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ALUMINIUM AND pH EFFECTS ON SOME OSMOREGULATORY AND HAEMATOLOGICAL PARAMETERS OF THE ACID-RESISTANT AMERICAN BULLHEAD *ICTALURUS NEBULOSUS* (LE SUEUR)

by

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ABSTRACT

Physiological experiments performed with the acid-resistant fish *Ictalurus nebulosus* gave evidence that :

1) Na^+-influx at high H^+- and Al-concentrations increased, in contrast to acid-sensitive fishes, which decrease their Na^+-influx in such conditions ;

2) high external Ca^{++}-concentrations have no beneficial effect on low pH and Al toxicity ;

3) differences in populations from acid compared to circumneutral lakes disappeared when acclimated to the same soft water of low ionic content (pH 6.8).

Key words : acidification, aluminium, bullhead, acid-resistance, calcium.

INTRODUCTION

Atmospheric emissions of SO_2 and NO_x are responsible for an acid deposition which can cause acidification of poorly buffered fresh waters (IRWIN and WILLIAMS, 1988). As a consequence of the H^+-ion input in the soil, especially leaching of Al can take place. This can result in elevated Al-concentrations in streams and surface waters (DICKSON, 1980 ; VANGENECHTEN, 1983).

Freshwater fishes exhibit physiological disturbances e.g. in ion balance, at conditions of high H^+- and Al-concentrations (MCDONALD and WOOD, 1981 ; MCDONALD *et al.*, 1983 ; DALZIEL *et al.*, 1985 ; WITTERS, 1986 ; GONZALEZ and

DUNSON, 1987 ; WITTERS *et al.*, 1987 ; FREDA and MCDONALD, 1988 ; HÔBE and MCMAHON, 1988). Elevated Ca^{++}-concentrations in the water sometimes can exercise an ameliorating effect on pH- and Al-stress (MCDONALD *et al.*, 1980, 1983 ; FREDA and MCDONALD, 1988). Most studies have been made on acid-sensitive fishes, especially Salmonids (MCDONALD and WOOD, 1981 ; MCDONALD *et al.*, 1983 ; DALZIEL *et al.*, 1985 ; WITTERS, 1986 ; WITTERS *et al.*, 1987 ; FREDA and MCDONALD, 1988).

American bullheads are found in neutral as well as in acid lakes. As far as is known, only the effect of low pH has been investigated on bullheads of a circumneutral water (VANGENECHTEN *et al.*, 1987). The latter study revealed that the physiological response to pH 4.0 was qualitatively similar but less pronounced compared to the reaction of acid-sensitive fishes.

In order to get more information on the effects of high H^+- and Al-concentrations on the American bullhead *I. nebulosus* (LE SUEUR), experiments focussed on the following questions :

1) What is the effect of low pH and elevated Al-concentrations on *I. nebulosus* ?

2) Do elevated external Ca^{++}-concentrations influence pH- and Al-sensitivity of *I. nebulosus* ?

3) Are *I. nebulosus* from an acid and a non-acid water physiologically different ?

MATERIALS AND METHODS

Bullheads were obtained from three lakes in the Campine region of Belgium (Table 1). Several experiments were conducted when the fishes remained on their natural water, while in some experiments an artificially made water was used (Table 1).

TABLE 1.

pH and ion concentrations (mmol/l) of the natural and artificially prepared waters.

	pH	Na^+	Ca^{++}	Al_{total}
Kooldries	6.1	0.26	0.13	0.007
Zwart Water	4.5	0.75	0.26	0.03
Zegge	7.0	0.53	0.51	B.D.
Artificially prepared water	6.8	0.44	0.03	B.D.

(B.D. = below detection limit of Al = < 0.001 mmol Al/l)

The methods used in our experiments to measure physiological parameters are given in detail in WITTERS (1986) and VANGENECHTEN *et al.* (1987). The measurements of Na^+ fluxes were performed over 4 hours. In the figures, results of Na^+-fluxes are presented at the start of the measurement period.

RESULTS AND DISCUSSION

(1) The influence of low pH (pH 4.0) and several Al-concentrations (7 ; 40 ; 110 µmol/l) was examined on the NaCl-balance, the haematocrit value and the plasma glucose and protein levels of the bullhead. Experimental animals were caught in the « Kooldries » lake (pH 6.1). They remained in their natural water for about 5 days and at t = 0, the water was acidified to pH 4.0 (Figure 1). After 84 hours at pH 4.0, two experimental groups were treated with elevated Al levels (40 and 110 µmol/l) while one group remained at pH 4.0 with the natural Al-concentration (7 µmol/l).

About 2 hours after acidification of the water to pH 4.0 a net whole body loss of Na^+ (Fig. 1 A)was established, which was entirely caused by a strongly increased efflux (Fig. 1 C). Most acid-sensitive fishes show both an increased Na^+-efflux and a decreased Na^+-influx at acute pH decreases (McDONALD and WOOD, 1981 ; McDONALD *et al.*, 1983 ; GONZALEZ and DUNSON, 1987 ; FREDA and McDONALD, 1988). Our results on the contrary showed an increase of the Na^+-influx (Fig. 1 B) after 84 hours exposure to pH 4.0 with low (7 µmol/l) Al levels. In this way, the bullhead seemed to be able to restore its Na^+-net flux. When Al levels were acutely raised up to 40 µmol/l or 110 µmol/l at 84 hours of acid exposure, Na^+-influx remained significantly higher than control values at pH 6.1 and significantly higher than acid exposure values at t = 2 hours. The negative net whole body Na^+-balance at 110 µmol Al/l was the result of a strongly increased Na^+-efflux (Fig. 1 C).

(2) In a second experiment, we investigated whether high external Ca^{++}-concentrations have an ameliorating influence on the physiology of *I. nebulosus* at low pH and high Al-concentrations.

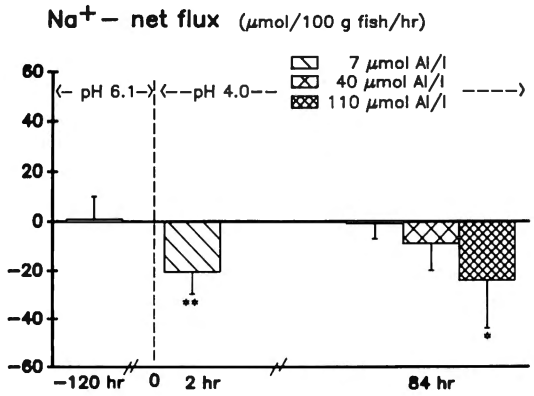
Fishes of the « Kooldries » lake which remained 3 to 4 months in artificially prepared water (Table 1) were used. After a pre-exposure to pH 4.0 for 4 days some ionoregulatory and haematological parameters were measured. Then, the physiological effects of 30 and 1000 µmol Ca^{++}/l, with and without addition of 40 µmol Al/l, were examined on the bullheads after 3 days exposure to these conditions. The exposure to low pH and to low pH with Al caused minor changes. The elevated Ca^{++}-level (1000 µmol/l) had no effect, neither at low pH, nor at low pH with Al.

(3) Finally, it was investigated whether pH-differences of natural waters can be an initiating factor in forming physiological strains of *I. nebulosus*. It was questioned whether bullheads from an acid lake are physiologically adapted to low pH and therefore exhibit some physiological differences with bullheads from a less acid lake.

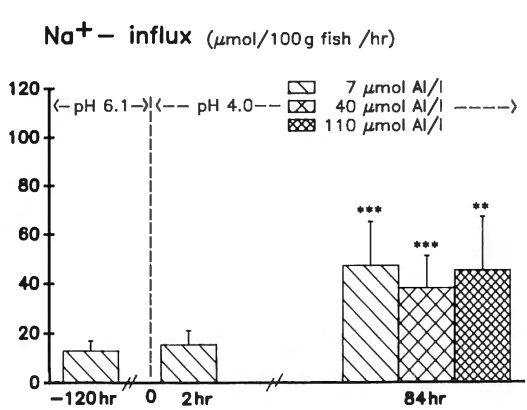
Samples of two populations of bullheads (Kooldries : pH 6.1 and Zwart Water : pH 4.5) were kept in their natural waters. Significant differences in plasma ion concentrations, haematocrit value and plasma glucose concentration were measured.

After an acclimation of 2 populations of bullheads (Zegge : pH 7.0 and Zwart Water : pH 4.5) to pH 6.8 for 5 weeks, these physiological differences disappeared,

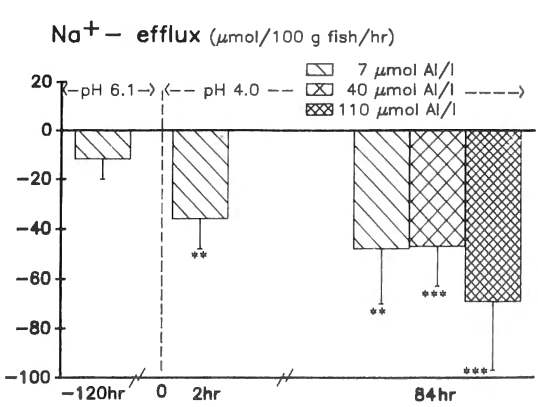
A


Na+ – net flux (μmol/100 g fish/hr)
7 μmol Al/l
40 μmol Al/l
110 μmol Al/l
<– pH 6.1–><––pH 4.0––
60
40
20
0
–20
–40
–60
–120 hr
0
2 hr
84 hr

B


Na+ – influx (μmol/100g fish /hr)
7 μmol Al/l
40 μmol Al/l
110 μmol Al/l
<– pH 6.1–><–– pH 4.0––
120
100
80
60
40
20
0
–120hr
0
2hr
84hr

C


Na+ – efflux (μmol/100 g fish/hr)
7 μmol Al/l
40 μmol Al/l
110 μmol Al/l
<–pH 6.1–><–– pH 4.0 ––
20
0
–20
–40
–60
–80
–100
–120hr
0
2hr
84hr

except for the haematocrit value. The physiological response of both populations to a subsequent acidification to pH 4.3 remained comparable during the whole course of the experiment (14 days). This is in contrast with the results of Na^+-influx measurements in the waterbug *Corixa punctata* (ILLIGER). Animals from a non-acid water exhibited a decreased Na^+-influx at low pH in contrast to animals from an acid water (VANGENECHTEN *et al.*, 1989).

CONCLUSIONS

Extremely high H^+- and Al-concentrations had a relatively small effect on the examined physiological parameters of the American bullhead. The increased Na^+-influx was a striking result. It can be an adaptive response to resist pH- and Al-stress by compensating Na+ losses.

Our second experiment indicated that *I. nebulosus* experienced no beneficial effect of high external Ca^{++}-concentrations. Some experimental evidence was obtained arguing that pH differences of natural waters have not yet given rise to physiological strains of *I. nebulosus*.

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Figure 1. — Na^+-netflux(A), Na^+-influx(B), and Na^+-efflux(C) (mean value ± 95% confidence limits) in *I. nebulosus* at several conditions of pH and Al. Significant differences were tested by a two-tailed Student 't'-test by comparison with the control value (pH 6.1) and are indicated by asterisks * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

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