Limnol. Oceanogr., 47(5), 2002, 1447–1455
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Interactive effect of temperature and food concentration on growth rate: A test case using the small freshwater ciliate *Urotricha farcta*

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Abstract

The combined effect of temperature and food concentration on the growth rate, cell volume, and production of the freshwater ciliate *Urotricha farcta* was investigated in laboratory batch cultures. Experimental temperatures ranged from 9 to 25°C and food levels ranged from 0.1 to 4.4 μg C ml^{-1}. The ciliates were fed the small cryptophyte *Cryptomonas* sp. The combined effect of temperature and food on growth and cell volume resulted in negative production rates at high temperatures and low to moderate food supply. Three main changes were observed in the shape of the numerical response (growth vs. food concentration) of *U. farcta* with temperature: change in the threshold level, where population net growth rates are zero; change in the initial slope of the numerical response curve; and change in maximum growth rate (μ_{max}). The threshold food concentration and μ_{max} were shifted up at the highest temperatures. The threshold level was also higher at the lowest experimental temperature. The initial slope of the numerical response curve was several-fold lower at both high and low temperatures. The analysis suggests that temperature altered the numerical response so that the species shifted from being adapted to low food concentrations at moderate temperatures to requiring, and potentially thriving at, high food concentrations at the temperature extremes. These findings support and extend conclusions previously obtained for metazooplankton and indicate that changes as small as 3°C could alter the role of protozoa in planktonic food webs.

Temperature and food are significant environmental factors that determine the abundance and productivity of plankton. The reaction norm of many proto- and metazoa has been studied in detail in response to each of these factors independently (*see* DeMott 1989; Montagnes 1996; Moore et al. 1996; Weisse and Montagnes 1998). Recently, interest in long-term temperature effects has been renewed because of concerns regarding global warming (Moore et al. 1996; Mitchell and Lampert 2000; Giebelhausen and Lampert 2001). Short-term local temperature changes of 2–3°C, however, also occur, especially in small, shallow water bodies in which water temperature closely follows changes in air temperature.

It might be assumed that a temperature increase will lead to increased productivity because rate processes are classically considered to increase with temperature (Bělehrádek 1935). However, food levels that support growth at low temperatures might be inadequate at high temperatures (Muck and Lampert 1984; Orcutt and Porter 1984; DeMott 1989). Thus, interaction between increased temperature and food resources can produce a counterintuitive decrease in plankton productivity (Lampert 1977*a*). In this paper, we use the small freshwater ciliate *Urotricha farcta* to examine the combined effect of food concentration and ambient temperature on growth, cell size, and production. This ciliate is widespread (Foissner et al. 1999) and has been used as a model organism in several ecophysiological studies (Weisse and Montagnes 1998; Montagnes and Weisse 2000; Weisse et al. 2001).

The response of growth rate to food concentration typically follows a rectangular hyperbolic numerical response (Fig. 1). This curve has three main attributes: (1) the food concentration where population growth equals mortality (the threshold level x'); (2) the maximum growth rate that reaches an asymptote at high prey levels (μ_{max}); and (3) the rate at which the growth rate approaches this maximum level, indicated by the initial slope of the curve (α). These important ecophysiological parameters can be used to assess the competitive ability of zooplankton (Lampert 1977*b*; Stemberger and Gilbert 1985; Gliwicz 1990; Montagnes 1996).

Threshold values of several major taxonomic categories of planktonic herbivores have been reviewed, and predictions have been made concerning their consequences for planktonic seasonal succession (Schiemer 1985; DeMott 1989; Duncan 1989; Walz 1995). *Daphnia*, for instance, are considered superior competitors to rotifers in lakes, mainly because they have lower threshold values than rotifers (DeMott 1989; Duncan 1989; Walz 1995); that is, they are better able to exploit low food concentrations typical of summer levels in many temperate, moderately eutrophic lakes. Maximum growth rates are also extensively used to estimate the potential zooplankton biomass and production in many

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Acknowledgments

Laboratory work was conducted at the Institute for Limnology of the Austrian Academy of Sciences, and some data analysis was conducted at Port Erin Marine Laboratory; we appreciate the use of these facilities. This research was partially supported by a travel grant from the Royal Society awarded to D.J.S.M., and by a NERC studentship and University of Liverpool travel funds awarded to S.A.K.

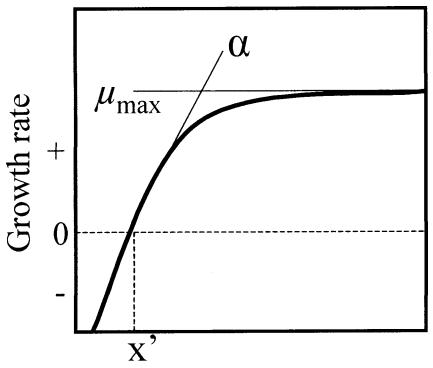

Fig. 1. The numerical response (growth rate vs. food concentration) indicating x', the point where population growth equals mortality (the threshold level); μ_{max}, the maximum growth rate that is predicted from the asymptote at high prey levels; and α, the rate at which growth rate approaches this maximum level (i.e., the initial slope of the curve at x').

systems. For instance, predictive equations have been developed to estimate maximum growth rates of ciliates of a given size at a given temperature (e.g., Montagnes 1996 and references therein). Likewise, the half saturation constant and the initial slope of the numerical response (Stemberger and Gilbert 1985; Rothhaupt 1990) are indications of an organism's ability to exploit low food concentrations; maximizing the ability to respond to low food levels has been used to predict competitive advantage between species (e.g., Stemberger and Gilbert 1985; Rothhaupt 1990).

All three of these aspects of the numerical response can be combined to compare the selective advantage of different taxa on a persistence versus opportunistic strategy continuum (Taylor 1978), analogous to the r/K selection theory (MacArthur and Wilson 1967; Stemberger and Gilbert 1985; Rothhaupt 1990; Walz 1993, 1995). That is, a persistent species, adapted to low food concentrations, would predictably have a low threshold concentration (x'), a rapid increase to its maximum growth rate (α), and a low maximum growth rate (μ_{max}). In contrast, an opportunistic species, adapted to high food levels, would possess a high x', a low α, and a high μ_{max}.

The persistence versus opportunistic and r/K concepts generally assume that the numerical response is species specific. However, the numerical response can alter with changing environmental conditions, resulting in an intraspecific acclimation. Temperature, for instance, might affect not only μ_{max} (Montagnes 1996; Weisse and Montagnes 1998) but also the x' and α, thereby shifting the position of the species along the persistence versus opportunistic continuum.

In spite of the wealth of information on the effect of temperature and food on growth, the combined effect of these factors has been little studied for metazoa and virtually ignored for planktonic protozoa. Significant temperature–food interactions have been found for several *Daphnia* and rotifer species (Orcutt and Porter 1984; Achenbach and Lampert 1997; Stelzer 1998; Giebelhausen and Lampert 2001); for example, threshold concentrations increased with increasing temperature in both cladocerans (Orcutt and Porter 1984; Achenbach and Lampert 1997) and rotifers (Stelzer 1998). However, few if any studies have examined the entire shape of the numerical response, as outlined above. Partially, this is because of the difficulties of working with organisms that have long generation times.

Ciliates, with generation times on the order of hours, are model organisms to use to test such hypotheses related to the influence of environmental factors on population growth. Furthermore, both empirical data (Müller et al. 1991; Macek et al. 1996; Weisse and Müller 1998) and predictions from carbon flow models (Gaedke and Straile 1994; Straile 1998) suggest that, in many temperate lakes, ciliates are food-limited in the warmer summer months, but we lack data to properly parameterize these arguments. Thus, there is a specific need for such studies on protozoa.

Earlier work has indicated significant interactive effects between temperature and food concentration on the growth rate of the common planktonic ciliate *Urotricha farcta* (two-way ANOVA, Kimmance 2001). The present study builds on these initial findings by assessing the combined effect of temperature and food on growth parameters using nonlinear curve-fitting methods; we then extend the results to make predictions about other planktonic groups. Specifically, we investigated whether temperature altered the threshold level (x'), the initial slope of the numerical response (α), and the maximum growth rate (μ_{max}). We then extend this work to assess the effect of temperature and food concentration on cell volume and production (i.e., the product of growth rate and cell volume).

Material and methods

Study organisms—The prostomatid ciliate *Urotricha farcta* (live cell size 20–30 × 15–20 μm, Foissner et al. 1999) was isolated from mesotrophic Lake Schöhsee, Germany (Weisse and Montagnes 1998). This species is common in temperate and subtropical ponds, lakes, and rivers. It occurs throughout the year and is abundant in oligotrophic, mesotrophic, eutrophic, and hypertrophic waters (*see* Foissner et al. 1999; Weisse et al. 2001).

The prey species used was *Cryptomonas* sp. strain 26.80 ($\sim$11 × 7 μm, average cell volume $\sim$280 μm^3, Weisse and Kirchhoff 1997) and was obtained from the Culture Collection of Algae in Göttingen (Germany). This flagellate has been used as a standard food in experiments with prostome ciliates (Müller and Geller 1993; Weisse and Montagnes 1998; Müller and Schlegel 1999; Montagnes and Weisse 2000). Both the ciliate and prey were maintained in modified Woods Hole medium (MWC medium, Guillard and Lorenzen 1972) at 15 ± 1°C. Ciliate cultures were not axenic, but *U. farcta* does not feed on bacteria if suitable flagellates are abundant (Weisse et al. 2001). For all experiments, both ciliate and flagellate cultures were harvested in the exponential phase.

Experimental design—Ciliates were taken from exponentially growing stock cultures maintained at 15°C and continuous light (100 μmol photons m^{-2} s^{-1}). An inoculum was transferred to 250-ml tissue culture bottles, containing 200 ml of MWC medium. Over 5–7 d, ciliates and prey were stepwise acclimated to experimental food levels and tem-

peratures (9, 12, 15, 18, 21, 24 ± 0.5°C). The target temperatures were reached by changing the incubation temperature by up to 3°C per day. The light level for ciliates during the acclimation period was ~70 μmol photons m^{-2} s^{-1}. Prey levels were monitored with an electronic particle counter (CASY 1-model TTC, Schärfe System, Reutlingen, Germany), daily (at 18–24°C), or every other day (at 9–15°C) during the acclimation period, and the ciliates were regularly fed to maintain food levels. The prey were maintained in exponential phase under the same temperature conditions as the ciliates but at 100 μmol photons m^{-2} s^{-1}.

After the temperature acclimation period, the ciliates were inoculated into 50-ml tissue culture bottles, containing 40 ml of medium and acclimated prey, at prey concentrations ranging from 1.0×10^4 to 2.5×10^5 cells ml^{-1}, corresponding to carbon levels from 0.1 to 4.4 μg C ml^{-1} (*see Fig. 2* for concentrations). To render this study comparable to previous investigations, we converted prey cell volume to carbon units using C (pg) = $0.109V^{0.991}$ (where V is μm^3) (Montagnes et al. 1994). Controls for prey growth, without ciliates, were run at food levels comparable to incubations at each temperature.

Ciliates in defined prey concentrations and the controls were maintained for 24 h at each temperature and food concentration. After 12 and 24 h, prey numbers in all treatments were determined, and prey concentrations were adjusted with temperature-acclimated prey or MWC medium if they deviated from the target prey levels by more than 20%. The experimental incubation began immediately after readjusting the food concentration in each container and lasted for 24 h. Light levels were identical to those used during the acclimation period. Each experiment was run with three to five replicates.

Samples were taken from containers at 6–12-h intervals and fixed. For flow cytometric analyses, samples were fixed with formalin (final concentration 2%, vol/vol); for microscopic analyses, samples were fixed with acid Lugol's iodine (final concentration 2%, vol/vol).

Prey and ciliate numbers were determined by flow cytometry according to the protocol of Lindström et al. (2002). Briefly, 60 μl of the DNA stain TO-PRO-1 (Molecular Probes Europe) was added to 2 ml of sample. Samples were stained in the dark at ~20°C for 60 min. Samples were then analyzed using a FACSCalibur flow cytometer (Becton Dickinson) equipped with an Argon-ion laser-emitting light at 488 nm. Measured parameters of the cells were forward scatter, side scatter, TO-PRO–induced green DNA fluorescence, and chlorophyll *a* Chl *a*–induced red fluorescence. Green fluorescence was used as the triggering parameter (i.e., only signals from particles with a green fluorescence intensity exceeding a given threshold value were measured). The data were analyzed with CELLQuest v3.0 and Attractors v3.0 (Becton Dickinson). The prey populations were detected and gated from the ciliate cultures based on forward scatter or side scatter versus red autofluorescence signals. Ciliates were enumerated using a gate in the side scatter versus green fluorescence dot plots (CELLQuest) or a combination of side scatter or forward scatter with green fluorescence and red fluorescence (Attractors) to distinguish them from other particles.

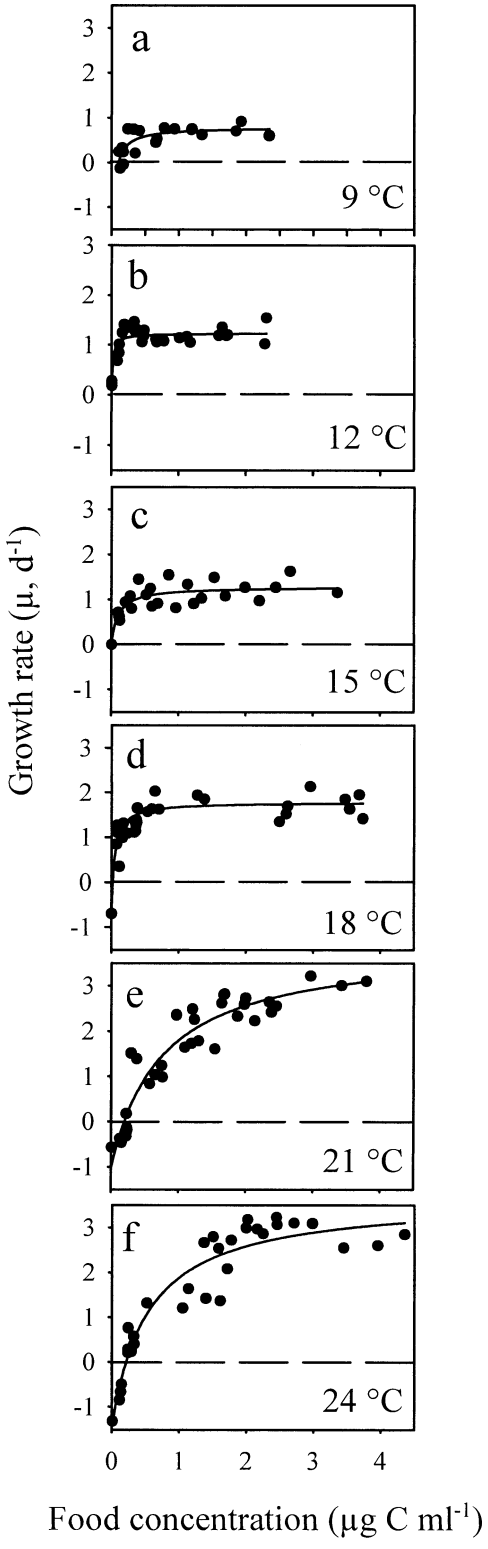

Fig. 2. The relationship between growth rate and prey concentration (numerical response) at six temperatures. Circles represent single estimates. Solid curves are the fit of Eq. 3 (*see text*) to the data. Dashed lines indicate zero growth. See Table 1 for the parameters and their error estimates for these curves.

Table 1. Parameter values (±SE) for the curves presented in Fig. 2 (numerical response), Fig. 4 (volume response), and Fig. 5 (production response); see text for the associated formulae.

Response	Temperature (°C)	Parameters ± SE		
Numerical response		μ_{max} (d^{-1})	k (μg C ml^{-1})	x' (μg C ml^{-1})
	9	0.79±0.12	0.14±0.11	0.10±0.03
	12	1.2±0.05	0.03±0.01	—*
	15	1.3±0.08	0.09±0.04	—*
	18	1.8±0.07	0.06±0.01	0.02±0.01
	21	3.9±0.40	0.95±0.24	0.19±0.03
	24	3.7±0.30	0.75±0.18	0.22±0.03
Volume response		V_{max} (μm^3)	a (μg C ml^{-1})	c (μm^3)
	9	4,460±1,050	1.2±0.80	2,490±310
	12	6,380±931	0.38±0.20	1,790±871
	15	3,860±639	0.59±0.38	1,570±579
	18	2,190±329	0.59±0.41	1,620±298
	21	4,050±613	1.7±0.73	1,170±205
	24	2,650±350	1.5±0.66	1,170±139
Production reponse		Pr_{max} (μm^3 d^{-1})	b (μg C ml^{-1})	x′ (μg C ml^{-1})
	9	3,520±842	0.17±0.17	0.11±0.04
	12	8,930±632	0.14±0.06	—*
	15	6,010±758	0.35±0.16	—*
	18	6,660±616	0.30±0.10	—*
	21	21,500±4,750	2.7±1.1	0.16±0.07
	24	24,800±5,440	4.4±1.5	0.20±0.06

* Values not significantly different from zero (SigmaPlot, $\alpha = 0.05$).

Ciliate volume was determined from length and width measurements assuming a prolate spheroid shape. Measurements were made on 50 ciliates obtained at the end of the experiment from each treatment, using an inverted microscope and an image analysis system. Ciliate size measurements were made on Lugol's fixed material, which might underestimate live volume by 30% (Jerome et al. 1993; Müller and Geller 1993). Cell size of the prey was measured for each temperature treatment at the beginning of each experiment with the electronic particle counter.

Ciliate growth rate was determined from end-point measurements of cell number, assuming exponential growth over the experimental period according to Eq. (1).

$$\mu = \ln(N_t/N_0)/t \quad (1)$$

N_0 and N_t are ciliate numbers at the beginning and end of the experiment, respectively; μ (d^{-1}) is the intrinsic rate of increase; and t is the duration of the experiment (d). Ciliate production (μm^3 d^{-1}) was calculated as the product of growth rate (μ) and the corresponding cell volume.

Ciliate growth rates were related to the geometric mean prey concentration (P) during the experimental period. The latter was calculated according to

$$P = (P_t - P_0)/\ln(P_t - P_0) \quad (2)$$

where P_0 and P_t are the initial and final prey concentrations, respectively, during incubations.

Numerical response data were fit to Eq. 3, which includes a positive x-axis intercept, using the Marquardt–Levenberg algorithm (SigmaPlot, SPSS).

$$\mu = \mu_{max}\,(P - x')/(k + P - x') \quad (3)$$

μ is the growth rate, μ_{max} is the maximum growth rate, P is the prey concentration (Eq. 2), k is a constant, and x' is the x-axis intercept (i.e., the threshold concentration, where $\mu = 0$).

For Eq. 3, the expression μ_{max}/k, is a measure of the initial slope of the numerical response curve (Stemberger and Gilbert 1985; Rothhaupt 1990). This slope is thus an indicator of the affinity between the prey and predator.

The response of ciliate cell volume to food concentration was fit to Eq. 4, where V is the cell volume (μm^3), P is the prey concentration, a is a constant, and c is the theoretical cell volume at zero food concentration.

$$V = [V_{max}P/(a + P)] + c \quad (4)$$

Production rate (Pr), the product of cell volume and growth rate, was fit to an equation similar to Eq. 3, but μ and μ_{max} were replaced by the parameters Pr and Pr_{max}, respectively; k was replaced by the constant b; and x' remained the prey concentration where growth, and hence production, was zero.

Results

At all temperatures, growth rate followed a rectangular hyperbolic response to food concentration (Fig. 2). Equation 3 was fit to the growth data independently at each temperature; the parameters and their error estimates are presented in Table 1.

There were three main changes in the shape of the numerical response with temperature: change in maximum growth rate (μ_{max}); change in the constant k, also reflected

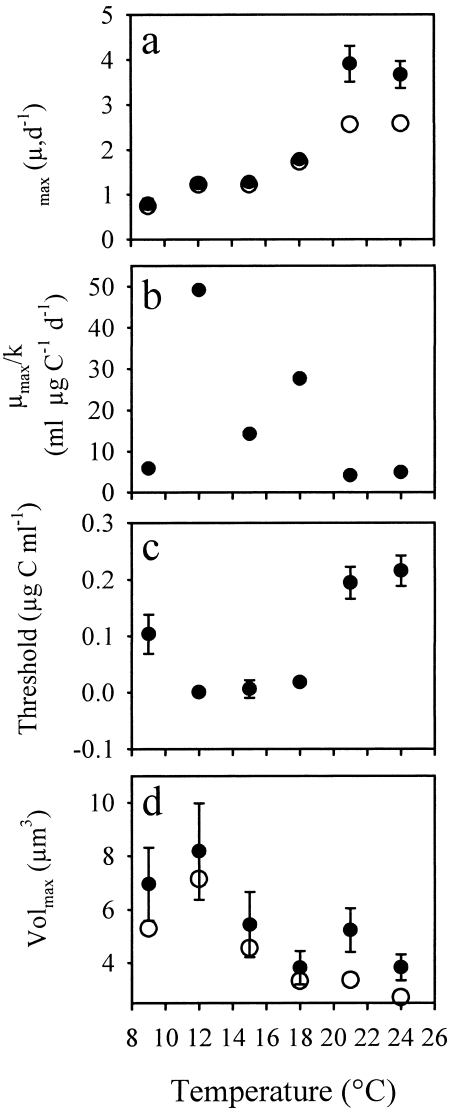

Fig. 3. The relationship between four parameters and temperature. (a) The relationship between maximum growth rate (μ_{max}, solid circles) and growth rate at a prey concentration of 2.0 μg C ml^{-1} (open circles) versus temperature; (b) the relationship between the initial slope (a) of the numerical response versus temperature; (c) the relationship between the threshold level (i.e., x', where growth rate is zero) versus temperature; (d) the relationship between maximum cell volume (V_{max}, solid circles) and cell volume at 2.0 μg C ml^{-1} (open circles) versus temperature. Error bars represent one SE.

in the change in the initial slope of the curve at x' (i.e., μ_{max}/k = α); and change in threshold level (x'). These are presented independently.

The maximum growth rate (μ_{max}, Eq. 3, Table 1) increased with increasing temperature (solid circles, Fig. 3a); maximum growth rate appeared to increase linearly between 9 and 18°C and then increased rapidly to a relatively stable level between 21 and 24°C. However, the parameter μ_{max} might not provide the best estimate of growth if an asymptote is not adequately predicted. This appears to be the case at both 21 and 24°C (Fig. 2e,f), where μ_{max} might thus be an overestimate. Consequently, for each temperature, we have calculated the growth rate, predicted by Eq. 3, at 2.0 μg C ml^{-1}. This carbon level corresponds to a Chl *a* concentration of ~0.05 μg ml^{-1} (i.e., to prey levels observed in many eutrophic lakes where this ciliate can be found, Foissner et al. 1999). These data (open circles, Fig. 3a) suggest a more linear increase in growth rate with temperature under eutrophic conditions.

For the numerical response, the constant k was 0.14 μg C ml^{-1} at 9°C; it decreased to low levels (<0.1 μg C ml^{-1}) between 12 and 18°C, and above 18°C, it rose to >0.7 μg C ml^{-1} (Table 1). The initial slope of the numerical response curve (μ_{max}/k = α) ranged from 14 to 40 ml μg^{-1} C d^{-1} between 12 and 18°C, and was low (<6 ml μg^{-1} C d^{-1}) both at 9°C and >18°C (Fig. 3b).

The threshold concentration, the prey level where ciliate population growth is zero, was 0.10 μg C ml^{-1} at 9°C. It decreased to a level statistically indistinguishable from zero between 9 and 15°C ($p > 0.05$, SigmaPlot) and remained low until 21°C, where it rose to, and remained near, 0.2 μg C ml^{-1} (Fig. 3c, Table 1).

Cell volume also increased with increasing food concentration at all temperatures. Equation 4 provided a good fit to these data, but an asymptote was rarely reached (Fig. 4, Table 1). Thus, like growth rate, the maximum volume (V_{max}, Eq. 4) might not offer a useful parameter to examine the response of cell volume to temperature. We have examined both the relationship between V_{max} (solid circles, Fig. 3d) and the volume predicted at 2.0 μg C ml^{-1} (*see arguments above*; Fig. 3d, open circles) versus temperature. In both cases, cell volume increased between 9 and 12°C and then decreased to a minimum at 24°C.

Production (μm^3 d^{-1}) increased with increasing food concentration at all temperatures (Fig. 5; Table 1). Maximum production increased with temperature. However, the highest food concentrations used in this study did not provide saturating levels at the highest temperatures (Fig. 5e,f). Thus, to illustrate the effect of temperature on production, production was calculated at 0.05 μg C ml^{-1} (ultraoligotrophic), 0.1 μg C ml^{-1} (oligotrophic), 0.2 μg C ml^{-1} (mesotrophic), 2.0 μg C ml^{-1} (eutrophic), and 5.0 μg C ml^{-1} (hypertrophic) (Fig. 6). Under highly eutrophic conditions, production generally increased with temperature (Fig. 6a). However, under eutrophic, mesotrophic, oligotrophic, and ultraoligotrophic conditions, production initially increased and then decreased with temperature (Fig. 6b–d).

Discussion

As indicated previously, initial work revealed significant interactive effects of temperature and food concentration on the growth and production of the freshwater ciliate *Urotricha farcta* (two-way ANOVA, Kimmance 2001). These results extended the ecophysiological effects observed for metazooplankton such as *Daphnia* (Achenbach and Lampert 1997; Giebelhausen and Lampert 2001) and rotifers (Stelzer 1998) to protozooplankton. They also provided the basis for this study, in which we examine in detail the response of growth parameters to changing food concentration and temperature. We can then use these data to predict the in situ response of

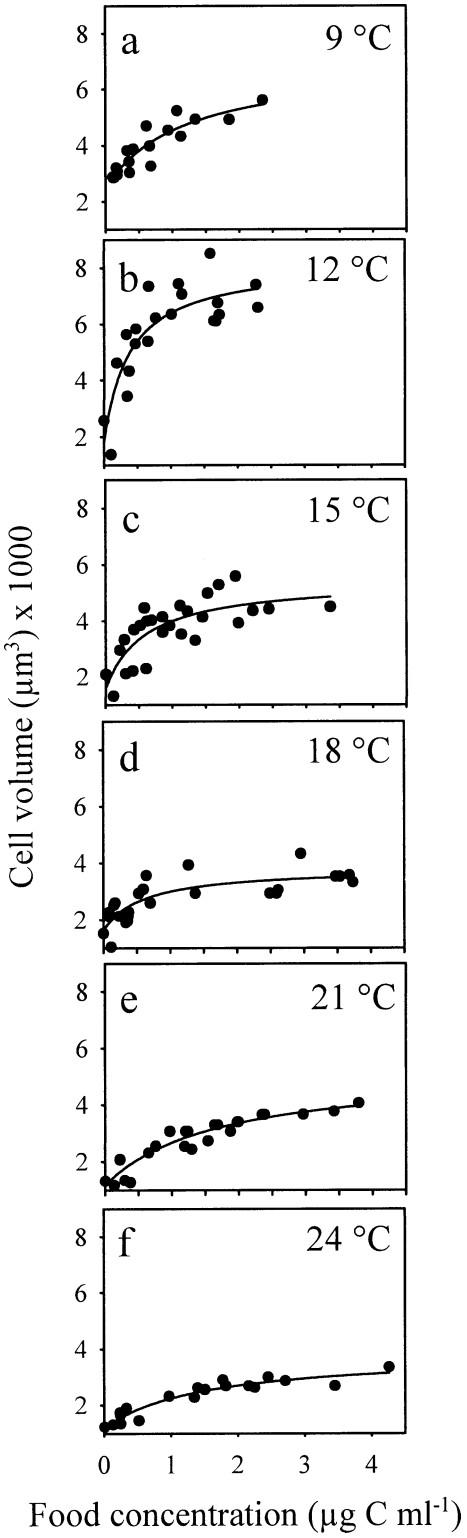

Fig. 4. The relationship between cell volume and prey concentration (volume response) at six temperatures. Circles represent single estimates based on 50 cells. Solid curves are the fit of Eq. 4 (*see text*) to the data. See Table 1 for the parameters and their error estimates for these curves.

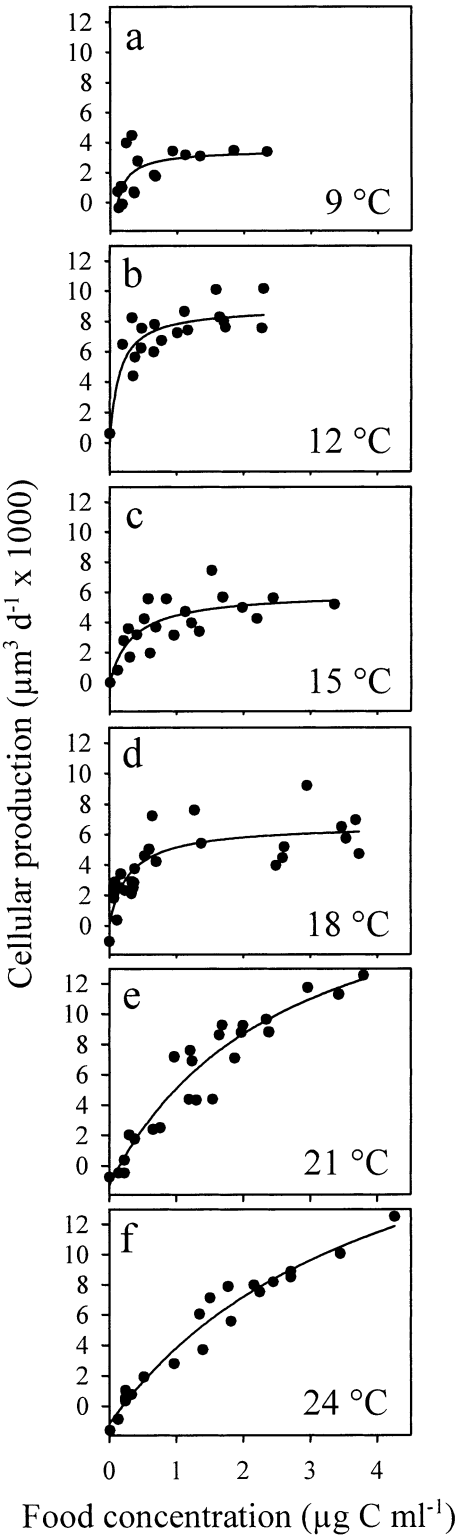

Fig. 5. The relationship between production and prey concentration (production response) at six temperatures. Circles represent single estimates. Solid curves are the fit of a modified version of Eq. 3 (*see text*) to the data. See Table 1 for the parameters and their error estimates for these curves.

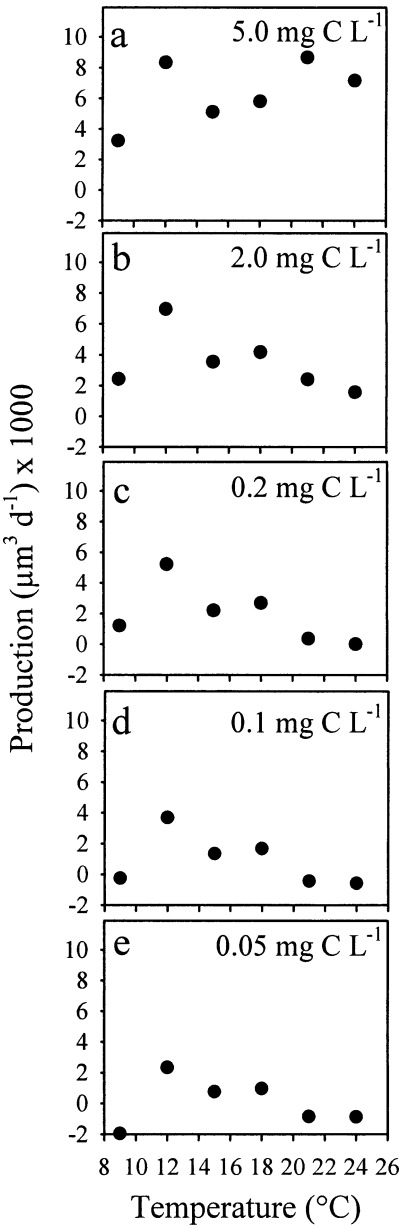

Fig. 6. The relationship between production and temperature at five food levels: (a) hypertrophic, (b) eutrophic, (c) mesotrophic, (d) oligotrophic, and (e) ultraoligotrophic waters. Production was determined using the modified version of Eq. 3 (described in the text) and the parameters presented in Table 1.

U. farcta to changing temperature and food levels. Furthermore, using this rapidly growing ciliate, we have provided an extensive data set that illustrates trends that might apply to other protozoa and to slower growing metazoa.

The nature of the interaction—If considered alone, both temperature and food concentration can increase the growth rates of *U. farcta.* The response to increased food levels followed a typical, rectangular hyperbolic, numerical response (Figs. 1, 2), and under eutrophic conditions, the response to temperature was roughly linear over a wide range of temperature (Fig. 3a, open circles). These data support earlier trends obtained for the same and similar taxa (Müller and Geller 1993; Montagnes 1996; Weisse and Montagnes 1998; Müller and Schlegel 1999). If, however, both environmental factors were combined, growth rate did not follow the trends indicated by previous studies.

At a combination of high temperature and moderate food levels, temperature can have a negative influence on growth rate and production; in fact, under some conditions, mortality occurred (Fig. 2d–f). At extremely high food concentrations, the relationship between temperature and maximum growth rate (μ_{max}) can deviate from linearity (Fig. 3a); either the response is exponential or there is a stepwise shift to a different, faster growing state. This shift at high temperatures is also reflected in production estimates (Fig. 6): only at high food levels does production continue to increase with temperature. However, we recognize that our data lack the resolution between 18 and 21°C to adequately assess this trend for μ_{max}, and our estimates of production, which are based on volume, might be confounded by a dependence of cell composition on temperature or food concentrations (e.g., cell carbon content might be altered by temperature or food availability). Thus, this proposed trend of a distinct shift is speculative and requires further elucidation.

Still, there does appear to be a change in the growth response of the ciliate with temperature. At temperatures ranging from 12 to 18°C, the threshold values (x') were near zero, and the initial slope (α) was at least threefold higher than at the temperature extremes (Table 1; Fig. 3). Thus, at these midtemperatures, the ciliate would exhibit a persistent strategy, surviving even at low food levels and rapidly reaching its maximum growth rate if food concentration did increase (i.e., the ciliate would survive under oligo- to eutrophic conditions). In contrast, at the two highest temperatures (21 and 24°C), growth rate (at hypertrophic levels) and the threshold concentration increased at least twofold, whereas the initial slope (α) decreased (Table 1; Fig. 3). Clearly, an increase in growth rate would confer a selective advantage on the species, whereas an increase in x' and a decease in α would confer a selective disadvantage. In essence, the ciliate shifted, on a relative scale, from exhibiting a persistent strategy at lower temperatures to an opportunistic strategy requiring high food concentrations (eutrophic to hypertrophic levels) at the two highest temperatures. At the lowest temperature, however, the pattern exhibited at higher temperatures did not hold; at 9°C *U. farcta* had a low growth rate, a small α, and an increased threshold concentration (Table 1; Fig. 3a–c). Furthermore, cell volume at 9°C did not continue to follow the trend to increase with decreasing temperature, and in fact, it appeared to decrease (Fig. 3d). The combination of these changes at the low end of the temperature range would confer a relatively poor selective advantage on the ciliate (at any food concentration). We suggest that, although *U. farcta* will survive at 9°C, at this temperature the ciliate is moderately temperature stressed.

In general, the trend exhibited by *U. farcta* at temperatures above 9°C is in accordance with the pronounced nonlinear increase of food requirements at high temperatures exhibited by four cladoceran species (Achenbach and Lampert 1997). Thus, our results extend the concept of the effect of tem-

perature–food interaction on the competitive ability of species.

The competitive ability of U. farcta—The maximum growth rates of *U. farcta* measured between 12 and 18°C (Fig. 3a) were higher than those of similar species at comparable temperature and food conditions (Müller and Geller 1993; Weisse and Montagnes 1998; Müller and Schlegel 1999), and production of *U. farcta* peaked in this temperature range at low to moderate food levels (Fig. 6). If temperature exceeds 20°C, this ciliate needs high food concentrations (>0.2 μg C ml^{-1} or ~0.05 μg Chl *a* ml^{-1}) to thrive. We conclude that *U. farcta* has the potential to grow at near-maximum rates in meso- and eutrophic temperate lakes in spring and early summer. Thus, under these conditions *U. farcta* could potentially outcompete other ciliates and contribute substantially to the total ciliate production. However, if extremely high food levels and temperatures occur, as can be typical of shallow, nutrient-rich, temperate lakes, *U. farcta* might shift to an opportunistic strategy with extremely high growth and production rates that could exceed other ciliate species (Weisse and Montagnes 1998; Jürgens et al. 1999; Müller and Schlegel 1999; Montagnes and Weisse 2000).

There is some direct and indirect evidence from natural populations supporting our findings. *U. farcta* has been recorded primarily from eutrophic or highly eutrophic ponds and lakes, where it can be abundant (Foissner et al. 1999; Jürgens et al. 1999; Weisse et al. 2001). For instance, in situ growth rates of small prostomatid ciliates in Lake Constance reached maximum growth rates when water temperature was moderate (6–16°C) and suitable prey concentrations were high (Weisse and Müller 1998). In summer, however, at high temperatures (>18°C) and low food conditions, in situ growth rates were distinctly lower than lab-based estimates of μ_{max}. Thus, by indicating interactive effects of temperature and food concentration, this study supports the earlier conjecture that herbivorous ciliates are food limited in this lake in summer (Müller et al. 1991).

Application of the observed trends—Previous work, used to model planktonic ecosystems, has often not appreciated the potential effect of temperature and food concentration interactions. For instance, ciliate production in Lake Constance was estimated using an allometric relationship based on volume-specific ciliate growth rate of food-saturated laboratory cultures (i.e., the equation in Montagnes et al. 1988), but the estimated summer ciliate production had to be reduced by a factor of five to balance carbon flow models (Gaedke and Straile 1994; Straile 1998). The apparent overestimation of ciliate production in summer could not be explained by predation. Possibly, the high summer temperatures interacted with the relatively low food levels to reduce summer production. In agreement with this interpretation, during the other seasons, the empirical equation (in Montagnes et al. 1988) provided a reasonable estimate of the ciliate production in Lake Constance (Gaedke and Straile 1994).

Further support for the interaction, observed here, is seen in other field studies. Macek et al. (1996) reported discrepancies between measured in situ and estimated maximum growth rates of small *Urotricha* spp. from a eutrophic reservoir and an oligo-mesotrophic lake. The relative deviation from predicted maximum growth rates was larger in the lake with the lower food supply. In another case, Jürgens et al. (1999) conducted enclosure experiments in a highly eutrophic lake; these authors measured maximum net growth rate of *Urotricha furcata*/*farcta* of 2.24 d^{-1} at phytoplankton biomass of ~1.2 μg C ml^{-1} (0.03 μg Chl *a* ml^{-1}) and temperatures ranging from 19.5 to 22.8°C. In a second experiment, phytoplankton carbon (5 μg C ml^{-1}, 0.12–0.14 μg Chl *a* ml^{-1}) and temperature were higher (21.2–25.2°C). In spite of the elevated phytoplankton biomass and temperature, the maximum net growth rate of *U. furcata*/*farcta* was lower (1.00 d^{-1}) than in the first experiment. Although the phytoplankton biomass in the enclosures overestimates the food available to ciliates, because some phytoplankton were likely inedible, and predation on the ciliates might have played some role, the scenario observed by Jürgens et al. (1999) clearly agrees with our measured growth rates (Figs. 3e,f, 4a) and supports the conclusion of a negative interaction between temperature and food concentration on growth at high temperatures.

At this point, we can only speculate whether our results are representative of most small freshwater ciliates and other microzooplankton. The few studies that have investigated the combined effect of temperature and food in cladocerans (Orcutt and Porter 1984; Achenbach and Lampert 1997; Giebelhausen and Lampert 2001) and rotifers (Stelzer 1998) consistently indicate an increase of the threshold food concentration with temperature, similar to our findings. The relative, combined temperature–food effect was, however, species specific (Achenbach and Lampert 1997; Stelzer 1998).

Considering the importance of protozoa in biogeochemical processes and the ongoing interest in both local and global warming events, there is clearly a need to further investigate the combined effect of temperature and food concentration on aquatic protozoa. Based on the results from this study, we propose the following hypotheses: (1) There is typically an interaction between temperature and food concentration on growth and production of planktonic protozoa; (2) as temperature increases, threshold concentration (x') and μ_{max} will increase, but the initial slope of the numerical response (α) will decrease; and (3) the change in these three parameters might be stepwise rather than incremental, shifting an organism's adaptive state from one level (e.g., mesotrophic) to another (e.g., hypertrophic). Finally, we speculate that these hypotheses will apply to both metazoa and protozoa.

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Received: 20 November 2001
Accepted: 9 May 2002
Amended: 27 May 2002