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Ecophysiological effects of temporary acidification on crucian carp, *Carassius carassius* (L.): a case history of a forest pond in eastern Finland

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During a dry summer, the pH of a small (0.13 ha), natural forest pond in eastern Finland decreased from > 6.0 to 4.0, returning to normal the following winter. Experimental lowering of the pH, using a strong acid, in a laboratory water-sediment system and in pond water revealed qualitatively similar changes to those in the naturally acidified pond: decreases in water colour, dissolved organic material and total Fe, and increases in total Ca, Mg and Al concentrations. The effects of acidification, together with the simultaneously increased bioavailability of aluminium, were assessed on crucian carp, the only fish species present, by comparison with data from a connected pond that retained its normal pH (> 6.0). The growth in the length of the fish did not differ significantly between the ponds, and the liver glycogen stores were actually slightly larger in the acidic pond. Yet, finds of dead fish after 4 months of acidification, suggested high mortality. In comparison with the carp in the reference pond, the crucian carp in the acidified pond suffered from ionoregulatory imbalance and chronic stress, indicated by decreased plasma chloride, and increased cortisol and glucose concentrations, respectively. In addition, their blood haematocrit and haemoglobin concentration were elevated and the relative size of their livers had increased.

1. Introduction

Because of winter anoxia the only fish species present in numerous small forest ponds in Scandinavia is the crucian carp. Besides surviving long-term anoxia (Blazhka 1960, Holopainen & Hyvärinen 1985), this cyprinid is also reported to tolerate a pH as low as 4.0 (Bryukhatova 1937, EIFAC 1969) and extreme water temperatures as well (Lenkiewicz 1964, Horoszewich 1973).

Although both the ecological and physiological effects of environmental acidification on fish and other freshwater biota have been studied intensively (Almer et al. 1974, Fromm 1980, Haines & Johnson 1982, Dillon et al. 1984, Schindler 1988, Kauppi et al. 1990), both approaches have only seldom been incorporated within the same study. This kind of dual approach became possible for us when, during a series of biomanipulation studies on fish population dynamics (Holopainen et al. 1992, Tonn et al. 1992), temporary natural acidification occurred in part of a manipulated pond (Holopainen 1991).

2. Study area, material and methods

The oligotrophic forest pond Hermanninlampi is situated in the Karelian schist area in eastern Finland (62°41′N, 29°41′S), ca. 10 km from the city of Joensuu. The pond formed 130 years ago in a flat-bottomed sandy depression in a glaciofluvial plain, when a 9.5 m man-induced decrease in the water level of Lake Höytiäinen isolated the pond from the lake. In the pond bottom, the transgressive littoral sand is underlain by a layer of strongly humic peat originating from an ancient mire (Hyvärinen & Alhonen 1970, Vesajoki 1980).

The pond is surrounded by pine forest and has a narrow paludified margin of grass, sedges and *Sphagnum*. Its surface area is 1.5 ha and its maximum depth 1.6 m (Holopainen & Pitkänen 1985). It is normally ice-covered from late October to early May. Due to the winter anoxia, the only fish species that it contains is the crucian carp (Piironen & Holopainen 1988).

In 1985, one year before the present study, the pond was divided for experimental purposes into four sections, using plastic fences. This pond has no clear inlets but drains through section M (referred to here as Pond M, area 3270 m^2, water volume 2470 m^3) into Pond A (area 1300 m^2, water volume 700 m^3) ca. 20 m away (Fig. 1 in Holopainen 1991). Pond A has a maximum depth of 1.0 m and is partly overgrown by sedge and *Sphagnum* inshore and by *Sparganium* sp. offshore. Due to the close proximity of Ponds M and A, their overall edaphic characteristics and water quality are similar.

Pond M, fishless after treatment with rotenon in May 1985, was stocked in the beginning of June 1986 with 785 crucian carp (tot. 5.2 kg with mean total length of 7.3 cm), which resulted in a fish density of 0.24/m^2. In autumn, the density was estimated to be 0.7/m^2, due to the large number of young-of-the-year fish. The growth and condition of the fish were checked by trapping seven times before mid-October.

Before the acidification was noticed, Pond A was emptied of fish in early June 1986 by the removal method (1007 fish were trapped) and restocked on June 18 with 422 crucian carp of the same origin as in Pond M (tot. 3.3 kg, mean length 8.2 cm), which gave a density of 0.32/m^2. After stocking, the growth and condition of the fish were checked by trapping at one-month intervals (four times).

In the beginning of October 1986, 45 crucian carp were trapped in Pond M and 25 in Pond A. The fish were kept in pond water, taken to the laboratory and allowed to recover overnight at the ambient temperature. For sampling, the fish were stunned with a blow on the head, weighed, their total length recorded, and a blood sample aspirated into a heparinized syringe by puncturing caudal vessels. The liver and spleen were dissected out and weighed to the nearest 1 mg. The alimentary canal was sampled by taking the whole tubular gut from oesophagus to anus. At that time the guts were empty because of the winter cessation of both locomotory and feeding activity (Penttinen & Holopainen 1992). Samples of the white muscle tissue were taken from the great lateral muscle beneath the dorsal fin. All tissue samples were frozen in liquid nitrogen and stored in polythene vials at –40°C for later analyses.

Immediately after collection of the blood sample, a subsample for haematocrit (Hct) measurement was centrifuged in a heparinized capillary (Clay Adams 1025), a portion of the whole blood was pipetted for haemoglobin determination, and the rest of the blood was centrifuged for 1 min in a Beckman Microfuge B (ca. 12000 g). The plasma and red cells were separated, and the top layer of red blood cells (RBC) was discharged. The plasma and the red cell pellet were saved for ion analyses. A plasma subsample for glucose and lactate analyses was

precipitated in 0.6 M perchloric acid. The blood haemoglobin concentration was determined by the cyanhaemoglobin method, and the mean erythrocytic haemoglobin concentration (MCHC) was calculated from the Hct/Hb ratio. Blood plasma electrolyte concentrations were measured by atomic absorption spectrophotometry (Na, K, Ca and Mg, Hitachi AAS) and by electrometric titration (chloride, Radiometer CTM10). The sodium concentration of RBC was determined by AAS and calculated without correction for extracellular space. Plasma lactate and glucose were determined using test kits (Boehringer Mannheim, No 139084 and 123986). The amount of cortisol was measured using a cortisol 125 RIA kit(100) system (No. 30CRT0100, Farmos Inc., Finland).

The method of Siu et al. (1970) was used for liver glycogen analysis. The tissue water concentration was measured by drying 200–300 mg pieces for 24 h at 105°C to a constant weight. Fulton's condition factor (CF) was calculated from the total weight (W) and total length (L) of the fish with the formula $CF = W(g) / L(cm)^3 \times 100$.

Surface water samples were taken with a tube sampler from both ponds during the summer, autumn and winter (Table 1) and analysed according to national standard methods (approved by National Board of Waters and the Environment, NBWE) in the analytical laboratory of the Karelian Institute, Section of Ecology at the University of Joensuu. On October 14 the surface water of both ponds was sampled for organic and minerogenic acidity analyses made by NBWE, Helsinki (Mannio & Kortelainen 1990, Forsius et al. 1990). The surface (top 5–10 cm) sediment at 1-m water depth was sampled on October 6, 1986 and March 3, 1987 by scooping up sediment with a cloth net and was stored frozen at –20°C. Before determination of organic matter (ignition loss at 500°C) and metals (Table 2), the sediment was dried at 105°C for 12 h.

For analysis of the total metal content in fish tissues and sediment, dry samples (ca. 0.5 g) were ashed at 500°C for 3 h, dissolved in warm HCl (18.5 % v/v) and HNO_3 (conc.), and dried at 55°C for 1 h. The material was redissolved in HCl (1.85 % v/v), filtered (Whatman 44) and diluted with 1.85% HCl to the appropriate concentration for AAS determinations. Measurements of metals were conducted with a Hitachi 2–6000 AAS according to the instructions and recommendations of the manufacturer.

In order to simulate the acidification history and the effects of pH on water colour, metal solubility and other factors, two experimental acidifications of pond water and a water-sediment system were conducted in the laboratory:

Table 1. Water quality in the acidified Pond A and the reference Pond M in late 1986 and early 1987.

	July 7		Sept. 9		Sept. 29		Jan. 26		May 5	
	A	M	A	M	A	M	A	M	A	M
pH	5.2	6.6	4.0	6.1	4.4	6.4	6.6	6.1	5.7	5.8
Oxygen, mg/l	4.8	8.1	8.2	9.3	10.7	11.7	0.0	10.1	4.5	3.1
Temperature, °C	19	19	–	9.5	4.0	4.6	1.5	0.7	–	
Conduct. mS/m	3.1	3.1	1.0	3.5	6.6	4.9	14.5	4.3	3.2	2.6
Gran alk. µmol/l	–	–	–	–	–46.5	58.4	–	–	138	120
Colour Pt mg/l	80	80	5	40	15	25	500	80	150	70
COD $_{Mn}$ O_2 mg/l	11.0	11.0	1.7	8.2	3.3	7.0	34.0	9.5	7.5	4.7
Tot. N µg/l	621	522	155	394	510	405	1180	445	346	282
Tot. P µg/l	28	29	14	43	9	12	51	8	18	8
Cl mg/l	1.6	1.4	1.4	1.5	1.5	1.3	–	–	–	
Tot. Fe µg/l	1890	1828	320	276	217	722	39	892	5591	1577
Mn µg/l	36	7	–	–	4	24	150	27	157	80
Ca+Mg, mg/l	2.1	2.2	6.2	2.9	4.1	2.7	3.5	3.4	–	
Na, mg/l	1.2	1.3	1.1	1.6	1.3	1.2	2.2	2.2	0.8	0.3
K, mg/l	0.2	1.2	0.5	1.1	0.6	0.5	1.1	1.6	0.5	0.5
Tot. Al µg/l	–	–	–	–	198	3	685	43	211	141
Tot. S mg/l	–	–	–	–	–	–	–	–	1.8	0.9

Experiment I: On March 3, 1987 ca. 1 kg (wet) settled sediment and ca. 3 l water (added on top of the sediment) from Pond A were allowed to settle in a polythene container at 2.8°C for one week. The container was then warmed to room temperature, and the water was aerated for two weeks to simulate the early summer oxygen conditions. At that time subsamples from the sediment and the water were collected for chemical analyses. On March 26, the pH of the water was titrated down from 6.9 to 4.0 over 24 hours, using a total of 7.2 ml of 0.2 N hydrochloric acid (HCl). During the next 10 days the water colour was followed at one-day intervals. On April 6, both the clear water (volume 3.3 l) and sediment (tot. 922 g) were sampled again for metal analyses.

Experiment II: On April 9, 1987 ca. 15 l of surface water were taken from Pond A (acidified) and Pond M (reference) and stored in polythene containers for five days at 2.5°C. Then, over a 3-h period, hydrochloric acid (0.2 N) was added in 0.2 ml portions to 1.3 l water samples until pH values of 6.0 (no acid addition), 5.2, 4.4 and 4.0 had equilibrated without any visible precipitations of humics or other colouring material. For each pH level, once a stable pH was attained, a subsample of 1.0 l was transferred to a glass bottle, i.e. four bottles for each pond. These subsamples were incubated under aerobic conditions but without aeration, at 6°C in the dark and examined on days 1, 2, 8 and 16. After 16 days, the clear water above the precipitate was sampled for metal analyses. The precipitate was centrifuged (12 000 rpm, 15 min. 4°C) and weighed (wet weight) before the analyses. The original large water samples (ca. 11 l), stored like the others, were also analysed as controls.

The SPSS programme package of the University of Joensuu Computer Center was used in the statistical analyses.

3. Results and discussion

3.1. Reason for the temporarily low pH

The ultimate reason for this spatially and temporally restricted acidification is not certain, but it was caused by both organic and minerogenic acidity. The dry, warm summer resulted in low water levels, which reduced the volume of the acidic pond A and isolated it from the main pond. Although these factors probably enhanced the change, they do not provide the ultimate cause. No foreign objects, e.g. metals, were found in the acidic pond.

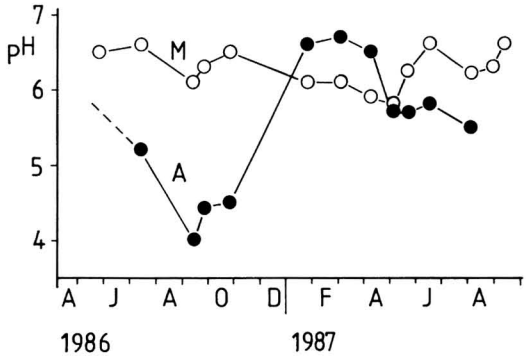

Fig. 1. Seasonal variation of water pH in the acidic Pond A and the main Pond M in 1986–1987.

A plausible explanation is a significant input of sulphate ions from surface runoff, originating from naturally accumulated sulphide compounds in the surrounding former lake bottom. These compounds could have been oxidized upon exposure after the lowering of the water table (cf. Alasaarela 1980, Hartikainen & Yli-Halla 1985, Holopainen 1991).

3.2. Water and sediment chemistry

The small acidic Pond A was characterised by drastic changes in water quality during 1986–87, varying from very clear, normoxic water with a low pH during summer and autumn 1986 (Fig. 1, Table 1) to water with a normal pH but strongly coloured and totally anoxic in January. Later in the winter (March 3, 1987), conductivity (20 mS/m) and colour (725 Pt mg/l) had further increased, but they decreased again in the spring (Table 1). The intense winter colour of the water was bluish grey in Pond A, in contrast to the brownish colour of humus in Pond M.

During the winter, until early March, Pond M was also anoxic, with a consequent increase in conductivity (5.5 mS/m) and colour (200 Pt mg/l). In late October 1986 the organic acidity of Pond A (160 μeq./l, pH 4.6) clearly exceeded that

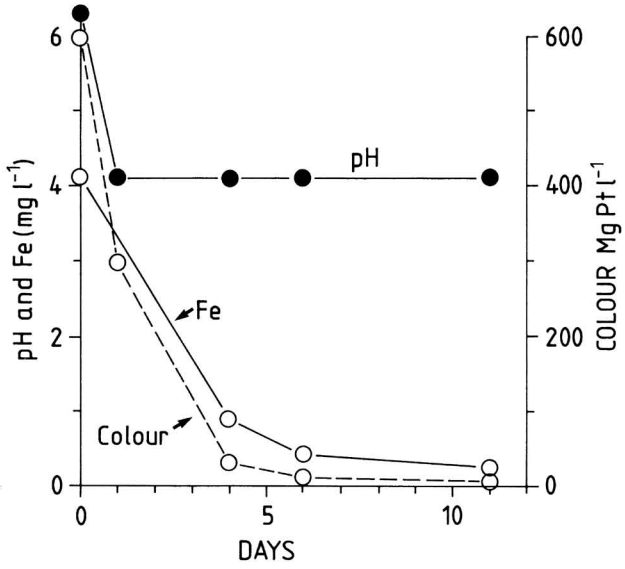

Fig. 2. The effects of experimental acidification (from pH 6.3 to 4.1) on water colour and iron content in a sediment-water sample. The sample was taken from the acidic pond A on March 3, 1987 and aerated 16 days at room temperature before the experiment.

of Pond M (26 µeq./l, pH 6.4). In addition, minerogenic acidity of 24 µeq./l was found in Pond A, which supports the idea that strong acids, such as sulphuric acid, were a possible cause of the low pH. No acid-neutralizing capacity (ANC) was left in Pond A, as revealed by the negative Gran alkalinity in late September (Table 1).

The solubility and sediment-water exchange rates of many nutrients and metals depend in a complex way on the pH, oxygen content, redox potential, temperature, etc. These interactions are made even more complex by the presence of humic substances (e.g. Wetzel 1983, Dillon et al.1988). Probably as a result of the acidification, Pond A was characterised in October by a somewhat different sediment chemistry from that in Pond M: the content of organic material was higher as were the amounts of several heavy metals (Al, Pb, Cd and Mo). However, the concentrations of Ca and Mg were lower in the sediment of Pond A (Table 2).

Table 2. Ash and metal concentrations (per unit dry weight, mean±*SD*, *n*) in surface sediments of the acidified Pond A and the reference Pond M. Combined data from 7 or 8 parallel subsamples collected in October 1986 and March 1987 are presented (none of the differences between dates were significant). The *P* values refer to the results of Mann-Whitney *U*-tests. *P*: *** <001, **< 0.01, * < 0.05, NS not significant.

	Pond A		Pond M		*P*
Ash, %	67.1±5.4	7	80.3±3.2	8	***
Mg, mg/g	0.8±0.1	7	3.5±0.3	8	***
Ca, mg/g	0.8±0.1	7	1.0±0.2	8	*
Al, mg/g	15.8±0.6	7	9.6±1.4	8	***
Mo, mg/g	24.1±2.9	7	14.3±3.2	8	***
Cd, mg/g	2.3±0.3	7	1.1±0.3	8	***
Pb, mg/g	91.3±5.7	7	76.4±16.2	8	*
Cr, µg/g	29.4±2.3	7	30.0±4.1	8	NS
Ni, µg/g	38.6±14.9	7	34.6±4.2	8	NS
Cu, µg/g	20.4±5.4	7	21.0±3.3	8	NS
Zn, µg/g	121±45.7	7	197±19.2	8	NS

In summary, the seasonal changes in the water chemistry of Pond A provided the fish with an abiotic environment differing greatly from that in Pond M for a large part of the year. The contents of soluble metals (Table 1), for example, even suggested toxic levels at times.

3.3. Experimental acidification in the laboratory

The experimental lowering of the pond water pH with HCl to 4.1 in the 1+3 l sediment-water system, sampled and reconstructed from Pond A in March, when the temporary acidification was over (Table 3), resulted in a drastic decrease of water colour and iron content (Fig. 2). The lowest colour value (5 Pt mg/l) was the same as

recorded from Pond A during the most intensive phase of the acidification six months earlier. Ca. 20 % more aluminium was released from the sediment to the water (Table 3). Like Al, the concentrations of Ca and Mg, and those of Ni and Pb as well, also decreased in the sediment phase, whereas those of Zn, Cu and Mo increased. All in all, the observations suggested that the changes in water chemistry resulting from the natural acidification were largely reproducible in the simulated laboratory system.

In winter, after the pH had recovered, the buffering capacity of Pond A water was, somewhat unexpectedly, higher than that in Pond M (Table 3). This may have been due to the higher concentration of buffering humics, as indicated by higher organic carbon, COD and colour. Although the initial pHs were equal in Ponds A and M (6.0, in April), the amount of acid needed to create the same decrease in pH was about twice as great in the water of Pond A as in Pond M (Table 3). Precipitation of the humics and other coloured substances was greatest at the lowest pH, but took place to some extent in all the bottles, including those with untreated water (Table 3).

The metal analyses, on the other hand, suggested higher solubility at low pH (higher contents in water, lower in the precipitate) for aluminium, zinc, nickel, magnesium and calcium in this experiment (Table 3).

3.4. Fish mortality, growth and winter energy reserves

In Pond A, crucian carp were exposed not only to an increased hydrogen ion concentration, but also to increased levels of total aluminium in the water (Table 1). Therefore, as has been shown in precipitation-induced acidification in which the pH falls below ca. 5 (Baker 1982), the crucian carp from Pond A suffered from both acid and aluminium toxicity. Under these conditions, other heavy metals may also have been involved (Baker 1982).

Visual examination of Pond A, promoted by the exceptionally clear water in late August, revealed motionless, but living crucian carp scattered close to the bottom. On Sept. 9, dead fish were observed for the first time, and on Sept. 25 ca. 20 dead fish, both adult and young-of-the-year, were seen. On Oct. 24 another 10 dead fish were found in Pond A. No dead fish were found in Pond M.

In summer 1986 the growth in length of the adult fish was the same in the two ponds (Fig. 3). However, Fulton's condition coefficient (Table 4) and the length-specific weight of the female fish (ANCOVA, $F = 4.83$, $P = 0.04$) were lower in Pond A in September–October. No differences were found in the weights of the males or of the combined material (Table 4). The mean length of the young-of-the-year fish in September–October was also almost the same in the two ponds (Fig. 3).

During summer growth followed the normal rate and pattern in both ponds: high rates in June–July, but no further increase in length from August onwards. Late summer and autumn are used for accumulation of winter reserves, which in this species consist of liver glycogen (Holopainen et al. 1988, 1991).

The energy reserves in the liver, expressed as the relative organ size and glycogen content, were

Table 3. Quality of water and precipitate in experimentally acidified (HCl addition) water samples after 16 days' incubation at +6°C. Four one-litre water samples with different pH values from both the acidic Pond A and the reference Pond M were created. The ponds were sampled on April 9, 1987. The precipitate mass is given as wet mass after centrifugation; precipitate quality is given per unit dry weight.

	Pond A				Pond M			
	1	2	3	4	1	2	3	4
Water								
pH, titrated	6.0	5.2	4.4	4.0	6.0	5.2	4.4	4.0
HCL,µmol/l	0	560	720	840	0	240	320	420
pH, end	5.8	4.1	3.9	3.9	6.1	5.4	4.8	4.2
DOC mg/l	31.2	18.7	16.6	17.7	18.7	19.2	18.7	25.0
Col. Pt mg/l	700	10	10	10	125	125	100	60
COD_{Mn} mg/l	24	3	3	4	8	8	9	6
Al µg/l	50	100	110	110	60	40	40	50
Precipitate								
mass, g/l	1.39	1.51	2.00	2.06	0.47	1.05	0.97	1.23
Mg, µg/g	856	47	56	198	1042	659	638	107
Ca, µg/g	2976	203	181	595	7604	5275	2793	857
Ni, µg/g	29	3	11	11	69	43	11	21
Zn, µg/g	147	0	8	21	854	214	351	118

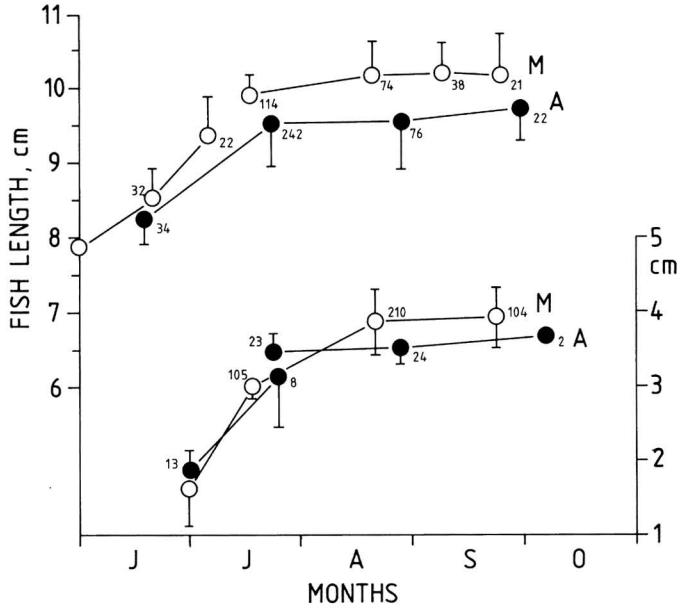

Fig. 3. The growth of a fin-clipped size class of crucian carp in the acidic Pond A and in the main Pond M in 1986. The lower figure is for the young-of-the-year. The vertical lines show *SD* and the numbers indicate sample size.

Table 4. Physiological characteristics (mean±*SD*, *n*) of crucian carp populations caught in water with pH 4.5 (Pond A) and pH 6.5 (Pond M) in October 1986, after ca. four months' acidification history. The values for aluminium are based on tissue wet weight. The *P* values refer to the Mann-Whitney *U*-test, except for weights, which were compared by ANOVA with length as a covariate. *P*: *** <001, **< 0.01, * < 0.05, NS not significant.

	Pond A		Pond M		*P*
Length, cm	9.6±1.4	25	10.6±3.4	45	
Weight, g	13.4±4.9	25	23.64±23.2	45	NS
Fulton's cond.	1.4±0.13	22	1.48±0.1	63	***
Muscle Al, µg/g	64.9±28.1	7	20.6±13.8	7	**
Intestine Al, µg/g	122.8±62.5	7	32.7±8.1	7	**
Liver size, %	10.3±2.2	25	6.6±2.2	45	***
Liver glycogen,%	27.2±4.9	25	25.4±4.2	45	*
Liver water,%	67.5±2.7	15	66.7±1.4	16	NS
Muscle water, %	82.3±2.0	15	81.4±1.0	16	NS
Spleen size, %	0.27±0.12	15	0.22±0.08	16	NS
Haematocrit, %	36.6±4.6	13	30.1±2.7	16	***
Haemoglobin,g/l	102.9±22.3	14	81.3±10.6	16	***
MCHC, g/l RBC	281±38	13	271±34	16	NS
RBC, Na mmol/l	18.1±6.3	11	19.6±5.7	14	NS
Blood plasma					
Glucose, g/l	1.8±0.6	10	0.8±0.2	15	***
Lactate, mg/l	93±21	7	100±60	6	NS
Cortisol,nmol/l	974±484	8	504±98	9	**
Cl, mmol/l	73±9.2	6	115±10.2	15	***
Na, mmol/l	145±31	3	137±20	7	NS
K, mmol/l	4.7±0.3	2	3.0±0.6	7	*
Mg, mmol/l	1.9	1	2.0±0.5	7	NS

slightly but significantly higher in Pond A fish, indicating sufficient availability of food and metabolic capability to prepare for winter hypoxia/anoxia in the way that is characteristic of this species (Hyvärinen et al. 1985).

The water quality changes were shown to affect both the phyto- and zooplankton from mid-July onwards (Holopainen 1991). Both the phytoplanktonic species diversity and the community biomass were lower in acidic Pond A. Similarly, the zooplankton mean biomass, especially that of Crustacea, was low in late summer. The effect of this temporarily low pH on benthic invertebrates was not measured but is assumed to have been smaller. Chironomid larvae, which in addition to micro-crustaceans serve as the main food source for crucian carp in this pond (Penttinen & Holopainen 1992), are generally reported to be tolerant of acidification and make up the major part of the macrozoobenthos in acidified Finnish lakes (e.g. Meriläinen & Hynynen 1990 and references therein).

As reviewed by Dillon et al. (1984), the long-term effects of acidification on fish populations include decreases in species richness and population sizes. Population length structures may change at an early stage due to reproduction failure (Haines 1981, Magnuson et al. 1984, Lappalainen et al. 1988). The effects on individual growth may be

either negative, due to the increased energy demand of ion balance regulation (Lee et al.1983), or positive, due to decreases in population density and a consequent decline in competition for food, as has been shown, e.g., for perch (Ryan & Harvey 1980, Rask & Raitaniemi 1988). The short-term effects of acidic pulses are strongly dependent on the species. Salmonidae and, e.g., roach (*Rutilus rutilus*; EIFAC 1969, Milbrink & Johansson 1975) have been shown in many studies to be sensitive species and are affected first. Perch (Lappalainen et al. 1988) and crucian carp are reported to be much more tolerant of low pH. Bryukhatova (1937) reports several months' tolerance of pH 4.0 in the laboratory for one-year-old crucian carp. The fish did not, however, increase their mass at pH 4.0, as they did with the same feeding at pH 5.0.

In the present study, the four-month acid exposure, with a documented minimum pH of 4.0, was lethal to a part of the crucian carp population. As the growth of the fish was unaffected in Pond A, the observed mortality is suggested to be due to physiological stress.

3.5. Fish physiology

At the time of fish sampling (end of September) the total Al concentration in the water reached 200 µg/l in Pond A (only 3 µg/l in the reference pond), which is lethally toxic to many fish at an ambient water pH near 4 (Baker 1982). The capacity of organic chelates to reduce aluminium toxicity (Driscoll et al. 1980) was also reduced, due to precipitation of humics at the low pH, as shown by the water chemistry (e.g. colour and COD, Table 1) and the simulation experiments (Fig. 2, Table 3). Interestingly, the total aluminium concentration in the water continued to increase towards winter (up to ca. 1000 µg/l in March) when the temporary acidification was already over. At that time, however, a large part of the Al was probably chelated with humics (Table 1), and largely lacking acute toxicity.

Analyses of muscle and intestine tissue indicated that the body burden of aluminium in crucian carp in Pond A was more than three times that of fish in the reference Pond M (Table 4). This indicates that the aluminium in the Pond A ecosystem had been biologically available to the crucian carp and led to chronic toxicity due to the combined acid and metal loadings.

At the end of September the crucian carp collected from the clear acidic Pond A were much darker in coloration than those from Pond M. This is probably a normal reaction to the clear water, helping the animals to blend in with the darkness of the organic bottom sediments. On the other hand, direct endocrinological reasons connected with chemical stress may also have been involved in the altered coloration.

Compared with those in Pond M, which had a pH above 6, the crucian carp in the acidified Pond A revealed characteristic physiological signs of acid and aluminium stress, especially distinct in soft waters (Leivestad & Munitz 1976, Fromm 1980, Wood & McDonald 1982, McDonald 1983). Most typically, the crucian carp suffered from ionoregulatory imbalance as indicated by their decreased plasma chloride concentration (Table 4). The mechanisms for this imbalance in acidic waters are well documented, and include both increased branchial loss and decreased uptake of ions (Wood & McDonald 1982, Freda & McDonald 1988). Unexpectedly, however, the dominant plasma osmolyte, sodium, remained unchanged. This discrepancy between the sodium and chloride responses is probably related to the suggested separation of regulation of these major osmolytes in teleost gills (Evans 1979). Compared with many other teleosts, however, crucian carp seemed to have better resistance to osmoregulatory failure at the organ level, as indicated by only insignificantly higher levels of tissue water in the liver and muscle. The lower condition factor of the crucian carp from Pond A supported the assumption that the volume regulatory stability was largely maintained in these fish. Red cell size, as indicated by unaltered MHCH, seemed to be regulated, too. The evidently high osmoregulatory capacity may furnish a partial explanation of the ability of this species to tolerate several months of acidification at around pH 4, as seen in the case of Pond A in Hermanninlampi.

Some physiological differences (Table 4) can even be seen as adaptive responses. Increased concentrations of haemoglobin in the blood counteract the Bohr effect caused by decreased blood pH

(Fromm 1980, Ultsch et al. 1981) and increase the blood buffering capacity as well (Nikinmaa et al. 1990). Higher liver glycogen reserves can be expected to improve the ability of crucian carp to withstand hypoxic and acidic winters as well.

On the other hand, the significantly increased levels of cortisol and glucose in the blood plasma (Table 4) can be considered indicative of chronic physiological stress in the crucian carp due to long-term exposure to natural acidification. Despite the fact that this species tolerates hypoxia very well at cold temperatures, a much lower oxygen concentration (Table 1) in the ambient water may have contributed to the physiological stress (Nikinmaa et al. 1990). The moribund behaviour and mortality of crucian carp observed at the end of September in the acidified Pond A were therefore associated with chronically expressed physiological stress and indices of osmoregulatory failure.

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