Acidity and associated water chemistry of amphibian habitats in Nova Scotia

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Received January 24, 1984

DALE, J. M., B. FREEDMAN, and J. KEREKES. 1985. Acidity and associated water chemistry of amphibian habitats in Nova Scotia. Can. J. Zool. **63**: 97–105.

One hundred and fifty-nine field sites consisting of ditches, bogs, marshes, ponds, and lakes in south and central Nova Scotia were surveyed for the presence of the adults, eggs, or larvae of 11 amphibian species. Water samples were analyzed for pH, alkalinity, color, conductivity, Na, K, Mg, Ca, SO_4, and Cl. Discriminant function analysis revealed that none of these variables predicted a species' presence. Two three-species groups were found to be significantly associated among themselves, but mutually exclusive of each other. The two groups were (*i*) *Ambystoma maculatum*, *Hyla crucifer*, and *Rana sylvatica* and (*ii*) *Rana clamitans*, *Rana catesbeiana*, and *Rana palustris*. *Rana sylvatica* and *A. maculatum* were observed breeding successfully in an acidic bog (mean pH 4.1). *Rana clamitans* adults and larvae were located in the field at pHs as low as 3.5 and 3.9, respectively. Field transplant studies, using eggs of *A. maculatum* and *R. sylvatica* (at pH 5.7 and 4.1) and *Bufo americanus* (at pH 6.3 and 4.1), revealed that *R. sylvatica* was least sensitive to acidity. There is considerable variation in acid tolerance among the various species of Nova Scotia amphibians. Nevertheless, successful breeding by some species is occurring at very low pHs.

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Cent cinquante-neuf sites du Sud et du centre de la Nouvelle-Écosse, fossés, tourbières, marécages, étangs et lacs, ont été échantillonnés dans le but de faire l'inventaire de onze espèces d'amphibiens, adultes, oeufs ou larves. L'analyse des échantillons d'eau recueillis en même temps a permis de déterminer le pH, l'alcalinité, la couleur, la conductivité et les concentrations de Na, K, Mg, Ca, SO_4 et Cl. Une analyse discriminante a révélé qu'aucun de ces paramètres ne permet de prédire la présence d'une espèce. Deux groupes d'espèces associées significativement entre elles s'excluent mutuellement. Ce sont, d'une part, *Ambystoma maculatum*, *Hyla crucifer*, *Rana sylvatica*, et, d'autre part, *R. clamitans*, *R. catesbeiana* et *R. palustris*. *Rana sylvatica* et *A. maculatum* se sont reproduites avec succès dans une tourbière (pH moyen = 4,1). Des adultes et des larves de *R. clamitans* ont été rencontrés à des pH très bas, 3,5 et 3,9 respectivement. Des expériences de transfert en nature, notamment d'oeufs d'*A. maculatum* et de *R. sylvatica* (à des pH de 5,7 et 4,1) et de *Bufo americanus* (à des pH de 6,3 et 4,1) ont révélé que *R. sylvatica* est l'espèce la moins sensible à l'acidité. Il existe une variation considérable de tolérance à l'acidité chez les diverses espèces d'amphibiens de la Nouvelle-Écosse. Néanmoins, certaines espèces réussissent à se reproduire à des pH particulièrement bas.

[Traduit par le journal]

Introduction

Relatively little published information is available concerning the impacts of acidic deposition on amphibians compared with other components of ecosystems (i.e, fish, forests). However, (*i*) amphibians are ecologically important in some forest–pond ecosystems (Burton and Likens 1975*a*; Wassersug 1975); (*ii*) they are important predators or primary consumers, especially in fishless water bodies (Dickman 1968; Dodson and Dodson 1971; Orser and Shure 1972; Seale 1980); (*iii*) they are extremely efficient at converting ingested energy into new biomass (Pough 1980); (*iv*) they are preyed heavily upon by birds and mammals (Debenedictis 1974; Burton and Likens 1975*a*, 1975*b*; Cecil and Just 1979; Racey and Euler 1983); and (*v*) they appear at breeding sites in the spring, a time that coincides with a period of low food availability for predators (Gerrell 1969).

Several fish studies have suggested that habitat acidity can reduce the population size or restrict the distribution of amphibians. Gosner and Black (1957) reported that the distributions of anurans in the New Jersey pine barrens were limited by water acidity (pH 3.6–5.2). Of the 11 species that occurred in the area, only five bred regularly. Two of these, the carpenter frog (*Rana virgatipes*) and the pine barrens tree frog (*Hyla andersoni*), were restricted to this area and were found to tolerate the lowest pHs (i.e., 3.8) used in laboratory bioassays. Gosner and Black (1957) suggested that the apparent toxicity of the bog waters was due to acidity produced by humic substances derived from *Sphagnum* peat. In Sweden, Hagstrom (1980) reported the elimination of the common frog (*Rana temporaria*) from a lake of pH 4.0–4.5. He suggested that in addition to acid precipitation, sphagnaceous organic acids were a contributing factor since eggs laid in *Sphagnum* had high mortality. Saber and Dunson (1978) showed that amphibian species richness was lower in an acidic bog in Pennsylvania compared with surrounding less acidic water bodies. They also noted that only five of the eight species that occurred in the bog were represented by both immature and mature life stages, indicating possible reproductive failure in the other three species. In England, Cooke and Frazer (1976) found that smooth newts (*Triturus vulgaris*) rarely bred in ponds below pH 6.0, whereas palmate newts (*Triturus helveticus*) occurred in ponds with pHs as low as 3.9. No newts were observed in ponds of pH 3.8 or lower. Also in England, Beebee and Griffin (1977) reported the absence of the natterjack toad (*Bufo calamita*) below pH 5. In the Netherlands, Strijbosch (1979) found that six anuran species avoided acidic waters with pHs less than 4.5. He also noted that the percentage of dead egg masses was higher in habitats with relatively low pH. In Ontario, Clark and Euler (1982) reported that the densities of both spring peeper (*Hyla crucifer*) calling males and yellow-spotted salamander (*Ambystoma maculatum*) egg masses were positively correlated with habitat pH. In New Hampshire, the experimental acidification (to pH 4) of a small stream caused salamanders to move out of the treatment area (Hall and Likens 1980). In addition to the above field studies, lab experiments have revealed that in 14 species of amphibians, pHs of 3.7–3.9 during embryonic development caused mortality exceeding 85%, and prolonged exposure to pHs below 4.0 caused mortality exceeding 50% (for a review of lab bioassays, see Tome and Pough 1982).

Most of the field studies to date have looked at geographically small areas or single water bodies. Only one study that we are aware of has measured all significant water chemistry variables in amphibian habitats (Clark and Euler 1982). Amphibian and water chemistry data that encompass diverse habitats over a relatively wide geographic area would allow generalizations about habitat selection by amphibians; these are not possible in one- or few-pond studies.

The objectives of the present study were (*i*) to survey a large number of aquatic sites in Nova Scotia with respect to the presence of amphibian adults, larvae, or egg masses, the viability of eggs, and water chemistry variables which are important from an acidic deposition perspective; (*ii*) to analyze these data to determine which variables might predict the presence of amphibians; and (*iii*) to test for associations between amphibian species.

Methods

Study locale

Certain areas of Nova Scotia, especially in the southern region, contain poorly buffered watersheds that are located on granite or other oligotrophic bedrocks (Kerekes *et al.* 1982). Soils are dominated by podzols developed on glaciofluvial or morainal deposits (Rubec 1981). Nova Scotia receives much of its precipitation from weather systems that originate in industrial areas of the northeastern United States and eastern Canada. The average pH of precipitation increases from ca. 4.5 in the southwest to ca. 5.0 in the north, but individual events can be more acidic than this (Freedman and Ogden 1981; Underwood 1982; Anonymous 1983).

Fieldwork

During the spring and summer of 1982 and 1983, 159 field sites were surveyed and sampled in south and central Nova Scotia, within an area of ca. 320 × 120 km (Dale and Freedman 1984). Many sites were located while driving along roads at night listening for frog calls and were resurveyed during daylight.

At each site, duplicate surface water samples were collected in 125-mL plastic bags. A visual and acoustic survey of the entire water body and (or) perimeter was made (excluding lakes, which were only partially surveyed). Observations were made of species presence and relative abundance as eggs, larvae, adults, and hatched egg masses (not all of these variables were assessed for all species at all sites). Finally, the site was categorized within one of the five following habitat types: (*i*) ditch, less than 0.5 m deep, usually clear water, ephemeral, little or no aquatic vegetation, generally less than 25 m^2 surface area; (*ii*) bog, less than 2 m deep, dark brown water, permanent to ephemeral, wide range in surface area, vegetation dominated by *Sphagnum*; (*iii*) marsh, less than 1 m deep, clear water, permanent to ephemeral, wide range in surface area, vegetation dominated by graminoids; (*iv*) pond, shallow to moderate depth, permanent, still water, <0.5 ha surface area, mixed vegetation; (*v*) lake, moderate depth to deep, permanent with outflow, dark or clear water, large surface area, vegetation dominated by various aquatic macrophytes.

Chemical analysis of water samples

In the laboratory, one set of water samples was analyzed for pH, total alkalinity, conductivity, and water color. pH measurements and fixed end point (pH 4.5 and 4.2) titrations for alkalinity determination were made under nitrogen on 40-mL aliquots according to standard methods (Anonymous 1975) using a Radiometer TTT60 titrator, an ABU 12 Autoburette, and a TTA60 titrator assembly. Samples for pH and alkalinity determination were taken from sealed, air-free plastic bags, which had been warmed to 25°C. The in-laboratory pH measurements did not differ significantly from *in situ* measurements; the former are reported in this paper. The mean pH values were calculated using hydrogen ion concentrations. Conductivity was measured with a Radiometer CDM model 2e conductivity meter. Water color was measured with a Hellige Aqua Tester equipped with 20-cm nessler tubes (Welch 1948) and color discs ranging from 0 to 100 Hazen units (A.P.H.A. platinum–cobalt color units). In situations where water color exceeded 100 Hazen units, samples were diluted and the resultant color measurement was adjusted using the dilution factor.

The second set of water samples was either frozen or stored at 2°C. Samples were thawed if necessary and then filtered through a plastic filter wool. Thirty-millilitre subsamples were stored in plastic vials for later Cl and SO_4 analysis. The remaining water sample was analysed for Na, K, Ca, and Mg, using a Perkin-Elmer 2380 atomic absorption spectrophotometer with an air–acetylene flame. Cl and SO_4 were analyzed using a Dionex I.C.10 ion chromatograph.

Statistical analysis

The entire data set (amphibian occurrence and water chemistry variables, by habitat) was analyzed using discriminant function analysis (statistical package for the social sciences (SPSS), subprogram Discrim; Nie *et al.* 1975) to determine whether any chemical features of an aquatic environment could predict a species' presence or absence. Sites were tested separately by habitat and together against the 10 water chemistry variables.

We also tested for associations between species, taking a similar approach to that of Collins and Wilbur (1979). Only the six most commonly encountered species were tested (present at ≥25 sites), as the other five species were insufficiently observed (present at ≤15 sites). Presence and absence observations were used in a 2 × 2 contingency table, and the association between each species pair was determined by the coefficient of association, phi (ϕ) (SPSS, subprogram Crosstabs), where

$$[1] \quad \phi = (\chi^2/N)^{0.5}$$

Phi has a value of 0 when no relationship exists and a value of 1 when the variables are perfectly associated, all cases falling on either the major or minor diagonal. Phi also incorporates the Yates corrected χ^2 for 2 × 2 contingency tables with small sample size (N < 200). By using the formula for phi of Collins and Wilbur (1979),

$$[2] \quad \phi = (ad - bc)/(mnrs)^{0.5}$$

where a, b, c, and d are the observed cell frequencies for a 2 × 2 contingency table and m, n, r, and s are the row and column tables, respectively (Pielou 1969), it was possible to determine the sign of the associations (positive or negative) that were statistically significant. Statistical significance was determined by testing the null hypothesis that the parametric value of ϕ was equal to zero, i.e., that the two species were independently distributed with respect to one another (Collins and Wilbur 1979). The resulting p values were corrected using the Bonnferroni correction (Wilks 1962), which adjusts significance levels to account for multiple tests (no. of tests = 15).

Since our data could be broken down into five different habitat types, we were also able to determine whether the amphibian species were nonrandomly distributed among habitats. The presence and absence data were used in a 2 × 5 contingency table to evaluate this. The test statistic in this case was the raw χ^2 test for association provided by the SPSS subprogram Crosstabs. The Bonnferroni correction was also applied to these p values (no. of tests = 9).

TABLE 1. Summary of statistics of all the locations sampled for presence of amphibians and water chemistry

(A) Presence of amphibians[a]

	A.m.	*A.l.*	*N.v.*	*H.c.*	*R.sy.*	*R.cl.*	*R.pa.*	*B.a.*	*R.ca.*	*R.pi.*	*R.se.*
Ditch (n = 38)	17	0	1	20	21	16	5	3	0	0	0
Bog (n = 25)	17	1	0	19	11	11	1	2	7	0	0
Marsh (n = 18)	6	0	0	18	11	5	1	1	1	0	0
Pond (n = 36)	17	2	4	21	16	17	4	5	14	1	4
Lake (n = 42)	1	0	5	7	0	24	14	3	18	2	6

(B) Water chemistry

	pH	Alkalinity (mg $CaCO_3$/L)	Color (Hazen units)	Conductivity (μmho/cm)	Na (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Cl (mg/L)	SO_4 (mg/L)
Ditch (n = 38)										
Mean	5.10	5.73	92	103	7.4	1.09	4.78	1.67	16.3	5.9
SD		7.47	120	154	11.4	0.92	4.91	3.42	28.4	19.0
Minimum	3.88	0	0	16	0.9	0.20	0.37	0.23	1.0	0.8
Maximum	7.58	31.32	750	780	64.0	5.35	21.63	21.51	115.8	119.3
Bog (n = 25)										
Mean	4.64	1.38	118	120	7.3	0.71	6.10	1.00	22.2	4.6
SD		2.62	83	181	9.8	0.62	10.57	1.15	46.2	7.6
Minimum	3.90	0	20	9	0.6	0.12	0.49	0.17	0.4	0.4
Maximum	6.64	12.05	296	803	45.1	3.08	43.70	4.84	223.0	30.1
Marsh (n = 18)										
Mean	6.48	23.35	57	396	24.7	1.39	33.74	6.02	61.9	38.8
SD		28.67	44	536	51.0	1.07	66.44	10.92	112.7	114.6
Minimum	5.75	1.00	13	23	1.8	0.20	0.81	0.32	1.9	0.9
Maximum	7.80	99.00	175	1550	212.0	4.64	299.7	46.00	370.1	481.8
Pond (n = 36)										
Mean	5.21	4.67	65	100	6.8	1.16	4.76	0.90	15.5	4.0
SD		6.41	41	140	7.3	0.66	5.33	0.55	23.1	4.4
Minimum	4.13	0	5	15	1.5	0.18	0.64	0.29	1.8	0.3
Maximum	8.95	28.74	200	670	40.3	3.03	18.80	2.38	97.9	21.7
Lake (n = 42)										
Mean	4.83	2.24	49	64	6.2	0.61	1.80	0.57	9.7	4.8
SD		7.06	40	74	6.9	0.60	1.81	0.42	11.9	6.7
Minimum	3.64	0	4	16	1.6	0	0.30	0.16	1.3	1.2
Maximum	7.00	44.00	150	369	32.8	2.68	6.83	2.50	58.4	42.8

[a] *A.m.*, *Ambystoma maculatum*; *A.l.*, *A. laterale*; *N.v.*, *Notophthalmus viridescens*; *H.c.*, *Hyla crucifer*; *R.sy.*, *Rana sylvatica*; *R.cl.*, *R. clamitans*; *R.pa.*, *R. palustris*; *B.a.*, *Bufo americanus*; *R.ca.*, *Rana catesbeiana*; *R.pi.*, *R. pipiens*; *R.se.*, *R. septentrionalis*.

Field transplant studies

Caging bags were constructed of 1-mm mesh fiber glass screening. Edges were sealed with a plastic heat sealer, such that the resultant bags were approximately 20 cm on a side. Differences between treatments were tested for significance using the paired Student's t-test; $p \leq 0.05$ was considered significant using one-tail probabilities.

Ambystoma maculatum – Rana sylvatica *transplants*

Julie's Pond, mean pH 5.7 (44°42′ N, 63°40′ W; located 8 km north of Halifax) and Drain Lake, mean pH 4.1 (44°47′ N, 63°45′ W; located 20 km northeast of Halifax) were chosen as caging sites for transplants of *A. maculatum* and *R. sylvatica* eggs (see Table A1 for water chemistry). Note that the low pH of Drain Lake was caused by the oxidation of pyrites in the bedrock of its watershed (Kerekes *et al.* 1984). Freshly laid egg masses were collected from a marsh at Carroll's Corner (mean pH 6.9; 45°00′ N, 62°23′ W, Halifax County) and maintained in the lab for several days in pH 7 dechlorinated tap water at 5°C. Three egg masses of each species were divided, one half of each mass was transported to Julie's Pond, and the other half was transported to Drain Lake. Each half mass was placed into a separate fiber glass bag and submerged in ca. 1 m of water. At this time, the eggs were at the following developmental stages: *A. maculatum*, blastula; *R. sylvatica*, midgastrula (Rugh 1962). The embryos were monitored periodically until either larvae had hatched or the embryos were dead. The assay lasted from May 3 to June 13, 1983.

Bufo americanus *transplants*

A single strand of *B. americanus* eggs was found in a ditch with a pH of 6.3 (44°40′ N, 63°43′ W; located 10 km west of Halifax). Six groups of 50 eggs each were placed into separate bags. Three bags were replaced in the pH 6.3 ditch. The remaining three bags were submerged in a nearby dystrophic *Sphagnum* pool with a pH of 4.1 (see Table A1 for water chemistry). All of the embryos were initially at the neurula stage (Rugh 1962). The transplants were monitored weekly until either hatching had occurred or the embryos were dead. The assay lasted from May 29 to June 13, 1983.

Results and discussion

Habitat characteristics by species

The following species were observed during our survey: *Ambystoma maculatum* (yellow-spotted salamander), *A. laterale* (blue-spotted salamander), *Notopthalmus viridescens* (red-spotted newt), *Hyla crucifer* (spring peeper), *Rana sylvatica* (wood frog), *R. clamitans* (green frog), *R. palustris* (pickerel frog), *R. catesbeiana* (bullfrog), *R. pipiens* (northern leopard frog), *R. septentrionalis* (mink frog), and *Bufo americanus* (eastern American toad).

Table 1 is a summary in which the various sites have been arranged into five habitat types: ditch, bog, marsh, pond, and

TABLE 2. Summary of statistics of water chemistry variables for sites where Nova Scotia amphibians were present. Many of the sites summarized were common to more than one species[a]

	pH	Alkalinity (mg $CaCO_3$/L)	Color (Hazen units)	Conductivity (μmho/cm)	Na (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Cl (mg/L)	SO_4 (mg/L)
A.m. (n = 58)										
Mean	5.02	5.66	80	130	10.6	0.88	6.14	1.39	23.7	3.9
SD		8.74	68	264	28.8	0.53	10.12	2.15	62.1	4.7
Minimum	3.90	0	0	19	0.9	0.16	0.52	0.37	1.2	0.3
Maximum	7.80	45.90	296	1430	212.0	1.98	58.90	16.10	370.1	21.7
A.l. (n = 3)										
Mean	6.77	15.95	109	215	6.7	0.91	13.32	1.34	31.2	8.1
SD		13.46	122	204	4.9	0.84	5.89	0.25	35.7	11.8
Minimum	6.56	1.91	30	56	1.9	0.41	6.97	1.07	10.6	0.7
Maximum	7.07	28.74	250	445	11.7	1.88	18.60	1.57	72.4	21.7
N.v. (n = 10)										
Mean	5.49	3.94	38	50	4.2	0.48	2.56	0.74	8.6	3.4
SD		5.46	38	53	4.3	0.39	3.23	0.57	12.7	1.9
Minimum	4.96	0	5	16	1.5	0.10	0.40	0.27	2.1	2.0
Maximum	7.00	12.83	98	191	16.0	1.26	9.72	2.03	44.4	8.1
H.c. (n = 85)										
Mean	5.06	7.62	81	154	10.4	0.99	10.95	2.04	23.8	11.2
SD		16.09	93	291	25.0	0.78	34.02	5.37	57.1	53.7
Minimum	3.90	0	0	9	0.6	0.12	0.30	0.17	1.0	0.3
Maximum	7.80	99.00	750	1550	212.0	4.64	299.7	46.00	370.1	481.8
R.sy. (n = 59)										
Mean	5.34	9.51	77	159	11.5	1.04	8.38	2.59	29.6	8.0
SD		18.42	67	298	30.2	0.93	13.38	6.71	68.9	22.7
Minimum	4.26	0	0	15	1.3	0.12	0.52	0.24	1.2	0.3
Maximum	7.80	99.00	296	1430	212.0	5.35	58.90	46.00	370.1	130.6
R.cl. (n = 73)										
Mean	4.95	3.17	76	91	8.1	0.91	4.19	1.14	15.2	5.4
SD		4.45	53	189	25.0	0.81	7.33	2.56	46.0	14.4
Minimum	3.88	0	5	9	0.6	0	0.30	0.17	1.0	0.5
Maximum	7.26	18.87	296	1375	212.0	5.35	47.60	21.51	370.1	119.3
R.pa. (n = 25)										
Mean	5.22	5.87	56	63	4.7	0.72	3.13	0.75	7.5	3.3
SD		20.37	36	110	6.0	0.55	7.92	1.23	11.3	4.1
Minimum	4.52	0	5	16	1.3	0	0.30	0.23	1.5	1.0
Maximum	7.80	99.00	140	510	23.1	1.98	38.20	5.60	44.4	22.5
B.a. (n = 14)										
Mean	5.10	2.86	133	113	4.8	1.42	6.63	2.40	17.0	11.1
SD		4.88	183	206	3.9	1.48	12.09	5.62	32.6	31.2
Minimum	4.40	0	5	18	1.5	0.10	0.30	0.29	2.0	0.8
Maximum	7.80	18.08	750	780	13.3	5.35	43.70	21.51	115.8	119.3
R.ca. (n = 40)										
Mean	5.06	2.38	70	62	4.7	0.80	2.66	0.60	9.1	3.2
SD		4.14	65	79	4.4	0.66	3.60	0.44	13.6	3.1
Minimum	4.01	0	5	9	0.6	0	0.30	0.17	1.0	0.5
Maximum	8.95	18.87	296	400	22.1	2.68	18.80	2.36	67.9	18.4
R.pi. (n = 3)										
Mean	4.91	0.91	50	83	7.1	0.58	2.21	0.57	17.6	3.8
SD		1.58	40	94	7.7	0.59	2.91	0.34	23.2	1.3
Minimum	4.60	0	5	24	2.7	0.20	0.30	0.33	3.9	2.8
Maximum	6.00	2.74	80	191	16.0	1.26	5.56	0.96	44.4	5.3
R.se. (n = 10)										
Mean	5.54	5.12	42	26	2.6	0.37	1.02	0.46	3.6	2.4
SD		13.70	36	7	0.7	0.23	0.39	0.10	1.2	0.5
Minimum	5.20	0	5	16	1.6	0.10	0.42	0.27	1.5	1.5
Maximum	6.40	44.00	100	38	4.1	0.77	1.53	0.57	5.4	3.5

[a]Species abbreviations as in Table 1.

lake. For each habitat, the number of observations of each individual species is noted. In addition, Table 1 lists the summary statistics by habitat for each of the 10 water chemistry variables that were measured. Table 2 is an overall summary of water chemistry variables for each amphibian species, summarizing the data among all of the habitats.

Of the 11 species encountered during the survey, two were observed infrequently. *Ambystoma laterale* has a range in Nova Scotia that is largely outside of the area where most of our sampling was done (Gilhen 1984). *Rana pipiens*, although

TABLE 3. Significant associations (positive and negative) between amphibian species (A × B). Listed are the number of sites for each pair where either A + B, A only, B only, or neither occurred. Coefficients of association (ϕ) and probabilities of random association (p) are also listed. The significance is based on corrected χ^2 with df = 1. Note that p corrected refers to the Bonnferroni correction which adjusts significance levels to account for multiple tests (Wilks 1962)

Species pair (A × B)	A + B	A	B	None	ϕ	p	p corrected
H. crucifer × *R. sylvatica*[a]	42	43	17	57	0.27	0.0010	0.0150
A. maculatum × *H. crucifer*[a]	42	16	43	58	0.29	0.0005	0.0075
R. clamitans × *R. catesbeiana*[a]	30	42	10	77	0.35	0.0001	0.0015
A. maculatum × *R. sylvatica*	35	23	24	77	0.37	0.0001	0.0015
R. palustris × *R. catesbeiana*	13	12	27	107	0.27	0.0018	0.0270
R. clamitans × *R. palustris*	18	54	7	80	0.23	0.0068	0.1020
H. crucifer × *R. catesbeiana*[a]	14	71	26	48	−0.22	0.0117	0.1755
H. crucifer × *R. clamitans*[a]	32	53	40	34	−0.16	0.0457	0.6855
R. sylvatica × *R. catesbeiana*	9	50	31	69	−0.18	0.0433	0.6495
R. sylvatica × *R. palustris*	3	56	22	78	−0.23	0.0092	0.1380
A. maculatum × *R. palustris*	3	55	22	79	−0.22	0.0110	0.1650

[a]Significant associations common to Collins and Wilbur (1979).

present in our sampling area, was observed at only three sites. The remaining nine species were observed more frequently (at from 10 to 85 sites).

Ambystoma maculatum was located at 59 sites, occurring frequently in ditches, bogs, and ponds, and to a lesser extent in marshes. It occurred over a pH range of 3.9−7.8, with a mean pH of 5.0. Alkalinities ranged from 0 to 46 mg $CaCO_3$/L; water color from 0 to 300 Hazen units. The most pH-extreme water body where we observed successful hatching of *A. maculatum* embryos was a sphagnaceous bog (mean pH 4.3; minimum pH 4.1; color, 300 Hazen units). Subsequent larval development was not monitored at this site.

Adult *N. viridescens* occurred at 10 sites, primarily in ponds and lakes; these ranged in pH from 5.2 to 6.9 and from 5.0 to 6.0, respectively. We did not locate any *N. viridescens* eggs during our survey.

Hyla crucifer were found at 85 sites in all habitat types, although infrequently in lakes. They were the most frequently encountered species, due in part to their conspicuous call. The overall range of pH where they occurred was 3.9−7.8, and alkalinity from 0 to 99 mg $CaCO_3$/L. Because *H. crucifer* eggs are relatively small and laid singly, none were located.

Rana sylvatica adults, larvae, and (or) egg masses were found at 59 sites, occurring frequently in all habitat types except lakes where they were absent. Overall, pH ranged from 4.3 to 7.8 and alkalinity ranged from 0 to 99 mg $CaCO_3$/L. Larvae appearing normal were seen to emerge from egg masses at the same site described above for *A. maculatum* (mean pH 4.3; minimum pH 4.1; color, 300 Hazen units). *Rana sylvatica* larvae were later observed actively swimming at this site.

Rana clamitans adults or larvae were found at 72 sites, although rarely in marshes. Adult *R. clamitans* were observed over a pH range of 3.5 to 7.3, and metamorphosing tadpoles were observed in one ditch with a pH of 3.9.

Adult *Rana palustris* were found at 25 sites in all habitat types, although mainly in ditches, ponds, and lakes, the last representing 14 of the 25 sites. Overall, it occurred over a pH range of 4.5−7.8. Only one egg mass was located, the pond having a pH of 6.0.

Adult *B. americanus* were observed at 14 sites representing all five habitat types. The overall pH range was 4.4−7.8. *B. americanus* eggs and larvae were observed at only one site, which had a pH of 6.3.

Adult and larval *R. catesbeiana* were found at 40 sites, most of which were large permanent water bodies. Adults occurred over a pH range of 4.0 to 9.0. The most acidic site where we observed larval *R. catesbeiana* had a pH of 4.5.

Adult *R. septentrionalis* were found at 10 sites, all ponds and lakes. The overall pH range was 5.2 to 6.4. We only observed *R. septentrionalis* larvae at two sites, the lower pH being 5.2.

Briefly summarizing these field observations with regard to successful reproduction at very low pHs, *R. clamitans* larvae were found at pH 3.9, *A. maculatum* and *R. sylvatica* embryos hatched at pH 4.1, and *R. catesbeiana* larvae were found at pH 4.5.

Discriminant function analysis showed that neither the water chemistry variables collectively nor selected combinations of them (stepwise discriminant analysis) could significantly discriminate between the presence and absence of any of the 11 amphibian species found in the five habitat types. These results suggest that other factors, e.g., competition (Wilbur 1972), predation (Morin 1983; Woodward 1983), and (or) habitat structure may be more important than water chemistry in influencing the occurrence of amphibian species. Note, however, that water chemistry variables such as oxygen tension and temperature have been shown to have effects on amphibians (Noland and Ultsch 1981; Petranka *et al.* 1982). These variables may have influenced species distribution in our sites, but they were not measured.

From the water chemistry summaries in Table 1, it can be seen that bogs and marshes had the lowest and highest mean pHs, respectively (4.6 and 6.5). In fact, marshes had the highest mean values for all of the chemical variables except color. Alternatively, lakes had the lowest mean values for all of the variables except pH, alkalinity, and SO_4. Although the mean values of the water chemistry variables varied considerably between the different habitat types, the minima and maxima indicate a wide range of values within any particular habitat.

Species associations

We tested for significant associations between the six most common species to see if one species could predict the presence of another. Table 3 lists the species pairs that were significantly

TABLE 4. Results of test for nonrandom distribution of amphibian species among the five habitat types (χ^2, df = 4); p values ≤ 0.05 are considered significant. Note that p corrected refers to the Bonnferroni correction which adjusts significance levels to account for multiple tests (Wilks 1962)

Species	χ^2	p	p corrected
A. maculatum	34.78	0.0001	0.0009
N. viridescens	7.42	0.1155	—
H. crucifer	43.98	0.0001	0.0009
R. sylvatica	35.93	0.0001	0.0009
R. clamitans	3.98	0.4094	—
R. palustris	14.59	0.0056	0.0504
B. americanus	1.60	0.8089	—
R. catesbeiana	27.15	0.0001	0.0009
R. septentrionalis	11.41	0.0223	0.2007

TABLE 5. Percent mortalities of the in-field egg transplant studies. Three *Ambystoma maculatum* and three *Rana sylvatica* egg masses were each divided, one half caged in Julie's Pond, the other half in Drain Lake. *Bufo americanus* eggs were removed from a single strand of eggs and caged at pH 6.3 and 4.1 (three replicates per treatment)

Species[a]	Location	No. of eggs	% mortality
A.m.	Julie's Pond (pH 5.7)	32	78
		45	23
		56	11
	Drain Lake (pH 4.1)	42	100
		37	100
		57	100
R.sy.	Julie's Pond (pH 5.7)	105	100
		160	83
		176	56
	Drain Lake (pH 4.1)	85	92
		58	93
		68	93
B.a.	Ditch (pH 6.3)	50	20
		50	58
		50	10
	Pool (pH 4.1)	50	100
		50	100
		50	100

[a]Species abbreviations as in Table 1.

associated (χ^2, df = 1, $p \leq 0.05$), their respective ϕ values, and their distribution among the sites. Both the p value and the Bonnferroni corrected p value are reported. Using the former, there were 11 significant associations, 6 positive and 5 negative. Using the latter probability, only the first five associations in Table 3 remained significant at the 5% level. However, we feel that the use of the Bonnferroni correction in this situation is rather conservative, and that the negative associations listed in Table 3 still have ecological meaning, as discussed below.

Inspection of the different combinations revealed that two three-species groups were mutually exclusive. *Ambystoma maculatum, H. crucifer*, and *R. sylvatica* (group I) were significantly associated with each other, as were *R. catesbeiana, R. clamitans*, and *R. palustris* (group II). Furthermore, the five negative associations of Table 3 occurred between these two groups.

These results are similar to those of Collins and Wilbur (1979). They examined 49 aquatic habitats in Michigan, and of the 14 amphibian species present, 9 also occurred in our study. Considering these species only, Collins and Wilbur (1979) had six positive and six negative associations that were significant (χ^2, $p \leq 0.05$). Of the six positive associations that we obtained, three were in common with those of Collins and Wilbur (1979), and of our five negative associations, two were in common with their study. The three common associations were *H. crucifer* × *R. sylvatica, H. crucifer* × *A. maculatum*, and *R. catesbeiana* × *R. clamitans*. The two common negative associations were *H. crucifer* × *R. catesbeiana* and *H. crucifer* × *R. clamitans*.

Concerning our two three-species groups I and II, one might consider the causative factors that could account for such a definitive split. It is apparent that group I is composed of spring breeders, while the group II species are summer breeders. Since there is seasonality in the availability of habitats, we tested whether our study species were randomly distributed among the five habitat types. Table 4 lists the raw χ^2 values and their significance values resulting from 2 × 5 contingency tables of presence or absence by the five habitat types. Six of the species exhibited significant ($p \leq 0.05$), nonrandom distributions among the five habitat types. *Ambystoma maculatum, H. crucifer*, and *R. sylvatica* were generally not found in lakes. Alternatively, *R. catesbeiana, R. palustris*, and *R. septentrionalis* were found almost exclusively in ponds and lakes (with the exception of *R. catesbeiana* and *R. palustris*, which were found occasionally in bogs and ditches, respectively). *Rana clamitans* and *B. americanus* were both distributed randomly among the five habitat types (see Table 1 for species distributions).

These results strongly suggest a lack of spatial overlap between the two groups. Thus, the group I species breed in the spring, utilizing small, relatively temporary aquatic habitats; alternatively, the group II species breed later in the year, and they almost exclusively utilize permanent ponds and lakes. The one partial exception to this is *R. clamitans*, which was found in all habitat types. However, since *R. clamitans* was negatively associated with *H. crucifer* of group I, the apparent spatial overlap between *R. clamitans* and group I is due to habitat type and not specific sites. A possible explanation for this type of negative relation is interspecific competition, as has been shown for *H. crucifer* and large *Rana* tadpoles (Morin 1983).

Field transplants

The results of the field transplant studies are in Table 5. The three *A. maculatum* half egg masses caged in Drain Lake (pH 4.1) suffered 100% mortality, exhibited as either the inability to develop as embryos or the inability to hatch as larvae. The three Julie's Pond (pH 5.7) transplants suffered 78, 23, and 11% mortality, which was significantly different from the Drain Lake mortalities ($p = 0.045$). The low hatching success for one of the three Julie's Pond egg mass transplants was surprising, and may have been due to either inherent egg inviability or some artifact of our experimental procedure.

Developing *R. sylvatica* embryos were more tolerant of acidity than *A. maculatum*. In Drain Lake, mortalities were 82, 93, and 93%, while the matched half egg masses in Julie's Pond had mortalities of 100, 83, and 56%, respectively. The 100% mortality replicate at Julie's Pond was particularly surprising, since the matched half egg mass at Drain Lake had only 82% mortality. Again, this low hatching success may be due to low egg viability or an artifact of our experimental procedure.

There was no significant difference between treatments ($p = 0.220$). Nevertheless, it is ecologically significant that *R. sylvatica* embryos successfully hatched at pH 4.1, and that some larvae survived for 3 weeks until the experiment was terminated.

The *B. americanus* transplants suffered 100% mortality at pH 4.1, while at pH 6.3 mortalities were 20, 58, and 10% (significantly different, $p = 0.020$). Since all of the eggs for this experiment were from a single clutch, the variation in mortality between and within groups is likely due to environmental factors rather than genetic variation. The between-group variation is likely due to differences in acidity and associated chemical factors between the two ponds.

The caging experiments suffered from several drawbacks, one of which was fouling of the mesh. This problem was encountered especially at Drain Lake, which is eutrophic (Kerekes *et al.* 1984). However, *R. sylvatica* embryos did hatch, and some caged larvae survived there. It is reasonable, therefore, to assume that the *A. maculatum* embryos caged alongside these *R. sylvatica* embryos failed to hatch due to the low pH or some other chemical factors, rather than the fouling of the cage.

Results similar to these have been reported from central Ontario using the same three species in caging studies (K. L. Clark, personal communication). Clark's results indicated that both *B. americanus* and *A. maculatum* had reduced survival at pH 4.6 and 4.4, respectively, while *R. sylvatica* survival was unaffected at a pH as low as 4.4.

It appears that at least some Nova Scotia amphibians may not be presently threatened by habitat changes caused in part by acidic deposition. The weighted mean annual pH of rain falling in Nova Scotia in 1980 was 4.5 (Anonymous 1983). Under this loading, clear water sites have pHs >5.0, but colored humic waters have pHs <5.0 due to the presence of naturally acidifying organic acids (Kerekes *et al.* 1982). Although precipitation of pH 4.5 is quite acidic, *A. maculatum*, *R. sylvatica*, *R. clamitans*, and *R. catesbeiana* are all capable of successfully breeding in habitats below this pH in Nova Scotia, and we have observed *A. maculatum* and *R. sylvatica* hatching at pH 4.1 in the field. Also, 1st-year *R. clamitans* larvae were collected from Drain Lake (mean pH 4.1, minimum pH 3.8), and from a ditch with a pH of 3.9.

Of the 159 water bodies sampled for amphibians during this study, only 11% had a pH <4.5 and only 3% had a pH <4.0. The lowest pHs are probably due to organic acids or pyrite oxidation. If our sampling was representative of available breeding sites for amphibians in south and central Nova Scotia, then approximately 90% of the available habitat is still suitable for breeding by most species.

This line of reasoning may not be applicable in other regions of North America. In 1980, the Ohio River valley in the United States had a weighted mean annual precipitation pH of <4.2 (Anonymous 1983). In that area, a larger proportion of breeding sites may have pHs <4.5. Pough and Wilson (1977) studied *A. maculatum* and *A. jeffersonianum* breeding in temporary ponds in the Ithaca, NY region. Many of these ponds had acidic pHs during the breeding season, some as low as 3.5. The annual mean pH of precipitation in that area during 1970–1971 was 4.0 (Likens and Bormann 1974), substantially more acidic than that in Nova Scotia. Pough and Wilson (1977) found that *A. jeffersonianum* tolerated much more acidic waters in laboratory assays than did *A. maculatum*. These different acid tolerances were reflected in a field study (unpublished data of Wilson 1976, cited in Tome and Pough 1982) in which *A. jeffersonianum* was found to produce six times as many juveniles per adult as *A. maculatum* during a 4-year study of an enclosed breeding pond (pH 5.0–6.5). Pough (1976) found that *A. maculatum* suffered an abrupt transition from low to high embryonic mortality below pH 6 in a study of five ponds in the Ithaca region (mortality rates of 1, 43, 73, and 63% at pHs 6.0, 5.5, 5.0, and 4.5, respectively). These authors concluded that the future of *A. maculatum* in northern New York appeared bleak.

However, Cook (1983) recently published different findings for these two species from the same general area. He monitored 14 vernal breeding ponds (mean pH range of 4.2–5.9) in Massachusetts and recorded the cumulative mortality of embryos. He found no significant correlation between pond pH and mortality. Moreover, mortality in acidic habitats was considerably less for *A. maculatum* than that reported by Pough (1976) in the acidic Ithaca ponds. Cook (1983) concluded that embryonic mortality in *Ambystoma* varies considerably between species, pond, and year owing to the interaction of biotic and abiotic factors. He suggested that the low mortality at acidic pHs was possible due to the evolution of long-term acid tolerance of the salamander populations that he studied.

Controlled laboratory bioassays which we have performed (J. Dale, B. Freedman, and J. Kerekes, in preparation) using eggs of native species collected in the field support this contention. *Rana palustris* and *H. crucifer* suffered drastic reductions in hatching success at pH 5 and lower. *Ambystoma maculatum* and *R. sylvatica* hatching success was reduced by 50% at pH 5.0–4.5 and pH 4.5–4.0, respectively. However, *B. americanus* exhibited 90% hatching at pH 4.0, while pH 3.75 allowed no hatching. Obviously, amphibians vary in their susceptibility to acidity in laboratory assays, and some (such as *B. americanus*) will tolerate extreme conditions. Others (such as *R. palustris*) are much less tolerant, and this may explain why we infrequently observed this species and its close relative *R. pipiens*.

Many of the watersheds in Nova Scotia, particularly the southern portion, are highly sensitive to acid loading, being situated on granite or other oligotrophic, slowly weathering bedrocks. If atmospheric acidic deposition rates are increased in the future, then additional aquatic habitats could acidify. Reductions in the populations of certain amphibian species might then be expected. It is notable, however, that of the seven sites sampled in this southern region with a pH range of 4.2–4.9, we found larvae of *A. maculatum*, *R. sylvatica*, *R. clamitans*, and *R. catesbeiana*, as well as adult *H. crucifer* and *R. palustris*. These observations indicate a rather high degree of acid tolerance in adults and (or) larvae of these species.

Acknowledgements

The following persons participated in the collection of field or laboratory data, and we gratefully acknowledge their help: J. Gilhen, E. Green, J. Hanken, M. Lannoo, N. Macnee, U. Prager, and T. Smith. We also express our appreciation to R. Wassersug and P. Daye, who critically reviewed the manuscript and provided many useful comments. This work was supported under a research contract with the Canadian Wildlife Service, Environment Canada.

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Appendix

TABLE A1. Water chemistry data of the field sites used in the egg transplant study. Eggs of *Ambystoma maculatum* and *Rana sylvatica* were collected from Carrolls Corner and transplanted into Drain Lake and Julie's Pond. Eggs of *Bufo americanus* were collected from the ditch and were returned to the ditch as well as being transplanted into the pool

	pH	Alkalinity (mg $CaCO_3$/L)	Color (Hazen units)	Conductivity (μmho/cm)[a]	Na (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	SO_4 (mg/L)	Cl (mg/L)
Drain Lake	4.1	0	17	301	22.1	2.68	6.83	1.63	39.8	18.4
Julie's Pond	5.7	1.21	15	99	9.9	1.27	3.77	1.87	15.8	8.9
Ditch	6.3	5.40	10	38	3.2	1.72	0.82	0.57	1.8	2.1
Pool	4.1	0	125	40	3.3	0.42	0.58	0.20	2.4	4.2
Carrolls Corner	6.9	17.20	20	1375	212.0	1.39	47.60	4.80	370.1	18.6

[a] 1 mho = 1 S.