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NOTE

Fluctuating temperatures affect growth and production rates of planktonic ciliates

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ABSTRACT: We measured the effect of fluctuating experimental temperatures on volume, growth rate, and production of 3 isolates of *Urotricha furcata* and 1 isolate of *U. farcta*. Laboratory experiments were conducted under constant (20°C) and daily fluctuating (12:12 h) temperatures (19:21, 17:23, 15:25°C). Experiments simulated temperature fluctuations that ciliates experience in natural environments. Fluctuating temperatures affected all parameters measured, suggesting that rate measurements at constant temperatures are not necessarily appropriate for modelling of processes in naturally fluctuating environments. Although there were significant effects of fluctuating temperature on parameter estimates, no universal trend was observed. Furthermore, effects under fluctuating conditions could not be predicted from mean estimates of measurements made over the temperature ranges. Finally, differences among isolates of *U. furcata*, from lakes 100s of km apart, were as large as differences between *U. furcata* and *U. farcta*; this suggests that various ecotypes exist among morphologically identical isolates.

KEY WORDS: Cell volume · Growth rate · Temperature response · Ecotypes

Urotricha, a small, planktonic ciliate, is abundant in freshwater plankton (Weisse & Müller 1998). We have previously examined the effect of constant temperature regimes on *Urotricha* isolates and species and have identified that distinct responses exist (Weisse & Montagnes 1998); similar findings have been demonstrated for other ciliates (e.g. Müller & Geller 1993, Pérez-Uz 1995). However, determining parameters at constant temperatures may be unreasonable, as temperature fluctuations are typical of most freshwater environments, e.g. temperature may fluctuate daily by 1 to 3°C in the top metre of the pelagic zone, and in the littoral zone fluctuations may be up to 5–10°C (see 'Discussion').

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The potential impact of fluctuating temperatures has been virtually ignored in previous studies on planktonic ciliates (e.g. Müller & Geller 1993, Pérez-Uz 1995, Weisse & Montagnes 1998). *In situ* growth or production rates are often predicted from temperature response curves measured in the laboratory (Montagnes et al. 1988, Macek et al. 1996), and ecosystem models (e.g. Gaedke & Straile 1994, Straile 1998) commonly assume that growth measured at a constant temperature yield the same growth rate as variable temperatures of the same mean value. We have investigated if this is a valid assumption by determining if *Urotricha* exhibits distinct inter- and intraspecific responses to fluctuating temperature. We provide data which should stimulate further studies on the effect of fluctuating daily temperatures on ciliate growth.

Material and methods. ***Study organisms:*** *Urotricha* species were isolated as described by Weisse & Montagnes (1998). *U. furcata* was isolated from the pelagic region of Lake Schöhsee (northern Germany) by K. Jürgens (Max Planck Institute for Limnology, Plön), Lake Constance (southern Germany) by H. Müller (Limnological Institute, Konstanz), and Lake Mondsee (Austria) by G. Pfister (Institute for Limnology, Mondsee); these lakes are of comparable mesotrophic status and similar temperature regime (Geller & Güde 1989, Hofmann 1989, Dokulil & Jagsch 1992) but are 100s of km apart. *U. farcta* was isolated from the littoral region of Lake Schöhsee by T.W. All 4 *Urotricha* strains were isolated during spring or early summer when water temperatures ranged between 7 and 17°C. The ciliates were kept in the laboratory at 15°C under a 12:12 h light:dark cycle. For brevity, these ciliates are referred to as isolates.

Prior to the experiment, all ciliate cultures were maintained in WC medium (Guillard & Lorenzen 1972) containing *Cryptomonas* sp. (cell volume ≈ 280 μm^3) at

~10 µmol photons m^{-2} s^{-1} and 15 ± 1°C. *Cryptomonas* sp. was grown at 100 µmol photons m^{-2} s^{-1} and 15 ± 1°C and was harvested in exponential phase for the experiment.

Experimental design: Ciliates were initially grown for 3 d at 10 µmol photons m^{-2} s^{-1} in 200 ml tissue-culture bottles containing ~65 ml of media/prey; ciliates were maintained at saturating prey levels (>1 × 10^5 cells ml^{-1}, Weisse & Müller 1998, Weisse unpubl.). During this time the 4 isolates were exposed to 4 treatments: constant 20°C and temperatures fluctuating between 19:21, 17:23, and 15:25°C. Temperatures were maintained on a 12:12 h cycle (i.e. mean temperature was 20°C for fluctuating treatments); shifts

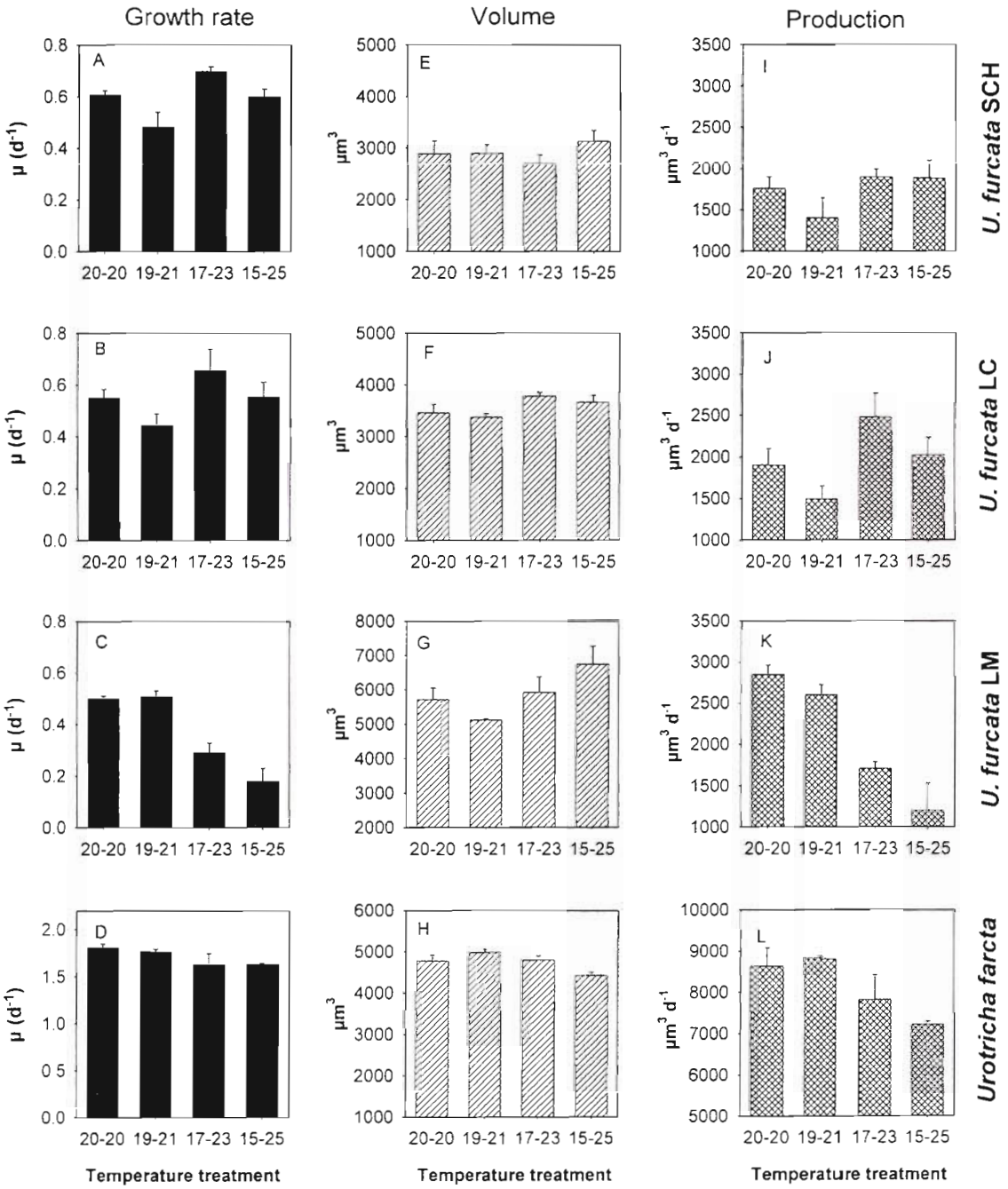

Fig. 1. Volume, growth rate, and production of 4 isolates of the ciliate *Urotricha* measured at constant (20:20 C) and fluctuating (19:21, 17:23 and 15:25 C) experimental temperatures. Error bars = 1 SD. *U. furcata* isolates from Lake Schöhsee (SCH), Lake Constance (LC) and Lake Mondsee (LM)

Table 1. Summary of the effects of fluctuating temperatures on volume, growth rates, and production of 4 *Urotricha* isolates. Effects are relative to values measured at constant temperatures (20:20°C) LC: Lake Constance, SCH: Lake Schöhsee, LM: Lake Mondsee, +: significant positive effect, –: significant negative effect, o: no effect. All tests are described in the text ($p < 0.05$)

Isolate	Growth			Volume			Production		
	19:21	17:23	15:25	19:21	17:23	15:25	19:21	17:23	15:25
U. furcata SCH	–	+	o	o	o	o	o	o	o
U. furcata LC	o	+	o	o	+	o	o	+	o
U. furcata LM	o	–	–	o	o	+	o	–	–
U. farcta	o	–	–	o	o	–	o	o	–

between temperatures occurred within ~3 h; and temperatures were ±0.5°C.

After the 3 d acclimation period, a known number of each acclimated isolate was placed in each of three 50 ml tissue-culture bottles, containing prey and medium (total volume 50 ml), i.e. each treatment was replicated 3 times. The prey and ciliates in the 50 ml tissue-culture bottles were then incubated for 3 d for *Urotricha furcata* and 2 d for the faster growing *U. farcta* under conditions identical to those of the acclimation period.

Ciliate abundance and volume were determined from 2% acid Lugol's iodine preserved samples. Ciliate volumes were calculated as prolate spheroids from length and width measurements made on ~50 cells, obtained at the end of the experiment; Lugol's fixation likely underestimates live volume by 30% (Müller & Geller 1993). Prey numbers were determined using a CASY 1-model TTC (Schärfe System, Reutlingen, Germany) electronic particle counter at the beginning and end of the experiments. Except for the experiments with *Urotricha farcta* in which the *Cryptomonas* sp. concentration had been reduced to $\sim 0.5 \times 10^5$ cells ml^{-1} at the end of the experiments, final prey concentration was $> 0.8 \times 10^5$ cells ml^{-1}.

Data analysis: Ciliate specific growth rate (μ, d^{-1}) was determined from end point measurements, assuming exponential growth over the 2 or 3 d. The error involved in measuring ciliate cell numbers microscopically restricts the sampling frequency (see 'Discussion'). We have chosen a sampling interval of 2 or 3 d in order to obtain statistically reliable results. Ciliate production was determined as the product of cell volume and the specific growth rate. Student's *t*-test, 2-way ANOVA, Tukey's test, and 1-way ANOVA were performed using SigmaStat (V 2.00 SPSS, Chicago, IL).

Results. There were significant interactive effects between isolate and temperature treatments on volume, growth rate, and production (2-way ANOVA), i.e. the different isolates exhibited unique responses. Results for volume, growth rate, and production changes with temperature are summarised in Fig. 1.

Growth rates: The growth response of the individual isolates revealed differences between treatments: growth rate of *Urotricha furcata* isolated from Lake Schöhsee (*U. furcata* SCH) was, relative to the constant temperature (20°C), significantly lower when temperature ranged between 19 and 21°C and significantly higher when temperature ranged between 17 and 23°C, but growth at the highest temperature variations (15:25°C) was not different from the constant temperature treatment (Fig. 1A). A similar pattern emerged for *U. furcata* isolated from Lake Constance (*U. furcata* LC, Fig. 1B), although growth rate at 19:21°C was not significantly different ($p = 0.074$) from the constant temperature. *U. furcata* from Lake Mondsee (*U. furcata* LM, Fig. 1C) decreased in growth rate when temperature fluctuations exceeded ±1°C. A similar, but less pronounced, pattern was observed for *U. farcta* (Fig. 1D), where growth at 17:23 and 15:25°C was also significantly lower than at constant 20°C.

Volume: Compared to growth rates, volume changed little with fluctuating temperatures. Differences between treatments were not significant for *Urotricha furcata* SCH (Fig. 1E). For *U. furcata* LC the volume measured at 17:23°C was significantly higher than at the constant temperature (Fig. 1F). *U. furcata* LM was significantly larger at the greatest temperature fluctuations (15:25°C, Fig. 1G). An opposite trend occurred for *U. farcta*, where cells were significantly smaller at 15:25°C than at the constant temperature (Fig. 1h).

Changes in volume were unrelated to growth rates for *Urotricha farcta* or any of the *U. furcata* strains individually. Volume was, however, significantly negatively related to growth rates of *U. furcata* if all 3 isolates were pooled (least-squares linear regression, $p < 0.01$).

Production: Similar to volume, production was unaffected by fluctuating temperatures in *Urotricha furcata* SCH (Fig. 1I) and significantly higher compared to the constant temperature for *U. furcata* LC at 17:23°C (Fig. 1J). *U. furcata* LM production followed the same trend that was observed for their growth: production was significantly lower at intermediate (17:23°C) and large (15:25°C) temperature fluctuations (Fig. 1K). The widest temperature fluctuations also yielded significantly lower production rates in *U. farcta* (Fig. 1L).

The various effects of fluctuating temperatures on the 3 measured parameters of the 4 isolates of *Urotricha* are summarised in Table 1.

To assess if the observed changes with temperature were a direct effect of temperature, we compared our results to the previously measured values of the same

3 isolates at the same, but constant, temperatures (Weisse & Montagnes 1998). Note that the general temperature response of *Urotricha furcata* LM has not yet been investigated.

In the previous study (Weisse & Montagnes 1998), growth rates of all 3 *Urotricha* isolates were linear between 15 and 20°C and then levelled off (*U. furcata* SCH, *U. farcta*) or declined slightly (*U. furcata* LC) between 20 and 25°C. The growth rate measured for *U. farcta* in the present study at constant 20°C was slightly higher than previous estimates at 20°C, and growth rates of *U. furcata* at 20°C were somewhat lower in the present study compared to the previous investigation (Weisse & Montagnes 1998, see 'Discussion'). To allow comparison with previous data, we assumed that the general temperature response remained unchanged, i.e. the same relative changes would occur at the respective temperatures in the present investigation.

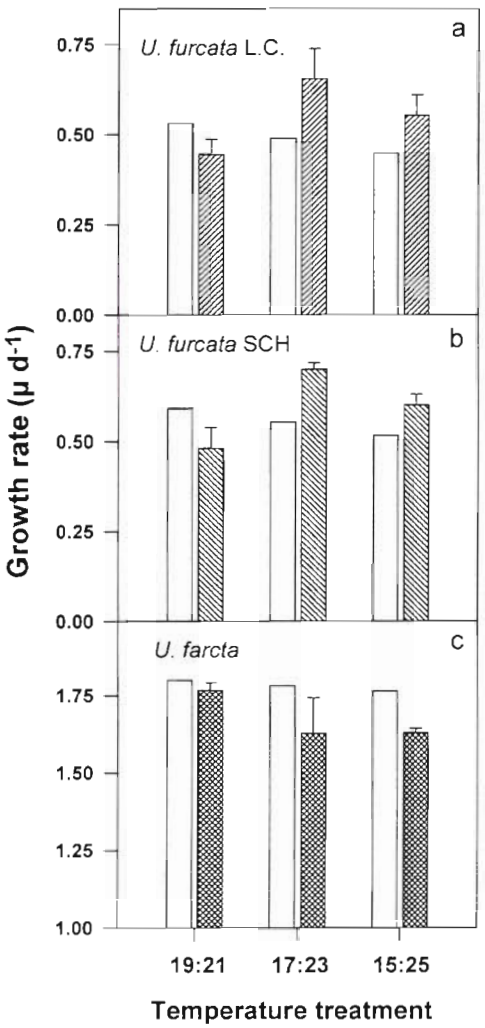

Fig. 2. Comparison of predicted (white bars) and measured (shaded bars) growth rates of 3 isolates of *Urotricha* in the various temperature treatments. (a) *U. furcata* from Lake Constance (LC), (b) *U. furcata* from Lake Schöhsee (SCH), (c) *U. farcta*. Predicted values indicate growth rates that would have been measured if growth was affected directly by fluctuating temperatures (see text for details). Error bars = 1 SD

For instance for *Urotricha furcata* SCH growth rate was 11% higher at 25°C and 32 % lower at 15°C compared to the growth rates at 20°C (Weisse & Montagnes 1998). For analysis, we assumed the same changes relative to the growth rates measured at 20°C would occur in the present investigation at the respective constant temperatures. Thus, we calculated theoretical growth rates (Fig. 2, open bars) and compared those to measured rates (Fig. 2, hatched bars). If the ciliates were subjected to fluctuating temperatures of, for example, 17:23°C, and the effect of temperature was immediate, the theoretical growth rate would be the arithmetic mean of the rates predicted at 17 and 23°C.

Predicted growth rates, however, differed from measured rates (Fig. 2). In both *Urotricha furcata* isolates, measured growth rates were significantly lower (*t*-test, $p < 0.05$) than predicted values in the 19:21°C treatments and higher than calculated rates at the wider temperature fluctuations. For *U. farcta*, measured rates tended to be lower than predicted rates. This effect was highly significant ($p < 0.001$) in the 15:25°C experiment. Similar disparities between measured and calculated values also occurred for volume and production of these isolates (data not shown).

Discussion. The effect of temperature on cell size, growth rate, and production of ciliates has been restricted mainly to studies at constant temperatures (e.g. Müller & Geller 1993). As constant temperatures are rarely experienced in nature, such work may be of questionable ecological significance (Cossins & Bowler 1987).

Day-to-day temperature variations of 1 to 2°C are typical for the lakes that our experimental ciliates were isolated from, especially during the onset of springtime stratification (e.g. Lampert et al. 1986, Bäuerle et al. 1998). Daily temperature fluctuations of 2 or 3°C occur for non-migrating epilimnetic organisms during summer (Geller 1986, Stich 1989). Furthermore, temperature changes of ~5°C occur in the epilimnion in summer during periods of rapid weather change (e.g. Geller et al. 1991), and even larger fluctuations may be regularly experienced by organisms living close to the thermocline, in particular in combination with transportation processes associated with internal seiches (Gaedke & Schimmele 1991). However, our results suggest that the effect of such fluctuating temperatures on volume, growth rate, and production cannot be predicted from the values measured at average, constant temperatures.

Our comparison of theoretical and measured growth rates (Fig. 2), admittedly, must be viewed with some caution. We assumed that the general temperature response of the respective strains determined earlier was valid, although the growth rates measured in this study at constant 20°C differed somewhat from the previous estimates (Weisse & Montagnes 1998). However, we have repeatedly measured the temperature response of both *Urotricha* species and have often found variation in the rates while the shape of the growth versus temperature curve remained unchanged (Weisse et al. unpubl.). The variation may originate from sampling bias, counting error, and some physiological changes of the ciliates in cultures. We can rule out that food limitation was important in the present study as we measured the highest growth rates (for *U. farcta*) at the lowest final food concentrations, and the remaining *Cryptomonas* sp. concentrations seemed still high enough to support unlimited growth of the ciliates. Furthermore, synchronous cell division which may affect growth rate calculations is of little importance in the ciliates studied under the experimental conditions (Weisse unpubl. res.). Thus, we consider our comparison of the 2 studies appropriate to assess differences in temperature responses.

The temperature effects we observed could not be consistently determined from temperature changes: in some isolates (*Urotricha furcata* LC and SCH) growth was accelerated at intermediate (±3°C) and wide (±5°C) temperature fluctuations but retarded at small (±1°C) temperature variations. In other isolates (*U. furcata* LM, *U. farcta*) growth was negatively affected if temperature fluctuations exceeded ±1°C. The stimulating effect of fluctuating temperatures was pronounced in 2 *U. furcata* isolates at moderately changing temperatures (17:23°C): *U. furcata* LC doubled every 25 h while the theoretically calculated value was 34 h (Fig. 2). Similarly, *U. furcata* SCH doubled every 24 h compared to the theoretical estimate of 30 h.

While the negative impact of temperature changes may be explained by stress effects (Cossins & Bowler 1987), the cause for a positive impact of temperature fluctuations is not obvious. However, data exist that show positive effects: there can be an acceleration effect of fluctuating temperatures on insect development (Kaufmann 1932) and the growth rate of *Daphnia* (Orcutt & Porter 1983) and rotifers (Halbach 1973); this is often referred to as the 'Kaufmann effect' (Cossins & Bowler 1987). The reasons for the Kaufmann effect and the often observed linear relationship between the rate of biological processes and temperature (Krogh 1914, Cossins & Bowler 1987, Weisse & Montagnes 1998) are, however, not well understood. The differential effect of fluctuating temperatures we found suggests that the temperature response of ciliates is a complex phenomenon composed of various physiological processes with specific relations to temperature. Regardless of the physiological basis of this response, it would appear that simply applying estimates from constant temperatures to fluctuating environments may be inappropriate.

A remarkable result of our study is that the effects of fluctuating temperatures on volume, growth rate, and production of *Urotricha* varied both inter- and intraspecifically. Although some differences existed, the temperature responses of the *U. furcata* isolates from Lakes Constance and Schöhsee were similar, supporting our earlier results (Weisse & Montagnes 1998). The effects of fluctuating temperatures on the *U. furcata* LM isolate were, however, more similar to those on *U. farcta* than to those on its conspecifics from Lakes Constance and Schöhsee.

The isolate from Lake Mondsee was unequivocally identified as *U. furcata*, both morphologically (W. Foissner pers. comm.) and genetically (Bruchmüller 1998). Our findings, therefore, support the earlier conjecture that large ecophysiological differences exist among morphologically identical ciliate isolates and species (Pérez-Uz 1995, Weisse & Montagnes 1998). Thus, although the total number of free-living ciliate species may be relatively small (Finlay & Fenchel 1999 and references therein), various ecotypes (Turesson 1922) undoubtedly exist within each species, and the functional biodiversity may be considerably larger than is at present assumed mainly based upon species numbers.

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