Competition in variable environments: experiments with planktonic rotifers

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SUMMARY

1. In a constant environment, competition often tends to reduce species diversity. However, several theories predict that temporal variation in the environment can slow competitive exclusion and allow competing species to coexist. This study reports on laboratory competition experiments in which two pairs of planktonic rotifer species competed for a phytoplankton resource under different conditions of temporal variability in resource supply.

2. For both species pairs, Keratella cochlearis dominated under all conditions of temporal variability, and the other species (Brachionus calyciflorus or Synchaeta sp.) almost always went extinct. Increasing temporal variation in resource supply slowed competitive exclusion but did not change competitive outcome or allow coexistence.

3. Rotifers show a gleaner–opportunist trade-off, because gleaner species have low threshold resource levels ($R^*$) and low maximum population growth rates, while opportunist species have the opposite characteristics. In the competition experiments, the gleaners always won and the opportunists always lost. Thus, a gleaner–opportunist trade-off was not sufficient to facilitate coexistence under conditions of resource variability. Instead, the winning species had both the lowest $R^*$ and the greatest ability to store resources and ration their use during times of extreme resource scarcity.

Keywords: competition, coexistence, temporal variation, rotifers, zooplankton

Introduction

The competitive exclusion principle predicts that competition will tend to reduce species diversity (Hardin, 1960). Mechanistic resource competition theory makes the same prediction: in environments with constant resource supply, the competitor with the lowest $R^*$ (the threshold resource level, where population growth rate equals zero) will exclude all other species (Tilman, 1982). Experiments using phytoplankton and rotifers support this prediction (Tilman, 1982; Rothhaupt, 1988). Freshwater herbivorous zooplankton often compete for limiting phytoplankton resources (DeMott, 1989; Rothhaupt, 1990). However, the low species diversity predicted by the competitive exclusion principle conflicts with the often high diversity of coexisting herbivorous zooplankton. For example, there are often 5–30 species of rotifers coexisting in a single lake (Stemberger et al., 1996).

Temporal variation in the environment is one of the processes that may facilitate coexistence between competing species. Hutchinson (1961) suggested that environmental fluctuations might allow many species of phytoplankton to coexist. Competitive exclusion takes time, so if different species are competitively superior in different environments, and if the environment changes before competitive exclusion can occur, then species might coexist. Hutchinson’s ideas have been elaborated on by Levins (1979), Armstrong & McGehee (1980) and many others (reviewed by Grover, 1997). The basic predictions of these theories are that different frequencies of temporal variation can change competitive outcome (the identity of the winning species), slow the rate of competitive
exclusion, and allow the coexistence of competitors. Under appropriate conditions, maximum species diversity is expected at an intermediate frequency of environmental variation (Hutchinson, 1961; Grover, 1997).

The idea that environmental variation can change competitive interactions between phytoplankton species has been tested in several laboratory experiments (reviewed in Grover, 1997). For example, Sommer (1984, 1985) found that two species of phytoplankton coexisted when inorganic nutrients were supplied continuously, while seven species coexisted when nutrients were supplied in weekly pulses. In contrast, Grover (1991a) examined competition between two species of green microalgae and found that the same species dominated under both constant and fluctuating resource supply. More recently, Litchman (1998) found that fluctuations in light intensity increased the diversity of coexisting phytoplankton species.

Many authors have speculated that variable food rations can affect competition between zooplankton species (e.g. Threlkeld, 1976; Stemberger & Gilbert, 1985; DeMott, 1989; Rothhaupt, 1990; Schulze, Zagarese & Williamson, 1995). However, there has been only one experimental test of this idea. MacIsaac & Gilbert (1991) examined the effect of different periods of food addition on competition between the rotifer *Keratella cochlearis* Gosse and the cladoceran *Daphnia ambigua* Scourfield. When food was supplied as pulses of high concentration every 2 or 4 days, *Keratella* was excluded by *Daphnia*. MacIsaac & Gilbert (1991) attributed this result to the greater ability of *Daphnia* to survive the episodes of starvation that occurred between food pulses. When food was supplied as low concentration pulses twice a day, the two species coexisted for 44 days. Thus, contrary to theoretical expectations, coexistence occurred only in the treatment with the least amount of temporal variation in resource supply.

Theories about competition in variable environments assume the existence of trade-offs between characteristics that confer success in different environments. One such trade-off is that between gleaners and opportunists (Grover, 1997). Gleaners are species with low $R^*$ that do well at chronically low food levels but, because of their low maximum population growth rates ($r_{max}$), are not able to grow rapidly during pulses of high food concentration. Opportunists are species with high $r_{max}$ that can rapidly exploit transient periods of high food availability, but have high $R^*$ and so do poorly when food levels are chronically low. The expectation is that gleaners will dominate under steady state conditions, while opportunists will dominate under conditions of variable resource supply.

Herbivorous planktonic rotifers make an interesting case study for testing theories of the effects of temporal variation in resource supply on competitive interactions. Rotifers exhibit a strong gleaner–opportunistic trade-off (Stemberger & Gilbert, 1985), and the abundance of their phytoplankton resources varies over time (Stewart & Wetzel, 1986; Merriman & Kirk, 2000). In this paper, I examine the influence of temporal variation in resource supply on the competitive interactions between two pairs of planktonic rotifer species. I test the predictions that increasing temporal variation of resource supply will change competitive outcome, slow competitive exclusion, and allow coexistence.

**Methods**

Three species of rotifers were used in the competition experiments: *Brachionus calyciflorus* Pallas (dry body mass 0.20 µg; Kirk, 1997a), *K. cochlearis* (0.02 µg) and an unidentified species of *Synchaeta* (0.03 µg). Hereafter, these species are referred to as *Brachionus*, *Keratella* and *Synchaeta*. The strains used here are the same as those used in my previous work (Kirk, 1997a, b; Kirk, Ellis & Taylor, 1999). These rotifer species were chosen for three reasons. First, they were all able to live on the same strain of phytoplankton, an unidentified species of *Cryptomonas*. Secondly, they occupy different points on the gleaner–opportunistic trade-off (Fig. 1). *Keratella* has the lowest $R^*$ and $r_{max}$ values, *Brachionus* is the highest, and *Synchaeta* is intermediate. Thirdly, the species respond very differently to starvation. *Keratella* has the longest mean starvation time (4.9 days), *Synchaeta* the shortest (0.4 days), and *Brachionus* is intermediate (2.2 days) (Fig. 1). In rotifers, starvation time is not correlated with body mass, but rather is largely determined by whether a species continues to reproduce when starved (Kirk, 1997a). *Keratella* reduces fecundity during starvation to only 6% of that at high food. In contrast, *Synchaeta* actually increases fecundity during starvation to 158% of fed controls. *Brachionus* is intermediate and reduces fecundity during starvation to 44% of fed controls (Kirk, 1997a).
Stock cultures of rotifers and phytoplankton were grown in modified MBL medium (Stemberger, 1981). All cultures and experimental containers were kept in an incubator at 20°C, under fluorescent illumination with a photoperiod of 16 h light : 8 h dark. To prevent significant phytoplankton growth during the experiments, illumination was kept to a low intensity (5 μmol photons m−2 s−1 of photosynthetically active radiation). When used as food in the competition experiments, the concentration of Cryptomonas cells in rapidly growing cultures was determined by in vivo fluorescence using a calibrated Turner model 450 fluorometer (Turner Designs, Sunnyvale, CA, USA). Cultures were then diluted with 0.22 μm-filtered MBL to the desired mass concentration, using the known dry mass of this strain of Cryptomonas (8.8 × 10^{-5} μg cell^{-1}) (Stemberger & Gilbert, 1985).

Competition experiments were conducted in tall glass beakers containing 250 mL of medium. The contents of the beakers were periodically stirred (200 rev. min^{-1} for 8 s every 4 min) with Teflon-coated raised magnetic stir bars. Medium and phytoplankton food were renewed every 2 days in the following manner. Rotifers were removed by filtering the contents of a beaker through 25-μm nylon mesh. Rotifers were saved in a small volume of medium. Seventy percent (175 mL) of the beaker contents was discarded and replaced with fresh medium containing the desired concentration of phytoplankton food. Rotifers were then placed back into the beaker. As all rotifers were replaced, the food renewal method did not impose an extrinsic mortality rate on the rotifers. The contents of the beakers were placed into clean beakers every 10 days.

There were three temporal variation treatments (Fig. 2). Temporal variability was defined by the period (T) of fluctuations in food renewal concentration. Where T = 0, medium containing a food concentration of 1 μg dry mass mL−1 was added every 2 days. Where T = 4 days, medium was added every 2 days at alternating food concentrations of 2 μg mL−1 and zero (0.22 μm-filtered medium). Where T = 8 days, medium was added containing 2 μg mL−1 food for 2 days, zero food for 2 days and then the pattern was repeated. All treatments resulted in the same total

Fig. 1 Illustration of the gleaner versus opportunist trade-off that exists between the three species of rotifers used in the competition experiments. R* is the threshold food concentration. r_{max} is the maximum population growth rate. Symbol diameter is proportional to mean starvation time (data from Kirk, 1997a). Note the lack of a trade-off between starvation time and either R* or r_{max}. R* and r_{max} data for Brachionus and Keratella are from Stemberger & Gilbert (1985). The r_{max} value for Synchaeta is from observations of population growth rate at high food concentrations (K.L. Kirk, unpublished data). The R* value for Synchaeta was estimated using the allometric equation given in Stemberger & Gilbert (1985).

Fig. 2 Temporal pattern of food supply for the three treatments. T is the period of food addition, in days. Food was added every 2 days at the concentration indicated on the vertical axis (see Methods).
amount of food being added to the beakers over the long-term.

Two pairs of species were tested against each other: *Keratella* versus *Brachionus*, and *Keratella* versus *Synchaeta*. In the *Keratella* versus *Brachionus* experiment, there were two food renewal treatments: \( T = 0 \) and 4 days. In *Keratella* versus *Synchaeta* experiment, there were three food renewal treatments: \( T = 0 \), 4 and 8 days. Control beakers contained a single species. There were two replicate control beakers for each species in the *Keratella* versus *Brachionus* experiment, and one control beaker for each species in the *Keratella* versus *Synchaeta* experiment. The sole purpose of the control beakers was to test whether the species could persist in a given food renewal treatment. The control beakers were not statistically compared with the competition beakers to determine the existence of competition; as there was only one food source, resource competition was inevitable. There were two replicate competition beakers for each food renewal treatment in the *Keratella* versus *Brachionus* experiment, and three replicate competition beakers in the *Keratella* versus *Synchaeta* experiment. Competition beakers were initiated by adding roughly equal biomass levels of each of two rotifer species. Control beakers were initiated by adding the same biomass of only one species. Rotifer abundance was determined every 3–6 days in the *Keratella* versus *Brachionus* experiment, and every 10 days in the *Keratella* versus *Synchaeta* experiment. After stirring, 20% of the contents of each beaker was filtered through a 25-μm mesh net, and the rotifers in this sample were counted under a dissecting microscope and then placed back into the beaker. If the abundance of a species dropped to zero in the 20% sample, its absence was confirmed by examining the entire contents of the beaker. In a community consisting of two species, the Shannon diversity index (\( H' \)) indicates the degree of evenness of abundance (Stiling, 1996). Shannon diversity index was calculated using the biomass of each species in the competition beakers.

**Results**

In the *Keratella* versus *Brachionus* experiment, population dynamics in the control beakers demonstrate that both *Keratella* and *Brachionus* were able to persist under both resource supply treatments (Figs 3a,b). In the competition beakers where \( T = 0 \), *Brachionus* was driven extinct in both replicate competition beakers in 14–18 days (Figs 3c,d). Under conditions of greater variability in resource supply (\( T = 4 \)), *Brachionus* persisted in one replicate beaker, although at low and declining abundance (Fig. 3e), and went extinct in the other replicate beaker in 24 days (Fig. 3f). The dynamics of the biomass-based \( H' \) illustrates the difference in competitive dynamics in the two treatments. \( H' \) declined at a faster rate when \( T = 0 \) than when \( T = 4 \) (Fig. 4). The time taken for \( H' \) to decline to the arbitrary value of 0.4 (using linear interpolation between time points) was used as an index of the rate of loss in community evenness. This value increased when \( T \) increased from 0 to 4 (Fig. 5a), but the trend
was not statistically significant (t-test, $P = 0.35$). In summary, increasing the temporal variability of resource supply by increasing $T$ from 0 to 4 days did not change the outcome of competition (Keratella always dominated) and did not allow coexistence.

In the Keratella versus Synchaeta experiment, the dynamics in the control beakers demonstrate that both species were able to persist under the three resource supply treatments (Fig. 6a-c). Synchaeta went extinct in all competition beakers regardless of the level of temporal variability (Fig. 6d–l). However, temporal variability did change the dynamics of competition. Evenness ($H'$) declined more slowly when $T = 8$ than when $T = 0$ or 4 days (Fig. 4). The time taken for $H'$ to decline to 0.4 was the highest at $T = 8$ (Fig. 5a) (one-way ANOVA, $P < 0.01$). In addition, competitive exclusion of Synchaeta took longer at $T = 8$ than at $T = 0$ or 4 days (Fig. 5b) (one-way ANOVA, $P < 0.01$). In summary, increasing the temporal variability of resource supply by increasing $T$ from 0 to 8 days slowed the rate of competitive exclusion of Synchaeta but did not change the outcome of competition (Keratella always won) and did not allow coexistence.
Discussion

The three rotifer species used in these experiments, and planktonic rotifers in general, exhibit a strong gleaner–opportunist trade-off (Fig. 1; Stemberger & Gilbert, 1985). Theories predict that a gleaner–opportunist trade-off can make it possible for environmental variability to change competitive interactions (Grover, 1997). Contrary to predictions, temporal variation in resource supply did not change competitive outcome or allow coexistence of competing rotifer species. One possible reason is that the environment was not sufficiently variable. It is possible that longer periods of variation in resource supply would have favoured Brachionus and Synchaeta. At higher $T$-values, the time periods when resource level is relatively high would be longer, potentially giving an advantage to species with higher $r_{max}$ values. However, the time periods when resource abundance is very low would also be longer, giving the species with the longest starvation time (Keratella) an advantage.

The method of food supply used in these experiments was somewhat different from that adopted previously. MacIsaac & Gilbert (1991) increased temporal variability by adding food less frequently but in pulses of increasing concentration. This results in a confounding of the period and amplitude of food renewal. When resources are added as infrequent pulses of very high concentration, this gives an advantage to opportunistic species. The method used here, in which food renewal concentration did not increase as period ($T$) increases from 4 to 8 days, results in less confounding of period and amplitude. Some confounding still exists, because food renewal concentration increases as $T$ increases from 0 to 4 days (Fig. 2). The lower peak resource concentrations used here would give less of an advantage to opportunistic species. This may be one reason why Brachionus and Synchaeta did not dominate even at high periods of variation in food supply.

As food was renewed every 2 days, rotifer grazing must have caused fluctuations in food concentration with a period of 2 days in all treatments. It is unlikely that a less variable environment, for example where food is added continuously as in a chemostat, would have changed the competitive outcome. Under steady state conditions, the species with the lowest $R^*$, Keratella, would probably have won.

Ideally, laboratory experiments should use patterns of temporal variation in resources that are similar to those experienced by organisms in nature. Unfortunately, the time scales of variation in resource abundance in nature are incompletely understood for any organism. Cryptomonad phytoplankton are a highly nutritious food for planktonic rotifers (Stemberger, 1981), and cryptomonad abundance can change rapidly in nature on time scales of a few days to a few weeks (Stewart & Wetzel, 1986). The results of food limitation experiments provide another index of phytoplankton availability. When natural rotifer populations are given high quality foods to supplement the phytoplankton available in nature, the population growth rate of rotifers usually increases (González & Frost, 1992; Merriman & Kirk, 2000). If adding
supplemental foods causes a large increase in rotifer population growth rate, then food limitation is intense and the availability (abundance and nutritional quality) of natural phytoplankton is low. The results of food limitation experiments indicate that phytoplankton availability can change significantly in only 1 week (Merriman & Kirk, 2000). Thus, the scale of temporal variation of resource supply used in these experiments (4–8 days) may have been similar to the scale of variation of phytoplankton resources in nature.

In addition to the temporal scale of resource variation, the amplitude of resource variation may also be important. It is likely that the maximum food supply concentration used in these experiments (2 μg mL\(^{-1}\)) was sufficient to saturate the numerical responses (i.e. maximise the population growth rates) of all three rotifer species. The resource concentration necessary to saturate the numerical response of *K. cochlearis* is about 1 μg mL\(^{-1}\) (Stemberger & Gilbert, 1985). Rothhaupt (1988) found that *B. calyciflorus* required about 2 μg mL\(^{-1}\), although Stemberger & Gilbert (1985) found that *B. calyciflorus* requires > 5 μg mL\(^{-1}\). The details of the numerical response of the species of *Synchaeta* used here is unknown, but Stemberger & Gilbert (1985) found that another small *Synchaeta* species, *S. oblonga* Ehrenberg, required about 1 μg mL\(^{-1}\) to maximise its population growth rate.

As predicted, increasing temporal variation in resource supply slowed the rate of competitive exclusion in the *Keratella* versus *Synchaeta* experiment. The gleaner–opportunist trade-off may have contributed to the slower rate of exclusion and decline in evenness under conditions of greater resource variation. *Synchaeta* has a higher \(r_{\text{max}}\) than *Keratella* (Fig. 1) and may have been able to exploit periods of high food abundance to delay its population decline.

The results of the competition experiments also confirm another theoretical prediction about the rate of competitive exclusion. Mechanistic resource competition theory predicts that the rate of competitive exclusion will decrease as the difference between the competitive ability (\(R^*\)) of two species decreases (Grover, 1997). When the environment is constant a pair of species with very similar \(R^*\) values will be able to coexist for a longer period of time than will a pair of species with very different \(R^*\) values. The difference in \(R^*\) values of *Keratella* and *Brachionus* is 0.22 μg mL\(^{-1}\), while the difference between \(R^*\) values of *Keratella* and *Synchaeta* is only 0.04 μg mL\(^{-1}\) (Fig. 1). In the treatment with the least temporal variation in food supply (\(T = 0\)), *Brachionus* went extinct in less time than did *Synchaeta* (Fig. 5b) (t-test, \(P < 0.01\)). In addition, evenness (\(H^'\)) declined at a faster rate in the *Keratella* versus *Brachionus* experiment than in the *Keratella* versus *Synchaeta* experiment (Fig. 4). The time taken for \(H^'\) to decline to 0.4 was shorter in the *Keratella* versus *Brachionus* experiment than in the *Keratella* versus *Synchaeta* experiment (Fig. 5a) (t-test, \(P < 0.01\)). Thus, as predicted, the rate of competitive exclusion was slower in the species pair with the smaller difference in \(R^*\) values.

In addition to \(R^*\) and \(r_{\text{max}}\), another characteristic that is important to success in variable environments is the ability to store resources internally and use them during periods of external resource scarcity. Sommer (1984) speculated that species with a high resource storage capacity may function as storage specialists. Because storage specialists allocate a relatively large portion of their assimilated resources to internal stored reserves, they are expected to have relatively high \(R^*\) and low \(r_{\text{max}}\) values (Sommer, 1984; Grover, 1997). Thus, the ability to be a storage specialist may trade-off against competitive ability at steady state and also against the ability to rapidly respond to transient peaks in resource abundance. Theories based on the Droop model of phytoplankton growth kinetics predict that storage capacity will often be critical to success in variable environments (Grover, 1991b).

The storage capacity of rotifers has been estimated as the fraction of total body mass that can be lost during starvation (Kirk *et al*., 1999). The storage capacity of *Brachionus* is 71% of body mass, while that of *S. pectinata* is only 42% of body mass. The storage capacity of the *Synchaeta* species used in these experiments is unknown, as is the storage capacity of *Keratella*. However, the starvation time of all three rotifer species used in the competition experiments is known (Kirk, 1997a). As storage capacity is positively correlated with starvation time (Kirk *et al*., 1999), starvation time can be used as an index of storage capacity. *Keratella* has a longer starvation time than either *Brachionus* or *Synchaeta* (Fig. 1), and so *Keratella* may have the greatest capacity to store resources. Contrary to expectations, in planktonic rotifers there is no evidence of a trade-off between storage capacity
and either $R^*$ or $r_{\text{max}}$ (Kirk et al., 1999), nor is there any evidence of a trade-off between starvation time and either $R^*$ or $r_{\text{max}}$ (Kirk, 1997a). In the competition experiments reported here, Keratella always won under all conditions of variation in resource supply. Thus, the winning species had both the lowest $R^*$ and the highest storage capacity, while the losing species had higher $r_{\text{max}}$ values. Keratella’s low $R^*$ gave it an advantage when food was supplied frequently, while its high storage capacity gave it an advantage when there was greater temporal variation in food supply. Thus, these experiments confirm the importance of resource storage in determining success in environments with variable resource levels.

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References


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