Sensitivity of Early Life Stages of Vendace, *Coregonus albula*, to Acid pH in Postmining Lakes: An Experimental Approach

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ABSTRACT: Since 1990, many highly acidic lakes with very hard water have formed in Lusatia, eastern Germany, following the decommissioning of most open-cast lignite mines. Even after neutralization of the water, the lakes may reacidify due to the high acidification potential of soil and groundwater. Hence, investigation of critical pH-levels for the respective fish species is required when planning to use the lakes for fisheries. Sensitivity of early life stages of vendace, *Coregonus albula*, to low pH was determined in reconstituted water with the hydrochemical characteristics of the postmining lakes. Eggs were transferred to test solutions with pH-values ranging from 3.50 to 7.40 either 10 min after artificial insemination (series A) or 7 h later (series B) and incubated using a static-renewal procedure. Water hardening of the eggs at exposure pH (series A) led to a severe reduction in egg diameter at pH $\leqslant$ 5.00 and to an earlier and stronger increase in egg mortality, but by the time of hatching, differences between both series were small. Hatching percentages at pH $\leqslant$ 5.00 were very low and the eleutheroembryos died shortly after hatching. In both series, hatching percentages at pH 5.50 did not differ from those at pH 7.40. Feeding activity of vendace exposed to pH 5.50 was, however, reduced and the fish did not survive. Hence, early life stages of vendace can be regarded as very sensitive to low pH and associated elevated concentrations of Al, and maintenance of neutral pH is essential when postmining lakes are stocked with vendace. Environ Toxicol 15: 214–224, 2000

Keywords: *Coregonus albula*; early life stages; postmining lakes; iron sulfides; acid pH; aluminum; laboratory study; water hardening; feeding

INTRODUCTION

Since 1990, many strongly acidic, artificial lakes have formed as a consequence of the decommissioning of most open-cast lignite mines in Lusatia, eastern Germany. The extreme low pH-values of the postmining lakes are caused by weathering of iron sulfides (pyrite, marcasite), which are associated with the lignite. During mining activity, when exposed to air and water, the iron sulfides are oxidized to sulfuric acid and Fe^{2+} which leads to acidification of soils and groundwater (Blowes et al., 1994; Reichel and Uhlmann, 1995). When mining activity is stopped and the basins fill with groundwater, Fe^{2+} is oxidized to Fe^{3+} which in turn precipitates as hydroxide, generating more acidity (Blowes et al., 1994; Wisotzky, 1998). Newly formed postmining lakes are not only very acidic (pH 2.5–3.5) and rich in Fe and SO_4^{2-} (Nixdorf et al., 1998b), but also contain high concentrations of Ca, Al, Mn and

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often trace metals mobilized from soil minerals (Reichel and Uhlmann, 1995).

Although there is still debate on future use of the postmining lakes and on restoration strategies, in the longer term circumneutral pH is generally aimed for (Klapper and Schultze, 1995; Klapper et al., 1998; Nixdorf et al., 1998a). Flooding with nutrient rich, circumneutral river water has been applied to neutralize several postmining lakes. However, even if neutral pH is reached, reduced supply of river water, land slides, and changes in the groundwater flow may cause reacidification of the lakes (Reichel and Uhlmann, 1995; Klapper et al., 1998; Werner, 1999). Concepts for future use of the postmining lakes include commercial and recreational fisheries and some aged, neutralized postmining lakes have already been stocked with fish, including vendace (*Coregonus albula* L.). This pelagic species which mainly feeds on zooplankton (Jacobsen, 1982) and does not need littoral macrophytes for spawning (Zuromska, 1982) appears to be appropriate for stocking of the postmining lakes, which often lack well structured, vegetated littoral zones.

With regard to possible reacidification, it is of crucial importance to know the lower threshold pH-value for the survival of vendace. There is not much information on critical pH-values for this species, because it typically occurs in relatively large lowland lakes which are not very sensitive to acid rain (Bergquist, 1991; Rask et al., 1995). Degerman et al. (1992) observed that reproduction was impaired at pH < 4.5–5.2 in soft water. However, as acid-tolerance depends on hydrochemistry (Leuven and Oyen, 1987; Lien et al., 1996), and hydrochemical conditions in the postmining lakes (hard, high conductivity waters; Reichel and Uhlmann, 1995; Nixdorf et al., 1998b) greatly differ from those in the soft, low conductivity waters affected by acid rain (Gjessing et al., 1976; Sayer et al., 1993), this threshold-value might not apply to the postmining lakes.

Acidification has greatest adverse effects on the early life stages of fish (von Westernhagen, 1988; Sayer et al., 1993). In various species including perch (*Perca fluviatilis* L.) and salmonids, effects of soft acid water on hatching were more pronounced when the embryos had already been exposed during early embryonic development and, especially, during water-hardening of the eggs (Rask, 1984; Rombough and Jensen, 1985; Sayer et al., 1993).

The aims of the present study were (1) to investigate the lower acceptable pH-limit for early life stages of vendace continuously exposed for 112 days to reconstituted water with the main hydrochemical characteristics of the Lusatian postmining lakes and (2) to assess the influence of the time of transfer to low pH (before vs. after water-hardening of the eggs) on acid tolerance.

MATERIALS AND METHODS

Experimental Media

Reconstituted media—hard, acidic water (pH 3.37, 1345 μS cm^{-1}) and neutral water (pH 7.40, 658 μS cm^{-1})—were prepared according to the hydrochemical characteristics of Lusatian postmining lakes and of river water used to flood several postmining lakes (Reichel and Uhlmann, 1995; Nixdorf et al., 1998b; Gelbrecht, personal communication; Lessmann, personal communication). Nominal concentrations (mg L^{-1}) were Na: 10, K: 4, Ca: 215, Mg: 25, Fe: 10, Al: 5, Mn: 2, Cl^-: 25, SO_4^{2-}: 680 in the reconstituted acidic water, and Na: 20, K: 7, Ca: 91, Mg: 13, Fe: 1.5, Al: 0.3, Mn: 0.5, Cl^-: 36, SO_4^{2-}: 241, HCO_3^-: 64 in the neutral water. Test solutions with pH-values of 3.50, 3.75, 4.00, 4.25, 4.50, 4.75, 5.00, 5.50, and 7.00 (simulating neutralized mining lake water) were prepared by mixing acid and neutral water. The reconstituted neutral (pH 7.40) water was used for incubation of the controls. The experimental media were aerated for at least 3 days before using them in the early life stage test, pH-values were readjusted daily with acidic or neutral medium. Ferric hydroxide was precipitating in all test solutions. Supernatant water was used for the incubation of the fish.

Hydrochemistry

For hydrochemical analysis, the experimental media were filtered (cellulose-acetate filters, pore size 0.45μm, Sartorius) and analyzed according to German standard methods (DEV, 1986–1998). Concentrations of Na and K were determined by atomic emission spectrometry, those of Ca, Mg, Mn, and total dissolved Al (Al_{td}) by flame atomic absorption spectrometry (Perkin-Elmer AAS 3300). Al concentrations below 0.2 mg L^{-1} were quantified by graphite furnace atomic absorption spectrometry (Perkin-Elmer HGA 600). Concentrations of Cl^- and SO_4^{2-} were measured by ion chromatography (Sykam), dissolved Fe was determined using the orthophenanthroline method.

Early Life Stage Test

Mature vendace were caught in the circumneutral lake Arendsee (Sachsen-Anhalt, Germany) on January 7, 1997 (day 0) at a temperature of 3°C. Hydrochemical characteristics (mean values from 0–20 m depth) of lake Arendsee were: pH 7.9, 488 μS cm^{-1}; Na: 22.3, K: 9.4, Ca: 60, Mg: 8.2, Fe: < 0.03, Cl^-: 47, SO_4^{2-}: 63 mg L^{-1} (Rönicke, personal communication). Immediately after capture, ova from about 30 females and sperm from about 30 males were obtained by stripping and mixed. Two experimental series were set up to assess

the influence of the time of transfer to low pH on acid tolerance of early life stages of vendace. One batch of eggs was transferred 10 min after insemination to the experimental media (direct transfer, series A). A second batch was incubated for 7 h in lake water and then transferred to the test solutions (7 h-transfer, series B). For each treatment, four replicates were carried out (series A: 40 eggs per replicate, series B: 30 eggs per replicate). All eggs were incubated in polystyrene Petri dishes (Greiner, 89 mm diameter) containing 20 mL of test solution. The solutions were renewed every other day during the embryonic period and daily after the onset of feeding; pH-values of the new test solutions were readjusted to the nominal pH of the respective treatment and pH-values of the old solutions were measured. Eggs were incubated at 4°C until day 50, then temperature was gradually increased to 10°C (5°C until day 59, 6°C until day 79, 7°C until day 80, 8°C until day 99, 9°C until day 107). Fish were fed *ad libitum* with cultured paramecia and newly hatched *Artemia* nauplii (AF 480, INVE Aquaculture). Larvae which fed on paramecia but did not yet regularly feed on *Artemia* were transferred to glass jars containing 50 mL of experimental medium. When feeding regularly on *Artemia*, the larvae were transferred to chambered aquaria containing 30 L of test solution. The aquaria were cleaned daily, the pH was adjusted, if necessary, and 10% of the water was changed.

Development of vendace was monitored. Mortalities were registered daily, embryos and larvae with gross deformities were classified as mortalities (Birge et al., 1985). Mortality before hatching was expressed as the percentage of the number of eggs at the beginning of exposure, mortality after hatching as the percentage of the number of hatched embryos. Egg diameters (to the outer margin of the chorion) were measured on days 2 (series A), 3 (series B), and 21 (both series) for five randomly selected eggs per replicate. The number of fully hatched embryos was counted daily. Embryos which died in a partly hatched state were registered separately. Both hatching and hatching mortality were expressed as percentages of the number of eggs at the beginning of exposure.

The terminology of Balon (1975), according to which the larval period begins with the onset of feeding, was applied to the early life stages.

Statistical Analyses

Percentages of mortality before hatch and hatching (arcsine-square root transformed data) as well as egg diameters were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey test. When analyzing mortalities before hatch and hatching percentages, treatments with 100% mortality and no hatch, respectively, in $\geqslant 1$ replicate were not included into ANOVA, as differences were obvious and homogeneity of variances would have been offended. Data on hatching mortality were evaluated using the Kruskall–Wallis test followed by the Mann–Whitney test. Comparisons between both series were carried out using either student's *t*-test or the Mann–Whitney test. All tests were conducted at the 5% level of significance using the SPSS statistical program.

RESULTS

Hydrochemistry

In Tables I and II, results of the chemical analyses of the experimental media are summarized. As test solutions were prepared by mixing acid and neutral media of different ion content, concentrations of dissolved Na, K, and Cl^- slightly increased with increasing pH,

TABLE I. Measured hydrochemical characteristics of the experimental media: conductivity ($\mu S\ cm^{-1}$) and concentrations of dissolved Na, K, Ca, Mg ($mg\ L^{-1}$)[a]

Nominal pH	Specific Conductivity	Na	K	Ca	Mg
3.50	1230 ± 37	15.3 ± 1.3	5.7 ± 0.1	223 ± 28	23.8 ± 2.6
3.75	1105 ± 35	16.1 ± 0.6	5.8 ± 0.3	190 ± 26	20.9 ± 2.6
4.00	1035 ± 32	16.9 ± 0.6	6.0 ± 0.3	181 ± 25	20.0 ± 2.3
4.25	993 ± 29	16.9 ± 0.7	6.1 ± 0.3	175 ± 25	19.6 ± 2.3
4.50	968 ± 29	17.0 ± 0.8	6.1 ± 0.3	172 ± 18	19.1 ± 2.2
4.75	959 ± 20	17.2 ± 1.0	6.2 ± 0.2	171 ± 21	18.9 ± 2.3
5.00	930 ± 26	17.7 ± 1.3	6.3 ± 0.2	166 ± 20	18.5 ± 2.3
5.50	874 ± 24	18.5 ± 2.4	7.0 ± 1.1	155 ± 20	17.5 ± 1.8
7.00	808 ± 64	17.6 ± 3.5	6.3 ± 1.3	119 ± 16	13.9 ± 2.6
7.40	662 ± 17	22.0 ± 3.5	8.1 ± 1.3	102 ± 6	12.8 ± 0.3

[a] Values are means ± SD; $n = 9$ for conductivity and $n = 4$ for all other measurements.

TABLE II. Measured hydrochemical characteristics of the experimental media: concentrations of dissolved Fe, Al, Mn, Cl^-, SO_4^{2-} (mg L^{-1})[a]

Nominal pH	Fe	Al	Mn	Cl^-	SO_4^{2-}
3.50	2.33 ± 1.37	3.51 ± 0.88	1.72 ± 0.46	29 ± 1	601 ± 135
3.75	0.62 ± 0.35	2.78 ± 0.85	1.46 ± 0.40	30 ± 2	526 ± 129
4.00	0.41 ± 0.50	2.45 ± 0.78	1.32 ± 0.34	35 ± 7	549 ± 144
4.25	0.55 ± 0.84	2.25 ± 0.74	1.29 ± 0.34	33 ± 5	535 ± 136
4.50	< 0.03 − 1.34	2.08 ± 0.60	1.21 ± 0.26	32 ± 1	488 ± 120
4.75	< 0.03 − 0.25	1.84 ± 0.60	1.19 ± 0.30	32 ± 2	473 ± 108
5.00	< 0.03 − 0.89	1.51 ± 0.73	1.13 ± 0.27	32 ± 3	445 ± 126
5.50	< 0.03 − 0.15	0.16 ± 0.12	1.03 ± 0.26	32 ± 2	424 ± 81
7.00	< 0.03 − 0.25	0.07 ± 0.08	0.70 ± 0.10	35 ± 1	364 ± 64
7.40	< 0.03 − 0.13	0.04 ± 0.02	0.50 ± 0.07	36 ± 2	242 ± 10

[a] Values are means ± SD except for Fe concentrations at pH 4.50–7.40, where the range is indicated, as some concentrations were below the detection limit; $n = 3$ for Al and $n = 4$ for all other measurements.

whereas those of Ca, Mg, Fe, Al, Mn, and SO_4^{2-} decreased. Marked reductions in the Al- and Fe-content at higher pH were caused by the pH-dependent solubility of both metals. Despite the use of supernatant water in the early life stage test, precipitation of ferric hydroxide on the egg envelopes occurred in all treatments. The amount of precipitate increased at lower pH-values.

During the 24 or 48 h interval between two changes of the test solutions in Petri dishes and glass jars, pH-values of the acidic media increased slightly (Table III). These pH-changes were generally small, but increased at pH 5.50 during the period of hatching and after the onset of exogenous feeding. Differences in pH-values measured after incubation between series A and B were not significant.

TABLE III. Measured pH-values of the old test solutions after incubation of vendace embryos and larvae in Petri dishes and glass jars[a]

	Measured pH	
Nominal pH	Series A	Series B
3.50	3.55 ± 0.03	3.56 ± 0.06
3.75	3.81 ± 0.04	3.83 ± 0.13
4.00	4.07 ± 0.04	4.10 ± 0.17
4.25	4.35 ± 0.06	4.38 ± 0.17
4.50	4.61 ± 0.09	4.65 ± 0.15
4.75	4.84 ± 0.09	4.87 ± 0.12
5.00	5.04 ± 0.08	5.06 ± 0.11
5.50	5.61 ± 0.20	5.63 ± 0.24
7.00	6.97 ± 0.11	7.02 ± 0.13
7.40	7.40 ± 0.11	7.45 ± 0.13

[a] When changing the test solutions in Petri dishes and glass jars, pH-values of the new solutions were adjusted to the nominal treatment pH, and pH-values of the old solutions were measured. The latter are presented as means ± SD.

Early Life Stage Test

Series A

Diameters of the eggs transferred directly to pH ⩽ 5.00 were significantly reduced compared to those at pH 5.50–7.40 (Fig. 1). At pH ⩽ 5.00, the perivitelline space was so small that the developing embryos were squeezed between the yolk and the chorion. At pH 3.75 and 4.00, the embryos could not move and vertebral deformities were occasionally observed. Development of the embryos subjected to low pH was retarded. At pH 3.50, development ceased at ⩽ 50% epiboly. Compared to the control (pH 7.40), optic primordia appeared with 5 days delay at pH 3.75 and with 1–2 days delay at pH 4.00–4.75.

For series A, the effects of hard, acidic water on survival and hatching are summarized in Table IV. At pH 3.50 and 3.75, mortality rapidly increased during the first days of exposure (Fig. 2). On day 21, 100% mortality was reached at pH 3.50. Mortality at pH 4.00 and 4.25 gradually increased during the first 40 days. A second phase of high mortality was observed at pH 4.00–5.00 immediately before, during, and shortly after hatching of the embryos kept at pH 7.40.

At pH-values below 4.75, hatching was prevented. Hatching percentages at pH 4.75 and 5.00 were very low (2 and 11%, respectively), those at pH 5.50 and 7.00 did not differ significantly from control values. Compared to the control, the onset of hatching was delayed by 6 days at pH 4.75, by 5 days at pH 5.00–5.50, and by 1 day at pH 7.00 (Fig. 3). At pH 4.00–5.00, a significant percentage of the embryos died in a partly hatched state (Table IV).

Vendace incubated at pH 7.00 and 7.40 started to ingest paramecia several days after hatching and began feeding on *Artemia* a week later. The fish at pH 5.50 started to feed on paramecia with 7 days delay com-

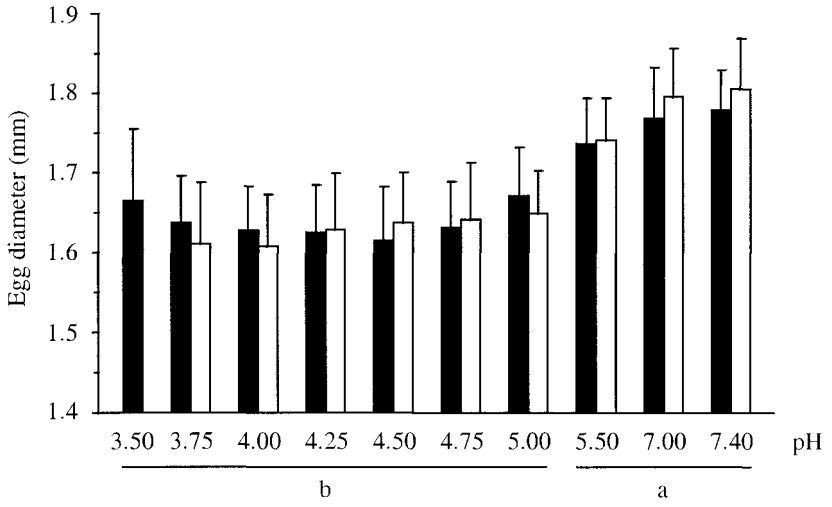

Fig. 1. Diameters of vendace eggs (means ± SD) transferred directly after insemination to the experimental media (series A) as measured on days 2 (solid bars) and 21 (open bars). On day 21, no eggs were left at pH 3.50. Means marked with "a" are significantly different from those marked with "b" (ANOVA, Tukey, $p < 0.05$).

pared to the controls. Apart from few exceptions, they did not ingest *Artemia*. Most of the observed attempts of larvae at pH 5.50 to catch *Artemia* nauplii were unsuccessful. Often, the larvae did not even react to the nauplii. They were less pigmented than the controls and gradually became emaciated. Five weeks after median hatch, all larvae at pH 5.50 had died. Eleutheroembryos exposed to pH 4.75 and 5.00 remained inactive, did not begin external feeding, and died within a few days after hatching.

Due to nonfeeding of the eleutheroembryos exposed to pH ⩽ 5.00 and restricted feeding of the larvae at pH 5.50, only vendace incubated at pH 7.00 and 7.40 were transferred to aquaria. As long as these larvae had been kept in Petri dishes (until day 86), mortality was low. The chosen type of aquaria, however, turned out

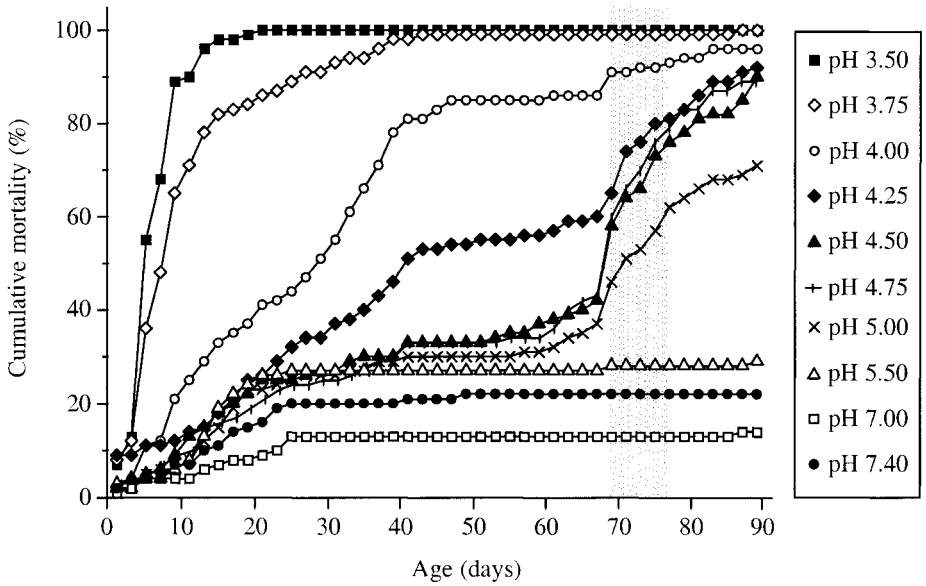

Fig. 2. Cumulative mortality percentages (mean values) prior to hatching, series A (direct transfer). The shaded area indicates the period of hatching at pH 7.40. For clarity of presentation, only data from every other day are plotted, although mortality was registered daily.

to be unsuitable for the constantly swimming vendace larvae: collisions of the fish with the glass and the gauze used to separate the replicate exposure groups were frequently observed. After transfer to the aquaria, mortalities increased to 85% at pH 7.00 and 82% at pH 7.40 at the end of the experiment (day 112). As the fish incubated at pH $\leqslant$ 5.50 never reached the developmental stage which those at pH 7.00 and 7.40 had when transferred to the aquaria, it appears appropriate to compare mortality at pH $\leqslant$ 5.50 to mortality rates at pH 7.00 and 7.40 until day 86 (Table IV).

Series B

In series B (7 h-transfer), egg diameters at pH 3.50–7.00 measured on day 3 did not differ significantly from those at pH 7.40. On day 21, only diameters at pH 3.50 and 3.75 were significantly reduced and the observed differences were much smaller than in series A (Fig. 4). As in series A, development of embryos exposed to acidic pH was retarded.

During the first days of exposure, only mortality at pH 3.50 increased rapidly. Until the onset of hatching of the control embryos, mortality at pH 3.75–4.25 remained much lower than in series A. The second phase of increased mortality began later, during and especially after the hatching period at pH 7.40 (Fig. 5). Although maximum survival times of the embryos at pH 3.50–5.00 were longer in series B, total egg mortalities did not differ much from those in series A (Table V).

Hatching occurred at pH $\geqslant$ 4.25 (i.e., 0.5 pH-units lower than in series A), but as in series A, hatching percentages at pH $\leqslant$ 5.00 were very low (Table V), whereas those at pH 5.50 and 7.00 did not differ significantly from the control. At pH $\leqslant$ 7.00, a delay in hatching similar to that described for series A was observed. Hatching mortality in series B (Table V) was consistently lower than in series A, differences between both series were significant at pH 4.00 and 5.00.

Eleutheroembryos at pH $\leqslant$ 5.00 remained inactive, did not start exogenous feeding, and did not survive longer than a few days. At pH 5.50, feeding was generally restricted to paramecia, and the last larvae died 4 weeks after median hatch. After transfer to the aquaria, mortality of vendace at circumneutral pH increased to 90% at pH 7.00 and 85% in the controls. The values for mortality after hatching, which are given in Table V, refer to mortality until transfer from Petri dishes to aquaria (day 86).

DISCUSSION

When exposing early life stages of vendace to acidic media with the hydrochemical characteristics of Lusatian postmining lakes, high mortality was observed during the first weeks after fertilization, throughout the hatching period, and after transition to exogenous nutrition. During the egg development, differences between both experimental series were pronounced. Hardening at exposure pH (series A) led to a substantial reduction in egg diameter at pH 3.50–5.00. Acid

TABLE IV. Effects of hard acid water on survival and hatching of early life stages of vendace: series A (direct transfer)[a]

Nominal pH	Mortality before Hatching (%)[b]	Hatching (%)[b]	Hatching Mortality (%)[b]	Mortality after Hatching (%)[c]
3.50	100 ± 0	—	—	—
3.75	100 ± 0	—	—	—
4.00	96 ± 3^{i}	—	4 ± 3*	—
4.25	$92 \pm 3^{h,i}$	—	8 ± 3*	—
4.50	$90 \pm 7^{h,i}$	—	10 ± 7*	—
4.75	89 ± 2^{h}	2 ± 1^{h}	9 ± 2*	100 ± 0
5.00	71 ± 5^{g}	11 ± 5^{g}	18 ± 2*	100 ± 0
5.50	29 ± 2^{f}	71 ± 2^{f}	—	100 ± 0
7.00	14 ± 4^{e}	86 ± 4^{e}	—	7 ± 4^{d}
7.40	$22 \pm 5^{e,f}$	$78 \pm 5^{e,f}$	—	11 ± 5^{d}

[a] Values are means $\pm$ SD.
[b] Percentages refer to the initial number of eggs.
[c] Percent of hatched embryos.
[d] Mortality rates prior to transfer to the aquaria (see text).
[e–i] Different letters as superscripts denote significant differences between treatments.
* Significant difference from the control (pH 7.40: no hatching mortality).

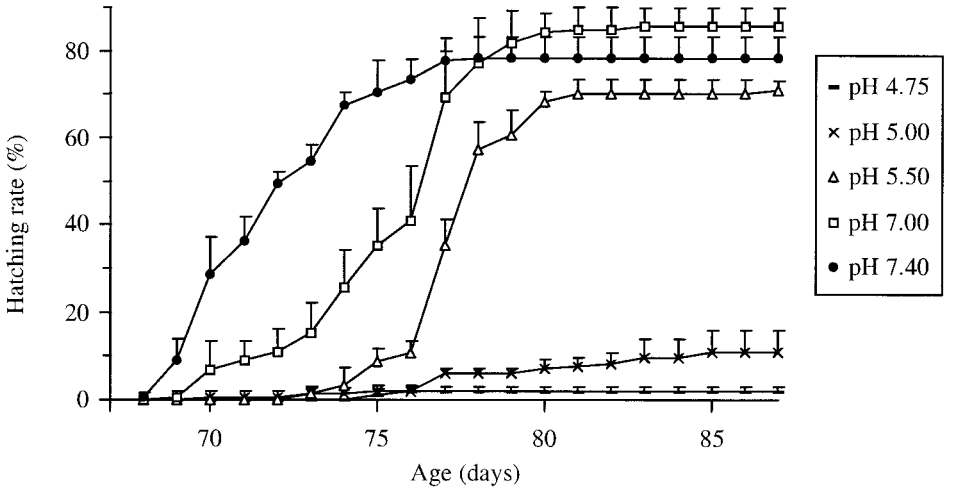

Fig. 3. Hatching of vendace embryos transferred directly after insemination to the experimental media (series A). Values are means ± SD.

pH may have reduced water uptake by interfering with exocytosis of the cortical alveoli or by affecting the osmotic activity of the perivitelline colloids (Peterson and Martin-Robichaud, 1982; Rombough and Jensen, 1985). Water uptake is restricted to a brief period immediately after insemination (Alderdice, 1988). Therefore, the perivitelline space had already formed and the process of chorion hardening, which involves structural changes leading to increased stability of the chorion (Riehl, 1996), had been completed when the eggs were transferred to the experimental media after having been incubated for 7 h in lake water (series B). Under these circumstances, only the eggs exposed to pH 3.50 and 3.75 experienced small reductions of volume.

In series A, the extremely small perivitelline space at the lowest investigated pH-values impaired embryo movements and presumably was also the cause for disturbed growth resulting in vertebral deformities. Moreover, exposure to low pH during the period of water hardening exacerbated mortality during the early embryonic period. This might be related to the high permeability of the vitelline membrane during the earliest developmental phase (Rombough and Jensen, 1985; Alderdice, 1988). Acid-induced changes in the structure and amount of the perivitelline colloids could have contributed to the high mortality, because the colloids act as buffering agents (Kügel and Peterson, 1989) and have ionoregulatory functions (Eddy and Talbot, 1985).

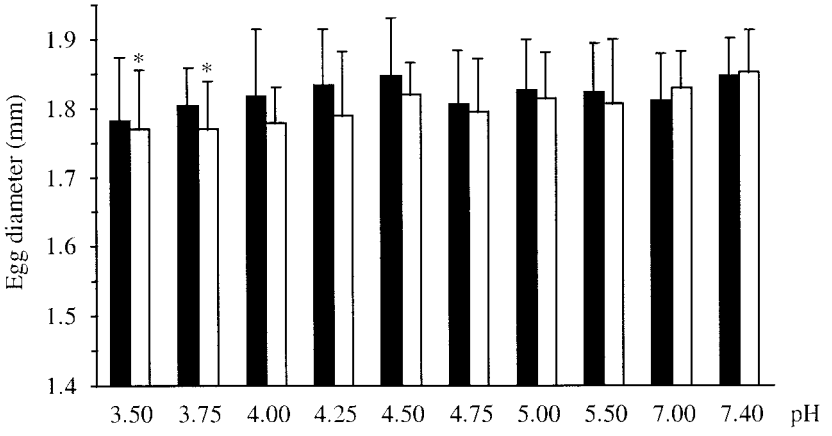

Fig. 4. Diameters of vendace eggs (means ± SD) transferred 7 h after insemination to the experimental media (series B) as measured on days 3 (solid bars) and 21 (open bars). Asterisks denote significant differences from the control (ANOVA, Tukey, $p < 0.05$).

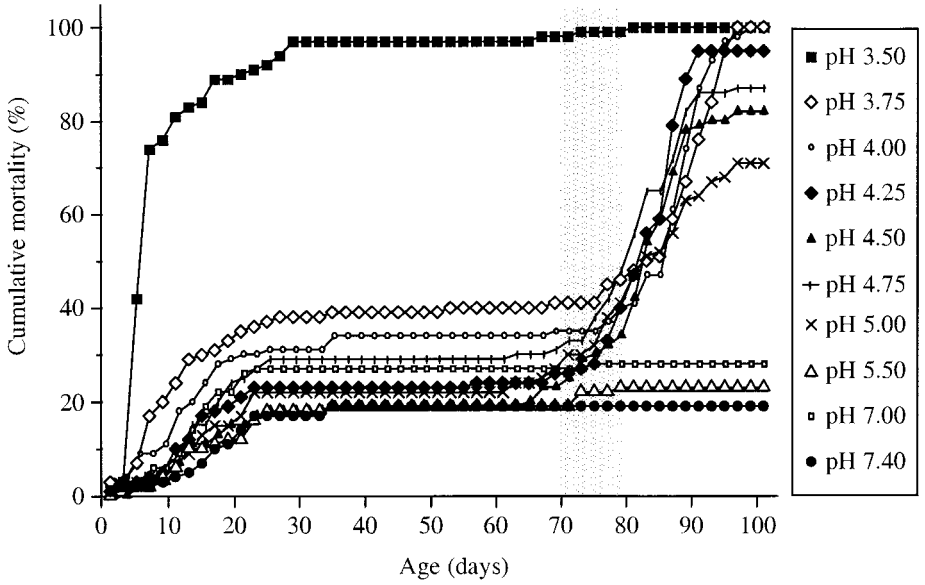

Fig. 5. Cumulative mortality percentages (mean values) prior to hatching, series B (7 h-transfer). The shaded area designates the period of hatching at pH 7.40. For clarity of presentation, only data from every other day are plotted, although mortality was registered daily.

Despite the earlier and stronger increase in mortality in series A, differences in total egg mortality between both series were small, since egg mortality in series B increased after a prolonged nonhatch period. Embryos prevented from hatching presumably died of oxygen deficiency (Peterson et al., 1980; Kügel et al., 1990). Considering the narrow pH-range required for activity of the hatching enzyme of vendace—at pH 6.0, activity was already reduced to 5% of its maximum activity at pH 8.5–9.0 (Luczynski et al., 1987; Luberda et al., 1992)—it is not surprising that in our study hatching percentages at pH < 5.50 were very low and that hatching at acid pH was consistently delayed. Due to the buffering capacity of the perivitelline fluid (which might, however, have been reduced in series A), pH-values in the eggs may remain about 0.5 units above

TABLE V. Effects of hard acid water on survival and hatching of early life stages of vendace: series B (7 h-transfer)[a]

Nominal pH	Mortality before Hatching (%)[b]	Hatching (%)[b]	Hatching Mortality (%)[b]	Mortality after Hatching (%)[c]
3.50	100 ± 0	—	—	—
3.75	100 ± 0	—	—	—
4.00	100 ± 0	—	—	—
4.25	95 ± 3	1 ± 2	4 ± 4	100 ± 0
4.50	83 ± 15	11 ± 13	6 ± 4*	100 ± 0
4.75	87 ± 4^{g}	4 ± 2^{g}	8 ± 5*	100 ± 0
5.00	71 ± 9^{f}	20 ± 8^{f}	9 ± 5*	100 ± 0
5.50	23 ± 4^{e}	77 ± 4^{e}	—	100 ± 0
7.00	28 ± 6^{e}	72 ± 6^{e}	—	4 ± 5^{d}
7.40	19 ± 3^{e}	81 ± 3^{e}	—	8 ± 4^{d}

[a] Values are means $\pm$ SD.
[b] Percentages refer to the initial number of eggs.
[c] Percent of hatched embryos.
[d] Mortality rates prior to transfer to the aquaria (see text).
[e–g] Different letters as superscripts denote significant differences between treatments.
* Significant difference from the control (pH 7.40: no hatching mortality).

those in the surrounding water (Peterson et al., 1980; Kügel and Peterson, 1989). Both, the loss of this protection when the chorion is ruptured and the embryos come into direct contact with the external medium, and difficulties in digesting and discarding the chorion (Haya and Waiwood, 1981; Sayer et al., 1993) were probably the causes for the mortality of partly hatched vendace embryos at pH $\leqslant$ 5.00.

Increased sensitivity after hatching was evident in both experimental series: mortality of eleutheroembryos exposed to pH 5.00 and below increased very rapidly. As the capability of the perivitelline fluid to accumulate cations is reduced at acid pH, the ion balance of the embryos might already have been disturbed at hatch (Eddy and Talbot, 1985; Peterson and Martin-Robichaud, 1987; Alderdice, 1988; McWilliams and Shephard, 1991). After hatching, ion losses were probably exacerbated by the large surface area of the eleutheroembryos which were directly exposed to the acidic medium. Furthermore, cutaneous respiration might have been affected (McDonald et al., 1989; Ingersoll et al., 1990b; Ostaszewska and Wojda, 1997).

The high Al concentrations of the reconstituted media at pH 5.00 and below were very likely a major factor contributing to the rapid increase of mortality after hatching. At pH 4.75 and 5.00, the experimental media contained as much as 1.84 and 1.51 mg L^{-1} Al_{td}, respectively. A detailed speciation of Al was not performed, but from the chemical characteristics of the experimental media it can be concluded that inorganic monomeric Al (Al_{im}), which is very toxic to fish (Rosseland and Henriksen, 1990; Lien et al., 1996), prevailed. At high ion concentration and low pH, polymeric Al species are of secondary importance (Miller and Andelman, 1987; Playle and Wood, 1990; Monterroso et al., 1994). Since no dissolved organic material was added to the media, Al was not organically complexed. During incubation of the fish, some complexation with substances released from the eggs and eleutheroembryos probably occurred, but with each change of the test solutions new Al_{im} was added.

In both experimental series, hatching percentages at pH 5.50 did not differ significantly from those at pH 7.40, and swimming and feeding activity initially appeared to be normal. Mortality only increased well after the onset of feeding. This gradual increase in mortality may be related to the transition from cutaneous to branchial respiration (Rosseland and Staurnes, 1994), as effects of acid exposure on the gills are more severe than those on the skin. In salmonids, gill development at acid pH was severely disturbed and elevated Al concentrations exacerbated these disturbances (Jagoe et al., 1987; Conklin et al., 1992). At pH 5.50, the mean concentration of Al_{td} in our reconstituted medium was 0.16 mg L^{-1}. Since respiratory toxicity of Al is highest at pH 5.0–6.0 (Poléo, 1995), this concentration might already have been critical to the larval stages, which are very sensitive to Al (Ingersoll et al., 1990a; Weatherley et al., 1990).

In addition, the severely reduced feeding activity certainly contributed to the gradual increase in mortality. Loss of appetite, which has often been observed at acid pH (Lacroix et al., 1985; Cleveland et al., 1986; Buckler et al., 1995), is thought to be related to impaired chemoreception (Lemly and Smith, 1987; Rosseland and Staurnes, 1994) and to increased concentrations of plasma glucose, which in turn result from elevated cortisol levels (Jones et al., 1987; Tam et al., 1988; Wilson et al., 1996). Since the vendace larvae in our study gradually ceased feeding, exhaustion was inevitable. Irrespective of the time of transfer to acid pH (before vs. after water-hardening of the eggs), pH 5.50 was ultimately lethal to the larvae.

The high Ca concentrations of the reconstituted media apparently provided only a partial protection from the toxic effects of acid and Al. The toxicity of Mn, however, significantly decreases with increased Ca content (Reader et al., 1988; Stubblefield et al., 1997). Nevertheless, a negative influence of Mn on vendace embryos and larvae cannot be excluded. This is also the case for Fe, although formation of a thick layer of ferric hydroxide was prevented, because the eggs were moved during changes of the test solutions. In the postmining lakes, precipitation of Fe might adversely affect egg development, especially when considering the long incubation time of vendace eggs.

Acid tolerance may increase as a consequence of long-term adaptation to low pH (McWilliams, 1982; Hurley et al., 1989; Vuorinen et al., 1994). However, since we focused on the consequences of reacidification on early life stages of vendace in previously neutralized postmining lakes, we used eggs from parental fish caught from a circumneutral lake. Vendace of the same origin were also used for stocking of postmining lakes.

In our experiment, early life stages of vendace were continuously exposed to acid pH. The effects of reacidification of a postmining lake on fish would of course depend on exposure duration and on the developmental stages exposed to reduced pH (Ingersoll et al., 1990a). Short-term reductions in pH may already have major deleterious impacts (Gunn and Noakes, 1987; Cleveland et al., 1991; van Sickle et al., 1996), and acid pH might interfere with oogenesis and spawning (Peterson et al., 1982; Rask et al., 1990). Moreover, mixing of acid groundwater and neutral lake water might lead to polymerization and precipitation of Al. It was demonstrated that conditions of ongoing precipitation of Al are extremely toxic to fish (Rosseland et al., 1992; Poléo and Muniz, 1993; Poléo et al., 1994). Re-

lease of free carbon dioxide might further increase toxicity (Alabaster and Lloyd, 1980). Considering the high sensitivity of early life stages of vendace to low pH, maintenance of neutral pH is of critical importance when postmining lakes have been stocked with vendace.

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