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Coastal degradation impacts on green turtle's (*Chelonia mydas*) diet in southeastern Brazil: Nutritional richness and health

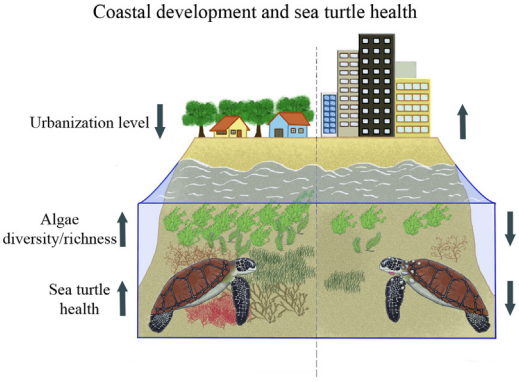
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HIGHLIGHTS

- Coastal degradation alters community composition, chemical diversity and nutritional composition of marine plants.
- A single genus of Chlorophyta represented 62% of the diet in the highly urbanized area.
- Primary metabolites showed higher concentration in the highly urbanized area.
- Sea turtles in urbanized area suffer from fibropapillomatosis; 24% of individuals are moderately/heavily affected.
- Little is known of urbanization effects on algae diversity and metabolite production.

GRAPHICAL ABSTRACT



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ABSTRACT

This study evaluated the influence of environmental degradation on the nutritional value of the main marine macrophytes consumed by green sea turtles (*Chelonia mydas*) in areas with different degrees of urbanization. Macrophyte assemblages in the highly urbanized area (HUa) showed lower richness compared to the lightly urbanized area (LUa) (Mann-Whitney *U* test: 10.0 ± 3.6 SD genera and 11.9 ± 4.2 taxa per transect vs. 20.1 ± 7.0 genera and 23.5 ± 9.2 taxa per transect) respectively. Also, diet was poorer with 4.0 ± 1.6 genera per turtle (vs. 8.5 ± 4.0 in HUa) and less diverse with Shannon index of diversity = 0.45 ± 0.29 (vs. 0.64 ± 0.46 in LUa). Body condition was similar in both areas. About half of individuals were classified as having normal body condition, 14–15% as underweight and 23–34% as being emaciated. Fibropapillomatosis prevalence ($\chi^2 = 8.720$; $n = 222$; df = 1; $p = 0.003$) was higher in the HUa but, in affected animals, severity was marginally non-significant ($\chi^2 = 5.721$; $n = 82$; df = 2; $p = 0.057$). Significant differences in energy content (kcal) were detected between areas in both summer (S) and winter (W). All ANOVAs on total lipids (F = 22.15 [S] and 30.39 [W]), total water-soluble proteins (F = 327.65 [S] and 64.42 [W]) and total carbohydrates (F = 70.90 [S] and 27.62 [W]) showed high significance ($p < 0.001$). Carotenoids concentration yielded significant results for *Halodule* in summer and *Hypnea* in winter (ANOVAs, F = 39.42 and 13.07, respectively). For both, tests revealed that concentration was higher in LUa than HUa. High levels of phycobiliproteins and proteins in this area probably reflect nitrogen accumulation. Frequency and severity of fibropapillomatosis suggest that urbanization-caused alterations in species diversity and in chemical composition of marine plants affect green turtles' health.

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Light abstract: The use of coastal areas by humanity is widespread and increasing. The impacts caused to the coastal environment, be it terrestrial, estuarine or marine, are important and affect numerous species. Our study evaluated the influence of environmental degradation on the nutritional value of the main algae eaten by the green turtle, one of the very few marine megaherbivores (those herbivores with body mass above 10 kg). Diet in the highly urbanized area was richer in proteins, lipids and carbohydrates (sugars) and lower in carotenoids (photosynthetic and photoprotectant pigments in algae and plants; precursors of vitamin A involved in oxygen transport in animals—animals do not synthetize such molecules). High levels in phycobiliproteins (photosynthetic pigments present in some algae) and proteins in the highly urbanized area probably result from organic pollution and nitrogen accumulation in coastal waters. Nitrogen compounds dissolved in water are a threat to vertebrates due to its toxicity and negative effects on the immune system. Our results suggest that algae chemical composition and severity of fibropapillomatosis (tumors caused by a herpesvirus in green sea turtles) are directly related through environmental alterations caused by urbanization.

1. Introduction

Coastal areas have long been occupied, and their resources heavily exploited, by humanity (Lotze et al., 2006). Urban development and resource use have strongly accelerated these last decades with coastal areas going through tremendous socio-economic and environmental changes (Neumann et al., 2015). Patterns of urban development and population spatial distribution differ among world regions partly due to a global tendency of developing countries to occupy more intensively coastal areas and to release higher volumes of untreated urban sewage (Lotze et al., 2006; Worm et al., 2006; Halpern et al., 2008; Neumann et al., 2015).

Sessile organisms depend upon specific habitats for growth and propagation and, thus, equilibrium and survival of communities are dependent upon physiological responses to environmental variation at the species level (Murray and Litter, 1978; Jiménez-Escrig and Cambrodón, 1999; Wong and Cheung, 2000; Scherner et al., 2012). In particular, the chemical composition of macroalgae is greatly influenced by external conditions (Marinho-Soriano et al., 2006; Marsham et al., 2007; Misurcová, 2011). Through a complex physiological and biochemical apparatus, the concentration of proteins, essential amino acids, minerals, vitamins and bioactive secondary metabolites, and other compounds (i.e., the nutritional value available to a primary consumer) is directed affected by the environment (Wong and Cheung, 2000, Misurcová, 2011). Thus, anthropogenic stressors can not only alter the composition and species richness of benthic marine communities (Martins et al., 2012) but also affect the chemical diversity and the nutritional composition of marine organisms (Misurcová, 2011).

Alterations in chemical and nutritional composition promote modifications in metabolite classes that possess ecological functions or participate in the ecophysiological equilibrium (Murray and Litter, 1978; Jiménez-Escrig and Cambrodón, 1999; Wong and Cheung, 2000; Misurcová, 2011). However, how environmental factors shape the chemical composition of basal trophic levels (e.g. macroalgae and seagrasses) and how this nutritional potential is used by consumers (e.g. herbivores) are poorly known.

Extant herbivorous marine megafauna is limited to sirenians (dugong and manatees), and green sea turtle, *Chelonia mydas* (Linnaeus, 1758); all are extremely dependent on shallow and coastal areas. Coastal urbanization and development are among the main threats to green turtle populations (Santos et al., 2011). Habitat degradation lowers diet complexity and turtles´ nutritional gain, which may affect their growth rate, reproductive capacity and demography (Carr and Carr, 1970; Bjorndal, 1982; Bjorndal, 1997). In addition, a worsening in environmental quality may also promote disease transmission (Smith et al., 2009) and lower immunocompetency (Selgrade, 2007).

While our knowledge on the ecological consequences of the degradation of coastal habitats is growing (Halpern et al., 2008; Bulleri and Chapman, 2010; Duarte et al., 2013), little is known of urbanization effects on metabolite diversity and production by marine algal communities and their potential effects on herbivores (Martins et al., 2014). In this context, the present study aims at (1) determining if the nutritional value (carbohydrates, lipids, pigments, proteins and energy) of macroalgae and angiosperms consumed by the green turtle varies with urbanization; (2) establishing turtle diet and describing macrophyte flora in feeding areas heavily or more lightly urbanized; (3) determining if green turtle health varies with urbanization; and (4) evaluate the potential impacts of diet quality on the health of these imperiled herbivores.

2. Materials and methods

2.1. Green turtle feeding areas and determination of urbanization level

The study sites were located on the Brazilian coast, southwestern Atlantic Ocean, where green turtle forages mainly on macroalgae (Santos et al., 2015). This region has low extent of marine seagrasses (Copertino et al., 2016) and human footprint is considered moderate to high (Sanderson et al., 2003; Martins et al., 2012). The green turtle feeding areas evaluated in this study are distinct in relation to human occupation (Fig. 1): a highly urbanized area (thereafter HUa), the municipality of Vitória (more specifically the areas near Ilha do Boi (20°30′77.65“ S; 40°28′03.37” W) and Ilha do Frade (20°30′08.42“ S; 40°28′51.00” W) with 3338.3 inhab./km^2, and a relatively lightly urbanized area (LUa), the municipality of Fundão (more specifically the areas near Enseada das Garças (20°03′09.18“ S; 40°15′83.60” W) with 58.97 inhab./km^2 (IBGE,2010).

Satellite nocturnal imagery was used to map the distribution and intensity of anthropic lighting (Elvidge et al., 2007) in the study area. Urbanization level was built using as *proxy* the night-light (NL) obtained from the satellite sensors DMSP/OLS (Defense Meteorological Satellite Program / Operational Linescan System) that operate in visible (and thermal infrared) between 440 and 940 nm with highest sensitivity between 500 and 650 nm. High-resolution pixel is 0.55 km. Because on-board calibration for visible light is lacking, DMSP/OLS stable-light layer data are relative values ranging between 0 and 63 (https://data.noaa.gov /metaview /page?xml = NOAA / NESDIS/NGDC /STP/DMSP /iso/xml /G01119.xml&view = getDataView &header = none). Study areas were manually delineated using Google Earth Pro 7.3.0. A 2-km wide buffer was generated around the geographic polygon of each area (HUa and LUa) to compute mean values for NL. Four degrees of urbanization classes were defined based on mean relative light intensity detected by the high-resolution telescope aboard DMSP/OLS satellites (Azevedo, 2013): 1–7 not urbanized, 8–23 low urbanization, 24–39 moderately urbanized e 40–63 highly urbanized. The underlying working assumption is that light intensity closely corresponds to human occupation (Azevedo, 2013).

2.2. Impacts of urbanization on macrophyte community

To evaluate the effects of urbanization on the macrophyte community, macroalgae and seagrass were collected at spring low tide in the intertidal zone where green turtles are feeding, using 50 × 50 cm quadrats in three parallel transects randomly set on the rocky reefs (Murray et al., 2002). Sampling was done in April 2016 (end of austral summer) and in August 2016 (winter) in the HUa (n = 16 transects, i.e., 48 quadrats) and in the LUa (n = 14 transects, i.e., 42 quadrats). Transect length varied due to differing reef nature and morphology between 9.7 m in the HUa (ranging 5.0–22.8 m) to 94 m (no within-area variation) in the LUa. For each transect, algae were scraped off the substrate of three quadrats; quadrats were

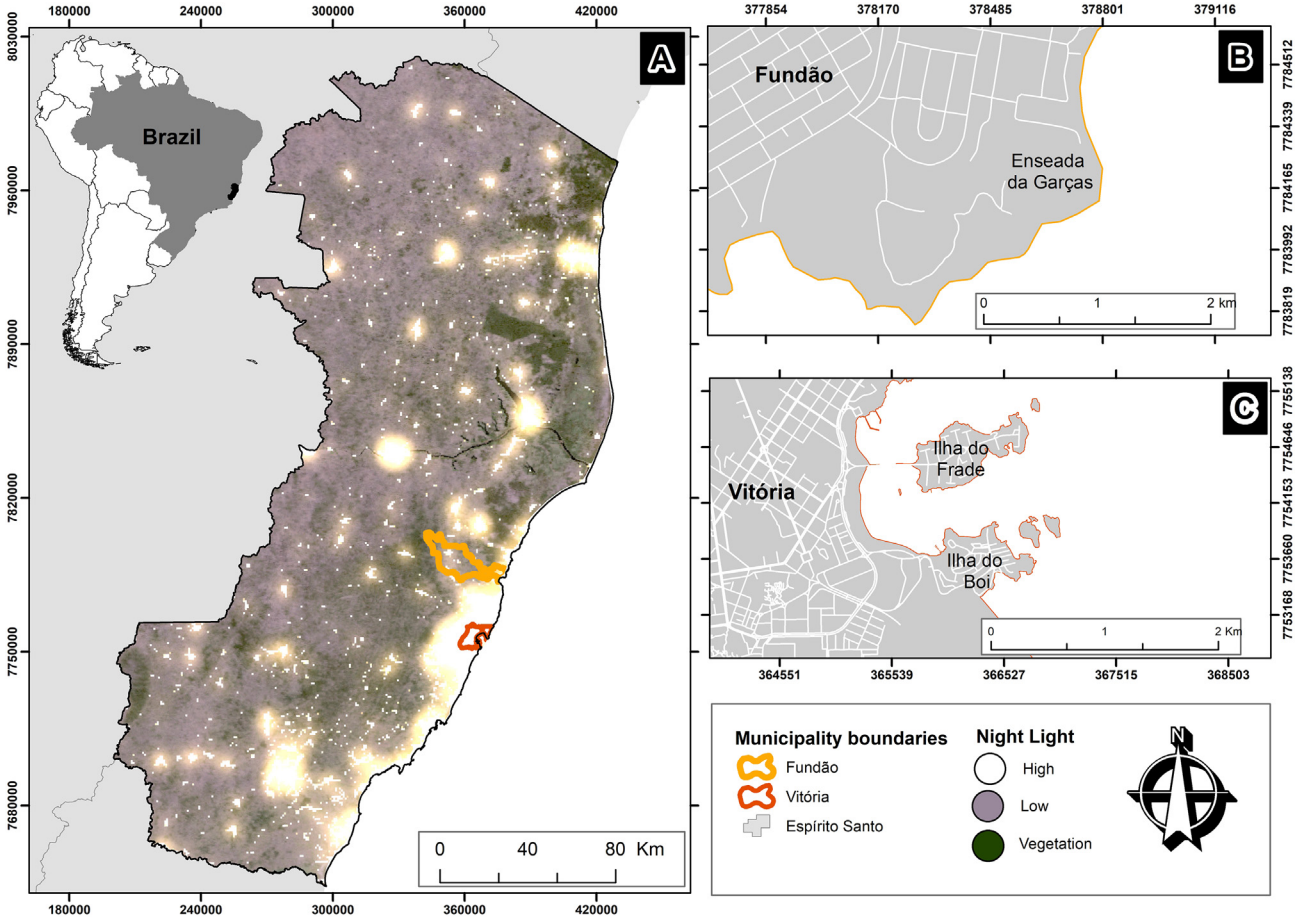


Fig. 1. A) Night-light in the study areas on the coast of the state of Espírito Santo (black in insert in A), southeastern Brazil; B) Enseada das Garças, municipality of Fundão, the relatively lightly urbanized area; C) Ilha do Boi and Ilha do Frade, municipality of Vitória, the highly urbanized area.

located at each end and in the middle of the transect. After sorting and identification at the lowest taxonomic level (Nassar, 2012), fresh wet mass was obtained by the volume displacement method assuming density = 1. All samples were preserved in buffered 4% formalin.

2.3. Green turtle diet

Diet samples were obtained from stranded dead green turtles found during beach monitoring (2010 to 2012) in HUa (n = 23) and LUa (n = 24). The diet samples from turtles were obtained by collection of their stomach and esophagus contents. The samples were preserved in 4% buffered formalin solution in plastic containers and analyzed in the following two months after collection. Diet taxa were identified at the lowest taxonomic level based on morphological criteria and, when necessary, on anatomical details observed microscopically on cross-sections (Nassar, 2012).

2.4. Nutritional parameters and pigment composition

Macroalgae and seagrass for biochemical analysis were collected at the same sites and time as macrophyte community surveys. To perform the nutritional analyses, we selected diverse food items available at the two sites (but not necessarily present in quadrats). Populational samples of 200 g (wet mass) of four taxa, *Halodule* sp., *Hypnea* sp., *Caulerpa* sp. and *Ulva* sp. were collected, washed under deionized water and identified at the lowest taxonomic level using morphological attributes and microscopy (Nassar, 2012). Samples were maintained at −20 °C until further analysis.

Photosynthesizing pigment content (carotenoids and phycobiliproteins), carbohydrate, protein and lipid concentrations and diet-containing energy (calories) per unit wet mass of marine plants collected in the two areas were quantified. Each analysis was done thrice for each populational sample. For all chemical analyses, an appropriate amount of freezer-frozen biomass was extracted and ground in liquid nitrogen before further processing.

Total non-structural carbohydrates concentration was determined following Dubois et al. (1956). Thus, 1 g of biomass was heated to 80 °C in 80% ethanol for 5 min for enzymatic inactivation. After manual homogenization followed by centrifugation at 3000 *g* for 15 min, an extract was obtained from the supernatant. This alcoholic extract was concentrated in a rotary evaporator and read using a spectrophotometer at 490 nm.

Lipid extraction was conducted following the Bligh and Dyer (1959) method. Each N_2-macerated frozen alga (200 mg) was suspended in chloroform:methanol:water (2:2:1). Total lipids were quantified by gravimetry.

Hydrosoluble proteins concentration was determined following Bradford (1976). Extraction was realized from 100 mg biomass suspended in buffer phosphate solution (pH 5.5) in the proportion of 0.1 $g.mL^{-1}$ biomass in 2 mL buffer solution. Total soluble protein concentration was obtained using a spectrophotometer at 595 nm after adding the *Quick Start TM Bradford Protein Assay* (Bio-Rad) solution. Five dilutions of bovine serum albumin, between 0.2 and 0.9 $mg.mL^{-1}$, were used to build the standard curve.

To determine carotenoids concentration, 100 mg extraction was suspended in 2 mL acetone and centrifuged at 4 °C. The concentration of carotenoids was measured according to Jeffrey and Humphrey (1975). To determine phycobiliproteins (allophycocyanin, phycocyanin and phycoerythrin) concentrations, 1 mL of phosphate buffer 50 mM, pH 5.5, was added to the supernatant after centrifugation at 4 °C, through the formulas described by Kursar et al. (1983). Readings (in $\mu g.mL^{-1}$) were converted in mg pigment per gram of fresh biomass considering the initial mass (100 mg) used for extraction.

Calories were computed according to Lajolo and Vanucchi (1987) where total energy is the sum of protein (4 kcal.g^{-1}), lipid (9 kcal.g^{-1}) and carbohydrate (4 kcal.g^{-1}) contents.

2.5. Green turtle health

Health data were extracted from a database on 230 immature green turtles stranded in the two study areas between 2009 and 2014. The average curved carapace length (CCL) for these turtles was 34.7 cm (range: 26.0 to 72.2 cm) and the average body mass was 5.8 kg (range: 1.4 to 31.0 kg). We used three proxies to evaluate green turtles´ health: subjective body condition score (BCS) (a visual assessment used in conservation monitoring), residual condition index (RCI, an index based on actual size and mass) and fibropapillomatosis (FP) severity (an index for external tumor development). The number of values for each variable used as a proxy for green turtle health varied slightly due to missing data (HUa: N = 133–184; LUa: N = 28–46). Individual BCS was evaluated visually according to Walsh (1999) 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. The individual RCI was computed to get an observer-independent health estimate; it corresponds to the value of the residual of the linear regression between the natural logarithm (ln) of mass and that of length (i.e., the difference, in logarithm, between the value observed for mass and that estimated by the linear model; e.g., Salvador and Muelbert, 2019). Positive RCI indicates the individual is above the mean weight-at-length and therefore displays higher body condition (Schulte-Hostedde et al., 2005). The index of FP severity (FP score or FPS) is based on an analysis of the number and size of tumors. FP severity index value is correlated to a number of blood and physiological alterations and pathologies reflecting health status (Work and Balazs, 1999; Aguirre and Balazs, 2000; Work et al., 2003, 2004). All specimens were photographed and examined for the presence of FP tumors externally and in the oral cavity. Tumors were classified into four categories based on their maximum diameter (CT): CT-A ≤ 1 cm, CT-B = 1–4 cm, CT-C = 4–10 cm and CT-D > 10 cm (Work and Balazs, 1999). Number and size of tumors are combined into the FP severity score (FPS_{SWA}; specific for the southwestern Atlantic) with three levels: mild, moderate and severe (Rossi et al., 2016).

2.6. Data analysis

Macrophyte communities were described using the mean wet mass per transect (± SD) for each genus, combining summer and winter transects. The Shannon index of diversity (H´) was computed over the mass and using natural logarithms. Differences in number of genera (richness) and diversity (Shannon) between areas were tested by non-parametric Mann-Whitney U test with 10,000 Monte Carlo resampling at $\alpha = 0.05$.

Diet was described as the mean (± SD) wet mass of each macrophyte genus per turtle, using esophagus and stomach data combined (when available). The Shannon index of diversity of each individual diet was computed using natural logarithms on mass. Diversity (Shannon) and richness (number of ingested genera) for the overall diet were computed by pooling all individuals. Composition, understood as diversity or richness of genera in diet, was tested by non-parametric Kruskal-Wallis tests with 10,000 Monte Carlo resampling at $\alpha = 0.05$.

Total non-structural carbohydrates, total lipids, total proteins, calories and carotenoids of four macrophyte genera were analyzed independently via analysis of variance (ANOVA). The sole variable was the combination between genus and area (4 genera * 2 areas = 8 levels, each with $n = 3$). Note that testing genera or areas per se (for example in an ANOVA that includes these two explanatory variables and their interaction) is out of the scope of the study (e.g., McDermid et al., 2007; Fiset et al., 2019). To test for differences between areas in a genus, Tukey *post-hoc* tests were run between all levels; differences between HUa and LUa were deemed significantly different when the two means for that genus belonged to different homogeneous groups ($\alpha = 0.05$). Note that due to sensitivity and precision of chemical analyses, analyses run at the genus level, use of populational samples and overall design, there was no need to lower alpha to account for the number of analyses; both ANOVAs and *post-hoc* tests were considered significant with $\alpha = 0.05$. Because phycobiliproteins were measured in one single genus, there was no need for *post-hoc* tests. Analyses for summer and winter were done separately (McDermid et al., 2007).

Difference in green turtle size (CCL) between the two areas were tested through non-parametric Mann-Whitney U tests with 10,000 Monte Carlo resampling at $\alpha = 0.05$. The appropriateness of visually-estimated body condition (BCS) to represent with accuracy the RCI of turtles was estimated using non-parametric Kruskal-Wallis tests with 10,000 Monte Carlo resampling at $\alpha = 0.05$. Test for difference in individual RCI between areas was done via analysis of variance (ANOVA) under type III sum of squares. RCI data normality was assumed as residuals from a linear regression should be symmetrically distributed around a mean close to zero (Zar, 1999). FP prevalence and severity in affected individuals (FPS_{SWA}) and body condition were tested by χ^2 tests on raw counts.

3. Results

3.1. Impacts of urbanization on macrophyte community and green turtle health

Based on satellite imagery, night-light intensity was 59 in Vitória and 22 in Fundão. The two study areas can therefore be classified as HUa and LUa, respectively, and are under distinct levels of anthropic impact.

Macrophyte assemblages were distinct between areas (Table 1). The HUa showed low richness (10.0 ± 3.6 SD genera and 11.9 ± 4.2 taxa per transect vs. 20.1 ± 7.0 genera and 23.5 ± 9.2 taxa per transect, excluding Cyanophyceae and undetermined macroalgae) compared to the LUa (Kruskal-Wallis U = 20.0; $p < 0.001$ and U = 27.5; $p < 0.001$ for genera and taxa respectively). Genera diversity per transect was also lower (H´ = 1.21 ± 0.24) in the former than in the latter (H´ = 1.84 ± 0.38; Kruskal-Wallis U = 19.0; $p < 0.001$). We found no difference in total wet mass per transect between the two areas (Kruskal-Wallis U = 91.0; $p = 0.400$). Note, however, that the HUa presented the five lowest (51.6–116.7 g/transect) and five of the six highest (1436.8 - 1853.9 g/transect) biomass values (out of 90 quadrats in total). This unexpected pattern was not investigated further.

Turtle size differed between areas (U = 3150; $n = 233$; df = 1; $p = 0.004$), but the differences in CCL (35.4 ± 5.9 cm in the more urbanized area and 33.0 ± 3.9 cm in the less urbanized area) do not appear relevant to our study (between one and two years of age according to Torezani et al., 2010). Body condition score (BCS), using both healthy and FP-affected individuals, was similar in both areas ($\chi^2 = 1.949$; n = 233; df = 2; $p = 0.377$). About half of individuals were classified as normal, 14–15% as underweight and 23–34% as being emaciated (Table 2). Individual RCI and BCS were correlated (Kruskall-Wallis $\chi^2 = 132.86$; $n = 231$; df = 2; $p < 0.001$), with the category Normal showing positive RCI = 0.1238 ± 0.1056 mean ± SD (Standard error of the mean SE = 0.0094). In contrast, underweight showed RCI close to zero (RCI = 0.0406 ± 0.1500; SE = 0.0261) and emaciated showed negative RCI (RCI = −0.2296 ± 0.1602; SE = 0.0187). No significant difference in RCI between areas was detected (ANOVA F = 1.997; n = 231; $p = 0.159$; $r^2 = 0.009$). FP prevalence ($\chi^2 = 8.720$; $n = 222$; df = 1; $p = 0.003$) was higher in the HUa but, in affected animals, severity was marginally non-significant ($\chi^2 = 5.721$; $n = 82$; df = 2; $p = 0.057$) (Table 2).

3.2. Green turtle diet

Macroalgae are the main food item consumed by green turtle at the study area. Terrestrial vegetal material accounted for 0.1 ± 0.5% to 0.2 ± 1.0% of diet mass in HUa and LUa, respectively, and animal material (mostly crustaceans) varied from traces to 0.8 ± 3.9%. In the HUa, diet was poorer (Table 1) with 4.0 ± 1.6 genera per turtle (vs. 8.5 ± 4.0; Kruskal-Wallis U = 76.0; $p < 0.001$). Diet also looked less diverse, either on an

Table 1
Macrophytes in stomach and esophagus of 47 necropsied green turtles and in macrophyte floristic surveys in HUa and LUa. Data shown refer to mean wet mass ± SD per turtle (g) or per transect (g) and the corresponding mean proportion (%; SD was omitted). Values are truncated to one (Mass) or two digits (Proportion). Data are presented by genus; unidentified phyta, cyanobacteria and calcareous encrusting Rhodophyta morphospecies were omitted. "T" indicates traces and "—" absence. Number of turtles and transects in HUa is 23 and 16, respectively, and 24 and 14 in LUa.

Taxa	HUa				LUa			
	Diet		Flora		Diet		Flora	
	Mass ± SD per turtle (g)	Proportion in diet (%)	Mass ± SD per transect (g)	Proportion of flora (%)	Mass ± SD per turtle (g)	Proportion in diet (%)	Mass ± SD per transect (g)	Proportion of flora (%)
Chlorophyta	46.5 ± 37.4	77.94	219.8 ± 250.2	35.02	33.1 ± 36.5	59.39	273.0 ± 200.5	30.35
Acicularia	–	–	–	–	0.0 ± 0.0	0.04	–	–
Anadyomene	–	–	0.7 ± 2.8	0.04	0.0 ± 0.0	0.04	6.8 ± 12.5	0.78
Bryopsis	–	–	3 ± 5.4	1.86	0.0 ± 0.1	0.05	0.1 ± 0.40	0.02
Caulerpa	6.0 ± 12.6	15.35	–	–	3.5 ± 14.5	3.52	106.4 ± 189.2	9.96
Chaetomorpha	0.0 ± 0.0	0.12	1.9 ± 5.6	0.25	–	–	0.0 ± 0.1	0.00
Cladophora	0.4 ± 1.5	0.85	6.2 ± 22.3	0.54	0.0 ± 0.1	0.10	4.4 ± 8.3	0.48
Codium	–	–	–	–	0.0 ± 0.1	0.07	0.5 ± 1.6	0.06
Halimeda	–	–	–	–	1.8 ± 6.1	3.99	10.6 ± 11.8	1.37
Ulva	40 ± 38	61.62	208 ± 253.4	32.34	27.6 ± 31.9	51.58	143.4 ± 45.4	17.59
Valonia	–	–	–	–	–	–	0.8 ± 1.6	0.09
Rhodophyta	6.5 ± 9.0	17.91	332.9 ± 425.5	43.53	13.2 ± 17.8	37.2	306.3 ± 181.8	35.28
Acrochaetium	–	–	–	–	T	n/a	–	–
Amphiroa	–	–	2.0 ± 7.8	0.11	0.1 ± 0.3	0.51	43.8 ± 56	5.23
Arthrocardia	–	–	37.0 ± 82.5	3.02	0.0 ± 0.1	0.16	8.7 ± 17.2	1.12
Asparagopsis	–	–	–	–	–	–	0.4 ± 1.6	0.06
Bryothamnion	–	–	–	–	0.1 ± 0.5	0.39	7.1 ± 16.9	0.78
Calliarthron	–	–	0.4 ± 1.5	0.02	–	–	–	–
Centrocera	–	–	13.5 ± 21.3	11.57	–	–	0.2 ± 0.6	0.05
Champia	–	–	1.7 ± 3.2	0.18	T	n/a	0.1 ± 0.3	0.01
Chondracanthus	1.5 ± 4.9	3.37	2.7 ± 6.8	3.34	4.5 ± 10.7	10.93	5.4 ± 11	1.01
Corallina	–	–	234.1 ± 317.3	17.68	–	–	35.4 ± 41.3	4.07
Cryptonemia	–	–	1.9 ± 5.1	0.17	–	–	0.6 ± 1.3	0.05
Dichotomaria	–	–	–	–	–	–	7.6 ± 14.8	0.98
Galaxaura	–	–	–	–	0.0 ± 0.1	0.09	6.5 ± 15	0.77
Gelidiopsis	–	–	0.4 ± 1.6	0.09	0.5 ± 1.6	0.9	–	–
Gelidium	3.1 ± 6.6	10.29	1.0 ± 4.2	0.25	4.3 ± 12.9	7.88	0.0 ± 0.1	0.00
Gracilaria	0.3 ± 1.0	1.83	0.5 ± 1.7	0.04	2.3 ± 7.6	7.25	6.4 ± 18.2	1.6
Gracilariopsis	0.0 ± 0.0	0.01	–	–	–	–		
Grateloupia			2 ± 7.3	0.24	–	–	0.3 ± 1	0.04
Gymnogongrus	0.1 ± 0.5	0.09	–	–	0.0 ± 0.2	2.92	–	–
Halymenia	1.3 ± 3.4	1.76	–	–	0.1 ± 0.5	1.18	–	–
Heterosiphonia	–	–	0.3 ± 0.9	0.09	–	–	0.2 ± 0.3	0.02
Hypnea	0.0 ± 0.1	0.03	19.5 ± 26.4	2.63	0.3 ± 1.3	0.23	32.1 ± 32.4	3.39
Jania	–	–	–	–	–	–	12.7 ± 28.5	1.43
Laurencia	–	–	–	–	0.0 ± 0.1	0.05	9.7 ± 14.0	1.8
Ochtodes	–	–	0.1 ± 0.3	0.01	–	–	0.4 ± 0.8	0.04
Osmundaria	–	–	–	–	0.1 ± 0.5	0.3	–	–
Palisada	–	–	–	–	–	–	1.5 ± 1.8	0.18
Peyssonnelia	–	–	–	–	0.0 ± 0.1	0.04	–	–
Plocamium	–	–	6.6 ± 15.9	0.49	0 ± 0	0.01	0.2 ± 0.4	0.03
Porphyra	–	–	1.9 ± 7.7	0.12	0.0 ± 0.1	0.06	–	–
Pterocladiella	0.0 ± 0.2	0.04	0.1 ± 0.3	0.01	0.7 ± 3.2	4.27	0.0 ± 0.0	0.00
Rhodymenia	0.2 ± 0.5	0.37	–	–	0.0 ± 0.0	0.01	–	–
Thuretia	–	–	–	–	0.0 ± 0.0	0.02	–	–
Sebdenia	0.0 ± 0.1	0.12	–	–	–	–	–	–
Solieria	–	–	–	–	–	–	3.0 ± 6.8	0.27
Vidalia	–	–	–	–	–	–	105.7 ± 132.9	10.22
Ochrophyta	0.0 ± 0.0	0.00	236.8 ± 300.4	24.8	1.6 ± 3.8	3.41	317.1 ± 176.7	36.46
Colpomenia	–	–	48.7 ± 79.9	8.78	–	–	18.0 ± 42.7	2.58
Dictyopteris	0.0 ± 0.0	0.00	0.5 ± 1.3	0.03	1.1 ± 3.2	1.68	5.7 ± 12.0	0.66
Dictyota	–	–	–	–	–	–	3.3 ± 7.4	0.29
Lobophora	–	–	0.0 ± 0.0	0.00	0.2 ± 0.7	0.27	0.0 ± 0.0	0.00
Padina	–	–	2.8 ± 7.5	0.31	–	–	70.5 ± 96.8	8.19
Sargassum	–	–	184.8 ± 307.6	15.67	0.3 ± 1.2	1.45	219.0 ± 161.4	24.69
Zonaria	–	–	–	–	–	–	0.6 ± 1.1	0.06
Phanerogama	2.4 ± 11.5	4.15	0.6 ± 2.2	0.11	0.0 ± 0.2	0.74	0.4 ± 0.9	0.04
Halodule	2.4 ± 11.5	4.15	0.6 ± 2.2	0.11	0.0 ± 0.2	0.74	0.4 ± 0.9	0.04

overall basis (H′ = 1.07 vs. H′ = 1.62; Table 1) or on an individual basis (H′ = 0.45 ± 0.29 vs. 0.64 ± 0.46 per turtle). However, Shannon diversity per turtle showed no difference between areas (Kruskal-Wallis U = 210.0; $p = 0.162$). This probably resulted, in part, from the diet being dominated by *Ulva* in both areas (62 ± 39% of diet in HUa vs. 53 ± 40% in LUa). Rhodophyta presented the highest richness in both areas (10 and 21 genera in HUa and LUa, respectively [Table 1], or 1.9 ± 1.2 and 3.9 ± 2.2 genera per turtle) and represented between 18 (± 29%) and 35% (± 39%) of the diet in highly and less urbanized areas, respectively. The only marine angiosperm detected in the diet was the seagrass *Halodule*. Together, *Halodule* sp., *Hypnea* sp., *Caulerpa* sp. and *Ulva* sp. composed about 85% of green turtle diet in both HUa and LUa.

Table 2
Fibropapillomatosis prevalence (Prev.; $n = 230$), severity score (following Rossi et al., 2016; n = 82) and body condition score (n = 233) in green turtles in highly and less urbanized areas. Values are percentages of the total number of specimens examined in each area. Probabilities associated with the statistical tests comparing LUa and HUa are given. n.s.: not significant; n/a: not applicable.

Area	n	Prev. (%)	$p(\chi^2)$	Severity score (%)			$p(\chi^2)$
				Mild	Moderate	Severe	
LUa	43	18.61	0.003	0.00	11.63	6.98	n.s.
HUa	187	41.34		17.32	12.85	11.17	(0.057).
				Body condition score (%)			$p(\chi^2)$
				Normal	Underweight	Emaciated	
LUa	47	n/a	n/a	61.70	14.89	23.40	n.s.
HUa	186	n/a		52.15	13.98	33.87	

3.3. Energy content, lipids, hydrosoluble proteins and carbohydrates

Significant differences in energy content (kcal) were detected between areas for *Halodule, Hypnea* and *Ulva* in summer (Fig. 2A; ANOVA, F = 72.88, $p < 0.001$) and winter (Fig. 2B; ANOVA, F = 27.92, $p < 0.001$). In all cases energy was higher in HUa. *Caulerpa* (in both seasons) and *Halodule* (in winter) did not show urbanization-related differences (Fig. 2A). *Ulva* in HUa presented the highest energy content (7.49 kcal/g in winter and 6.25 kcal/g in summer).

All ANOVAs on total lipids (F = 22.15 and 30.39 respectively for summer [S] and winter [W], both $p < 0.001$), total water-soluble proteins (F = 327.65 [S] and 64.42 [W], both $p < 0.001$) and total carbohydrates (F = 70.90 [S] and 27.62 [W], both $p < 0.001$) showed high significance. Higher concentration in HUa than LUa was detected by Tukey post hoc tests ($p < 0.05$) in summer and winter *Ulva* for lipids (Fig. 3A, B), proteins (Fig. 3C, D) and carbohydrates (Fig. 3E, F). *Hypnea* was more variable with HUa > LUa for summer lipids, winter proteins and in both seasons for carbohydrates. Most tests showed a lack of significant difference between HUa and LUa in both seasons (lipids and carbohydrates in *Halodule* and *Caulerpa*; proteins in *Caulerpa*). Significantly higher concentration in LUa than HUa was limited to winter lipids in *Halodule* and summer proteins in *Halodule* and *Hypnea*.

3.4. Photosynthetic pigments

Phycobiliproteins are water-soluble pigments solely found in Rhodophyta and Cyanobacteria. They were, therefore, only analyzed in *Hypnea*. In both summer (S) and winter (S), ANOVAs detected higher concentration of phycocyanin ([S] F = 45.96, $p = 0.003$; [W] (F = 22.29, $p = 0.009$) and of phycoerythrin ([S] F = 64.30, $p = 0.001$; [W] F = 27.88, $p = 0.006$) in the HUa than LUa (Fig. 4). Allophycocyanin concentration did not differ between areas ([S] F = 0.48, $p = 0.527$ and [W] F = 1.32, $p = 0.315$).

ANOVAs on carotenoids concentration yielded significant results for *Halodule* in summer and *Hypnea* in winter (ANOVAs, F = 39.42, $p < 0.001$ and F = 13.07, $p = 0.002$, respectively) (Fig. 5). In both cases, *post-hoc* Tukey tests revealed that concentration was higher in LUa than HUa.

4. Discussion

We observed a strong decrease in the diversity of the macroalgae community, turtle diet and health status in the HUa that are likely due to degradation of this foraging area. The absence or low abundance of diverse food items may have led turtles to foraging mainly (62%, vs. 52% in LUa) on a single genus, *Ulva* and much less on Rhodophyta (18%, vs. 37% in LUa). Ochrophyta appeared poorly attractive to green turtles (but see Awabdi et al., 2013 for a counter-example), probably due to indigestibility (Witherington et al., 2012) or low protein content (Fiset et al., 2019). Low diet richness and diversity in the HUa contrast with the pattern of relatively higher numbers of diet items reported in foraging areas relatively less degraded (Sazima and Sazima, 1983; Seminoff et al., 2002; López-Mendilaharsu et al., 2005; Makowski et al., 2006; Arthur and Balazs, 2008; Awabdi et al., 2013; Santos et al., 2015), and may have an impact on turtles' nutritional balance and consequently on their health (Santos et al., 2011, 2015).

While marine plant communities are sensitive to environmental challenges brought by seasonal variation (McDermid et al., 2007) and habitat degradation (Ortega, 2000), few studies have assessed how anthropic stress impacts the nutritional values of macrophytes and how this reflects upon herbivores´ diet (Olsen et al., 2010; Scherner et al., 2012). In the present study, all primary metabolites (proteins, including phycobiliproteins, lipids and carbohydrates) showed higher concentration in the HUa. Macroalgae tissue concentration of proteins and phycobiliproteins is positively influenced by nitrogen availability in the environment (Bird et al., 1982; Lobban and Harrison, 1997), and higher levels of nitrogen are usually related to coastal degradation (Bricker et al., 1999; Fisher and Oppenheimer, 1991). High nitrogen concentration in macroalgae tissues, particularly in those from the HUa, may increase the energy provided by the food item but may also have an impact on the health of green turtles. Habitat eutrophication alters the gut microbiome of marine herbivores, probably through the nutrient enrichment of the diet items (Degregori et al., 2021). The alteration of microbiome diversity may also have an impact in their function, which

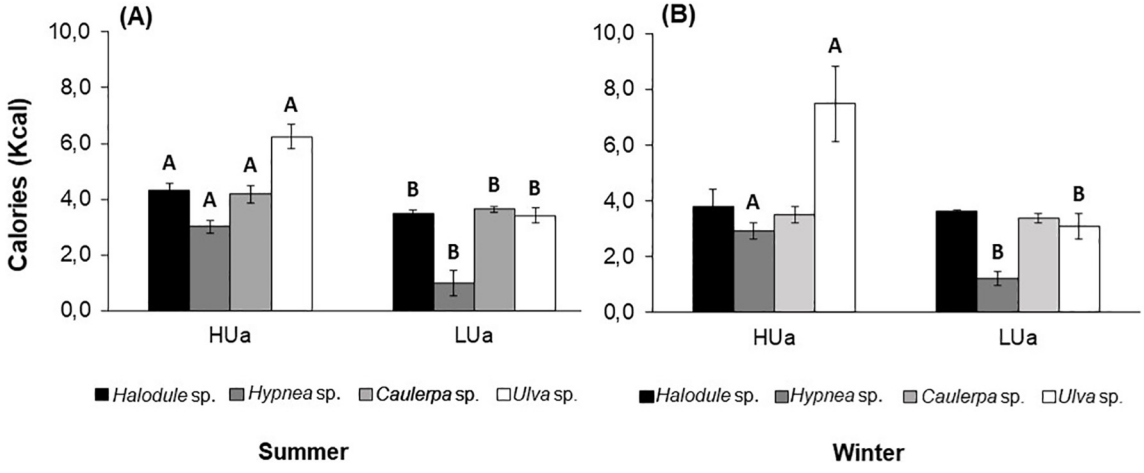


Fig. 2. Energy content (kcal.g^{-1}) wet mass for plant genera consumed by green turtles in summer (A) and winter (B). Error bars represent the standard deviation from three repetitions. Uppercase letters above bars indicates significant differences detected by Tukey post hoc tests ($p < 0.05$) between HUa and LUa within the same season (A is significantly higher than B for the same species). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

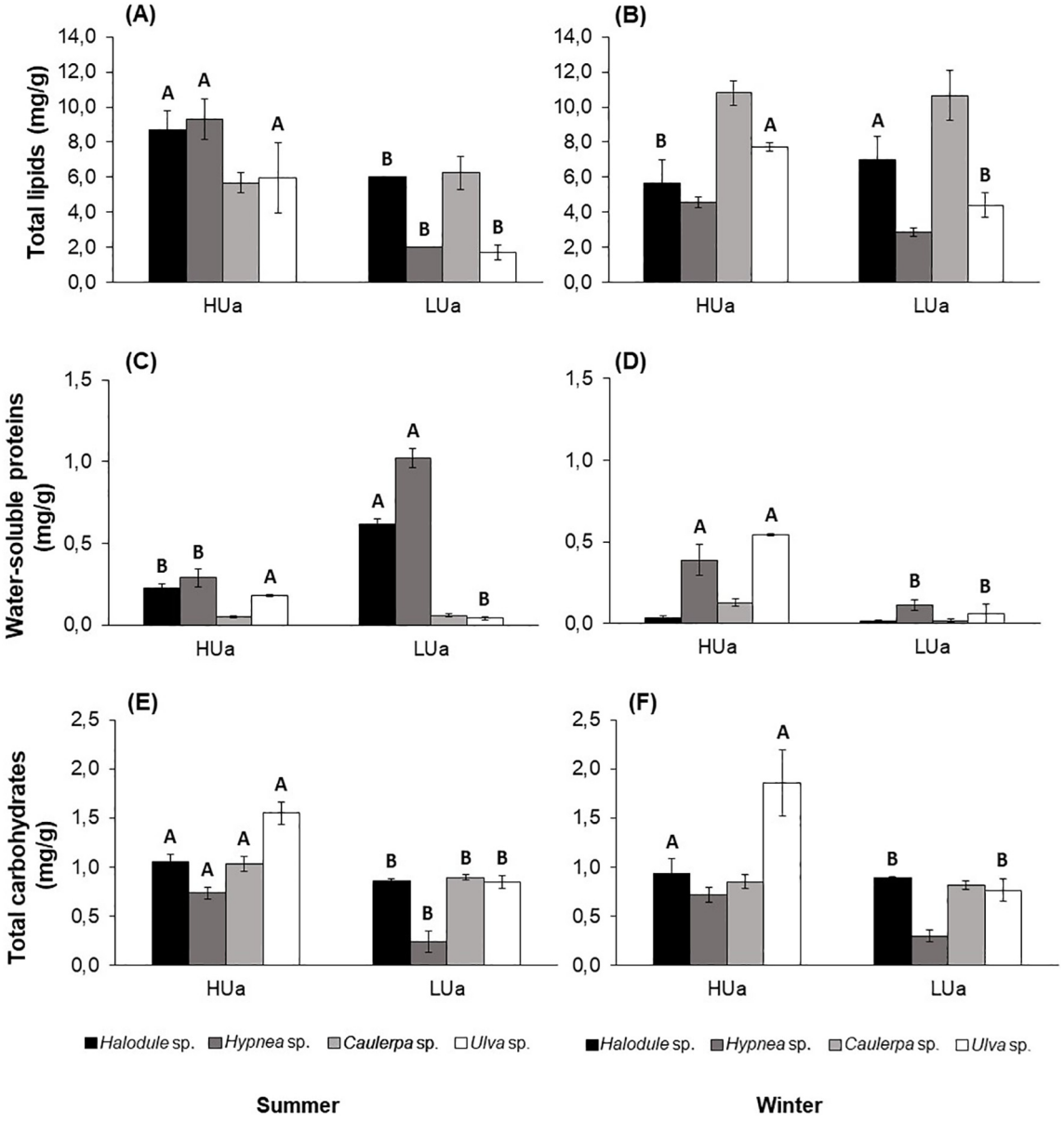


Fig. 3. Nutritional parameters for marine plants: lipid concentration (mg/g) in summer (A) and winter (B); protein concentration (mg/g) in summer (C) and winter (D); carbohydrate concentration (mg/g) in summer (E) and winter (F). Error bars represent the standard deviation from three repetitions. Uppercase letters above bars indicate significant differences detected by Tukey post hoc tests ($p < 0.05$) between HUa and LUa within the same season (A is significantly higher than B for the same species).

may affect the immune response as microbiome plays an important role in the immune system (Zheng et al., 2020). Additionally, high nitrogen levels in diet items have been related to effects in the immune response of vertebrates (Fairbrother et al., 2004; Noyes et al., 2009).

Changes in nutrient availability affect the physiological performance and the production of primary and secondary metabolites in marine plants (Murray and Litter, 1978; Jiménez-Escrig and Cambrodón, 1999; Wong and Cheung, 2000; Misurcová, 2011). However, responses to the addition of nutrients are species-specific (Schaffelke, 1999). Alterations in environmental conditions promote changes in classes and quantities of metabolites that have different ecological functions and play an important role in the ecophysiological balance (Murray and Litter, 1978; Jiménez-Escrig and Cambrodón, 1999; Wong and Cheung, 2000; Misurcová, 2011). We observed lower carotenoids concentration in the HUa. These compounds have antioxidant and anticarcinogenic capacity and are used preventively in humans against various infirmities, such as cancer, cardiac diseases and inflammatory disorders (Wollgast and Anklam, 2000; Zhou et al., 2017). In addition to carotenoids, algae produce a wide array of metabolites that may have some influence on the health of animals (Gama et al., 2008). Some are used in the treatments of virus infections that afflicts humans such as herpes simplex types 1 (HSV-1) and 2 (HSV-2), Human immunodeficiency virus (HIV), Human cytomegalovirus (HCMV), Dengue virus (DENV), among others (Laillea et al., 1998; Cirne-Santos et al., 2008; Hidari et al., 2008). Therefore, we may expect an alteration of the capacity of animals to respond to disease as the chemical diversity of their food items are altered by environmental degradation.

The loss of chemical diversity and the decrease in species diversity may influence green turtle nutritional balance and health in HUa, because nutritional diversity is required to optimize a number of life cycle processes such as growth, survival and fertility (Worm et al., 2006). The effects of decrease in macroalgae nutritional richness due to impacts on ecosystems are still poorly understood, and may induce negative effects on turtle health, including reduced growth rate, late sexual maturity and lower efficiency of

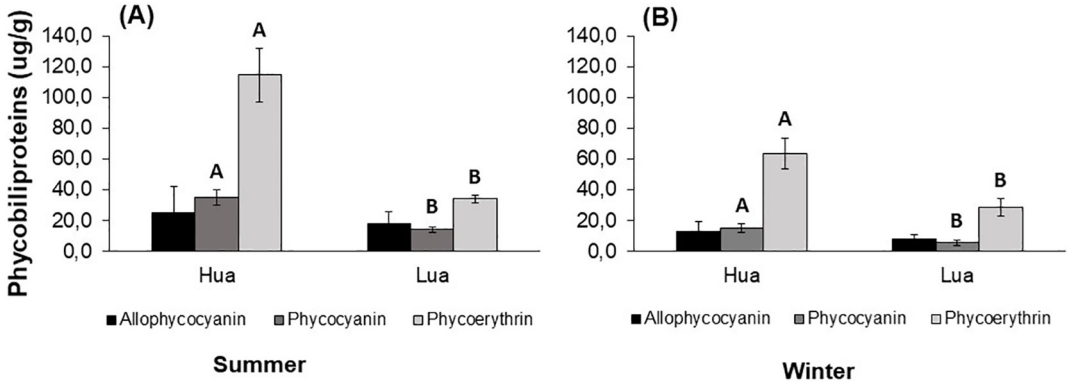


Fig. 4. Mean phycobiliprotein concentration in *Hypnea* sp. in summer (A) and winter (B). Bars represent the standard deviation from three repetitions. Error bars represent the standard deviation from three repetitions. Uppercase letters above bars indicates significant differences detected by Tukey post hoc tests ($p < 0.05$) between HUa and LUa within the same season (A is significantly higher than B for the same species).

the immunological system that could let the species be more susceptible to diseases such as FP (Santos et al., 2011).

We found relatively high prevalence and severity of FP in animals foraging in the HUa, suggesting that high intake of nitrogen compounds (via *Ulva* proteins), lipids or carbohydrates and lower food diversity may have chronic impacts on green turtle health. Under such a scheme, rapid coastal degradation poses a long-term threat to green turtle populations. In fact, previous works have evidenced fecundity variation in the Caribbean and Suriname (Bjorndal, 1982) and differences in juvenile growth rate in the Hawaiian Islands (Balazs and Chaloupka, 2004) associated with differences in diet. Despite the high prevalence and severity of FP and the lower food diversity found in the HUa, we did not find differences in the condition between areas, and we attribute this lack of difference to the higher energy level found in macroalgae in the HUa.

5. Conclusion

We find that habitat degradation is altering green turtles nutritional balance and health. Considering that coastal habitat degradation is accelerating worldwide (Scherner et al., 2013), future research should focus on expanding our knowledge of the ecological impacts caused by changes in nutritional balance of key species. Studies should be designed to understand, within a broad health perspective, impacts brought by decreases in diet and chemical diversities and alterations of gut microbiome. On the conservation side, high-quality feeding areas are fundamental to ensure juvenile recruitment success and development to the adult phase in green turtles (Rees et al., 2016) because herbivory already imposes a nutrient-poor diet, slow growth and late sexual maturity (Bjorndal, 1997). Therefore, we suggest 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). A diverse and nutritive diet should result in higher body growth, reproductive output, and population growth, thus directly influencing conservation prospects for the green turtle (Bjorndal, 1997; Mazaris et al., 2017). On the other hand, such megaherbivores help to maintain the health and the resilience of their foraging habitats while, among other, limiting their canopy-shading growth and competition with other sessil organisms. Thus, areas of relevant diversity and richness of benthic community should receive the highest priority for protection to offer prospects to the green turtle and other marine herbivores.

CRediT authorship contribution statement

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Design of methodology: R.G. Santos, L.P. Machado, J.C. Joyeux and K.V. Bastos.

Field sampling: K.V. Bastos, J.S. Ferreira, L.P. Machado and R.G. Santos.

Formal analysis Application of statistical: J.C. Joyeux, K.V. Bastos. L.P. Machado, F.P. Miltão.

Laboratory procedures: K.V. Bastos, F.P. Militão, J.S. Ferreira.

Writing - Original Draft Preparation: K.V. Bastos.

Writing - Review & Editing: All authors.

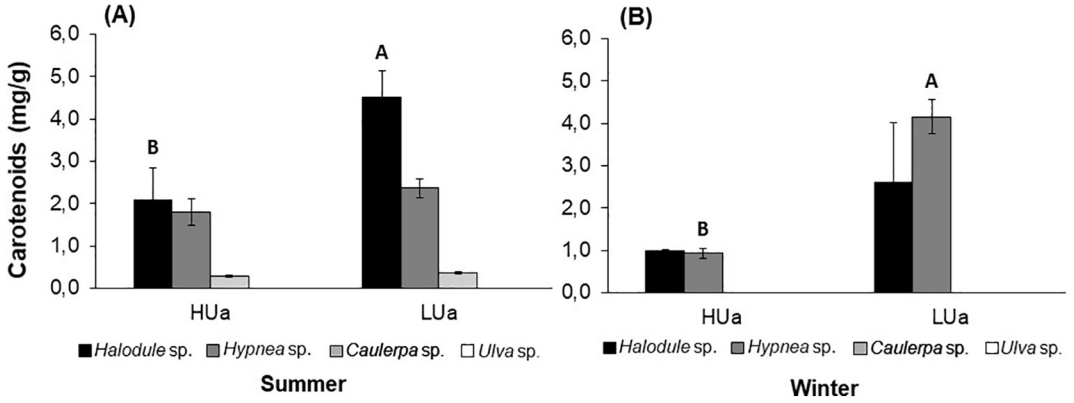


Fig. 5. Mean carotenoids concentration in marine plants in summer (A) and winter (B). Bars represent the standard deviation from three repetitions. Error bars represent the standard deviation from three repetitions. Uppercase letters above bars indicates significant differences detected by Tukey post hoc tests ($p < 0.05$) between HUa and LUa within the same season (A is significantly higher than B for the same species).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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