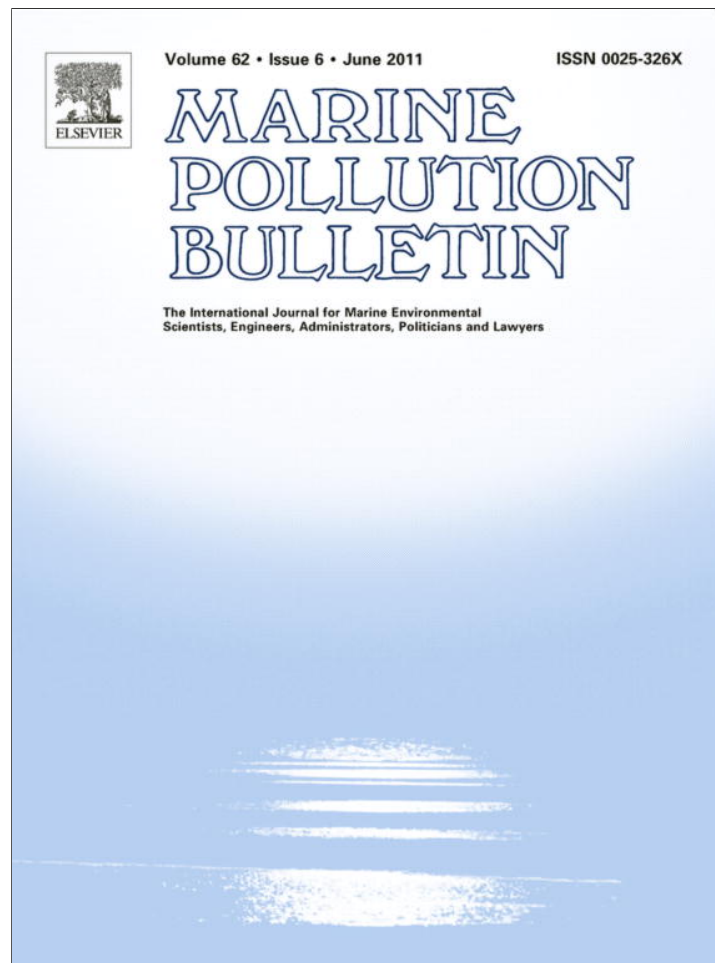


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Coastal habitat degradation and green sea turtle diets in Southeastern Brazil

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ABSTRACT

To show the influence of coastal habitat degradation on the availability of food for green turtles (*Chelonia mydas*), we assessed the dietary preferences and macroalgae community at a feeding area in a highly urbanized region. The area showed low species richness and was classified as degraded. We examined stomach contents of 15 dead stranded turtles (CCL = 44.0 cm (SD 6.7 cm)). The diet was composed primarily of green algae *Ulva* spp. (83.6%). In contrast, the macroalgae community was dominated by the green alga *Caulerpa mexicana*. We found a selection for red algae, seagrass and *Ulva* spp., and avoidance for *C. mexicana* and brown alga *Dictyopteris delicatula*. The low diversity of available food items, possibly a result of environmental degradation, likely contributed to the low dietary diversity. The nutritional implications of this restricted diet are unclear.

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1. Introduction

Coastal regions worldwide are impacted by increasing human populations and intense socioeconomic activities. As of 1990, nearly one billion people, roughly 23% of the world population, were settled within 100 km of coastlines (Small and Nicholls, 2003). Estuaries, coastal wetlands, and seagrass beds are particularly vulnerable (Lotze et al., 2006; Waycott et al., 2009). Lotze et al. (2006) reconstructed causes and consequences on 12 once-diverse and productive estuaries and coastal seas worldwide and found that more than 90% of formerly important species have disappeared from these areas while more than 65% of seagrass and wetland habitats have been destroyed. Likewise, these areas have experienced degraded water quality and greater rates of nonnative species invasions. All coastal areas are now affected by human activities, and a large fraction (41%) is affected by multiple drivers like development, organic and inorganic pollution, fisheries and invasive species (Halpern et al., 2008). Although destruction of these ecosystems clearly results in a loss of local biodiversity, the impacts of such losses can be difficult to quantify (Raffaelli, 2004).

Coastal habitat degradation particularly affects marine macroalgae as a result of their rapid uptake of water-borne contaminants

and their dependence on specific benthic habitat types for growth and propagation. These primary producers have an important role in the coastal ecosystem food webs and serve as a shelter for fish and invertebrates (e.g., Anderson, 1994). Marine macroalgae communities respond to ecosystem changes by integrating the long-term exposure effects to pollutants and nutrients that manifest as modification in community structure modification. This is usually exemplified by the favoring of more resistant and opportunistic species and the exclusion of late successional and fragile species (Murray and Littler, 1978). Because of their sensitivity to habitat alteration and contamination, marine macroalgae are valuable bio-indicators of environmental quality (e.g., Ballesteros et al., 2007; Borowitzka, 1972; Gorostiaga and Díez, 1996; Littler and Murray, 1975; Orfanidis et al., 2001; Santos et al., 2010).

Despite the difficulty in elucidating the effects of reduced coastal biodiversity (Raffaelli, 2004), the direct impacts on marine macroalgae communities would be expected to be manifested in the form of reduced species richness, which in turn would reduce dietary complexity of generalist marine herbivores. Among the marine herbivores that make use of coastal areas is the green sea turtle, *Chelonia mydas*, globally threatened by many anthropogenic activities, with loss of feeding habitat being a major threat (Bjorndal, 1999; Lutcavage et al., 1997; Seminoff et al., 2002). In many parts of the world, the feeding biology and the habitat requirements of this species remain poorly understood (Bjorndal,

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1997), and with the exception of Russell and Balazs (2009), few published works on green turtle diet and feeding preferences have focused on a highly degraded and urbanized area. Knowing the effects of environmental degradation on sea turtle diets may be critical to their management. The goal of the present study was to characterize the food availability, diet intake, and feeding preference of green turtles in a highly urbanized coastal marine habitat along the east coast of Brazil.

2. Methods and materials

The study site was located at Espírito Santo Bay in the center of a metropolitan region (Vitória) consisting of ca. 1.5 million inhabitants with human density surpassing 3300 inhabitants/km². It has a large commercial port, water discharge from domestic residences, and light and heavy coastal industry. The study was conducted between Frade Island and the mainland (Fig. 1), in an area with water depth of 1–3 m and sea water temperature of 22–26 °C where hazards to turtles such as recreational boat traffic, entanglement nets, and hook and line are common. The study area is a recognized green turtle (*C. mydas*) pasture with a juvenile aggregation highly affected by fibropapillomatosis (FP), a debilitating neoplastic disease (Santos et al., 2010).

Vegetation sampling of marine algae biomass (dry weight) was estimated in the summer of 2007 (December) and in the winter of 2008 (July). The samples were collected once in each season using eight randomly placed 25 × 25 cm quadrates deployed on five 50-m parallel transects randomly distributed within the study area (Murray et al., 2002). Samples were preserved in 4% buffered formalin and identified to the lowest possible taxonomic level.

Environmental characterization and quality evaluation of the phyto-benthic community structure was described by the total abundance (biomass), number of species (*S*), Shannon-Wiener diversity index (*H'*), and Pielou's evenness factor (*J'*) (Krebs, 1999). After confirming homogeneity of variances using the Cochran test, we performed ANOVA tests to verify the differences in each of the descriptors between sampling periods. Multidimensional scaling (MDS) was employed to show the spatial distribution of samples (Clarke and Warwick, 1994). PERMANOVA analyses, a powerful approach of multivariate data, (Anderson, 2001) were accomplished to test for significance of factors period and transects using 9999 permutations. Pairwise tests among factors were analyzed to determine if and when period and transects caused significant differences in seaweed community biomass. MDS ordination was used to visualize similarities in phyto-benthic community structure across seasons and over transects. The MDS procedure

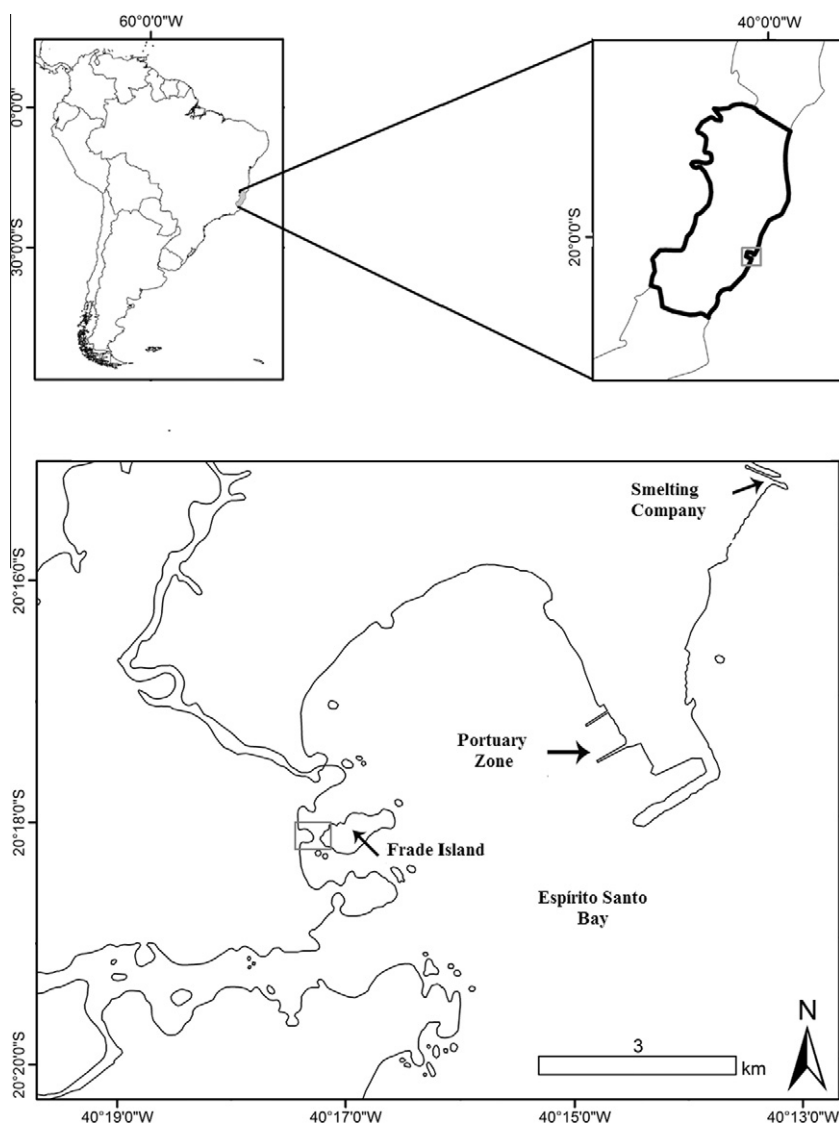


Fig. 1. Study area, between Frade Island and the mainland, located in Espírito Santo Bay, Southeastern Brazil.

displays samples in two-dimensional space by preserving the ranked similarities among all samples; thus, the closer together two points are the more similar they are in composition and abundance. Analyses of similarity (ANOSIM) were used to contrast and test difference in stomach contents between turtles afflicted and not afflicted by FP. The similarity matrix used in MDS, PERMANOVA and ANOSIM was obtained through the Bray–Curtis index and the values of abundance were square-root transformed to control for the influence of the highly abundant species.

Assessment of environmental quality was determined using the ecological evaluation index (EEI) (Orfanidis et al., 2001, 2003) which uses abundance of marine benthic macrophyte (seagrass and seaweed) species as bioindicators of ecosystem shifts due to anthropogenic stress. This approach codifies the ecological status of an area, from the pristine state with late-successional species (e.g., high ecological status class; ESC) to the degraded state with opportunistic species (“bad” ESC; Orfanidis et al., 2001). Each species is placed into one of two ecological state groups (ESG): ESG I includes algae with a thick or calcareous thallus, low growth rates and long life cycles (i.e., late-successionals); ESG II includes algae with sheet-like or filamentous morphology, high growth rates and short life cycles (i.e., opportunistic). Using the abundance (%) of species in each ESG, an ecological status class (ESC) is generated representing the environmental quality of the area (Orfanidis et al., 2001, 2003).

Diet and feeding preference analysis was conducted on necropsied stranded turtles, and stomach contents were preserved in 4% buffered formalin solution. The stranded turtles were found in the beaches immediately adjacent to the vegetation sample area, marked (gray square) in the map of study area (Fig. 1). The curved carapace length (CCL; ± 0.1 cm) was measured with a flexible plastic metric tape. Individuals with CCL ≤ 101 cm were considered sexually immature, based on the smallest registered size for nesting females in the largest and closest nesting area for the species, Trindade Island, Brazil (Moreira et al., 1995). The body condition of the individuals was evaluated according to Walsh (1999), which categorized body condition as normal, underweight or emaciated considering the decrease in muscle and fat tissue in the neck and flippers area and characteristics of the eyes and the plastron. All of the turtles were examined for presence/absence of FP. The ingesta were identified to the lowest possible taxonomic level with the use of magnifier lenses, microscopes and specialized taxonomic keys.

For each diet item we determined the frequency of occurrence, relative sample volume (0.5 ml; via water displacement), and dry weight biomass (0.0001 g). These were determined as follows:

$$\text{Frequency}(\%) = \frac{\text{Number of samples containing diet item} \times 100}{\text{Total number of samples}}$$

$$\text{Volume}(\%) = \frac{\text{Total volume of diet item in all of the samples} \times 100}{\text{Total volume of all of the samples}}$$

$$\text{Biomass}(\%) = \frac{\text{Total dry weight of the diet item of all samples} \times 100}{\text{Total dry weight of all samples}}$$

Feeding preference was determined by comparing food consumed with food available. Diet selection was ascertained using the Waller–Duncan test for rank differences in relation to selection, which indicates preference of diet items (Johnson, 1980). This procedure shows a measure in relation to the availability of the component and the usage of this diet, which is expressed with Tbar values (average rank difference). Tbar < 1 indicates that component was selected, Tbar = 0 indicates that the component was consumed in the same proportion of its availability and Tbar > 1 indicates that

the component was not selected. Analyses were conducted using Prefer 5.1: statistical package for comparisons of resource preference, USGS Northern Prairie Research Center, Jamestown, North Dakota.

3. Results

3.1. Marine algae community structure

Fourteen marine algae species were identified along transects: six Rhodophyta, six Chlorophyta and two Ochrophyta (Phaeophyceae). The relative biomass for species that reached more than 0.5% of the total is shown in Fig. 2. The marine algae species *Bryopsis pennata*, *Ceramium comptum*, *Ceramium luetzelburgii*, *Chaetomorpha aerea*, *Champia parvula*, *Cladophora vagabunda*, *Hypnea musciformis* and *Sargassum* spp. were found in the area; however, their summed biomass did not reach 1%. Species richness was higher during the summer (14 species) than the winter (eight species).

Based on the biomass (relative dry weight) the predominant summer species was *Caulerpa mexicana* (89.8%). During winter there was a 2.3-fold increase and 98.7% dominance in *C. mexicana* biomass.

Significant differences were seen when comparing the community structure in the different sampling periods. Biomass was significantly higher whereas species richness, Pielou's evenness, and Shannon diversity were significantly lower in winter (Table 1). Winter and summer communities were segregated significantly between seasons (Fig. 3) (PERMANOVA, $F = 27.9749$, $P = 0.0001$). Despite no significant differences to be observed among transects (PERMANOVA, $F = 1.5304$, $P = 0.0953$), the interactions analyses (PERMANOVA, $F = 2.1806$, $P = 0.0113$) reinforces the influence of seasons in the community structure patterns.

3.2. Environmental quality evaluation

All marine algae species found in the study site belonged to the ecological state group II (ESG II) consisting of species with fast growth and short lifecycles (opportunistic; Orfanidis et al., 2003), generating an EEI = 2 indicating a degraded ecosystem.

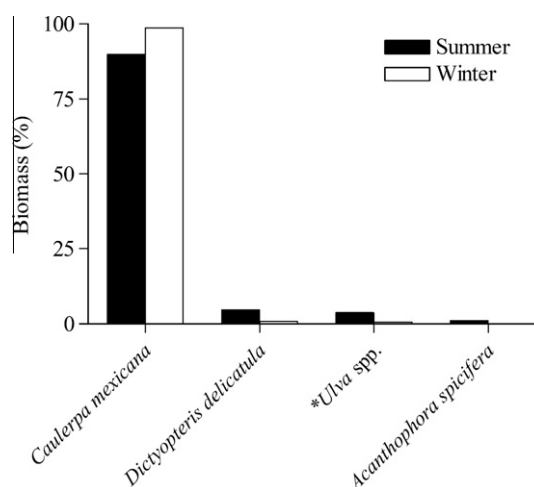


Fig. 2. Seasonal variation (summer and winter) of algae species collected in a degraded feeding area for juvenile green turtles (*Chelonia mydas*) in Espírito Santo Bay, Southeastern Brazil. Values represent the relative biomass (dry weight). **Ulva* spp. = *U. fasciata*, *U. lactuca* and *U. rigida*.

Table 1
The macroalgae community descriptors from the green turtle foraging area in two seasons.

	Winter	Summer
Biomass (g sample ⁻¹)	28.08	13.61
Species richness (S) (sp sample ⁻¹)	2.26	5.79
Pielou's evenness (J')	0.09	0.29
Shannon-Wiener diversity (H')	0.09	0.45

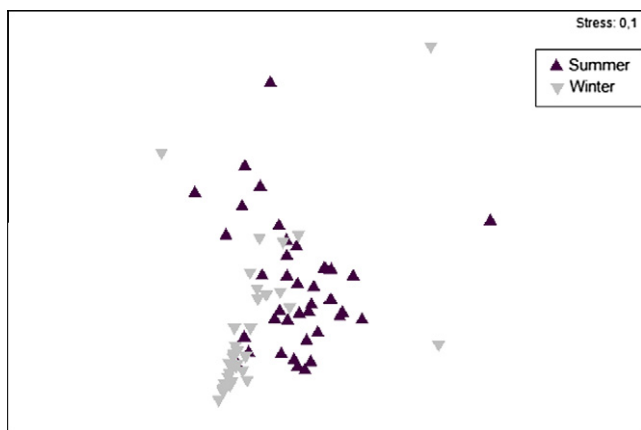


Fig. 3. MDS from biomass matrix of macroalgae community from a degraded feeding area for juvenile green turtles (*Chelonia mydas*).

3.3. Green turtle diet and preference

We examined 15 dead turtles found in the study area, four of which had been tagged during prior capture efforts in the same area (Santos et al., 2010). These turtles had a mean CCL of 44.0 cm (SD = 6.7; range = 35.1–60 cm). Prevalence of FP was 46.7%, and all were in good body condition, except for one emaciated individual that upon internal examination had an obstruction in the digestive system caused by a nylon line, that went from the esophagus to the cloaca. Ascertaining the exact cause of death was not possible from necropsies; however, some individuals exhibited lesions compatible with net entanglements and boat impact. The FP cases were not considered a primary cause of stranding because the tumor scores were low and all FP turtles were in good body condition.

The diet was dominated by marine algae, which represented 96% of the total biomass (Table 2). Traces of seagrass (*Halodule wrightii*) were also found in three stomach contents except for one turtle where *H. wrightii* predominated (95% of the biomass). Among the marine algae in the diet, red algae were the most diverse (10 species). However, they represented only 4.6% of the biomass whereas green algae (four species, dominated by *Ulva* spp.) made up 91.4% of the biomass. We found three species of *Ulva* (*U. fasciata*, *U. lactuca* and *U. rigida*) in the study area, but based on impracticality of identifying each fragment to species, we grouped them as *Ulva* spp. No brown algae were found in the stomach contents. Three individuals had small amounts of nylon line 5–15 cm in length and one had a 1.5 cm diameter rubber ring in its stomach none of which presented with associated pathology. No significant difference in the diet was found between turtles with and without FP ($R = 0.022$; $P = 0.275$).

The diet of the sea turtles did not reflect the availability of each item within the study area. The Johnson (1980) preference index showed an apparent preference for red algae, followed by *H. wrightii*, and *Ulva* spp. and avoidance of the brown alga, *Dictyopteris delicatula* and the dominant green alga, *C. mexicana*.

Table 2
Relative biomass (dry weight), relative volume and frequency of occurrence (FO) of diet items found in stomachs 15 dead stranded juvenile green turtles (*Chelonia mydas*) found in the study area. T denotes presence in trace levels.

Diet items	Biomass (%)	Volume (%)	FO (%)
Algae			
Rhodophyta			
<i>Halymenia floresii</i>	0.5	2.6	53.3
<i>Gracilariopsis tenuifrons</i>	T	T	6.7
<i>Gelidium floridanum</i>	2.5	1.8	26.7
<i>Rhodymenia pseudopalmeta</i>	T	T	6.7
<i>Chondracanthus elegans</i>	0.2	1.2	6.7
<i>Hypnea musciformis</i>	T	T	40.0
<i>Pterocladia capillacea</i>	0.9	0.7	13.3
<i>Gymnogongrus griffithsiae</i>	0.2	0.2	13.3
<i>Gracilaria mammillaris</i>	0.3	0.2	6.7
<i>Laurencia</i> spp.	T	T	6.7
Total Rhodophyta	4.6	6.8	73.3
Chlorophyta			
<i>Caulerpa mexicana</i>	7.1	10.1	73.3
<i>Ulva</i> spp.	83.6	76.9	100.0
<i>Cladophora vagabunda</i>	0.6	0.9	66.7
<i>Chaetomorpha aerea</i>	T	T	6.7
Total Chlorophyta	91.4	87.9	100.0
Seagrass			
<i>Halodule wrightii</i>	3.9	4.9	26.7
Trash	T	T	20.0

Table 3

Diet preference of juvenile green turtles (*Chelonia mydas*) from a degraded feeding area. Diet items are listed from most preferred to least preferred. $T_{bar} < 1$ indicates that diet item was selected, $T_{bar} = 0$ indicates that diet item was consumed in same proportions as its availability and $T_{bar} > 1$ indicates that diet item was not selected. Test of H_0 : all diet items are equally preferred; $F_{9,6} = 256.93$. Critical value for the Waller-Duncan procedure with $K = 100$ is $W = 3.08$ ($\alpha = 0.05$).

Diet Items	Average difference in ranks for diet items	
	Tbar	Rank
Gelidiaceae ^a	-2.7	1
<i>Halymenia floresii</i>	-2.5	2
<i>Ulva</i> spp.	-1.7	3
<i>Cladophora vagabunda</i>	-1.3	4
<i>Halodule wrightii</i>	-1.3	5
<i>Gymnogongrus griffithsiae</i>	-1.3	6
<i>Hypnea musciformis</i>	1.1	7
<i>Acanthophora spicifera</i>	2.3	8
<i>Caulerpa mexicana</i>	2.3	9
<i>Dictyopteris delicatula</i>	5.2	10

^a Gelidiaceae = *Gelidium floridanum* and *Pterocladia capillacea*.

For red algae, the exceptions included *H. musciformis*, which was found only in trace levels, and *Acanthophora spicifera*, which was absent in turtle diets despite representing 1.5% of the summer biomass of the macro-algal community. The preference ranking and its significance is shown in Table 3.

4. Discussion

Low species diversity in our study site with a predominance of opportunistic algae and dominance of *C. mexicana* contrasted with the nearby more pristine areas in this transition region situated between a tropical and a warm temperate province which is considered one of the most diverse regions for macroalgal flora along the Brazilian coast, typically dominated by a diverse assemblage of 400 macroalgal species (Figueiredo et al., 2008; Guimarães, 2003; Horta et al., 2001). We suspect environmental degradation could explain this phenomenon because only opportunistic marine algae were found at our study site thus characterizing the study area as degraded, based on EEI. In contrast, similar sampling of algae in nearby (within 20–30 km) green turtle foraging pastures with

minimal human impact, revealed a higher species richness (60 Rhodophyta, 7 Chlorophyta and 7 Ochrophyta) and a dominance of red algae (45.95% of coverage) rather than *C. mexicana* despite similarities in benthic substrate, water temperature, tidal conditions between both areas (P.A. Horta unpublished manuscript).

In addition to the EEL, the presence of green turtle FP, a debilitating neoplastic disease (Work et al., 2001; Work et al., 2003) commonly associated with degraded coastal areas (Aguirre, 1991; Aguirre and Lutz, 2004; Herbst, 1994; Herbst and Klein, 1995; George, 1997;) also supports the observation that our study area is degraded. Captures performed from March 2007 to April 2008 in the same study area revealed a high prevalence of FP (58.3%), which is higher than comparable regions in Brazil with most turtles moderately to heavily affected (Santos et al., 2010).

Although our sample size for turtle diets was relatively small, and we cannot ascertain completely where stranded turtles had foraged, we suspect that stomach contents of stranded green turtles were representative of animals in the study site based on the following: (1) the examined stomachs were collected throughout the year, thus providing diet information for all seasons; (2) 26% of the turtles included in the diet study were marked during the study period; (3) our use of stomach contents provides more representative information than esophageal lavage samples, where only part of the food items are recovered (Seminoff et al., 2002); and (4) we saw no difference in stomach contents between animals with and without FP suggesting that disease does not affect foraging behaviors of green turtles.

Turtles in this study showed a diet consisting primarily of marine algae (96% of sample biomass and 94.7% of sample volume) with seagrass comprising a minority of samples except for one turtle where *H. wrightii* dominated. In other studies where green turtles showed a diet based on algae, Rhodophyta predominated in the diet globally: Australia (André et al., 2005; Fuentes et al., 2006; Forbes, 1996), Brazil (Ferreira, 1968; Sazima and Sazima, 1983), Florida (Makowski et al., 2006), Hawaii (Arthur and Balazs, 2008; Russell and Balazs, 2009) and Mexico (López-Mendilaharsu et al., 2005; Seminoff et al., 2002). In contrast, we found that Chlorophyta predominated in green turtle diets made up 91.4% of total biomass (Table 2).

Many species of the genus *Caulerpa* have life history traits such as growth rate, thallus morphology and dispersal strategy that promote invasiveness (Davis et al., 2005; Ceccherelli et al., 2002). The poor environmental quality coupled with the highly competitive capacity of *C. mexicana* probably contributed to its dominance in our study area and contrasted with its low occurrence in resident turtle stomachs. Marine algae of the order Cauleriales are often avoided by macroherbivores (Hay, 1981; Littler et al., 1983; Lobel and Ogden, 1981; Ogden, 1976; Tsuda and Bryan, 1973) perhaps as the result of toxic/distasteful metabolites such as caulerpenyne (Paul and Fenical, 1986; Paul and Hay, 1986) or their poor nutritional quality (Paul and Fenical, 1986). For example, parrotfish that were fed *C. mexicana* had reduced survival rates (Lobel and Ogden, 1981), and Balazs (1985) showed that juvenile green turtles in Johnston Atoll ate *Caulerpa racemosa* only when more preferred marine algae species were absent, thus resulting in reduced growth rates of the turtles.

The extent to which the diet of green turtles is determined by selective feeding or food availability has been discussed in several studies (Bjørndal, 1997). Our study showed that the green turtles had a selective feeding, which agree with recent studies (Fuentes et al., 2006; López-Mendilaharsu et al., 2008). The preponderance of red algae in the diets of green turtles in previous studies (André et al., 2005; Arthur and Balazs, 2008; Forbes, 1996; Fuentes et al., 2006; López-Mendilaharsu et al., 2005; Makowski et al., 2006; Seminoff et al., 2002) agrees with our study; however, they were found in small quantities probably reflecting their paucity in the

foraging area, which may be due to habitat degradation. Another preferred item in turtles from our study was the green algae *Ulva* spp., which was the main food item found in the stomach contents. The consumption of *Ulva* by green turtles was reported in other studies (e.g. André et al., 2005; Arthur and Balazs, 2008; Ferreira, 1968), but rarely predominates in diet save for a few exceptions (López-Mendilaharsu et al. 2008).

The absence of the red algae *A. spicifera* and *H. musciformis* in turtles from our study contrasted with their presence in green turtle diets in other areas (André et al., 2005; Fuentes et al., 2006; Russell and Balazs, 2009). This absence can be explained by the low biomass of these algae in the study area and their presence as epiphytes on *C. mexicana* which was avoided by green turtles. The absence of *D. delicatula* in green turtles diet may be due to this alga's low digestibility. Although there are few data on food avoidance in green turtles, low digestibility was cited by Seminoff et al. (2002) as the primary reason for the avoidance of the brown alga *Sargassum johnstonii* by green turtles inhabiting a temperate foraging area in the Gulf of California, Mexico. Digestibility has also been reported as a contributing factor to diet composition for green turtles in Bahia Magdalena, Mexico (López-Mendilaharsu et al., 2005).

The low number of available food items within our study site, coupled with the possible secondary metabolite production by the dominant marine alga in the environment (*C. mexicana*), may force green turtles to base their diet on only one genus of marine algae (*Ulva*). McDermid et al. (2007) showed that the marine algae, seagrasses, and algal turfs known to be consumed by Hawaiian green turtles have considerable variation in nutritional composition. Restricted diets may adversely affect turtles because different food items are required to optimize different process of the life cycle such as growth, survival, and fecundity (Worm et al., 2006). For example, variation in fecundity of wild Caribbean and Surinam green turtle populations was credited to differences in diet (Bjørndal, 1982), nesting numbers have been correlated with food availability in the months prior to breeding (Broderick et al., 2001; Limpus and Nichols, 1988), and differences in growth rates of juveniles were attributed to dietary differences the in Hawaiian Islands (Balazs and Chaloupka, 2004).

The potential nutritional deficit combined with high prevalence of fibropapillomatosis and the continuous and increasing degradation of the coastal environments throughout the world, create a high risk scenario for green turtles. Based on our findings and given the high degradation rates of coastal ecosystems, we recommend that conservation programs for green turtles focus not only on the turtles, but also on the entirety of coastal habitats within which these turtles live (Worm et al., 2006). Feeding areas that have a high diversity of marine algae should be seen as having a high priority for protection. Conservation measures that benefit green turtle populations will also help other herbivores and organisms that use these areas.

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