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CLADOCERA

Colonization of acidic mining lakes: *Chydorus sphaericus* and other Cladocera within a dynamic horizontal pH gradient (pH 3–7) in Lake Senftenberger See (Germany)

Maria Belyaeva · Rainer Deneke

Abstract Acidic mining lakes are man-made habitats, which differ greatly from natural acidic lakes in their water chemistry. In order to study the potential for colonization of mining lakes by Cladocera we investigated their in situ distributions within a pH gradient from 3 to 7.5 occurring in Lake Senftenberger See (Germany). We found that species in situ pH minima were higher and the overall diversity at the respective pH values was lower in the investigated mining lake in comparison to natural acidic lakes. Possible explanations involve the specific water chemistry in mining lakes. *Chydorus sphaericus* was the most acid-tolerant species and occurred along the entire pH gradient. We experimentally tested the effect of lake water of different pH values on *C. sphaericus*. Surprisingly, its survival was the highest at low pH (3–4), while moderately acidic and neutral pH (5–7) had a well-expressed toxic effect on the animals. About 20% of *C. sphaericus* survived when transferred from pH 3 to pH 7 and vice versa, which suggests that this species is a generalist in relation to pH. As Cladocera display species-specific pH tolerances, we suggest that they could be a useful group for ecological quality assessment of acidified mining lakes.

Keywords Acidic stress · Extreme environment · Acute toxicity · Acclimation · Survival · Littoral Cladocera

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M. Belyaeva (✉) · R. Deneke
Department of Freshwater Conservation, Brandenburg University of Technology at Cottbus, Research Station, Seestr. 45, 15526 Bad Saarow, Germany
e-mail: Maria.Belyaeva@tu-cottbus.de

Introduction

Acidic mining lakes provide a harsh environment for zooplankton organisms (Nixdorf et al., 1998). Only a few species of metazoan zooplankton are able to colonize those lakes, in spite of the abiotic stress imposed by the combination of low pH and high concentrations of toxic metal ions (Deneke, 2000; Wollmann et al., 2000; Nixdorf et al., 2005). Cladocera are the most acid-tolerant crustaceans and are important colonizers at pH above 3 (Nixdorf et al., 1998; Steinberg et al., 1998; Deneke, 2000; Wollmann et al., 2000; Nixdorf et al., 2005). However, little is known about the community composition and physiological thresholds of colonizing species, especially in moderately acidic and neutral mining lakes (Nixdorf et al., 1998). On the other hand, there have been many reports, considering zooplankton communities in acidified soft-waters and naturally acidic bog lakes, which discussed the influence of species-specific pH tolerance ranges and additional stressors on the species colonization success (e.g., Brett, 1989; Fryer, 1993; Havas & Rosseland, 1995). Although low pH is considered to be the most important factor limiting colonization of soft-water acidic lakes, it has been also shown that additional stressors, such as metal pollution, lack of calcium and high content of humic substances, can further decrease in situ tolerances of colonizing species (Fryer, 1993; Kappes & Sinsch, 2005). Some of the potential stressors are highly correlated with pH, what made it difficult to separate the effects of individual factors. Acidic mining lakes, which represent hard-water acidic habitats, can help to assess the limiting role of pH in a different combination of abiotic conditions. Namely, these water bodies are characterized by elevated concentrations of iron, aluminium and sulphuric ions, resulting in a very high conductivity, as well as by high calcium concentrations and a lack of humic substances (Geller et al., 1998; Nixdorf et al., 1998).

Littoral Cladocera display species-specific tolerances in relation to a number of factors, and are therefore useful indicators of present and past environmental conditions (Krause-Dellin & Steinberg, 1986; Schartau et al., 2000; Walseng et al., 2003; De Eyto et al., 2003). This group is also both species-rich and includes many tolerant species (Smirnov, 1971), and thus they can be especially useful for water quality assessment in habitats subjected to multiple abiotic stress, such as mining lakes, where the number of potential biological indicators is generally low. Lake Senftenberger See, with its water chemistry typical of mining lakes (Table 1), is well suited to study the response of the littoral cladoceran community to these multiple factors. A peculiar pH gradient from highly acidic (pH 3) to neutral (pH 7.5) has been formed between its two basins, which enables the study of almost the entire pH range occurring in mining lakes. Given the age of the lake (40 years) and the lack of physical barriers within the pH gradient, all potential colonizers should have reached the lake and distributed according to their pH tolerances.

In our study, a single cladoceran species – *Chydorus sphaericus* (O. F. Müller, 1776) – was found along the entire pH gradient in the lake. Therefore, we decided to test its pH tolerance in mining lake water experimentally. This species was previously reported as an important colonizer of acidic mining lakes (Nixdorf et al., 1998; Wollmann et al., 2000), although its optimum pH range is unknown. Generally, the ability of a single cladoceran species to live under a wide range of conditions was doubted by Frey (1980, 1986), who discovered a number of cryptic chydorid species complexes consisting of ecologically distinct but morphologically similar species. Hence, he proposed that a wide distribution and eurytopy indicates plurality of species (Frey, 1980, 1986).

Table 1 Characteristic data of Lake Senftenberger See (data of water chemistry are given separately for the North Basin (Elsterfeld) and the South Basin (Südfeld))

Catchment area (km^2)	779	
Area of lake (km^2)	11	
Area of island (km^2)	3.5	
Maximum depth (m)	23	
Mean depth (m)	7.0	
Volume (10^6 m^3)	80	
Water level (mNN)	97.8–99.25	
	North	South
pH	7.4*	3.2*
Conductivity ($\mu S\ cm^{-1}$)	577*	941*
Acidity ($K_{B\ 8.2}$) ($mmol\ l^{-1}$)	0.06*	1.72*
Chlorophyll *a* ($\mu g\ l^{-1}$)	2 - 6	≈ 1
Total nitrogen ($mg\ l^{-1}$)	4	3
Total phosphorus ($\mu g\ l^{-1}$)	6	2
Sulfate ($mg\ l^{-1}$)	266	300
Ca ($mg\ l^{-1}$)	85	79
Total Fe ($mg\ l^{-1}$)	0.27	3.1
Total Al ($mg\ l^{-1}$)	<0.01	0.31

Data indicated by an asterisk are original measurements; all other data are taken from Nixdorf & Hemm (2001)

The present study of littoral Cladocera in Lake Senftenberger See aimed to assess their in situ pH tolerances in a hard-water acidic mining lake in order to predict the potential for colonization of these waters. In order to investigate the pH range at which *C. sphaericus* can survive we experimentally assessed the acute toxicity of mining lake water for *C. sphaericus*, as well as its within-lake variation in pH tolerance.

Description of the study site

Lake Senftenberger See (51°29′03″ N 14°01′14″ E) is located near the town of Senftenberg in the Lusatian mining area (Germany) (Fig. 1). The lake was formed by flooding of the abandoned open-cast

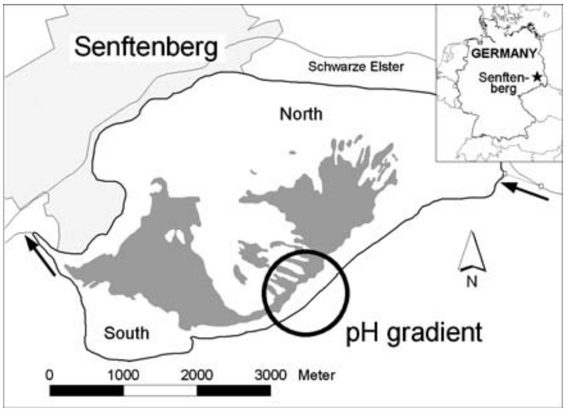

Fig. 1 Map of Lake Senftenberger See in Lusatia (Germany). Inset: location of the lake in Germany, circle: location of the pH gradient in the channel connecting the neutral North and the acidic South Basin, grey area: islands. Arrows indicate the in- and out-flow of water from the River Schwarzer Elster

mining pit Niemtsch with groundwater and water from the River Schwarze Elster from 1967 to 1972. Due to the mining technique applied, several small and larger islands divide the lake into two main parts, connected by a shallow channel: the large North Basin with a maximum depth of 23 m, and the small South Basin. As the ground water in the region is extremely acidic (ca. pH 2) due to pyrite oxidation, neutral water from the River Schwarze Elster has been used for continuous flushing of the lake, in order to prevent acidification. However, the South Basin, which is not influenced by the river, acidified. Mixing of water from the highly acidic South Basin and the neutral North Basin occurs within the connecting channel forming a horizontal pH gradient. The mixing zone is a shallow littoral habitat with dense stands of macrophytes dominated by *Juncus bulbosus*.

Water chemistry is essentially different at both sides of the gradient (Table 1). The hydrogen ion concentration is about 10,000 times higher in the acidic part (ca. pH 3.2) that is strongly buffered by high concentrations of iron (3.1 mg l^{-1}), resulting in an acidity of 1.72 mmol l^{-1}. The neutral part is buffered by the carbonate system, and metal ion concentrations are much lower here, which corresponds to the higher pH. Furthermore, high concentrations of sulphate, carbonate, calcium and magnesium were detected on both sides of the gradient and are characteristic of mining lakes in general (Nixdorf et al., 1998). The measured concentrations of heavy metal ions, such as cadmium and copper, are negligible. Within the mixing zone the chemical conditions are unstable due to continuous chemical processes, such as the oxidation and precipitation of Fe (II) and Al, which leads to the loss of buffer capacity. These processes presumably account for visible changes in water colour and clarity as well as for the formation of a dense layer of precipitate covering macrophytes within the mixing zone.

The acidic part of the lake is oligotrophic, while the neutral part seasonally becomes more productive reaching a mesotrophic level. Apart from tourism and fishery the lake plays an important role in the water management of the mining region, in particular, for flood control and has been used as a reservoir.

Materials and methods

Sampling of Cladocera and recording of physico-chemical parameters within the pH gradient in Lake Senftenberger See were carried out in 2003 (8.5., 3.9.) and 2005 (19.4., 28.4.). Horizontal profiles of temperature, conductivity and pH were taken at ca. 0.5 m depth by a multi-parameter probe (Hydrolab H20) connected to a field computer (Husky Hunter). Simultaneously locations of point measurements were recorded on three dates (3.9.2003, 19.4.2005, 28.4.2005) using a hand-held GPS receiver (Garmin GPS 72). On each date about 200 points were measured over a transect of ca. 1.5 km. GPS coordinates were transformed from WGS 84 to DHDN GK5 format using WGEO 3.0 software. Contour maps were created using the GIS software package ArcView 3.2a with 3D extension.

Samples of Cladocera were taken from up to 1 m depth using a 100 µm mesh dip net. A total of 37 samples were collected, 6–14 samples on each respective date, and preserved with 37 % formalin to a final concentration of approximately 4 %. Cladocera species were identified at 400× magnification under a light microscope according to Flößner (2000), Lieder (1996) and Smirnov (1996). For calculation of relative abundances each sample was examined under a stereomicroscope using a Bogorov chamber. 100 specimens were counted starting from the second row of the chamber.

In a first set of experiments, we assessed the acute toxicity of lake water from the pH gradient in Lake Senftenberger See for *C. sphaericus* in relation to in situ environmental conditions at the sampling localities. Both *C. sphaericus* and lake water were sampled on 28 April 2005 at pH values of 7.4, 6.1, 5.3, 4.3 and 3.4, taken to the laboratory and used for the experiments within the next 10 h. We used two different experimental setups to test for the influence of acclimation on survival rates. First, in a sequential setup the treatments consisted of stepwise transfers of test animals from their respective in situ pH to the final pH in steps of ca. 1 pH unit every 18 h to account for acclimation. This procedure was applied to all possible combinations of in situ pH and final pH using water from the gradient and amounted to overall 60 vials (20 pH combinations with 3 replicates each). Given that the most extreme transfers consisted of four 18h-steps, the duration of the whole experiment was 72 h. Survival rates were calculated separately for each single transfer step. Only those transfer steps with at least 10 individuals were included into the statistical analysis, which resulted in overall 81 data points. In the second setup animals were transferred from their respective in situ pH in one step to the final pH without prior acclimation. This procedure was restricted to the transfers from pH 3.4 and 5.3 to 7.4 and from 7.4, 6.1 and 5.3 to 3.4, respectively, and summed up to overall 15 vials (5 pH combinations with 3 replicates each). The same controls were used for both setups, and they consisted of *C. sphaericus* kept at their original in situ pH, media being renewed and dead animals removed at 18 h intervals, so the handling was the same as in the treatments. Overall 15 vials (5 pH values with 3 replicates each) were used as controls. All replicates in both treatments and controls consisted of 20 individuals per vial, which were randomly selected from field samples. All the tests were performed in glass vials filled with 25 ml 30 µm filtered lake water and kept under a 8:16 h light/dark regime at ca. 11°C (the recorded in situ temperature varied from 10 to 14°C among the sampling localities). No additional food was added, apart from phytoplankton that naturally occurred in the lake water. Survival was calculated as the ratio of the final and the initial numbers of live animals. The controls were included into calculations as 'zero treatments', rather than used for treatments' correction, because of an unexpected mortality of the controls at pH 6.1 and 7.4.

In order to address this phenomenon, we conducted a second set of experiments to investigate factors, diminishing the survival of *C. sphaericus* at circumneutral pH. The setup of the first set of experiments was then simplified in the following way: *C. sphaericus* was sampled on the 10.05.2005 at two locations, at pH 6.5 within the pH gradient and at pH 7.4, 800 m off the gradient. The treatments consisted of the transfers of animals in one step from their respective in situ pH to 3.2, 6.5, 7.4 (Lake Senftenberger See) and to pH 8.1 (a natural eutrophic Lake Scharmützelsee, 52°17′27″ N, 14°03′04″ E, for the description of the water chemistry parameters see Hämmerling et al., 2006). Five replicates each for the treatments and controls were incubated for 48 h without prior acclimation at ca. 17°C, the standard temperature for laboratory cultures, overall resulting in 30 vials for treatments and 10 for controls. A shorter incubation time of 48 h was applied because no additional mortality occurred after this period in the first experiment.

Statistical analysis

For statistical tests (χ^2, *t*-test for independent samples) we used the software package SPSS 12.0G according to Backhaus et al. (2003). The nonlinear regression analysis was based on the Levenberg–Marquardt algorithm and used the nonlinear least-squares curve-fitting procedure implemented in the OriginPro 6.1 (OriginLab Corporation) software. Survival data in the experiments, as well as presence/absence field data of species occurrences were fitted to the logistic (logit) regression model $p = A * (1 + \exp(-Z))^{-1}$ with $Z = k * (X - X_c)$, where k is the regression coefficient, $(k * X_c)$ is the intercept of the logit-linearized function, X_c is the centre value of the regression line, and A is the amplitude which was set to 1. In order to test for significant differences between the regression coefficients a Monte Carlo resampling simulation (bootstrapping) procedure was applied using Microsoft Excel (Simon, 1997). Randomized regressions of the experimental data with treatment pH as the independent variable were calculated by drawing 1,500 replicate samples from the original survival data (reshuffling with replacement), each time calculating regression coefficients, as well as the differences between them. The probability to erroneously reject the null hypothesis, i.e. the differences between coefficients are random, was calculated as the ratio of cases with a higher difference in comparison to the original data and the sum of all simulated cases.

Results

Cladocera species richness and distribution within the pH gradient

We found a total of 25 species of Cladocera, the majority of them (14) from the family of Chydoridae (Table 2). The most common genera were *Alona* Baird, 1843 (5 species) and *Ceriodaphnia* (Dana, 1853) (3 species). Only 2 species – *C. sphaericus* and *Scapholeberis mucronata* (O. F. Müller, 1776) – were found at pH 3, whereas other species occurred starting at pH 4.9. *C. sphaericus* was the most abundant and the only species present in all the samples (*N* = 37). In 32 samples (91 %) its share of the total abundance of Cladocera was higher than 10% (Table 2) and in many cases close to 100%. Other occasionally dominant taxa were *Acroperus harpae* (Baird, 1835) (8 times), *Ceriodaphnia pulchella* Sars, 1862 (2 times), *Sida crystallina* (O.F. Müller, 1776) (2 times) and *Scapholeberis mucronata* (2 times). None of the species found was restricted to the acidic conditions, but all acid-tolerant species were found at neutral pH as well.

Table 2 Comparison of the minimum in situ pH recorded for 25 Cladocera species within the pH gradient in Lake Senftenberger See on four sampling dates and the minimum pH values given in the literature for the respective species

pH	Cladocera	*N*	Dominance	Minimum in situ pH	Other locations	Citations
3	*Chydorus sphaericus*	37	32	3.1	3.2	Flößner (2000)
	Scapholeberis mucronata	13	2	3.0	3.8	Bērziņš & Bertilsson (1990)
5	*Eurycercus lamellatus*	15	–	4.9	3.9	Flößner (2000)
	Pleuroxus truncatus	2	–	4.9	4.2	Flößner (2000)
	Bosmina longirostris	15	1	5.0	4.3	Bērziņš & Bertilsson (1990)
	Sida crystallina	14	2	5.0	4.6	Fryer (1993)
	Alona affinis	7	–	5.0	4.0	Flößner (2000)
6	*Acroperus harpae*	12	8	5.7	4.6	Bērziņš & Bertilsson (1990)
	Alona costata	10	–	5.7	4.7	Bērziņš & Bertilsson (1990)
	Ceriodaphnia pulchella	9	2	5.7	5.0	Bērziņš & Bertilsson (1990)
	Polyphemus pediculus	7	–	5.7	3.9	Flößner (2000)
	Alona guttata	3	–	5.7	3.8	Bērziņš & Bertilsson (1990)
	Diaphanosoma brachyurum	2	–	5.7	3.9	Flößner (2000)
	Simocephalus vetulus	8	–	6.1	4.4	Flößner (2000)
7	*Camptocercus rectirostris*	2	–	6.8	4.7	Uimonen-Simola & Tolonen (1987)
	Daphnia galeata	2	–	6.8	5.0	Flößner (2000)
	Bosmina coregoni	1	–	6.8	3.3	Almer et al. (1974)
	Monospilus dispar	1	–	6.9	5.5	Flößner (2000)
	Alona rustica	3	–	7.0	3.3	Fryer (1993)
	Pleuroxus trigonellus	1	–	7.0	5.0	Flößner (2000)
	Alona quadrangularis	1	–	7.0	5.0	Flößner (2000)
	Alonella excisa	2	–	7.2	3.8	Flößner (2000)
	Ceriodaphnia quadrangula	2	–	7.2	3.8	Bērziņš & Bertilsson (1990)
	Acroperus angustatus	1	–	7.4	–	
	Ceriodaphnia megops	1	–	7.4	5.0	Flößner (2000)

N: frequency of occurrence in 37 samples, Dominance: frequency of records with a share >10% of the total abundance

Therefore, they tended to occur more often, than the species that were found only at neutral pH. Most of the species found are generally common in the littoral zone. *B. longirostris* was the most tolerant pelagic species, having been found at pH 5.0, whereas the other three pelagic taxa occurred at pH 5.7 or higher in the following order: *Diaphanosoma brachyurum* (Liévin, 1848), *Bosmina coregoni* Baird, 1857 and *Daphnia galeata* Sars, 1863 (Table 2).

Figure 2 illustrates the highly dynamic nature of the pH gradient and the spatial distribution of the species richness. Depending on the wind direction, the pH gradient changed its location within the connecting channel by approximately 800 m, and its length varied from 100 to 600 m. The decrease in species numbers was towards the acidic part, concordant with the gradient. The mean species richness per sample declined from 8.2 ± 3.2 under neutral conditions to 1.5 ± 0.7 below pH 4.6 (Fig. 3A). The concomitant reduction of variation within the groups indicates the growing importance of pH as a limiting factor towards more acidic conditions.

In order to estimate if the potential for cladoceran colonization differs between mining and soft-water lakes we used cumulative species richness curves based on the minimum in situ tolerances of species. We fitted a nonlinear (logistic) regression to our field data, as well as to the in situ pH minima given in the literature for the same species. A substantial discrepancy exists between the two curves (Fig. 3B), the centre values X_c of the regression line differing by more than 2 pH units, and the slope k of the regression being steeper for the literature values. Thus, the cumulative species richness of Cladocera found in our study was considerably lower than predicted from the species in situ pH minima in soft-water acidic lakes.

Is the pH gradient a toxic environment for *Chydorus sphaericus*?

In the first experimental setup using sequential transfers we aimed to test the null hypotheses that the probability of survival of *C. sphaericus* within the pH gradient is (1) equal at all tested pH values and (2) independent of the location, where the animals were collected. A χ^2-test, assuming an even distribution of survival data over the observed pH range, revealed highly significant differences ($P < 0.01$) compared to the distribution of experimental data. Hence, the probability of survival is significantly different among the tested pH values, and the first null hypothesis can be rejected. The evidence to reject the second null hypothesis comes from plotting the survival rates of *C. sphaericus*, grouped according to the in situ pH, against the treatment pH as the independent variable (Fig. 4). The overall response pattern can be described by fitting a logistic (logit) regression model to the experimental data. The two most divergent regression coefficients (in situ pH 5.4 and 3.4) were tested for significant differences by a Monte Carlo re-sampling method (see Methods for the test procedure). The second null hypothesis (differential response among the sampled localities)

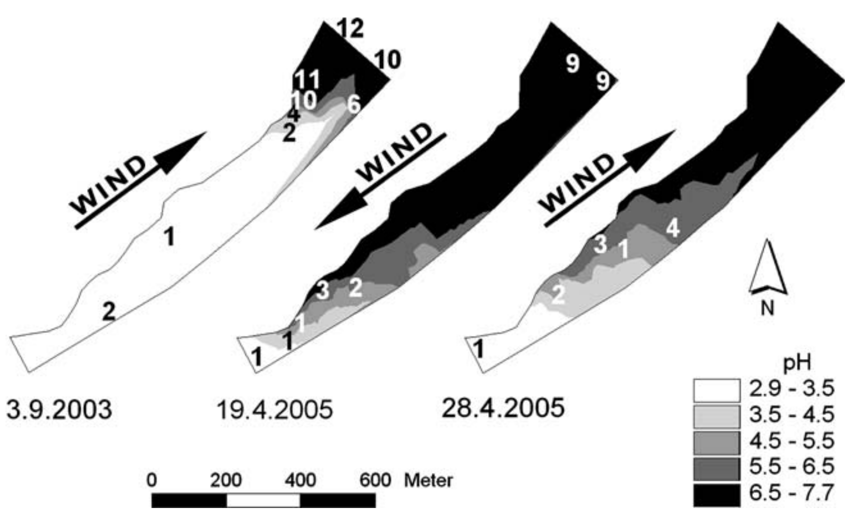

Fig. 2 Contour plots of pH within the transitional zone between the neutral and the acidic basins of Lake Senftenberger See (Lusatia, Germany) on the 3 dates (3.9.2003, 19.4.2005, 28.4.2005). Numbers: species richness of Cladocera species, arrows: main wind direction at the respective date

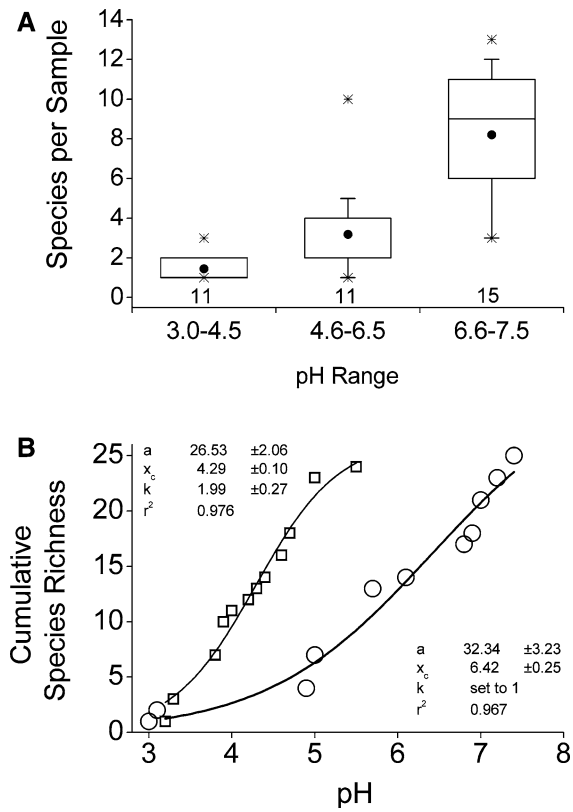

Fig. 3 Species richness of Cladocera within the pH gradient in Lake Senftenberger See (Germany). (**A**) Box-plots of species richness on the basis of 37 samples aggregated to three pH ranges between 3 and 7.5 with similar sample sizes. Cross: maximum and minimum values, whisker: 10 and 90 percentile box: interquartile range (50 %), solid circle: mean value, horizontal line: median, numbers above the X-axis give the numbers of samples examined. (**B**) Nonlinear regression curves for the potential cumulative species richness of Cladocera. Data represent the in situ pH minima at which species were found at the study site (open circles, bold solid line) as well as the lowest in situ pH values reported for these species in the literature (open squares, thin solid line, for data see Table 2). For explanation of the model parameters see Methods

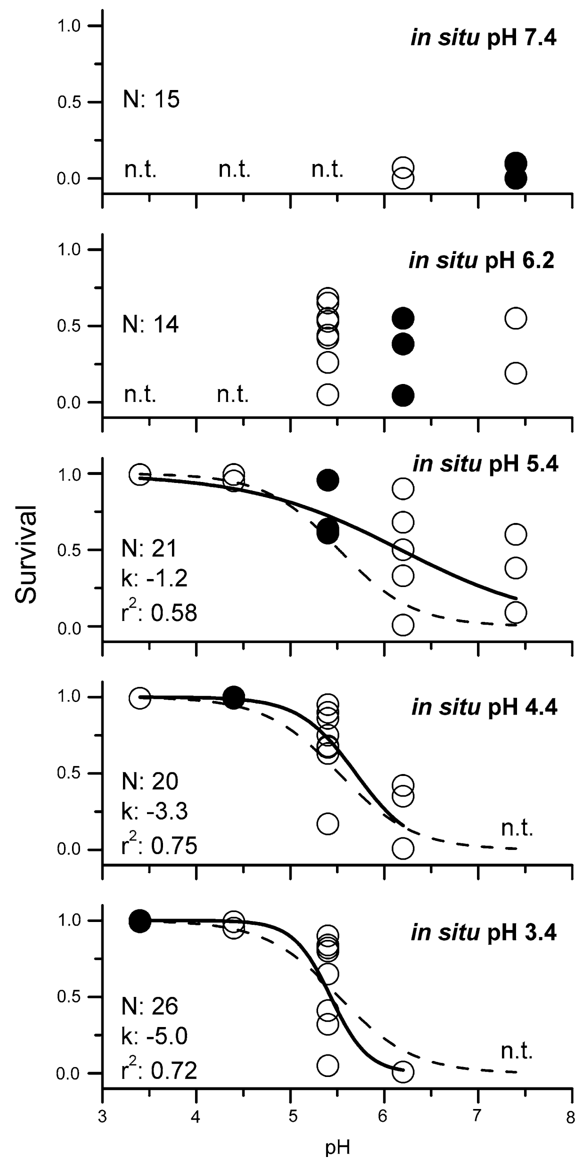

Fig. 4 Survival of *C. sphaericus* in acute toxicity experiments with mining lake water (Senftenberger See, Germany) of 3.4, 4.4, 5.4, 6.2 and 7.4 pH. Test animals have been transferred from the respective in situ pH by a stepwise method (1 pH unit every 18 h) to all other pH to account for acclimation. Results are grouped according to the in situ pH, i.e. the original pH at the sampling station. Solid circles: controls, hollow circles: survival in treatments after ca. 18 h of exposure, "n.t.": no transfer possible because less than ten individuals survived in the previous transfer step, solid lines: logistic regressions fitted to the data of the respective in situ pH, dashed lines: logistic regression line fitted to the pooled data, N: number of observations, k: regression coefficient of the linearized function, r^2: correlation coefficient

could not be rejected because no significant differences were found ($P > 0.05$).

In order to explore the general response pattern the data from all experiments were pooled and a combined regression model was calculated describing the observed asymmetrical response over the entire pH gradient (dashed line in Fig. 4). The centre value X_c of the pooled regression line is at pH 5.5 and it assigns the place in the gradient with a 50%

probability of survival (*k*: −2.5, r^2: 0.69). No regression could be fitted to the data of in situ pH 6.2 and 7.4, because no animals could pass further than a pH of 5.4. Furthermore, an increased mortality was observed in all treatments and controls at weakly acidic and neutral conditions (Fig. 4). The survival rates were extremely low at pH 6.2 and 7.4 (median values of 0 and 0.15 respectively), indicating nearly no chance for survival, while 100% survival occurred at the allegedly stressful pH 3.4 and 4.4. Lake water of intermediate pH 5.4 also caused an intermediate effect with a high variability. Transfers starting from here were successful in both directions with the highest survival rates towards acidic pH (Fig. 4).

We applied the second setup using one-step transfers in order to study the effect of abrupt pH changes, which may occur within the gradient, for example, via intensive mixing. In the most extreme transfers bridging 4 pH units we observed survival rates of about 0.2 (Fig. 5). When both transfer methods were compared, survival was significantly higher in one-step transfers from pH 7 to 3 ($P < 0.05$, *t*-test), but no significant differences were observed in pH 3 to 7 transfers. At a smaller pH increment of 2 units, there were no significant differences related to the method used. In both stepwise and one-step transfers survival was higher under acidic compared to neutral conditions ($P < 0.05$, *t*-test, Fig. 5). At an intermediate pH increment of 3 units (pH 6.1 to 3.4) the survival rate was intermediate as well (mean 0.88 ± 0.03, data not shown).

In the second set of experiments, we tested the null hypotheses that (1) neutral pH rather than other chemical characteristics of the tested lake water caused the mortality of *C. sphaericus* in the previous experiments and (2) that there is no difference in the sensitivity of *C. sphaericus* collected from different circumneutral locations in the lake. The results confirmed the strong lethal effect at pH 6.4 and 7.4 with mean survival rates of 0.02 and 0.04 (Fig. 6), respectively. When the test animals were transferred to water from a natural lake with a pH of 8.1, the survival rate increased significantly to 0.57 ± 0.24 (pooled mean for both test groups, *t*-test, $P < 0.05$), suggesting that other chemical characteristics of the Lake Senftenberger See water, rather than pH, caused the increased mortality of *C. sphaericus*.

No significant difference in survival was found between the test groups (*t*-test: $P > 0.05$), suggesting that there is no difference in response to pH 6.4 and 7.4 between the two sampling localities. However, a significant difference in survival between the two groups was found at a highly acidic pH of 3.2 (*t*-test:

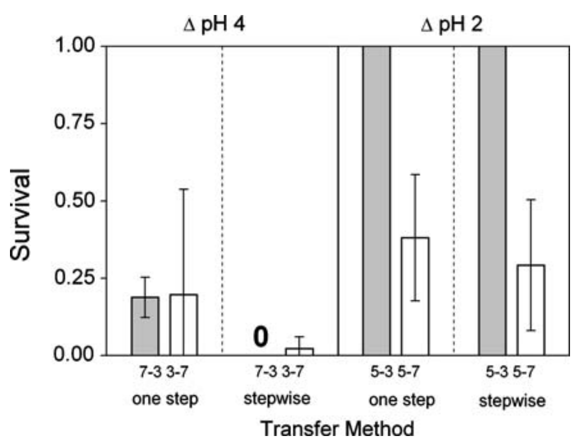

Fig. 5 Survival of *C. sphaericus* in acute toxicity experiments using two different transfer methods: Comparison of transfers over 2 and 4 pH units, respectively. (method 'one step': transfers in one step without prior acclimation, method 'stepwise': transfer in steps of ca. 1 pH unit per 18 h, grey bars: transfers to acidic pH 3.4, white bars: transfers to neutral pH 7.4, error bars: standard deviation of 3 replicates). Numbers below the X-axis indicate the pH increment and direction of change, e.g., 7–3 means transfer from pH 7.4 to 3.4

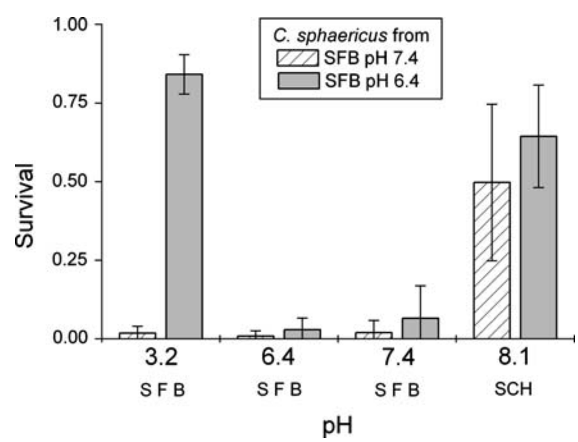

Fig. 6 Survival of *C. sphaericus* collected at two circumneutral locations in Lake Senftenberger See: at pH 6.4 within the gradient and at pH 7.4 800 m off the gradient. The test animals were exposed for 48 h to lake water of pH 3.2, 6.4 and 7.4 from Lake Senftenberger See (SFB) and water of pH 8.1 from a natural, eutrophic lake (SCH: Lake Scharmützelsee). Error bars: standard deviations

$P < 0.01$). Animals collected at a pH of 6.5 within the gradient survived much better under the harsh acidic conditions, which were lethal to those collected 800 m further off the gradient at a pH of 7.4 (Fig. 6). The mean survival rates were not significantly different in the transfers from pH 6.4 to 3.2 in this set of experiments as compared with the corresponding one-step transfers in the previous experimental setup (0.84 ± 0.07, and 0.88 ± 0.03 respectively). Meanwhile, *C. sphaericus* collected 800 m off the gradient survived significantly worse when transferred to acidic pH than those animals which were collected at pH 7.4 close to the gradient (mean survival rates 0.02 ± 0.02 and 0.19 ± 0.07, respectively; *t*-test: $P < 0.05$). Our findings suggest that both the effect of mining lake water chemistry as well as variation in tolerance among circumneutral locations within the lake should be incorporated to account for the response of *C. sphaericus*.

Discussion

Unfavourable conditions in mining lake water at pH 5–6

Acidic mining lakes represent hard-water acidic habitats that are poorly investigated in respect of their cladoceran community composition and species-specific physiological thresholds. In Lake Senftenberger See, the unique situation of a within-lake pH gradient from 3 to 7 enabled us to study the community response of Cladocera to acidic water with a chemical composition typical of mining lakes. Our results show that the acidic pH alone is neither sufficient to explain the decreased cumulative species richness; nor does it explain the observed mortality of an acid-tolerant species *C. sphaericus* at weakly acidic and neutral pH values. In mining lakes at a pH below 4, water is strongly buffered by the iron buffer system, whereas mixing with neutral water results in a loss of buffer capacity. This causes chemical instability, and intense precipitation of metal compounds occurs, as the buffer system changes towards the aluminium and afterwards to the carbonate system (Nixdorf et al., 2003). In the mean time, precipitation of Al and Fe was shown to be harmful for fish in acidic streams which were limed to achieve a neutral state (Weatherley et al., 1991). In our study the centre value of the logistic regression at pH 5.5 and the high variation in survival of *C. sphaericus* indicate unfavourable conditions at this pH range. It seems likely that it is mainly precipitation of Al compounds that prevents expansion of cladoceran species towards more acidic conditions and that this caused the mortality of *C. sphaericus* at this pH in the experiments. While high toxicity at moderately acidic conditions seems to be related to the specific water chemistry typical of mining lakes, this effect would be only weakly expressed in soft-water acidic lakes, where relatively diverse cladoceran communities have been reported at this pH (e.g., Fryer, 1980; Walseng et al., 2003). A possible explanation for this might be that much higher concentrations of inorganic dissolved Al are achieved in mining lakes accompanied with a lack of metal-binding humic substances, which have been shown to decrease toxicity and prevent precipitation of inorganic metal compounds in soft-water lakes (Steinberg, 2003).

Due to geogenic acidification, most of the mining lakes in the Lusatian Area (Germany) are highly acidic, with a pH of 2.5–3.5, unless they have been flushed with neutral water and achieved circumneutral conditions (Geller et al., 1998). However, the latter tend to be unstable, due to continuous influx of acidic groundwater and once the flushing is stopped the lake may revert to an acidic state. Our findings predict that during such a re-acidification, as well as in mixing zones, the conditions can become highly unfavourable even for acid-tolerant species.

Community response of Cladocera in a changing environment

In Lake Senftenberger See the investigated cladoceran community consisted of wide spread species (Flößner, 2000), which are also common in nearby neutral lakes (unpubl. authors' data). Communities comprised of generalist species and forming simple food webs indicate environmental stress (Simon, 2002), and acidic mining lakes can be regarded as extreme environments, where animals are subjected to multiple abiotic stressors.

The cumulative species richness of Cladocera was essentially lower in Lake Senftenberger See at the respective pH values than it is predicted from the in situ pH minima taken from literature. Assuming that our sampling was sufficient to reveal species in situ distributions and no physical barrier prevents dispersal within the gradient, we can hypothesize that some lake water characteristics hamper colonization by cladoceran species. It has been previously shown that additional stressors, such as metal pollution, can decrease species pH tolerances (Fryer, 1993, Kappes & Sinch, 2005). At pH 3–5 relatively high concentrations of Al and Fe in lake water are likely to contribute to its toxic effect on cladocerans. At a pH range of 5–6 precipitation of metal compounds discussed above probably plays the most important role in the extinction of even acid- and metal-tolerant species. Furthermore, also at neutral pH we have found much lower diversity, than is commonly found in temperate lakes. The reasons are unclear and are probably related to the specific water chemistry in mining lakes.

Dynamic conditions within the gradient is another factor, which may bias the correspondence between the observed in situ species distributions and the true species preferences, as water shifts could be too fast for small cladocerans to avoid unfavourable conditions and to occupy space that becomes less acidic. Thus, the question arises: Can small species actively adjust their horizontal position according to their preferences despite of the dynamic nature of the gradient? Considering the average speed of the slowest cladocerans – chydorids of 2.5–7 mm s^{-1} (Smirnov, 1971), we can roughly estimate the distance potentially covered by a chydorid during one day. Using the lowest value of 2.5 mm s^{-1} and assuming 8 h of movement in one direction the distance amounts to ca. 72 m, what exceeds by far the observed shift of ca. 21 m d^{-1} of the pH gradient between the two sampling dates in April 2005, when the wind direction changed. The spatial pattern of species richness corresponded well to the gradient extension, and no irregularity was detected suggestive of possible impact of other environmental factors such as water mixing or micro-habitat structure. Thus, we can hypothesize that despite the dynamic nature of the gradient Cladocera are capable to adjust their position within the gradient according to their preferences. It signifies that in situ distributions of cladocerans are likely to reflect their true tolerances.

As cladocerans in the investigated mining lake displayed differential species-specific tolerances, they can be useful indicators of environmental conditions in mining lakes. Supplementary to the suggestions by Nixdorf et al. (2005) to use zooplankton as bio-indicators in ecological quality assessments, we recommend using the species richness and composition of littoral Cladocera as a valuable parameter, particularly, under neutral and weakly acidic conditions.

Chydorus sphaericus—a specialist in tolerance?

In Lake Senftenberger See *C. sphaericus* has been found across the entire pH gradient, however, in the experiments its survival varied greatly among the treatment pH values. Acidic conditions seem to be most favourable for the studied population, as survival in the controls was absolute, and of all treatments those at pH 3.4 and 4.4 caused the lowest mortality irrespective of the origin of the specimens. While the decrease in survival at a pH range of 5–6 is likely to be an effect which also occurs in situ (see above), high mortality at pH around 7 was apparently an artefact. This is suggested by the high survival of the test animals in natural lake water of pH 8.1. Besides, abundant populations were collected in the neutral part of the Lake Senftenberger See far from the gradient zone. Presumably, this effect can be attributed to the chemical instability of the lake water at pH 6.4 and 7.4 and some undetected changes that had occurred during the transport or laboratory incubations in small volumes. Although pH in the incubation vials, measured every 18 h, remained stable, self-toxification of lake water apparently occurred in a few hours after the experiments had started.

The response pattern in the acute toxicity experiments was not significantly different among *C. sphaericus* collected at pH 3.4, 4.4 and 5.4. However, animals collected at pH 6.2–6.4, 7.4 near the gradient and pH 7.4 800 m off the gradient displayed significant differences in survival, when transferred to the acidic pH in one step. The proportion of individuals, which survived the transfers to acidic mining lake water, was lowest for the animals collected 800 m off the gradient. This may be due to either environmentally induced or genetic variation in tolerance of *C. sphaericus* and further investigations are required to distinguish between these two cases.

The results of the acute toxicity experiments suggest that the C. *sphaericus* population from Lake Senftenberger See is capable of enduring both extremes of the pH range of the gradient. We found that ca. 20% of the individuals survived the most extreme transfers. A possible explanation could be that those individuals survived, which were in the insensitive stage of their moulting cycle. Some investigations suggest that resistance to low pH is related to the effectiveness of ionic regulation (Havas & Likens, 1985; Havas & Advokaat, 1995). Moulting of crustaceans was shown to be accompanied by changes in ionic fluxes (Ahearn et al., 2004), and thus life cycle stages are likely to be differentially sensitive to a high-ambient protons' concentration. Essentially, the results of the experiments have shown that *C. sphaericus* can cope both with fast environmental changes and with a wide pH range, though a chemical barrier, represented by a highly toxic zone at pH 5–6, should to a great extent limit the exchange between the sub-populations at the two ends of the gradient in Lake Senftenberger See. However, this barrier for dispersal is not likely to be absolute. According to the calculations above a specimen of *C. sphaericus* should theoretically be able to pass the chemical barrier within the gradient in 5 to 20 h depending on the length of the zone on the respective day. An exposure time of that duration allows a high probability of survival, as it has been shown for *C. sphaericus* in the acute toxicity experiments. Thus, exchange of animals between both sides of the gradient should be possible, however, limited, and thus it might allow an existence of two sub-populations at each side of the gradient. The selection pressure should be very different between these two lake parts, as they exhibit essential differences in water chemistry, trophic state, food web structure and interactions (Table 1; Wollmann et al., 2000). It seems probable, that this might have led to colonization of each part by distinct genotypes with adaptations to the local conditions, which existed prior to the colonization or/and have been developed during the 40 years of the site's existence. Alternatively, we even have to consider the presence of sibling species, each with a restricted tolerance range, which would be suggested by the non-cosmopolitanism hypothesis that regards existence of eurybiotic cladoceran species as unlikely (Frey, 1980, 1986). Yet, contrary to this hypothesis, our study experimentally confirms the generalism of *C. sphaericus* in relation to pH, therefore, showing the principal possibility of the existence of generalists among cladoceran species. Overall, *C. sphaericus* displays to our knowledge the broadest range of pH tolerance among crustaceans, though some rotifer species are known to be even more eurybiotic (Deneke, 2000). Some studies also report on the wide tolerance ranges of this species to other environmental factors such as temperature, trophic state and salinity (Belyaeva, 2003; Boronat et al., 2001; De Eyto et al., 2003), so *C. sphaericus* seems to be a 'specialist' in tolerance to a wide range of abiotic conditions.

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