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Effect of acidic conditions on cadmium kinetics and electrolyte balance in the freshwater clam *Unio pictorum*

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Accumulation of cadmium by a unionid freshwater clam, *Unio pictorum*, was studied under acidic (pH 4.0–5.0) and circumneutral (pH 8.0–8.3) conditions. Cd uptake in all organs studied (gills, kidney, mantle, midgut gland, the rest, and total soft parts) was lower in the acidic medium than in the neutral medium. After two weeks of exposure the effect was significant. After four weeks, the Cd concentration in most organs of *Unio pictorum* was three times higher in the specimens in the neutral medium than in those in the acidic medium. Shells and calcium concretions bound less Cd when clams were exposed in acidic conditions. The rate of elimination in the acidic medium was not significantly higher than that in the neutral medium.

Acid exposure did not affect the amount of calcified concretions in the clams. Therefore, most of the calcium needed for buffering was probably mobilised from the shell and mantle. Exposure to acid alone for 10 days caused a transitory increase in haemolymph calcium concentration, while a Cd exposure of 3 weeks in both a neutral and an acidic medium decreased haemolymph Ca^{2+} concentration. Haemolymph Na^{+} and K^{+} concentrations were not influenced during 4 weeks of exposure.

1. Introduction

Increased sulphur dioxide emissions during the last few decades have markedly changed fresh water chemistry as well as the community structure in large areas of the northern hemisphere (Kenttämies et al. 1985, Økland & Økland 1986). In addition to lowering pH, acid rain and snowfall lead to leaching of metals from the watershed, which can cause an overall elevation of metals in lake water (Schindler et al. 1980). The impact of acidification upon the aquatic environment is

greatest in certain areas of Scandinavia and North America, where poorly buffered watersheds are common. Increase in the H^+ ions and heavy metal concentrations in small lakes can cause severe physiological stress to aquatic organisms.

Acidification can change metal-organism interactions in different ways by affecting metal speciation and availability or by affecting biological sensitivity at the level of the cell surface (Campbell & Stokes 1985). Experiments with algae (Peterson et al. 1984) and fish (Cusimano et al. 1986) indicate that H^+ may alter cadmium toxicity. Laurén & McDonald (1986) observed that a pH decrease from neutral to pH 5.0 reduced copper uptake by about 50 % in *Salmo gairdneri*. Acidified water may also change behavioural activity (Scherer et al. 1986, Gunn et al. 1987), and affect sensitivity to metals and metal accumulation. Graney et al. (1984) observed that lowering the pH from 7.8 to 5.0 reduced Cd uptake at 21 °C in the Asiatic clam, *Corbicula fluminea*, although the effect was only minor at 9 °C. In the acidified field situation during the snowmelt runoffs, no significant bioaccumulation of Cd, Zn or Al was found in the bivalve *Elliptio complanata* (Servos et al. 1987).

Sensitivity of molluscs to acidification varies among species, depending on the structure and composition of the shell (Kat 1982). Shell dissolution, leading to microbial attack (Kat 1982), and impaired reproduction (Servos et al. 1985) have been suggested as reasons for absence of molluscs in acidified waters.

Large calcium carbonate and calcium phosphate reserves located in the shell and calcium concretions (Silverman et al. 1983, Pynnönen et al. 1987) give the unionids a good buffering capacity. These, together with a welldeveloped anoxic metabolism (Holwerda & Veenhof 1984), help freshwater unionids to withstand short periods of severe acidification. Malley et al. (1988) report that exposure to acid and aluminium in the field caused a transitory increase in the haemolymph Ca^{2+} concentration.

In recent studies on the kinetics of cadmium accumulation, *Anodonta* and *Unio* sp. accumulated Cd in high tissue concentrations (Hemelraad et al. 1986a, b). The role of calcified concretions in Cd sequestration in neutral conditions has been studied (Pynnönen et al. 1987), and found to be of minor importance. Also the accumulation kinetics of Al in the unionids under acidified conditions has been investigated recently (Pynnönen 1990b). In the present study, the effect of acidity on the accumulation and elimination of Cd in *Unio pictorum* was studied. In addition, the effects of Cd and Cd/low pH exposures on the electrolyte homeostasis and gill calcium concretions are reported.

2. Materials and methods

2.1. Animals

Specimens of *Unio pictorum* L. were collected in June from the Haringvliet river area (tributaries of the Rhine) south of Rotterdam. Clams from the Haringvliet area are known to contain reasonably high metal concentrations in their tissues (Hemelraad 1988). The animals were stored in aquaria with a sand substrate in running tapwater for 3 months prior to the onset of the Cd exposure. It was assumed that during the storage in the unspiked tapwater some of the accumulated Cd was eliminated. The clams were not fed during storage or the experimental period.

2.2. Exposure system

Clams were exposed to $CdCl_2$ in glass aquaria at a nominal concentration of 50 µg/l (Fig. 1). A mixture of tapwater and $CdCl_2$ solution were supplied with pumps at rates of 7 l/h in the neutral (Experimental tank 1) and acidified (Experimental tank 2) medium. The pH of the acidified medium was kept between 4 and 4.5 by adding concentrated H_2SO_4 to the stock tank.

The nominal conditions for the exposure groups were (experimental tanks are given in parentheses):

A. 42 clams exposed to 50 µg/l Cd, pH 4.0–4.5 (Tank 1)
B. 42 clams exposed to 50 µg/l Cd, pH 8.0–8.3 (Tank 2)

After exposure, both groups A and B were divided into two equal subgroups (A_1, A_2, and B_1, B_2) and relocated in Tanks 1 and 2 for elimination of Cd as follows:

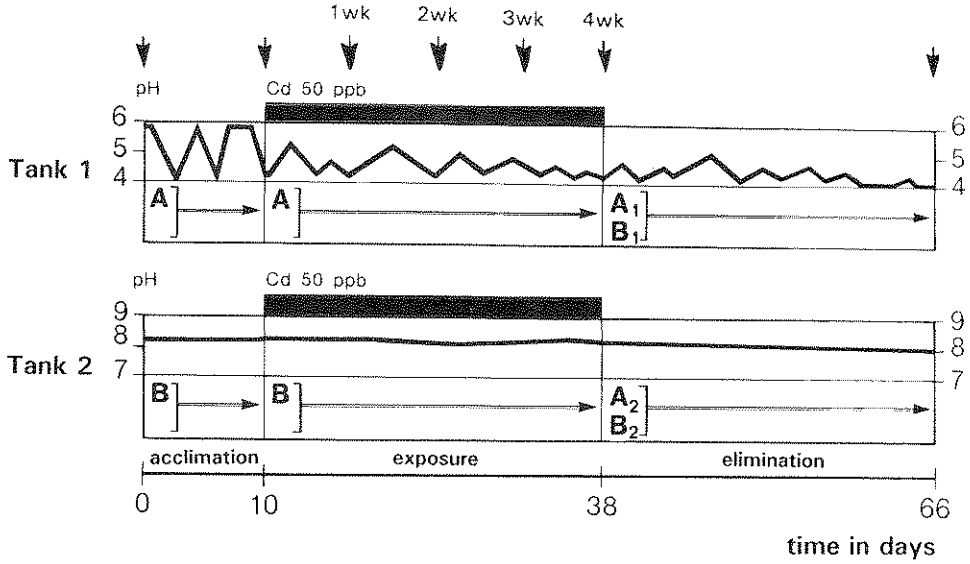

Fig. 1. Schematic representation of the experiment. The measured pH values and Cd concentrations (in µg/l = ppb) are given in Table 2 . A and B are groups comprising 42 *Unio pictorum* each. A_1, A_2, B_1 and B_2 are elimination groups. Further explanation given in the text. Arrows give the sampling times.

A_1 18 clams, elimination at pH 4.0-4.5 (Tank 1)
A_2 18 clams, elimination at pH 8.0-8.3 (Tank 2)
B_1 18 clams, elimination at pH 4.0-4.5 (Tank 1)
B_2 18 clams, elimination at pH 8.0-8.3 (Tank 2)

Water quality parameters are given in Table 1, and the measured pH and Cd concentrations in the aquaria during the exposure and the elimination periods in Table 2. Before exposure to Cd, animals to be exposed to Cd at low pH were acclimated to low pH for 10 days. Altogether 84 *Unio pictorum* were exposed for 4 weeks to 50 µg/l Cd, either at pH 8.0-8.3 or at pH 4.0-4.5 in aquaria of 150 l. The temperature was kept between 11 and 12°C. During the exposure, 24 specimens from both experimental tanks were sampled.

Cd concentration in the water was measured daily by atomic absorption spectrophotometer (AAS). The instrument (Instrumentation Laboratory, type IL 451) was equipped with a deuterium lamp for background correction. The pH was determined by KCl-electrode pH-meter (Philips PW9414 digital ion activity meter).

Table 1. Properties and composition of the tap water used in the experiments.

	Minimum	Maximum
Total hardness, mmol/l	0.84	1.45
Chloride, mg/l	14	26
Bicarbonate, mg/l	95	115
Sodium, mg/l	11	20
Potassium, mg/l	0.7	0.7
Phosphate, mg/l	0.07	0.25
Calcium, mg/l	29	52
Iron, mg/l	0.1	0.1
Magnesium, mg/l	3	4
Zinc, µg/l	10	10
Cadmium, µg/l	0.1	0.1
Copper, µg/l	Not detectable	
pH	8.0	8.3

2.3. Sampling

During the exposure period, tissue samples for Cd analysis were taken weekly from the gills, kidney, mantle and midgut gland separately from 6 specimens of *Unio pictorum*. The remaining tissue was handled as one fraction, called "the rest". The total body burden of Cd in the soft parts of the animals is calculated and given as "soft parts". After the

Table 2. Water cadmium (µg Cd/l) and pH levels during acclimation, exposure to 50 µg Cd/l (weeks 1–4), and elimination (weeks 5–8), and mortality, during experiments 1 and 2. Means ± *SE* of the Cd concentration and pH in experimental tank 1 are calculated from 6–7 measurements per week. pH in experimental tank 2 was measured once a week. NM = not measured.

		Cd in exp. 1	Cd in exp. 2.	pH in exp. 1	pH in exp. 2	Mortality
Acclimation		0.1	0.1	5.4 ± 0.4	8.3	1, 1
Exposure	1st week	34.7 ± 2.3	47.8 ± 1.3	4.8 ± 0.3	8.2	—
	2nd week	49.8 ± 1.3	47.3 ± 1.2	4.7 ± 0.2	8.3	—
	3rd week	51.0 ± 2.4	50.4 ± 2.1	4.4 ± 0.1	8.2	—
	4th week	47.0 ± 3.1	45.8 ± 1.4	4.2 ± 0.1	8.1	1, 1
Elimination	5th week	16.5 ± 5.2	15.5 ± 4.5	4.6 ± 0.3	NM	—
	6th week	2.5 ± 1.0	2.3 ± 1.0	4.3 ± 0.1	NM	—
	7th week	Not detectable		4.0 ± 0.1	NM	—
	8th week	Not detectable		4.0 ± 0.1	NM	—

4-week elimination period, tissue samples (gills, kidney, mantle and midgut gland) were taken from each elimination group (A_1, A_2, B_1, B_2). Unexposed animals and the animals acclimated to pH 4-6 for 10 days were analysed for their Cd concentration.

Haemolymph samples were taken from the sinus of the anterior adductor muscle (unexposed, low pH acclimated, groups A and B), prior to organ dissection, using a syringe fitted with a 22 gauge needle. Clams in the elimination groups (A_1, A_2, B_1, B_2) were not sampled. Mucus excreted by animals exposed to Cd (in acid) was collected for Cd analysis. Tissue samples and mucus were lyophilized and decomposed in concentrated HNO_3 according to the method of Hemelraad et al. (1986a).

For analysis of calcium concretions, tissue samples were taken from the gills, mantle and midgut gland from 4 unexposed *Unio pictorum*, specimens acclimated to pH 4–5 for 10 days, and specimens exposed for 4 weeks to 50 µg Cd/l at pH 4.0–4.5 (group A) and to 50 µg Cd/l at pH 8.0–8.3 (group B). Concretions were isolated from weighed, lyophilized tissue samples of gills, mantle and midgut gland using the method described in Pynnönen et al. (1987). Concretions were decomposed in HNO_3 for Cd and Ca analysis. Shells from non-exposed animals and from animals exposed for 4 weeks to Cd (groups A and B) were collected for Cd analysis. Shells were decomposed in HNO_3.

2.4. Metal and ion analysis

Cd (from organs, haemolymph, shells and calcium concretions of the gills) and Ca (from calcium concretions) were determined by atomic absorption spectrophotometer (Instrumentation Laboratory, type IL 451). Prior to dissection, the animals were kept in tapwater for 24 h in order to eliminate adherent cadmium. Of the gills that contained glochidia, only the inner demibranches (without glochidia) were used. The excised organs as well as isolated calcium concretions were lyophilized for 48 h and weighed. Dry tissue and concretion fraction were decomposed with nitric acid, according to Hemelraad et al. (1986a). Water and haemolymph samples were assayed without prior decomposition. All samples were assayed tenfold.

The potassium and sodium ion concentrations in haemolymph samples were determined by flame photometry, and calcium concentration by using an atomic absorption spectrophotometer (Instrumentation Laboratory, type IL 451). Since no appropriate method was available, the pH of the haemolymph was not measured.

2.5 Condition and shell weight

Shell length of all specimens was measured and shells were weighed after sampling the soft parts. The total dry weight of the soft parts was com-

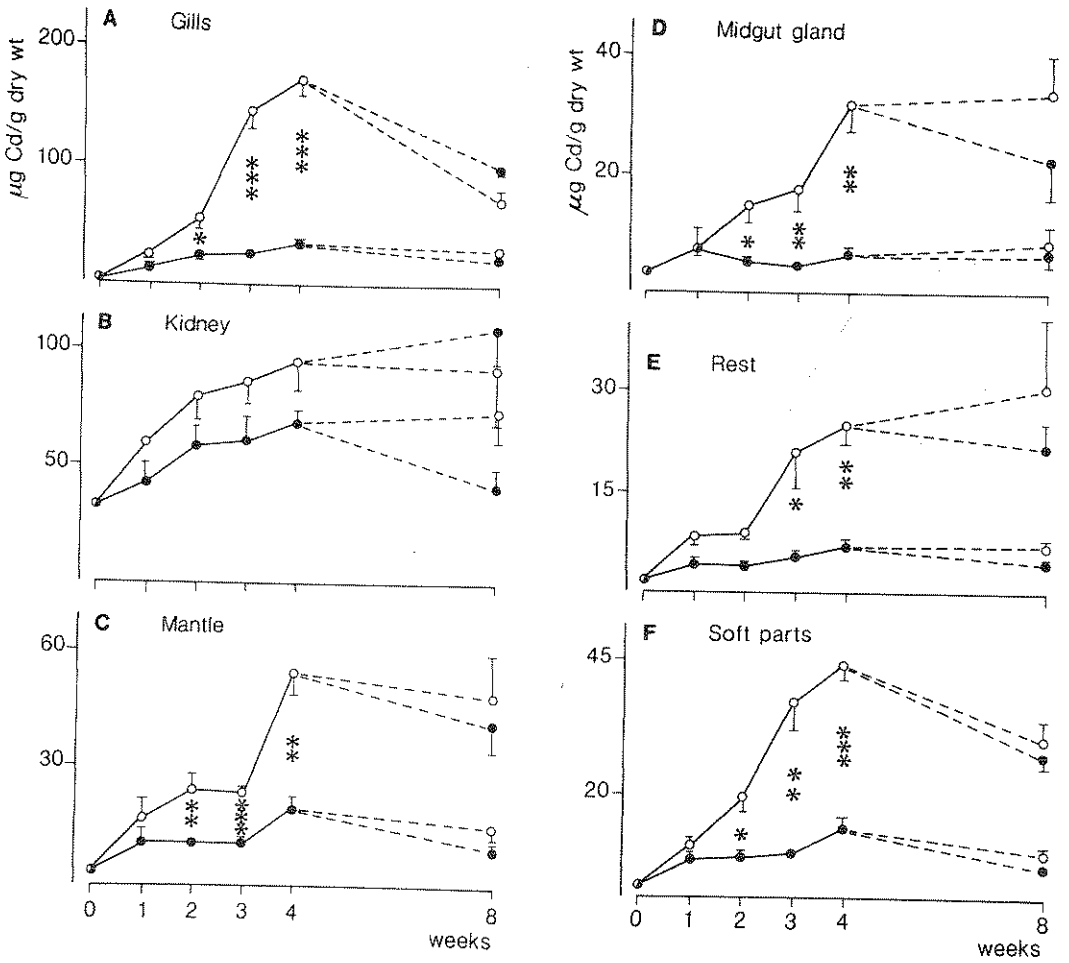

Fig. 2. Cd concentration in body parts of *Unio pictorum* during exposure (continuous lines) to Cd (50 µg/l Cd) in a neutral (unfilled circles, group B) and an acidic (filled circles, group A) medium, and elimination (dotted lines, groups A1 and B1 in acidic, B2 and A2 in neutral medium). Mean of 6 animals, ± *SE*. Asterisks indicate significant differences in Cd accumulation (*** = $P \leq 0.001$, ** = $P \leq 0.01$, * = $P \leq 0.05$).

pared with the shell length in order to evaluate the condition of the clams.

2.6. Statistical analysis

The differences in Cd accumulation and other physiological parameters analysed were tested for significance using a 2-way Student's *t*-test accompanied by the Cochran method when sample variance was unequal. Differences were considered significant at the 0.05 probability level.

3. Results

3.1. Cd accumulation and elimination

The concentration of Cd in the various organs or in the whole soft part of *Unio pictorum* did not change significantly during the acid acclimation period of 10 days. After 2 weeks of exposure to 50 µg/l Cd (Fig. 2), the Cd concentration in all the organs, except the kidney and the rest, was significantly higher in the slightly alkaline than in the acidified medium. After 3 weeks of exposure, also

the rest fraction possessed a significantly higher Cd concentration. In the kidney, no significant difference in the Cd accumulation between clams of groups A and B was observed in the course of the exposure. The distribution of Cd among organs was different when exposed in the acidic medium: the kidney bound more and the gills less Cd than in the clams exposed to the slightly alkaline medium. The Cd concentration in the kidney of unexposed animals measured prior to exposure was relatively high (about 35 mg Cd/g dry weight), since the clams had been collected from the severely polluted Haringvliet area. This pre-exposure, however, does not change the metal accumulation pattern typical to unionid clams (reported earlier by Hemelraad et al. 1986a, b)

After 4 weeks of exposure, the Cd concentration in the shell and haemolymph was significantly higher in the animals which had been exposed to the metal in the neutral medium (group B, see Table 3). Cd was not detected in the shells of unexposed *Unio pictorum* individuals.

In gills of *Unio pictorum*, Cd elimination was slightly more rapid in slightly alkaline (pH 8.0–8.3, group B_2) than in acidic (pH 4.0–4.5, group B_1) conditions (Fig. 2), but no significant difference was measured. Groups A_1 and A_2, with lower Cd concentrations (exposed in pH 4–5) showed no difference in Cd elimination in the acidic vs. the slightly alkaline medium.

Table 3. Cd concentrations (mean ± *SE*) in gill calcium concretions (CaCo) and shells (µg Cd/g dry weight) and haemolymph (µg Cd/ml) of unexposed and exposed (4 weeks, 50 µg Cd/l) in acidic (group A, pH 4.0-4.5) and neutral (group B, pH 8.0-8.3) water) *Unio pictorum*. All differences in Cd concentration between acidic and neutral medium are significant at0.05 level. ND = not detected by flame AAS, NM = not measured.

	n	Unexposed	Acidic (A)	Neutral (B)
CaCo	4	NM	7.70 ± 1.55	21.60 ± 6.37
Shells	6	ND	0.56 ± 0.23	4.63 ± 1.73
Haemo-lymph	6	1.23 ± 0.04	1.22 ± 0.13	2.99 ± 0.59

3.2. Calcium concretions and haemolymph ions

There were no significant changes in the amount of calcium concretions in the different organs of *Unio pictorum* (Fig. 3) analysed after 10 days in pH 4-6 and 4 weeks in 50 µg/l Cd (acidic and neutral, groups A and B). Calcium concentration of the gill concretions decreased significantly during acid exposure ($P \leq 0.05$, decrease from 33.2 ± 1.1 % (w/w) to 24.2 % ± 2.7). In animals exposed to a slightly alkaline medium, calcium concentration remained constant (32.3 % ± 3.6).

Clams exposed to Cd in acidic water accumulated about 3 times less Cd in their gill calcium concretions (Table 3), contrary to total gill tissue, where the Cd concentration was 4 times lower (Fig. 2A). No significant change in the calcium concentration of the gill concretions was measured in the clams exposed to the acid medium.

Table 4. The concentrations (meq/l) of calcium, sodium, and potassium in the haemolymph of *Unio pictorum* in unexposed animals, in acclimated animals (10 days in acidic medium), and during the 4-week exposure to Cd (50 µg/l) in acidic (pH 4.0-4.5) and neutral (pH 8.0–8.3) medium. Means ± *SD* are calculated from 4 individuals. 1st and 4th week Ca values differ significantly from unexposed and acclimation values (*t*-test, $P \leq 0.001$).

	Ca	Na	K
Unexposed	13.0 ± 2.0	16.2 ± 3.0	0.5 ± 0.2
Acclimated	19.5 ± 5.0	15.7 ± 5.4	0.5 ± 0.3
Exposure			
1st week			
acidic	7.0 ± 1.0	15.4 ± 5.6	0.4 ± 0.2
neutral	7.0 ± 1.0	14.4 ± 7.4	0.3 ± 0.2
2nd week			
acidic	9.0 ± 2.0	15.8 ± 4.4	0.4 ± 0.2
neutral	9.0 ± 1.0	14.4 ± 7.4	0.4 ± 0.2
3rd week			
acidic	9.0 ± 1.0	17.0 ± 8.0	0.5 ± 0.2
neutral	10.0 ± 1.0	16.2 ± 4.6	0.4 ± 0.2
4th week			
acidic	7.5 ± 1.0	16.7 ± 7.8	0.4 ± 0.3
neutral	12.5 ± 0.4	17.0 ± 3.9	0.5 ± 0.1

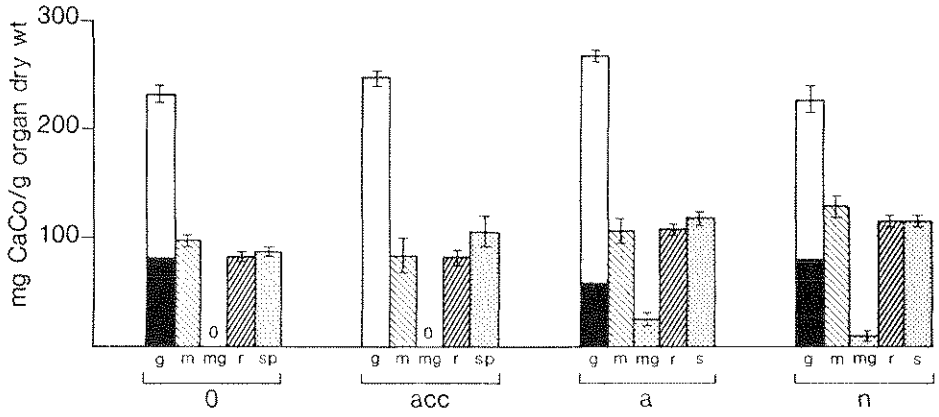

Fig. 3. Amount (mg/g dry weight) of calcium concretion (CaCo) in gills (g), mantle (m), midgut gland (mg), the rest (r) and soft parts (sp) of *Unio pictorum*. Mean of 4 animals, ± SEM. 0 = unexposed, acc = acclimated 10 days in pH 4-6, a = exposed for 4 weeks to 50 µg/l Cd in an acidic medium (group A) and n = exposed for 4 weeks to 50 µg/l Cd in a neutral medium (group B). The dark portion of the gill column gives the amount of Ca (w/w %) in the concretions. The amount of Ca of the gill calcium concretions of the acclimated animals was not measured.

Ca^{2+} concentration in the haemolymph of *Unio pictorum* increased slightly during 10 days exposure in acid (pH 4-5) water (Table 4). Even one week of Cd exposure in neutral and acidic water caused a significant decrease in haemolymph Ca^{2+} concentration. The decrease was transitory in pH 8.0-8.3/Cd exposure. After 4 weeks of exposure, Ca^{2+} concentration in haemolymph was still significantly lower in clams exposed to acidic water. Haemolymph Na^+ and K^+ concentrations did not change during the exposure (Table 4).

3.3. Condition, behaviour and mortality

The acclimation to pH 4–6 or exposures to Cd in acidified (pH 4.0–4.5) or a slightly alkaline (pH 8.0–8.3) water did not significantly change the relative dry weight of soft parts (Table 5). A white mucus, which possessed high concentrations of Cd (60–90 µg/g dry mucus), was observed on the mantle edge of the clams from group A during the last two weeks of exposure.

Damage of the shell periostracum was not observed. No significant differences were measured in shell weights (relative to the shell length) between the different experimental groups.

During the first 3 weeks of exposure no deaths occurred. In the 4th week of exposure and during the acclimation period one clam died in the acidified medium. All animals in the acidic as well as in the neutral medium survived the elimination period of 4 weeks.

Table 5. Shell length (cm) and weight (g), weight/length ratio (g/cm), and dry weight of soft parts (g) of unexposed, control, and exposed (see Table 4) *Unio pictorum*. Means ± *SE* of 6 animals.

		Length (l)	Weighth (w)	w/l	Dry weight (dw)	dw/l
Unexposed		7.00 ± 0.14	11.18 ± 0.74	1.59 ± 0.09	1.25 ± 0.08	0.18 ± 0.01
Acclimated		7.05 ± 0.15	10.47 ± 0.83	1.76 ± 0.09	0.91 ± 0.12	0.13 ± 0.02
Exposed, 4th week	acidic	7.03 ± 0.09	10.90 ± 0.42	1.55 ± 0.05	1.02 ± 0.08	0.15 ± 0.01
	neutral	7.03 ± 0.28	11.57 ± 1.44	1.56 ± 0.16	0.97 ± 0.09	0.16 ± 0.01

4. Discussion

4.1. Cd accumulation and elimination

Freshwater clams (*Anodonta* and *Unio* species) accumulate Cd in a biphasic way (Hemelraad et al. 1986a and b) reaching saturation after 10 weeks of exposure in 25 µg Cd/l. In this study, Cd accumulation in *Unio pictorum* was followed during the first linear period of 4 weeks. The first plateau of accumulation was reached in most of the organs of *Unio pictorum* after 2 to 3 weeks (Fig. 2). It has been shown earlier that the distribution of Cd is dependent on the total body burden (Hemelraad 1986a). The difference in the Cd distribution between the organs of animals expose to acidic and slightly alkaline mediums can also be explained in this way because the total body burden of Cd in animals exposed to the neutral medium was about 2 to 3 times higher than in those exposed to the slightly alkaline.

In the acidic conditions, accumulation of cadmium was reduced in all organs, especially in the gills (Fig. 2). The concentration factor of Cd in *Unio pictorum* exposed to 50 µg/l Cd was 3 500 in slightly alkaline and 800 in acidic conditions for the gills. Since gills play an important role in ion uptake (Hemelraad et al. 1986a), are the first target organs for Cd accumulation and a route for Cd penetration into the clam, it was expected that acid stress would influence accumulation. Also the amount of Cd bound in the shells was lower in acid exposure. This could be a result of a decreased rate of shell formation in the acidic (+Cd) medium. The low Cd concentration in the haemolymph reflects the decreased uptake in the acidic medium.

The difference between Cd accumulation in the neutral and the acidic medium cannot be due to differences in Cd speciation because Cd, in contrast to the most heavy metals, does not differ between pH 4 and 7 (Hahne & Kroontje 1973). Two possible explanations may lie behind the difference in accumulation. First, behavioural responses, such as valve closure, can affect the filtration rate and thus diminish the amount of Cd in tcontact with the gill epithelium. The second possible reason for lowered Cd uptake is the change in the cellular uptake mechanism. Sakaguchi et al. (1979) suggest that the Cd adsorption on the cell is pH sensitive and report that from pH 7 to pH 4 there is a linear decrease of about 95 % in surface bound Cd.

When clams were transferred into an acidic Cd-free medium, Cd concentration in the gills and kidney did not decrease as quickly as in neutral conditions (Fig. 2). This could be a result of increased transport of Cd from the other organs to the gills and kidney, which under pH stress could not effectively eliminate Cd. These results, together with the results from the accumulation experiments, indicate a disturbed Cd movement across the gill epithelium in both directions.

4.2. Calcium concretions and haemolymph ions

In the acidic conditions the amount of Cd in the gills bound in the calcium concretions was significantly lower than in the slightly alkaline conditions (Table 3). In both exposures the amount of Cd bound in the concretions was less than 10 % of the total amount of tissue Cd when the granules were 22–26 % of gill dry weight of *Unio pictorum*. It has been shown earlier (Pynnönen et al. 1987) that the gill calcium concretions bind less Cd than their mass proportion would imply. Gill calcium concretions are the maternal source of glochidial shell calcium (Silverman et al. 1985, 1987) and they are mobilized during the glochidial development. Therefore it would be disadvantageous to developing glochidia if the concretions worked as sequestration sites for heavy metals.

During severe acidification of the internal environment, the calcium concretions could play a role in the haemolymph buffering. The amount or composition of calcium concretions in the gills (w/w dry) did not, however, change during the 4-week exposure to the acidic medium. According to the studies on their mobility (Silverman et al. 1983, 1985) and heavy metal sequestration (Pynnönen et al. 1987, Silverman et al. 1987) they seem to be inert and well protected against environmental influence.

The ionic changes in the haemolymph under pH stress (see Table 4) have been reported earlier not only for the clams (Malley et al. 1988) but also for fishes (McDonald 1983, Witters 1986) and crustaceans (Nikinmaa et al. 1983, Wood & Rogano 1986). The rise of blood [Ca^{2+}] shortly

after the onset of exposure (10 days in pH 4–4.5) might indicate a ventilation change and metabolic acidosis caused by valve closure. A good correlation between the decrease in the haemolymph pH and increase in the haemolymph Ca^{2+} concentration was shown recently (Pynnönen 1990a). Presumably the majority of Ca used for buffering the haemolymph originates from shell and mantle Ca-reserves. Increased Ca uptake from the medium, is less likely under severe pH stress, since it has been shown earlier that low pH causes a decrease in Ca uptake in crustaceans (Malley & Chang 1985).

It is hypothesized that the decrease of the blood $[Ca^{2+}]$ as a result of Cd exposure can affect the $[Ca^{2+}]$ influx. This has been earlier shown in the rainbow trout, *Salmo gairdneri*, exposed for 24 h to 36 ppb Cd (Reid & McDonald 1988). In a chronic exposure to 50 µg Cd/l, however, a significant increase of Ca^{2+} concentration in the haemolymph of *Anodonta cygnea* was seen up to 8 weeks of exposure (Hemelraad et al. 1988).

In a chronic Cd exposure (50 µg/l), a decrease in the haemolymph $[Na^+]$ was seen after 8 weeks of exposure in *Anodonta cygnea* (Hemelraad 1988). The 4 weeks exposure to Cd was apparently too short to exhaust Na^+ and K^+ reserves, because no decrease in the blood concentration of these ions was measured. Unexpectedly, the depletion of the haemolymph Na^+ and K^+ often found in the unionids after 3 to 4 weeks of acid exposure in moderately hard water (18 mg Ca/l, Pynnönen 1990a) was not found when clams were exposed simultaneously to Cd and acid. Lack of this response might be a result of the hardness (35 mg Ca/l) of the exposure water. Brown (1983) demonstrated that water Ca concentration exceeding 2 mg/l can effectively ameliorate the toxic effects of H^+ ions.

4.3. Condition and behaviour

According to the condition index, no significant dry weight decrease in relation to shell length was observed (Table 5).

An excess of mucus, which could be produced either by mantle or gill cells, may work as a barrier to Cd accumulation as well as to elimination, since high amounts of Cd were measured in the mucus. This could partly explain the lowered Cd uptake in the animals exposed to the acidified Cd-rich medium.

Shells of the animals remained relatively unchanged during the exposures (Table 5). Only a slight decrease in shell weight in relation to shell length was measured after 9 weeks of exposure to the acidic medium. If the periostracum is undamaged, it offers good protection against the destructive effect of the acid. In contrast, dissolution of the shell can occur within 4 weeks in animals with shell erosion in the umbonal region.

Valve closure responses of the Asiatic clam *Corbicula fluminea* in the presence of Cd and Zn have been reported by Doherty et al. (1987). In this study, both the decrease in Cd accumulation and the increase in blood Ca might be due to the changes in ventilation rate and valve closure, but more investigations are needed to verify these assumptions.

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References

Brown, D. J. A. 1983: Effect of calcium and aluminum concentrations on the survival of brown trout (Salmo trutta) at low pH. — Bull. Environ. Contam. Toxicol. 30:582–587.

Campbell, P. G. C. & Stokes, P. M. 1985: Acidification and toxicity of metals to aquatic biota. — Can. J. Fish. Aquat. Sci. 42:2034–2049.

Cusimano, R. F., Brakke, B. F. & Chapman, G. A. 1986: Effects of pH on the toxicities of cadmium, copper, and zinc to steelhead trout (Salmo gairdneri). — Can. J. Fish. Aquat. Sci. 43: 1497–1503.

Doherty, F. G., Cherry, D. S. & Cairns Jr, J. 1987: Valve closure responses of the Asiatic clam Corbicula fluminea exposed to cadmium and zinc. — Hydrobiologia 153:159–167.

Graney, R. L., Cherry, D. S. & Cairns Jr, J. 1984: The influence of substrate, pH, diet and temperature upon cadmium accumulation in the Asiatic clam (Corbicula fluminea) in laboratory artificial streams. — Water. Res. 18:833–842.

Gunn, J. M., Noakes, D. L. G. & Westlake, G. F. 1987: Behavioural responses of lake charr (Salvelinus namaycush) embryos to simulated acidic runoff conditions. — Can. J. Zool. 65:2786–2792.

Hahne, H.C.H. & Kroontje, W. 1973: Significance of pH and chloride concentration on behavior of heavy metal pollutants: mercury(II), cadmium(II), zinc(II), and lead(II). — J. Environ. Quality 2:444–450.

Hemelraad, J. 1988: Accumulation and effects of cadmium in freshwater clams (Unionidae). — Ph.D. Thesis. University of Utrecht.

Hemelraad, J., Holwerda, D. A. & Zandee, D. I. 1986a: Cadmium kinetics in freshwater clams. I. The pattern of cadmium accumulation in Anodonta cygnea. — Arch. Environ. Contam. Toxicol. 15:1–7.

Hemelraad, J., Holwerda, D. A., Teerds, K. J. Herwig, H. J. & Zandee, D. I., 1986b: Cadmium kinetics in freshwater clams. II. A comparative study of cadmium uptake and cellular distribution in the unionidae Anodonta cygnea, Anodonta anatina, and Unio pictorum. — Arch. Environ. Contam. Toxicol. 15:9–12.

Holwerda, D. A. & Veenhof, P. R. 1984: Aspects of anaerobic metabolism in Anodonta cygnea L. — Comp. Biochem. Physiol. 78B:707–711.

Kat, P. W. 1982: Shell dissolution as a significant cause of mortality for Corbicula fluminea (Bivalvia: Corbiculidae) inhabiting acidic waters. — Malacol. Rev. 15:129–134.

Kenttämies, K., Haapaniemi, S., Hynynen, J., Joki–Heiskala, P. & Kämäri, J. 1985: Biological characteristics of small acidic lakes in southern Finland. — Aqua Fennica 15:21–33.

Laurén, D. J. & McDonald, D. G. 1986: Influence of water hardness, pH, and alkalinity on the mechanism of copper toxicity in juvenile rainbow trout, Salmo gairdneri. — Can. J. Fish. Aquat. Sci. 43:1488–1496.

Malley, D. F. & Chang, P. S. S. 1985: Effects of aluminum and acid on calcium uptake by the crayfish Orconectes virilis. — Arch. Environ. Contam. Toxicol. 14:739–747.

Malley, D. F., Huebner, J. D. & Donkersloot, K. 1988: Effects of ionic composition of blood and tissues of Anodonta grandis grandis (Bivalvia) of an addition of aluminum and acid to a lake. — Arch. Environ. Contam. Toxicol. 17:479–491.

McDonald, D.G. 1983: The effects of H^+ upon the gills of freshwater fish. — Can. J. Zool. 61:691–703.

Nikinmaa, M., Järvenpää, T., Westman, K. & Soivio, A. 1983: Effects of hypoxia and acidification on the haemolymph pH values and ion concentrations in the freshwater crayfish (Astacus astacus L.). — Finnish Fish. Res. 5:17–22.

Økland, J. & Økland, K. A. 1986: The effects of acid deposition on benthic animals in lakes and streams. — Experientia 42:471–486.

Peterson, H.G., Healey F.P. & Wagemann, R. 1984: Metal toxicity to algae: a highly pH dependent phenomenon. — Can. J. Fish. Aquat. Sci. 41:974–979.

Pynnönen, K. 1990a: Physiological responses to severe acid stress in four species of freshwater clams (Unionidae). — Arch. Environ. Contam. Toxicol. 19:471–478.

— 1990b: Aluminium accumulation and distribution in the freshwater clams (Unionidae). — Comp. Biochem. Physiol. C. in press.

Pynnönen, K., Holwerda, D. A. & Zandee, D. I. 1987: Occurrence of calcium concretions in various tissues of freshwater mussels, and their capacity for cadmium sequestration. — Aquat. Toxicol. 10:101–114.

Reid, S. D. & McDonald, D. G. 1988: Effects of cadmium, copper, and low pH on ion fluxes in the rainbow trout, Salmo gairdneri. — Can. J. Fish. Aquat. Sci. 45:244–253.

Sakaguchi, T., Tsuji, T., Nakajima, A. & Horikoshi, T. 1979: Accumulation of cadmium by green microalgae. — Eur. J. Appl. Microbiol. Biotechnol. 8:207–215.

Scherer, E., Harrison, S. E. & Brown, S. B. 1986: Locomotor activity and blood plasma parameters of acid–exposed lake whitefish, Coregonus clupeaformis. — Can. J. Fish. Aquat. Sci. 43: 1556–1561.

Schindler, D. W., Hesslein, R. H. & Wagemann, R. 1980: Effects of acidification on mobilization of heavy metals and radionuclides from the sediments of a freshwater lake. — Can. J. Fish. Aquat. Sci. 37:373–377.

Servos, M. R., Rooke, R. J. & Mackie, G. L. 1985: Reproduction of selected mollusca in some low–alkalinity lakes in south-central Ontario. — Can. J. Zool. 63:511–515.

Servos, M. R., Malley, D. F., Mackie, G. L. & LaZerte, B. D. 1987: Lack of bioaccumulation of metals by Elliptio complanata (Bivalvia) during acidic snowmelt in three south–central Ontario streams. — Bull. Environ. Contam. Toxicol. 38:762–768.

Silverman, H., Steffens, W. L. & Dietz, T.H. 1983: Calcium concretions in the gills of a freshwater mussel serve as a calcium reservoir during periods of hypoxia. — J. Exp. Zool. 227:177–189.

Silverman, H., Steffens, W. L. & Dietz, T. H. 1985: Calcium from extracellular concretions in the gills of freshwater unionid mussels is mobilized during reproduction. — J. Exp. Zool. 236:137–147.

Silverman, H., McNeil, J. W. & Dietz, T. H. 1987: Interaction of trace metals Zn, Cd, and Mn, with Ca concentrations in the gills of freshwater unionid mussels. — Can. J. Zool. 65:828–832.

Witters, H. 1986: Acute acid exposure of rainbow trout, Salmo gairdneri Richardson: effects of aluminium and calcium on ion balance and haematology. — Aquat. Toxicol. 8:197–210.

Wood, C. M. & Rogano, M. S. 1986: Physiological responses to acid stress in crayfish (Orconectes): haemolymph ions, acid-base status, and exchanges with the environment. — Can. J. Fish. Aquat. Sci. 43:1017–1026.