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Establishment success in young cladoceran communities: An experimental test

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Abstract

We tested the relative importance of regional and local factors in determining zooplankton community composition in an enclosure experiment. In this experiment, we assessed establishment success of immigrant cladoceran zooplankton species in young communities in the first and second year of existence in five newly created pools. In both years, we created four treatments, representing a gradient of strength in biotic interactions with the resident communities, to explore differences in establishment success of immigrant species. In general, species diversity increased when immigrant species were added, suggesting dispersal limitation. However, this increase was lower in the second than in the first year, indicating a declining effect of regional factors during the course of community assembly. No significant difference in establishment success between the experimental treatments could be detected in the first year. In the second year, immigrant species were more often present in the treatment without resident species than in the treatments with resident species, at least during the first weeks. Our results demonstrate that species sorting and biotic interactions, mainly competition among zooplankton species and predation by *Chaoborus*, become important in determining the zooplankton community composition early in community assembly.

Both regional and local processes determine the assemblage of local communities. On the regional scale, individuals must arrive by dispersal to the habitat to establish viable populations. Locally, the presence of suitable habitat (structural facilities and abiotic conditions that species can tolerate), competition, and predation can play major roles in the success of a colonizing species (Caley and Schluter 1997; Shurin et al. 2000). Communities in newly formed habitats are assembled by dispersal of species from regional sources. The colonization curve of such new habitats initially follows a linear pattern, the slope of which is determined by the dispersal capacity (the product of propagule production rate and vagility) and the density of source habitats. After a certain time period, the colonization curve typically exhibits a saturation level (Preston 1960). The decreasing rate of colonization can have two causes. First, it can be caused by limits imposed by regional species diversity. The increasing number of species in the local communities results in a decrease of the probability that an immigrant belongs to a new species, and this effect becomes stronger as the local community grows more similar to the regional species pool. Also, the first immigrants are likely to be species with a high dispersal capacity, high regional abundance, or both. A decreasing number of colonizing species through time can, however, also result from local factors, in that an increase in the strength of biotic interactions can result in a decreased establishment success of immigrants. The most straightforward way to differentiate between these mechanisms is experimental manipulation.

In the current literature, there is some disagreement on the relative importance of local and regional factors in structuring communities. On the one hand, Jenkins and Buikema (1998) indicated that the species composition among 1-yr-old habitats differed substantially. The authors concluded that stochastic effects associated with low dispersal rates were most important in these systems. On the other hand, Shurin (2000) indicated that local factors are most important in structuring communities because establishment success of immigrant species was observed to be low in old communities.

Our research positions itself between these two previously performed studies. Through an experimental design, we assessed the relative importance of dispersal limitation and local interactions in the assemblage of cladoceran zooplankton communities in newly created habitats under natural conditions. Cladoceran zooplankton are a key component of lentic freshwater systems (Scheffer 1998) and are known to have good dispersal capacities over short spatial scales (Bilton et al. 2001; De Meester et al. 2002; Havel and Shurin 2004), which leads to the assembly of multispecies communities in a few months (Louette and De Meester 2004, 2005). We hypothesized that establishment success of immigrant species declines with the age of the habitat because aging of a habitat results in an increase in the number of resident species. Furthermore, by manipulating the densities of the resident communities, we tested whether there is a difference in establishment success of immigrant species because of altered biotic interactions with the resident community. Although our experimental design is very similar to that employed by Shurin (2000), there are two important differences. Whereas Shurin (2000) performed his experiments on older communities (ponds were at least a few decades old), we focused on young communities. This makes a comparison of our results with those of Shurin (2000) very informative with respect to understanding the changes in establishment success of immigrant species during community

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Table 1. The four different treatments used in the experiment, with all potential outcomes in the establishment success of immigrant species (after artificially neutralizing dispersal limitation) and their respective limiting local factor.

Code	Treatment	Establishment success of immigrant species			
RI	Resident + immigrant	High	Low	Low	Low
RRI	Reduced resident + immigrant	High	High	Low	Low
I	Immigrant	High	High	High	Low
R	Resident	—	—	—	—
		Limiting local factor			
		None	Biotic	Biotic	Abiotic

development. Second, we carried out experiments on geographically separated sites (study area of 3,000 km^2) and thus cover a wider range in regional characteristics than earlier colonization studies (Jenkins and Buikema 1998; Shurin 2000). This allows an assessment of the degree to which regional factors, reflected in a different composition of the resident community in the pools and of the inoculum of regional species, influence establishment success.

Materials and methods

We performed enclosure experiments in the summers of 2002 and 2003 in five fishless pools (surface area of 100–250 m^2, maximum depth of 150 cm). The pools are part of nature restoration projects that aim at creating suitable habitats for amphibian populations in Flanders (Belgium) and were dug in late September 2001. All pools were created on sites with no previous wetland history and filled naturally within 2 months with rain- and groundwater. Because all pools were new, there was no resting egg bank (Vandekerkhove et al. 2005). Because the pools were isolated from all other surface waters, natural colonization could only be achieved through external dispersal. For more details on the environmental variables and the detailed colonization history of the pools, see Louette and De Meester (2005).

Experimental design—The experiment consisted of four treatments, with three replicates in each of the five pools in both years (Table 1). The first treatment (RI, resident + immigrant) consisted of the resident community enriched with an inoculum containing cladoceran species occurring in waterbodies from the direct neighborhood of the pool (regional species pool). This treatment allowed us to assess establishment success in the natural community. The second treatment (RRI, reduced resident + immigrant) consisted of a reduced (50%) density of the original community and an inoculum of the regional species pool. Comparison of the establishment success of this treatment with that in the RI treatment allowed testing of the effect of reduced biotic interactions on establishment success of new species. In the third treatment (I, immigrant), the original community was removed and replaced by an inoculum from the regional species pool. This third treatment allowed an assessment of the possibility of immigrant species to survive in local conditions in the absence of interactions with resident species. A comparison of the establishment success in the I treatment with that in the RI treatment provides insight into the relative importance of biotic interactions and abiotic environmental conditions in determining establishment success. Finally, we created an additional control treatment (R, resident). In this treatment, the zooplankton community consisted exclusively of the resident community, both in terms of species composition and density. This treatment allowed us to assess the fate of the natural community in the experimental units.

We conducted the experiments in green plastic cylindrical rainwater containers (height 100 cm, diameter 55 cm), which were positioned in the center of each pool under study (experimental units were removed between both years). They were in direct contact with the atmosphere and were closed at the bottom. We filled the containers (water volume 180 liters) by pumping natural pool water twice through a 64-μm plankton net, thus removing all resident zooplankton. We assigned treatments to containers in a randomized block design. Subsequently, we inoculated the resident crustacean zooplankton in densities according to the specified treatments. For each pool, three additional samples were preserved to determine the density and species composition of the resident community. On the same day, we collected a regional inoculum from two long-standing ponds in the immediate vicinity ($<$3 km) of each focal pool. For each regional waterbody, we balanced the volume of filtered water such that approximately equal densities of cladocerans of the two waterbodies were obtained. We collected three additional samples of the inoculum of each of the regional species pools to determine density and species composition of the inocula.

When submerged, macrophytes were present in the resident pool at the time of the experiment; we mimicked this in the containers. This was only the case for one pool (KO1) and only in the second year, when *Chara globularis* occurred. In this case, we collected *C. globularis* from the pool 1 month before the start of the experiment, kept it in the laboratory, and rinsed it regularly so as to ensure that adding the macrophytes to the experimental units would not result in contamination with zooplankton.

Sampling—In all five pools and both years, the experiment started in the beginning of July and ran until the end of September (90 d). All experimental units were sampled every 2 weeks, resulting in a total of six sampling dates per year. We sampled the zooplankton community during the day with a tube sampler (length 1 m, diameter 75 mm), which was positioned vertically in the water column and then closed at the bottom by a magnetic lid. This resulted in a

water column of 80 cm (volume 3.5 liters), which was filtered through a 64-μm plankton net. We took four samples from random locations in each container, resulting in 14 liters of collected water. To prevent contamination, we used strictly different plankton nets for each treatment and rinsed the tube sampler thoroughly between each sampling event. We preserved zooplankton in 4% formaldehyde saturated with sucrose, counted all cladocerans and copepods present in the zooplankton sample, and identified cladocerans to species level following Flössner (2000). *Chaoborus* was also present in all the experimental units, and the number of third and fourth instars were counted in all samples. We transformed cladoceran density values to biomass values with published length–weight relationships (McCauley 1984) on the basis of the measurement of 20 randomly chosen individuals from each species in each pool.

In both years, environmental variables (pH, temperature, oxygen concentration, chlorophyll *a* [Chl *a*] levels, and conductivity) were measured on three different sampling dates (day 1, day 45, and day 90) in each of the experimental units.

Data analysis—Establishment success in this study is quantified with two indices: the total biomass (μg L^{-1}) of inoculated species that were not yet present in the resident community, and the percentage (%) of successful establishments. We calculated the percentage of successful establishments for each sample separately as the number of new species (i.e., not present in the resident community) present in the sample divided by the total number of new species that were present in the inoculum. Patterns through time in total biomass ($\log[x + 1]$ transformed to match the assumptions of normality of the data and homogeneity of variances) of newly inoculated species and the percentage of successful establishments (%, not transformed) were analyzed for each year separately by repeated measures analysis of variance over the six sampling dates (days 15–90), with pools and experimental treatments as independent factors (the R treatment was excluded from this analysis because immigrant species were absent in this treatment).

Fisher's α diversity was calculated on the total species composition of each sample separately and averaged over the six sampling dates and replicates to obtain an overall estimate for each pool–treatment combination in each year (Magurran 2004).

To test for changes in a number of variables as the pool habitats aged, we performed dependent *t*-tests on the number of resident species, resident diversity, immigrant biomass, and percent establishment success between both years. Average values over the six sampling dates and experimental units of the RI treatment were used as input values for these tests.

Changes in community structure between the different treatments were determined by multivariate analysis. We used biomass (μg L^{-1}, $\log[x + 1]$ transformed) data of the different species occurring in the different treatments (R, RI, RRI, and I) at a given sampling occasion (averaged over the three replicates) to construct a similarity matrix (Bray–Curtis similarity) involving all treatments and pools. This analysis was done for each year separately. Subsequently, we performed a multiple dimensional scaling (MDS) analysis on the similarity matrix for each year. In the representation of the MDS, data points of the time–treatment samples are thus located against each other proportional to the similarity relations between samples.

Results

Environmental conditions remained quite stable in the experimental units and did not differ among treatments. Levels approximated those of natural waterbodies (i.e., dissolved oxygen 8.5 $\pm$ 0.3 mg L^{-1} [mean $\pm$ SE], range 7.4–9.7 mg L^{-1}; temperature 18°C $\pm$ 0.4°C, range 15.5–20°C; conductivity 341 $\pm$ 39 μS cm^{-1}, range 204–517 μS cm^{-1}; pH 7.3 $\pm$ 0.3, range 5.1–8.3). Chl *a* levels were 13 $\pm$ 3 μg L^{-1} (range 4–28 μg L^{-1}) if treatment R of pool BI1 in the first year is left out. The R treatment of BI1 in the first year contained much higher Chl *a* concentrations (256 μg L^{-1}) than the other treatments, most likely because of the absence of large resident cladocerans in this treatment.

The inocula (species originating from the regional species pool) added to the different experimental treatments consisted of an average of 833 $\pm$ 97 individuals (range 305–1,187 individuals), representing an average of 13 new species in the first year and 10 new species in the second year to the respective resident communities. A detailed overview of the species composition of the inocula from the regional species pool is given in Table 2. The number of individuals that were inoculated differed considerably among species. Three species (*Bosmina longirostris, Ceriodaphnia pulchella,* and *Chydorus sphaericus*) were added, with an average of more than 100 individuals in the experimental units, whereas almost all other species had very low numbers in most of the inocula (1–20 individuals). The resident communities consisted of one to three species during the first year and two to six species during the second year. Some species that were present in the regional inocula were also found in the resident community (e.g., *C. sphaericus* in all pools both in the first and second year and *Simocephalus vetulus* in all five pools in the second year). Although one could argue that the presence of *S. vetulus* in the resident communities during the second year might have been from escapees from experimental manipulations in the first-year experiment, a regional colonization survey in Flanders (Belgium) indicated that *S. vetulus* successfully colonized 14 of 20 pools by their second year. Furthermore, identical pools constructed at the same time and in the immediate neighborhood of the studied pools harbored *S. vetulus* within only 15 months after their construction.

In the first year, we observed a significant effect of pool and time on both biomass (μg L^{-1}) and percent successful establishments (%) of new inoculated species, but there was no significant treatment effect (RI, RRI, and I; Table 3). In the second year, the effect of treatment was significant, in addition to that of pool and time. In this second experiment, the I treatment had a significantly higher immigrant biomass and establishment success than the RI and RRI treatments. There was, however, also a significant time–treatment interaction effect, reflecting that the differences in immigrant biomass and establishment success between the I and the other

Table 2. Number of inoculated individuals for each species of the inoculum and number of individuals for each species of the resident cladoceran zooplankton community in the five pools (HB1, HB2, BI, KO1, and KO2) and 2 consecutive yr.

	HB1		HB2		BI		KO1		KO2	
	1	2	1	2	1	2	1	2	1	2
Inoculum										
Acroperus harpae							12	47	25	47
Alona affinis	5	13	4	15			1	5	5	10
A. guttata	1	2	5				2	2		
A. rectangula	1	5		2	1	13	7	2	12	7
Alonella excisa					9					
Bosmina longirostris	306	475	245	403	61	143	480	45	372	43
Camptocercus rectirostris								2		3
Ceriodaphnia laticaudata		3								
C. pulchella	1	10	1	22			388	678	325	720
Chydorus sphaericus	100	273	128	347	124	170	14	47	23	57
Daphnia ambigua		8	2	8						
D. cucullata	1	33	2	12			39	13	22	7
D. galeata					1	3				
D. parvula					1	30	166	22	175	53
Disparalona rostrata	3	2		2						
Eurycercus lamellatus							1	2		7
Graptoleberis testudinaria	63	25	138	52	9	10			2	
Iliocryptus agilis	1	2	1	2						
I. sordidus								2		
Leydigia leydigi							3		8	3
Macrothrix laticornis	1	2					1	2		
Pleuroxus aduncus					7	27	3	2	8	3
P. denticulatus							8	5	10	10
P. truncatus	3	3	4	7		3	9	108	7	87
P. uncinatus							2		2	7
Scapholeberis mucronata	1	2	1	3	1		44	95	58	73
Sida crystallina							3			
Simocephalus vetulus	11	8	12	28	91	310	2	25	3	27
Total	496	867	543	902	305	710	1,187	1,102	1,057	1,163
New species*	13	11	10	10	9	6	18	13	15	12
Resident community										
Alona guttata		67	15	13						13
Chydorus sphaericus	1,793	4,747	66	1,553	2	3,733	5	2,980	20	2,587
Daphnia obtusa	710	40	97				224			
D. pulex									9,743	
Graptoleberis testudinaria				47						
Macrothrix laticornis									40	13
Pleuroxus aduncus										27
P. denticulatus				53				13		
P. truncatus		53						7		13
Scapholeberis mucronata		40								
Simocephalus vetulus		67		2,113		2,667		1,073		160
Total	2,503	5,013	179	3,780	2	6,400	229	4,073	9,802	2,813
Species	2	6	3	5	1	2	2	4	3	6
Chaoborus sp.	106	27	103	794	149	347	58	700	20	239

* The number of species in the regional inoculum that are not members of the resident community.

treatments were only significant on day 15, and not on any of the subsequent sampling days (post hoc tests; Table 3). Analyses of covariance with Chl *a* levels as covariate yielded the same results, with the effect of the covariate being nonsignificant.

The number of resident species and resident diversity was significantly higher in the second year compared with the first year ($p < 0.05$). The biomass of immigrant species was significantly lower in the second year compared with the first year ($p < 0.05$). The average establishment success was 16% ± 1% in the first year, whereas it was reduced to 10% ± 2% in the second year; however, this difference was only marginally significant ($p = 0.08$; Fig. 1). The cumulative establishment success of inoculated species, determined as the sum of records of all immigrant species that were observed on at least one sampling occasion over the different

Table 3. Repeated measures ANOVA on the effects of pool, treatment (RI, RRI, and I), and time on (A) immigrant biomass and (B) percent establishment success of the inoculated species in the enclosures in both years. Post hoc tests are given for days 15, 30, 45, 60, 75, and 90 during the second year of the experiment to indicate which of the experimental treatments (I, RI, and RRI) differed significantly.

	Year 1				Year 2				Year 2 post hoc			
	df	SS	*F*	*p*	df	SS	*F*	*p*	Day	I–RI	I–RRI	RI–RRI
(A) Immigrant biomass												
Between subjects												
Pool	4	50.251	34.389	<0.0001	4	14.994	22.125	<0.0001	15	<0.0001	<0.0001	0.900
Treatment	2	0.861	1.178	0.322	2	5.348	15.783	<0.0001	30	0.127	0.147	0.936
Pool × treatment	8	2.866	0.981	0.470	8	1.428	1.053	0.420	45	0.958	0.593	0.630
Error	30	10.959			30	5.083			60	0.172	0.063	0.596
									75	0.292	0.148	0.683
Within subjects									90	0.093	0.090	0.989
Time	5	20.201	29.749	<0.0001	5	2.421	5.004	0.0003				
Time × pool	20	14.227	5.238	<0.0001	20	18.443	9.529	<0.0001				
Time × treatment	10	2.507	1.846	0.057	10	3.484	3.600	0.0003				
Time × pool × treatment	40	6.200	1.141	0.281	40	4.829	1.247	0.173				
Error	150	20.371			150	14.516						
(B) Percent establishment success												
Between subjects												
Pool	4	0.211	3.306	0.023	4	0.783	20.817	<0.0001	15	0.003	0.002	0.931
Treatment	2	0.040	1.241	0.304	2	0.169	8.997	0.001	30	0.677	0.628	0.945
Pool × treatment	8	0.039	0.303	0.959	8	0.019	0.253	0.976	45	0.977	0.979	0.998
Error	30	0.479			30	0.282			60	0.539	0.363	0.765
									75	0.347	0.086	0.121
Within subjects									90	0.092	0.070	0.629
Time	5	0.313	10.806	<0.0001	5	0.112	3.941	0.002				
Time × pool	20	0.325	2.806	0.0002	20	0.205	1.803	0.025				
Time × treatment	10	0.051	0.876	0.557	10	0.119	2.104	0.027				
Time × pool × treatment	40	0.261	1.126	0.300	40	0.259	1.139	0.284				
Error	150	0.869			150	0.852						

df, degrees of freedom; SS, sum of squares.

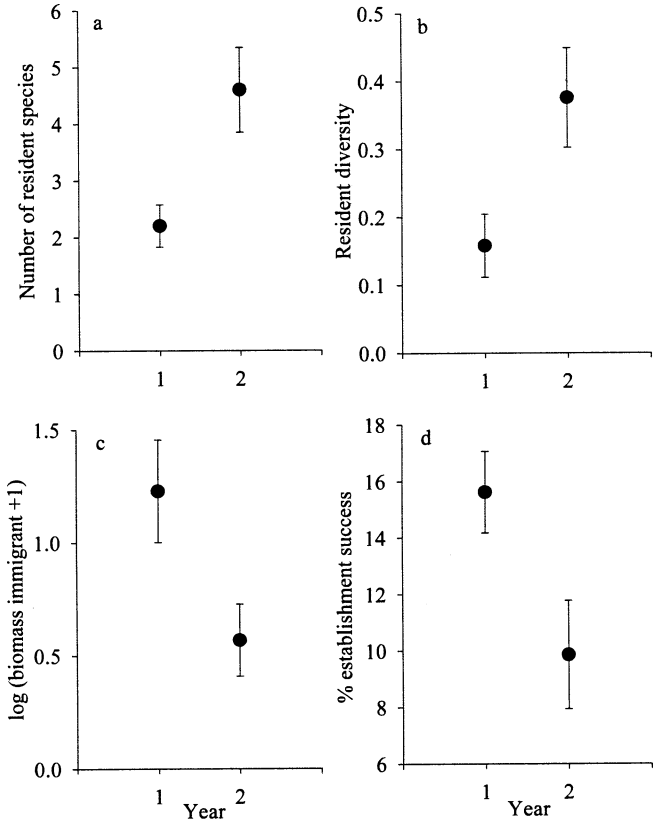

Fig. 1. (a) Number of resident species, (b) resident diversity (Fisher's α), (c) immigrant biomass, and (d) percent establishment success for both years. All data points are averages of the RI treatment over all pools. Error bars represent the standard error of the mean.

pools, six sampling dates, and replicates in the RI treatment, was 63% ± 7% in the first year and 46% ± 7% in the second year.

Species that were initially inoculated in very low numbers were in some cases very successful in colonizing and assembling viable populations. For example, *Alona affinis* was inoculated in very low numbers (<20 individuals in all pools) but was a successful species during the experiment. Also, *S. vetulus* was inoculated in very low numbers (<15 individuals) in four of the five pools during the first year, but became the most successful colonist in the experimental units. The opposite was true for some of the species that were inoculated in high numbers, such as *B. longirostris* and *C. pulchella* (>100 individuals). These species were only capable of maintaining a viable population during the first month.

The development of immigrant species biomass through time for both the RI and the I treatment is presented in Fig. 2. We distinguished between two types of immigrant species (pelagic and littoral; Flössner 2000). Pelagic species (*B. longirostris, C. pulchella, Daphnia ambigua, Daphnia cucullata, Daphnia galeata,* and *Daphnia parvula*) seemed only to be successful during the first 15 d, and in many cases, disappeared thereafter. The opposite was true for littoral and benthic species (chydorids, *S. vetulus, Scapholeberis mucronata,* and macrothricids), for which strong increases in biomass during the course of the experiment were observed. The most dominant species in the littoral group were *Acroperus harpae, Alona affinis, Graptoleberis testudinaria, Pleuroxus aduncus, Pleuroxus truncatus, S. mucronata,* and *S. vetulus.* The last species, especially, was very successful, representing on average 60% of the total immigrant biomass.

Community diversity increased substantially in the treatments in which an inoculum of the regional species pool was added (dependent *t*-test on Fisher's α diversity in the R and RI treatments; $p < 0.05$). No significant differences were found between the experimental treatments RI, RRI, and I (Fig. 3). Because of natural colonization of the pools, resident diversity had increased in year 2 compared with year 1. However, diversity in the experimental treatments (RI, RRI, and I) did not differ among years (Fig. 3).

Multivariate analysis indicates that adding the inocula with immigrant species resulted in strong changes in community composition in the different pools during the first year (Fig. 4). The communities in the R treatment shifted in a similar direction in all pools during the course of the experiment. Adding the regional pool resulted in dramatic changes in similar directions for all pools and resulted in quite similar community compositions, even though the regional inocula were different for all pools. In the second year, adding the inocula did not cause changes in community structure. Adding inocula of different regions did not result in strongly divergent communities. For both experiments, the community of almost all experimental treatments (RI, RRI, and I) in the five pools on days 75 and 90 was dominated by *A. affinis, C. sphaericus, G. testudinaria, P. truncatus,* and *S. vetulus.*

Predatory *Chaoborus* (*crystallinus* and *obscuripes*) larvae were observed in all experimental units. The first year, *Chaoborus* was initially present at low densities but increased in density throughout the experiment. No differences were found among treatments. The second year, *Chaoborus* density was initially considerably higher, and there was a significant difference between the I treatment (filtered water and no resident community added) and the other treatments until day 30 ($p < 0.05$; Fig. 5). Overall, the *Chaoborus* density did not differ between years (average of the six sampling dates of the RI treatment).

Copepods represented a substantial part of the zooplankton community biomass in the experimental units in both years, but there was a tendency toward a decrease in their density over time in both years. Copepod densities did not differ among the four treatments in either the first or the second year experiment, and no difference was observed in the average copepod density among years (Fig. 6).

Discussion

Establishment success is expected to decline with increasing diversity of the native resident community because, in more diverse communities, immigrating species have fewer opportunities to occupy empty niches. This pattern has been demonstrated experimentally on several occasions in both terrestrial and aquatic communities (Tilman 1997; Shurin

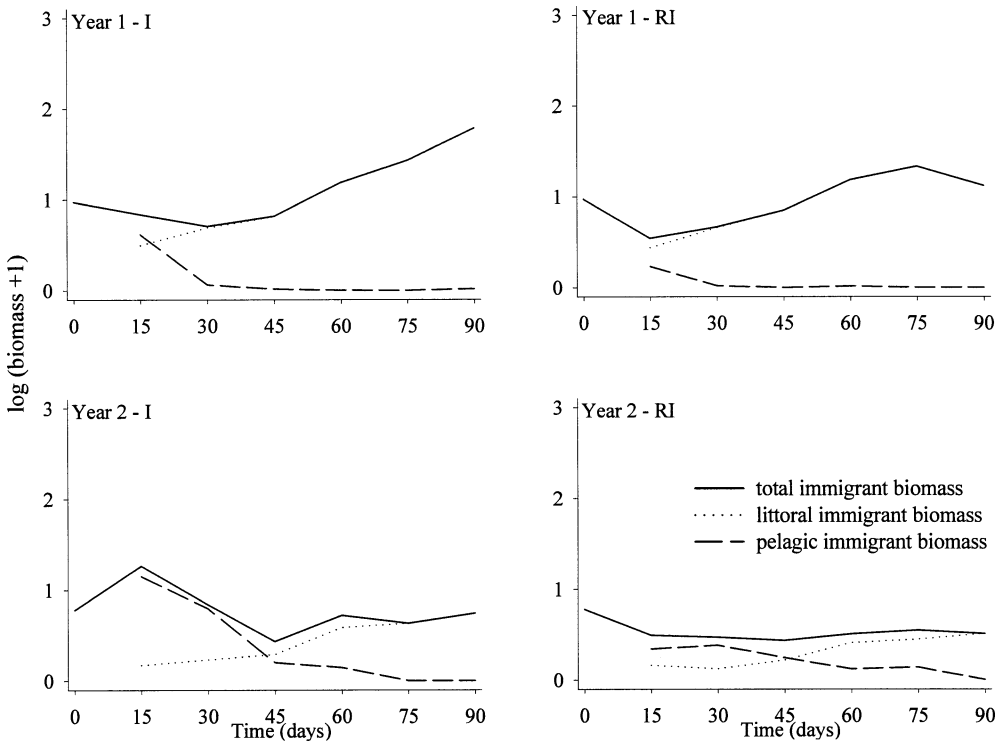

Fig. 2. Biomass (μg L^{-1}) development (average over pools) of different groups of cladoceran zooplankton during the experiment. Total immigrant biomass, biomass of pelagic immigrant species (*Ceriodaphnia*, *Daphnia* spp. and *Bosmina longirostris*), and biomass of littoral immigrant species (chydorids, *Simocephalus vetulus* (year 1), *Scapholeberis mucronata*, and macrothricids) are shown for both the first and second year of existence of the pools. Only the I (without resident community) and RI (with resident community) treatments are presented (RRI was not significantly different from the RI treatment).

2000). In many field surveys, however, the reverse pattern has been observed, with more diverse resident communities showing a higher number of invasive species. In these cases, the pattern of invasibility has been shown to depend on propagule pressure, habitat type, availability of resources, and species-specific responses to predation or disturbances (Stohlgren et al. 1999; Levine 2000).

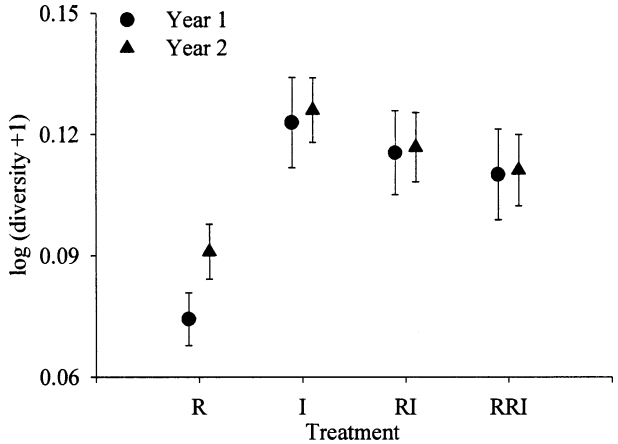

Fig. 3. Diversity (Fisher's α) in the different treatments, averaged over the six sampling dates, replicates, and five pools for both years. Error bars represent the standard error of the mean over pools.

We observe that in the same habitats and localities, more diverse communities are more resistant to establishment of new species than younger and less diverse communities. In the first year, young communities display a high vulnerability to establishment of new species once these reach the habitat (proportion of immigrant species biomass to the total biomass: 65% $\pm$ 10%). During the second year, as resident communities accumulate more species by natural colonization of the habitat, the proportion of immigrant species biomass is strongly reduced (7.5% $\pm$ 2.5%). Shurin (2000) performed similar experiments on older (>10 yr old) zooplankton communities. In his invasion experiment, an even lower biomass (0–2.5%) of introduced zooplankton species (rotifers, copepods, and cladocerans) compared with total zooplankton community biomass was observed. Establishment success of nonresident cladoceran species, expressed cumulatively (i.e., considering all species that were detected at least once during the experiment) shows a similar pattern, dropping from 63% in the first to 46% in the second year. This illustrates that establishment success is already strongly reduced in the second year of the existence of zooplankton communities. In the invasion and resistance experiments of Shurin (2000), an average of 5.5 nonresident cladoceran species were introduced, of which only 30% was detected on one or more of the sampling dates. Taking both our experiment and the results of Shurin (2000) together, it is clear that local biotic interactions become very important

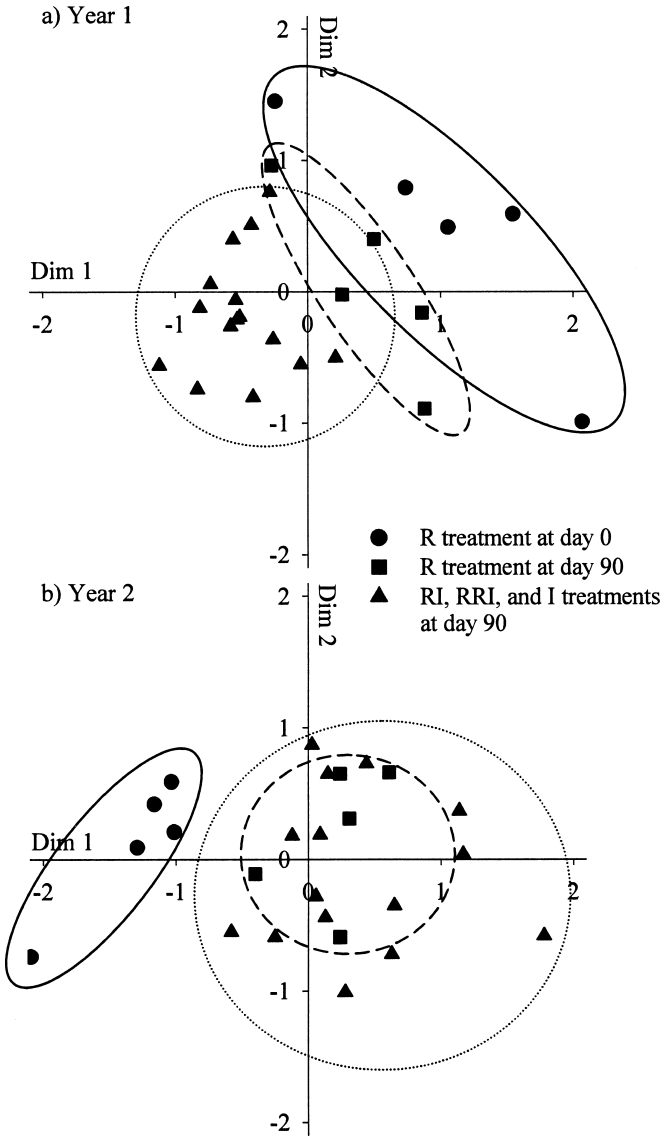

Fig. 4. Representation of a multiple dimensional scaling (MDS) analysis performed on the biomass of the different species occurring in the experiments performed in (a) year 1 and (b) year 2. Every data point is the average value for one pool. Solid lines refer to the R treatments at day 0; long dashed lines refer to the R treatments at day 90; and dotted lines delineate the RI, RRI, and I treatments at day 90. RI, RRI, and I treatments are not shown for other dates (days 0, 15, 30, 45, 60, and 75) to keep the figure sufficiently simple to interpret.

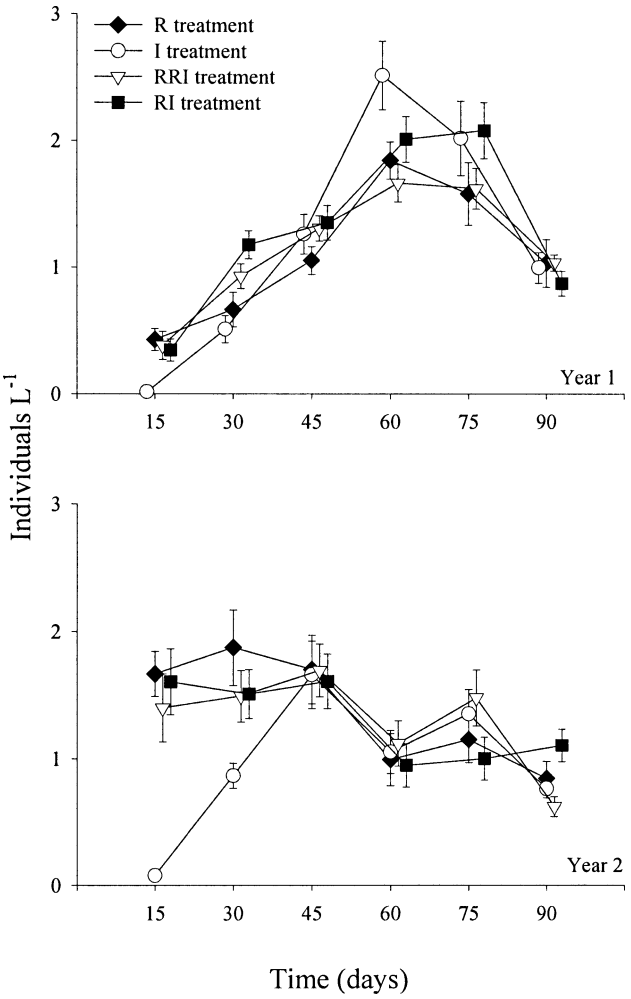

Fig. 5. Changes in *Chaoborus* third and fourth instar density (individuals L^{-1} averaged over pools and replicates) through time for the different treatments in the first year and second year. Error bars represent the standard error of the mean over pools.

in limiting establishment success in zooplankton communities on a short timescale.

The different treatments (RI, RRI, and I) were designed to quantify the effect of biotic interactions with resident species on establishment success of immigrant species. In the first year, no differences were observed among treatments, indicating that the effect of the resident communities in reducing establishment success was minimal. Given the strong difference in establishment success between the first and the second year, one expects a strong effect of the local resident community in the second year. Yet, we observed only a significant difference between the treatments RI, RRI, and I during the first sample occasion after the start of the experiment (day 15). On further sampling occasions (days 30–90), there was no significant treatment effect. These results contrast with those of Shurin (2000), who observed a lasting effect (40 d) of treatment in his resistance experiment. In the experiment reported by Shurin (2000), the disturbed resident community treatment had 3.8 times more immigrant zooplankton species than the invasion treatment during the duration of the experiment.

At first sight, our observation on the decrease in establishment success of immigrant species over time contrasts with the absence of a long-lasting treatment effect in the second year. Closer inspection of our data, however, offers an explanation for this apparent conflict. *S. vetulus,* a large littoral cladoceran species, appears to strongly dominate the zooplankton community in all experimental units. Irrespective of its initial density, *S. vetulus* became dominant within a few weeks, resulting in a convergence of the species composition of all treatments. This convergence of species com-

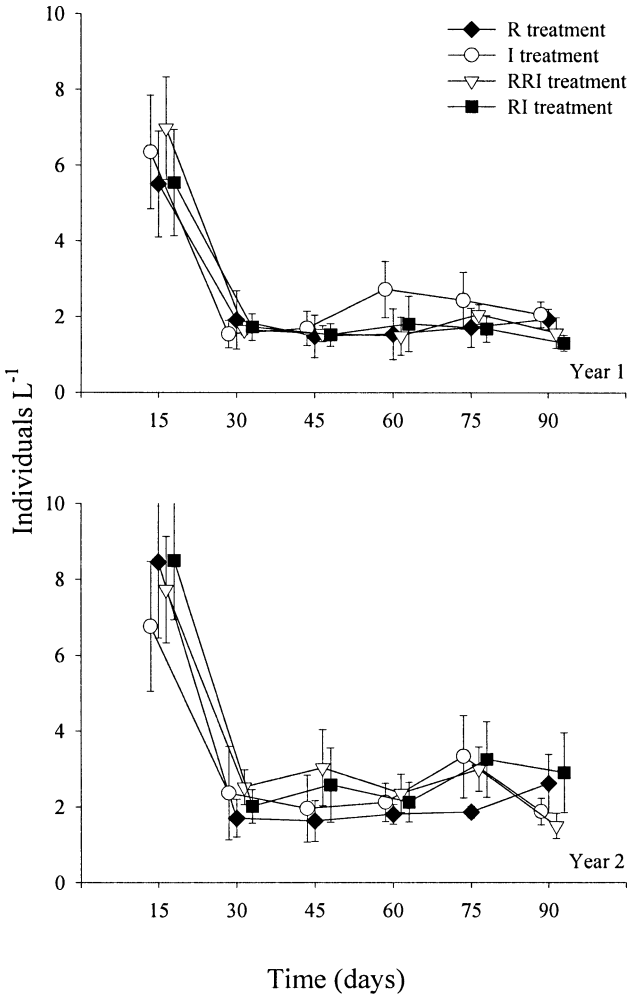

Fig. 6. Changes in copepod density (individuals L^{-1} averaged over pools and replicates) through time for the different treatments in the first year and second year. Error bars represent the standard error of the mean over pools.

position illustrates the strikingly fast species sorting in zooplankton communities that has already been reported by Cottenie and De Meester (2004). Also, community assembly experiments in outdoor containers indicate that *S. vetulus* rapidly dominates the zooplankton community in the presence of *Chaoborus* predation, whereas *Daphnia* dominates over *Simocephalus* in the absence of *Chaoborus* (Louette and De Meester unpubl. data). Our results are in concordance with these observations because *Chaoborus* was observed in all experimental units.

This suggests that establishment success of immigrant species is strongly affected by competition with local resident species. Competition with other members of the community is, however, not the sole mechanism limiting establishment success. Some species inoculated in high numbers (*Bosmina, Ceriodaphnia,* and *Daphnia*) declined in biomass during the first weeks, before strong populations of *Simocephalus* developed, perhaps because of predation by *Chaoborus,* which is known to be a very important predator of zooplankton in fishless environments (Sutor et al. 2001). *Chaoborus* is a fast disperser, has a short life cycle during warm periods (45 d), and feeds on cladocerans and copepods from the third instar onward (Saether 1997; Berendonk and Bonsall 2002). It is known to prey preferentially on small and agile species like *Bosmina,* small *Daphnia,* and *Ceriodaphnia* species when food resources are plenty (Pastorok 1980; Riessen et al. 1988). *Chaoborus* species of fishless habitats (*C. crystallinus* and *C. obscuripes*) can even suppress populations of larger *Daphnia* species (Wissel and Benndorf 1998). The density of *Chaoborus* in the experimental units was quite high and might have had a prominent effect on the composition of the cladoceran community. Most pelagic species didn't manage to assemble large populations in the experimental units during the entire time of the experiment in any of the 2 yr. Moreover, during the first 15 d of the experiment, they were more successful in the I treatment than in the RI and RRI treatments, the difference being especially pronounced in the second year. The nature of the I treatment (the entire resident zooplankton community as well as *Chaoborus* was removed) strongly reduced initial *Chaoborus* density. The success of littoral immigrant species in relation to pelagic immigrant species could thus be explained by the high *Chaoborus* numbers in the type of experimental units used. Because we applied small units to mimic the habitat of the natural pools, little pelagic habitat was available, and *Chaoborus* predation rates could have been very intense. One can expect these negative effects on pelagic species to be less pronounced in larger systems.

Copepods were also observed to be an important member of the zooplankton communities in the experimental units. Most copepod species are herbivorous and compete with cladocerans for algae and other protists; however, some species are known to be carnivorous (Fryer 1957). These predatory species feed mainly on small or juvenile cladocerans, such as *Bosmina, Ceriodaphnia,* and *Daphnia* (Brandl 1998). Because we did not detect any difference in copepod densities among treatments and years, it is unlikely that the copepods influenced the observed differences in establishment success among pools and years substantially. Rather, copepod densities themselves declined fast in most experimental units.

By studying the establishment success of regional zooplankton species in 1- and 2-yr-old pool communities, we documented the transition from dispersal-limited communities to communities in which interactions with resident species strongly determine establishment success. Our results also clearly provide evidence for rapid species sorting and point to the importance of biotic interactions, such as predation and competition, in determining species composition in zooplankton communities. Jenkins and Buikema (1998) showed that stochastic effects associated with low dispersal rates determine establishment success during initial zooplankton community assembly. Shurin (2000) illustrated that local environmental control is very important in determining the establishment success in old communities. Our results demonstrate that the transition between these two states occurs quite early (in the second year). In general, we conclude that the results of our experiments clearly indicate that establishment success declines as the resident diversity increases through natural succession and that an increased strength of biotic interactions with the resident communities

can reduce successful establishments of immigrant species. Furthermore, our results suggest that predation by *Chaoborus* is an important structuring factor and that *S. vetulus* is a key competitor in the type of habitat studied.

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