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CONTRIBUTION TO THE STUDY OF THE GREEN TURTLE (*CHELONIA MYDAS*) DIET IN THE SOUTH ATLANTIC, NORTHEAST BRAZIL

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ABSTRACT

This work presents information about the digestive tract contents of the green turtles (*Chelonia mydas*) on the southern coast of Pernambuco, Northeast Brazil. Between November 2016 and October 2018, the gastrointestinal tract contents were collected from 44 stranded dead individuals. The diet consisted mainly of red algae (65.90%), especially the species *Pterocladiella beachiae*, *Gelidium lineare* (52.72%) and *Gelidiella acerosa* (47.72%). Some animal items were also found, such as the soft coral *Palythoa caribaeroum* (first record for this region), the planktonic mollusks *Jhantina jhantina* and *Spirula* sp., as well as the sponges *Geodia gibberosa* and *Steletta* sp. The contents of the digestive tract showed a strong herbivorous pattern, with indications that green turtles feed mainly on marine macroalgae.

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INTRODUCTION

The species *Chelonia mydas*, known as green turtle, is a testudine widely distributed in tropical and subtropical regions, presenting coastal habits, and is rarely found in temperate waters. This species is typically nektonic and solitary, but occasionally forms aggregations in feeding areas (Márquez, 1990). This characteristic makes it the most common sea turtle species found in feeding areas along the Brazilian coast (Sanches & Bellini, 1999, Grossman *et al*., 2007; Almeida *et al*., 2011; Gallo *et al*., 2006).

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During their life cycle, green turtles perform long-distance migrations and inhabit different habitat types, depending on their life stages (neonates, juveniles and adults) (Bolten, 2003). From the moment they begin to visit coastal sites to forage, their diet converts from the omnivorous one of a pelagic turtle to a herbivorous one, feeding almost exclusively on benthic algae and marine grasses (Bolten, 2003; Cardona *et al*., 2010). Understand the trophic ecology of turtle populations allows inferences about other population parameters, such as growth patterns and size at sexual maturity (Sampson *et al*., 2018), supporting the management and execution of strategies for conservation of the species. Although it is known that green turtles play an important ecological role in the food web

(Bjorndal, 1997), few studies of trophic ecology have been conducted in the northeastern region of Brazil. The goal of this study was to assess their diet by examining the recovered food items from the gastrointestinal tracts of dead stranded turtles collected in the state of Pernambuco, for the first time.

MATERIAL AND METHODS

Between November 2016 and November 2018, green turtles found dead along the beaches of the southern coast of the state of Pernambuco were analyzed, (between the coordinates - 8.078341/-4.875720 and -8.606433/-35.046698), comprising the municipalities of Recife, Jaboatão dos Guararapes, Cabo de Santo Agostinho and Ipojuca (Fig. 1).

was still able to illuminate that content. In this way, subsamples of contents were analyzed in Petri dishes, with a volume of approximately 50 ml individually, totaling 300 ml per sample (6 Petri dishes). This number was chosen, until the samples were stabilized, in identified food items, thus determining an aliquot pattern for analysis. The taxonomic characteristics were observed with the help of a Zeiss stereoscopic microscope. For the taxonomic studies, references of Rios (1994) was used to identify the molluscs, for cephalopods we had the support of the Laboratory of Biology and Conservation of Pelagic Organisms of the São Paulo State University, while the other items were counted with the collaboration of experts from the Macroalgae Laboratory of the Oceanographic Museum of Federal University of

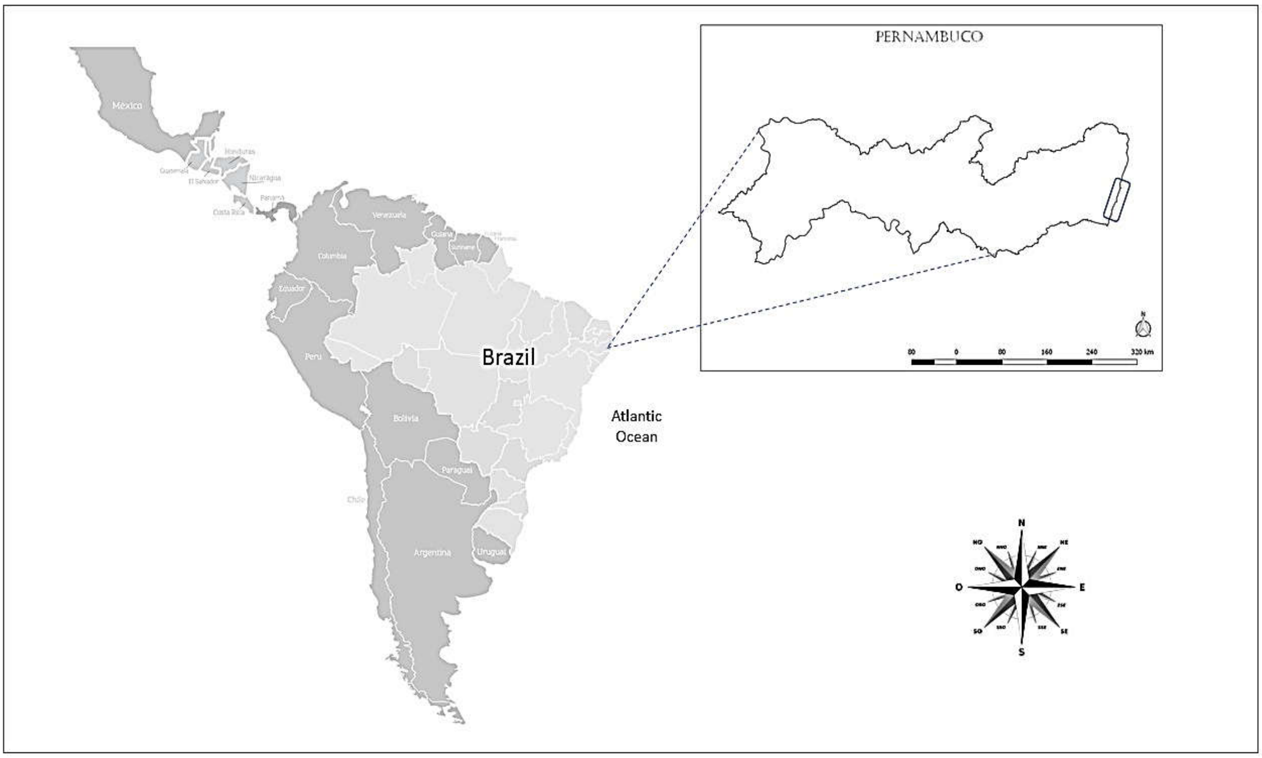

Figure 1. State of Pernambuco. Stomachs of stranded green turtles were colleted along the south coast

The animals were transported to the Laboratory of Herpetological and Paleoherpetological Studies of Federal Rural University of Pernambuco, where necropsies were performed to remove the gastrointestinal contents. Biometric measurements, such as curved carapace length (CCL) and curved carapace width (CCW), were used to determine the development stage of the turtles (Bolten, 1999). The coastal region where these animals were collected is characterized by a narrow and shallow continental shelf formed by carbonic deposits and several reef formations. Turtles were found in the various formations of sandy beaches along the southern coast of the Pernambuco. To study the diet, the digestive tracts of estranded animals were used, collected from the esophagus to the rectum. All the gastrointenstinal samples were placed in labeled plastic bags and frozen for subsequent sorting and identification of food items, to the lowest possible taxonomic level. These contents were analyzed according to the method adapted from Forbes (1996) and Awabdi *et al*., (2013). All contents of each animal were emptied entirely into a white plastic vessel and then mixed with distilled water until homogenized. After this, the samples were placed in Petri dishes until reaching a volume at which the depth of the light

Pernambuco; Botanical Institute (SP); Laboratory of Remaining Environments of Federal Rural University of Pernambuco, and Laboratory of Porifera of Federal University of Pernambuco. Among the analyzed material, some items remained unidentified because they were very fragmented due to the digestive processes, being considered as "remains". The quantitative representativeness of the food items consumed was calculated by the frequency of occurrence (FO), dividing the number of gastrointestinal samples that contained a given food by the total number of samples with food items (Fig. 2). The visual predominance (Awbadi *et al*., 2013), of the digestive tracts analyzed was used to make inferences about the dietary preferences of the species, when the individual samples contained amounts equal to or greater than 30% of the total analyzed (300ml for each gastrointestinal content). Analyses were carried out regarding the dispersion of variables, considering two factors. First, correlations between the factors of food diversity and total weight of the animal were tested. In this case, the correlation value was not significant ($p = 0.02$). Then the factors of food diversity and length of the animal carapace were correlated. In this case, the correlation value found was also not significant ($p = 0.06$).

$$Fo = \frac{n^1 \ldots x\ 100}{N}$$

Where:
n^1 = Number of samples where the food item was found.
N = Total number of samples.

Figure 2. Equation to calculate the quantitative representativeness of the food items

To confirm the positive correlation index between two parameters, the equation below was used to determine the sample correlation coefficient (Fig. 3).

$$r = \frac{n\sum Xi.Yi-(\sum Xi).(\sum Yi)}{\sqrt{[n\sum Xi^2].[n\sum Yi^2-(\sum Yi)]2}}$$

Where:
X = Sum of the equivalent value of the first data pattern.
Y = Sum of the equivalent value of the second data pattern.

Figure 3. equation below was used to determine the sample correlation coefficient, to confirm the positive correlation index between two parameters

RESULTS

In total 44 turtles ranging from 32 to 111 cm CCL (mean 49.7, SD ± 7.7) were analysed. Regarding the 44 gastrointestinal samples examined, macroalgae were found in 42 of these (FO = 95.4%). Traces of marine phanerogams were found in 5 individuals (FO = 11.36%). In 8 (FO = 18.2%) of the studied specimens, items of animal matter were found. From this percentage, N = 1 (FO = 2.27%) contained pelagic molluscs; N = 1 (FO = 2.27%) contained cephalopod jaws, N = 4 contained cnidarians and N = 2 contained sponges (FO = 4.54%). All the turtle sampled had food contents in the oesophagus or stomach. Three animals probably had drowned after entanglement in fishing nets and twelve had injuries resulting from interaction with fishing activity. Seven of these presented signals of poor health conditions. Of 44 animals found stranded dead on beaches, 7 were male and 37 female. Only 6.82% (N = 3) of anthropogenic debris were identified, such as nylon lines, pieces of plastic bags and other plastic materials. The diet was marked by the presence of macroalgae, mainly representatives of red algae, Rodophyta.

This phylum was present in 56.4% of the samples, followed by brown algae (29.6%) and green algae (14%). The red alga Pterocladiella beachiae was the most commonly eaten food, with 65.90% occurrence, followed by rhodophytes as well as Gelidiales, Gelidium lineare (52.27%) and Gelidiella acerosa (47.72%). Regarding brown algae, the most frequent occurrence was the species Dictyopteris delicatula (45.45%), followed by the genera Sargassum sp (38.63%) and Dictyopetris sp (31.81%). Regarding green algae, the highest frequency was Caulerpa prolifera (15.90%) and Anadyomne (15.90%) (Table 1). Half of the invertrebate food samples consisted of plankton (Mollusca). The other half was composed of benthic animals (Porifera, Cnidaria and Crustacea) (Table 2). The correlation analyses between the size of the animal (related to its maturity) and the diversity of its food items showed no correlation between its size or body weight with food sources.

Table 1. Frequency of occurrence of prey groups recovered from stomachs analysed from south coast of Pernambuco state. This frequency is the number of digestive tracts in which the item was found

Taxonomic organization	Species	Fo (%)
Rhodophyta	*Pterocladiella beachiae*	65.90
	Gelidium lineare	52.27
	Gelidiella acerosa	47.72
	Amansia multifida	22.72
	Cryptonemia crenulata	20.45
	Cryptonemia seminervis	6.81
	Cryptonemia sp	4.54
	Hypnea sp	20.45
	Gracilaria sp	20.45
	Bryothamnion seaforthii	9.09
	Palisada perforata	4.54
	Chondrophycus sp	4.54
	Ceramium sp	4.54
	Hypnea pseudomusciformis	13.63
	Graacilaria cervicornes	4.4
	Gracilaria domigensis	2.27
	Chondria sp	2.27
	Amphiroa	4.54
	Jania sp	2.27
	Cryptonemia sp	2.27
	Bryothamniom triquetum	2.27
	Hypnea spinella	2.27
	Polysiphonia sp	4.54
	Botryocladia sp	2.27
Ocrophyta	*Dictypteris plagiorama*	4.54
	Dictyopteris sp	31.81
	Sargassum sp	38.63
	Lobophora variegata	29.54
	Dictyota sp	27.27
	Padina sp	2.27
	Dictyopteris delicatula	45.45
Chlorophyta	*Caulerpa prolifera*	15.90
	Bryopsis sp	13.63
	Ulva sp	9.09
	Caulerpa racemosa	6.81
	Bryopsis pennata	4.54
	Valonia sp	2.27
	Cladophora sp	2.27
	Codium sp	2.27
	Chamaedoris penicullum	2.27
	Caulerpa sertularioides	2.27
	Anadyomne sp	15.90
	Caulerpa lanuginosa	2.27
Phanerogamae	*Halodule wrightii*	11.36
Porifera	*Geodia gibberosa*	2.27
	Stelleta sp	2.27
Cnidaria	*Palythoa caribaeorum*	4.54
	Halocordyle sp	4.54
Mollusca	*Spirula* sp	2.27
	Janthina janthina	2.27
	Chiroteuthis veranyi	2.27
Crustacea	Shrimp sp	4.54
Anthropogenic debris	Plastic items	6.81
Remains	Non-identified items	6.81

Table 2. Origin of invertrebate items ingested by stranded green turtles analysed. CCL = curved carapace length; CCW = curved carapace width

Sex	CCL (cm)	CCW (cm)	Origin of food	
			Benthos	Plankton
female	42	39		X
female	34	31	X	
female	33.5	31		X
male	33	32.5		X
female	48	44.3	X	
female	56	52.5	X	
female	42	38		X
female	43	40.5	X	

The dispersion diagrams did not present logical groupings, as shown in Figures 4 and 5, and the Pearson correlation coefficient values were insignificant, being p = 0.06 for the animal length ratio and food diversification, and p = 0.02 for the weight of the animal and its food diversification.

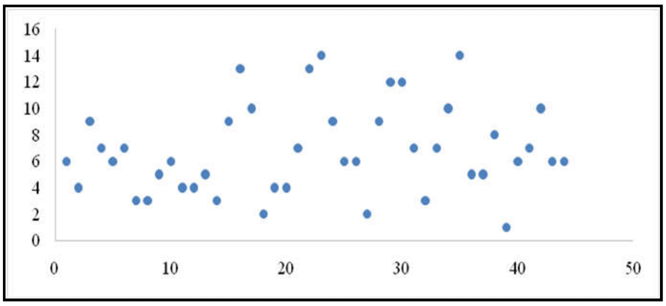

Figure 4. Scatter diagram for the correlation between animal size and its diet diversification

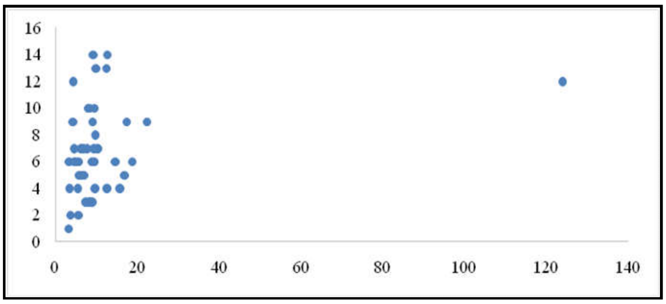

Figure 5. Scatter diagram for the correlation between the weight of the animal and its diversification of diet

DISCUSSION

The content of the digestive tract showed a strong tendency to herbivory, with indications that the turtles fed mainly on marine macroalgae (N = 42). Only traces of marine grasses were found in some of them (N = 5). This tendency for consumption of macroalgae has been reported in other studies of the Brazilian coast: Ubatuba, São Paulo state (Sazima & Sazima, 1983); Espírito Santo Bay (Santos *et al.*, 2011) southern coast of São Paulo state (Nagaoka, 2012), Ceará state (Ferrreira, 1968); Arvoredo Reserve in Santa Catarina state (Ressier *et al.*, 2013), Rio de Janeiro state (Awabdi, *et al.*, 2013) as well as in studies from other countries, such as Mexico (Seminoff *et al.*, 2002; López-Mendilaharsu *et al.*, 2005), Hawaii - USA(Arthur & Balazs, 2009), Australia (Limpus *et al.*, 1994), Arabia (Ross, 1985) and Oman (Ferreira *et al.*, 2006). The most predominant macroalgae in the digestive tract of the animals in our study area were Pterocladiella beachiae, Gelidium lineare and Gelidiella acerosa, all Rhodophytes of the Gelidiales order. In the same way, other studies have also presented red algae as the group most consumed by C. mydas (Ferreira, 1968; Sazima & Sazima, 1985; Brand-Gardner *et al.*, 1999; Fuentes *et al.*, 2006; Arthur & Balazs, 2009; Russell; Balazs, 2009; Reisser etal., 2013; Awabdi *et al.*, 2013; Karam-Martínez, *et al.*, 2017) The red algae, especially those of the order Gelidiales, the main macroalga consumed in this study, are known to be a group with high protein and energy values (Patterson 1977; Rubenstein & Wikelski, 2003), which suggests that green sea turtles in southern Pernambuco have positive selectivity for these, enabling them to extract greater nutritional value from the available foods.

The genus Pterocladiella has also appeared as one of the main items of the green turtle diet at other feeding sites, such as Hawaii (Arthur & Balazs 2009), Uruguay (López - Mendilaharsu *et al.*, 2006), southeastern Brazil (Santos *et al.*, 2011; Awabadi *et al.*, 2013) and south Brazil (Ressier, *et al.*, 2013). Paiva *et al.*, (2017) reported that one species of the genus Pterocladiella and another of the genus Gelidium, both found having high FO levels in this work, are sources of high-quality nutrients as well as bioactive components. In this respect, McDermid *et al.*, (2007), studying the nutritional values of algal species consumed by C. mydas in Hawaii, concluded that P. capillacea presented the highest values of energy, soluble carbohydrates, proteins, vitamin A and some minerals, in agreement with our findings. In contrast to Seminoff *et al.*, (2002), López-Mendilaharsu *et al.*, (2005) and Santos *et al.*, (2011), who found possible negative selectivity of green turtles in relation to the brown algae *Sargassum horridum* and *Dictyopteris delicatula*, we found a high frequency of brown algae, with three species surpassing 30%, including D. delicatula, which was found in 20 of 44 animals analyzed. It is known that the diet of green turtles can be influenced by palatability (Guebert-Bartholo *et al.*, 2011), quality (Sazima & Sazima, 1983; Bjornda1, 980) and digestibility of the food, since turtles seem to avoid certain algal species, even in places where they are abundant (Santos *et al.*, 2011). However, our evidence shows that for the region studied, green turtles do not avoid brown algae species, which were the second group of macrolagae most consumed (29.6%), presenting species with considerable presence in the gastrointestinal tracts: D. delicatula (N = 20), Sargassum sp (N = 17) and Dictyopteris sp (N = 14). Although we did not analyze the plant cover (sea grass and macroalgae) in the places where the dead turtles were found, other factors may influence the feeding choice of turtles, according to the peculiarities of the feeding area (food availability, environmental conditions). In addition, observations made on the island of Fernando de Noronha by Fernandes and Moura (2018) reinforce the idea that green turtles feed on the brown macroalgae of the genus Dictyopteris in that region.

Like in other studies (Seminoff *et al.*, 2002; Ferreira *et al.*, 2006; Fuentes *et al.*, 2006; López-Mendilaharsu *et al.*, 2008, Carrión *et al.*, 2010), we also noted the incidental consumption of some items that are adhered to algae, appearing at low frequency levels, such as epiphytic algae and animal material. However, it is recognized that analyzing only the FO of some items can generate erroneous interpretations, since in this study, relatively significant amounts of animal items were found in some samples, but with low FO.In one of the samples (FO = 2.27), eight squid beaks were found; in another two (FO = 4.54%), 17 pieces of *Palythoa caribaeorum* were found, while in another three (although it was not possible to count them), we visually identified *Geodia gibberosa* and *Stelleta* sp, occupying large portions of the total volume analyzed. Therefore, visual and interpretative analysis of the stomach contents of each dead turtle is important (Awabdi *et al.*, 2013). In one of the turtles analyzed in this study (33.5 cm CCL), no macroalgae or foods of plant origin were found, only shells of the pelagic molluscs Janthina janhtina and Spirula spirula. Hugs (1974), while analyzing a green turtle (25.7 cm SCL) found inside a shark in Africa, found the stomach to be full of J. janthina. The mean carapace length (CCL) indicated as marking the change from pelagic to neritic habitat of green turtles is between 20 and 25 cm in the Northwest Atlantic (Musick & Limpus, 1996) and between 30 and 35 cm for

Hawaii and Australia (Limpus *et al*., 1994). It can be inferred that this juvenile animal was still occupying pelagic foraging areas, with carapace length closer to that found for the United States and Australia. Bolten (2003) confirmed that pelagic juveniles use opportunistic feeding strategies, predating planktonic organisms. The results presented here are reinforced by the observations of Ressier *et al*. (2013), in Santa Catarina, who when analyzing the grazing behavior of C. mydas found several individuals feeding on the coral Palythoa caribaeorum. The authors pointed out that the bite marks on the colonies of these cnidarians were extensive to the point of exposing the rocky portion below them. As far as we know, this is the first report of the consumption of P. caribaeorum by a green turtle in northeastern Brazil. Therefore, the evidence presented here indicates there is deliberate consumption of some items of animal matter, such as sponges, cnidarians and mollusks, corroborating the findings of Seminoff *et al*., (2002); Amorocho and Reina (2007); Arthur *et al*. (2009); Lemons *et al*. (2011); González Carman *et al*., (2012); Ressier *et al*., (2013). Similarly, Bugoni *et al*., (2003) suggested that ingestion of invertebrates by green turtles may be incidental, but there appears to be selectivity in some cases. It is also believed that although some of these items are accidentally consumed as a result of the green turtle's eating habit, they may contribute to their diet by providing vitamins, minerals and essential amino acids (Bjorndal, 1990).

The consumption of squid by green turtles has also been reported (Seminoff *et al*., 2002; Morais *et al*., 2012). However, cephalopods should not be considered a common prey for green turtles, because their association with deep waters (Vaske Jr, 2011; Perez, 2014). In this case, Andrades *et al*., (2019) suggest a oportunisticscavenging behavior to explain this and make correlations with debris ingestion. The majority of floating material on the ocean surface is plastic debris, thus the behavior to forage on floating material can be dangerous (Law *et al*., 2010; Andrades *et al*., 2019). This corroborates our findings, once several plastic debris were observed in the stomach where the squid beaks were found. As also found by Santos *et al*., (2011) and Awabdi *et al*., (2013), although we were unable to be absolutely certain where the turtles foraged before dying, our analyses of the digestive content of beached animals were significant, since more than 85% of the animals collected had recently died, indicating that the contents found represented the local diet. Furthermore, our analysis during all seasons over a two-year period (2017 and 2018) provides information about the feeding habits of green turtles at all times of the year. In this study, it was not possible to determine a correlation between the maturity or weight of the studied animals with their diets. On the other hand, the frequency of occurrence of the red algae found, mainly of the Gelidiales Order, compared with the visual analyses of the volume of contents, in agreement with the literature, showed a strong tendency of the green turtles to feed selectively on macroalgae. Differences in diet composition can be related to local availability of food (Sazima & Sazima, 1983; Garnett *et al*., 1985; López-Mendilaharsu *et al*., 2008; Carrión-Cortez *et al*., 2010, Guebert-Bartholo *et al*., (2011), as well as a certain level of selectivity (Bjorndal, 1980) Brand-Gardner *et al*., 1999); Seminoff *et al*., 2002; Fuentes *et al*., 2006; López-Mendilaharsu. *et al*., 2005; Russell; Balazs, 2009; Santos *et al*., 2011; Ressier *et al*., 2013).

Implications for Conservation: Studies related to sea turtles on the Brazilian coast have been encouraged since this animal group has often been included in lists of species threatened with extinction. Studies are carried out related to their reproductive and alimentary activities. Research on feeding habits of turtles generally lists items consumed and their distribution in the marine environment. This research amplifies significantly the knowledge of the feeding habits of these animals. Thus, the information about their behavioral process contributes, in a significant way, to the indication of priority areas for the protection of food stocks for these animals. In addition, complementary studies on the toxic composition of the stomach contents of the turtles studied could predict the areas with the highest risk indices related to the turtle's dietary health.

Conclusions

The survey of the contents of the digestive tracts of 44 green turtles reinforces the high preference for foods of plant origin, predominated by macroalgae of the Rhodophyta division, followed by the Ocrophyta division. Pearson's correlation tests did not reveal more selective feeding activities. However, our analyses show that for the southern coast of Pernambuco, despite the diversity of macroalgae, there is a preference for a few species as main food items, namely: (1) Pterocladiella beachiae; (2) Gelidium lineare; and (3) Gelidiella acerosa, all of the order Gelidiales. In this sense, it is important to conduct a survey of the marine vegetation in the studied sites, thus allowing a better evaluation of the existence or not of selectivity in the algal intake found in our analysis of C. mydas diet. It is also worth noting that knowledge of the feeding sites of this endangered species is important for the formulation of conservation proposals, focusing mainly on coastal areas with large macroalgae vegetation.

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