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Effect of pH on growth, cell volume, and production of freshwater ciliates, and implications for their distribution

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Abstract

We investigated the effect of pH on growth, cell volume, and production of the freshwater ciliates *Urotricha farcta*, *U. furcata*, and *U. castalia* in laboratory cultures with *Cryptomonas* sp. as food. Overall, pH had a significant, species-specific effect on all parameters investigated. The food alga, *Cryptomonas* sp., showed a wide pH tolerance, with positive growth rates between pH 4.4 and pH 9.65. Among the ciliates, *U. farcta* was the most pH-tolerant and *U. castalia* was the most pH-sensitive species, with positive growth being confined to pH 6.5–8.2. The pH optimum was derived from cellular production rates. The pH optima of the three ciliate species were shifted; their production rates peaked at pH 4.4–5.3 (*U. farcta*), pH 5.9–7.3 (*U. furcata*), and pH 6.8–7.9 (*U. castalia*). The pH effect on growth and survival of the ciliates was minor at circumneutral and moderately alkaline pH values, relative to the effect of temperature and food measured in earlier experiments. The widths of the pH tolerances of the ciliates were positively related to the widths of their temperature niches and to their natural distributions. *U. farcta* and *U. furcata* were characterized as euryoecious species, with broad pH and temperature tolerances and ubiquitous distribution; *U. castalia* is a rare, stenoecious species, requiring specific pH and temperature conditions.

pH is a major environmental factor of aquatic ecosystems at the interface of physicochemical and biological processes. It is regulated by carbonate equilibrium, both in the ocean and in most inland waters, and is impacted by biological processes such as photosynthesis and respiration. Although pH is relatively constant in the ocean, 8 ± 0.5 (Lalli and Parsons 1993), it varies between <2 and 12 in lakes and rivers (Wetzel 2001), in close relation to the geology (rock type) and hydrology of their drainage basins. Weathering of soils and rocks primarily controls the ion supply and, thus, the pH of inland waters. The effects of biological processes are less important when comparing the pH across different ecosystems, but may largely control seasonal pH fluctuations within a given water body. Biologically-driven seasonal variation in temperate, moderately-productive and moderately–hard-water lakes is typically ≤ 1 pH unit (e.g., BfW 2002). Larger pH fluctuations, up to >2 pH units, occur in lakes where the buffering capacity of the carbonate system is less efficient, in highly productive small water bodies, and in the littoral zones of shallow lakes with intense primary production (Talling 1976; Krambeck et al. 1994; Edmonson 2005).

Effects of hydrogen ion activity on aquatic biota have received the most attention at the extremes of the pH range; in particular, the impact of lowered pH in poorly buffered waters as a consequence of acidic deposition was studied in great detail in Northern Europe and North America during the closing decades of the last century (Schindler 1988; Battarbee 1990; Charles 1991). Similarly, interdisciplinary studies investigated the impact of acid mine drainage on aquatic communities (Geller et al. 1998). As a result of those efforts, the general reduction of species diversity with decreasing pH and the tolerance limits for low pH are known for major aquatic taxa such as fish, zooplankton, and algae (Schindler 1988; Baker and Christensen 1991). Algal taxa with solid cell walls, such as diatoms and chrysophytes, are used by paleolimnologists to infer the natural and man-made changes of pH in lakes over the past 10,000 years (Smol et al. 1986; Psenner and Schmidt 1992). With the exception of the pioneering work by Beaver and Crisman in Florida lakes (Beaver and Crisman 1981; Bienert et al. 1991), protozoa, although they are important components of all aquatic food webs (Sherr and Sherr 1984; Laybourn-Parry 1992; Weisse 2003) and may dominate at low pH (Packroff 2000), were largely neglected in the previous investigations cited above. Beaver and Crisman (1981) reported a shift in the taxonomic composition of ciliates with decreasing pH, with oligotrichs becoming the dominant group at pH <5. A recent review concluded that Prostomatida, Hypotricha, and Peritricha are the dominant ciliate orders in acidic lakes (Packroff 2000).

Although there is some evidence for species-specific pH tolerance of planktonic freshwater ciliate species, primarily originating from cursory field measurements (compiled by Foissner et al. 1999), an experimental laboratory investigation of the pH reaction norm of common species is still lacking. Temperature, food, and predators are assumed to control the population dynamics of natural ciliate communities (Fenchel 1987; Laybourn-Parry 1992; Weisse 2003) and are responsible for niche partitioning among sympatric, closely related taxa (Weisse et al. 2001). The significance of pH for the occurrence and competitiveness of planktonic freshwater ciliates is virtually unknown. This may result from the assumption that daily and seasonal

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fluctuations in pH are minor, relative to those of temperature and biological interactions. However, pH fluctuations by 1–2 units imply 10- to 100-fold changes in free hydrogen ion activity. It is well known that hydrogen ion concentration gradients affect many transport processes across cellular membranes and metabolic functions in the cytoplasm and cellular organelles (Anderson 1988; Prescott et al. 2002). Further, pH has a strong impact on the solubility, bioavailability, and toxicity of ammonium/ammonia, iron, aluminum, and some other heavy metals (Anderson 1988; Wetzel 2001). Physiological mechanisms and adaptations to withstand fluctuating pH have been studied in some protist species (e.g., Knitt and Herschlag 1996; Davis et al. 1998). Irrespective of the specific cellular strategy for coping with changing pH, to maintain a stable pH gradient under fluctuating external hydrogen ion concentrations requires energy that is unavailable for anabolic processes. It is, therefore, likely that pH changes affect the growth rates of aquatic protists negatively. This effect has already been demonstrated at high pH for several marine protist species (Hansen 2002; Pedersen and Hansen 2003) and for some freshwater algae, mainly at low pH (Gerloff-Elias et al. 2005 and references therein).

The present study investigated the pH reaction norm of ecologically contrasting planktonic freshwater ciliates under standard laboratory conditions. We chose two common and one rare small species of the genus *Urotricha* (Foissner et al. 1999; Weisse et al. 2001) to test for interspecific differences among closely related ciliates. The common species *U. furcata* and *U. farcta* seem to be tolerant of a wide range of pH values; these and/or morphologically similar small *Urotricha* species were recorded in lakes at pH <2 to >9 (Foissner et al. 1999; Packroff 2000; Packroff and Woelfl 2000). Little is known of the ecology of the third species, *U. castalia* (Weisse et al. 2001). Our investigation had two goals: first, we tested the hypothesis that the pH tolerance of the species would be related to their occurrence and distribution, i.e., that the two ubiquitous species would have broader pH reaction norms than the rare species; second, we wanted to assess the extent of minor pH fluctuations in the circumneutral and moderately alkaline range on the growth and production rates of the respective species. The latter aimed at evaluating the potential significance of temporal and spatial pH fluctuations in the natural environment, relative to the effects of temperature and food known from earlier experiments with the same species (Weisse and Montagnes 1998; Weisse et al. 2001, 2002).

Material and methods

Study organisms—All protist species investigated in this study have been reared in our laboratory as nonclonal, nonaxenic batch cultures for several years. Ciliate stock cultures were maintained in modified Woods Hole Medium (MWC; UKNCC 2001) on a 12 : 12 light : dark (LD) cycle at an irradiance of 30–40 μmol photons m^{-2} s^{-1} and 15 $\pm$ 1°C. The small cryptophyte *Cryptomonas* sp. (SAG strain No. 26.80, provided by the Culture Collection of Algae in Göttingen, Germany) served as food. Earlier experiments with the same combination of ciliates and food investigated the effect of temperature and food on growth and survival of the ciliates (Weisse and Montagnes 1998; Weisse et al. 2001, 2002).

The strain of *Urotricha furcata* Schewiakoff 1893 used in this study was isolated from surface waters of Lake Schöhsee, northern Germany, during summer 1995. *U. farcta* Claparède and Lachmann 1859 was isolated from the littoral zone of the same lake in spring 1996. *Urotricha castalia* Muñoz, Télez, and Fernandez-Galiano 1987 is the largest of the 3 prostome ciliate species investigated. The species was first described by Munoz et al. (1987) from an artificial Spanish pond and was redescribed by Foissner and Pfister (1997) with material provided by H. Müller from Lake Constance. This isolate was also used in the present study. With a maximum length of 42 μm of Lugol's-fixed cells, *U. castalia* is the largest of the three *Urotricha* species investigated (Table 1). The pH in all ciliate stock cultures ranged from 7.4 to 8.1.

pH measurements—pH was measured using a microprocessor pH-mV meter (model pH 526, WTW) to the nearest 0.01 unit. The pH sensor was two-point calibrated with standard buffer solutions of pH = 6.87 and pH = 9.18 prior to each series of measurement. The pH in each experimental flask was adjusted by the addition of 0.1 mol L^{-1} NaOH or HCl.

Experimental design—Experiments were carried out in 50-mL culture tissue flasks in dim light (10–30 μmol photons m^{-2} s^{-1}) under a 14 : 10 LD cycle. Experimental volume in each flask was 40 mL. Experimental temperature was 15 $\pm$ 0.2°C. The food and ciliate species were stepwise acclimated to the experimental conditions over a period of 2–5 d; the longest period was applied at the extremes of the pH range. We used this acclimation period to render this study comparable to earlier experimental investigations with the same ciliate and algal species (Weisse et al. 2001, 2002). At the beginning of each experiment, algae were diluted with MWC medium and distilled water to yield saturating food concentrations for the ciliates (60,000–110,000 cells mL^{-1}; Weisse et al. 2001, 2002). Between 2 and 20 mL of the acclimated ciliate cultures were added to yield initial ciliate levels of 100–500 cells mL^{-1}. Each pH experiment was run in triplicate. Containers with the same algal levels and pH but without ciliates served as controls.

The experimental duration was 24 h; pH was measured in each flask at 0, 3, 6, 12, and 24 h after the beginning of the experiment. If the pH differed by more than 0.2 from the target pH, it was adjusted by addition of small amounts (15–35 μL) of 0.1 mol L^{-1} NaOH or HCl. At pH <6, small amounts (3–5% final concentration, vol/vol) of sterilized soil and peat extract were added as buffer. Preliminary experiments with an oligotrich ciliate species had shown that this addition stabilizes the pH without negatively affecting the food algae (Weisse et al., unpubl.). Algal abundance in each container was measured at the beginning and end of each experiment in unfixed samples with an electronic particle counter (CASY® 1-Model TTC, Schärfe System) and in formalin-fixed samples by flow

Table 1. Range of cell length, pH tolerance, and pH optimum of *Cryptomonas* sp. and three *Urotricha* species (*n* denotes number of Lugol's-fixed cells measured for size; cell length of *Cryptomonas* sp. was measured over the pH range 5.1–9.3).

Species	Cell length (μm)	*n*	pH tolerance	pH optimum
Cryptomonas sp.	9.9–16.4	140	4.4–9.65	5.8–8.1
Urotricha farcta	13.2–28.3	1,050	4.1–9.5	4.4–5.3
Urotricha furcata	11.1–25.2	900	5.4–9.2	5.9–7.3
Urotricha castalia	18.7–42.0	600	6.5–8.2	6.8–7.9

cytometry (FCM; FACSCalibur, Becton Dickinson Austria; Weisse et al. 2002). Samples for ciliates were taken at the beginning and end of each experiment and fixed with acid Lugol's.

Analyses and calculation of experimental results—The average pH in each experimental flask was estimated from the logarithms of the reciprocals of the concentration of free hydrogen ions measured at 0, 3, 6, 12, and 24 h after the beginning of the experiment, i.e., from log pH, and subsequent power transformation. Ciliate cell numbers were measured microscopically using a Sedgewick Rafter cell (1 mL volume) or settling chambers of 3 mL volume. Algal concentrations were also measured in some of these samples to check for the precision of the electronic and optical (FCM) cell count measurements. Cell volume of ciliates was determined from length and width measurements of Lugol's-fixed material, assuming a prolate spheroid shape with circular cross-section. Measurements were made on 50 ciliates obtained at the end of the experiment from each experimental series at a given pH, using an inverted microscope and an image analysis system (LUCIA version 4.51, Laboratory Imaging Ltd.). Subsamples for the ciliate size measurements were pooled if pH differed by <0.05 units.

Algal and ciliate population growth rates were determined from end-point measurements of cell numbers, assuming exponential growth over the experimental period according to

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

where N_0 and N_t are algal or 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. Ciliate production ($\mu m^3\ d^{-1}$) was calculated as the product of growth rate (μ) and the corresponding cell volume.

A second order polynomial regression of the form $y = y_o + ax + bx^2$ (Eq. 2) was used to fit the curves of the ciliate growth and volume response versus pH. If the shape of the curves was inadequately represented by the second order polynomial regression, least-squares linear regression and Student's *t*-test were used to analyze differences within a given species. All statistical analyses were performed with SigmaStat for Windows, version 2.03 (SPSS Inc., Chicago).

Results

pH effect on Cryptomonas *sp.*—Our investigation of the pH impact on the ciliates was limited by the pH tolerance of their prey. We therefore report the response of *Cryptomonas* sp. to pH measured in the control bottles without ciliates first. *Cryptomonas* sp. showed positive population growth rates over a wide pH range, from 4.4 to 9.65 (Fig. 1A). The cell volume of the algae remained constant over the same pH range (Fig. 1B); it increased rapidly at pH <4.4. Visual inspection under the microscope revealed that the cells looked inflated under highly acidic conditions. Cells died quickly at pH >10, and it was impossible to accurately estimate growth rate and cell volume under highly alkaline conditions.

pH effect on growth rates of Urotricha *spp.*—Among the 3 *Urotricha* species investigated, population numbers of *U. farcta* increased over the widest pH range (4.1–9.5) and reached the highest maximum growth rates (μ_{max}), 1.8 d^{-1} at pH 5.2 (Fig. 2A). Average growth rates of this species at its pH optimum (pH 4.4–5.3, Table 1) were 1.41 $\pm$ 0.29 d^{-1}, i.e., significantly higher (Student's *t*-test, $p<0.01$) than at pH 6.0–8.0 (1.00 $\pm$ 0.34 d^{-1}). From pH 6.3 to pH 9.4, growth rate declined linearly (μ = 3.042–0.281 pH, $r^2 = 0.613$, $n = 25$, $p < 0.001$; Eq. 3). Positive population growth of *U. furcata* was confined to the pH range of ~5.4–9.2 (Fig. 2B); highest growth rates of ~0.6 d^{-1} were measured between pH 6.0 and pH 7.0. Mean growth rates in this range (0.43 $\pm$ 0.10 d^{-1}) were significantly higher than at pH 7.5–8.1 (0.24 $\pm$ 0.08 d^{-1}). As with *U. farcta*, growth rates declined linearly between pH 7.0 and 9.2 (μ = 1.562–0.168 pH, $r^2 = 0.650$, $n = 20$, $p < 0.001$; Eq. 4). The largest species investigated, *U. castalia*, grew over a narrow pH range only, from pH 6.5 to pH 8.2 (Fig. 2C). Its growth rates peaked at pH 6.9–7.2; μ_{max} of *U. castalia* was close to 0.4 d^{-1}.

The shape of the pH response curves of all three species was irregular and difficult to model. A second-order polynomial regression yielded, in each case, the best curve fit (data not shown), but left 29% (*U. farcta*) to 44% (*U. castalia*) of the variance of the ciliate growth rates unexplained. In particular, the second-order polynomial regressions did not adequately model the linear part of the growth curve of *U. furcata* (Fig. 2B), and the peak of the growth rate of *U. castalia* (Fig. 2C).

pH effect on cell volume of Urotricha *spp.*—Cell volume of all 3 *Urotricha* species peaked close to the pH at which each species' respective μ_{max} was recorded (Fig. 3), but we did not measure a significant overall relationship between μ and cell volume in any of the species. A second-order polynomial regression (Eq. 2) yielded a significant association between pH and cell volume for *U. farcta* (Fig. 3A)

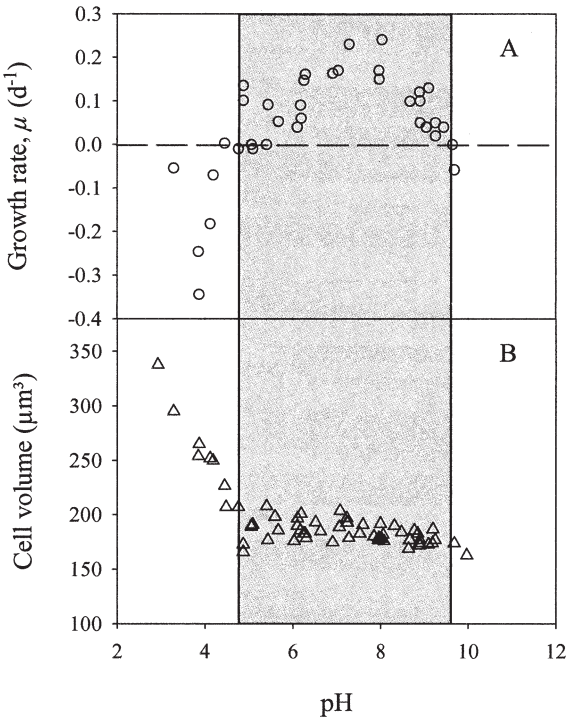

Fig. 1. (A) Population growth rate, μ, and (B) cell volume of *Cryptomonas* sp. versus pH. The shaded area indicates the pH range in which positive population growth was measured.

and *U. furcata* (Fig. 3B), whereas the fit was insignificant for *U. castalia* (Fig. 3C). The relative pH impact on cell volume was highest in *U. farcta*; its mean volume varied by a factor of 3, whereas relative differences in the cell volumes of the other two species were <1.5. The cell sizes of *U. farcta* (2,600 ± 560 μm^3) and *U. furcata* (2,020 ± 230 μm^3) overlapped in the circumneutral range, but they were significantly different at pH <6 and >8 (Student's *t*-test, $p<0.01$).

Our volume and production estimates are conservative estimates, because Lugol's fixation may underestimate live volume by 30% (Jerome et al. 1993; Müller and Geller 1993).

pH effect on cell production of Urotricha *spp.*—Cellular production illustrates the combined pH effect on growth rates and cell volume of *Urotricha* spp. Figure 4 shows mainly positive production rates for the 3 species, i.e., over the pH range in which their respective growth rates were positive (cf. Fig. 2). Therefore, and because cells from the growth rate versus pH experiments were pooled for the size measurements, there are fewer data points in Fig. 4 than in Fig. 2. In *U. castalia*, we included the slightly negative production rates measured at pH 6.7 and pH 8.3 to illustrate more clearly the drastic decline at both sides beyond the pH optimum (Fig. 4C). If the pH optimum is defined as the range at which production is >80% of the maximum production, the pH optima of the three *Urotricha* species (shaded areas in Fig. 4) overlap not at all (*U. farcta* vs. *U. furcata* and *U. castalia*) or only little

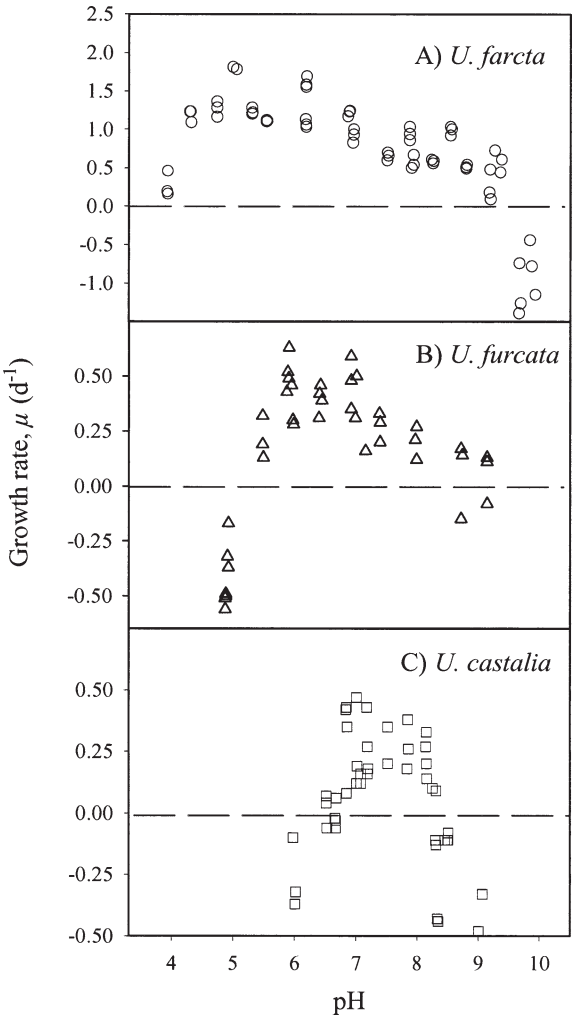

Fig. 2. Population growth rate, μ, of three *Urotricha* species versus pH. (A) *U. farcta*, (B) *U. furcata*, and (C) *U. castalia*.

(*U. furcata* vs. *U. castalia*). For *Cryptomonas* sp., the pH optimum thus defined ranged from 5.8 to 8.1 (Table 1).

Discussion

Methodological constraints—acclimation and food quality may affect species-specific physiological response to changing pH—It appears obvious that we have not covered the full ranges of pH tolerance, i.e., the fundamental pH niches of the species in our experiments. This is primarily because we used only one food species at particular light, temperature, and medium conditions. More research with different food and experimental conditions is needed before the in situ pH responses of the respective species can be predicted based on an empirical model. We can rule out that food limitation was responsible for declining ciliate growth rates, because, in all experiments, the food concentration was kept at the saturation range known for each species from previous experiments (Weisse et al. 2001, 2002). Furthermore, growth rates of all three ciliate species measured in the present study were not different from earlier results obtained with the same species under

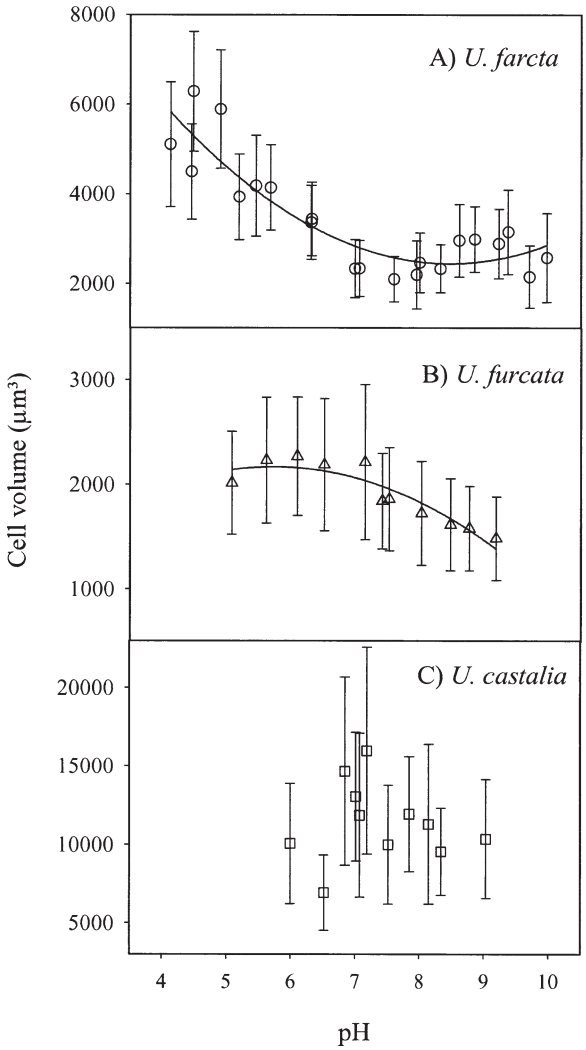

Fig. 3. Mean cell volume of three *Urotricha* species versus pH. Error bars denote 1 SD. Solid lines in (A) and (B) indicate the fit to a nonlinear polynomial regression (see text). (A) *U. farcta*, (B) *U. furcata*, and (C) *U. castalia*.

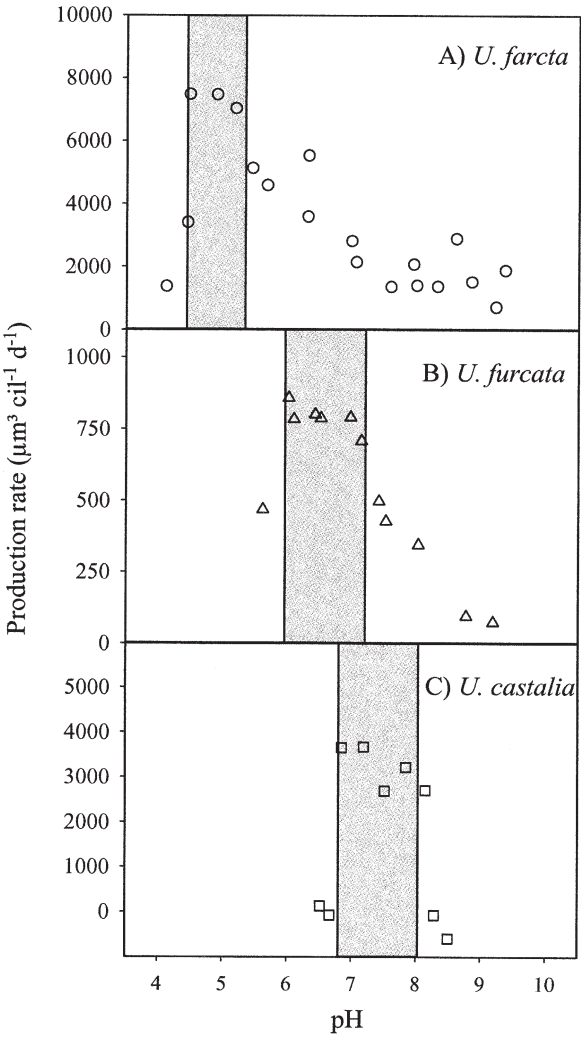

Fig. 4. Cellular production rate of three *Urotricha* species versus pH. The shaded area indicate the pH optimum at which production reached >80% of the respective maximum. (A) *U. farcta*, (B) *U. furcata*, and (C) *U. castalia*.

comparable experimental conditions (Weisse et al. 2001, 2002).

We measured the impact of pH on population growth rate because, in asexually reproducing protists, increase in cell numbers may be used as a proxy for Darwinian fitness. The pH response might have been affected by long-term adaptation in the laboratory, because the pH in our stock cultures usually varies between 7.6 and 7.9. Similarly, longer acclimation to the experimental conditions may expand the pH tolerance and/or yield somewhat higher growth rates at the extremes of the pH range. The shift in the pH optimum of *U. farcta* and, less obviously, of *U. furcata* towards acidic conditions (Table 1) illustrates, however, that adaptation and acclimation cannot explain the pH reaction norm of these species.

The physiological mechanisms by which the protist species maintained internal (cytoplasmic) pH homeostasis under changing external pH were beyond the scope of this study. If the ability to regulate cell size is taken as a crude indicator of pH stress, species-specific differences between the ciliates and their prey become apparent. The food alga, *Cryptomonas* sp., kept its cell size constant and small over a wide range (pH 4.5–9.6, Fig. 1), i.e., relatively high growth rates were associated with small cell size. The constant cell size does not necessarily imply that food quality did not change, because algal growth rates varied over this range. The pH responses of the ciliates were most likely determined by a combination of direct pH effects and indirect effects through the nutritional quality of their food.

In the two smaller of the three ciliate species investigated, cell volume was significantly affected by pH changes, whereas there was no significant pH effect on the cell size of *U. castalia*. The data basis of our study is, however, too limited, and the scattering around the mean intrinsically too high in cell volume, to reveal any more statistically significant effects between pH tolerance and cell size. The high standard deviation of cell volume results from the fact that in actively growing protist populations

there are always large, ''old'' cells present, ready to divide, and small, ''young'' cells that have just completed cell division. The relative pH effect on cell volume was twice as high in *U. farcta* as in their congeners (Fig. 3), indicating species-specifically different regulatory processes.

We used one nonclonal isolate for each species in this study; recent evidence suggests that intraspecific ecophysiological differences may be pronounced in ciliates and other protists (Weisse and Montagnes 1998; Weisse 2003; Weisse and Rammer 2006). Our own preliminary experiments with several clones of an oligotrich freshwater ciliate under comparable experimental conditions as used in the present study revealed intraspecific differences in the pH reaction norm by up to 0.5 pH units (Weisse et al. unpubl. data). The pH tolerance of the three *Urotricha* species reported in this study (Table 1) may therefore be expanded if several clonal cultures of each species obtained from different environments are measured.

The significance of pH as an environmental factor for freshwater ciliates—To our knowledge, ours is the first experimental study that reports the pH reaction norm not only of three small *Urotricha* species, but of any ecologically relevant freshwater ciliate. The pH tolerance of planktonic ciliates and other free-living protists is poorly documented in the literature, although it has been known for a long time that pH is an important physicochemical environmental parameter affecting ciliate species composition and species richness (Noland 1925; Lackey 1938; Doflein and Reichenow 1949). Similar to investigations on the pH effect on metazoans and at the ecosystem level (Schindler 1988; Charles 1991), the impact of pH on protists has been studied primarily at the extremes, such as in acid mining drainages, humic lakes, or soda lakes (Laybourn-Parry 1992; Packroff 2000). With some remarkable exceptions (Anderson 1988), the ecological impact of pH on freshwater protists has been largely neglected in recent textbooks and reviews (e.g., Fenchel 1987; Laybourn-Parry 1992).

To some extent, our results seem to support the view of little ecological significance of hydrogen ion concentration for freshwater protists. At circumneutral and moderately alkaline pH values, which are characteristic of most natural fresh waters, the effect of pH on *Cryptomonas* and the three prostome ciliate species investigated was minor. All species showed positive growth rates at pH values ranging from 6.5 to 8 (Table 1). We used *U. farcta* as a model organism to evaluate the potential significance of seasonal pH changes in the natural environment on growth rates of planktonic ciliates, relative to the effect of seasonally fluctuating temperature and food levels. An increase in pH from 7.4 to 8.4 reduced mean growth rates of this species from 0.96 d^{-1} to 0.68 d^{-1}. Previous investigations in our laboratory with the same ciliate and food alga revealed that the growth rate of *U. farcta* declined from 2.95 d^{-1} at 24°C to 0.04 d^{-1} at 5°C (Weisse et al. 2001) and from 1.67 d^{-1} at a food concentration of 1 mg C L^{-1} to 0.61 d^{-1} at 0.05 mg C L^{-1} (measured at 18°C, Weisse et al. 2002). These are the ranges of seasonal variation of pH, temperature, and food levels typically encountered in Lake Schöhsee and other meso-

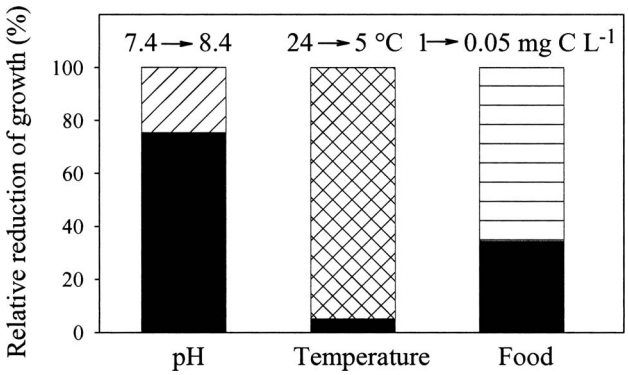

Fig. 5. The effect of pH, temperature, and food on growth rates of *Urotricha farcta*. The solid bars indicate the relative reduction of growth from near-optimal (hatched bars, growth = 100%) to suboptimal conditions for the three variables; the shift of each environmental factor from near-optimal to suboptimal conditions is given on top of the respective bars.

trophic, temperate, hard-water lakes where *U. farcta* is prominent (Foissner et al. 1999). The relative effects of these environmental variables on growth rates of *U. farcta* are illustrated in Fig. 5. In each case, ciliate cell numbers predicted from Eq. 1 after 24 h under less favorable environmental conditions (i.e., pH = 8.4, T = 5°C, food = 0.05 mg C L^{-1}) were compared to ciliate cell numbers obtained under more favorable or near-optimum conditions (pH = 7.4, T = 24°C, food = 1 mg C L^{-1}). To render the results comparable, the impact of the three environmental variables is expressed in terms of percentage reduction of growth under the suboptimal conditions. It is obvious that the potential effect of seasonal pH changes is minor relative to that of temperature and food (Fig. 5).

This general conclusion holds true for ciliates with a wide pH tolerance, such as *U. farcta* and *U. furcata*. Note that for pH-sensitive species, such as *U. castalia*, pH may limit their distribution in many inland waters. The ciliate fauna has been investigated in detail in north German Lake Plußsee (Reck 1987), which is located less than 3 km from Lake Schöhsee, and from which two of the three ciliate species used in this study had been isolated. Seasonal changes of the ciliate community were not studied in Lake Schöhsee. In contrast to the latter and most other lakes in the Schleswig-Holstein area, Lake Plußsee is less strongly buffered by the carbonate system, and seasonal pH changes are, therefore, wider, ranging from 7.3 to 9.5 in the epilimnion (Reck 1987; Krambeck et al. 1994). In accordance with the experimental results of the present study, *U. farcta* was the only *Urotricha* species that was recorded during all seasons in Lake Plußsee, whereas *U. castalia* was not found (Reck 1987). In prealpine Lake Mondsee, where all three *Urotricha* species occur (Foissner and Weisse unpubl. data), seasonal changes in pH are confined to the range 7.6–8.5 (BfW 2002).

The pH niches of Urotricha *spp.*—Relative to the other two species, *U. castalia* had a narrow niche width, with positive growth rates limited to the circumneutral range. In contrast to this stenoecious species, both *U. farcta* and *U.*

furcata are euryoecious species with a wide pH tolerance. Similar to the effect of temperature (Weisse et al. 2002), the highest growth rates of ciliates measured under saturating food conditions may not necessarily indicate their pH optima. We suggest the use of cellular production, which includes the pH effect on cell volume, to characterize the pH optimum of the various ciliate species. According to the pH optima thus defined, *U. castalia* and *U. furcata* are neutrophil species, whereas *U. farcta* is an acidophil species (e.g., Prescott et al. 2002). Similar to the effect of temperature investigated earlier (Weisse et al. 2001), *U. farcta* was the most competitive of the three *Urotricha* species with respect to the effect of pH; it not only tolerated the widest pH range of all three *Urotricha* species, but also showed the highest growth rates at all pH levels investigated. The results of the present study expand the pH range of *U. farcta* hitherto reported in the literature (6.4–9.2, Foissner et al. 1999). According to our results, *U. farcta* is a candidate species to be encountered in acidic lakes. Note, however, that the species identity of small *Urotricha* species recorded from highly acidic water bodies (pH <3) remained, in most cases, obscure (Packroff 2000; Packroff and Woelfl 2000).

In conclusion, the extent of pH tolerance was positively correlated to the occurrence and distribution of the species, i.e., the two common species were pH-tolerant and the rare species was pH-sensitive. The pH tolerance may thus limit the distribution of ciliate species across freshwater ecosystems with widely different pH. The relative tolerance towards temperature and pH were coupled, declining in the order *U. farcta* > *U. furcata* > *U. castalia*. Further research is needed to reveal whether this coupling between temperature and pH tolerance is a general trait of planktonic ciliates.

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