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<https://escholarship.org/uc/item/51r1026m>

Journal

Ecology, 86(9)

ISSN

0012-9658

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Publication Date

2005-09-01

Peer reviewed

MULTIPLE MUTUALISTS PROVIDE COMPLEMENTARY BENEFITS TO THEIR SEAWEED HOST

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Abstract. Although the importance of mutualisms in structuring communities is becoming increasingly appreciated, relatively little is known about the degree to which potential mutualists are interchangeable with respect to the services they provide their host. We examined the degree to which potential mutualists of a seaweed provide complementary vs. redundant benefits to their host to begin to assess the effects of mutualist diversity on host performance. The gastropods *Anachis lafresnayi* (*Costoanachis lafresnayi*) and *Mitrella lunata* (*Astyris lunata*) are common associates of the red alga *Chondrus crispus* in subtidal locations in southern New England. Field surveys showed that where these snails were rare, *Chondrus* was sparse and covered with a heavy growth of sessile invertebrates, but where snails were common, *Chondrus* was abundant and free from fouling overgrowth. In manipulative field experiments, plants with no snails had the most overgrowth (measured both as percent cover and total mass) and lost 25% of their mass in five weeks. Plants with either species of snail alone were also heavily fouled and lost mass. Those with only *Mitrella* were overgrown by solitary ascidians, whereas those with only *Anachis* were overgrown by bryozoans, suggesting that both snails remove epibionts, but they differ in the suite of epibionts they are capable of removing. As a result, only *Chondrus* with both species of snail present remained unfouled and gained mass over the course of the experiment. Field tethering experiments showed that both snail species can gain a refuge from crab predation by associating with *Chondrus*. Our results show that these two snail species are not interchangeable and they provide complementary benefits to their host because they differ in their prey preferences. If this type of differentiation in resource use often mediates coexistence among mutualists, then complementary effects of mutualists on their hosts may be common.

Key words: competition; complementarity; diversity–ecosystem function; epiphytes; foundation species; marine invertebrate; mutualism; predation; redundancy; resource partitioning; seaweed.

INTRODUCTION

The renaissance of interest in positive interactions (mutualisms, commensalisms, and facilitation) has highlighted their ubiquity and the critical role they play in ecosystems worldwide. In particular, reviews have highlighted the key ecological role of habitat-forming species in facilitating a diverse array of associated flora and fauna (Stachowicz 2001, Bruno et al. 2003). For example, canopy-forming plants and reef-building invertebrates provide refuge from abiotic or biotic stresses for associated species, enhancing the persistence of a variety of associated species and increasing community diversity (e.g., Dayton 1975, Suchanek 1986, Hacker and Bertness 1999, Stachowicz and Hay 1999). There is no doubt that these hosts (foundation species, sensu Dayton 1972) play a key role in their respective ecosystems. However, few studies examine whether the diversity of organisms facilitated by foundation species

plays any role in ecosystem maintenance or if these species are functionally irrelevant.

How might “associated” species play a role in the persistence of their host? While foundation species are almost by definition dominant species, they are susceptible to several stressors including overgrowth by epibionts or fast-growing weedy species and consumption by predators or herbivores (review in Stachowicz 2001). Associated species that benefit from shelter provided by foundation species may thus benefit their host by reducing the load of these epibionts (Witman 1987, Coen 1988, Stachowicz and Hay 1996, 1999) or deterring consumers (Janzen 1966, Glynn 1976). In some terrestrial systems, mutualisms with seed dispersers can be critical for maintaining the diversity of habitat-forming species, and the removal of these dispersers has dramatic community consequences (Christian 2001). In each of these cases, the habitat-forming species and the ecosystem that depends on them depend on the presence of associated species (i.e., mutualists). Associated species, as well as their hosts, are often threatened by human activities such as habitat fragmentation or biological invasions (Kearns and Inouye

Manuscript received 13 May 2004; revised 21 January 2005; accepted 1 February 2005. Corresponding Editor: P. D. Steinberg.

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PLATE 1. Representative pictures of *Chondrus* plants from experimental treatment. (Top left) *Mitrella* + *Anachis*: note lack of fouling and healthy appearance. (Top right) *Mitrella* only: note heavy fouling by solitary ascidians. (Bottom left) No snails: plants are covered by colonial and solitary ascidians. (Bottom right) *Anachis* only: note heavy fouling by encrusting bryozoans and the reduced size of the plant due to the breakage of fronds. Only plants with both snail species exhibited positive growth (see Fig. 3C). A color version of Plate 1 is available in Appendix B. Photo credit: J. J. Stachowicz.

1997, Christian 2001), highlighting the need for a better understanding of the effects of multiple mutualists on their hosts, and on ecosystem structure and function in general.

Because host species are typically subject to multiple stresses simultaneously (e.g., predation, competition, and abiotic stresses), and different associates will be suitable to alleviating each stress, it follows that a given host species may benefit from simultaneously supporting multiple mutualists. For example, corals that depend on symbiosis with dinoflagellate microalgae as a major carbon source also enter into associations with mobile invertebrates to protect themselves from predators or competitors (e.g., Glynn 1976, Coen 1988, Stachowicz and Hay 1999). Similarly, legumes depend on bacterial root symbionts for a nitrogen supply but also require the services of mutualistic pollinators (e.g., Heithaus et al. 1982, Fenster 1991). Each of these mutualistic services can be thought of as being provided by a functional group of mutualists, defined by the benefit they provide to their host. Just as there is growing appreciation that functional group richness enhances ecosystem processes (e.g., Hooper and Vitousek 1997, Tilman et al. 1997), it seems likely that the performance of some host species will be enhanced by the presence of multiple functional groups of mutualists.

Less clear, however, is whether species within a functional group or guild of mutualists perform complementary or redundant roles. We suggest that similar species within a mutualist guild might commonly provide complementary benefits to the host as a byproduct of selection to minimize competitive overlap. Coexistence among competitors can often occur due to various forms of resource partitioning (Schoener 1974, Tilman 1982, Abrams 1983, Chesson 1991), and given that mutualists on a single host do compete for resources (e.g., Fonseca 1999, Palmer et al. 2003), coexistence among mutualists might be facilitated by differentiation in host utilization. If, as a result, no single mutualist completely alleviates the stress experienced by the host, then increasing mutualist species richness might lead to enhanced host performance, although this hypothesis remains untested. The growing realization that the presence of multiple mutualists on each host is the rule rather than the exception (Stanton 2003) suggests that this may be a critical gap in our understanding of mutualistic interactions.

In this study we examine the ecological role of marine gastropods that associate with dominant habitat-forming algae (*Chondrus crispus*) in shallow subtidal waters of the cold-temperate western North Atlantic (see Plate 1). *Chondrus* is a bushy, clonal, red seaweed

that provides habitat for numerous species of invertebrates (Rogers 1998). However, in subtidal areas *Chondrus* is prone to overgrowth by a suite of sessile invertebrates, including ascidians (sea squirts) and bryozoans. *Mitrella lunata* (*Astyrus lunata*) and *Anachis lafresnayi* (*Costoanachis lafresnayi*) are small gastropods in the family Columbellidae that are common within *Chondrus* turfs and are known to consume early life history stages of at least some of these fouling organisms (Osman and Whitlatch 1995). Using field surveys and experiments, we ask: (1) Do snails benefit *Chondrus* by removing potentially harmful epibionts? (2) Does *Chondrus* benefit the snails by reducing their susceptibility to predation? (3) Are the benefits provided by the two snails complementary or redundant? We interpret our results in the context of the role of mutualist diversity for host persistence and ecosystem functioning.

METHODS

Field surveys

Surveys of the subtidal abundance and distribution of *Chondrus*, *Mitrella*, and *Anachis* were conducted in summer (July–August) in the Poquonnock River estuary and surrounding open coast near Avery Point, Connecticut, USA, in Long Island Sound. Ten sites (four named sites, plus the six marked with an “x” in Appendix A) were sampled for *Chondrus* percent cover in ten 0.25×0.25 m quadrats. Within each quadrat a 15 cm diameter circular area was sampled for snails by vacuum suctioning the area for 30 seconds (see Miles and Whitlatch 1997 for a description and validation of these methods). Four sites, representing the extremes of *Chondrus* and snail abundance, were chosen for more intensive sampling: the University of Connecticut breakwater (UConn), Avery Point, and the exposed and protected sides of Pine Island (see Appendix A for a map). Sites range from 2 to 6 m in depth and consisted of rocky, bouldery substrate that should be appropriate habitat for *Chondrus* (soft sediment sites were excluded). At each of these sites we collected 15 randomly selected *Chondrus* plants, placed them in resealable plastic bags underwater, and returned them to the lab where we counted the number of gastropods on each plant and identified them to species. We also measured the wet mass of both the *Chondrus* and its sessile epifauna, sorted to the lowest taxonomic level possible. The three most abundant gastropods in these samples were *Lacuna vincta*, *Mitrella lunata*, and *Anachis lafresnayi* (species will hereafter be referred to by genus). Previous experiments showed that *Mitrella* and *Anachis* are voracious consumers of newly recruited sessile invertebrates and can alter adult community abundance and species composition (Osman et al. 1992), but that *Lacuna* does not consume these potential *Chondrus* competitors (Osman and Whitlatch 1995). *Lacuna* appears to be primarily herbivorous, but does not appear

to readily consume *Chondrus* itself (Chavanich and Harris 2002), probably consuming algal epiphytes instead. Because *Chondrus* at our study sites rarely had algal epiphytes, regardless of the presence or absence of *Lacuna*, we did not consider this species further.

As a preliminary indication of whether propagule supply might underlie survey patterns, at each of the four sites we measured larval availability of potential mutualists (gastropods) and foulers (ascidians, bryozoans), using tube traps modified from the design of Yund et al. (1991). Four traps were deployed at each site for two weeks each during late July and early August, after which they were recovered and the contents examined under a dissecting microscope. This period of time was chosen for larval sampling because it corresponds with the timing of settlement of *Mitrella* and *Anachis* at these sites (late July–early September; Rogers 1998), and the peak recruitment of the dominant fouling organisms on *Chondrus* (July–early September; J. Stachowicz and R. Whitlatch, unpublished data). The effect of site on the density of larval and adult snails and epibionts was tested using a one-way nonparametric ANOVA. Other field survey data were analyzed via one-way ANOVA with site as a random factor. Data were transformed using appropriate transformations (e.g., arcsin square-root transform for percent cover) only when assumptions were violated.

Field manipulations

To examine whether the snails benefit *Chondrus* by grazing on fouling invertebrates, we caged *Chondrus* plants with and without snails at the site where fouling was the highest (UConn breakwater; see Results). We reasoned that if snails could prevent fouling at this site, then they could effectively do it at any of the other sites in the area. Snails and *Chondrus* plants were placed within 1 L plastic containers at 1.0 m depth. The sides of each container were cut out and covered with window screen with a 1.7×1.4 mm mesh. This mesh size was small enough to enclose snails but large enough to permit larvae of sessile invertebrates to enter (J. Stachowicz, unpublished data). Preliminary experiments at this site found no difference in the growth of *Chondrus* inside vs. outside cages (unpaired *t* test, $P = 0.79$, $N = 34$), so cage controls were not considered further. Four different cage treatments were employed (no snails, 20 *Mitrella*, 5 *Anachis*, 5 *Anachis* + 20 *Mitrella*). Snail densities were chosen to mimic those found on natural substrates, as estimated using suction sampling in the field (see Results; Rogers 1998). We opted to maintain natural densities of both species and did not attempt to control for overall snail biomass or density in these experiments. We did this in part because the abundance of these two species is consistently positively correlated in the field (log–log correlation $r = 0.90$, $P < 0.0001$, see also Rogers 1998), suggesting that under natural conditions removing one species might not lead to an increase in

the other. Also, the nearly five-fold size difference between the two species (*Mitrella* adults were 3–5 mm; *Anachis* were 15–18 mm) would have made it impossible to control for both the total number of snails and snail biomass in these manipulations while also maintaining realistic ratios of *Mitrella* : *Anachis*. In the discussion we address the relative influence of snail density vs. species composition on among treatment differences.

The exterior of each cage was scrubbed twice weekly to remove encrusting organisms that were growing directly on the cages that might impede water motion through the mesh. Experiments were terminated after five weeks, because plants with no snails were completely overgrown and some had begun to disintegrate. At this time, plants were removed from their cages and photographed so that image analysis (IMAGE J, National Institutes of Health, Bethesda, Maryland, USA) could be used to determine the percent cover of each group of epibionts on each plant. After photographing, the epibionts on each plant were stripped, sorted, and weighed, and then the *Chondrus* was reweighed. Experiments were conducted in the summer (July–early September) to cover the most intense period of seasonal recruitment of fouling invertebrates. Data were analyzed by one-way ANOVA with treatment as a fixed factor.

To assess whether snails might gain a predation refuge from associating with *Chondrus*, we deployed a series of tethered snails in the field at sites of low and high predator abundance. Green crabs (*Carcinus maenas*) appeared to be the dominant predators on these small gastropods, so we chose sites with low–moderate (Pine Island exposed) and high (UConn) crab densities (Rogers 1998) to deploy our experiments. Snails were tethered using monofilament line, which was fastened to snail shells with a small drop of superglue; the free end of the line was attached to lead fishing weights. Tethered *Anachis* were placed in pairs in the field with one individual immediately adjacent to a *Chondrus* plant and the second in an adjacent open area sufficiently large to prevent them from reaching a *Chondrus* plant. Because *Mitrella* were smaller, we placed them in five groups of 20 tethered snails with and without access to *Chondrus* at the same two sites. Additionally, we placed five groups of 20 *Mitrella* in separate cages at each site to estimate loss of snails due to tether failure. After one week, all tethers were recovered and the presence of snails and the condition of the tether was noted. Each group of 20 snails was treated as a replicate, and percentage lost was compared among treatments using ANOVA with treatment as a fixed factor.

RESULTS

Field surveys

The abundance of each snail and *Chondrus* were positively associated in the field (Fig. 1; $r = 0.88$, $P =$

FIG. 1. Correlation between adult snail abundance (log scale) and *Chondrus* percent cover from field surveys. Analysis is by simple linear correlation ($r = 0.88$, $P = 0.003$ for *Anachis*; $r = 0.812$, $P = 0.003$ for *Mitrella*).

0.0030 for *Anachis*; 0.812, $r = 0.812$, $P = 0.003$ for *Mitrella*). Where *Chondrus* did occur, the amount of fouling by epiphytic invertebrates was less in areas where snails were abundant (Fig. 2A, B). The abundance of both *Mitrella* and *Anachis* varied among sites ($P < 0.0001$, ANOVA) and was greatest at the exposed Pine Island site ($P < 0.05$; all post-hoc tests by Ryan's Q, as per recommendation of Day and Quinn 1989). Both snails were less abundant at Avery Point than at the exposed Pine Island site, but were more abundant than at the protected Pine Island site or the UConn breakwater, where there were very few snails. The abundance of ascidians, bryozoans and other epibionts (primarily sponges) on *Chondrus* varied among sites in similar ways (Fig. 2B, ANOVA, $P < 0.0001$). At the two sites at which *Chondrus* had few or no snails (protected Pine Island and UConn breakwater), plants supported 60–110% of their own biomass of epibionts. These were dominated by colonial and solitary ascidians and bryozoans (Fig. 2B). In contrast, *Chondrus* from the two sites with higher snail densities (Avery Point and exposed Pine Island) supported less than 10% of their mass in fouling organisms, and these were primarily small bryozoan colonies.

Adult snail and epibiont abundances were not correlated with the abundance of larvae of these taxa (Fig. 2C), suggesting post-settlement processes were responsible for the differences in fouling of *Chondrus* among sites. The number of gastropod larvae found in tube traps was highest at the UConn site (Kruskal-Wallis test; $P = 0.053$, $N = 4$ traps per site, Fig. 2C), the location where the abundance of adult gastropods was lowest (Fig. 2A). Gastropod larvae could not be identified to species, but the few other adult gastropods present at these sites showed a similar spatial pattern of abundance, so it does not appear that larval supply determines among-site differences in adult gastropod population size. We found no significant differences in

FIG. 2. Field abundance of gastropods and epibionts on *Chondrus* (means $\pm$ SE): (A) density of adult gastropods, (B) epibiont mass as a proportion of *Chondrus* mass, and (C) gastropod and ascidian larval abundance per trap. Statistical analysis is by ANOVA for panels A and B. When overall ANOVA showed a significant effect of site on the response variable ($P < 0.05$), Ryan's Q post hoc tests were conducted to assess differences among sites (Day and Quinn 1989). Sites with different letters above them were significantly different ($P < 0.05$); tests only compare levels of a given response variable (e.g., *Mitrella* abundance, bryozoan mass) among sites. For example, letters refer to statistical tests done on the data for ascidians, lowercase letters refer to tests done on bryozoans, and those marked with a prime are for other. Analysis in (C) was by Kruskal-Wallis test ($P > 0.05$).

the abundance of ascidian larvae among sites (Kruskal-Wallis test, $N = 4$ traps per site, $P = 0.196$). We could not identify any bryozoan larvae in our traps. Low replication hindered detection of differences among

sites, but the trend was toward a higher abundance of ascidian larvae in traps at exposed Pine Island, the site at which fouling of *Chondrus* was lowest. The timing of the deployment of these traps corresponded to the seasonal occurrence of peak settlement season for both snails and fouling organisms, however we also deployed one trap at the site with highest adult snail abundance (Pine Island) and one at the site with the highest density of foulers (UConn breakwater) for successive two week periods from July 1–September 26. Although these data are unreplicated, they generally reflect similar trends to those in Fig. 1 (biweekly mean $\pm$ SE for ascidian larvae, UConn breakwater 19.8 ± 1.1 , Pine Island 48.3 ± 5.0 ; for gastropod larvae, UConn breakwater 75.8 ± 12.3 , Pine Island 22.5 ± 2.2 ; $N = 5$ for UConn, $N = 6$ for Pine Island).

Benefits to the plant

Only *Chondrus* with both *Anachis* and *Mitrella* remained unfouled and increased in mass. Exclusion of both snail species from *Chondrus* increased sessile invertebrate overgrowth by several orders of magnitude, resulting in substantial loss of plant mass (Fig. 3; overall ANOVA $P < 0.001$, post-hoc comparisons by Ryan's Q , $P < 0.05$, see Plate 1 and Appendix B for representative pictures of plants from each treatment). These plants were completely overgrown by fouling organisms. *Mitrella* alone did reduce the mass and cover of epibionts on *Chondrus* relative to no snail treatments ($P < 0.05$, Fig. 3A, B), but coverage was still near 100%, and host mass change was slightly negative (but not significantly different from zero: one-sample t test, $P = 0.33$). *Anachis* reduced both the mass and total cover of invertebrates to lower levels than *Mitrella* ($P < 0.05$, Fig. 3A, 3B), but change in plant mass was still indistinguishable from *Mitrella* (Ryan's Q post-hoc test, $P > 0.05$, Fig. 3C) or zero ($P \geq 0.44$, one-sample t test). Change in plant mass in the *Mitrella* only and *Anachis* only treatments was significantly less negative than controls only at the $P = 0.10$ level. Plants with both snails grew the fastest (Fig. 3C) and had the lowest cover of epibionts (Fig. 3A) of all treatments ($P < 0.05$, Ryan's Q test).

In addition to the among-treatment differences in the total mass and cover of fouling organisms on *Chondrus*, species composition of the epibionts also differed among treatments (Fig. 4). Bryozoan and solitary ascidian mass and cover differed most strongly among treatments (Fig. 4A–D, ANOVA $P < 0.0001$), whereas differences in colonial ascidians among treatments were weaker (Fig. 4E, F, ANOVA $P = 0.023$ for mass and $P = 0.003$ for cover). Plants enclosed with *Mitrella* were covered mostly by solitary ascidians (Fig. 4C, D), such as *Molgula manhattensis* and *Ascidia aspersa*, whereas those enclosed with *Anachis* were covered mostly by encrusting, calcified bryozoans such as *Schizoporella unicornis* and *Cryptosula pallasiana* (Fig. 4A, B). *Mitrella* reduced the abundance of solitary as-

FIG. 3. Effect of gastropod presence and species composition on the (A) cover and (B) mass of epibionts on *Chondrus* and (C) on the growth of *Chondrus* itself. Bars represent means $\pm$ SE. Statistical analysis was by ANOVA with post hoc comparisons using the Ryan's *Q* method. Treatments with different letters above bars were significantly different ($P < 0.05$). Sample sizes were as follows: Control, $n = 19$ replicate plants; *Mitrella*, $n = 19$; *Anachis*, $n = 18$; *Mitrella* and *Anachis*, $n = 18$.

cidians relative to plants with no snails only slightly ($P < 0.05$, Ryan's *Q*), whereas there were no solitary ascidians on plants that had *Anachis* or both *Anachis* and *Mitrella* (Fig. 4C, D). In contrast, bryozoans were nearly completely absent from plants with *Mitrella* or *Mitrella* + *Anachis*, and were most abundant on plants with *Anachis* only (Fig. 4A, B; $P < 0.05$, Ryan's *Q*). This result suggests that the snails differ in that *Anachis* is effective at removing solitary ascidians but not bryo-

zoans, and *Mitrella* is effective at removing bryozoans but not solitary ascidians.

Bryozoans were also lower in abundance on control plants than on those with *Anachis* and did not differ from *Mitrella* or *Mitrella* + *Anachis* plants ($P < 0.05$), but this is likely because the high solitary ascidian abundance on plants without snails prevented establishment and growth of bryozoans (e.g., Osman and Whitlatch 1996). Colonial ascidians were in lower cover and mass in all the treatments with snails relative to plants from which snails had been excluded ($P < 0.05$, Fig. 4E, F), so either snail alone appears effective at reducing the load of colonial ascidians on *Chondrus*.

Benefits to the snails

The effectiveness of *Chondrus* as a refuge from predation varied between the two sites where tethering experiments were conducted (Fig. 5). At the exposed Pine Island site where green crab (*Carcinus*) abundance was lower, predation on *Mitrella* and *Anachis* was reduced by 50–80% for snails tethered in *Chondrus* patches relative to those tethered in the open (*Mitrella*, ANOVA with Ryan's *Q* post-hoc test at $P < 0.05$, $N = 5$; *Anachis* Fisher's Exact Test $P = 0.015$, $N = 21$ pairs of snails). At the UConn breakwater, where green crab abundance was an order of magnitude higher, >80% of snails were consumed in one week, regardless of whether they were tethered within or outside of *Chondrus* patches. At both sites, loss rates of *Mitrella* tethered in cages were low (<15%) indicating that most loss from tethers was likely due to predator-induced mortality (always different from bare substrate treatment $P < 0.05$, Ryan's *Q* test).

DISCUSSION

In our experiments two mutualistic gastropod species were required to keep their host plant free from fouling organisms and maintain positive net growth of the host. Whether each snail species is mutualistic (provides a fitness benefit to its host) depends on the presence of the other because each fills a different functional role in the removal of host epibionts. Only *Anachis* reduces the abundance of solitary ascidians whereas only *Mitrella* reduces host encrustation by bryozoans (Fig. 4), such that both species must be present for plants to remain free of epibionts and exhibit positive net growth (see Plate 1, Fig. 3, and Appendix B). Both snails can gain refuge from predation in *Chondrus* (Fig. 5A, B), suggesting that by benefiting a shared host, the snails are indirect mutualists of one another (Fig. 6, see also Schmitt and Holbrook 2003). The experimental data, in combination with the negative correlation between the abundance of snails and that of sessile invertebrates on plants in field surveys (Figs. 1, 2A, B) suggests that these two snails jointly enhance *Chondrus* growth and abundance in the field. It is difficult to assess how common these sorts of multiple, complementary mutualists might be because most experimental studies of

FIG. 4. Taxonomic breakdown of percent cover and wet mass of fouling organisms as a function of snail presence and species composition (means $\pm$ SE). Statistical analysis and symbols are as in Fig. 2. Sample sizes were as follows: Control, $n = 19$ replicate plants; *Mitrella*, $n = 19$; *Anachis*, $n = 18$; *Mitrella* and *Anachis*, $n = 18$.

mutualism manipulate only a single species or an entire guild of potential mutualists and thus are unable to assess the potential for complementarity and redundancy among mutualists. However, we suspect the phenomenon may be widespread because: (1) many hosts support multiple mutualists (Stanton 2003), and (2) functional differentiation among mutualists may be a common by-product of selection to reduce overlap and promote coexistence among similar co-occurring species (e.g., Schoener 1974, Tilman 1982).

The strong qualitative differences that emerged in the epibiont community in the *Mitrella* only and *Anachis* only treatments suggest that these species differ in their feeding preferences and that this contributes to the cumulative effects of these species on their host. The sessile invertebrates studied here become less susceptible to predation with increasing size/age, ultimately reaching a size refuge from small gastropods within weeks to a month (Osman and Whitlatch 1996). Because *Anachis* is much larger than *Mitrella*, and the range of prey items taken by a predator is often constrained by predator size, juvenile sea squirts likely reach a refuge from *Mitrella* predation earlier than from *Anachis*. Although both snails can consume solitary ascidian recruits (Osman and Whitlatch 1995), when recruitment is high *Mitrella* may not be able to consume all recruits before they reach a size escape. In this case, as found in our experiments, the larger *Anachis* would be more likely to be able to control fouling of *Chondrus*

by solitary seasquirts because it can consume them further into their ontogeny. Background settlement of solitary ascidians during our experiments was typical of that found over the last 15 years (J. Stachowicz, R. Whitlatch, and R. Osman, *unpublished data*), suggesting that solitary ascidians often have sufficiently high recruitment to escape complete predation by *Mitrella*.

However, some taxa may become invulnerable to predators at a relatively small size due to rapid ontogenetic changes in morphological or chemical defenses that occur shortly after metamorphosis (e.g., Harvell 1986, Lindquist 2002). Because larger predators typically focus on larger prey, these taxa may become relatively invulnerable before they reach a size at which they are perceived by larger predators (e.g., *Anachis*) as prey. Smaller predators like *Mitrella* may be more effective at controlling the abundance of these species on *Chondrus*. For example, in the encrusting bryozoans studied here, the initial zooid (ancestrula), which metamorphoses from the swimming larval stage, is poorly calcified and thus is susceptible to predation by small gastropods; subsequently produced daughter zooids are heavily calcified and relatively invulnerable to predators (Osman and Whitlatch 2004). Thus bryozoan colonies may be susceptible to snail predation for only a few days post-settlement, prior to the production of more heavily calcified daughter zooids. At this small stage, bryozoans likely appear in the size range of prey taken by the smaller *Mitrella*, which then consumes

FIG. 5. Predation on tethered (A) *Mitrella* and (B) *Anachis* at two sites: UConn breakwater (higher natural crab abundance) and Pine Island exposed (lower natural crab abundance). Statistical analysis was by ANOVA for panel A ($P < 0.05$ for both sites); data are means ($\pm$ SE) of the number of *Mitrella* consumed per group of 20 snails. Letters above each bar indicate statistical difference by Ryan's Q tests ($P < 0.05$). Analysis was by Fisher's exact test for panel B (P values as shown in Fig.).

them before they advance to the relatively invulnerable multiple zooid stage. But bryozoans may not appear in the size range of prey items taken by the larger *Anachis* until they are sufficiently calcified to be relatively invulnerable to predation. This would explain why calcareous bryozoans fouled *Chondrus* with only *Anachis* present, whereas plants with both *Anachis* and *Mitrella* remained healthy (Plate 1, Fig. 3B, and Appendix B).

Alternatively, lower fouling and greater growth in the plants with *Mitrella* + *Anachis* could be a function of the greater total snail density in this treatment relative to the single-species treatments. Increasing the density of *Mitrella* might allow it to consume a greater fraction of solitary ascidians before they reach a size escape. However, this does not appear to be the case, as a large number of solitary ascidians did escape predation by *Mitrella* (Fig. 4, see picture in Appendix B), and the snail densities used are representative of the site where field density is highest (Fig. 1, also Rogers 1998). Thus, it seems unlikely that the greatly increased density of *Mitrella* that might be needed to completely consume solitary ascidians would occur under field conditions. Increases in *Anachis* density, even if they did occur in the field, would be unlikely to decrease

bryozoan cover on *Chondrus* because it appears that bryozoans are only vulnerable to snail predation when they are outside the size range of prey consumed by *Anachis*. Thus, we conclude that the low epibiont load in the *Mitrella* + *Anachis* treatment was due to the complementary feeding abilities of the two snails and not the increased overall snail density.

This result suggests that the loss of either species of snail would have dramatic ecosystem consequences. Enhanced growth of sessile invertebrates might shift the system from macroalgal to sessile invertebrate dominance, altering habitat complexity. Although there are other potential predators of sessile marine invertebrates at these sites (e.g., small crabs, fishes), these do not consume sessile invertebrates until they reach a relatively large size (Osman and Whitlatch 1996) at which point they are likely to have already had a negative effect on *Chondrus*. By increasing the growth and survival of structurally complex species, associates like these snails can indirectly affect other associated species (e.g., Estes and Palmisano 1974, Bertness 1985, Stachowicz and Hay 1999). Thus the loss of *Mitrella*, for example, might indirectly lead to a loss of *Anachis* due to the positive effects of each snail on their shared host, making *Mitrella* and *Anachis* indirect mutualists. *Chondrus* also serves as habitat for a diversity of mobile invertebrates from at least four different phyla (Rogers 1998), so reduction in habitat availability that would follow the loss of either snail could result in a cascading loss of associated species whose ecosystem roles are generally unknown. Although few of these species use *Chondrus* for food, the likelihood of survival of these invertebrates is low in the absence of *Chondrus* or other structurally complex organism, due to predation by small fishes and large crabs (Fig. 6).

Of the two sites at which tethering experiments were conducted, the one with higher predator density (UConn breakwater) also had the lowest abundance of snails and *Chondrus* (Figs. 1, 2A), suggesting that predation by crabs and the effectiveness of *Chondrus* as

FIG. 6. Diagram depicting the interactions elucidated by the experiments. Arrows lead from the species causing the effect to the species experiencing it. Signs (+/-) next to arrows indicate the direction of the effect. Solid lines indicate direct effects; dashed lines indicate indirect effects. Note that snails have complementary positive effects on their host, and that the snails are indirect mutualists of one another.

a refuge may play a role in determining snail distribution and abundance. Although replication at a much greater number of sites of varying predator densities is needed, this result is consistent with the idea that the effectiveness of associational refuges from predation should decline at high predator density. Differences in physical conditions among sites might also affect snail abundance, although the site with more rigorous physical conditions (Pine Island) had more snails, suggesting that these do not play a direct role in determining differences in snail abundance among sites, although they could play an indirect role by mediating predator abundance or predation intensity.

Earlier, we suggested that multiple, functionally differentiated mutualists might be common in a range of mutualistic systems. However, even when mutualists within a guild provide redundant benefits, mutualist diversity may serve as a buffer against environmental changes. Reef-building corals, for example, rely on mutualistic dinoflagellate algae for the bulk of their food. It is now known that most corals harbor more than one species (or genotype) of dinoflagellate, and some evidence suggests that different genotypes of symbiotic dinoflagellate perform better under different light regimes (reviewed in Knowlton and Rowher 2003). Given that light quantity and quality can vary significantly with position within a single coral colony, harboring a diversity of symbionts might enhance total colony productivity. Also, algal genotypes are differentially susceptible to the phenomenon known as coral bleaching in which corals expel their symbionts during environmentally stressful periods (Rowan et al. 1997). The possession of bleaching-resistant genotypes would be a great advantage during periods of extreme physical conditions, even if these genotypes provided redundant (or perhaps even reduced) benefits under "normal" conditions. Thus increased mutualist diversity may enhance community persistence in the same way that species diversity more broadly appears to enhance the consistency and predictability of ecosystems (Tilman and Downing 1994, Naeem and Li 1997, Stachowicz et al. 2002, Elmqvist et al. 2003).

The role of mutualist diversity is an understudied aspect of the broader relationship between biodiversity and ecosystem processes. Our data, and that on coral-zooxanthellae associations, suggest that diversity of what would appear to be functionally similar mutualists may enhance the performance and persistence of host species. Because the hosts in question (corals, seaweeds) often form the habitat on which the rest of the community depends, mutualist diversity may be important for the maintenance of these ecosystems. Habitat loss and fragmentation as a result of human activities could reduce the density of host foundation species below that required to maintain populations of associated species (e.g., Bruno and Kennedy 2000). When the loss of these associated species matter to the fitness of the host, the resulting feedback could accelerate the

decline of the ecosystem based on the foundation species. Thus, understanding the role of the many "associated" and potentially mutualistic species and the degree to which species provide complementary versus redundant benefits should be a high priority for ecology and conservation biology. Studies that explicitly and systematically manipulate mutualist diversity in the same way that others have manipulated plant diversity should prove particularly useful in assessing the importance of mutualist diversity to host persistence and the degree to which mutualisms are truly pairwise versus multispecies interactions.

ACKNOWLEDGMENTS

We thank the National Sea Grant College and National Science Foundation Biological Oceanography (OCE 0082049) programs for funding. A. Lohrer, K. Heinonen, and H. Fried provided field assistance. Data on larval abundance for gastropods and ascidians and some adult snail abundance data were kindly provided by E. Rogers. Field tethering data for *Mitrella* were collected by M. Berger. Comments from J. Byrnes, R. Hughes, S. Olyarnik, R. Osman, J. Witman, P. Steinberg, and an anonymous reviewer improved the manuscript.

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APPENDIX A

A map of field sites showing the location of Groton, Connecticut, USA, and the location of the field sites within Groton, is available in ESA's Electronic Data Archive: *Ecological Archives* E086-127-A1.

APPENDIX B

Color versions of the photographs in Plate 1 depicting *Chondrus* plants from each experimental treatment are available in ESA's Electronic Data Archive: *Ecological Archives* E086-127-A2.