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Physiological Responses to Severe Acid Stress in Four Species of Freshwater Clams (Unionidae)

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Abstract. Four species of freshwater clam, *Anodonta anatina, A. cygnea, Unio pictorum,* and *U. tumidus* were exposed for 2 weeks to acidified soft water (pH 4.0–4.5, Ca 4.6 mg/L) and for 4 weeks to acid in hard water conditions (Ca 18.5 mg/L). The exposures caused a decrease in Na^+, K^+, and Cl^- ion and a rapid increase of Ca^{2+} in the hemolymph. The elevation of the hemolymph Ca^{2+} was positively correlated with the decrease in the hemolymph pH in all species studied. Low ambient [Ca^{2+}] level accelerated the pH decrease and Ca^{2+} increase in the hemolymph. Na^+ and Cl^- ion concentrations changed less rapidly in the soft conditions. Although there were minor changes in the mineral composition of the calcium concretions in the gills, the amount of calcium in the concretions did not change during the exposure. There was no correlation between the thickness of the shell and the ionic response, but all four species responded to low ambient pH in the same way.

Hemolymph from freshwater clams has one of the lowest solute concentrations known (Potts 1954) and 99.71% (±0.04) of the volume is reported to be water (Malley *et al.* 1988). The low ionic concentration might be due to both the high permeability of the body wall and to the low metabolic rate, which is insufficient to maintain high osmotic potential in diluted medium. In soft water, much energy is needed to maintain the osmotic balance. With a slight decrease in the ambient pH, this problem will be aggravated and a rapid loss of the hemolymph and tissue ions follows. The H^+ ions from the acidic water can intrude into the animal and cause severe acidosis.

The physiology of acid stress has been studied in the crustaceans and teleost fishes. In teleosts, acid exposure causes ionoregulatory disturbances (McDonald 1983a; Hõbe *et al.* 1984; Witters 1986) caused by a leakage of Na^+ and Cl^- ions via paracellular channels as well as by a reduction in uptake of Na^+ and Cl^-. The response of crustaceans to acid stress is similar to the response in fishes, but the higher Ca^{2+} levels in the blood change the specific nature of the response (Wood and Rogano 1986). Clams are similar to crustaceans in regard to their high Ca reserves and they mobilize Ca from the shell and mantle (Istin and Girard 1970; Silverman *et al.* 1987). Rapid release of Ca into the hemolymph and accumulation in the calcium concretions of the gills are reported as responses to metabolic acidosis (Silverman *et al.* 1983).

Fishes and crustaceans detect and avoid low ambient pH (France 1985; Pedder and Maly 1987). Clams are relatively sessile animals with restricted mobility. Their only way to avoid acidity is by closing the valves and changing to anaerobic metabolism. *Anodonta* species are able to withstand anoxic conditions for at least five days (Holwerda and Veenhof 1984), which could be sufficient to protect them against episodic pH depressions caused by snow melt and heavy rainfalls.

Økland and Økland (1986) reported a decrease in the number of mollusc species with decreasing pH. No snails were found below pH 5.2 and no clams below pH 4.7. In small freshwater gastropods, a pH of 5.5 caused impaired egg development and reduced growth (Servos *et al.* 1985), whereas at the same pH, pisidiid clams were not affected. Effects of acid and aluminum stress on the ionic balance of unionids (Malley *et al.* 1988) and effect of low pH on metal uptake (Servos *et al.* 1987; Pynnönen *et al.* 1987) have been studied. Very little is known about physiological reactions of clams in acidified waters and about differences in the sensitivity among species. Only one study (Chang *et al.* 1988) on acid stress in freshwater bivalves under laboratory conditions has been reported.

The purpose of this study was to compare the physiological responses to severe acid stress of four common freshwater unionids inhabiting European inland waters. Two different water hardnesses were used in exposures of 2 and 4 weeks. Besides the hemolymph parameters, attention was paid to the composition of the calcified concretions in the gills.

Material and Methods

Animals

Freshwater clams, *Anodonta anatina* L., *Anodonta cygnea zellensis* Gmelin, *Unio pictorum* L., and *Unio tumidus* L. were collected by SCUBA diving and by using a net from lakes and rivers in

Table 1. Dimensions of the animals used in the experiment and description of the sampling areas

Species	Shell length (age in years) mean ± SEM	Shell weight/ area (g/cm²) mean ± SEM	Ca-granules in gills (%) mean ± SEM	Sampling site (coord.)	Type of water	pH (summer)	Ca (mg/L)
Anodonta anatina	8.2 ± 0.7 (6 ± 1)	0.38 ± 0.03	53.3 ± 14.7	Lake Hiidenvesi (60°19'N, 24°8'E)	big, eutrophic lake	6.9–7.1	1.1
Anodonta cygnea	12.8 ± 1.7 (9 ± 1)	0.27 ± 0.07	45.6 ± 12.0	Lake Ekträsket (59°59'N,23°18'E)	small, oligotrophic pond (humus rich)	6.5–6.6	1.3
Unio pictorum	6.9 ± 0.5 (5 ± 1)	0.51 ± 0.05	28.3 ± 1.4	The Vantaa River (60°16'N,25°18'E)	grassland draining river (eutrophic)	6.8–7.0	28.1
Unio tumidus	7.9 ± 0.6 (14 ± 1)	0.80 ± 0.05	19.9 ± 3.3	Lake Haukkajärvi (61°16'N,27°58'E)	big, oligotrophic lake (humus rich)	6.5–6.9	3.9

Table 2. Chemical composition of laboratory tapwater measured for the hard water exposure and calculated for the soft water exposure

Parameter	Hard water	Soft water
pH, range	6.6–7.0	6.4–7.0
Chloride, mg/L	5.8	1.5
Sodium, mg/L	4.6	1.2
Potassium, mg/L	1.4	0.4
Calcium, mg/L	18.5	4.6
Magnesium, mg/L	1.6	0.4
Aluminum, mg/L	0.07	0.02
Temperature, °C	10–11	10–11
Alkalinity, mmol/L	0.62	—

Table 3. The experimental groups and conditions

Species	Duration of exposure (weeks)	pH (exposed)	pH (controls)	Water
A. cygnea	4	4.0–4.5	6.6–7.0	Hard
A. anatina	4	4.0–4.5	6.6–7.0	Hard
U. tumidus	4	4.0–4.5	6.6–7.0	Hard
A. cygnea	2	4.0–4.5	6.4–7.0	Soft
A. anatina	2	4.0–4.5	6.4–7.0	Soft
U. pictorum	2	4.0–4.5	6.4–7.0	Soft
U. tumidus	2	4.0–4.5	6.4–7.0	Soft

southern Finland in June to July, when the water temperature varied between 15 and 20°C. The *A. anatina* individuals were collected in June from Lake Hiidenvesi and *A. cygnea* in June from Lake Ekträsket. *Unio* species were collected from the Vantaa River and from Lake Haukkajärvi in July. A description of the sampling sites is given in Table 1.

The animals were kept in aquarium without substratum with continuously-flowing filtered tapwater for three weeks preceding the exposures. Chemical composition of tapwater is given in Table 2. During the acclimation period animals were not fed.

Exposure System

From June to August 1987 the clams were exposed to acid in stainless steel aquaria in static volumes of 150 L for hard water exposures and 86 L for soft water exposures. The entire water volume was changed once a week. Control and experimental tests were run simultaneously in identical light (an astronomical regimen) and temperature conditions (10–11°C). The pH was adjusted to 4.0–4.5 using 1M sulphuric acid and was measured daily, using a KCl electrode. The elevation of pH caused by the $CaCO_3$ buffering of the molluscs was corrected by adding small amounts of 1M H_2SO_4. The pH in the hardwater tests varied between 6.6 and 7.0 (tapwater) and between 6.4 and 7.0 (1:4, tapwater:distilled water). The water quality parameters are given in Table 2.

The experiments were initiated with 36 animals (18 *A. anatina* and 18 *A. cygnea*) in 150 L hard water, and 16 animals (8 *Aa* and 8 *Ac* or 8 *U. pictorum* and 8 *U. tumidus*) in 86 L soft water. Twenty-four *U. tumidus* were exposed as one group to acidic hard water. The experimental groups and the experimental conditions are described in Table 3. No deaths occurred during the exposures.

Sampling

After 1, 2, 3, and 4 weeks from the onset of the acidic exposure in hard water, and after 1 and 2 weeks in soft water, four animals from each aquarium (exposure and control) were taken for the hemolymph analysis. Animals acclimated for 3 weeks to the aquarium conditions were also sampled.

The hemolymph samples were taken from the sinus of the anterior adductor muscle (AAM) and from the pericardium (PK) using a 1 mL syringe fitted with a 22 gauge needle. The hemolymph of the individuals with a shell length less than 5 cm, was sampled only from the pericardium. The total sample volume taken from one individual was 1 to 2 mL. The hemolymph pH was measured immediately after sampling. The remainder of the sample was frozen (at −20°C) for later analysis.

Unexposed animals (animals bled after a 3-week acclimation period in the aquarium), and *A. anatina* and *A. cygnea* exposed for 4 weeks in pH 4.0–4.5, were studied for the amount of the calcium concretions in the gills and midgut gland. The gills and a piece of the midgut gland were excised and dried for 24 hr at 80°C. The tissue was weighed and 1M NaOH was added (tissue volume:NaOH volume 1:10), and the mixture was incubated at 60–70°C for at least 1 hr. After several washes with NaOH and distilled water, concretion material was separated by centrifugation (10 min at 5,000 g). The pellet was dried at 80°C to a constant dry weight.

The weight, length, and breadth (measured at the umbo region) of the shell were determined from the unexposed animals and from animals exposed for 4 weeks in hard acid water. The shell index was calculated as follows:

$$i = \frac{\text{shell weight (g)}}{\text{shell length (cm)} * \text{shell breadth (cm)}}$$

Chemical Analysis

The hemolymph pH was measured at room temperature with a Radiometer BMS3 Mk2 pHM 72 system thermostated at 22°C, with a total delay time of 50 sec (sampling 20 sec + delayed hold 30 sec). The Na^+ and K^+ concentrations were determined simultaneously from diluted samples with an EEL flame photometer, Cl^- concentrations, using a Radiometer CMT 10 chloride titrator and Ca^{2+} concentration by colorimetric method for calcium (WAKO test kit B 997-21809). The mineral composition of the gill calcium concretions was determined by the induced coupled plasma (ICP) method from samples dissolved in HNO_3 and diluted 1 to 10 in triple-distilled water.

Statistical Analysis

The differences in hemolymph pH and ionic composition, as well as the differences in the mineral composition of the gill calcium concretions, were tested for significance using the Student's t-test, or Cochran method when sample variances were unequal. The control (pH 6–7) clams were tested against the acid exposed (pH 4.0–4.5) animals. The hemolymph parameters of the control animals were tested against those of animals sampled after a 3-week acclimation in the aquarium to examine the possible influence of the test arrangement on the condition of the animals. For Cl^- values lower than the detection limit (3 meq/L), 3 meq/L was used for calculations.

Results

There was no difference between the pH of the hemolymph samples taken from the AAM or the pericardium of *Unio* sp.. But for the aquarium acclimated *A. anatina* and *A. cygnea*, the pH of the pericardium sample (*A.a.* 7.90 ± 0.18, *A.c.* 8.02 ± 0.09) was higher than the pH of the AAM sample (*A.a.* 7.81 ± 0.17, *A.c.* 7.95 ± 0.13). The difference was, however, not significant ($P \leq 0.05$). The hemolymph of the aquarium acclimated *Unio* individuals was above 8.0 (*U. pictorum* 8.03–8.24, *U. tumidus* 8.03–8.20), whereas in *A. anatina* it varied between 7.67 and 8.02, and in *A. cygnea* between 7.67 and 7.78, respectively. This difference in pH could be explained by the difference in the hemolymph Ca^{2+} concentration, which was highest in *U. tumidus* and lowest in *A. anatina* (see Figure 1). When compared to the hemolymph taken from the clams before the experiments (kept for 3 weeks in the aquarium), the hemolymph pH of the *A. cygnea* changed markedly during the 4 week aquarium exposure in circumneutral pH (Table 4). In other species, the hemolymph pH remained constant during this period.

The Cl^- concentrations did not differ between the species, while K^+ concentrations were highest in *Unio* species. The lowest Na^+ concentrations after a 3-week aquarium acclimation were measured in *U. tumidus*, but during the exposure the concentration increased significantly in the control animals and reached the same level as the other species (Figure 1).

Of the four bivalve species, *U. tumidus* had the thickest shells (index 0.80 ± 0.07 g/cm²) and *A. cygnea* the thinnest (index 0.27 ± 0.07 g/cm²). The relationship between shell weight and shell length did not change during exposure. The amounts of the calcium granules (% of the dry weight of the gills, Table 1) were typical for these species (Pynnönen *et al.* 1987). Measurable amounts of calcium concretions were found only in the gills.

A decrease in the hemolymph pH of *A. anatina* was noted after 4 weeks of acid exposure in hard water, but due to the wide variation among the individuals the change was not significant (Table 4). In *A. cygnea*, the difference in hemolymph pH between the controls and acid exposed animals was significant (Table 4), but during the exposure the pH also changed in the control animals. The hemolymph pH of *U. tumidus* decreased significantly only after 2 weeks exposure in the hard acid water, but at the end of the 4 weeks exposure the difference between the acid exposed and control clams was not significant (Table 4). In soft acid water, the hemolymph pH declined significantly only in *A. anatina* (Table 5).

After 2 weeks of exposure in acid soft water, the calcium concentration in the hemolymph was 3 times greater in *Anodonta anatina*, *A. cygnea* and *Unio tumidus*, and 4 times greater in *U. pictorum* (Figure 1). The elevation of hemolymph calcium was slower in hard water (Figure 2), and an exposure time of 3 weeks was necessary to cause a significant increase in Ca concentration in *Anodonta* sp.. In *U. tumidus*, no significant [Ca_2^+] increase was registered in acid hard water during 4 weeks of exposure (Figure 3). The correlation between the calcium increase and pH decrease in the hemolymph was highly significant ($P \leq 0.001$) in *U. tumidus* exposed in acid soft water and *A. anatina* exposed in acid hard water (Figure 4). For *A. cygnea* and *U. tumidus* exposed in acid hard water and *A. anatina* exposed in acid soft water, no correlation was seen (Figure 4).

In acid soft water, the *Unio* species reacted with a significant decrease of hemolymph Na^+ and Cl^- ion concentrations after 2 weeks of exposure. *A. anatina* and *A. cygnea* did not show any ionic response after 2 weeks exposure under soft conditions, but they responded after only one week of exposure in acid hard water. K^+ concentration varied greatly between the individuals within the same group, which resulted in a significant decrease after 4 weeks of exposure only in *A. cygnea* (Figure 5).

No change in *Anodonta* species in the amount of calcium concretions in the gills (% of dry weight) was seen after 4 weeks of acid hard exposure. The granule composition, however, changed slightly (Table 6). The change in silicon level was significant in both species. Although a slight elevation was seen in Mn, Fe, Mg, K, Al, Cu and Cd concentrations in *A. cygnea* and in Mg, Zn, and Cd concentrations in *A. anatina*, the changes were not significant. None of the species possessed measurable amounts of granules in the midgut gland.

Discussion

A negative correlation between hemolymph pH and Ca^{2+} concentration has been shown in the freshwater unionids. The increase in hemolymph [Ca^{2+}] as a result of acid exposure could be due to two factors: a metabolic acidosis caused by anaerobic metabolism due to valve closure or di-

Table 4. The hemolymph pH of AAM-samples from aquarium acclimated (for 3 weeks) and acid exposed *A. anatina*, *A. cygnea*, and *U. tumidus* (pH 4.0–4.5, in hard water, Ca 18.5 mg/L). Controls were exposed in pH 6.6–7.0. Mean (± SEM) of 4 individuals is given

	A. anatina	*A. cygnea*	*U. tumidus*
Acclimated	7.83 ± 0.08	7.70 ± 0.03	8.11 ± 0.08
1 week exposed	7.84 ± 0.10	7.92 ± 0.03	7.82 ± 0.13
control	7.83 ± 0.06	7.83 ± 0.04	—
2 week exposed	7.81 ± 0.08	7.88 ± 0.10	7.80 ± 0.10*
control	7.97 ± 0.05	7.91 ± 0.04	8.07 ± 0.03
3 week exposed	7.60 ± 0.06	7.98 ± 0.05	7.90 ± 0.12
control	7.75 ± 0.11	7.84 ± 0.07	—
4 week exposed	7.65 ± 0.05	7.69 ± 0.06[a]	7.95 ± 0.21
control	7.86 ± 0.11	8.05 ± 0.06	8.01 ± 0.06

[a] Significantly different from the control (t-test, $p \leq 0.05$)

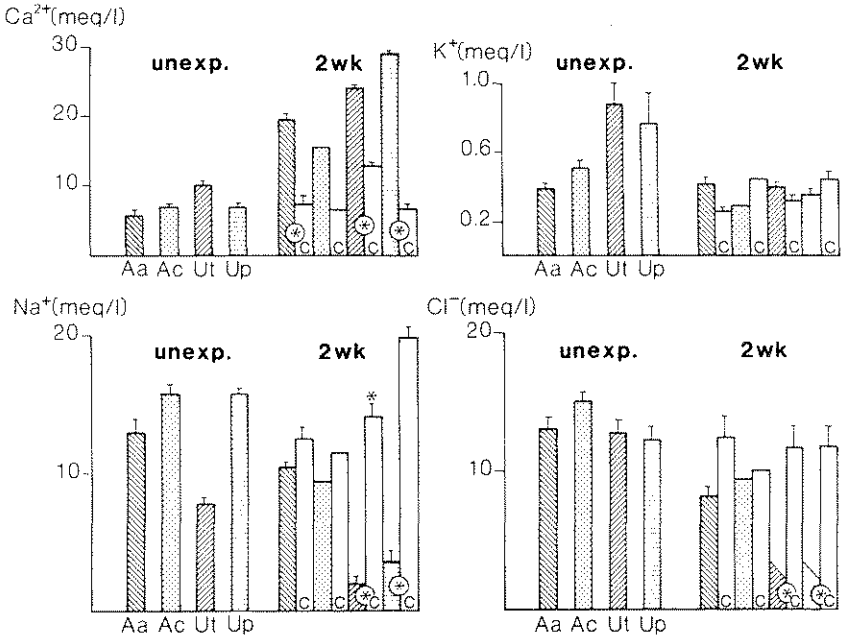

Fig. 1. The hemolymph Ca^{2+}, K^+, Na^+, and Cl^- concentrations (meq/L) of *A. anatina* (Aa). *A. cygnea* (Ac), *U. pictorum* (Up) and *U. tumidus* aquarium acclimated (unexp.) and exposed for 2 weeks (2 wk). The animals were exposed in the soft conditions (Ca 4.6 mg/L). White bars represent the controls (c) exposed simultaneously to circumneutral (pH 6.4–7.0) pH. Cut bars indicate values below the detection limit (less than 3 meq Cl^-/L). Data are means of 4 animals, ± SEM (*U. pictorum*) and 2 animals (*A. cygnea*). Asterisks between bars indicate a significant difference between the controls and the exposed animals, while asterisks above the bar value significantly different from the aquarium acclimated animals.

Table 5. The hemolymph pH of the AAM-samples from aquarium acclimated (for 3 weeks), in acid (4.0–4.5) and in circumneutral (6.4–7.0) exposed *A. anatina*, *A cygnea*, *U. pictorum*, and *U. tumidus* (soft water, Ca 4.6 mg/L). Mean (± SEM) of 4 individuals is given for all species except *A. cygnea* (n = 2), controls and exposed

	Acclimated	2 Weeks exposed	2 Weeks control
A. anatina	7.83 ± 0.08	7.56 ± 0.03[a]	7.66 ± 0.01
A. cygnea	7.70 ± 0.03	7.68	7.67
U. pictorum	8.12 ± 0.04	7.78 ± 0.16	8.11 ± 0.11
U. tumidus	8.11 ± 0.04	7.87 ± 0.12	8.07 ± 0.12

[a] Significantly different from the control (t-test, $P \leq 0.05$)

rectly by the influx of H^+ from the environment. Bivalves react to noxious environmental conditions (such as heavy metals) by valve closure (Doherty *et al.* 1987). Similarly, animals exposed to acid might close their valves and use anaerobic metabolism. This would cause an accumulation of acid metabolites and lowered hemolymph pH as reported for the marine carpet shell clam, *Venerupis decussata* during acid exposure (Bamber 1987). Alternatively, H^+ ions diffusing through the epithelium could directly cause a decrease in the hemolymph pH. It is possible that both factors contribute simultaneously to lower hemolymph pH and increase hemolymph $[Ca^{2+}]$.

The origin of the increased Ca^{2+} in the hemolymph could be internal or external. But because preliminary results show that the Ca uptake from the soft water is reduced below 7 in *Unio pictorum* and the experiments on crustaceans show the same effect at pH 5.5 (Malley and Chang 1985), the external origin is less likely. Freshwater bivalves

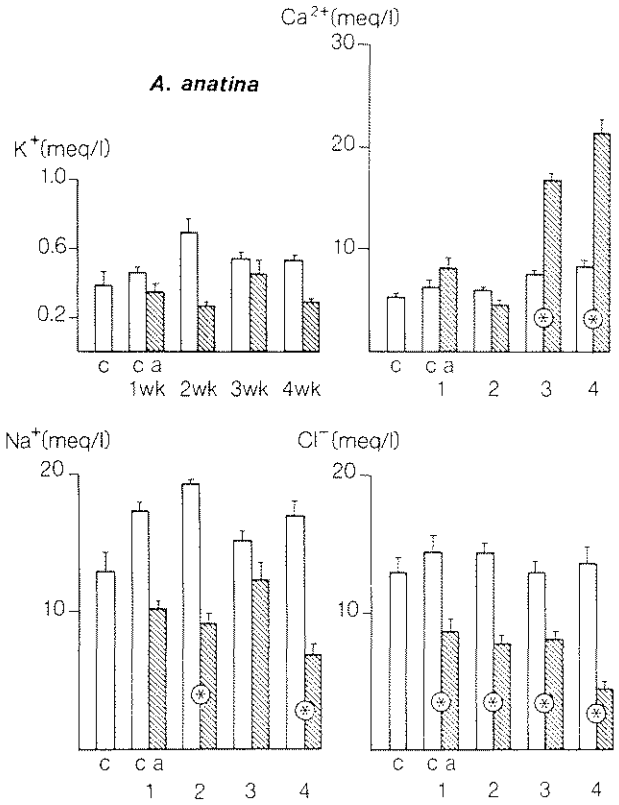

Fig. 2. The hemolymph K^+, Ca^{2+}, Na^+, and Cl^- concentrations (meq/L) in circumneutral (c) (pH 6.6–7.0) and acid (a) (1, 2, and 3 weeks in pH 4.0–4.5) exposed *A. anatina*. The animals were exposed in hard water (Ca 18.5 mg/L). White bars represent the controls, hatched bars the acid exposed clams. The first white bar gives the ionic composition of the hemolymph of aquarium acclimated animals and the white bars next to the hatched bars the hemolymph ionic concentrations of the controls. Data are means of 4 animals, ± SEM. Asterisks indicate a significant difference between the controls and the exposed animals.

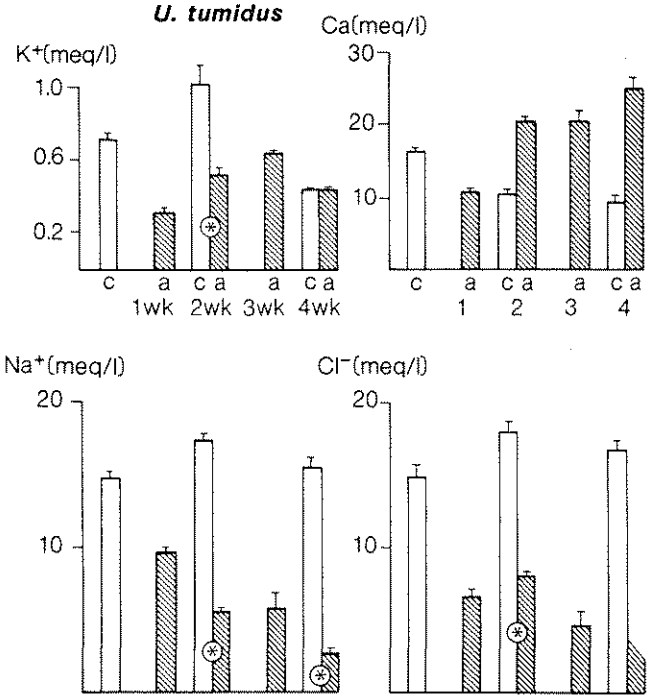

Fig. 3. The hemolymph K^+, Ca^{2+}, Na^+, and Cl^- concentrations (meq/L) in circumneutral (c) (pH 6.6–7.0) and acid (a) (1, 2, 3 weeks in pH 4.0–4.5) hard water exposed *U. tumidus*. Other details as in Fig. 2.

possess large $CaCO_3$ reserves in their gills, mantle and shell. In the mantle and shell, they are often in the form of calcified granules. According to Silverman *et al.* (1983), under hypoxic conditions, the amount of gill concretions as well as their Ca concentration is positively correlated with the increase of hemolymph $[Ca^{2+}]$. In this study, no significant change was measured in the concretion amount or in their Ca concentration. The changes in the granule composition were of minor importance, which matches with the results reported earlier by Silverman *et al.* (1987) on their role in the glochidial shell formation. Chronic exposure or repeated acid pulses might, however, lead to different results. These observations confirm that Ca is not liberated from the gill $CaCO_3$. Calcium, therefore, most likely originates from the shell and mantle reserves.

During exposure, the Na^+ and Cl^- concentrations decrease significantly after only one week exposure in acid water. The hemolymph of freshwater unionids is very dilute in both mineral solutes as in the amino acids which play a minor role in maintaining the osmotic balance in marine bivalves (Potts 1954). The strong ionic concentration of $Ca^{2+} + Na^+ + Cl^-$ was kept constant during the acid exposure, maintaining the electrolyte balance. The decrease of hemolymph $[Na^+]$ and $[Cl^-]$ were compensated for by the increase in hemolymph $[Ca^{2+}]$. If the excess of hemolymph Ca^{2+} originated from the internal $CaCO_3$ reserves, a simultaneous excess of HCO_3^- can be expected. The K^+ level, important to normal cellular function, was relatively constant during the exposure.

Salt depletion caused a decline of hemolymph solutes of *Ligumia* (Murphy and Dietz 1976). $[Ca^{2+}]$ and $[HCO_3^-]$ increased while $[Na^+]$ and $[Cl^-]$ decreased in the deionized water. Since the *Anodonta* and *Unio* species used in this experiment came from soft water, it is not surprising that 2 week exposure to soft water caused no change in hemolymph Ca^{2+}. Agrell (1949) showed that the thickness of the shell in *Unio* species, in contrast to *Anodonta* species, does not increase with a rise in the trophic level and Ca content in the water. This observation points to the possible differences in Ca metabolism.

According to the results presented here, adult animals can stand severe acidification in hard water for as long as 4 weeks. Not even under severe acid stress is the ionic balance disturbance lethal. A prolonged exposure will, however, damage the periostracum which leads to microbial contamination (Kat 1972). Undamaged periostracum offers a good barrier against the destructive effect of the acid. In the *Anodonta cygnea* individuals collected for the experiments, the periostracum in the umbonal region was worn off by the mechanical action of the coarse sediment. Due to the poor water buffering capacity in Lake Ekträsket ($[Ca^{2+}] \leq 2$ mg/L), the shells of the clams were also extremely thin. In none of the 4 species were the shells of exposed animals lighter than the shells of the control ones.

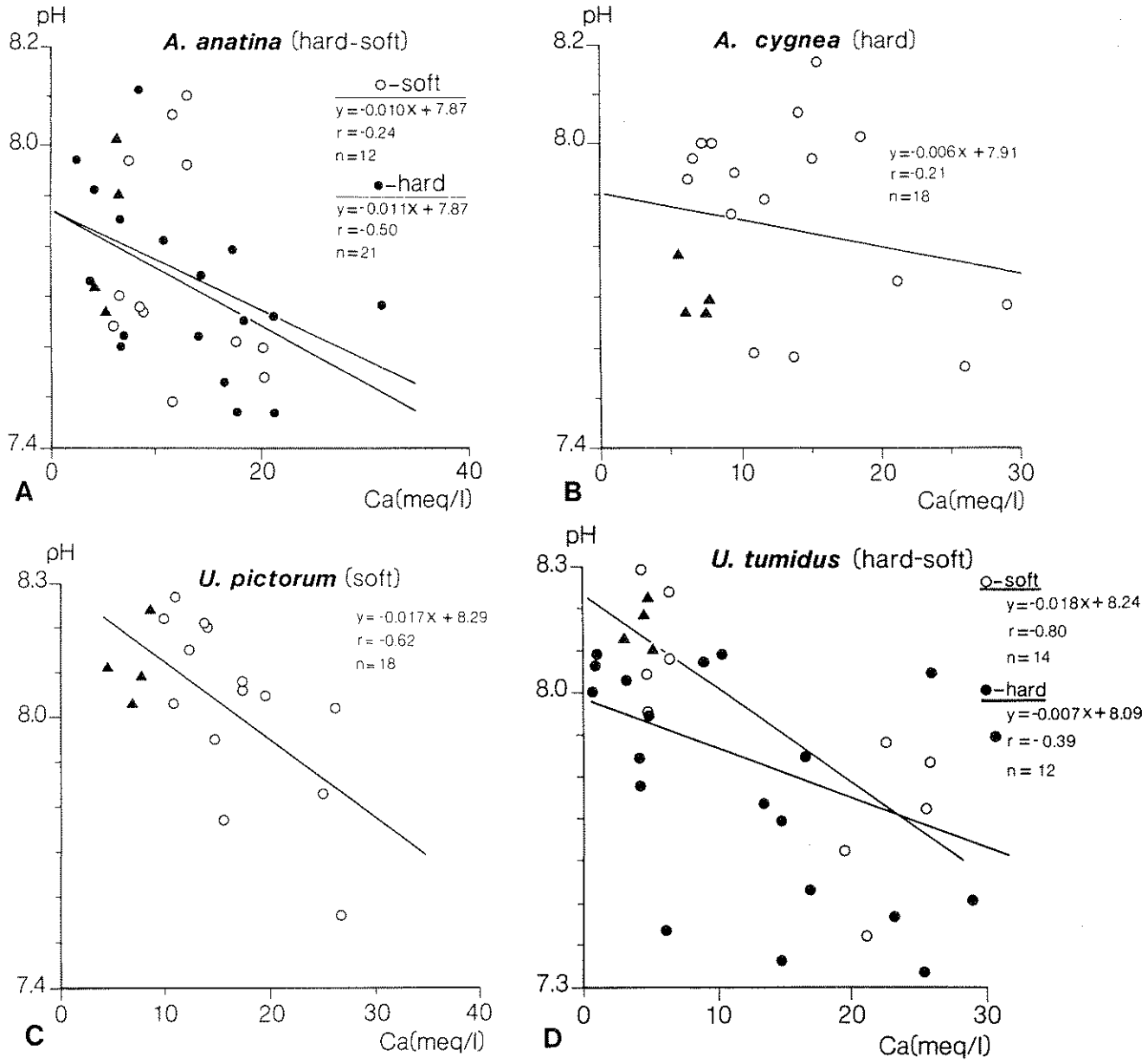

Fig. 4. The linear regression relationships between the hemolymph pH and Ca^{2+} concentration in **A,** *A. anatina,* **B,** *A. cygnea,* **C,** *U. pictorum,* and **D,** *U. tumidus*. All data from the hard and soft water exposure were used. The points given by triangles indicate the data from the aquarium acclimated (for 3 weeks) animals.

Suffocation due to the excessive mucus production by the gills can lead to death in acid exposed fishes. The threshold pH for mucus production is 5.6 (Daye and Garside 1976). Excess mucus produced by gills works as a barrier to O_2 uptake. Together with a decreased ventilation rate, it could lead to hypoxia induced acidosis, which can be measured as a pH decrease in the hemolymph, accompanied by the increase of the hemolymph $[Ca^{2+}]$ in molluscs and crustaceans. However, during short periods of acidification, freshwater clams can effectively be protected from suffocation by anaerobic metabolism.

The process of reproduction could be the most sensitive to acidification. Developing embryos of the freshwater bivalves are not in direct contact with the outside (Silverman *et al.* 1987), and therefore are protected against short pH-depressions. Mature glochidia will, by contrast, be exposed to acid run-offs when they are released into the water in order to attach to a fish host. The effect of acidification on the viability of free glochidia is currently under investigation.

Under acid stress, clams respond similar to crustaceans than to fish. Both clams and crustaceans release Ca into the environment suggesting carapace (Wood and Rogano 1986) or shell as the origin of the buffering material ($CaCO_3$). The fish gill is more permeable to H^+ ions in soft water than in the hard water (McDonald 1983b; McDonald *et al.* 1983). Also in *A. anatina* the hemolymph pH decreased more rapidly in the soft water, which points to an increased influx of

Table 6. Composition (μg/g dry concretion) of the calcium concretions isolated from the gills of aquarium acclimated (for 3 weeks) and for 4 weeks in pH 4.0–4.5 exposed individuals of *A. anatina* and *A. cygnea*. Values represent the mean (± SEM) of four (aquarium acclimated) or five (exposed) individuals

Component	*A. anatina* acclimated	*A. anatina* exposed	*A. cygnea* acclimated	*A. cygnea* exposed
Ca	262 233 ± 7 270	264 205 ± 6 856	270 305 ± 28 051	262 590 ± 13 569
Mn	29 302 ± 3 537	27 778 ± 4 347	46 416 ± 4 025	39 780 ± 4 347
Fe	5 088 ± 688	4 403 ± 634	14 809 ± 4 646	2 990 ± 494
Mg	3 925 ± 236	3 110 ± 70	3 415 ± 685	2 462 ± 177
K	1 855 ± 422	1 789 ± 193	3 756 ± 1 726	1 093 ± 113
Zn	1 390 ± 131	875 ± 58	981 ± 181	584 ± 47
Si	857 ± 58	559 ± 35	1 034 ± 146	561 ± 53
Al	345 ± 44	372 ± 66	442 ± 157	242 ± 35
Cu	41 ± 5	75 ± 14	71 ± 25	53 ± 19
Cd	25 ± 13	9 ± 1	17 ± 3	7 ± 2

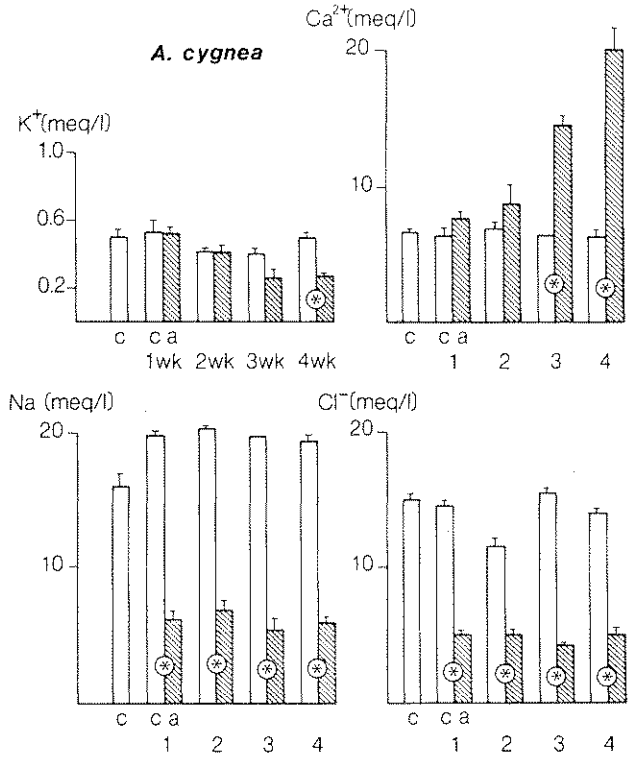

Fig. 5. The hemolymph K^+, Ca^{2+}, Na^+, and Cl^- concentrations (meq/L) in circumneutral (c) (pH 6.6–7.0) and acid (a) (1, 2, 3 weeks in pH 4.0–4.5) hard water exposed *A. cygnea*. Other details as in Fig. 2.

H^+ ions in the soft medium. In *A. cygnea*, however, the external Ca concentration did not affect the intensity of acidosis.

If Ca, which helps to keep junctional impermeability, is removed from the external medium, the Na^+ efflux is increased ten times in goldfish (Eddy and Bath 1979). In contrast, in *Anodonta* species, hemolymph Na^+ and Cl^- did not change in acid soft water, but were decreased by 50% in acid hard water. Similarly, the more rapid decrease of Na^+ when compared to Cl^- in acid hard water observed in crayfish (Wood and Rogano 1986) was not apparent in clams. The soft water response in crustaceans and in *Anodonta* resembles the hard water response in fish. Fishes are obviously more dependent on external Ca and HCO_3^- as buffering agents, while clams and crayfishes possess rapidly mobilizable $CaCO_3$ reserves in the shell and carapace.

The reason for fish mortality in the acidified water is usually suffocation due to excessive mucus production or a severe ionoregulatory disturbance (McDonald 1983a). In crustaceans, failures in moulting and reproduction explain their elimination from acid water (Malley and Chang 1985). The large freshwater clams proved rather insensitive to severe acidification. Ionoregulatory disturbance occurred, but it did not lead to death. Due to their detoxification mechanisms, adult clams are resistant to the heavy metals (Hemelraad *et al.* 1986). These results indicate that the reason for their disappearance from the acidified waters might be due to reproductive failure.

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