Resource competition between sympatric sibling rotifer species

Jorge Ciros-Pérez,[1] *María José Carmona, and Manuel Serra*
Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, A. O. 22085, València 46071, Spain

Abstract

   Mechanisms underlying competitive interactions are important in understanding the structure of planktonic communities, particularly the coexistence of similar species. Here we present experimental results of exploitative competition among three sympatric sibling species of the *Brachionus plicatilis* complex for two differently-sized species of food microalgae. These three rotifer species are to be found in seasonal succession in brackish ponds on the Spanish Mediterranean coast; they can, however, co-occur for long periods. The functional and numerical responses of the three *Brachionus* species to both food microalgae, although similar, differed. Neither rotifer population growth nor grazing rates showed a clear positive correlation between rotifer and alga sizes. Tilman's models were applied to these results in order to predict the outcome of competition in two rotifer, two resources systems. For testing predictions and addressing the possibility of rotifer coexistence, we performed pairwise competition experiments in semicontinuous cultures, which introduces periodic disturbance as an additional factor. Results confirmed the pattern expected from Tilman's models regarding the competitive superiority of each rotifer species, when food composition was biased toward one of the two resources. This shows the relevance of the food threshold concept to predict the superior competitor. However, coexistence of rotifers was found in conditions in which Tilman's models had predicted an unstable equilibrium, with the winning species depending on the initial conditions. We hypothesize that variance in food availability mediated coexistence. Our results suggest that both food partitioning and disturbance are important in explaining the coexistence of these sibling species in nature.

   Natural communities are structured by a network of interactions among species and of the species with their environment. Among the most important are those of a trophic nature, known as consumer-resource interactions. Food limitation and resource competition can clearly exert a strong influence on a herbivorous zooplankton community structure in natural environments (e.g., DeMott and Kerfoot 1982; Sommer et al. 1986). Resource competition is an indirect interaction that affects competitors via exploitation of common resources, which can lead finally to coexistence or exclusion. In exploitative competition, displacement occurs when one species reduces the limiting resource below that level required for sustained growth by the competing species (Tilman 1982). Exclusion of the inferior competitive species limits the community diversity by restricting the number of co-occurring species with similar use of one or more resources from their fundamental niches (Hutchinson 1957).

   Because closely related species may have considerable overlap in their food requirements, strong competition is to be expected. It has been proposed that zooplankton can avoid competition by spatial-temporal niche separation (e.g., Makarewicz and Likens 1975). However, spatial-temporal

niche separation has usually been inferred from seasonal field patterns, which in turn may be affected by abiotic conditions. Nevertheless, very similar species may synchronically coexist in a homogeneous environment because of resource partitioning, which seems to be caused by moderate interspecific differences in resource use (e.g., Rothhaupt 1988; Cartes 1998). Differential use of resources would explain the abundant patterns of herbivorous zooplankton, which depend on food particle sizes (e.g., Rothhaupt 1990*a*) or food quality (e.g., DeMott and Kerfoot 1982; Rothhaupt 1995).

   The mechanism for resource competition can be analyzed by use of Tilman's models (Tilman 1982). These models can predict the outcome of competition from prior knowledge of (1) the kinetics of the growth potential of each competitor to resource availabilities and (2) how competitors remove those resources from the environment (i.e., numerical response and functional response, respectively, sensu Holling 1959). As a result, these models can explain how coexistence is possible, on the basis of the differential use of the same resources. These models have been used successfully while explaining phytoplankton (e.g., Tilman et al. 1982; Sommer 1989; Huismann et al. 1999) and zooplankton interactions (Rothhaupt 1988; Boraas et al. 1990; Kreutzer and Lampert 1999). Using Tilman's graphic models for a two-consumer, two-resource system, Rothhaupt (1988) was able to predict the outcome: either the coexistence of two species, or competition, to the point of exclusion, by one species over the other, in 11 of 12 cases.

   On the other hand, coexistence of zooplankton competitors can be promoted by fluctuating environmental conditions (e.g., Hebert and Crease 1980; Chesson 1986; see also Nisbet et al. 1997; McCauley et al. 1996 and references therein). For instance, the variability of resources availability is a factor that can strongly influence zooplankton commu-

nities (DeMott 1989; Rothhaupt 1990*d;* Kirk 1997; Nisbet et al. 1997) by relaxing competition and thus enhancing the diversity of coexisting species. Furthermore, it has been observed that in other communities (e.g., Connell 1978; Huston 1979, 1995) systems approach equilibrium, and competition exclusion reduces diversity to minimal levels in the absence of disturbance. Accordingly, the Huston's postulation (1979), that disturbance and competition exclusion are the two essential processes controlling diversity, becomes relevant.

Sibling or cryptic species (complexes of closely related and morphologically similar species) have been effectively used as case studies in interspecific competition research (e.g., Hedrick and King 1996). Sibling species are expected to be common in invertebrates with nonvisual mate-recognition systems (Knowlton 1993), and there is increasing evidence for their occurrence in zooplankton, including rotifers (e.g., Serra et al. 1997). Suspension-feeding rotifers are an important group of zooplankters in freshwater and coastal brackish environments. *Brachionus plicatilis* is a rotifer that has been described as an ecological generalist, with a cosmopolitan distribution in coastal marine and inland habitats. However, several lines of evidence suggest that this rotifer taxon is a cluster of various sibling species (Serra et al. 1998; Gómez and Carvalho 2000; Ortells et al. 2000). Recent studies on molecular markers in *B. plicatilis* at Torreblanca Marsh (Gómez et al. 1995; Serra et al. 1998), a coastal brackish area of Eastern Spain, revealed a pattern of three co-occurring biological species, with no hybrids found between them. The three sibling species have been identified as *B. plicatilis* Müller, 1986, *B. rotundiformis* Tschugunoff, 1921, and *B.* sp. nov. (Ciros-Pérez et al. in press), which are differentiated by several genetic markers, morphology (i.e., body size and shape), and sexual reproduction patterns (Carmona et al. 1995; Gómez et al. 1995, 1997), showing an assortative mating behavior (Gómez and Serra 1996). These species, involved in a regular pattern of seasonal succession, were found in a single marsh pond. Although the three taxa present a differential response to ecological parameters (i.e., salinity and temperature; Gómez et al. 1997), this specialization cannot explain completely all events of coexistence and exclusion that take place in the field. Because the three sibling species are so similar morphologically, with no important differences in the grazing and trophic structures, it is to be expected that they would exploit a similar fraction of the available resources (i.e., microalgae) with intense competitive interactions that may be relevant in explaining patterns of species substitution in nature.

In the present article, we examine the exploitative competition among three sympatric sibling species of rotifers of the *B. plicatilis* complex (i.e., *B. plicatilis, B. rotundiformis,* and *B.* sp.nov.) for two species of food microalgae, on the basis of Tilman's mechanistic theory. We analyzed the numerical and functional responses of the three rotifer species to the two food algae. We applied Tilman's graphical models to the results of both responses, in order to predict the competition outcomes for pairs of consumers and to perform competition experiments, which were done in a regimen of semicontinuous culture. The aim of our study is to analyze the possibility of a differential use of a common resource base as a starting point to explain the seasonal succession patterns of these sibling species in nature. We discuss the role of this factor and the perturbations in mediating coexistence of these taxa.

Material and methods

Food algae—*Tetraselmis suecica* (Prasinophyceae) and *Nannochloris atomus* (Chlorophyceae) were cultured at a temperature of $19 \pm 1°C$, 11 g L^{-1} salinity water and constant light conditions (photosynthetically active radiation $\sim 35 \ \mu E \ m^{-2} \ s^{-1}$). The salinity and temperature values used were the same in all experiments and were chosen because co-occurrence of the three rotifer species had been observed with these conditions in the field (Gómez et al. 1995). *T. suecica* is a flagellate alga, ovoid in shape (equivalent spherical diameter, ESD = 9 μm), whereas *N. atomus* is nonflagellated and spherical (ESD = 2.5 μm). Both algae are in that size range of edible food particles preferred by *Brachionus* spp. (Rothhaupt 1990*b,* Vadstein et al. 1993) and are also in the size range of the phytoplankton dominant in a pond where the three sibling rotifer species have been observed (unpubl. data). The cultures originally came from the collection of the Instituto de Ciencias Marinas de Andalucía (Cadiz). Saline water was made with commercial sea salt (Instant Ocean®, Aquarium Systems) and fertilized with *f*/2 modified medium (Guillard and Ryther 1962). Both algae were grown in a semicontinuous culture system, with a dilution rate (*D*) of 0.65 and 0.70 d^{-1}, respectively. Light extinction (750 nm) of algal cultures was measured to estimate carbon content (mg C L^{-1}) with a previously established calibration curve. Carbon content was determined with an elemental analyzer (Perkin-Elmer 2400CHN). The algae ESD was estimated with a Coulter® counter Z2 by use of a standard 70-μm tube aperture.

Experimental animals—We used a representative clone (see Gómez et al. 1997) of each of the three sibling rotifer-species of the *B. plicatilis* complex: *B. plicatilis* clone L1 (adult lorica length, $237.5 \pm 5.8 \ \mu m$), *Brachionus* sp. nov. clone SM2 (adult lorica length, $162.3 \pm 3.5 \ \mu m$), and *B. rotundiformis* clone SS2 (adult lorica length, $117.4 \pm 2.0 \ \mu m$; all measurements were taken from Gómez et al. 1995). The three clones came from the rotifer culture collection at the University of Valencia and were originally isolated from single amictic females collected in Torreblanca Marsh, Spain (Gómez et al. 1995). Pre-experimental cultures were maintained in 250-ml glass flasks fed on *T. suecica* ($\sim$3.0 mg C L^{-1}) every 3–4 d, and the medium was renewed weekly.

Numerical response—Standard food concentrations used were 0.03, 0.06, 0.13, 0.25, 0.5, 1, 2, 4, and 8 mg C L^{-1} of *N. atomus* and 0.035, 0.07, 0.15, 0.3, 0.55, 1.1, 2.25, 4.5, and 9 mg C L^{-1} of *T. suecica.* Experiments were run in duplicate jars. Fifty female rotifers, chosen randomly from healthy stock cultures (at exponential growth phase), were put into stoppered glass tubes holding 55 ml food suspension and kept in darkness on a rotating wheel (22 cm of diameter) at $\sim$6 r.p.m. At 24-h intervals, the food suspension was completely renewed, and rotifers were counted. Fifty animals of every experimental tube (or all those alive when the number

was <50) were randomly chosen and pipetted back into the new medium. Because rotifers are cyclical parthenogens, sexual and asexual females may be present in the cultures. Thus, this random procedure prevents biases in the proportion between both female types, as well as in the age distribution. The experiment was terminated when the intrinsic growth rate (r, d^{-1}) stabilized for at least 3 d (coefficient of variance ≤ 0.1). The first 4 d were not considered, because this was a reasonable time period for the rotifers to adapt to the environmental food (Rothhaupt and Lampert 1992). r for daily intervals was calculated as

$$r = \ln N_t - \ln N_{t-1},$$

where N_t and N_{t-1} are rotifer numbers at consecutive days. Monod curves (Monod 1950), with a threshold for zero-growth, were fitted by iterative nonlinear regression (SPSS Inc., 1999) on the basis of the equation

$$r = r_{max}(C - C_0)/(C - C_0 + K_C),$$

where the variables are r, the population growth rate (d^{-1}), and C, the environmental food concentration, and the parameters are r_{max}, the maximal population growth rate, C_0, the minimum food concentration for zero population growth, and K_C, the Monod constant (Rothhaupt 1993).

The significance of the threshold for zero growth (C_0) was tested by use of a nonlinear analogue of ANCOVA (see Rothhaupt 1990*c*). The sum-of-squares for error (SSE) of the full model and the SSE of a reduced model (without threshold) were determined for each rotifer species and food alga. The significance was then assessed by an F test on both SSEs of the full and the reduced model.

A two-way ANOVA was performed to assess the experimental growth rates where there was no food concentration trend (plateau region of Monod curves), by use of the rotifer and alga species as factors. Multiple comparisons among means were made with the Student-Newman-Keuls test (Sokal and Rohlf 1969).

Clearance rates in mono-algal cultures—The clearance rates were determined by short-term feeding experiments that used the closed-bottle method (Peters 1984) for the combinations of three food levels, two algae species, and three rotifer species. Food concentrations used were 0.07, 0.14, and 0.21 mg C L^{-1} *N. atomus* and 0.16, 0.24, and 0.32 mg C L^{-1} *T. suecica.*

Before the experiments, the rotifers were adapted for 4 d to the respective food concentration in slowly rotating bottles (~6 r.p.m.) and transferred into fresh food suspension daily. Care was taken to avoid depletion of food resources by keeping the densities below 1.5–2 rotifers per milliliter. Rotifers were transferred again to the experimental food concentration at least 2 h before starting the experiment. Experiments were performed by adding 40 rotifers when fed on *N. atomus* and 20 when fed on *T. suecica* in 1 ml of standard medium (see above), with the appropriate food concentration. Cultures were kept in the dark for a 2-h incubation period on a rotating wheel at ~6 r.p.m. Four experimental cultures and four controls—without rotifers—were run for each experimental combination. After incubation, cultures were fixed with acid Lugol's solution. Algae were

counted under an inverted microscope by use of standard methods (Lund et al. 1958). At least 800 cells of each sample were counted to obtain a 95% confidence limit of $\pm 7\%$.

We tested whether the experimental food concentrations were below the incipient limiting level (ILL; Rigler 1961), the critical food concentration up to which clearance rates are constant for brachionids (Rothhaupt 1990*b*). For this purpose, an additional experiment was performed for the highest food concentration of both algae following the procedure above, except that an incubation of 1 h was used. Were the food concentration below the ILL, the log-food concentration of algae would decrease linearly with feeding time.

Clearance rates (CR, μl ind^{-1} h^{-1}) were estimated as the slope of the relationship between log-food concentration and incubation time:

$$CR = (\ln C_{t-1} - \ln C_t)/(N \times t),$$

where $\ln C_{t-1}$ and $\ln C_t$ are, respectively, the log-food concentration before and after the incubation time (t), and N is rotifer density (Peters 1984). The slope was estimated by use of linear regression when 2 incubation times were available (i.e., in the case of the highest food concentration of each alga species).

Selectivity and clearance rates in mixed food—Feeding selectivity was determined following the methods described above for clearance rates, with 20 females ml^{-1} and a 2-h incubation time. Tested food concentrations (*N. atomus* mg C L^{-1} : *T. suecica* mg C L^{-1}) were 0.08 : 0.15 and 0.25 : 0.25. Selectivities were expressed as D indexes (Jacobs 1974). Selectivity for *T. suecica* (D_{Ts}) is defined as

$$D_{Ts} = (CR_{Ts} - CR_{Na})/(CR_{Ts} + CR_{Na}),$$

where CR_{Na} and CR_{Ts} are the clearance rates of *N. atomus* or *T. suecica*, respectively. D varies from -1 to 0 for negative selection and from 0 to $+1$ for positive selection. Clearance rates in mixed food were estimated as described for mono-algal cultures.

Differences in maximal clearance rates caused by food algae and culture type (single alga or mixed algae cultures) were tested by a two-way ANOVA. Multiple comparison among means was performed with a Student-Newman-Keuls test (Sokal and Rohlf 1969).

Pairwise competition experiments—Resource competition experiments were designed on the basis of exploitative competition graphical analysis, as proposed by Tilman (1982). Graphic models were parameterized by use of empirical information obtained in our study. The experimental design simulated resource supply points, resource supply rates, and additional nonspecific rotifer mortality rates. The values for these features were chosen to critically discriminate the conditions for competitive exclusion or coexistence (see "Results" for further explanation). The additional mortality accounts for the extrinsic rotifer mortality factors acting in nature. In our experiments, in contrast to Tilman's assumptions, resource supply and additional rotifer mortality were not continuous but discrete and performed by daily removal and replacement of a fraction of the experimental cultures. This also implies that, because of practical constraints, the

Table 1. Estimates ($\pm$ SE) for the parameters of the modified Monod equation describing the relationship between population growth and food concentration for the three *Brachionus* species on the two food algae. Monod curves were fitted by iterative nonlinear regression ($P < 0.05$). C_0 and K_C in mg C L^{-1}; r_{max} in d^{-1}. Asterisk indicates that C_0 is different to zero ($P \leq 0.05$) tested by use of a nonlinear analog of ANCOVA (see "Material and methods" for details).

	Food algae							
	N. atomus				*T. suecica*			
Rotifer species	C_0	K_C	r_{max}	R^2	C_0	K_C	r_{max}	R^2
B. plicatilis	0.04 ± 0.02	0.60 ± 0.13	1.01 ± 0.05	0.98	$0.20 \pm 0.01^*$	0.20 ± 0.04	0.81 ± 0.04	0.99
Brachionus sp. nov.	$0.06 \pm 0.02^*$	0.31 ± 0.08	1.09 ± 0.07	0.97	$0.05 \pm 0.01^*$	0.21 ± 0.05	1.15 ± 0.05	0.98
B. rotundiformis	$0.04 \pm 0.01^*$	0.07 ± 0.02	0.73 ± 0.04	0.95	$0.11 \pm 0.01^*$	0.22 ± 0.06	0.99 ± 0.06	0.97

two rates were linked in our experiments. The parameter defining these two rates is the dilution rate, D (d^{-1}), which is

$$D = \ln[1/(1 - F)],$$

where F is the fraction of the culture replenished per unit time (Boraas et al. 1990). D was 0.4 d^{-1} for the pairwise competition experiments *B. rotundiformis*/*B.* sp. nov. and *B.* sp. nov./*B. plicatilis* and 0.7 d^{-1} for *B. rotundiformis*/*B. plicatilis.* Several resource supply concentrations were tested for each rotifer species pair (5 for *B. rotundiformis*/*B.* sp. nov. and *B. rotundiformis*/*B. plicatilis* and 3 for *B.* sp. nov./ *B. plicatilis*), with two replications performed for each combination.

Experiments followed the same basic procedure as that explained for the numerical response experiments. However, as mentioned above, to generate the determined dilution rate and the resource supply point, a constant fraction of each culture (rotifers included) was removed daily and replaced with fresh suspension of algae—a mixture of *N. atomus* and *T. suecica* at the concentrations defined by the respective supply point. Cultures were started with 3 females ml^{-1} of

each rotifer species (randomly chosen from the pre-experimental cultures), and experimental glass tubes were changed daily at the dilution event. Daily removed culture fractions were fixed with formaldehyde (~2% 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 versus time for the rarer species was not significantly different from zero for a time span of at least 3 d.

Results

Numerical response—Relationships between rotifer population growth rate and algal concentrations, for all the rotifer and algae species tested, are well described by the modified Monod equation (Table 1; Figs. 1, 2), with R^2 values ranging from 0.95 to 0.99. The observed curves are characterized by a curvilinear increase with food concentration asymptotically approaching a maximal growth rate, as expected from the Monod model. Fig. 1 shows that *Brachionus* sp. nov. is the species with the highest growth rate over the
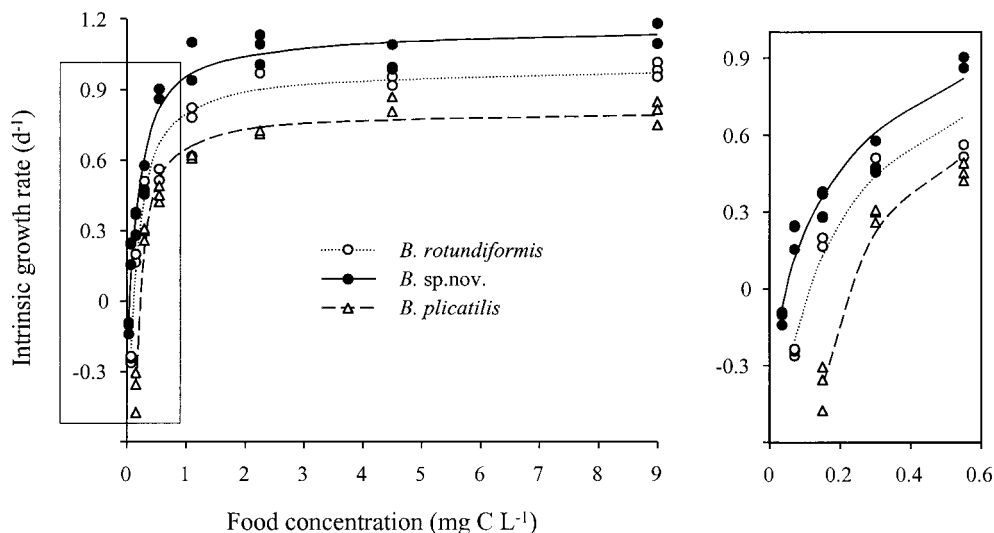

Fig. 1. Relationship between environmental concentration of *T. suecica* and intrinsic growth rates for the three sibling *Brachionus* species. The curves were fitted by iterative nonlinear regression calculated from a modified Monod equation. Inlet at the right side shows details for low food concentrations.
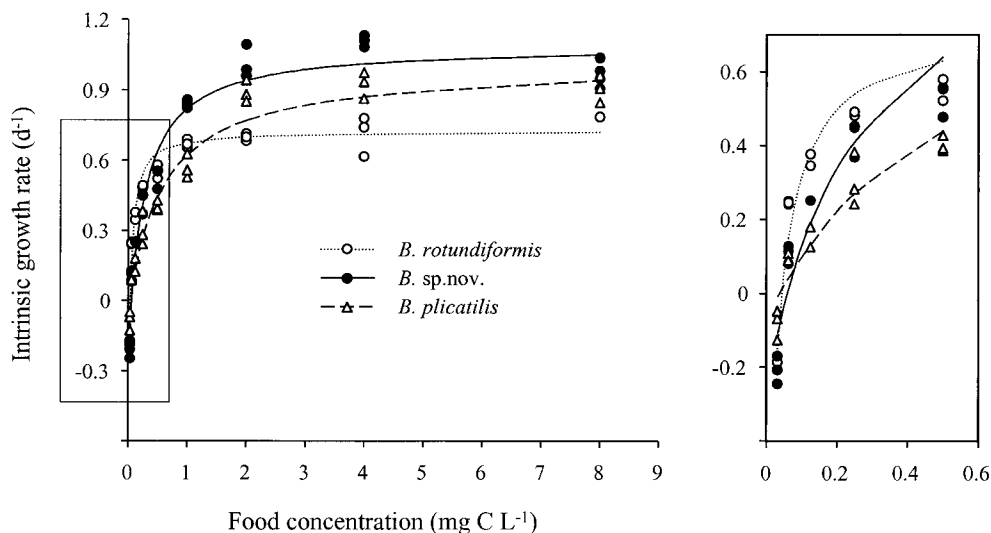
Fig. 2. As Fig. 1, but for *N. atomus* as food algae.

whole range of resource concentrations when fed on *T. suecica.* When, on the other hand, rotifers were fed on *N. atomus,* response curves intersected (Fig. 2). In this case, *B. rotundiformis* was the fastest growing species at low food concentrations, but, as environmental food increased, this pattern reversed and both *B.* sp. nov. and *B. plicatilis* had higher growth rates than *B. rotundiformis, B.* sp. nov. being the species that showed the highest growing rate.

The threshold food concentrations for zero population growth (C_0) were significantly different from 0 in all cases, except for *B. plicatilis* feeding on *N. atomus* (Table 1). Thresholds for *B. rotundiformis* and *B. plicatilis* were several times higher when fed on *T. suecica* than when fed on *N. atomus* (Table 1). In contrast, *B.* sp. nov. showed rather similar requirements for both algae (Table 1; Figs. 1, 2). Maximum growth rates (r_{max}) for each of the three rotifer species were, in all cases, higher than 0.7 d^{-1}. Differences of r_{max}

Table 2. Two-way ANOVA (fixed effects) on the maximal intrinsic growth rates. These rates were estimated from the plateau region observed in the *r* vs. food concentration curves (asymptotic region of Monod curves; * $P \leq 0.05$, ** $P \leq 0.001$). Results of a multiple comparison Student-Newman-Keuls test are shown on the lower part of the table, where a horizontal bar clusters those combinations which were not significantly different ($P > 0.05$; BR = *B. rotundiformis*; BP = *B. plicatilis*; BSN = *B.* sp. nov.; NA = *N. atomus*; TS = *T. suecica.*)

Source	SS	df	F
Rotifer species	0.45	2	55.18**
Algae species	0.02	1	6.03*
Rotifer × algae	0.25	2	30.50**
Error	0.17	41	

Student-Newman-Keuls test:

BSN	BSN	BP	BR	BP	BR
NA	TS	NA	TS	TS	NA

were tested by use of the experimental growth rates from the plateaus observed in the *r* versus food concentration curves (Table 2). ANOVA showed interactive effects between algae and rotifer species on maximal growth rate (Table 2). Accordingly, differences between algae-rotifer combinations were analyzed by use of a multiple-comparison test, which showed that *Brachionus* sp. nov. had significantly higher r_{max} than the other two rotifer species, regardless of the algae used as food (Tables 1 and 2). The maximal growth rate of *B.* sp. nov. was not different when feeding on either algae; *B. rotundiformis* reached its highest maximum growth rate when fed on *T. suecica,* whereas *B. plicatilis* had a higher maximum growth rate with *N. atomus* as food.

The r_{max}/K_C coefficient, which is a measure of the initial slope (i.e., nearby C_0) of the numerical response curve (Rothhaupt 1990*c*), was the highest for *B. rotundiformis* fed on *N. atomus* (Fig. 3). This finding, in combination with the low threshold shown for this rotifer, is relevant in assessing the advantages of this species at low food concentrations. r_{max}/K_C values were similar for all species when using *T. suecica* as food alga, *B.* sp. nov. having the highest value.

Functional responses—When we estimated clearance rates for the highest tested food concentrations (0.21 mg C L^{-1} for *N. atomus* and 0.32 mg C L^{-1} for *T. suecica*), we found that the log concentration of algae per rotifer decreased linearly with feeding time ($R^2 > 0.90$) in all combinations of algae and rotifer species. On the other hand, the rates did not show a trend in any algae-rotifer combination (R^2 for linear regression <0.15), when clearance rates were compared among the three concentrations tested for each alga. According to these results, tested food concentrations were below the ILL (Rigler 1961), and, consequently, the measured clearance rates were estimations of the respective maximum clearance rates (CR_{max}). The averages of all clearance rates measured within each combination are shown in Table 3, and the results of the statistical analysis performed on them are summarized in Table 4. Because interactions
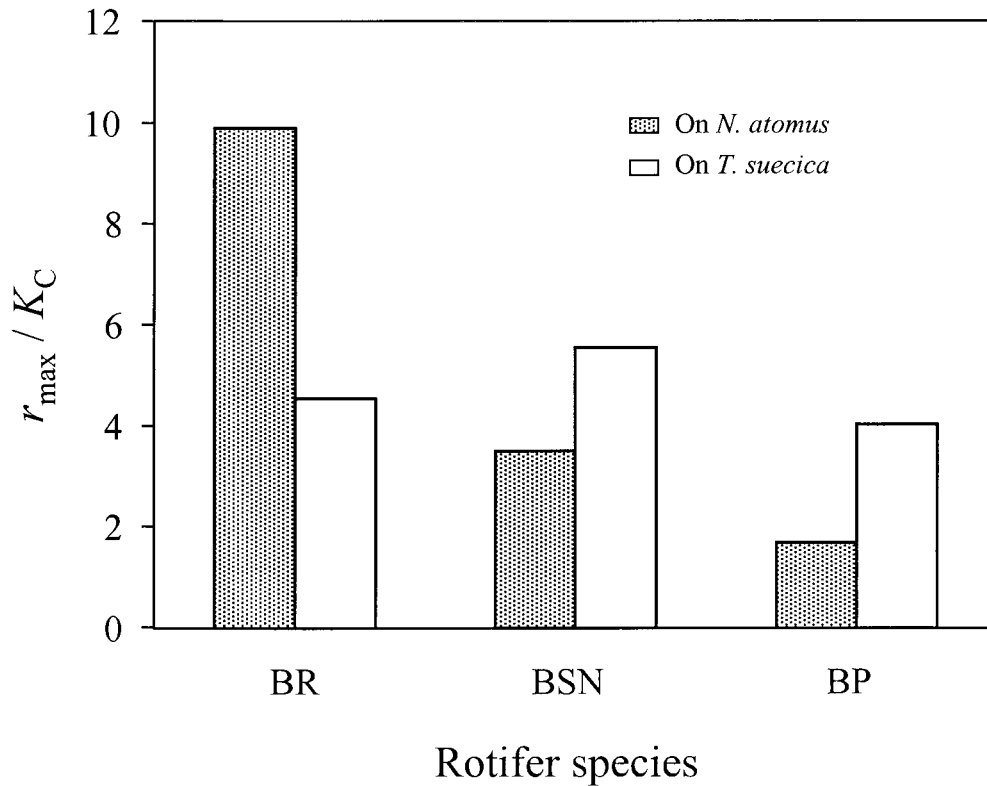
Fig. 3. Estimations for initial slopes of the Monod curve (r_{max}/K_C) for the three rotifer species.

between factors were significant, we performed a multiple-comparison test on the maximum clearance rates for each possible combination.

All rotifer species cleared *T. suecica* faster than *N. atomus,* regardless of the fact that algae were provided in a monoalgal or mixed diet. When mono-algal feedings are compared, maximum clearance rates on *T. suecica* were 8–15 times higher than that on *N. atomus*. The differences, although significant, were lower for clearance rates in a medium where both algae were present. *B. plicatilis* removed *T. suecica* significantly more efficiently than the other two rotifers. *B.* sp. nov. was the rotifer with the highest clearance efficiency on *N. atomus.*

Clearance rates on *N. atomus* were always higher when a mixture of both algae was provided rather than on a monoalgal diet (Tables 3 and 4). The opposite pattern was found for *T. suecica*. Grazing on the larger alga decreased in a mixed diet. This effect was significant for *B. rotundiformis* and *Brachionus* sp. nov. According to selectivity indexes, all

rotifer species preferred *T. suecica* to *N. atomus* (Table 3), this selectivity being highest in *B. plicatilis* and lowest in *B.* sp. nov.

Competition predictions—We applied the competition graphical models developed by Tilman (1982), to predict the outcome of competition between rotifer species. Parameters of Tilman's models can be estimated from the numerical and functional responses. We assumed that both resources (i.e., algae) were perfectly substitutable, according to Tilman's classification (Tilman 1982), and that resources were consumed by individuals of each species proportionally to their concentration in the environment, the constant of proportionality being the maximum clearance rate as estimated in mixed algae cultures. A critical input in Tilman's models is the resource level at which population growth rate is zero (C_0 of Monod's model). Because our predictions are intended for natural populations, we assumed that rotifer mortality could be partitioned into two mortality rates: an intrinsic one,

Table 3. Maximal clearance rates (μl ind^{-1}h^{-1}) on the two food algae determined on monoalgal and mixed cultures. Feeding selectivity in mixed algae diet is expressed as Jacobs' index D. D range from -1 to $+1$. Positive D indicates selection for *T. suecica*. Numbers are means $\pm$ SE (sample size in parentheses).

Rotifer species	Mono-algal		Mixed algae		
	N. atomus	*T. suecica*	*N. atomus*	*T. suecica*	$D_{T. suecica}$
B. plicatilis	3.15 ± 0.12 (14)	47.66 ± 1.38 (12)	6.69 ± 0.07 (8)	45.84 ± 0.49 (8)	0.74 ± 0.01
Brachionus sp. nov.	4.38 ± 0.13 (14)	27.00 ± 0.57 (12)	10.07 ± 0.08 (8)	22.02 ± 0.27 (8)	0.37 ± 0.01
B. rotundiformis	3.21 ± 0.14 (14)	26.15 ± 1.19 (12)	7.75 ± 0.19 (8)	20.24 ± 0.32 (8)	0.45 ± 0.01

Table 4. Two-way ANOVA (fixed effects) on the maximal clearance rates (* $P \leq 0.05$, ** $P \leq 0.001$). Results of a multiple comparison Student-Newman-Keuls test are shown on the lower part of the table, where a horizontal bar clusters those combinations which were not significantly different ($P > 0.05$; BR = *B. rotundiformis*; BP = *B. plicatilis*; BSN = *B.* sp. nov.; NA = *N. atomus*; TS = *T. suecica*; S = one alga species in the medium; M = two algae species in the medium).

Source	SS	df	F
Rotifer species	3094.11	2	439.10**
Algae species	19403.87	1	5507.39**
Single alga (S) vs. mixed algae (M)	0.60	1	0.17
Rotifer × Algae	3914.15	2	555.48**
Rotifer × S/M	13.53	2	0.15
Algae × S/M	585.94	1	166.31**
Rotifer × Algae × S/M	46.47	2	6.60*
Error	401.65	114	

Student-Newman-Keuls test:

BR	BSN	BP	BR	BP	BSN	BR	BSN	BR	BSN	BP	BP
NA	NA	NA	NA	NA	NA	TS	TS	TS	TS	TS	TS
S	S	S	M	M	M	M	M	S	S	M	S

depending on food levels, and an extrinsic one due to ecological factors, such as nonselective predators, parasitism, temperature drops, etc. Intrinsic mortality is that assumed to be acting in our numerical response experiments. In order to account for the extrinsic mortality causes, we assumed an additional mortality rate (D). If such an additional mortality is assumed, the food level for $r = 0$ can be calculated from the numerical response experimental results by looking at the food concentration at which rotifers had a growth rate equal to D ($r = D$). This is because the birth rate should balance the intrinsic mortality plus the additional mortality.

Accordingly, the outcome of competition for a given resource supply point (resource concentrations to which the environment, in the absence of consumption by competing species, tends) in the bidimensional resource space (i.e., environmental food algae concentrations) can be predicted. Figure 4 shows, by use of an example, the type of predictions that can be done with this approach. In Fig. 4A, an unstable equilibrium point (where both zero net growth isoclines [ZNGI] cross) exists when *B. rotundiformis* and *B.* sp. nov. compete. Above the ZNGIs, the consumption vectors define three sectors. The competition outcome is predicted to be dependent on the location or sector of the resource supply point.

Similarly, an unstable equilibrium is predicted for *B. rotundiformis* and *B. plicatilis* (Fig. 4C). By contrast, Fig. 4B shows that, wherever the resource supply point is, *B. plicatilis* cannot out-compete *B.* sp. nov., because the latter is assumed to be able to grow at lower food concentrations than *B. plicatilis.*

In our graphical analysis, we focused particularly on assessing whether equilibrium between species was found. We tried different D values (from 0.0 to 0.8, stepped 0.1), to derive predictions on the existence of equilibrium points for each rotifer-species pair. We found no equilibrium point for the pair *B.* sp. nov/*B. plicatilis*. For *B. rotundiformis*/*B. plicatilis,* equilibrium was found to be possible only if D was 0.7, because for lower D values, *B. rotundiformis* always

out-competed *B. plicatilis,* whereas for *B. rotundiformis*/*B.* sp. nov., equilibrium was predicted for $D < 0.6$.

Summarizing, when the diet was biased toward one of the two algae, the ranking of competitive performance predicted was *B.* sp. nov. > *B. rotundiformis* > *B. plicatilis* when *T. suecica* was prevalent, whereas when *N. atomus* was prevalent, the ranking predicted was *B. rotundiformis* > *B.* sp. nov. > *B. plicatilis* at low D rates and *B.* sp. nov. > *B. plicatilis* > *B. rotundiformis* at high D rates.

Competition experiments—In order to test some of the predictions found in the previous section, we analyzed the situations outlined in Fig. 4, simulating experimentally an array of resource supply points (5, 3, and 5 points for the conditions described in Fig. 4A–C, respectively). The experimental simulation of the supply points and the corresponding resource supply vectors (see Tilman 1982) can be done simultaneously by removing a fixed culture fraction, where the species are growing and competing, and adding the same volume of fresh medium containing the food concentration at the assumed supply point. The fraction of volume removed determines $D,$ which is the assumed additional rotifer mortality.

Time courses of population dynamics for competition experiments are shown in Figs. 5–7. As predicted by the models (Fig. 4B), *B.* sp. nov. was the dominant competitor when growing with *B. plicatilis* in all three tested resource supply concentrations at $D = 0.4$ d^{-1} (Fig. 6A–C). For the two other rotifer pairs (*B. rotundiformis*/*B.* sp. nov. and *B. rotundiformis*/*B. plicatilis*), experimental results (Figs. 5 and 7) were not in complete agreement with predictions (Fig. 4A, 4C). As predicted, at relatively high concentrations of *T. suecica,* and thereby relatively low *N. atomus* concentrations, *B.* sp. nov. out-competed *B. rotundiformis* (Fig. 5A), whereas the opposite was true when *N. atomus* was at the highest relative frequency (Fig. 5E). However, in the intermediate concentrations (Fig. 5B–D), observed outcomes differed from those predicted. On one hand, it was found that the two species
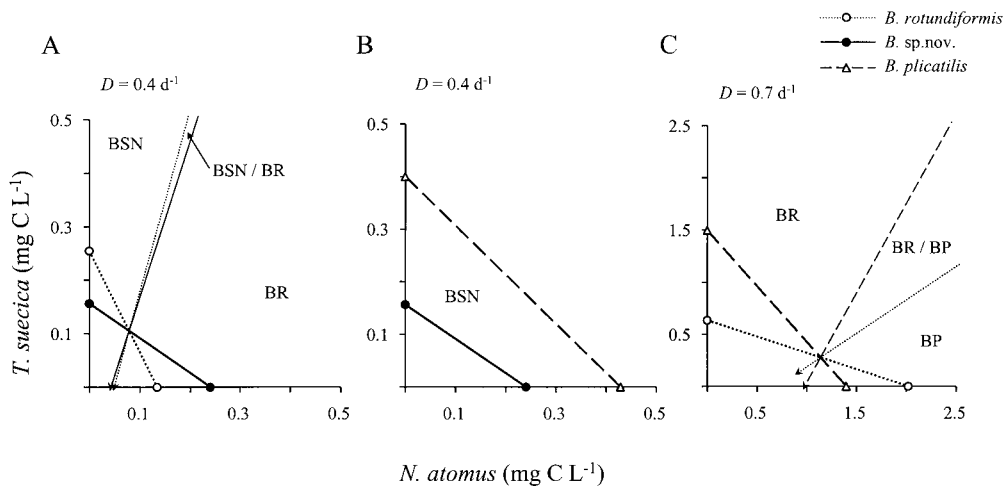
Fig. 4. Theoretical predictions from Tilman's graphical analysis for the exploitative competition outcome between pairs of rotifer species assuming (1) perfectly substitutable resources, (2) a constant dilution rate (D), and (3) that clearance rates estimated from mixed-algae cultures can be extrapolated to the equilibrium point. ZNGI (negative-slope lines linking the axes) and consumption vectors (their slopes being extrapolated toward high resource concentrations) for *B. plicatilis* (BP), *B.* sp. nov. (BSN) and *B. rotundiformis* (BR) are represented in a bidimensional resources space. D values are 0.4 d^{-1} for (A) and (B) and 0.7 d^{-1} for (C). According to Tilman's model, a rotifer species cannot achieve positive growth if the resource supply point (RSP) is below its ZNGI. If the RSP is below the ZNGI of one of the species but above the ZNGI of the other, the latter is predicted to out-compete the former. When ZNGIs cross each other, the superior competitor is dependent on which sector (delimited by the consumption vectors slopes) in which the RSP is located, but an equilibrium point (at the intersection) exists. The predicted superior competitor in each sector of (A) and (C) is shown. Because equilibrium is predicted to be unstable, when two species are marked in a sector, it means that the winning species would depend on initial rotifer densities.

were able to grow and finally reach a steady state, in contrast to Tilman's model predictions (*see* Fig. 5). Moreover, the range of resource supply points that allows stable coexistence is wider than that predicted for unstable coexistence. A similar pattern was found for experimental competition between *B. rotundiformis* and *B. plicatilis* (Fig. 7). *B. rotundiformis* displaced *B. plicatilis* at relatively high *T. suecica* concentrations (Fig. 7A). In contrast, *B. plicatilis* was dominant at the opposite relative food concentrations (Fig. 7E), whereas, at the intermediate concentrations, stable coexistence occurred (Fig. 7B–D). A summary of comparison between predictions and actual outcome of competition is shown in Fig. 8A–C.

Discussion

Numerical and functional responses—As has been outlined previously (Rothhaupt 1988, 1990c; Boraas et al. 1990; Hansen et al. 1997), we found that a modified Monod model describes the effect of food quantity on the rotifer population growth rates well. Maximum growth rates (r_{max}) and thresholds for zero growth (C_0) found for the three species are similar to those reported in previous studies for *Brachionus* spp. (e.g., Doohan 1973; Stemberger and Gilbert 1985, 1987; Starkweather 1988; Miracle and Serra 1989; Rothhaupth 1990c; Hansen et al. 1997). However, the expected pattern of higher growth rates, when rotifer size and food particle sizes are correlated (Stemberger and Gilbert 1985; Rothhaupt 1990c), was not, in most cases, found (e.g., *B.* sp. nov.,

the intermediate size species, grew the fastest in most conditions).

Because *B. rotundiformis* had a low C_0, a relatively low r_{max} (Table 1; Fig. 2), and a high r_{max}/K_C (Fig. 3) when feeding on *N. atomus,* it could be considered as an "affinity" specialist (sensu Crowley 1975). By contrast, the other two species could be considered as "velocity" specialists, because they had higher reproductive rates and a low r_{max}/K_C when cultured at high concentrations of *N. atomus.* Accordingly, if food particles were small, *B. rotundiformis* would have advantages at low food level, a condition expected at high rotifer population density. The other species, and particularly *B.* sp. nov., would have fast growth if food were abundant and might be adapted to grow before competition prevailed.

Maximum clearance rates found in this study are similar to those reported by Rothhaupt (1990a,b) for other *Brachionus* fed on similarly sized algae. The higher clearance on *T. suecica* (Table 3) found in our study is consistent with the optimal prey size reported for *Brachionus* ($\sim$3.5–8.5 μm; Rothhaupt 1990b; Hansen et al. 1997). However, by contrast to Rothhaupt's (1990a) findings, maximum clearance rates on the two food-algae cells were not clearly related to rotifer size. The middle-size *B.* sp. nov. was significantly more efficient at removing the small *N. atomus* than the other two rotifer species, which, on the other hand, did not vary in this respect. Bogdan and Gilbert (1984) achieved similar conclusions. They showed that the efficiency with
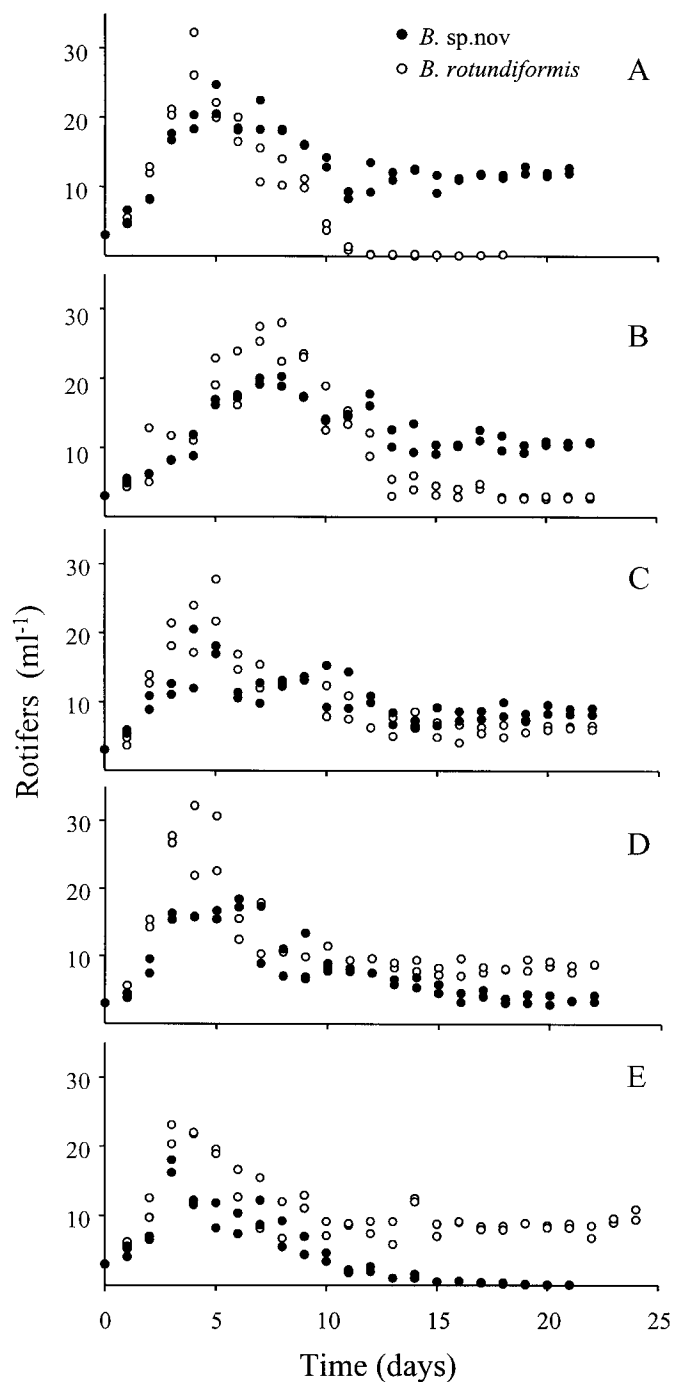
Fig. 5. Time course of competition experiments between *B. rotundiformis* and *B.* sp. nov. at a 0.4 d^{-1} dilution rate (*D*) and different resource supply concentrations. Food concentrations (*T. suecica* mg C L^{-1}:*N. atomus* mg C L^{-1}) were (A) 7.5:0.5, (B) 6.8:1.2, (C) 6:2, (D) 5.5:2.5, and (E) 4:4.

Fig. 6. Time course of competition experiments between *B.* sp. nov. and *B. plicatilis* at *D* = 0.4 d^{-1}. Resource supply concentrations (*T. suecica* mg C L^{-1}:*N. atomus* mg C L^{-1}) were (A) 7:1, (B) 4:4, and (C) 1:7.

which a species eats different-sized cells can not be predicted solely by body length.

A close correspondence between functional and numerical responses has been described in some *Brachionus* species (Rothhaupt 1990*c*): higher growth rates should be expected on the more intensively grazed algae. This argument neglects
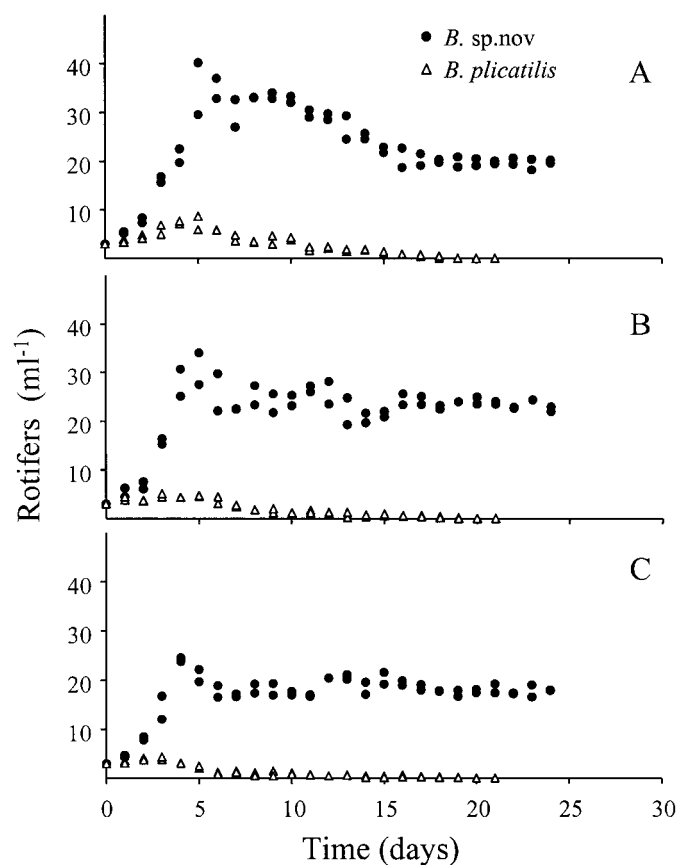
differences in the nutritional quality of food resources The pattern observed by Rothhaupt was only found for *B. rotundiformis.* We presume that *T. suecica,* despite being more efficiently grazed, has a lower nutritional quality or digestibility than *N. atomus.* This last possibility is consistent with the observation of some apparently undigested cells in fecal pellets from those rotifers fed on *T. suecica* (Ciros-Pérez, pers. obs.).

Resource competition—Our experiments on single rotifer species show that the three species overlap but also differ while responding to a common resource base. Using numerical and functional responses, we applied the theory for resource-based competition developed by Tilman (1982) to predict competition outcomes. An important mismatch exists, however, between this theory's assumptions and our experimental approach, because Tilman's theory, which primarily describes the equilibrium conditions, does not consider the effect of the disturbance caused by the semicontinuous culture techniques (i.e., pulses in both food supply and rotifer unspecific mortality). Tilman's theory predicted that only one species from each pair would survive, with the consequent complete exclusion of the other competitor. The winning species were dependent on the food conditions and, to a lesser extent, on the action of unspecific
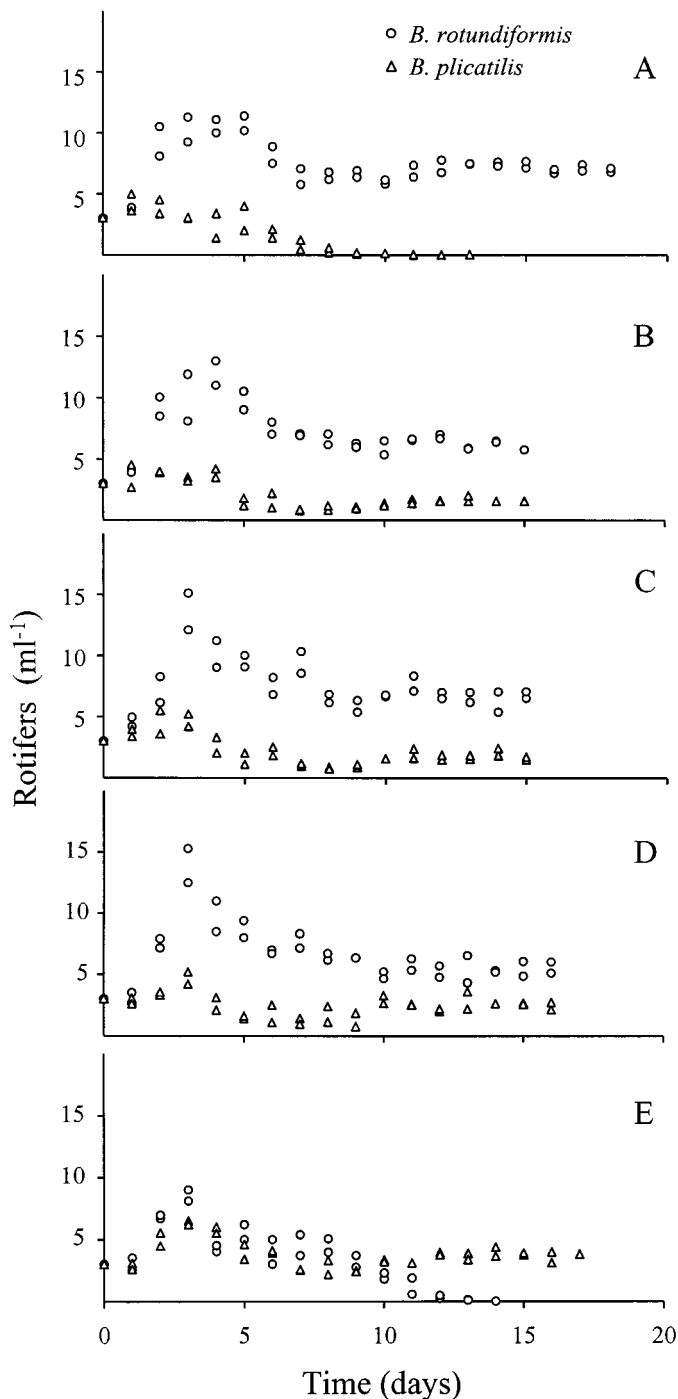
Fig. 7. Time course of competition experiments between *B. rotundiformis* and *B. plicatilis* at $D = 0.7$ d^{-1}. Resource supply concentrations (*T. suecica* mg C L^{-1}:*N. atomus* mg C L^{-1}) were (A) 7:1, (B) 5:3, (C) 4:4, (D) 3:5, and (E) 1:7.

mortality causes (i.e., additional mortality rate, D). In some cases, an unstable equilibrium was predicted where the initial conditions would determine which species would win.

We experimentally explored 13 competitive scenarios out of the indefinite number that could be theoretically analyzed. However, some conclusions can be drawn. When the diet

was biased toward one of the two algae, the winning species was correctly predicted by Tilman's theory. These results prove the relevance of the food threshold concept (see, e.g., Stemberger and Gilbert 1985; Kreutzer and Lampert 1999) in predicting the superior competitor, because the rotifer species that was able to maintain a positive growth rate, with the lowest algal requirement, out-competed the other. Results also confirmed that the competitive abilities in some mixed diets are quite balanced for the pairs *B. rotundiformis*/*B.* sp. nov. and *B. rotundiformis*/*B. plicatilis*. Moreover, the proportion of algae controlling which rotifer species was superior, or whether the species' capabilities were equivalent, was rather well predicted. It is noteworthy that disturbance does not qualitatively affect the competition output and cannot mediate coexistence if one resource largely constitutes the diet. On the other hand, this agreement between prediction and experimental outcome suggests that the incidence of sexual reproduction, producing males and resting eggs that do not contribute to the current population growth and could change the competition dynamics, was similar in both numerical response and competition experiments. As a result, growth rates estimated in the former were rightly extrapolated to the latter.

However, Tilman's theory was unable to explain all actual outcomes. There were situations where an unstable equilibrium, with the winning species dependent on the initial conditions, was expected but where species coexistence was, in fact, observed. Likewise, the resource supply range, for which steady states were found, was slightly wider than that for which unstable equilibrium had been predicted. These findings could have two alternative explanations: (1) disturbance due to semicontinuous culture promotes coexistence, as might be expected from nonequilibrium theories (Nisbet et al. 1997; McCauley et al. 1996) and (2) disturbance does not significantly affect the dynamics, but the equilibrium predicted as unstable is actually stable, which could be explained in turn in several ways. First, parameter estimation could be inaccurate—in particular, the estimation of consumption vector slopes may be very sensitive to errors in clearance rates calculations. Second, clearance rates estimated in some food concentrations were extrapolated to other concentrations, but the clearance rate might be dependent on algal concentration (Starkweather 1980). Third, the assumption that the two algae used were perfectly substitutable might not hold. If the algae contain different proportions of essential components, the resources would be complementary (Tilman 1982); consequently ZNGIs would not be linear, affecting the location of the equilibrium point. In our view, these three explanations are unlikely to account for a reversion from unstable equilibrium to coexistence, because their effects would be expected to be quantitative (e.g., location of the resource supply sector defining an equilibrium point). Fourth, incidence of sexual reproduction could be different in competition dynamics than in mono-specific rotifer cultures. If so, the growth rates estimated in the latter could not be extrapolated to the former. Sexual reproduction would promote coexistence if it were positively density dependent (Snell and Boyer 1988; Carmona et al. 1995), because the rarer species would invest relatively more in asexual reproduction (current population growth), whereas the
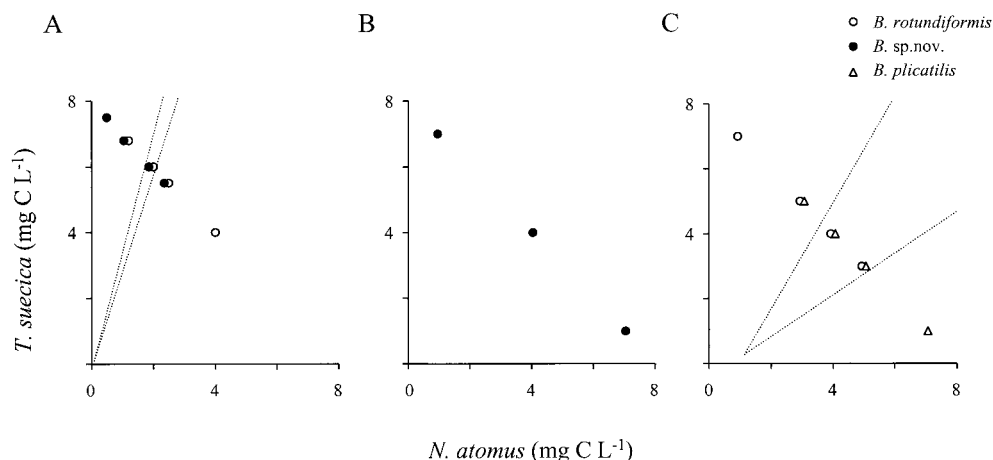
Fig. 8. Comparison between the theoretical and actual outcome of competition between pairs of rotifer species. Food concentrations in the middle sectors between the dotted lines are expected to lead to the exclusion of a species (one or the other, depending on initial conditions). A single symbol denotes experiments in which only one species persisted, and two symbols side by side indicate coexistence.

opposite would be true for the other competitor. However, this explanation is inconsistent with our observations during the experiments' time course, because sexual females were generally more frequent for the inferior competitor (data not shown).

By contrast, disturbance due to semicontinuous culture is a likely explanation for coexistence. Semicontinuous culture, although periodic dilutions were small and frequent, implies changes in the resource availability, with high food pulses that are bound up with subsequent phases of food depletion. Phases of over- and under-exploitation of food resources alternate (Boraas 1980; Rothhaupt 1993), and populations will not be at an exact equilibrium but will be oscillating around an average density. This variance in food availability could have an effect on species coexistence (Goulden et al. 1982; Gaedeke and Sommer 1986; DeMott 1989). Coexistence between competing cladocerans seems to be promoted when some species prevent their exclusion at low food concentrations by efficiently collecting and storing energy during periods of high food availability (Romanovsky and Feniova 1985; Matveev 1985, 1987). Nisbet et al. (1997) theoretically showed that coexistence of *Bosmina* and *Daphnia* observed in experimental cultures fed with a single resource (see Goulden et al. 1982) can be explained as just a consequence of a fluctuating food supply regime (see also McCauley et al. 1996). Saturation of functional response during phases of high food availability can also promote coexistence (Rothhaupt 1990*d*).

The observed numerical responses suggest mechanisms about how variance in food availability can promote coexistence. For instance, *B. rotundiformis,* as an ''affinity'' strategist, would be adapted to low *N. atomus* levels, while *B. plicatilis,* as a ''velocity'' strategist, would be superior at high food levels. A similar explanation can be proposed for the coexistence of *B.* sp. nov. and *B rotundiformis.* In this case, high population densities were achieved, and possibly the resource depletion was larger between dilution events. If

so, a differential ability of each competitor to withstanding starvation could be an important coexistence determinant.

The sympatric occurrence of sibling species belonging to the *B. plicatilis* complex is a frequent phenomenon in small brackish ponds in Eastern Spain (Gómez et al. 1995; Ortells et al. 2000). Although these species have differential responses to temperature and salinity, their specialization does not completely explain the patterns of species substitution and co-occurrence (Gómez et al. 1995). In this sense, our results provide insights into what food conditions (supply rates and particle quality) would favor the dominance of different species in the complex and offer an explanatory hypothesis about the patterns of succession (i.e., exclusion) in the field, to be tested in the future. On the other hand, given the seasonal overlap between species, which can last as long as 4 months, and the short generation time of rotifers, this overlap is unlikely to be due to the transition between the dominance from one species to another but rather to a phase of steady-state coexistence. Our analysis offers an explanation for the coexistence of such similar species. Results suggest that both differential resource use and small disturbances such as those due to daily drops in temperature, daily fluctuations in food availability, predation pressure, etc., play a role in coexistence.

Sibling species are a common phenomenon in rotifers and other aquatic invertebrates (Knowlton 1993; Serra et al. 1997; Cerný and Hebert 1999), and their occurrence in sympathy is not rare. Thus, diversity of zooplankton assemblages might largely have been underestimated in standard field samples. If so, a higher diversity than previously thought poses a new challenge to ecological theory, in order to explain what mechanisms allow high diversity in a spatially homogeneous environment, particularly because very similar species are involved. Our results, by suggesting a role of both small disturbances and differential use of shared resources, shed light on this problem.

References

BOGDAN, K. G., AND J. J. GILBERT. 1984. Body size and food size in freshwater zooplankton. Proc. Natl. Acad. Sci. USA **81:** 6427–6431.

BOORAS, M. E. 1980. A chemostat system for the study of rotifer-algal-nitrate interactions, p. 173–182. *In* W. C. Kerfoot [ed.], The evolution and ecology of zooplankton communities. University Press of New England.

⸺, D. B. SEALE, AND J. B. HORTON. 1990. Resource competition between two rotifer species (*Brachionus rubens* and *B. calyciflorus*): An experimental test of a mechanistic model. J. Plankton Res. **12:** 77–87.

CARMONA, M. J., A. GÓMEZ, AND M. SERRA. 1995. Mictic patterns of the rotifer *Brachionus plicatilis* Müller 1786 in small ponds. Hydrobiologia **313/314:** 365–371.

CARTES, J. E. 1998. Feeding strategies and partitioning of food resources in deep-water decapod crustaceans (400–2300 m). J. Mar. Biol. Assoc. U. K. **78:** 509–524.

CERNÝ, M., AND P. D. N. HEBERT. 1999. Intercontinental allozyme differentiation among four holarctic *Daphnia* species. Limnol. Oceanogr. **44:** 1381–1387.

CHESSON, P. L. 1986. Environmental variation and the coexistence on species, p. 240–256. *In* J. Diamond and T. Case [eds.], Community ecology. Harper and Row.

CIROS-PÉREZ, J., A. GÓMEZ, AND M. SERRA. In press. On the taxonomy of three sympatric sibling species of the *Brachionus plicatus* (Rotifera) complex from Spain, with the description of *B. ibericus* n. sp. J. Plankton Res.

CONNELL, J. H. 1978. Diversity in tropical rain forest and coral reefs. Science **199:** 1302–1310.

CROWLEY, P. H. 1975. Natural selection and the Michaelis constant. J. Theor. Biol. **50:** 461–475.

DEMOTT, W. R. 1989. The role of competition in zooplankton succession, p. 195–252. *In* U. Sommer [ed.], Plankton ecology. Succession in plankton communities. Springer.

⸺, AND W. C. KERFOOT. 1982. Competition among cladocerans: Nature of the interactions between *Bosmina* and *Daphnia*. Ecology **63:** 1949–1964.

DOOHAN, M. 1973. An energy budget for adult *Brachionus plicatilis* Muller (Rotatoria). Oecologia **13:** 351–362.

GAEDEKE, A., AND U. SOMMER. 1986. The influence of the frequency of periodic disturbances on the maintenance of phytoplankton diversity. Oecologia **71:** 25–28.

GÓMEZ, A., M. J. CARMONA, AND M. SERRA. 1997. Ecological factors affecting gene flow in the *Brachionus plicatilis* complex (Rotifera). Oecologia **111:** 350–356.

⸺, AND G. R. CARVALHO. 2000. Sex, parthenogenesis and genetic structure of rotifers: Microsatellite analysis of contemporary and resting egg bank populations. Mol. Ecol. **9:** 203–214.

⸺, AND M. SERRA. 1996. Mate choice in male *Brachionus plicatilis* rotifers. Funct. Ecol. **10:** 681–687.

⸺, M. TEMPRANO, AND M. SERRA. 1995. Ecological genetics of a cyclical parthenogen in temporary habitats. J. Evol. Biol. **6:** 601–622.

GOULDEN, C. E., L. L. HENRY, A. J. TESSIER. 1982. Body size, energy reserves, and competitive ability in three species of Cladocera. Ecology **63:** 1780–1789.

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.

HANSEN, B., T., WERNBERG-MØLLER, AND L. WITTRUP. 1997. Particle grazing efficiency and specific growth efficiency of the rotifer *Brachionus plicatilis* (Muller). J. Exp. Mar. Biol. Ecol. **215:** 217–233.

HEBERT, P. D. N., AND T. J. CREASE. 1980. Clonal coexistence in *Daphnia pulex* (Leydig): Another planktonic paradox. Science **207:** 1363–1365.

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.

HOLLING, C. S. 1959. The components of predation as revealed by a study of small-mammal predation of the European sawfly. Can. Entomol. **91:** 293–320.

HUISMAN, J., R. R. JONKER, C. ZONNEVELD, AND F. J. WEISSING. 1999. Competition for light between phytoplankton species: Experimental test of mechanistic theory. Ecology **80:** 211–222.

HUSTON, M. A. 1979. A general hypothesis of species diversity. Am. Nat. **113:** 81–101.

⸺. 1995. Biological diversity: The coexistence of species on changing landscapes. Cambridge University.

HUTCHINSON, G. E. 1957. Concluding remarks. Cold Spring Harbour Symp. Quant. Biol. **22:** 415–427.

JACOBS, J. 1974. Quantitative measurement of food selection. A modification of the forage ratio and Ivlelv's electivity index. Oecologia **14:** 413–417.

KIRK, K. L. 1997. Life-history responses to variable environments: Starvation and reproduction in planktonic rotifers. Ecology **78:** 434–441.

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.

LUND, J. W. G., C. KIPLING, AND E. D. LE CREN. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia **11:** 143–170.

MAKAREWICZ, J. C., AND G. E. LIKENS. 1975. Niche analysis of a zooplankton community. Science **190:** 1000–1003.

MATVEEV, V. F. 1985. Competition and population time lags in *Bosmina* (Cladocera, Crustacea). Int. Rev. Gesamten. Hydrobiol. **70:** 491–508.

⸺. 1987. Effect of competition on the demography of planktonic cladocerans *Daphnia* and *Diaphanosoma*. Oecologia **74:** 468–477.

MCCAULEY, E., R. M. NISBET, A. M. DE ROSS, W. W. MURDOCH, AND W. S. C. GURNEY. 1996. Structured population models of herbivorous zooplankton. Ecol. Monogr. **66:** 479–501.

MIRACLE, M. R. AND M. SERRA. 1989. Salinity and temperature influences in rotifer life history characteristics. Hydrobiologia **186/187:** 81–102.

MONOD, J. 1950. La technique de culture continue; Théorie et applications. Ann. Inst. Pasteur **79:** 390–410.

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., 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.

PETERS, R. H. 1984. Methods for the study of feeding, grazing and assimilation by zooplankton, p. 336–412. *In* J. A. Downing and F. H. Rigler [eds.], A manual on methods for the assessment of secondary productivity in fresh waters. 2nd ed. IBP Handbook 17. Blackwell.

RIGLER, F. H. 1961. The relation between concentration of food and

the feeding rate of *Daphnia magna* Straus. Can. J. Zool. **39:** 857–868.

ROMANOVSKY, Y. E., AND I. Y. FENIOVA. 1985. Competition among Cladocera: Effects of different levels of food supply. Oikos **44:** 243–252.

ROTHHAUPT, K. O. 1988. Mechanistic resource competition theory applied to laboratory experiments with zooplankton. Nature **333:** 660–662.

————. 1990*a*. Differences in particle size-dependent feeding efficiencies of closely related rotifer species. Limnol. Oceanogr. **35:** 16–23.

————. 1990*b*. Changes of the functional responses of the rotifers *Brachionus rubens* and *Brachionus calyciflorus* with particle sizes. Limnol. Oceanogr. **35:** 24–32.

————. 1990*c*. Population growth rates of two closely related rotifer species: Effects of food quality, particle size, and nutritional quality. Freshwater Biol. **23:** 561–570.

————. 1990*d*. Resource competition of herbivorous zooplankton: A review of approaches and perspectives. Arch. Hydrobiol. **118:** 1–29.

————. 1993. Rotifers and continuous culture techniques: Model systems for testing mechanistic concepts of consumer-resource interactions, p. 178–192. *In* N. Walz [ed.], Plankton regulation dynamics. Ecological Studies 98, Springer-Verlag.

————. 1995. Algal nutrient limitation affects rotifer growth rate but not ingestion rate. Limnol. Oceanogr. **40:** 1201–1208.

————, 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. GALIANA, AND A. GÓMEZ. 1997. Speciation in monogonont rotifers. Hydrobiologia **358:** 63–70.

————, A. GÓMEZ, AND M. J. CARMONA. 1998. Ecological genetics of *Brachionus* sympatric sibling species. Hydrobiologia **387/388:** 373–384.

SNELL, T. W., AND E. M. BOYER. 1988. Thresholds for mictic-female production in the rotifer *Brachionus plicatilis* (Müller). J. Exp. Mar. Biol. Ecol. **124:** 73–85.

SOMMER, U. 1989. The role of competition for resources in phytoplankton succession, p. 57–106. *In* U. Sommer [ed.], Plankton ecology. Succession in plankton communities. Springer.

————, Z. M. GLIWICZ, W. LAMPERT, AND A. DUNCAN. 1986. The PEG-model of seasonal succession of planktonic events in fresh waters. Arch. Hydrobiol. **106:** 433–471.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry, 1st. ed. W. H. Freeman.

STARKWEATHER, P. L. 1980. Aspects of the feeding behavior and trophic ecology of suspension-feeding rotifers. Hydrobiologia **73:** 63–72.

————. 1988. Reproductive and functional response of the rotifer *Brachionus plicatilis* to changing food density. Int. Ver. Theor. Angew. Limnol. Verh. **23:** 2001–2005.

STEMBERGER, R. S., AND J. J. GILBERT. 1985. Body size, food concentration, and population growth in planktonic rotifers. Ecology **66:** 1151–1159.

————, AND ————. 1987. Rotifer threshold food concentration and size-efficiency hypothesis. Ecology **68:** 181–187.

TILMAN, D. 1982. Resource competition and community structure. Princeton Univ. Press.

————, S. S. KILHAM, AND P. KILHAM. 1982. Phytoplankton community ecology: The role of limiting nutrients. Annu. Rev. Ecol. Syst. **13:** 349–372.

VADSTEIN, O., G. ØIE, AND Y. OLSEN. 1993. Particle size feeding by the rotifer *Brachionus plicatilis*. Hydrobiologia **255/256:** 261–267.