

The following supplement accompanies the article

Effect of seagrass nutrient content and relative abundance on the foraging behavior of green turtles in the face of a marine plant invasion

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Text S1

Focal follows

During a follow, trained observers used GoPro® Hero 1+ video cameras attached to a 2 m extendable pole at a 90° angle. Observers entered the water at haphazardly selected points along the shoreline and swam for a predetermined, haphazardly selected amount of time before actively searching for a turtle to avoid following only turtles nearest to shore. Upon spotting a turtle, the video recording was initiated, the pole was extended to ~ 2 m, and the camera was oriented to record the turtle's head. If the turtle reacted to the presence of the observer or the camera, the observer moved 1-2 m further away from the turtle but continued recording. If the turtle resumed normal behavior (e.g., foraging, resting) within a few minutes, the observer would re-approach and continue the recording; if not, the follow was terminated, and another turtle was located. During follows, the observer attempted to record the species turtles were consuming and attempted to minimize their movements and noises. Observers remained at the surface throughout follows to minimize disturbance. We followed each turtle for 35 min to allow for 5 min for the turtle to become comfortable with being followed and 30 min of data collection. The 5 min adjustment period was abandoned when it was apparent that turtles resumed foraging within moments of the start of follows. Follows were terminated early if human disturbance caused the turtle to flee or the turtle moved into areas that were unsafe to snorkel (e.g., channels with high boat traffic).

Water depth, distance from turtle, and water visibility varied across focal follows. However, videos were assessed for clarity prior to data extraction. Videos in which an observer could not distinguish among seagrass species within the field of view would have been excluded from analysis.

Text S2

Methods

We estimated the straight carapace length of turtles from multiple still frames of each follow when a linear view (i.e., not angled and distorted by distance) of both the turtle carapace and the width of *Halophila stipulacea* leaves were possible. The width of *H. stipulacea* leaves was used as the standard because the flat leaves provide an identifiable and measurable surface in most of the focal follow videos. Microsoft Power Point was used to find the measurements of each turtle carapace and leaf width from still frames. We measured 374 *H. stipulacea* leaves from the samples collected for nutrient content analysis in October 2016. The length of the turtles was estimated by:

$$\frac{TL_v}{Hs_v} \times Hs_s,$$

where TL_v is the length of the turtle in a video still frame, Hs_v is the width of the *H. stipulacea* leaf in a video still frame, and Hs_s is the median width of *H. stipulacea* leaves in the samples collected. To test for the effect of turtle size on forage selection, turtles were grouped into three size classes: < 40 cm, 40-60 cm, and > 60 cm. We used a Chi-square test of independence between the turtle size class and forage selection.

Results

Videos of 29 turtles provided linear views of both the turtle carapace and *H. stipulacea* leaves for size estimation in 1 to 6 still frames with a mean standard deviation of 8.99 cm. The mean (± 1 SD) width of *H. stipulacea* leaves from the samples collected for nutrient content analysis was 6.38 ± 0.86 cm with a median and mode of 6. The lengths of 29 turtles are given in Table S1. Observed and expected values and the contributions to Chi-squared are given in Table S2.

Table S1. Mean and standard deviation (Sd) of estimated carapace lengths (SCL), selection ratios for *H. stipulacea* (Hs) and *S. filiforme* (Sf), and the selected resource of all measured turtles

SCL		Selection ratio		Selection
Mean	Sd	Hs	Sf	
33.7	4.2	1.2	0.0	Hs
38.9	11.6	1.1	NaN	Hs
24.4	3.0	0.1	1.5	NA
32.2	1.1	0.0	2.0	Sf
44.6	4.0	0.5	2.2	NA
46.6	9.6	0.5	4.1	NA
54.5	10.0	0.8	1.5	NA
40.6	6.4	0.1	5.8	Sf
48.7	7.8	0.0	3.5	Sf
50.6	16.4	0.6	3.6	Sf
52.1	7.0	0.0	2.6	Sf
52.6	NA	0.2	4.2	Sf
54.9	9.5	0.0	3.4	Sf
55.5	6.5	0.2	3.5	Sf
70.1	0.9	1.3	0.0	Hs
61.0	10.5	0.0	2.4	Sf
65.3	1.0	0.0	3.8	Sf
67.0	14.7	0.0	4.1	Sf
67.5	11.6	0.2	8.8	Sf
68.3	15.0	0.0	2.8	Sf
68.4	1.1	0.0	5.7	Sf
69.1	9.9	0.2	5.1	Sf
73.3	7.0	0.1	5.2	Sf
74.5	9.9	0.0	7.6	Sf
74.7	11.7	0.0	7.0	Sf
77.0	NA	0.0	6.0	Sf
79.4	17.8	0.0	5.1	Sf
97.5	13.9	0.1	4.6	Sf
114.8	20.7	0.1	7.1	Sf

Table S2. Observed and expected numbers of turtles within each size class with preferences for *H. stipulacea* (Hs), neither (NA), and *S. filiforme* (Sf). The Chi-square contribution of each size class is provided.

Size	Selection	Exp.	Obs.	Obs. - Exp.	χ^2
< 40	Hs	0.41	2	1.59	7.89
	NA	0.55	1	0.45	
	Sf	3.03	1	-2.03	
40 - 60	Hs	1.03	0	-1.03	2.98
	NA	1.38	3	1.62	
	Sf	7.59	7	-0.59	
> 60	Hs	1.55	1	-0.55	2.87
	NA	2.07	0	-2.07	
	Sf	11.38	14	2.62	
Total			n = 29		13.74

Text S3

The direct measurement of ambient nutrients is unnecessary because it is widely accepted that nutrient contents of seagrass leaves reflect relative nutrient availability (Atkinson & Smith 1983; Duarte 1990), and have even been used to identify sources of nitrogen inputs to the system (Fourqurean et al. 1997). We used ANOVA to test for differences in nutrient content among macrophyte species. All nutrient content data were tested for normality using Shapiro-Wilk's tests. The non-normal distribution in %C and %N of macroalgae were rectified using a \log_{10} transformation on all %C and %N data. Back transformations were performed for display of results. We used Tukey's HSD corrections, with alpha value set at 0.05, for all pairwise comparisons.

Table S3. Post-hoc comparisons among macrophytes using the Tukey's test (alpha = 0.05) for %C, %N, C:N, C:P, and N:P

Dependent Variable	(I) Species	(J) Species	Mean Difference (I-J)	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
%N	<i>H. stipulacea</i>	macroalgae	1.65	<0.0001	1.37	1.99
%N	<i>S. filiforme</i>	macroalgae	1.89	<0.0001	1.55	2.31
%N	<i>S. filiforme</i>	<i>H. stipulacea</i>	1.15	0.19	0.95	1.38
%C	<i>H. stipulacea</i>	macroalgae	1.52	<0.0001	1.41	1.64
%C	<i>S. filiforme</i>	macroalgae	1.69	<0.0001	1.56	1.83
%C	<i>S. filiforme</i>	<i>H. stipulacea</i>	1.11	0.01	1.03	1.20
%P	<i>H. stipulacea</i>	macroalgae	0.12	<0.0001	0.10	0.15
%P	<i>S. filiforme</i>	macroalgae	0.11	<0.0001	0.08	0.14
%P	<i>H. stipulacea</i>	<i>S. filiforme</i>	0.01	0.54	-0.01	0.04
C:N	macroalgae	<i>H. stipulacea</i>	1.94	0.37	-1.56	5.44
C:N	macroalgae	<i>S. filiforme</i>	2.69	0.19	-1.05	6.43
C:N	<i>H. stipulacea</i>	<i>S. filiforme</i>	0.75	0.86	-2.75	4.25
C:P	macroalgae	<i>H. stipulacea</i>	780.59	<0.0001	545.44	1015.74
C:P	macroalgae	<i>S. filiforme</i>	687.50	<0.0001	436.12	938.89
C:P	<i>S. filiforme</i>	<i>H. stipulacea</i>	93.09	0.59	-142.06	328.24
N:P	macroalgae	<i>H. stipulacea</i>	31.70	<0.0001	21.27	42.13
N:P	macroalgae	<i>S. filiforme</i>	26.14	<0.0001	14.99	37.29
N:P	<i>S. filiforme</i>	<i>H. stipulacea</i>	5.56	0.40	-4.87	16.00

References

- Atkinson, A. M. J., and Smith, S. V. (1983). C : N : P ratios of benthic marine plants. *Limnology and Oceanography*, 28(3), 568–574.
- Duarte, C. M. (1990). Seagrass nutrient content. *Marine Ecology Progress Series*, 67(April), 201-207–207. <https://doi.org/10.3354/meps067201>.
- Fourqurean, J. W. et al. (1997). Spatial and temporal variation in C:N:P ratios, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ of eelgrass *Zostera marina*. *Marine Ecology Progress Series*, 157(October), 147–157.

Text S4

To test our four mutually-exclusive hypotheses (main text Figure 1), we quantified the effects of seagrass community composition to our proxy for preference, proportion of bites taken of each species (per bout and cumulative). Data on percent cover of each macrophyte were tested for normality with Shapiro Wilk's tests. The inability to achieve a normal distribution after multiple transformation attempts and high dispersion of our data led us to use generalized linear regression (GLM, family = quasipoisson). Akaike's Information Criterion (AIC) scores were used to determine best model fit.

The combination of predictor variables that produced the lowest AIC score for all GLMs included the percent cover (as percent macrophyte cover) of the native seagrass *S. filiforme*, the invasive seagrass *H. stipulacea*, and macroalgae (Table S4). No single predictor variable or combination of predictor variables significantly affected the number of bites taken per bout (bite rate) of the invasive *H. stipulacea* (Table S4). The percent cover of *H. stipulacea* did significantly affect bite rate, and the bite rate of native *S. filiforme* was significantly affected by the percent covers of *H. stipulacea* and *S. filiforme* as well as the interaction between the percent covers of *H. stipulacea* and *S. filiforme*.

Table S4. *P* values from GLM testing the effect of percent cover on the number of bites taken (Factor); Hs (*H. stipulacea*), Sf (*S. filiforme*), and Algae (macroalgae) and all possible interactions were tested; asterisks represent significant affects

Factor	df	Hs	Sf	Algae	Hs x Sf	Hs x Algae	Sf x Algae	Hs x Sf x Algae
Total bites	124	0.001*	0.160	0.685	0.120	0.811	0.334	0.501
Bites of Hs	124	0.370	0.866	0.234	0.995	0.350	0.270	0.457
Bites of Sf	124	<0.001*	<0.001*	0.662	0.016*	0.804	0.786	0.624

Text S5

Individual selectivity; 30 of the 35 turtles foraged selectively, three selected for the invasive seagrass (*H. stipulacea*), 26 positively selected for the native seagrass (*S. filiforme*), and one selected both seagrasses; test of habitat selection:

Table S5. Selectivity test statistic (Khi2Lj), degrees of freedom (df), *P*; Selection Ratios (*Wi*) for each resource: *H. stipulacea* (Hs), *S. filiforme* (Sf), macroalgae (Algae); Resource with positive selection: NA indicates turtles that did not feed selectively

TurtleID	Test of habitat selection			Selection Ratios (<i>Wi</i>)			Selection
	Khi2Lj	df	pvalue	Hs	Sf	Algae	
G1501	32.02	1	0.0000	0.11	4.63	0.00	Sf
G1502	1.12	0	0.0000	1.13	NaN	0.00	Hs
G1503	1.70	0	0.0000	1.17	0.00	0.00	Hs
G1504	31.80	1	0.0000	0.19	8.79	0.00	Sf
G1505	47.13	0	0.0000	0.00	6.96	0.00	Sf
G1506	32.37	1	0.0000	0.01	5.68	0.00	Sf
G1507	2.20	0	0.0000	1.27	0.00	0.00	Hs
G1508	41.16	0	0.0000	0.00	5.75	0.00	Sf
G1509	29.05	1	0.0000	0.14	7.09	0.00	Sf
G1510	34.44	0	0.0000	0.00	5.11	0.00	Sf
G1601	23.07	1	0.0000	0.22	4.23	0.00	Sf
G1602	31.83	1	0.0000	0.06	5.76	0.00	Sf
G1603	41.15	1	0.0000	0.02	8.07	0.00	Sf
G1604	20.44	2	0.0000	0.24	5.09	0.02	Sf
G1605	25.11	0	0.0000	0.00	3.75	0.00	Sf
G1606	34.85	0	0.0000	0.00	6.02	0.00	Sf
G1608	13.52	2	0.0012	0.02	2.35	0.13	Sf
G1609	5.47	2	0.0650	0.51	2.22	0.31	NA
G1610	5.75	1	0.0165	1.39	1.43	0.00	Hs/Sf
G1611	5.52	2	0.0634	0.48	4.05	0.05	NA
G1612	4.83	2	0.0893	0.35	1.86	0.21	NA
G1613	15.01	2	0.0005	0.64	3.57	0.15	Sf
G1614	47.67	1	0.0000	0.00	7.56	0.28	Sf
G1615	20.69	2	0.0000	0.03	9.39	0.29	Sf
G1617	16.57	1	0.0000	0.02	1.98	NaN	Sf
G1618	2.70	0	0.0000	0.00	1.78	0.00	Sf
G1619	27.54	1	0.0000	0.00	4.07	0.56	Sf
G1620	0.97	1	0.3259	0.78	1.46	0.00	NA
G1621	18.43	1	0.0000	0.00	3.52	0.01	Sf
G1622	2.31	1	0.1282	0.08	1.47	0.00	NA
G1623	10.63	0	0.0000	0.00	2.56	0.00	Sf
G1624	27.13	2	0.0000	0.05	5.24	0.18	Sf
G1625	14.00	2	0.0009	0.22	3.53	0.08	Sf
G1626	24.13	0	0.0000	0.00	2.77	0.00	Sf
G1627	16.14	0	0.0000	0.00	3.41	0.00	Sf

Text S6

We tested individual selectivity of resources using data collected on six turtles that were followed on more than one occasion. Individual selectivity was stable across multiple follows for 4 individuals that all consistently selected for *S. filiforme*. Selection indices changed between follows for 2 turtles, however, they also positively selected for native *S. filiforme* during at least one follow.

Fig. S1. Selectivity indices for *H. stipulacea* (Hs), *S. filiforme* (Sf), and macroalgae (Algae) during multiple follows of individual turtles; G1501, G1504, G1608, and G1621 were followed twice each; G1604 and G1611 were followed four times each; values above 1.0 indicate positive selection, values below 1.0 indicate avoidance, and values near 1.0 indicate no apparent preference; asterisks denote differences in selection among follows of the same individual

