

# Effects of pH on the wintering of the common frog (*Rana temporaria* L.)

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*Received 5 January 1998, accepted 26 March 1998*

The aim of the present study was to establish whether acid water affects the behaviour and physiology of the wintering common frog. Frogs were caught from two biotopes ("neutral" and "low-pH" populations). Initially frogs were kept in containers in water with different pH where they could choose whether to be in or out of water. Then altogether 210 frogs were over-wintered at different pH and after winter the liver and muscle glycogen contents were determined from 106 frogs. In the biomonitoring experiments we tested if changes in water pH had had an effect on the total activity of wintering frogs. The same monitoring apparatus was also used to measure the heart rates of the frogs at pH 4 and pH 6.5. Following metamorphosis the common frog is very well adapted to cope with environmental acidity. It does not avoid water at pH 4 and wintering success occurs also in acid environment. In fact it is a matter of concern that mature frogs react so slightly to water acidity and therefore they obviously do not avoid acid habitats.

## 1. Introduction

Around the turn of the current decade numerous articles appeared in international literature reporting declines in frog populations across the world (e.g. Bradford 1989, 1991, Barinaga 1990, Phillips 1990, Wyman 1990, Tyler 1991, Wake 1991). One reason for the general reduction in amphibian numbers has been the overt destruction of their terrestrial and aquatic habitats. However, additional factors responsible for the decline, also influencing those regions that have not been destroyed, may include global climatic change, pollution, ultraviolet radiation or habitat fragmentation.

A reduction in amphibian populations has also been reported in Finland in the popular press but as yet there is no scientific evidence of this phenomenon. Although there are occasional and local observations of the decline (e.g. Terhivuo 1981, Raatikainen 1989, Pasanen & Sorjonen 1994), no clear changes were detected in national surveys between 1960–79 and 1980–92 (Terhivuo 1981, 1993). However, it should be emphasized that surveys of this kind are extensive and indeterminate.

If there was a widespread decline in amphibian populations in Finland, one possible and perhaps most likely cause could be environmental acidification (Anon. 1983, Kauppi *et al.* 1990).

Acid fallout has increased in Finland since the 1960s (Kauppi *et al.* 1990). According to Forsius *et al.* (1990) approximately 17% of Finnish lakes are acid.

There are five amphibian species in Finland which are likely to vary in their sensitivity to low pH. In a study carried out in Holland, the most susceptible of the Finnish species were newts (*Triturus vulgaris* and *T. cristatus*), but the populations of *Rana temporaria*, *R. arvalis* and *Bufo bufo* also decreased in acid ponds and lakes (pH < 4) (Leuven *et al.* 1986). Egg-cell fertilization and embryo development are the most sensitive phases. For instance, low pH negatively affects tadpoles of the common frog (Cummins 1986), but it is possible that acidification also effects other stages. After metamorphosis, the common frog (*Rana temporaria*) becomes mainly terrestrial in summer but seeks principally aquatic habitats for wintering (Koskela & Pasanen 1974). It is possible, for instance, that the activity of kidneys and integument of frogs in acid environment increases, and the frogs have to utilise more of their energy reserves. Wintering in an acid environment may not be as successful as in neutral water.

The aim of the present study was to establish whether acid water affects the behaviour and physiology of the wintering common frog. We tried to answer the following questions: How do young and old common frogs react and behave in autumn and winter in acid water? Can they overwinter in acid water (with or without aluminium)? Do frogs utilise more stored glycogen in acid than in neutral water?

## 2. Material and methods

Frogs, *Rana temporaria*, were captured in September 1995 from two biotopes (Rutajoki 62°N, 26°E and Iso-Tenhetty 61°34'N, 25°E) using a fence-with-traps method (Koskela & Pasanen 1974). The population of Rutajoki (pH 6.6–6.8) is referred to in this paper as the “neutral” population and that of Iso-Tenhetty (pH 5.1–5.3) as the “low-pH” population. The frogs were transferred to the Tammen Mylly Laboratory of the University of Jyväskylä in Leivonmäki where they were held prior to the experiments, the first of which began in November. Water from the River Rutajoki at ambient pH and temperature was passed through the tanks in which the frogs were kept. The frogs were marked by toe clipping (different toe for “neutral” and “low-pH” populations). The material was grouped as follows: mature indi-

viduals (length greater than 60 mm), under 1-year-old individuals (length less than 30 mm) and other immature individuals (length 30–60 mm). The total number of frogs from the “neutral” and “low-pH” populations were 161 and 94, respectively.

### 2.1. Selection of wintering site

The first experiments were carried out in November 1995. Frogs were placed in containers (length × breadth × height = 40 × 30 × 35 cm, volume 8 litres water) where they could select their position either in or out of water. Each experiment involved 12 containers and lasted 35–40 hours during which the location of the frogs was recorded nine times. Frogs from both populations were exposed to four pH levels (3.5, 4.5, 5.5 and 6.5) either with aluminium (500 mg l<sup>-1</sup>) or without. There were 6–7 frogs in each container. In total, 72 frogs from the “low-pH” population and 84 frogs from the “neutral” population were tested. Temperature during these experiments was kept below +4°C. Numbers of frogs in and out of water were expressed as percentages. The null hypothesis, H<sub>0</sub>, was that acidity alone or with aluminium would have no effect on the proportion of frogs selecting a wintering site in or out of water.

### 2.2. Wintering experiments

These experiments were carried out between 1 December 1995 and 16 April 1996. Frogs from both populations were divided among six containers with mesh covers (length × breadth × height = 26 × 35 × 17 cm, outflow pipe 12.5 cm from the bottom) through which water flowed at a rate of 22 l day<sup>-1</sup>. Each two containers received water at one of three pH levels (4.0, 5.0 and 6.5). Twenty-one frogs from the “neutral” population were placed in each of the 6 containers. In addition, further 21 frogs from the “low-pH” population were added to four of the containers (pH 4 and 6.5). Altogether, in the wintering experiment, there were 126 frogs from the “neutral” population and 84 frogs from the “low-pH” population, distinguishable by their clipped toes. Water temperature, oxygen concentration and pH were measured throughout the winter. Temperature varied between 0 and 1°C, the pH within 1–2 decimals and oxygen content was between 7 and 9 mg l<sup>-1</sup>. In April 1996 after the experiment, 106 frogs were killed and their liver and muscle glycogen contents were determined spectrophotometrically (Lo *et al.* 1970). The null hypothesis, H<sub>0</sub>, was that water acidity has no effect on the utilization of glycogen resources by wintering frogs.

### 2.3. Biomonitoring experiments

These experiments were carried out in January 1996 using a bioelectronic monitoring system earlier applied to fish

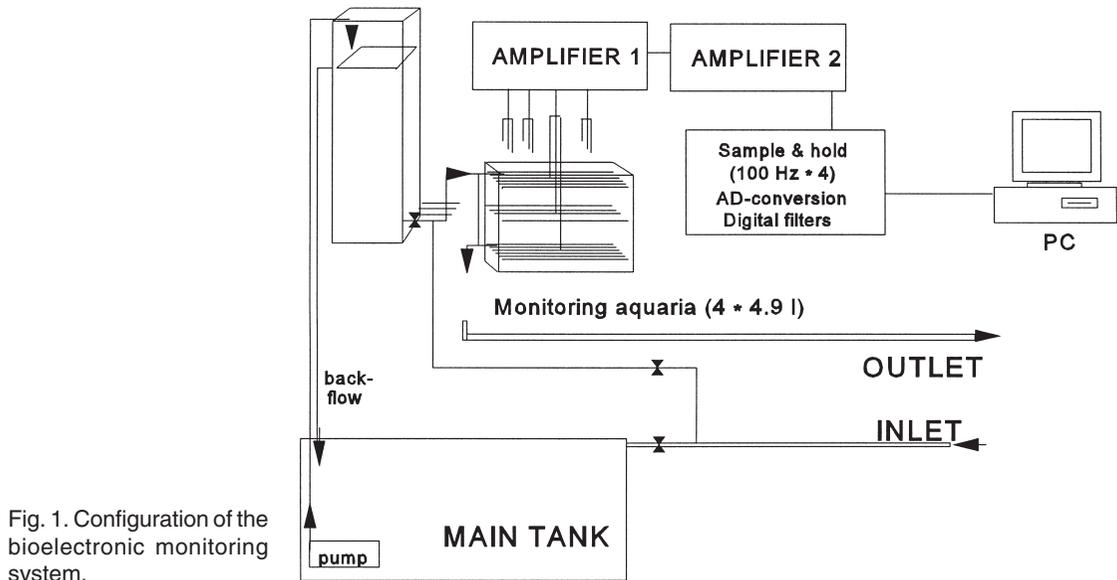


Fig. 1. Configuration of the bioelectronic monitoring system.

(Laitinen & Valtonen, 1995) (Fig. 1). The bioelectronic monitoring system (Microvolt Oy, Helsinki) can detect the muscular action potentials of one to four frogs simultaneously in an on-line manner by means of non-contact electrodes. Frogs were monitored each in an individual aquarium ((length  $\times$  breadth  $\times$  height = 27  $\times$  13  $\times$  14 cm) in which the positive and negative electrodes were placed above and below the frog and a neutral electrode between them. The frog could freely change position between the electrodes during measurement without affecting the signal quality. Signals only a few microvolts in strength are amplified and obtained at a rate of 100 Hz. They were then converted to digital mode before processing with a digital signal processing card in a microcomputer. Interference from the line frequency of 50 Hz and its harmonics were removed during the signal processing. Digital filters separated the signal into total muscular activity and an ECG (contains components from 4.5 to 50 Hz) which were saved to a file on the computer at one-minute intervals. Total activity is expressed as counts per minute i.e. the number of signal samples from a total of 6 000 per minute that exceeded the resting limits and heart rate as beats per minute, respectively.

The frogs were allowed 2–3 days of recovery from the transfer to experimental aquaria after which the water was acidified to pH 4. The frogs were then monitored for 4–5 days followed by their transfer to neutral water and further monitoring for 2–3 days. Six mature frogs from each population were studied and were kept in the dark throughout the experiments. The null hypothesis,  $H_0$ , was that changes in water pH have no effect on the total activity of wintering frogs.

In April 1996, this same monitoring system was used to measure the heart rates of frogs that had been wintering at pH 4 and pH 6.5. Five frogs from the “neutral” popula-

tion and eight frogs from the “low-pH” population were monitored at pH 6.5, and correspondingly five frogs from each population were monitored at pH 4. Frogs were allowed a 24h period of recovery from transfer to the experimental aquaria before taking measurements. Automatic recording of heart rate was possible only when the frog was at the bottom of the aquarium and because the frogs liked to stay most of the time against the wall of the aquarium, the heart rate had to be counted manually from the on-line ECG at the screen of the PC. This was carried out 3–4 times for each frog and the results were converted to beats per minute. The null hypothesis,  $H_0$ , was that water acidity has no effect on the heart rate of wintering frogs.

## 2.4. Statistical methods

Observed frequencies (in water/out of water) relative to control groups in both populations were compared using the log-likelihood statistic ( $G^2$ ) with the Williams correction. Results from the wintering experiments were tested with ANOVA and the  $t$ -test for independent samples. Differences in heart rates were tested using the Kolmogorov-Smirnov test. Individual results for frog activity were expressed graphically without statistical testing.

## 3. Results

### 3.1. Selection of wintering site

As water pH decreased, the proportion of mature frogs out of water increased in the “neutral” popu-

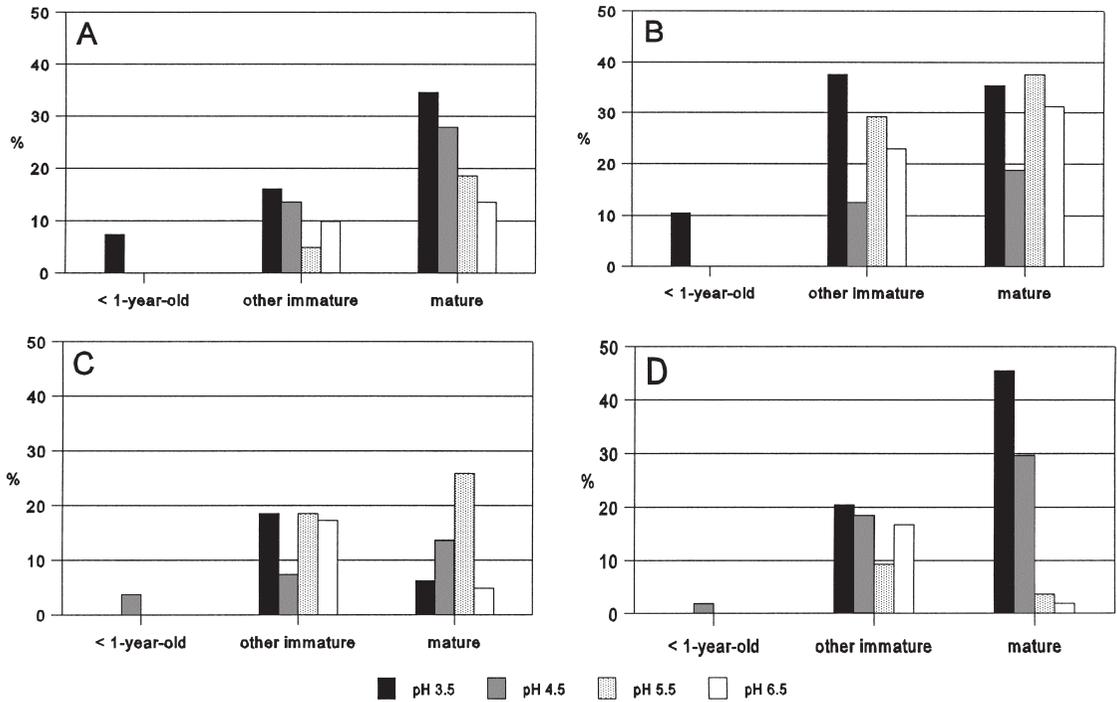


Fig. 2. Selection of wintering site by frogs, *Rana temporaria*. Bars = frogs out of water (as percents). A = "neutral" population/ no aluminium, B = "low-pH" population/ no aluminium, C = "neutral" population/ aluminium 500 mg l<sup>-1</sup>, D = "low-pH" population/ aluminium 500 mg l<sup>-1</sup>.

lation, being significant at pH 3.5 ( $x = 9.886$ ,  $g = 1.014$ ,  $p < 0.01$ ) and pH 4.5 ( $x = 4.604$ ,  $g = 1.016$ ,  $p < 0.05$ ) when compared with pH 6.5 (Fig. 2). No such trend was apparent in the frogs from the "low-pH" population (Fig. 2C). However, when acid stress was accompanied with aluminium, the frogs from the "low-pH" population showed a significantly greater preference for a wintering site out of water at pH 4.5 ( $x = 17.902$ ,  $g = 1.030$ ,  $p < 0.001$ ) and pH 3.5 ( $x = 32.033$ ,  $g = 1.021$ ,  $p < 0.001$ ) than at pH 6.5 (Fig. 2D). The mature frogs from the "neutral" population were significantly more out of water with aluminium at pH 5.5 ( $x = 14.491$ ,  $g = 1.021$ ,  $p < 0.001$ ) than at pH 6.5 (Fig. 2B). In immature frogs, there were no significant differences in wintering site preference between different pH (Fig. 2). The youngest frogs seemed to be almost always in water, except at pH 3.5.

### 3.2. Wintering experiments

The frog survived easily over winter at different water pH. Acidity had no marked effect on the

usage of glycogen reserves in the liver or muscle during wintering (Table 1). Only in the youngest frogs at pH 4 were liver glycogen content and total body glycogen content significantly lower than in pH 6.5 ( $p < 0.05$  and  $p < 0.01$ , respectively) (Table 1). No significant differences existed between the populations or between the sexes within populations.

### 3.3. Biomonitoring experiments

The reaction to a change in acid conditions varied between individual frogs (Fig. 3). Activity increased in some (frogs no. 2, 3 and 7), decreased in others (no. 4, 8, 10 and 12,) and remained unchanged in the rest (no. 1, 5, 6, 9 and 11). The heart rates of the frogs from the "low-pH" population were significantly higher in pH 4 than in pH 6.5 ( $p = 0.041$ ) (Table 2), but the corresponding values for frogs from the "neutral" population did not differ significantly ( $p = 0.320$ ). No significant differences were observed between the two populations in total activity or heart rate at pH 4 ( $p = 0.400$ ) or pH 6.5 ( $p = 0.367$ ).

#### 4. Discussion

As early as the 1950s, environmental acidity was reported to affect amphibian reproductive success and consequently the size of the population (Gosner & Black 1957). This conclusion has since been supported by many field observations (e.g. Pough 1976, Hagström 1977, Clausnitzer 1979, Leuven *et al.* 1986), and by field and laboratory experiments (e.g. Clark & Hall 1985, Freda & Dunson 1985, Andren *et al.* 1988, Frisbie & Wyman 1992, Tattersall & Wright 1996). Embryos of different amphibian species have been used as bioindicators for environmental acidification (e.g., Fernandez *et al.* 1989, Melekhova 1994).

Despite this wealth of studies the effects of acidity on adult amphibian specimens remain partly unknown and questionable. According to some studies, it seems that mature frogs are not sensitive to acidity (e.g., Pierce 1985, Aston *et al.* 1987), but are also reported observations that adult frogs avoid

acid places (e.g. Leuven *et al.* 1986, Wyman 1988). In some cases, the aluminium content of the water was found to increase the injurious effects of acidity (e.g., Cummins 1986, Leuven *et al.* 1986, Olsson *et al.* 1987, Andren *et al.* 1988).

The present study supports the assumption that frogs after metamorphosis are not very sensitive to acidity. In the wintering site experiments, mature frogs were out of water a little more often at pH 4 than at pH 6.5. Aluminium does not seem to increase this effect significantly. It is interesting that frogs from the "neutral" population were more sensitive to acidity than the adapted frogs from the "low-pH" population, and that the opposite pattern was observed in the presence of aluminium, i.e. frogs from the "low-pH" population reacted more strongly. Immature frogs and especially the youngest ones seem to remain almost constantly in water irrespective of the pH.

Frogs wintered successfully at both pH 4 and pH 6.5. According Pasanen & Koskela (1974) gly-

Table 1. Glycogen contents of the liver and muscle of the frogs from the neutral and low pH populations after laboratory wintering at different pHs.

	"Neutral" population							"Low-pH" population						
	muscle			liver				muscle			liver			
	N	x (%)	SD	x (%)	SD	x (mg g <sup>-1</sup> )	SD	N	x (%)	SD	x (%)	SD	x (mg g <sup>-1</sup> )	SD
<b>Mature</b>														
pH 4	12	0.29	0.18	2.89	1.25	0.39	0.17	10	0.22	0.13	3.70	1.18	0.45	0.17
pH 5	11	0.22	0.14	4.22	2.02	0.51	0.25							
pH 6.5	12	0.25	0.06	3.93	1.49	0.50	0.20	9	0.23	0.06	3.96	1.53	0.48	0.25
<b>Immature</b>														
pH 4	6	0.09	0.03	1.91	0.48	0.32	0.10	6	0.07	0.02	1.40	0.31	0.19	0.05
pH 5	6	0.07	0.04	1.48	1.05	0.25	0.15							
pH 6.5	6	0.08	0.02	1.38	0.34	0.31	0.17	6	0.08	0.02	1.23	0.07	0.15	0.01
<b>Under 1-year old</b>														
pH 4	2	0.04	0.03	0.22	0.05	0.03	0.01	6	0.09	0.06	0.036	0.15	0.05	0.03
pH 5	5	0.11	0.10	0.59	0.23	0.09	0.04							
pH 6.5	6	0.07	0.03	0.41	0.14	0.06	0.01	6	0.12	0.05	0.87	0.47	0.16	0.06

Liver glycogen is expressed as a percentage and as mg g<sup>-1</sup> (total glycogen content in liver/body weight)

Table 2. The heart rate of frog at two pHs.

	pH 6.5			pH 4.0		
	N	x	SD	N	x	SD
"Neutral" population	5	3.97	0.58	5	4.34	0.16
"Low-pH" population	7	3.72	0.83	4	5.35	1.26

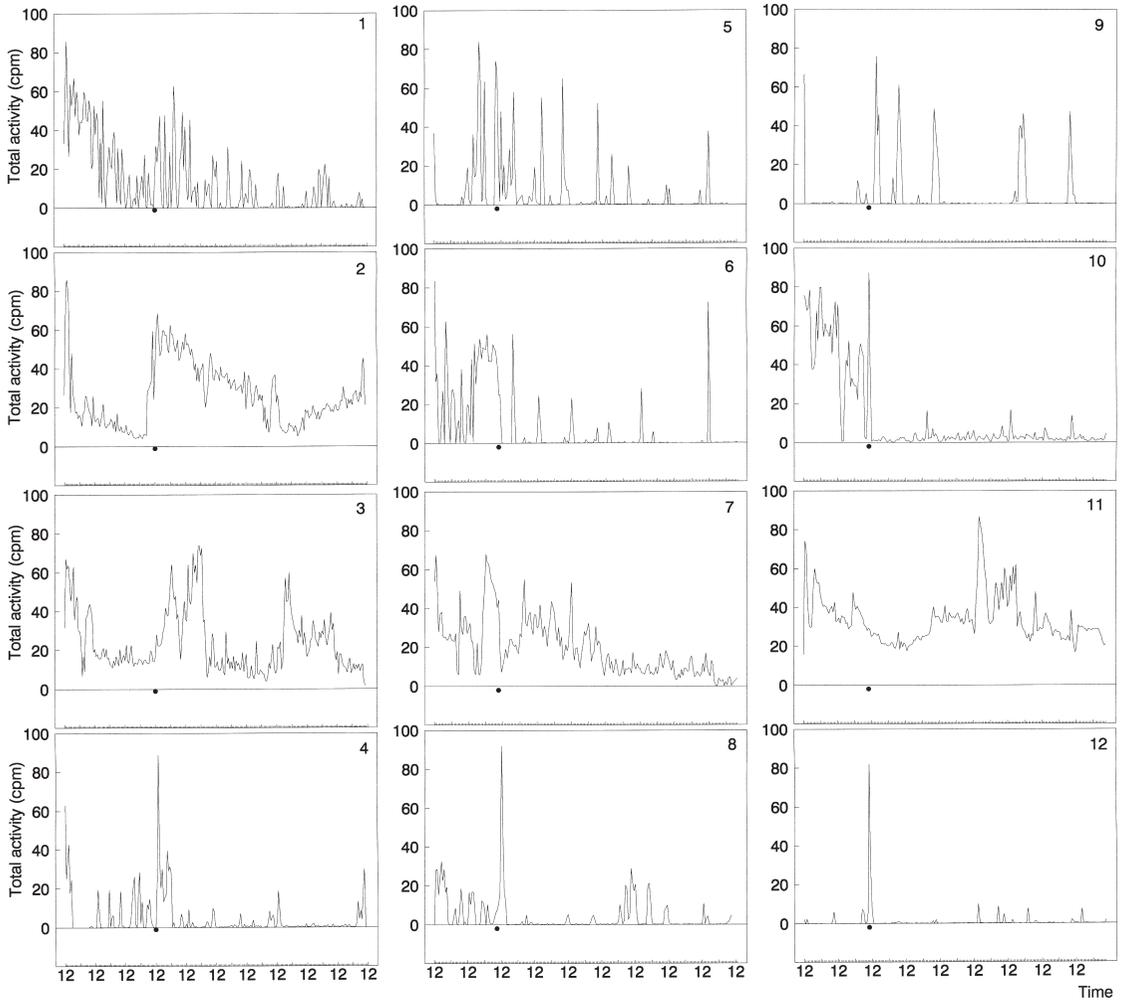


Fig. 3. Total muscular activity of frogs, *Rana temporaria*, from the "low-pH" population (1–6) and the "neutral" population (7–12) after exposure to pH 4. The asterisk below the x-axis indicates the time of change from pH 6.7 to 4.0.

cogen contents of mature frogs after the winter were about 10% in the liver, and 1.5–2% in the muscle. In the present study, the corresponding values were 4–5% and 0.2%. The differences are great, but it has to be emphasized that different methods were used in these two studies. In every case, there were no clear differences in glycogen storage between frogs in different water pH or between "neutral" and "low-pH" frog populations.

The behaviour of frogs in the biomonitoring experiment did not differ between the two populations. Acidification of the water did not cause any systematic reactions. In other words, frogs from the "low-pH" population do not seem to be better adapted to acidity than frogs from the "neu-

tral" population. However, there was one interesting finding: heart rate at pH 4 was higher in the frogs from the "low-pH" population than those from the "neutral" population. This does not appear to reflect differences in energy consumption because glycogen contents were nearly identical in frogs from the two populations.

## 5. Conclusions

Following metamorphosis the common frog is very well adapted to cope with environmental acidity. It does not avoid water at pH 4 and wintering is also successful in an acid environment.

Furthermore, aluminium content (until 500 mg l<sup>-1</sup>) has no dramatic effect.

Acidity is obviously of much greater significance to the embryo phases and this can result in declines in frog populations. As matter of fact it is worrying that mature frogs show so little reaction to acidity or aluminium content of the water and do not avoid acid places. Selection of acid waters for spawning will have serious consequences for the reproductive success of frog populations.

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