The effects of low water pH on the ionic balance in freshwater mussel *Anodonta anatina* L.

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The freshwater mussel *Anodonta anatina* was exposed for 8 or 10 days in water (3 mg Ca/l) acidified with sulphuric acid to give pH values ranging from 4.8 to 2.3 and 7.3. Exposures at pH 2.3 and 2.6 resulted in a mortality rate of 63% in 8 days but exposure at a higher pH did not affect survival. No statistical differences were detected in the shell index (shell weight / shell length × breadth) or the body condition index (100 × dry weight / shell length) between exposure groups. However, dissolution of the shell periostracum layer was observed at all pHs below 3.9. Acidic exposure, in general, caused a decline in the hemolymph sodium and chloride concentrations, and a decrease in the soft tissue sodium. An increase of calcium and potassium concentrations in the hemolymph was also found.

1. Introduction

The hydrogen-ion concentration of inland waters has been reported to increase with sulphuric and nitrogen oxide deposition in Scandinavia, as well as in wide areas of eastern Canada and north-eastern United States (Wright et al. 1980, Heines 1981, Forsius et al. 1990). Episodic runoff events caused by spring snow-melt and heavy rains can cause short-term decreases in pH (Overrein et al. 1980). In temperate regions, the duration of increased acidity (pH 4 to 5) after spring snow-melt usually lasts from 1 to 8 weeks and after heavy rains from a few hours to two weeks (Leivestad & Muniz 1976, Reader & Dempsey 1989).

The water pH level is a major environmental factor in determining the distribution of limnetic species and, thus, acute reductions in water pH have a profound impact on aquatic life. Reduced primary production and changes in the composition of species caused by acidification has been documented in several investigations (Haines 1981, Havas & Hutchinson 1982, McCahon & Pascoe 1986, Rask & Tuunainen 1990). A critical pH, below which the survival of many limnetic organisms is significantly reduced, lies in the pH range of 5 to 6 (Økland & Økland 1980).

The major concern in acidification studies has been species composition, but until recently we have had rather limited knowledge of the

physiological changes in sensitive freshwater invertebrates, like mussels, in acidic conditions. The hemolymph ionic composition of Unionidae at various water pHs (down to pH 4) has been studied by Pynnönen (l990a, b). Changes in the ionic composition of tissues, however, have only been reported by Malley et al. (1988) for field-incubated mussels in water with pH levels down to 4.5.

During the last ten years, freshwater mussels have been increasingly used in environmental monitoring, and several studies with Unionidae have been carried out (Heit et al. 1980, Adams et al. 1981, Herve et al. 1988, Elder & Collins 1991). The primary object has been to measure the body burden of various xenobiotics, although some studies have been concerned with the biological effects of environmental stress on these animals (Foe & Knight 1986, Day et al. 1990). In future, the physiological parameters of these robust and tolerant mussels can be valuable indicators in assessing the biological status of an aquatic environment under anthropogenic stress, such as acidification, or heavy metal and organic pollution.

In this study we used the freshwater mussel *Anodonta anatina* L., a species having an important role in the benthic community of European streams and lakes, and which can locally make up over 90 percent of the total benthic biomass (Økland 1962, Haukioja & Hakala 1974). Our purpose was to assess the tolerance of *A. anatina* to a wide range of pHs and determine the level at which lethality is encountered in subacute exposures. The acidity resistance was evaluated by measuring the ability of the organism to maintain osmotically important elements: sodium, calcium, potassium, magnesium and chloride. The ionic composition of both the hemolymph and homogenized soft tissue was analyzed.

2. Material and methods

2.1. Animals

Duck mussels (*Anodonta anatina*) were obtained by scuba diving from Lake Höytiäinen in eastern Finland (62°51′N, 29°47′E) in the middle of May. Lake Höytiäinen is a large (695 km²) Finnish lake

with average water quality parameters: pH 6.6, colour 40 Pt/l and conductivity 50 µS/m.

Animals were transported to the laboratory in 25-litre plastic buckets immediately after collection and were maintained in static aerated conditions (12:12 h photo period), without substratum, in unchlorinated Joensuu city tap water. The water temperature in aquaria (15×33×55 cm) was 13°C, the pH was 7.3, and the water depth was about 10 cm. Mussels were fed twice a week with an algae-protozoan culture. The animals were acclimated in the laboratory for 2 to 4 weeks prior to experiments. During the last five days of the acclimation period the animals were not fed. Twenty-four hours before the start of an experiment their shells were scrubbed to remove all debris and they were moved to clean water. The experiments were initiated with mussels that had a shell length of 5.0 to 7.5 cm, and an average soft tissue wet weight of 5.5±2.0 g (*SD*).

2.2. Exposures

Two sets of experiments were conducted. In Experiment I mussels were incubated for 8 days at exposure pHs of 4.8, 2.6, and 2.3; and in Experiment II, for 10 days at pHs 3.9, 3.5, and 3.0. The control group in both experiments was maintained at pH 7.3. Incubations were made in continuously flowing water in 40-litre glass aquaria which were immersed in a 13°C-water bath. Oxygenated and temperature-adjusted inflowing water was acidified at the desired pH level by adding diluted sulphuric acid (p.a.) with a peristaltic pump. The inflow rate of dilution water was 0.5 l/min. The control and experimental exposures were run simultaneously and the fluctuation range of the water pH and temperature during the experiments were, in all cases, less than 0.2 pH units and 0.5°C, respectively. The chemical composition of the Joensuu city tap water used in experiments is shown in Table 1.

Both experiments were initiated with 16 mussels at each pH level. Animals were exposed without a sediment substratum or any feeding. The water pH level was measured twice a day, both for the inflowing water and the water in each aquarium, using a KCl electrode pH-meter (Radiometer PHM80). Water temperature was recorded simultaneously.

Table 1. Chemical composition of Joensuu city tap water.

Chloride mg/l	0.01–0.02
Sodium mg/l	< 0.5
Potassium mg/l	< 0.5
Calcium mg/l	2.70–3.31
Magnesium mg/l	< 0.5
Alkalinity meq/l	0.58
pH	7.2–7.4

2.3. Sampling and analyses

At the end of an exposure animals were weighed. A 1-ml sample of hemolymph was taken from the pericardium using a 1-ml syringe fitted with a 22-gauge needle. The shell adductor muscles were dissected, and the mantle cavity water was drained away. The total soft tissue was weighed with the rest of the hemolymph and homogenized using the Ultra-Turrax device. A one-gram subsample of the soft tissue homogenate was taken and extracted for two days in 10 ml of 0.1 M HNO_3 (p.a.) using the Loenn & Oikari (1982) method. The extract was centrifuged, filtered through ash-free filtration paper (Machery-Nagel, 205009) and the ionic analyses were carried out. The mussel soft tissue dry weight as a percentage of wet weight was measured by desiccating a subsample of the homogenate of known weight at 105°C for 24 hours.

Sodium, potassium, calcium and magnesium concentrations in the hemolymph and in the soft tissue extract were measured using an atomic absorption spectrophotometer (Hitachi 180-60/80). Chloride in the hemolymph was analyzed with a chloride titrator (Radiometer CMT 10). All instruments used in the dissection and glassware used in analyses were acid washed. All chemicals used were of analytical grade.

Two indexes of the mussel condition were calculated from weights (g) and lengths (cm): the shell index (SI = shell weight / shell length × breadth) and condition index (CI = 100× dry weight / shell length). The shell index (Pynnönen 1990) gives a measure of the shell condition. The body condition index (Graney & Giesy 1988) is the relationship between the soft tissue dry weight and the shell length.

2.4. Statistical analysis

The differences in mean ionic concentration between the control and exposure groups were tested by one-way analysis of variance (ANOVA) followed by the Turkey HSD test between the control and exposure groups for differing parameters. Differences were considered significant at the 0.05 probability level. All computations were carried out using the SYSTAT statistical computer package (Wilkinson 1990).

3. Results

The two lowest pHs (2.3 and 2.6) were lethal: two-thirds (63%) of the mussels died within eight days. However, both of these pHs are clearly beyond the extremes found in nature. At lower acidity levels, i.e. at pH 3.0 and above, no deaths were observed in 10 days.

The shell index (SI) did not differ statistically among exposures (Table 2). This was also the

Table 2. Mean ± *SD* for soft tissue dry weight (DW g, 105°C), the condition index (CI) and the shell index (SI) of the freshwater mussel *Anodonta anatina* after 8 or 10 days exposure at various pH levels. The values differing statistically ($P < 0.05$) from the respective control groups at pH 7.3 are marked with an asterisk.

	pH	DW	CI	SI
Experiment I	7.3	13.58 ± 2.03	0.60 ± 0.19	0.38 ± 0.07
(8 days, *n* = 6)	4.8	15.08 ± 0.86	1.15 ± 0.18*	0.32 ± 0.05
	2.6	12.73 ± 1.84	0.45 ± 0.06	0.28 ± 0.03
	2.3	12.44 ± 3.01	0.52 ± 0.14	0.40 ± 0.06
Experiment II	7.3	13.12 ± 1.21	0.42 ± 0.03	0.31 ± 0.05
(10 days, *n* = 10)	3.9	11.63 ± 1.92	0.34 ± 0.07	0.28 ± 0.04
	3.5	13.15 ± 1.61	0.45 ± 0.08	0.31 ± 0.08
	3.0	12.40 ± 1.60	0.35 ± 0.10	0.29 ± 0.05

case for the dry weight and condition index (CI), except at pH 4.8, where mussels had a significantly higher CI than the other groups.

The hemolymph sodium concentration decreased significantly from the average of 8 mmol/l to less than 3 mmol/l at a pH level of below 3.0 (Fig. 1A). Chloride behaved very similarly to sodium (Fig. 1B), but it declined even more sharply below pH 3.9 than sodium did — from about 10 mmol/l to less than 2 mmol/l. The calcium concentration remained unchanged down to pH 3.5, below which it increased, to two to six times higher at the three lowest pH levels (2.3–3.0) than in the control group (7 mmol/l; Fig. 1C). The average potassium concentration in the hemolymph was doubled below pH 3.0. No effect on magnesium was detected (Table 3).

At the range of low pH levels used, duck mussels were unable to maintain ionic stability under laboratory conditions. The soft tissue sodium and magnesium concentrations decreased significantly when the mussels were exposed to below pH 3.9 (Table 4). In Experiment I, sodium dropped from 1.31 to 0.57 mg/g dw and below, and in Experiment II, it declined from 1.12 to about 0.80 mg/g dw. The concentrations of calcium, potassium and magnesium, in the soft tissue remained unaltered (Table 4).

4. Discussion

The survival of the duck mussel was markedly reduced at pH 2.6 and below, but no deaths were observed at pH 3.0 or above. The erosion of shell periostracum was evident below pH 3.9. Shell erosion caused by acid incubation water has also been reported by Kat (1972) in *Corbicula flu–minea* and by Pynnönen (1991) in *Anodonta anatina*. However, no significant decline in the shell index could be detected in this study. The dissolution of shell calcium was evidenced by white powder easily detaching from the outer shell surface. This did not seem to increase the fragility of the shells in the 8 to 10-day exposure periods, but it probably would have led to this with longer incubation periods.

The high tolerance of adult mussels (Unionidae) to acidic short-term exposure has been reported earlier in literature. In a soft-water lake (1.70 mg Ca/l) the addition of aluminum sulfate

Table 3. Mean ± *SD* for potassium and magnesium concentration (mmol/l) of the freshwater mussel *Anodonta anatina* hemolymph after 8 and 10 days exposure at various pH levels. The values differing statistically from the respective control groups are marked with an asterisk.

	pH	K	Mg
Experiment I	7.3	0.26 ± 0.05	0.58 ± 0.18
(8 days, n = 6)	4.8	0.38 ± 0.30	0.58 ± 0.08
	2.6	0.61 ± 0.23*	0.76 ± 0.20
	2.6	0.67 ± 0.35*	0.42 ± 0.12
Experiment II	7.3	0.35 ± 0.14	0.58 ± 0.10
(10 days,	3.9	0.28 ± 0.04	0.42 ± 0.12
n = 10)	3.5	0.36 ± 0.04	0.44 ± 0.04
	3.0	0.37 ± 0.15	0.54 ± 0.12

Table 4. Mean ± *SD* for ionic composition (mg/g DW) of the freshwater mussel *Anodonta anatina* soft tissue after 8 and 10 day exposure at various pH levels. The values differing statistically from the respective control groups are marked with an asterisk.

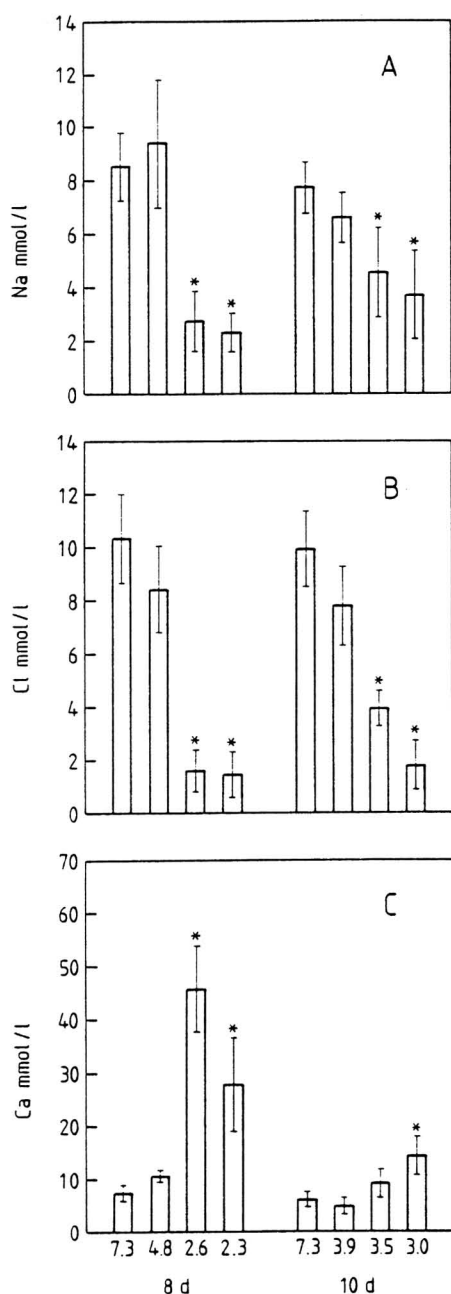|	pH	Na	Ca	K	Mg
Experiment I	7.3	1.31 ± 0.27	41.89 ± 15.77	1.70 ± 0.34	1.01 ± 0.17
(8 days, n = 6)	4.8	1.27 ± 0.25	24.38 ± 14.38	1.92 ± 0.32	0.88 ± 0.04
	2.6	0.56 ± 0.41*	36.54 ± 8.24	2.00 ± 0.18	0.98 ± 0.12
	2.3	0.48 ± 0.23	36.63 ± 5.38	1.68 ± 0.11	0.88 ± 0.12
Experiment II	7.3	1.12 ± 0.26	31.89 ± 1.42	1.99 ± 0.30	1.01 ± 0.14
(10 days, n = 10)	3.9	1.21 ± 0.28	48.38 ± 20.34	2.39 ± 0.61	1.32 ± 0.30*
	3.5	0.79 ± 0.19*	24.72 ± 4.40	2.33 ± 0.29	0.95 ± 0.13
	3.0	0.80 ± 0.38*	26.43 ± 10.15	2.26 ± 0.15	1.02 ± 0.17

Fig. 1. Effects of acute acidic exposure on the hemolymph of the freshwater mussel *Anodonta anatina*. Sodium, chloride, and calcium concentrations (mmol/l) after eight days of exposure (Experiment I, $n = 6$) and ten days of exposure (Experiment II, $n = 10$). The values (mean ± *SD*) differing statistically from the respective control groups (pH 7.3) are marked with an asterisk.

lowered the pH level to 4.5, but did not affect the survival of caged *Anodonta grandis* (Malley et al. 1988). *Anodonta* spp. and *Unio* spp. have survived several weeks' exposure to a pH range of 4 to 5. However, if the pH level of the ambient water (4.0 mg Ca/l) reached values below 3.0, death followed in 24 hours (Pynnönen 1991).

Although condition indexes have correlated with changes in the nutritional reserves of mussels (Gabbott & Stephenson 1974), and they have been successfully applied in pollution studies (Axiak & George 1987), the condition index did not show any response in our acute acidification experiments. It is likely that the period of exposure in the present study was too short.

The reason for the death of animals at the very acidic conditions of pH 2.3 and 2.6 was probably the loss of ions. According to McCorkle & Dietz (1980), a blood ionic loss of 50 to 90% of the original values is intolerable to most freshwater molluscs. In this study, such a loss was recorded for sodium (41 and 52%) and chloride (60 and 92%) even at pH 3.0 and 3.5 when no lethality was recorded. This is in accordance with the observation of Pynnönen (1991) that Unionidae can tolerate an acute decline of hemolymph sodium and chloride to less than 20% of the control value. The decrease in the soft tissue sodium may have been caused by a surplus of H^+ in the hemolymph compensated with a Na^+/H^+ exchange between the hemolymp and tissue.

In this study the decline in the hemolymph sodium concentration was associated with an increase in the hemolymph calcium, similar to the finding reported by Chang et al. (1988) and Malley et al. (1988), but no increase in the soft tissue calcium was detected. An increase in the hemolymph calcium concentration, accompanied by an increase in the hemolymph HCO_3 was also reported by Pynnönen (1991) to be the first ionic response to acidic stress.

According to Malley et al. (1988), the decrease in the hemolymph sodium concentration is due to increased passive loss in response to the increased hemolymph calcium related to blood buffering. The malfunction of the Na^+/H^+ exchange caused by an increased external H^+, another hypothesis presented by Malley et al., may also have an important role.

Hemolymph calcium also rises in anoxia, due to dissolution of $CaCO_3$, while it buffers against the acidosis. It has been hypothesized by Malley et al. (1988) that adaptations by mussels to anoxia (i.e. internal acidification) preadapt them to withstanding enternal acidification. In *Ligumia subrostrata* this increase during anoxia can be as high as eightfold (Burton 1983). The increase of hemolymph calcium concentration in molluscs may originate from the shell (Dugal 1939) or from $CaCO_3$-containing cells in the connective tissue (Istin & Girard 1970a, b). These $CaCO_3$ reserves in the shell and mantle enable clams to buffer the acidification of their body fluids (Akberali 1977). In general, the increase in the hemolymph calcium decreases the permeability of cellular membranes to water, sodium, and chloride (Burton 1983), and thus, decreases the ion depletion.

Elevation in the hemolymph calcium concentration in this study may partly be due to periods of valve closure and subsequent anoxia. Pynnönen (1991) reported that acid exposure shortens the periods that valves are open and lengthens the periods they are closed. In her study the threshold for avoidance by increased valve closure was around pH 5; most of our experimental pH levels were below that level. Valve closure does not, however, produce total isolation of the mussel tissues from the surrounding water. For example, Salanki & V-Balogh (1984) have recorded metal accumulation in closed clams. That may explain why some degree of mortality was observed in a shorter period of time than has been reported for *Anodonta* to be able to survive in total anoxia (Holwerda & Veenhof 1984).

In conclusion, based on our observations and those of Pynnönen (1991), we can agree with the hypothesis presented by Malley et al. (1988) that adult Unionidae are able to survive episodes of relatively low pH levels up to several weeks because of physiological adaptation to anoxia, but not without changes in the hemolymph ionic composition.

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