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Influence of Aluminum and H^+ on the Electrolyte Homeostasis in the Unionidae *Anodonta anatina* L. and *Unio pictorum* L.

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Abstract. Two species of freshwater clams, *Anodonta anatina* and *Unio pictorum*, were exposed to aluminum (300–900 μg/L) and acid (pH 4–5 and 6.6–8.3) in hard (35 mg Ca/L) and soft (3.5 mg Ca/L) water. Long- and short-term pH depressions of 2 and 3 weeks and intermittent, repetitive pulses of 3 days were used. The pattern of change in the hemolymph electrolyte balance was different in *U. pictorum* and in *A. anatina*. In general, an increase in hemolymph $[Ca^{2+}]$, and a decrease in $[Na^+]$, $[Cl^-]$, $[K^+]$ and $[Mg^{2+}]$ as a result of acid exposure was seen in both species. Hemolymph $[Ca^{2+}]$ of *U. pictorum* was reduced after 3 days of exposure to acid water, whereas an exposure of one week was needed to affect the other hemolymph ions. In circumneutral, hard water Al had no effect on the electrolyte balance. Intermittent pulses of low pH and Al produced a transitory increase in hemolymph $[Ca^{2+}]$, whereas $[Na^+]$ and $[K^+]$ were not affected.

High Al and H^+ concentrations cause different physiological disturbances in freshwater organisms (in fish: Wood and McDonald 1982; McDonald 1983; Witters 1986; in crustaceans: Havas 1985; Wood and Rogano 1986; in molluscs: Malley *et al.* 1988). Ion-regulatory disturbance and suffocation due to excessive mucus production are common causes of death in acid-stressed freshwater fish. Reduced skeleton calcification and feeding activity are reported as latent effects of exposure to Al and low pH (Gunn and Noakes 1987). In trout, Al and acid cause gill lesions (Karlsson-Norrgren *et al.* 1986). Al often increases the H^+ toxicity (Baker and Scholfield 1982; Witters 1986), but is also known to decrease the toxic effects of low pH (Heming and Blumhagen 1988).

The mechanisms of Ca regulation in animals with calcified shells, such as crustaceans and molluscs, differ from those of fish. Because Ca reserves in the form of $CaCO_3$ are easily mobilized, a decrease in hemolymph pH is rapidly buffered (Silverman *et al.* 1983). In clams, acid exposure often leads to a rapid, sometimes transitory increase in hemolymph $[Ca^{2+}]$. Freshwater clams also possess large amounts of calcium concretions in their gills, even in the mantle and hepatopancreas (Pynnönen *et al.* 1987). These reserves, however, function exclusively as a Ca-source for developing larvae (Silverman *et al.* 1987) and acid exposure has little or no effect on the granule composition.

The relatively impermeable carapace of crustaceans and the shell of molluscs can protect these organisms during short-term pH depressions. The clam shell is covered with periostracum, which, when undamaged, can effectively protect the shell against the destructive effect of acid water. Unionids can withstand anoxia for up to 6 days (Holwerda and Veenhof 1984), and by closing their valves they may reduce the passive loss of hemolymph solutes. Long-term acid exposure (9 weeks at pH 4) dissolves shells of the freshwater clam (Kat 1972) and leads to a microbial contamination of the mantle. Marine clams are more vulnerable, measurable shell dissolution in *Venerupis decussata* occurring at pH ≤ 7.55 (Bamber 1987).

The hemolymph of freshwater clams has one of the lowest osmotic concentrations known (Potts 1954; Malley *et al.* 1988), consisting of 99.71% water. A considerable amount of energy is needed to preserve the electrolyte balance and to concentrate Ca for shell growth. Therefore, it is suggested that ion-regulatory stress in the form of low ambient pH could soon lead to electrolyte imbalance and exhaustion. Earlier investigations, however, have proved that unionid clams can withstand severe acidification for four weeks in water containing 18 mg Ca/L. Both in hard and in soft water, clams show a decrease in hemolymph Na^+ and Cl^-, and an increase in hemolymph Ca^{2+} following an exposure to acid of 2 to 4 weeks. In the field, an increase in hemolymph $[Ca^{2+}]$ and a decrease in hemolymph $[Na^+]$ and $[Cl^-]$ were observed as a result of exposure to acid and Al (Malley *et al.* 1988).

The present work extends our knowledge of the effects of acidification and elevated Al concentrations of aquatic organisms. Two unionid species (*Anodonta anatina, Unio pictorum*), originating from hard water (50 mg Ca/L) were compared. The effects of water hardness, low pH and high Al (300 and 900 μg/L) were investigated separately and in com-

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bination. The Al concentrations match the concentrations measured in poorly buffered watersheds of North America and Scandinavia during the period of snowmelt (Hall and Likens 1984; Sharpe *et al.* 1984).

Material and Methods

Animals

Anodonta anatina L. and *Unio pictorum* L. were collected from ditches in the Maarsseveen Lake district near Utrecht in the autumn of 1987 and 1988. The animals were kept in 150-L aquaria supplied with a sandy substrate, plants, fish and snails. The chemical composition of the Cu-free tapwater used in the aquaria is given in Table 1. The clams were kept at a temperature of 13°C ± 1° and under a natural light schedule, and were fed weekly on *Chlorella*.

Table 1. Chemical composition of the experimental water. Cl^- was measured with a chloride titrator. pH was measured with a KCl electrode, and the other elements with a flame-AAS for hard water and with a ICP-AES for soft water

Element	Hard water	Soft water
Ca mg/L (mmol/L)	35 (0.88)	4.6 (0.12)
Na mg/L (mmol/L)	11 (0.47)	0.3 (0.01)
Cl mg/L (mmol/L)	15 (0.45)	NM[a]
K mg/L (mmol/L)	0.7 (0.02)	ND
Mg mg/L (mmol/L)	3.5 (0.15)	1.0 (0.04)
Fe mg/L (mmol/L)	0.1 (0.02)	0.3 (0.06)
Al mg/L (mmol/L)	ND[a]	ND
pH	8.0–8.3	6.6–7.0

[a] ND = not detected; NM = not measured

Exposures

Constant Flow Exposures: Specimens of *Unio pictorum* were exposed for three weeks in hard (35 mg Ca/L), acid (pH 4–5) water to a nominal Al concentration of 300 µg/L. Twenty *A. anatina* or *U. pictorum* individuals were exposed in glass aquaria with a volume of 100 L. Water was pumped through the aquaria at a speed of 5.5 L/hr. The water pH was regulated with sulphuric acid (0.8–1.2%) and a dosage pump connected to the water pump. Aluminum was added as $AlCl_3{:}3H_2O$ (Merck n. 1081) together with the sulphuric acid. The water pH was checked daily with a KCl electrode pH-meter and the Al concentrations were checked by atomic absorption spectrophotometry (AAS, Varian SPECTRAA-10, nitrous oxide—acetylene flame). The total Al concentrations measured by atomic absorption spectrophotometry were 166.8 µg/L ± 67.9 (SD) (1st week of exposure), 378.0 µg/L ± 64.1 (2nd week) and 414.3 µg/L ± 11.8 (3rd week). Following exposure, the elimination of Al from *U. pictorum* was followed in the circumneutral pH of 7–8 for 12 weeks.

Semi-static Exposures: Eight different combinations of water hardness, pH and Al concentration were used:

	Al concentration (µg/L ± SD)	pH	Ca (mg/L)
1.	—	4–5	35
2.	—	6.6–7.0	3.5
3.	—	4–5	3.5
4.	—	8.0–8.3	35
5.	336 ± 73	4–5	35
6.	320 ± 87	6.6–7.0	3.5
7.	257 ± 49	4–5	3.5
8.	319 ± 104	8.0–8.3	35

Four *A. anatina* and 4 *U. pictorum* individuals were exposed simultaneously in the aquarium containing 50 L water. All exposures lasted for 2 weeks. Every third day, 90% of the water volume was changed. Before and after the water was changed, the Al concentrations of the water from the exposure tanks 5, 6, 7 and 8 were measured by AAS. Two different water hardnesses were used in the exposures: Cu-free tapwater containing 35 mg Ca/L and Cu-free tapwater diluted with demineralized water in a proportion of 1:10 to reach a Ca concentration of 3.5 mg Ca/L. Prior to the addition of acid and/or Al, animals were acclimated for one week to an appropriate water hardness.

Fluctuating Exposure: In order to simulate the spring snowmelt with acid run-offs, a group of 24 *U. pictorum* were exposed to a fluctuating pH (from 8 to 4) and to Al concentrations (from ≤70 to 900 µg/L) (Figure 1). Cu-free tapwater was pumped, at a continuous rate of 7.2 L/hr, into the control (10 clams) and experimental aquaria (24 animals), both having a volume of 100 L. During a period of 3 days an acidified Al solution was pumped three times into the experimental aquarium to achieve gradually a pH between 4 and 5 and a nominal Al concentration of 900 µg/L (Figure 1). After each exposure period the pump dosing the acid Al solution was turned off and the pH was allowed to normalize gradually. Before the next exposure period, clams were allowed to recover for 5 days in Al-free (≤70 µg/L), circumneutral (pH 7–8) water. It requires about 12 h to reach the circumneutral pH and the background level of Al.

Clams were exposed without a sediment substratum and feeding. No deaths occurred during the exposures. Exposures lasted from September until October. In this period, *Unio* individuals were carrying ripening eggs and sperm, and *Anodonta* were carrying immature glochidia, respectively.

Sampling

At 1, 2, and 3 weeks following the onset of the exposure, 6 animals from the flowing system were sampled for hemolymph ion analysis. Untreated animals were sampled as controls after a 3-week aquarium acclimation. Animals from each semi-static experimental arrangement (4 *A. anatina*, 4 *U. pictorum* in each) were all sampled after 2 weeks of exposure. From the fluctuating exposure, samples were taken after each pH depression of 3 days, after each recovery period of 5 days, and from controls before and after the exposure period of 24 days. A hemolymph sample of 0.8–1.0 mL was taken from the sinus of the anterior adductor muscle (AAM) using a 1 mL syringe fitted with a 22 gauge needle. The hemolymph sample was frozen (−20°C) for later analysis.

Chemical Analysis

An atomic absorption spectrophotometer (Varian SPECTRAA-10) was used to determine the hemolymph $[Ca^{2+}]$ and $[Mg^{2+}]$ from samples diluted with concentrated HNO_3 in a proportion of 1:100. Na^+ and K^+ concentrations from the hemolymph were determined

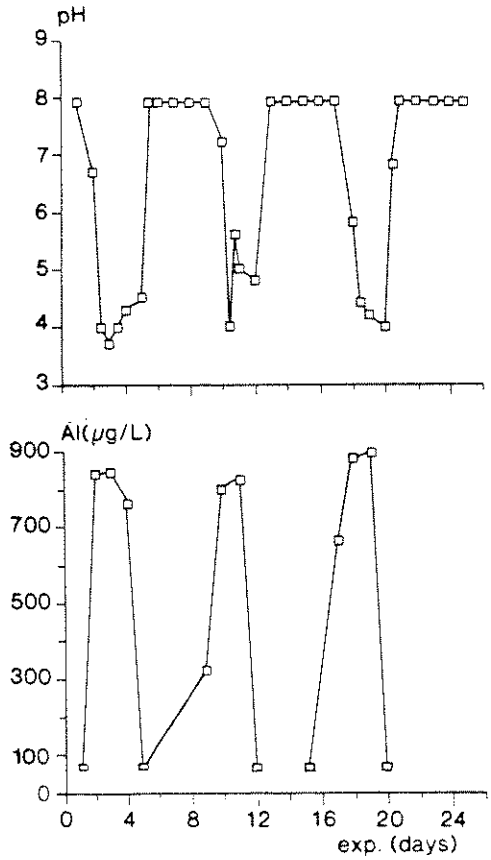


Fig. 1. Water pH and Al concentrations during the intermittent Al-rich pulses. The detection limit of the flame-AAS for Al was 70 µg/L. The chemical composition of the exposure water is given in Table 1

with an EEL flame photometer. Cl^- concentrations were measured with a Radiometer CMT 10 chloride titrator.

Statistical Analysis

Data from the semi-static exposures were statistically evaluated in order to find the significance of differences in the hemolymph ionic concentrations. One-way analysis of variance (ANOVA) was used. A probability limit of $P \leq 0.05$ was considered as significant. A 2-way Student's t-test was used to compare the differences in the ionic composition of the hemolymph during the flowing and fluctuating exposures.

Results

Constant Flow Exposures—Low pH with or without Al

Hemolymph electrolyte concentrations in unexposed animals did not differ significantly in the unionid species (Figure 2). During an exposure of 3 weeks to pH 4–5 in hard

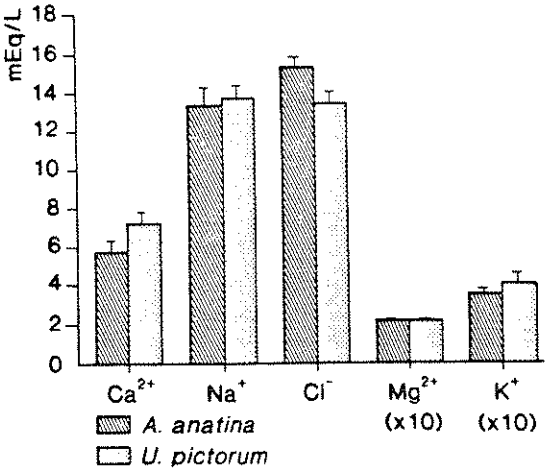


Fig. 2. Hemolymph electrolytes of unexposed *U. pictorum* and *A. anatina*. The K^+ and Mg^{2+} concentrations are drawn in 10-fold. Mean of 6 clams, ± SEM is given

water, however, *Anodonta* and *Unio* species reacted differently as far as their hemolymph Ca concentration is concerned. In *U. pictorum*, the increase in hemolymph $[Ca^{2+}]$ was transitory (Figure 3A), reaching the highest value, twice as high as the control value (Figure 2) after one week of exposure. After 3 weeks of exposure, the hemolymph Ca concentration had reached the level measured in the unexposed animals. In *A. anatina*, the hemolymph $[Ca^{2+}]$ was elevated after 2 weeks of exposure (Figure 3A) and did not change during the last week of exposure.

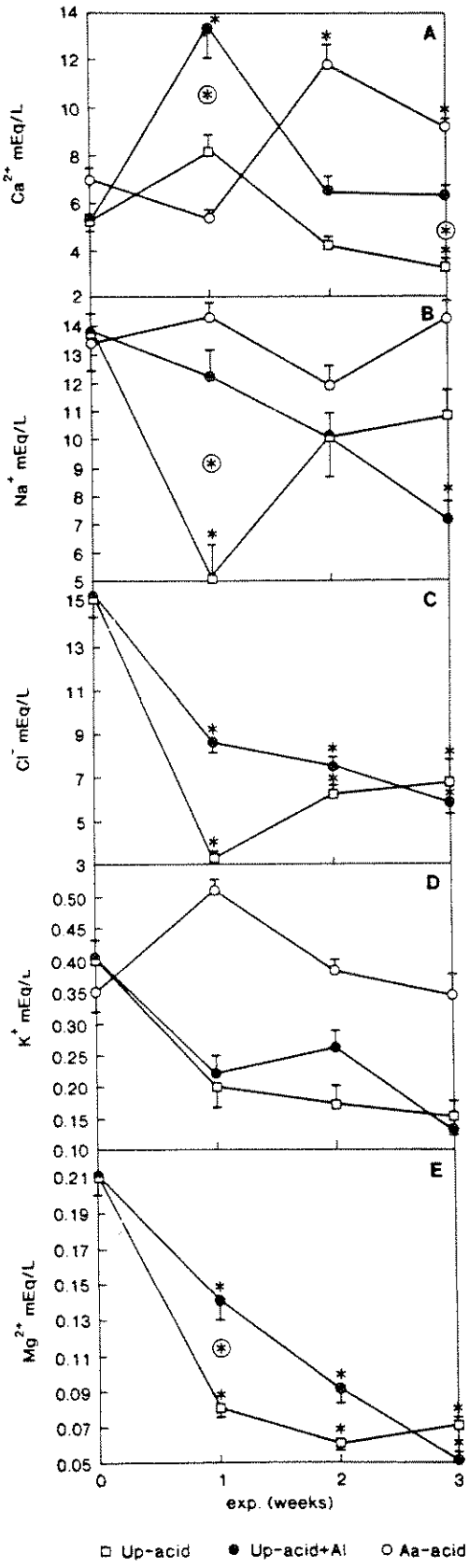
Hemolymph Na^+ and Cl^- concentrations were both decreased, $[Cl^-]$ being more affected than $[Na^+]$ (Figures 3B and C). The decrease in the Na^+ concentration in the hemolymph of *U. pictorum* was transitory following the same pattern as $[Ca^{2+}]$ increase. $[Na^+]$ and $[K^+]$ in the hemolymph of *A. anatina* (Figures 3B and D) were not significantly affected by the 3-week acid exposure.

After a recovery period of 4 weeks, specimens of *U. pictorum* exposed for 3 weeks to 300 µg Al/L at pH 4–5 showed a normalized electrolyte balance. Ca^{2+}, Na^+, Cl^-, K^+ and Mg^{2+} concentrations in the hemolymph were at the same level measured in the untreated animals (Figure 2).

Semi-static Exposures—Combinations of Water Hardness, pH, and Al

In 6 of the 8 experimental conditions, $[Ca^{2+}]$ was decreased to below the control level (Figure 4). Exceptionally, in both hard and soft water, the $[Ca^{2+}]$ increased in *A. anatina* as a result of the simultaneous low pH and Al exposure (Figure 4). The change was most clear-cut in soft water; the hemolymph $[Ca^{2+}]$ of the clams kept in soft water was about three times higher than in the control clams. In general, the Ca balance in *A. anatina* was more sensitive to these ambient factors than the Ca balance in *U. pictorum* (Table 2).

Hemolymph Na^+ and Cl^- concentrations of the two un-



ionid species were decreased in the soft water (Table 2). A decreased hemolymph Na^+ concentration was found in acid soft water (*U. pictorum*), as well as in acid hard water (*A. anatina*) (Figure 4). Acidification in hard and soft water decreased hemolymph $[Cl^-]$ both in *A. anatina* and in *U. pictorum* (Figure 4). In soft water, the Al concentration of 300 μg/L caused a decrease in the hemolymph $[Cl^-]$ of *U. pictorum*. The three external factors did not modify K^+ concentration in the hemolymph of *A. anatina*. In *U. pictorum*, in contrast, both low pH and salt depletion from the medium increased hemolymph $[K^+]$. The hemolymph Mg^{2+} concentration was generally lower in both of the species studied in soft water (Figure 4). Low water pH decreased the Mg^{2+} concentration in the hemolymph of *A. anatina*, as did Al added to the soft water (Table 2).

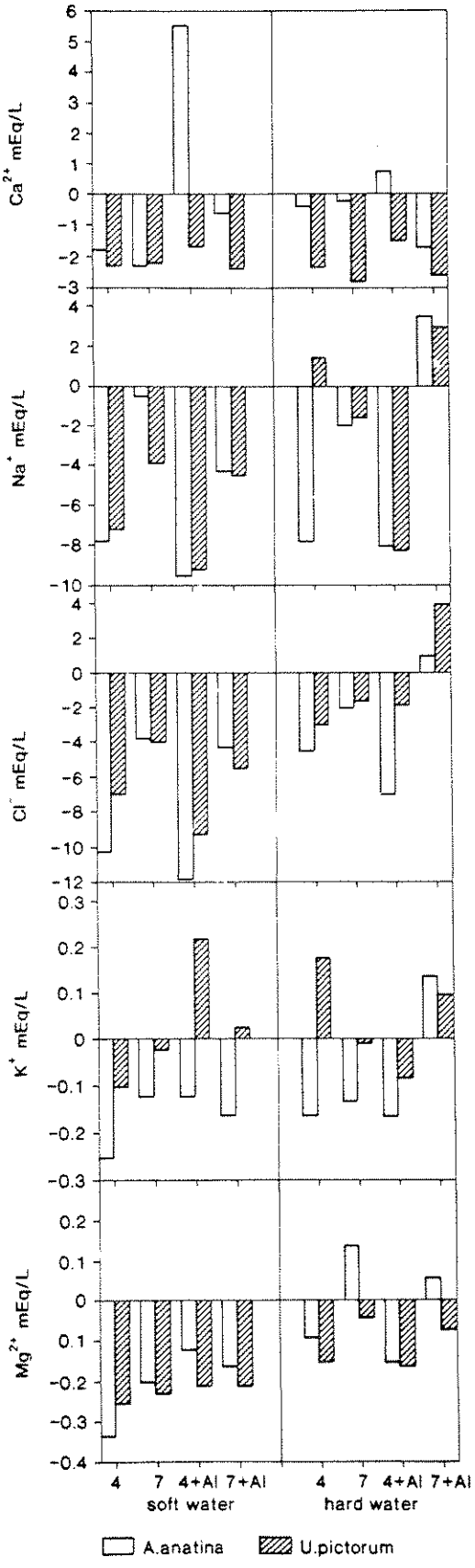
Fluctuating Exposure—Intermittent Low pH and High Al

The hemolymph $[Ca^{2+}]$ of *U. pictorum*, measured directly after an acid, Al rich (900 μg/L) pulse of 3 days, was clearly increased after each of the three pulses (Figure 5). Following each peak, the hemolymph $[Ca^{2+}]$ level was 2.5 to 3 times higher in the exposed animals than in the unexposed controls. Intermittent pulses were obviously too short to affect the hemolymph $[Na^+]$ and $[K^+]$. The increase in the hemolymph $[Ca^{2+}]$ was transitory, the Ca level returning to the control level during each recovery period of 5 days (Figure 5).

Condition of the Animals

Mortality did not occur in any of the three experiments during the experimental period. One *U. pictorum* of the group exposed for 3 weeks to pH 4–5 had a completely perforated shell umbonal region. The disturbance in the electrolyte balance of this specimen was, however, no more severe in the animals with undamaged shells. After 2 to 3 weeks of exposure at pH 4–5, loosening of the periostracum was seen in *A. anatina* specimens. CO_2 apparently liberated from the shell of $CaCO_3$ by the acid medium, accumulated under the periostracum causing local loosening of the periostracum. Mucus production was accelerated in the clams exposed to low pH or to low pH/Al. After 24 h exposure the onset of the mucus accumulation on the shell and mantle edges had already increased markedly.

Fig. 3. Hemolymph Ca^{2+} (A), Na^+ (B), Cl^- (C), K^+ (D), and Mg^{2+} (E) concentrations during a 3-week flowing, low pH (pH 4–5) and low pH + Al (300 μg/L) exposures. Open squares = *U. pictorum* exposed to acid only, closed circles = *U. pictorum* exposed to acid and Al, and open circles = *A. anatina* exposed to acid. Mean of 6 clams, ± SEM is given. Asterisks (uncircled) indicate significant differences ($P \leq 0.05$) when compared to the controls and the circled asterisks significant differences between acid- and acid/Al-exposed specimens of *U. pictorum*



Discussion

It has been demonstrated that specimens of *A. anatina* and *U. pictorum* transferred into a medium 20 times more diluted than their natural medium (50 mg Ca/L) showed a clear decline in hemolymph ions (Na^+, Cl^-, K^+, Mg^{2+}) after 2 weeks of exposure (Figure 4). In *Ligumia subrostrata*, almost identical results were presented earlier as a result of 30 days of exposure to deionized water (Murphy and Dietz 1976). In *Ligumia*, salt depletion caused an elevation of hemolymph [Ca^{2+}], which was not found in this unionids. The elevation in hemolymph Ca in the extremely diluted medium is obviously a result of metabolic or respiratory acidosis stemming from shell closure.

A general decline of hemolymph ion concentrations due to low external pH has been described earlier for fish, as well as for crustaceans. The data reported for freshwater clams in this study agree with the earlier observations made on fishes and crustaceans. The gills of the freshwater clams, which contains both HCO_3^--Cl^--ATPase and Na^+-K^+-ATPase activity, are a major site of Na^+/Cl^--uptake (Dietz and Findley 1980). The decline in hemolymph Na^+ in fish can be explained by the means of reduced gill Na^+-K^+-ATPase activity as reported earlier by Nieminen *et al.* (1982), or by the increased loss of Na^+ ions across the gill epithelium (Wood and McDonald 1982). The decline of hemolymph [Cl^-], by contrast, could be a result of a lower branchial HCO_3^-/Cl^- exchange in order to ameliorate the net base loss (Heming and Blumhagen 1988). These two mechanisms found in fish could also explain the electrolyte imbalance in the acid-stressed clams.

The data concerning the effect of Al on the survival and ion-regulation of the aquatic animals are contradictory. Both antagonistic and synergistic toxic action of acid and Al have been reported. Al exacerbates the ion-toxic effects of H^+ in fish (Muniz and Leivestad 1980; Baker and Scholfield 1982). On the other hand, Al mitigates toxic effects of acid (France and Stokes 1987). In this study, Al added to the acid water strengthened the electrolyte disturbance measured in the hemolymph of *U. pictorum* during a continuous exposure of 3 weeks. Not only low pH, but also Al, reduces Na^+-K^+-ATPase activity (Staurnes *et al.* 1984). Different combinations of water pH and hardness and the Al in circumneutral hard water had only a minor effect on the hemolymph electrolytes. It is suggested that in the pH range used, the toxic effect of H^+ dominated the possible toxic effect of Al.

Under acid stress, animals with a large reserve of easily mobilizable $CaCO_3$, such as crustaceans and molluscs, react

Fig. 4. Differences in the hemolymph electrolyte concentrations of unexposed and exposed *U. pictorum* and *A. anatina*. Hemolymph Ca^{2+}, Na^+, Cl^-, K^+,and Mg^{2+} concentrations after a 2-week exposure to acid (4), acid and Al (300 µg/L) (4 + Al), circumneutral (7) and circumneutral with Al (7 + Al) in soft or hard water (3.5 vs. 35 mg Ca/L) are given. The hemolymph electrolyte concentrations in unexposed clams are given (0-level) in Figure 1. The statistical significances of the changes in the electrolyte concentration in relation to the three ambient factors (hardness, pH and Al) are given in Table 1. In each experiment, the mean of 4 clams is used for the calculations

species reflect differences in the ion-regulatory physiology (Wood and Rogano 1986). An *Orconectes*-species, that was less acid tolerant, showed elevated hemolymph [Ca^{2+}] and declined [Na^+] following the low pH exposure, whereas in the more tolerant species, no change in hemolymph [Ca^{2+}] or [Na^+] was found. *Anodonta* and *Unio* species are reported to inhabit different types of waters (Agrell 1949). *U. pictorum* survives in waters of low alkalinity, which are highly vulnerable to atmospheric sulphur emissions. The ability of *U. pictorum* to normalize the hemolymph Ca^{2+} level soon after the onset of the acid exposure could save internal Ca^{2+} reserves needed for reproduction and shell growth. This points to a better resistance to hemolymph acidosis in *U. pictorum* than in *A. anatina*, which under acid stress continuously buffers the hemolymph with Ca^{2+} liberated from the internal Ca reserves.

Severe pH depressions in the natural waters are often of short duration. This could be advantageous for freshwater bivalves which can avoid short periods of acidification by closing the valves. Short, repetitive pulses could stress the animals more than a slight continuous pH depression. Animals in constantly fluctuating pH conditions are constantly forced to re-adapt to the new ambient situation. In the fluctuating exposure, mortality was not seen during the exposure period of 24 days. Acid, Al rich pulses (900 μg Al/L) of 3 days caused only transitory hemolymph [Ca^{2+}] elevation. Contradictory results have been reported for brook trout (Siddens *et al.* 1986) and for white suckers (Höbe and McMahon 1988). Repetitive intermittent pulses of acid water with Al were more detrimental than continuous low pH exposure.

Due to their large Ca reserves and their ability to avoid an unpleasant medium temporarily by means of shell closure, adult freshwater bivalves are relatively resistant to short-term pH depressions both in the laboratory conditions and in the field (Malley *et al.* 1988). No mortality occurred even in severe acidity in the continuous exposures of 3 weeks or as a result of several intermittent repetitive pulses. Al increases the pH toxicity, but in the concentrations of Al measured in the acidified waters the effect is only of minor importance. Different sensitivity of juveniles and young animals could explain the disappearance of unionid clams from acidified waters. The effect of the size of the adult clam and the sensitivity of the larvae (glochidia) to aluminum and acid need further investigation.

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Table 2. Probability values derived from ANOVA for the ionic composition of the hemolymph. P values are given for the water pH (4–5 vs. 6.6–8.3), hardness (35 mg vs. 3.5 mg Ca/L), Al concentration (≤70 vs. 300 μg Al/L), and the interaction of these factors. Values for $P \leq 0.05$ are printed in bold type

Unio pictorum

	Hemolymph Ca^{2+}	Na^+	Cl^-	K^+	Mg^{2+}
pH	**0.003**	0.923	**0.002**	**0.005**	0.368
Hardness	0.308	**0.000**	**0.000**	**0.000**	**0.001**
Al	0.408	0.246	0.590	0.754	0.489
pH × hardness	0.216	**0.011**	0.545	**0.004**	0.057
pH × Al	0.126	0.655	0.101	0.330	0.107
Hardness × Al	0.650	0.138	**0.005**	0.330	0.107

Anodonta anatina

	Hemolymph Ca^{2+}	Na^+	Cl^-	K^+	Mg^{2+}
pH	**0.001**	**0.000**	**0.000**	0.197	**0.000**
Hardness	0.571	0.100	**0.032**	1.000	**0.000**
Al	**0.001**	0.537	0.436	0.703	0.396
pH × hardness	**0.047**	0.912	0.736	0.703	0.052
pH × Al	0.126	0.655	0.101	0.372	0.315
Hardness × Al	**0.000**	0.181	0.879	0.259	**0.004**

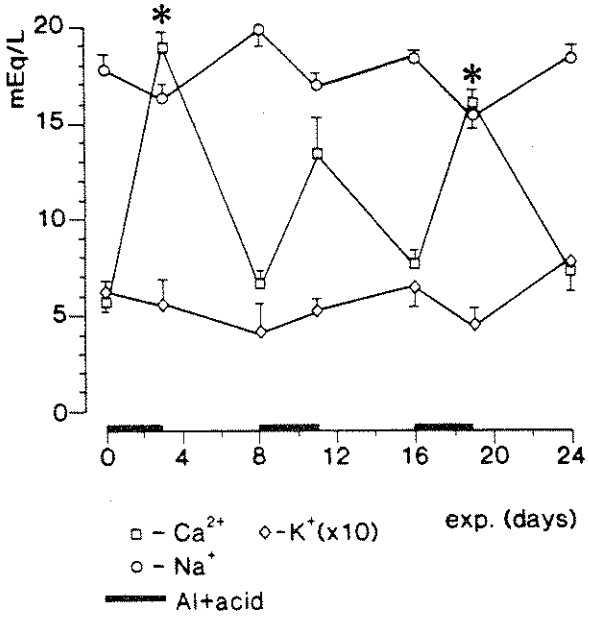

Fig. 5. Hemolymph Ca^{2+}, Na^- and K^+ of *U. pictorum* during an intermittent Al-rich (900 μg/L) exposure. The acid, Al-rich peaks are indicated by the black bars on the x-axis. The K^+ concentration is drawn in 10-fold. Mean of 6 clams, ± SEM is given. The asterisks indicate the significant differences ($P \leq 0.05$) in the electrolyte concentration of the control (I) and exposed clams. Controls I and II (unexposed animals dissected before and after the 24-day experimental period) did not differ significantly

differently in relation to their Ca balance than animals with more restricted or less easily mobilizable Ca reserves. In fish, hemolymph $[Ca^{2+}]$ tends to decrease as a result of a low pH exposure, whereas in clams (Malley *et al.* 1988) and crustaceans (Wood and Rogano 1986) acid exposure is followed by a rapid elevation of hemolymph $[Ca^{2+}]$. The origin of the hemolymph Ca^{2+} is most probably external, because an increased uptake from the medium is not obvious (Malley and Chang 1985). The effect of low pH on the Ca fluxes in the freshwater clams is under investigation.

The Ca balance in the two species of freshwater clams studied responded differently to a low external pH. In *A. anatina*, an exposure period of 2 weeks was necessary to evoke an increase in the hemolymph $[Ca^{2+}]$. The elevated Ca level was stable, pointing to a continuous release of Ca^{2+} from the internal Ca reserves. In *U. pictorum*, the Ca elevation was transitory, showing up 3 to 4 days after the onset of the exposure. When the exposure was continued, the original hemolymph $[Ca^{2+}]$ level became reestablished after 7 to 8 days of exposure. The hemolymph Ca^{2+} peak recorded during the first week of exposure was obviously due to shell closure in the acid medium. After three weeks of exposure, the hemolymph Ca^{2+} level was slightly below that of the controls. The acid stressed clams showed a tendency to compensate the decrease in the hemolymph $[Na^+]$ by an increase in $[Ca^{2+}]$.

Simultaneous exposure to acid and 300 μg Al/L exacerbated electrolyte disturbances in *U. pictorum*. Exposing the clams simultaneously to low pH and Al intensified the $[Ca^{2+}]$ increase (Figure 3). Also, the pattern of change in hemolymph $[Na^+]$ and $[Cl^-]$ was different when animals were exposed to low pH and Al than to low pH only. In both cases, hemolymph $[Cl^-]$ was affected more severely. After 3 weeks of exposure, the Na^+ and Cl^- concentration in the hemolymph were twice as high in specimens of *U. pictorum* exposed only to low pH than in the animals exposed to low pH and Al. Hemolymph Mg^{2+} and K^+ concentrations had decreased by 50% at the end of the 3-week exposure in both experimental conditions.

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