esophagus and stomach samples were submitted to a second screening, using morphology and staining as a criterion, with the aid of the Leica EZ24 HD stereomicroscope with an increase between 10 and 30 times and when necessary to assist in morphological visualization, the Nikon Eclipse e200 optical microscope was used. The frequency of each macroalgae phylum was calculated. Tables and graphs were developed using the Microsoft Excel Software program.

3 RESULTS

The data collection obtained during the project can be divided into two parts: monitoring of the beaches and analyzing of the individuals collected in the period.

In the monitoring of the beaches, as illustrated in Figure 2, was observed the presence of a large amount of solid waste. It was possible to verify that these materials may be associated with the food components of sea turtles. Another important factor was to visualize the growth of macroalgae present in the rocky shores and according to Figure 3, may grow associated with bivalve molluscs.

Figure 2 - (A) **-** Inorganic constituents found in Guaraú Beach Solid waste with algae and (B) - Inorganic constituents collected during monitoring on the rocky shores and Guaraú Beach (Picture courtesy of Alexandre de Carvalho Junior, 2019).

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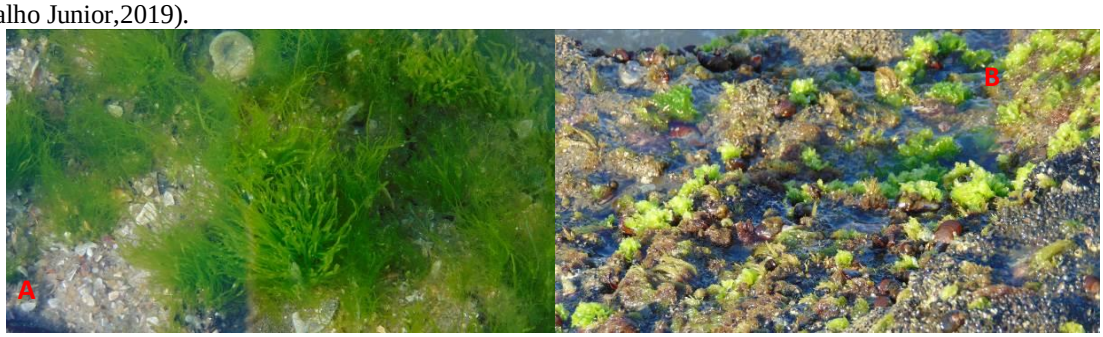
Figure 3 - (A and B) **-** Marine macroalgae observed on the rocky shore of Guaraú Beach. (Picture courtesy of Alexandre de Carvalho Junior,2019).



In the analysis of biometric measurements, juveniles were considered with CCL ranging between 26 and 44.5 cm (table 1).

Table 1 - Biometry data collection and information regarding the strandings of the individuals of the green turtles studied.

Sample	Body Mass (kg)	CCL (cm)	CCW (cm)
T1	4,400	38	36,2
T2	5,300	39	35
T3	4,428	35,5	32,5
T4	3,968	33	31
T5	1,602	26	25
T6	7,852	44,5	39,4
T7	6,500	40	35

The samples of the organic constituents were analyzed and the macroalgae specimens found in each sample were separated and weighted in a precise scale (0.01g), being used as a of color typical and morphology. For the morphological separation of the different groups of algae, the illustrated identification guides were used: Algas Marinhas Bentônicas do Estado de São Paulo of the Avanzo Neto e Fujii (2016), Algas do Estado de São Paulo: Chave artificial para identificação de alguns gêneros of the Oliveira Filho (2019) and Raven (2014).

The contents of the gastrointestinal tracts were separated according to the respective organs and were weighed, the values are described in Table 2. One of the individuals, due to the decomposition stage of the dead and it did not present any content for analysis. The other individuals found presented of esophageal food content (4.62g to 58.58g), stomach food content (18.30g to 92.40g) and intestinal content (11g to 731g).

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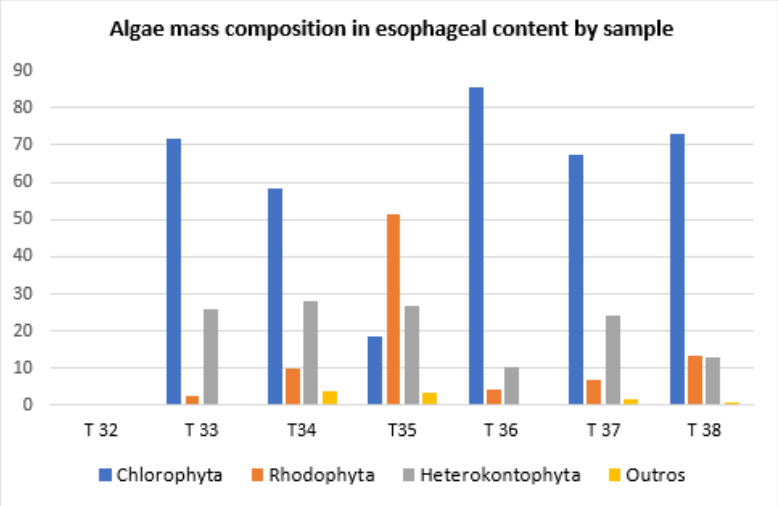
Table 2 - Value of food content obtained according to the harvest in the organs studied.

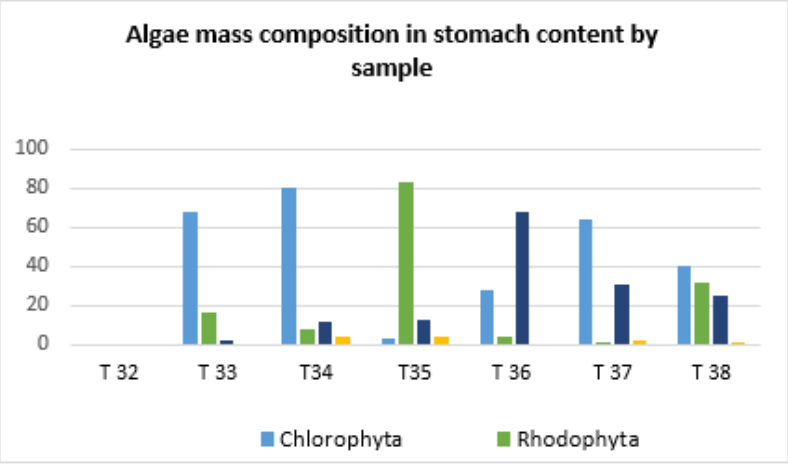
Sample	Esophagus (g)	Stomach (g)	Intestine - complete (g)
T1	0	0	0
T2	45,19	32,01	33
T3	8,11	18,30	236
T4	58,58	92,40	331
T5	4,62	31,33	11
T6	34,63	31,24	731
T7	20,88	87,13	435
Total	172,01	292,41	1777

Esophageal and stomach contents contained green algae (Chlorophyta), red (Rhodophyta) and brown (Heterokontophyta - Phaeophyta class), as well as fragments of leaves, arthropod exoskeletons and fragments of molluscs (others). The mass composition of each group/phylum of macroalgae present in the contents were calculated, as shown in graphs 1 and 2 and the graph 3 shows the algae present in esophageal and stomach content in all analyzed samples.

Graph 1 Algae present in esophageal content by sample.



Graph 2 Algae present in esophageal and stomach content by sample.



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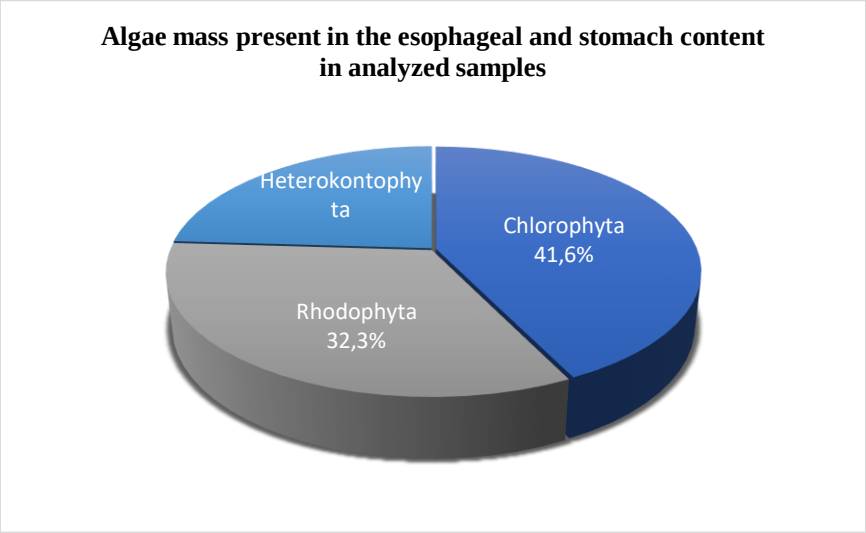
Graph 3 Algae present in esophageal and stomach content in all analyzed samples.



Besides the macroalgae, it was possible to observe the presence of marine microalgae – diatoms – phylum Heterokontophyta), it can be visualized in figure 4.

Figure 4 - Diatoms - marine microalgae - indicated by the arrow. Optical microscopy image with100X zoom (AUTHOR,2018).

The inorganic constituents were categorized according to the nature of the material and the frequency of the total constituents found. As a result, it was found: polypropylene fiber (raffia bag) with 2.22%; plastic bag (garbage bag and market bag) representing 35.56%; transparent adhesive tape being 2.22%; polypropylene fragment (gift packaging bow) 6.67%; hard plastic packaging of 2.22%; cassette tape (magnetic tape) in a percentage of 2.22%; net mesh shown by 4.44%; plastic waste fishing net found in 28.89%; plastic rope was 4.44% and others (unidentified) completed with 11.11% (figure 5).

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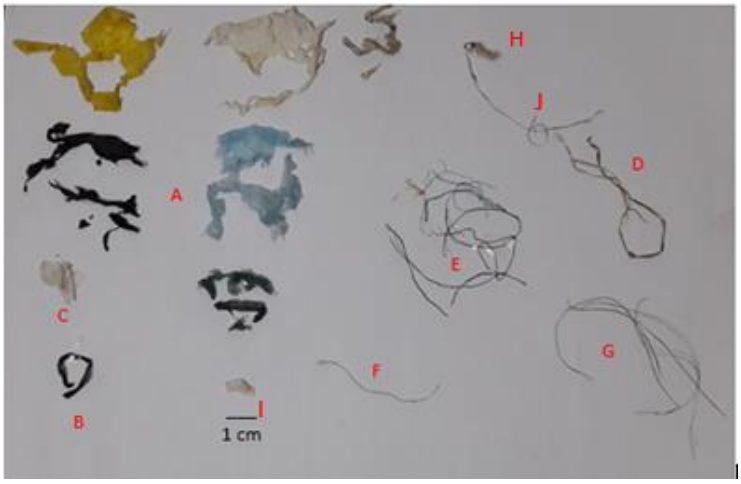
Figure 5 - Inorganic constituents found in specimens analyzed *C. mydas.* (A) garbage bag/market bag; (B) cassette tape (magnetic tape); (C) transparent adhesive tape; (D) polypropylene fragment (gift packing bow); (E) plastic waste fishing net; (F) polypropylene fiber (raffia bag); (G) others and (H) net mesh and; (I) hard plastic packing and (J) plastic rope. (AUTHOR, 2019).



4 DISCUSSION

The growth stage of the individuals analyzed confirmed that the studied region is a feeding area, as mentioned by Lopes *et al.*, (2018 e 2020), so all the information obtained regarding the maintenance of these animals can help preserve of the local ecosystem, which is also an environmental protection area.

The fragments of molluscs and crustaceans exoskeleton were found in the esophageal content and it can be justified, as observed during the monitoring of the beaches, that marine macroalgae can be found mostly fixed to a solid substrate, that is, they have benthic habits and consequently can use bivalve molluscs as substrate for growth and the green turtles when are feeding can accidentally ingest these food items, this can be suggested due to the low presence of animal material found in the stomach and esophageal contents. This result was also addressed by Nagaoka *et al*., (2012) who pointed the ingestion of invertebrates as incidental.

The exoskeletons of crustaceans found were possibly accidentally ingested together with algae because they, due to their morphology, determine the structure of various microhabitats, offering support and shelter to the fauna associated (OLIVEIRA FILHO; BERCHEZ, 1978) therefore, it can be suggested that the feeding behavior of C*helonia mydas* individuals from the coast of Peruíbe is mostly herbivorous. This study had an amount of 7 individuals and the continuity of the research can better elucidate the behavior the individuals who feed there.

The results obtained confirm what was mentioned by Hirth (1997) that the composition of food content can be justified according to variation in the habitat and/or region in which they feed, so eating habits may vary with the populations of green turtles and can be compared with a survey

conducted in Rio Grande do Sul State, whose diet of the specimens analyzed can be considered opportunistic and generalist, because they did not tend to herbivory (BARROS *et al.*, 2007). The researchers suggested that variety of diet may be associated with the limitation of plant resources and/or macroalgae in the region or due to the size of the studied individuals who could be in the transition range of habitat and feeding.

Comparing the frequency of macroalgae present in the São Paulo State's ficoflora with the frequencies of algae ingested by individuals it is possible to agree with Sazima and Sazima (1983) and it can indicate that green turtles may have selective behavior of "inspect" and do not simply consume the most abundant alga species, because the percentage of algae groups varied according to individuals studied.

In the screening of macroalgae, the predominance of the Chlorophyta taxon was obtained, the same result was described by Awabdi *et al.*, (2013) and Souza *et a.l.* (2014). The predominance of green algae may occur due to abundance in much of the Brazilian coast (GUIMARAENS and COUTINHO, 1996; OLIVEIRA *et al.*, 2002) and because it is associated with genus that can be considered opportunistic and tolerant to organic pollution, being able to rapid colonize and grow in unfavorable environmental conditions (LITTLER, 1980).

Diatoms microalgae found in the esophageal and stomach contents studied can be considered as an additional source of energy for sea turtles, because as algae are associated, in a relationship of epiphytism, based on fixation strategies (COSTA, 2015) and were accidentally ingested.

Intestinal contents were not separated due to loss of algae coloring related to digestive processes and also by the high fragmentation level that occurs according to the feeding process, because individuals of the species *Chelonia mydas*, after catching the food, they perform mandibular movements before ingesting food (REISSER *et al.*, 2013). This process is optimized by keratinous beaks of the upper and lower jaws in the marine turtles, the jaws that has a corneal and serrated structure, called rhamphotheca that assists in the fractionation of algae and rupture of the cell walls, exposing its contents, favoring the digestive process (ROMANINI, 2014).

There are currently several awareness campaigns to reduce marine debris on beaches since plastics are one of the main polluters of the marine environment and represents about 85% of the total solid waste found on beaches around the world (ANDRADE NETO, 2010). The ingestion of solid waste can be associated with predominantly herbivore eating behavior and perform the passively ingestion, because they entangle to algae (ANDRADE NETO, 2010), this was observed in one of the individuals studied due to the large presence of hair in sample T7 that was totally tangled in algae and which may hinder the digestive process.

The inorganic material was found entirely in the intestinal portion of the animals analyzed; a result also observed by Romanini (2014). Most of the items found representing 73.33% are associated with plastic bags and plastics forfishing activities. The ingestion of residues can cause several harms to the health of these animals such as obstruction of the gastrointestinal tract causing fecalomas formation (represent firm organized form of impacted feces in intestine); the false feeling of satiety, which can reduce the feed frequency, affecting buoyancy regulation due to the gases generated after ingestion and accumulation of solid waste in the gastrointestinal tract (BJORNDAL, 1996; LUTZ, 1990; LAIST, 1987).

The natural feeding characteristics of the *Chelonia mydas*, has been altered due to anthropic action, on the coast of Peruíbe. The animals that feed in the region are ingesting solid waste, mostly plastic material, intentionally or accidentally, which can cause diseases and even the death of the animal (LOPES *et al.*, 2018 e 2020).

5 CONCLUSIONS

The data obtained in the present study were important to evaluate the behavior of individuals of the species *Chelonia mydas* in Guaraú Beach and the predominance of macroalgae found in the organs of the gastrointestinal tract confirms herbivory as a feeding pattern of the species, and it may be proposed that green turtle presents selective eating behavior, according to the variation in the food composition of individuals, as it shows that they have preference for several kinds and species of seaweeds. It is suggested the continuity of this project for a deeper understanding of the feeding behavior of *Chelonia mydas* that feed in the region.

The results show that the Peruíbe region is an important food and development habitat for the species. As the region is in a federal conservation unit of sustainable uses and integrates the Atlantic Forest biome, it would be importance that other researchers aimed at identifying algae species that are feeding base of the green turtles, in order to verify their richness and abundance, as the integrity of the habitat in the face of human actions. A prolonged study of this nature can help to define the food composition of turtle populations that use the region as a feeding area.

The analysis of the individuals studied showed the problem related to pollution by plastic waste from activities like fishing, tourism and human occupation, because all contained materials of anthropogenic origin in their gastrointestinal tract. In monitoring of the beaches, a large amount of solid waste was also found in the sand and entangled to algae, consequently it was verified the need to develop environmental education work with fishers, population and tourists oriented to recycling and the reduction of anthropogenic actions on site.

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