

## Predation as a factor mediating resource competition among rotifer sibling species

Jorge Ciros-Pérez,<sup>1</sup> María José Carmona, Sara Lapesa, and Manuel Serra

Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, A. O. 22085, València 46071, Spain

### Abstract

The relevance of predation as a factor mediating the competitive interaction among ecologically very similar species is investigated by experimentally analyzing the effect of the copepod predator *Diacyclops bicuspidatus odessanus* on three sibling rotifer species belonging to the *Brachionus plicatilis* species complex. These rotifer species are similar in shape but show notable differences in body size. Predator and prey species co-occur in brackish waterbodies close to the Mediterranean coast of Spain. First, we characterized differential vulnerability of rotifers to predation. A consistent tendency of higher predation rates on smaller prey (i.e., smaller species and younger individuals) was observed. Analysis of predation showed that predator contact rate did not differ significantly among prey species, but that attack, capture probabilities, and handling time did. Second, we performed population dynamics experiments with two prey species competing for a single resource at different levels of predation. Predation extended coexistence of the competing rotifers, whereas the inferior competitor was excluded in the absence of the predator. In some pairwise experiments, we found that the greater the predation level, the larger the relative increase in density of the inferior competitor. Our results suggest that predation can affect the dynamics of very similar competing species in natural aquatic communities, promoting coexistence.

A central challenge in ecology is to understand which factors make possible the coexistence of similar species, which affects community organization and biodiversity. Compared to other ecological systems (e.g., terrestrial or benthic), pelagic systems are relatively spatially homogeneous and nutritionally diluted environments. Both characteristics promote strong competition between zooplankton species (Lampert 1997). Differential resource requirements of similar competitors (i.e., the resource ratio hypothesis; Tilman 1982) has been crucial in understanding the effect of resource limitation in structuring natural communities (DeMott and Kerfoot 1982; Rothhaupt 1990). On the other hand, it has long been thought that predation causes an increase of species richness (e.g., Darwin 1859; Paine 1966). For instance, a positive effect on richness is expected if the predator selects the superior competitor, so that exclusion of inferior competitors is prevented. However, experimental evidence of predator-mediated coexistence is scarce for mobile aquatic prey (Spiller and Schoener 1998).

Sibling or cryptic species are complexes of closely phylogenetically related and morphologically similar species. Because of their similarity, they represent critical cases in

the appreciation of which factors might promote coexistence (e.g., Hedrick and King 1996; Ciros-Pérez et al. 2001a). Sibling species are thought to be common among aquatic invertebrates (Knowlton 1993) and have been reported in amphipods (Witt and Hebert 2000), miscidaceans (Vainöla et al. 1994), anostracans (Pérez et al. 1994), and rotifers (Gómez et al. 2002). These findings have caused revision of the previous idea of wide ecological tolerance and geographic distribution of some aquatic invertebrates. On the other hand, studies on sibling species are showing that they frequently have a sympatric distribution (e.g., Knowlton 1993; Witt and Hebert 2000; Ortells 2002). The commonness of sibling species presents a new challenge: how to explain the coexistence of potentially strong competitors.

Among rotifers, *Brachionus plicatilis* (Müller 1786) is probably one of the best studied monogonont taxa (Rotifera, Monogononta). It has been widely used as a model for physiological, ecological, and ecotoxicological studies, as well as being employed worldwide in the marine aquaculture (reviewed by Ciros-Pérez et al. 2001b). This taxon has recently been recognized as a complex of several sibling species (Gómez et al. 1995, 2002; Ortells et al. 2000). Three of these sibling species, differing in body size (see *Methods*), have already been formally described (i.e., *B. plicatilis* Müller 1786, *B. rotundiformis* Tschugunoff 1921, and *B. ibericus* Ciros-Pérez et al. 2001; see Ciros-Pérez et al. 2001b). These species are common in the zooplankton of brackish ponds and pools in eastern Spain, where they are often sympatric. The analysis of 22 waterbodies from this region (Ortells 2002; Ortells et al. 2000) revealed the co-occurrence of more than two species belonging to this complex from at least 13 sites. Their populations are temporary and have different, but overlapping, seasonal distributions (~4 months for at least two of the species; see Gómez et al. 1997; Ortells 2002).

Laboratory experiments have shown competitive exclusion between these species (Ciros-Pérez et al. 2001a). When occurring in sympatry, their seasonal distribution seems to

<sup>1</sup> To whom correspondence should be addressed. Present address: Tropical Limnology Project, PILT-UIHCSE; FES Iztacala, Universidad Nacional Autónoma de México, A.P. 314, C.P. 54090, Tlalnepantla, Edo. Mex., Mexico (ciros@servidor.unam.mx).

### Acknowledgments

We thank E. Aparici, J. Armengol, J. M. Conde-Porcuna, M. Macek, and T. W. Snell for their valuable comments on early drafts of the manuscript. J.C.-P. thanks J. Alcocer for his support and encouragement during the manuscript's preparation at the National Autonomous University of Mexico. We also acknowledge two anonymous reviewers for their detailed and constructive comments. This research was founded by the Ministry of Science and Education (Spain) grant PB96-0771 to M. Serra, by Conselleria de Cultura, Educació i Ciència (Generalitat Valenciana) fellowship FPI99-03-118 to S. Lapesa, and by Mexico's National Council of Science and Technology (CONACYT) grants 010665 and I39224V to J.C.-P.

be narrower than allowed by abiotic factors (Gómez et al. 1997; Serra et al. 1998). These observations suggest that competition in nature can occur, as expected by their similar feeding apparatus structures (Ciros-Pérez et al. 2001b). However, considering the short generation time of rotifers, the long seasonal overlap observed is unlikely a result of competitive exclusion as competitive dominance switches from one species to another.

Recently, Ciros-Pérez et al. (2001a) examined the relevance of competition to explain coexistence and exclusion in these species by applying Tilman's (1982) mechanistic theory of competition (*see also* Rothhaupt 1988). These authors concluded that food partitioning and the effect of disturbances, such as daily fluctuation of food availability, might explain coexistence of these taxa. Moreover, when the available resources are not diverse, competitive exclusion remains the rule.

In aquatic systems, invertebrate predators have been recognized as a major factor in structuring zooplankton communities (e.g., Lampert and Sommer 1997). Cyclopoid copepods are selective, omnivorous predators that can have a substantial effect on their zooplankton prey (reviewed in Brandl 1998). Cyclopoid copepods detect prey by mechano- and chemoreception, and most of them manipulate captured prey before ingestion. Therefore, prey selection relies on detection capability and capture efficiency after detection, both of which are greatly affected by relative prey-predator body sizes. It has been argued that prey body size is an important component of prey selection when only morphologically similar prey species are available (e.g., Kerfoot et al. 1980; Stemberger 1985).

In this study, we address the relevance of predation as a factor mediating competitive interaction among rotifer sibling species. We have conducted laboratory experiments with the three sibling rotifer species belonging to the well-characterized *B. plicatilis* complex (Ciros-Pérez et al. 2002). As a predator, we used the cyclopoid copepod *Diacyclops bicuspidatus odessanus* (Schmankevitch 1875), one of the most abundant microcrustaceans inhabiting the ponds where the studied rotifers occur (Rodrigo et al. 2001; unpubl. data). In the first part of our study, we describe differential predation of *D. bicuspidatus odessanus* on the three rotifer species. In a series of experiments, we analyze (1) the differential predation on the three prey species by adult copepods of both sexes; (2) the predation electivity on pairwise prey species, offered at different relative densities; and (3) the predation steps from contact to ingestion. The results of these experiments, in addition to the earlier information on resource use by rotifers (Ciros-Pérez et al. 2001a), were used to design the experiments in the second part of our study, in which we analyze the effect of predation on the resource competition dynamics of the three sympatric rotifer species.

## Methods

*Experimental conditions and organisms*—All experiments were performed at  $19 \pm 1^\circ\text{C}$ ,  $11 \text{ g L}^{-1}$  salinity, in a temperature-controlled room. These salinity and temperature values were chosen because co-occurrence of the three ro-

tifer species had been observed in these conditions in the field (Gómez et al. 1995). Artificial seawater was made with commercial sea salt (Instant Ocean®, Aquarium Systems) dissolved in autoclaved, deionized water.

As food for rotifers, we used the microalgae *Tetraselmis suecica* (Prasinophyceae) and *Nannochloris atomus* (Eustigmatophyceae), which originally came from the Instituto de Ciencias Marinas de Andalucía, Cádiz (strain TSH and strain OCU, respectively). Microalgae were cultured at the same temperature and salinity, in constant light (photosynthetically active radiation,  $\sim 35 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ), with *f/2* modified medium (Guillard and Ryther 1962) in a semicontinuous culture system and a dilution rate (*D*) of  $0.65 \text{ d}^{-1}$  (*T. suecica*) and  $0.70 \text{ d}^{-1}$  (*N. atomus*). Light extinction (750 nm) in algal cultures was measured to estimate carbon content ( $\text{mg C L}^{-1}$ ) with a previously established calibration curve. Carbon content was determined with an elemental analyzer (Perkin-Elmer 2400CHN).

As competing prey, the following rotifer strains were used (*see* Gómez et al. 1997): *B. plicatilis*, clone L1; *B. ibericus*, clone SM2; and *B. rotundiformis*, clone SS2 (adult lorica length:  $237.5 \pm 5.8$ ,  $162.3 \pm 3.5$ , and  $117.4 \pm 2.0 \mu\text{m}$ , respectively). Detailed descriptions of the experimental clones have been reported elsewhere (e.g., Gómez et al. 1995; Ciros-Pérez et al. 2001b). *Brachionus* spp. are cyclical parthenogens, whose populations are dominated by asexual females; males are nonfeeding, dwarf organisms. Hereafter, rotifer counts and densities refer to females. Each clone was founded by parthenogenetic reproduction from a single female collected in Cabanes-Torreblanca Marsh, Spain (Gómez et al. 1995). The clones have been maintained in our laboratory for several years. Stock cultures were maintained in 250-ml glass flasks and fed on *T. suecica* (about  $3.0 \text{ mg C L}^{-1}$ ) every 3–4 d, and the medium was renewed weekly.

As predator, we used adult copepods of *D. bicuspidatus odessanus* (adult age: 10–14 d). In the experimental conditions, the body length (excluding the caudal setae) was  $780 \pm 10 \mu\text{m}$  ( $n = 15$ ) for females and  $635 \pm 5 \mu\text{m}$  ( $n = 15$ ) for males. *D. bicuspidatus odessanus* was cultured in the laboratory, starting with a single ovigerous female originally isolated from plankton samples collected from Cabanes-Torreblanca Marsh. Copepod cultures were maintained in 250-ml glass flasks, and fed on a mixture of microalgae (*T. suecica*;  $\sim 15 \text{ mg C L}^{-1}$  every 3–4 d) and rotifers. For practical reasons (overgrowth of *B. plicatilis* in the copepod cultures), we used a mixture of only *B. ibericus* and *B. rotundiformis* in a 1:1 ratio (about 5–10 rotifers  $\text{ml}^{-1}$ ). Culture medium was renewed weekly. Copepod cultures were kept in the laboratory for at least 1 yr before being used in the experiments.

*Differential predation experiments*—In the first experiment, we determined the predation rate of both female and male predators fed on ovigerous females of the three rotifer species. The experiments followed a three-way factorial design, resulting from the combination of three factors: (1) predator sex, (2) prey species, and (3) prey density (1, 2, 5, 10, 15, 20, 30, and 40 rotifers  $\text{ml}^{-1}$ ). For each experimental combination (i.e., two predator sexes  $\times$  three rotifer species  $\times$  eight prey densities), 4 replicates plus two controls (without predators) were performed. Copepods were isolated from

stock cultures, individually placed into wells (Nunc<sup>®</sup> polystyrene 12-well plates) containing 1 ml of artificial seawater, and maintained in culture conditions without food supply. After 18–20 h of starvation, copepods were transferred individually to new wells with 1 ml fresh medium, containing the desired type and prey density. After 4 h of predation, the copepods were removed, and the number of remaining prey was determined by counting live rotifers, plus rotifer carcasses (remains of more than ~50% of the body). The number of predated rotifers was computed as the difference between initial and final counts (neonate rotifers discounted) in a well. In a few cases, the number of neonates was statistically different between controls and treatments (one-way ANOVA,  $P > 0.1$ ), or mortality in control cultures was  $>10\%$  (i.e., an order of magnitude below nominal density). Then the experimental combination was repeated. All statistical analyses were performed with the SPSS program, release 10.0.6 (SPSS).

In the second experiment, we tested differential predation on neonate rotifers by female and male *D. bicuspidatus odessanus*. General experimental procedure was similar to that described above, but a single prey density (40 individuals  $\text{ml}^{-1}$ ) was tested. For each experimental combination (i.e., two predator sexes  $\times$  three rotifer species), eight replicates plus two controls (without predators) were performed. Mortality in control cultures was never  $>10\%$ ; neither was the number of neonates statistically different between control and treatments (one-way ANOVA,  $P > 0.1$ ). Consequently, no experimental combination was repeated.

In the third experiment, differential predation in cultures with two prey species was estimated (i.e., three species pairwise combinations: *B. rotundiformis*/*B. ibericus*, *B. rotundiformis*/*B. plicatilis*, and *B. ibericus*/*B. plicatilis*, henceforth abbreviated as BR/BI, BR/BP, and BI/BP, respectively). Total prey density was 40 rotifers  $\text{ml}^{-1}$ , resulting from the sum of the two rotifer species at five different relative densities (expressed as percentages: 100/0, 75/25, 50/50, 25/75, and 0/100). Adult copepod females were used as predators. For each experimental combination (i.e., three pairwise prey species  $\times$  five relative densities of prey), eight replicates plus two controls (without predators) were performed. General procedure was that described previously (i.e., 18–20-h starvation period of predators, 1 ml experimental volume, and 4 h predation time). Mortality in control cultures was not  $>10\%$ , nor was the number of neonates statistically different between control and treatments (one-way ANOVA,  $P > 0.1$ ). Consequently, no experimental combination was repeated. From the number of prey consumed in pairwise prey cultures (i.e., 75/25, 50/50, 25/75), prey selection was measured using the electivity index  $\varepsilon_i$  for the  $i$ th prey (Chesson 1983). The index is

$$\varepsilon_i = 2 \left\{ \frac{\ln[(n_{i0} - r_i)/n_{i0}]}{\sum_{j=1}^m \ln[(n_{j0} - r_j)/n_{j0}]} \right\} - 1$$

where  $n_{i0}$  is the number of prey of type  $i$  present at the beginning of the experiment,  $r_i$  is the number of individuals of prey type  $i$  in the predator's diet, and  $m$  is the number of prey types (in this case,  $m = 2$ ). The electivity index  $\varepsilon_i$

varies from  $-1$  for negative preference to  $1$  for positive preference ( $\varepsilon_i = 0$  indicates unselective predation). An analysis of variance was used to test the effect of the relative prey density on the electivity values ( $\varepsilon_i$ ) of *D. bicuspidatus odessanus*. We tested two null hypotheses: (1) there was no selective predation ( $\varepsilon_{\text{average}} = 0$ ) and (2) selectivity was not affected by the relative prey density ( $\varepsilon_{75:25} = \varepsilon_{50:50} = \varepsilon_{25:75}$ ).

In the fourth experiment, predation cycle steps were studied for all rotifer species by direct observations of predator–prey behavior as described elsewhere (e.g., Roche 1987; Williamson 1987). All experiments were conducted using starved (see above) adult *D. bicuspidatus odessanus* females as predators and ovigerous female rotifers as prey. Predators were individually placed into round-bottomed glass wells containing 0.5 ml of artificial seawater, with 20 rotifers of a given species randomly taken from stock cultures. The number of different predators used for each species was 23 for *B. rotundiformis*, 26 for *B. ibericus*, and 18 for *B. plicatilis*. These numbers of predator were used to obtain at least six ingestions for each rotifer species. In order to maintain a constant temperature ( $19 \pm 1^\circ\text{C}$ ) throughout the course of each experiment, wells were introduced into a water bath, and observations were made in a walk-in environmental chamber.

All behavioral observations were made with a binocular stereomicroscope (Nikon SMZ1000;  $\times 12$  magnification). Four steps of predation cycle (contact, attack, capture, and ingestion) and the handling times (using a 1/100-s stopwatch; Latitude) were scored. Contact was defined as a prey physical contact with any part of the predator's body (excluding the caudal rami). Contacts were classified as attacking contacts and nonattacking contacts. Attacking contacts were those where a predator lunges directly toward the prey prior to contact or when grasping movements of the mouth appendages following the contact were noted. Attacking contacts resulted eventually in capture. Capture was defined as a prey handling by the mouthparts of the predator  $>3$  s from contact. If  $<3$  s, it was not considered a capture. If the time between a transitory prey loss and its recovery was less than 10 s, it was considered a single capture. If this time was longer than 10 s, it was recorded as a new event. For those contacts resulting in captures, handling time was the time span in which predator and prey (or the rest of a prey) kept physical contact with interruptions of  $<10$  s. Ingestion was recorded when the predator ingested  $>\sim 50\%$  of the prey. Behavioral observations on a given predator lasted until the first prey handling was completed. Observation ceased in those cases where no captures were recorded after 15 min.

The probability of each step in the predation cycle (e.g., probability of attack after contact, etc.), the mean contact rate (mean number of prey contacted per predator per searching time), and the mean handling time were estimated. Statistical significance of the predation cycle steps was tested using contingency chi-square tests. Mean contact rates and handling times were analyzed using one-way analyses of variance, plus comparisons planned a priori (Sokal and Rohlf 2001) to test the differences (1) between BP (the largest prey species) and the other two prey species and (2) between BI and BR (the two smaller prey species).

*Competition plus predation experiments*—Experimental design for the three pairwise combinations of competing prey species (i.e., BR/BI, BR/BP, and BI/BP) was based on results obtained from (1) resource competition experiments between pairs of the three rotifer species reported by Ciro-Pérez et al. (2001a) and (2) differential predation of the copepod *D. bicuspidatus odessanus* on the three rotifer species (see Results section). Ciro-Pérez et al. (2001a) found that the threshold food levels required by the three rotifer species to maintain zero population growth (Tilman 1982) were ranked as follows: *B. ibericus* < *B. rotundiformis* < *B. plicatilis* (fed on the algae *T. suecica*) and *B. rotundiformis* = *B. plicatilis* < *B. ibericus* (fed on the alga *N. atomus*). The experimental conditions used here correspond to conditions where (1) the competitive exclusion of one rotifer species is predicted as a result of its higher food threshold requirements (see details in Ciro-Pérez et al. 2001a) and (2) the species predicted as excluded is the one less vulnerable to predation.

Experimental cultures were semicontinuous (Rothhaupt 1988) so that a constant fraction of each culture (rotifers included) was removed. This fraction was replaced with a fresh food suspension of algae. The fraction of the culture replenished per unit time ( $F$ ) defines the dilution rate ( $D = \ln[1/(1 - F)]$ ; see Boraas 1993). Dilution rate accounts for both a constant resource supply for rotifers and a nonspecific rotifer mortality rate additional to the intrinsic one. One could suppose that this additional mortality simulates ecological death in natural conditions. For all experiments,  $D = 0.4 \text{ d}^{-1}$  was used, supplying a constant food concentration of  $8 \text{ mg C L}^{-1}$  of *T. suecica* for the pairs BR/BP and BI/BP (expected superior competitor BR and BI, respectively; Ciro-Pérez et al. 2001a) and  $8 \text{ mg C L}^{-1}$  of *N. atomus* for the pair BR/BI (expected superior competitor BR). Algae were maintained exponentially in semicontinuous cultures (see above). This method was used to keep the physiological state of algae constant and, thus, its nutritional quality.

Experiments had two phases. The first one corresponded to the first 5 d, and the second lasted from the day 6 until the experiments were finished. During the first phase, experiments followed the same basic procedure as described in the resource competition experiments performed by Ciro-Pérez et al. (2001a). Cultures were started with three females  $\text{ml}^{-1}$  of each rotifer species (randomly chosen from stock cultures that were in an exponential growth phase) in 100-ml glass flasks holding 50 ml food suspension and kept in darkness on an orbital platform-shaker ( $\sim 40 \text{ rpm}$ ). Six replicates cultures were set up for each combination of competing prey species. Darkness was used to prevent algae growth, which could have altered the experimental resource supply. Experimental glass flasks were changed daily at the dilution event. Duration of the first phase was long enough to acclimatize rotifers to the food conditions and experimental procedure (Rothhaupt and Lampert 1992) but short enough to prevent exclusion of the rotifer predicted to be competitively inferior (see Ciro-Pérez et al. 2001a).

From day 6 (the beginning of the second phase), the six culture replicates of each pairwise combination were randomly separated into three groups, which were assigned to three different treatments (i.e., three rotifers-pairs  $\times$  three

treatments  $\times$  two replicates). Treatment 1 consisted of the addition of five adult females of *D. bicuspidatus odessanus* (i.e.,  $0.1 \text{ copepods ml}^{-1}$ ). Treatment 2 consisted of the addition of seven adult females of *D. bicuspidatus odessanus* (i.e.,  $0.14 \text{ copepods ml}^{-1}$ ). In treatment 3 (control), no copepods were added. Copepods were isolated from the stock cultures by selecting noncarrying egg-sac females of healthy appearance. For treatments with predators, cultures were revised under a stereomicroscope before the daily dilution event to check predator status, to renew them, and to detect the production of nauplii larvae resulting from copepod reproduction. For these purposes, fractions of the cultures were gently poured into sterilized petri dishes. After checking, these fractions were carefully placed in new sterilized culture flasks. When nauplii larvae were observed, they were removed with a micropipette. Every other day, all copepods were replaced. On alternate days, when all copepods were not replaced, new ones were added to replace the dead. Following this procedure, we tried to maintain an approximately constant predation rate by preventing the senescence effects in the predators' efficiency, as well as avoiding the possible accumulation of nauplii larvae, which could compete for resources with the rotifer species. Once all copepods were transferred or substituted, we diluted the cultures by eliminating the corresponding fraction of the culture ( $F$ ), including rotifers but not copepods, and replenishing with fresh medium ( $D = 0.4 \text{ d}^{-1}$ ) at the experimental food algae concentration.

For control cultures, the same procedure was followed, including fractionated pouring of the cultures into petri dishes, despite the absence of copepods from this treatment. Daily removed culture fractions were fixed with formaldehyde ( $\sim 2\% \text{ v/v}$  final concentration), and rotifer population densities were estimated. These data constituted a time series accounting for the competition dynamics. The experiment finished when the slope of the regression of population densities of the rarer species, versus time, did not differ from zero significantly for a time span of at least 3 d.

We tested the effects of predator density, time, and their interaction by analysis of variance for repeated measures (ANOVAR; von Ende 1993) on the arcsine relative rotifer densities. Only the species of each pair that was the inferior competitor in control experiments (without copepods) were analyzed statistically. Statistical analysis only included the period from experimental day 6 (from introduction of predators) until the last day in which data of the two replicates of each treatment were available. The probability values ( $P$ ) of the main effects and their interactions were obtained by the Greenhouse-Geisser adjustment (von Ende 1993). If an ANOVAR test indicated significant differences, a post hoc Student-Newman-Keuls test (Sokal and Rohlf 2001) was carried out.

## Results

*Differential predation experiments*—*D. bicuspidatus odessanus* differentially preyed on the three sibling species of rotifers of the *B. plicatilis* complex. In single-species prey cultures, copepod predation was dependent on prey species,

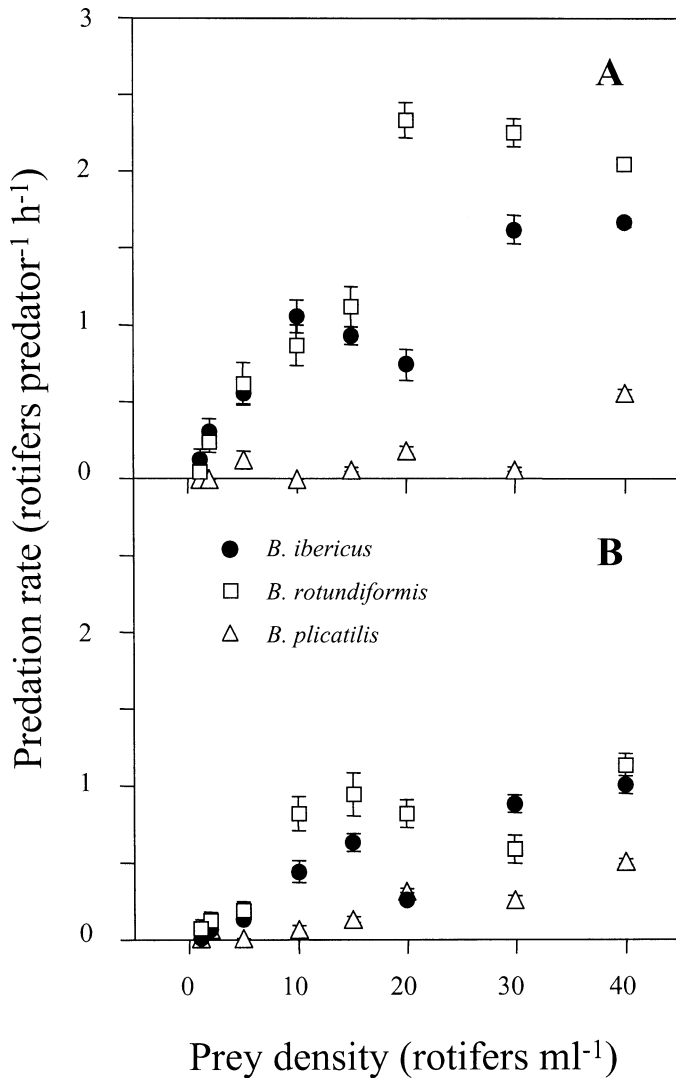


Fig. 1. Rotifer predation rate by adult *D. bicuspidatus odessanus* (A) females and (B) males in relation to the density of the three prey species (ovigerous females; single-species prey cultures) belonging to the *B. plicatilis* complex. Vertical bars are the standard error of the mean assuming a binomial distribution of the captured prey. Values are means of the different replicates.

prey density, and predator sex (Figs. 1, 2; Table 1). These results can be summarized as follows. (1) The general pattern of rotifer predation vulnerability was *B. rotundiformis* > *B. ibericus* > *B. plicatilis*. (2) Predation rates on the three rotifer species by copepod females were generally higher than by copepod males. (3) Predation rates of both predator sexes decreased as species prey size increased. The tendency of higher predation rates on smaller prey is well observed in the experiments, comparing the percentage of predation on neonate and ovigerous rotifers (Fig. 2; Table 1), although this effect was marginally significant.

Selection on pairwise experiments is shown in Fig. 3. According with the above reported results, there was a positive selection for the smallest size *B. rotundiformis*, whatever other prey species was offered (i.e., BR/BI, BR/BP). This happened even when *B. rotundiformis* was offered in rela-

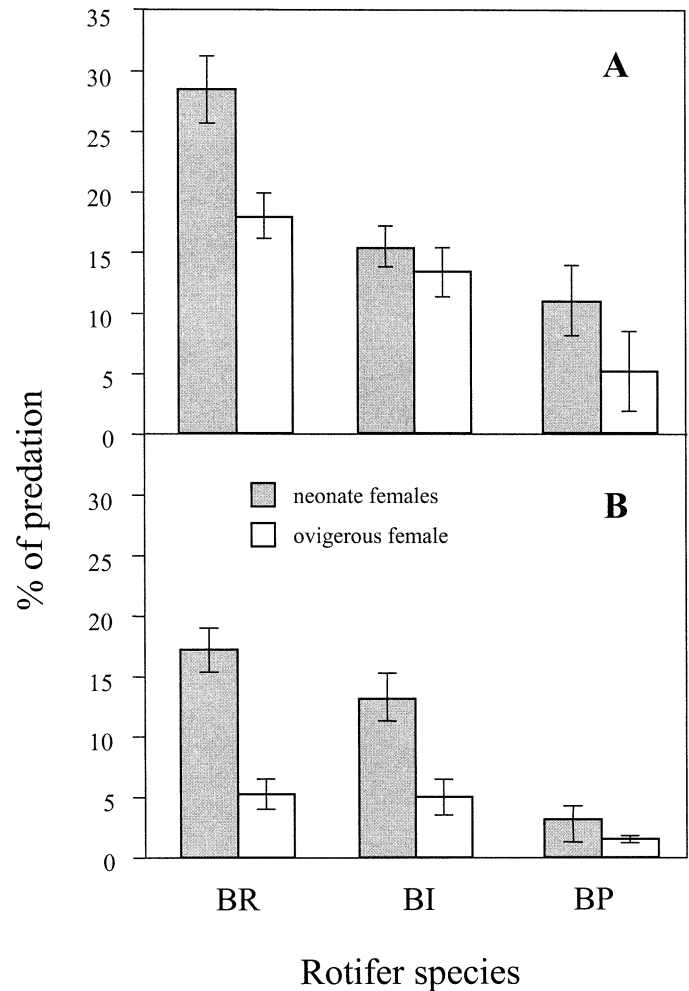


Fig. 2. Predation percentage (number of predated rotifers/total number of rotifers  $\times$  100) by *D. bicuspidatus odessanus* (A) females and (B) males on neonates and ovigerous females of the three *Brachionus* rotifer species. Values are means of the different replicates. Vertical bars are the standard error of the mean assuming a binomial distribution. Initial prey density was 40 rotifers  $\text{ml}^{-1}$ . BR, *B. rotundiformis*; BI, *B. ibericus*; BP, *B. plicatilis*. Results of the three differential predation experiments corresponding to ovigerous females (40 rotifers  $\text{ml}^{-1}$ , single-prey species) were grouped for this figure.

tively low densities (Fig. 3; Table 2). The higher the prey size differences between rotifers, the higher the prey selection for *B. rotundiformis*. However, *D. bicuspidatus odessanus* showed low selection for the mid-sized *B. ibericus* in mixed cultures when the largest, *B. plicatilis*, was also available (Fig. 3).

Results of the different steps of the predation cycle of *D. bicuspidatus odessanus* on the three rotifer species are summarized in Table 3. There were no differences among species in the mean contact rate. However, significant differences by prey species were observed in the postcontact interactions. *B. ibericus* was the rotifer species with the lowest probability of being attacked after contact. *B. plicatilis* and *B. rotundiformis* had similar attack probability after contact, about two times higher than that for *B. ibericus*, but once they were

Table 1. ANOVA (fixed effects) on the predation percentages (number of predated rotifers/total number of rotifers  $\times$  100) of the copepod *D. bicuspidatus odessanus*. Initial prey density was 40 rotifers ml<sup>-1</sup>. Results of the three differential predation experiments corresponding to ovigerous females (40 rotifers ml<sup>-1</sup>, single-prey species) were joined for this analysis.

Source	SS	df	F	P
Predator sex (PS)	0.101	1	6.892	0.010
Prey species (P)	0.798	2	27.162	<0.001
Prey age (ovigerous vs. neonate; A)	0.045	1	3.076	0.082
PS $\times$ P	0.016	2	0.555	0.576
PS $\times$ A	0.036	1	2.472	0.119
P $\times$ A	0.052	2	1.198	0.306
PS $\times$ P $\times$ A	0.006	2	0.219	0.804
Error	1.587	108		

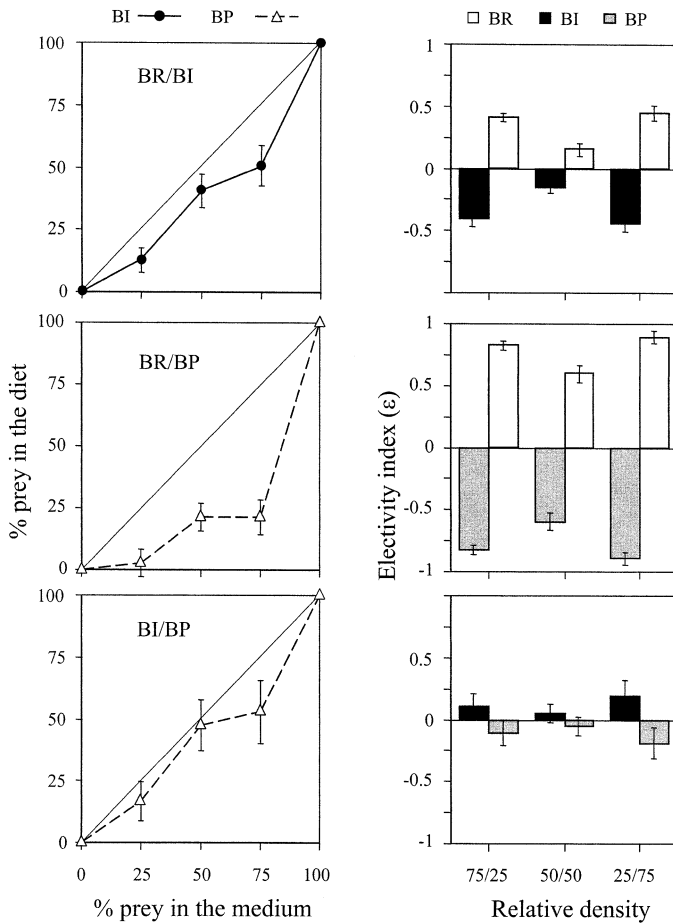


Fig. 3. Prey selection by *D. bicuspidatus odessanus* females in mixed cultures of *Brachionus* pair species measured at different relative prey densities (total prey density: 40 rotifers ml<sup>-1</sup>). Left-hand panels show the relation between (1) percentages (mean  $\pm$  SE) in the predator diet of the less vulnerable prey of each rotifer pair and (2) percentage of prey in the medium. Prey in the diet were estimated as the difference between the initial and the final prey counts in the medium. Tendency lines represent the percentage of each species in the diet if the predator would have no prey preferences. Right-hand panels show the Chesson's electivity index ( $\epsilon$ ; mean  $\pm$  SE) for each species pair. BR, *B. rotundiformis*; BI, *B. ibericus*; BP, *B. plicatilis*.

attacked, their capture probabilities were different. If attacked, *B. rotundiformis* was captured significantly more frequently ( $\sim$ 5 times) than *B. ibericus* and *B. plicatilis*. Ingestion probability after capture was similar for the three prey species. By contrast, prey handling time was significantly different among the three rotifer species, being longer for larger prey.

**Competition plus predation experiments**—Figure 4 shows that, during the first phase of the experiment (i.e., in the absence of predators), population dynamics of the two competing prey species were characterized by a high population increase because food resources were abundant. The species having the lowest food threshold for the maintenance of population growth (see Methods) was dominant from the first days, having higher population density than its respective competitor at the end of this phase. When each rotifer species was analyzed separately, there were not significant differences in population densities among all the cultures at the end of the first phase of the experiment (day 6;  $P > 0.2$  for the six one-way ANOVAs). At the time predators were introduced, the two rotifer species in a culture had relatively high population densities (Fig. 4).

Competitive exclusion in the control experiments (i.e., without predator), as well as a competitor being superior, was correctly predicted with a priori data and the exploitative competition theory (see Methods). In the control cultures, superior competitors reached approximately constant density values, whereas the displaced species, after reaching the maximum population numbers, systematically decreased their population densities until they were completely excluded.

Very different results were found when predators were present. Predation caused the superior competitor to have lower density compared with controls, and the opposite was observed in the species excluded from controls (Fig. 4). Coexistence for a relatively long period ( $\sim$ 18 d after predator addition) was observed in the two treatments with predators of the rotifer pair BR/BI (Fig. 4B,C). For the pairs BR/BP (Fig. 4E,F), and BI/BP (Fig. 4H,I) coexistence was longer when predators were present. Nevertheless, in these two latter species pairs, both prey densities decreased in the experimental cultures, except in one culture (BI/BP pair, predator density 0.14 copepods ml<sup>-1</sup>; Fig. 4I).

Table 2. ANOVAs on the prey electivity indices ( $\epsilon_i$ ) of the copepod *D. bicuspidatus odessanus*. For each pair of prey species offered at three different relative densities (in percentage, i.e., 75:25, 50:50, 25:75), two null hypotheses were tested: (1) there is no positive selection for a prey ( $\epsilon_{\text{average}} = 0$ ) and (2) the selection is not dependent on the prey relative density ( $\epsilon_{75:25} = \epsilon_{50:50} = \epsilon_{25:75}$ ). BR, *B. rotundiformis*; BI, *B. ibericus*; BP, *B. plicatilis*.

Species pair	Source	SS	df	F	P
BR/BI	$\epsilon$ average = 0	0.742	1	12.574	0.002
	Prey relative density	0.069	2	0.581	0.568
	Error	1.240	21		
BR/BP	$\epsilon$ average = 0	3.562	1	121.753	<0.001
	Prey relative density	0.096	2	1.646	0.217
	Error	0.614	21		
BI/BP	$\epsilon$ average = 0	0.083	1	0.767	0.391
	Prey relative density	0.020	2	0.091	0.913
	Error	2.278	21		

Figure 5 shows the dynamics of relative prey densities for those prey that were displaced in the control cultures (i.e., the inferior competitors). A significant divergence between controls and treatments with predators was found (ANOVAR, interaction between predator density and time, all  $P \leq 0.01$ ; Table 4). When predators were present, the dynamics tended to be more stationary, and the inferior competitors attained nonmarginal relative frequencies (~50% or higher). When *B. rotundiformis* was a competitor (i.e., pairs BR/BI and BR/BP; Fig. 5A,B; Table 4) we found that the greater the predation level, the larger the relative increase in density of the inferior competitor.

## Discussion

**Differential predation**—In contrast to several other rotifer species, species of the *B. plicatilis* complex do not have marked morphological or escape defenses. Thus, the protection against cyclopoid predators probably relies on a com-

bination of strategies, including body size or swimming behavior (e.g., Williamson 1980; Stemberger 1985; Lapesa et al. 2002). These defenses might be quite effective because our results show a rather low predation efficiency (Table 3). However, despite its low efficiency, predators can significantly affect rotifer population dynamics (Figs. 4, 5).

We found that a raptorial copepod is able to differentially prey on sibling rotifer species belonging to the *B. plicatilis* complex. The general pattern of prey vulnerabilities in single-prey species cultures suggests that prey size is an important factor; the smaller the prey, the higher the vulnerability. This size effect is also supported by higher predation on neonates. Furthermore, copepod males, which are smaller than females, were less efficient predators. This pattern remained qualitatively similar when two rotifer species were offered to copepods.

These results support the hypothesis that relative body size plays a major role in the interactions between cyclopoid copepods and their prey. However, the predation cycle analysis showed that prey vulnerability does not have a simple relationship with size. Prey body size is expected to negatively affect prey capture, but to positively affect both prey detection and handling time. So, for large prey, the predator might require shorter searching times, but longer manipulation times (Gilbert and Williamson 1978; Roche 1987).

Our results confirm that larger handling times are needed for larger prey. However, no evidence for a positive effect of size on detection was found. In fact, image analysis data (Lapesa et al. 2002) showed that *B. rotundiformis* (the smallest species) elicited attacks by copepods from a greater distance than *B. ibericus* and *B. plicatilis*. The higher vulnerability of *B. rotundiformis* could be explained by its higher probability of capture after attack, as is shown in our predation cycle analysis. However, according to this analysis, *B. ibericus* should be less vulnerable than found in our other experiments, those assessing prey mortality. Interestingly, Lapesa et al. (2002) reported attack probabilities on *B. ibericus* to be intermediate between those for *B. plicatilis* and

Table 3. Predation cycle analysis of *D. bicuspidatus odessanus* on the three rotifer species of the *B. plicatilis* complex, where [C→A] = probability of attack after contact; [A→CP] = probability of capture after attack; [CP→I] = probability of ingestion after capture. Mean contact rate is the mean number of contacts per predator per searching time. P, probability for the corresponding statistical test.

Prey species	Mean contact rate $\pm$ SE (rotifers predator <sup>-1</sup> s <sup>-1</sup> )	[C→A]	[A→CP]	[CP→I]	Mean handling time $\pm$ SE (s)
	<i>B. rotundiformis</i>	0.05 $\pm$ 0.04	0.19	0.48	0.59
<i>B. ibericus</i>	0.07 $\pm$ 0.04	0.09	0.07	0.75	85 $\pm$ 16
<i>B. plicatilis</i>	0.05 $\pm$ 0.02	0.21	0.09	0.60	237 $\pm$ 50
Statistical analysis					
P	0.4*	<0.001†	<0.001†	0.7†	<0.001*‡
n	33*	46†	22†	13†	19.5*

\* One-way ANOVA; n is the average number of contacts used to compute the contact rate of each species.

† Contingency chi-square test (species vs. event result; e.g., attack without capture vs. attack with capture); n is the total number of attacks, captures, and ingestions, respectively.

‡ Significant differences  $P < 0.05$  were found between *B. plicatilis* and the other two species and between *B. ibericus* and *B. rotundiformis* according to planned comparisons tests.

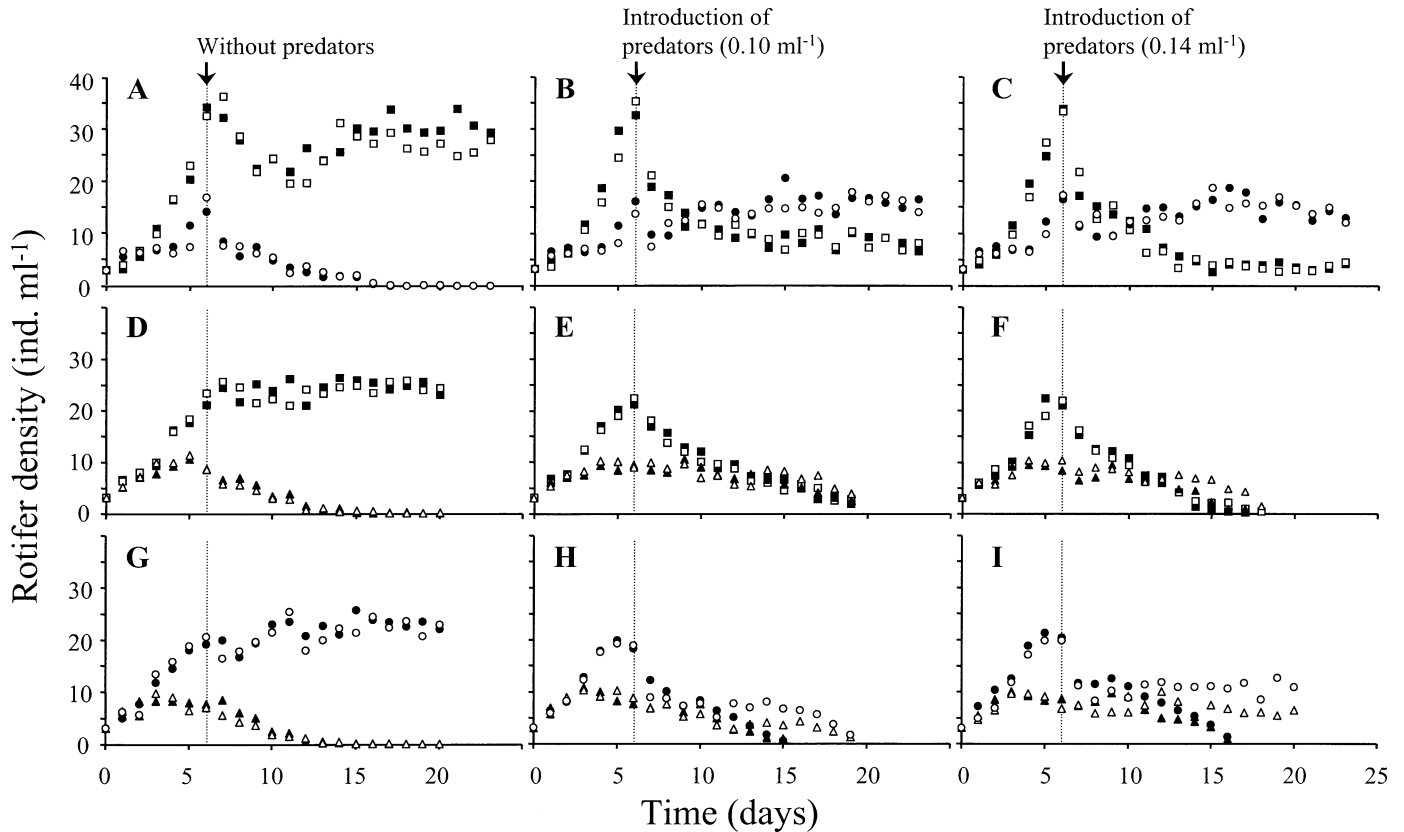


Fig. 4. Time course of competition between rotifer species pairs for single resources ( $D = 0.4 \text{ d}^{-1}$ ) under three levels of predation by the copepod *D. bicuspidatus odessanus* (adult females). BR, *B. rotundiformis* (squares); BI, *B. ibericus* (circles); BP, *B. plicatilis* (triangles). Resource supply was  $8 \text{ mg C L}^{-1}$ ; (A–C) BR/BI was fed with *N. atomus*, whereas the other two rotifer pairs (D–F) BR/BP and (G–I) BI/BP were fed with *T. suecica*. Filled and unfilled symbols correspond to different replicates. Dotted vertical lines indicate the separation between the two experimental phases, and arrows are the predator introduction events.

*B. rotundiformis*, whereas captures of *B. ibericus* and *B. rotundiformis* were similar and higher than for *B. plicatilis*.

**Competition plus predation dynamics**—Competitive exclusion of one rotifer in each pairwise experiment happened in control cultures as predicted by exploitative competition theory (see Ciroso-Pérez et al. 2001a). These findings confirmed the relevance of the threshold food concept (Stemberger and Gilbert 1985) to predict the superior competitor (e.g., Rothhaupt 1988; Kreuzer and Lampert 1999; Ciroso-Pérez et al. 2001a) and provided additional support for the conclusions of Ciroso-Pérez et al. (2001a) that strong competition and exclusion occur if resources are not diverse. Hence, competition is likely an important factor affecting the co-occurrence of these sibling species.

The effect of predation on consumers competing for the same algal resource changes the rotifer population dynamics significantly. The general effect of predation was to increase the length of rotifer co-occurrence. This can be interpreted as the result of compensating for the competition superiority with a higher predation vulnerability. Predators mediated coexistence between *B. rotundiformis* and *B. ibericus* where no evidence for rotifer species exclusion was found during the relatively long experimental period. For the other two pairs of rotifer species, predation caused a lengthening of the co-

existence periods between competitors, with a tendency of the two rotifer species being excluded by the predator. Despite being selective, *D. bicuspidatus odessanus* showed some degree of polyphagy. The tendency to exclude both prey suggests that predation requires an intermediate level to mediate coexistence (e.g., Paine 1966; Lubchenco 1978; Menge 1995).

A lengthening of the coexistence period might be important for the long-term persistence of these rotifer species in natural systems. On one hand, rotifer populations are usually seasonal, and their long-term persistence in a habitat depends on the production of diapausing eggs, enabling the population to be refounded when the habitat becomes suitable. Because of the severe time constraints acting on diapausing egg production (Snell et al. 1999), a longer coexistence period might be critical for producing the number of diapausing eggs needed for long-term population survival. On the other hand, rotifers inhabit a fluctuating environment, where population persistence could depend on the rate of environmental change, as related to the rate of competitive exclusion. Therefore, any factor delaying exclusion is a potential factor for the long-term persistence of competing species.

In our experimental design, we selected conditions in which a positive correlation between competitive ability and predation vulnerability of the rotifer species existed. Thus,

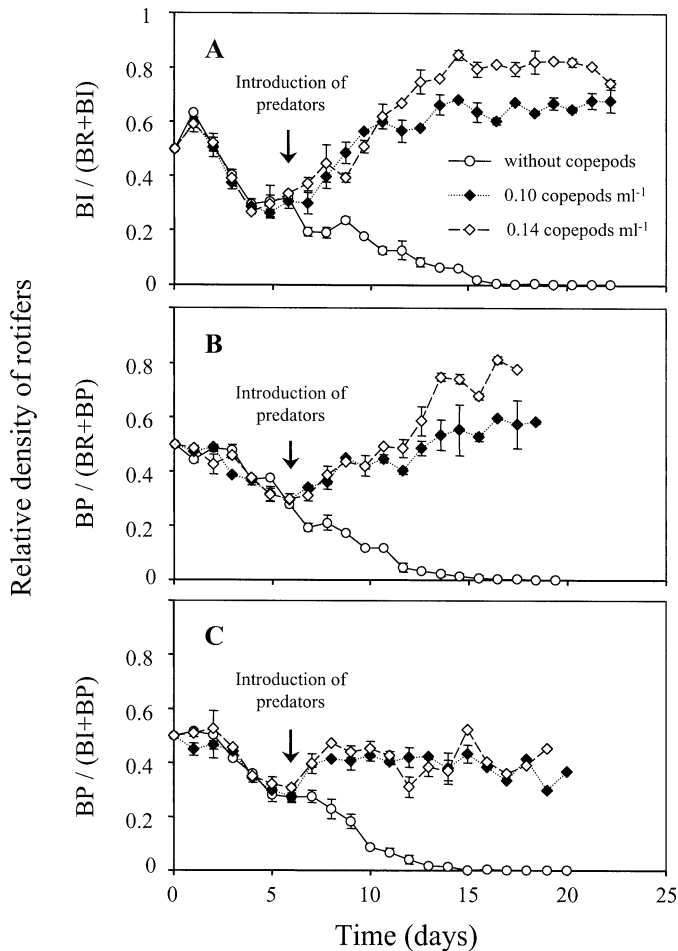


Fig. 5. Time course of rotifer relative densities in the competition experiments between pairs of competitors under three predation levels. Values are means of two replicates and standard errors. Only the densities of those species that in the controls (without copepods) were displaced (see Fig. 4) are shown. Introduction of predators (beginning of second experimental phase) is indicated. BR, *B. rotundiformis*; BI, *B. ibericus*; BP, *B. plicatilis*.

compensation could be expected, and a process promoting coexistence could be shown. Consequently, the outcome of the predator-mediated interactions could be very different if other conditions were chosen. For example, for *B. rotundiformis* and *B. ibericus* competing for the alga *T. suecica*,

predation by *D. bicuspidatus odessanus* is expected to be a factor of exclusion, rather than a factor of coexistence, because the inferior competitor is also the most vulnerable prey. However, to some extent, the correlation present in our experiments might also exist in nature. *B. plicatilis* is both the least vulnerable prey and the inferior competitor in most of the conditions explored by Ciros-Pérez et al. (2001a). This suggests the evolutionary hypothesis that large body size is a trade-off between predation protection and competitive efficiency. Zooplankton defenses against predation are costly, requiring allocation of limited resource, which ultimately reduces some other fitness component (Jacobs 1978). Thus, the relatively large size of *B. plicatilis*, the most evident trait of this species to avoid copepod predation, could be associated with a reduction of its competitive ability.

The mechanism whereby predation promotes competitor coexistence has long been analyzed both theoretically (e.g., Holt et al. 1994; Abrams 1999) and experimentally (e.g., Paine 1966; Lawler and Morin 1993). When theoretical work takes into consideration realistic, complicating assumptions on the predatory interaction, it can be shown that predation can either intensify or reduce competition (Grover and Holt 1998; see also Chase et al. 2002). This makes experimental tests more crucial. There are few experimental studies about the effect of predation on zooplankton species with little differences in resource use and in which the effect of both resource competition and predation can be clearly separated. For example, Denno et al. (1995), in a review of competition in insects, concluded that most experimental works evaluating the predator-mediated competition offer conflicting evidence (see also Spiller and Schoener 2001). Our experiments offer nonambiguous, experimental evidence about how predators promote the coexistence of very similar species. The experimental design for this finding was possible after the experimental identification of a positive correlation between competitive capabilities and vulnerabilities to copepod predation. Our experimental system does not account for the copepod demographic response, and then our results do not exclude more complex, long-term effects.

The coexistence of ecologically similar species in a comparatively spatially homogeneous habitat poses a challenge to the ecological theory of competition and community structure. The finding that sympatric sibling species are more common than previously thought stresses that challenge.

Table 4. Mean relative density ( $\pm$ SE) of rotifer species in the competition experiments, with three different predator levels. Only the densities of those species displaced in controls are shown (see Fig. 4). Results of the analysis of variance for repeated measures (ANOVAR) are also shown. The probabilities of the main effects and their interactions are indicated. Analyses were performed with the data corresponding to the time from the predators' introduction until the last experimental day. BR, *B. rotundiformis*; BI, *B. ibericus*; BP, *B. plicatilis*.

Relative densities	Predator density (copepods ml <sup>-1</sup> )			Period (d)	Probability		
	0.0	0.10	0.14		Predator density	Time	Interaction
BI/(BR+BI)	0.088 $\pm$ 0.016	0.575 $\pm$ 0.021	0.674 $\pm$ 0.030	18	<0.001	0.005	<0.001
BP/(BR+BP)	0.102 $\pm$ 0.015	0.451 $\pm$ 0.017	0.533 $\pm$ 0.032	12	<0.001	0.025	<0.001
BP/(BI+BP)	0.118 $\pm$ 0.024	0.398 $\pm$ 0.012*	0.409 $\pm$ 0.021*	9	0.002	0.031	0.01

\* Means were statistically insignificant after a post hoc Student–Newman–Keuls test ( $P \geq 0.05$ ).

Several factors and processes could explain coexistence of similar zooplankton species, as the so-called storage effect (the combined effect of among-year random variation and resting stage banks; Cáceres 1997), the differential use of resources (Rothhaupt 1988; Ciroso-Pérez et al. 2001a), and the effect of disturbances, as variation in resource availability (Rothhaupt 1990; Nisbet et al. 1997). We have shown that, despite competing for resources of low diversity, sibling species coexistence can be promoted by a selective predator.

### References

- ABRAMS, P. A. 1999. Is predator-mediated coexistence possible in unstable systems? *Ecology* **80**: 608–621.
- BORAAS, M. E. 1993. Semicontinuous culture methods, p. 13–20. *In* N. Walz [ed.], *Plankton regulation dynamics*. Ecological Studies 98. Springer-Verlag.
- BRANDL, Z. 1998. Feeding strategies of planktonic cyclopoids in lacustrine ecosystems. *J. Mar. Syst.* **15**: 87–95.
- CÁCERES, C. E. 1997. Temporal variation, dormancy and coexistence: A field test of storage effect. *Proc. Natl. Acad. Sci. USA* **94**: 9171–9175.
- CHASE, J. M., AND OTHERS. 2002. The interaction between predation and competition: A review and synthesis. *Ecol. Lett.* **5**: 302–315.
- CHESSON, P. L. 1983. The estimation and analysis of preferences and its relationship to foraging models. *Ecology* **64**: 1297–1304.
- CIROSO-PÉREZ, J., M. J. CARMONA, AND M. SERRA. 2001a. Resource competition between sympatric sibling rotifer species. *Limnol. Oceanogr.* **46**: 1511–1523.
- , A. GÓMEZ, AND M. SERRA. 2001b. On the taxonomy of three sympatric sibling species of the *Brachionus plicatilis* (Rotifera) complex from Spain, with the description of *B. ibericus* n. sp. *J. Plankton Res.* **23**: 1311–1328.
- , M. J. CARMONA, AND M. SERRA. 2002. Resource competition and patterns of sexual reproduction in sympatric sibling rotifer species. *Oecologia* **131**: 35–42.
- DARWIN, C. R. 1859. On the origin of species by natural selection, or the preservation of favoured races in the struggle for life. John Murray.
- DEMOTT, W. R., AND W. C. KERFOOT. 1982. Competition among cladocerans: Nature of the interactions between *Bosmina* and *Daphnia*. *Ecology* **63**: 1949–1964.
- DENNO, R. F., M. S. McCLURE, AND J. R. OTT. 1995. Interspecific interactions in phytophagous insects: Competition reexamined and resurrected. *Annu. Rev. Entomol.* **40**: 297–331.
- GILBERT, J. J., AND C. E. WILLIAMSON. 1978. Predator–prey interaction behavior and its effect on rotifer survival in associations of *Mesocyclops edax*, *Asplanchna girodi*, *Polyarthra vulgaris* and *Keratella cochlearis*. *Oecologia* **37**: 13–22.
- GÓMEZ, A., M. TEMPRANO, AND M. SERRA. 1995. Ecological genetics of a cyclical parthenogen in temporary habitats. *J. Evol. Biol.* **6**: 601–622.
- , M. J. CARMONA, AND M. SERRA. 1997. Ecological factors affecting gene flow in the *Brachionus plicatilis* complex (Rotifera). *Oecologia* **111**: 350–356.
- , M. SERRA, G. R. CARVALHO, AND D. H. LUNT. 2002. Molecular phylogeny of the rotifer *Brachionus plicatilis* reveals an ancient cryptic species complex. *Evolution* **56**: 1431–1444.
- GROVER, J. P., AND R. D. HOLT. 1998. Disentangling resource and apparent competition: Realistic models for plant–herbivore communities. *J. Theor. Biol.* **191**: 353–376.
- GUILLARD, R. R. L., AND J. H. RYTHER. 1962. Studies on marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve). *Can. J. Microbiol.* **8**: 229–239.
- HEDRICK, P. W., AND E. KING. 1996. Genetics and the environment in interspecific competition: A study using the sibling species *Drosophila melanogaster* and *Drosophila simulans*. *Oecologia* **108**: 72–78.
- HOLT, R. D., J. P. GROVER, AND D. TILMAN. 1994. Simple rules for interspecific dominance in systems with exploitative and apparent competition. *Am. Nat.* **144**: 741–771.
- JACOBS, J. 1978. Coexistence of similar zooplankton species by differential adaptations to reproduction and escape in an environment with fluctuating food and enemy densities. III. Laboratory experiments. *Oecologia* **35**: 35–54.
- KERFOOT, W. C., D. L. KELLONG, JR., AND J. R. STRICKLER. 1980. Visual observations of live zooplankters: Evasion, escape and chemical defenses, p. 10–27. *In* W. C. Kerfoot [ed.], *Evolution and ecology of zooplankton communities*. University Press of New England.
- KNOWLTON, N. 1993. Sibling species in the sea. *Annu. Rev. Ecol. Syst.* **24**: 189–216.
- KREUTZER, C., AND W. LAMPERT. 1999. Exploitative competition in differently sized *Daphnia* species: A mechanistic explanation. *Ecology* **80**: 2348–2357.
- LAMPERT, W. 1997. Zooplankton research: The contribution of limnology to general ecological paradigms. *Aquat. Ecol.* **31**: 19–27.
- , AND U. SOMMER. 1997. *Limnoecology: The ecology of lakes and streams*. Oxford University Press.
- LAPESA, S., T. W. SNELL, D. M. FIELDS, AND M. SERRA. 2002. Predatory interactions between a cyclopoid copepod and three sibling rotifer species. *Freshw. Biol.* **47**: 1685–1695.
- LAWLER, S. P., AND P. J. MORIN. 1993. Food web architecture and populations dynamics in laboratory microcosms of protists. *Am. Nat.* **141**: 675–686.
- LUBCHENCO, J. 1978. Plant species diversity in a marine intertidal community: Importance of herbivore food preference and algal competitive abilities. *Am. Nat.* **112**: 23–39.
- MENGE, B. A. 1995. Indirect effects in marine rocky intertidal interaction webs: Patterns and importance. *Ecol. Monogr.* **65**: 21–74.
- NISBET, R. M., E. MCCAULEY, W. S. C. GURNEY, W. W. MURDOCH, AND A. M. DE ROSS. 1997. Simple representation of biomass dynamics in structured populations, p. 61–79. *In* H. G. Othmer, F. R. Adler, M. A. Lewis, and J. C. Dillon [eds.], *Case studies in mathematical modeling: Ecology, physiology, and cell biology*. Prentice-Hall.
- ORTELLS, R. 2002. Diversidad genética y ecológica en especies crípticas de rotíferos: patrones y procesos. Ph. D. thesis, Univ. of Valencia, Spain.
- , T. W. SNELL, A. GÓMEZ, AND M. SERRA. 2000. Patterns of genetic differentiation in resting egg banks of a rotifer species complex in Spain. *Arch. Hydrobiol.* **149**: 529–551.
- PAINE, R. T. 1966. Food web complexity and species diversity. *Am. Nat.* **100**: 65–75.
- PÉREZ, M. L., J. R. VALVERDE, B. BATUECAS, F. AMAT, R. MARCO, AND R. GARESSE. 1994. Speciation in the *Artemia* genus: Mitochondrial DNA analysis of bisexual and parthenogenetic brine shrimps. *J. Mol. Evol.* **38**: 156–168.
- ROCHE, K. 1987. Post-encounter vulnerability of some rotifer prey types to predation by the copepod *Acanthocyclops robustus*. *Hydrobiologia* **147**: 229–233.
- RODRIGO, M. A., X. ARMENGOL-DÍAZ, R. OLTRA, M. J. DASÍ, AND W. COLOM. 2001. Environmental variables and planktonic communities in two ponds of El Hondo wetland (SE Spain). *Int. Rev. Hydrobiol.* **86**: 297–313.
- ROTHHAUPT, K. O. 1988. Mechanistic resource competition theory

- applied to laboratory experiments with zooplankton. *Nature* **333**: 660–662.
- . 1990. Resource competition of herbivorous zooplankton: A review of approaches and perspectives. *Arch. Hydrobiol.* **118**: 1–29.
- , AND W. LAMPERT. 1992. Growth-rate dependant feeding rates in *Daphnia pulicaria* and *Brachionus rubens*: Adaptation to intermediate time-scale variations in food abundance. *J. Plankton Res.* **14**: 737–751.
- SERRA, M., A. GÓMEZ, AND M. J. CARMONA. 1998. Ecological genetics of *Brachionus* sympatric sibling species. *Hydrobiologia* **387/388**: 373–384.
- SNELL, T., M. SERRA, AND M. J. CARMONA. 1999. Toxicity and sexual reproduction in rotifers: Reduced resting egg production and heterozygosity loss, p. 170–185. *In* V. E. Forbes [ed.], *Genetics and ecotoxicology*. Taylor and Francis.
- SOKAL, R. R., AND F. J. ROHLF. 2001. *Biometry: The principles and practice of statistics in biological research*, 3rd ed. W. H. Freeman.
- SPILLER, D. A., AND T. W. SCHOENER. 1998. Lizards reduce spider species richness by excluding rare species. *Ecology* **79**: 503–516.
- , AND ———. 2001. An experimental test for predator-mediated interactions among spider species. *Ecology* **82**: 1560–1570.
- STEMBERGER, R. S. 1985. Prey selection by the copepod *Diacyclops thomasi*. *Oecologia* **65**: 492–497.
- STEMBERGER, R. S., AND J. J. GILBERT. 1985. Body size, food concentration, and population growth in planktonic rotifers. *Ecology* **66**: 1151–1159.
- TILMAN, D. 1982. *Resource competition and community structure*. Princeton Univ. Press.
- VAINÖLA, R., B. R. RIDDOCH, R. D. WARD, AND R. I. JONES. 1994. Genetic zoogeography of the *Mysis relicta* species group (Crustacea: Mysidacea) in northern Europe and North America. *Can. J. Fish. Aquat. Sci.* **51**: 1490–1505.
- VON ENDE, C. N. 1993. Repeated-measures analysis: Growth and other time-dependent measures, p. 113–137. *In* S. M. Scheider and J. Gurevitch [eds.], *Design and analysis of ecological experiments*. Chapman and Hall.
- WILLIAMSON, C. E. 1980. The predatory behavior of *Mesocyclops edax*: Predator preferences, prey defenses and starvation induced changes. *Limnol. Oceanogr.* **25**: 903–909.
- . 1987. Predator–prey interaction between omnivorous diaptomid copepods and rotifers: The role of prey morphology and behavior. *Limnol. Oceanogr.* **32**: 167–177.
- WITT, J. D. S., AND P. D. N. HEBERT. 2000. Cryptic species diversity and evolution in the amphipod genus *Hyalella* within central glaciated North America: A molecular phylogenetic approach. *Can. J. Fish. Aquat. Sci.* **57**: 687–698.

Received: 13 January 2003

Accepted: 6 August 2003

Amended: 15 August 2003