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# Pronounced ecophysiological clonal differences of two common freshwater ciliates, *Coleps spetai* (Prostomatida) and *Rimostrombidium lacustris* (Oligotrichida), challenge the morphospecies concept

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*Cell volume, growth and production rates of two common planktonic freshwater ciliates, the prostomatid* Coleps spetai *and the oligotrich* Rimostrombidium lacustris, *were investigated in clonal laboratory cultures. The clones were isolated from oligo-mesotrophic alpine Lake Mondsee, Austria, during summer and kept in culture with the small cryptophyte* Cryptomonas *sp. as food. All parameters investigated revealed significant clonal differences among both species. The extent of the clonal differences was comparable to differences observed earlier between similar planktonic ciliate species. The ecological relevance of varying clonal growth rates was evaluated using a simple numerical model. The model outcome suggests that differences in growth rates by 10% may significantly alter the clonal composition in the course of a ciliate peak in temperate lakes. The experimental results and the model outcome are discussed in the context of the functional diversity of freshwater ciliates. It is concluded that the morphospecies concept, which is the most widely used concept by both ciliate taxonomists and ecologists, may severely underestimate the ecological plasticity and the functional diversity of aquatic ciliates.*

## INTRODUCTION

Some ecologists assume that most free-living ciliate and other microbial species and genera have cosmopolitan distributions without biogeography, and that the variety of niches available for ciliates is restricted (Finlay *et al.*, 1996, 2002; Fenchel and Finlay, 2004). The total number of morphologically defined, free-living ciliate species may, therefore, be rather small (Finlay *et al.*, 1996, 2002; Finlay and Fenchel, 1999). Although this general view is strongly opposed (Foissner, 1999, 2004; Coleman, 2002; Lachance, 2004; Nanney, 2004, 2005), the low species diversity seems to hold true for planktonic freshwater species (Foissner *et al.*, 1999). While the total number of ciliate species found in the freshwater plankton is close to 700, only about 180 are euplanktonic (Foissner *et al.*, 1999). In spite of their contrasting views on the total number of free-living ciliate species, both Finlay and colleagues (Finlay *et al.*, 1996) and Foissner and co-workers (Foissner *et al.*, 1999) share the assumption that the morphospecies concept is a theoretically sound and pragmatic species concept, since morphology is closely related to ecological function (Finlay *et al.*, 1996; Fenchel and Finlay, 2004).

It is, however, obvious that cosmopolitan freshwater ciliate species will experience a range of environmental conditions. Morphologically indistinguishable populations can be sufficiently isolated to allow genetic divergence (Dini and Nyberg, 1993; Nanney, 1999). Thus, differing environmental regimes should create niches for ciliate species and clones; such clonal diversity is common in other freshwater taxa that reproduce

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primarily asexually (Carvalho, 1994; De Meester, 1996). Morphological intraspecific differences have been demonstrated for several ciliate species (Gates, 1978; Eigner, 1990, 1999); the recent application of molecular techniques revealed genotypic variances among different clones of the same or sibling species obtained from different geographical locations (Kusch, 1998; Fokin *et al.*, 1999; Kusch *et al.*, 2000). Similarly, there is evidence emerging that intraspecific ecophysiological variation is common among aquatic ciliates and other protists (Weisse, 2002, 2003). Considerable growth rate variability has been reported for planktonic ciliates isolated from different marine (Pérez-Uz, 1995) and freshwater (Weisse and Montagnes, 1998; Montagnes and Weisse, 2000) localities. Recent research revealed that common prostomatid ciliates of the genera *Urotricha* and *Balanion* isolated from similar, but geographically distant, lakes may differ significantly with respect to their cell size, ingestion, growth and production rates (Weisse *et al.*, 2001). Thus, although the total number of planktonic ciliate species may be relatively small, various ecotypes (Turesson, 1922) may exist within each species, and the functional diversity may be considerably larger than is at present assumed based mainly upon morphologically defined species numbers (Weisse, 2002; Nanney, 2005).

The goal of this study was to measure the extent of ecophysiological variation among ciliate populations obtained from a single lake within a few weeks. We chose temperature as a major environmental parameter to document ecophysiological variation among clones of two common planktonic species, the prostomatid *Coleps spetai* and the oligotrich *Rimostrombidium lacustris*. We did not apply molecular techniques to differentiate the clones, because there is at present no meaningful genetic marker available to characterize complex physiological processes such as growth rate. The potential ecological significance of our laboratory findings is illustrated using a simple mathematical model. Finally, the implications of the observed clonal variation for the ciliate species concept and the assessment of microbial diversity will be discussed.

## METHOD

### Study organisms and experimental design

Ciliate 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). Clonal cultures were obtained by pipetting of individual cells into separate 12-mL volume, tissue-plate wells, containing algae and modified woods hole medium (MWC, Guillard and Lorenzen, 1972). We repeated this step three times for each clone. All clones used in this investigation were identified unequivocally according to the taxonomic key properties listed in Foissner *et al.* (Foissner *et al.*, 1999). We can, therefore, rule out that we have mixed different (morpho)species in our study.

*Coleps spetai* Foissner was isolated from various sampling locations and different depths in Lake Mondsee during early summer 2000. Mondsee is an oligo-mesotrophic, deep (maximum depth 68 m), alpine lake located in the 'Salzkammergut' lake district in Austria. Water temperature during isolation ranged from 15 to 20°C. *Coleps spetai* is common in the plankton of L. Mondsee throughout the season (W. Foissner, University of Salzburg, personal communication).

*Rimostrombidium lacustris* Foissner, Skogstad and Pratt (syn. *Strobilidium lacustris*, Petz and Foissner, 1992; Foissner *et al.*, 1999) was isolated from Lake Mondsee during early summer 2001 at similar water temperatures as in the previous year.

All ciliate cultures were maintained in WC medium containing *Cryptomonas* sp. (cell volume ~280 $\mu m^3$) at 10–30 μ mol photons $m^{-2}$ $s^{-1}$ and 15 ± 1°C. *Cryptomonas* sp. was grown in WC medium at 100 μ mol photons $m^{-2}$ $s^{-1}$ and 15 ± 1°C.

All experiments were carried out with relatively fresh material, i. e. within 3–4 months after isolation and gradual acclimation to the experimental conditions over several generations. All clones of each species were kept and investigated under identical conditions. Ciliate growth experiments were conducted in 12-mL volume, tissue-plate wells, containing 8–10 mL of media/prey, or 50-mL tissue-culture bottles over 2 to 5 days. All experiments were performed in triplicate. A container without ciliates served as control for algal growth in each treatment. The ciliates were maintained at saturating prey levels ranging from 0.7 to 2.0 × $10^8$ cells $L^{-1}$, depending on species and temperature. The experiments were performed in darkness because *C. spetai* contains symbiontic algae that affect its growth rates when illuminated (own unpublished observation). The temperature range investigated was 9–21°C (*C. spetai*) and 9–24°C (*R. lacustris*). Subsamples were taken at 24-h intervals, and ciliate cell numbers were measured microscopically in a Sedgewick-Rafter counting chamber of 1-mL volume. Ciliate growth rates were calculated from changes in cell numbers assuming exponential growth.

Ciliate abundance and volume were determined from 2% acid Lugol's iodine preserved samples. Ciliate production was calculated as the product of cell volume and growth rate. Size measurements were performed using a semi-automatic image analysis systems (SIS and

LUCIA, Laboratory Imaging Ltd.). Ciliate volumes were calculated as prolate spheroids from length and width measurements made on 50 cells each, obtained at the end of each experiment. Lugol's fixation likely underestimates live volume of *R. lacustris* by 30–40% (Müller and Geller, 1993), whereas the armoured *C. spetai* does not shrink significantly upon fixation (Pfister *et al.*, 1999 and own unpublished observation). Prey numbers were determined using a CASY 1-model TTC (Schärfe System, Reutlingen, Germany) electronic particle counter. We did not measure ciliate cell volume by the electronic particle counter, because this procedure requires a minimum abundance of ~500 ciliates $mL^{-1}$ (Weisse, 2004), which was not reached in most experiments during this study.

Further details of the experimental design have been reported by Weisse and Montagnes (Weisse and Montagnes, 1998), Weisse *et al.* (Weisse *et al.*, 2001) and Weisse (Weisse, 2004).

## Population dynamics model

We used a simple numerical model to simulate the ciliate population dynamics in the course of a typical phytoplankton spring peak in a mesotrophic, temperate lake such as Lake Mondsee. We assumed that temperature would increase from 6.9 to 14.3°C in the course of one month; this increase in surface temperature was measured in Lake Mondsee between 20 April and 20 May, 1999. Since daily measurements were unavailable during this period and for the sake of simplicity, we assumed that temperature would rise linearly, i.e. by 0.24°C per day, over a period of 30 days. We then calculated a linear regression between ciliate growth rate (μ) and temperature (T) taking the data reported in Fig. 1 for *R. lacustris* and including results from Müller and Geller (Müller and Geller, 1993) for the temperature range from 5.5 to 18°C. The least-squares linear regression revealed the equation

$$\mu\left(\text{day}^{-1}\right) = -0.088 \times 0.0694T \quad \left(r^2 = 0.88\right) \qquad (1)$$

Since both zooplankton grazing on ciliates and food limitation of the latter gradually increase in temperate lakes during spring (Weisse *et al.*, 1990), we introduced a loss term ($g$) which was initially low (0.06 $\text{day}^{-1}$) and then increased at a rate of 25% per day. Population net growth rates ($k$) of the ciliates were thus computed according to

$$k\left(\text{day}^{-1}\right) = \mu - g \qquad (2)$$

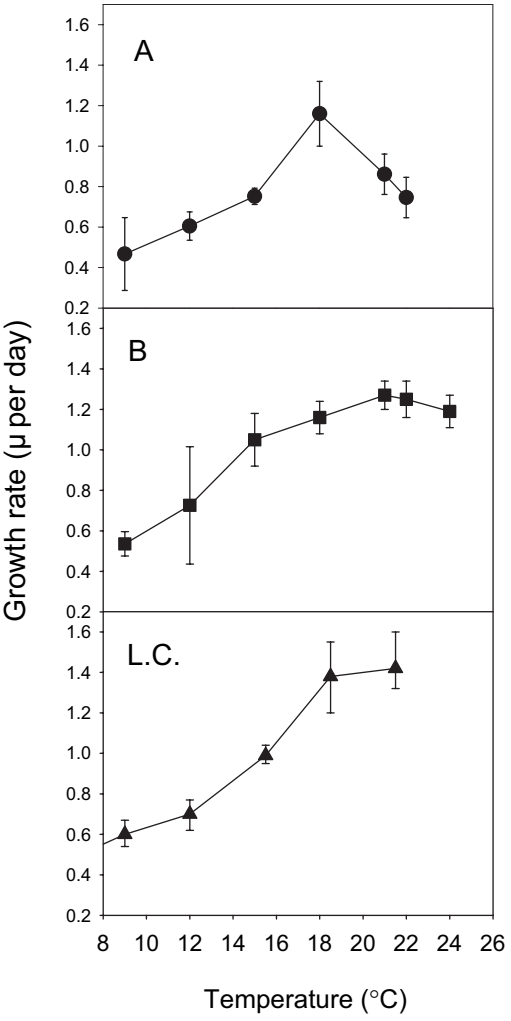


**Fig. 1.** Growth rates of two clones (**A** and **B**) of *Rimostrombidium lacustris* from Lake Mondsee and a non-clonal, mixed population of the same species from Lake Constance (L.C.) versus temperature. Error bars denote 1 SD of the mean values.

Changes in cell numbers were then calculated over a period of 30 days as

$$N_t = N_0 \times \mathrm{e}^{kt} \qquad (3)$$

Equation (3) was used to calculate cell numbers of a non-clonal, 'mixed' ciliate population and a 'fast growing' clone which would grow at a rate 10% faster than the average. Both ciliate fractions combined yielded the total ciliate population stock. We assumed an initial abundance of our hypothetical population of 1 ciliate $mL^{-1}$, and that 1% of this population was composed of the 'fast growing clone'. The starting conditions of the model have been summarized in Table I.

*Table I: Initial parameter values used in the population growth model*

| Parameter | Mixed population | Fast growing clone |
|---|---|---|
| Temperature (°C) | 6.88 | 6.88 |
| $\mu$ (day$^{-1}$) | 0.39 | 0.43 |
| $g$ (day$^{-1}$) | 0.06 | 0.06 |
| $N_0$ (cells mL$^{-1}$) | 0.99 | 0.01 |

### Statistical analyses

We used two-way analysis of variance (ANOVA) and *post-hoc* tests (Tukey's test, Student-Newman-Keuls test) to assess significant effects of temperature and clone on volume and growth rates of the study organisms. All statistical analyses were performed using SigmaStat (V 2.00 SPSS, Chicago, IL, USA).

## RESULTS

### Cell size, growth and production rates of *C. spetai* versus temperature

The cell size of *C. spetai* varied with temperature and clone. The mean length of the fixed cells ranged from 50.3 ± 2.4 (SD) µm (clone A, at 21°C) to 56.0 ± 3.2 µm (clone C, at 15°C); since length and width were closely correlated, these clones and temperature treatments exhibited also the maximum and minimum cell volume, 20 270 ± 3 850 µm$^3$ and 32 810 ± 6 750 µm$^3$, respectively (Fig. 2, left panels). Both temperature and clone impacted the cell volume of *C. spetai* significantly (two-way ANOVA,

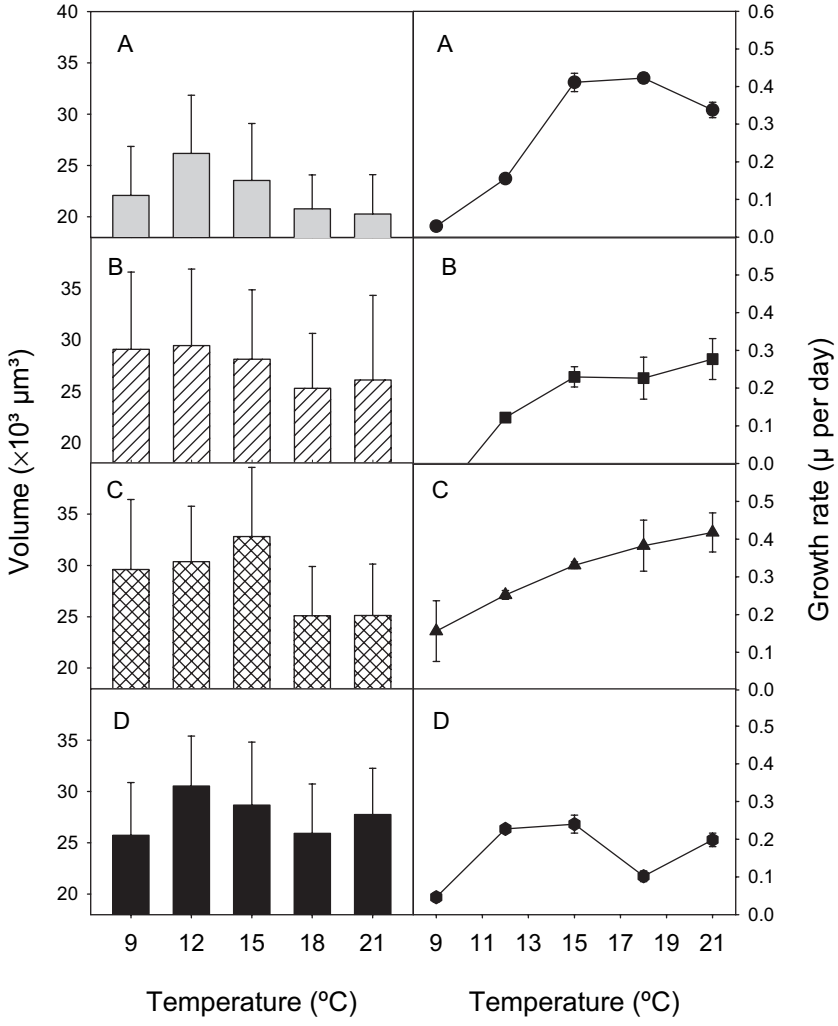


**Fig. 2.** Cell volume (**left** panels) and growth rates (**right** panels) of four clones (**A**–**D**) of *Coleps spetai* versus temperature. Error bars denote 1 SD of the mean values.

*Table II: Comparison of volume, growth and production rates of four clones of* Coleps spetai

| Clone | Volume ($\mu m^3$) | | | | $\mu_{max}$ ($day^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D |
| A | – | ++ | ++ | + | – | NS | NS | ++ |
| B | ++ | – | NS | NS | NS | – | + | NS |
| C | ++ | NS | – | + | NS | + | – | ++ |
| D | ++ | NS | NS | NS | NS | NS | + | NS |

All parameters were measured at five temperatures ranging from 9 to 21°C in triplicate each. Clonal differences were analysed by two-way analysis of variance (ANOVA) and Student-Newman-Keuls test. NS, not significant.
+, differences at $P < 0.05$; ++, significant differences between clones at $P < 0.01$.

$P < 0.001$). Overall, the volume was significantly decreased at 18 and 21°C, while there was no significant difference at the lower temperatures. Averaged over the experimental temperatures, clone A was significantly smaller than the other three clones investigated (Table II).

There were also significant effects of temperature and clone on growth rates of *C. spetai* ($P < 0.001$). Overall, growth rates increased with temperature; the extent of the temperature effect, the shape of the temperature response curve, and the temperature at which growth rates peaked were, however, clone specific (Fig. 2, right panels and Table II). Clones A and C were the fastest growing ones; both reached maximum growth rates of 0.42 $day^{-1}$, either at 18°C (clone A) or at 21°C (clone C). The latter was significantly different from all other clones but clone A (Table II). Negative growth at the lowest temperature was obtained for clone B (Fig. 2; right panel B). Averaged over the temperature range from 9 to 21°C, the mean growth rate of clone C (0.31 $day^{-1}$) was twice as high as that of clone D (0.16 $day^{-1}$).

The relative temperature impact was stronger on growth rate than on cell volume of *C. spetai*, with cells becoming smaller at higher temperatures; accordingly, the temperature response of cellular production, which is the product of growth rate and cell volume, closely followed that of growth rate (data not shown). Overall, positive production rates ranged from 640 $\mu m^3$ $day^{-1}$ (clone A, at 9°C) to 10,860 $\mu m^3$ $day^{-1}$ (clone C, at 15°C). The mean production rate between 12 and 21°C was lowest in clone D (5490 ± 2010 $\mu m^3$ $day^{-1}$) and highest in clone C (9660 ± 1430 $\mu m^3$ $day^{-1}$).

## Temperature response of *R. lacustris*

Similar to the prostomatid *C. spetai*, we found significant clonal differences in cell volume and growth rates of the oligotrich ciliate *R. lacustris*. Both temperature and clone significantly impacted the measured growth rate (two-way ANOVA). Clone A was, with an average cell volume of 99 990 ± 6 650 $\mu m^3$ at temperatures ranging from 9 to 22°C, significantly larger than clone B (mean volume 85 010 ± 9140 $\mu m^3$; Fig. 3). The average ciliate volume was significantly larger at 15 C than at 21 C and remained constant at temperatures <15°C.

The growth response to temperature was also different between the two clones of *R. lacustris* from Lake Mondsee, and these clones differed from a non-clonal *R. lacustris* population isolated from Lake Constance and investigated by Müller and Geller (Müller and Geller,

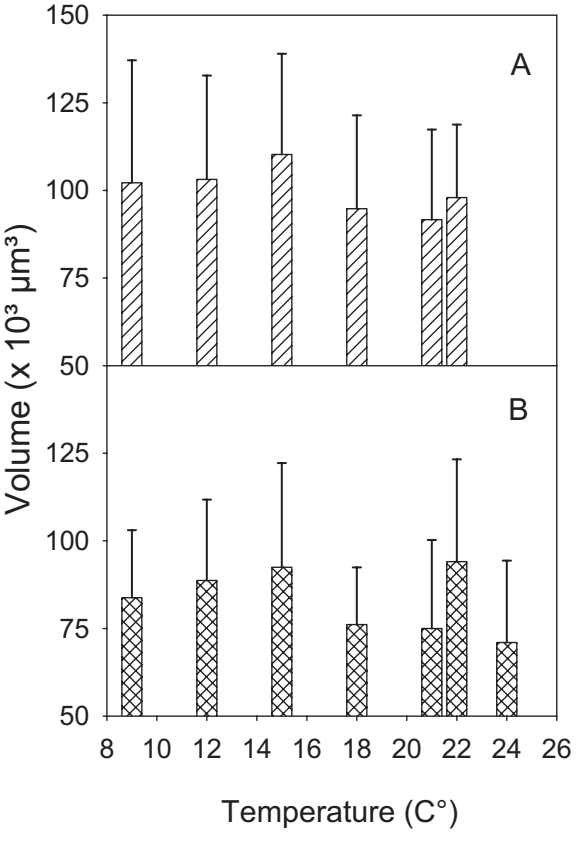


**Fig. 3.** Cell volume of two clones (**A** and **B**) of *Rimostrombidium lacustris* versus temperature. Error bars denote 1 SD of the mean values.

1993) under comparable laboratory conditions over the temperature range from 5.5 to 21.5°C (Fig. 1). Among the Mondsee isolates, the smaller clone B (cf. Fig. 3) grew significantly faster and better at higher temperatures than the larger clone A. Growth of the latter declined linearly at temperatures >18°C; at 22°C growth rate was identical (0.75 $day^{-1}$) to that obtained at 15°C. Growth rates of clone B peaked at 21°C and were not different at 22°C. We therefore extended the temperature range for this clone to 24°C, at which temperature growth was slightly decreased.

Cellular production was similar in both clones (data not shown). The average production of the Mondsee clones (80 200 ± 22 940) was also similar to the one of the Lake Constance population for the same temperature range [73 100 ± 27 090; recalculated from Müller and Geller (Müller and Geller, 1993).

### The ecological significance of clonal difference in growth rates—a simple model

Growth rates between the four *Coleps* clones and the two *Rimostrombidium* clones were significantly different, even under standardized laboratory conditions. To explore if seemingly minor differences in growth rates may be relevant under natural conditions, we compared the population dynamics of a hypothetical 'fast growing clone' to an undefined, 'mixed' ciliate assemblage using a simple model (Fig. 4). The model is based on our experimental data obtained for *R. lacustris* and several assumptions (see *Method* and Table I); the model should reflect the situation typically encountered in Lake Mondsee and comparable temperate lakes during spring, when the lake stratifies and the populations of algae and protozoa increase exponentially over several weeks (Weisse *et al.*, 1990; Salbrechter and Arndt, 1994).

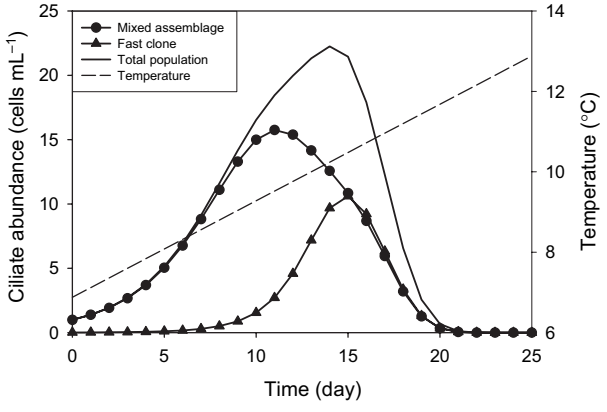


**Fig. 4.** Population dynamics of a hypothetical non-clonal ciliate population (mixed assemblage), a fast growing clone, and the combined total ciliate population during model simulations over 25 days. Temperature was assumed to increase linearly over the study period. See *Method* for a description of the model.

The model outcome illustrates that clonal differences in growth rates may be ecologically relevant. It took only 15 days for the fast growing clone, which initially contributed only 1% to total ciliate numbers, to reach similar cell numbers as the slower growing, mixed population (Fig. 4). The combined stocks of the two populations yielded a maximum ciliate abundance of 22 cells $mL^{-1}$, reached 2 weeks after the beginning of the ciliate peak. Thereafter, the losses exceeded the intrinsic growth rates of the ciliates, and their abundance began to decline. The model suggests a duration of such a ciliate 'bloom' of ~3 weeks.

## DISCUSSION

Growth and production rates of *C. spetai* have not been investigated previously. The cell size we measured was in the range reported by Foissner *et al.* (Foissner *et al.*, 1999) for this species. Pfister and co-workers (Pfister *et al.*, 1999) reported a higher live cell volume of 49 700 ± 9960 $\mu m^3$ for natural *C. spetai* obtained from Lake Mondsee in spring, 1997. It appears unlikely that this difference is an effect of fixation, because *C. spetai* is little sensitive to shrinkage (Pfister *et al.*, 1999). Similar cell volumes and growth rates as reported in this study were measured for the closely related *C. hirtus* (Madoni *et al.*, 1990), which does not bear symbiotic algae. Cell volume and growth rates of *R. lacustris* (syn. *Strombilidium lacustris*, Petz and Foissner, 1992) were similar to earlier estimates (Müller and Geller, 1993; Müller and Schlegel, 1999) obtained with a non-clonal culture from Lake Constance under comparable laboratory conditions (Fig. 1).

### Ecological implications of clonal differences

This study demonstrates that intraspecific ecophysiological differences may be of similar magnitude as interspecific differences among common planktonic ciliates, even within a given lake. The temperature, for instance, at which growth rate of *C. spetai* peaked differed clone specifically by up to 6°C (Fig. 2). Our results, therefore, corroborate and extend recent findings of intraspecific differences among mainly marine scuticociliates (Pérez-Uz, 1995) and freshwater prostomatids (Weisse and Montagnes, 1998; Montagnes and Weisse, 2000; Weisse *et al.*, 2001). Similar intraspecific differences were found among freshwater and marine (dino)flagellates (Kim *et al.*, 2004; Lowe *et al.*, 2005). These previous results were obtained mainly with geographically distant clones of the same species. Concerning the functional diversity of herbivorous ciliates and their ability to respond to short-term environmental changes, the as yet unknown within-lake

clonal variability may be of even larger ecological significance. The ecological performance of a nominal species may differ significantly in space and time, in relation to the changing clonal composition. Accordingly, the assumption of a species-specific growth response to temperature (Montagnes, 1996; Jakobsen and Hansen, 1997) may be an oversimplification for ciliates and other protists which reproduce primarily or exclusively asexually (Weisse, 2002). It appears that the reaction norm of those ciliates to temperature is a mosaic of individual clones whose reaction norms do not completely overlap. This conclusion was supported by a recent study on the ecophysiological and genetic intraspecific variability of the marine flagellate *Oxyrrhis marina* (Lowe *et al.*, 2005). Clonal differences may become even more pronounced if the effects of fluctuating temperatures, rather than constant temperatures, as in this study, are considered (Montagnes and Weisse, 2000).

Intraspecific differences have also been found among prostomatid ciliates for other ecophysiological parameters such as the response to predators (Weisse and Frahm, 2001). Apparently, there is a continuum in physiological as well as in some morphological parameters (Gates, 1990; Eigner, 1999) within a given species. Intraspecific genotypic variation has been demonstrated for several ciliate species (Diggles and Adlard, 1997; Kusch, 1998; Fokin *et al.*, 1999; Kusch *et al.*, 2000), for dinoflagellates (Kim *et al.*, 2004; Lowe *et al.*, 2005) and for some other heterotrophic protist taxa (reviewed by Schlegel and Meisterfeld, 2003). As already noted by Dini and Nyberg (Dini and Nyberg, 1993), extensive intraspecific variation is a fact in ciliates and other protists (Schlegel and Meisterfeld, 2003; Lowe *et al.*, 2005). It is, however, at present impossible to link genotypic variation observed within nuclear ribosomal DNA or protein-coding gene regions to phenotypic divergence measured in complex ecophysiological processes such as growth or feeding rates. The extent of genotypic and phenotypic clonal variation among common aquatic protists, and the widths and boundaries of their respective ecological niches, need to be better characterized.

The ecological relevance of our experimental findings was supported by the model simulation. Based upon our experimental results and realistic assumptions of the environmental parameters (temperature, abundance of ciliates, ciliate losses) encountered in Lake Mondsee and similar mesotrophic lakes, the model revealed that differences in growth rates in the order of 10% may significantly alter the clonal composition of ciliates within 2 weeks. The model results are in accordance with observations from (pre)alpine and other temperate lakes (Weisse and Müller, 1998; Weisse, 2003). Both the extent, i.e. the total ciliate abundance and the duration of the ciliate bloom are close to empirical observations of the wax and wane of the spring peak in lakes (Weisse *et al.*, 1990; Salbrechter and Arndt, 1994; Weisse and Müller, 1998) and coastal marine environments (Smetacek, 1981; Montagnes *et al.*, 1988).

Our model used growth rates and the temperature response measured for the oligotrich *R. lacustris*. Similar results have been reported for the marine oligotrich *Strombidinopsis multiauris* (Montagnes and Lessard, 1999). These authors simulated the population dynamics of this species in relation to food and predators during a ciliate 'bloom' in coastal waters. As in this study, they assumed an initial abundance of 1 ciliate $mL^{-1}$; maximum ciliate levels predicted by Montagnes and Lessard (Montagnes and Lessard, 1999) were 35 cells $mL^{-1}$, and the typical duration of the ciliate bloom was 2 to 3 weeks. It seems, therefore, likely that clonal differences in growth rates may also affect the population dynamics of coastal ciliates.

## Conclusions—implications for the (morpho) species concept and ciliate biodiversity

Protists with predominant or exclusive asexual reproduction have a (multi-)clonal population structure (Kusch, 1998; Kusch *et al.*, 2000; Lowe *et al.*, 2005) with divergent genotypes and phenotypes. This intraspecific variability is obvious at the physiological but usually hidden at the morphological level. The concept that each ciliate morphospecies has a discrete phenotype and a unique ecological niche and that, therefore, biodiversity can be assessed in terms of (morpho)species numbers (Finlay *et al.*, 1996; Finlay and Fenchel, 2004), needs to be rejected. The ecologically relevant realized or partial niche (Hutchinson, 1965; Vandermere, 1972) of a given, asexually reproducing ciliate species may vary considerably in space and time, primarily depending on the clonal composition of the population. This will inherently affect ecosystem function, since, e.g. clone-specific production rates provide the amount of ciliate food available for metazoan predators. We agree with Nanney (Nanney, 1999, 2004, 2005) that the functional diversity of ciliates is considerably larger than it is obvious at the morphospecies level, and that adherence to the morphospecies concept will grossly underestimate the number of species, the number of niches and the complexity of the ecosystem. The taxonomic rank of divergent clones within a nominal species is an open question that requires further research, and the quest for the most adequate species concept will thus continue.

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