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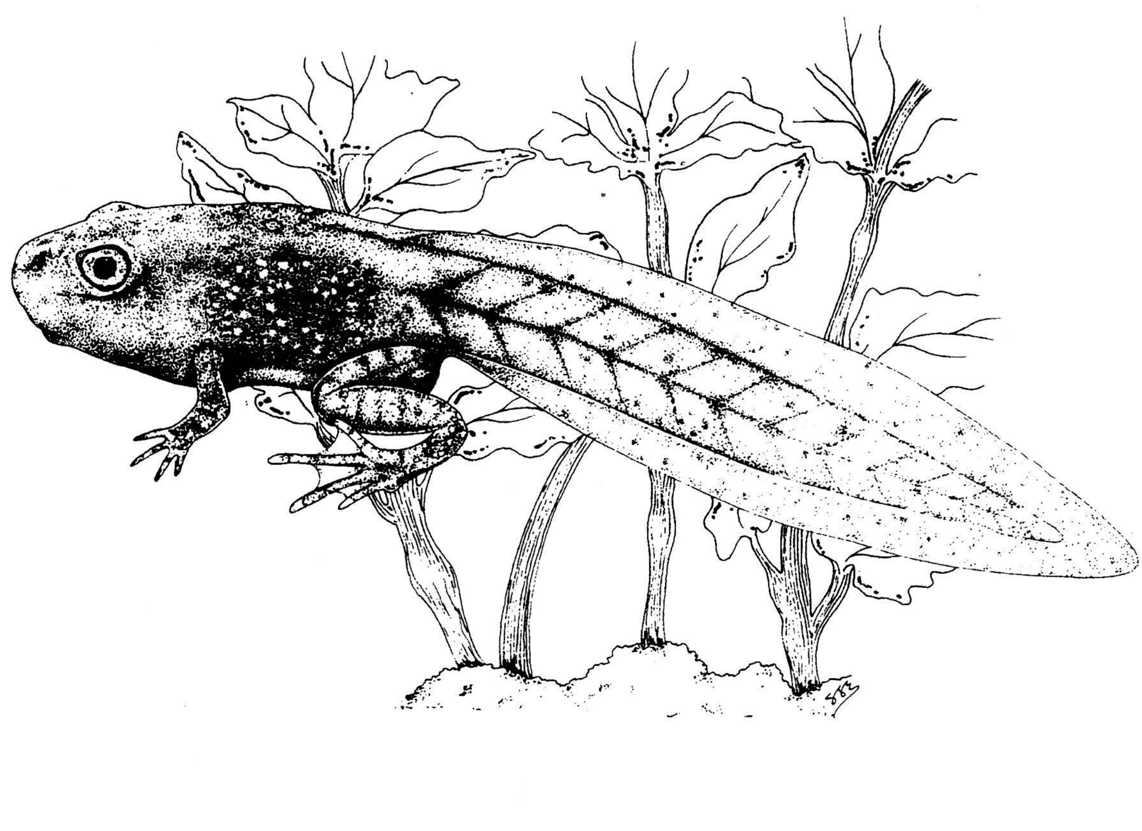

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EMBRYONIC AND LARVAL SURVIVAL OF THE COMMON FROG (*RANA TEMPORARIA* L.) IN ACIDIC AND LIMED PONDS

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ABSTRACT

Limestone was added to two acidic ponds in upland, northern England in an attempt to improve the survival of embryos and larvae of the common frog (*Rana temporaria* L.). As expected, the addition of limestone to the ponds resulted in a significant increase in both the pH and the dissolved calcium concentration of the pond water. Fertilization success of common frog eggs was approximately 87% in acidic water and increased to 100% following liming. Embryonic survival in the two acidic ponds increased from 0% and 22% to 69% and 93% respectively following liming. A year after liming, embryonic survival in one pond had decreased significantly from 93% to 79%. It was estimated that at least 2.1% of the eggs deposited in a limed pond gave rise to metamorphs. The environmental implications of liming acidic frog breeding ponds are discussed.

INTRODUCTION

The common frog (*Rana temporaria* L.) is known to breed in oligotrophic waters which are susceptible to anthropogenic acidification (Aston, Beattie & Milner, 1987; Hagström, 1981; Leuven *et al*., 1986). A number of laboratory studies indicate that acid water, with or without high concentrations of aluminium, can cause sublethal and lethal effects in common frog eggs and larvae (Andrén *et al*., 1988; Beebee & Griffin, 1977; Cummins, 1986, 1989; Linnenbach, Marthaler & Gebhardt, 1987; Olsson *et al*., 1987; Tyler-Jones, Beattie & Aston, 1989). There is concern that amphibians might be declining in areas of Britain (Fry, unpubl., cited in Fry & Cooke, 1984) and Sweden (Hagström, 1981) subjected to acidification.

The addition of limestone ($CaCO_3$) to acid waters can increase the pH and reduce the concentration of toxic aluminium species (Underwood, Donald & Stoner, 1987) and consequently increase the abundance and diversity of aquatic organisms (Eriksson *et al*., 1983; Hasselrot, Andersson & Hultberg, 1984; Raddum *et al*., 1986; Rosso, 1977). It should be noted, however, that the abundance of certain organisms can decline after the addition of limestone to acid waters, due to a variety of factors such as increased survival of predatory fish (Evans, 1989; Hultberg & Andersson, 1982).

In the present study, limestone was added to two acidic, frog breeding-ponds in northern England in an attempt to improve the survival of common frog embryos and larvae. The main aim of the present study was to assess the survival of common frog embryos and larvae in acidic ponds, before and after liming.

MATERIALS AND METHODS

DESCRIPTION OF SITES

Two acidic ponds (1.4 km apart) in northern England were used in this study, both being on open moorland with a substratum of peat. Pond 1 (altitude, 617 m OD; latitude 54° 46' 56" N. longitude 2° 18' 42" W; National Grid reference NY 800 432) and Pond 2 (altitude, 600 m OD. latitude 54° 47' 40" N, longitude 2° 19' 7" W; National Grid reference NY 796 445) were similar in depth and had surface areas of 70 m^2 and 160 m^2 respectively. Frogs have spawned in pond 1 recently (30 clumps in 1985, 22 clumps in 1988), whereas frogs stopped spawning in pond 2 around 1975.

WATER CHEMISTRY AND TEMPERATURE

Field measurements of pH and conductivity were made using a Radiometer M80 portable pH meter and an EIL MC-1 conductivity meter. Water samples from ponds 1 and 2 were filtered through a 0.45 μ membrane filter and samples for cation analysis were acidified to 1% v/v with Aristar nitric acid.

The concentrations of different aluminium species (acid soluble aluminium; total monomeric aluminium; labile, inorganic, monomeric aluminium and non-labile, organic, monomeric aluminium) were measured in ponds 1 and 2, using a modification of the catechol violet method (Dougan & Wilson, 1974; Seip, Muller & Naas, 1984).

The concentrations of Na and K were measured by flame emission spectrophotometry. Ca, Mg, Fe, B, Cu, Cd, Hg, Mn, Mo, Ni, Pb, S, Si, Ti, and Zn concentrations were measured with a Bausch and Lomb Inductivity Coupled Plasma Atomic Emission Spectrophotometer (Series No. 34000). Cl, SO_4, NO_3, and NH_4 concentrations were measured using an Ion Chromatograph (Dionex).

Maximum/minimum thermometers were placed in both ponds, next to the egg clumps, and were read and reset nine times during the study.

LIMING

Limestone ($CaCO_3$) was used because it is cost-effective and has been shown to be the best material for improving the water quality for fish (Underwood, Donald & Stoner, 1987). On 6 April 1988, powdered limestone was uniformly spread over pond 1 at a dose equivalent to 250 g m^{-2}, while pond 2 was left as an unlimed control. On 22 February 1989, a similar rate of liming was applied to pond 2. Given that both ponds were similar in depth, they each received approximately 333 mg of limestone per litre.

Fertilization Success Following Liming

In the spring of 1988, frogs laid two egg clumps in pond 1 after liming. Samples of approximately 50 eggs were taken from each clump shortly after spawning and reared in the laboratory, in conditions suitable for normal development (i.e. dechlorinated tap-water, pH 7, temperature 15°C), so that fertile eggs could be easily distinguished from infertile ones.

Embryonic and Larval Survival

Field-based experiments were performed to estimate the survival of frog embryos in 1985 (pond 1 only), 1988 (ponds 1 and 2) and 1989 (ponds 1 and 2). In 1988, estimates of embryonic and larval survival at different developmental stages were made. Details of the methods are given below.

On 16 April 1985, samples of approximately 50 eggs (all at the two- or four-cell stage; stages 3 to 4, Gosner, 1960) were taken from 30 egg clumps deposited in pond 1. These samples were placed in numbered vessels which allowed the free circulation of water, and were then returned to pond 1. On 9 May (after 23 days in pond 1), the normal embryos had reached gill circulation (stage 20, Gosner, 1960) and had hatched. The vessels were removed from pond 1 and the numbers of normal embryos hatching successfully and the numbers of dead and abnormal embryos were recorded.

On 6 April 1988, all egg clumps (23) were removed from pond 1 before it was limed. Twenty of these egg clumps (at stages 3 to 4, Gosner, 1960) were each divided into two approximately equal portions. The number of eggs in each half-clump was assessed gravimetrically and a numbered tag was attached to each half-clump. On 7 April, twenty-clumps were replaced in pond 1 (after it had been limed) and the other corresponding twenty half-clumps were placed in pond 2 (unlimed control). No eggs were deposited naturally in pond 2 in the spring of 1988.

To assess embryonic survival at different developmental stages, approximately 20 eggs were taken from each of the 40 half-clumps in ponds 1 and 2 after 7, 14 and 19 days from the start of the experiment (i.e. on 14, 21, and 26 April 1988). These eggs were returned to the laboratory and reared in conditions suitable for normal development (dechlorinated tap-water, pH 7, temperature 15°C) until all the normal embryos had reached stage 20 and had hatched. The numbers of normal embryos hatching successfully and the numbers of dead and abnormal embryos resulting from laboratory culture were recorded.

On 12 July and 8 August 1988, estimates of the numbers of frog larvae in ponds 1 and 2 were made using the successive removal method (Southwood, 1980).

On 10 April 1989, twenty spawn clumps (stages 3 to 4, Gosner, 1960) were taken from pond 1 and divided into approximately equal halves. Each half-clump was then placed in an individually numbered vessel which allowed the free circulation of water over the eggs. One half of each of the twenty clumps was then replaced in pond 1 and the corresponding twenty half-clumps placed in the recently-limed pond 2. On 8 May 1989 (28 days from the start of the experiment) the normal embryos had reached gill circulation (stage 20, Gosner, 1960) and had hatched. The vessels were removed from ponds 1 and 2 and the numbers of normal embryos hatching successfully and the numbers of dead and abnormal embryos were recorded.

Statistical Methods

The numbers of normal embryos hatching successfully and the numbers of dead and abnormal embryos were expressed as percentages. To normalize the distribution of these data, percentages were arcsin transformed (Sokal & Rohlf, 1981). Paired *t* tests and *t* tests for independent samples were the statistical tests used.

RESULTS

Water Chemistrty and Temperature

Ponds 1 and 2 were both acidic, oligotrophic ponds before liming (Table 1). The differences between ponds 1 and 2 in pH, conductivity, total monomeric Al, labile inorganic monomeric Al, Ca, K, Mg, Fe, Cl, SO_4 and NO_3 were tested statistically before and after liming (post-lime data for pond 1 in 1988 and 1989 being combined). Before liming, there was significantly more Ca, Mg and total monomeric aluminium in pond 2 than in pond 1 (t=2.64, df=10, P=0.025; t=2.60, df=9, P=0.029; t=2.68, df=5, P=0.044 respectively). Sample sizes were too small to carry out statistical tests on the other water chemistry parameters listed in Table 1. The concentration of Si in pond 2, however, appears to have been much higher than in pond 1.

In pond 1, pH and conductivity were significantly higher after liming (t=6.54, df=3, P=0.0073; t=4.05, df=10, P=0.0023 respectively) as were the concentrations of Ca, K and Mg (t=3.69, df=7, P=0.0078; t=4.17, df=8, P=0.0032; t=4.55, df=9, P=0.0014 respectively).

The pH of pond 1 increased to a maximum value of 8.04 on 14 April 1988 (eight days after liming) and it subsequently fell to a minimum value of 5.90 on 22 February 1989 (322 days after liming). From February to May 1989, the pH of pond 1 increased again to 6.53.

The conductivity of pond 1 increased to a maximum value of 204 $\mu S\ cm^{-1}$ on 14 April 1988 (eight days after liming) and it subsequently fell to a minimum value of 52 $\mu S\ cm^{-1}$ on 30 September 1988 (177 days after liming). From September 1988 to May 1989, the conductivity of pond 1 fluctuated around 76 $\mu S\ cm^{-1}$.

The Ca concentration of pond 1 rose to a maximum value of 32.8 mg l^{-1}, on 14 April 1988 (eight days after liming) and it subsequently fell to a minimum value of 2.2 mg l^{-1} on 10 April 1989 (369 days after liming). From April to July 1989, the Ca concentration of pond 1 increased steadily to 9.0 mg l^{-1}.

In pond 2, pH and the concentration of Ca increased significantly after liming (t=12.42, df=2, P=0.0064; t=4.40, df=2, P=0.048 respectively).

Both total monomeric and labile, inorganic, monomeric aluminium concentration decreased after liming, but these reductions were not statistically significant (P>0.05), probably due to the small sample sizes.

In 1985, the minimum and maximum temperatures in pond 1 during the embryonic development period (16 April - 9

	POND 1			POND 2	
	pre-lime (1985-88)	post-lime (1988)	post-lime (1989)	pre-lime (1985-89)	post-lime (1989)
pH	4.84 (3)	7.34 (12)	6.23 (4)	3.92 (13)	7.39 (3)
COND.	42 (3)	134 (9)	76 (4)	74 (10)	107 (3)
TAl	0.065 (3)	0.065 (4)	0.022 (3)	0.127 (8)	0.063 (2)
MAl	0.026 (2)	0.006 (4)	0.004 (3)	0.119 (7)	0.035 (2)
Ca	0.627 (4)	28.6 (4)	5.63 (4)	1.096 (9)	39.57 (3)
Na	4.33 (4)	3.5 (4)	5.108 (4)	4.352 (9)	5.293 (3)
K	0.583 (4)	2.690 (3)	1.333 (4)	0.493 (8)	0.943 (3)
Mg	0.451 (4)	1.033 (4)	0.903 (4)	0.681 (9)	1.227 (3)
Fe	1.357 (4)	1.183 (4)	2.695 (4)	0.554 (9)	0.343 (3)
Cl	7.3 (4)	4.918 (4)	10.37 (4)	8.25 (9)	10.293 (3)
SO_4	4.52 (4)	7.103 (3)	2.918 (4)	5.613 (8)	6.84 (3)
NO_3	0.166 (4)	0.105 (3)	0.123 (4)	0.114 (8)	0.306 (3)
NH_4	0.57 (3)			0.74 (3)	
B	0.20 (3)			0.012 (3)	
Cu	0.006 (3)			0.004 (3)	
Cd	0.006 (2)			0.006 (2)	
Hg	0.01 (2)			0.01 (2)	
Mn	0.038 (3)			0.024 (3)	
Mo	0.01 (3)			0.01 (3)	
Ni	0.011 (3)			0.011 (3)	
Pb	0.042 (3)			0.046 (3)	
S	1.67 (3)			1.92 (3)	
Si	0.099 (3)			0.271 (3)	

TABLE 1. Mean values and sample sizes (*n*) are given for pH, conductivity ($\mu S\ cm^{-1}$) and ion concentration ($mg\ l^{-1}$), measured in ponds 1 and 2. COND, conductivity; TAl, total monomeric aluminium; MAl, labile inorganic monomeric aluminium. Pond 1, pre-lime, water chemistry measurements were taken in April 1985, 1986 and 1988; post-lime measurements weretaken between April and September 1988 and between February and July 1989. Pond 2, pre-lime, water chemistry measurements were taken in April 1985 and 1986, between April and September 1988 and also in February 1989; post-lime measurements were taken between March and July 1989.

May), were 10 and 15°C respectively. In 1988, the minimum temperatures in ponds 1 and 2 during the embryonic development period (7 - 26 April) were 0 and 1°C respectively, and the maximum temperature in both ponds was 15°C. In 1989, the minimum temperatures in ponds 1 and 2 during the embryonic development period (10 April - 8 May) were 0 and 2°C and the maximum temperatures were 11 and 17°C respectively.

Embryonic Survival

When limestone was added to ponds 1 and 2, the mean percentage survival of embryos increased in the following season, from 22% to 93% and from 0% to 69.3% respectively (Table 2). Embryonic survival in pond 1 decreased from 93% to 79% a year after liming (Table 2).

In 1988, a significantly higher percentage of embryos (at all stages) survived in pond 1 (limed) than survived in pond 2 (unlimed). There was no significant difference between ponds in the percentage of abnormal eggs after seven days, but there was a significantly higher percentage of abnormal eggs in pond 1 after 14 days (Table 2).

In April 1989, a significantly higher percentage of embryos survived in pond 1 (limed in 1988) than survived in pond 2 (limed in 1989) (Table 2). There was no significant difference between ponds in the percentage of abnormal eggs (Table 2).

Larval Survival

On 7 April 1988, 6311 eggs (ie. 20 half-clumps) were placed in pond 1. On three occasions, a total of 1305 eggs were taken from pond 1 to assess embryonic survival at various developmental stages, leaving 5006 eggs in pond 1 on 26 April. On 12 July 1988, there were estimated to be 285 frog larvae (most with hind limbs; stages 36-39, Gosner, 1960) in pond 1 (ie. 5.7% survival from 5006 eggs). On 8 August 1988, there were estimated to be 106 larvae (most with front limbs: stages 42-46, Gosner, 1960) in pond 1 (ie. at least 2.1% survival). Several froglets were also found around the pond, thus survival at this time was probably underestimated due to froglets leaving the pond.

DISCUSSION

As might be expected, there was a significant increase in pH in both ponds following liming. Dissolved limestone neutralizes the acids in the water by a series of chemical reactions outlined by Underwood, Donald & Stoner (1987). The dissolved calcium concentrations in both ponds 1 and 2 increased significantly following liming by approximately 28 and 38 $mg\ l^{-1}$ respectively. A year later, the mean concentration of dissolved calcium in pond 1 had declined by 23 $mg\ l^{-1}$ to 5.6 $mg\ l^{-1}$. The concentrations of total monomeric and labile, inorganic, monomeric aluminium decreased following liming, although these reductions were not statistically significant, presumably due to the small sample sizes. The aluminium concentration in

	% normal hatch		% dead		%abnormal	
	Pond 1	Pond 2	Pond 1	Pond 2	Pond 1	Pond 2
1985	unlimed	unlimed	unlimed	unlimed	unlimed	unlimed
Stage 20	21.5 (30)	-	76.0 (30)	-	2.5 (30)	-
1988	limed	unlimed	limed	unlimed	limed	unlimed
Stage 14	89.8 (20)	9.2 (19)	7.3 (20)	88.0 (19)	3.0 (20)	2.4 (19)
	t=16.5, n=19, P<0.00001		t=15.8,n=19,P<0.00001		t=1.3, n=19,P=0.2	
Stage 17	92.5 (16)	1.8 (11)	5.9 (16)	97.7 (11)	1.6 (16)	0.5 (11)
	t=22.1, n=11, P<0.00001		t=20.8, n=11, P<0.00001		t=2.8, n=11, P=0.018	
Stage 18	93.2 (13)	-	3.6 (13)	-	3.2 (13)	-
1989	limed (1988)	limed	limed (1988	limed	limed (1988)	limed
Stage 20	79.09 (20)	69.3 (20)	16.4 (20)	27.3 (20)	4.6 (20)	3.4 (20)
	t=2.95,n=20,P<0.0082		t=4.71, n=20, P=0.0002		t=0.66, n=20, P=0.52	

TABLE 2. The fate of eggs in pond 1 (limed once in April 1988) and pond 2 (limed once in April 1989). In 1985 and 1989, newly fertilized eggs were placed in pond 1 and ponds 1 and 2 respectively, and removed when they had developed to stage 20 (gill circulation). In 1988 newly fertilized egg clumps were placed in ponds 1 and 2. Egg samples were taken from the clumps in both ponds after seven days (when the eggs were at the neural fold stage of development [stage 14]), 14 days (tail bud [stage 17]) and 19 days (muscular response [stage 18]) from the start of the experiment. The mean percentage of normal eggs hatching successfully, dead and abnormal eggs are given with the number of clumps sampled (n). Paired t test values, on arcsin transformed percentages, show the significance of the differences between ponds 1 and 2. In 1988 no values were available for pond 2 after 19 days as all the eggs had decomposed. Several of the egg clumps disappeared from pond 1 but the reason for this is unknown.

acidic waters normally decreases following liming (Hasselrot & Hultberg, 1983; Hasselrot, Andersson & Hultberg, 1984; Raddum *et al.*, 1986; Underwood, Donald & Stoner, 1987), due to the precipitation of aluminium compounds following the increase in pH (Underwood, Donald & Stoner, 1987; Wright, 1982).

The mean number of eggs (±1SE) in the clumps produced by upland *R. temporaria* was 631±38.2 (maximum 1134, minimum 403), less than half the number normally produced by lowland frogs in this area (Beattie, 1987). This has implications for the potential recruitment from upland ponds.

When *R. temporaria* eggs were laid in pond 1 after liming, infertility was estimated to be <1%. An earlier study on common frogs in this area (Beattie, Aston & Milner, 1991) showed that fertilization success in pond 1 prior to liming was 87%. Thus fertilization success increased following liming, but in general, fertilization success in the common frog is not greatly reduced in acidic conditions.

Prior to the addition of limestone, the survival of *R. temporaria* embryos in ponds 1 and 2 was 21% and 0% respectively. Mortality in these ponds probably resulted from the combined effects of low pH and high aluminium concentration (forming toxic inorganic, monomeric aluminium) (Andrén *et al.*, 1988; Clark & Hall, 1985; Clark & LaZerte, 1985; Freda & McDonald, 1990; Olsson *et al.*, 1987; Tyler-Jones, Beattie & Aston, 1989). Survival was probably lower in pond 2 because it was more acidic and had a higher aluminium concentration than pond 1. Most mortality occurred in the early stages of development (prior to the formation of the neural fold; stage 14, Gosner, 1960). In 1985, only 21% of the common frog embryos survived to hatching in pond 1. This survival value was lower than would have been predicted from laboratory studies (Tyler-Jones, Beattie & Aston, 1989), given the pH and aluminium concentration of the water in pond 1. It has been shown that pond water can be more toxic than artificial soft water under certain conditions, particularly when the pond water has a high concentration of dissolved organic carbon (Freda, Cavdek & McDonald, 1990). Other variables such as temperature may also be important.

The survival of frog embryos increased in both ponds after liming. Increased survival presumably resulted from a rise in pH. The increase in calcium concentration may have had an ameliorative effect by reducing sodium efflux at low pH (Cummins, 1988; Freda & Dunson, 1984).

A year after liming, embryonic survival in pond 1 had decreased significantly by 14%. Whether this was related to the reduction in the dissolved calcium concentration in the pond is unclear. Pond 1 had a mean pH of 6.23 and a mean total monomeric aluminium concentration of 0.022 mg l^{-1}, during the development period, which should pose no significant threat to embryonic survival. This suggests that other factors may have been responsible for this decline.

More abnormal frog embryos occurred in pond 1 after liming than in pond 2 when unlimed. This is probably because abnormal embryos are able to survive in the limed water whereas they are killed and decompose at an early stage of development in acidic water.

Larval development appeared normal and froglets successfully emerged from limed pond 1. There were estimated to be 106 larvae in pond 1, at the time of emergence (ie. 2.1% survival). This is similar to some lowland, neutral ponds where the survival of common frog larvae at emergence is approximately 1% (Savage, 1961).

Uplands ponds in this area have probably always been acidic due to the surrounding peat. In addition, they have a poor buffering capacity which makes them susceptible to anthropogenic acidification. The frogs in this area are comparatively acid-tolerant ecotypes (Tyler-Jones, Beattie & Aston, 1989). Such intraspecific variation in acid-tolerance has also been recorded in other frogs (Andrén, Mårdén & Nilson, 1989; Pierce & Harvey, 1987). Nevertheless, several ponds are too acidic to support frogs and survival is reduced in others (Aston, Beattie & Milner, 1987; Beattie, Aston & Milner, 1991).

The frogs that live in this upland area appear to be specialized ecotypes (Beattie, 1987) worthy of protection. It is important, however, that other acidophilic species in these ponds are not lost. A compromise would be to lime selected highly acidic ponds, which would restore them for use for both frogs and invertebrates (Rosso, 1977). A balance would have to be struck between saving threatened populations and the danger of diluting the acid tolerant gene pool. Liming acidic waters can be undertaken economically (Blake, 1981) and would provide a reservoir of upland frog-ecotypes in this area.

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