

WHOLE-COMMUNITY MUTUALISM: ASSOCIATED INVERTEBRATES FACILITATE A DOMINANT HABITAT-FORMING SEAWEED

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Abstract. Many habitat-forming, or foundation, species harbor diverse assemblages of associated taxa that benefit from the refuges from predators or harsh physical conditions that foundation species provide. Growing numbers of studies show how specific taxa associated with foundation species can benefit their hosts, but the aggregate effects of the entire community of associated species remain poorly understood. Here, we evaluate the role that a diverse assemblage of invertebrates plays in mediating the dominance of a foundation species, the green filamentous seaweed *Cladophora columbiana* Collins, in rocky intertidal habitats. *Cladophora* is a fast-growing seaweed with a high nitrogen demand, and we suggest that it persists in nutrient-limited high-intertidal pools because of local-scale nitrogen excretion by the invertebrate taxa living within its filaments. Removal of associated invertebrates resulted in a fourfold increase in the rate of water-column nitrogen depletion by *Cladophora*, and ammonium concentrations inside *Cladophora* turfs with invertebrates present were seven times higher than in the adjacent tide-pool water. The ammonium excreted by invertebrate meiofauna far surpassed the nitrogen used by *Cladophora*, suggesting that all of *Cladophora*'s nitrogen requirements could be met by the invertebrates associated with it. This study links host performance to the total aggregate biomass of mutualists rather than the particular traits of any one species, suggesting the potential for important feedbacks between individual hosts and the communities of associated species that they support.

Key words: ammonium; *Cladophora columbiana*; facilitation; foundation species; mutualism; nitrogen; positive interactions; rocky-intertidal communities; seaweeds.

INTRODUCTION

The physical structure and consequent amelioration of harsh conditions provided by foundation species (*sensu* Dayton 1972) enhance the diversity and abundance of organisms in many terrestrial and marine ecosystems (Bruno and Bertness 2001, Stachowicz 2001). In fact, it has been argued that the community structure of most benthic marine systems is dependent on the physical structure provided by organisms such as reef-building corals (Done et al. 1997), kelps (Graham 2004), seagrasses (Heck and Crowder 1991), oysters (Grabowski et al. 2005), and mangroves (Ellison et al. 1996). Many foundation species harbor diverse assemblages of associated taxa (Dayton 1975, Seed 1996, Bruno 2000, Ellison et al. 2005), and few studies have simultaneously examined the combined roles of these species and their effects on host performance (Stanton 2003, Stachowicz and Whitlach 2005). This is especially true for small invertebrates, which are a diverse and abundant, but under-appreciated, component of many ecological sys-

tems. Invertebrate meso- and meiofauna are commonly associated with marine foundation species (Taylor and Rees 1998, Duffy et al. 2003, Grabowski et al. 2005, Stachowicz and Whitlach 2005). Despite their small size, these organisms can provide important benefits to their hosts. For example, invertebrate mesograzers remove epiphytes from seagrasses and algae, enhancing their hosts' growth rates (Williams and Ruckelshaus 1993, Stachowicz and Whitlach 2005).

The seaweed *Cladophora columbiana* Collins (hereafter, *Cladophora*) acts as a foundation species in marine rocky intertidal habitats, where it supports a distinct assemblage of invertebrate meiofauna (0.1–2.0 mm in length). *Cladophora* typically grows as a tangled green turf composed of thin filaments and is often found in high-intertidal pools on northeastern Pacific shorelines (Abbott and Hollenberg 1976). Its occurrence in these pools is paradoxical; *Cladophora* is a fast-growing, opportunistic algal species with a high nitrogen demand (Pedersen and Borum 1996), and seaweeds growing in high-zone tide pools are often nitrogen limited (Bracken and Nielsen 2004). This nitrogen limitation can be ameliorated by sessile, suspension feeding invertebrates (primarily mussels and sea anemones) living in tide pools. These invertebrates excrete inorganic nitrogen (ammonium) as a waste product, and both growth and

Manuscript received 26 May 2006; revised 18 December 2006; accepted 25 January 2007. Corresponding Editor: P. D. Steinberg.

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diversity of tide pool seaweeds are higher in pools where invertebrates are abundant (Bracken 2004, Bracken and Nielsen 2004).

Our previous work (Bracken and Nielsen 2004) indicates that in high-intertidal pools where invertebrate biomass and invertebrate-mediated ammonium levels are low, most seaweeds tend to be slow-growing species with low nitrogen requirements. Based on their height on the shore (i.e., without taking into consideration wave splash), these pools are isolated from the ocean at low tide for 87% of the time, integrated over the entire year, so local-scale nitrogen regeneration is likely to be an important contributor to the nitrogen requirements of seaweeds living in them (Bracken and Nielsen 2004). As invertebrates increase in abundance and nitrogen becomes more available, the slow-growing species are joined by fast-growing ephemeral species with higher nitrogen requirements. The exception to this pattern is *Cladophora*, which, despite being a fast-growing species characterized by high nitrogen uptake rates, is found in virtually all high-intertidal pools, regardless of nitrogen availability and macroinvertebrate abundance. We hypothesize that *Cladophora*'s role as habitat for microinvertebrates may help explain this apparent contradiction. Specifically, the matrix of tangled filaments within *Cladophora* turfs provides habitat for an assemblage of invertebrate meiofauna (Dodds and Gudder 1992, Hull 1997), and nitrogen excreted by these invertebrates may decrease *Cladophora*'s reliance on other (external) nitrogen sources. Such an interaction would be functionally similar to the relationship between many terrestrial plants and their microbial endosymbionts; just as mutualistic bacteria transform atmospheric nitrogen into a fixed form which can be assimilated by their plant hosts (e.g., Parker 1995), we hypothesize that mutualistic meiofauna transform particulate organic nitrogen, which cannot be used by seaweeds, into readily usable dissolved inorganic nitrogen.

In this study, we use a combination of field and laboratory observations and experiments to quantify the abundances of *Cladophora* and its associated invertebrates and evaluate the contribution of the invertebrates to *Cladophora*'s nitrogen demand. More generally, we use our data to discuss the mechanisms by which the assemblage of organisms supported by a given foundation species may influence that species' distribution and abundance and the consequent potential feedbacks between foundation species and the assemblages which they facilitate.

MATERIALS AND METHODS

Surveys of algae and invertebrates

We surveyed abundances of seaweeds and their associated invertebrates in high-intertidal pools in the Bodega Marine Reserve, northern California, USA (38°19'0" N, 123°4'6" W; see Plate 1). Earlier studies in similar high-intertidal pools on the Oregon coast suggested that *Cladophora* dominated algal cover (Bracken and

Nielsen 2004). To verify this pattern, we measured the total rock surface area and the surface area covered by each algal species in 49 high-intertidal pools (just above the mean high water mark) in the reserve. We found that seven macroalgae (*Cladophora*, coralline crusts, *Endocladia muricata* (Endlicher) J. Agardh, *Mastocarpus papillatus* (C. Agardh) Kützing [including both upright and *Petrocelis* forms], *Mazzaella flaccida* (Setchell et N. L. Gardner) Fredericq, *Porphyra perforata* J. Agardh, and *Prionitis lanceolata* (Harvey) Harvey; referred to hereafter by genus) were common inhabitants of the pools. We therefore divided the 49 sampled tide pools into seven blocks of seven pools each, based on geographic proximity. We then randomly assigned each seaweed species to one of the pools in each block, which provided us with $n = 7$ independent estimates of the cover of each species.

To characterize the meiofauna associated with *Cladophora* and other common seaweed species, we collected 15 samples of *Cladophora*, coralline crusts, *Endocladia*, *Mastocarpus*, *Mazzaella*, *Porphyra*, and *Prionitis*. We also sampled invertebrates in the upright coralline alga *Corallina vancouveriensis* Yendo, which is occasionally found in high-intertidal pools. We quantified all invertebrates in 1–2 g (wet mass) portions of each of the 15 samples of each species. Algal samples were counted by placing them in a Petri dish containing artificial sea water, gently separating algal filaments or branches (where applicable), and counting every invertebrate associated with the alga. All invertebrates were identified to the lowest practical taxonomic group. Individuals were then removed from the turf, dried to constant mass at 60°C, and weighed on a microbalance (Model MX5, Mettler Toledo, Columbus, Ohio, USA). These mass measurements were averaged for each taxon and used with the abundance data to calculate the biomass of each invertebrate species per gram of wet algal tissue.

Ammonium uptake by Cladophora turfs

To quantify the contribution of invertebrate meiofauna to the nitrogen requirements of *Cladophora*, we measured ammonium uptake rates of *Cladophora* with and without associated invertebrates. Intact *Cladophora* turfs were collected from high-zone pools in the Bodega Marine Reserve immediately prior to running uptake trials. Half of the collected algal biomass (randomly selected) was thoroughly rinsed with seawater to remove organisms and sediments trapped in the algae, whereas the other half was neither rinsed nor disturbed, to ensure that it retained its natural meiofaunal assemblage. Because the mass of invertebrates and sediments within a *Cladophora* turf is roughly equivalent to the amount of algal biomass in the turf (C. Gonzalez-Dorantes, unpublished data), approximately 24 g (wet mass) of algae from the “–invertebrates” treatments, and 48 g (wet mass) of combined algae, invertebrates, and sediment from the “+invertebrates” treatments were then placed in acid-washed glass microcosms ($n = 16$ for



PLATE 1. Tide pools in the Bodega Marine Reserve, northern California, USA. High-zone pools on northeastern Pacific shores are dominated by the seaweed *Cladophora columbiana*, which provides habitat for a diverse and abundant assemblage of invertebrate meiofauna. Photo credit: M. Bracken.

each treatment). Microcosms contained 400 mL of artificial seawater at eight initial ammonium concentrations (2, 4, 8, 12, 20, 30, 40, and 60 $\mu\text{mol/L}$), with two replicates at each concentration. Our previous work indicates that nitrogen dynamics in microcosms of this size accurately reflect excretion and uptake rates in natural tide pools (Bracken and Stachowicz 2006).

We situated microcosms in an outdoor shallow seawater tank under ambient light intensities ($1559 \pm 17 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ [mean \pm SE]) and seawater temperatures ($16.7^\circ \pm 0.04^\circ\text{C}$ [mean \pm SE]). We then took 1 mL water samples from each microcosm at 0, 15, 30, and 45 minutes. Ammonium concentrations of these samples were determined using the phenol-hypochlorite method (Solórzano 1969). We then removed all sediment and invertebrates from “+invertebrates” treatments and dried the algae from each of the microcosms to constant mass at 60°C . Finally, we calculated uptake based on rates of nitrogen depletion from the water, normalized by dry algal tissue mass ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$). Because of still-water conditions in the microcosms (as would occur in natural tide pools), uptake rates increased linearly with increasing ammonium concentration (Bracken and Nielsen 2004). We therefore compared uptake of *Cladophora* turfs with and without associated invertebrates using an analysis of covariance, with initial ammonium concentration as a covariate. This comparison allowed us to assess the reliance of *Cladophora* on ammonium supplied by associated invertebrates (the presence of which reduced its uptake from the surrounding water) relative to its actual nitrogen usage.

To verify that these differences in uptake were due to invertebrate meiofauna, and not simply due to removal of sediment, we conducted an identical experiment to the one described above, but instead of rinsing the *Cladophora* to remove invertebrates and sediment, we used the insecticide Sevin (1-naphthyl n-methylcarbamate; Ortho, Marysville, Ohio, USA) to reduce the abundances of associated invertebrates (Duffy and Hay 2000). We collected additional samples of *Cladophora* from high-intertidal pools, placed the samples into two buckets filled with seawater, and randomly selected one of the buckets for treatment with a dilute insecticide solution ($\sim 1 \mu\text{L/L}$) for 30 min. We assessed the effectiveness of the insecticide by counting invertebrates associated with the *Cladophora* before and after adding Sevin. We found that whereas different invertebrate taxa were affected to varying degrees, adding the insecticide reduced overall invertebrate abundance (no. individuals/g) by 72% and biomass (mg/g) by 47%. After the insecticide treatment, we gently emptied and refilled both buckets several times with seawater, then evaluated the ammonium uptake abilities of the treated and untreated algal turfs.

Effects of macroalgae on ammonium fluxes and concentrations

We conducted sequential manipulations to evaluate in situ ammonium uptake by turfs of *Cladophora*, which harbor numerous invertebrates, and by the red alga *Prionitis*, which lacks fine branching structures and is therefore relatively free of small invertebrates (see

Results), but was still common in many of the surveyed tide pools. By comparing uptake of these two seaweed species, we assessed the degree to which the ability to harbor associated invertebrates alters reliance on external sources of ammonium. Manipulations were conducted in two sets of 12 pools (one set for each algal species) in the Bodega Marine Reserve. Sample collection began at the time when pools were isolated by the receding tide and was repeated every hour over a four-hour period, and the water samples were then analyzed for ammonium concentrations (Solórzano 1969). After the samples were taken, all target algae (*Cladophora* or *Prionitis*) present in the sampled tide pools were removed. The following day, the ammonium measurements were repeated in the same pools to quantify ammonium fluxes in the absence of either *Cladophora* or *Prionitis*. Changes in ammonium concentrations over time and effects of algae on ammonium concentrations were analyzed using repeated-measures ANOVA.

Finally, to assess the in situ concentration of ammonium and the degree to which regenerated nitrogen was used within algal turfs vs. exported to the rest of the tide pool, we measured ammonium concentrations within and outside of *Cladophora* turfs in an additional set of tide pools ($n = 10$) in the reserve. We used B-D 5cc^{21G1} disposable syringes (Becton Dickinson and Co., Franklin Lakes, New Jersey, USA) to collect water samples four hours after pools were isolated by the receding tide. We collected samples within the *Cladophora* turf and ~5 cm above the turf in each pool, and separate syringes were used for each sample. Concentrations inside and above *Cladophora* turfs were compared using paired *t* tests after log-transformation.

Ammonium contribution of associated invertebrates

Previous work on nitrogen excretion by seaweed-associated invertebrate fauna (Taylor and Rees 1998) provided us with a formula relating ammonium excretion (A , in $\mu\text{mol/h}$) to the biomass (B , in g ash-free dry mass) of individual invertebrates ($A = 0.147 \times B^{0.602}$). This relationship explained 89% of the variance in ammonium excretion rates, and the slopes and intercepts of the relationship did not differ among taxonomic groups. Note, however, that this formula was empirically verified by Taylor and Rees (1998) for invertebrates >0.4 mm in length. Since the meiofauna within *Cladophora* turfs were as small as 0.1 mm, we assumed that this relationship held for smaller individuals. We used this relationship and our invertebrate abundance and biomass data to calculate the amount of ammonium excreted ($\mu\text{mol/h}$) by the meiofauna within one gram of *Cladophora* tissue. We then calculated the ammonium uptake capacity ($\mu\text{mol/h}$) per gram of *Cladophora* using data from our microcosm experiments and the concentrations of ammonium we measured within *Cladophora* turfs in the field. Based on these values, we calculated the percentage of *Cladophora*'s

ammonium usage which was supplied by associated invertebrate meiofauna.

Specifically, the total excretion rate of the 14 invertebrate taxa within a turf (ΣE) was calculated as a function of the total abundances (no. individuals/g, A_n) and average individual biomasses (B_n) of each taxon:

$$\Sigma E = \sum_{n=1}^{14} A_n [0.147 (M_n B_n)^{0.602}]. \quad (1)$$

This is the formula from Taylor and Rees (1998), with a taxon-specific term (M_n) converting dry biomass to ash-free dry mass (Brey 2001). The relationship between ammonium use (U) by *Cladophora* and initial ammonium concentration (C), was determined using our measurements of water column ammonium depletion by *Cladophora* in the “-invertebrate” tide pool microcosms (see *Results*):

$$U = 0.085 \times C - 0.092. \quad (2)$$

We then used these calculations and our empirical measurements of invertebrate abundances (A_n) and biomasses (B_n) and ammonium concentrations inside *Cladophora* turfs (C) to quantify the proportion of *Cladophora*'s ammonium uptake capacity that was provided by the invertebrate meiofauna (I):

$$I = \frac{\Sigma E}{U}. \quad (3)$$

RESULTS

Surveys of algae and invertebrates

When compared to other common tide pool macroalgae, *Cladophora* was clearly the dominant species, averaging 67% of algal cover ($F_{6,36} = 9.13$, $P < 0.012$ after Tukey adjustment) (Fig. 1a). When we averaged algal cover in all 49 pools instead of separating them into seven blocks of seven species, we observed a similar pattern: cover of *Cladophora* was 60%, and cover of *Mastocarpus*, the next-most-abundant species, was 22%. *Cladophora* also harbored a far more abundant and diverse invertebrate assemblage. Both biomass ($F_{6,98} = 237.89$, $P < 0.001$, after Tukey adjustment) and abundance ($F_{6,98} = 316.06$, $P < 0.001$) of associated invertebrates were higher on *Cladophora* than on any other common tide pool species (Fig. 1b). In fact, *Cladophora* harbored many more invertebrates (179.7 ± 18.8 individuals/g) than either *Corallina* (22.5 ± 4.9 individuals/g [mean \pm SE]; $F_{1,28} = 65.43$, $P < 0.001$) or *Endocladia* (6.1 ± 0.8 individuals/g; $F_{1,28} = 85.17$, $P < 0.001$), both of which have structurally complex thallus morphologies and have previously been shown to host abundant meiofaunal assemblages (Glynn 1965, Hicks 1980).

Close examination of *Cladophora* samples and entrapped sediments revealed high abundances of invertebrates in 14 major taxonomic groups, which represented seven different animal phyla (Fig. 2). Few of these taxa (e.g., some of the amphipods, polychaetes, and insect

larvae) have the potential to actually consume *Cladophora* (Morris et al. 1980, Trowbridge 1993; M. Bracken and J. Stachowicz, *personal observation*). Instead, most of the invertebrate biomass in the turfs (>80%) was composed of groups that feed on detritus (e.g., ostracods, bivalves, tanaids, and some amphipods), periphyton (e.g., copepods, nematodes, and gastropods), or other invertebrates (e.g., nematodes, flatworms, and some of the polychaetes) (Fig. 2). Ostracods (*Xestolebris* sp.; A. Cohen, *personal communication*) were the most abundant taxonomic group, found in densities of up to ~450 individuals/g of wet algal mass. Based on our counts and the average biomass of each taxon, the total dry biomass of invertebrates per gram of *Cladophora* turf was 34.5 ± 14.6 mg, with ostracods comprising the largest fraction (39%) of the biomass (Fig. 2).

Ammonium uptake by *Cladophora* turfs

We found that removing the invertebrates from *Cladophora* turfs by thoroughly rinsing them in seawater had profound effects on the turfs' use of external water-column ammonium. As initial ammonium concentrations increased, ammonium uptake by *Cladophora* turfs without invertebrates increased more rapidly than uptake by turfs containing invertebrates (initial concentration \times invertebrates interaction: $F_{1,28} = 34.19$, $P < 0.001$; Fig. 3), suggesting that *Cladophora* turfs containing invertebrates are not as reliant on water-column ammonium because invertebrates within the turfs are important contributors to the nitrogen used by the algae. Reduction of invertebrate biomasses and abundances via addition of Sevin insecticide resulted in similar, albeit less pronounced, results (initial concentration \times invertebrates interaction: $F_{1,28} = 5.18$, $P = 0.030$). Because Sevin only reduced invertebrate biomasses (mg/g) by ~50%, the increase in water-column ammonium use associated with insecticide addition was only about half that of the "rinsing" treatment. However, the increase in ammonium use by *Cladophora* when the invertebrates were reduced but the sediment was left in place demonstrates that the effect we describe was due to the removal of invertebrates, not to the removal of sediment.

Rates of ammonium depletion from the water column by *Cladophora* turfs containing invertebrates increased as external ammonium concentrations increased, indicating that even when invertebrates are present, external ammonium can be important in meeting the nitrogen demands of *Cladophora* turfs when ammonium concentrations are high. However, net positive uptake of water-column ammonium (a depletion rate significantly greater than zero) was only apparent when external ammonium concentrations exceeded $30.7 \mu\text{mol/L}$, which is higher than any water-column ammonium concentrations we observed in tide pools in the Bodega Marine Reserve, even those containing high biomasses of sessile invertebrates. This suggests that under natural conditions, *Cladophora* requires no inorganic nitrogen from

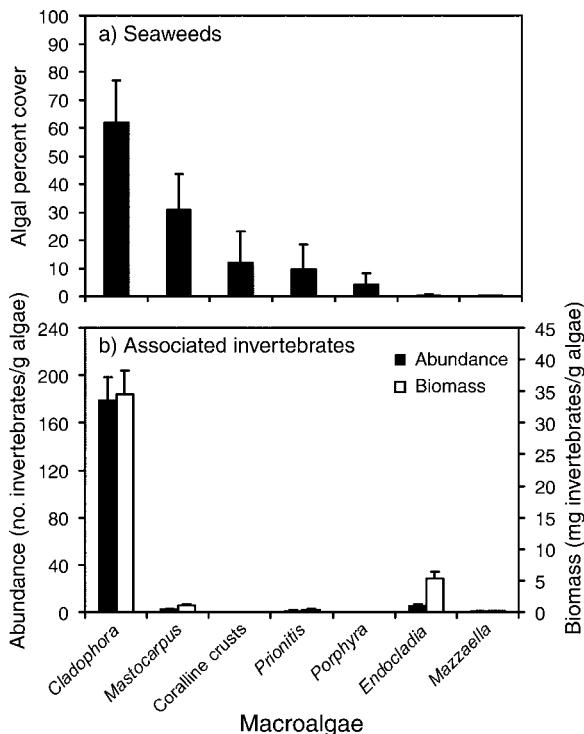


FIG. 1. Abundances of seaweeds and associated invertebrates in high intertidal pools. (a) *Cladophora columbiana* was the dominant macroalgal species in these pools ($P < 0.012$ after Tukey adjustment). Values are algal cover estimates (mean + SE) from surveys of 49 tide pools. (b) Abundance and biomass of associated invertebrates were highest in *Cladophora* ($P < 0.001$ for both measurements after Tukey adjustment). Values (mean + SE) are based on counts and mass of invertebrates in $n = 15$ samples of each algal species.

the water column of the tide pool (e.g., ammonium excreted by macroinvertebrates).

Effects of macroalgae on ammonium fluxes and concentrations

In order to evaluate the reliance of *Cladophora* turfs on water-column ammonium, we quantified the effects of *Cladophora* removal on ammonium concentrations in tide pools. In all treatments, the ammonium concentration increased over time due to excretion by tide pool macroinvertebrates. Removal of *Cladophora* resulted in no change in ammonium concentrations in the pools (repeated-measures ANOVA, $F_{4,88} = 1.60$, $P = 0.181$; Fig. 4a). In contrast, removal of *Prionitis*, which virtually lacks associated invertebrates (Fig. 1b), resulted in a doubling in water-column ammonium concentrations over four hours (repeated-measures ANOVA, $F_{4,88} = 5.84$, $P < 0.001$; Fig. 4b). These differences were not due to differences in the algal biomass removed or in the different species' ammonium uptake abilities. In fact, the average biomass of *Cladophora* removed (9.6 ± 2.0 g/L) was four times higher than the biomass of *Prionitis* (2.3 ± 0.4 g/L), and *Cladophora* is more effective at

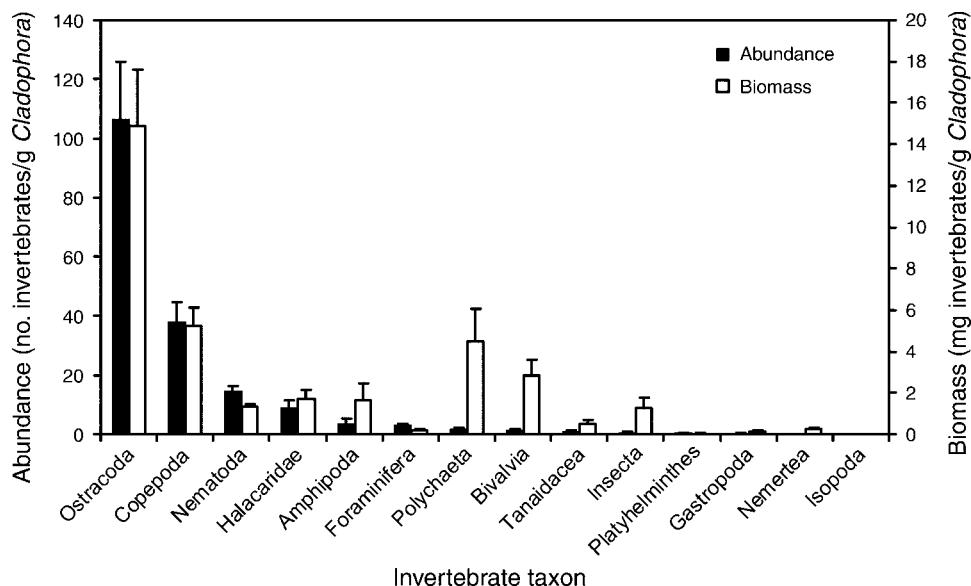


FIG. 2. Abundances and biomass measurements of invertebrates in *Cladophora* turfs. Abundances and biomasses of major taxonomic groups were quantified by counting and weighing (dry-tissue mass) all invertebrates per gram (wet-tissue mass) of *Cladophora*. Values are means (\pm SE) of $n = 15$ samples of algal turf.

ammonium uptake than *Prionitis* (Bracken and Stachowicz 2006). These comparisons suggested that the ammonium excreted by invertebrates within a turf was sufficient to meet the nitrogen demands of *Cladophora*. We verified this by measuring ammonium concentrations within *Cladophora* turfs in the field. We found that ammonium concentrations within the turfs averaged $61.1 \pm 22.6 \mu\text{mol/L}$ and were seven times higher than

those found in the water column just 5 cm above the turfs (paired t test, $t = 2.62$, $df = 9$, $P = 0.028$). The average water-column concentration above the turfs was no different than ammonium concentrations in tide pools without algae ($t = 1.55$, $df = 20$, $P = 0.136$), indicating that there is minimal export of regenerated nitrogen from *Cladophora* turfs or that any exported ammonium is rapidly used by other algal species.

Ammonium contribution of associated invertebrates

Our data on biomass of associated invertebrates, *Cladophora* uptake rates, concentrations of ammonium within *Cladophora* turfs, and ammonium fluxes in tide pools enabled us to evaluate the contribution of the invertebrates to the nitrogen requirements of *Cladophora*. The average ammonium contribution of invertebrates, per gram dry *Cladophora* mass, was $63.3 \mu\text{mol/h}$ (Eq. 1). Based on their biomass and abundance, ostracods contributed 52% of this total, copepods contributed 21%, and the remaining taxa (especially nematodes, polychaetes, and mites) were responsible for the remainder. Using our measurements of ammonium concentrations within *Cladophora* turfs ($C = 61.1 \mu\text{mol/L}$) and the relationship between ammonium concentration and uptake in *Cladophora* turfs lacking invertebrates (Fig. 3), we found that *Cladophora* took up ammonium at an average rate of $5.1 \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$ under field conditions (Eq. 2). Based on these calculations, the rate of nitrogen excretion by associated invertebrates was 12.5 times the rate of nitrogen use by *Cladophora* (Eq. 3), which is consistent with the high concentrations of ammonium (up to $192 \mu\text{mol/L}$) we observed within *Cladophora* turfs.

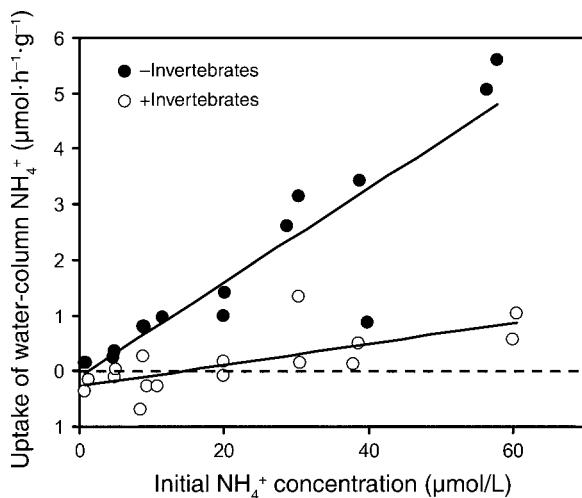


FIG. 3. Removal of associated invertebrates ("–invertebrates") increases the reliance of *Cladophora* turfs on water-column ammonium (initial concentration \times invertebrates interaction, $F_{1,28} = 34.19$, $P < 0.001$). The dashed horizontal line indicates the point at which *Cladophora* switches from reliance on internal ammonium regeneration by invertebrates (negative ammonium uptake) to external water-column ammonium (positive ammonium uptake).

DISCUSSION

These results suggest that *Cladophora* may achieve dominance in high intertidal pools (Fig. 1a) because it facilitates a unique assemblage of associated invertebrates (Fig. 2). Specifically, the invertebrates living within *Cladophora* turfs excrete substantial quantities of ammonium (Fig. 3), providing all of the nitrogen the seaweed requires and potentially allowing it to thrive in an otherwise nitrogen-limited high-intertidal habitat (Bracken and Nielsen 2004). Most of the taxa we identified consume epiphytes or detritus, and very few consume macroalgae (see *Results*). Based on our surveys of other tide pool seaweeds, most of these invertebrates are rare or absent on co-occurring algae (Fig. 1b), so we suspect that they also derive some benefit from the association, likely due to *Cladophora*'s provision of unique habitats such as trapped sediment and fine filaments that provide refuge and a substrate for the periphyton on which some of the meiofauna feed.

Growth of seaweeds in high-intertidal pools is a function of a variety of factors, including grazing pressure (Lubchenco 1978, Nielsen 2003), disturbance (Dethier 1984), and nutrient availability (Nielsen 2003, Bracken 2004). These tide pools typically contain high abundances of molluscan grazers, predominately the turban snail *Tegula funebris* A. Adams (Nielsen 2003, Bracken and Nielsen 2004), and increased snail abundance is associated with reduced total algal biomass (g/L) in the pools (Bracken and Nielsen 2004). However, snail grazing primarily impacts ephemeral algal species such as *Porphyra* and *Ulva*, which are rare in tide pools unless grazer abundances are reduced (Fig. 1a; see Lubchenco 1978, Nielsen 2003). Cover of the most abundant algal species in these pools (*Cladophora*, the encrusting *Petrocelis* form of *Mastocarpus*, coralline crusts, and *Prionitis*) was not associated with *Tegula* abundances (M. Bracken and J. Stachowicz, unpublished data), likely because many of these species are defended against or tolerant of herbivory (Lubchenco and Cubitt 1980, Dethier 1982, 1984, Sousa 1984, Steneck et al. 1991).

We propose that the dominance of *Cladophora* in high-zone tide pools is therefore due to a combination of factors. First, the lack of a relationship between *Cladophora* cover and herbivore abundance suggests that its growth rates are sufficient to compensate for any grazing impacts. Second, provided that it has sufficient nitrogen, *Cladophora*'s growth rate is higher than that of either *Mastocarpus* or *Prionitis* (Bracken and Nielsen 2004), which likely allows it to effectively compete with these other common seaweeds for space. Finally, unlike other species in the pools, *Cladophora* harbors a diverse and abundant assemblage of invertebrate meiofauna (Figs. 1b and 2), which contribute far more ammonium than it uses, despite its high uptake rates relative to other common tide pool algae (e.g., *Mastocarpus* or *Prionitis*; Bracken and Stachowicz 2006). *Cladophora* has a limited ability to store nitrogen and a high nitrogen demand for

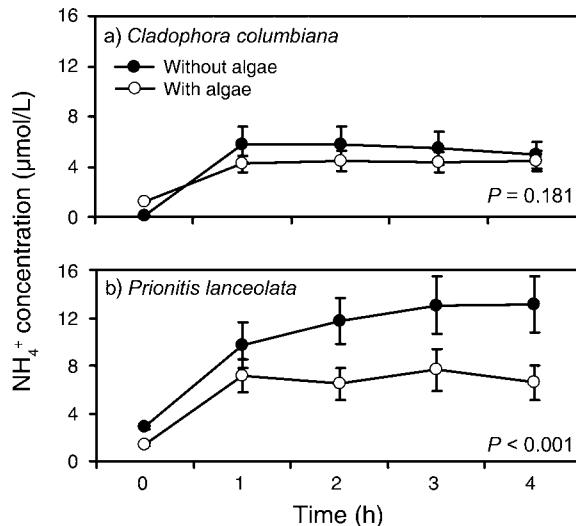


FIG. 4. Influences of macroalgal removal on ammonium concentrations in tide pools. Values are ammonium concentrations (mean + SE) measured at one-hour intervals after isolation of pools ($n = 12$ for each species) by the receding tide. (a) Removal of *Cladophora* from pools had no effect on ammonium concentrations ($F_{4,88} = 1.60$, $P = 0.181$). (b) Removal of *Prionitis*, however, resulted in increases in tide pool ammonium concentrations ($F_{4,88} = 5.84$, $P < 0.001$).

growth (Pedersen and Borum 1996). We therefore suggest that *Cladophora* relies on local-scale nitrogen inputs from associated invertebrates to sustain its growth in high-intertidal pools, which are isolated from oceanic nitrogen inputs for long periods of time—up to five days during neap tides and calm weather (Bracken and Nielsen 2004)—at low tide. Furthermore, *Cladophora* appears to have a limited ability to take up nitrate, especially in the still-water conditions found in tide pools (Bracken and Stachowicz 2006), so invertebrate-excreted ammonium may be even more important to *Cladophora* than to other tide pool seaweeds.

Whereas most research describing the interactions between foundation species and the organisms they shelter has focused on the benefits provided by the hosts to the associated species (Bruno and Bertness 2001, Stachowicz 2001), our data suggest that increasing the abundances of associated species leads to an increase in the benefits provided to their seaweed host, creating a positive feedback between the foundation species and the associated species that live in it. Many studies have described the negative effects of small invertebrate grazers on algal biomass (e.g., Brawley and Fei 1987, Graham 2002). However, our work joins a growing body of evidence indicating that associated invertebrates can provide benefits to their sessile hosts, be they plants, algae, or colonial invertebrates, in a variety of different systems. These benefits include removal of epiphytic and epizootic competitors (Williams and Ruckelshaus 1993, Stachowicz and Hay 1999, Stachowicz and Whitlatch 2005), provision of limiting nutrients (Hurd et al. 1994,

Ellison et al. 1996, Taylor and Rees 1998), and protection against natural enemies (Janzen 1969, Glynn 1976, Ellison and Farnsworth 1990). Most of these examples involve cases where particular species, among the many associated with a host organism, benefit their host. However, given the growing recognition that multiple associated species can play mutualistic or antagonistic roles (Horvitz and Schemske 1990, Stanton 2003, Ness et al. 2006), we suggest that it is important to consider the net effect of the entire community of associates on their host. Indeed, we show that the benefits to *Cladophora* likely increase with the total biomass of associated species and do not depend on the presence of particular invertebrate taxa.

The nutritional interactions between seaweeds and the species associated with them are similar to the ubiquitous relationships between microscopic endosymbionts and their hosts. These symbioses, which include the associations between unicellular algae and marine invertebrates, nitrogen-fixing bacteria and legumes, and cellulose-digesting protozoans and termites, all involve physical interactions between species by which the host gains access to a novel metabolic capability (e.g., carbon fixation, nitrogen fixation, or cellulose degradation) (Douglas 1994). In our study, the invertebrate meiofauna living within *Cladophora* turfs mediate the conversion of particulate nitrogen, which is useless to their seaweed host, into ammonium, which can be readily taken up and assimilated (Lobban and Harrison 1994). An analogous interaction occurs between tropical seaweeds (*Sargassum* spp.) and their microbial epiphytes. Growth rates and tissue nitrogen levels of *Sargassum* are higher when the seaweeds are exposed to particulate matter, because the epiphytic microbial community remineralizes the particulate nitrogen, making it available to the host seaweed (Schaffelke 1999).

In similar associations between plant roots and nitrogen-fixing endosymbionts, benefits provided to the host plants predictably decline as soil nitrogen levels increase (Kiers et al. 2002). Based on this, we predict that the contribution of associated invertebrate meiofauna should be most important in habitats in which nitrogen is limiting for growth, such as high-intertidal pools (Bracken 2004), and may diminish in lower-intertidal habitats where nitrogen inputs (due to excretion by macroinvertebrates and oceanic nitrate availability) are higher. The role that these invertebrates play in allowing *Cladophora* to thrive in an otherwise inhospitable environment suggests that cryptic meiofauna, which have been largely ignored in ecological studies due to their small size and difficulty of collection (Underwood and Petraitis 1993, Hull 1997), can have important influences on the community structure and dynamics of macroscopic organisms.

ACKNOWLEDGMENTS

We thank S. Williams for providing advice, lab space, and equipment; A. Carranza, A. Fish, K. Sellheim, and members of the Stachowicz lab for research assistance; and A. R. Hughes

and two anonymous reviewers for helpful comments on the manuscript. This work was supported by the National Science Foundation (DBI-0453251 to S. Williams for the Bodega Marine Laboratory Research Experience for Undergraduates Program, OCE-0351778 to J. Stachowicz, and OCE-0549944 to S. Williams and M. Bracken). This is contribution number 2371 of the Bodega Marine Laboratory, University of California–Davis.

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