Hydrobiologia **387/388:** 349–353, 1998.
E. Wurdak, R. Wallace & H. Segers (eds), Rotifera VIII: A Comparative Approach.

Population growth in planktonic rotifers. Does temperature shift the competitive advantage for different species?

Claus-Peter Stelzer
Max-Planck-Institut für Limnologie, P.O. Box 165, D-24306 Plön, Germany

Key words: competition, threshold food level, Rotifera, temperature, *Brachionus*, *Synchaeta*

Abstract

The numerical response of populations to different food concentrations in an important parameter to be determined for a mechanistic approach to interspecific competition. Theory predicts that the species with the lowest food level (TFL) should always be the superior competitor if only one food source is offered. However, TFLs are not species specific constants but may change along environmental gradients such as food size or temperature.

The hypothesis that temperature differentially affects the TFLs of three planktonic rotifers (*Asplanchna priodonta, Brachionus calyciflorus* and *Synchaeta pectinata*) was tested in laboratory experiments. Numerical responses were assessed for all three rotifers at 12, 16, 20, 24 and 28°C with *Cryptomonas erosa* as food alga. Growth rates of all three rotifers at high food concentrations (1 mg C l^{-1}) increased as temperature increased until the limits of thermal tolerance were reached. This increase was very pronounced for *Brachionus*, but less for *Synchaeta* which already had relatively high growth rates at 12°C. Along the temperature gradient, the TFLs of *Synchaeta* increased from 0.074 to 0.66 mg C l^{-1}, whereas those of *Asplanchna* and *Brachionus* stayed relatively constant at 0.3 and 0.2 mg C l^{-1}, respectively. Hence, the zero net growth isocline (ZNGI) of *Synchaeta* crossed those of *Brachionus* and *Asplanchna* at 16 and 20.5°C, respectively. The results suggest that *Synchaeta* is better adapted to low temperatures than the other two rotifers and should be the superior competitor below 16°C.

Introduction

Resource limitation is regarded as one of the most important factors for structuring plankton communities (Gliwicz, 1985). According to the 'threshold hypothesis' (Lampert, 1977; Lampert & Schober, 1980) the superior species in a resource limited environment is the one with the lowest food requirement needed to maintain growth (threshold food level, TFL). Lampert & Schober (1980) distinguished between a threshold for the individual and a threshold for a population.

Threshold food levels in rotifers have usually been determined on the population level and are defined as the food concentration at which population growth is zero. Stemberger & Gilbert (1985) showed that TFLs in eight planktonic rotifer species varied by a factor of 17 and that small rotifers tend to have lower TFLs than large rotifers. However, TFLs are not species-specific constants and may change along various environmental gradients. For example Rothhaupt (1990) found considerable differences in the TFLs of two *Brachionus* spp. along a gradient of food size, with the lowest TFLs for food algae in the most readily ingested size range for the respective species. Achenbach & Lampert (1997) determined TFLs of four cladoceran species along a temperature gradient from 16–28°C and found an increase in the TFLs above 20°C for all species. This did not change the competitive abilities among the different species because the species with the lower TFLs at 16 and 20°C still had the lower TFLs at 24 and 28°C.

The aim of this paper is to investigate how the population growth and the TFLs of three different planktonic rotifers (*Asplanchna priodonta, Brachionus calyciflorus, Synchaeta pectinata*) change along a temperature gradient of 12–28°C. According to their maximum abundance in the field, the three species may have different thermal preferences. *Synchaeta* and *Asplanchna* are most abundant at 12 and 15°, respectively, and *Brachionus* at 20°C (Berzins & Pejler, 1989). The hypothesis tested was that the 'cold wa-

ter' rotifers have lower TFLs than the 'warm water' rotifers at low temperatures and vice versa.

Materials and methods

The rotifers *Asplanchna priodonta* and *Synchaeta pectinata* were isolated from Schöhsee (northern Germany) and cultured as clones. *Brachionus calyciflorus* was obtained from K.O. Rothhaupt and is identical to the *B. calyciflorus* used in his work (e.g., Rothhaupt, 1990).

All rotifers were raised on *Cryptomonas erosa* var. *reflexa*, which was obtained from J.J. Gilbert, New Hampshire. In all experiments the rotifers were cultured in ADaM medium (Klüttgen et al., 1994a, b), which was supplemented with 2.2 mg l^{-1} Na_2EDTA and Woods Hole MBL (Guillard, 1975) medium (9:1) to improve the conditions for the *Cryptomonas* food (Kirk & Gilbert, 1990). *C. erosa* was cultured in MBL medium in semicontinuous culture (dilution rate = 0.25 day^{-1}) and was continuously illuminated (100 μmol quanta m^{-2} s^{-1}). The carbon content of *Cryptomonas erosa* cells was determined in a C/N analyser (NA 1500, Carlo Erba). Samples were filtered on precombusted (5 h at 500°C) glass-fibre filters (Whatman GF/F) and dried overnight. No corrections for associated bacteria were made. Food concentrations were determined with an electronic particle counter (CASY).

The experimental treatments were combinations of five different temperatures (12, 16, 20, 24, and 28°C) and various food concentrations ranging from 0.075 to 3 mg C l^{-1}. At least three replicates were used for each combination of food and temperature.

Populations of rotifers were acclimated to the experimental temperatures for at least 2 weeks in 2-l or 3-l Erlenmeyer flasks at high food concentrations (3 mg C l^{-1}). During the acclimation phase, the rotifers were cultured in fed batch cultures and medium was replaced every 4–5 days by filtration through 30-μm gauze, except for the 24 and 28°C treatments, where the replacement interval was 2 days. At these temperatures there was rapid population growth and enhanced grazing particularly by *Brachionus*, so that the algae were depleted much faster than at the lower temperatures. In the experiments, the rotifers were cultured in 100-ml glass test tubes sealed with a double layer of Parafilm (American Can Co.). The test tubes were placed on plankton wheels, rotating every 15 min for 2 min at 1 rpm, and set in an incubator with low illumination (5 μmol quanta m^{-2} s^{-1}). Every day, the food algae and medium in each test tube were exchanged by filtration with a Plexiglas tube covered with a 30-μm gauze. The tube was outfitted with a device which permitted a low flow rate and left 10 ml of medium in the bottom of the tube, so that the rotifers would not become stuck to the gauze. The tube was refilled with 90 ml of fresh ADaM to ensure that most of the old algae were removed. The rotifers were then poured into a new test tube which was filled with medium and *Cryptomonas* in the desired concentrations.

After 3–4 days of acclimation to the different food concentrations, population growth was measured for another 3–4 days. Initial population sizes during acclimation were around 50 to 100 animals per test tube (100 ml), depending on expected growth at the various food concentrations. Visual inspections were made to ensure that the populations did not exceed 150–200 rotifers per test tube to prevent overgrazing during the acclimation period. At the start of the growth measurements, 50–100 rotifers were selected randomly from the acclimated populations and transferred into new test tubes. When high clearance rates were expected (low food concentrations combined with high temperatures) the initial concentration of rotifers was always 0.5 ind. ml^{-1}. On the following days, samples of ~40% volume were taken from these experimental populations, fixed with Lugol's solution, transferred to 50-ml sedimentation chambers, and enumerated under an inverted microscope. In cases of very slow population growth at low temperatures and low food concentrations samples were taken at 2-day intervals. The remaining 60% of rotifer culture medium was exchanged in the way described above. At the end of the growth measurements, all animals of one test tube were concentrated to 50 ml, fixed and enumerated. Threshold food levels were defined as the intersections of the regression lines fitted through near-zero growth rates with the x-axis (food concentration). Intersections of the 95% confidence limits with the x-axis were calculated as 'fiducial limits' according to Draper & Smith (1980).

Previous tests with all three rotifer species at 20°C showed that no animals got lost or damaged during the filtration procedure and that the population growth rates obtained by this method were comparable to those obtained by other methods (e.g., Rothhaupt, 1990). During the experiments it became obvious that the growth rates of *Synchaeta* at the low temperatures (12 and 16°C) were underestimated by the above de-

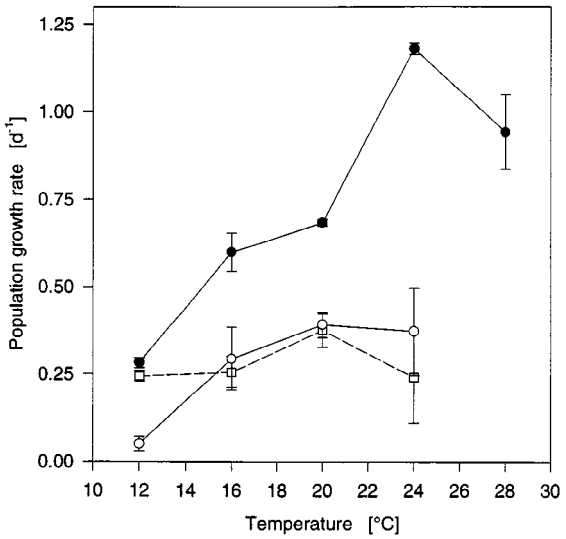

Figure 1. Growth rates of *Asplanchna priodonta* (circles), *Brachionus calyciflorus* (dots) and *Synchaeta pectinata* (squares) at a food concentration of 1 mg C l^{-1} and at different temperatures. Means and S.D.s (n=3).

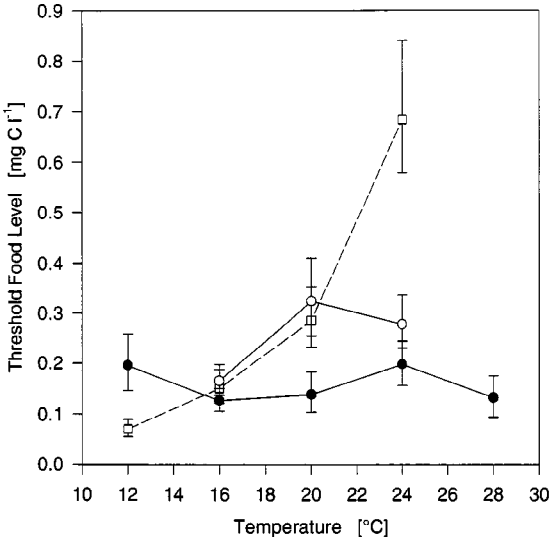

Figure 2. Threshold food levels of *Asplanchna priodonta* (circles), *Brachionus calyciflorus* (dots) and *Synchaeta pectinata* (squares) at different temperatures. TFLs are the intersections of the regression lines fitted through near-zero growth rates with the x-axis (food concentration) and error bars are the 95% fiducial limits.

scribed method. This was most likely because some of the eggs, which were laid freely into the culture medium, were found stuck to the walls of the culture vessel. Hence some of these eggs may have been lost during the daily medium exchange, and this effect must have been more pronounced at the low temperatures because egg development times are longer. Thus it was necessary to culture *Synchaeta* in six-well polystyrene culture plates (containing 8 ml medium), with daily transfers of all rotifers and eggs via glass pipettes (Stemberger & Gilbert 1985). To prevent overgrazing, not more than 30 rotifers were cultured per well. If necessary, the numbers of rotifers were reduced and care was taken that the egg ratio was not changed by this procedure.

Results

The carbon content of *Cryptomonas erosa* was 219 ± 8.7 pg/cell (n=12) and the average cell volume was 885 μm^3.

Brachionus was able to grow at all of the experimental temperatures, whereas *Synchaeta* and *Asplanchna* could not be cultured at 28°C for long time periods. Even at food concentrations above 3 mg C l^{-1} the populations died after some days. No numerical response curves could be assessed for *Synchaeta* and *Asplanchna* at 28°C.

In all three rotifer species, population growth rates at 1 mg C l^{-1} generally increased with temperature, apart from slight decreases in *Brachionus* above 24°C and *Synchaeta* above 20°C (Figure 1). For *Brachionus* a 4.2-fold increase in the population growth rate was found from 12 to 24°C. In *Asplanchna* the increase in population growth was highest between 12 and 16°C (5.9-fold), lower between 16 and 20°C (1.3-fold), and zero between 20 and 24°C. *Synchaeta* had a relatively high growth rate at 12°C and its increase with temperature (1.5-fold from 12 to 20°C) was not as pronounced as in *Brachionus* or *Asplanchna*.

The TFL of *Asplanchna* was 0.3 mg C l^{-1} at 20 and 24°C, but lower (0.16 mg C l^{-1}) at 16°C (Figure 2). At 12°C population growth rates in *Asplanchna* were not significantly different from zero (t-test, P>0.05) for the tested food concentrations (0.3–3mg C l^{-1}), hence no TFL could be calculated. The significantly positive value for the growth rate at 1 mg C l^{-1} (Figure 1) is probably an artefact of the small number of replicates. For *Brachionus* the TFLs stayed relatively constant between 0.15 and 0.2 mg C l^{-1} along the investigated temperature gradient. The small differences between the TFLs were not significant as can be seen from the overlapping 95% fiducial limits. The TFLs of *Synchaeta* increased dramatically with temperature, from 0.074 mg C l^{-1} at 12°C to 0.66 mg C l^{-1} at

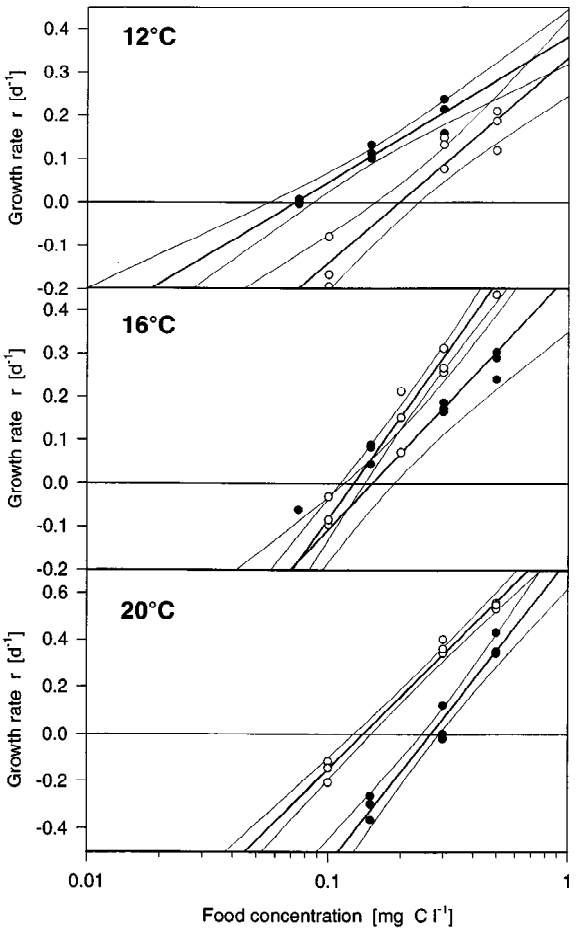

Figure 3. Population growth rates near the threshold food levels of *Brachionus calyciflorus* (circles) and *Synchaeta pectinata* (dots) at 12, 16 and 20°C. Regression lines (thick lines) and 95% confidence intervals (thin lines).

24°C. Along its increase from 12 to 24°C the zero net growth isocline (ZNGI) of *Synchaeta* intersected the ZNGIs of the other two rotifers. The intersections were at 16°C for *Synchaeta–Brachionus* and at 20.5°C for *Synchaeta–Asplanchna*. Below these intersections *Synchaeta* had the lower TFLs for *Cryptomonas* and above them the other rotifer had the lower TFLs. This situation is shown in Figure 3 for *Synchaeta* and *Brachionus*.

Discussion

Growth rates measured in this study are comparable to those found in previous studies (e.g., Rothhaupt, 1990; Seale et al., 1993; see Stemberger & Gilbert, 1985). Since most of the previous studies have been conducted at temperatures around 20°C, comparisons are restricted to these temperatures. Population growth of *Brachionus calyciflorus* is most comparable to the work of Rothhaupt (1990), since the rotifers originate from the same clone. Both, TFL and maximum growth rates (determined at 1 mg C l^{-1} in this study) are consistent with his results for *Chlamydomonas sphaeroides* (an alga of comparable size to *Cryptomonas erosa*). Assuming a 50% carbon content of dry weight, comparisons can be made to the data in Stemberger & Gilbert (1985). The TFLs of *Synchaeta pectinata* and *Asplanchna priodonta* are consistent with their data, whereas the growth rates of *Synchaeta* at 1 mg C l^{-1} and 20°C were about 50% lower than theirs. These differences are most likely clone specific, since food algae and culture methods for *Synchaeta* were identical with those in Stemberger & Gilbert's work.

Growth rates at the relatively high food concentration of 1 mg C l^{-1} generally increased with temperature (Figure 1). As rotifers are ectothermic organisms their metabolism is directly exposed to the temperature of their environment, hence the speed of biochemical reactions is enhanced at higher temperatures. The decrease in the growth rates at the highest experimental temperatures may have occurred because the upper limit of thermal tolerance was reached. Additionally, in *Synchaeta* it may also be an effect of the high TFL at 24°C (Figure 2). The relatively high growth rates of *Synchaeta* at 12°C and their small increase with temperature support the hypothesis that this rotifer is more adapted to cold temperatures than *Asplanchna* and *Brachionus*.

The most surprising result of this study was the strong increase of *Synchaeta* TFLs with temperature, leading to a ZNGI that intersected the ZNGIs of the other two rotifer species. According to theory of exploitative competition such intersections are points where superiority can switch between two competitors (Tilman, 1982). Tilman et al. (1981) demonstrated this for two diatoms, whose ZNGIs (at a dilution rate of 0.11 day^{-1}) intersected at one point along a temperature gradient. Below 20°C *Asterionella formosa* had lower requirements for silicate to maintain population growth than *Synedra ulna*, whereas above 20°C the situation was reversed. In competition experiments Tilman et al. (1981) showed that *Asterionella* displaced *Synedra* below 20°C and that *Synedra* displaced *Asterionella* above 20°C. Similar changes in competitive superiority may also occur if pairs of the rotifers used in this study were to compete at different

temperatures for their food *Cryptomonas*. The order of superiority should be: *Synchaeta* > *Brachionus* > *Asplanchna* below 16°C, *Brachionus* > *Synchaeta* > *Asplanchna* between 16 and 20°C and *Brachionus* > *Asplanchna*>*Synchaeta* at 24°C. It may be difficult to test these predictions for *Asplanchna priodonta*, since this species is an omnivore and can also prey on juveniles of *Synchaeta* or *Brachionus* (Pourriot, 1977).

The competitive relationships between *Brachionus* and *Synchaeta* determined in laboratory can apply in field, but under special circumstances since both species can use other food sources than *Cryptomonas*. *Brachionus calyciflorus* can use a very broad array of food particles such as bacteria (Starkweather et al., 1979), many different kinds of planktonic algae and even small ciliates (Gilbert & Jack, 1993). *Synchaeta pectinata* seems to be restricted to large particles like *Cryptomonas erosa* or some ciliates (Gilbert & Jack, 1993). Hence, overlap in the diets of the two rotifer species is relatively small and competitive interactions in the field can only be expected for particular food conditions like dominance of cryptomonads.

In conclusion, the results of this study suggest that temperature can shift the competitive advantage for the rotifers *Synchaeta pectinata* and *Brachionus calyciflorus*. However, only well-controlled competition experiments can test the robustness of these findings. Thus this problem needs further study.

Acknowledgements

I thank M. Brewer and N. Weider for constructive comments on the manuscript and linguistic help. This work was supported by DFG Grant LA 309/12–1.

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