



Positive versus negative effects of an invasive ecosystem engineer on different components of a marine ecosystem

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Habitat-forming invasive species have complex impacts on native communities. Positive above ground and negative below ground impacts are reported, suggesting that habitat-forming invasive species may affect community components differently. Furthermore, such effects may vary depending on the density of the invader. We determined the responses of community components to different densities of the invasive green alga *Caulerpa taxifolia* in southeastern Australia. Initially we investigated differences in soft-sediment faunal communities (above and below ground) across a biomass gradient at two invaded sites. *Caulerpa taxifolia* biomass was positively associated with the composition and abundance of the epifaunal community, but negatively correlated with the abundance of infauna. To examine the response of common community members in more detail, we caged two species of mollusk (the infaunal bivalve, *Anadara trapezia* and the epifaunal gastropod, *Batillaria australis*) across the same biomass gradient to determine lethal and sublethal effects of *C. taxifolia* biomass on individuals. Survivorship of *A. trapezia* was low when *C. taxifolia* was above 300 g m⁻². Negative sublethal effects were also density-dependent with *A. trapezia* tissue weight being lowest above this same *C. taxifolia* biomass. The proportion of *B. australis* surviving was unaffected by *C. taxifolia* biomass. However, the total number of live *B. australis* recovered in cages increased as *C. taxifolia* biomass increased, providing further evidence of positive density dependent effects (in line with the survey data) of *C. taxifolia* on epifauna. Finally, we removed *C. taxifolia* from plots of differing *C. taxifolia* biomass and followed community change for 5 months. Community change following *C. taxifolia* removal was also density dependent as recovery 5 months post-removal depended on the initial biomass of *C. taxifolia*, suggesting a lag in the recovery of communities due to residual environmental effects post-removal (i.e. hysteresis). We have shown that the effects of a habitat-forming invasive species are biomass dependent and also affect community components differently, suggesting that, globally, the impact of these types of invaders may be context dependent.

Habitat-forming invasive species often have pervasive effects on entire ecosystems because of their strong control of structure and resource availability (i.e. via ecosystem engineering, sensu Jones et al. 1994, 1997). However, the responses of native communities to habitat-forming invasive species can be varied, with both positive and negative effects reported (Crooks 2002, Rodriguez 2006). Habitat-forming invasive species often facilitate above ground communities by providing a refuge from predation or suitable habitat for colonization where they add structure to previously unstructured habitats, or where native habitat-forming equivalents have been lost (Van Riel et al. 2000, Zavaleta et al. 2001, Neira et al. 2005, Wonham et al. 2005). However, below ground modification of the abiotic environment (e.g. changes in nutrient cycling, reducing dissolved oxygen content and salt accumulation) by habitat-forming invasive species can have negative impacts on competitors and within

soil communities (Vitousek 1986, Kourtev et al. 2003, Brusati and Grosholz 2006, Neira et al. 2006, Thorpe and Callaway 2011). Typically, investigation of the impacts of invasive habitat-forming species focuses on only one aspect of the community (i.e. above or below ground), or determines the net effect on the entire community without consideration of the responses of different community components. Because habitat-forming invasive species have the capacity to simultaneously 'engineer' both above and below ground factors, determining the response of only one aspect of the community or the entire community per se may misconstrue the impacts of the invader. However, the intra-specific effects of habitat-forming invasive species on different community components remain obscure.

In a recent review, Thomsen et al. (2011) highlighted the strong density dependent impacts of aquatic invasive species on native biota. Interestingly, 37 of the 39 studies

included in their analysis considered how different densities of the invasive species affected native species through direct biotic interactions (e.g. competition or predation). In contrast, empirical investigations of the impacts of habitat-forming invasive species on native species that occur via abiotic changes typically employ limited manipulations of the invader, often comparing treatments with no invader with those of high invader density only (Gribben et al. 2009, Posey et al. 1993, Neira et al. 2006). These manipulations often represent worst-case scenarios (Elgersma and Ehrenfeld 2011), thus we know little about the density dependent effects of habitat-forming invasive species, particularly when they are at low density (Parker et al. 1999, Ricciardi 2003). However, the impacts of Japanese barberry, *Berberis thunbergii*, leaf litter on microbial community structure was density dependent, with even small additions of litter eliciting large changes in the relative abundances of bacteria (Elgersma and Ehrenfeld 2011). Low abundances of the invasive alga, *Caulerpa racemosa*, altered benthic community structure of algal dominated subtidal rocky reefs in the Mediterranean (Bulleri et al. 2010). Similarly, a few examples show that the facilitation of native macrophytes and epibiota by habitat-forming invasive species is also density dependent (Thomsen 2010, White and Shurin 2011). Understanding the density dependent impacts of habitat-forming invasive species will clearly benefit the development of appropriate management strategies for these organisms (Yokomizo et al. 2009, Elgersma and Ehrenfeld 2011).

Density dependent effects of invaders will also have important individual level consequences. For example, sublethal effects on the key life-history traits of individuals may be particularly important in the context of habitat-forming invasive species because of their strong effects on the abiotic environment (Neira et al. 2005, 2006, Hacker and Dethier 2006) and life-history traits are often very sensitive to environmental change. Generally, however, little is known about the sublethal impacts of invasive species on native biota (but see Levine et al. 2003 for review of terrestrial plant invaders). In a marine example, Crooks (2001) demonstrated that the growth of the native soft-sediment bivalves, *Chione* spp., was significantly decreased in the presence of invasive mussel *Musculista senhousia*. Investigating sublethal effects is important as they will likely manifest themselves at lower invader densities than those at which changes in the abundance of native species occur via mortality. If sublethal effects exist then managing invader density to a level at which community change occurs may be insufficient for maintaining healthy populations (Gribben and Wright 2006b).

The invasive green alga *Caulerpa taxifolia* has invaded several temperate regions worldwide where it covers large areas of soft-sediment habitat, forms high density beds (Meinesz et al. 2001, Anderson 2005, Wright 2005) and modifies chemical and physical sediment and water properties. Typically, there are high densities of epibionts and low densities of infauna associated with *C. taxifolia* in southern and southeastern Australia (Gribben and Wright 2006b, Gribben et al. 2009, McKinnon et al. 2009, Tanner 2011). The traits of a native bivalve (size, reproductive output) are also reduced in *C. taxifolia* (Gribben and Wright 2006a,

Wright et al. 2007, 2012, Wright and Gribben 2008). Similar positive above ground and negative below ground effects linked to abiotic modification have been demonstrated for other habitat forming invasive species in soft-sediment marine ecosystems (Crooks 1998, 2002, Crooks and Khim 1999, Hedge and Kriwoken 2000, Wonham et al. 2005, Levin et al. 2006, Neira et al. 2006). These effects may be density (or biomass) dependent and even upon removal, the prior density of *C. taxifolia* may influence the community because of hysteresis and residual environmental effects. To date, however, the impacts of *C. taxifolia* have included limited density manipulations.

Using *C. taxifolia* we tested the responses of different community components (above and below ground) and different levels of community organization (communities and individuals) to different invader densities. Specifically we investigated the following hypotheses: 1) *C. taxifolia* will have differing density dependent effects on different community components (positive for epifauna and negative for infauna); 2) Sublethal and lethal effects on individuals will be density dependent; 3) The recovery of the abiotic environment and communities following its removal will be dependent on initial *C. taxifolia* density.

Methods

Study site and organisms

The study was conducted at Sponge Bay and Yooralla Bay, Lake Conjola (35°15'44.3"S, 150°26'47.8"E) southeastern Australia. Detailed descriptions of the organisms and study sites are found in Gribben and Wright (2006a, b) and Wright et al. (2007). We present only brief descriptions here. Lake Conjola is a temperate barrier estuary heavily invaded by *Caulerpa taxifolia*. *Caulerpa taxifolia* was first discovered in Lake Conjola in April 2000, but may have invaded the estuary between 1987 and 1995 (Creese et al. 2004). It is not known when *C. taxifolia* invaded Sponge Bay, although it has been in the bay since at least 2002 (Wright unpubl.). The alga spread throughout Lake Conjola to cover greater than 150 ha of the lake floor (Creese et al. 2004, Wright 2005) at the time of the experiments. In Lake Conjola, native seagrasses (e.g. *Zostera capricorni*) are patchily distributed and restricted to very shallow subtidal regions, and there is no other vegetated habitat, so we did not compare biomass effects of *C. taxifolia* with native seagrasses. In Lake Conjola and other estuaries in New South Wales, Australia, *C. taxifolia* commonly invades bays which contain large populations of the ark shell *Anadara trapezia* (Arcidae, Deshayes 1840) and the mudwhelk *Batillaria australis* (Wright et al. 2007, McKinnon et al. 2009). At both bays studied, *A. trapezia* and *B. australis* occur in both *C. taxifolia* invaded and unvegetated sediments.

Responses of community components and abiotic conditions to *C. taxifolia* density

Biotic communities and key abiotic characteristics (water column dissolved oxygen (DO), sediment redox potential,

sediment organic content) were determined across a naturally occurring biomass gradient in both Sponge Bay and Yooralla Bay ($n = 15$ plots site⁻¹) in February 2008. Because the biomass of *C. taxifolia* is patchy, plots (0.5 × 0.5 m) were placed in a contiguous area of intermingling biomasses of *C. taxifolia* between 1–2 m water depth thus limiting any potential confounding effects of gradients in biomass with water depth. We established plots that encompassed the full range of *C. taxifolia* density. Once each plot was established, DO was determined for a single water sample which was collected 3–5 cm above the sediment surface using 20 ml syringes. Following collection of water samples, two sediment cores were taken for each plot and used for analyses of redox (200 ml core) and organics (50 ml core). Redox was measured on site, cores for organics were labelled, placed on ice and transported to the Univ. of Technology, Sydney (UTS), and stored in a freezer until analysed. All samples were collected between 11 a.m. and 1 p.m. to limit changes that may occur over the course of day/night cycles. Procedures for quantifying DO, redox and sediment organics are described below. All *C. taxifolia* was then carefully removed from each plot, placed in labeled plastic bags and transported to UTS where it was thoroughly searched for associated biota. (Note: these plots also formed the basis of community recovery experiment described below). Any epifauna visible on the surface of each plot were identified and recorded. Infauna were sampled by collecting a single sediment core (10 cm diam. × 10 cm deep) and sorted on a 500 µm sieve. All organisms were identified to the lowest taxonomic level possible and their abundances determined. Epifaunal abundances are expressed as numbers of organisms/0.25 m², infauna are expressed as numbers of organisms/core.

For DO, collected samples were immediately wrapped in foil and DO measured within 15 min of sample collection using oxygen-sensing 100 µm optodes (Precision Sensing). The optodes were calibrated linearly to 100% air saturation using methods outlined in Ulstrup et al. (2005). Redox was measured using a Pt-wire electrode with Ag/AgCl reference electrode and pH meter. The electrode was calibrated using Orion Application Solution Standard and all redox readings corrected for $E_{\text{H}}^{\text{Ref}} = -210 \pm 3$ mV, i.e. reported redox potentials are versus the hydrogen electrode, $E_{\text{H}}^0 = 0$ mV. Percent organics was determined by placing 2 g from each of the sediment samples in a drying oven at 60°C for 24 h, and then weighing them. The samples were then placed in a muffle furnace at 440°C at 24 h and reweighed. The proportion of organics was then determined from the differences in sample weight pre- and post-furnacing.

Community composition was compared between sites using ANOSIM (square root transform and Bray–Curtis similarities). Multivariate and univariate Pearson correlations were used to examine relationships between invertebrate communities (infauna or epifauna) and *C. taxifolia* biomass or abiotic variables. Multivariate analyses were done with the BIOENV routine in the PRIMER software package using Bray–Curtis similarities calculated from square-root transformed biotic data, or normalised Euclidian distances for abiotic data. The DISTLM sub-routine in PRIMER was used to examine the relationship (linear

regression) between Shannon diversity and *C. taxifolia* biomass, using square-root transformed data Euclidian distances and 9999 permutations of the data.

Sublethal and lethal effects of *C. taxifolia* density on individuals

To examine the sublethal and lethal effects of *C. taxifolia* biomass on two abundant native species (*Anadara trapezia* and *Batillaria australis*), we exposed these species to a gradient of *C. taxifolia* density for five months in Sponge Bay. For *B. australis* we investigated density dependent effects on mortality only; animals were lost because of a faulty freezer. *Anadara trapezia* and *B. australis* were collected from unvegetated sediments in St Georges Basin in February, 2008, to avoid any prior influence of *C. taxifolia* on the traits of both these species. In addition, *A. trapezia* are now rare in Sponge Bay and sufficient numbers could not be found in available unvegetated habitat. Organisms were transported to Sponge Bay and randomly assigned to cages within 2 h of collection. Clams ($n = 30$) and gastropods ($n = 20$) were added to each of 15 cages (50 cm long × 50 cm wide × 20 cm high; 9 mm² mesh size) placed in *C. taxifolia* within 1 m of each of the *C. taxifolia* removal plots described above. (Note: we lost one cage in the process of the experiment.) Cages were inserted to a depth of 10 cm. We did not record the initial size of clams, but all were in the range of 40–60 mm shell length (anterior–posterior axis) and final lengths were measured. The placement of cages within 1 m of the *C. taxifolia* removal plots was done to expose the native molluscs to a known density of *C. taxifolia* that was well quantified in the initial survey described above. Any *A. trapezia* or *B. australis* naturally occurring in *C. taxifolia* were removed prior to the addition of the experimental animals. Cages were left untouched for 5 months, although they were groomed monthly to remove fouling (note, cages did not experience extensive fouling on a month to month basis). Because all plots were treated the same (i.e. all had cages), the mesh size was large enough to maintain water flow (but prevent snails from escaping), and we regularly groomed the cages, we believe any caging effects, if there were any, were minimal. (Note, at the end of the experiment, the abundance of *Caulerpa* in the cages appeared visibly similar to that surrounding *Caulerpa* cages.) After five months, the percent survival of clams and gastropods was determined. Sampling of all remaining clams and gastropods coincided with sampling of the removal plots in July. After collecting all clams and gastropods, all *C. taxifolia* was removed from the cages and dry weight (g) determined for each cage. The proportion of clams and gastropods surviving was determined from the total number of individuals for each species (alive + dead) recovered. Any mortality of animals was attributed to the effects of *C. taxifolia* biomass as the common predators of *A. trapezia* and presumably *B. australis* (blue swimmer crabs, *Portunus pelagicus*; rays and stingarees, *Urolophus* spp.; octopods, *Octopus tetricus*; Byers et al. 2010) were excluded by cages. In addition, we found no evidence of predation (crushed or chipped shells) on animals in cages. For all individual clams recovered alive, we recorded the final shell length

(anterior–posterior axis), dry shell weight, and dry tissue weight. Dry shell and tissue weights were determined by separating all tissues from their shells and drying the separate components in an oven for 48 h at 60°C.

Nested analysis of variance (ANOVA) followed by Tukey's tests were used to test the effect of *C. taxifolia* biomass on *A. trapezia* shell length, dry shell weight and dry tissue weight. Numbers of replicate *A. trapezia* recovered per plot ranged from 16–27. A bonferroni adjusted p-value of 0.017 used to account for the multiple tests. For all analyses, plot (6 low, 5 medium, 3 high) was a random factor nested within fixed *C. taxifolia* biomass categories: < 200 g m⁻² dry weight (low), 200–300 g m⁻² dry weight (medium) and > 300 g m⁻² dry weight (high). These biomass categories were based on Gribben and Wright (2006a; Fig. 2) who showed that the number of recruits of *A. trapezia* increased up to 200 g m⁻² dry weight, was very high between 200 and 300 g m⁻² dry weight, and low at biomasses higher than >300 g m⁻² dry weight. For all analyses, assumptions were checked by examining the distributions of residuals and plots of residuals versus means (Quinn and Keough 2002). The effect of *C. taxifolia* biomass on the proportion of *A. trapezia* and *B. australis* surviving as a function of total number of each species recovered (alive + dead) was explored using linear regression. The proportion surviving/plot was regressed against initial *C. taxifolia* biomass for each corresponding plot.

Density dependent effects of initial *C. taxifolia* density on community recovery following its removal

The recovery of communities following the removal of *C. taxifolia* was investigated at Sponge Bay. Following sampling of the community components and removal of *C. taxifolia* to estimate biomass in each of the plots for the initial community study (described above), we further removed *C. taxifolia* from all plots to expand the area cleared to 2 × 2 m, so that we could create enough area to subsample the plot over time without resampling the same place. (These expanded plots did not impinge on the *B. australis* and *A. trapezia* cages described above which were always 1–2 m away for the adjacent removal plots.) The abundance of epibiotic and infaunal organisms were identified and enumerated in each of the 15 plots as described above, after five months (July 2008). All plots were gardened monthly to prevent encroachment of *C. taxifolia* into plots. To ensure all plots were handled similarly, plots that had little or no starting biomass of *C. taxifolia* were prodded by hand to mimic *C. taxifolia* removal. We could not include unmanipulated unvegetated controls due to the rarity of naturally unvegetated areas at our site and the continued expansion of *C. taxifolia* into them, although we did have plots of very low biomass. To account for any temporal variation in communities in *C. taxifolia* that could have contributed to changes in communities in our recovery plots, after five months, we also sampled communities in *C. taxifolia* outside four of our 15 removal plots (methods as described above for the initial biomass survey). Each control and removal plot were within 2 m of each other. The communities were

compared to those found in removal plot in the initial biomass survey. The four control/removal plot pairs covered the entire biomass range found in plots from the initial survey.

We used nMDS ordination to compare infaunal communities in plots 1) with *C. taxifolia* at the start of the experiment (February; filled circles), 2) with *C. taxifolia* in July (closed triangles), 3) without *C. taxifolia* in July (five months post removal; open triangles). We also graphed the multivariate similarity of infaunal community composition in individual plots at time 0, individual plots at five months after removal, and control plots without *C. taxifolia* biomass removal. Changes in communities after removal of *C. taxifolia* (before removal vs five months post removal) were investigated using ANOSIM and by comparing Bray–Curtis similarities between communities in the same plots from before to after *C. taxifolia* removal.

Results

Responses of community components and abiotic conditions to *Caulerpa taxifolia* density

Infaunal and epibiotic communities did not differ significantly between Sponge Bay and Yooralla Bay (infauna: Global R = 0.018, p = 0.26, epibenthic: Global R = 0.008, p = 0.31). Data were therefore combined to test for correlations between communities and *Caulerpa taxifolia* biomass. There were positive associations between *C. taxifolia* biomass and Shannon diversity of epifauna ($r^2 = 0.303$, p = 0.002) and number of epibenthic species ($r = 0.32$, p = 0.008). Similarly, *C. taxifolia* biomass was positively associated with the numbers of epibenthic individuals ($r = 0.51$, p < 0.01; Fig. 1). The latter association was driven by the numerically dominant (69% of all individuals) mussel *Musculus varicosa* ($r = 0.48$, p < 0.01). The next three most abundant species, *Cathalotia fragum* (9.7% of individuals), *Nassarius burchadi* (8.9%) and *Anadara trapezia* (4.2%), showed no significant correlation with *C. taxifolia* biomass ($r = 0.06$, 0.06 and 0.07, respectively, p > 0.1 for each). The gastropod *Batillaria australis*,

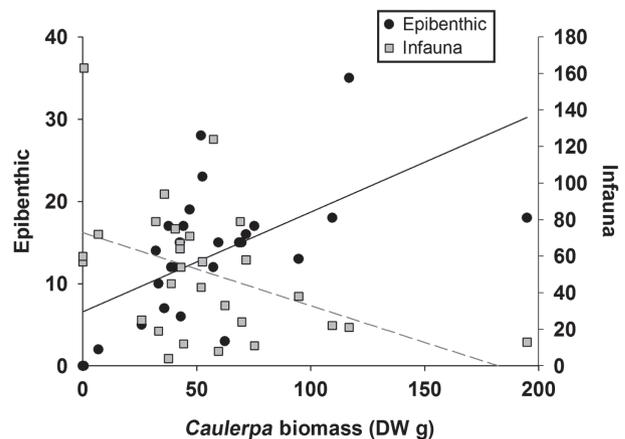


Figure 1. Correlations between *C. taxifolia* biomass and number of epibenthic individuals (black circles, solid black line) and number of infaunal individuals (grey squares, grey dotted line).

although uncommon (2.6% of all individuals, occurring in 20% of samples), was positively associated with *C. taxifolia* biomass ($r = 0.52$, $p < 0.01$).

No significant relationship was detected between *C. taxifolia* biomass and Shannon diversity of infauna ($r^2 = 0.028$, $p = 0.376$) or the number of infaunal species and ($r = 0.11$, $p = 0.131$). The number of infaunal individuals was, however, negatively correlated with *C. taxifolia* biomass ($r = -0.45$, $p < 0.05$, Fig. 1). The strongest relationship with *C. taxifolia* for an individual infaunal taxon was for the nematode *Metoncholaimus* sp. ($r = -0.50$, $p < 0.01$), which accounted for 72% of all individuals. Abundances of larvae of the insect *Pontomyia* sp., which accounted for an additional 23% of infaunal individuals, did not correlate significantly with *C. taxifolia* biomass ($r = -0.07$, $p > 0.1$).

There was no significant multivariate correlation between abiotic variables and infaunal or epibenthic communities. Correlations between abiotic sediment variables and individual infaunal and epibenthic taxa were typically weak or non-existent. There were, however, significant negative relationships between *C. taxifolia* biomass and water column dissolved oxygen ($r = -0.66$, $p < 0.001$) and *C. taxifolia* biomass and sediment redox ($r = -0.47$, $p < 0.01$). There was a non-significant negative association between *C. taxifolia* biomass and organic content ($r = -0.35$, $p > 0.05$), which is notable given suggestions that *C. taxifolia* tends to thrive in organically rich sediments (Chisholm et al. 1997).

Sublethal and lethal effects of *C. taxifolia* density on individuals

There was a significant effect of *Caulerpa* biomass on *A. trapezia* survivorship ($F_{1,12} = 51.045$, $p < 0.001$; $r^2 = 0.810$, Fig. 2A). Because we were concerned about the undue influence of the one extremely high density plot (779 g m⁻²), we re-ran the analysis without this plot. The results were still highly significant although biomass now explained less of the variation in clam survivorship ($F_{1,11} = 15.389$, $p < 0.005$; $r^2 = 0.583$).

There was no significant relationship between *C. taxifolia* biomass and *B. australis* survivorship with the highest density *Caulerpa* plot included ($F_{1,12} = 0.410$, $p = 0.534$; $r^2 = 0.033$) or excluded ($F_{1,11} = 0.041$, $p = 0.843$; $r^2 = 0.020$) (Fig. 2B). The lack of fit may have been influenced by the unequal numbers of *B. australis* recovered from each plot (total lost 62 of 280, range lost in individual plots 5–20). *Batillaria australis* may have escaped by burrowing under the cages. However, the total number of gastropods recovered from each plot was positively correlated to *C. taxifolia* biomass ($F_{1,12} = 4.884$, $p = 0.047$; $r^2 = 0.289$).

Final dry tissue weight was significantly different among *C. taxifolia* biomass categories ($F_{2,14} = 7.593$, $p < 0.001$) (Fig. 3A). Tissue biomass was significantly lower in the high *C. taxifolia* biomass treatment compared to the other two treatments, and significantly lower in the low *C. taxifolia* biomass treatment compared to the middle *C. taxifolia* biomass treatment (all Tukey's $p < 0.002$). There

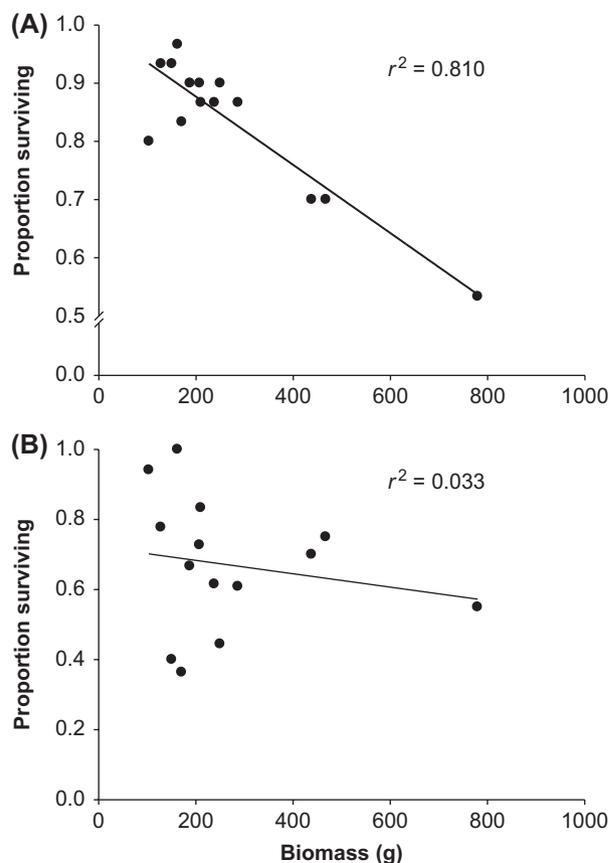


Figure 2. Relationship between (A) *A. trapezia* and (B) *B. australis* proportion surviving and *C. taxifolia* biomass (dry weight m⁻²).

were also significant differences among plots nested within *C. taxifolia* biomass categories ($F_{12,333} = 5.289$, $p < 0.001$).

There was no difference in final shell length among *C. taxifolia* biomass categories ($F_{2,14} = 1.843$, $p = 0.183$) or among plots within categories ($F_{12,333} = 1.680$, $p = 0.07$) (Fig. 3B). There was also no difference in final dry shell weight among *C. taxifolia* biomass categories ($F_{2,14} = 2.412$, $p = 0.109$) or among plots within categories ($F_{12,333} = 1.08$, $p = 0.376$) (Fig. 3C).

Density dependent effects of initial *C. taxifolia* density on community recovery following its removal

The most dramatic effect of *C. taxifolia* removal occurred for epibenthic species, which had disappeared from all plots within five months of removing *C. taxifolia*. There was some evidence for a change in infaunal communities five months after removal of *C. taxifolia*, particularly for the low initial biomass plots. Infaunal communities in plots from which *C. taxifolia* had been removed for five months (sampled in July) were significantly different from those in undisturbed plots where *C. taxifolia* was still present in July (Global R = 0.49, $p = 0.006$, Fig. 4). Infaunal communities in plots that had not had *C. taxifolia* removed did not change significantly over the five month experiment (Global R = 0.117, $p = 0.16$; Fig. 4). However, the communities in plots from which *C. taxifolia* had been removed

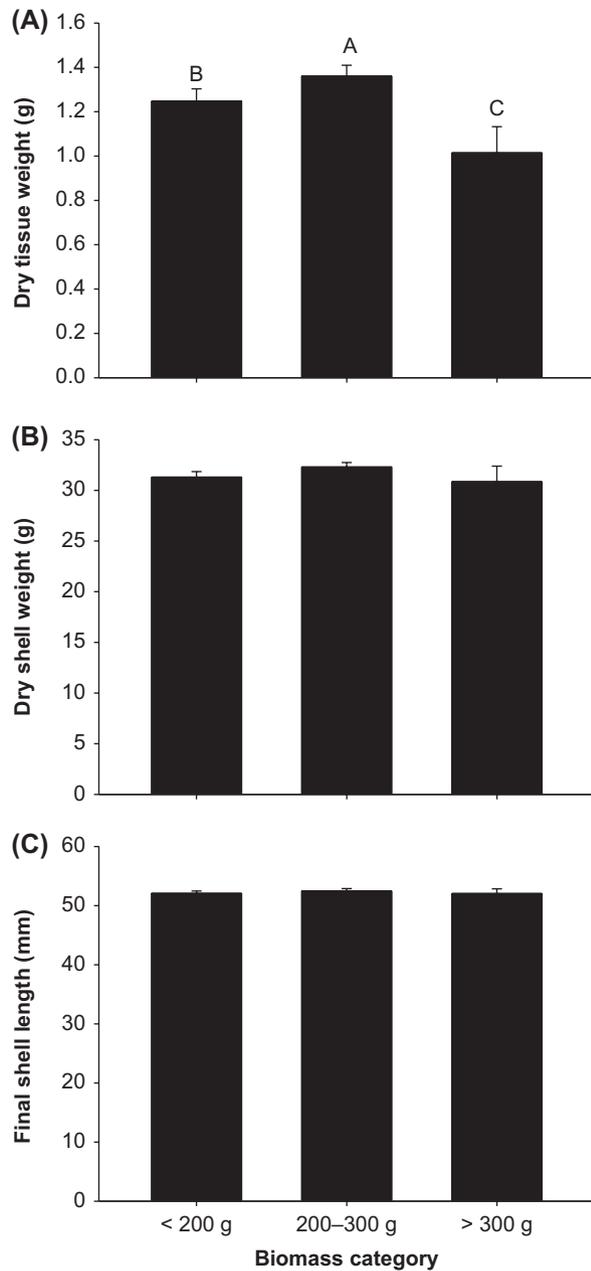


Figure 3. Mean (\pm SE) dry tissue weight (g), dry shell weight (g) and final shell length (mm) of live *Anadara trapezia* recovered from plots ($n = 15$ in total) containing different biomasses of *Caulerpa taxifolia* after six months. Plots were placed into three *C. taxifolia* biomass categories (200 g, 200–300 g and > 300 g dry weight; $n = 3$ –6 plots/biomass category), and category means were determined from plot averages for each trait measured. ($n = 16$ –29 clams recovered alive from plots). Different letters indicate significant differences among treatments (Tukey's tests, $p < 0.05$).

for five months were still not particularly different from those amongst *C. taxifolia* at the start of the experiment (Global $R = 0.182$, $p = 0.004$; note that the small Global R value is a more reliable indicator of significance than the p -value when there are very large numbers of possible permutations, Clarke and Gorley 2006). As for the initial survey, there was no significant relationship between *C. taxifolia* biomass and diversity for infauna ($r^2 = 0.022$, $p = 0.801$).

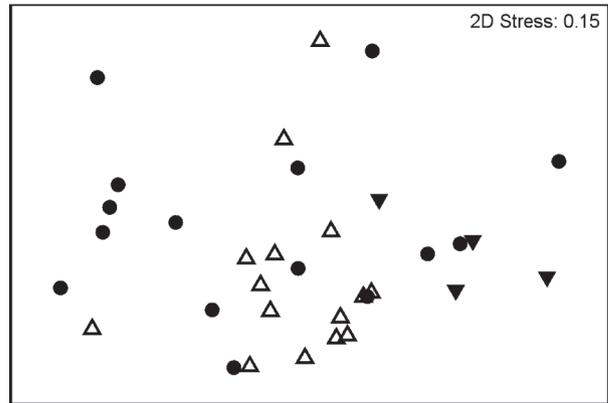


Figure 4. nMDS ordination comparing infaunal communities in plots 1) with *C. taxifolia* at the start of the experiment (February; filled circles), 2) with *C. taxifolia* in July (closed triangles), 3) without *C. taxifolia* in July (five months post removal; open triangles).

SIMPER analyses indicated that at the end of the experiment, 69% of the differences between infaunal communities with versus without *C. taxifolia* were caused by nematodes (*Metoncholaimus* sp.), insect larvae (*Pontomyia* sp.) and gastropods (*Assimineea buccinoides*) being more abundant in plots where *C. taxifolia* had been removed. (Note that *A. buccinoides* may be epifaunal but we only found it shallowly buried in plots so it was deemed infaunal. Its categorisation as infauna does not affect the overall results.) These three species accounted for 95% of all individuals sampled. *Assimineea buccinoides* was not present in any plot with *C. taxifolia* at either the start or end of the experiment but was present (1–2 individuals) in 47% of plots without *C. taxifolia*. *Metoncholaimus* sp. mean abundance per plot where *C. taxifolia* had been removed for five months was 27.7 ± 5.6 (SE) compared to 10.3 ± 1.1 in plots that still had *C. taxifolia*. There was a far less marked difference in numbers of *Pontomyia* sp. between plots with (1.5 ± 0.6) versus without (0.3 ± 0.3) *C. taxifolia*. An unidentified oligochaete was found only in plots where *C. taxifolia* had been removed (0.4 ± 0.2), whilst the gastropods *Cathalotia fragum* and *Nassarius burchadi* were not present in plots from which *C. taxifolia* was removed, but rare in plots with *C. taxifolia* (0.5 ± 0.3 and 0.3 ± 0.3 individuals per plot, respectively). These latter three species accounted for an additional 23% of multivariate dissimilarity between infaunal communities with versus without *C. taxifolia*.

Changes in infaunal communities following the removal of *C. taxifolia* were also investigated by graphing, as a function of initial *C. taxifolia* biomass, the multivariate similarity between plots at the start vs five months post removal of *C. taxifolia*. This plot–plot similarity showed a significant positive correlation with initial biomass of *C. taxifolia* ($r = 0.57$, $p < 0.05$), indicating that the change in infaunal communities after five months decreased as a function of increasing initial *C. taxifolia* biomass (Fig. 5). This community pattern was primarily a function of differences in the composition of communities, rather than changes in total abundances of infauna. After five months the mean number of individuals core⁻¹ (32.7 ± 5.8 SE)

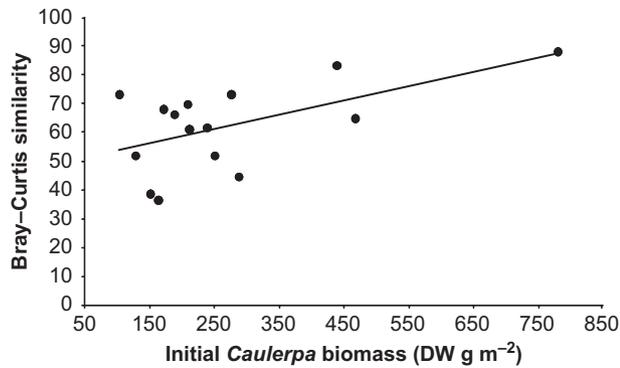


Figure 5. Initial *C. taxifolia* biomass plotted against Bray–Curtis multivariate similarity between individual plots for the start of the experiment vs five months after *C. taxifolia* removal.

had not changed significantly from the start of the experiment (38.9 ± 6.8) and the magnitude of change in numbers of individuals was not correlated with initial *C. taxifolia* biomass ($r = -0.22$, $p > 0.05$).

Discussion

Where invasive species add habitat complexity they generally facilitate biodiversity (Crooks 2002). Whilst our data suggests that some species are facilitated, others are not. *Caulerpa taxifolia* had divergent impacts on different community components – positive for epifauna but negative for infauna. In addition, *C. taxifolia* density had important consequences for community structure (particularly in the *C. taxifolia* removal experiment) and on sublethal and lethal effects on individuals.

Understanding how communities change in the response to invasion by habitat-forming species is an important component of determining their impacts. This study suggests that simply describing changes in overall community structure associated with invasion does little to improve our understanding of their overall impacts, and in soft-sediment ecosystems may in fact be misleading. Importantly, changes in sediment characteristics may have broader implications for ecosystem function if infaunal communities are replaced by different functional groups. Although we did not measure ecosystem function, species commonly associated with degraded conditions (e.g. capitellid polychaetes associated with organic pollution) were abundant at high *C. taxifolia* biomass. Negative effects on infauna – and potentially ecosystem function (Waldbusser et al. 2004) – may be a general attribute of invasive habitat-forming species in soft-sediment ecosystems as several studies show negative effects on infauna even when overall positive effects on diversity are described (Crooks 1998, Crooks and Khim 1999, Hedge and Kriwoken 2000).

Our study indicates that the density of a habitat-forming invasive species has important implications for native biota across multiple levels of community organization (i.e. for individuals and communities). For example, the highest observed mortality of *A. trapezia* occurred at *C. taxifolia* abundance of more than 300 g m^{-2} (Fig. 2A). Although high, biomass $> 300 \text{ g dw m}^{-2}$ is observed in estuaries

invaded by *C. taxifolia* in New South Wales (Wright 2005, Gribben and Wright 2006a, Wright and Davis 2006). Tissue weight of surviving clams was also lowest in the high biomass category ($> 300 \text{ g m}^{-2}$; Fig. 2A). By contrast, dry tissue weight of clams was highest in the middle biomass category (200–300 g). Recruitment of *A. trapezia* to *C. taxifolia* is also highest at these intermediate biomasses of *C. taxifolia* (Gribben and Wright 2006a). Thus a threshold value of $> 300 \text{ g m}^{-2}$ may be required to induce both sublethal and lethal effects which increase as density increases. The reasons for high *A. trapezia* tissue biomass at intermediate *C. taxifolia* biomass are unclear, but at intermediate *C. taxifolia* biomass, food supply may increase due to greater trapping of particles (Gribben et al. 2009) and the environmental conditions within *C. taxifolia* may not yet be degraded enough to affect clam health.

Positive relationships between macrophyte density and the number of *B. australis* have been demonstrated (Thomsen et al. 2012), so it is not surprising we found a similar relationship between *C. taxifolia* biomass and the number of *B. australis* recovered from cages. Being detritivores, *B. australis* may be exploiting enhanced detrital input of *C. taxifolia* into sediments. In addition, *B. australis* may be better adapted to the reduced DO and redox conditions resulting in high survivorship across the range of *C. taxifolia* biomass it was exposed to – the gastropod is very abundant in seagrasses where sediment conditions can be reduced compared to unvegetated sediments (Gribben et al. 2009, McKinnon et al. 2009).

Community change following *C. taxifolia* removal also appeared density dependent with the degree of recovery five months post removal related to the initial biomass. As for the lethal and sublethal responses of *A. trapezia*, there appeared to be a threshold of around 300 g dw m^{-2} above which there was a lag in community recovery, possibly due to the severity of the initial disturbance of *C. taxifolia* at high biomasses. The species that drove the community change were *Metoncholaimus* sp. (nematode), *Pontomyia* sp. (insect) and *Assiminea buccinoides* (gastropod), all of which began to increase after *C. taxifolia* was removed. These results mirror the patterns from the initial descriptive work – the *Metoncholaimus* sp. and *Pontomyia* sp. were most abundant where *C. taxifolia* biomass was the least, while *A. buccinoides* was absent from all plots with *C. taxifolia*. Overall, our results are consistent with the model that *C. taxifolia* changes communities and that removing *C. taxifolia* allows the infaunal community composition to ‘recover’, but it takes five or more months to do so. Abundances of infauna, however, showed little evidence of recovery after five months. Unfortunately, we cannot comment on the extent to which the communities have truly recovered because there was no comparison with plots that never had *C. taxifolia* (due to the almost complete coverage of *C. taxifolia* at comparable depths). However, the recovery of infaunal communities (number of individuals and taxa) is similar to that reported by Glasby et al. (2005) six months after *C. taxifolia* was killed by smothering with salt (a managerial response to the invasion).

Changes in community components per se were surprisingly unrelated to abiotic modification – at least at the temporal and spatial scales studied here – although

C. taxifolia biomass was negatively correlated with some key abiotic variables (redox and DO) but not all (sediment organic content). McKinnon et al. (2009) showed a significant positive relationship between redox and infaunal abundance (but not species richness) among habitats (including *C. taxifolia*) across four sites in southeastern New South Wales. In addition, large negative effects of *C. taxifolia* on *A. trapezia* have previously been linked to low DO and redox within the *C. taxifolia* canopy (Gribben et al. 2009, Wright et al. 2010): bivalves generally respond strongly in terms of life-history traits, behaviour and survivorship to changes in dissolved oxygen (Diaz and Rosenberg 1995, Tallqvist 2001, Altieri 2008). Therefore, the absence of significant relationships between abiotic variables and infauna in this study is unusual. We suspect that this result may relate to sediments in Lake Conjola being naturally high in organics with associated low redox values. For example, in this study the average redox in Yooralla Bay and Sponge Bay was -256 mV. Even the three plots with the lowest initial *C. taxifolia* biomass (range 0 to 27 g dw m^{-2}) had strongly negative redox values (range -57 to -165 mV), whereas McKinnon et al. (2009) reported redox values in *C. taxifolia* of no less than -180 mV in Burrill Lake and St Georges Basin. This is supported by the fact that in the present study there was a strong positive association ($r=0.70$) between redox and infaunal abundance for the five samples with the highest redox potentials (ranging from -50 to -150 mV), possibly indicating density dependent responses of abiotic variables to *C. taxifolia* density. Alternatively, infauna may be responding to other variables not measured in this study including sediment sulfides which are higher in *C. taxifolia* invaded sediments compared to unvegetated sediments (Chisholm and Moulin 2003, Gribben et al. 2009) or other factors such as reduced water flow which may affect food supply to organisms resulting in poor survivorship (Allen and Williams 2003). However, it is unknown whether sublethal and lethal effects of *C. taxifolia* biomass are correlated with associated changes in abiotic variables because we did not determine these in our experimental plots. Moreover, we could not explore the relationship between *A. trapezia* and DO in the initial survey as few *A. trapezia* were recovered – Lake Conjola is at risk of localized extirpations of *A. trapezia* resulting from chronic exposure to *C. taxifolia*.

Our results have several implications for the management of habitat-forming invasive species. First, in circumstances where the management of invaders is possible, consideration needs to be given to how different community components respond to the presence of the invader and not just the effects on overall community structure. Our study indicates that focusing only on community structure may lead to incorrect decisions about the extent of the impacts of habitat-forming invasive species. Second, given strong sublethal density dependent effects of *C. taxifolia* biomass on *A. trapezia*, intervention by managers may be required at much lower densities than when changes in community structure occur. Obviously, determining sublethal effects on all species within a community is impractical, but it may be important to understand sublethal effects on species that respond strongly to environmental perturbations, are rare or threatened, are commercially

valuable or contribute disproportionately to ecosystem function. Third, our results indicate the community recovery is dependent on invader density, and that areas with different biomasses of habitat-forming species may have different temporal recovery trajectories. Understanding how the hysteresis of a system is affected by the abundance of invasive species prior to their removal is a seldom recognized, yet key aspect of restoration ecology (Byers et al. 2006).

An obvious limitation of this study is that it was conducted in one estuary only. Understanding how density dependent effects vary spatially and under different environmental contexts is an important next step. However, we have shown that a habitat-forming invasive species can have contrasting effects on different community components and density-dependent effects on individuals and communities. We encourage researchers to consider the use of multiple-density manipulations and to incorporate individual level responses, in uncovering the impacts of these types of invaders on native biota.

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