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Aquatic Amphibians in the Sierra Nevada:

Current Status and Potential Effects of Acidic Deposition on Populations

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

AIR RESOURCES BOARD
Research Division

AQUATIC AMPHIBIANS IN THE SIERRA NEVADA: CURRENT STATUS AND POTENTIAL EFFECTS OF ACIDIC DEPOSITION ON POPULATIONS

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ABSTRACT

This study focuses on several species of amphibians as indicators of adverse ecological effects of acidic deposition in the Sierra Nevada. Goals of the study were (1) to provide a basis for evaluating the potential future effects of acidic deposition on aquatic-breeding amphibians in the Sierra Nevada at high elevation, and (2) to evaluate evidence that acidic deposition may have been a factor in causing recent population declines of amphibians throughout the Sierra Nevada. We conducted laboratory dose-response studies to determine the sensitivity to low pH and elevated aluminum content of water for early life stages of four species, and we also conducted field surveys to characterize the abundance and associated water chemistry of populations at high elevation.

A standard toxicity testing approach (i.e., survival endpoints determined using reconstituted soft water) indicated that amphibians are at little risk from low pH in waters acidified to an estimated extreme of pH 5.0 in surface waters due to acidic deposition. This approach also indicated that amphibians are at little risk from the aluminum levels tested, i.e., 39-80 ug/l. Nevertheless, the possibility exists that observed sublethal effects due to pH as high as 5.25 or elevated aluminum at the above levels, such as reduced growth rate and earlier hatching, may represent significant threats to amphibian populations. However, there are no field data indicating the above aluminum levels are likely to be reached or exceeded during episodes of acidification.

We tested the hypothesis that acidification of habitats in the field has resulted in elimination of populations from waters most vulnerable to acidification, i.e., low in pH or ANC, or from waters low in ionic strength, a condition that increases the sensitivity of amphibians to low pH. We surveyed potential breeding sites for two declining and one non-declining species at high elevation within 30 randomly selected survey areas, and compared the above chemical

parameters between sites containing a species and sites lacking the species. No significant differences were found that were consistent with the hypothesis, and water chemistry did not differ among sites inhabited by the three species. These findings imply that acidic deposition is unlikely to have been a cause of recent amphibian population declines in the Sierra Nevada.

DISCLAIMER

The statements and conclusions in this report are those of the authors and not necessarily those of the California Air Resources Board. The mention of commercial products, their source or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

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SUMMARY AND CONCLUSIONS

Surface waters of the Sierra Nevada are often very low in acid neutralizing capacity (ANC), and thus are potentially vulnerable to changes in water chemistry due to acidic deposition. In this study we use several species of amphibians as indicators of adverse ecological effects of acidic deposition. These organisms were chosen for study largely because their life stage most sensitive to low pH (typically the embryo) is exposed to episodes of low pH that occur early in snowmelt.

The primary goal of the study was to provide a basis for evaluating the potential future effects of acidic deposition on aquatic-breeding amphibians in the Sierra Nevada at high elevation. We also conducted the study to evaluate evidence that acidic deposition may have been a factor in causing recent population declines of amphibians throughout the Sierra Nevada. We conducted laboratory dose-response studies to determine the sensitivity to low pH and elevated aluminum content of water for early life stages of four species: Rana muscosa (mountain yellow-legged frog), Bufo canorus (Yosemite toad), Pseudacris (=Hyla) regilla (Pacific treefrog), and Ambystoma macrodactylum (long-toed salamander) We also conducted field surveys to characterize the abundance and associated water chemistry of a statistically representative sample of populations at high elevation. We use information from both approaches to evaluate the potential for both past and future effects of acidic deposition on amphibians.

EFFECTS OF LOW pH

Embryos and hatchling larvae (referred to as either tadpoles or larvae for frogs and toads) were kept for 7 d in reconstituted soft water (RSW) at pH 4.0 to 6.0, and subsequently for a post-treatment period of 0 to 16 d in RSW at pH 6.0 (no aluminum). Survival was high for all four

species at the higher pH levels, but declined dramatically as a function of pH in the mid to low 4's. LC_{50} values for the most sensitive survival endpoint, post-treatment survival, averaged 4.4 and <4.0 for embryos and tadpoles of R. muscosa, respectively; 4.7 and 4.3, respectively, for B. canorus; and 4.3 for both stages of P. regilla and A. macrodactylum. The total length of embryos and larvae of all species, except A. macrodactylum embryos, was reduced at low pH in comparison to pH 6.0. This effect was significant at pH 5.25 and below for embryos of R. muscosa, and at 4.75 or below for the others. Hatching time was significantly reduced at pH ≤ 5.0 in B. canorus, whereas it was increased significantly at one pH, 4.75, in P. regilla. Hatching time was not significantly affected by pH in R. muscosa and A. macrodactylum.

EFFECTS OF ELEVATED ALUMINUM CONCENTRATION IN WATER

Inorganic aluminum (75 ug/L) was added to reconstituted water at pH 6.0, 5.5, and 5.0, resulting in measured levels of dissolved aluminum of 39, 70, and 80 ug/L at pH 5.8, 5.3, and 4.9, respectively. Experimental levels of aluminum did not cause a decrease in survival endpoints for embryos or larvae of any species. Nevertheless, aluminum did cause a significant reduction in total length of B. canorus tadpoles at pH 5.3 and 5.8, but not at pH 4.9, and for A. macrodactylum larvae at pH 5.3, but not 4.9 and 5.8. This pattern is surprising because aluminum is more soluble at lower pH, and measured concentrations were greatest at pH 4.9. The addition of aluminum also caused earlier hatching in embryos of B. canorus and P. regilla, but not the other species.

EFFECTS OF IONIC STRENGTH OF WATER

The ionic strength of many Sierra Nevada surface waters is among the lowest in the world (Landers et al. 1987), and low ionic strength increases the sensitivity of amphibians to

low pH (Freda and Dunson 1984). Thus, this factor may be important in evaluating the potential effects of field water chemistry on amphibian populations. The effects of ionic strength on sensitivity to pH was tested on embryos of R. muscosa at four pH levels (4.25, 4.5, 4.75, 5.0) and three solute levels (i.e., 1, 4, and 16 times the concentrations used for experiments above). LC_{50} values for post-treatment survival decreased with increasing solute concentrations from a mean pH of 4.44 at solute level 1 to below 4.25 at solute level 16.

COMPARISONS TO OTHER AMPHIBIANS

The sensitivities for amphibians to low pH in this study are similar to the majority of amphibians studied elsewhere, including most other species from high elevation studied in dilute water. The sensitivities to aluminum cannot be compared because the aluminum dose in this study was much lower than nearly all other studies.

RELATIVE ABUNDANCE, DISTRIBUTION, AND ASSOCIATED WATER CHEMISTRY OF AMPHIBIAN POPULATIONS IN THE FIELD

Breeding amphibians were found in 25 of the 30 randomly selected survey areas (15 km^2) at high elevation. P. regilla occurred in 25, R. muscosa in 6, B. canorus in 6, B. boreas in 2, and A. macrodactylum in 1. A total of 141 water samples were collected from amphibian breeding sites, and 94 were collected from sites considered potential breeding habitat that lacked amphibians. pH of the 141 sites containing amphibians ranged from 5.4 to 7.9 (median = 6.2), ANC ranged from -1.5 to 1100 ueq/l (median = 89), and conductivity ranged from 2.6 to 194 uS/cm (median = 11.1). In comparison to two other studies of water chemistry throughout the Sierra Nevada at high elevation, the sites in the present study were generally lower in pH, but slightly higher in ANC. The lower

pH may have been due to sampling earlier in the summer season in the present study, as pH typically increases following snowmelt (Melack and Stoddard 1991).

For sites lacking amphibians pH was ≥ 5.4 except for two sites (pH 4.6 and 4.9) in one survey area. The source of acidity here appears to be from iron pyrite deposits (G. A. Mahood, pers. comm.).

HYPOTHESIS THAT ACIDIC DEPOSITION HAS RESULTED IN AMPHIBIANS NOT OCCURRING IN WATERS LOW IN pH, ACID NEUTRALIZING CAPACITY, OR ELECTRICAL CONDUCTIVITY

If anthropogenic acidification of habitats has been a factor influencing the distribution of amphibians in the Sierra Nevada, either by itself or in combination with other factors, it would be expected that amphibians have been eliminated from waters most vulnerable to acidification, i.e., low in pH and ANC. In addition, low EC may have contributed to such elimination, because low ionic strength increases the sensitivity of amphibians to low pH (Freda and Dunson 1984).

The present study does not support this hypothesis. Water chemistry in the field for two declining species (R. muscosa and B. canorus) and one non-declining species (P. regilla) showed no significant differences between sites containing and sites lacking the species, both within and between survey areas.

HYPOTHESIS THAT ACIDIC DEPOSITION HAS RESULTED IN AMPHIBIANS DIFFERING IN WATER CHEMISTRY OF BREEDING SITES IN THE SAME MANNER THAT THEY DIFFER IN SENSITIVITY TO ACIDIC CONDITIONS IN THE LABORATORY

This pattern would be expected if anthropogenic acidification of habitats has been a factor influencing the distribution of amphibians in the Sierra Nevada. LC_{50} pH values for embryos differ significantly among the three

species used to test this hypothesis, and rank in the following order: P. regilla < R. muscosa < B. canorus. The embryo is the life stage chosen for comparison because (1) embryos typically develop in waters fed by snowmelt immediately following thawing of the water surface, which coincides with the time of lowest pH and ANC, and (2) embryos of these species are similar to or greater than tadpoles in sensitivity to low pH.

This study does not support this hypothesis. No significant differences were found in water chemistry among the three species analyzed, either within survey areas or between survey areas.

AMPHIBIAN FAUNA AND ASSOCIATED WATER CHEMISTRY OF WATERSHEDS INCLUDED IN THE "SEVEN-WATERSHEDS" PROJECT

P. regilla was found in or near 5 of the 7 watersheds: Crystal, Ruby, Lost, Topaz, and Emerald. A. macrodactylum was found in or near the Lost Lake watershed. No amphibians were found in or near Spuller Lake watershed or Pear Lake watershed. Relatively few sites (i.e., 10) were found with amphibians probably in large part because the watersheds are relatively small, and predatory fish were present in 6 of the 7 watersheds. pH, ANC, and EC for the 10 amphibian breeding sites were similar to the majority of sites containing amphibians found throughout the Sierra Nevada at high elevation.

EVIDENCE FOR PAST AND POTENTIAL FUTURE EFFECTS OF ACIDIC DEPOSITION ON AMPHIBIANS IN THE FIELD

The potential future effects of acidic deposition on amphibians in the Sierra Nevada are evaluated for an estimated extreme acidification event of pH 5.0. This pH is used because it is the lowest pH measured to date from snowmelt water prior to contact with substrate, and the time of

snowmelt is when the lowest pH levels have been measured in Sierra Nevada surface waters. Moreover, the life stage usually the most sensitive to low pH, i.e., embryo, occurs at this time. A standard toxicity testing approach (i.e., survival endpoints) indicated that amphibians are at little risk from low pH in waters acidified to pH 5.0 due to acidic deposition. This approach also indicated that amphibians are at little risk from aluminum levels of 39, 70, and 80 ug/l at pH 5.8, 5.3, and 4.9, respectively. Nevertheless, the possibility exists that observed sublethal effects due to pH as high as 5.25 or elevated aluminum at the above levels, such as reduced growth rate and earlier hatching, may represent significant threats to amphibian populations. However, there are no field data indicating that the above aluminum levels are likely to be reached or exceeded during episodes of acidification.

The proposition that acidic deposition has been a factor in causing amphibian populations in the Sierra Nevada in the past is not supported by the above argument based on laboratory studies, nor on the test of two hypotheses concerning amphibian distribution and water chemistry in the field (see two previous sections).

RECOMMENDATIONS

A number of additional studies would increase the validity of using dose-response experiments as the basis for assessing the risk from acidic deposition in the field. These include:

1. Conduct dose-response experiments in the field. Such experiments would better represent the natural conditions of water chemistry, temperature, light, etc. in comparison to simulated natural conditions in the laboratory. If this is not feasible, dose-response experiments could be conducted in the laboratory using water derived from the field, rather than using reconstituted water. This would probably necessitate conducting experiments at a laboratory facility near a high-elevation site.

2. Obtain from ongoing or future studies of water chemistry an estimate of aluminum levels that potentially could occur in the field due to acidic deposition. Conduct dose-response experiments using these levels.

3. Further evaluate the significance of sublethal effects. This would probably entail monitoring growth and survival of experimental amphibians until metamorphosis is completed.

4. Test effects of exposure to low pH and elevated aluminum on amphibian immune function. Several studies suggest that potential stressors such as low pH may depress immune function, which can result in infection and mortality some time after exposure (Carey, in press).

CHAPTER 1 -- INTRODUCTION

STATEMENT OF THE PROBLEM

The integrity of some aquatic ecosystems in California may be at risk due to acidic deposition from anthropogenic sources (CARB 1989; Melack and Stoddard 1991). Resources of primary concern include high-elevation surface waters of the Sierra Nevada and their biological populations. These waters typically are extremely low in acid neutralizing capacity (ANC), and thus may be exceptionally vulnerable to changes in water chemistry due to acidic deposition (Landers et al. 1987; Eilers et al. 1989; Melack and Stoddard 1991). At present surface waters in the Sierra Nevada experience depressions in pH and ANC during snowmelt and summer storms (Melack and Stoddard 1991; Williams and Melack 1991).

The Atmospheric Acidity Protection Act of 1988 requires the California Air Resources Board (CARB) to quantify the potential for damage to aquatic ecosystems due to acidic deposition and develop techniques for early detection of changes in these ecosystems (CARB 1989). An investigation of both vertebrate and invertebrate populations in one area of the southern Sierra Nevada was completed under the Kapiloff Program funded by the CARB (e.g., Melack et al. 1989; Cooper et al. 1988a, b; Soiseth 1992). A data base is now needed that applies to a broader region so that predictions can be made for the extent of biological damage that might accompany changes in surface-water chemistry due to acidic deposition throughout the Sierra Nevada.

To achieve this goal, information is needed on the extent and condition of vulnerable biological populations in the Sierra Nevada, and the dose-response relationships for the effects of episodic acidification (defined herein as reduction in ANC) on these populations (CARB 1989). Two groups of organisms are of concern: "indicator" organisms likely to be affected by environmental changes associated with acidic

deposition, and species of economic importance, particularly trout. The present study focuses on amphibians as a group likely to be sensitive bioindicators of environmental changes resulting from acidic deposition in the Sierra Nevada. A companion study was focused on fish (Jenkins 1990).

AMPHIBIANS AS BIOINDICATORS OF ENVIRONMENTAL CHANGE

A number of physiological and ecological characteristics of amphibians render them especially sensitive to environmental change or degradation (Blaustein and Wake 1990; Vitt et al. 1990; Wyman 1990). For example, most amphibians have complex life cycles with both aquatic and terrestrial phases; the highly permeable skin of amphibians is exposed to substances in water, air, and terrestrial substrate; adult amphibians are high in the food web, whereas most larvae are low in the food web; and metamorphosis between larva and adult is a physiologically and ecologically sensitive time.

Aquatic amphibians are especially pertinent to examine in the Sierra Nevada for other reasons as well. First, amphibians were historically the only native aquatic vertebrates throughout most of the high Sierra, and individuals were generally abundant (Storer 1925; Christenson 1977). Subsequently, many amphibian populations were eliminated by introduced fishes (Grinnell and Storer 1924; Bradford 1989a). Nevertheless, the original five species still occur in the high Sierra Nevada in many areas: Pseudacris (=Hyla) regilla (Pacific treefrog), Rana muscosa (mountain yellow-legged frog), Bufo canorus (Yosemite toad), B. boreas (western toad), and Ambystoma macrodactylum (long-toed salamander). Second, all aquatic amphibians at high elevation in the Sierra Nevada breed during or shortly after snowmelt, thereby exposing the early life stages that are the most sensitive to acidification (Pierce 1985; Freda 1990) to conditions of depressed pH and ANC. Third, the abundant literature for amphibians in eastern North America indicates

that detrimental effects of acidification may occur at pH levels observed in the Sierra Nevada, e.g., at pH in the mid 5's for some species (Pierce 1985).

Perhaps the greatest justification for focusing attention on amphibians in the high Sierra is that dramatic population declines appear to have already occurred or are in progress for at least two of the five species (Phillips 1990). These are Rana muscosa and Bufo canorus, both of which are restricted to high elevation. Many of the population declines of these species have occurred in seemingly pristine environments, including areas within Sequoia, Kings Canyon, and Yosemite National Parks, and numerous wilderness areas. R. muscosa formerly ranged throughout the high Sierra Nevada and the mountains of southern California. Prior to the introduction of various game fishes, this frog was probably the most prevalent aquatic vertebrate in mid and high elevation lakes in the Sierra Nevada (Bradford 1989a). Subsequently, it remained a strikingly abundant species in many high elevation lakes throughout the Sierra Nevada that had not been stocked with introduced fishes (Zweifel 1955; Bradford 1989a). However, recent surveys show that this species has disappeared from many of its former sites during the past 10 to 20 years (Phillips 1990; L. Cory, pers. comm.; Bradford et al., ms. in review). Population disappearances appear to represent true extinctions, rather than a pattern resulting from limited population monitoring (Pechmann et al. 1991), because populations observed to go extinct between 10 and 20 years ago have remained so (Bradford et al., ms. in review). R. muscosa has almost entirely disappeared from the mountains of southern California (M. R. Jennings, pers. comm.).

Recent surveys for B. canorus show that it has disappeared from approximately 50% of its former sites in the past two decades (D. L. Martin, pers. comm.). Populations that have been observed regularly since the 1950's in the Tioga Pass area near Yosemite National Park have had little or

no reproductive success since 1982 (M. L. Morton, C. K. Sherman, pers. comm.). In contrast, populations of a third widespread species, Pseudacris regilla, do not appear to be declining at high elevation (Cooper et al. 1988a; Bradford et al., ms. in review; M.R. Jennings, pers. comm.). The population status of the remaining two species at high elevation, Bufo boreas and Ambystoma macrodactylum is largely unknown (M.R. Jennings, pers. comm.).

INFLUENCE OF ACIDIC DEPOSITION ON AMPHIBIAN POPULATIONS

Dramatic population declines of many amphibians have become apparent throughout the world in recent years (National Research Council 1990; Barinaga 1990; Wake 1991). These declines are feared by many biologists to represent significant environmental degradation and threats to the survival of some species. A number of causes for these population declines have been implicated or hypothesized, including habitat alteration, acidic deposition, predation from introduced fishes and other animals, heavy metal and pesticide poisoning, increased ultraviolet radiation, disease, and drought. The acidic deposition hypothesis is frequently suggested because many of the declining amphibians inhabit montane environments, such as the Sierra Nevada and Rocky Mountains, where waters are extremely low in ANC. Moreover, many of the montane species, and some lowland species, have disappeared over large geographic areas that have not undergone noticeable environmental change.

Acidic deposition appears to have affected amphibian populations in some areas of eastern North America and Europe (Freda 1990; Dunson et al. 1992). In the western U.S., however, the potential effects of acidic deposition on amphibian populations have not been well studied. Ambystoma tigrinum (tiger salamander) in the Rocky Mountains of Colorado appears to be adversely affected by acidic deposition in some years (Harte and Hoffman 1989). However, Corn et al. (1989)

found that the pH during snowmelt in the one year studied (pH $\geq$ 6.0) was well above the level (pH < 5.0) at which embryonic mortality occurs in the two declining species of anurans in the region, Bufo boreas (boreal toad) and Rana pipiens (leopard frog). Moreover, one of these species, R. pipiens, does not occur in the habitats most susceptible to acidification at high elevation.

In addition to low pH, the inorganic aluminum content (especially monomeric aluminum) of water during episodes of acidification may also pose a toxic threat to amphibians. Many acid surface waters (pH<5.5) have elevated concentrations of inorganic monomeric aluminum reflecting the increased solubility of aluminum at low pH (Baker et al. 1990). Numerous studies have documented the toxicity of aluminum to amphibians, fish, and other organisms (Baker et al. 1990; Freda 1986, 1991). However, the effects of aluminum on amphibians are highly variable. For example, in some species, aluminum is toxic only at low pH (<4.5), whereas in others, toxicity becomes apparent only at higher pH levels (>4.5) (Freda 1991).

SPECIFIC OBJECTIVES OF THE STUDY

The study focuses on 9 objectives listed below, which are described more fully in individual chapters:

Objective 1. Determine the extent to which embryos and larvae are adversely affected by episodes of low pH in the laboratory.

Objective 2. Determine the extent to which embryos and larvae are adversely affected by episodes of elevated aluminum content of water in the laboratory.

Objective 3. Determine if the sensitivity of Sierra Nevada amphibians to low pH is affected by the ionic strength of the water.

Objective 4. Determine if Sierra Nevada amphibians are similar to amphibians elsewhere in their sensitivity to low pH or elevated aluminum content.

Objective 5. Characterize the relative abundance, distribution, and associated water chemistry of a statistically representative sample of amphibian populations in the Sierra Nevada at high elevation.

Objective 6. Test the hypothesis that amphibians generally do not occur in waters low in pH, ANC, or EC.

Objective 7. Test the hypothesis that species differ in the water chemistry of breeding sites in the same manner that they differ in sensitivity to acidic conditions in the laboratory.

Objective 8. Characterize the amphibian fauna and associated water chemistry of the watersheds included in the CARB-sponsored "seven-watersheds" project.

Objective 9. Evaluate evidence for past and potential future effects of episodes of acidic deposition on amphibians in the field.

STUDY ANIMALS

The study focuses on four of the five species that breed in water at high elevation in the Sierra Nevada. Bufo boreas is excluded from laboratory studies because its distribution and abundance at high elevation are limited in comparison to the other four species, and its biology is likely to be

generally similar to its congener, B. canorus. Background biology for all five species is summarized in Table 1-1. Between two and four of these species inhabit the same regions, and often two species coexist in the same waters.

Larvae of these animals are referred to as "tadpoles" or "larvae" for anurans (frogs and toads), but only as "larvae" for the salamander, A. macrodactylum.

Table 1-1. Selected biological traits of the five species of aquatic amphibians occurring at high elevation in the Sierra Nevada.

Species	Mountain Yellow-legged Frog (Rana muscosa)	Yosemite Toad (Bufo canorus)	Western Toad (Bufo boreas)	Pacific treefrog (Hyla regilla)	Long-toed Salamander (Ambystoma macrodactylum)
Distribution	Throughout Sierra, 4,600-12,000 ft. elevation, mostly above 9,000 ft	Central Sierra, 4,800-12,000 ft. elevation, mostly above 9,000 ft	Southern and northern Sierra, 200-11,800 ft elevation	Throughout Sierra, 200-11,500 ft elevation	Northern Sierra, 6,000-9,000 ft elevation
Time of Breeding	Snowmelt	Snowmelt	Soon after snowmelt	Soon after snowmelt	Snowmelt
Larval Habitat	Permanent water of lakes and streams	Usually in temporary waters, e.g., meadows	Usually in temporary waters, e.g., meadows	Shallow ponds, shallow portions of lakes	Ponds and lakes
Duration of Larval Stage	2 to 3 summers, tadpoles overwinter in water	One summer	One summer	One summer	1-2 summers; larvae sometimes over-winter in water
Adult Habitat When not breeding	In and adjacent to water, esp. lakes; spends nights and winter in water	On land near water in meadows and forest borders, especially since 1978	On land near water in meadows and forest borders	On land, e.g., talus or vegetation	On land in vegetation and debris near water
Population Status	Declining, esp. during past 20 years; recolonization largely precluded by introduced fish in streams connecting lakes	Declining, especially since 1978; no successful breeding in Tioga Pass area since 1982	No information	Apparently stable	Little information; apparently stable
References*	Bradford (1983, 1984, 1989, unpubl.); Zweifel (1955)	Karlstrom (1962); Sherman (1980); Martin, Sherman, Morton, pers. comm.	Karlstrom (1962); Martin, Jennings, pers. comm.	Cooper et al. (1988a); Bradford (unpublished)	Jennings, pers. comm.; Bradford (unpublished)

* General references are Grinnell and Storer (1924), Storer (1925), and Stebbins (1962, 1985).

CHAPTER 2 -- LABORATORY DOSE-RESPONSE STUDIES

OBJECTIVES

Objective 1. Determine the extent to which embryos and larvae are adversely affected by episodes of low pH. For amphibians in general, the embryo is usually the stage most sensitive to reduced pH, whereas the larva is usually the most sensitive to elevated concentrations of aluminum (Pierce 1985; Freda 1986, 1991). Both embryos and larvae are investigated because they occur at the times of lowest pH in the field, i.e., at snowmelt and during summer storms (Melack and Stoddard 1991; Williams and Melack 1991).

Objective 2. Determine the extent to which embryos and larvae are adversely affected by episodes of elevated aluminum content of water.

Objective 3. Determine if the sensitivity of Sierra Nevada amphibians to low pH is affected by the ionic strength of the water. The ionic strength of many Sierra Nevada surface waters is among the lowest in the world (Landers et al. 1987), and low ionic strength increases the sensitivity of amphibians to low pH (Freda and Dunson 1984). Thus, this factor may be important in evaluating the potential effects of field water chemistry on amphibian populations.

Objective 4. Determine if Sierra Nevada amphibians are similar to amphibians elsewhere in their sensitivity to low pH or elevated aluminum content. It is possible that amphibians in the Sierra Nevada are adapted to the chemical characteristics of the water (e.g., low ionic strength) such that they are no more sensitive to low pH than amphibians elsewhere.

METHODS

Water Chemistry Measurements. -- pH was measured at room temperature using a Fisher Acumet 910 meter and Ross (Orion) combination electrode (Sickman and Melack 1989). The electrode was calibrated daily with standard buffers and checked periodically using dilute solutions of HCl (10^{-4} and 10^{-5} N). To further verify pH and ionic strength, pH was remeasured for all treatment waters 6 times during the experiments, and electrical conductivity (EC) was measured simultaneously with a YSI 35 conductance meter (cell constant = 0.1 cm). Major cations and anions were analyzed in filtered water samples according to Sickman and Melack (1989). Samples for measurement of total dissolved aluminum were filtered through 0.1 μm Nucleopore filters into 30 ml HDPE bottles (previously rinsed in deionized water, 18 megaohm/cm) using an all-plastic filter holder and a peristaltic pump. Samples were acidified with 0.1 ml of ultra-pure (Ultrex) nitric acid, and analyzed using an atomic absorption spectrometer with graphite furnace and auto sampler. Some samples were also analyzed for inorganic monomeric aluminum (MIBK method; USEPA 1987).

Experimental Animals. -- Adults of the P. regilla, B. canorus, and A. macrodactylum were collected at snowmelt prior to breeding from 3, 3, and 1 sites, respectively (Table 2-1). Adult R. muscosa were either collected at snowmelt prior to breeding (Sites 1 and 2, Table 2-1), or were collected in October and kept in simulated hibernation conditions in dechlorinated Los Angeles tap water at 4°C for 7 months (Sites 3 and 4, Table 2-1).

Males and females of R. muscosa and P. regilla were injected subcutaneously in the groin or lower back with 0.1 mg/kg synthetic luteinizing hormone ([Des-Gly^{10},Pro^{9}]-LH-RH, ethyl amide; Bachem Inc., Torrance, California). Eggs were subsequently obtained and fertilized

Table 2-1. Localities of adults or egg clutches used to provide embryos (E) and tadpoles (T) for experiments. Individuals from the same clutch were used for all experimental treatments, except for R. muscosa tadpoles from site 5. In this case, the 5 "clutches" represent 5 replicates of groups of tadpoles randomly selected from a mixture of tadpoles from approximately 6 egg clutches collected in the field.

Species	No. Clutches Used	Stage	Locality	
Rana muscosa	3	E	1.	1.5 km NE State Peak, Fresno Co., Calif., 3510 m elev.
	1 5	E T	2.	0.6 km SW Taboose Pass, Fresno Co., Calif., 3400m elev.
	2	E, T	3.	1.3 km NW Ebbett's Pass, Alpine Co., Calif., 2700 m elev.
	1	E, T	4.	2.5 km NE Badger Pass Ski Area, Yosemite Nat. Park, Mariposa Co., Calif., 2005 m elev.
	1	E, T	5.	Cross between female from site 4 and male from site 1.
Bufo canorus	4	E, T	6.	2.4-4.5 km (by air) S Ebbett's Pass along road to Highland Lakes, Alpine Co., Calif., 2440-2560 m elev.
	1	E, T	7.	Near Mildred Lake, 5 km SSW Convict Lake, Mono Co., Calif., 3110 m elev.
	3	E, T	8.	1.6 km N Pine Creek Pass, Inyo Co., Calif., 3210 m elev.
P. regilla	1	E, T	9.	Ebbett's Pass, Alpine Co., Calif., 2660 m elev.
	4	E, T	10.	Crescent Meadow, Sequoia National Park, Tulare Co., Calif., 2040 m elev.
	5	E, T	11.	Tuolumne Meadows, Yosemite National Park, Tuolumne Co., Calif., 2620 m elev.
A. macrodactylum	6	E, T	9.	See above

artificially (Rugh 1962). Male and female B. canorus were also injected as above, and placed together in pairs in shallow reconstituted soft water (described below) at pH 6.0 in 1.5-L polypropylene containers at 15°C. Female and male A. macrodactylum were not injected and were placed together in pairs in approximately 4 L reconstituted soft water at pH 6.0 at 10°C. For both B. canorus and A. macrodactylum, spontaneously deposited, fertilized eggs were collected within 18 h of oviposition.

Fertilized eggs were separated into small clumps (e.g., 2-5 eggs), and infertile or deformed eggs were removed. Eggs from each clutch were distributed among experimental containers (9-20 eggs/container for R. muscosa, 20 for B. canorus, 19-20 for P. regilla, 13-19 for A. macrodactylum), which were subsequently filled with treatment water and kept at 15°C with 14:10 L:D cycle. Initial embryonic stages of the three anurans were Gosner stages 6 to 9 (i.e., 6-cell to late cleavage stages; Gosner 1960), and for A. macrodactylum, Harrison stages 6 to 8 (mid cleavage stages; Rugh 1962). Hatchling larvae used in experiments were reared as embryos in approximately 2 L of aerated reconstituted soft water at pH 6.0 or dechlorinated tap water (some R. muscosa only) at 15°C. Within a few days of hatching, individuals from each clutch were randomly allocated to the various treatment conditions as described for embryos (10-20 tadpoles per container for R. muscosa, 11-20 for B. canorus, 15-20 for P. regilla, 9-11 for A. macrodactylum).

Treatment Regimes. -- Experiments were conducted in reconstituted soft water (RSW) using ratios of constituents recommended for amphibian toxicity testing (ASTM 1988). The amount of each constituent was calculated so that Ca^{+2} concentration was 25 μeq/L, i.e., approximately the concentration of the dilute water characteristic of much of the Sierra Nevada (Melack et al. 1989). Constituents were ACS-certified $NaHCO_3$, $CaSO_4 \cdot 2\ H_2O$, $MgSO_4 \cdot 7\ H_2O$, and KCl at

concentrations 29% of those for "very soft" water in ASTM (1988). Constituents were mixed in 20-L HDPE carboys of deionized distilled water, which were then aerated for several hours. pH was adjusted in each carboy by adding a 1:1 ratio of H_2SO_4 (diluted from 36 N) and HNO_3 (diluted from 16 N) (Fisher ACS grade) until desired pH was reached. pH levels used were 4.0, 4.25, 4.5, 4.75, 5.0, 5.5, and 6.0. Carboys were stored at 15°C for a few days (higher pH's) to 3 weeks (lower pH's) until used. pH, ANC, and EC remained stable during this time.

Cation and chloride concentrations (μeq/L) averaged: Na = 37.5, Mg = 34.0, Ca = 25.1, K = 4.26, and Cl = 3.2 (N=19 samples). These values were well within the range observed in the Sierra Nevada (Landers et al. 1987). Nitrate and sulfate ions increased in concentration with the addition of acid from approximately 12 and 75 μeq/L, respectively, at pH 6.0 to approximately 85 and 150 μeq/L, respectively, at pH 4.0. Aluminum treatment water was mixed daily in 1-L batches from above solutions at pH 5.0, 5.5, and 6.0. Aluminum (nominally 75 μg/L) was added as $Al_2(SO_4)_3 \cdot 18\ H_2O$ (ACS reagent), which resulted in solutions of pH 4.9, 5.3, and 5.8. Measured background levels of total dissolved aluminum averaged 2 μg/L (N=14); treatment samples averaged 39, 70, and 80 μg/L at pH 5.8, 5.3, and 4.9, respectively (N=6 at each pH). Virtually all aluminum was in inorganic monomeric form (N=13). During the 1-d exposure between water changes, aluminum concentration decreased by averages of 42, 28, and 13% at pH 5.8, 5.3, and 4.9, respectively (N=5 at each pH).

Treatment containers consisted of 10-cm diameter polypropylene cups with perforated lids, which held approximately 150 ml of treatment water. Treatment water was changed daily for 7 d (defined as "treatment period"). This duration was selected to represent an episode of acidification at snowmelt in the field (Melack et al. 1989). Subsequently, treatment water was replaced with RSW at pH 6.0 (no aluminum), which was changed at 3-4 d intervals. The duration of this

"post-treatment period" was defined for each species and life stage as the period during which unfed individuals at pH 6.0 survived well, apparently living on yolk reserves. The duration of the post-treatment period was as follows: R. muscosa (12 d for embryos, 4 d for tadpoles), B. canorus (14 d for embryos, 7 d for tadpoles), P. regilla (11 d for embryos, 0-1 d for tadpoles), and A. macrodactylum (16 d for embryos, 2 d for larvae).

To test for effects of ionic strength of the water, embryos from three clutches of R. muscosa were tested as above at four pH levels (4.25, 4.5, 4.75, 5.0) and three solute levels. One solute level was comprised of RSW; the other two levels were made in the same fashion as RSW except that salts added were 4 and 16 times as concentrated as the RSW. pH was adjusted as for RSW.

Experimental Endpoints. -- All containers were checked daily and dead individuals removed. Survival endpoints were determined as: Treatment Survival = percent survival of 7-d exposure to experimental conditions; Survival to Hatching = percent of embryos that hatched (hatching typically occurred during the post-treatment period); and Post-Treatment Survival = percent survival to end of the post-treatment period. Other endpoints were: Total Length = tip of snout to end of tail at end of post-treatment period (determined by measuring individual on moist paper toweling to nearest 0.1 cm with a ruler and dissecting microscope); and Hatching Time = time between onset of treatment and hatching.

LC_{50} (reported as pH) was calculated for each clutch or series of replicates from H^+ concentrations by interpolation because fewer than two pH levels killed between 0 and 100% of the individuals in many cases (ASTM 1988). "Critical pH" was defined as the highest pH at and below which an endpoint value was significantly reduced below that at pH 6.0 (no aluminum) (Freda 1986).

Statistical Analysis. -- Data for all geographic locations were combined in analyses because differences in LC_{50} among sites were small. The effects of pH on each endpoint were analyzed by ANOVA using clutch and pH as category variables (Wilkinson 1990). Survival data (%) were arcsine square-root transformed prior to analysis (Steel and Torrie 1960). Critical pH was determined by post hoc comparisons between values at each pH and pH 6.0, using the Bonferroni procedure to maintain an overall $\alpha = 0.05$ (Wilkinson 1990). To test for aluminum effects, paired t-tests were done between values at the three pH levels with aluminum and the values interpolated at these pH's using data for which aluminum was absent. The Bonferroni procedure was used to maintain an overall $\alpha=0.05$ for comparisons at the three pH levels (Wilkinson 1990).

RESULTS

pH Effects. -- For all four species survival was high at the higher pH levels and declined dramatically as a function of pH in the mid to low 4's (Figs. 2-1 to 2-4). LC_{50} values for the most sensitive survival endpoint, post-treatment survival, averaged 4.4 and <4.0 for embryos and tadpoles of R. muscosa, respectively; 4.7 and 4.3, respectively; for B. canorus; and 4.3 for both stages of P. regilla and A. macrodactylum (Table 2-2). The critical pH for post-treatment survival was 4.5 or below for both embryos and tadpoles of all species (Figs. 2-1 to 2-4). Interestingly, post-treatment survival for B. canorus tadpoles at pH 5.5 was significantly reduced below that at pH 6.0, but not at pH 4.75 and 5.0, although the magnitude of this effect was not large (Fig. 2-2).

The total length of embryos and larvae of all species, except A. macrodactylum embryos, was also affected by pH (Figs. 2-5 to 2-8; ANOVA, $P<0.01$ in all cases except A. macrodactylum embryos [$P=0.09$]). Critical pH values for total

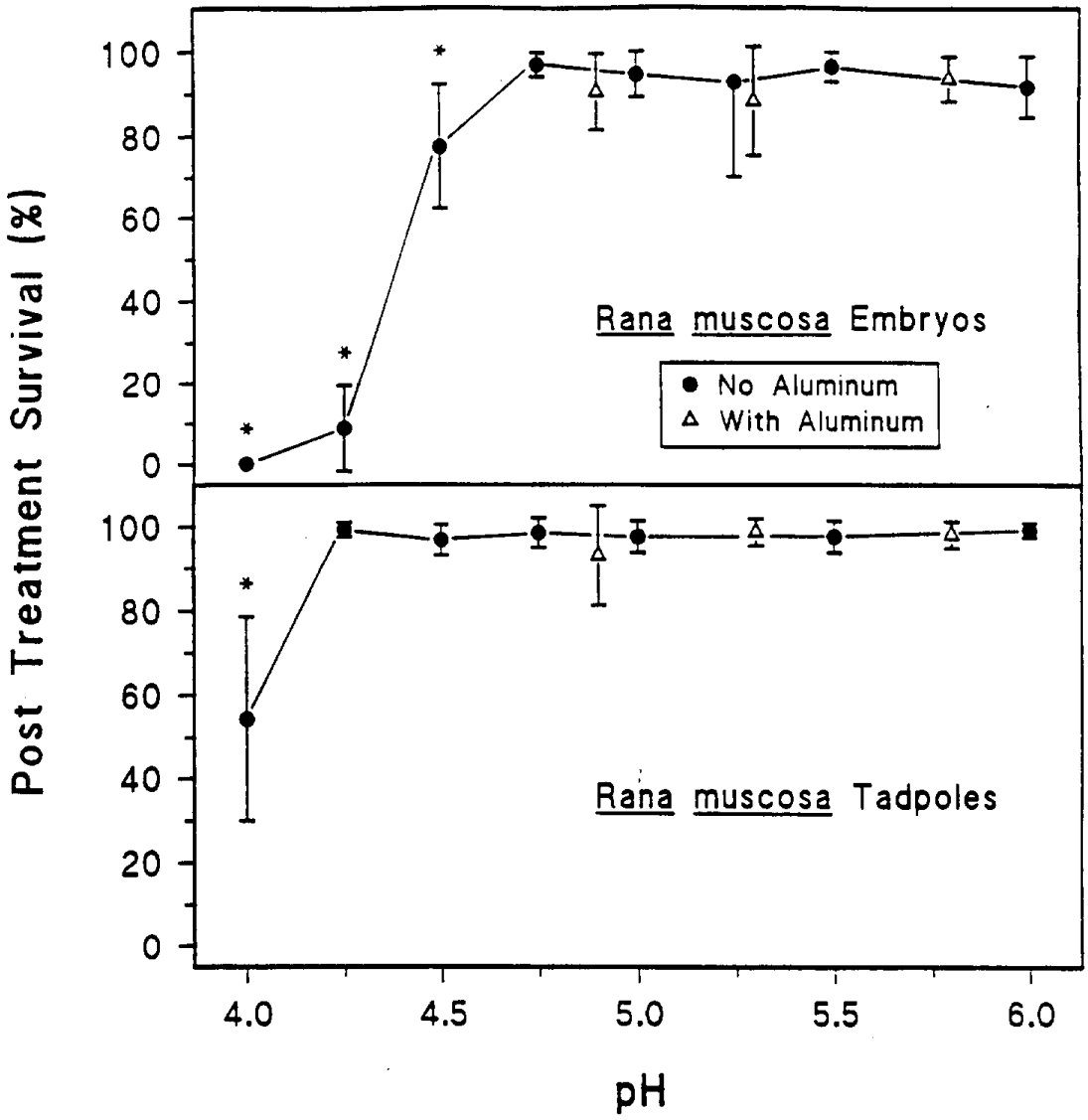

Figure 2-1. Post-treatment survival for Rana muscosa embryos and tadpoles as a function of pH and aluminum concentration (effectively 0 or 39-80 μg/L). Symbols and vertical lines represent clutch/replicate means and 95% confidence limits of mean (based on untransformed data). A line connects symbols for treatments without aluminum. Asterisks above a "No Aluminum" symbol indicate significant difference (overall α=0.05; Bonferroni procedure for multiple comparisons; see text) from value at pH 6.0 (no aluminum); asterisks below a "With Aluminum" symbol indicate significant difference (overall α=0.05) from interpolated value at same pH, aluminum absent (see text). For embryos N = 8 clutches represented in all treatments except pH 5.25, for which only 4 were represented. For tadpoles N = 7, which consists of a combination of individual clutches and replicates from a group of clutches (see Table 2-1).

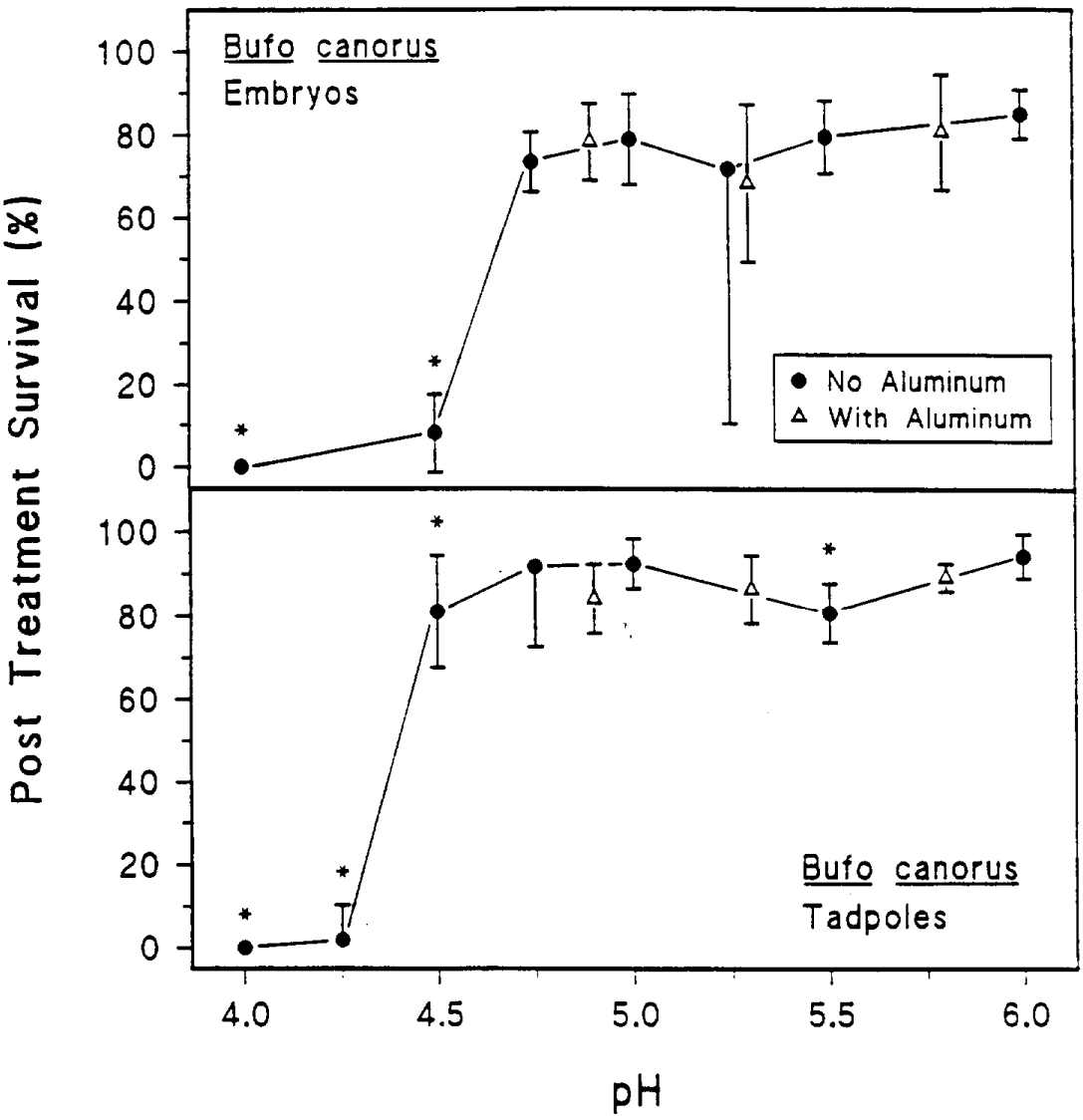

Figure 2-2. Post-treatment survival for Bufo canorus embryos and tadpoles as a function of pH and aluminum concentration. Symbols as in Fig. 2-1. For embryos N = 8 clutches represented in all treatment conditions except pH 4.75 and 5.25, for which only 3 were represented. For tadpoles N = 9 clutches in all treatments except pH 4.25 and 4.75, for which only 3 were represented.

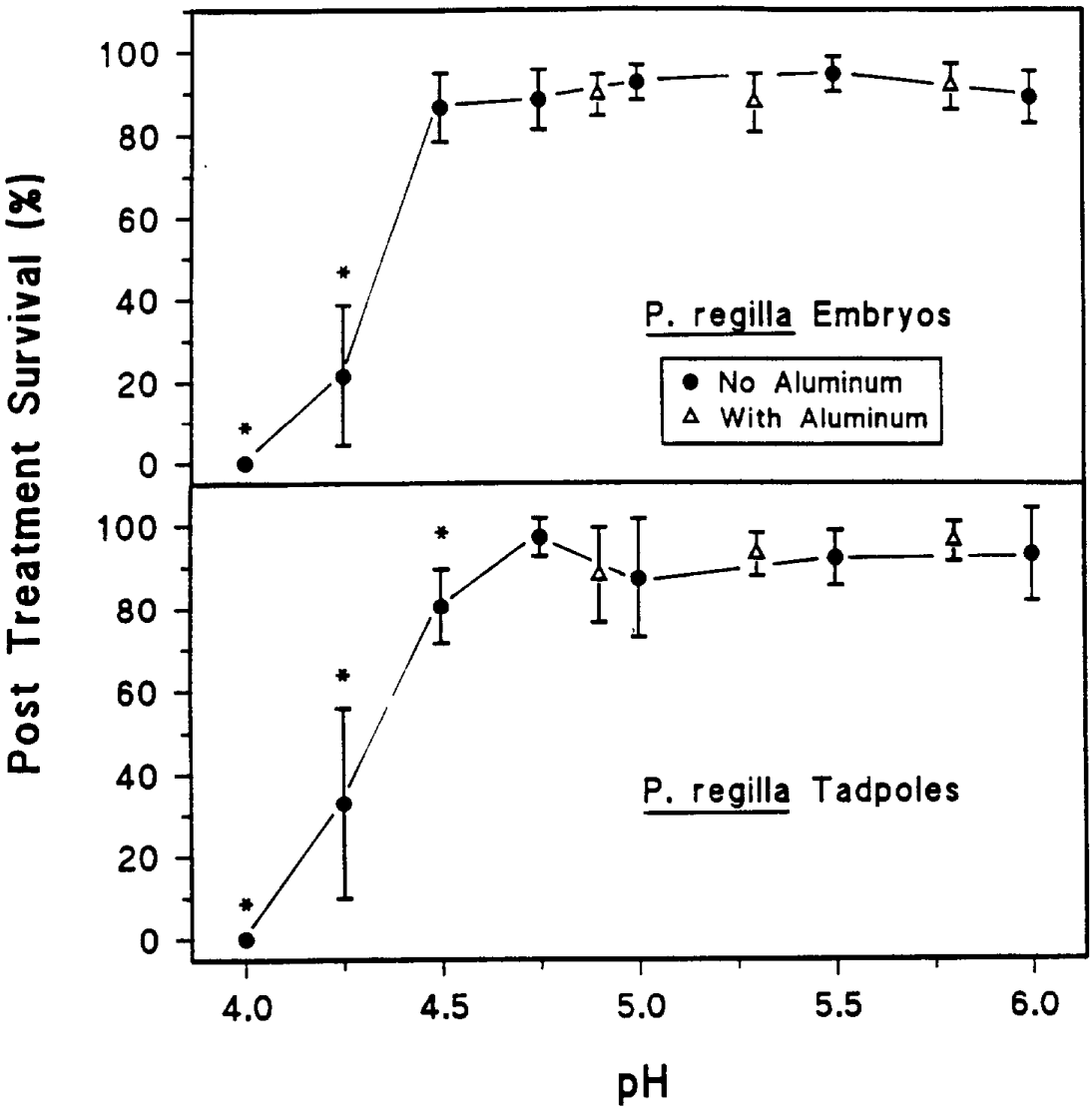

Figure 2-3. Post-treatment survival for Pseudacris regilla embryos and tadpoles as a function of pH and aluminum concentration. Symbols as in Fig. 2-1. For both embryos and tadpoles N = 8 clutches represented in all treatment conditions.

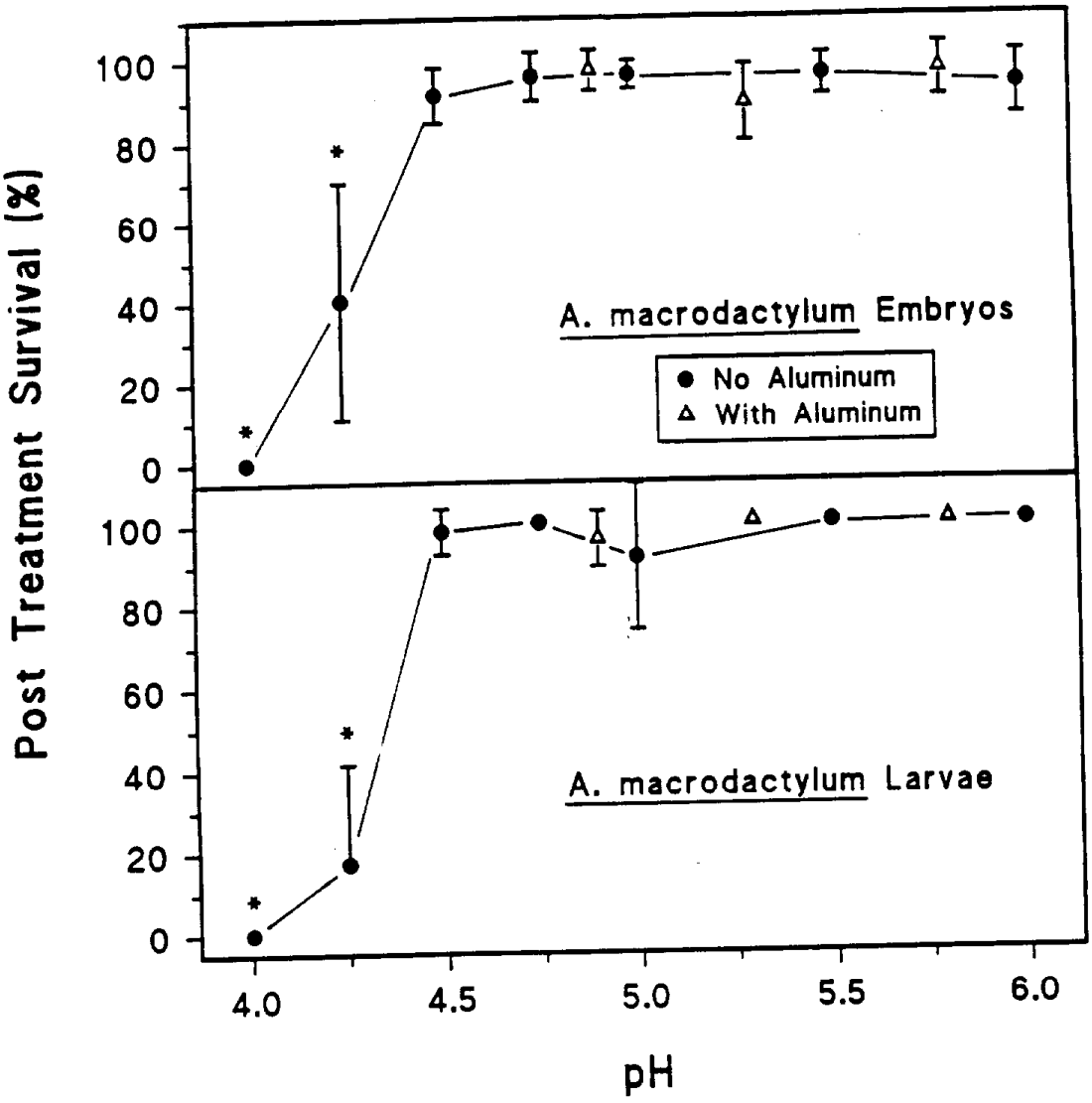

Figure 2-4. Post-treatment survival for Ambystoma macrodactylum embryos and tadpoles as a function of pH and aluminum concentration. Symbols as in Fig. 2-1. For embryos N = 6 clutches represented in all treatment conditions; for tadpoles N = 5 clutches.

Table 2-2. LC_{50} values (pH) for treatment survival (TS), survival to hatching (HS), and post-treatment survival (PTS), based on data shown in Figs. 2-1 to 2-4. Aluminum is not present. Values are mean (S.D.) and range.

Species	Stage	N	TS	HS	PTS
R. muscosa	Embryo	8	4.37 (0.08)	4.38 (0.08)	4.38 (0.08)
			4.28 - 4.50	4.28 - 4.50	4.28 - 4.50
	Tadpole	7	<4.00	-	<4.00
			<4.00 - 4.10	-	<4.00 - 4.11
B. canorus	Embryo	8	4.58 (0.15)	4.62 (0.06)	4.70 (0.08)
			4.22 - 4.68	4.50 - 4.68	4.62 - 4.87
	Tadpole	7	4.25 (0.09)	-	4.32 (0.12)
			4.18 - 4.37	-	4.19 - 4.56
P. regilla	Embryo	10	4.23 (0.08)	4.31 (0.07)	4.32 (0.07)
			4.11 - 4.36	4.18 - 4.36	4.18 - 4.38
	Tadpole	10	4.30 (0.10)	-	4.32 (0.12)
			4.11 - 4.38	-	4.11 - 4.50
A. macrodactylum	Embryo	6	4.14 (0.02)	4.26 (0.09)	4.27 (0.09)
			4.11 - 4.17	4.14 - 4.37	4.14 - 4.37
	Larva	5	4.33 (0.04)	-	4.33 (0.04)
			4.27 - 4.37	-	4.27 - 4.37

length were 4.75 or below for embryos and tadpoles of all species, except for embryos of R. muscosa, which was 5.25 (Figs. 2-5 to 2-8). Critical pH was higher for embryos than tadpoles of R. muscosa, B. canorus, and P. regilla, but the reverse was true for A. macrodactylum.

Hatching time was not significantly affected by pH in R. muscosa and A. macrodactylum (Figs. 2-9 to 2-10; ANOVA, $P>0.3$ in both cases), whereas it was in B. canorus (ANOVA, $P<0.001$) and P. regilla ($P<0.05$). For B. canorus the critical pH for this effect was 5.0 (Fig. 2-9). For P. regilla critical pH could not be defined because hatching time differed significantly from the control pH only at 4.75, and not at the lower pH of 4.5 (Fig. 2-10). Interestingly, low pH reduced hatching time in B. canorus, but its effect in P. regilla was to increase it at pH 4.75.

Aluminum Effects. -- The addition of aluminum to treatment waters did not significantly affect post-treatment survival in embryos or tadpoles of any species (Figs. 2-1 to 2-4). Moreover, total length was not affected by aluminum in embryos of any species, but it caused a significant reduction in total length of B. canorus tadpoles and A. macrodactylum larvae (Figs. 2-5 to 2-8). For B. canorus tadpoles this effect was evident at pH 5.3 and 5.8, but not at 4.9, whereas for A. macrodactylum larvae the effect was evident only at pH 5.3 (Figs. 2-6 and 2-8). Hatching time was not affected by aluminum in R. muscosa and A. macrodactylum, but it was significantly reduced by aluminum at all pH's used for B. canorus and P. regilla (Figs. 2-9 and 2-10).

Life Stage Differences. -- Embryos of R. muscosa and B. canorus were more sensitive to low pH than tadpoles (Figs. 2-1 and 2-2). LC_{50} values for treatment survival (i.e., the endpoint taken at identical times for the two life stages) averaged >0.4 and 0.3 pH units lower in tadpoles than embryos

of R. muscosa and B. canorus, respectively (Table 2-2; t-test,

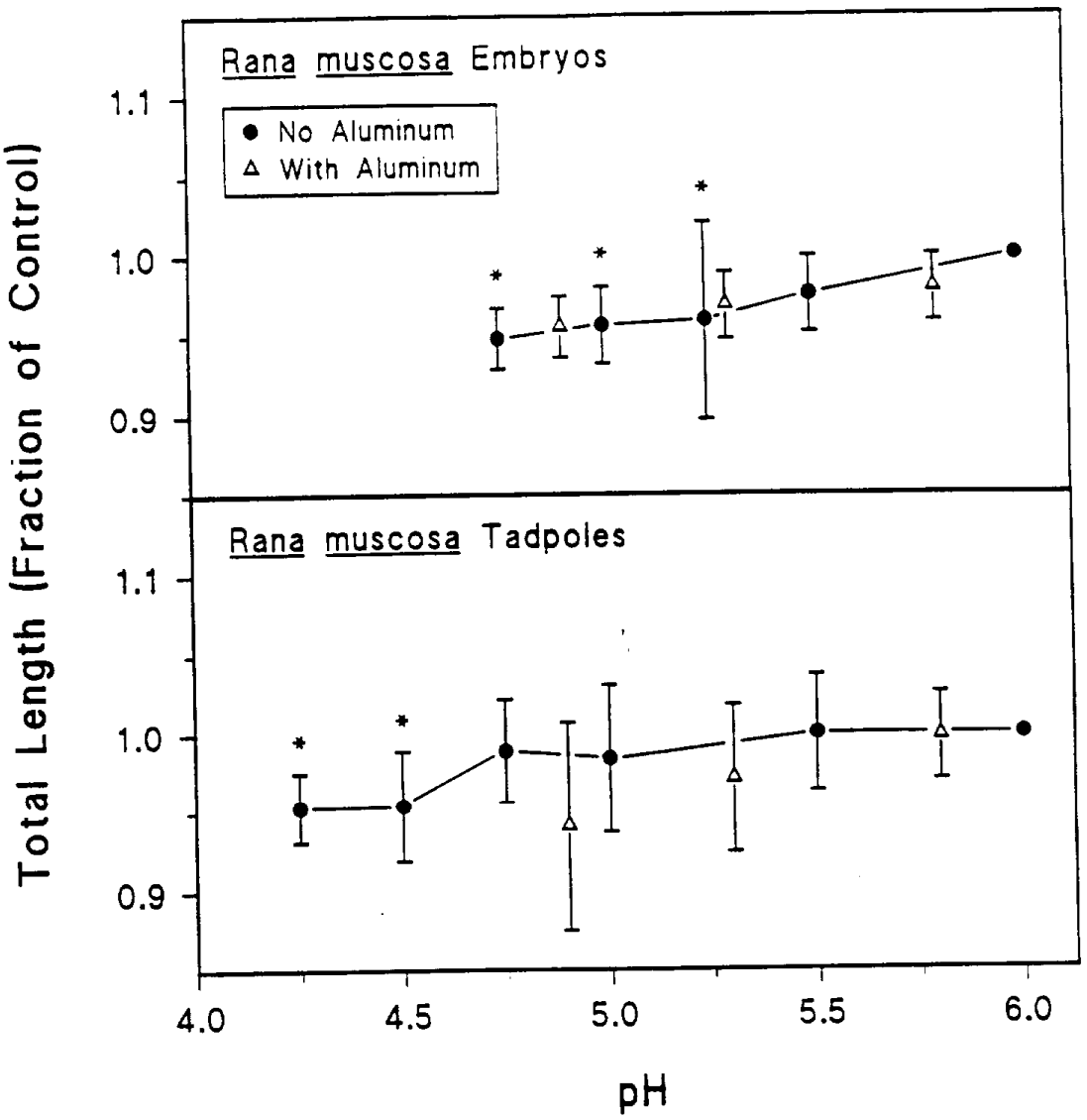

Figure 2-5. Total length of R. muscosa embryos and tadpoles as a function of pH and aluminum concentration at end of post-treatment period. Symbols represent clutch/replicate means as a fraction of values at pH 6.0 (no aluminum). Vertical lines represent 95% confidence limits of mean. Asterisks and sample sizes as in Fig. 2-1. Data are restricted to pH levels at which survival frequency was high (Fig. 2-1). Absolute values for total length of embryos and tadpoles at pH 6.0 (no aluminum) averaged 13.1 and 14.8 mm, respectively.

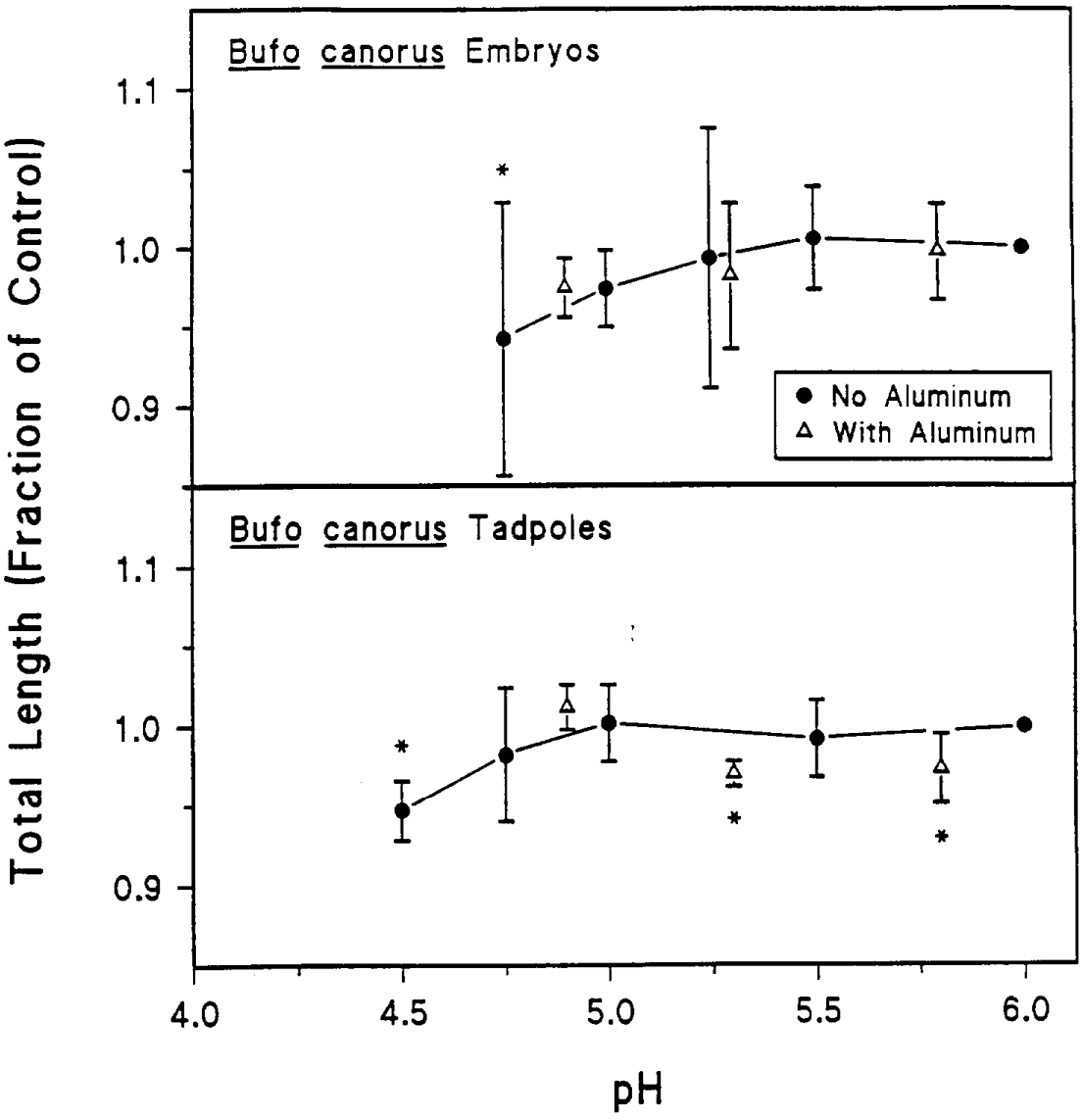

Figure 2-6. Total length of B. canorus embryos and tadpoles as a function of pH and aluminum concentration at end of post-treatment period. Symbols as in Fig. 2-5; sample sizes as in Fig. 2-2. Absolute values for total length of embryos and tadpoles at pH 6.0 (no aluminum) averaged 10.7 and 11.5 mm, respectively.

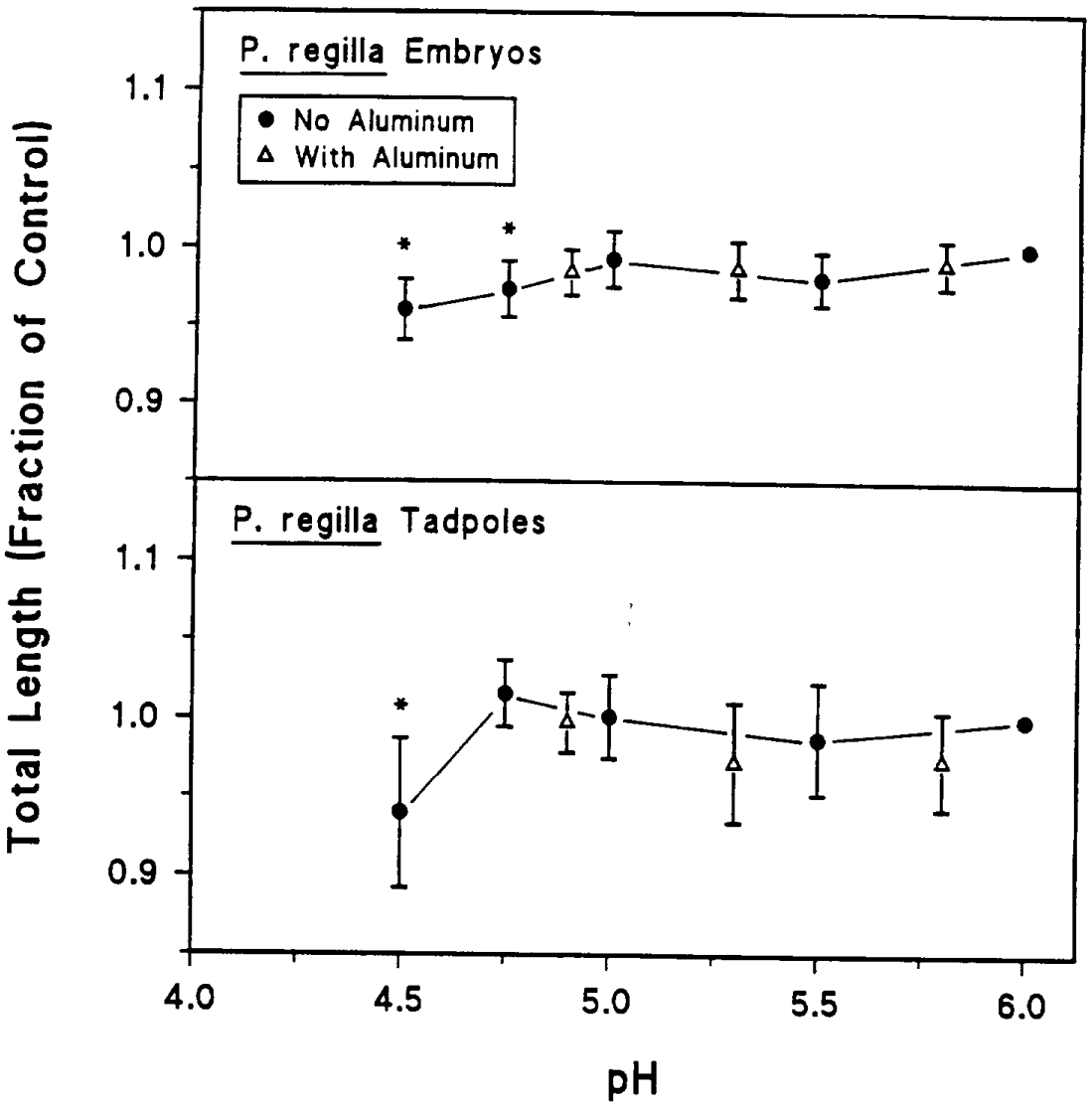

Figure 2-7. Total length of P. regilla embryos and tadpoles as a function of pH and aluminum concentration at end of post-treatment period. Symbols as in Fig. 2-5; sample sizes as in Fig. 2-3. Absolute values for total length of embryos and tadpoles at pH 6.0 (no aluminum) averaged 9.7 and 9.9 mm, respectively.

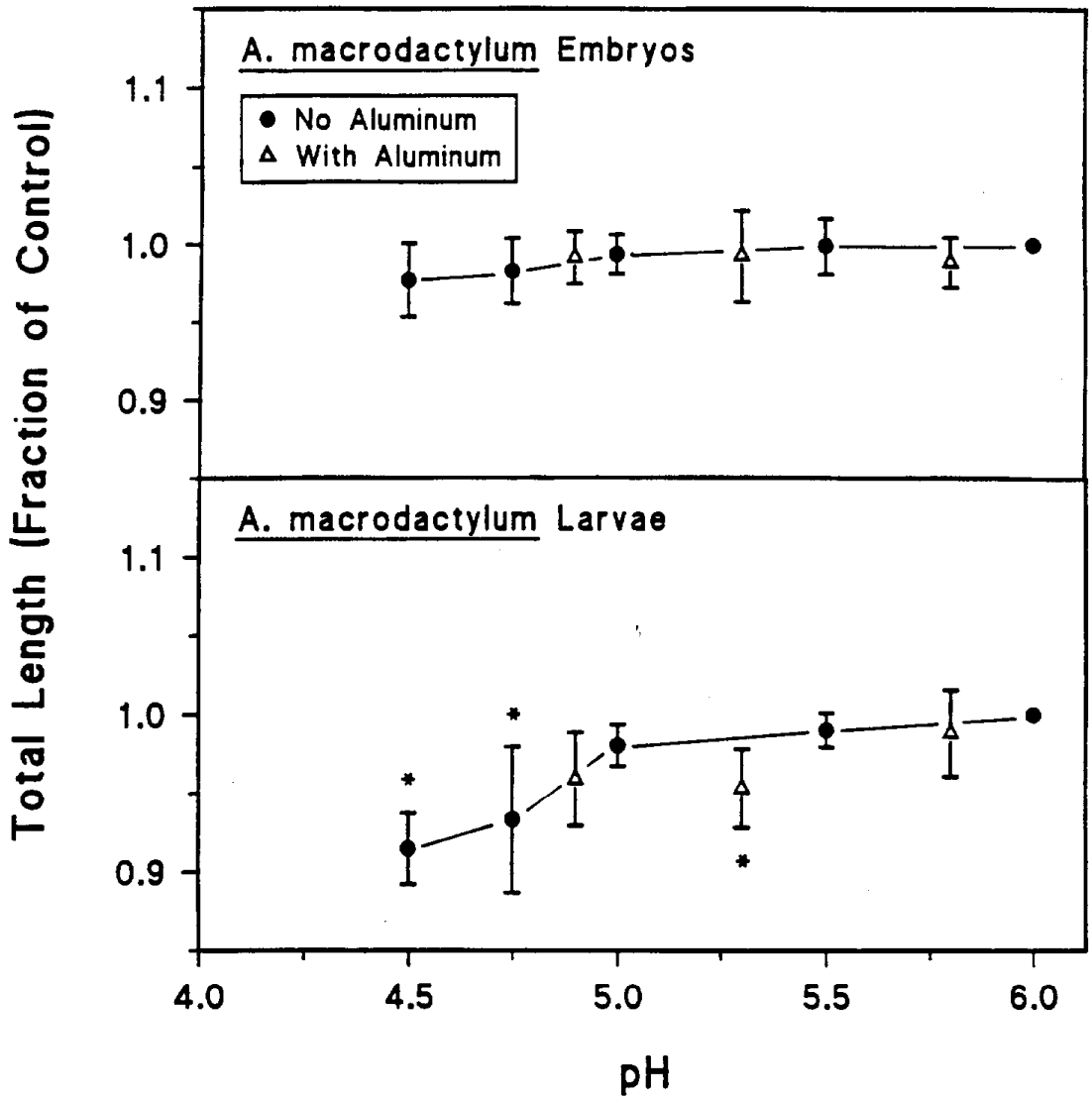

Figure 2-8. Total length of A. macrodactylum embryos and tadpoles as a function of pH and aluminum concentration at end of post-treatment period. Symbols as in Fig. 2-5; sample sizes as in Fig. 2-4. Absolute values for total length of embryos and tadpoles at pH 6.0 (no aluminum) averaged 12.2 and 12.7 mm, respectively.

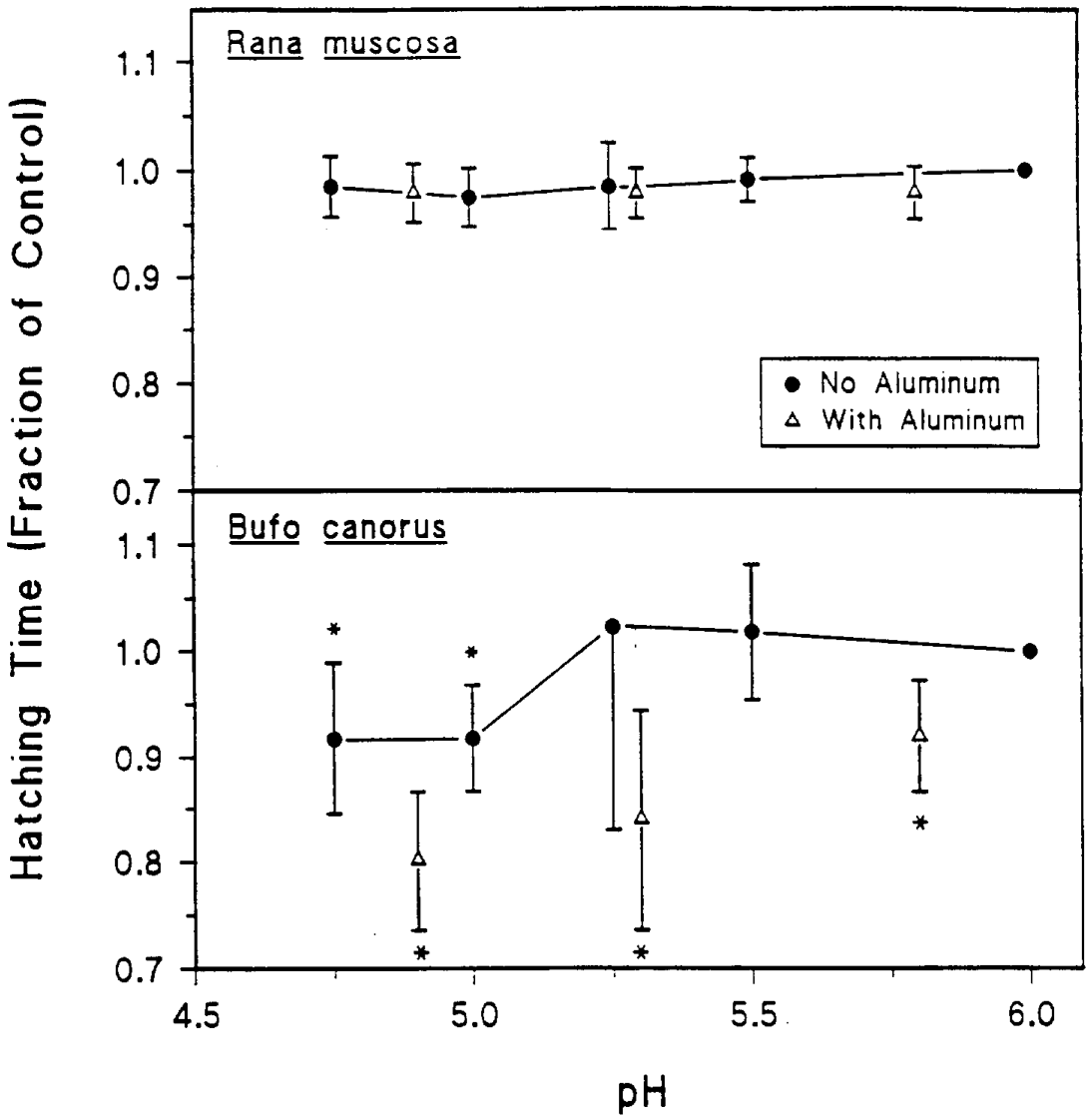

Figure 2-9. Time to reach hatching for R. muscosa and B. canorus as a function of pH and aluminum concentration. Symbols as in Fig. 2-5; sample sizes as in Figs. 2-1 and 2-2. Absolute values for hatching time at pH 6.0 (no aluminum) averaged 15.5 d in R. muscosa and 9.3 d in B. canorus.

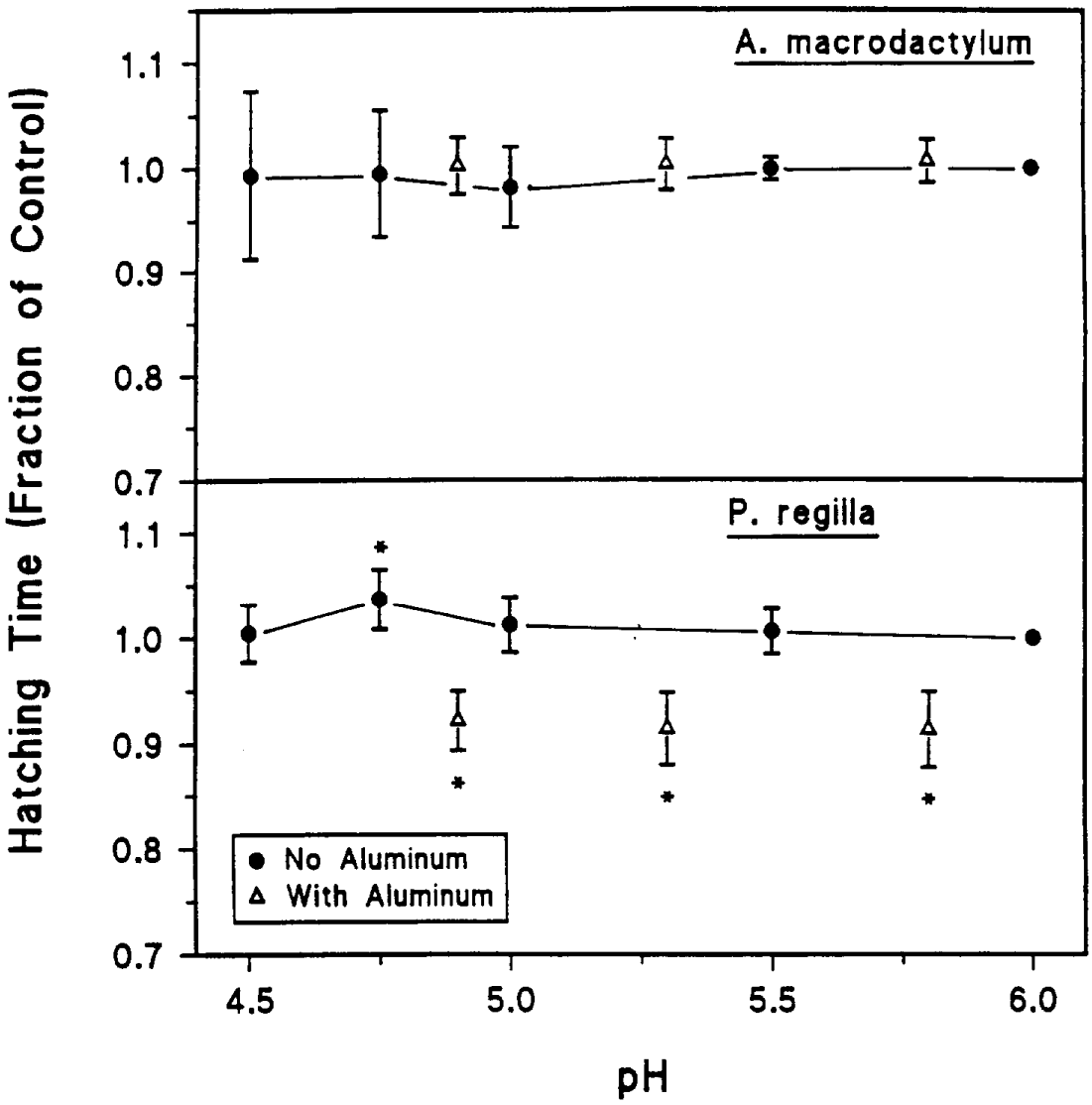

Figure 2-10. Time to reach hatching for P. regilla and A. macrodactylum as a function of pH and aluminum concentration. Symbols as in Fig. 2-5; sample sizes as in Figs. 2-3 and 2-4. Absolute values for hatching time at pH 6.0 (no aluminum) averaged 10.5 d in P. regilla and 16.4 d in A. macrodactylum.

$p<0.001$ in both species). Total length was also more strongly affected by reduced pH in embryos than tadpoles (Figs. 2-5 and 2-6). Critical pH for embryos of R. muscosa and B. canorus was 5.25 and 4.75, respectively, whereas it was 4.5 for tadpoles of both species.

In contrast to these two species, P. regilla and A. macrodactylum did not show such distinct differences between life stages in sensitivity to low pH. For P. regilla LC_{50} values for treatment survival were not significantly different between embryos and tadpoles (Table 2-2), although the critical pH for total length was lower for tadpoles than embryos (Fig. 2-7). For A. macrodactylum LC_{50} values for treatment survival were significantly higher for larvae than embryos (t-test, $P<0.001$; Table 2-2) and total length of larvae was affected by low pH whereas it was not for embryos (Fig. 2-8).

Larvae of B. canorus and A. macrodactylum were more sensitive to aluminum than embryos, showing a significant reduction in total length at some pH levels (Figs. 2-6 and 2-8). In R. muscosa and P. regilla, however, aluminum did not significantly affect total length of either life stage (Figs. 2-5 and 2-7).

Species Differences. -- The four species differed in their sensitivity to low pH. LC_{50} values for treatment and hatching survival of embryos (i.e., the two survival endpoints that were identical for embryos of all species) differed significantly among species (ANOVA, $P<0.0001$; Tables 2-2) and ranked in the following order: A. macrodactylum < P. regilla < R. muscosa < B. canorus (Table 2-3). All pairwise comparisons for treatment survival of embryos were significantly different except for A. macrodactylum versus P. regilla (Table 2-3). For hatching survival, however, fewer pairwise comparisons were significantly different. Based on critical pH values for embryo total length and hatching time, R. muscosa and B. canorus again ranked as the most sensitive

Table 2-3. Comparison of LC_{50} pH values among species. Mean values below each species are derived from Table 2-2. Bars above species represent no significant difference among species (P>0.05). For all comparisons other than those with R. muscosa tadpoles, Tukey's HSD multiple comparisons test was used (Wilkinson 1990). R. muscosa was compared to the other species by Mann Whitney U test (Siegel 1956), with Bonferonni procedure to maintain overall α=0.05, because LC_{50} for most clutches was below 4.0 and could not be calculated (Table 2-2).

EMBRYOS: TREATMENT SURVIVAL

A. macrodactylum < P. regilla < R. muscosa < B. canorus

4.14 < 4.23 < 4.37 < 4.58

EMBRYOS: HATCHING SURVIVAL

A. macrodactylum < P. regilla < R. muscosa < B. canorus

4.26 < 4.31 < 4.38 < 4.62

TADPOLES: TREATMENT SURVIVAL

R. muscosa < B. canorus < A. macrodactylum < P. regilla

<4.0 < 4.25 < 4.33 < 4.30

species to low pH. Critical pH for total length was highest for R. muscosa whereas critical pH for hatching time was greatest for B. canorus (Figs. 2-5 to 2-10).

For larvae the ranking of LC_{50} values for treatment survival differed from that for embryos: R. muscosa < B. canorus < A. macrodactylum < P. regilla (Table 2-3). In this case R. muscosa was significantly more tolerant of low pH than all of the other species, but the other species did not differ significantly from each other. Based on critical pH for total length, larvae differed little among the species (Figs. 2-5 to 2-8). Critical pH for total length was 4.5 for all species except A. macrodactylum, which was 4.75.

Sensitivity to aluminum also differed among the species, although the differences were less distinct than for sensitivity to pH. Survival was not significantly affected for any of the species by aluminum at the pH and aluminum levels tested (Figs. 2-1 to 2-4). However, total length was reduced in the presence of aluminum at some pH levels for B. canorus tadpoles and A. macrodactylum larvae (Figs. 2-6 and 2-8). Moreover, hatching time was reduced in the presence of aluminum at all pH levels used for B. canorus and P. regilla (Table 2-9 and 2-10).

Effects of Ionic Strength of Water. -- Embryos of R. muscosa tolerated lower pH as solute concentration increased (data not shown). Post-treatment survival at pH 4.25 increased from an average of 0% at solute level 1 to 74% at solute level 16 (2-way ANOVA, $P<0.0001$). LC_{50} values for post-treatment survival decreased with increasing solute concentrations from a mean pH of 4.44 at solute level 1 to below 4.25 at solute level 16 ($P<0.01$, Kruskal-Wallis 1-way ANOVA by ranks, Siegel [1956]).

DISCUSSION

Adverse Effects of Low pH. -- Survival of all life stages and species was reduced at pH 4.5 and below (i.e., critical pH for post-treatment survival was 4.5 or below). LC_{50} for post-treatment survival among the species and stages ranged as high as 4.7 for B. canorus embryos; however, this value is probably inflated because only 3 of the 8 clutches were represented at pH 4.75.

Low pH also had sublethal effects on nearly all species and stages. Total length was reduced at low pH for all species and stages except for A. macrodactylum embryos. The general decline in total length with decreasing pH is a common pattern in amphibians (Pierce and Wooten 1992), and presumably indicates that growth is inhibited as pH declines. This sublethal effect was evident in many cases in the present study at higher pH levels than effects on survival. For example, embryos were smaller than controls (i.e., pH 6.0) at pH $\leq$ 5.25 for R. muscosa, and at pH $\leq$ 4.75 for B. canorus and P. regilla. Another sublethal effect of low pH, reduced hatching time, was evident at pH $\leq$ 5.0 for B. canorus. However, no effect was evident in R. muscosa and A. macrodactylum, and hatching time was increased at pH 4.75 for P. regilla.

The life stage most sensitive to low pH differed among the species. Embryos of R. muscosa and B. canorus were more sensitive to low pH than tadpoles, based on LC_{50} values and total length. LC_{50} for embryos of R. muscosa and B. canorus were higher than values for hatchling tadpoles, by about $\geq$ 0.3 pH units. Greater sensitivity of embryos than tadpoles is the common pattern observed in amphibians (Pierce 1985; Freda 1986). However, the opposite pattern was found for A. macrodactylum, based both on LC_{50} values and total length. For P. regilla the pattern was equivocal; embryos were more sensitive based on total length, whereas LC_{50} values did not differ significantly between the two life stages.

Adverse Effects of Aluminum. -- Experimental levels of aluminum did not cause a decrease in survival endpoints for embryos or tadpoles/larvae of any species. This result is not surprising given that the LC_{50} for total dissolved aluminum for embryos and tadpoles of other amphibian species is generally much higher than 80 μg/L (Freda 1991). Nevertheless, aluminum did cause a significant reduction in total length of B. canorus tadpoles at pH 5.3 and 5.8, but not at pH 4.9, and for A. macrodactylum larvae at pH 5.3, but not 4.9 and 5.8. This pattern is surprising because aluminum is more soluble at lower pH (Baker et al. 1990), and measured concentrations were greatest at pH 4.9. The addition of aluminum also caused earlier hatching in embryos of B. canorus and P. regilla, but not the other species.

It is not possible to generalize about which life stage is more sensitive to aluminum. Total length of larvae was reduced with aluminum present at some pH levels for B. canorus and A. macrodactylum, whereas length was not affected for embryos. However, embryos of B. canorus and P. regilla were affected by aluminum by having reduced hatching times.

Comparisons to Other Amphibians. -- The sensitivities to low pH for amphibians in this study are similar to most amphibians studied elsewhere. LC_{50} for survival to hatching, which is the endpoint most frequently used in chronic toxicity tests on amphibian embryos, and critical pH for post treatment survival, provide the basis for comparison to other species. These values are, respectively, 4.4/4.5 for R. muscosa, 4.6/4.5 for B. canorus, and 4.3/4.3 for P. regilla, and 4.3/4.3 for A. macrodactylum. These values are similar to those for the majority of about two dozen other species of anurans (frogs and toads) and four other Ambystoma salamander species tested from eastern North America, Europe, and Africa (Pierce 1985; Freda 1990), although experimental conditions vary among these studies. LC_{50} values for the three anurans in the present study are also similar to values obtained for four

anurans inhabiting dilute water at high elevation in Colorado (Corn et al. 1989). However, the LC_{50} for A. macrodactylum is considerably below that reported for A. tigrinum (5.3) in the Rocky Mountains (recalculated by Corn and Vertucci [1992] from data of Harte and Hoffman [1989]).

The sensitivities of amphibians to aluminum in the present study cannot be compared to other species because the dose used in this study was much lower than nearly all other studies (Freda 1991).

Effects of Low Ionic Strength of Water. -- Most of the surface waters in the Sierra Nevada are exceptionally low in ionic strength, among the most dilute surface waters in the world (Melack et al. 1985; Landers et al. 1987). Low ionic strength typically renders amphibians and fish more sensitive to low pH because of its effect on sodium balance (Freda and Dunson 1984). This sensitivity was evident in the present study, as embryos of R. muscosa tolerated lower pH levels when the solute concentration in water was increased. Despite this effect, however, the dilute waters of the Sierra Nevada appear to not have rendered the species in the present study especially sensitive to low pH in comparison to other species. Their LC_{50} values, determined in dilute water, appear to be similar to the majority of other species, which generally inhabit waters with greater ionic strength (Freda 1990).

CHAPTER 3 -- WATER CHEMISTRY OF AMPHIBIAN HABITATS IN RANDOMLY SELECTED SURVEY AREAS IN THE FIELD

OBJECTIVES

Objective 5. Characterize the relative abundance, distribution, and associated water chemistry of a statistically representative sample of amphibian populations in the Sierra Nevada at high elevation.

Objective 6. Test the hypothesis that amphibians generally do not occur in waters low in pH, ANC, or EC. If anthropogenic acidification of habitats has been a factor influencing the distribution of amphibians in the Sierra Nevada, either by itself or in combination with other factors, it would be expected that amphibians have been eliminated from waters most vulnerable to acidification, i.e., low in pH and ANC. In addition, low EC may have contributed to such elimination, because low ionic strength increases the sensitivity of amphibians to low pH (Freda and Dunson 1984).

Objective 7. Test the hypothesis that species differ in the water chemistry of breeding sites in the same manner that they differ in sensitivity to acidic conditions in the laboratory. This pattern would be expected if anthropogenic acidification of habitats has been a factor influencing the distribution of amphibians in the Sierra Nevada. LC_{50} pH values for embryos differ significantly among the three species used to test this hypothesis, and rank in the following order: *P. regilla* < *R. muscosa* < *B. canorus* (Chapter 2). The embryo is the life stage chosen for comparison because embryos typically develop in waters fed by snowmelt immediately following thawing of the water surface, which coincides with the time of lowest pH and ANC, and (2) embryos of these species are similar to or greater than tadpoles in sensitivity to low pH.

METHODS

Survey Areas

Thirty survey areas were included in this study, each consisting of a 15 km^2 circle (diameter = 4.4 km), exclusive of an average of 7% of each circle that was below 2,440 m (8,000 feet) elevation. The center of each circle was randomly selected from a uniform grid established as part of the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP), with a minimum elevation of 2,440 m. Specific locations and maps for each survey area are provided in Appendix A. The 30 survey areas were divided at random into two subsets of 15 areas, each of which was surveyed during either 1990 or 1991. All survey areas were potentially within the geographic ranges of P. regilla and R. muscosa, 23 were potentially within the range for B. canorus, 7 for B. boreas, and 3 for A. macrodactylum (Zweifel 1955, Karlstrom 1962, Stebbins 1985).

Survey, Sampling, and Measurement Procedures

Each survey area was searched by two individuals in a manner designed to provide data for sites that contain each species and sites that do not, and to ascertain whether a breeding population of each species exists in the survey area. Survey areas were searched during the daytime in early and mid summer (31 May to 23 July 1990; 12 June to 2 August 1991) when amphibian larvae are present and most visible and abundant in shallow water near shore (Bradford 1984). The survey areas were searched in a non-random manner until five "different or separate" sites containing larvae (or eggs) of each species were found, or until enough of the survey area had been searched to be confident that a breeding population of a species did not exist in the survey area. "Different or separate" sites were defined as ones separated by more than

200 m or ones that appeared to differ in water characteristics or sources (e.g., a pool in a stream versus an isolated pond). Sites were surveyed for amphibian larvae by walking near or in shallow water near shore. Also surveyed were up to five "different or separate" sites that appeared to represent potential breeding habitat for each amphibian species, but which lacked the species. Judgements concerning potential breeding habitat were based largely on (1) geographic range of each species and elevational limit of 3660 m (Zweifel 1955, Karlstrom 1962, Mullaly and Cunningham 1956); (2) depth or other characteristics suggesting that the time interval during which standing water is available could be sufficient for tadpoles to reach metamorphosis, and (3) absence of predatory fishes unless emergent vegetation was extensive in area (Bradford 1989a). Species identification of Bufo tadpoles was based on geographic ranges for B. canorus and B. boreas described in Karlstrom (1962).

At each site an unfiltered water sample was taken in a clean, rinsed 125-ml high-density polypropylene bottle. Some samples were filtered for comparison to unfiltered samples using a 1 μm Nucleopore filter and an all-plastic filter holder and plastic syringe. Approximately 10% of samples were taken as duplicates. Water samples were kept cool and in the dark until analyzed for pH, ANC, and electrical conductivity (EC) at the Sierra Nevada Aquatic Research Laboratory within 6 days of collection. Environmental parameters for each site were elevation (derived from 7 1/2-minute U.S.G.S. topographic maps), water temperature (to 0.1°C, using a thermometer calibrated against a NIST-traceable thermometer), and maximum depth of the water body (estimated visually from shore in one of five categories: <0.50 m, 0.51-1.00 m, 1.01-1.50 m, 1.51-2.0 m, >2.0 m).

pH was measured at room temperature using a Fisher Acumet 910 meter or Orion SA250 meter, and Ross (Orion) combination electrode (Sickman and Melack 1989). The electrode was calibrated daily with standard buffers and checked

periodically using dilute solutions of HCl (10^{-4} and 10^{-5} N). ANC was determined by incremental titration with 0.1N HCl (Gran titration; Talling 1973). EC was measured with a YSI model 35 conductance meter (cell constant = 0.1 cm) at room temperature.

A comparison of 9 filtered versus unfiltered samples revealed no significant differences ($P>>0.05$) in pH, EC, and ANC. Fourteen unfiltered duplicate samples differed from each other by averages of 0.1 pH unit and 8.3 ug/L (ANC) between the two meters ($P<0.001$). However, samples were randomly assigned to each meter for analysis.

Statistical Analysis

Statistical analyses were limited to the three common species (P. regilla, R. muscosa, and B. canorus) because sample sizes were very small for the other two species. As discussed previously, R. muscosa and B. canorus are considered declining species, whereas P. regilla is considered a non-declining species. For the three species water chemical characteristics were compared between sites containing breeding individuals (as evidenced by eggs or tadpoles) and sites considered potential breeding habitat for the species, but lacking the species. Comparisons were made both "within" and "between" survey areas. "Within survey areas" comparisons were made using the ANOVA model (Wilkinson 1990):

$$Y = \text{Constant} + \text{Survey Area} + \text{Species Presence} + \text{Elevation} + \text{Depth} \quad (1)$$

where Y = pH, log ANC, or log EC; Constant = a value generated by the analysis; Survey Area = a category variable representing each survey area containing the species (1, 2, etc.); Species Presence = a category variable for larvae present or absent at a site (0 or 1); and Depth is a category variable as defined above (1, 2,... or 5). Elevation and

depth are included in the analysis because the three water chemistry parameters (Y, above) were negatively correlated with elevation within survey areas ($P<0.01$ for each parameter), and log EC differed significantly among depth categories ($P=0.03$). Temperature was omitted because it did not correlate significantly with any of the water chemistry parameters. Wilks' Lambda statistic (Wilkinson 1990) was computed to test for multivariate effects of pH, log ANC, and log EC. "Between survey areas" comparisons were made using the following ANOVA model:

$$Y = \text{Constant} + \text{Species Presence} + \text{Elevation} \qquad (2)$$

where Y = mean pH, geometric mean (= mean log) ANC, or geometric mean EC of sites within each survey area; and Species Presence = a category variable for larvae present or absent within the survey area. For survey areas containing a species, sites used to compute means were those containing the species, whereas for survey areas lacking the species, sites used were those considered potential breeding habitat for the species. Depth is omitted from model 2 because the loss of degrees of freedom in the analysis would be substantial.

In a similar manner water chemical characteristics were compared among species by replacing "Species Presence" in models 1 and 2 with "Species," where Species = a category variable representing each of the three species. These new models are referred to as models 3 and 4. The "within survey areas" "among species" comparison (model 3) was limited to pairwise comparisons between P. regilla and each of the other species, because only one survey area contained more than two species. In the analysis of all of the above models, three sites were excluded as statistical outliers (pH <5.0 and/or ANC<0). Sites with such values are extremely rare in the Sierra Nevada (Melack and Stoddard 1991).

RESULTS

Amphibians Found

Amphibians were found in 25 of the 30 survey areas (Table 3-1). The five survey areas lacking amphibians contained relatively few water bodies, and fish were usually present in the few sites that otherwise appeared suitable for amphibians. P. regilla was present in all 25 survey areas containing amphibians, whereas R. muscosa and B. canorus were each found in six, B. boreas in two, and A. macrodactylum in one. Two species (P. regilla plus another) occurred together in 13 survey areas, and three species (P. regilla, R. muscosa, and B. canorus) occurred together in one.

Specific locations of sites sampled are shown on maps in Appendix A, and species occurrence and water chemistry for each site are provided in Appendix C.

General Characteristics of Waters Sampled

pH of the 141 sites containing amphibians ranged from 5.4 to 7.9 (median = 6.2), ANC ranged from -1.5 to 1100 ueq/l (median = 89), and conductivity ranged from 2.6 to 194 uS/cm (median = 11.1) (Fig. 3-1). Temperature of sites containing amphibians ranged from 9 to 30°C (median = 11°C), and elevation ranged from the minimum included in the survey (2,440 m; 8,000 feet) to 3,520 m (11,550 feet) (median = 2,800 m; 9,200 feet). Values for sites lacking amphibians were similar to sites containing amphibians (Fig. 3-1); detailed comparisons by species are presented below.

pH was exceptionally low (4.6 and 4.9) in two sites in one survey area, both of which lacked amphibians. The source of acidity here appears to be from iron pyrite deposits (G. Mahood, pers. comm.). These two sites, and an additional one in which ANC was essentially 0, were omitted from statistical analyses as outliers.

Table 3-1. Amphibian species and water samples represented in survey areas (15 km^2).

	No. Survey Areas	No. Sites Sampled
Amphibians Present	25	141
Amphibians Absent	5	94
TOTAL	30	235
Species Present:		
P. regilla	25	122
R. muscosa	6	21
B. canorus	6	17
B. boreas	2	3
A. macrodactylum	1	5

Characteristics of Waters Containing Versus Lacking Amphibians

In general, water chemistry parameters overlapped considerably between sites containing a species and sites lacking the species (Figs. 3-1 to 3-6). When analyzed "within survey areas" for the three common species according to model 1, multiple R^2 values were highly significant for all species ($R^2>0.5$ and $P<0.001$ for all species and all "Y" parameters). However, Wilks' Lambda statistic for multivariate effects of presence/absence on pH, log ANC, and log EC was not significant for any of the three species, and a significant univariate difference for presence/absence was found only for log EC in P. regilla (P=0.046; Table 3-2). In this case, EC (untransformed data) was greater for sites lacking P. regilla by a factor of 1.20 (based on coefficients derived for model 1), an effect opposite to the hypothesis in Objective 6. This difference is not evident in Fig. 3-2 because of pronounced effects on log EC from Survey Area and Elevation found in the analysis of model 1, which are not represented as variables in the 2-dimensional figure.

Mean values for survey areas containing or lacking each species are shown in Figs. 3-7 to 3-11. In the "between survey areas" comparison of presence/absence (model 2) for the three common species, Wilks' Lambda was again not significant for any of the three, and a significant univariate effect for presence/absence was found only for log EC in B. canorus (P=0.041; Table 3-2). In this case, EC (untransformed data) was greater for sites lacking B. canorus by a factor of 2.27 (based on coefficients derived for model 2). This effect is evident in Fig. 3-7 because elevation (which is not represented as a variable in the figure) varied little between sites containing and sites lacking the species. Again, this effect is opposite to that predicted in Objective 6.

In species comparisons (Objective 7) "within survey areas" for the three common species, neither R. muscosa nor B. canorus differed significantly from P. regilla in pH, log ANC,

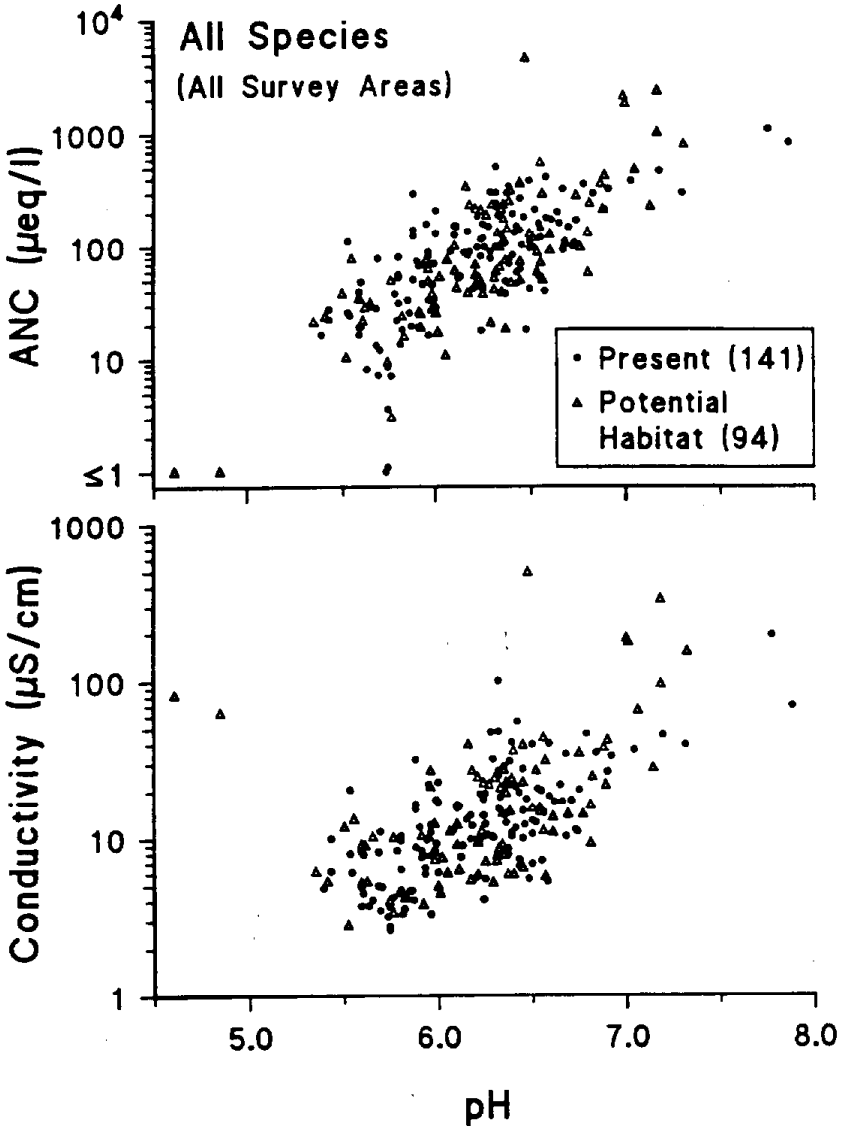

Figure 3-1. pH, ANC, and EC for amphibian breeding sites and sites considered potential breeding habitat but lacking amphibians. Numbers in parentheses represent total number of sites within all survey areas. Three sites are excluded from statistical analyses as outliers. Specific values for pH, ANC, and EC at these three sites were, respectively, 4.61, -13.1, 81.2 (site 1); 4.85, 0.1, 62.2 (site 2); and 5.73, -1.5, 3.18 (site 3).

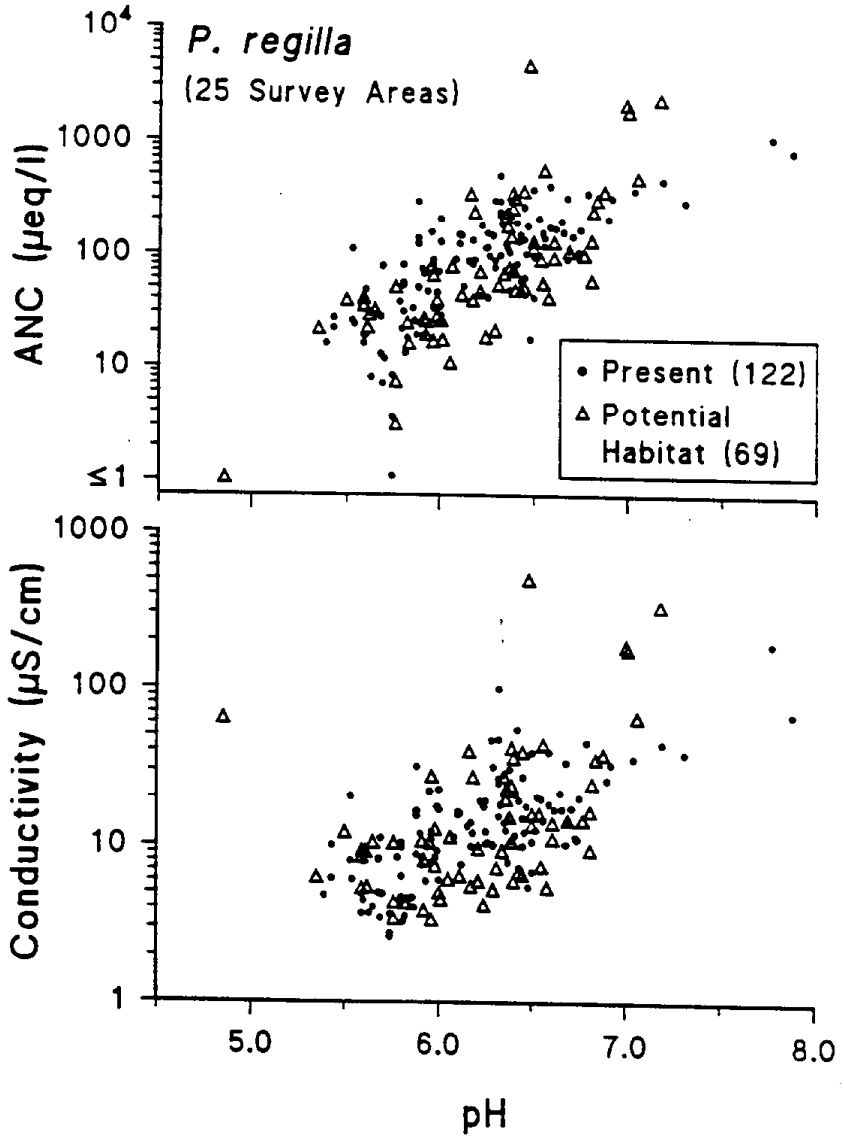

Figure 3-2. pH, ANC, and EC for P. regilla breeding sites and sites considered potential breeding habitat but lacking the species. Survey areas represented are only those containing the species. Numbers in parentheses represent total number of sites for species within the survey areas. Sites excluded from statistical analyses as outliers are described in Fig. 3-1.

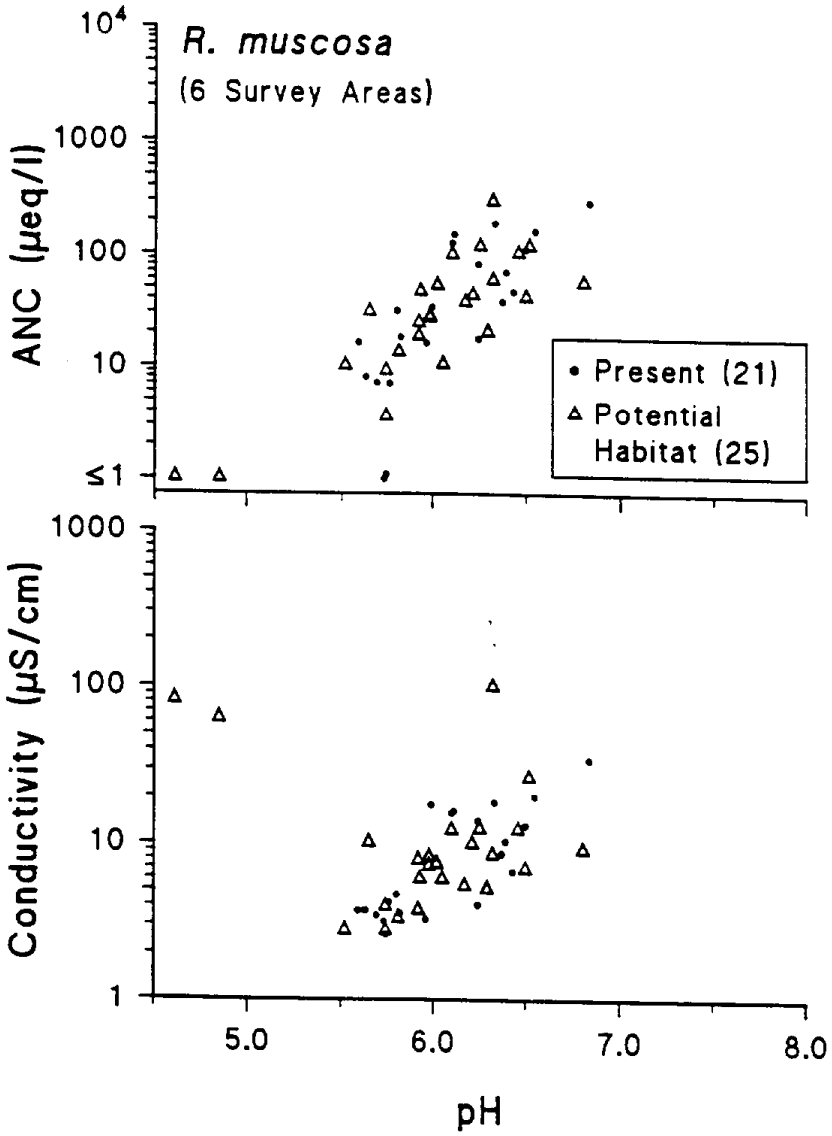

Figure 3-3. pH, ANC, and EC for R. muscosa breeding sites and sites considered potential breeding habitat but lacking the species. Details as in Fig. 3-2.

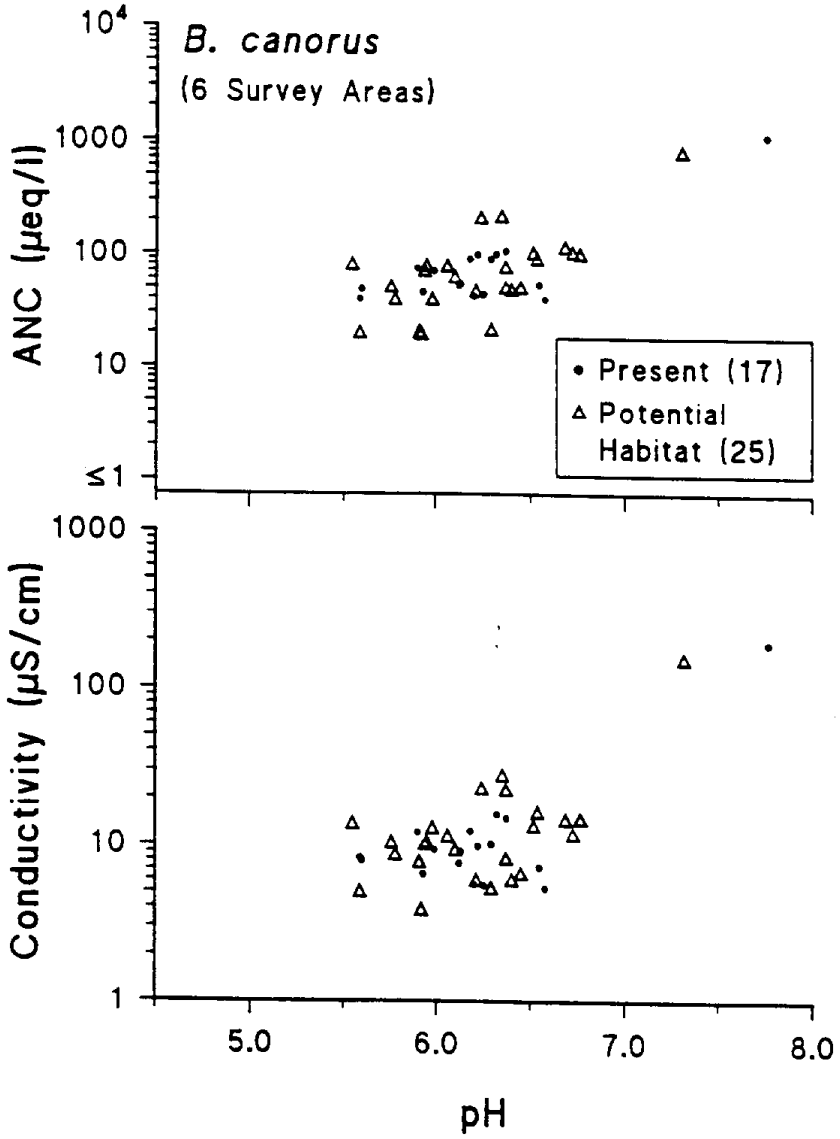

Figure 3-4. pH, ANC, and EC for B. canorus breeding sites and sites considered potential breeding habitat but lacking the species. Details as in Fig. 3-2.

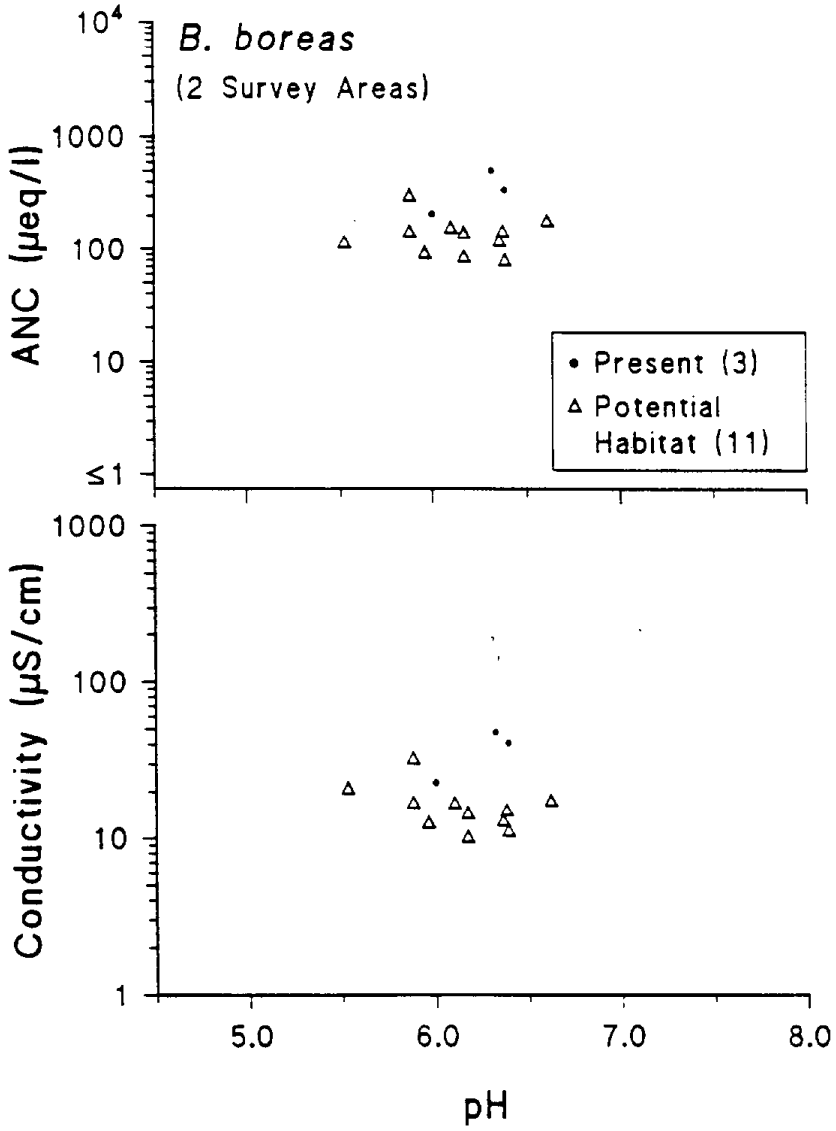

Figure 3-5. pH, ANC, and EC for B. boreas breeding sites and sites considered potential breeding habitat but lacking the species. Details as in Fig. 3-2.

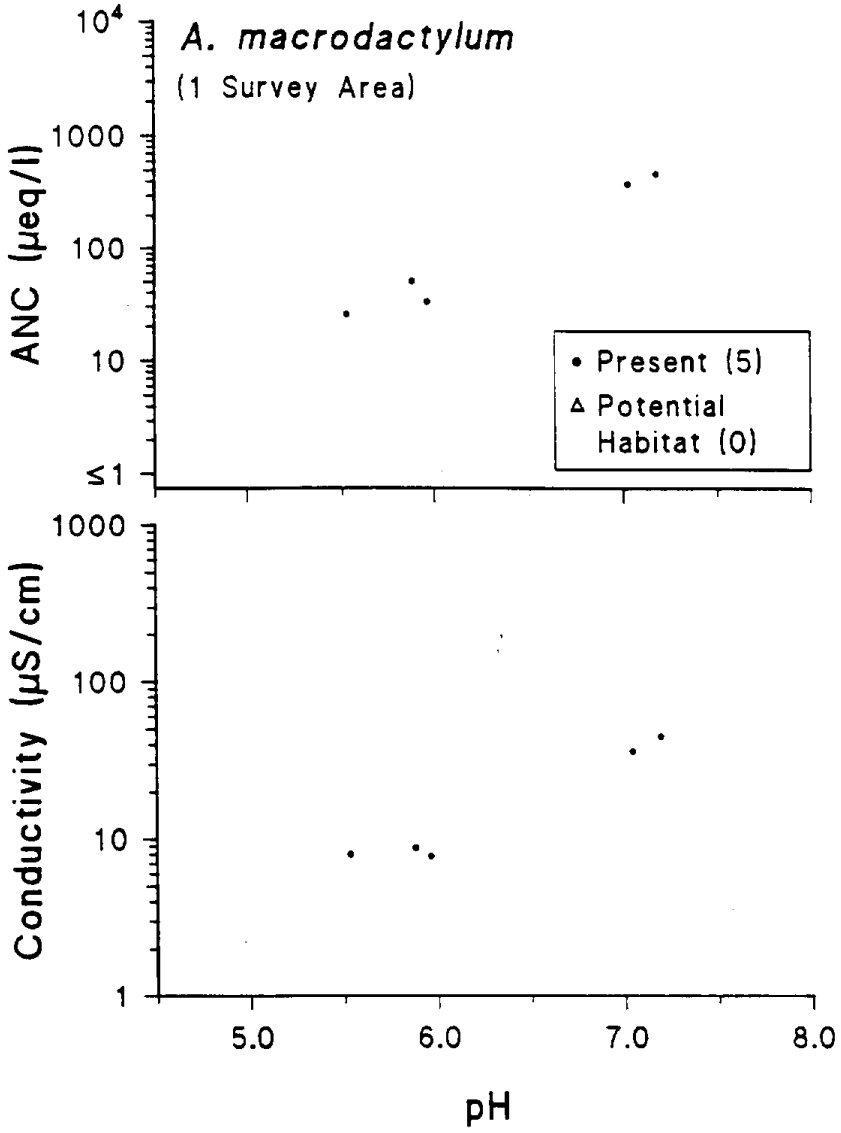

Figure 3-6. pH, ANC, and EC for A. macrodactylum breeding sites and sites considered potential breeding habitat but lacking the species. Details as in Fig. 3-2.

Table 3-2. Probability values (P) for effects of amphibian presence/absence on water chemical parameters: within survey areas (equation 1) and between survey areas (equation 2). Lambda is Wilks' Lambda statistic (Wilkinson 1990) for multivariate effects of species presence/absence on pH, log ANC, and log EC. "df" represents degrees of freedom.

	P. regilla				R. muscosa				B. canorus			
	"Within"		"Between"		"Within"		"Between"		"Within"		"Between"	
	P	df	P	df	P	df	P	df	P	df	P	df
PH	.879	1,159	.770	1,25	.966	1,31	.152	1,24	.720	1,30	.099	1,17
log ANC	.190	1,159	.821	1,25	.664	1,31	.318	1,24	.941	1,30	.095	1,17
log EC	.046	1,159	.814	1,25	.768	1,31	.261	1,24	.467	1,30	.041	1,17
Lambda	.185	3,157	.994	3,23	.967	3,29	.498	3,22	.479	3,28	.191	3,15

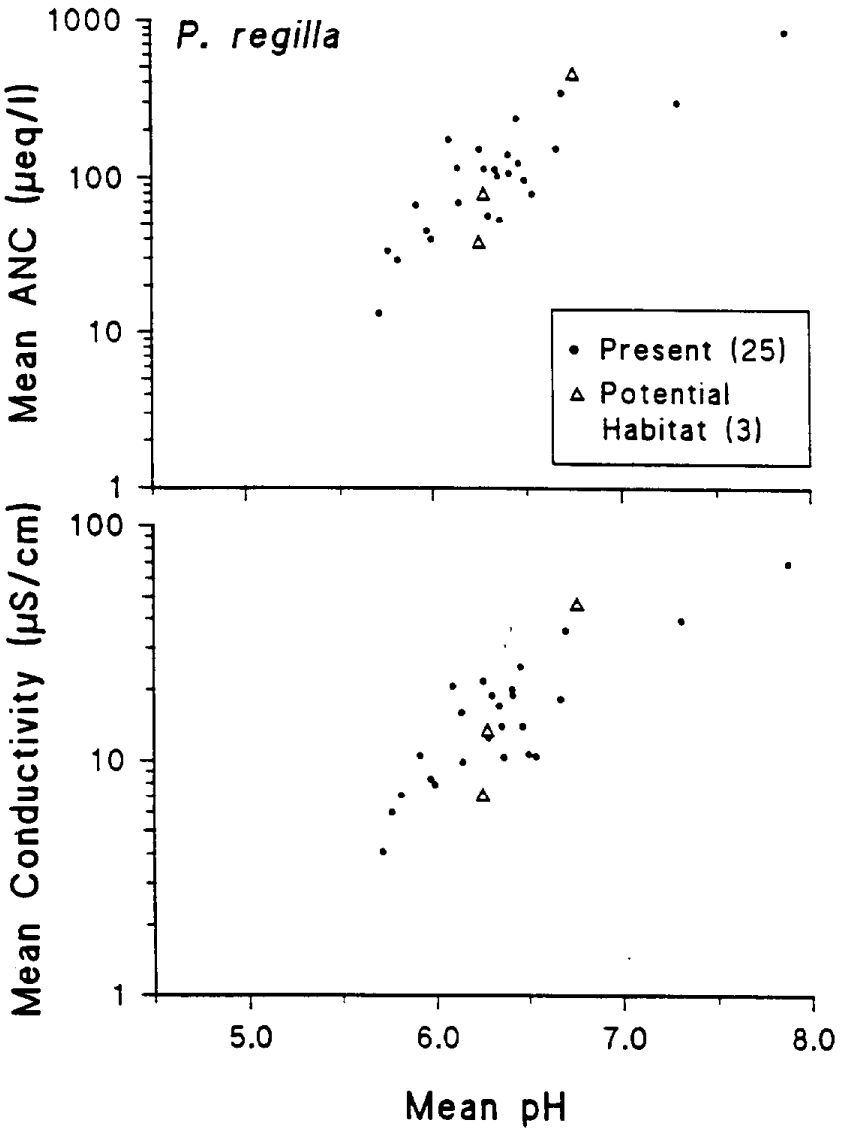

Figure 3-7. Mean pH, and geometric means for ANC and EC, for survey areas containing breeding P. regilla, and for survey areas containing potential breeding habitat but lacking the species. Numbers in parentheses represent number of survey areas. Three sites were excluded from computation of means as described in Fig. 3-1.

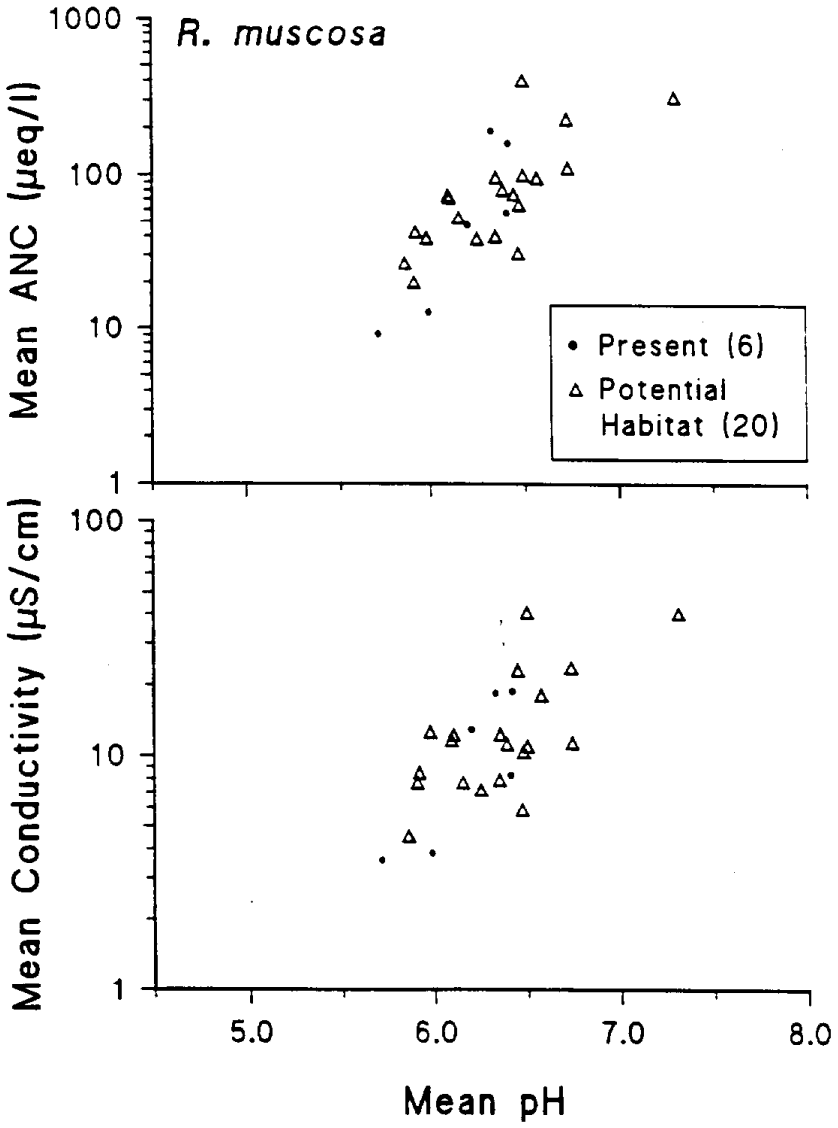

Figure 3-8. Mean pH, and geometric means for ANC and EC, for survey areas containing breeding R. muscosa, and for survey areas containing potential breeding habitat but lacking the species. Details as in Fig. 3-7.

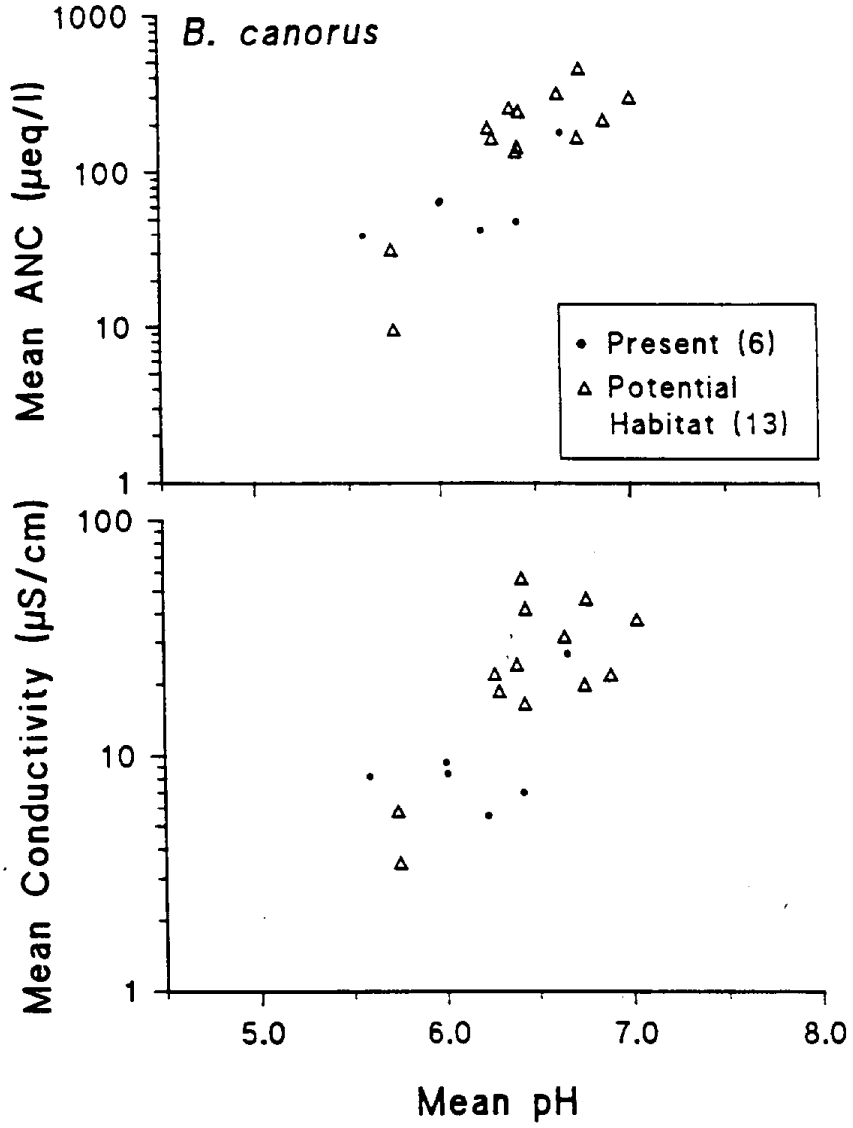

Figure 3-9. Mean pH, and geometric means for ANC and EC, for survey areas containing breeding B. canorus, and for survey areas containing potential breeding habitat but lacking the species. Details as in Fig. 3-7.

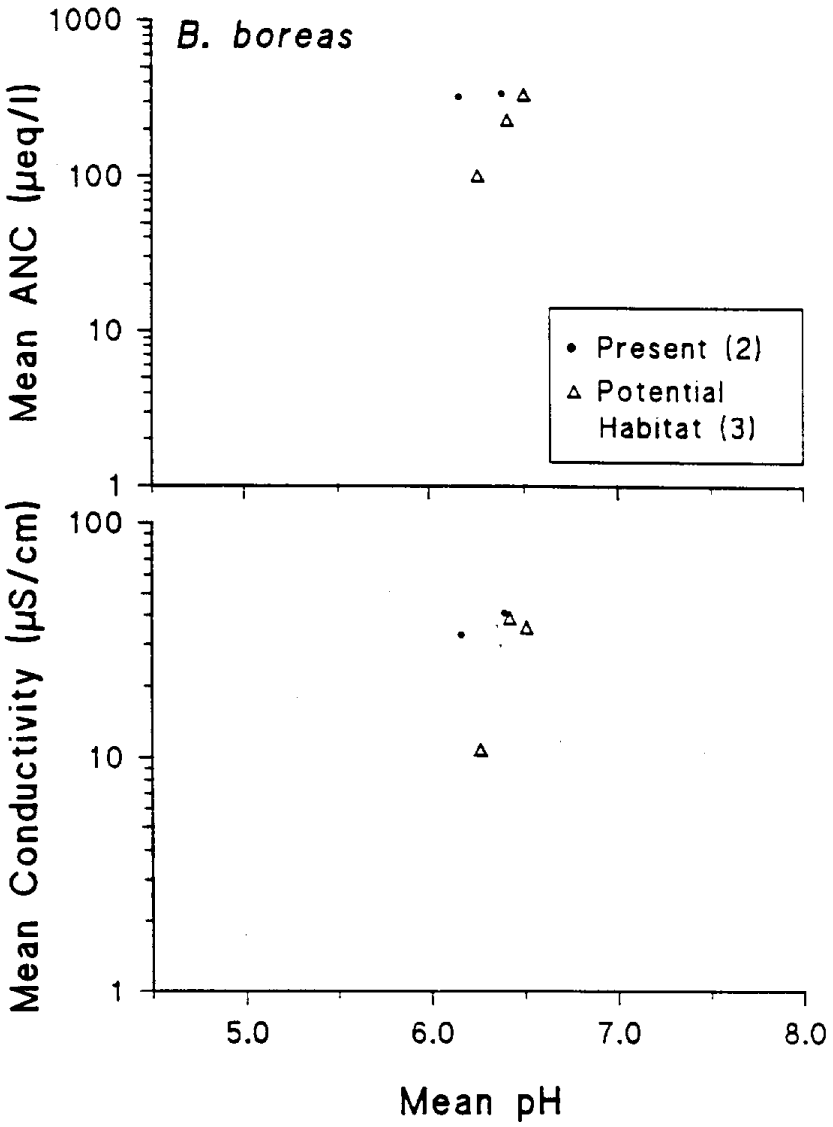

Figure 3-10. Mean pH, and geometric means for ANC and EC, for survey areas containing breeding B. boreas, and for survey areas containing potential breeding habitat but lacking the species. Details as in Fig. 3-7.

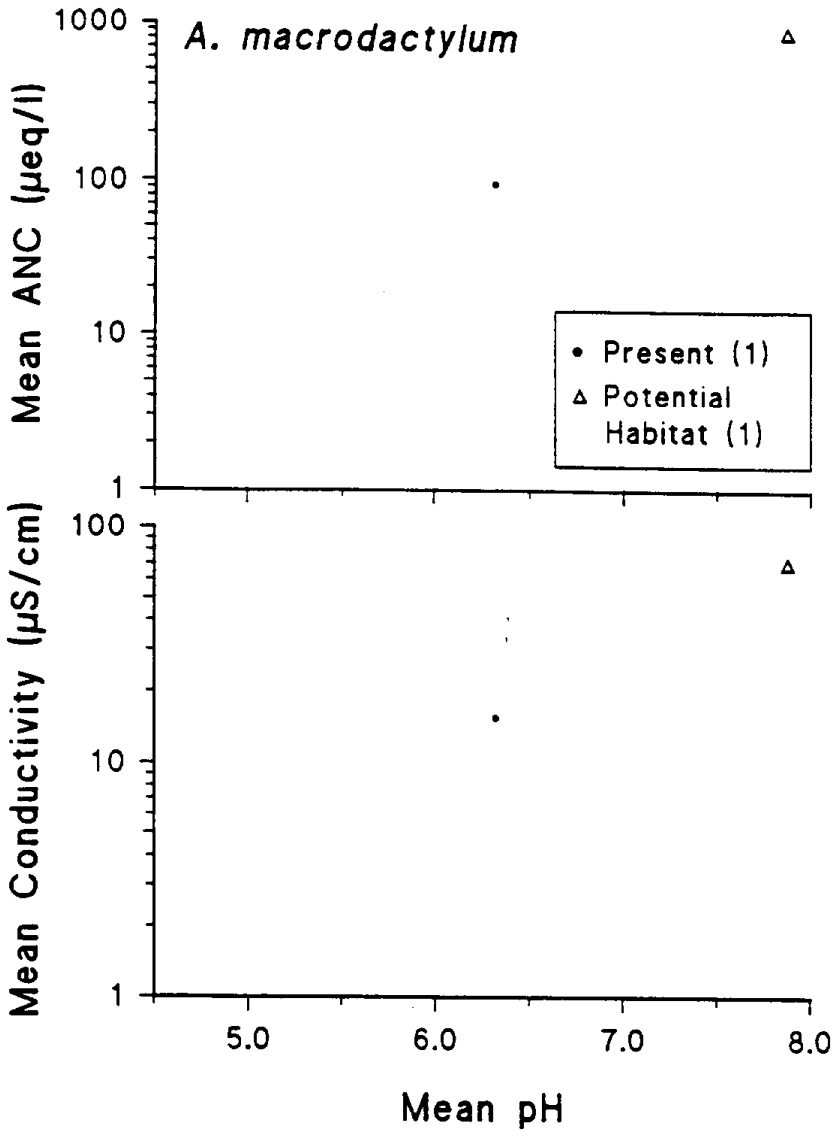

Figure 3-11. Mean pH, and geometric means for ANC and EC, for survey areas containing breeding A. macrodactylum, and for survey areas containing potential breeding habitat but lacking the species. Details as in Fig. 3-7.

or log EC (Table 3-3). In the "between survey areas" comparisons (Fig. 3-12), none of the three parameters (pH, log ANC, log EC) differed significantly among the three common species, and Wilks' Lambda was also not statistically significant. A suggested difference in log ANC among the species (P=0.088) is due to a slightly higher ANC for P. regilla than R. muscosa and B. canorus (based on coefficients for model 4; and evident in Fig. 3-12).

Table 3-3. Probability values (P) for significant differences in water chemistry among species: within survey areas (equation 3) and between survey areas (equation 4). Lambda and df are defined in Table 1.

	Within Survey Areas				Between Survey Areas	
	P. regilla vs. R. muscosa		P. regilla vs. B. canorus		All Species	
	P	df	P	df	P	df
pH	.813	1,42	.206	1,33	.402	2,33
log ANC	.516	1,42	.443	1,33	.115	2,33
log EC	.468	1,42	.856	1,33	.088	2,33
Lambda	.899	3,40	.333	3,31	.285	6,60

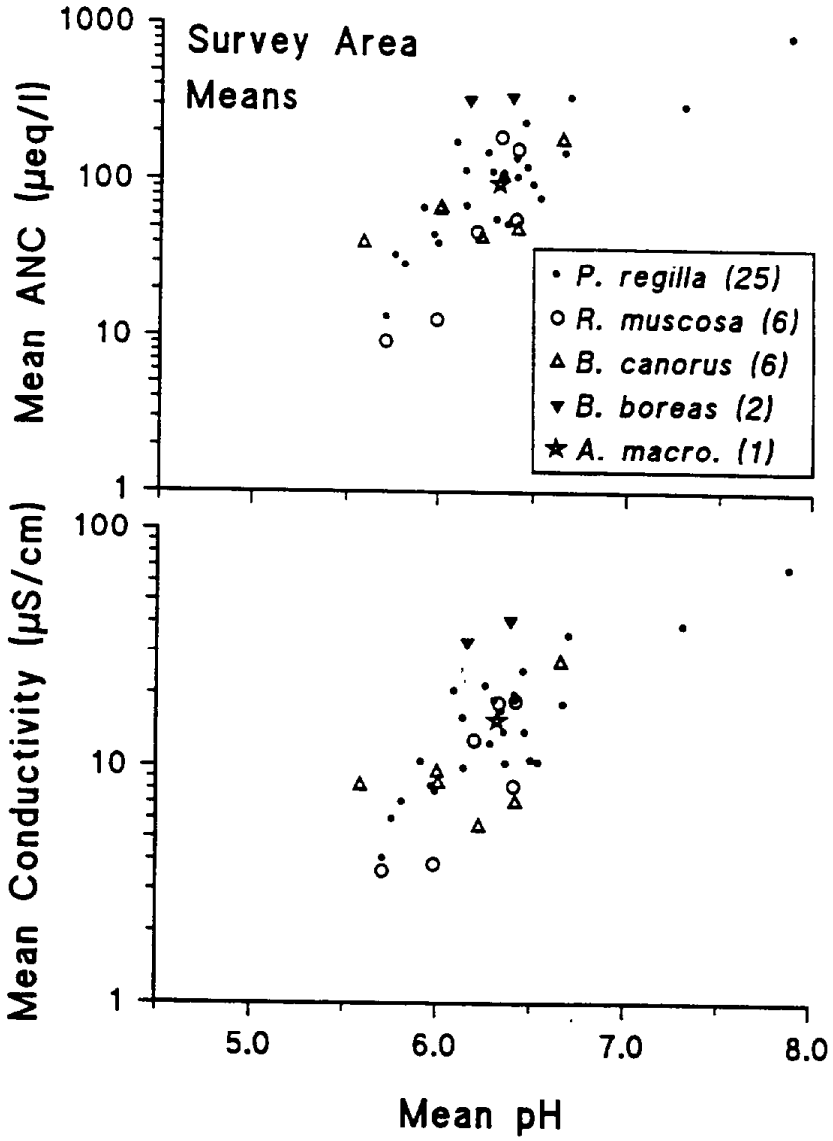

Figure 3-12. Mean pH, and geometric means for ANC and EC, for survey areas containing each of the five species. Details as in Fig. 3-7.

DISCUSSION

Amphibian Abundance and Distribution

P. regilla was widespread and abundant in the areas surveyed. It occurred in all survey areas that contained any amphibians, and it usually was found at more sites than any other species within a survey area. R. muscosa and B. canorus occurred in fewer survey areas than P. regilla (6 of 30, each), probably in large part because these species have more restricted habitat requirements than P. regilla, and the distribution of at least R. muscosa in the Sierra Nevada has been substantially reduced by introduced fish (Zweifel 1955; Karlstrom 1962; Bradford 1989a). Moreover, R. muscosa and B. canorus have disappeared for unknown reasons from many historic sites (see Chapter 1). B. boreas and A. macrodactylum were found in very few survey areas (2 and 1, respectively), largely because the geographic ranges of these species include limited portions of the study area. B. boreas ranges to high elevation in the southern Sierra (e.g., south of 36 or 37° S latitude), and elsewhere it is generally replaced by B. canorus at high elevation (Karlstrom 1962). A. macrodactylum occurs only in the northern half of the Sierra Nevada (i.e., north of approximately 38° 30′ N latitude), and only three survey areas occur in this portion of the Sierra (Appendix A). Moreover, A. macrodactylum does not appear to range as high in elevation as the other species (Stebbins 1985). Nevertheless, this species was commonly encountered in the one survey area where it occurred.

Water Chemistry in Comparison to Other Studies

Relevant water chemistry parameters have been surveyed throughout the Sierra Nevada by Melack et al. (1985) and Landers et al. (1987) (Fig. 3-13). Almost all of the lakes in these studies were above 2,440 m elevation as in the present

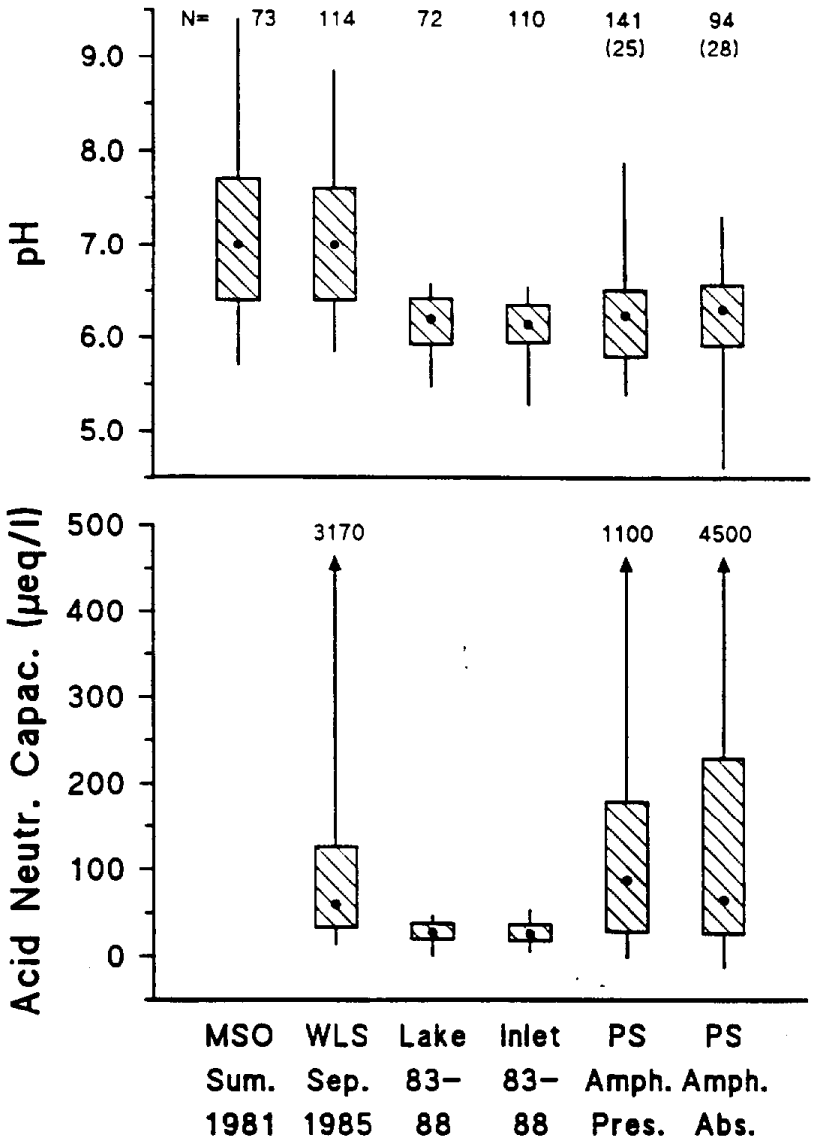

Figure 3-13. Comparison of pH and ANC among several studies of surface waters in the Sierra Nevada. MSO = Melack et al. (1985), June to October 1981; WLS = Western Lakes Survey, Sierra Nevada portion, September 1985 (Landers et al. 1987); Lake and Inlet = Emerald Lake and its inlet, year-round spot sampling (Melack et al. 1989); PS = present study, amphibians present and absent. Dots represent medians, boxes represent 20th and 80th percentiles, and bars represent range. Sample size (N) refers to number of sites sampled; parenthetic numbers refer to number of survey areas in present study.

study. However, samples in the present study were taken earlier in the ice-free season than in the other two studies, i.e., within approximately 1 week to 1 1/2 months following snowmelt on adjacent land. Sites sampled in the present study were also usually smaller in area and shallower than those in the other studies.

The sites in the present study were generally lower in pH, but slightly higher in ANC, than sites in the other two studies (Fig. 3-13). The lower pH may be due to sampling earlier in the summer season in the present study, as pH typically increases following snowmelt (Melack and Stoddard 1991).

Emerald Lake and its inlet, sampled throughout the year over a 5-year period, is representative of the low-ANC surface waters that are common in much of the Sierra Nevada (Fig. 3-13; Melack and Stoddard 1991). Sites in the present study were about the same in pH but higher in ANC than Emerald Lake and its inlet. The higher ANC in the present study is somewhat puzzling, as low pH is typically associated with low ANC in Sierra Nevada waters (Landers et al. 1987; Melack et al. 1989).

Comparison of Water Chemistry by Amphibian Presence/Absence

The present study does not support the hypothesis that amphibians do not occur in waters most vulnerable to acidification, i.e., low in pH and ANC, or in waters low in EC (Objective 6). This finding is based on analyses for the three most common species, two of which are believed to be declining in numbers in the Sierra Nevada. For *P. regilla* and *R. muscosa* no significant differences in water chemistry were found when analyzed both within and between survey areas. For *B. canorus* the only observed difference (i.e., greater EC for survey areas lacking the species) was opposite that predicted by the hypothesis. Moreover, a suggested difference for ANC for *B. canorus* was also inconsistent with the hypothesis,

i.e., higher ANC where the species was absent. In a separate study of P. regilla in a localized area of Sequoia National Park, pH and ANC also did not differ between sites containing the species and sites lacking the species (Soiseth 1992).

Nevertheless, amphibians were not found in the two sites with pH < 5.0 that were excluded from statistical analyses as outliers, whereas they were present in many nearby sites with pH ≥ 6.0. These sites were found in one survey area (No. 8) which has since become the focus of another CARB-sponsored study (Bradford et al. 1992; Mahood 1992). The source of acidity in waters in this region appear to be iron pyrite deposits (G.A. Mahood, pers. comm.). Based on data from the present study, and from other systematic surveys of water chemistry in the Sierra Nevada (Melack et al. 1985; Landers et al. 1987), surface waters with pH < 5.0 are extremely rare (Fig. 3-13). The absence of amphibians at the two acidic sites suggests that amphibians cannot exist in these rare conditions.

Comparison of Water Chemistry Among Species

This study also does not support the hypothesis that species differ in water chemistry of breeding sites in the same manner that they differ in sensitivity to acidic conditions in the laboratory (Objective 7). No significant differences were found in water chemistry among the three species analyzed, whereas the prediction was that P. regilla would occur at lower pH levels than both R. muscosa and B. canorus. A suggested difference in log ANC among species is due to a higher ANC for P. regilla than both R. muscosa and B. canorus, which is inconsistent with the above prediction.

CHAPTER 4. WATER CHEMISTRY OF AMPHIBIAN HABITATS IN THE "SEVEN-WATERSHEDS" STUDY AREAS

OBJECTIVES

Objective 8. Characterize the amphibian fauna and associated water chemistry of the watersheds included in the CARB-sponsored "seven-watersheds" project.

METHODS

The seven watersheds are those for the following lakes: Crystal, Ruby, Lost, Spuller, Topaz, Pear, and Emerald. Each of these watersheds was surveyed for amphibians in either 1990 or 1991 in the same manner as described in Chapter 3. Some sites near these watersheds were also sampled. Location of the seven lakes and specific sample sites are shown on maps in Appendix B.

RESULTS

A total of ten amphibian breeding sites were sampled, and 15 additional sites were sampled that represented potential breeding habitat, but lacked amphibians. Seven other samples were also taken from water bodies containing fish; but these sites were not considered suitable habitat for amphibians because of the presence of fish.

P. regilla was found in or near five of the watersheds: Crystal, Ruby, Lost, Topaz, and Emerald. A. macrodactylum was found only in or near the Lost Lake watershed. No amphibians were found in or near Spuller Lake watershed or Pear Lake watershed. Fish were observed in all watersheds other than Lost Lake and Topaz Lake watersheds, and they are known to occur in the Lost Lake watershed based on information from fishermen.

Water chemistry for sites containing amphibians is shown in Figs. 4-1 and 4-2. Values for these sites are similar to the majority of sites containing amphibians found throughout the Sierra Nevada (see Figs. 3-1, 3-2, and 3-6). Details for water chemistry and presence of amphibians at each sample site are described in Appendix C.

DISCUSSION

Only a few sites were found containing amphibians in the vicinity of the seven watersheds. This is probably largely because the watersheds are relatively small, and fish were present in 6 of the 7 watersheds. Fish usually preclude the existence of breeding amphibians in a water body (Bradford 1989a).

In former times many of these watersheds may have contained R. muscosa. This species was recorded in Emerald Lake in 1937 (Univ. of Calif. Museum of Vertebrate Zoology No. 21824), a time that may have antedated stocking with fish. In the 1960s and 1970s this species was abundant in Topaz Lake, in ponds in its watershed, and in many fishless lakes in the surrounding area (H. Basey, pers. comm.; Bradford 1989a, 1989b). However, adults disappeared from this and other nearby lakes between the summer of 1977 and spring of 1978, and all stages were absent in 1989 (Bradford, unpublished).

Water chemistry for ponds containing P. regilla in the Emerald Lake watershed was described in greater detail by Cooper et al. (1988a) and Soiseth (1992).

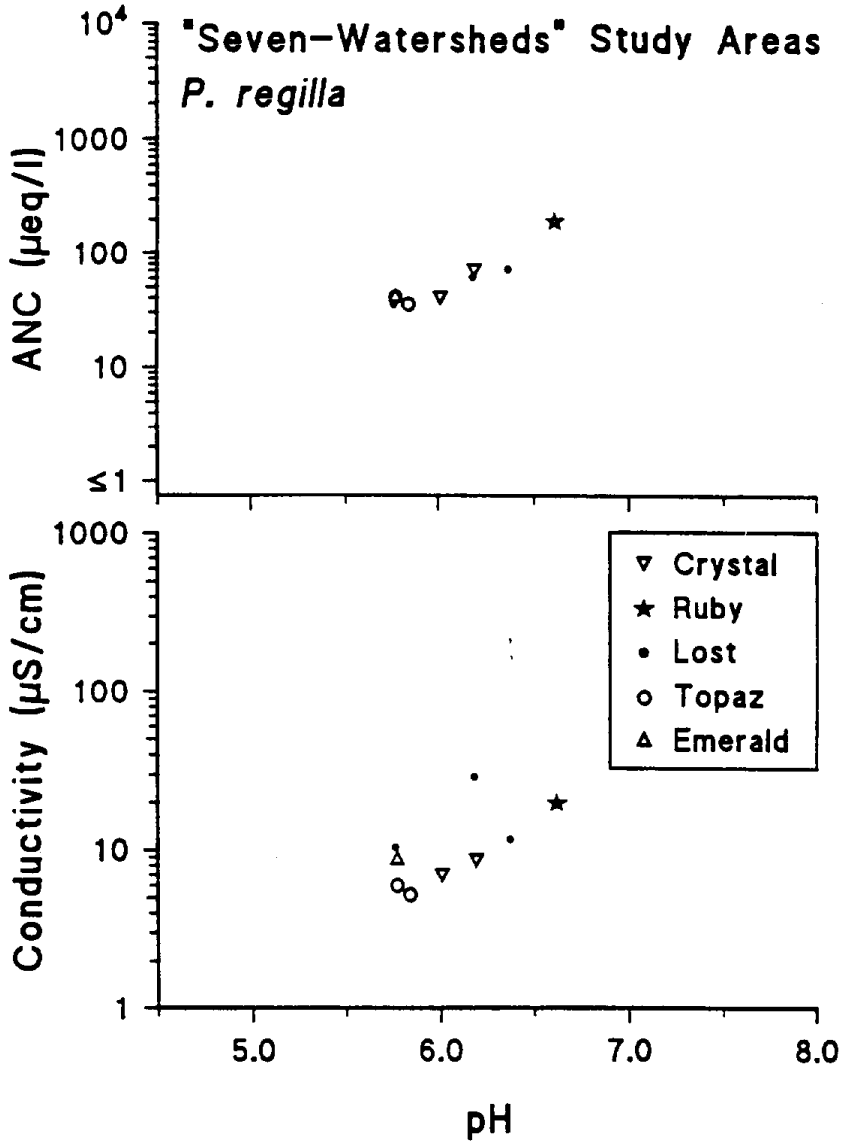

Figure 4-1. pH, ANC, and EC for P. regilla breeding sites found in or near the "Seven-watersheds" study areas. Names refer to lakes at the bottom of the watersheds. P. regilla was not found in or near Spuller and Pear Lake watersheds.

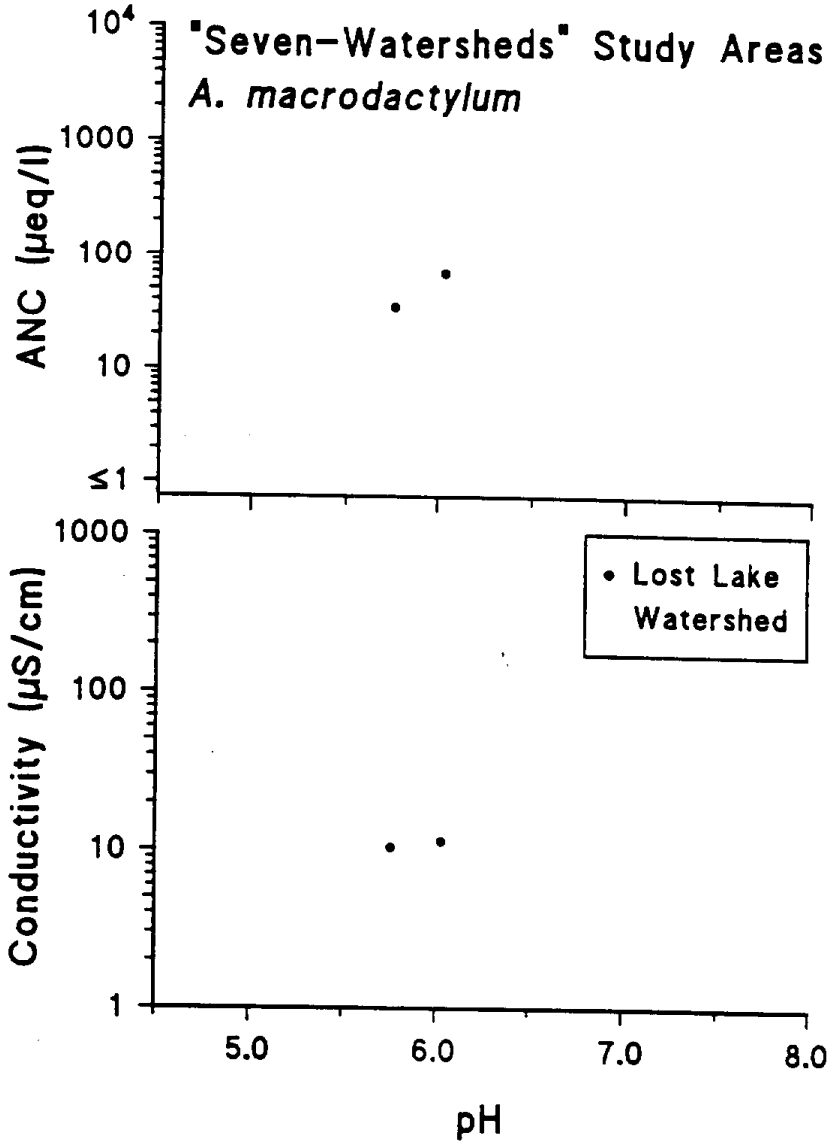

Figure 4-2. pH, ANC, and EC for A. macrodactylum breeding sites found in or near the "Seven-watersheds" study areas. A. macrodactylum was found only in the Lost Lake watershed.

CHAPTER 5 -- EVIDENCE FOR PAST AND POTENTIAL FUTURE EFFECTS OF ACIDIC DEPOSITION ON AMPHIBIANS IN THE SIERRA NEVADA

OBJECTIVES

Objective 9. Evaluate evidence for past and potential future effects of episodes of acidic deposition on amphibians in the field.

ACIDIFICATION OF SIERRA NEVADA SURFACE WATERS

Acidification of surface waters due to atmospheric deposition in the Sierra Nevada is likely to be most pronounced during snowmelt and major summer storms (Melack and Stoddard 1991; Williams and Melack 1991). To date, the lowest pH and ANC values measured in surface waters during such episodes are 5.3 and 0, respectively (Melack et al. 1989; Melack et al. 1991). This occurred during snowmelt in an inlet stream to Emerald Lake, Sequoia National Park, a lake characterized by extremely low ANC. During most of the rest of the year, pH at Emerald Lake and its inlet streams is typically in the low to mid 6's, which is similar to that for the many lakes in the Sierra Nevada that are low in ANC (Melack et al. 1985; Landers et al. 1987). The present study did not capture events of pH below 5.4, except for two sites with a geological source of acidity.

An estimate of the minimum pH likely to be experienced by amphibians in the Sierra Nevada can be derived from data for snowpack meltwater. This is because the ephemeral and shallow water bodies frequently used by amphibians for breeding are entirely flushed with snowmelt water each spring, and the pH of snowmelt water increases rapidly after it comes in contact with soil or rock (Melack et al. 1989; Williams and Melack 1991). Snowmelt water from the Emerald Lake basin is relevant because it is extremely low in ANC. To date, the lowest pH

measured in such water is 5.0 (Williams and Melack 1991). Thus, pH 5.0 is used as the basis for the following evaluation of past and potential future effects of acidic deposition on amphibians in the Sierra Nevada. Ongoing and future studies of surface water chemistry in the Sierra Nevada (e.g., Melack et al. 1991) may provide a better estimate for this value.

The duration of an episode of acidification causing adverse effects may be much shorter than the 7 days used in dose-response experiments. Mortality of embryos and tadpoles of all species at LC_{50} and below occurred mostly during the first day of treatment. Thus, although episodes of acidification in the field conceivably could last longer than 7 days, an episode of only one day may be equally detrimental.

POTENTIAL FUTURE EFFECTS OF ACIDIC DEPOSITION IN THE SIERRA NEVADA: pH EFFECTS

In the laboratory, acidification to the estimated extreme of 5.0 in the field had little effect on embryos and hatchling larvae of all species. Post-treatment survival was significantly affected only for B. canorus tadpoles at pH 5.5, but this may be a spurious finding because the effect was small and no effect of pH on survival was evident at pH 4.75, 5.0, and 6.0. For embryos and larvae of all species, survival did not show a decreasing pattern with pH until pH was below 5.0, and LC_{50}'s were in the low to mid 4's. Low ionic strength of water in the field is unlikely to increase the sensitivity of amphibians beyond these levels because dose-response experiments were conducted in waters very low in ionic strength. Elsewhere in the western U.S., six amphibian species at high elevation in the Rocky Mountains also appear to not be affected by pH levels expected in the field (Corn et al. 1989; Corn and Vertucci 1992).

Nevertheless, embryos of R. muscosa were smaller at pH $\leq$ 5.25, and embryos of B. canorus hatched at an earlier time at pH 5.0. The general decline in total length with decreasing

pH in embryos and tadpoles of all species (except A. macrodactylum embryos) is a common pattern in amphibians (Pierce and Wooten 1992), and presumably indicates that growth is inhibited as pH declines.

The ecological significance of these more subtle or sublethal effects is poorly known, but it may be substantial (Pierce and Wooten 1992). For example, small differences in growth rate in the field may have dramatic consequences on the time to reach metamorphosis and the size of the individual at metamorphosis (Smith-Gill and Berven 1979). In contrast, reduced hatching time may have little adverse effect, as amphibian embryos often hatch at earlier stages without apparent adverse effects when exposed to certain natural environmental conditions such as low oxygen level or elevated temperature (Petranka et al. 1982; Seymour et al. 1991).

In summary, the evidence using a standard toxicity testing approach (i.e., survival endpoints) indicates that amphibians are at little risk from acidification of waters to pH 5.0 due to acidic deposition. Nevertheless, the possibility exists that observed sublethal effects at pH $\leq$ 5.25, such as reduced growth rate and earlier hatching, may represent significant threats to amphibian populations.

POTENTIAL EFFECTS OF ACIDIC DEPOSITION IN THE SIERRA NEVADA: EFFECTS OF ALUMINUM

Concentrations of inorganic monomeric aluminum as high as those used in this study (39-80 μg/L) do not chronically occur in Sierra Nevada surface waters. The highest extractable aluminum concentration measured in the field (i.e., the aluminum measurement most similar to that represented here[J.L. Stoddard, pers. comm.]) is 36 μg/L (Landers et al. 1987). Although the possibility exists that aluminum levels may increase during episodes of acidification, the magnitude of this effect is expected to be small (J. L. Stoddard, pers. comm.).

Experimental levels of aluminum did not cause a decrease in survival endpoints for embryos or hatchling larvae of any species. Nevertheless, aluminum did cause a significant reduction in total length of B. canorus tadpoles at pH 5.3 and 5.8, and in A. macrodactylum larvae at pH 5.3, but not at pH 4.9 for either species. This pattern is surprising because measured concentrations were greatest at pH 4.9. The addition of aluminum also caused earlier hatching in embryos of B. canorus and P. regilla. As discussed above, the possible ecological consequences of these apparently sublethal effects are not clear, but should not be ignored.

In summary, the evidence using a standard toxicity testing approach (i.e., survival endpoints) indicates that amphibians are at little risk from aluminum levels of 39, 70, and 80 ug/l at pH 5.8, 5.3, and 4.9, respectively, that may result from acidification of waters due to acidic deposition. Nevertheless, the possibility exists that observed sublethal effects at the above aluminum and pH levels, such as reduced growth rate and earlier hatching, may represent significant threats to amphibian populations. However, there are no field data indicating the these levels are likely to be reached or exceeded during episodes of acidification.

EVIDENCE FOR PAST EFFECTS OF ACIDIC DEPOSITION ON AMPHIBIANS IN THE SIERRA NEVADA

Acidic deposition has been an attractive hypothesis to explain amphibian population declines in the Sierra Nevada in part because it could account for the broad geographic distribution of affected populations, including those near human activities as well as isolated populations in remote locations. However, the present study does not support this hypothesis.

It is not possible to directly address the proposition that acidic deposition has affected amphibian populations in the Sierra Nevada because there are virtually no data for the

timing of population declines, and few data for water chemistry during the brief episodes of acidification (Melack and Stoddard 1991). However, two related hypotheses have been addressed in the present study, both of which are predictions based on the assumption that acidic deposition has been a factor in causing recent populations declines: (1) declining species generally do not occur in waters low in pH, ANC, or EC (Objective 6, Chapter 3); and (2) species differ in water chemistry of breeding sites (Objective 7, Chapter 3) in the same manner that they differ in sensitivity to acidic conditions in the laboratory (Objective 1, Chapter 2).

Neither of these hypotheses was supported. Water chemistry in the field for two declining species (R. muscosa and B. canorus) and one non-declining species (P. regilla) showed no significant differences between sites containing and sites lacking the species, both within and among survey areas, and no significant differences were found among the three species. Moreover, data from the laboratory were not compelling that R. muscosa and B. canorus would be adversely affected by episodes of acidification of an extreme of pH 5.0, which has never been reported in Sierra Nevada surface waters (discussed above). The acidic deposition hypothesis for amphibian population declines has also not been supported in low-ANC waters in the Rocky Mountains, based on comparisons between field water chemistry and laboratory dose/response studies, and comparison of water chemistry between sites in which amphibians have recently disappeared and sites where they remain (Corn et al. 1989; Corn and Vertucci 1992).

The recent declines of many amphibian populations around the world may be caused by a multiplicity of factors that vary among species, regions, and time (Barinaga 1990; Blaustein and Wake 1990; Wake 1991). Aside from acidic deposition, pertinent hypotheses for amphibian population declines in the Sierra Nevada include habitat fragmentation by introduced predatory fishes, increased ultraviolet radiation, disease, immunosuppression, and livestock grazing (Hayes and Jennings

1986; Bradford 1989a; Bradford et al., in press; Bradford et al., ms. in review; Carey, in press). The present study suggests that attention should be given to these other hypotheses, in addition to acidic deposition. However, investigation of such hypotheses may be difficult because several factors may be operating simultaneously, key events may be episodic rather than chronic, and there may be substantial time lags in population responses.

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