

# THE INFLUENCE OF ACIDIC POND WATER ON AMPHIBIANS: A REVIEW

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**Abstract:** Acidic pond water may influence the reproduction of amphibians by causing direct mortality of embryos and larvae, and/or by disrupting trophic relationships between amphibians and other aquatic organisms. The embryo is the most sensitive stage of development and may abort soon after exposure to very low pH or eventually fail to hatch at a more moderate pH. Larvae are killed by disruption of Na and Cl balance. The toxicity of pond water is governed by complex interactions of pH, temperature, and the concentrations of Al, Ca, and organic acids. The reduction in recruitment associated with acidification of ponds affects both the local distribution and abundance of amphibians. The relative importance of different mechanisms of acidification of amphibian breeding sites is unknown. Amphibian breeding ponds are generally small, low in buffering capacity, and darkly stained by humic substances, making it difficult to discriminate between the contribution of acid from atmospheric input and natural sources (such as sphagnum and organic acids). It is however quite clear that temporary ponds are extremely vulnerable to acidification by rain and that they require continued monitoring in order to detect future changes in biological and/or chemical characteristics.

## 1. INTRODUCTION

The toxic nature of surface waters low in pH and the resulting influence on the distribution and abundance of aquatic organisms have been recognized for several decades (Jewell and Brown, 1924; Gosner and Black, 1957; Smith, 1960). These early studies were conducted within the naturally acidic sphagnum and cedar bogs of Michigan and the New Jersey pine barrens. Pough (1976) was the first to report a possible effect of acidic precipitation on amphibians. He observed high mortality of embryos of the spotted salamander (*Ambystoma maculatum*) in acidic ponds near Ithaca, New York. These observations paralleled results of laboratory bioassays at similar pH's, implicating low pH as the source of toxicity. In the years following these published observations, an explosion of research investigating possible effects of low pH water on amphibians has occurred. The main objective of this review is to consolidate and evaluate all current information describing the effects of low pH water on amphibians. Special emphasis will be placed on the possible effects of acidic precipitation on amphibian populations.

## 2. THE TOXICITY OF ACIDIC WATER TO AMPHIBIANS

*Inter-specific Differences in Embryonic Tolerance* - Amphibian development passes through a series of discrete life stages: embryo, larva, and adult. Most species (in temperate North America) require water for oviposition and larval development, but adults can be aquatic, semi-aquatic, or terrestrial. As with other aquatic organisms, reproductive failure is the major effect of acidic water on amphibians (Haines, 1981). Schlichter (1981) reported that water with a pH below 6.3 decreased sperm motility and

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Table I. Summary of lethal and critical pH's of embryos of 26 species of amphibians determined under laboratory conditions. Lethal pH causes 100% mortality of embryos; the point where hatching success declines below levels in neutral water is termed the critical pH.

Species	Lethal pH	Critical pH	Investigator(s)
<b>FROGS (Anura)</b>			
<i>Hylidae</i>			
<i>Acris gryllus</i>	4.1	4.6	1
<i>Hyla andersoni</i>	3.4-3.7	3.8	1, 15
<i>Hyla crucifer</i>	3.8-4.2	4.2	1, 8, 11
<i>Hyla versicolor</i>	3.8	4.3	1
<i>Pseudacris nigrata</i>	3.8	4.1	1
<i>Pseudacris triseriata</i>	4.2	-	8
<i>Bufonidae</i>			
<i>Bufo americanus</i>	3.8-4.2	4.0-4.2	8, 11, 12, 13
<i>Bufo boreas</i>	-	4.0	3
<i>Bufo bufo</i>	4.25	4.5	2, 16
<i>Bufo calamita</i>	4.5	4.75	2
<i>Bufo woodhousei</i>	4.0	4.2	13, 15
<i>Pipidae</i>			
<i>Xenopus laevis</i>	3.0-4.1	3.5-4.5	5, 6, 7, 11
<i>Ranidae</i>			
<i>Rana arvalis</i>	3.5	4.0	16
<i>Rana catesbeiana</i>	3.9	4.3	1, 5
<i>Rana clamitans</i>	3.7-3.8	4.1	1, 15
<i>Rana esculenta</i>	4.0	4.5	16
<i>Rana palustris</i>	4.0-4.5	4.3-4.5	1, 11
<i>Rana pipiens</i>	4.5	-	8, 13
<i>Rana sylvatica</i>	3.5-4.0	3.9-4.25	1,7,8,10,11,12,13,14
<i>Rana temporaria</i>	4.25	4.5	2, 16
<i>Rana utricularia</i>	3.7	4.1	1
<i>Rana virgatipes</i>	3.4	3.8	1
<b>SALAMANDERS (Urodela)</b>			
<i>Ambystomatidae</i>			
<i>Ambystoma jeffersonianum</i>	4.0-4.5	4.5-5.0	4, 13
<i>Ambystoma laterale</i>	4.2	-	8
<i>Ambystoma maculatum</i>	4.0-4.5	4.5-5.0	4, 11, 13
<i>Ambystoma texanum</i>	4.0	5.0	9

Investigators:(1) Gosner and Black, 1957, (2) Beebee and Griffin, 1977, (3) Porter and Hakanson, 1976, (4) Pough and Wilson, 1977, (5) Saber and Dunson, 1978, (6) Dunson and Connell, 1982, (7) Tome and Pough, 1982, (8) Karns, 1983, (9) Punzo, 1983, (10) Pierce et al., 1984, (11) Dale et al., 1986, (12) Clark and Lazerte, 1985, (13) Freda and Dunson, 1985b, (14) Pierce and Sikland, 1985, (15) Freda and Dunson, 1986, (16) Leuven et al., 1986.

fertilization success in the leopard frog (*Rana pipiens*), but these results are misleading because toxic sodium acetate/acetic acid buffers were used. Dale *et al.* (1986) observed complete mortality of embryos of the African clawed frog (*Xenopus laevis*) in solutions buffered by sodium acetate/acetic acid. Karns (1983) reported 100% fertilization of eggs of the wood frog (*Rana sylvatica*), the american toad (*Bufo americanus*), and the leopard frog (*R. pipiens*) in pH 4.2 bog water. These embryos subsequently died from developmental abnormalities induced by the continued exposure to bog water. Similarly, most embryonic mortality in acidic ponds occurs during the latter stages of development, indicating little effect of acidic pond water on fertilization.

The majority of studies investigating toxic properties of acid water have focused on embryonic development because this life stage is the most sensitive to low pH (Gosner and Black, 1957; Pierce *et al.*, 1984; Dale *et al.*, 1986). The results of laboratory bioassays conducted at low pH on 26 species of North American and European amphibians are presented in Table I. I have assigned two values to each species: 1) the pH at which hatching success decreased below levels normally occurring in neutral water (critical pH), and 2) the pH at which complete mortality occurred (lethal pH). Large interspecific differences (> 1.0 pH unit) in tolerance to low pH exist and are generally correlated with habitat acidity. For example, the pine barrens treefrog (*Hyla andersoni*) and the carpenter frog (*Rana virgatipes*) are the most tolerant species tested thus far and are endemic to the naturally acidic coastal plain of the eastern United States. Documentation of inter-specific differences is also essential in predicting which species of amphibians are most vulnerable to acidic precipitation.

Differences in breeding behavior among species may also contribute to inter-specific variability in hatching success of embryos. Intra- and inter-pond variability in pH can be exploited by species able to discriminate between toxic and non-toxic oviposition sites. The pH within a pond can exhibit considerable spatial variation (> 1.0 unit; Beebee and Griffin, 1977; Pierce *et al.*, 1984), and can fluctuate weekly as well as increase gradually during spring and summer (Freda and Dunson, 1985c). The span between the critical and lethal pH of embryos of many species is only 0.2 to 0.5 units (Table I). Therefore microhabitat placement of egg masses and timing of oviposition are critical in ponds near the edge of a species' tolerance range. Salamanders can differentiate between acidic and neutral substrates, but whether they can resolve relatively small differences (< 0.5 pH units) is not known (Mushinsky and Brodie, 1975). Beebee and Griffin (1977) observed that populations of natterjack toads (*Bufo calamita*), native to naturally acidic heathland regions, refused to breed in low pH ponds lethal to embryos. Individuals transplanted to heathland areas from neutral coastal regions failed to discriminate and oviposited in lethally acidic ponds. *Rana sylvatica* and *B. americanus* were also able to discriminate between odors of high pH fens and acidic bogs in olfactometer experiments (Karns, 1983). A behavioral trait of *R. sylvatica* which may alter survival of embryos in acidic ponds is the construction of communal egg rafts containing several dozen egg masses. Hatching success in acidic bog water was higher in large egg rafts (> 65 egg masses) in comparison to small rafts (< 10 egg masses; Karns, 1983).

*Intra-specific Variation in Embryonic Tolerance* - A high degree of intra-specific variation in tolerance to low pH has also been documented. At the most basic level, considerable variability in tolerance can exist within a single egg mass. Pough (1976) noted that within a single pond, some egg masses were viable while 100% mortality occurred in others. Pierce and Sikand (1985) were the first to examine whether these inter-clutch differences had a genetic basis. They crossed 4 *R. sylvatica* females to 5 different males, resulting in 20 different male/female combinations. At pH 3.75 and 4.0, no significant differences in hatching of embryos derived from crosses of different males to a single female were found. However highly significant differences occurred between embryos taken from different females. This maternal effect may have a genetic basis or reflect environmental influences such as diet, physiological condition of female, and/or maturity of eggs.

Inter-populational variability in embryonic tolerance to low pH was first hypothesized by Cook (1983). He observed little mortality of *Ambystoma* salamanders in ponds with a pH of 4.2 in Massachusetts, whereas Pough (1976) observed high mortality

Table II. Percent mortality of wood frog embryos *Rana sylvatica* measured by different investigators under laboratory conditions.

Investigator(s)	Mortality (%)	
	at pH 3.5	at pH 4.0
Gosner and Black, 1957	100%	0%
Tome and Pough, 1982	45%	10%
Karns, 1983	-	89% <sup>c</sup>
Pierce et al., 1984	100%	0%
Clark and Lazerte, 1985	-	19% <sup>b</sup>
Freda and Dunson, 1985b	-	100%
Dale et al., 1986	-	88%
Pierce and Harvey, 1986	58% <sup>a</sup>	13%

a at pH 3.75

b at pH 4.14

c at pH 4.20

in ponds with pH's below 6.0. Unfortunately, toxic elements such as Al in pond water were not measured in these studies. Table II summarizes the results of controlled laboratory studies which have tested the low pH tolerance of *R. sylvatica* with the intent of making comparisons between geographic regions. Large intra-specific differences in lethal pH (> 0.5 pH units) are immediately apparent. Drawing conclusions from these comparisons is also risky because experimental protocols differed considerably. Under carefully controlled conditions, Pierce and Harvey (1986) tested whether this inter-population variation had a genetic basis by making half-sibling crosses of *R. sylvatica* as previously described, but using individuals from different populations. They found that different populations were different in tolerance to low pH, but as before, the degree of tolerance was not genetically determined and could be attributed to maternal factors. Tolerance of embryos from different ponds was significantly different but was not correlated with pond pH (Pierce and Harvey, 1986). The pH tolerance of embryos of *A. maculatum* in New York also was not correlated with pond pH (Tome and Pough, 1982).

The investigations of Tome and Pough (1982), and Pierce and Harvey (1986) were all conducted in the laboratory under controlled but somewhat different conditions. Differences in mortality of embryos under field conditions at a specific pH are difficult to interpret since the toxicity of pond water is governed by more than just the concentration of H<sup>+</sup>. Under field conditions, the complex interactions of pH, temperature, and the concentrations of Al, Ca and organic compounds may ultimately determine hatching success. Comparisons among laboratory bioassays which have employed vastly different experimental protocols should also be made cautiously. The concept that several independent variables interact with the physiological and biochemical adaptations of each species to produce a unique toxicological response is the major theme of this review and the subject of the next section.

*Physiological and toxicological responses of embryos* - Amphibian embryos killed by low pH display characteristic morphological traits depending on pH, species, and

developmental stage. Embryos stop developing and die within 12 hr of exposure to water less than 0.25 pH units below the pH of 100% lethality (Pough and Wilson, 1977; Freda and Dunson, 1985b). A mottled appearance of the surface layer of cells and a shrunken, rubbery texture of egg jelly and membranes are characteristic of mortality of young embryos at these extremely low pH's. The most commonly observed abnormality at slightly higher but still lethal pH (within 0.25 units of 100% lethal level) is a distinct curling of embryos within the vitelline membrane; termed the "curling defect" (Tome and Pough, 1982; Freda and Dunson, 1985b). The vitelline membrane normally lifts off the embryo at neurulation, creating the perivitelline space (Salthe, 1965; Carroll and Hedrick, 1974). At low pH, the perivitelline space never enlarges, forcing the elongating embryo to fold over itself. The degree of curling can be moderate, only causing a slight curvature, or severe enough to compress the embryo into a solid ball (Freda and Dunson, 1985b). Embryos do not curl or die if the vitelline membrane is removed (Dunson and Connell, 1982; Karns, 1983). This result suggests that mortality associated with curling results from vitelline membrane dysfunction rather than a general toxic effect on the embryo itself. It has been hypothesized that  $H^+$  invade the perivitelline space and deactivate the hatching enzyme (Gosner and Black, 1957; Dunson and Connell, 1982). The hatching enzyme of *X. laevis* *in vitro* is totally inhibited at pH 4.0; the pH of perivitelline fluid in fish embryos parallels environmental pH (Urch and Hedrick, 1981; Peterson *et al.*, 1982). The hatching enzyme is responsible for the changes in the vitelline membrane which allow it to expand in response to the osmotically driven influx of water (Salthe, 1965; Carroll and Hedrick, 1974). The digestive action of the hatching enzyme also facilitates the rupture of the vitelline membrane by muscular contractions of the embryo at hatching (Carroll and Hedrick, 1974; Yoshizaki, 1978). Curled embryos that do manage to hatch often have curved spines. These abnormal larvae are only capable of swimming in tight circles with a doubtful prospect for survival. Staining techniques have revealed that vertebrae of larvae with bent spines are actually fused (Dale *et al.*, 1986). Other less commonly observed abnormalities induced by acidic water include swelling of the thoracic region, stunted gills, failure to retract the yolk plug, and deformation of the posterior trunk (Pough and Wilson, 1977; Karns, 1983; Freda and Dunson, 1986a).

In acidic ponds (< pH 5.0), the solubility of Al increases to toxic levels (Clark and Hall, 1985; Freda and Dunson, 1986a). Dissolved Al can lower hatching success of fish and amphibians at very low concentrations (0.05 to 0.2 mg L<sup>-1</sup>) and its toxicity varies with pH and the inorganic and organic complexes that it forms (Baker and Schofield, 1980; Clark and Lazerte, 1985). The toxicity of Al to fish is attributable to Al<sup>+3</sup> and Al(OH)<sub>3</sub> while AlF is intermediate and the organically bound form is harmless (Driscoll *et al.*, 1980). Clark and Lazerte (1985) have discovered a completely different relationship between pH and Al toxicity in amphibians. *Rana sylvatica* and *B. americanus* embryos did not respond differently to Al<sup>+3</sup>, Al(OH)<sub>3</sub>, or AlF. At pH 4.75, concentrations of inorganic monomeric Al up to 100 µg L<sup>-1</sup> had no effect. However, when pH was lowered to 4.32 or 4.14, a dual threshold phenomenon was observed. Small but statistically significant increases in mortality (19 to 20%) occurred when Al was increased from 0 to 10 or 20 µg L<sup>-1</sup>, whereas no further significant increases resulted unless the concentration was elevated to 200 µg L<sup>-1</sup> (Clark and Lazerte, 1985). It is unfortunate that the toxicity of organically bound Al has not been determined because a large percentage of amphibian breeding ponds contain high concentrations of organic compounds (Freda and Dunson, 1986a). Attributing the mortality of amphibian embryos in field situations to a specific toxic element is difficult because pH and the concentrations of Al and organic compounds (DOC) are correlated. To circumvent these inter-relations, Clark and Hall (1985) artificially acidified a stream and were able to manipulate pH and Al while DOC remained constant. Hatching of *B. americanus* at pH 4.3 was reduced from 72 to 11% when inorganic monomeric Al increased from 35 to 46 µg L<sup>-1</sup>, whereas only small non-significant declines in hatching of the more low pH tolerant *R. sylvatica* occurred (91 to 83%). The physiological effects of Al have not been studied in amphibians, but this element does increase the severity of the curling defect (Clark and Lazerte, 1985). Acidified lakes can also have elevated concentrations of other heavy metals (Cu, Fe, Mn, Zn, etc.). These heavy metals have been shown to be a

major source of toxicity to amphibians in acidic mine drainage and leachates from pyritic formations (Porter and Hakanson, 1976; Mathews and Morgan, 1982). Glooschenko *et al.*, (1985) have measured toxic levels of Fe, Mn, and Al in acidic amphibian breeding ponds near Sudbury, Ontario.

Low pH and high concentrations of Al are the major causes of mortality in acidic water, but several other physical and chemical variables can modify their action. Calcium, Mg and Na have a species-specific influence on hatching success at low pH (Dale *et al.*, 1986; Freda and Dunson, 1985b). In all species tested, these cations (especially Ca) at concentrations above  $10 \text{ mg L}^{-1}$  block the inhibition of development that occurs at very low pH's. Cations alone, however, can inhibit the hatching enzyme and intensify curling in acidic water (Salthe, 1985; Urch and Hedrick, 1981; Katagiri, 1975; Freda and Dunson, 1985b). A species-specific response to the combined effects of low pH and high Ca ( $> 10 \text{ mg L}^{-1}$ ) results from a differential ability of embryos to hatch when curled. For example, Ca reduces mortality during early development at low pH in both *R. sylvatica* and *A. maculatum*, but *R. sylvatica* never hatches when severely curled whereas *A. maculatum* is able to do so (Freda and Dunson, 1985b). The concentrations of single cations needed to influence hatching success ( $> 10 \text{ mg L}^{-1}$ ) rarely occur in acidic ponds, but preliminary evidence suggests that the interaction of several cations at realistic natural concentrations ( $1 \text{ mg L}^{-1}$ ) can influence mortality at low pH (Freda and Dunson, 1985b, 1986a). Calcium also has an important role in reducing the toxicity of Al and other heavy metals in fish (Sprague and Ramsey, 1965; Brown and Lynam, 1982).

Organic compounds which commonly occur at very high concentrations in temporary forest ponds may also influence embryonic mortality at low pH. Dunson and Connell (1982) reported that the critical and lethal pH for embryos of *X. laevis* were 0.4 pH units higher in darkly stained bog water as compared to clear creek water, but these media were different in ionic composition. *Rana clamitans*, *B. woodhousei*, and *H. andersoni* also experienced high mortality in acidic bog water as compared to laboratory solutions with a similar pH and chemical composition (Freda and Dunson, 1986a).

Water temperature can also influence hatching of embryos in acidic ponds. Species-specific temperature optima exist, and a departure from optimal temperatures increases mortality at all pH's but to a higher degree at low pH (Pough and Wilson, 1977; Dale *et al.*, 1986). Mortality of early spring breeding species increases sharply below 5 C and above 15 C. Hatching success of embryos of *R. sylvatica* in a Minnesota bog was several fold higher during a warm spring (5 to 27 C) than in a cold spring (3 to 14 C), whereas pH was similar (Karns, 1983).

*Physiological effects of low pH on larvae* - Inter-specific differences in tolerance of embryos to low pH carry over into the larval stage, accompanied by a general increase in tolerance. For example, embryos of *R. pipiens* can not hatch below pH 4.5, but the lethal pH for larvae is pH 4.0 (Freda and Dunson, 1984, 1985b). In comparison, the lethal pH for embryos and larvae of the more low pH tolerant *R. sylvatica* are 4.0 and 3.5, respectively (Pierce *et al.*, 1984). Low pH tolerance of larvae also increases with age, but it is not known whether this is due to an increase in size or physiological changes during development (Pierce *et al.*, 1984, Freda and Dunson, 1985a). In contrast with studies of embryos, inter-populational differences in low pH tolerance of *R. sylvatica* larvae are genetically based and inversely correlated with pond pH (Pierce and Harvey, 1986).

Because larvae can withstand lower pH's than embryos, direct mortality of larvae in acidic ponds may not be a major limiting factor. For early spring breeding species, the pH experienced by embryos is also usually lower than for larvae, because pond pH increases during spring and summer (Freda and Dunson, 1985c). Direct mortality of larvae would therefore only occur after episodic declines in pH resulting from rainfall or snowmelt (Gascon and Bider, 1985). However, both Karns (1983) and Freda and Dunson (1986a) observed successful hatching of embryos in acidic ponds but failed to find any larvae even though pond pH remained the same or increased.

The primary physiological effect of low pH on larvae is the disruption of Na and Cl balance (Freda and Dunson, 1984, 1986b; McDonald *et al.* 1985). Acute exposure to lethal pH's inhibits active uptake of Na and Cl at the gills and promotes massive losses of these ions. The stimulation of Na efflux in fish at low pH is thought to result from

displacement of bound Ca from inter-cellular diffusion channels (in gills) by  $H^+$  (McDonald, 1983). The depression of sodium influx results from a disruption of  $Na^+/H^+$  exchange mechanisms. The inhibition of influx is quantitatively insignificant and death ensues when 50% of the body Na content is lost (Freda and Dunson, 1984). Intra- and inter-specific differences in sensitivity to low pH are correlated with the magnitude of Na efflux. Increasing the concentration of Ca in acid water increases the survival time of larvae or prevents mortality because this ion slows the lethal loss of body Na (Freda and Dunson, 1984).

Chronic sublethal exposure to low pH also reduces body sodium concentration by 10 to 20% and compensatory mechanisms which maintain ion balance are stimulated (Freda and Dunson, 1984). Sodium influx (in pH 4.5 test water) in *R. pipiens* tadpoles pre-exposed (7 d) to pH 4.5 was higher than in pH 5.8 maintained animals in pH 5.8 test water. Sodium efflux was also reduced by low pH pre-exposure (7 d; Freda and Dunson, 1984). In field studies, *R. sylvatica* tadpoles from a low pH pond (4.1 to 4.9) were 10 to 20% lower in body Na and Cl concentration than tadpoles collected from a high pH pond (5.7 to 6.4). Tadpoles from both ponds also lost Na at a faster rate when placed in the acidic pond than in the neutral pond (Freda and Dunson, 1985a)

### 3. THE INFLUENCE OF POND PH ON AMPHIBIAN POPULATIONS

Gosner and Black (1957) were the first to report an association between a species' tolerance to low pH and its abundance in acidic ponds. *Hyla andersoni* and *R. virgatipes* have a lethal pH of 3.4 and commonly breed in the lowest pH ponds (< 4.0) in the naturally acidic New Jersey Pine Barrens. *Rana catesbeiana* (lethal pH of 3.9) was restricted to isolated areas containing high pH water. Saber and Dunson (1978) reported a lowered species diversity within a sphagnum bog in central Pennsylvania (pH 4.2). *Rana clamitans* was the most abundant species whereas *R. catesbeiana* was noticeably absent. Laboratory and field exposures of embryos and larvae of both species to bog water (pH 4.2) resulted in complete survival for *R. clamitans*, and 100% percent mortality for *R. catesbeiana* (Saber and Dunson, 1978). In Great Britain, the natterjack toad (*B. calamita*) did not breed in ponds with a pH below 5.0, supporting laboratory tests which revealed high embryonic mortality below this pH (Beebee and Griffin, 1977). Cooke and Frazier (1976) reported that smooth and palmate newts (*Triturus vulgaris* and *T. helvetica*, respectively) were never found in ponds with pH's below 3.9. Smooth newts were also rarely encountered in ponds below pH 6.0. Numbers of breeding adults, egg masses, larvae, and metamorphosing frogs, and embryonic hatching success were significantly lower in acidic bogs in comparison to neutral fens (Karns, 1983). These previously mentioned investigations all dealt with naturally acidic ecosystems. In 1976, Pough reported that declines in the pH of amphibian breeding ponds were having a detrimental effect on *Ambystoma* salamanders in New York. Clark and Euler (1982) surveyed 20 streams and 21 ephemeral meltwater ponds in central Ontario for pH and species of amphibians present. Principal component analysis revealed negative associations between densities of *R. catesbeiana*, *H. crucifer*, and *R. sylvatica* and habitat acidity. Gascon and Planas (1986) conducted similar surveys of *R. sylvatica* in Quebec, but also measured hatching success of embryos. Acidity and DOC were inversely correlated with density of egg masses and percent hatching was directly correlated with pond pH. In the Netherlands, Leuven *et al.* (1986) found pond pH to have a large influence on the distribution and abundance of amphibians. In contrast, Cook (1983) found no correlation between hatching success of *Ambystoma* salamanders and pond pH, since little mortality occurred even in the most acidic ponds. Dale *et al.* (1985) also found no correlation between presence of an amphibian species and several chemical variables in 169 locations in Nova Scotia. However, in this study, calling adults were used as an indicator of presence, which does not necessarily indicate oviposition or recruitment (Freda and Dunson, 1986a).

Another powerful approach to studying the exclusion of amphibians from acidic ponds is to transplant embryos of missing species into uninhabited ponds low in pH. Low pH or Al toxicity can be confirmed by examining dead embryos for the characteristic curling defect. In central Pennsylvania, *A. jeffersonianum* did not breed in ponds with a pH

below 4.6, whereas the more low pH tolerant *R. sylvatica* was found in most ponds regardless of pH (Freda and Dunson, 1986a). Embryos of both species were transplanted into the 12 ponds surveyed. Hatching of *R. sylvatica* was not correlated with pond pH. However mortality of *A. jeffersonianum* did increase with declining pond pH and high mortality (62 to 100%) occurred in 5 of 6 ponds uninhabited by this species.

Direct mortality of embryos and larvae of amphibians in acidic ponds has an obvious effect on reproductive success, but other more subtle mechanisms may be operating. Changes in temporary pond food chains may also influence recruitment. Community structure and populations of benthic invertebrates and zooplankton on which larval salamanders feed and the phytoplankton on which tadpoles are dependent can be dramatically affected by acidification (see review by Haines, 1981). Water low in pH can also directly reduce the growth rate of tadpoles and salamander larvae (Freda and Dunson, 1985a, 1986a; Ling *et al.*, 1986). Slower growing individuals in acidic temporary ponds may not metamorphose before the time of pond drying. Smaller larvae are also more susceptible to gape-limited predators such as newts and salamander larvae (Wilbur, 1984). The outcome of intra- and inter-specific competition among amphibian larvae is heavily dependent on body size (Wilbur, 1984).

In summary, acidic water influences the distribution and abundance of amphibians through complex interactions of several chemical and physical variables (pH, temperature, Al, Ca, organic acids) and the physiological, biochemical, behavioral, and ecological attributes of each species. Predictions of embryonic mortality in acidic ponds are further complicated by temporal variation in both the chemical environment and sensitivity of embryos.

#### 4. SOURCES OF ACIDITY IN AMPHIBIAN BREEDING PONDS

I have summarized chemical surveys of amphibian breeding sites in North America and Europe (Table III). Ponds were categorized into 3 groups based on their lowest observed pH: below pH 4.5, between pH 4.5 and 5.0, and above pH 5.0. The relative distribution of pond pH's in each geographic region varies considerably. For example, 81% of ponds in the naturally acidic New Jersey Pine Barrens are below 4.5 in pH, whereas other regions range from 5 to 29%. These inter-regional differences in general levels of acidity have not been addressed but probably involve differences in geology, soils, vegetation type, and  $H^+$  loading from acidic precipitation and/or natural chemical and biological processes. In most non-agricultural regions, a large percentage of ponds have a pH below 5.0 (15 to 87%) indicating that toxicity of pond water could be a major force structuring amphibian communities.

Multivariate toxicity studies and detailed biological and chemical surveys of amphibian habitat have been conducted but no study has rigorously ascertained the source of acidity in ponds of low pH. Acidic precipitation has been identified as the major source of hydrogen ions in many lakes and streams and may also be responsible for the acidification of temporary ponds. However, the same characteristics that make temporary ponds vulnerable to acidic precipitation (small volume and low alkalinity) are also associated with vulnerability to acidification by natural processes. Sphagnum moss can acidify water by exchange of endogenous  $H^+$  for environmental Ca (Clymo, 1967; Kilham, 1982). In several studies, the ponds lowest in pH contained or were surrounded by abundant sphagnum (Freda and Dunson, 1985c; Ling *et al.*, 1986; Gascon and Planas, 1986). The sphagnum, however, may have simply invaded ponds acidified by rain or somehow increased the vulnerability of ponds to acidic precipitation (ie. lower Ca conc.). The high concentrations of organic acids in temporary ponds also can not be ignored (Dale *et al.*, 1985; Freda and Dunson, 1986a). These compounds are capable of influencing the pH of surface water and are the primary source of  $H^+$  in many naturally acidic aquatic habitats (Patrick *et al.*, 1979; Hemond, 1980). Many acidic temporary ponds also have a pH well below that of rain or snowmelt, thus lending support to the idea that natural sources of acidity are a contributing factor (Dale *et al.*, 1985; Freda and Dunson, 1985c; Gascon and Planas, 1986). Because no historical data for temporary ponds are available, one approach to assessing the influence of acid rain on pond chemistry is to measure pond pH before and after rainstorms. Pough (1976) and Cook



Table III. Summary of the distribution of pond pH in 13 regions of North America and Europe.

Location	Total no. of Ponds Surveyed	The % of Ponds with a pH		Source
		< 4.5	4.5-5.0 > 5.0	
<b>NORTH AMERICA</b>				
Massachusetts	14	28%	43%	28% Cook, 1983
Nova Scotia	153	5%	10%	85% Dale and Freedman, 1983
C. Pennsylvania	39	18%	18%	64% Freda and Dunson, 1985c
N.E. Pennsylvania	36	11%	6%	83% Freda and Dunson, 1985c
S. New Jersey	16	81%	6%	13% Freda and Dunson, 1986
E. Ontario	49	10%	14%	76% Glooschenko et al., 1985
C. Ontario	20	5%	15%	80% Clark, 1984
Quebec	15	7%	20%	73% Gascon and Planas, 1986
<b>EUROPE</b>				
England				
Hampshire	52	37%	6%	57% Cooke and Prazer, 1976
South, Agricultural	81		2%	98%
N.W., Coastal Dune	31		10%	90% Beebee and Griffin, 1977
South, Heathland	93		48%	52%
The Netherlands	96	29%	32%	39% Leuven et al., 1986

a pH 4.0-5.0

b pH < 4.0

(1983) observed large declines in pH (0.25 to 0.75) after rainstorms, but Freda and Dunson (1985c) and Pierce (1985) found no correlation between weekly pond pH and atmospheric  $H^+$  loading and did not observe significant declines in pH after rainstorms. This type of assessment is, however, too simplistic because percolation of rain water through peat deposits or organically enriched, water saturated soils can acidify ponds (Hemond, 1980). The fact that ponds are lowest in pH early in spring is not, however, diagnostic of acidification by snowmelt because the pH of naturally acidic ponds also increases during spring and summer due to higher temperatures and biological activity. The major point of this discussion is to illuminate possible difficulties in rigorously determining the relative contribution of acidic precipitation to the acidity of temporary ponds. Detailed bio-geochemical studies measuring ion and water budgets of a large series of ponds are needed before accurate conclusions can be drawn. It is however quite clear that temporary ponds are extremely vulnerable to acidification because of their very low alkalinity (0 to 2 mg  $L^{-1}$   $CaCO_3$ ; Clark and Euler, 1982; Dale *et al.*, 1985; Freda and Dunson, 1985c; Gascon and Planas, 1986). Due to the pervasive character of acidic precipitation and the finite buffering capacity of soils and waters, future declines in pH should be anticipated. Even further modest declines in pH (0.2 units) may have substantial effects on amphibian populations because large percentages of ponds are already below pH 5.0, close to the lethal pH of many species.

Much more information is needed if we are to detect and deal with current and future acidification of amphibian breeding ponds. For example, many extensive areas of North America which experience acidic precipitation have not been surveyed. In addition, virtually all studies have narrowly focused on amphibians which breed in temporary ponds. Plethodontid salamander communities are equally at risk because many species within this taxonomic group live and breed in mountain headwater springs and seeps, which experience drastic episodic declines in pH after snowmelt and rainstorms (Corbett and Lynch, 1982; DeWalle *et al.*, 1984).

In conclusion, pond acidity is currently having a major effect on the local distribution and abundance of temporary pond amphibians. Continued monitoring is necessary to determine the precise role of natural and man-made processes in pond acidification.

#### LITERATURE CITED

- Baker, J.P. and Schofield, C.L.: 1980, *Water Air Soil Pollut.* **18** , 289.
- Beebee, T.J.C. and Griffin, J.R.: 1977, *J. Zool., Lond.* **181** , 341.
- Brown, D.J.A. and Lynam, S.: 1982, *Bull. Envir. Contam. Toxicol.* **30** , 582.
- Carroll, E.J. and Hedrick, J.L.: 1974, *Devel. Biol.* **38** , 1.
- Clark, K.L.: 1984, unpublished observations.
- Clark, K.L. and Euler, D.: 1982, *The Importance of pH and Habitat Disturbance in Amphibian Distributions in Central Ontario*. Technical Report of the Wildlife Habitat Component, Lakeshore Capacity Study. Ontario Ministry of Natural Resources, Queen's Park, Toronto.
- Clark, K.L. and Hall, R.J.: 1985, *Can. J. Zool.* **63** , 116.
- Clark, K.L. and Lazerte, B.D.: 1985, *Can. J. Fish. Aquat. Sci.* **42** , 1544.
- Clymo, R.S.: 1967, 'Control of cation concentrations, and in particular of pH, in *Sphagnum* dominated communities'. p. 273-284. In: *Chemical Environment in the Aquatic Environment*. H.L. Golterman and R.S. Clymo eds. N. V. Noord-Hollandsche Uitgevers Maatschappij - Amsterdam 1967.
- Cook, R.P.: 1983, *Biol. Cons.* **27** , 77.

- Cooke, A.S. and Frazer, J.F.D.: 1976, *J. Zool., Lond.* **178** , 223.
- Corbett, E.S. and Lynch, J.A.: 1982, 'Rapid fluctuations in streamflow, pH, and associated water quality parameters during a stormflow event'. p. 461-464. *Proc. Inter. Sympo. Hydrometeorol. Am. Water Resour. Assoc.* June 13-17, Denver, CO.
- Dale, J. and Freedman, B.: 1983, *Effects of Acidity on Nova Scotia Amphibians*. First Annual Report for the Canadian Wildlife Service and Institute for Resource and Environment Studies. Dalhousie University, Halifax, Nova Scotia.
- Dale, J.M., Freedman, B. and Kerekes, J.: 1985, *Can. J. Zool.* **63** , 97.
- Dale, J.M., Freedman, B. and Kerekes, J.: 1986, *Proc. N.S. Inst. Sci., in press.*
- DeWalle, D.R., Sharpe, W.E., Leibfried, R.T., Dinicola, R.S., Kimmel, W.G. and Sherwin, L.S.: 1984, *J. Environ. Qual.* **13** , 619.
- Driscoll, C.T., Baker, J.P., Bisogni, J.J. and Schofield, C.L.: 1980, *Nature* **284** , 161.
- Dunson, W.A. and Connell, J.: 1982, *J. Herp.* **16** , 314.
- Freda, J. and Dunson, W.A.: 1984, *Physiol. Zool.* **57** , 435.
- Freda, J. and Dunson, W.A.: 1985a, *Copeia* **1985(2)** , 414.
- Freda, J. and Dunson, W.A.: 1985b, *Can. J. Zool.* **63** , 2649.
- Freda, J. and Dunson, W.A.: 1985c, *The Effect of Acidic Precipitation on Amphibians breeding in Temporary Ponds in Pennsylvania*. U.S. Fish and Wildlife Service, Eastern Energy and Land Use Team. Biol. Report 80(40.21), Box 705, Kearneyville, WV, 25430.
- Freda, J. and Dunson, W.A.: 1986a, *Copeia* **1986(2)** , 454.
- Freda, J. and Dunson, W.A.: 1986b, *J. Comp. Physiol. B*, in press.
- Gascon, C. and Bider, J.R.: 1985, *Can. Field-Nat.* **99** , 259.
- Gascon, C. and Planas, D.: 1986 *Can. J. Zool.* **64** , 543.
- Glooschenko, V., Weller, W. and Eastwood, C.: 1985, *Amphibian Abundance and Distribution with Respect to Environmental Contaminants and pH Stress Adjacent to Sudbury, Ontario*. Technical report of Wildlife branch, Ontario Ministry of Natural Resources, Queen's Park, Toronto.
- Gosner, K.L. and Black, I.H.: 1957, *Ecology* **38** , 256.
- Haines, T.A.: 1981, *Trans. Am. Fish. Soc.* **110** , 669.
- Hemond, H.F.: 1980, *Ecol. Monogr.* **50** , 507.
- Jewell, M.E. and Brown, H.W.: 1929, *Ecology* **10** , 427.
- Karns, D.R.: 1983, *Toxic Bog Water in Northern Minnesota Peatlands: Ecological and Evolutionary Consequences for Breeding Amphibians*. Ph.D. Thesis, University of Minnesota, Minneapolis, MN.
- Katagiri, C.: 1975, *J. Exp. Zool.* **193** , 109.
- Kilham, P.: 1982, *Michigan Botanist* **21** , 159.

- Leuven, R.S.E.W., den Hartog, C., Christiaans, M.M.C and Heijligers, W.H.C.: 1986, *Experientia*, in press.
- Ling, R.W., VanAmberg, J.P. and Werner, J.K.: 1986, *Ambystoma maculatum* and *Rana sylvatica* in Upper Michigan'. *J. Herpetol.*, in press.
- Mathews, R.C. and Morgan, E.L.: 1982, *J. Environ. Qual.* **11** , 102.
- McDonald, D.G.: 1983, *Can. J. Zool.* **61** , 691.
- McDonald, D.G., Ozog, J.L. and Simons, B.P.: 1985, *Can. J. Zool.* **62** , 2171.
- Mushinsky, H.R. and Brodie, E.D.: 1975, *Amer. Midl. Nat.* **93** , 440.
- Patrick, R., Matson, B. and Anderson, L.: 1979, 'Streams and lakes in the Pine Barrens'. p. 169-193. In: *Pine Barrens: Ecosystems and Landscape*. R.T.T. Forman, ed. Academic Press, N.Y.
- Peterson, R.H., Daye, P.G., Lacroix, G.L. and Garside, E.T.: 1982, 'Reproduction in fish experiencing acid and metal stresses'. p. 177-196. In: *Acid Rain / Fisheries*. R.E. Johnson, ed. Am. Fish. Soc., Bethesda, MD.
- Pierce, B.A.: 1985, Unpublished observations.
- Pierce, B.A. and Harvey, J.M.: 1986, *Copeia*, in press.
- Pierce, B.A., Hoskins, J.B. and Epstein, E.: 1984, *J. Herp.* **18** , 159.
- Pierce, B.A. and Sikand, N.: 1985, *Can. J. Zool.* **63** , 1647.
- Porter, K.R. and Hakanson, D.E.: 1976, *Copeia* **1976(2)** , 327.
- Pough, F.H.: 1976, *Science* **192** , 68.
- Pough, F.H. and Wilson, R.E.: 1977, *Water Air Soil Pollut.* **7** , 307.
- Punzo, F.: 1983, **31** , 467.
- Saber, P.A. and Dunson, W.A.: 1978, *J. Exp. Zool.* **204** , 133.
- Salthe, S.N.: 1965, *Physiol. Zool.* **38** , 80.
- Schlichter, L.C.: 1981, *Can J. Zool.* **59** , 1693.
- Smith, R.F.: 1960, *An Ecological Study of an Acid Pond in the New Jersey Coastal Plain*. Ph.D. Thesis, Rutgers University, New Brunswick, N.J.
- Sprague, J.B. and Ramsey, B.A.: 1965, *J. Fish. Res. Bd. Canad.* **22** , 425.
- Tome, M.A. and Pough, F.H.: 1982, 'Responses of amphibians to acid precipitation'. p. 245-254. In: *Acid Rain / Fisheries*. R.E. Johnson, ed. Amer. Fish Soc. Bethesda, MD.
- Urch, U.A. and Hedrick, J.L.: 1981, *Arch. Biochem. Biophys.* **206** , 424.
- Wilbur, H.M.: 1984, 'Complex life cycles and community organization in amphibians'. p. 195-224. In: *A New Ecology - Novel Approaches to Interactive Systems*. P.W. Price, C.N. Slobodchoff, and W.S. Gaud, eds. John Wiley and Son, N.Y.
- Yoshizaki, N.: 1978, *J. Exp. Zool.* **203** , 127.