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Food selection and habitat use patterns of immature green turtles (*Chelonia mydas*) on Caribbean seagrass beds dominated by the alien species *Halophila stipulacea*

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ABSTRACT

Marine herbivores face rapid changes in the coastal ecosystems where they forage. In the Caribbean, the recent and fast expansion of the invasive phanerogam species *Halophila stipulacea* is threatening native seagrass ecosystems. So far, *H. stipulacea* is escaping most Caribbean herbivores, certainly because of its recent introduction or lower nutritional value. We investigated

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Foraging behavior
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Martinique

the impact of *H. stipulacea* invasion on fine-scale foraging habitat selection and food resource selection of immature green turtles at critical foraging sites in Les Anses d'Arlet, Martinique. The analysis of seagrass distribution and nutritional content, together with turtle behaviour and resource selection, showed that *H. stipulacea* may be of contrasting interest to green turtles. Compositional analysis confirmed the lower nutritional value of *H. stipulacea* compared to the native species, but the invasive species showed higher digestibility than native ones, which calls into question the energetic advantage of consuming the native plants over the exotic plant. Thus, although green turtles mostly selected the native seagrass *Thalassia testudinum* in multispecies seagrass beds, some individuals fed on *H. stipulacea*. Accordingly, in bays entirely invaded by *H. stipulacea*, one possibility for resident green turtles is to increase foraging on this species, but, if so, the consequences on their growth and survival still remain to be determined. As the expansion of *H. stipulacea* may have been facilitated by factors such as shipping, anchor scarring and fishing activities, protection of native seagrass beds and immature green turtles from human disturbances is urgently required to ensure the long-term adaptation of green turtles to this new foraging environment.

1. Introduction

Investigating foraging habitat use, food selection and underlying strategies in threatened species is essential to understand their ecological roles and trophic relationships and thus develop appropriate conservation measures. Optimal foraging theory postulates that fitness (survival and reproductive success) depends on foraging efficiency and that natural selection has favoured behaviours that maximise energy acquisition (Pyke et al., 1977). Therefore, foraging strategies have evolved in adaptation with the environment, i.e. in response to factors like predation risk, intra- and interspecific competition, as well as food availability and quality (Chevallier et al., 2008; Fritz et al., 1996; Pyke et al., 1977). Rapid changes in the environment can challenge these foraging strategies and affect individuals' fitness, threatening the future of the population. This is especially true for species spending considerable amounts of time on feeding sites over their lifetime, i.e. those with delayed sexual maturity and for which individuals in advanced life stages have high reproductive value or are essential for the survival of young (Braby et al., 2011; Heppell et al., 2002). Thus, foraging ground quality should be considered critical for population recovery.

Marine herbivores have developed adaptive morphological, physiological and behavioural traits to consume algae and seagrass and maximise the nutritional value they get from them. For example, sea urchins, parrotfish and surgeonfish use powerful mouthparts to break plant fibres and cell walls, while green turtles and some fishes that do not masticate food use a low stomach pH to break certain algae or fibres like hemicellulose (Bjorndal, 1985; Steneck et al., 2017; Thayer et al., 1984). Sirenians and green sea turtles also depend on their rich gut microflora (composed of cellulolytic bacteria) which ensures the partial digestion of plant fibres such as cellulose and hemicellulose, by microbial fermentation (Bjorndal, 1980, 1985; Thayer et al., 1984). At the behavioural level, one foraging strategy is to regularly revisit the same seagrass patches, which allows herbivores to cultivate plots of young leaves (Bjorndal, 1980). Since regrowths generally have higher protein levels and lower lignin levels (Bjorndal, 1980; Moran and Bjorndal, 2007), and digestibility of forages is negatively correlated with lignin concentration (Moore and Jung, 2001), this strategy improves nutritional content and digestibility of the targeted plants. In addition, bite sizes that favour a high surface-to-volume ratio of ingesta particles, and thus greater exposure to microbial attack, are likely to maximise digestion rates (Bjorndal et al., 1990; Gulick et al., 2021). Reducing ingesta particle size also provides a strategy to meet energy requirements in the face of declining food availability, whether due to anthropogenic threats or overgrazing (Gulick et al., 2021). Thus, marine herbivores have adapted to the plant resources that thrive in coastal marine environments, and are largely dependent on the well functioning of these ecosystems. In turn, these herbivores, and especially megaherbivores such as manatees, dugongs and green turtles, can induce structural changes in their foraging habitats and influence their evolution (Thayer et al., 1984). Understanding interactions between these endangered megaherbivores and their ecosystem is therefore crucial for successful conservation strategies.

Coastal marine ecosystems conservation is even more important given that they face strong anthropogenic pressures and are jeopardised by a rapid reduction in their distribution. This is the case for seagrass meadows, which have suffered a global loss of 29% since 1879, mainly due to coastal human activities, pollution and eutrophication, and climate change (Waycott et al., 2009). The expansion of invasive species such as sea urchins and exotic plants also hinders the development of native seagrasses (Williams, 2007). In particular, the recent implantation of the exotic seagrass species *Halophila stipulacea* in the Caribbean has raised new worries. Originating from the Red Sea, it reached the Western Atlantic Ocean probably transported by boats, and spread rapidly around the Eastern Caribbean islands after a first record in 2002 (Ruiz and Ballantine, 2004; Willette et al., 2014). *H. stipulacea* has been considered an invasive species in the Caribbean due to its rapid expansion and strong competitive ability (Winters et al., 2020), thus contributing to rapidly modifying native plant communities.

The green turtle (*Chelonia mydas*) is the only sea turtle species with a plant-dominated diet (Bjorndal, 1980; Jones and Seminoff, 2013). Upon recruitment to neritic habitats, juvenile green turtles are indeed known to shift from a carnivorous to a predominantly herbivorous diet (Jones and Seminoff, 2013). Green turtles have been classified as Endangered on the IUCN Red List, mainly due to ongoing harvest and bycatch, and the rapid change in their foraging habitats may represent an additional threat (Seminoff, 2004). While conservation efforts worldwide began in the 1950 s, management actions have primarily targeted nesting beaches and protected eggs and hatchlings, as at-sea monitoring of populations remains difficult (Heppell et al., 2002). Yet, stage-based population models on

long-lived turtle species have highlighted the importance of reducing mortality in the late life stages (large juveniles, subadults, adults) to promote population recovery (Heppell et al., 2002). Therefore, estimating the impact of changes, particularly in the developmental range of immature green turtles, is important to adapt protection measures for these key habitats.

Only a few studies mention the presence of non-native plant species in green turtles' diet. In Hawaii, it took 10–12 years for highly invasive species and 20–30 years for slow-growing algae, to become the majority of green turtles' diet (Russell and Balazs, 2015). In the Eastern Caribbean the first occasional grazing events on *H. stipulacea* by green turtles were observed in 2013 (Becking et al., 2014), but the relatively short time since its arrival (Willette et al., 2014) may explain why it was not yet meaningful in turtles' diet. Moreover, these grazing events were always observed within assemblages dominated by the native seagrass *T. testudinum*, which was significantly preferred over *H. stipulacea* (Christianen et al., 2018; Whitman et al., 2019). A single study conducted in the U.S. Virgin Islands in 2017–2018 (i.e. within 2 years of the arrival of *H. stipulacea*), highlighted the prevalence of this introduced seagrass in the diet of juvenile green turtles, although it was not a dominant plant in the environment (Gulick et al., 2021). In Martinique (Lesser Antilles), *H. stipulacea* now covers most of the foraging habitats available along the Caribbean coast (Ortolé, 2012). After a first record in 2006, nearly 90% of the changes observed in the benthic communities within 4 years benefited *H. stipulacea* (Maréchal et al., 2013). While conservation plans still take little account of invasive plants (Giakoumi et al., 2016), it is important to assess the impact of *H. stipulacea* invasion on foraging habitat and food resource selections of immature green turtles. This could provide a better understanding of how turtles have adapted to this rapid change, so that protective measures can be implemented for key seagrass habitats.

Our study focused on the seagrass beds of Les Anses d'Arlet, Martinique, a green turtle foraging hotspot where immatures spend many years until reaching sexual maturity (Chambault et al., 2018; Siegwalt et al., 2020). We assessed seagrass bed composition and determined nutritional content and digestibility of plants. In parallel, we recorded turtle behaviour and movements to analyse foraging habitat selection, and analysed turtle bite counts to infer diet and food preferences. Because Martinique's multispecies seagrass meadows are dominated by *H. stipulacea*, and given the high fidelity of immature green turtles to their feeding grounds (Siegwalt et al., 2020), we hypothesised that they might now consume significant proportions of *H. stipulacea*.

2. Materials and methods

2.1. Study site

We conducted our study in Martinique, French West Indies, France, in five bays of Les Anses d'Arlet (14°30'9.64"N, 61°5'11.85"W): Anse Noire, Anse Dufour, Grande Anse, Anse du Bourg and Anse Chaudière (see Fig. S1 and S2 for details). Grande Anse (~75 ha), Anse du Bourg (~30 ha) and Anse Chaudière (~40 ha) are large bays where algae, and the three main phanerogams found in Martinique (i.e. two native species, *Syringodium filiforme* and *Thalassia testudinum*, and one invasive species, *Halophila stipulacea*) are present. Anse Noire (~8 ha) and Anse Dufour (~10 ha) are only covered by *H. stipulacea* and algae.

2.2. Benthic composition and resource availability

Macroalgae and seagrass species composition of each bay was assessed during 2016 and 2018. No precise mapping was done in Anse Noire and Anse Dufour, as *H. stipulacea* covered nearly 100% of the meadows, with few algae on the rocky banks. In Grande Anse, Anse du Bourg and Anse Chaudière, meadows between 2 and 15–20 m depth were entirely surveyed, at predefined points located every ten metres along GPS-based transects. At each sampling point, a close picture of the ground was taken within 1 m² quadrats, either using an underwater camera linked to a monitor on a boat or by scuba-divers using GoPro Hero 4 Silver cameras. The benthic vegetal composition and cover percentages of Algae (which, in our study, mainly include the following macroalgae species: *Acanthophora spicifera*, *Avrainvillea fulva*, *Avrainvillea nigricans*, *Caulerpa cupressoides*, *Caulerpa sertularioides*, *Cerarium ciliatum*, *Cladophora prolifera*, *Dictyota prolifera* and other *Dictyota* sp., *Halimeda incrassata*, *Peniculus capitatus*, *Sargassum fluitans*, *Sargassum histrix* var. *buxifolium*, *Ulva fasciata*), and seagrass species (*H. stipulacea*, *S. filiforme*, and *T. testudinum*), were recorded on the pictures *a posteriori*. To determine coverage of algae and each seagrass species, we adapted the Braun-Blanquet scale (Braun Blanquet, 1932) and defined different categories according to the percentage of species coverage. To the initial categories, we added the two categories 0% and 100% to indicate that a species was either absent or covered a whole quadrat (as it was important for us to identify areas without or only with *H. stipulacea*). Finally, rather than using one '5–25%' category and several '< 5%' categories, two categories between 1% and 25% ('1–12%' and '13–25%') were created to facilitate the use of an interpolation scheme in QGIS. As a result, on each plot, we estimated and classified plant coverage in seven categories (0%, 1–12%, 13–25%, 26–50%, 51–75%, 76–99%, 100%).

Using the 'natural neighbor' interpolation scheme (Cueto et al., 2003) available in QGIS (version 3.16.5, QGIS Development Team, 2022), we created raster maps for each plant and for both years. Once vectorised, surface areas of all polygons composing each of the percentage classes were extracted and multiplied by the corresponding median class percentage to determine the total area available equivalent to a 100% cover. Maps of algae and each seagrass species were then combined into a single map representative of the coverage of macroalgae and seagrass species. Finally, Voronoi polygons were constructed in QGIS using the sampling points as references to map the presence/absence of the different plants and thus represent the different vegetation communities on a composition map.

2.3. Video recording of feeding events

Field observations were performed in the five bays from May to September 2016, from December 2016 to May 2017, in February,

April and June 2018, and from June to October 2019. Three snorkelers prospected a bay daily either 7:00–12:00, or 11:00–14:00 or 14:00–19:00 to ensure data collected were evenly distributed across daylight hours. Once a turtle was spotted, the snorkelers video-recorded its behaviours with GoPro Hero 4 Silver cameras, using the focal sampling technique (Altmann, 1974), at a minimal distance of 4 m. The bays being highly frequented, most turtles are used to human presence and their behaviour usually seems unaffected by the presence of snorkelers. Nevertheless, if snorkelers' presence appeared to stress a turtle (which displayed a change in behaviour, location or direction), they moved away. Snorkelers' GPS location (recorded 30–60 times/min) was used as a proxy for turtle location. In 2018–2019, a few individuals were also equipped with on-board cameras with GPS as part of the study of Jeantet et al. (2020, 2021), which allowed us to obtain additional behavioural data. From all video recordings, we identified six behavioural categories: Swimming, Surfacing, Resting, Feeding, Interacting, and Scratching (Table S1). Video recordings were analysed by observers and behaviours were labelled with exact start and end times annotated. A total of 235 h of recordings were obtained during 254 follows, of which 117 h of sequences with feeding activity were extracted and analysed.

2.4. Foraging habitat selection

Foraging habitat selection was investigated for turtles foraging in bays with multispecific seagrass beds (Grande Anse, Anse du Bourg/Anse Chaudière) and not in the bays where only monospecific seagrass meadows of *H. stipulacea* were present (Anse Noire/Anse Dufour). Using behavioural and trajectory data obtained from direct observations, GPS-derived locations were associated with the behavioural category recorded at the same time. Data from individuals monitored less than one minute were discarded. The tracks of 62 individuals monitored in Grande Anse in 2016–2017 ($n = 44$) and 2018–2019 ($n = 12$), and in Anse du Bourg/Anse Chaudière in 2016–2017 ($n = 2$) and in 2018 ($n = 4$) were analysed to investigate fine-scale foraging habitat selection.

We performed a compositional analysis of habitat use (Aebischer et al., 1993), with R software (v.4.0.0, R Core Team, 2021) and adehabitatHS package (Calenge, 2020), to test whether immatures preferentially selected specific vegetation communities during foraging activities. Based on the Voronoi composition maps (see 2.2) we identified fifteen distinct plant communities that we gathered into four main habitat types: monospecific seagrass meadows of *H. stipulacea* (H), algal meadows (A), communities composed of *H. stipulacea* and algae (HA), and all other compositions with at least one native species in a monospecific or mixed meadow (M). The composition maps were used to calculate the relative abundance (so-called 'availability') of the different habitat types at the site scale, while habitat use by each GPS-tracked turtle was calculated using the proportion of locations within each of these habitat types. A habitat was considered 'preferred' if individuals used it more than expected from its availability. Random habitat use (i.e. no habitat preference) was tested with a chi-square test before communities were ranked in order of preference based on pairwise t-tests.

2.5. Diet composition and food selectivity

Individual-level diet composition was inferred from bite count. A bite corresponds to a directed head/jaws movement towards a food item with evident intent to remove and ingest all or most of it, and exclude movements associated with chewing only (Thomson et al., 2018). From the video recording analysis and for each foraging bout, bites were counted and assigned to a prey category: *H. stipulacea*, *S. filiforme*, *T. testudinum*, Algae (any species of macroalgae) or Other (typically animal prey, and occasionally squid or fish discards, or food scraps such as eggshells). We did not consider bites for which prey could not be identified and according to Thomson et al. (2018), only individuals with ≥ 30 confidently identified bites were considered ($n = 61$: 27 in monospecific sites, and 34 in multispecific sites, see Table S2 for exact total observation time, total feeding time and time over which bites were counted for each individual).

In the multispecific bays (Grande Anse and Anse du Bourg/Anse Chaudière), to investigate food selection of immature green turtles at the population level, we used the compositional analysis similarly to foraging habitat selection ($n = 34$). Except for the category Other, for which availability was unknown, we compared the proportion of bites on each resource to their availability at the site scale based on the composition maps of the corresponding year. At the individual level, resource use was also tested using the Manly's selection ratio w_i (Manly et al., 2002), with the 'widesIII' function of the adehabitatHS R package (Calenge, 2020). For each individual, this index was calculated as $w_i = \frac{o_i}{\pi_i}$, where o_i is the proportion of bites on the i item and π_i the proportion of i in the study site. The index gives values between zero and ∞ . Values equal to 1 indicate random feeding, values under 1 indicate negative selection, or avoidance, and values above 1 indicate positive selection, or preference.

2.6. Nutritional composition and digestibility of native and invasive plants

2.6.1. Plant sampling and processing

Between 2015 and 2020, we collected macroalgae (see 2.2 for species details) and seagrass species (leaves of *T. testudinum*, *S. filiforme* and leaves and rhizomes of *H. stipulacea*, as its rhizomes are easily accessible for feeding) at several sampling points in Grande Anse and Anse Noire to assess nutritional composition. *S. filiforme* and algae being rarer, fewer samples were collected to avoid affecting their dynamics. Once collected, samples were rinsed three times in fresh water to remove debris, invertebrates, epiphytes and excess salt. Excess water was removed using a salad spinner and a clean cloth, and the fresh weight (FW) of each sample was determined (± 0.1 g). Samples were dried in an oven at 50 °C for 48 h and packed in airtight bags for transport to the laboratory. Samples were then freeze-dried until constant mass, i.e. successive weighing did not differ by more than 0.1%, to determine the dry weight (DW). Dried samples were ground to pass through a 1 mm grid in a Retsch ultra-centrifugal mill (model ZM200), in the same

way as in Moran and Bjorndal (2007).

2.6.2. Nutritional composition and digestibility determination

Samples were freeze-dried again before analyses to eliminate any traces of water that might have appeared during grinding. They were analysed in duplicate except when their mass was too low. For analyses, samples were weighed to the nearest 0.001 g:

- a) Energy content was determined on 1 g pellets using a Parr 6200 calorimeter (Parr Instrument Company) calibrated with benzoic acid.
- b) Total mineral content: 1 g samples were burned at 500 °C for 24 h and reweighed to quantify remaining ashes.
- c) Carbon (C) and nitrogen (N) content: we used a Flash (2000 or EA 1112) elemental analyser (Thermo Fisher Scientific), which performed the dynamic flash combustion method (derived from Dumas method, see Dumas, 1831), followed by gas detection using a thermal conductivity detector. These measurements were translated into %C and %N in the sample using Eager software, which allowed the calculation of C:N ratios.

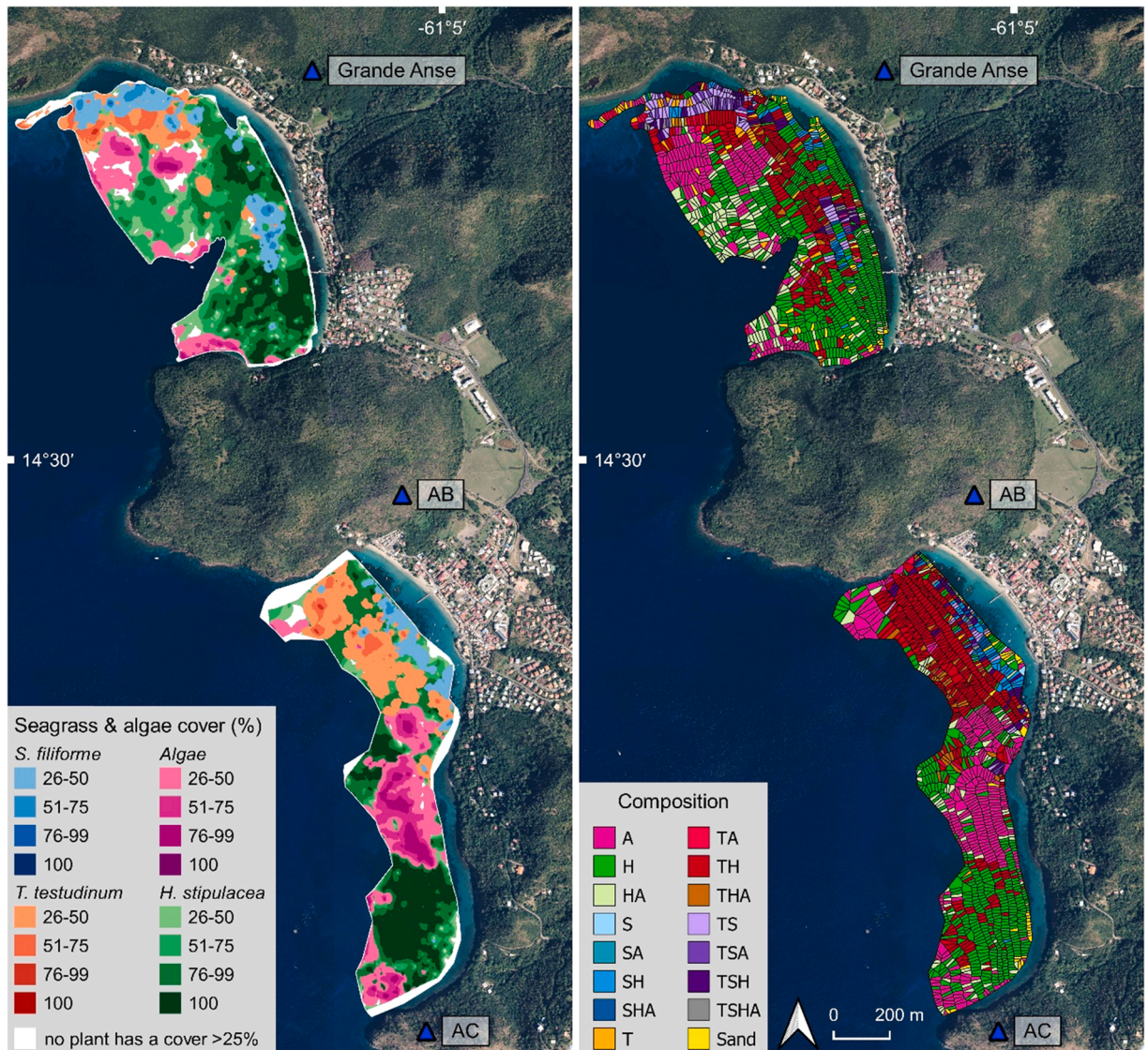


Fig. 1. Benthic composition maps showing: macroalgae and seagrass species cover (left) and plant communities (right) of Grande Anse and Anse du Bourg (AB)/Anse Chaudière (AC) from surveys conducted in 2018. In the left panel, plants are superimposed in order: *H. stipulacea* in background, *T. testudinum*, Algae, and *S. filiforme* in foreground. In the right panel, the Algae (A), and seagrasses *T. testudinum* (T), *S. filiforme* (S), *H. stipulacea* (H) and their combinations form fifteen distinct plant communities.

- d) Soluble carbohydrates content: soluble carbohydrates (polysaccharides) were measured on a continuous flow chain, using the neocuproin method, adapted from [Brown \(1961\)](#). The method consists in extracting the water-soluble sugars from the plant tissues and then hydrolysing them with an acid to obtain simple carbohydrates. Monosaccharides' reducing power is then used to reduce, after heating, a cupric-neocuproin chelate into a red copper-neocuproin complex, which absorbs at 460 nm. The determination of reducing sugars is carried out using an auto-analyser and a glucose standard curve, allowing the results to be expressed in glucose equivalent.
- e) Fibre content: the Van Soest sequential fibre solubilisation method, adapted from [Goering and Van Soest \(1970\)](#), classifies the cell wall components of a plant into three types of insoluble residues: neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) residues. Neutral soluble detergent (NDS) added with amylase (which allows starch to be degraded) first allows separating cytoplasmic compounds from all insoluble fibres (NDF). After filtration, soluble acid detergent (ADS) helps extracting soluble fibres (ADF), before sulphuric acid is used to retain ADL only. After each of the three phases, the fractions are passed through a desiccator and then weighed. Until the last stage, mineral matter is present in all three fractions NDF, ADF and ADL. A mineralisation stage at 500 °C thus allows the proportion of ash to be determined in relation to the proportion of lignin. Once the ash-free ADL is expressed as a percentage of dry matter, the quantity of the three types of fibre can be determined as follows: Lignin = (ash-free) ADL, Cellulose = ADF – ADL, Hemicellulose = NDF – ADF.
- f) In vitro enzymatic digestibility: based on a method adapted from [Aufère et al. \(2007\)](#), we estimated the potential digestibility of plant samples through the action of enzymes: pepsin, cellulase, amyloglucosidase. These enzymes were chosen to replace the ruminant rumen fluid originally used to measure organic matter fermentability in vertebrates (see [Moran and Bjørndal, 2007](#)). The samples were first incubated at 40 °C with pepsin (a gastric enzyme whose role is to break down proteins in the food bolus). The starch was then gelatinised at 80 °C before being degraded by amyloglucosidase during a second incubation. This led to the release of glucose molecules. Meanwhile, cellulose was degraded by cellulase. Finally, solubilised fractions were incubated at 70 °C for 48 h and then, weighed. The proportion of solubilised plant material represents the fraction degraded by the enzymes.

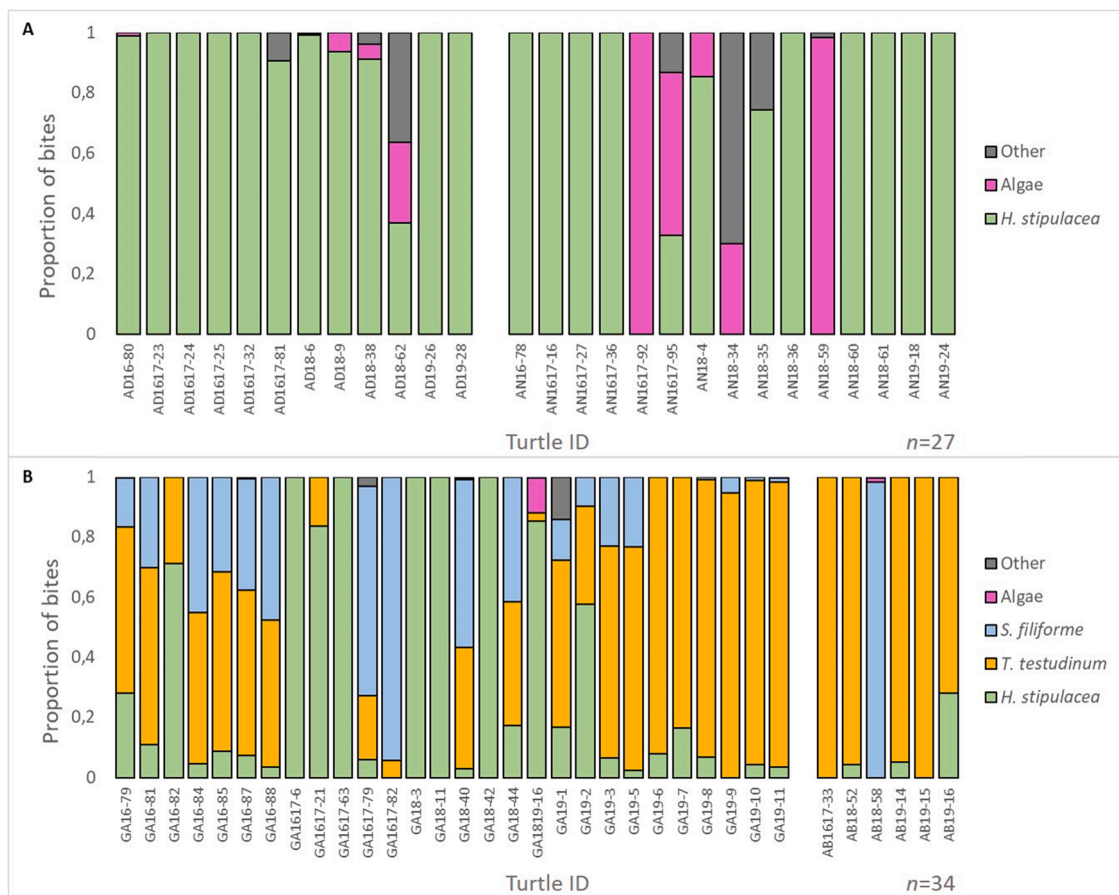


Fig. 2. Green turtle diet in the monospecific sites of Anse Noire (AN) and Anse Dufour (AD) (panel A) with *H. stipulacea*, Algae, and Other (non-flora items) as food resources and in the multispecific sites of Grande Anse (GA) and Anse du Bourg/Anse Chaudière (AB) (panel B), with *H. stipulacea*, *T. testudinum*, *S. filiforme*, Algae, and Other as food resources. Bar charts show the proportion of bites taken on each food resource by each turtle monitored. Turtle AB18–58 consumed *H. stipulacea* in proportion too small to be visible on the graph.

Differences in plant nutritional content and digestibility between groups were analysed with R software (R Core Team, 2021) using Student's t tests and Kruskal-Wallis tests to consider non-normality of some variables. When a significant difference was raised when comparing more than two groups, we performed pairwise Mann-Whitney-Wilcoxon tests. P-values for multiple tests were corrected using Holm's method (Holm, 1979) from rstatix package (Kassambara, 2021). We used alpha values set at 0.05. Results are presented

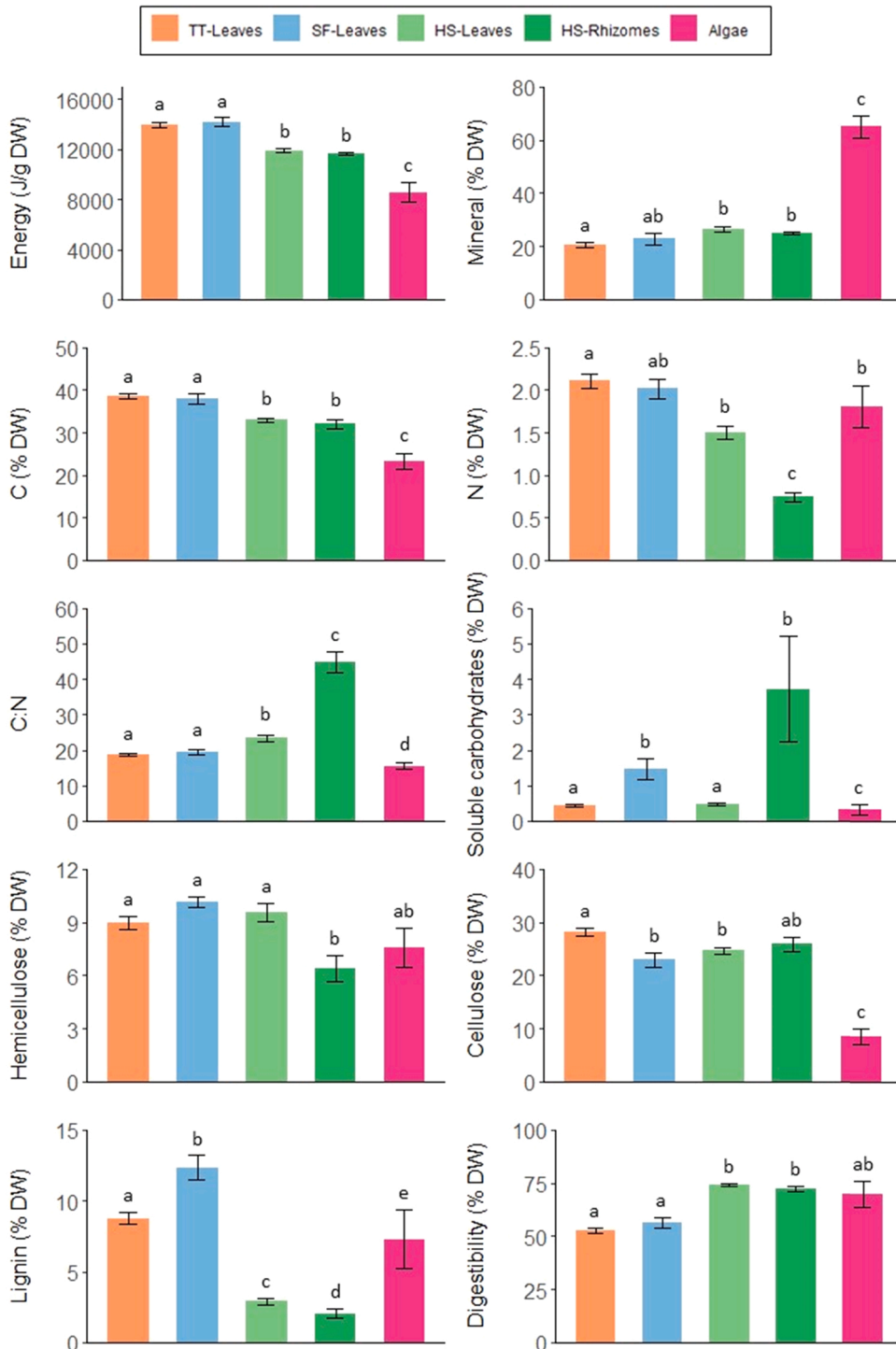


Fig. 3. Comparison of nutritional composition (mean ± SE) expressed on a dry weight (DW) basis, of seagrasses (TT: *T. testudinum*; SF: *S. filiforme*, HS: *H. stipulacea*) and Algae. Refer to Table S5 for exact values and sample sizes. Kruskal-Wallis tests evidenced significant differences in each case (p < 0.001). Groups with the same letter on top are not significantly different (based on multiple pairwise-comparisons).

as means \pm standard errors (SE).

3. Results

3.1. Benthic composition maps of the multispecific bays

Marine plants covered 89% of Grande Anse area in 2016, and 84% in 2018, while they covered 100% of Anse du Bourg/Anse Chaudière in 2016, and 91% in 2018. From 2016–2018, we found only small positive or negative variations in terms of coverage of the total area by each plant, ranging from 0.4% to 7.5%. Thus, in 2018, *H. stipulacea* covered 56% and 59%, *T. testudinum* 12% and 12%, *S. filiforme* 7% and 3%, and Algae 9% and 16%, of the total area of Grande Anse and Anse du Bourg/Anse Chaudière, respectively (Fig. 1).

3.2. Foraging habitat selection

At the population level, in the multispecific bays (i.e. Grande Anse and Anse du Bourg/Anse Chaudière), the random habitat use hypothesis was rejected ($\lambda = 0.2305$, $\chi^2 = 90.98$, $p < 0.0001$), meaning that turtles favoured some habitats regardless of habitat availability. The pairwise t-tests and the ranking matrix (Table S3) showed that habitats with at least one native species (M) were significantly preferred over habitats composed of *H. stipulacea* or/and algae (H, A, HA). There was no detectable difference in the selection of habitats H and HA, while A was the least selected habitat of the four habitats.

3.3. Diet composition and food selection

The time over which bites considered in the diet assessment were counted was approximately 30 min per individual (Table S2). With 61 individuals retained in the analyses, this equates to over 30 h of feeding sequences.

In sites with only monospecific stands of *H. stipulacea* (i.e. Anse Noire and Anse Dufour), 24 of the 27 monitored individuals foraged on *H. stipulacea* (Fig. 2A). That was overall the most represented item in the diet. In sites with multispecific seagrass meadows (i.e. Grande Anse and Anse du Bourg/Anse Chaudière), 30 of the 34 monitored individuals foraged on *H. stipulacea* (Fig. 2B), with high variability among individuals. Native seagrasses were the most represented in the diet. Algae and Other items were very rare.

At the population level, in the multispecific bays (i.e. Grande Anse and Anse du Bourg/Anse Chaudière), the random food use hypothesis was rejected ($\lambda = 0.1766$, $\chi^2 = 58.99$, $p < 0.0001$), with *T. testudinum* significantly preferred over *S. filiforme*, *H. stipulacea* and Algae, the latter being the least selected. At the individual level, about 47% of the turtles positively selected more than one food resource (Table S4). *T. testudinum* and *S. filiforme* were selected positively by 76% and 44% of the monitored turtles, respectively, while *H. stipulacea* and Algae were positively selected by 24% and 3% of them, respectively.

3.4. Comparison of nutritional composition and digestibility between native and invasive plants

Results are expressed on a dry weight basis (refer to Fig. 3, Table S5). Native seagrasses had a similar energetic value ($14\,004 \pm 211$ J/g DW for *T. testudinum* and $14\,204 \pm 391$ J/g DW for *S. filiforme*). There was no significant difference in C, N and hemicellulose between them. However, cellulose content was higher, and conversely lignin content lower, in *T. testudinum* than *S. filiforme* (5.2 points higher and 3.6 points lower, respectively). Compared to *T. testudinum* and *S. filiforme*, the invasive species *H. stipulacea* had a lower energy content (approximately 2 330 J/g DW lower than *T. testudinum* and *S. filiforme*), with lower C contents but higher C:N ratio. *H. stipulacea* rhizomes had also 3 times lower N content and 2 times higher C:N ratio than *T. testudinum*. The invasive species contained up to 6 times less lignin than *T. testudinum* and *S. filiforme*. Focusing on *H. stipulacea*, rhizomes had lower N, hemicellulose, and lignin content than leaves (0.8, 3.2, and 0.9 points lower, respectively). There was no significant difference in energy, mineral, C, nor in cellulose content between leaves and rhizomes. However, rhizomes had eight times more soluble carbohydrates and a C:N ratio twice as high as in leaves. The nutritional composition of Algae showed higher variability than seagrasses because of the inclusion of several species. Compared to seagrasses, Algae contained notably more minerals (about 40 points higher), less cellulose (14–20 points lower) and had the lowest C/N ratio.

Water content (refer to Fig. S3, Table S5) was similar in *S. filiforme* and *H. stipulacea* (around 90%). It was lower in *T. testudinum* and Algae (around 87% and 75% FW, respectively). Since the hydrated samples are more representative of the food actually ingested by green turtles, results expressed in fresh weight are also given in the Supplementary material (Fig. S3, Table S5). For example, despite leaves of *T. testudinum* and *S. filiforme* had a similar energy content when expressed on a dry weight basis, it was 1.4 times higher in the former than in the latter when expressed on a fresh weight basis because of the difference in water content.

Regarding digestibility (refer to Fig. 3, Table S5), *T. testudinum* and *S. filiforme* showed similar values for this parameter ($52.9 \pm 1.3\%$ DW for *T. testudinum* and $56.5 \pm 2.5\%$ DW for *S. filiforme*). However, *H. stipulacea* appeared to be more digestible than both native seagrasses (approx. 20 points higher), and digestibility was similar in leaves and rhizomes of the invasive species ($74.3 \pm 0.7\%$ DW for leaves and $72.2 \pm 1.4\%$ DW for rhizomes). Algae had an intermediate digestibility ($69.9 \pm 6.2\%$ DW) with values between those of native and invasive seagrasses.

4. Discussion

4.1. Resource selection in immature green turtles

Foraging habitat selection analyses showed that immature green turtles favoured habitats with at least one native species. Moreover, they preferentially selected *T. testudinum* in their diet when available (Fig. 2B). These results are consistent with other studies conducted in the Caribbean. Christianen et al. (2018) showed that green turtles selected *T. testudinum* when presented with two other species (*S. filiforme* and *H. stipulacea*) in Bonaire. They also highlighted a change in grazing locations to shallower areas where native seagrass species were still predominant. In Malendure Bay (Guadeloupe), where *T. testudinum* was rarely found in close proximity to foraging green turtles, Whitman et al. (2019) highlighted turtle preference for *S. filiforme* in multispecies seagrass beds. Thus, in these studies, green turtles were still consuming native species about six years after the introduction of *H. stipulacea* and were mainly avoiding the invasive species. Nevertheless, similar to Christianen et al. (2018) and Whitman et al. (2019), we found high inter-individual variability in diet composition, with *H. stipulacea* accounting for 0–100% of the diet, regardless of native plant availability. In multispecies sites, we showed positive selection for *H. stipulacea* in 24% of individuals monitored. However, for the first time, we investigated green turtle food selection at a site where *H. stipulacea* completely replaced native seagrass species. At Anse Noire and Anse Dufour, we observed that the majority of green turtles fed on *H. stipulacea* while some ate Algae (Fig. 2A). In addition, a previous study highlighted the high fidelity of green turtles to their capture site in Martinique (Siegwalt et al., 2020). This suggests that individuals observed at Anse Noire and Anse Dufour may spend several years growing in these bays and do not change bays to feed. Therefore, this population appears acclimatised to environmental change and has integrated *H. stipulacea* into its diet.

Selectivity for food resources may be influenced by several factors, such as forage relative availability (López-Mendilaharsu et al., 2008), nitrogen and phosphorus content (Bjorndal, 1980), and energy content (Arthur and Balazs, 2008). On a dry basis, compared to native seagrasses, *H. stipulacea* had globally lower energy, C, and N content (Fig. 3). Similarly Christianen et al. (2018) found lower N content and higher C:N ratio for *H. stipulacea* compared to *T. testudinum* and *S. filiforme*. It is likely that the strong preference for native seagrasses, and in particular for *T. testudinum*, could be related to their nutritional benefits. However, Whitman et al. (2019) found no significant differences in these nutritional parameters between *H. stipulacea* and *S. filiforme* at Malendure Bay. To explain the similar nutritional composition of both plants and their high C and N values, Whitman et al. (2019) hypothesised that their feeding area was certainly a very nutrient rich environment. We also expressed the nutritional composition on a fresh basis as it is more representative of the food actually ingested by turtles, and consequently of their foraging effort to meet energetic needs. On this basis, *T. testudinum* had higher energy and N content than the other seagrasses (Table S5, Fig. S3), which could explain the frequent positive selection for this species, especially in this context of growing juvenile turtles. Nevertheless, at Grande Anse and Anse du Bourg/Anse Chaudière, we observed that some individuals fed on *H. stipulacea* (Fig. 2B), while native species were available (Fig. 1). If these results can be extrapolated over the long term, this could indicate an advantage to feeding on *H. stipulacea*, first, in terms of energy balance (perhaps related to higher digestibility; or higher ingestion rate, at the expense of longer feeding time), and second, in terms of intraspecific competition (which could be lowered since *H. stipulacea* is abundant in the environment). Moreover, at Anse Noire/Anse Dufour where native seagrasses were no longer present, the majority of the monitored green turtles foraged exclusively on *H. stipulacea* (Fig. 2A). Nevertheless, due to its lower nutritional value, the consequences of the massive inclusion of *H. stipulacea* in the diet of green turtles on their growth and survival need to be assessed.

Fibre content and digestibility are two other important parameters of plant nutritional value that can influence food selectivity of sea turtles (Bjorndal, 1980; Brand-Gardner et al., 1999). Interestingly, we found that *H. stipulacea* was more digestible and had up to 6 times less lignin content than the native species (Fig. 3, Table S5). For *T. testudinum* leaves, we found an average digestibility (53% DW) higher than fermentability values previously reported (25–34% DW, Moran and Bjorndal, 2007) (the enzymes we selected being potentially more stable than the rumen juice formerly used for this type of analysis), but consistent with Bjorndal's (1980) apparent organic matter digestibility values. Regarding fibre content, Moran and Bjorndal (2007) found somewhat lower results for *T. testudinum* blades (ligno-cellulose fraction: 25–31% DW), with lower lignin contents than in our study (about 2% DW in their study and 9% DW in ours), while Bjorndal (1980) and Vicente et al. (1980) lignin values accord with ours. According to Moran and Bjorndal (2007), the variability in lignin content may be due to the current flow characteristics of each study site. Yet, while lignin has a higher energy density than cellulose and hemicellulose (between 21 and 27 kJ/g DW, compared to 17 kJ/g DW for cellulose and hemicellulose, see Agrawal et al., 2014; Frei, 2013; Murphy and Masters, 1978; Welker et al., 2015), the low digestibility that lignin confers on food (due to its own low digestibility and because it prevents digestive enzymes from breaking down cellulose and hemicellulose completely by complexing with these fibres) precludes it from being an efficient nutrient (Moore and Jung, 2001; Van Soest, 1982). Therefore, although *H. stipulacea* showed lower energy content than the native species through nutritional analysis (Fig. 3), its low lignin content and higher digestibility calls into question the measured energetic advantage of native plants over the exotic one. Nevertheless, the digestibility we measured in vitro cannot account for all chemical reactions and biological interactions involved in digestion at the whole-body level. In particular, the gut microbiome of green turtles evolves during their growth and notably at their recruitment to the neritic zone with the acquisition of bacteria involved in plant digestion (Campos et al., 2018; Price et al., 2017). Bjorndal (1980) showed that in vivo digestibility of hemicellulose and protein increased significantly with turtle's body size, while Campos et al. (2018) highlighted an increase in the taxonomic richness of the gut microbiome as green turtle grow. *H. stipulacea* may therefore be of particular nutritional value for small turtles that have not yet fully adapted their gut microflora to herbivory, but this may diminish for larger individuals. Further research on overall digestibility is needed to accurately test for a difference between native and invasive species considering the body size of individuals.

Les Anses d'Arlet are considered a critical foraging and developmental habitat for immature green turtles, which spend many years

growing until they reach sexual maturity and eventually migrate (Bonola et al., 2019; Chambault et al., 2018; Siegwalt et al., 2020). Due to the lower nutritional value of *H. stipulacea*, inclusion of this seagrass in turtle's diet may have important consequences for the population. Moreover, nitrogen and energy contents of plants have been linked to turtle growth rate and reproductive parameters (Bjørndal, 1980; Wood and Wood, 1981). In Bermuda, Gulick et al. (2021) observed a decline in body mass of growing green turtles related to a decrease in available resources, while Bjørndal et al. (2017) showed a decline in growth rates in three sea turtle species throughout the West Atlantic, linked to an ecological regime shift and probably to an associated decrease in quantity and quality of food resources. Here, we showed that immature green turtles preferentially foraged on the native species when possible, but in bays fully invaded by *H. stipulacea* as in Anse Noire/Anse Dufour, green turtles foraged actually on *H. stipulacea* (Fig. 2A). Therefore, for this population, we can expect that a shift from native plants to lower nitrogen and energy plants may reduce turtle's growth rate and delay their sexual maturity. In addition, a previous study showed that residence time in Anse Noire was lower than in Grande Anse (Siegwalt et al., 2020). It has been showed that developmental migrations may be triggered by nutrient limitation and/or reduced growth rates (Bjørndal et al., 2000, 2019; Chaloupka and Limpus, 2001). Thus, we can hypothesized that lower nutrient quality of *H. stipulacea* and potentially sub-optimal growth experienced by immature green turtles in Anse Noire/Anse Dufour may force them to move to other more profitable foraging areas. The capture-mark-recapture studies currently conducted in Martinique will allow long-term comparison of growth rate between these multi- and monospecific bays.

The rapid expansion of *H. stipulacea* in Les Anses d'Arlet have modified the foraging ground of the immature green turtles, which have thus adopted different energetic strategies. Indeed, at Grande Anse, we found a high inter-individual variability in diet composition, with immature green turtles that preferentially foraged for the native species, but also individuals that fed mainly on *H. stipulacea* while native species were available (Fig. 2B). It would be interesting to assess whether these individuals feed on *H. stipulacea* occasionally or permanently. Indeed, forager fitness and diet breadth may be affected by increased intraspecific competition for food resources (Svanbäck and Bolnick, 2005). While most turtles selected native plants, whose coverage represented < 20% in both multispecific sites (Fig. 1, Fig. 2B), individuals integrating *H. stipulacea* in their diet or specialising on it may benefit from a lower inter-individual competition for resources. Finally, we can also expect changes in green turtle behaviour when integrating the invasive species into their diet. Thus, turtles may modify their activity budget to adapt to lower energy acquisition by decreasing the time allocated to energy-demanding activities and increasing rest time, or alternatively by increasing the proportion of time devoted to feeding, and thus, the amount of material consumed to meet energy requirements. It would be interesting to compare the activity budget of individuals between Grande Anse and Anse Noire/Anse Dufour to identify potential different energetic strategies used by green turtles to adapt to changes in their environment.

In this study, we used bite counts from direct observations to estimate diet composition and food selection of immature green turtles at the individual level. Direct observation has the advantage of being non-invasive and causing little disturbance, but it is still difficult to monitor free-ranging green turtles over the long term as they move quickly and change environments often. This leads to observation times depending on the goodwill of the green turtles. In this study, we performed behavioural observations during around 100 min per follow, and recorded feeding behaviours over an average of 52 min (with standard deviation of 55 min, Table S2). Thus, this large variability in the recorded duration of feeding activity may result in a bias in diet composition estimation at population level. In addition to the difficulty of monitoring sea turtles, this variability is also due to a high inter-individual variability in the expressed behaviours, with some individuals feeding very little and others feeding during most of the day (Rice et al., 2000, pers. obs.). In addition, the direct observations represented a short period of the turtle's daily time and we cannot exclude that other items were ingested during the rest of the day. Therefore, these limitations highlight the complexity of studying green turtle foraging strategy while direct observation remains an essential first step in identifying the inter-individual variability in diet composition and feeding strategies with minimal disturbance. Thus, although the total time used in our study for the assessment of the diet (approx. 30 h) was quite high compared to the literature (e.g. approx. 8 h in Whitman et al., 2019), further research is needed to improve the precision of these results. Oesophageal content analysis (see in Gulick et al., 2021), is another complementary method that could be implemented to obtain the precise amount of food ingested. However, this method is much more invasive than direct observation and requires capture of the turtles and oesophageal lavage. Stomach and oesophageal contents can also be collected during necropsy, but the body of the dead turtle must not be decomposed, which is rarely the case in Martinique. Finally, the development of on-board cameras with an increasing autonomy represents a promising new method to follow individuals over a longer period of time and thus to go further in the study of feeding strategy of immature green turtles.

4.2. Expansion of *H. stipulacea* in Martinique and conservation implications

Benthic composition maps highlighted the dominance of *H. stipulacea* in Les Anses d'Arlet, with a cover > 55% in Grande Anse and Anse du Bourg/Anse Chaudière in 2018, while it was the only remaining seagrass species in Anse Noire and Anse Dufour. *H. stipulacea* was first recorded in Martinique in 2006 and spread from North to South (Ortolé, 2012). Its exact time of arrival in Les Anses d'Arlet is unclear, but its presence was reported in Anse Noire in 2011 (DEAL Guadeloupe et Martinique, 2011). This fast expansion in Martinique has been observed on other neighbouring islands and is characteristic of the Caribbean (Willette et al., 2014; Winters et al., 2020).

The introduction and expansion of *H. stipulacea* appears primarily due to anthropogenic factors. While pleasure yachts probably carried *H. stipulacea* from the Mediterranean to the Caribbean, inter-island vessel transit and fishing activities also contributed to its expansion throughout the region by creating vacant spaces for it, and by generating and dispersing fragments (Ruiz and Ballantine, 2004; Willette et al., 2014; Willette and Ambrose, 2012). Some of our study sites where *H. stipulacea* was found in monospecific beds in 2018 (Fig. 1) seem to have suffered from bottom-scraping anchors and chains. While the Southern area of Grande Anse is a past

authorised anchorage zone (Sermage, 2006), Anse Chaudière still experiences heavy boat traffic (pers. obs.). Indeed, *H. stipulacea* has high ability to colonise bare sand, especially where conditions are not favourable for native species (Winters et al., 2020). In sites with multispecific seagrass meadows, its expansion may have been more difficult and some areas remain dominated by native species (Fig. 1). Areas where native meadows were certainly dense and less subject to mechanical disturbances (Sermage, 2006) have been preserved from total invasion by *H. stipulacea*.

Today, it seems urgent to limit the pressures of anthropic origin on the native meadows in order to slow down the expansion of *H. stipulacea*. Thus, prohibiting anchorages and setting up a defined mooring zone (with multiple floating buoys connected to cement blocks by ropes) outside the zones that include native meadows could be a solution. An exhaustive survey at the scale of Martinique, both on the progression of *H. stipulacea* and on the factors likely to favour its expansion (absence of anchorage area, high boat traffic, pollution of anthropic origin) should be conducted annually to take preventive and curative measures to limit its development.

Furthermore, the effects of climate change on *H. stipulacea* expansion are still very little studied. A recent study showed different responses to sea warming among populations (Nguyen et al., 2020). Increasing sea temperature would reduce *H. stipulacea* meadows in its native regions while facilitating its spread in the Mediterranean Sea, a region invaded 150 years ago. It is therefore difficult to predict the future range expansion of *H. stipulacea* in the Caribbean. Species distribution models could help predict the potential range expansion of the invasive species but require accurate data on the current distribution and environment. Consequently, rigorous monitoring of *H. stipulacea*, not only in Martinique but also in the whole Caribbean, should be implemented. Improving knowledge of seagrass is an essential prerequisite to ensure their preservation. This may facilitate a proper growth and adaptation of immature green turtles to changing environments and promote the protection of this threatened species. In turn, this will benefit the entire seagrass ecosystem.

5. Conclusion

This study provides a precise assessment of the cover of *H. stipulacea* in an area of South-West Martinique, which is a hotspot of immature green turtles. For the first time, their diet was investigated at sites where native plants were still present, but also at sites where the invasive species had completely replaced native seagrasses. We show that, although green turtles preferentially chose native seagrasses when available, they included *H. stipulacea* in their diet at sites completely or partially covered by this species. We also show that *H. stipulacea* was less energetic but more digestible than native plants. In this context, new questions about the impacts of this dietary change on the growth and survival of immature green turtles may be raised. Our study highlights the importance of in-depth dietary analysis (composition, energetics, digestibility, etc.) in ecosystems where resource availability may vary, to gain a broader picture of the causes and consequences of food choices for endangered species like the green turtle.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Damien Chevallier reports financial support was provided by CNRS. Damien Chevallier reports a relationship with National Centre for Scientific Research that includes: employment. n.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.gecco.2022.e02169](https://doi.org/10.1016/j.gecco.2022.e02169).

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