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Impacts of an abundant introduced ecosystem engineer within mudflats of the southeastern US coast

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Abstract Invasive ecosystem engineers can have far-reaching effects on systems, especially if they provide structure where none was before. The non-native seaweed *Gracilaria vermiculophylla* has proliferated on estuarine mudflats throughout the southeastern US, including areas (South Carolina and Georgia) that historically were extremely low in seaweed biomass. Quantitative field surveys across 150 km of high salinity estuaries revealed that the density of the native onuphid polychaete *Diopatra cuprea* and the aboveground height of its biogenic tubes, which *Diopatra* decorates with drifting debris

and seaweed, positively influenced *Gracilaria* biomass. The abundance of *Gracilaria* epifauna, composed primarily of amphipods and small snails, increased with *Gracilaria* biomass at many locations in our field surveys. To examine whether epifauna were facilitated by *Gracilaria* we experimentally manipulated *Gracilaria* biomass in two locations. Consistent with the field surveys, we found that increasing *Gracilaria* biomass facilitated epifauna, particularly amphipods and snails. Epifaunal densities on *Gracilaria* were higher than on a biologically-inert structural mimic of *Gracilaria* (plastic aquarium alga), indicating that epifauna colonize *Gracilaria* because *Gracilaria* provisions both physical structure and a biological resource. We also quantified the seaweed's net rate of productivity and decomposition. Primary production of *Gracilaria* was variable, but massive in some areas (up to 200 % net biomass increase in 8 weeks). The seaweed rapidly degraded upon burial in silty sediments (79 % loss in mass within 10 days) and thus may represent an important new addition to detrital foodwebs. As a copious, novel source of primary production, detritus, and desirable habitat for epifauna, *Gracilaria* has the potential to transform southeastern US estuaries.

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Introduction

Introduced species with the highest impact are often those that alter ecosystem functions (e.g. Parker et al. 1999; Hooper et al. 2005). Such alterations are typically generated by a relatively small number of invaders that create physical structure, including important biogenic habitat, de novo (Jones et al. 1994; Crooks 2002; Byers 2007; Hastings et al. 2007). By altering physical structure, these non-native ecosystem engineers alter local abiotic conditions, interactions between species, and species composition. If such a structurally important invasive species is also highly productive or qualitatively different in nutrient composition from native species, the invader may affect ecosystem functions even more strongly (Allen 1998; Kennedy and Hobbie 2004; Ricciardi and Atkinson 2004).

A non-native seaweed (*Gracilaria vermiculophylla*, hereafter *Gracilaria*) has recently established itself in nearshore habitats in Georgia and South Carolina. Originally from east Asia, *Gracilaria* has colonized the U.S. west and east coasts and northern Europe (Freshwater et al. 2006; Thomsen et al. 2006, 2007, 2009). *Gracilaria* is rapidly proliferating along the mudflats in many Georgia and South Carolina estuaries. The seaweed invasion in these states is particularly novel and noteworthy because these mudflats were historically devoid of macrophyte-based primary production and structure (e.g., Blackwelder 1972; Sandifer et al. 1980). Atlantic coast estuaries of the southeastern USA are highly turbid, and the soft-sediment substratum is often too fluid to facilitate seaweed growth and attachment. These physical characteristics are particularly pronounced in Georgia and South Carolina, which have a lower abundance and diversity of macrophytes than areas in North Carolina or Florida (Stephenson and Stephenson 1952; Sandifer et al. 1980) including a virtual absence of seagrass beds. It is not clear why *Gracilaria* succeeds where other benthic seaweeds and seagrasses largely fail, though *Gracilaria* appears to be tolerant of stresses in salinity, light and temperature within some experimental tests (Thomsen and McGlathery 2007; Weinberger et al. 2008; Nyberg and Wallentinus 2009). Its success is apparently facilitated in some locations by native tube worms (genus *Diopatra*; family Onuphidae) which attach the seaweed to hardened tubes (Thomsen and McGlathery 2005;

Berke 2012) and allow *Gracilaria* to maintain high intertidal abundance on mudflats without hard substrata (Fig. 1).

Because *Gracilaria* has few to no native analogues on mudflats of the southeastern U.S., this system represents a unique opportunity to examine the consequences of invasions within a previously unexploited niche of a community. (Note: Although not abundant on mudflats, there are several *Gracilaria* species native to the eastern US, therefore we sequenced several samples collected from our study region and confirmed that they were all *G. vermiculophylla*; Appendix A in Supplementary material). We predict that the seaweed's novelty coupled with its current abundance is increasing the primary productivity of the region's mudflats, generating inputs into detrital foodwebs, and facilitating secondary production of organisms not often found in these areas of the estuary because they rely on aboveground physical structure.

As an initial effort to understand the extent of *Gracilaria*'s impact and which taxa it affects, we designed an observational study to inform: (1) the macro-spatial distribution of *Gracilaria* abundance in Georgia and South Carolina, (2) the association of aboveground tubes of the polychaete *Diopatra cuprea* with *Gracilaria* abundance on a finer scale, and (3) the biomass-dependent effects of *Gracilaria* on epifaunal species. We coupled these field surveys with a field experiment in which we manipulated *Gracilaria* abundance to isolate its influence on community structure and development. As part of this experiment, we used a structural mimic of *Gracilaria* to parse the effects of *Gracilaria*'s physical structure from its biotic influences on epifauna. To further quantify the extent of *Gracilaria*'s impact on the community we quantified its net productivity (biomass change over time) and decay rate when buried.

Methods

Field survey

To examine the influence of *Gracilaria* on native epifaunal communities of southeastern mudflats and to determine the factors associated with increased *Gracilaria* biomass, in May 2009 we selected 11 sites within multiple estuaries along 150 km of the Georgia and



Fig. 1 *Gracilaria vermiculophylla* on the mudflat and lower marsh at Wappoo Cut, a high density site in Charleston Harbor, SC. Nearly every algal piece is held into place on this mudflat by the onuphid worm *Diopatra cuprea*, which actively decorates its

South Carolina coast. Specifically, within each of the 3 regional target areas of Savannah, Hilton Head, and Charleston we sampled 4–5 sites separated by at least 1 km and usually at least 2 km. (Two pairs of sites at Hilton Head were later determined to be 0.6 km apart and so the data for each pair were treated as a single site). This sampling design ensured that we

tube with the seaweed. *Lower panel* shows the 3–4 cm long aboveground portion of a *Diopatra* tube with *Gracilaria* attached

systematically spread our effort along the coastline, but still captured smaller scale factors potentially affecting *Gracilaria* (Fig. 2).

At each site during low tide, we set five transects perpendicular to the water line that extended from the bottom edge of the *Spartina*-marsh and intertidal oyster beds and to approximately 0 m MLLW. To

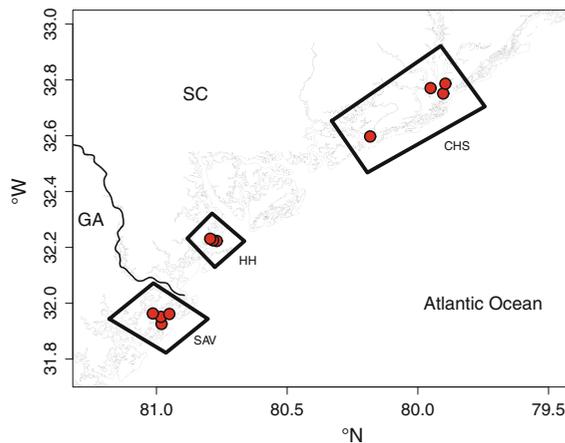


Fig. 2 Sites of the 2009 field survey of *Gracilaria vermiculophylla* along the Georgia and South Carolina coastline. CHS Charleston, HH Hilton Head and SAV Savannah. Site coordinates given in Appendix B in Supplementary material)

ensure systematic coverage over the whole lower intertidal area, along each transect we placed three evenly spaced 1 m² quadrats, for a total of 15 quadrats per site. (Exceptions were Charleston sites 2, 3, and 4 which for logistical reasons were sampled with 10, 14, and 10 quadrats respectively). We photographed the quadrat, counted all tube worms (which were predominately *Diopatra cuprea*, hereafter *Diopatra*), measured above-ground tube heights to the nearest 0.5 cm, and estimated *Gracilaria* percent cover visually. We positioned a 0.25 m² quadrat in a haphazardly-chosen corner within the 1 m² quadrat and collected all *Gracilaria* (and its associated biota) into a sealed plastic bag. Thus our subsequent use of the word epifauna is meant to refer to species living in *Gracilaria*, not on the mud surface. In the laboratory, we rinsed the *Gracilaria* in freshwater for 1 min, strained the emerged epibiota on a 500 micron mesh and quantified the blotted wet biomass of *Gracilaria*. For comparative purposes these epifaunal and *Gracilaria* biomass measurements were scaled to abundance or mass per m². Using a dissecting microscope we enumerated and identified the organisms to broad taxonomic categories (amphipods, gastropods, etc.).

We first analyzed these data to understand how the density and tube heights of *Diopatra* predicted *Gracilaria* percent cover, and whether the worm's influence was consistent across sites. For this we used an analysis of covariance (ANCOVA) to examine the effects of *Diopatra* density and tube height

(covariates) and site (categorical factor) on *Gracilaria* percent cover (logit-transformed). One quadrat at the Hilton Head 1 site was excluded from this analysis as an outlier. We examined all terms and interactions in the model including plots of residuals versus predictor variables for the absence of any pattern, i.e., no evidence of nonlinearity or heteroscedasticity. When significant interactions of the categorical factor (site) with the covariates existed, these terms were left in the model and post hoc tests were used to examine the site-specific effects of the covariates. Second, to examine the effects of *Gracilaria* biomass on epifauna we used generalized linear models (GLM) in an analysis of deviance framework. For these, we focused on all epifaunal gastropods and amphipods because these taxonomic groups represented $81 \pm 3\%$ of total epifauna (Mean \pm S.E.). These untransformed data were highly non-linear with many zeros, and thus, GLMs were performed with Poisson, quasi-Poisson or negative binomial distributions (depending on the degree of data dispersion; following Zuur et al. 2009). (We tested and found that no data set in the field survey for epifauna was grossly zero-inflated, with the exception of gastropods. Apparently due to the large number of sites, we cannot run the zero-inflated analysis on gastropods (i.e., df are insufficient). Thus, we retained our analysis that use a quasi Poisson fit for gastropods). Analysis of deviance models used site as a random categorical factor and *Gracilaria* biomass as a continuous covariate. Because nearly all such models revealed a significant interaction term, we generated posthoc analyses to explore response variables within individual sites. For analyses of epifaunal responses, two Charleston sites (3 and 4) were excluded because they had two or fewer 0.25 m² quadrats that contained *Gracilaria*.

Manipulative field experiment

To determine if the patterns we saw in the diversity and abundance of epibiota in the field survey were driven by *Gracilaria* abundance per se, we initiated an experiment that manipulated *Gracilaria* biomass. Furthermore, we sought to measure the net productivity of *Gracilaria* and whether it was biomass dependent. To address the spatial consistency of our findings, we conducted this experiment at two separate sites (Wassaw Sound, near Savannah, GA and Charleston Harbor, SC; see Appendix B in

Supplementary material) for coordinates of sites SAV2 and CHS1, respectively).

We constructed cylindrical cages 0.5 m in diameter and 0.3 m in height from PVC-coated chicken wire. The mesh size (2.5 cm) was large enough to allow settlement and movement of most members of the benthic community, retain larger pieces of algae that might break off and otherwise drift away, but prohibit access by macropredators and herbivores. We inserted the cages into the estuarine mudflat in the intertidal zone (approximately +0.5 m above MLLW) centered on a cluster of 3–5 *Diopatra* worm tubes since almost all intertidal *Gracilaria* is attached to *Diopatra*. We collected *Gracilaria* and rinsed it in freshwater for 1 min to defaunate it. Using a 30-cm garden staple we secured *Gracilaria* into the center of seven replicate cages of three treatment levels of low (12 g), medium (60 g), and high biomass (120 g). These appropriate treatment biomass values were determined from our field surveys. The *Diopatra* attach the *Gracilaria* to their tubes overnight (personal observations), although staples were left in place to minimize disruption to treatments. One replicate of the medium biomass treatment in Charleston was lost during the experiment.

We also employed seven replicates of a treatment designed to parse the biological effects of *Gracilaria* from its purely structural effects. Specifically, we used a structural mimic treatment that was a plastic aquarium seaweed with a very similar form to *Gracilaria*. We desired to standardize the volume of the *Gracilaria* mimic to match the volume of the medium biomass treatment of real *Gracilaria*. Thus we lightened the mass of the *Gracilaria* mimic to 43 g due to its slightly lighter density of material. This seaweed mimic was pinned down next to *Diopatra* in the cages, and the worms attached it to their tubes within 1–2 days. Because the plastic seaweed has similar structure to its live analogue, but is biologically inert, by comparing it to live *Gracilaria* treatments we could infer the degree to which associations of biota are due to the physical structure alone versus both physical and biological aspects (e.g. food or moisture provisioning).

After 8 weeks, (25–29 June to 17–19 August 2009) we removed all *Gracilaria* and plastic mimics and bagged them to retain associated epifauna. To characterize the substratum at each experimental site, we took sediment cores within each cage (20 ml, 2.1 cm

diameter). In the lab, we quantified the final biomass of the wet and dried *Gracilaria* and identified and quantified all biota using the same methods as above for processing of field survey samples. We quantified sediment grain size by analyzing cores using a combination of wet sieving for the sand fraction and pipette analysis for the silt and clay fraction as described in Buchanan (1984).

To examine whether *Gracilaria* exhibits biomass dependent growth or loss, we used a 2-way ANOVA to analyze the effects of the initial biomass treatments (low, medium, and high) and site (Charleston and Savannah) on relative growth rate (or RGR; Hoffmann and Poorter 2002). RGR appears normally distributed and homoscedastic. Next, because the biomass of *Gracilaria* changed over the experiment we used regression based analyses to examine how the abundance of epifauna responded to final *Gracilaria* wet mass. Specifically, we used analysis of deviance to examine the effects of *Gracilaria* biomass on the total abundance of associated epifauna and on the major constituent taxonomic groups of the epifauna, all fit with negative binomial distributions. Another advantage of this regression-based approach is that we could more readily compare side by side the experimental results to the survey results, which similarly examined epifaunal responses as a function of continuous variation in *Gracilaria* wet mass. Finally, we compared epifaunal responses in those replicates with a final medium biomass (~60 g) of real *Gracilaria* to those in the similarly sized *Gracilaria* mimic treatment. For these comparisons we used analyses of deviance to analyze the responses of taxonomic groups of epifauna (amphipods, snails, crab larvae, and all epifauna combined) fit with Poisson distributions.

Decomposition

Experimental *Gracilaria* replicates grew less at Savannah relative to Charleston experimental sites, and field observations suggested sediment accumulation may accelerate *Gracilaria* decomposition rates. To test whether burial is a plausible means for *Gracilaria* to lose mass during our experiment (and thus also contribute to the detrital food webs), we quantified rates of degradation of live tissue upon burial. We collected clumps of *Gracilaria* at our Savannah site from the intertidal zone and rinsed them

in freshwater to remove animals and sediment. We then blotted the alga dried and weighed it. We buried 8 intact clumps of *Gracilaria* in masses that spanned a continuum of clump sizes seen in the field (10–155 g). We buried these distinct clumps ~3 cm beneath the sediment surface at ~+0.5 m MLLW and allowed them to sit for 10 days. After 10 days we recovered the remaining *Gracilaria*, rinsed it, blotted it dry and weighed it. We calculated the difference in wet weight before and after burial and the percent mass loss for each replicate.

Results

Field survey

In our regional field survey we measured a maximum of 250 g/0.25 m² of *Gracilaria*, a mean of 10.6 g/0.25 m², and a median of 1.1 g/0.25 m². The density and average tube height of *Diopatra* tubes significantly affected *Gracilaria* percent cover, however, their effects were highly site specific ($R^2 = 0.75$; $P < 0.0001$, Table 1). Post hoc analysis of the ANCOVA indicated that the more influential factor of *Diopatra* density had a significantly positive effect at 8 out of 11 sampled sites and was neutral at the remaining three sites (Table 1; Fig. 3, Appendix C in Supplementary material). Tube height significantly affected *Gracilaria* percent cover at two sites, both positively (Appendix D in Supplementary material).

Table 1 ANCOVA of the field surveys examining the effect of *Diopatra* density, tube height, and site on *Gracilaria* percent cover (logit transformed)

Factor	df	Sum of squares	F	P
Site	10	12.08	7.42	<0.0001
Density	1	3.08	18.91	<0.0001
Site × density	10	4.39	2.69	0.0048
Tube ht	1	0.38	2.36	0.13
Site × tube ht	10	3.89	2.39	0.012
Density × tube ht	1	0.18	1.08	0.30
Site × density × tube ht	10	1.10	0.68	0.75
Error	137	22.31		

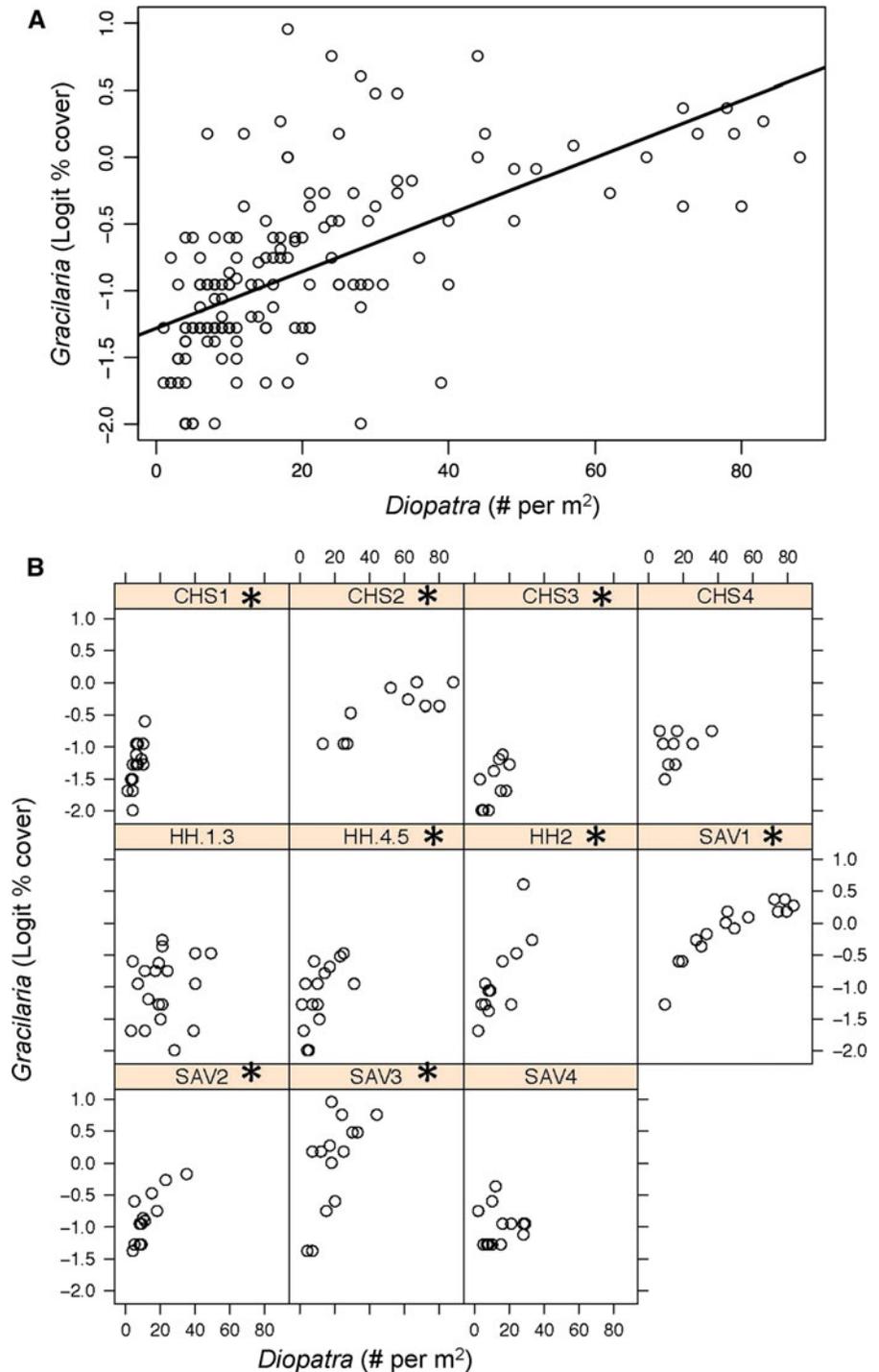
Gracilaria biomass significantly influenced total epifaunal abundance, though the strength of the effect was site-specific (Table 2). Three sites out of 9 tested (HH1&3; HH4&5; SAV2) showed significantly positive patterns, while the effect was neutral or trending positive in the remaining six sites (Fig. 4, Appendix E in Supplementary material). Amphipods (mostly *Gammarus* sp.) comprised most of the epifaunal abundance, accounting for 71 % of the total. The trends and significance patterns for amphipods were thus identical to those for total epifauna (Fig. 5; Table 2, Appendix F in Supplementary material). Gastropods (mostly *Astyrus lunata*, *Costoanachis avara*, *Ilyanassa obsoleta*, and *I. trivittata*) were the second most numerous epifauna at 10 % of total. Gastropods also showed a positive association with increasing *Gracilaria* biomass, however, the pattern was again site specific, driven in particular by strong effects at two sites (HH2, SAV2) (Fig. 6, Appendix G in Supplementary material).

Manipulative field experiment

At the end of our 2-month experiment, the biomass of *Gracilaria* correlated with the initial amount added at the beginning of the experiment; that is, cage treatments that started with the most algae ended with the most. Surprisingly, there was a tendency to lose far more *Gracilaria* biomass in Savannah, and *Gracilaria* even gained biomass in Charleston in the lowest initial biomass treatment (Table 3; Fig. 7). In Charleston, *Gracilaria* initiated at low biomass levels increased its biomass 200 % after two months (or a growth rate between 1 and 2 % per day), while *Gracilaria* did not have a positive growth rate in the mid or high biomass treatment. In Savannah *Gracilaria* lost biomass across all treatments (nearly 1–3 % per day) with higher proportional losses the greater the initial biomass.

Because final biomass was better characterized as a continuous variable than as discrete categories of low, medium and high treatments, we analyzed epifaunal abundances using an ANCOVA regression approach. As was seen within several sites of the field survey, we documented positive non-linear effects of *Gracilaria* mass on amphipods, gastropods and all epifaunal combined (Bottom panels of Figs. 4, 5, 6). The effect of *Gracilaria* was universally positive (Table 4).

Fig. 3 The influence of *Diopatra* (#/m²) on *Gracilaria* percent cover (per m²) (logit transformed) in field surveys at all sites combined (*top panel*) and separated by location (*bottom panel*). Asterisks in the *bottom panel* indicate sites that show a significant relationship, all positive



The plastic *Gracilaria* treatment in Charleston had significantly fewer epifauna relative to real *Gracilaria*, a pattern seen across amphipods, gastropods, crab larvae, and all epifauna combined. Epifaunal densities

were nearly equivalent in real and plastic *Gracilaria* in Savannah (Table 5; Fig. 8).

Upon take down of the experiment in Savannah (but not Charleston) we observed some burial of *Gracilaria*

Table 2 Analysis of deviance of the field surveys for all epifauna, gastropods, and amphipods

Factor	df	Deviance	Residual df	Residual deviance	$P(> \chi^2)$
Epifaunal amphipods. Negative binomial fit					
NULL	–	–	94	180.02	–
<i>Gracilaria</i> mass	1	17.23	93	162.79	<0.0001
Site	8	28.70	85	134.09	0.00036
<i>Gracilaria</i> mass × site	8	18.80	77	115.29	0.016
Epifaunal gastropods. Quasi-poisson fit					
NULL	–	–	94	1680.13	–
<i>Gracilaria</i> mass	1	377.23	93	1302.90	<0.00001
Site	8	573.94	85	728.96	<0.00001
<i>Gracilaria</i> mass × site	8	286.26	77	442.69	<0.00001
All epifauna. Negative binomial fit					
NULL	–	–	94	197.71	–
<i>Gracilaria</i> mass	1	26.54	93	171.38	<0.00001
Site	8	27.44	85	143.93	0.00059
<i>Gracilaria</i> mass × site	8	29.75	77	114.19	0.00023

CHS 3 and 4 missing from analyses due to too few quadrats with *Gracilaria*

under the seemingly muddier sediment. A subsequent analysis of grain size confirmed that Savannah substrata had a greater proportion of finer sediments (<64 μm ; mean proportion \pm SD = 0.26 ± 0.043) than did Charleston (0.061 ± 0.011). *Gracilaria* decays quickly when buried in these muddy sediments. After 10 days of burial, all *Gracilaria* replicates lost a minimum of 43 % of initial mass, with an average loss of 79 % (± 16.7 , SD). The *Gracilaria* recovered was black and slimy. Some large pieces remained, but mostly the initial clumps were reduced to small pieces.

Discussion

The introduced *Gracilaria vermiculophylla* provides extensive intertidal vegetative structure on mudflats that are otherwise devoid of macrophyte structure for most of the year. Estimates from our field surveys indicate that 90–100 % of macroalgal biomass on these mudflats is introduced *Gracilaria*. The remainder is the green seaweed *Ulva* spp., which occurs within intertidal habitats during winter, but is rarer during the warmer months because of herbivory, temperature stress or both. Although it has been known that the infaunal worm *Diopatra cuprea* decorates its tube with available debris and flotsam

(Bell and Coen 1982; Berke and Woodin 2008), including *Gracilaria* (Thomsen and McGlathery 2005), here we demonstrate that at larger spatial scales the worm appears to be an important driver in the introduced alga's distribution. Combined, the density and the average aboveground height of *Diopatra* worm tubes and a general site effect explained 75 % of the variability in *Gracilaria*'s abundance (logit transformed). Although not as influential as *Diopatra* density, tube height presumably adds to the model's explanatory power because it reflects a greater surface area to which *Gracilaria* can be attached. Site remains an important determinant of *Gracilaria* abundance likely due to spatial variation in a number of influential factors beyond what we measured, including propagule supply, exposure, dissolved nutrients, or sedimentation.

The interaction between the intertidal native *Diopatra* and the non-native *Gracilaria* contributes to the first of three major impacts of the alga that our field surveys and manipulative experiment have revealed. Specifically, by anchoring the seaweed in favorable photic zones of the intertidal and shallow subtidal, the worm can facilitate high levels of net primary productivity. Our measure of net productivity (i.e., biomass gain after any micrograzing or fragmentation) implies that our measures of primary productivity are underestimates of gross productivity. Even so, *Gracilaria*'s

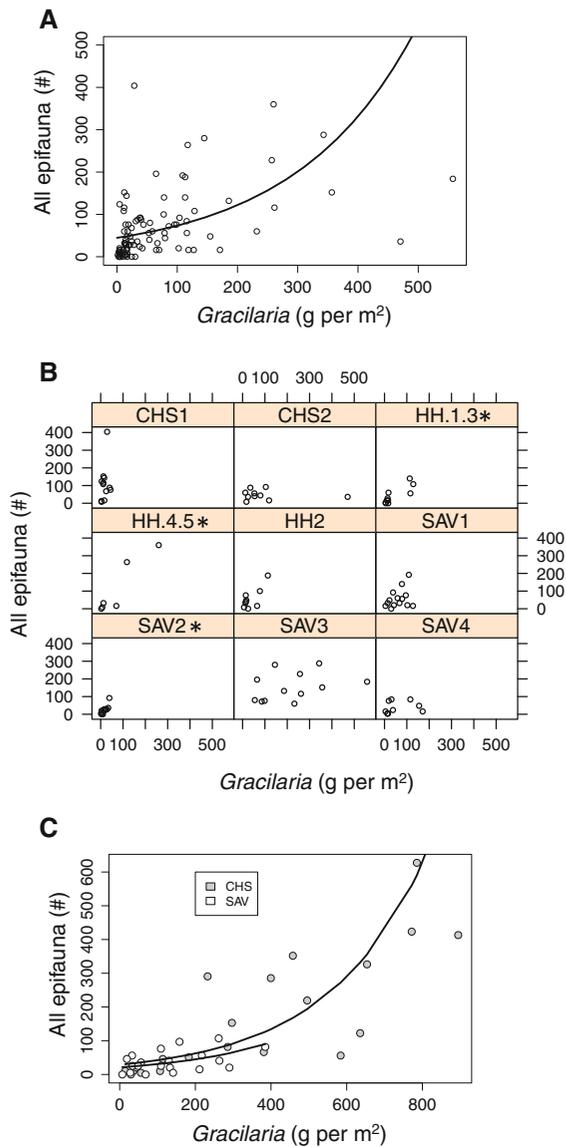


Fig. 4 The effect of *Gracilaria* biomass (g/m^2) on all epifauna ($\#/\text{m}^2$) in the field survey (*top and middle panels*) and in the experiment (*bottom panel*). Field survey model was fit using an analysis of deviance (GLM) with a negative binomial distribution. *Top panel* shows overall relationship; *middle panel* shows relationships by site with asterisks denoting sites with significant relationships (all positive) between epifauna and *Gracilaria*. *Bottom panel* plots the result of an analysis fitting epifauna with a negative binomial. *Gracilaria* biomass was a significant factor (Table 4)

maximum observed rate of growth measured in our experiment was large (200 % over two months). When high productivity of *Gracilaria* is coupled with the local biomass we measured in our regional surveys (up to 1 kg per m^2 ; mean = 40 g per m^2), we infer that

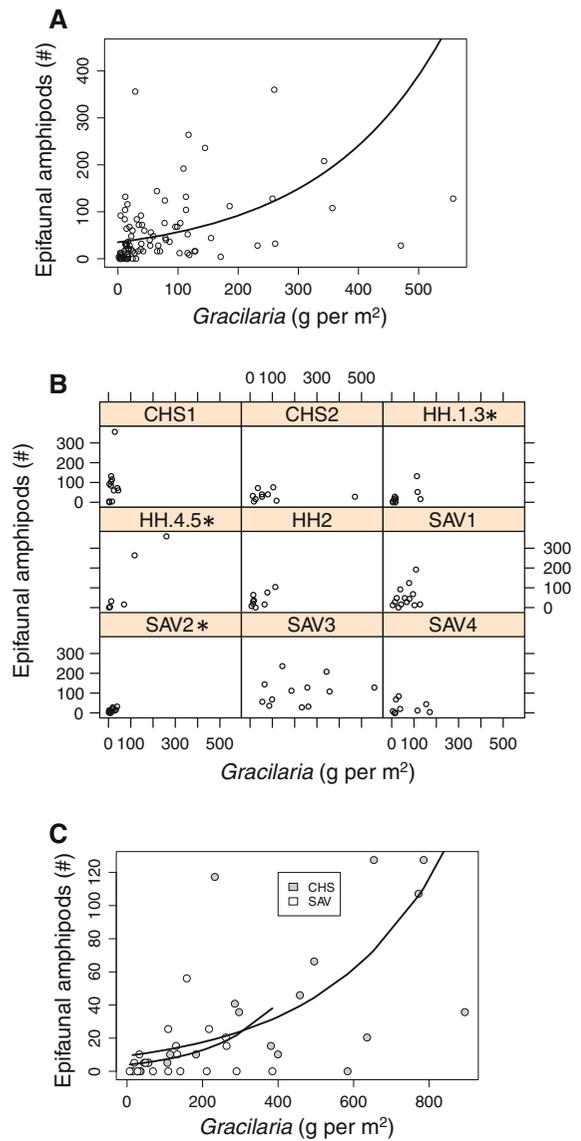


Fig. 5 The effect of *Gracilaria* biomass on epifaunal amphipod abundance in the field survey (*top and middle panels*) and in the experiment (*bottom panel*). Field survey amphipod data were fit with an analysis of deviance (GLM) using a negative binomial. *Top panel* shows overall relationship; *middle panel* shows relationships by site with asterisks denoting sites with significant relationships (all positive) between amphipods and *Gracilaria*. *Bottom panel* shows the result of an analysis fitting amphipods with a negative binomial. *Gracilaria* biomass was a significant factor (Table 4)

Gracilaria represents an enormous, novel source of primary production in a mudflat habitat previously devoid of emergent fleshy structure. Thus, under proper conditions, *Gracilaria* likely has profound effects on cycling of materials within estuarine food webs.

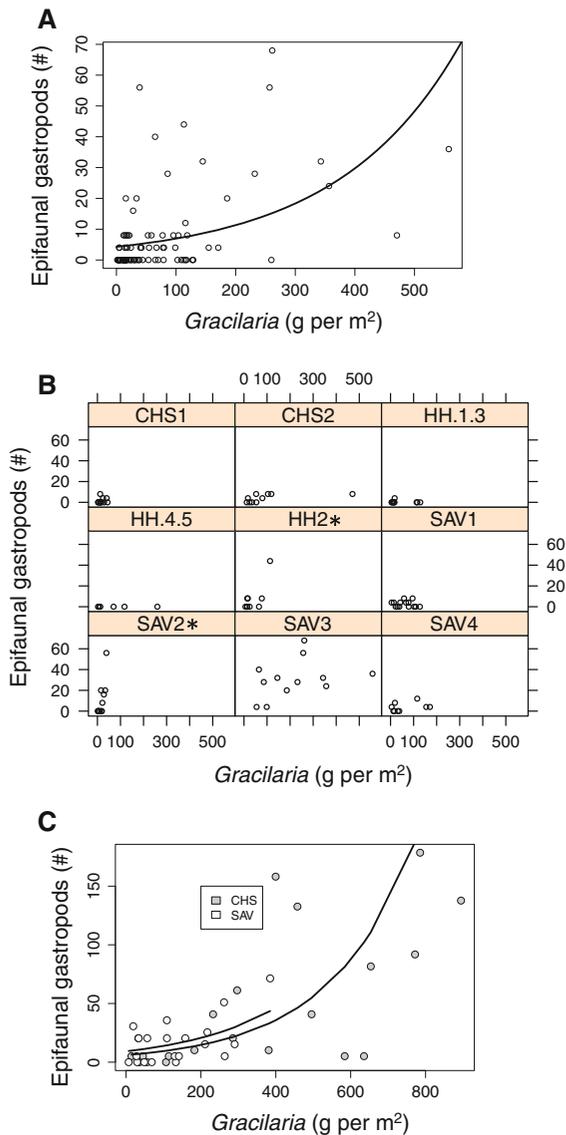


Fig. 6 The effect of *Gracilaria* biomass on epifaunal gastropods in the field survey (*top* and *middle panels*) and in experiment (*bottom panel*). Field survey gastropod data were fit using GLM and a quasi-Poisson distribution. *Top panel* shows overall relationship; *middle panel* shows relationships by site with asterisks denoting sites with significant relationships (*both positive*) between gastropods and *Gracilaria*. *Bottom panel* shows the result of an analysis fitting gastropods with a negative binomial. *Gracilaria* biomass was a significant factor (Table 4)

Whether and how *Gracilaria*'s primary production on the mudflats affects that of benthic microalgae, which prior to *Gracilaria* invasion were likely the principal source of mudflat primary productivity for most of the year, remains an ongoing topic of our investigation.

Table 3 Results of the 2-way ANOVA depicting the experimental effects of initial *Gracilaria* biomass and site on relative growth rate (% per day)

Variable	df	SS	F	P
Initial mass	2	24.9	3.78	0.033
Site	1	33.8	10.2	0.003
Initial mass × site	2	1.1	0.16	0.85
Error	35	115.4		

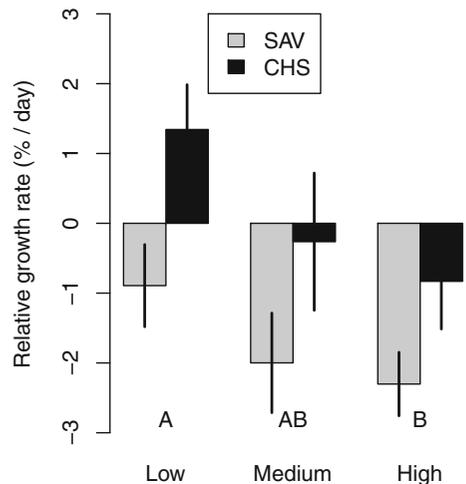


Fig. 7 Relative growth rate of *Gracilaria vermiculophylla* (\pm SE) at both experimental sites as a function of the three treatments of initial *Gracilaria* biomass (Low 12 g, Medium 60 g, and High 120 g). Letters below the bars represent significant differences in *Gracilaria* growth rates among initial biomass treatments

The algal's primary production appears to be biomass dependent, with its growth inhibited at medium and high biomasses. Specifically, individual clumps of seaweed greater than 60–100 g wet weight declined in biomass over the 2 month experiment. The highest rates of mass increase were observed in the low biomass treatments that started at 12 g and only at one of our two experimental sites. Such biomass (density) dependence/self limitation is common in seaweeds and often results from self-shading (e.g. Reed 1990; Arenas et al. 2002; Kotta et al. 2008).

The second insight from our survey and experiments is that, in addition to its rapid capacity for increase, *Gracilaria* breaks down quickly in sedimentary environments. As we saw in the depositional environment at our Savannah experimental site and in our burial trials, the seaweed loses mass quickly (see

Table 4 Analysis of deviance examining the effects of *Gracilaria* biomass on the total abundance of epifaunal amphipods and gastropods, and the total associated epifauna in the field experiment

Factor	df	Deviance	Residual df	Residual deviance	$P(> \chi^2)$
Epifaunal amphipods					
NULL	–	–	40	61.23	–
<i>Gracilaria</i> mass	1	13.97	39	47.26	0.00019
Site	1	0.033	38	46.72	0.46
<i>Gracilaria</i> mass × site	1	0.27	37	46.45	0.60
Epifaunal gastropods					
NULL	–	–	40	71.68	–
<i>Gracilaria</i> mass	1	21.96	39	49.72	<0.00001
Site	1	0.50	38	49.21	0.48
<i>Gracilaria</i> mass × site	1	0.02	37	49.19	0.87
All epifauna					
NULL	–	–	40	97.88	–
<i>Gracilaria</i> mass	1	47.91	39	49.98	<0.00001
Site	1	0.81	38	49.16	0.37
<i>Gracilaria</i> mass × site	1	0.002	37	49.16	0.97

All response variables were fit to negative binomial distributions

Table 5 Analysis of deviance comparing epifaunal responses in those replicates with a final medium biomass (~60 g) of real *Gracilaria* to those in the similarly sized *Gracilaria* mimic treatment in the field experiment

Factor	df	Deviance	Residual df	Residual deviance	$P(> \chi^2)$
Epifaunal amphipods					
NULL	–	–	24	125.42	–
Site	1	9.43	23	116.00	0.0021
Real/mimic	1	14.01	22	101.99	0.00018
Site × real/mimic	1	15.31	21	86.68	<0.0001
Epifaunal gastropods					
NULL	–	–	24	140.62	–
Site	1	4.53	23	136.09	0.033
Real/mimic	1	10.82	22	125.27	0.0010
Site × real/mimic	1	4.13	21	121.14	0.042
Epifaunal crab larvae					
NULL	–	–	24	65.80	–
Site	1	22.73	23	43.06	<0.00001
Real/mimic	1	2.54	22	40.52	0.11
Site × real/mimic	1	1.17	21	39.36	0.28
All epifauna					
NULL	–	–	24	212.86	–
Site	1	9.93	23	202.92	0.0016
Real/mimic	1	24.82	22	178.10	<0.00001
Site × real/mimic	1	25.64	21	152.46	<0.00001

All response variables were fit to Poisson distributions

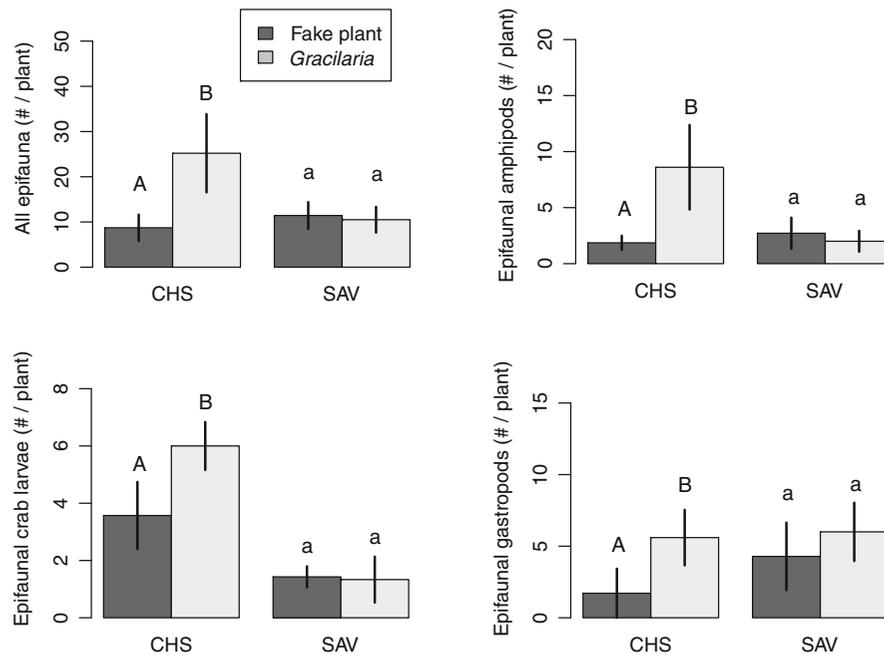


Fig. 8 'Mimic' versus 'Real' *Gracilaria*: A comparison of replicates with a final medium biomass (~60 g) of 'Real' *Gracilaria* against similarly sized 'Mimic' *Gracilaria*. All response variables were fit with Poisson distributions. Total epifaunal analysis does not include barnacles, which settled on only 'Mimic' *Gracilaria* in Charleston. Sample sizes = 7, 5, 7

also Tenore 1977a). We recognize that there are several ways by which the breakdown and detrital input of *Gracilaria* may occur, including physical abrasion of subtidal drift algae, desiccation after deposition as wrack, and leakage of nutrients from live algae. Thus, we do not consider our decomposition measurements from burial as the full picture of detrital production. However, burial is likely after storms and appears to represent one of the fastest means of decomposition in a depositional environment. *Gracilaria*'s breakdown after burial occurs at a faster rate relative to that of *Spartina alterniflora*, the salt marsh angiosperm that has traditionally been the dominant source of plant-based detritus in these estuaries (Tenore 1977a; Andersen and Hargrave 1984). *Gracilaria* has high levels of nitrogen, much higher than *Spartina* (Tenore 1977a, b). Thus, *Gracilaria*'s rapid breakdown, coupled with its different chemical and nutrient composition relative to *Spartina*, may make *Gracilaria* an important addition to detrital foodwebs.

and 6 for Charleston Mimic and Real *Gracilaria* and Savannah Mimic and Real *Gracilaria* (respectively). All comparisons within Charleston were significantly different as indicated by different grouping letters above each bar; comparisons within Savannah were not significantly different

Third, *Gracilaria* is a novel biogenic habitat that is readily colonized by amphipods and snails (Figs. 4, 5, 6; see also Nyberg et al. 2009; Thomsen 2010; Thomsen et al. 2010). Whether *Gracilaria* is ultimately boosting net rates of secondary productivity in the system or just attracting these epifaunal species is unclear. For example, the epifaunal species could be redistributing from adjacent saltmarsh or mudflat habitats. Teasing these mechanisms apart will require estimates of secondary production, as one can glean from size-distribution data on amphipods (Edgar 1993). However it would seem that the copious presence of *Gracilaria* and its heavy use as habitat is likely boosting overall regional population sizes.

The congruence of a similar positive effect in both the field survey and the experiment demonstrates the deterministic influence of *Gracilaria* on epifauna abundance. The roughly equivalent abundance of epifauna in the experiment and the field survey indicates that the build-up of epifauna is rapid (i.e., within weeks and not months). If we had not observed

results in the experiment similar to the survey, it would have suggested either that more time was needed to generate the epifaunal patterns seen in the survey, or that other process like local environmental effects, predation, or herbivory are more important in driving observed survey patterns. The fact that the two study approaches had qualitatively and quantitatively similar outcomes helps to establish *Gracilaria* biomass per se as a fundamental influence on epifauna and a sufficient mechanism to describe patterns observed in the field surveys.

The use of *Gracilaria* by epifauna appears to be due to more than just structure per se. Specifically, our plastic *Gracilaria* structural mimic in Charleston had fewer epifaunal associates than real *Gracilaria*. One of the most likely reasons for this pattern is the lack of moisture in the plastic alga. Additionally, real *Gracilaria* likely has much higher food value; herbivores consume the *Gracilaria* itself and epiphytic diatoms and microalgae that colonize its surface (Aikins and Kikuchi 2002). Our experimental cages protected epifauna from macropredators, so differential efficacy in predator protection between the real seaweed and the mimic did not influence the epifaunal pattern we quantified. The positive, though reduced value, of the mimic suggests that even apart from moisture and food *Gracilaria* has some value as structure, presumably in providing habitat and keeping the epifauna from washing away. Because we observed no differences between epifauna in plastic and real *Gracilaria* in Savannah, the structural value seems to be singularly important at that site.

Gracilaria's invasion of soft sediment habitats and subsequent effects on the native system are reminiscent of the green alga *Caulerpa taxifolia*, one of the world's best studied invasive ecosystem engineers. Like *Gracilaria*, *C. taxifolia* is capable of invading large areas of soft sediment habitat and rapidly expanding its biomass. The conversion of habitat by *C. taxifolia* from open soft substrate to dense algal canopy has been shown to affect the behavior, condition, predator susceptibility, and population dynamics of infaunal bivalves (Byers et al. 2010; Wright et al. 2010; Wright et al. 2012), as well as wholesale changes to fouling communities (Gribben et al. 2009). Once *Caulerpa* establishes, it provides three dimensional structure where little to none previously existed, on which epiflora and fauna thrive (Gribben et al. 2009).

Gracilaria's presence on mudflats is stark. As we show here, *Gracilaria* can be highly abundant and productive under the right conditions. The novel habitat it provides benefits epifauna that live among its structure in an area otherwise devoid of macrostructure. The full extent of *Gracilaria*'s impact requires more investigation, but for certain taxa, its effects are positive. Its high productivity coupled with rapid break down under the right conditions suggests a potentially important role in detrital foodwebs. As a widespread, novel source of primary production, detritus, and desirable habitat for epifauna, *Gracilaria* has the potential to transform southeastern US estuaries.

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