Proximate response of fish, conch, and sea turtles to the presence of the invasive seagrass *Halophila stipulacea* in Bonaire

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Summary

In this report we examined the proximate response of fish assemblages, queen conch, and sea turtles on *H. stipulacea* meadows in Lac Bay, Bonaire, Caribbean Netherlands. Here we primarily focused on the differences between the invasive species *H. stipulacea* and the principal species of native sea grass in Lac Bay, namely turtle grass *Thalassia testudinum* .

We addressed the following questions:

- Has *H. stipulacea* expanded in area since 2011 and what effect is observed on *T. testudinum* cover?
- How does the structural complexity, in terms of average height and density of vegetation, differ between meadows dominated by *T. testudinum* and those by *H. stipulacea*?
- Do monospecific fields of *H. stipulacea* differ from monospecific fields of native *T. testudinum* in terms of fish assemblages and abundances?
- Does the queen conch avoid *H. stipulacea* meadows?
- Will green sea turtles in Lac Bay graze on *H. stipulacea*?

In 2011 *H. stipulacea* was present in 7 of the 45 stations across the bay where the seagrass cover was recorded, while this increased to presence in 12 quadrats in the year 2013. From 2011 compared to 2013 the cover of *H. stipulacea* had generally increased, while the cover of the native *T. testudinum* had generally decreased in these quadrats. These results indicate that the invasive species is expanding in area in Lac Bay. It is unclear whether *H. stipulacea* is actively pushing out the native seagrass species, or whether the native seagrass cover is declining due to other causes and *H. stipulacea* is rapidly taking over areas that are left open.

The two species of seagrass differed significantly in habitat complexity, reflected by a difference in the number of shoots and the length of shoots. *H. stipulacea* had significantly shorter shoots compared to *T. testudinum.* Higher habitat complexity due to the seagrass canopy (i.e. higher seagrass density, leaf surface or aboveground biomass) is assumed to result in higher faunal abundance due to reduced predation risk and enhanced food supply. Invasive macrophytes can impose changes on native communities via mechanisms that modify the habitat and cause variation in indigenous faunal composition.

There was a large and significant difference in fish abundance between meadows dominated by *T. testudiunum* and those dominated by *H. stipulacea;* the abundance of fish was almost half in the *H. stipulacea* meadows. This result may be due to the reduced complexity of the invasive seagrass meadows, but also be due to underlying factors that were not measured. There was also a significant difference in the composition of fish species assemblage between *T. testudiunum* and *H. stipulacea* meadows. No Pomacentridae, Mullidae, and Sphyraenidae were recorded in the transects placed in *H. stipulacea* meadows, while these were present in *T. testudinum* meadows. If this is a long-term trend, the expansion in *H. stipulacea* may possibly result in a diminished nursery function of certain fish species in Lac Bay.

Queen conch was equally present on *H. stipulacea*, native seagrass meadows, and sandy patches. Hence, in the area of observation of the present study, it did not appear to avoid the invasive seagrass. These results only refer to part of Lac Bay, therefore we are cautious with making general conclusions.

We establish that Caribbean green turtles can feed on the invasive *H. stipulacea*. This was documented using a cafeteria experimental set-up. All three seagrass species were selected at least once.

Thus the present study indicates that the green sea turtle does not necessarily avoid *H. stipulacea* as a food source. While *H. stipulacea* is new to the Caribbean, *H. stipulacea* forms an important food species in its native distributional range for the green turtle in the Red Sea. *H. stipulacea* is increasing in cover in Lac Bay and may become the main food source for green sea turtles. What is more, when sea turtles consume seagrass the associated invertebrates are an important component of the diet. Invertebrates that are associated with seagrass differ per seagrass species (e.g. Willete & Ambrose 2012) and this could result in a different nutritional uptake for the turtles. It is important to understand the nutritional difference this shift in diet may cause, before it can be concluded what the effect of long term consumption of *H. stipulacea* on green turtle health may be.

1. Introduction

Lac Bay is a clear-water shallow tropical lagoon on the east coast of the island Bonaire, Caribbean Netherlands. The bay has been declared a RAMSAR site and it is a nursery site for fish, habitat for conch and forage area for sea turtles. This ecological importance of Lac Bay is carried predominantly by the mangrove and seagrass ecosystem in the lagoon, which contains the largest seagrass beds of the island and of the Caribbean Netherlands. At present the native seagrass species are threatened by a rapid expansion of the invasive seagrass *Halophila stipulacea* (Forsskål 1775) (Engel 2008, Debrot et al. 2012). *H. stipulacea* was first recorded in Lac Bay in 2010. In this study we examined the expansion of *H. stipulacea* since 2011 and the proximate response of conch, fish assemblages and sea turtle grazing on *H. stipulacea* meadows in Lac Bay.

1.1 Invasion of *H. stipulacea* in the Caribbean

Native to the Red Sea and western Indian Ocean, *H. stipulacea* spread to the Mediterranean Sea in the late 1800s and became established in the eastern Caribbean in 2002 (Ruiz and Ballantine 2004). The species has dispersed north and south of its first sighting in Grenada (Willette et al. 2014), most likely facilitated by a combination of commercial and recreational boat traffic. The continuing range expansion of *H. stipulacea* (Fig. 1) indicates the species has successfully acclimated to the Caribbean environment, which warrants further investigation into its ecological interactions with the indigenous seagrasses (Willette et al. 2014). The indigenous seagrass species of Bonaire are *Thalassia testudinum* and *Syringodium filiforme, Halodule beaudettei* and *Ruppia maritima* (Wagenaar Hummelinck & Roos 1969, Engel 2008, 2013).

H. stipulacea forms monocultures as well as multispecies assemblages throughout its native and extended range (Ruiz and Ballantine 2004, Short et al. 2007). The combination of aggressive growth of *H. stipulacea* (Willette and Ambrose, 2009) along with its potential capacity to disperse both short-range via fragments created by e.g. fish traps, grazing, waves, currents, via ship traffic (e.g. Ruiz and Ballantine, 2004; Willette et al. 2014) put this invasive species in a position to alter the seagrass communities throughout the region. *H. stipulacea* competes with the native Caribbean seagrass *S. filiforme*, *T. testudinum* and *Halodule beaudettei* for space and has been shown to alter the abundance and composition of seagrass-associated organisms in addition to the local seagrass community (Willette and Ambrose 2012, Engel 2013); however, further investigation of this interaction is needed. Here we primarily focus on the differences between *H. stipulacea* and the native turtle grass *Thalassia testudinum,* which is dominant seagrass species in Lac Bay, Bonaire.

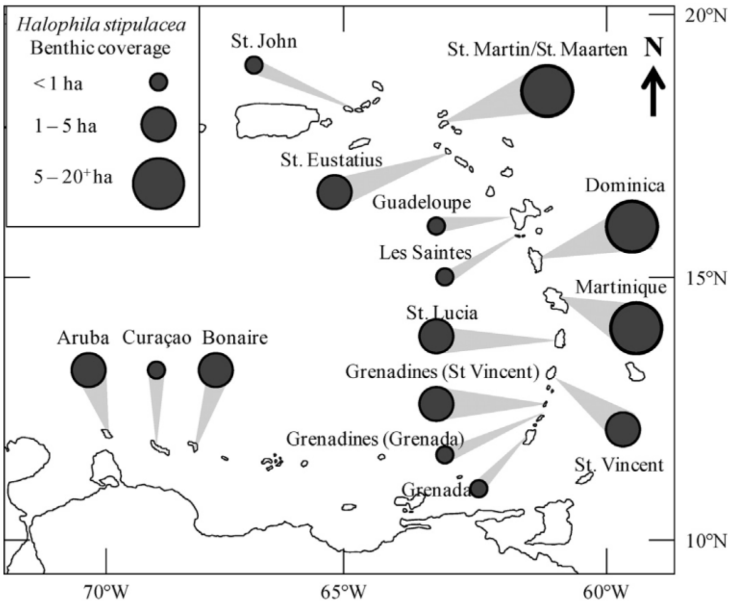


Figure 1. Geographic distribution of invasive Halophila stipulacea in the Caribbean with annotation of estimated benthic cover (ha) (taken from Willette et al. 2014).

1.2 Questions

- Has *H. stipulacea* expanded in area since 2011 and what effect is observed on *T. testudinum* cover?
- How does the structural complexity, in terms of average height and density of vegetation, differ between meadows dominated by *T. testudinum* and those by *H. stipulacea*?
- Do monocultures of *H. stipulacea* differ from monocultures of native *T. testudinum* in terms of fish species assemblages and abundances?
- Does the queen conch avoid *H. stipulacea* meadows?
- Will green sea turtles in Lac Bay graze on *H. stipulacea*?

1.3 Collaboration with institutes outside of IMARES

Tineke van Bussel from the Vrije Universiteit Amsterdam and the Sea Turtle Conservation Bonaire conducted the fieldwork and aided in the experimental design, and writing of the report. Sabine Engel did the thought out the experimental design, conducted the fieldwork and obtained the data on *H. stipulacea* expansion in Lac Bay. Marjolijn Christianen from the Rijksuniversiteit Groningen aided in the experimental design, analyses, and writing of the report.

2. Materials and Methods

All data related to expansion of *H.stipulacea* between the years of 2011 and 2013 was collected by Sabine Engel; fieldwork conducted in March-May 2011 and in March-April 2013. All data on fish composition, conch preference and sea turtle cafeteria experiment was collected by Tineke van Bussel in Lac Bay, Bonaire, between November 20 – December 18, 2013. Fluctuations in environmental conditions were minimal within this experimental period: (http://www.weather-and-climate.com/average-monthly-Rainfall-Temperature-Sunshine,Bonaire,Bonaire).

2.1 *H. stipulacea* expansion since 2011

Previous studies (Wagenaar Hummelinck and Roos 1969; Lott 2001 and Engel 2008) have shown that only the area near the mangroves and the shallow part at the inside of the fringing reef showed some level of uniformity regarding the benthic assemblages. The main, central part of Lac Bay is a mosaic of sand, and different algal and seagrass compositions. For this reason we have opted for a large number of stations equi-distanced throughout Lac. The shortest distance between two stations is 290 m. Using Google Earth, Garmin Basecamp and a handheld GPS (using Garmin eTrex-H or Garmin eTrex 10. Accuracy of handhelds in field is adequate for horizontal position with accuracy often of less than 5 m) a grid system with 48 stations has been set up in the main basin of Lac Bay (Fig.2)

At each station six 1 m^2 quadrats divided in 100 subsections were laid out to determine relative cover/occupancy by the different seagrass species.

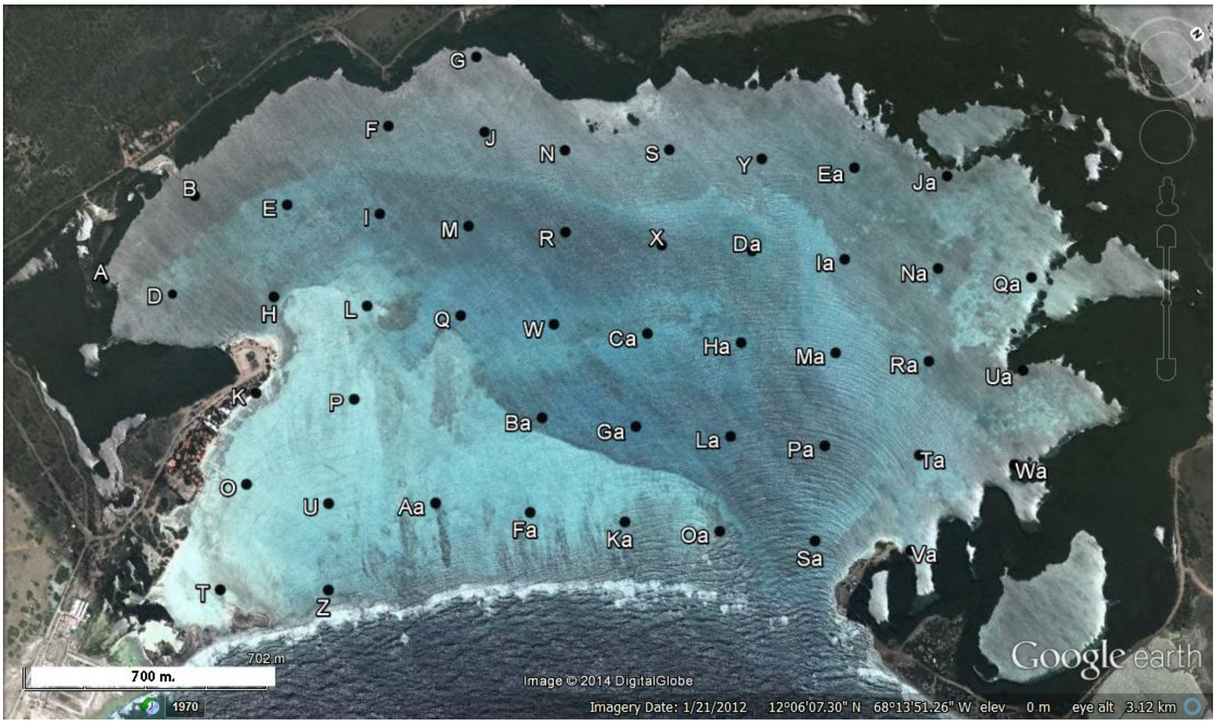


Figure 2. 45 stations equi-distanced throughout Lac Bay where seagrass cover was observed. At each station the percentage cover of seagrass species in six 1m^2. quadrats.

2.2 Fish composition and density in different seagrass beds

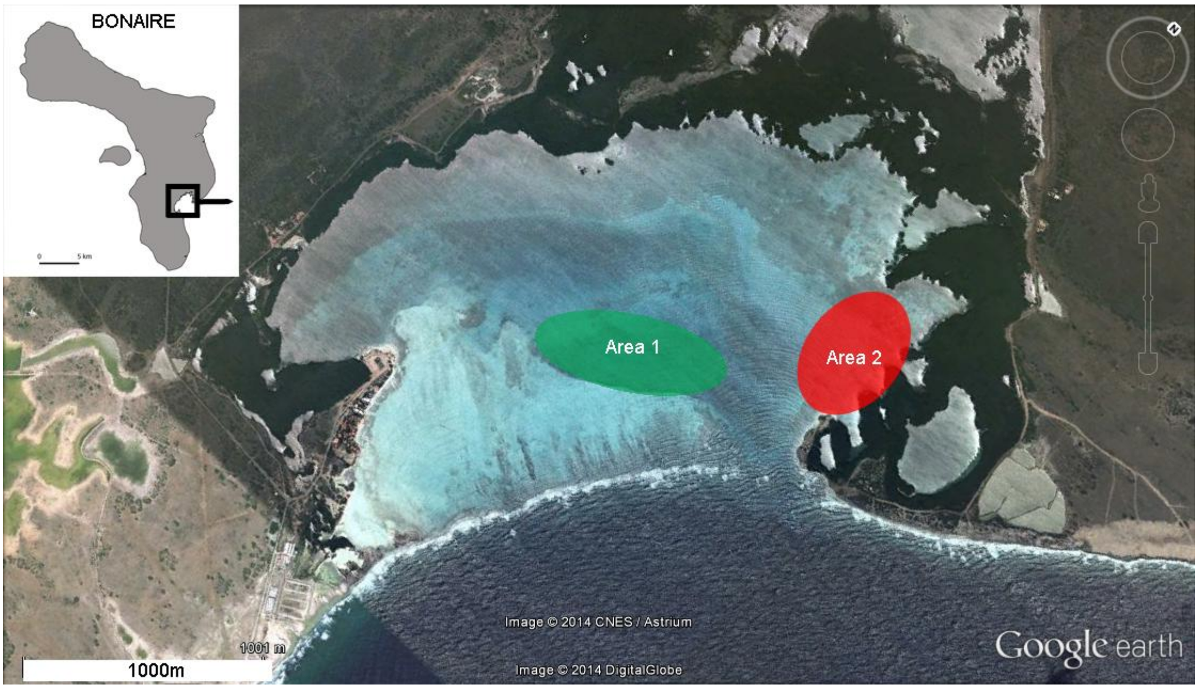


Figure 3. Study sites in Lac Bay, Bonaire: Area 1 (green) and Area 2 (red). The size of each area was approximately 1.2 ha.

For proper experimental design in community comparison, given the short time frame of study, areas were required with both high seagrass cover as well as presence of monocultures of both species to facilitate clear comparisons between meadows dominated by either *T. testudinum* or *H. stipulacea* while controlling for environmental variables. Most areas in the bay have high sand cover or mixed beds, yet two particular areas were found where both monospecific meadows were present, indicated as Area 1 and Area 2 in Figure 3. The areas were approximately 1.2 ha. Based on observations, within one particular area environmental conditions (e.g. depth, temperature, salinity, currents) of monoculture *T. testudinum* and monoculture *H. stipulacea* beds seemed equal, but between areas environmental differences were present (e.g. distance to mangrove, algae species, currents). Therefore, for statistical analysis differences among areas were tested. The general waterquality was good in both sites.

40 transects were recorded in the locations provided in Figure 3, according to the following scheme:

- Ten transects in *Thalassia testudinum* dominated meadows in the central area of the bay (Area 1)
- Ten transects in *Halophila stipulacea* dominated meadows in the central area of the bay (Area 1)
- Ten transects in *T. testudinum* dominated meadows near the mangroves (Area 2)
- Ten transects in *H. stipulacea* dominated meadows near the mangroves (Area 2)

Transects were laid out with a minimum distance of 3 meters from each other. Fish assemblage composition was investigated along the transects by means of underwater visual census using SCUBA and stationary point-count method between 8:00AM and 11:00AM (Polunin & Roberts 1993, Watson & Quinn 1997). The fish abundance, size-category and species composition was quantitatively sampled. A transect line of 5 meters was used as a visual reference for the transect size and laid down in a homogenous patch of seagrass patch at least 5 meters from the edge, to prevent edge-effects.

While laying out the transect larger more skittish fish were immediately recorded. Subsequently the observer waited on the edge of the transect for at least 5 minutes to allow most fish to return to the area. Care was taken to avoid double-counting by not counting individuals or groups of fish that moved in and out of the quadrat more than once. The species, their abundance, and size was recorded along the transect line within 1m on either side of the transect. The fish were identified according to the following families:

Labridae (wrasse)
Lutjanidae (snapper)
Haemulidae (grunt)
Pomacentridae (Damselfish)
Scaridae (parrotfish)
Chaetodontidae (butterflyfish)
Carangidae (jacks)
Acanthuridae (surgeonfish)
Gerreidae (mojarras)
Mullidae (goatfish)
Sphyraenidae (barracuda)

Size category (cm)	Index
0-10	1
10-20	2
20-30	3
30-40	4
40-50	5

Since fish densities are often correlated with the degree of habitat complexity (Luckhurst & Luckhurst 1978) the total seagrass cover and maximum height of the seagrass (in centimeters) were visually quantified for each quadrat (following methods described in Short 2001) once fishes had been counted. Seagrass cover was recorded in three 75cm x 75cm quadrats along the transect, following figure 4. Within the quadrat, the percentage cover was recorded of the different seagrass species, sand and other substrate. The average height and density of vegetation was recorded by laying down a smaller 10cm x 10cm quadrat within the larger quadrat and counting the number of shoots and measuring the length of the longest leaf of the first 25 shoots.

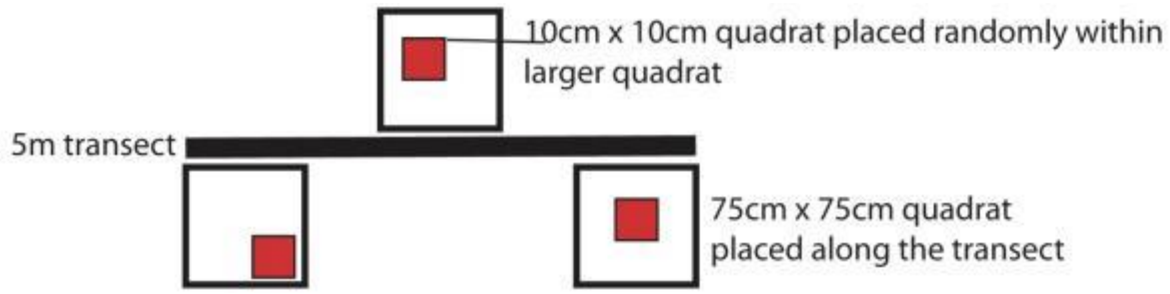


Figure 4. Experimental set up of underwater visual census of fish and quadrats to assess seagrass cover and vegetation complexity.

Data analysis
Data matrices of species abundance per transect were square root transformed and distance matrices were constructed using the Bray-Curtis coefficient with PRIMER-E version 6. The Bray-Curtis index is one of the most frequently applied (dis)similarity indices used in ecology (Purvis & Hector 2000, Legendre & Gallagher 2001). To test for similarities in fish species composition among meadow type an Analysis of Similarities (ANOSIM) was performed in PRIMER-E. Finally a non-metric multidimensional scaling plot (MDS) was created based on the Bray-Curtis similarity coefficient to visualize the resulting fish assemblages. Differences in vegetation characteristics (seagrass cover, sand cover, number of shoots, length of shoots) and fish size among meadows of the two seagrass species (*T. testudinum* and *H. stipulacea*) and among the two areas (1 and 2) were assessed with the Mann-Whitney U test (for non-normal distributed data) using SPSS 20.

2.3 Conch presence (habitat preference)

Habitat preference of the queen conch was studied by actively seeking conches within the study area indicated in Figure 6. Subsequently the conch was overlaid with a 75 x 75 cm quadrat. Within the quadrat the percentage cover of the dominant seagrass species was recorded. The average height & density of vegetation was recorded by laying down a smaller 10cm x 10cm quadrat within the larger quadrat. The number of shoots and the length of the longest leaf of the first 25 shoots was recorded. In addition the distance from the conch to the nearest patch of other type of patch was measured in the four compass-directions. For example, if the conch was in a *Thalassia* bed, then the distance was measured to the nearest *Halophila* bed and sandy patch in all four wind directions.

2.4 Cafeteria experiment: Food preference of seagrass species by sea turtles

To assess whether green turtles graze on *H. stipulacea,* a cafeteria experiment was set up, a method that was adapted from Iongh (1996) by M. Christianen (Fig. 5). Cafetaria experiments were set up randomly within an area with high year-round green turtle grazing pressure, predominantly near the channel in front of Cai (Fig. 6, Sea Turtle Conservation Bonaire pers. comm).

Figure 5. Experimental set up of cafeteria experiment. From left to right: T.testudinum , H.stipulacea, S. filiforme

Three seagrass tethers, each with a thick bush of a single species, were places on sticks in the sand (Figure 5). With each deployment, one stick per species was presented to the turtles and sticks were placed in random order containing:

a. *T. testudinum*
b. *H. stipulacea*
c. *S. filiforme*

A GOPRO camera was placed at 2m distance from the sticks and was left to film unattended as long as the battery lasted (1-2hrs).

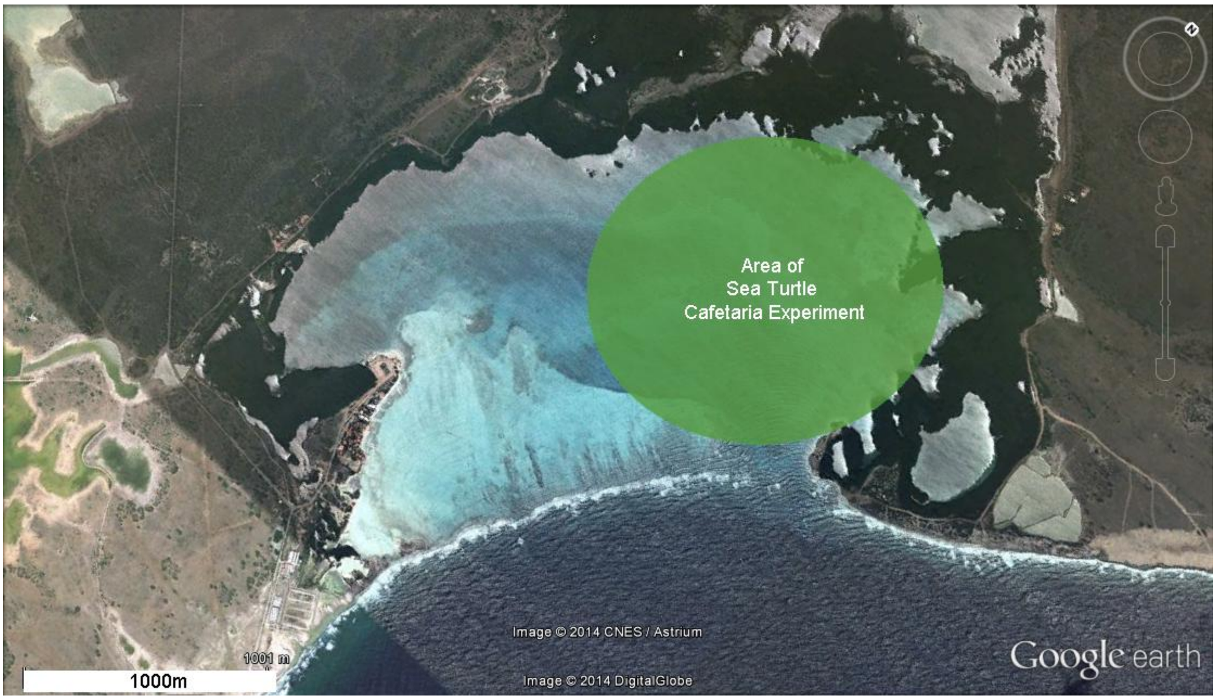


Figure 6. Location of sea turtle cafeteria experiment in Lac Bay, Bonaire. Green area indicates important feeding grounds exploited year-round by immature green turtles (pers. comm. Sea Turtle Conservation Bonaire). In this area the cafeteria experiments were haphazardly set up and conch were observed.

3. Expanse of *H.stipulacea* from 2011 to 2013

In 2011 *H. stipulacea* was present in 7 of the 45 stations where the seagrass cover was recorded(Fig. 7A); in 2013 *H. stipulacea* was present in 12 (Fig. 7B) and the cover of this species within the quadrats generally increased – the exception being in Stations Ba and Ha (Fig 8A). The cover of the native *T. testudinum* had generally decreased in 2013 compared to 2011 in the transects where *H. stipulacea* was present or had become present during that time period (Fig. 8B). These results indicate that the invasive species is expanding in location and cover within Lac Bay and may be pushing out the native seagrass species.

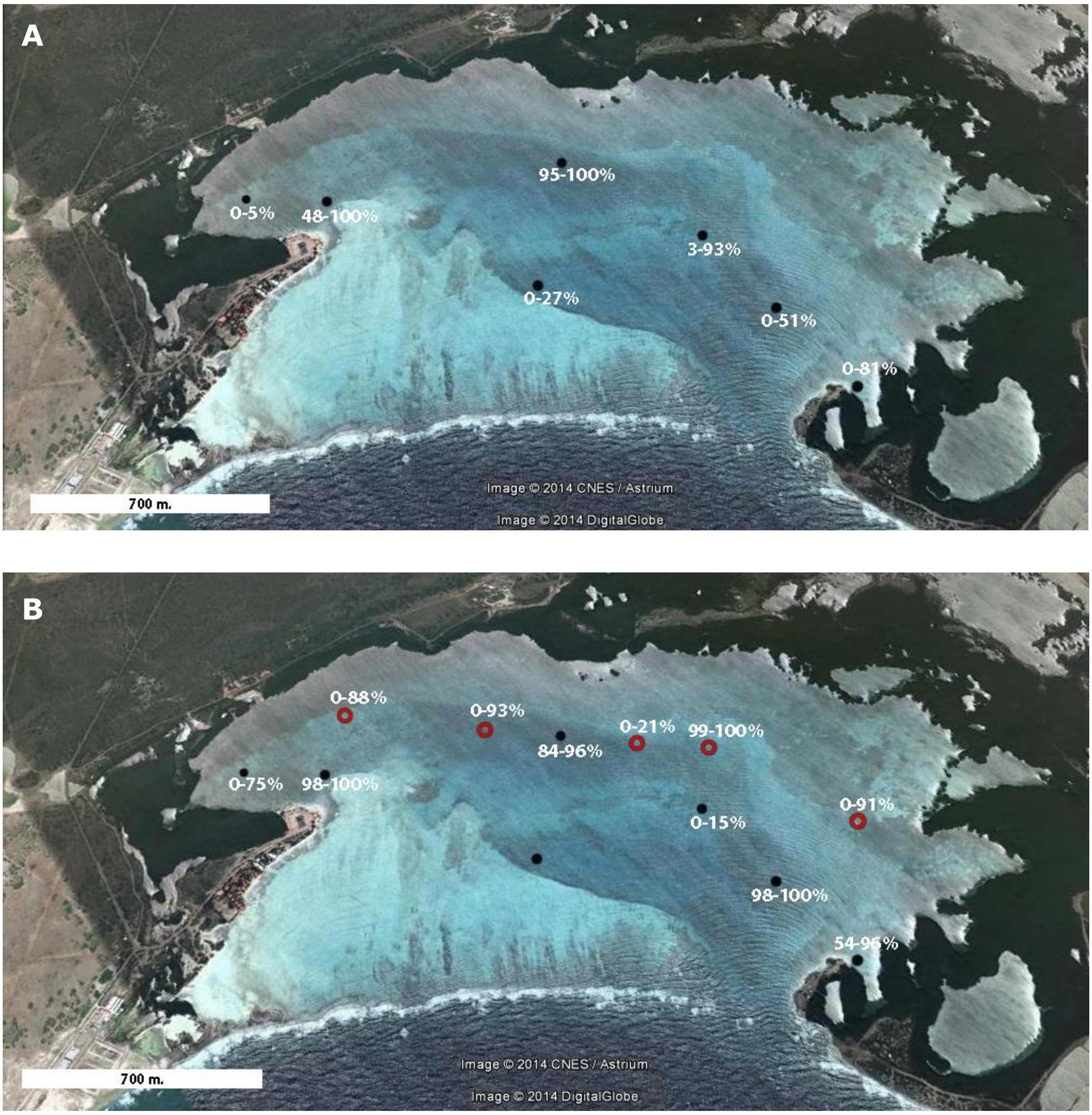


Figure 7. Halophila stipulacea *distribution in Lac Bay in* ***A.*** *2011, present in seven quadrats,* ***B.*** *2013, present in 12 quadrats. Red symbols indicate new areas that H.stipulacea expanded to since 2011. At each station the cover of seagrass was recorded in six* 1m^2 *quadrats, in this figure the minimum-maximum is provided.* At one station in the middle of the bay *H.stipulacea* was not present any more in 2013. *See Figure 2 for locality of all stations in the bay that were recorded.*

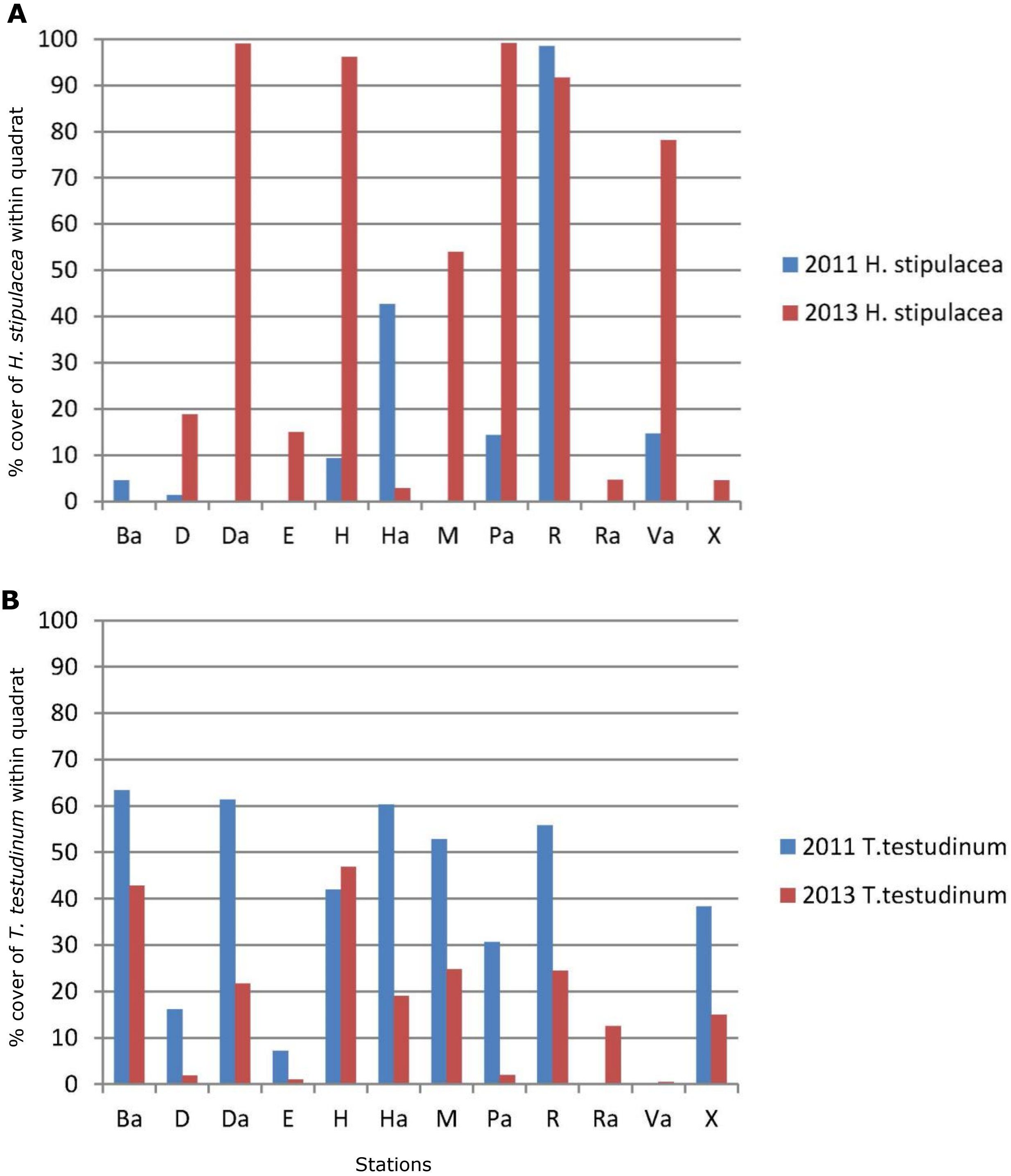
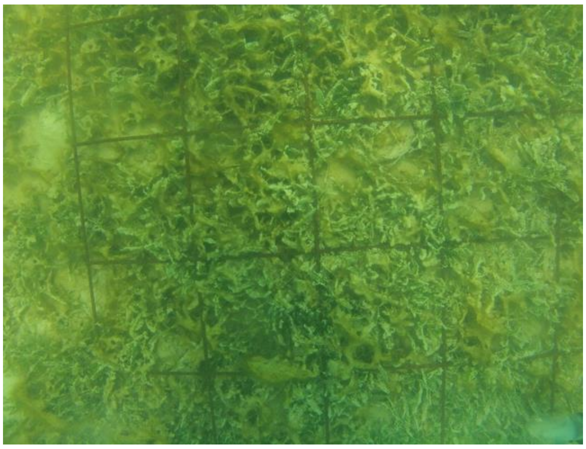


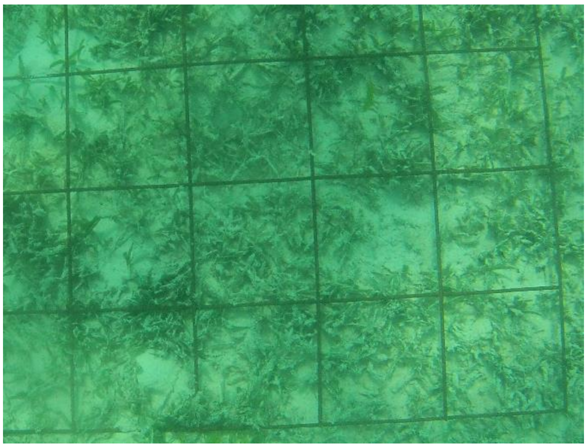
Figure 8. Percentage cover of seagrass recorded in 2011 and 2013 in stations where H. stipulacea *was present in 2011 or had become present between 2011-2013(for locations of the stations in Lac Bay see Figures 2 and 7)* ***A.*** *represents cover of* H.stipulacea, **B.** *represents cover of* T. testudinum.

4. Vegetation differences

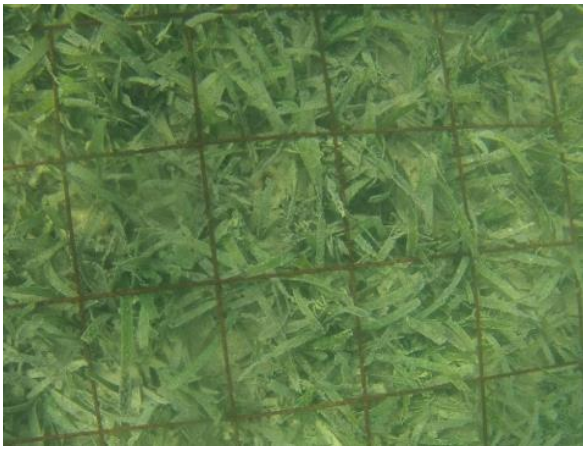
The cover of seagrass in the transects in the meadows of the two species of seagrass did not differ significantly. The majority of transects had a seagrass cover of 90-100%. Despite the cover being equal, there was a significant difference in habitat complexity between the species (Figure 9), reflected by a difference in the number of shoots ($p<0.001$) and the length of shoots ($p<0.001$) (Figure 10 AB).



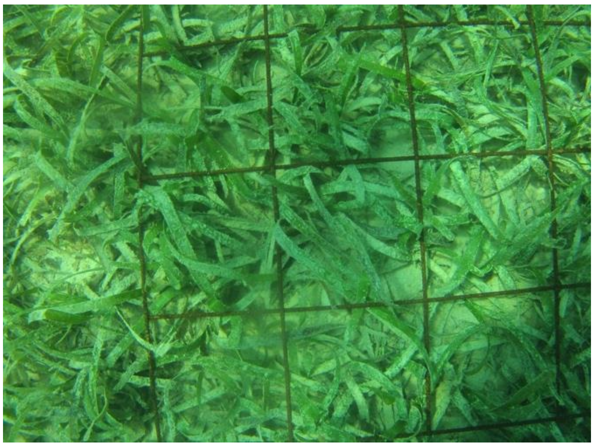
H. stipulacea meadow in Area 1



H. stipulacea meadow in Area 2



T. testudinum meadow in Area 1



T. testudinum meadow in Area 2

Figure 9. In situ images of H. stipulacea and T. testudinum in Area 1 and 2.

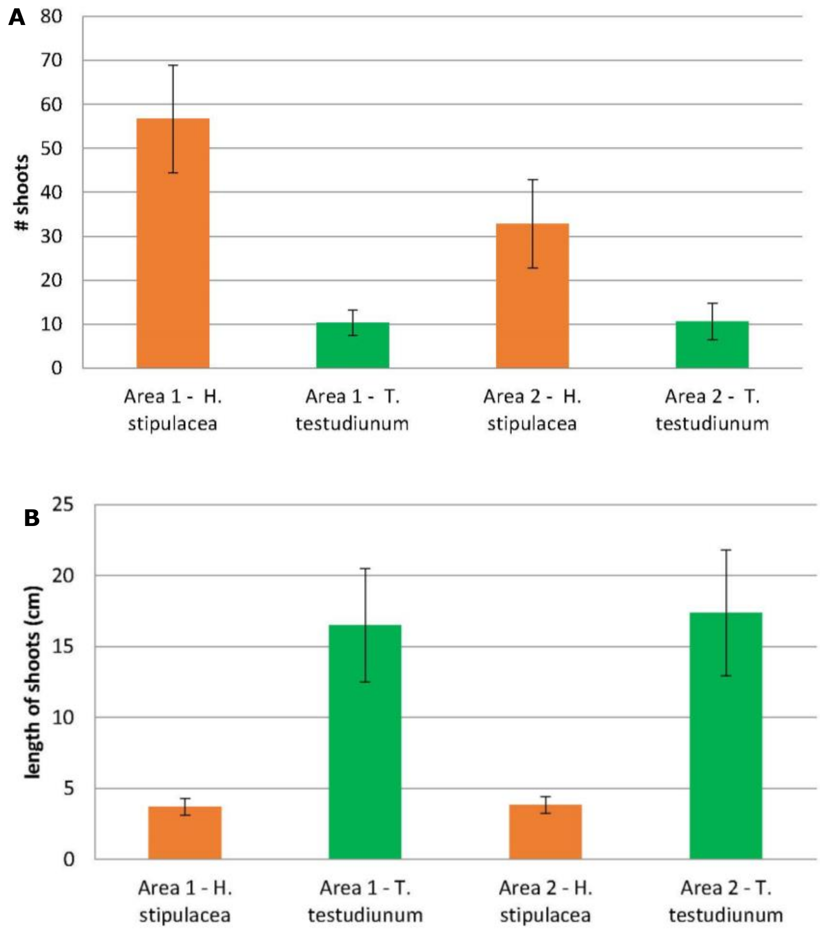


Figure 10. Habitat characteristics in monospecific T. testudinum and H. stipulacea transects of equal seagrass cover, in Area 1 and 2. (n=30 per species per area) ***A.*** *Average number of shoots with standard deviation,* ***B.*** *average length of shoots. The two se agrass meadows differ significantly in both the number of shoots and lengths (both p<0.001)*

5. Fish assemblages

Fish counts were conducted in *T. testudinum* beds and *H. stipulacea* beds in 20 transects each. Transects were only conducted in monospecific beds where the seagrass cover in the majority of the transects was 90-100% (thus controlling for potential effects of surficial cover) (Appendix Table 1). The total number of fish counted in *T. testudinum* bed transects was 420 (Table 1), with an average of 21 fish per transect (max=41; min=11). The total number of fish counted in *H. stipulacea* bed transects was 224, with an average of 13 fish per transect (max=26; min=0). The number of fish recorded in *H. stipulacea* was significantly lower compared to *T. testudinum* beds ($p<0.001$).

The majority of the fish were Scaridae in both seagrass meadows (Figure Table 1, Figure 11AB). No Pomacentridae, Mullidae, and Sphyraenidae were recorded in the transects placed in *H. stipulacea meadows* (Table 1, Figure 8B), while these were present in *T. testudinum* beds (Table 1, Figure 11A). The most notable species contrast concerned the Pomacentridae, because they were recorded in eight transects in *T. testudinum*, while none were recorded in *H. stipulacea.* Mullidae, and Sphyraenidae were only encountered in one transect in *T. testudinum* beds (Table 1, Figure 11AB).

The species assemblages differed significantly but not strongly between the two seagrass species (ANOSIM R=0.113, p=0.011); there was a stronger difference in assemblages between the two areas (ANOSIM R=0.409, p=0.001). Four transects in *H. stipulacea* beds stuck out due to lower diversity and abundance of fish (Figure 12, Appendix Table 2)

There were higher numbers of small fish (0-10 cm) in *T. testudinum* fields compared to *H.stipulacea* fields, but the proportion of fish length classes did not differ significantly between seagrass species (Figure 13). There was, however, a significant difference between area, with Area 1 having more fish counts with fish and significantly more fish in smaller sizeclasses (0-10 cm and 10-20cm) compared to Area 2.

Table 1. Total number of fish recorded per fish group and minimum-maximum number of fish per transect (5 m^2) in T. testudinum (n=20 transects) and H. stipulacae (n=20 transects) meadows. For raw data of underwater visual census see Appendix Table 2.

	T. testudinum		*H. stipulacea*	
	total # recorded	Min-max # per transect	total # recorded	Min-max # per transect
Labridae (wrasses)	28	0-4	15	0-3
Lutjanidae (snapper)	50	0-8	19	0-4
Haemulidae (grunt)	13	0-4	9	0-4
Pomacentridae (damselfish)	18	0-5	0	0
Scaridae (parrotfish)	263	5-28	147	0-20
Chaetodontidae (butterflyfish)	8	0-3	5	0-2
Carangidae (jacks)	16	0-4	17	0-5
Acanthuridae (surgeonfish)	18	0-3	9	0-3
Gerreidae (mojarras)	1	0-1	3	0-2
Mullidae (goatfish)	4	0-4	0	0
Sphyraenidae (barracuda)	1	0-1	0	0
total # of fish	**420**		**224**	

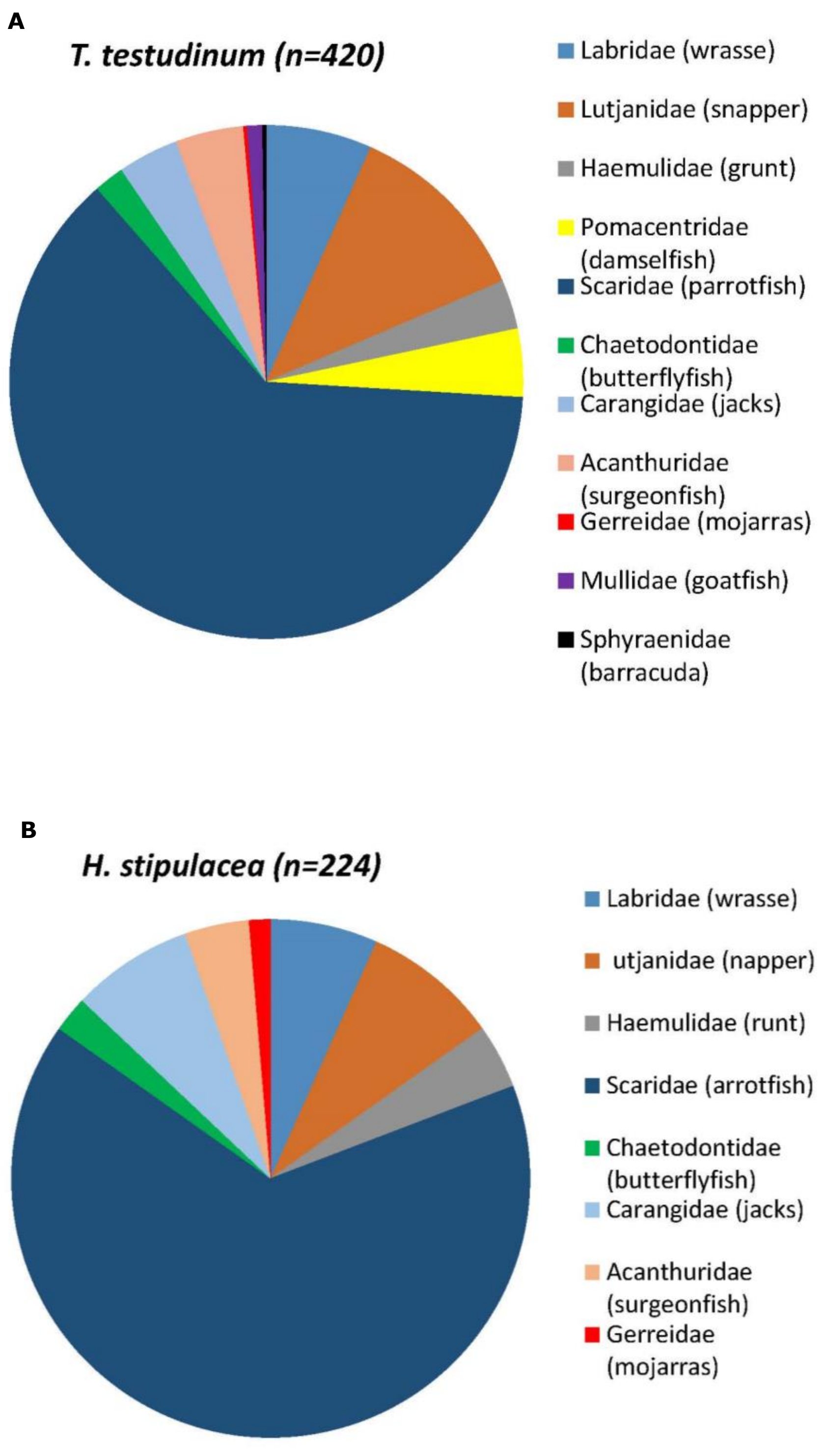


*Figure 11. Proportion of fish families in transects (based on fish counts from 20 plots per field type) in meadows of **A.** T. testudinum and **B.** H. stipulacea. Note that Pomacentridae (yellow), Mullidae (purple), and Sphyraenidae (red) were not recorded in transects in H. stipulacea, while these were present in T. testudinum.*

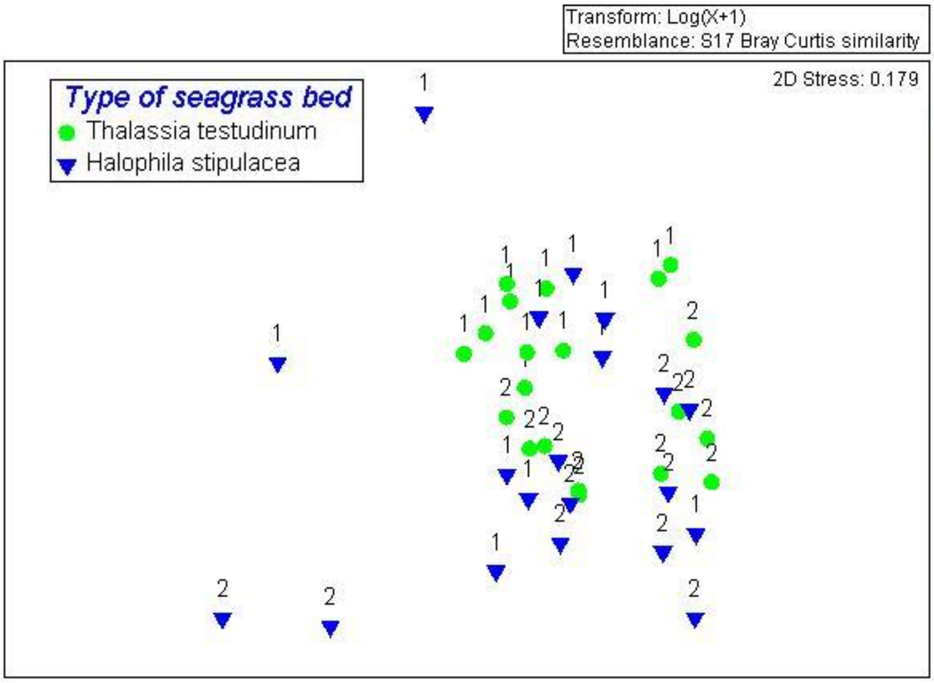


Figure 12. Comparison of fish species assemblages among T. testudinum and H. stipulacae. Nonmetric Multidimensional Scaling plot based on the Bray-Curtis similarity index between pairs of transects; each symbol represents a transect and symbols closer to each other are more similar to each other in species composition; numbers '1' and '2' indicate the two areas from Figure 3. Four transects in H. stipulacea beds stuck out due to lower diversity and abundance of fish.

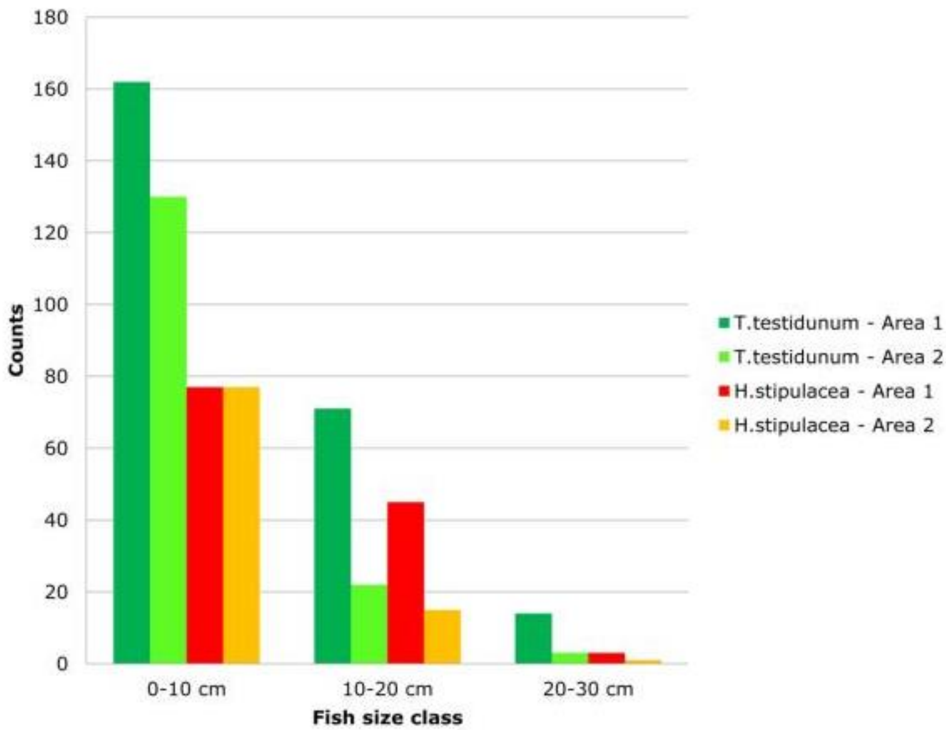


Figure 13. Size class distribution of fish in monospecific stands of T. testudinum *and* H.stipulacea *in Lac Bay (see Figure 3 for locality)*

6. Conch

In total 27 individual conchs (*Lobatus* giga) were observed within the research area indicated in Figure 6 and within a four day time frame. The conch occurred in all seagrass types and did not appear to avoid *H. stipulacea* meadows (Figure 14, Figure 15, Table 2). Occurrence was highest on *H. stipulacea* meadows (n=8) and sandy bottoms (n=6), followed by meadows with *T. testudinum* and *S.filiforme.* The conch had equal occurrence on meadows with *H. stipulacea* (n=10) and meadows with only native seagrass species (n=11) (Figure 14, Table 2).

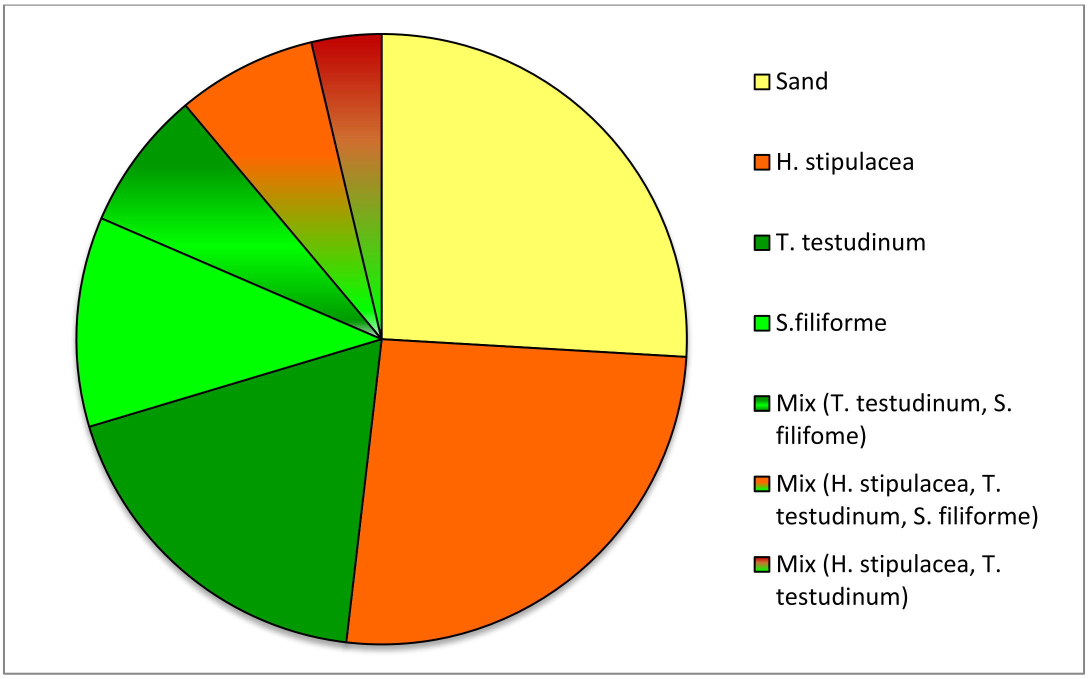


Figure 14. Occurrence of conch on different types of substrate. Green colors indicate native seagrass species, red-orange colors indicate presence of H. stipulacea. See Table 2 for percentage cover of seagrass.

There was an aggregation of conch (20 live individuals in a 50 x 50m area) in a sandy patch in Area 1 where Conch#2 was observed (Appendix Table 3).

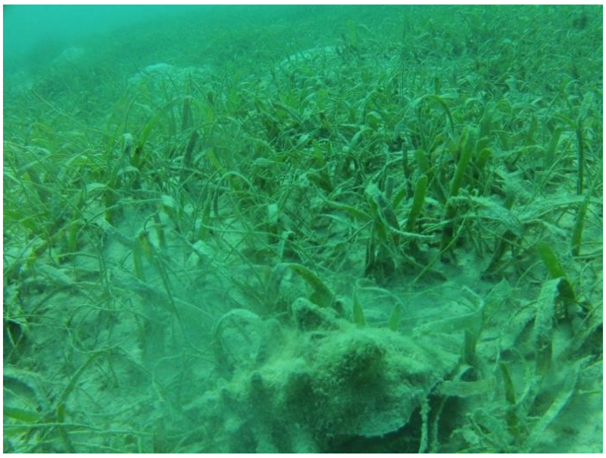
The nearest distance of conchs to a different type of habitat is indicated in Appendix Table 2. To summarize, the conch observed in *H. stipulacea* beds were located at 40-200 m distance from native seagrass beds; those observed in native seagrass beds were also 40-200m from *H. stipulacea* beds. This seems to indicate that there was no particular preference of either seagrass type and also no evident avoidance of *H. stipulacea* by conch in Lac Bay. These results only refer to part of Lac Bay, therefore we are cautious with making general conclusions.

Halophila stipulacea meadow

Halophila stipulacea meadow

Thalassia testudinum meadow

Syringodium filiforme meadow



Mixed bed

Sand

Figure 15. Conch in meadows of different species of seagrass and in sand.

Table 2. Type of substrate that conch occurred on in in Lac Bay, Bonaire.

Conch #	Present on substrate	% seagrass	% sand
1	*H. stipulacea*	100	0
2	Sand	0	100
3	Sand	0	100
4	Sand	0	100
5	Sand	0	100
6	Sand	0	100
7	*H. stipulacea*	90	10
8	*H. stipulacea*	95	5
9	*T. testudinum*	100	0
10	*T. testudinum*	98	2
11	*T. testudinum*	50	50
12	Mix *(T. testudinum, S. filifome)*	93	7
13	Mix *(T. testudinum, S. filifome)*	97	3
14	*H. stipulacea*	80	20
15	*H. stipulacea*	92	8
16	Mix *(H. stipulacea, T. testudinum, S. filiforme)*	95	5
17	*Mix (H. stipulacea, T. testudinum, S. filiforme)*	97	3
18	*Sand*	0	100
19	*S. Filiforme*	100	0
20	*S.filiforme*	100	0
21	*S. filiforme*	98	20
22	*H. stipulacea*	93	7
23	*H. stipulacea*	94	6
24	*T. testudinum*	100	0
25	*T. testudinum*	100	0
26	*H. stipulacea*	89	11
27	Mix *(H. stipulacea, T. testudinum)*	100	0

7. Grazing by green turtles

The cafeteria experiment was conducted 20 times. Eighteen green turtles (*Chelonia mydas*) were observed, with five grazing events (Table 3). All seagrass species were grazed on by the green turtles at least once (Table 1, Figures 16-18) and the turtles did not appear to show a preference for any species. While sample sizes in this experiment were limited, the results do not suggest any overt dislike of *H. stipulacea.* However, further study is required to establish any potential diet preference.

Table 3. Results of the cafeteria experiment.

	Duration filmed	Order of species	# turtles observed passing	# grazing events	seagrass species grazed
16-oct-13	52min	S-H-T	0	0	-
29-oct-13	1hr 18min	S-T-H	3	2	*H. stipulacea* & *S. filiforme*
30-oct-13	35min	T-H-S	0	0	-
4-nov-13	58min	T-H-S	1	0	-
5-nov-13	1hr 18min	H-T-S	0	0	-
7-nov-13	1hr 45min	S-T-H	1	0	-
11-nov-13	1hr 19min	H-T-S	5	1	*T. testudinum*
12-nov-13	1hr 33min	S-H-T	0	0	-
13-nov-13	1hr 21min	H-S-T	0	0	-
20-nov-13	1hr 18min	S-H-T	1	1	*S. filiforme*
16-dec-13	1hr18min	H-S-T	5	0	-
17-dec-13	52min	H-T-S	1	1	*T. testudinum*
18-dec-13	1hr18min	H-T-S	1	0	-

Figure 16. Grazing T. testudinum (17 dec 2013)

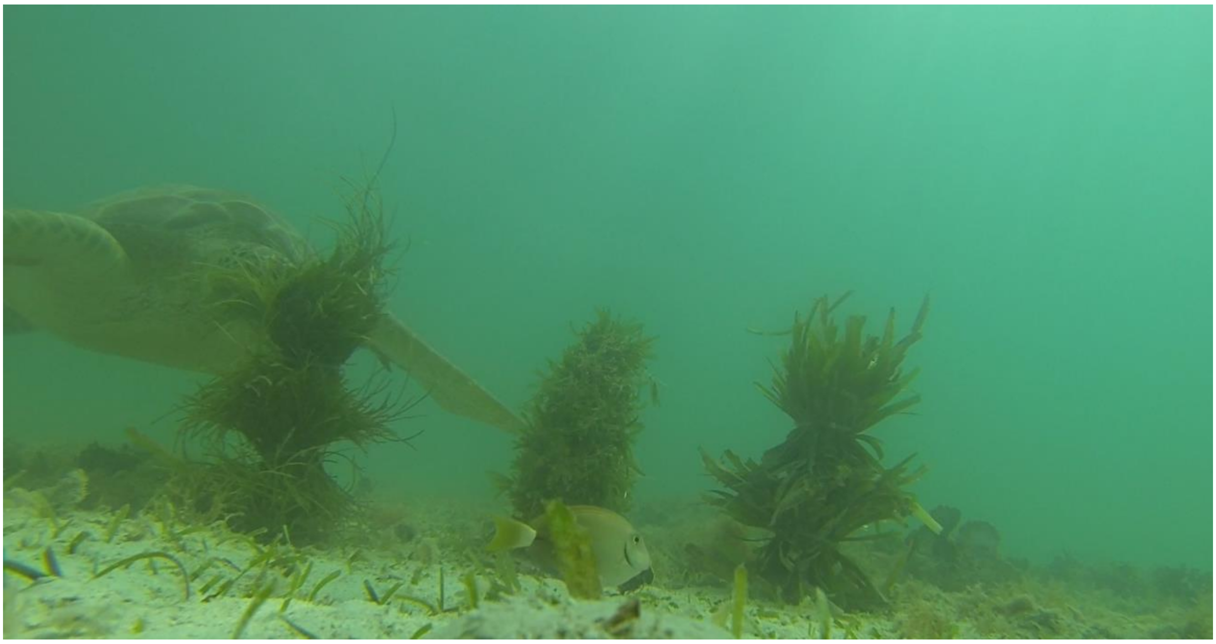


Figure 17. Grazing S. filiforme (20 nov 2013)

Figure 18. Grazing H. stipulacea (29 Oct 2013)

8. Discussion and conclusions

8.1 How does vegetation complexity differ between meadows of *T. testudinum* and those of *H. stipulacea*?

The two species of seagrass differed significantly in habitat complexity (Figure 9), reflected by a difference in the number of shoots and the length of shoots (Figure 10 AB). The presence of the above and below ground portion of the plant adds structure to an otherwise homogeneous sandy bottom. Seagrass beds may be continuous stands consisting of one or more species or be a heterogeneous array of sandy areas interspersed among the vegetation (e.g. Orth et al. 1984). Increase in habitat complexity due to the seagrass canopy (i.e. higher seagrass density, leaf surface or aboveground biomass) is assumed to result in higher faunal abundance due to reduced predation risk and enhanced food supply (Howard et al. 1989, Hyndes et al. 2003, Unsworth et al. 2007). Invasive macrophytes can impose changes on native communities via mechanisms that modify the habitat and vary indigenous faunal composition. For example, Willette & Ambrose (2012) conducted an *in situ* transplant experiment in Dominica to examine the effect of *H. stipulacea* within beds of the dominant native seagrass *S. filiforme*. They found the invasive seagrass was capable of rapid expansion, with the displacement of the native seagrass beginning in 10–12 weeks. Furthermore, *H. stipulacea* altered the abundance and composition of seagrass-associated organisms and the local seagrass community.

8.2 Do monospecific fields of *H. stipulacea* differ from monospecific fields of native *T. testudinum* in fish species assemblages and abundances?

The observed increase in cover of *H.stipulacea* (Fig. 7) and of reduced habitat complexity of *H.stipulacea* (i.e. significantly shorter shoots) could have an effect on the associated fauna such as fish. Therefore we investigated whether monospecific fields of *H. stipulacea* differ from monospecific fields of native *T. testudinum* in fish abundance and species composition. We did find a significant difference in fish abundance and species composition between meadows dominated by *T. testudiunum* and those dominated by *H. stipulacea.* Most notable was that the abundance of fish was almost half in the *H. stipulacea* meadows and that no Pomacentridae were present. If this is a long-term trend, the expansion in *H. stipulacea* may result in a diminished nursery function for (selected) fish in Lac Bay. This is particularly disconcerting because of the tendency of *H. stipulacea* to form monospecific stands, possibly to the exclusion of most other macrophytes (e.g. Willette & Ambrose 2012, Debrot et al. 2012b, Debrot et al. *in prep.*).

We suggest that this difference in fish density and community structure may be a result of active habitat selection as influenced by differences in habitat structure. *T. testudiunum* is significantly longer and may provide more three-dimensional shelter to small fish and invertebrates that they feed on than *H. stipulacea* can provide. Our results seem to follow observations by previous studies (Orth et al. 1984, Vonk et al. 2010) that suggests that the abundance of many species, both epifauna and infauna, is positively correlated with two distinct aspects of plant morphology: 1) the root-rhizome mat, and 2) the morphology of the plant canopy and 3) seagrass biomass and associated epiphytes.

Our results seem to align with a previous study in Dominica of fish traps that were placed in monospecific fields of the invasive seagrass and in fields of native seagrass *S. filiforme,* which showed that *S. filiforme* supported twice the proportion of juvenile stage fish compared to the invasive seagrass (Wilette & Ambrose 2012).

8.3 Does the queen conch avoid *H. stipulacea* meadows?

For the queen conch (*Lobatus gigas*), one of Bonaire's flagship conservation species, the seagrass beds of Lac Bay form their most important food source. The conch do not eat the actual leaves of the seagrass, but the organisms living on and among it as well as the dead organic material. Previous studies have shown that the invasive seagrass *H. stipulacea* supports a different abundance and composition of seagrass-associated organisms (particularly Crustacea) than the native species (Willette & Ambrose 2012), which in turn could potentially affect the distribution/behaviour of conch.

Our observations, however, suggest that the queen conch did not avoid *H. stipulacea* meadows in Lac Bay in the area that we studies. If just comparing meadows that are either monospecific or a mix of native seagrass species (n=11) with those containing *H. stipulacea* (n=10), the conch had equal occurrence on both types of meadows. The nearest distance between conch and a contrasting habitat type also suggests no avoidance. In our observations the conch that were present in *H. stipulacea* beds were located at 20-400m distance from native seagrass beds; this would indicate that the *H. stipulacea* beds are within the home-range of the conch (Bissada-Gooding & Oxenford 2009). What is more, if the conch were to avoid *H. tipulacea* we would expect to find them predominantly on native seagrass beds and only at the edge of the *H.stipulacea* beds in close proximity of the native seagrass beds. Nevertheless, the sample size is small therefore we must be cautious with conclusions and possibly there may be differences in habitat preference related to lifestage (which was not recorded).

8.4 Will green turtles in Lac Bay graze on *H. stipulacea*?

Lac Bay contains the largest seagrass beds of the Caribbean Netherlands and is an important forage area for green sea turtles. There is raised concern that the Caribbean green sea turtles that graze in Lac Bay might avoid the invasive seagrass *Halophila stipulacea,* which originates from the Red Sea and the western Indian Ocean.

We document that in the Caribbean, green turtles will graze on *H. stipulacea* if actively presented. All three seagrass species were selected at least once. Thus this study indicates that the green sea turtle does not necessarily avoid *H. stipulacea* as a food source*.* This result is not surprising, as *Halophila stipulacae* is an important food source for green turtles throughout the native range of *H. stipulacea* (Price et al. 1988, Turkozan & Durmus 2000, Spalding et al. 2003). Some preference feeding tests were carried out by Al-Ajzoon (1993) in the Gulf of Oman with young adults of the green turtle, which were offered clumps of macroalgae (preference: Sargassopsis > Sargassum > Ulua) and seagrasses (preference: Halophila > Syringodium > Halodule) (Al-Ajzoon 1993). Sea turtles are described to have a foraging preference for seagrass species with the highest palatability and nutrient content (thesis Christianen 2013, Bjorndal 1997) which are characteristics attributed to fast growing species, such as *H. stipulacea*. Generally green turtles favor fast-growing species (such as *H. stipulacea*) over slower-growing species (such as *T. testudinum*) (thesis Christianen 2013).

If turtle grazing pressure would increase in Lac Bay it may even stimulate invasion of *H. stipulacea.* It is already known that green turtles may induce stimulated production of seagrass (Valentine et al. 1997; Moran & Bjorndal 2005), especially if these species are able to recover fast after they are grazed upon, such as fast growing species like *H. stipulacea.*

Finally, *H. stipulacea* is increasing in cover in Lac Bay and may become the main food source for green sea turtles. It is important to understand the nutritional difference this shift in diet may cause before it can be concluded what the effect on green turtle health is.

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10. Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 124296-2012-AQ-NLD-RvA). This certificate is valid until 15 December 2015. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 1th of April 2017 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

11. Acknowledgements

We would like to thank Mabel Nava and the staff at Sea Turtle Conservation Bonaire and STINAPA, as well as Dr. Peter Bodegom at the University of Amsterdam for their support.

12. Justification

Report number: C118/14
Project Number: 4308701033

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved: Dr. H.W.G. Meesters
Researcher

Signature:

Date: September 11th, 2014

Approved: Drs. F.C. Groenendijk
Head Maritime Department

Signature:

Date: September 11th, 2014

13. Appendix

Table 1. percentage of seagrass and sand cover in the 40 transects of fish counts in Lac Bay.

Transect #	Area	Type of seagrass bed	% seagrass	% sand
1	1	*T. testudinum*	98	0
2	1	*T. testudinum*	95	2
3	1	*T. testudinum*	100	0
4	1	*T. testudinum*	93	0
5	1	*T. testudinum*	89	4
6	1	*T. testudinum*	99	0
7	1	*T. testudinum*	95	1
8	1	*T. testudinum*	96	0
9	1	*T. testudinum*	92	2
10	1	*T. testudinum*	92	2
11	1	*H. stipulacea*	93	7
12	1	*H. stipulacea*	92	8
13	1	*H. stipulacea*	66	34
14	1	*H. stipulacea*	87	13
15	1	*H. stipulacea*	87	13
16	1	*H. stipulacea*	99	1
17	1	*H. stipulacea*	100	0
18	1	*H. stipulacea*	93	7
19	1	*H. stipulacea*	92	8
20	1	*H. stipulacea*	100	0
21	2	*T. testudinum*	90	10
22	2	*T. testudinum*	95	5
23	2	*T. testudinum*	92	8
24	2	*T. testudinum*	92	8
25	2	*T. testudinum*	92	8
26	2	*T. testudinum*	93	7
27	2	*T. testudinum*	94	6
28	2	*T. testudinum*	98	2
29	2	*T. testudinum*	92	8
30	2	*T. testudinum*	100	0
31	2	*H. stipulacea*	98	2
32	2	*H. stipulacea*	100	0
33	2	*H. stipulacea*	100	0
34	2	*H. stipulacea*	99	1
35	2	*H. stipulacea*	99	1
36	2	*H. stipulacea*	97	3

37	2	*H. stipulacea*	100	0
38	2	*H. stipulacea*	95	5
39	2	*H. stipulacea*	100	0
40	2	*H. stipulacea*	100	0

Table 2. Raw data of underwater visual census of fish assemblages.

Transect	Area	Type of seagrass	Labridae (wrasse)	Lutjanidae (snapper)	Haemulidae (grunt)	Pomacentridae (damselfish)	Scaridae (parrotfish)	Chaetodontodae (butterflyfish)	Carangidae (jacks)	Acanthuridae (surgeonfish)	Gerreidae (mojarras)	Mullidae (goatfish)	Sphyraenidae (barracuda)
1	1	*T. testudinum*	0	6	0	5	12	0	0	0	0	0	0
2	1	*T. testudinum*	0	3	0	4	20	3	0	0	0	0	0
3	1	*T. testudinum*	2	4	0	0	10	0	1	0	0	0	0
4	1	*T. testudinum*	1	2	0	1	17	0	2	0	0	0	0
5	1	*T. testudinum*	1	2	2	1	5	2	0	1	0	0	0
6	1	*T. testudinum*	3	5	2	3	14	0	1	0	0	0	0
7	1	*T. testudinum*	4	7	1	0	28	0	0	1	0	0	0
8	1	*T. testudinum*	3	6	4	2	15	1	1	0	0	0	0
9	1	*T. testudinum*	4	4	1	0	5	0	2	0	0	0	0
10	1	*T. testudinum*	1	8	1	2	20	2	1	2	0	0	0
11	1	*H. stipulacea*	1	1	0	0	5	0	0	0	0	0	0
12	1	*H. stipulacea*	0	3	0	0	10	0	2	1	0	0	0
13	1	*H. stipulacea*	0	2	1	0	0	1	5	0	0	0	0
14	1	*H. stipulacea*	0	0	0	0	6	2	0	0	0	0	0
15	1	*H. stipulacea*	1	4	2	0	15	0	3	0	1	0	0
16	1	*H. stipulacea*	0	2	3	0	5	0	3	2	0	0	0
17	1	*H. stipulacea*	2	4	0	0	0	0	0	0	0	0	0
18	1	*H. stipulacea*	0	2	1	0	20	0	2	0	0	0	0
19	1	*H. stipulacea*	3	0	0	0	3	0	0	0	0	0	0
20	1	*H. stipulacea*	2	0	1	0	7	2	0	1	0	0	0
21	2	*T. testudinum*	0	0	0	0	10	0	0	1	0	0	0
22	2	*T. testudinum*	2	0	0	0	11	0	0	0	0	0	0
23	2	*T. testudinum*	0	0	0	0	20	0	0	1	1	0	0
24	2	*T. testudinum*	1	1	0	0	5	0	2	2	0	0	0
25	2	*T. testudinum*	0	0	0	0	12	0	0	0	0	0	1
26	2	*T. testudinum*	3	0	0	0	9	0	2	3	0	0	0
27	2	*T. testudinum*	0	0	2	0	18	0	0	1	0	0	0
28	2	*T. testudinum*	0	2	0	0	14	0	0	2	0	4	0
29	2	*T. testudinum*	1	0	0	0	8	0	4	3	0	0	0
30	2	*T. testudinum*	2	0	0	0	10	0	0	1	0	0	0
31	2	*H. stipulacea*	0	0	0	0	10	0	0	0	0	0	0
32	2	*H. stipulacea*	0	1	0	0	20	0	0	0	0	0	0
33	2	*H. stipulacea*	1	0	0	0	5	0	0	0	0	0	0
34	2	*H. stipulacea*	0	0	0	0	3	0	0	1	2	0	0
35	2	*H. stipulacea*	0	0	0	0	5	0	0	0	0	0	0
36	2	*H. stipulacea*	1	0	0	0	8	0	2	0	0	0	0
37	2	*H. stipulacea*	0	0	0	0	0	0	0	0	0	0	0
38	2	*H. stipulacea*	0	0	1	0	15	0	0	3	0	0	0
39	2	*H. stipulacea*	2	0	0	0	0	0	0	1	0	0	0
40	2	*H. stipulacea*	2	0	0	0	10	0	0	0	0	0	0

Table 3. Distance to other type of habitat in four with directions.

Conch #	Present on substrate	neighboring substrate type North	Distance North (m)	neighboring substrate type East	Distance East (m)	neighboring substrate type South	Distance South (m)	neighboring substrate type West	Distance West (m)
1	H. stipulacea	T. testudinum	100	T. testudinum + H. stipulacea	80	H. stipulacea	160	T. testudinum	50
2	Sand	T. testudinum + S. filiforme	50	S. filiforme+ T. testudinum	30	sand	50	H. stipulacea	20
3	Sand	S. filiforme	40	H. stipulacea + s. filiforme+ T. testudinum	40	S. filiforme+ T. testudinum	40	T. testudinum	30
4	Sand	T. testudinum + S. filiforme	70	H. stipulacea	20	H. stipulacea + S. filiforme+ T. testudinum	70	T. testudinum	70
5	Sand	H. stipulacea	120	H. stipulacea	100	sand	30	T. testudinum + S. filiforme	30
6	Sand	H. stipulacea	50	S. filiforme+ T. testudinum		H. stipulacea + S. filiforme+ T. testudinum			
7	H. stipulacea	T. testudinum	60	T. testudinum + H. stipulacea	200	H. stipulacea	50	T. testudinum	40
8	H. stipulacea	T. testudinum	80	T. testudinum + H. stipulacea	120	H. stipulacea	80	T. testudinum	70
9	T. testudinum	Land	50	T. testudinum + H. stipulacea	100	H. stipulacea+ T. testudinum+ S. filiforme	170	Land	100
10	T. testudinum	T. testudinum	160	H. stipulacea + T. testudinum	140	H. stipulacea	70	T. testudinum	200
11	T. testudinum	T. testudinum	80	H. syipulacea	120	T. testudinum +H. stipulacea	200	T. testudinum	90
12	Mix (T. testudinum, S. filifome)	H. stipulacea	200	H. stipulacea	300	S. filiforme	80	T. testudium + S. filiforme	120
13	Mix (T. testudinum, S. filifome)	H. stipulacea	150	T. testudinum + H. stipulacea	150	S. filiforme	40	T. testudinum	70
14	H. stipulacea	T. testudinum + H. stipulacea	200	T. testudinum	80	H. stipulacea	100	T. testudinum + s. filiforme	60
15	H. stipulacea	T. testudinum	50	T. testudinum	40	H. stipulacea	80	T. testudinum + S. filiforme	140
16	Mix (H. stipulacea, T. testudinum, S. filiforme)	S. fifome	60	H. stipulacea	100	Sand (further--> reef)	500	T. testudinum	120
17	Mix (H. stipulacea, T. testudinum, S. filiforme)	T. testudinum	80	T. testudinum + H. stipulacea	70	Sand (further --> reef)	450	H. stipulacea	110
18	Sand	T. testudinum	130	H. stipulacea	140	T. testudinum	200	T. testudinum	80
19	S. Filiforme	T. testudinum	80	H. stipulacea	200	T. testudinum	60	T. testudinum + s. filiforme	90
20	S.filiforme	T. testudinum	80	H. stipulacea	200	T. testudinum + H. stipulacea	60	T. testudinum + s. filiforme	90
21	S. filiforme	T. testudinum	60	H. stipulacea	250	T. testudinum + H. stipulacea	70	S. filiforme	40
22	H. stipulacea	t. testudinum	120	H. stipulacea	40	T. testudinum	120	H. stipulacea	50
23	H. stipulacea	T. testudinum	70	H. stipulacea	100	H. stipulacea	80	T. testudinum + H. stipulacea	30
24	T. testudinum	Land	60	T. testudinum + H. stipulacea	50	T.testudinum + H. stipulacea	40	Land	160
25	T. testudinum	T. testudinum	20	H. stipulacea + T. testudinum	100	T. testudinum + s. filifome	30	T. testudinum	300
26	H. stipulacea	H. stipulacea	80	H. stipulacea + s. filiforme+ T. testudinum	50	T. testudinum	25	T. testudinum + H. stipulacea	60
27	Mix (H. stipulacea, T. testudinum)	T. testudinum	50	H. stipulacea	100	sand	120	H. stipulacea	30