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Acid-Shock, Aluminium, and Presence of *Sphagnum aurantiacum:* Effects on Embryological Development in the Common Frog, *Rana temporaria* and the Moor Frog, *Rana arvalis*

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During the last two decades, several effects of acidification have been shown, e.g., enhanced leaching of metals from sediments and soil. Futhermore, an increased growth of Sphagnum aurantiacum frequently occurs in acidified waters.

The aim of the present study is to investigate some effects of acidification on the embryological development on two Anurans.

Temporary drops in pH of minor waters occur in Sweden during thawing and periods of acid rain. If these periods correspond to sensitive stages in the embryogenesis of amphibians, an increase in larvae mortality might follow. In the present study eggs of Rana temporaria were exposed to acid water during different stages of development.

The toxicity of aluminium is thought to vary with pH. The highest toxicity of aluminium in the hydroxyl form, have been found at pH 5 (Haines 1981). Below pH 5.5, Al has been shown to have lethal and sublethal effects on salmonid fry (Baker and Schofield 1980). In the present study a laboratory experiment was performed to investigate the toxicity of Al to frog embryos in water with pH 5.0.

In acidified waters Sphagnum and especially S. aurantiacum, is competitive and quickly becomes established (Overrein et al. 1980). It has been indicated that frog spawn deposited on Sphagnum show an unusually high mortality (Fryer 1973) and questions have been raised if Sphagnum reinforces the detrimental effects of acidification on Anuran reproduction.

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MATERIALS AND METHODS

The water used in all experiments was aerated, aged Göteborg tap water which has the following characteristics:

colour	<5 mgPt/L
conductivity (25°)	15.8 mS/m
hardness	18 mgCa^{2+}/L
calcium	15.0 mgCa^{2+}/L
magnesium	1.9 mgMg^{3+}/L
aluminium	<0.05 mgAl^{+}/L
natrium	8 mgNa^{-}/L
hydrogencarbonate	18 mgHCO_3/L
cloride	10 mgCl^{2-}/L
sulfate	31 mgSO_4/L*
total chlorine	<<0.25 mgCl_2/L*
mercury	<0.1 µgHg/L

*measured before aeration.

In order to detect any differenses in sensitivity to acid water between different stages of embryological devlopment, groups of eggs of Rana temporaria were submitted to a 24 hour acid shock at different stages of development.

Frogs in amplexus were collected from three localities with Rana temporaria as the only Anuran present. Spawn clumps from these amplexus-pairs and additional, newly deposited, spawn-clumps collected at the same localities were used in the experiment.

The eggs were distributed into 35 polypropylen containers, containing 1.1 L water where they were allowed to develop until all surviving eggs were hatched.

The 35 containers, with 75 eggs in each, were divided into five groups. All groups were acid shocked as they reached a predetermined stage of development. The stages were: 2-9 (n=7), 11 (n=5), 13 (n=5), 17 (n=5) and 19 (n=5) according to the table of staging Anuran embryos by Gosner (1960). The onset of acid shock was made by reducing pH from 5.5 to 4.0. Dead eggs and embryos were removed each day and pH in treatment water measured whereafter the water was exchanged. Eight replicates that were not subjected to acid shock during the embryogenesis, served as a control group.

The eggs were exposed to pH 4 for 24 h, whereafter the pH was reset to 5.5. During the acid shock, the eggs went through several stages of development. Therefore, all stages from stage 2 until hatching were tested for sensitivity to low pH. Water temperature was 14.5°C during the experiment.

In one experiment the toxic interaction between low pH and aluminium on the embryological development of two Anuran species was investigated.

Newly deposited spawn clumps were collected from several ponds inhabited by both Rana temporaria and Rana arvalis (Species recognition was not possible). Five parallel series were arranged with 0, 100, 200, 400, 800 and 1600 µg Al/L (as $AlCl_3$ 6 H_2O), respectively. The Al exposure lasted for 8 days, which includes the entire embryological development. Each day, with the exception of the 7th day, pH was measured whereafter the Al solutions were completely renewed with the appropriate Al concentrations. Finally, pH was adjusted to 5.0.

Morphological defects on larvae were noted and dead embryos and larvae were removed daily. At four occations during the experiment (the 8th, 9th, 11th and 19th day) studies of larvae behavior were performed. The containers were agitated for five seconds which made the animals give up their foraging on the walls. The animals that did not return to the walls to feed within 30 sec were classified as larvae with disturbed feeding behavior. Water temperature was 14.5°C during the experiment.

The impact of Sphagnum on the hatching frequency of R. temporaria in acid water, was examined in a third experiment.

Ten spawn clumps of R. temporaria were collected at different localities and placed in separate 12 L plastic aquaria with water adjusted to pH 4.5. In five aquaria the bottom was covered with S. aurantiacum and the spawn clumps, containing between 586 and 1039 eggs, were placed on top. Five additional aquaria without Sphagnum were prepared with between 507 and 1222 eggs as a control group. To facilitate counting and removal of dead eggs, nylon nets separated spawn clumps from Sphagnum. Nylon nets were also present in the control aquaria. Every second day pH was measured and the water was renewed and on alternate days pH was measured and adjusted to 4.5. The experiment period lasted for 10 days and the water temperature was 18.0°C.

The study was performed during April and early May.

RESULTS AND DISCUSSION

In the present study, no altered hatching frequency, due to a 24-h reduction in pH to 4.0, could be found for any stage of development (Fig. 1).

Hatching success showed a considerable variation between different samples. All samples from a certain spawn clump showed same hatching frequency independently of the stage of development at which they had been exposed to acid shock. Thus, variation in hatching frequency between samples in a group seemed to depend more on the spawn clump from which the eggs originated, than on the developmental stage at which they were shocked by low pH.

The mean of the pH measurements each day before water exchange are presented in Table 1.

Table 1. pH before water exchange in the acid chock experiment. Values are expressed as mean ± SE. Number of analyses in each group are given within parenthesis.

Acid shocked in stage no.	pH after 24 h of acid shock:	pH when not exposed to acid shock:
2-9	4.39±0.04 (7)	5.85±0.05 (46)
11	4.41±0.02 (5)	5.81±0.06 (27)
13	4.43±0.01 (5)	5.94±0.05 (30)
17	4.30±0.03 (5)	5.96±0.06 (23)
19	4.21±0.05 (5)	5.99±0.06 (24)
control	-	5.98±0.03 (33)

Different sensitivity to low pH between separate stages during the embryological development has been observed in brown trout, Salmo trutta (Edwards and Gjerdrem 1979) and a study of American Ambystoma (Urodeles) showed that low pH caused disturbance of gastrulation and hatching but had no effect on the larvae development between these stages (Pough 1976; Pough and Wilson 1980).

However, in our investigation it seems more likely that low hatching frequencies were caused by reduced rates of fertilization due to laboratory conditions, than by acid shock during a particular stage of the embryogenesis. Low hatching frequencies occurred mainly among spawn clumps produced in the laboratory by pairs captured in amplexus. The artificial conditions may have disturbed reproduction and caused a lower fertilization percentage in these spawn clumps.

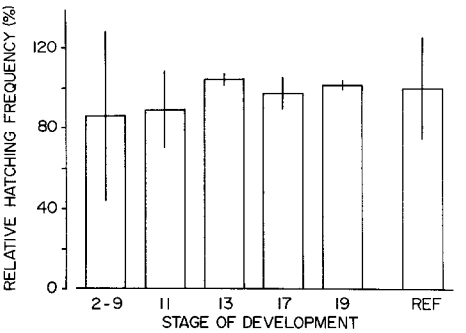

Figure 1. Relative hatching frequency of eggs from Rana temporaria subjected to acid shock during different stages of development. The relative hatching frequency was calculated as percentage hatched eggs in a sample divided by the percentage hatched eggs totally in the spawn clump from which the sample was taken. The values are expressed as the mean of (n) different samples, acid shocked at the same stage of development. Vertical lines indicate the 95 % confidence limits.

Elevated Al concentrations increased the rate of morphological defects in larvae (Fig. 2). The observed defects consisted of spinal curvatures and vesicles on head and thorax. At later stages the vesicles ruptured and caused ulceration. In addition to the morphological alterations, elevated Al concentrations caused disturbances in feeding behavior of the larvae. Animals in the control groups were constantly foraging, whereas larvae exposed to high concentrations of Al mostly lay passive on the bottom of the container (Fig. 3). The frequency of foraging individuals decreased significantly with an increase in Al concentration. No decrease in hatching frequency with elevated Al concentrations was observed.

Several studies have observed lethal or sublethal effects of Al on fish (Muniz and Leivestad 1980; Grahn 1980). The toxicity of Al to fish seems to be a combined effect of an impaired osmoregulation and a decreased respiratory capacity, which is due to mucus clogging of the gills (Muniz and Leivestad 1980). A recent study (Staurnes et al. 1984) has shown that a decrease in pH in combination with elevated Al levels inhibits the activity of Na/K-ATPase and carboanhydrase in fish gills where these enzymes are essential for the ion- and gas-exchange. This may be an explanation for the reduced viability in the exposed larvae, while the hatching frequency remained high.

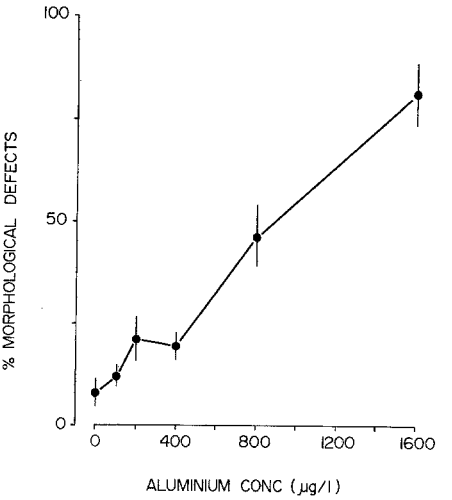

Fig. 2. Increased rate of morphological defects in larvae of Rana temporaria and R. arvalis as a function of increased aluminium concentration ($p<0.00023$, Equality against ordered alternatives (Lehman 1975)). Each group consisted of five samples. Vertical lines indicate SE.

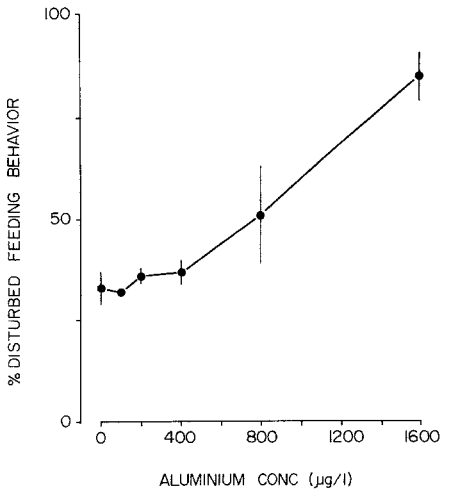

Figure 3. Increased percentage larvae of Rana temporaria and R. arvalis with disturbed foraging behavior as a function of increased aluminium concentration ($p<0.00005$, Equality against ordered alternatives (Lehman 1975)). Each group consisted of five samples. Vertical lines indicate SE.

The pH determinations before each water exchange are presented in Table 2.

Table 2. pH before water exchange in the aluminium toxicity experiment. values are expressed as mean ± SE. Number of analyses in each group are given within parenthesis.

Aluminium concentration (µg/L)	pH
0	5.58±0.05 (47)
100	5.52±0.06 (49)
200	5.56±0.05 (48)
400	5.50±0.06 (48)
800	5.41±0.05 (47)
1600	5.21±0.03 (45)

Spawn clumps incubated on Sphagnum aurantiacum showed significantly ($p<0.001$, Chi-square) lower hatching frequency (35.2 %) than control spawn clumps (67.6 %). It is notable that the water in the control aquaria showed a significant rise in pH (from 4.50 to 4.97±0.07, $p<0.001$, Mann-Whitney U-test), probably due to the carbonate buffering capacity of the water. This never happened in the aquaria containing Sphagnum. The apparent property of Sphagnum to keep pH low in its surroundings might be the reason for the decreased hatching success in frog spawn deposited on Sphagnum aurantiacum.

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