LONG TERM MONITORING OF AMPHIBIAN POPULATIONS WITH RESPECT TO THE EFFECTS OF ACIDIC DEPOSITION

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Abstract. Amphibians breed in a variety of aquatic habitats in the United States. While the tolerance of low pH has been examined for many species of amphibians, those that breed in temporary ponds have been the most studied in terms of acidic precipitation. Some of the latter have been shown to have lethal limits for low pH that are close to or above the pH's found in many breeding ponds. Temporary ponds occur in large numbers in the areas of the country most affected by acidic precipitation. Many of these ponds are susceptible to acidification and reduced reproductive success of sensitive species of salamanders has been observed in acidic ponds. Additional practical and logistical considerations clearly make temporary ponds, and certain salamanders that breed in them, the best candidates for monitoring the long term effects of acidic precipitation on amphibian populations. We recommend a cost efficient monitoring scheme across three categories of acidic temporary ponds (low, medium, and high) that incorporates (a) chemical analyses of pond samples, (b) a census of the annual deposition of egg masses, as well as assessment of embryonic mortality in enclosed and unenclosed egg masses in the ponds, and (c) sampling of larval success. This plan offers the best opportunity for detecting changes in amphibian populations that are correlated with the acidity of precipitation, and would provide desperately needed long term data on the abundance of these amphibians.

1. Introduction

The objective of this report is to present the most practical monitoring plan for detecting future changes in amphibian populations that result from continued or reduced rates of acidic deposition. Before such a plan is discussed in detail, a very brief review of the toxicological effects of acidity on amphibians is given. These toxicological mechanisms are complex and incompletely known, but they ultimately define sampling strategies. This review is intended to update a previous summary (Freda, 1986) and provide justification for the inclusion of particular parameters in the monitoring plan.

2. Amphibian Breeding Habitats

Amphibians inhabit and reproduce in a wide variety of habitats, ranging from rain puddles to lakes. In order to conduct a long term monitoring program, specific habitat types and the species assemblages which inhabit them must be carefully chosen. The chemical and biological responses to a reduction in pH of each habitat type are likely to be different. Each habitat also requires different sampling methods. Discussed below are the major habitat types in which amphibians are known to breed, the sensitivity of these habitats to acidification, and the species which breed in them.

2.1. Temporary ponds

Temporary ponds, also referred to as ephemeral, vernal, or autumnal ponds, are usually small (<30 m diameter), shallow (<1 m) bodies of water that dry during the summer and refill by rainfall and snowmelt in either the fall or late winter/ early spring. In spite of this harsh hydrological regime, temporary ponds harbor a diverse fauna (Wiggins *et al.*, 1980). These ponds can become acidified regionally via anthropogenic and/or endogenous sources of acidity. In the eastern U.S. they are often low in Ca (<5 mg L^{-1}) and alkalinity (0 to 2 mg L^{-1}); the latter makes them susceptible to acidification (Clark, 1986a, b; Freda and Dunson, 1986; Gascon and Planas, 1986; Albers and Prouty, 1987; Freda *et al.*, 1990a, b). In the northeastern U.S. the pH's of temporary ponds are usually lowest in spring immediately after snowmelt, and increase throughout the spring and summer until drying occurs (Pierce *et al.*, 1984; Freda and Dunson, 1985a, 1986; Freda *et al.*, 1990a).

The effect of large rainstorms on the pH's of temporary ponds may be specific to each pond. Pough (1976) and Cook (1983) observed large declines (0.25 to 0.75 of a unit) after rainstorms, but Freda and Dunson (1985c) and Pierce *et al.* (1984) found no correlation between pond pH, the volume of precipitation, or H^+ loading during the previous week. Albers and Prouty (1987) reported that 5 of 11 ponds exhibited a significant positive relationship between pH and the volume of precipitation that they received.

Other findings suggest that natural sources of acidity may be important in determining the pH of some temporary ponds. In two studies (Freda and Dunson, 1986; Gascon and Planas, 1986), all ponds with a pH below 4.5 contained dense growths of *Sphagnum* moss. *Sphagnum* is well known for its ability to acidify water (see reviews by Given, 1975; Clymo and Hayward, 1982; Kilham, 1982). Another source of naturally generated acidity in temporary ponds is dissolved organic acids derived from decaying vegetation. Many temporary ponds with a low pH also have high concentrations (>5 mg L^{-1} DOC) of organic acids (Clark and Hall, 1985; Clark, 1986a, b; Gascon and Planas, 1986; Freda *et al.*, 1990a).

In North America, it has been estimated that 30% of all salamander species and 50% of all frog species use temporary ponds for breeding (Pough and Wilson, 1977). This includes, for example, salamanders (*Notophthalmus* spp., *Ambystoma* spp.), the wood frog (*Rana sylvatica*), toads, (*Bufonidae*), and tree frogs (*Hylidae*), with many such species breeding exclusively in ephemeral ponds. In the northeastern U.S., eggs are deposited early in the spring, thereby providing adequate time for development and metamorphosis before the ponds dry. The life stages most sensitive to low pH are embryos and young larvae. Unfortunately, these life stages are present

TABLE I

Summary of lethal and critical pH levels of embryos of 27 species of North American and European amphibians determined with laboratory toxicity tests. Lethal pH causes 100% mortality of embryos and critical pH refers to highest pH which significantly increases mortality above control levels (from Freda 1986, with updates)

Species	Lethal pH	Critical pH	References
FROGS (Anura)			
Hylidae			
Acris gryllus	4.1	4.6	1
Hyla andersoni	3.4–3.7	3.8	1,15
H. crucifer	3.8–4.2	4.2	1,8,11
H. versicolor	3.8	4.3	1
Pseudacris nigrita	3.8	4.1	1
P. triseriata	4.2	–	8
Bufonidae			
Bufo americanus	3.8–4.2	4.0–4.2	8,11,12,13,17,21
B. boreas	–	4.0	3
B. bufo	4.25	4.5	2,16
B. calamita	4.5	4.75	2
B. woodhousei	4.0	4.2	13,15
Pipidae			
Xenopus laevis	3.0–4.1	3.5–4.5	5,6,7,11
Ranidae			
Rana arvalis	3.5	4.0	16,18
R. catesbeiana	3.9	4.3	1,5
R. clamitans	3.7–3.8	4.1	1,15
R. dalamtina	4.0	–	18
R. esculenta	4.0	4.5	16
R. palustris	4.0–4.5	4.3–4.5	1,11
R. pipiens	4.2–4.5	4.6	8,13,19,20
R. sylvatica	3.5–4.0	3.9–4.25	1,7,8,10,11,12,13,14
R. temporaria	4.0–4.25	4.5	2,16,18
R. utricularia	3.7	4.1	1
R. virgatipes	3.4	3.8	1
SALAMANDERS (Caudata)			
Ambystomatidae			
Ambystoma laterale	4.2	–	8
A. jeffersonianum	4.0–4.5	4.5–5.0	4,13
A. maculatum	4.0–4.5	4.5–5.0	4,11,13,17
A. texanum	4.0	5.0	9

References: 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, Punzon, 1983; 10, Pierce *et al.*, 1984; 11, Dale *et al.*, 1985a; 12, Clark and Lazerte, 1985; 13, Freda and Dunson, 1985b; 14, Pierce and Sikand, 1985; 15, Freda and Dunson, 1986; 16, Leuven *et al.*, 1986; 17, Clark and LaZerte, 1987; 18, Andren *et al.*, 1988; 19, Freda and McDonald, 1990; 20, Freda *et al.*, 1990a; 21, Freda and McDonald, 1990.

in the ponds when the water is lowest in pH, sometimes at or below lethal levels (Table I).

Temporary ponds have been the most intensively studied amphibian breeding habitat due to their a) susceptibility to acidification, b) small size and accessibility, c) array of breeding amphibians that lay highly visible egg masses within a short period of the year, making it feasible to estimate adult populations and quantify the number and survival of embryos, and d) suitability for experimental community simulations with micro- and mesocosms.

2.2. Lakes, rivers, and permanent ponds

Lakes and streams inhabited by amphibians have been acidified by acidic deposition in some regions. Detailed information on the sensitivity to acidification and the regions most impacted is discussed in detail in Baker *et al.* (1991).

Within the northeastern US, the bullfrog (*R. catesbeiana*) and the green frog (*R. clamitans*) commonly inhabit lakes and permanent ponds, where their territoriality and protracted breeding season are in stark contrast to the explosive breeding behavior of temporary pond amphibians. Therefore, embryos are not present immediately after snowmelt when pH's might be lowest. Laboratory toxicity tests have shown that the eggs and larvae of the bullfrog and the leopard frog (*R. pipiens*) are more sensitive to acidity relative to other species tested (Freda and Dunson, 1984, 1985b). Only one study has investigated the effects of acidity on amphibians which inhabit permanent ponds. Harte and Hoffman (1989) reported that an adult tiger salamander population in the Rocky Mountains of Colorado has declined by 65% over the past 7 yr. They attribute this decline to acidic precipitation as they found that the LC_{50} for these salamanders' eggs was similar to pond pH's during annual snowmelt.

2.3. Bogs

The dense growth of sphagnum moss and high concentrations of organic acids in bogs cause them to be naturally acidic (Gorham *et al.*, 1985). In many bogs, the pH is low enough to exclude acid-sensitive species of amphibians by direct toxicity (Saber and Dunson, 1978; Karns 1983; Freda and Dunson, 1986). Indeed some bog dwelling frogs such as *Hyla andersoni* and *Rana virgatipes* are among the most acid tolerant amphibians known (Table I). Humic and fulvic acids have been shown to be toxic to amphibian embryos, but only at pH's below 4.5 (Freda *et al.*, 1990a). Therefore, if the acidity of a bog is increased as a result of acidic precipitation (Gorham *et al.*, 1984), embryos might be killed directly by the H^+ or indirectly from the heightened toxicity of the endemic humic and fulvic acids.

2.4. Headwater streams and seeps

Many species of plethodontid salamanders live in headwater streams, seeps, and springs (Bishop, 1967), habitats that are susceptible to precipitation induced acid and aluminum pulses (Corbett and Lynch, 1982; Sharpe *et al.*, 1984; Hooper and

Shoemaker, 1985). Yet scant information is available concerning the effects of acidity on these stream-dwelling salamanders (Huckabee *et al.*, 1975; Mathews and Morgan, 1982; Roudebush, 1988).

3. Mechanisms of Acid Toxicity

This next section is intended to illustrate the current difficulty of predicting the toxicity of breeding waters solely on the basis of their chemical parameters. Therefore, our subsequently suggested monitoring plan includes concomitant chemical and biological measurements for an adequate evaluation of the toxicity of pond waters.

3.1. Mechanism of Action

In pH's $>$ 0.25 units below lethal limits, amphibian embryos tend to die early in development. In pH's $<$ 0.25 units below lethal pH's, embryos may develop normally, but due to insufficient expansion of the vitelline membrane (which immediately surrounds the embryo), the growing embryos become tightly curled, a syndrome called the 'curling defect'. In most species, such embryos do not hatch, or if they hatch are deformed. The curling defect is not a result of direct toxicity to the embryo itself, but may result from osmotic constraints on the volume of the perivitelline space, in conjunction with deactivation of a hatching enzyme that digests the vitelline membrane prior to hatching (Urch and Hedrick, 1981; Dunson and Connell, 1982; Robb and Toews, 1987).

The physiological effects of acidity on amphibian larvae have been well studied (Freda and Dunson, 1984, 1985b; McDonald *et al.*, 1984; Freda and McDonald, 1990) and are similar to effects observed in fish and invertebrates (McDonald, 1983; Hollett *et al.*, 1986). Variation in the acid tolerances of different species of amphibian larvae is the result of the differential rate of ion loss at low pH (Freda and Dunson, 1984). M. Frisbie and R. Wyman (unpub. obs.) have observed a very similar effect in terrestrial salamanders placed on acidic substrates.

3.2. Relative Sensitivity of Particular Stages in the Life History

For aquatic-breeding species, embryos are clearly the life stage most sensitive to acidity. In studies that used similar methodology to test both embryos and larvae, the larvae were able to survive at pH levels from 0.25 to 0.75 units lower that the lethal limits for embryos (Freda and Dunson, 1984, 1985a, b; Clark and LaZerte, 1985; Freda and McDonald 1990). After hatching, tolerance to acidity increases throughout larval development (Pierce *et al.*, 1984; Freda and Dunson, 1985b). Only a few studies have tested the toxicity of acidity to adult amphibians, but these studies have found adults to be very tolerant, with pH levels below 3.5 generally required to induce mortality (Dale *et al.*, 1985a, b; Wyman and Hawksley-Lescault, 1987).

3.3. The influence of calcium

The addition of Ca^{2+}, and other cations (Mg^{2+}, Na^{+}) to a lesser degree, can ameliorate the toxic effects of acidic water in embryos of some species. For other species, Ca may exacerbate acid toxicity (see Katagiri, 1976; Freda and Dunson, 1984; Dale *et al.*, 1985a; Freda and Dunson, 1985b; Gascon *et al.*, 1987).

3.4. The influence of aluminum

The toxicity of pond water to amphibians is not controlled solely by pH and Ca ions alone; the concentration of Al may increase to toxic levels in acidic ponds. Aluminum, an abundant element in soils, enters aquatic systems through acidified surface or subsurface runoff after rainstorms, or from direct leaching from the pond's sediments. The interpretation of Al toxicity is difficult because of its complex speciation which is controlled by pH and the presence of inorganic and organic ligands. The literature on Al toxicity in amphibians (summarized by Freda, 1990) is inconsistent. Some field studies have clearly demonstrated Al toxicity to amphibians (Clark and Hall, 1985; Freda *et al.*, 1990b), whereas others have found no correlation between Al and embryonic mortality or amphibian abundance (Dale *et al.*, 1985a; Clark, 1986a, b; Gascon and Planas, 1986). Still others have shown that Al can ameliorate the toxic effects of low pH (Freda and Dunson, 1985b; Clark and LaZerte, 1987; Freda and McDonald, 1990). We recognize the potential importance of the Al $\times$ pH $\times$ species $\times$ life stage interactions and recommend that only total Al concentrations be assayed in any monitoring program while we await further information on these interactions.

3.5. The influence of dissolved organic acids

High concentrations of natural organic compounds may be present in temporary ponds and bogs, generally as the functionally defined groups known as humic and fulvic acids. Studies have shown that these organic acids have a high binding affinity for metals, thereby reducing the bioavailability and toxicity of metals in solution (Driscoll *et al.*, 1980; Neville, 1985; Hutchinson and Spraque, 1987). Investigators who measured Al toxicity in diluted natural pond water and artificial soft water reported significantly reduced toxicity of Al to tadpoles in the presence of certain concentrations of dissolved organic acids (Freda *et al.*, 1990a). The mean concentration of DOC generally exceeds 5 mg L^{-1} in temporary ponds (Clark and Hall, 1985; Clark, 1986a; Gascon and Planas, 1986; Freda *et al.*, 1990b), which is high enough to significantly decrease Al toxicity (Freda *et al.*, 1990a). However, at higher concentrations, organic acids can become toxic, as several laboratory and field studies have reported increased mortality of amphibian embryos in water with high DOC (Saber and Dunson, 1978; Dunson and Connell, 1982; Freda and Dunson, 1986; Karns, 1983; Freda *et al.*, 1990a, b).

3.6. Sublethal toxic effects

In laboratory experiments, chronic exposure of tadpoles or salamander larvae to acidic water slows their growth, the severity depending on the water pH (Karns, 1983; Freda and Dunson, 1985b; Cummins, 1986; Freda and Dunson, 1986; Ling *et al.*, 1986). Although no studies have investigated growth inhibition in natural ponds, it has been demonstrated that tadpoles in sublethally acidic ponds become chronically stressed. Freda and Dunson (1985b) reported that wood frog tadpoles in an acidic pond had body Na^+ levels reduced by 20% as compared to tadpoles in a high pH pond. A 20% reduction in body Na^+ was shown to be symptomatic of sublethal acid stress in laboratory experiments (Freda and Dunson, 1984). The possibility that tadpoles may be sublethally stressed by acidity so that their growth is inhibited has far reaching consequences for amphibian community structure and dynamics in acidified ponds. For tadpoles or salamander larvae living in temporary ponds, survival and ultimately recruitment into the adult population are critically dependent on maintaining a high rate of growth for three principal reasons: (1) to insure metamorphosis before pond drying, (2) to escape predators or to capture prey, and (3) to gain a competitive advantage. Thus, any depression in growth rate due to pH induced disruptions of larval physiology or community food web interactions can ultimately preclude recruitment.

4. Field Studies in Acidic Ponds

In order to devise a successful monitoring program, it is essential to understand the types of biological and chemical data that can be collected. Field studies conducted thus far on amphibians may be broadly divided into three types: (1) chemical surveys, (2) biological surveys of abundance or presence/absence, and (3) *in situ* toxicity tests. Each of these types are discussed below, with a consideration of some of their advantages and limitations.

4.1. Chemical Surveys

As discussed in the previous sections, the toxicity of acidic water depends on physico-chemical factors, including pH, and the concentrations of Ca, Al, and organic acids. Biological factors such as species and life stage are important in determining the severity of the toxic response. Therefore, it is difficult to specify an exact pH value at which predictable effects on amphibian embryos, larvae, or ultimately populations will occur. Although exceptions certainly exist, it is generally true that water with a pH below 4.5 may be directly toxic to embryos of the most sensitive species of amphibians (Table I), and a pH between 4.5 and 5.0 may inhibit the growth of tadpoles and salamander larvae (Freda, 1986). Water with a pH between 4.0 and 5.0 may also have direct lethal effects if the concentration of total inorganic Al is above 200 $\mu g\ L^{-1}$ (Clark, and LaZerte, 1985, 1987; Freda *et al.*, 1990a,b). In chemical surveys of three regions of North America, between 10 and 27% of ponds had a pH below 5.0 (Dale *et al.*, 1985b; Freda and Dunson, 1985c; Clark, 1986d; Glooschenko and Stevens, 1986). Based on the findings of these studies, it is unlikely that large scale extinctions of any species will occur in the immediate future as a result of acidic deposition. However, within each region, the populations of sensitive species may have already been severely impacted in an unknown percentage of acidic temporary ponds (<pH 5.0), while others are close to lethal limits (W. Sadinski and W. Dunson, unpub. obs.). Mortality of amphibian embryos may rise from 0 to 100% over a decrease in pH as small as 0.25 to 0.40 units (Freda and Dunson, 1985b; Freda and McDonald, 1990). Therefore, even small depressions in pond pH (< 0.4 units) may cause the loss of species from ponds with pH levels near the edge of their tolerance ranges.

Chemical surveys are useful descriptions of the range of chemical conditions to which amphibians are exposed. They do however have the following limitations. (1) The chemical conditions in amphibian breeding waters can vary over time, and temporary ponds are no exception. Therefore, unless regular sampling is undertaken (including before and after major precipitation events), a single water sample represents only a snapshot of conditions at that time. (2) Because of the complex toxic interactions in acidic water, the chemical conditions in a pond alone are currently not accurate predictors of the ability of amphibians to successfully breed in that pond. (3) Samples collected for chemical analyses require specialized handling. (4) Chemical analyses are expensive.

4.2. Biological surveys

Biological surveys are relatively easy to conduct, but suffer the limitations imposed by observational data in that they cannot elucidate the mechanism of causation. For example, if adults are surveyed, one cannot assume that those observed at a particular site were born, or can successfully reproduce there. Another source of potentially misleading data is the natural annual variation in the number of breeding adults, eggs deposited, or recruitment (Heyer, 1979). Thus, short term observations can lead to erroneous conclusions. In addition, a myriad of factors, such as pond size and hydroperiod, seasonal climatic variation, occurrence of predators and prey, and historical factors can control the distribution of amphibians. Variance associated with such factors makes it difficult to detect significant effects directly related to water chemistry. Also, it is often difficult to correlate patterns of amphibian distribution with chemical variables because most of the important chemical variables (pH, Al, Ca, organic acids) are intercorrelated. In deference to these confounding factors, biological surveys must attempt to quantify survival under known physical/chemical conditions for comparison with survival predicted by rigorous toxicity tests for one or more of these conditions.

Gosner and Black (1957) were the first to report that acidic water was toxic to the embryos of amphibians and that the pH of the naturally acidic waters of the New Jersey Pine Barrens influenced the distribution of amphibians. Saber and Dunson (1978) later demonstrated that acidic water (pH 3.8 to 4.0) draining from a sphagnum bog was lethal to the embryos and tadpoles of the bullfrog (*R. catesbeiana*). Pough (1976) first suggested that acidic precipitation may be killing amphibians when he observed high mortality of spotted salamander (*Ambystoma* *maculatum*) embryos in acidified ponds in New York. Many studies have since confirmed that acidity can control the distribution and abundance of amphibians. In surveys of Ontario amphibians, Clark (1986a,b) found that the population size of several species of amphibians was positively correlated with pond pH. In Quebec, acidity and total organic C were also correlated with the density of *R. sylvatica* egg masses (Gascon and Planas, 1986). Karns (1983) found that the number of breeding adults, egg masses, hatching success, larval amphibians, and newly metamorphosed animals were significantly reduced by acidic water (pH 4.0 to 4.2) in northern Minnesota peatlands. European researchers have also reported that acidity can affect the distribution and abundance of amphibians (Cooke and Frazier, 1976; Beebee and Griffin, 1977; Strijbosch, 1979; Leuven *et al.*, 1986). In contrast, Dale *et al.* (1985a) did not find any correlation between water pH and presence of several species of amphibians in Nova Scotia. Cook (1983) also reported normal development of spotted salamanders in ponds of Massachusetts, which ranged in pH from 4.2 to 5.6.

4.3. *In situ* Toxicity tests

In situ bioassays are particularly important in determining if the absence of amphibians from acidic ponds is due to direct toxicity of the pond water. In 12 Pennsylvania ponds, the acid-tolerant wood frog (*R. sylvatica*) bred successfully in all ponds regardless of pH, whereas the acid-sensitive Jefferson salamander (*A. jeffersonianum*) was absent from ponds with pH values below 4.5 (Freda and Dunson, 1986). When embryos of the Jefferson salamander were transplanted into ponds or laboratory solutions with a pH below 4.5, they were not able to hatch, indicating that acid toxicity was the primary factor excluding them from these ponds. In another *in situ* test, Albers and Prouty (1987) found that spotted salamander embryos suffered complete mortality in an acidic pond (pH 3.66) in coastal Maryland, but were able to hatch in ponds which ranged from 4.0 to 5.1. In an experimental stream acidification, hatching success of three species was negatively correlated with Al and DOC and positively correlated with pH (Clark and Hall, 1985). A new method of assessing the effects of low pH that is showing promise is the use of mesocosms (S. Warner, W. Sadinski and W. Dunson, unpub. obs.) to simulate temporary ponds under more controlled conditions. This technique has been used extensively by ecologists to demonstrate the community interactions of amphibians in temporary ponds (Wilber, 1980; Hairston, 1989).

5. Long Term Monitoring Plan: Variables

5.1. Which habitat to study?

Amphibians breed in virtually every type of aquatic habitat (temporary ponds, permanent ponds and lakes, streams, bogs). Determining the best combination of habitat and species for the purpose of assessing effects of acidic deposition depends on many factors, including: (1) sensitivity of the habitat to acidification, (2) the acid-sensitivity of resident species, (3) life history characteristics of resident species, (4) availability of historical data, (5) geographic distribution of the habitat and its resident species, (6) importance of the habitat to the successful breeding of target species, and (7) logistics, including ease of access and long term protection of the site.

Based on these criteria, temporary ponds have some distinct advantages. One of the most significant is the availability of large numbers of ponds within a restricted geographic area, from which a statistically valid sample can be chosen. Many temporary ponds also have low alkalinities due to their direct dependence upon precipitation for filling. As previously discussed, in the few regions that have been surveyed, 0 to 15% of temporary ponds have a pH between 4.0 and 4.5, and 10 to 14% have a pH between 4.5 and 5.0. The embryos of the most sensitive species of amphibians have a lethal pH of 4.5 (Table I). Therefore, if acidification were to continue, sensitive species would be lost from ponds which currently have a pH between 4.5 and 5.0. In contrast, if ponds become less acidic, populations of sensitive species might recover in ponds with a pH between 4.0 and 4.5. Temporary ponds are the major breeding habitats for a wide diversity of amphibians and are ubiquitous within most regions impacted by acidic deposition. Because of the ephemeral nature of temporary ponds, the breeding season and larval periods are relatively abbreviated. In the northeastern U.S., where acidic precipitation is well-documented, easily collectable egg masses of acid-sensitive species are deposited within a short time after the ponds fill, which facilitates the determination of the number and condition of embryos. These embryos generally take two to four weeks to hatch due to cold water temperature, allowing for the collection, observation and experimental manipulation of large numbers of naturally deposited embryos. Another advantage is that the vast majority of previous research on the effects of acidic deposition on amphibians has utilized temporary ponds.

Since very little is known about the effects of acidic deposition on amphibians which breed in lakes of the eastern U.S., it is currently not known if populations of any species have been reduced. Two of the major species breeding in lakes of the northeastern U.S., the green frog (*Rana clamitans*) and the bullfrog (*Rana catesbeiana*) are territorial and have protracted breeding seasons that last most of the summer. Since these populations' egg production is dispersed in time and space, and their embryos can hatch rapidly in the usually warm water, it is difficult to collect or sample a sufficient number of egg masses for the determination of reproductive effort or embryonic mortality. The adult males defend breeding territories with vocalizations, making it easy to quantify the number of adult males in a lake. However, adults can migrate from lake to lake, and the number of adults would not necessarily be reflective of water quality for embryos or recruitment in previous years. Another lake species, the leopard frog (*Rana pipiens*), is an explosive breeder that breeds erratically depending on the temperature and rainfall. Obtaining its eggs, except by artifical spawning, would be quite unpredictable.

Mountain headwater streams are susceptible to pulses of acidity and metals after snowmelt and rainstorms, and are the preferred habitat for many species of salamanders. These habitats are not good monitoring locations because relatively little is known about the effects of acidic deposition on these species. No studies have measured stream salamander populations over a pH gradient or determined the acid tolerance of adults or embryos of any species. In addition, the eggs of many species are deposited under rocks in or alongside the stream bed, rendering the determination of embryonic survival difficult.

Although several studies have investigated amphibian populations in acidic bogs, the natural acidity of bogs makes it difficult to separate any toxic effects of acidic deposition from those resulting from the internal generation of acidity. Such difficulties, in conjunction with the fact that those species that successfully reproduce in bogs are generally very acid tolerant, make them less desirable locations for monitoring the impacts of acidic deposition on amphibian populations.

5.2. Which species to study?

An indicator species in a long monitoring study should have the following characteristics : (1) the embryos and larvae are sensitive to acidity levels in the monitoring area, (2) be regionally common and/or widespread in distribution, (3) be predictable in the use of breeding sites, and (4) have embryos and larvae that are easy to collect and study.

The spotted salamander (*Ambystoma maculatum*) is one species that fulfills all of these criteria. Several laboratory and field studies report the inability of this species to breed below pH 4.5 (Pough and Wilson, 1977; Freda and Dunson, 1985b; Clark and LaZerte, 1987), although some field studies have observed successful breeding at pH's as low as 4.0 to 4.2 (Cook, 1983; Dale *et al.*, 1985b; Albers and Prouty, 1987). The spotted salamander has a widespread distribution ranging from Canada to the Gulf Coast (Anderson, 1967), which encompasses all areas of the eastern U.S. impacted by acidic deposition. The large globular egg masses are deposited immediately after snowmelt in the north, or during winter rains in the south (where the breeding season may be more protracted), when pond pH is the lowest, and are easily located. The adults have high fidelity to their breeding site, making the size of a pond's breeding population responsive to long term trends in the chemical conditions in that pond. Population size of adults, as reflected in the number of egg masses, would not reflect short term changes because females might not breed every year and adults are long lived and may continue to breed even though the pond cannot support recruitment. Due to a polymorphism in their jelly coats, the egg masses of spotted salamanders may appear either as clear or milky (opaque). Only the former permit observations of the embryos without removing them from the jellied mass, while the latter do not.

An even more promising indicator species is the Jefferson salamander (*Ambystoma jeffersonianum*). Jefferson and spotted salamanders are similar in life history traits, except that Jefferson salamanders breed 1 to 2 weeks earlier, produce only relatively small, clear egg masses, and their distribution is restricted to the northeastern U.S., the area most heavily impacted by acidic precipitation in the entire United States. In Pennsylvania, Jefferson salamanders are more sensitive to low pH than spotted salamanders (Freda and Dunson, 1986; W. Sadinski and W. Dunson, unpub. obs.) and are known to be currently unsuccessful in reproducing in some ponds of low pH (W. Sadinski and W. Dunson, unpub. obs.). One potential problem with using the Jefferson salamander is that in some portions of its range, triploid hybrid complexes exist with the bluespotted salamander (*Ambystoma laterale*) (Bogart, 1989). Studies on Jefferson salamanders should be restricted to areas not inhabited by bluespotted salamanders or the hybrids, unless the pH tolerances of all of these forms are found to be similar.

If monitoring studies are conducted in the southeastern, central or western U.S., the tiger salamander (*Ambystoma tigrinum*) would be an excellent study species. It possesses many of the life history traits of the spotted and Jefferson salamanders and is sensitive to low pH (Harte and Hoffman, 1989), although in some regions its tendency to deposit embryos singly or in small masses would pose collecting and sampling problems.

Species of temporary ponds which would not be good indicator species include the wood frog (*Rana sylvatica*), Hylid frogs (*Hyla* spp.), and toads (*Bufo* spp.), due to their high tolerance to acidity, difficulty in obtaining embryos, and variable breeding period, respectively.

5.3. What ponds and geographic locations to study?

As with all systems, temporary ponds can vary with respect to many factors such as size, chemistry, and the number of breeding amphibians. Therefore, it is imperative that enough ponds are located in close geographic proximity to minimize such variability. This is usually not a problem given the large number of these ponds in many areas. A monitoring study should include at least 15 ponds (minimal – see the statistical section below) within each geographic region studied. Geographic regions which should certainly be studied are New England and the Central Appalachians (e.g. PA, OH, MD, NJ etc.). The pH of the precipitation is lowest in these areas, and a majority of the aforementioned studies on amphibians have been conducted there. Other regions which also deserve attention are the Rocky Mt. States and the Pacific Coastal ranges. Studies in each region should examine an equal number of ponds from each of three acidic pH ranges: high, medium, and low, as defined for each region. For example, in central PA such pH ranges would be between 4.0 and 4.5, 4.5 and 5.0, and greater than 5.0, respectively. Loss of populations from ponds within the medium acidic range, correlated with a concomitant decline in pH, would signal that the effects of acidic deposition are worsening. By measuring the mortality of embryos and water chemistry in these acidic ponds ($<$ pH 4.5), it should be possible to determine if low pH dependent breeding success is improving or worsening.

Temporary ponds are best located by using National Wetland Inventory and

USGS topographic maps, and discussions with local biologists. Many, but by no means all, temporary ponds are indicated on these maps. In any previously unstudied areas, an entire spring prior to the start of the monitoring program would be necessary for locating and selecting ponds of the desired pH's and containing the amphibian species under study. Thus it would be highly desireable to utilize previously studied sites in protected areas where long term monitoring would not be interrupted.

6. Recommended Monitoring Plan

As previously stated, temporary ponds are the most obvious habitat choice for monitoring amphibian populations affected by low pH. Moreover, relatively simple, proven technology exists for initiating a quality monitoring program in temporary ponds with minimal start-up time. Our recommended general protocol is as follows.

6.1. Chemical parameters

In each monitoring region, at least five ponds in each of the low, medium, and high acidic pH categories should be located and chemically screened to confirm their appropriateness. In addition to pH, total Al, DOC, Ca, Na, Mg, K, So_4, No_3 and alkalinity should be measured. Ponds within each category, and within each region, should be generally similar for these parameters. The above parameters should be measured in all study ponds as follows. In northern areas, thawing of the ponds can serve as a set reference point for year to year comparisons if initial sampling (minimum of three per pond) and analyses are conducted immediately after the ice melts, which is when ponds are usually most acidic. In southern areas, initial sampling should begin approximately two weeks prior to the expected breeding date of the species being monitored. This will require vigilant observations of local weather conditions. In all areas, pond water should be analyzed at least two more times: 1) when the total number of egg masses is counted, and 2) when embryonic mortality is quantified. A more abbreviated set of chemical parameters (pH, alkalinity, Ca, total Al, and DOC) could be measured once more during the mid-larval period. A simple physical description of the ponds (length × width × maximum depth (preferably at standard positions) should be obtained at the time of breeding, and precipitation data (quantities and pH's of snow and rain) collected from late winter through metamorphosis.

6.2. Breeding determination and transplants

The breeding use, or lack thereof, of monitored ponds by the local amphibians, and especially the indicator species, must be established at the first opportunity. If indicator species are absent from such ponds for no apparent reason, replicated transplants of embryos (Freda and Dunson, 1986) must be conducted from other local ponds into the depauperate ponds.

6.3. Survivorship curves

Survivorship curves, as a function of a pH gradient, should be established for the species of interest in each region, preferably under standardized laboratory conditions using artificial soft water of ionic concentrations similar to those of local ponds (Freda and Dunson, 1986).

6.4. Counting egg masses

The total number of egg masses laid by the species of interest in each of the study ponds should be counted at the conclusion of breeding.

6.5. Unenclosed egg masses

In each pond, a random, statistically adequate sample (at least 15) of egg masses (similar in size) of the indicator species should have (a) their fertilization success scored, (b) their locations marked (e.g. with a flag), and (c) their developmental success quantified at two different embryonic stages, at post neurula/tail bud and at hatching. Any egg mass evaluations should be done with minimal disturbance to the natural positions of the masses. In central PA, many ponds are sufficiently clear and shallow to allow observation of Jefferson and spotted (clear morph only) masses while they are still attached to submerged grasses and branches. The percentage of developmental abnormalities and hatching success will be the response parameters.

6.6. Enclosed egg masses

A number of egg masses equal to that in 6.5 should be isolated in enclosures that are porous and spacious enough to allow water movement around the masses, while still preventing the escape of newly hatched larvae. Disturbance of these masses should be minimized. Enclosing them will prevent potentially confounding factors such as predation from interfering with the effects of low pH. The data from the enclosed masses should be compared with those in section 6.5 to indicate whether any enclosure or 'cage' artifacts are present. Sampling methods and response parameters are as in 6.5.

6.7. Larval sampling

Since all evidence indicates that larvae are more tolerant of low pH than embryos (Freda, 1986), we feel that embryonic hatching success should be the main focus of any monitoring efforts. However, if researchers suspect that pH's are low enough during the larval period to affect larvae directly, then an optional larval sampling program could be initiated. The best way to quantitatively sample larval survival would be via a throw sampler (Kushlan, 1981; Harris *et al.*, 1988), or metamorphs can be captured by enclosing ponds with drift fences (Gibbons and Bennett, 1974). Both of these methods have their associated problems, such as basin obstructions and a bias for sedentary organisms in the former and labor intensity in the latter, but under certain conditions they can be very effective.

If only a qualitative assessment of larval survival is desired, we recommend that a standardized number (e.g. 20) of sweeps with a stout, longhandled net of appropriate mesh size (and bag dimensions so that most of the water column and upper layers of detritus can be simultaneously sampled) be performed in each pond near the end of the larval period. During this premetamorphic period, the larvae of interest will be large and active enough such that a sample of them can be obtained if they are present. If larvae are present at this late stage, their tolerance of the pond conditions during that season is a foregone conclusion.

In spite of the fact that indirect effects of low pH on larval survival, whether they be reduced primary production or disruption of food webs, are almost certainly operating, attempting to measure such parameters in a monitoring study is ill-advised. This would require measurements of primary production, the richness and abundance of zooplankton and aquatic insects, as well as numbers and sizes of amphibian larvae and adults. Yet the results of such an elaborate procedure could not establish causation of observed abundances and would not justify such a costly effort.

6.8. Statistical analyses

In order to determine which parameters were primarily responsible for differences among ponds in the chemical measurements of 6.1, Principal Components Analysis would be a good choice (Johnson and Wichern, 1982). Any test of associations between water chemistry and reproductive success could be easily conducted via the use of Kendall's tau (Daniel, 1990). Statistical comparisons of reproductive success in 6.2 (as a result of transplants), 6.5, 6.6, and 6.7, should be undertaken using analysis of variance, along with appropriate transformation and multiple comparisons (Neter *et al.*, 1985). Alternatively, nonparametric methods, such as Kruskal-Wallis or Page's test for ordered alternatives could be used in these analyses (Daniel, 1990). As always, statistical power is critical. We recommend a bare minimum of fifteen ponds, but obviously the greatest number of ponds, that is

TABLE II

A proposed annual budgetary outline for an amphibian monitoring study on 15 temporary ponds (costs specific to each study)

1. Chemical Analyses	
4 replicates per pond × 15 ponds × 3 sampling dates = 180 samples	9 parameters/sample
2. Transportation	
Rental of 1 4WD vehicle for 5 month	
3. Personnel (d = actual work days)	
A. Half time Biologist (Ph.D.) for 6 month	10 d/month = 60 d
B. 3 technicians for 5 month ea.	20 d/month ea. = 300 d
4. Equipment (pH meters)	
5. Miscellaneous supplies (pH electrodes, nets, enclosures, sampling bottles, waders, etc.)	

economically and logistically feasible, should be studied. For example, doubling the number of ponds in each pH category to ten would provide a much greater probability of detecting any changes in reproductive success, without sacrificing logistical feasibility or concerns for quality in the execution of the monitoring plan. It would cost more.

6.9. Cost/Effort

We estimate that the proposed monitoring plan should involve 360 total work days of effort annually, with additional costs for transportation, chemical analyses, supplies, and equipment (Table II).

7. Conclusion

There has been considerable public discussion of the decline of amphibian populations in many different geographic locations. The scientific issue is to provide a reliable data base on which to test such a premise and to establish causation. We do know that some amphibians are unsuccessfully attempting to reproduce in temporary ponds that have pH's below their lethal limits, while other ponds are very close to such pH limits. Any concomitant changes in the pH of precipitation and the ponds could, and may already have had dramatic effects on the reproduction of these amphibians. Our proposed monitoring plan is based upon our collective experience with temporary ponds in the eastern and southeastern United States. We are confident that, all things considered – habitats and species, feasibility and cost efficiency, and historical justification – this plan offers the best opportunity for successfully monitoring any long term changes in the toxicity of acidic precipitation to amphibian populations.

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