Biological Invasions (2005) 7: 711–722
DOI 10.1007/s10530-004-0997-8

Resource limitation, biodiversity, and competitive effects interact to determine the invasibility of rock pool microcosms

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Received 21 November 2003; accepted in revised form 30 June 2004

Key words: aquatic, *Dasyhelea*, invasion, nutrient limitation, rock pools, species richness, zooplankton

Abstract

The success of species invasions depends on both the characteristics of the invaded habitat and the traits of the invasive species. At local scales biodiversity may act as a barrier to invasion; however, the mechanism by which biodiversity confers invasion resistance to a community has been the subject of considerable debate. The purpose of this study was to test the hypothesis that productivity and diversity affected the ability of a regionally available species to colonize communities from which it is absent. We hypothesized that the invasibility of rock pool invertebrate communities would increase with increasing nutrients and decrease with increasing diversity. We tested this possibility using naturally invaded outdoor aquatic microcosms. We demonstrated that the invasibility of an experimental multi-trophic aquatic community by a competitive native midge species (Ceratopogonidae: *Dasyhelea* sp.) was determined by an interaction between resource availability, diversity, and the densities of two competitive ostracods species. Nutrient enrichment increased invasion success; however, within nutrient-enriched microcosms, invasion success was highest in the low-diversity treatments. Our results suggest that resource availability may in fact be the principal mechanism determining invasibility at local scales in multi-trophic rock pool communities; however resource availability can be determined by both nutrient input as well as by the diversity of the biotic community.

Introduction

Species invasions are considered to be a leading cause of the global biodiversity crisis (Drake et al. 1989; Sala et al. 2000). The consequences of species invasions have been particularly dramatic in aquatic communities (MacIsaac et al. 2001), however little is known about why some aquatic communities are more invasible than others. This is in part due to the complexity of factors potentially involved in making a community susceptibility to invasion. For example, characteristics of communities that have been shown to alter susceptibility to invasion include climate (Stachowitz et al. 2002a), disturbance (Cohen and Carlton 1998), ecosystem size or position in the landscape (Debinski and Holt 2000), competitive or facilitative abilities of resident species (Maron and Connors 1996; Lonsdale 1999; Callaway and Aschehoug 2000), presence of herbivores, pathogens or mutualists (Crawley 1987; D'Antonio 1993; Lonsdale 1999; Marler et al. 1999; Christian 2001), resource limitation and utilization (Tilman 1997, 1999; Stachowicz et al.

1999; Davis et al. 2000), and diversity (Elton 1958; Dukes 2001; Smith and Knapp 2001).

Of the above factors, diversity and the effects of diversity on resource availability are generally thought to play primary roles in the susceptibility of a community to invasion (Elton 1958; Jenkins and Buikema 1998; Levine 2000; Naeem et al. 2000; Davis and Pelsor 2001; Shea and Chesson 2002; Stachowicz et al. 2002b). In vascular plant communities, the primary factors that affect community invasibility are thought to be stress in terms of resource fluctuations (Davis et al. 2000), and interactions between resource limitations and diversity (Tilman 1997). In their general theory of invasibility, Davis et al. (2000) suggest that conditions of resource enrichment and release are key to controlling invasibility. They further suggest that there is no necessary relationship between invasibility and either species richness or community productivity (in terms of biomass produced per unit time). This lack of a mechanistic link between diversity or productivity and invasibility is thought to occur because complete resource-use (and very incomplete resource exploitation) can occur in both species-rich and species-poor communities. In contrast to Davis et al. (2000), Tilman (1997) has suggested that species diversity may play an important role in inhibiting invasion in the absence of covarying extrinsic factors such as disturbance. For example, diversity may directly affect the success of invasions if higher resident species richness leads to crowding or decreased availability of light (Naeem et al. 2000; Kennedy et al. 2002).

Thus, in spite of considerable attention, the question of whether diversity affects invasibility has been the subject of considerable debate (Loreau et al. 2001). This debate has been driven by two main issues. First, studies have reported idiosyncratic effects of diversity on invasibility, with diversity (i) increasing resistance to invasion (Elton 1958; Knops et al. 1999; Levine and D'Antonio 1999; Shurin 2000; Lyons and Schwartz 2001; Kennedy et al. 2002), (ii) decreasing resistance to invasion (Levine 2000), and (iii) having no effects on invasibility (Cahill 2003). However, these idiosyncratic results are due primarily to scale (Shea and Chesson 2002). At local scales both experiments and models generally suggest that diversity does inhibit invasion success (Shea and Chesson 2002). Second, resource limitation is the primary mechanism by which diversity has been thought to affect invasibility (Elton 1958; Jenkins and Buikema 1998; Levine 2000; Naeem et al. 2000; Davis and Pelsor 2001; Stachowicz et al. 2002b), implying that diversity *per se* may have only negligible effects on the observed patterns (Davis et al. 2000). This lack of a direct connection between diversity and invasibility is also suggested by Shea and Chesson (2002) who cite resources, natural enemies, and environmental factors as the three primary determinants of establishment and success of invaders.

The purpose of this study was to test the hypothesis that productivity and diversity affected the ability of a regionally available species to colonize communities from which it is absent. We hypothesized that the invasibility of rock pool invertebrate communities would increase with increasing nutrients and decrease with increasing diversity. Specifically, the success of invasion, which is defined as the relative numerical dominance of the invader, would be (1) higher in low-diversity microcosms than in high-diversity microcosms, and (2) higher in high-nutrient microcosms than in low-nutrient microcosms. We further hypothesized that there would be (3) an interaction between diversity and nutrient limitation where the success of the invader would be greatest in low-diversity high-nutrient microcosms. We tested these ideas experimentally using microcosm communities derived from natural rock pool invertebrate communities that were naturally invaded by a native midge species (Ceratopogonidae: *Dasyhelea* sp.) which must colonize existing communities to complete their life cycle.

Experimental rock pool and other natural and artificial aquatic microcosm experiments (see Srivastava et al. 2004) have been intensively used in diversity-invasibility studies (McGrady-Steed et al. 1997; Law et al. 2000; Shurin 2000; Miller et al. 2002; Lennon et al. 2003) as many features of microcosms (e.g. small size, ease of manipulation, fast generation times of organisms, constant environmental condition, see Srivastava et al. 2004 for a review) lend themselves to complex process-oriented experiments. However, little is known about the role of diversity or resource limitation on the invasibility

of aquatic communities composed of zooplankton and benthic invertebrates.

Materials and methods

We conducted this experiment on the grounds of the Discovery Bay Marine Laboratory (DBML) on the north coast of Jamaica (18°28′ N/ 77°25′ W) from September 27th to November 5th, 2001. We used a 5 × 3 factorial design with five levels of diversity and three levels of nutrient additions. Artificial 'rock pools', hereafter called microcosms, were plastic cups, 8 cm in diameter and 15 cm deep. The microcosms were filled to a depth of 10 cm (volume 500 ml) with water and were set up in September of 2001. The microcosms were covered loosely with a 2 mm mesh fiberglass screening.

Microcosms used in this experiment were representative of smaller sized natural rock pools from which the invertebrates were collected (Kolasa et al. 1996; Kolasa and Drake 1998; Therriault and Kolasa 1999; Romanuk and Kolasa 2001, 2002a, b). In 15 years of sampling 49 of the natural rock pools on a yearly basis we have identified 75 different species of micro- and meio-invertebrates in the pools with species richness in any one pool ranging from 0 to 16 species at any one time (Romanuk and Kolasa 2002b).

To assemble the experimental communities, water was collected from five freshwater rock pools. Every species that was present in the rock pool water was used in the experiment. The initial experimental communities consisted of 10 species retained on a 63 μm mesh net: three species of ostracods (*Candona* sp., *Cypridopsis* sp., *Potamocypris* sp.), a chydorid (*Alona* sp.), a daphnid (*Ceriodaphnia* sp.), a copepod (*Orthocyclops modestus* Herrick), a larval decapod (*Armases miersii* Rathbun), two worms (*Nematode* sp., *Oligochaete* sp.), and a dipteran larvae (*Culex* sp.). Generation times for these taxa are rapid due to high water temperature (Gillooly 2000). For example, based on Gillooly (2000), zooplankton of similar sizes would have generation times ranging from ~8 to 20 days at 20 °C. At ~30 °C, which was the average daytime water temperature of the microcosms (personal observations), these generation times would decrease substantially. For example *Alona affinis* has a generation time of 73 days at 5 °C, 39 days at 10 °C, 26 days at 15 °C and 17 days at 20 °C (Gillooly 2000).

After the first week of exposure, microcosms were naturally invaded by a biting midge (Diptera: Ceratopogonidae) from the genus *Dasyhelea* (the invader), which has an aquatic larval stage. *Dasyhelea* is a common native midge species that uses rock pools, container habitats, rain pools, phytotelmata, and other small bodies of water to oviposit. *Dasyhelea* was the only species that invaded and established itself in the experimental communities. Midges only use the rock pools during their larval and pupal stages, functioning as temporary invaders. While *Dasyhelea* is not a regionally novel species, we have defined it as an invader as it was not initially present in the microcosm array. Furthermore, adult insects are capable of flight and habitat choice making them a suitable species with which to test hypotheses of community invasibility.

The microcosms were fertilized on day 0 with soluble phosphorous (KH_2PO_4) and nitrogen (NH_4NO_3) with an N:P ratio of 20:1. Nutrient level I (NT = 1) was obtained by adding nutrients once at the start of the experiment ($n = 15$). Nutrient level II (NT = 2) was obtained by adding nutrients at the start of the experiment and again after the first week ($n = 15$). The other 15 microcosms had no nutrients added and served as controls (NT = 0) for the nutrient manipulations. Nutrients were measured on November 5th for one of each diversity treatment/nutrient treatment (DT/NT) combination (e.g. $n = 1$ of NT = 0/DT = 0, $n = 1$ of NT = 0/DT = 25, etc.).

Diversity was manipulated on day 0 using a dilution method (Franklin et al. 2001; Giller et al. 2004). To experimentally manipulate diversity, we collected 30 l of rock pool water with a standard salinity of 0 ppt and filtered half of the water through a 63 μm mesh filter. This procedure effectively manipulated the abundance and richness of the target communities, however species smaller than 63 μm such as rotifers, protozoans, and the juvenile stages of the target communities such as copepod nauplii would not have been affected by this manipulation. This manipulation resulted in 15 l of 'filtered' rock

pool water from which all organisms larger than the mesh size had been removed, and 15 l of 'natural' rock pool water with all the invertebrates in natural proportions. The diversity levels (DT) were created by mixing the 'filtered' water and the 'natural' water together to create five diversity levels (DT = 0, 25, 50, 75, and 100%). The percent given represents the amount of natural 'unfiltered' water in each diversity level. For example, the DT = 0% level was composed of only filtered water (i.e. control), and the DT = 25% level contained 1/4 'natural' water and 3/4 'filtered' water. Likewise, the DT = 50% level contained 1/2 'filtered' water and 1/2 'natural' water.

Samples were enumerated using a dissecting microscope and individuals were identified to species or genus. Where a taxon is only identified to genus, the taxon represents one species only and not a number of different species in the same genus.

We sampled the microcosms at the beginning of the experiment to provide baseline data. After 7 days each microcosm was sampled by gently stirring the water with a glass stirrer and using a 50 ml dip container to collect 30 ml of water. This water was sieved through a 63 μm mesh sieve and then stored in a centrifuge tube in 50–70% ethanol. Sampling was repeated every week for 4 weeks.

Statistical analyses

Diversity, H', was calculated using the Shannon–Weiner index. Diversity, H' was calculated as the $H' = \sum p_i \ln(p_i)$ where p_i represents the proportional contribution of the ith species to the community abundance, N. Species richness (S) was evaluated as the number of species in each microcosm observed in the 30 ml sample. Community density ($N \log_{10}$) was calculated as the abundance of each individual of all species in each microcosm. Invasion success was calculated as the dominance of the invader (percent abundance) relative to the rest of the community and was arcsine transformed prior to analysis to achieve normality.

Differences in N, P, and N:P ratio across nutrient levels (NT) were assessed using t-tests. To determine the effectiveness of the diversity manipulation we used linear regression and analysis of variance with Tukey *post-hoc* tests. Tukey *post-hoc* tests were used to determine whether these were significant differences in species richness, density and diversity, H' between diversity treatments across DT = 0–100. To determine the effects of the nutrient treatment (NT = 0, 1, 2) and diversity levels (DT = 0, 25, 50, 75, and 100%) on invasion success we used repeated measures ANOVA with NT and DT as categorical predictors and invasion success (dominance of the invader at each sampling date) as the dependent variable. To determine which components of the diversity treatment (DT) affected invasion success, we used a general linear model (GLM) with nutrient treatment as the categorical variable and mean diversity, H', species richness, S, and community density, N (minus the invader), as continuous variables and mean invasion success as the dependent variable. This analysis was done across DT = 0–100 as well as across DT = 25–100. To determine whether the density of any particular species affected mean invasion success we performed a separate univariate regression' with mean density ($N \log_{10}$) for each species as the independent variable and invasion success as the dependent variable. These univariate regressions were done across all DT levels as well as only across DT = 25–100. To test whether NT and DT affected the number of microcosms invaded throughout the experiment we used a log-linear analysis for invasion frequency with NT and DT as the categorical factors. Significant differences between observed and expected frequencies were assessed using a maximum-likelihood χ^2 test (Sokal and Rohlf 1981).

Results

Nutrient enrichment

The control treatment (NT = 0) averaged 0.105 μg P (SD 0.015) and 1.61 μg N (SD 0.426). NT = 1 averaged 3.35 μg P (SD 0.225) and 11.83 μg N (SD 0.752). NT = 2 averaged 5.483 μg P (SD 0.923) and 26.5 μg N (SD 1.87). These differences were all significantly different across nutrient treatments at $P < 0.001$. The N:P ratio averaged 16.8 for NT = 0, 3.45 for NT = 1, and 4.48 for NT = 2 (Figure 1). N:P

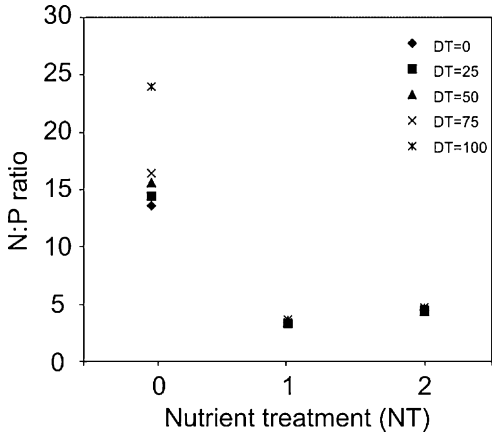

Figure 1. N:P ratio for the three nutrient treatments (NT = 0, 1, 2) and for each diversity treatment (DT = 0, 25, 50, 75 and 100).

ratio was significantly different across nutrient treatments at $P < 0.001$.

Diversity treatment

The dilution series used to manipulate community composition significantly affected initial species richness, abundance, and diversity, H' (Figure 2A; Table 1), and this general trend of higher species richness, abundance, and diversity, H' in the higher diversity treatments, was retained throughout the experiment (Figure 2B–D; Table 1). However, the observed mean values of species richness, abundance, and diversity, H', over time did not increase linearly with DT (Figure 3). Instead, mean species richness and abundance were highest across the three sampling dates at DT = 50. Across all three sampling

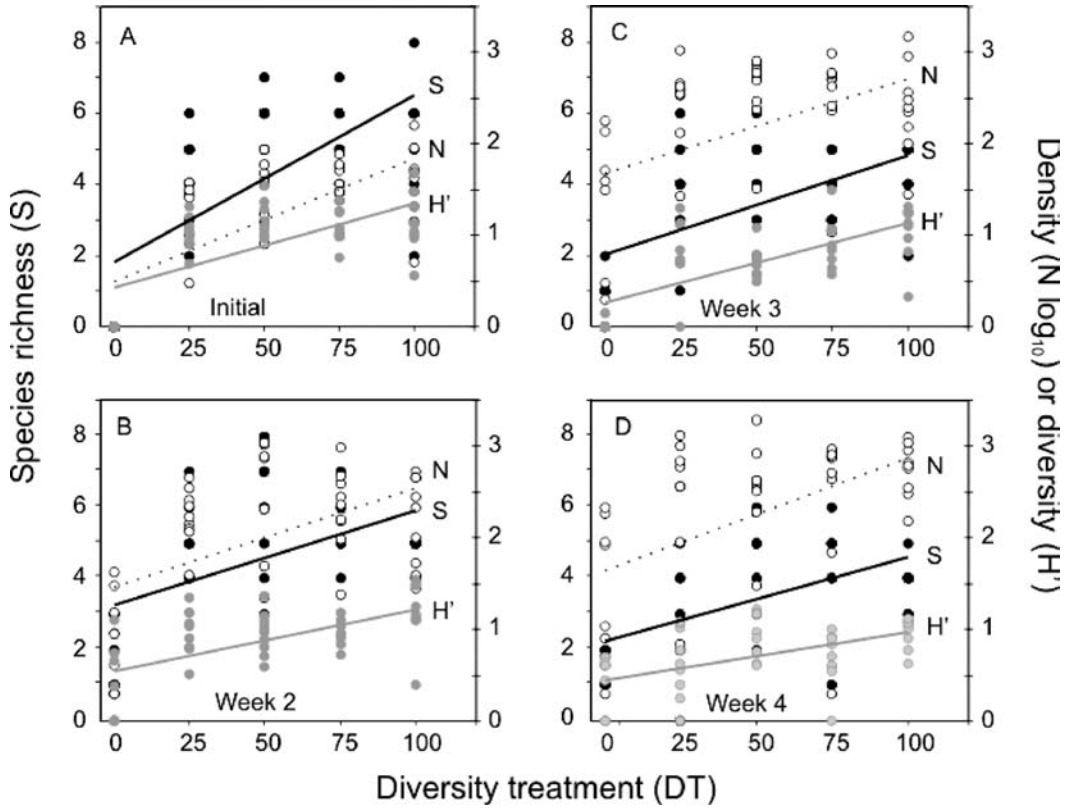

Figure 2. Effects of the diversity treatment (DT) manipulation on species richness (S, black circles, solid line), density (N, open circles, hatched line), and diversity, (H', grey circles, grey line) for (a) initial S, $R = 0.704$; N, $R = 0.711$; H', $R = 0.679$; (b) week 2 S, $R = 0.522$; N, $R = 0.522$; H', $R = 0.598$; (c) week 3 S, $R = 0.580$; N, $R = 0.470$; H', $R = 0.704$, and (d) week 4 S, $R = 0.566$; N, $R = 0.502$; H', $R = 0.513$ (all $P < 0.001$).

Table 1. Results of GLM showing the effect of the diversity treatment (DT) manipulation on mean species richness, density, and diversity, H', with time as a categorical factor.

Source	df	SS	MS	*F*	*P*
Mean species richness (*S*)					
Time	3	45.533	15.178	12.022	<0.001
DT	4	384.144	96.036	76.068	<0.001
Time × DT	12	40.522	3.377	2.675	0.003
Error	160	202.000	1.263		
Total	179	672.200			
Mean density (N $\log_{10}$)					
Time	3	33.870	11.290	35.374	<0.001
DT	4	53.553	13.388	41.947	<0.001
Time × DT	12	1.812	0.151	0.473	0.928
Error	160	51.067	0.319		
Total	179	140.302			
Mean diversity (H')					
Time	3	1.481	0.494	6.919	<0.001
DT	4	18.427	4.607	64.570	<0.001
Time × DT	12	2.397	0.200	2.800	0.002
Error	160	11.415	0.071		
Total	179	33.720			

Mean species richness (mean *S*), mean density (mean N $\log_{10}$), and mean diversity, H' (mean H').

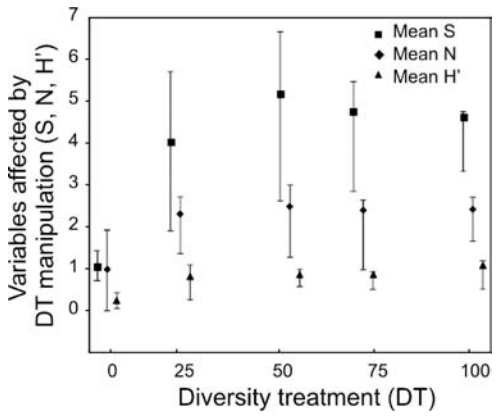

Figure 3. Effects of the diversity treatment (DT) manipulation on mean species richness (*S*), density (*N*), and diversity, (H'). Bars are 95% standard deviations from the mean.

dates, mean density was only significant different between DT = 0 and all the other diversity levels (Table 2). In contrast, species richness differed significantly between DT = 0 and all other DT levels as well as between DT = 25 and 50, and diversity, H' differed significantly between DT = 0 and all other DT levels as well as between DT = 25 and 100 (Table 2).

Initial species richness in the microcosms ranged from 0 to 8 species (mean 4.15 ± 2.39 SD; Figure 2A) and decreased over time (Figure 3). By week 4, a maximum of six species was seen in any one replicate. This decrease in species richness was due to molting of the crab larvae *A. miersii*, which enabled them to escape from the microcosms, and the death of the oligochaete, which likely died from high water temperature (<30 °C). Initial mean abundance in each microcosm ranged from 0 to 159 (mean 30.47 ± 30.95 SD; Figure 2a) and increased over time (Figure 3). By week 4, abundance ranged from 0 to 1932 (mean 469.42 ± 469.32 SD). Initial mean diversity, H', ranged from 0 to 1.67 (mean 0.88 ± 0.49 SD; Figure 2A) and decreased with time (Figure 3).

Invasion

Of the 45 microcosms, 37 were invaded at least once during there experiment. The invader became the numerical dominant in 6 of the 45 microcosms: 5 of 9 replicates of DT = 0 and 1 of 9 replicates of DT = 25. There was no significant difference in the proportion of microcosms invaded by either DT ($P = 0.65$) or NT ($P = 0.749$).

Invader success was highest in DT = 0. In DT = 0 the invader comprised 56.4% (±45.2% SD) of the individuals in the community, whereas in the DT = 100, the invader only comprised 2.6%

Table 2. Results of Tukey *post-hoc* test for significant differences in species richness, density, and diversity, H' by diversity treatment (DT = 0, 25, 50, 75, and 100). Also shown are the mean values of species richness, density, and diversity (H') calculated over the three sampling dates.

	DT = 0	DT = 25	DT = 50	DT = 75	DT = 100
Mean S	$S = 1.407$	$S = 3.889$	$S = 4.889$	$S = 4.556$	$S = 4.333$
DT = 0		<0.001	<0.001	<0.001	<0.001
DT = 25	<0.001		0.029	0.294	0.694
DT = 50	<0.001	0.029		0.868	0.485
DT = 75	<0.001	0.294	0.868		0.967
DT = 100	<0.001	0.694	0.485	0.967	
Mean N	$N = 1.100$	$N = 2.294$	$N = 2.501$	$N = 2.419$	$N = 2.440$
DT = 0		<0.001	<0.001	<0.001	<0.001
DT = 25	<0.001		0.736	0.946	0.908
DT = 50	<0.001	0.736		0.989	0.997
DT = 75	<0.001	0.946	0.989		1.000
DT = 100	<0.001	0.908	0.997	1.000	
Mean H'	$H' = 0.225$	$H' = 0.808$	$H' = 0.851$	$H' = 0.856$	$H' = 1.065$
DT = 0		<0.001	<0.001	<0.001	<0.001
DT = 25	<0.001		0.985	0.977	0.015
DT = 50	<0.001	0.985		1.000	0.071
DT = 75	<0.001	0.977	1.000		0.083
DT = 100	<0.001	0.015	0.071	0.083	

Mean species richness (mean S), mean density (mean N $\log_{10}$), and mean diversity, H' (mean H').

($\pm 5.1\%$ SD) of the community. In NT = 0 the invader made up 4.7% ($\pm 15.5\%$ SD) of the community, whereas in NT = I and II the invader comprised 22 ($\pm 36.4\%$ SD) and 19.7% ($\pm 34.1\%$ SD) of the community respectively.

There was a significant effect of time on observed invasion success ($F_{3,176} = 5.207$, $P = 0.002$, Figure 4). Repeated measures ANOVA for each week showed that the success of the invader increased with increasing NT (Table 3) and decreased with increasing DT (Table 3). There was a significant NT × DT interaction for the success of the invader (Figure 4; Table 3). Within nutrient-enriched microcosms, invader success

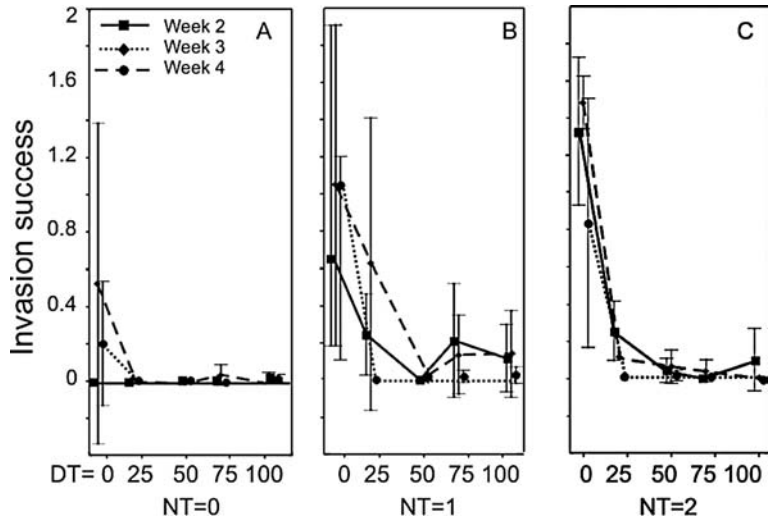

Figure 4. Effects of nutrient treatment (NT) and diversity treatment (DT) on invasion success over time. Panels represent nutrient treatments (a) NT = 0, (b) NT = 1, and (c) NT = 2. Lines represent weeks. Bars are 95% standard deviations from the mean.

Table 3. Multi-variate results of repeated measures ANOVA for effects of diversity treatment (DT), nutrient treatment (NT), and DT × NT interaction on invasion success over three sampling dates.

Source	Test	Value	*F*	Effect	Error	*P*
DT	Wilks	0.310	3.443	12	74.37	<0.001
NT	Wilks	0.574	2.981	6	56.00	0.013
DT × NT	Wilks	0.283	1.857	24	81.80	0.021

was strongly limited in the higher diversity microcosms.

Mean species richness and mean diversity, H', both had a significant effect on invasion success across all DT levels (Table 4). In contrast, mean density was unrelated to invasion success. However, when DT = 0 was removed from the analysis, none of the DT components were retained in the model for invasion success across all nutrient conditions (Table 4). Instead, across DT = 25–100, only time and NT showed significant effects on invasion success.

Univariate regressions for each species showed that the densities of two species were correlated with invasion success. Invasion success was negatively related to the densities of two of the ostracods, (*Cypridopsis* sp. $r^2 = 0.126$, $P = 0.033$; *Potamocypris* sp. $r^2 = 0.15$, $P = 0.019$). Separate univariate regressions excluding the DT = 0 treatment showed that invasion success was negatively related to the density of *Potamocypris* sp. ($r^2 = 0.11$, $P = 0.043$).

Discussion

Some have argued that greater diversity leads to greater productivity (see Loreau et al. 2001). Greater productivity should result in more complete use of limiting resources (Tilman 1997), and this, in turn, should increase the resistance of a habitat to invasion by novel species (Fox and Fox 1986; Case 1990; Robinson et al. 1995; Tilman 1997, 1999; Stachowicz et al. 2002b). However, complete resource-use can occur in both species-rich and species-poor communities (Davis et al. 2000), suggesting that there is no necessary

Table 4. (A) Univariate results of GLM for effects of observed mean species richness, mean density, and mean diversity, H' on mean invasion success across all nutrient treatments. (B) Univariate results of GLM for effects of observed mean species richness, mean density, and mean diversity H' on mean invasion success with the lowest diversity treatment (DT = 0) removed from the analysis.

Source	df	SS	MS	*F*	*P*
(A) Invasion success in DT = 0–100					
Mean *S*	1.830	1	1.830	14.385	<0.001
Mean *N*	0.343	1	0.343	2.694	0.103
Mean H'	1.165	1	1.165	9.156	0.003
Time	1.405	2	0.703	5.524	0.005
NT	1.669	2	0.834	6.558	0.002
Time × NT	0.296	4	0.074	0.581	0.677
Error	15.647	123	0.127		
(B) Invasion success in DT = 25–100					
Mean *S*	0.054	1	0.054	1.863	0.175
Mean *N*	0.025	1	0.025	0.872	0.353
Mean H'	0.016	1	0.016	0.557	0.457
Time	0.209	2	0.105	3.585	0.032
NT	0.354	2	0.177	6.065	0.003
Time × NT	0.124	4	0.031	1.063	0.379
Error	2.805	96	0.029		

Mean species richness (mean *S*), mean abundance (mean $N \log_{10}$), and mean diversity, H' (mean H').

relationship between diversity and invasibility (Davis et al. 2000).

Although previous studies have suggested reduced invasibility in high-diversity communities (Elton 1958; Knops et al. 1999; Levine and D'Antonio 1999; Naeem et al. 2000; Prieur et al. 2000; Kennedy et al. 2002) and have suggested resource limitation as the primary mechanism by which diversity inhibits invasibility (Davis et al. 2000; Stachowitz et al. 2002b), few previous studies have been able to conclusively separate direct (resource additions) and indirect (effects of diversity on resources) effects of resource limitation. Stachowitz et al. (2002b) suggested that the mechanism by which diverse plots of marine subtidal communities inhibited invasion was a reduction in space (the limiting resource in their system). When they held diversity constant and manipulated space, they found that available space was the primary determinant of invasibility.

Our results suggest that in rock pool communities resource limitation is a likely mechanism limiting the success of species invasions. However, resource limitation can be achieved in at least two ways. First, resource limitation can have a direct effect on the success of invasion. We found that invasion success was lower in low-nutrient microcosms than in high-nutrient microcosms. Second, resource limitation can be indirectly affected by diversity. Invasion success was lower within high-diversity high-nutrient microcosms than in low-diversity high-nutrient microcosms. Thus, while resource limitation is likely the mechanism inhibiting the success of invaders, resource limitation can be achieved through either direct reductions in resources, or indirectly through increases in the diversity of the community and, presumably, more thorough partitioning and consumption of the available resources.

Dilution methods have been successfully used to manipulate bacterial (Morales et al. 1996; Garland and Lehman 1999; Garland et al. 1999; Franklin et al. 2001; Kisand and Wikner 2003), fungal (Taylor and Bruns 1999), and plant (Goldberg et al. 2001) diversity. The premise behind using a dilution series to manipulate diversity is that dilution of a diverse community will result in the exclusion of rarer species as the dilution progresses. Subsequent re-growth of diluted mixtures should then result in cultures of roughly the same biomass or abundance while maintaining differences in diversity (Franklin et al. 2001). Both simulation and empirical experiments have shown that dilution series manipulations are effective at manipulating species richness of mixtures. For example, Franklin et al. (2001) used simulations and a dilution experiment to show that dilutions of bacteria resulted in mixtures that differed significantly in species richness. Recently, Giller et al. (2004) discussed the potential of using dilution series to manipulate species richness in diversity–ecosystem function experiments in aquatic systems. They concluded that dilution experiments are well suited to diversity–ecosystem function studies as they simulate the consequences of natural species loss in experimental treatments and may be preferable to random loss methods with multiple comparisons per diversity level.

One potential problem with using dilution series to manipulate diversity is that the initial manipulation affects species richness, density, and potentially, species abundance distributions. Thus, re-growth of the mixtures to similar biomass levels following the initial manipulation is necessary to conclusively determine what component of the diversity manipulation is driving the observed patterns. In the present experiment, the DT manipulation significantly affected initial species richness, density, and diversity, H'. However, the only significant difference in mean density between DT levels was between DT = 0 and 100. In contrast, mean species richness and mean diversity, H' differed significantly between DT = 0 and some of the intermediate DT levels and between DT = 0 and 100. Thus, after initial population growth, the components of the initial DT manipulation (i.e. species richness, density, diversity, H') that remained significantly different in a greater number of treatments were species richness and diversity, H'.

While it is possible that density may still have had an effect on invasion success, two other lines of evidence argue against this. First, across DT = 0–100, species richness and diversity, H' were the only components of DT retained in the ANOVA model for invasion success. Across DT = 25–100, no component of DT was retained in the model for invasion success. Second, invasion success was lowest at DT = 50, which had

the highest mean species richness, and did not differ in density from DT = 25, 75, or 100. However, we caution that it is not possible to conclusively unravel the correlative contributions of species richness, diversity, H', and density using our diversity manipulation protocol.

Regardless of the above considerations, however, the role of species richness in inhibiting invasion success appears to be limited to the importance of a low level of species richness. The strongest effect of species richness on invasion success existed in the transition between DT = 0 and 25 and no component of DT was retained in the ANOVA model for invasion success across DT = 25–100. Wardle (2001) argued that a clear effect of plant species richness on ecosystem function has never been demonstrated beyond very low levels of richness (2–3 species). Our study supports this argument in terms of the influence of species richness in rock pool communities on invasibility; however, these rock pool communities have a relatively low species richness in their natural state, ranging from 0 to 16 species in any one pool (mean 5.78 ± 2.65 SD, Romanuk and Kolasa 2002b).

Particular species also appeared to inhibit invasion success. Separate regressions for each species showed that success of the invader was negatively related to the densities of the ostracods *Cypridopsis* sp. and *Potamocypris* sp. for DT = 0–100 and to the density of *Potamocypris* sp. for DT = 25–100. The role of competitive dominants in inhibiting invasion success has been stressed by Wardle (2001) as a primary mechanism for limiting species invasions in plant communities. Particular species, both predators and competitive dominants, have also been shown to inhibit invasions success in zooplankton and protist communities (McGrady-Steed et al. 1997; Miller et al. 2002; Lennon et al. 2003).

In conclusion, the diversity-invasibility hypothesis predicts that greater diversity inhibits invasibility. The most commonly cited mechanism is that more diverse assemblages more fully utilize available resources, thus leaving little resource space for individuals of a new species (Tilman 1997; Knops et al. 1999; Levine and D'Antonio 1999). Our results lend some empirical support for this hypothesis in aquatic consumer communities. However, our results also suggest that beyond a very low level of species richness, diversity may have little effect on invasibility. In rock pool communities, nutrient enrichment and sampling effects (due to competitive dominants) are likely the determinants of the success of invasion across a wide range of diversity.

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