Effect of temperature on inter- and intraspecific isolates of *Urotricha* (Prostomatida, Ciliophora)

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ABSTRACT. Ecological models often presume that all members of a functional guild, e.g. herbivorous ciliates, respond identically to temperature changes. To test this general assumption, we investigated if planktonic ciliates within the genus *Urotricha* exhibit distinct inter- and intraspecific responses to temperature. We examined the response of growth rate, cell volume, and production to changing temperature, using 6 temperatures between 5 and 30°C. This experiment made 3 comparisons using: (1) different species isolated from the pelagic and littoral regions of the same lake; (2) different clones of the same species isolated from lakes of the similar trophic status, but different latitudes; and (3) different clones of the same species isolated from the pelagic region of a single lake. Using ANCOVA and ANOVA procedures (α = 0.05) to examine the data we demonstrated that: (1) ciliate species within a single genus may exhibit distinct responses to temperature, suggesting that treating ciliates as a single functional group is an oversimplification; (2) clones isolated from a laboratory culture of a single species, isolated from a single location, differ only to a small extent; and (3) clones isolated from different localities, but belonging to the same species differ considerably in their responses. Our data indicate that (1) temperature regimes may be an environmental niche that separates species and clones and (2) even where apparent morphological and molecular differences do not distinguish taxa, functional differences may still exist.

KEY WORDS: Ciliate · Temperature response · Clonal variation · Growth rate · Production · Cell volume

INTRODUCTION

As we become aware of the microbial food web in freshwater systems (see Stockner & Porter 1988, Weisse 1998), we begin to recognize the need to further characterize the species-specific response of protozoa to abiotic environmental factors. Changes in water temperature affect mixing and stratification and thus constrain the physical environment in many freshwater lakes. Temperature is, however, not only a dominant physical variable but also influences physiological processes of aquatic organisms. As temperature changes both temporally and spatially in lakes, it impacts pelagic food web dynamics, especially those processes mediated by the plankton which cannot easily avoid changes. The specificity of the temperature response of pelagic protozoa such as ciliates is, with a few exceptions (e.g. Müller & Geller 1993), currently unknown.

Considerable growth rate differences with respect to temperature have already been demonstrated within the same ciliate genus (Lee & Fenchel 1972, Pérez-Uz 1995). In spite of these findings, the potential significance of species-specific physiological differences has been ignored in many ecological investigations. Ecosystem studies which model pelagic systems often group ciliates into size or nutritional categories, and such studies often even apply a single temperature versus growth response for all ciliates (see Montagnes 1996). Modellers commonly presume that all members of one functional guild, e.g. herbivorous ciliates, respond identically to temperature changes (e.g. Gaedke
Materials and Methods

Study organisms. Urotrocha species were collected by enriching natural samples with potential prey (Cryptomonas sp. strain 26.80, obtained from the Culture Collection for Algae in Göttingen, Germany). Each Urotrocha culture was established by maintaining a mixture of cell isolates that were identified as a single morphotype, i.e. each culture was composed of a single species but, most likely, not of a single clone; these are henceforth referred to as ‘mixed clones’. Urotrocha turcata was isolated from the pelagic region of Lake Constance (southern Germany) and Lake Schöøsee (northern Germany); both lakes are of comparable mesotrophic status (Geller & Güde 1989, Hofmann 1989) and are ~1000 km apart, 47° and 54° N, respectively. Urotrocha farcata was isolated from the littoral region of Lake Schöøsee.

After mixed clones were established, 3 ‘true clonal’ cultures of Urotrocha turcata were isolated from the Lake Constance mixed clone by a minimum of 3 serial, single cell isolations. Note: our clones could have been composed of strains best adapted to the laboratory conditions which had outcompeted all other strains. Thus, our results do not test the specific differences between the initially isolated ciliates but examine the potential differences. Furthermore, as rate processes (e.g. growth, grazing) are typically estimated from laboratory cultures such as ours, our results reveal the possible errors associated with assuming isolates to be the same.

Prior to the experiment, all ciliate cultures were maintained in WC medium (Guillard & Lorenzen 1972 containing Cryptomonas sp. strain 26.80, at 10 μmol photons m⁻² s⁻¹ and 14±1°C. This cryptophyceae was formerly named Rhodomonas sp. strain 26.80 (e.g. Müller 1991) and has an average cell volume of ~280 μm³ (Weisse & Kitchhoff 1997).

Experimental design. For the experiment, the ciliates were grown in the dark in 12 ml capacity, polystyrene, tissue-plate wells, containing 8 to 10 ml of media/prey; the ciliates were always maintained at saturating prey levels (>1 x 10⁶ cells ml⁻¹ for Urotrocha spp.; Weisse et al. unpubl.). The prey (Cryptomonas sp.) were grown at 180 μmol photons m⁻² s⁻¹ and 14 ± 1°C and were harvested in exponential phase for the experiment. We minimised the effect of temperature on food quality by introducing the ciliates into new prey that was adapted to the experimental temperature for only 0.5 to 1 h.

All 6 ciliate isolates (Table 1) were acclimated for 48 h to 6 temperatures (5, 10, 15, 20, 25, and 30 ± 0.5°C); ciliates at the extreme temperatures were step-wise acclimated to intermediate temperatures for 1 h, rather than immediately transferring them to the final temperatures. After the 48 h acclimation, a known number of ciliates was introduced to replicate (n = 3) new wells (which contained saturating prey levels for the duration of the experiment) and these wells were incubated for a further 48 h to determine ciliate growth rates and cell volumes at the 6 temperatures.

Ciliate abundance was determined from preserved samples (2% acid Lugol’s iodine) by enumerating cells in a Sedgewick-Rafter counting chamber. Ciliate volumes were calculated as prolate spheroids from length and width measurements made on 15 to 30 cells (obtained at the end of the experiment), using a semi-automatic SISe image analysis system. All size measurements were made on Lugol’s fixed material which likely underestimates live volume by 60 to 70% (Jerome et al. 1993, Müller & Geller 1993). Prey numbers were determined using a CASY 1-model TTC (Schärfe System) electronic particle counter.

Data analysis. Ciliate growth rate (μ, d⁻¹) was determined from exponential growth rates (μ₀ and μ₉), assuming exponential growth over 48 h. Ciliate production was determined as the product of cell volume and the specific, net, daily change in abundance (i.e.
net cell volume per day, \( \mu m^3 \text{ d}^{-1} \). ANOVA, t-tests, Duncan's multiple range test, and ANCOVA were performed using Statistica 5.1 for Windows (\( \alpha = 0.05 \) for all tests). Data for all tests were homoscedastic (\( \alpha = 0.05 \), Bartlett chi-squared test). The relationships of (1) growth rate versus temperature and (2) production versus temperature were assumed to be linear over either 5 to 15°C or 5 to 20°C; this assumption is supported by the high \( r^2 \) values for these relationships (Table 1).

For regions of the temperature versus growth and temperature versus production response curves that appeared linear (indicated by regression lines, see Figs. 1 & 3), ANCOVA comparisons were made between the following groups: the 2 Urotricha species isolated from the same lake but different habitats; the 2 U. furcata mixed clones isolated from different latitudes; and the 3 U. furcata true clones isolated from the same lake. Note: as production is the product of growth rate and cell volume, and both of these varied from linearity at high temperatures (see below), the linear portion of the response occasionally differed between treatments. At 25°C, where the response curves were no longer clearly linear, ANOVA comparisons were made using the same comparisons listed above.

RESULTS

Growth rate

The growth rate of all ciliate isolates increased with increasing temperature between 5 and 20°C, and all ciliates had positive growth rates between 10 and 25°C (Fig. 1). At 5°C mortality (\( \mu < 0 \)) occurred for most clones, and at 30°C only Urotricha farcta grew (Fig. 1).

ANOVA revealed the following significant differences (\( \alpha < 0.05 \)) in the growth versus temperature responses: mixed clones of Urotricha farcta and U. furcata (both from Lake Schöhsee; Fig. 1B, C) had different slopes; the slope of the mixed clones of U. furcata from Lake Schöhsee (U. furcata Lake Schöhsee; Fig. 1B) and Lake Constance (U. furcata Lake Constance; Fig. 1A) were not different, but their adjusted means differed; there was no difference between the 3 U. furcata Lake Constance clones, A, B, and C (Fig. 1D–F).

ANOVA, Duncan's multiple range test, and t-tests revealed the following significant differences (\( \alpha < 0.05 \)) in growth rate at 25°C: Urotricha farcta Lake Schohsee > U. Eurcata Lake Schohsee; U. furcata Lake Schöhsee = U. furcata Lake Constance; U. furcata clone A < U. furcata clone B = U. furcata clone C.

The parameters for the above regressions and the mean values at 25°C are presented in Table 1.

Volume

The volume of all isolates decreased with increasing temperature, and this response was not clearly linear (Fig. 2). Lake Constance Urotricha furcata isolates were in general larger than the isolate from Lake Schöhsee, and U. farcta was larger than U. furcata. These data will be discussed in detail elsewhere, with reference to ciliate volume changes in general (Weisse & Montagnes unpubl.); here they are presented primarily to establish production responses.

Table 1. Regression parameters of the lines and the mean values of the responses at 25 and 30°C (Figs. 1 & 3) for growth and production (\( \mu m^3 \)) in estimates of 6 isolates of the genus Urotricha from Lake Schöhsee and Lake Constance. b(0) = the y intercept; b(1) = the slope; \( r^2 \) = the \( r^2 \) value of the regression. Where analysis of slopes, adjusted mean or means were not significantly different (see 'Results', \( \alpha > 0.05 \)) a single value is provided for that group, these values are shaded.

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<td>Equation parameters of growth versus temp. response</td>
<td>b(0): -0.422 b(1): 0.108 ( r^2 ): 0.961</td>
<td>b(0): -0.545 b(1): 0.070 ( r^2 ): 0.833</td>
<td>b(0): -0.483 b(1): 0.075 ( r^2 ): 0.971</td>
<td>b(0): -0.325 b(1): 0.072 ( r^2 ): 0.920</td>
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<td>Growth at 25°C (d⁻¹)</td>
<td>1.79</td>
<td>0.914</td>
<td>0.905</td>
<td>No growth</td>
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<td>Growth at 30°C (d⁻¹)</td>
<td>1.75426</td>
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<td>Equation parameters of net production versus temp. response</td>
<td>b(0): -1870 b(1): 475 ( r^2 ): 0.839</td>
<td>b(0): -645 b(1): 89.9 ( r^2 ): 0.866</td>
<td>b(0): -863 b(1): 144 ( r^2 ): 0.893</td>
<td>b(0): -1150 b(1): 203 ( r^2 ): 6.902</td>
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<td>Net production at 25°C</td>
<td>8720</td>
<td>1140</td>
<td>1750</td>
<td>2290</td>
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<td>Net production at 30°C</td>
<td>8860</td>
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Production

The production of all ciliate isolates increased with increasing temperature between 5 and 15°C or 5 and 20°C, and production was positive for all ciliates between 10 and 25°C (Fig. 3). At 5°C production was negative for most clones (Fig. 3). ANCOVA revealed the following significant differences (α < 0.05) in the production versus temperature responses: mixed clones of *Urotricha furcata* Lake Schönhsee and *U. furcata* Lake Schönhsee (Fig. 3B, C) had different slopes; *U. furcata* Lake Schönhsee and *U. furcata* Lake Constance had different slopes (Fig. 3A, B); there was no difference between the 3 *U. furcata* Lake Constance clones, A, B, and C (Fig. 3D–F).

**DISCUSSION**

Is there one temperature response curve per species?

Temperature affects both growth rate (e.g. Müller & Geller 1993) and cell size (Atkinson 1994) of ciliates, and our data support this observation. Thus, variation in temperature is an important factor to consider when...
examining freshwater ciliate ecology. This holds in particular for estimating ciliate production which depends on both growth rate and the individual cell size (see 'Why examine growth rate (µ) and production?' below).

It is commonly assumed that the temperature dependent growth rate of a given species can be represented by a single curve. Ecosystem studies which model pelagic systems often group protists (e.g. ciliates) into size or morphotype categories, and such studies often then apply a single temperature versus growth response for all ciliates (see Montagnes 1996). Our study may challenge this assumption. To test for temperature specificity we have examined the response to changing temperature of ciliates isolated from: (1) the pelagic and littoral regions of the same lake (Urotricha furcata and U. farcta, respectively); (2) different lakes of similar trophic status, but different latitudes (U. furcata from Lakes Constance and Schohsee); and (3) the pelagic region of a single lake (U. furcata clones A, B, and C).

We have demonstrated that ciliate species within a single genus may exhibit distinct responses to temperature, suggesting that treating ciliates as a single functional group may be an oversimplification. We have also demonstrated that clones isolated from a laboratory culture of a single species, isolated from a single location, differ only to a small extent (Table 1). This is not surprising, since laboratory cultures maintained for many generations will select for a reduced number of adapted strains. Of greater interest, however, is that clones isolated from different localities but belonging to the same species differ considerably in their responses (Table 1). Thus, our data show that even where morphological and obvious molecular differences (see below) do not distinguish taxa, functional differences may still exist.

Potential for inter- and intraspecific ecological differences in Urotricha

Lakes possess regions where temperature is relatively stable and others where it varies considerably, and regions of relatively high and low temperature occur within and between lakes. Many ciliate species and genera have cosmopolitan distributions (Finlay 1990, Finley et al. 1996) and will consequently experience a range of temperature conditions. At the same time morphologically indistinguishable populations can be sufficiently isolated to allow genetic divergence (Finlay 1990, Dini & Nyberg 1993). Thus, differing temperature regimes should create niches for ciliate species and clones; such clonal diversity is common in other freshwater taxa that reproduce primarily asexually (reviewed by Carvalho 1994, De Meester 1996). Considerable growth rate variability has already been reported for marine ciliates isolated from different localities (Perez-Uz 1995). Ours is the first study to indicate how sympatric, coexisting, freshwater ciliates react to temperature changes.

Urotricha furcata and U. farcta coexist in small lakes in northern Germany (Skibbe 1991 in Foissner et al. 1994) and Denmark (Jürgens et al. unpubl.), and both species are considered cosmopolitan (Foissner et al. 1994). Although they have different cell volumes (Fig. 2), these 2 species are similar in appearance, and even a trained observer would have difficulties identifying them in a typical plankton sample. Only advanced taxonomic techniques, e.g. silver staining (Foissner 1994) and molecular methods such as denta-
turning gradient gel electrophoresis and ssu-rDNA sequencing, have distinguished these species (Buchmuller 1998). However, there are significant ecological differences between *U. furcata* and *U. tarcta*: (1) they differ in their ability to grow at 25 and 30°C and (2) they differ in the slopes of their growth and production responses to temperature (Figs. 1 & 3, Table 1). Data suggest that at saturating food concentrations (1.2 mg C ml⁻¹; Weisse et al. unpubl.) *U. furcata* is the less successful competitor, with lower growth and production rates at all temperatures and a poor ability to cope with high temperatures (Table 1). However, these 2 species likely occupy different spatial niches.

*Urotricha farcta* is euryokous, i.e. it is able to live under widely varying environmental conditions. *U. farcta* occurs in ponds, lakes, and rivers throughout the year, and it is abundant in eutrophic and hypertrophic waters; in these waters temperature varies between 0 and 36°C (Foissner 1994). *U. furcata* occurs in hypertrophic lakes (Foissner et al. 1994, Jürgens et al. unpubl.) and eutrophic reservoirs (Šimek et al. 1995, Mecek et al. 1996) as well as in some rivers and creeks (Foissner et al. 1994), but appears to be typically found in oligo-eutrophic lakes (Müller 1991, Sommaruga & Psenner 1993, Schönberger 1994); these locations are likely to have a limited temperature range compared to the large range that *U. tarcta* experiences. Thus, although these 2 species may co-occur (Skibbe 1991 in Foissner et al. 1994), there is some evidence to suggest they occupy different ‘temperature niches’; this agrees with our findings. Similar interspecific differences with respect to temperature occur for the coexisting prosto-mes *U. furcata* and *Balanion planctonicum* (Müller et al. 1991, Sommaruga & Psenner 1993, Schönberger 1994) from both laboratory (Müller & Geller 1993) and field investigations (Weisse & Müller 1998). Thus, niche separation with respect to temperature may be common in closely related, coexisting prosto-mes *ciliates.*

**Why examine growth rate (μ) and production?**

The intrinsic rate of increase (μ) is often used as the dependent variable when determining temperature versus rate responses (e.g. Müller & Geller 1993, Montagnes 1996). Growth rate provides information useful for estimating population dynamics. For instance, from data presented in Table 1 we can show that at 25°C a single cell of *Urotricha farcta* from Lake Schöllsee would produce 5 new cells in 1 d while a population of *U. furcata* from the same lake would only produce 1.5 cells. In contrast, the new volume that these 2 ciliates produced in 1 d would be 8720 and 1140 μm³ for *U. farcta* and *U. furcata*, respectively (Table 1). Note that there is a 3.3-fold difference in the increase in numbers and a 7.6 difference in the potential production.

Considerable changes in the average cell volume of ciliates with temperature have been known for some time (Lee & Fenchel 1972). Details of the volume changes among *Urotricha* and other pelagic ciliates and their significance for production estimates will be reported elsewhere (Montagnes et al. unpubl., Weisse & Montagnes unpubl.). It is appropriate to use growth estimates when studying population growth and the subsequent dispersal of clonal organisms like ciliates; such information might be used to assess the exploitative ability of an organism. However, in studies where the main interest is in the amount of biomass available for transfer within the food web, production is a more appropriate measurement.

**CONCLUSIONS**

The above data support the prediction that different species within a genus behave differently, but they also indicate that clones that are morphologically indistinguishable may also differ. These differences may seem minor, but ciliates like *Urotricha* may double several times per day, and small initial differences between species or clone abundance will rapidly be amplified by exponential population growth. Therefore, we must be aware of this natural variability, and the growth response of *Urotricha* to temperature exemplifies such differences.

How then can we apply our awareness of clonal variations in growth and production rates? Besides recognizing the evolutionary implications of natural variation, which in themselves are intriguing, awareness of these responses might be applied to ecosystem models. However, it will be impractical for modellers to consider all the permutations of clonal (and even species) variability. We instead suggest that models should not only consider mean values but also the range of variation in response to a given abiotic parameter, i.e. the reaction norm. Future studies should pay more attention to the extremes at both ends of the reaction norm since natural selection will annihilate the worst adapted and favour the best adapted specimens within a population.

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