

Invasive décor: an association between a native decorator worm and a non-native seaweed can be mutualistic

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ABSTRACT: The likelihood of invasion success increases when non-native species engage in mutualisms with a native or non-native species. Mutualisms formed between native and non-native species have been termed 'novel mutualisms', and research in terrestrial systems has advanced our understanding of the ecological processes involved in their formation and persistence. However, documentation of novel mutualisms in marine systems is rare. In Atlantic estuaries of the southeastern USA, the native polychaete worm *Diopatra cuprea* actively decorates its tube with the non-native red seaweed *Gracilaria vermiculophylla*. We used field and laboratory experiments to test whether the *Diopatra*–*Gracilaria* interaction is mutualistic. We found that *Diopatra* facilitates *Gracilaria* by securing the seaweed onto the soft-sediment benthos within a favorable tidal elevation for growth and where hard substrata for attachment are otherwise rare. A combination of laboratory and field experiments also suggests that *Gracilaria* can enhance the growth of *Diopatra* by increasing access to epifaunal crustacean prey. However, field removal experiments showed that the benefits of *Gracilaria* to *Diopatra* only occurred in some sites and years. We found no evidence that this invader has a significant negative effect on *Diopatra*, and it appears in some instances to even benefit the worms, which suggests that *Gracilaria* (and its associated impacts on ecosystems) are likely to remain an important component of southeastern USA estuaries for the foreseeable future.

KEY WORDS: Biological invasion · Decoration association · Novel mutualism · *Diopatra cuprea* · *Gracilaria vermiculophylla*

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INTRODUCTION

The effects of non-native species on native species range from antagonistic to facilitative. Most ecological studies have focused on antagonistic interactions with non-native species (competitive, parasitic, and consumptive effects), while the roles and effects of facilitative interactions during biological invasions have been less thoroughly explored (Simberloff & von Holle 1999, Richardson et al. 2000, Bruno et al. 2005, Rodriguez 2006, Simberloff 2006). Within na-

tive communities, facilitative interactions often structure biological communities and enhance biodiversity by ameliorating abiotic stresses, or provisioning new habitat, food resources, or predator refuges (Stachowicz 2001, Bruno et al. 2003). Facilitation between native and non-native species can operate through analogous mechanisms with similarly large impacts on local communities and ecosystems (Richardson et al. 2000, Bruno et al. 2003, Altieri et al. 2010, Gribben et al. 2013). These impacts can be further enhanced when facilitation results in mutu-

alisms that generate positive feedbacks between native and non-native partners (Richardson et al. 2000, Aslan et al. 2015), or between non-native partners in novel habitats (Simberloff & von Holle 1999, Simberloff 2006).

Mutualisms between a native and non-native species have been termed ‘novel mutualisms’ (see Richardson et al. 2000, Aslan et al. 2015) and reflect the fact that the species share no evolutionary history of interaction. Novel mutualisms arise in real-time and therefore provide an opportunity to examine the early stages of mutualism formation and consequences for ecological communities and ecosystem function (Richardson et al. 2000, Aslan et al. 2015). Despite notable progress in terrestrial systems (reviewed by Richardson et al. 2000, Traveset & Richardson 2014), a recent review indicates that our understanding of the role of novel mutualisms in the sea is limited by a rarity of examples (Aslan et al. 2015).

Decoration associations (interactions in which a decorator species actively attaches a second species to its body or associated structure; reviewed in Berke et al. 2006, c.f. Ruxton & Stevens 2015) may be prime candidates for novel mutualisms because the associations commonly benefit the decorator, the décor, or both partners. For example, the decorator can gain refuge by attaching chemically-defended seaweeds that deter larger consumers, as in the urchin *Sterechinus neumayeri* (Amsler et al. 1999), and the crab *Libinia dubia* (Stachowicz & Hay 1999). Tube-building polychaete worms decorate to improve the structural integrity of the tube, to enhance the animal’s ability to detect physical disturbances (Brenchley 1976), and to ‘garden’ macrophytes to gain both direct and indirect food sources (Mangum et al. 1968, Woodin 1977, Bell & Coen 1982). Reciprocally, decoration behavior can facilitate macrophyte growth and distribution by anchoring the plant in a favorable light regime (Amsler et al. 1999, Thomsen & McGlathery 2005), increasing nutrient availability via nitrogenous waste output (Stachowicz 2001, Thomsen & McGlathery 2005), removing damaging epifauna through consumption (Stachowicz 2001), and, in the case of mobile decorators, facilitating dispersal (Amsler et al. 1999). Although these previous studies predicted decoration associations to be mutu-



Fig. 1. Native polychaete worm *Diopatra cuprea* decorates its tube cap with the non-native seaweed *Gracilaria vermiculophylla* on high-salinity mudflats of the southeastern USA. Diameter of tube opening is ~1 cm. Photo credit: Edna Diaz-Negron

alisms, most did not measure the effects of the association on the fitness of both partners (but see Amsler et al. 1999). Moreover, no previous study tested for a novel mutualism in a decoration association with a non-native species.

In this study, we tested for amutualism between the native decorator polychaete worm *Diopatra cuprea* (Bosc; hereafter *Diopatra*) and the non-native red seaweed *Gracilaria vermiculophylla* ([Ohmi] Papenfuss; hereafter *Gracilaria*) (Fig. 1). On mudflats of eastern North American estuaries, the locally abundant *Diopatra* builds infaunal tubes and actively decorates the 2–3 cm portion of its tube that projects aboveground (i.e. the tube cap) with shell debris, detritus, and drifting macrophytes, including seaweeds (Thomsen & McGlathery 2005, Berke & Woodin 2008, Berke 2012, Byers et al. 2012). Thomsen & McGlathery (2005) and Thomsen et al. (2009) hypothesized that this decoration behavior facilitates drifting seaweeds by anchoring the seaweeds within a favorable light regime. In turn, the seaweeds can serve an engineering role by ameliorating abiotic stresses (Bell & Coen 1982) and providing structure for a diverse and abundant invertebrate community (for *Gracilaria*, see Thomsen et al. 2009, 2010, Byers et al. 2012, Wright et al. 2014). Within high-salinity mudflats of South Carolina and Georgia, native seaweeds are historically rare due to a lack of appropriate substrate for attachment and high turbidity (Byers et al. 2012 and

references therein, Byers & Grabowski 2014). Yet, the highly stress-tolerant *Gracilaria* (Thomsen & McGlathery 2007) presently dominates these mudflat communities, but only where *Diopatra* are present (Berke 2012, Byers et al. 2012).

We used laboratory and field experiments to assess the net effect of *Gracilaria* and *Diopatra* on one another. We demonstrate that the anchoring of *Gracilaria* by *Diopatra* (Thomsen & McGlathery 2005, Thomsen et al. 2009) maintains the alga in a favorable light regime for growth. Decoration with *Gracilaria* appears to benefit *Diopatra* by increasing the worm's access to epifaunal crustacean prey; any benefits of *Gracilaria* as a direct food source and refuge from predation are relatively weak.

MATERIALS AND METHODS

Study sites

We conducted field surveys and experiments near Charleston, South Carolina (SC) (Fort Johnson: 32.751305°N, 79.90142°W; Stono River: 32.75253°N, 80.0076°W) and Savannah, Georgia (GA) (Priest's Landing: 31.96012°N, 81.01223°W; Bull's River: 31.97458°N, 80.92287°W) in the USA. The hydrodynamic forces in southeastern estuaries generate high turbidity and fluid soft sediments which reduce light attenuation and thus create a habitat that is largely inhospitable to macrophyte attachment and persistence (Byers et al. 2012 and references therein). *Gracilaria* invaded SC and GA estuaries in the early 2000s (E. E. Sotka unpubl. data), and on intertidal mudflats where *Diopatra* worms are common, *Gracilaria* presently represents 90–99% of the total macroalgal biomass (Byers et al. 2012). The green alga *Ulva* sp. can be found in colder months and attached to oyster shells, wooden debris, the hard calcareous tubes of the soda-straw worm and only rarely on *Diopatra* tubes (Berke 2012; N. M. Kollars, E. E. Sotka & C. Plante pers. obs.). Other macroalgae present in the system include red algae that are epiphytic on *Gracilaria* (of the genera *Polysiphonia* and *Ceramium*; Berke 2012, C. E. Gerstenmaier and E. E. Sotka pers. obs.) and rarely, *Gracilaria tikvahiae* (Berke 2012, N. M. Kollars & E. E. Sotka pers. obs.). The non-native *Gracilaria* and native *Diopatra* are both rare within the salt marshes and oyster beds that fringe the upper-intertidal edge of these mudflats. We performed all laboratory experiments within the Grice Marine Laboratory (College of Charleston, SC, USA).

Tidal distribution and growth assays

Previous tagging experiments in the coastal bays of Virginia, USA showed that attachment by *Diopatra* increases the retention of *Gracilaria* on a mudflat (Thomsen & McGlathery 2005, Thomsen et al. 2009) and hypothesized that this attachment retains *Gracilaria* in a favorable light regime for growth. We aimed to estimate the magnitude of this effect by assessing the distribution of *Gracilaria* and *Gracilaria* growth along a tidal gradient. Specifically, we sought to compare *Gracilaria* growth rates at the depth at which it is attached by *Diopatra* versus depths where the algae would otherwise be drifting (i.e. the subtidal) or accumulating on top of the sediment (i.e. the high intertidal). In late August 2013, we surveyed the abundance of *Diopatra* and *Gracilaria* on the Fort Johnson mudflat, Charleston, SC at 5 tidal heights (~+0.61, +0.09, 0.0, -0.09, and -0.91 m mean lower low water [MLLW]) that span the upper and lower tidal distributions of both species. At each tidal height, we sampled 5 replicate 3.05 × 0.5 m (~1.52 m²; long end parallel to the shore) quadrats separated by ~1.5 m. We specifically used a large sample frame in order to capture the patchy densities of *Diopatra* (and therefore *Gracilaria*) that are particularly common in the lower intertidal. Within each quadrat, we counted the number of *Diopatra* tube caps (the presence of a tube cap is an excellent proxy for a live worm; Peckol & Baxter 1986) and collected all *Gracilaria*. We removed macroscopic epifauna and epiphytes present on *Gracilaria* and dried *Gracilaria* tissue at 60°C until no change in mass occurred.

We measured the growth of *Gracilaria* at 3 tidal heights of the Fort Johnson mudflat ('high', approx. +0.61 m MLLW; 'mid' approx. +0.09 m MLLW; and 'low' approx. -0.02 to -0.09 m MLLW) during the spring (March–April, n = 3 per tidal height), summer (July–August, n = 5 per tidal height), and fall (September–October, n = 8 per tidal height) of 2013. At each tidal height, we strung a 3.00 g (± 0.05, acceptable range of variation) blotted wet mass piece of *Gracilaria* (predominantly collected from the mid-intertidal at ~0.0 m MLLW) through the end of a 15 cm piece of a 0.76 cm diameter 3-strand rope, attached the rope to a 30 cm long × 0.76 cm diameter PVC-post, and drove the post into the sediment until the seaweed laid on the surface of the benthos (see photograph of a replicate before transplantation in Supplement 1A at www.int-res.com/articles/suppl/m545p135_supp/). During the spring assay, we enclosed the seaweed within flexible mesh bags (1.5 cm mesh size) constructed from wildlife fencing and zip-

ties to protect the biomass from potential loss due to water flow. We observed few broken-off *Gracilaria* fragments present in the bags at the end of the spring assay, and therefore, we did not enclose the seaweed in the summer or fall assays. After 4–6 wk, we recovered and defaunated the seaweed and calculated change in wet mass as relative growth rate per week (hereafter RGR; Hoffmann & Poorter 2002).

To measure *Gracilaria* growth along a water-depth gradient of the subtidal zone, we weaved seaweed pieces of 3.00 g (± 0.05 , acceptable range of variation) blotted wet mass through rope and attached the rope pieces ($n = 5$) at 2.5 m intervals along a 10 m rope strung vertically between a surface buoy and a cement block. We enclosed the seaweed within mesh bags (1.5 cm mesh size) as in the spring intertidal assay. We lowered the buoy-rope-block system onto the benthos at 9 replicate locations of ~ 10 m depth (at high tide) in Charleston Harbor within 7.3 km of the Fort Johnson site. With this design, the pieces of seaweed at the '0 m' mark remained just below the surface of the water, regardless of fluctuations in tidal height. Because Charleston Harbor has an average tidal range of ~ 2 m, the other 4 chosen depths fluctuated with the tidal cycle and the true depth ranges were estimated at 0.5–2.5, 3–5, 5.5–7.5, and 8–10 m. After 8 wk (February through April 2013), we recovered and defaunated the seaweed pieces, measured blotted wet mass, and calculated RGR as before.

Because the residuals of the field survey and the intertidal and subtidal RGR assays were non-normally distributed, we analyzed differences among factors with non-parametric permutation tests and 1000 permutation replicates (Anderson 2001). We removed from analyses replicates in which all seaweed biomass was lost during the experiment (only 4% of replicates per assay) under the assumption that any biomass loss was due to methodological errors rather than a reflection of habitat conditions. In the intertidal RGR assay, we tested the interaction between season and tidal height with unrestricted data (Anderson 2001). Post-hoc significant differences were determined using a series of pairwise permutation tests. All statistical analyses were performed in R (version 2.15.1; R Core Team 2012).

Laboratory experiment

Using laboratory and field-based experiments, we tested whether *Gracilaria* increased the survivorship and growth of *Diopatra* through direct or indirect provisioning of food, or through refuge from predation.

Diopatra tubes extend up to 1 m below the benthos and it is difficult to collect *Diopatra* bodies without incidentally severing the worm. Severing, however, does not cause mortality because these animals are capable of both anterior and posterior regeneration (Berke et al. 2009). To standardize the initial size of the worm and create a point of posterior regeneration, we cut the field-collected worms to ~ 3 cm in length. We buried the worm in field-collected sediment contained in a 15 cm long \times 3 cm diameter plastic tube in the laboratory. Tubes were held in racks in a recirculating seawater table at 22°C and a salinity of 30 ppt to allow the worms to regenerate their sediment-based tubes before experimentation, which usually happened within 24 h (Berke et al. 2009, N. M. Kollars pers. obs.).

In the laboratory, we offered *Gracilaria*-associated diet items to *Diopatra* and measured survivorship and growth after 6 wk. *Diopatra* were collected from the Fort Johnson mudflat in January 2013, and placed into plastic tubes that were encircled with window screen to create feeding chambers that still allowed water flow. *Diopatra* were randomly offered one of 4 diets: sediment-only control, *Gracilaria*, amphipods, or *Gracilaria* and amphipods ($n = 24$ per treatment). *Gracilaria* was offered ad libitum and replaced weekly. Three frozen, field-collected amphipods were offered daily (primarily *Gammarus mucronatus*, the most abundant amphipod species on *Gracilaria*; Wright et al. 2014). Though we offered *Diopatra* dead amphipods due to logistical constraints, we do have video evidence that shows that, in the laboratory, *Diopatra* are capable of pursuing and catching amphipods (see video Supplement 2 at www.int-res.com/articles/suppl/m545p135_supp/). After 6 wk, we removed *Diopatra* from their sediment tubes and dried them at 60°C until no change in mass occurred and measured the final body mass. To examine the effects of diet treatment on *Diopatra* final dry weight, we used a 1-way ANOVA followed by a post-hoc Tukey's test.

To assess the incorporation of the supplied food source into the new worm tissue, we quantified the carbon and nitrogen stable isotopic signatures in the posteriorly regenerated tissue of 5 worms from each diet treatment. We randomly selected 5 individuals from each diet treatment and sampled the dried posteriorly regenerated tissue (targeting muscle tissue and avoiding the digestive tract or fecal pellets). For comparison, we also analyzed 3 samples of each resource (collected from the field in April 2013): *Gracilaria* and amphipods. We generated the isotopic signature data using a Delta V plus spectrometer

(ThermoFinnigan), with a Thermo Flash EA as the interface at the Skidaway Institute Scientific Stable Isotope Laboratory (Savannah, GA, USA).

Field experiments

To quantify the effect of *Gracilaria* on *Diopatra* growth and survivorship in the field, we manipulated *Gracilaria* density and predator access to *Diopatra* in a fully factorial and blocked field experiment over 4 wk in June 2012. We prepared worms in plastic tubes as above and out-planted them along a transect parallel to the shore at $\sim +0.15$ m MLLW in one of 4 treatments: ambient control, *Gracilaria* addition, *Gracilaria* addition and predator exclusion cage, and cage only. Two worms were added per plot and 10 plots were generated per treatment. We report the final body masses of surviving worms (if both survived we used the average of the 2 worms). Treatments of a given replicate block were within 1 m of each other and blocks were separated by at least 1 m. We added *Gracilaria* to a plot by weaving 50.00 g (± 0.05 g, acceptable range of variation) blotted wet mass of the seaweed through 30 cm of 0.76 cm 3-strand rope and placing the rope in the plot center. A pre-experimental survey indicated that the maximum seaweed biomass on a *Diopatra* individual in the field was ~ 25.0 g wet mass (data not shown). Cages were 0.5 m wide by 0.3 m tall, constructed with PVC-coated chicken wire (2.5 cm mesh size) and embedded ~ 10 cm into the sediment. Plots without cages were marked with 3 PVC posts extending <10 cm out of the sediment and separated by ~ 30 cm. After 4 wk, we removed the worms and severed the regenerated tissue from the rest of the body (see photograph in Supplement 1B at www.int-res.com/articles/suppl/m545p135_supp/) using a razor blade. We dried and measured the original body mass and regenerated tissue mass at 60°C until no change in mass occurred (see Supplement 1B for details). We collected all *Gracilaria* biomass within the plot, defaunated it, measured its wet mass and dry mass, and counted the number of whole, epifaunal amphipods.

To assess spatial and temporal variation in the effect of *Gracilaria* on *Diopatra*, we manipulated *Gracilaria* density in replicated field experiments in June 2013 at 2 sites near Charleston, SC (Fort Johnson, Stono River) and 2 sites near Savannah, GA (Priest's Landing and Bull's River). The 2013 experiments did not include a cage treatment because we did not detect a predator-exclusion effect in 2012 (see

'Results'). Relative to the 2012 experiment, the 2013 experiments used only one worm per plot, had 30 replicates per treatment, and we reduced the initial level of *Gracilaria* biomass in the addition treatment from 50 g down to 25 g wet mass. We randomly distributed plots 1 m apart along a transect parallel to the shore at approx. $+0.09$ m MLLW. In addition, we attached the seaweed-embedded ropes to PVC posts using zip-ties and pushed the posts into the mudflat to increase seaweed stability (see Supplement 1A). Plots where we did not add *Gracilaria* still contained a PVC post and empty rope. After 4 wk, we processed the worms, seaweed, and epifauna as in the 2012 experiment. Because of logistical constraints, we counted epifauna for only 2 of the 4 sites (Fort Johnson and Priest's Landing) used in 2013.

We analyzed survivorship of *Diopatra* as a function of treatment (*Gracilaria* addition and presence or absence of cage [2012 only]) using a χ^2 test. Because we did not measure *Gracilaria* final density for treatments in which worms died, we cannot pursue a logistic regression. For growth analyses, we excluded worms with evidence of sublethal predation on *Diopatra* (c.f. Berke et al. 2009; seen only in 2013 and in GA; 5 of 239 worms removed) and worms that were damaged during collection and processing (2 damaged worms out of 80 in June 2012; 15 damaged worms out of 239 in June 2013). Although the *Gracilaria* manipulation was initially designed as a categorical variable, we treated *Gracilaria* final biomass as an independent, continuous variable in our growth analysis because plots gained or lost *Gracilaria* biomass from *Diopatra* decoration activity, *Gracilaria* growth, and *Gracilaria* detachment. Moreover, epifauna abundance—an important proxy for *Diopatra* growth in the laboratory growth assay—scales positively with *Gracilaria* abundance (Supplement 1C at www.int-res.com/articles/suppl/m545p135_supp/, Byers et al. 2012). The response of *Diopatra* final size (as well as other growth metrics, Supplement 1B) was analyzed using ANCOVA with *Gracilaria* density as a covariate and cage presence as a categorical variable for the 2012 experiment, and *Gracilaria* density as a covariate and site as a categorical variable for the 2013 experiment. To assess the potential for *Gracilaria* as an indirect food source in the field (i.e. an attractor of prey species for *Diopatra*), we examined the relationship between counts of epifaunal amphipods and *Gracilaria* biomass using a generalized linear model with a quasi-Poisson distribution (Zuur et al. 2009). Finally, we used ANCOVA to examine the relationship between *Diopatra* size and the count of epifaunal amphipods.

RESULTS

Tidal distribution and growth assays

Across a tidal gradient of a single mudflat, *Gracilaria* abundance was positively correlated to *Diopatra* density ($R^2 = 0.93$, $n = 25$), and both species were most abundant in the mid intertidal (Fig. 2A). RGR of *Gracilaria* varied significantly with tidal height ($df = 2$, $F = 8.98$, $p < 0.01$) and season ($df = 2$,

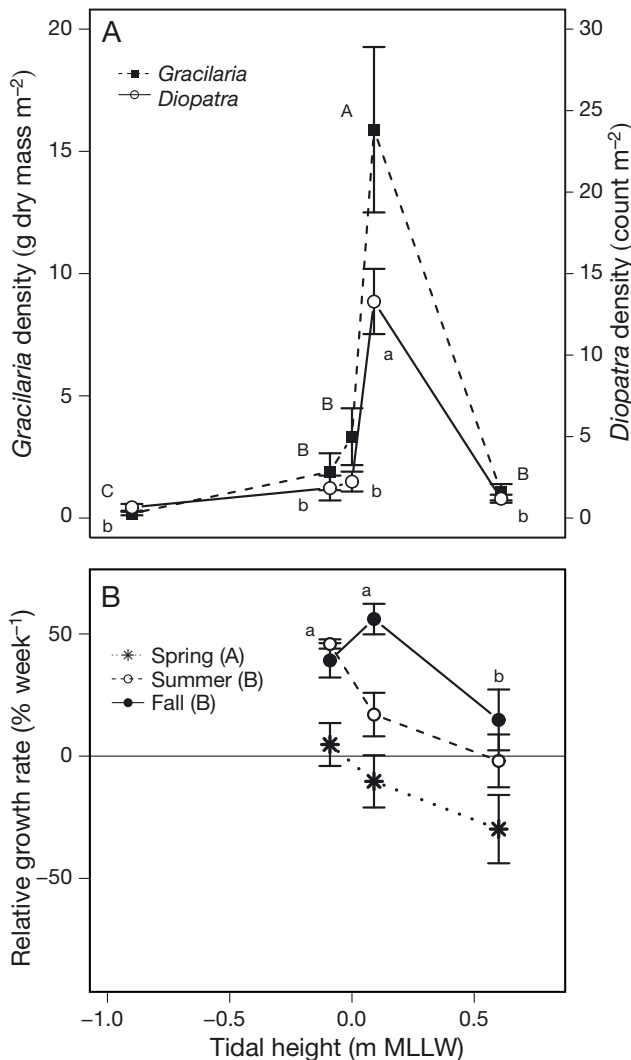


Fig. 2. (A) Mean (\pm SE) abundance of the non-native seaweed *Gracilaria vermiculophylla* and native decorator worm *Diopatra cuprea* along a tidal mudflat in Charleston Harbor, SC, USA ($n = 5$ quadrats per tidal height). Uppercase and lowercase letters reflect post-hoc differences in *Gracilaria* and *Diopatra* density, respectively ($\alpha = 0.05$). (B) Mean (\pm SE) relative growth rate (%RGR per week) of *Gracilaria* at Fort Johnson across 3 seasons. Sample sizes averaged 5 replicates per season/tidal height combination (range = 2–8). Uppercase and lowercase letters reflect post-hoc differences among seasons and tidal height, respectively ($\alpha = 0.05$)

$F = 14.61$, $p < 0.01$) with no tidal-height-by-season interaction ($df = 4$, $F = 1.71$, $p = 0.18$) (Fig. 2B). Post-hoc analyses indicated that RGR was significantly higher in the low and mid relative to high intertidal, and there was no significant difference in RGR between the low and mid intertidal. Fall and summer RGR generally exceeded spring RGR. In the subtidal, *Gracilaria* had the highest RGR (50% per week) when consistently submerged within a few centimeters of the surface, and RGR declined exponentially with depth ($df = 4$, $F = 28.3$, $p < 0.01$; Fig. 3).

Growth and survivorship

In the laboratory, access to amphipods increased *Diopatra* final size ($df = 1$, $F = 24.71$, $p < 0.01$). However, we detected no significant effect of *Gracilaria* ($df = 1$, $F = 2.25$, $p = 0.14$) nor an interaction between amphipods and *Gracilaria* on *Diopatra* final size ($df = 1$, $F = 1.18$, $p = 0.28$; Fig. 4A). Survivorship was high (only one death in the amphipod treatment) and did not differ among treatments ($df = 4$, $\chi^2 = 0.033$, $p = 0.99$). Stable isotope signatures of *Diopatra* offered

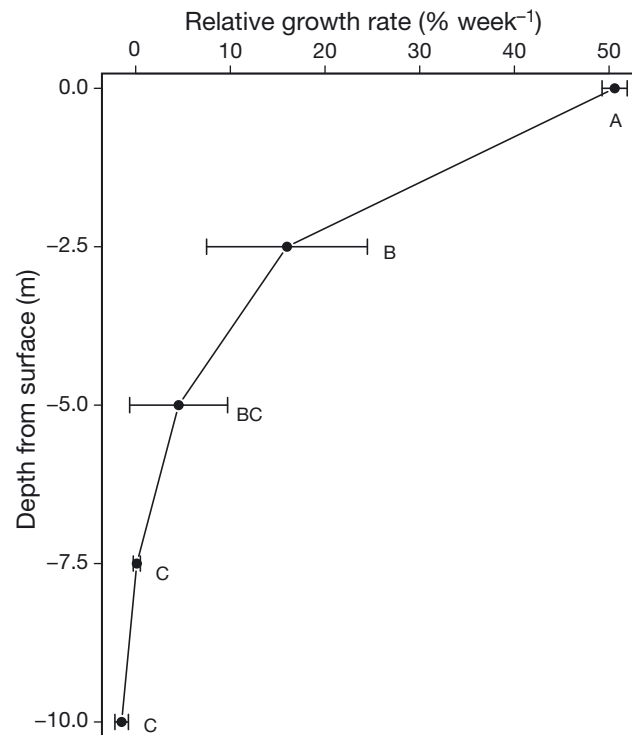


Fig. 3. Mean (\pm SE) growth rate of *Gracilaria* (%RGR per week) with depth below the water surface ($n = 4$ –5 per depth) from subtidal growth assays in Charleston Harbor, SC, USA. Letters reflect post-hoc differences in depth ($\alpha = 0.05$)

both *Gracilaria* and amphipods more closely resembled those of *Diopatra* offered only amphipods than *Diopatra* offered only *Gracilaria* (Fig. 4B).

In the 2012 manipulative field experiment, we detected no effect of *Gracilaria* addition, cage, nor their interaction, on survivorship (75–90% survivorship across treatments; $df = 1$, $\chi^2 = 0.059$, $p = 0.81$). An increase in *Gracilaria* density significantly increased *Diopatra* final size (β_1 : 0.0045; Fig. 5A; see Supplement 1B for ANCOVA statistical tables and a com-

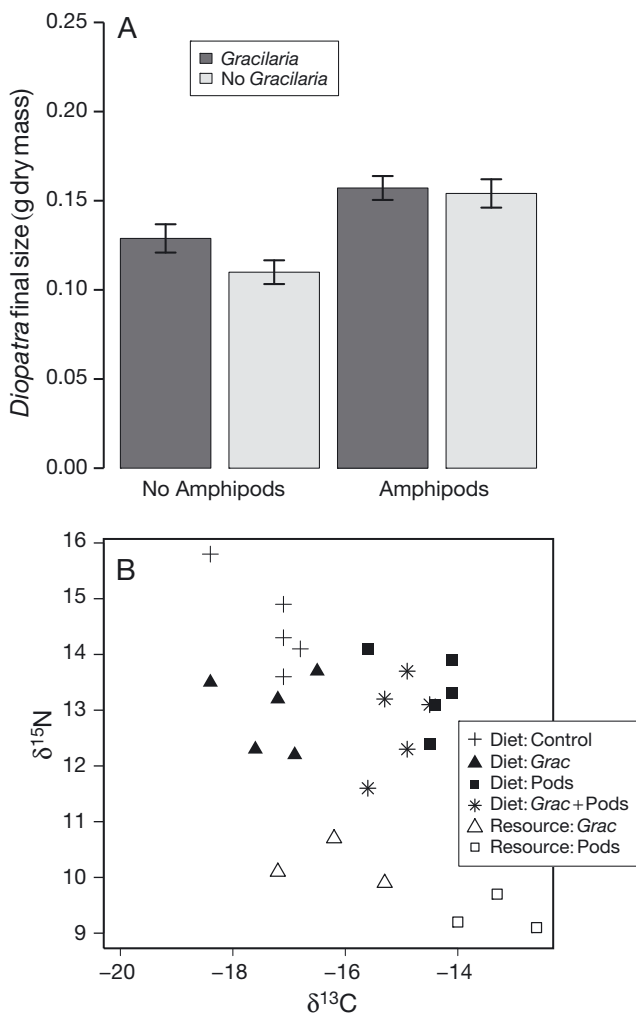


Fig. 4. Effects of *Gracilaria vermiculophylla*-mediated resource items on *Diopatra cuprea* in laboratory experiments. (A) Mean (\pm SE) final size of *Diopatra* after isolation for 6 wk with a sediment-only control, *Gracilaria*, amphipods, or *Gracilaria* and amphipods ($n = 19$ –24 per treatment). There was a significant effect of amphipods but not of *Gracilaria* on *Diopatra* body size. (B) Results of stable isotope analysis of the posteriorly regenerated muscle tissue of *Diopatra*. Diet: tissue taken from a *Diopatra* individual offered that particular diet during the experiment. Resource: field-collected samples for *Gracilaria* and amphipods. Treatments—*Grac*: *Gracilaria*; Pods: amphipods; Control: sediment-only

parison of final size, original mass, and regenerated tissue). Cage, and the interaction of cage and *Gracilaria* density, did not significantly affect *Diopatra* final size. When the experiment was repeated in 2013 at 4 sites, the *Gracilaria* density manipulation did not significantly affect survivorship (70–87% survivorship across treatments and among sites; $df = 3$, $\chi^2 = 0.438$, $p = 0.93$) or *Diopatra* final size (Fig. 5B, Supplement 1B), but there was a weak and marginally significant interaction effect between site and *Gracilaria* density ($p = 0.07$). We observed a slightly negative relationship between *Gracilaria* density and

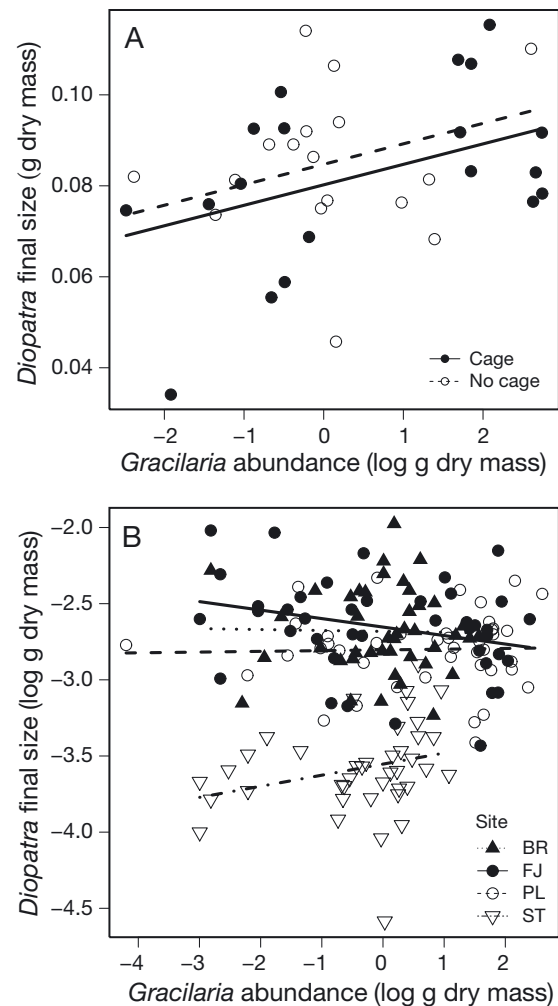


Fig. 5. *Diopatra cuprea* final size in manipulative field experiments. (A) The response of *Diopatra* final size to *Gracilaria vermiculophylla* abundance and the predator-exclusion cages after 4 wk in 2012. (B) Response of *Diopatra* final size to *Gracilaria* abundance after 4 wk in 2013 at 4 sites in the USA: BR = Bull's River, Savannah, GA; FJ = Fort Johnson, Charleston, SC; PL = Priest's Landing, Savannah, GA. ST = Stono River, Charleston, SC. See Supplement 1B at www.int-res.com/articles/suppl/m545p135_supp/ for statistical details

Diopatra growth at the Fort Johnson site, no relationship at Priest's Landing or Bull's River, and a slightly positive relationship between *Gracilaria* density and *Diopatra* growth at the Stono River (Fig. 5B). For both the 2012 and 2013 experiments, there was a positive relationship between *Gracilaria* density and epifaunal amphipod counts (Supplement 1C) but not between epifaunal amphipod counts on *Diopatra* final size (Supplement 1D at www.int-res.com/articles/suppl/m545p135_supp/).

DISCUSSION

We have quantified the reciprocal effects of an interaction between the native *Diopatra* worm and non-native *Gracilaria* seaweed on the mudflats of the southeastern USA. Evidence from the present study, combined with previous efforts (Thomsen & McGlathery 2005, Thomsen et al. 2009), indicates that the decoration behavior of *Diopatra* consistently facilitates *Gracilaria* survivorship and growth. We also found that an increase in the density of *Gracilaria* décor increased *Diopatra* growth by attracting epifaunal amphipods that serve as food, but we found evidence for this benefit only at some sites and in some years.

Gracilaria densities within (Fig. 2) and across mudflats (Byers et al. 2012) tightly correlate with *Diopatra* densities. This co-occurrence is generated by *Diopatra* decoration behavior, which secures *Gracilaria* onto the intertidal benthos where *Gracilaria* survivorship and growth rates are relatively high. Previous work by Thomsen and colleagues (Thomsen & McGlathery 2005, Thomsen et al. 2009) demonstrated that attached *Gracilaria* in the *Diopatra* zone had highest survival relative to drifting algae. Our study shows that individual growth rates are also greater within the *Diopatra* zone than in the high intertidal or deep subtidal (Fig. 2 & 3). Additionally, *Gracilaria* that is experimentally anchored via ropes in the intertidal zone (Supplement 1A) has similar growth rates when out-planted next to *Diopatra* and when out-planted in bare mud (Kollars 2014), which suggests that nitrogenous wastes from *Diopatra* do not facilitate algal growth. Taken together, these data suggest that the primary mechanism by which *Diopatra* facilitates *Gracilaria* is by preventing dispersal of the negatively buoyant *Gracilaria* into the deep subtidal where turbid water makes an unfavorable light regime for growth, or into the superlittoral marsh where desiccation during prolonged emersion can be stressful (see also Thomsen et al. 2009). In

southeastern USA estuaries, *Diopatra* decoration is the only means of benthic attachment for *Gracilaria* because there are few to no hard-bottom sites for spore recruitment (Byers et al. 2012, S. A. Krueger-Hadfield and E. E. Sotka pers. obs.). Although oysters dominate local high-intertidal habitats, *Gracilaria* does not appear to recruit successfully to these reefs (N. M. Kollars & E. E. Sotka pers. obs.).

Among all native and non-native populations of *Gracilaria* that have been surveyed, this association with a decorator worm is largely confined to Atlantic estuaries of the southeastern USA (i.e. Georgia through Delaware; though see Abreu et al. 2011 for a Portuguese estuary) and unique to the *Diopatra* genus. In a survey of ~30 populations in its native Japan, *Gracilaria* was not associated with *Diopatra* or other decorator worms and instead recruited to pebbles, rocks, and shells (Terada et al. 2010, Muangmai et al. 2014, S. A. Krueger-Hadfield and E. E. Sotka pers. obs.). Previous studies (Weinberger et al. 2008, Thomsen et al. 2009) and our own observations of non-native populations of Europe, New England and California in the USA and British Columbia, Canada reveal that *Gracilaria* persist in low-energy and soft-sediment habitats by accumulating and drifting on top of the sediment surface. These observations show that the success of the *Gracilaria* invasion globally did not depend on *Diopatra*, and it is possible that southeastern USA estuaries would have *Gracilaria* present even if *Diopatra* were absent. It seems equally clear, however, that *Diopatra* facilitates the *Gracilaria* invasion where the partners co-occur because of *Diopatra*'s positive effect on *Gracilaria* survivorship and growth.

Reciprocally, we show that *Gracilaria* can benefit *Diopatra* by indirectly enhancing the worm's growth, likely by increasing the worm's access to prey. Our laboratory-based assay showed a significant effect of including amphipods, but not *Gracilaria*, in the diet of *Diopatra* on the worm's final size (Fig. 4A). This is consistent with previous studies that showed that *Gracilaria* is a low-preference food for invertebrate herbivores because of secondary metabolites that serve as feeding deterrents (Nylund et al. 2011, Hammann et al. 2013, Murden, N. M. Kollars & E. E. Sotka unpubl.). Additionally, stable isotope data from the laboratory experiment suggest that when given a choice in prey items between *Gracilaria* and amphipods, the worms integrate the amphipod resource rather than the *Gracilaria* resource (Fig. 4B). Translating the results of the laboratory experiment into predictions for the field, we hypothesize that *Gracilaria* facilitates *Diopatra* by providing habitat for an

epifaunal community that *Diopatra* consume. This supports previous hypotheses that decorating with native seaweeds provide *Diopatra* with an indirect food supply (Mangum et al. 1968, Bell & Coen 1982) and further indicates that decoration with non-native seaweeds serves the same functional role.

In our first manipulative field experiment (Fort Johnson in 2012), we found that *Diopatra* final size was greater in plots with greater densities of *Gracilaria*. Given the positive association between epifaunal abundance and *Gracilaria* density during the experiment (Supplement 1C), and the results of our laboratory experiment (Fig. 4), we suggest that the greater final *Diopatra* size is due to *Gracilaria* providing *Diopatra* with increased access to prey. Thus, although seemingly context-specific, the *Diopatra*–*Gracilaria* interaction represents one of the first examples of a novel mutualism in a marine ecosystem (Aslan et al. 2015) and is among the few mutualisms documented within a marine decoration system (see also Amsler et al. 1999).

However, our replicated field experiments in 2013 suggested the strength and direction of the *Diopatra*–*Gracilaria* interaction is both temporally and spatially variable and likely conditional on other biotic or abiotic factors. Unfortunately, we cannot identify what factor(s) might generate such conditionality. In theory, large fish and crab predators, which profoundly alter many soft-sediment communities (e.g. Meyer & Byers 2005, Lindsay 2010, Byers & Grabowski 2014) may decrease the positive effect of *Gracilaria* if *Gracilaria* attracts *Diopatra* predators, or conversely, magnify the benefit of *Gracilaria* if *Gracilaria* provides refuge. However, excluding large fish and crab predators in our 2012 field experiment did not alter *Diopatra* survivorship nor growth rates. Previous research also indicated that *Diopatra* decoration behavior does not generate a refuge through crypsis (Berke & Woodin 2008). Thus, it is unlikely that predation profoundly alters this association and, thus contrary to other decoration systems (e.g. Amsler et al. 1999), the *Diopatra*–*Gracilaria* association does not appear to provide the decorator with protection.

Variation in epifaunal densities may also alter the *Diopatra*–*Gracilaria* interaction, because it is epifaunal crustaceans and not the *Gracilaria* per se that *Diopatra* principally consumes. We found no evidence of this, given that epifaunal amphipods scale in abundance with *Gracilaria* biomass both in 2012 and 2013 (our Supplement 1D; Byers et al. 2012). However, it remains possible that epifaunal recruitment was higher in 2012 than 2013, but was sub-

sequently consumed during the course of the experiment and before we surveyed. In future experiments, we suggest that researchers either manipulate gammarid densities experimentally (e.g. Poore et al. 2009) or survey epifauna more frequently.

While we do not yet understand the mechanism driving the spatial and temporal variation we observed, the presence of variability in this mutualism is not surprising. Theory predicts that mutualisms should show variability when (1) the association is facultative, (2) fitness benefits depend on the density of one of the interacting partners, (3) additional species influence the pairwise association of interest (Bronstein 1994), (4) the association is embedded within a heterogeneous environment (Chamberlain et al. 2014), and (5) the interaction is in the early stages of formation (Aslan et al. 2015). The *Diopatra*–*Gracilaria* interaction satisfies all of these conditions. We predict the facultative and context-dependent nature of the interaction will slow any co-evolution of traits that reinforce this mutually-beneficial relationship. At the very least, we have not observed any significant negative effect of the invader on *Diopatra* nor the associated epifaunal community (our Supplement 1C; Byers et al. 2012), suggesting that the *Diopatra*–*Gracilaria* association will remain an important component of the intertidal ecosystem in SC and GA for the foreseeable future.

This association between *Gracilaria* and *Diopatra* is transforming community dynamics of southeastern USA mudflats in several ways. *Gracilaria* fuels the detrital food web as a novel source and modifier of local nitrogen cycles (Tyler & McGlathery 2006, Gonzalez et al. 2013, Gulbransen & McGlathery 2013) and through increasing bacterial abundance on sediment and thallus surfaces (Gonzalez et al. 2014, C.E. Gerstenmaier & E. E. Sotka unpubl. data). *Gracilaria* also provides enhanced structure for a diverse and abundant epifaunal community (Thomsen et al. 2009, Byers et al. 2012), and facilitates juvenile blue crabs (Johnston & Lipcius 2012), mud snails (Guidone et al. 2014) and amphipods (Wright et al. 2014). We now suggest that *Gracilaria* can also facilitate the growth of *Diopatra* worms by allowing a greater access to epifaunal prey, potentially generating a positive feedback loop between populations of the decorator worm and its invasive décor.

Rigorous description of interactions formed between native and non-native species could critically enhance the prediction of invasion impact. Facilitative interactions can yield fundamentally different population and community level outcomes relative to systems where antagonistic interactions predominate

(Bruno et al. 2003). For example, mutualisms formed with non-native species can actually enhance invasion success (Richardson et al. 2000), which challenges theory that assumes biodiversity provides only biotic resistance (Bruno et al. 2003). However, empirical tests for mutualisms between native and non-native species are rare, especially in marine systems. Given the global prevalence of marine invaders (Rius et al. 2012), and that mutualisms between native marine species are ubiquitous (reviewed in Hay et al. 2004, Byers et al. 2015), it is likely that mutualisms formed between native and non-native species in the sea remain under-described.

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