BOG WATER TOXICITY
AND
AMPHIBIAN REPRODUCTION

Final Report

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Department of Natural Resources

Attention: Norm Aaseng

Submitted by: Daryl R. Karns
Bell Museum of Natural History
10 Church Street S.E.
University of Minnesota
Minneapolis, MN 55455

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1.0. INTRODUCTION

During the course of research on the general ecology of the herpetofauna of the boreal peatlands (Karns, 1979) it became apparent that bog water deleteriously affected the reproductive success of amphibians breeding at bog water sites. The toxicity of bog or blackwater habitats to a variety of organisms had been noted previously. Janzen (1974) reviews a variety of reports from both tropical and temperate areas which strongly suggest that blackwater habitats are deleterious relative to whitewater areas for a wide range of taxa including fish, mollusks, various microorganisms, plants, amphibians, and insects. These studies are largely descriptive and show a reduction in species richness, abundance, and general productivity in the blackwater areas. Relatively little experimental work has been done on bog water toxicity; experimental studies are reviewed in Janzen (1974), Crawford (1978) and Karns (1979).

During the spring of 1978, 1979, and 1980 the toxicity of bog water as relates to amphibian reproduction was investigated in a series of laboratory and field experiments and observations. The goals of the research were as follows:

1) To establish the relative tolerances of the resident amphibian species to bog water as a breeding medium.
2) To determine the phase of the life cycle at which the toxicity manifests itself. Amphibians have a complex life cycle. Bog water might affect fertilization, egg development, egg hatching, larvae, metamorphois or adults. The stage at which the organism is affected is of obvious ecological significance.
3) Elucidation of factors important in the toxicity of bog water (e.g. temperature, acidity). The chemistry of bog water is exceedingly complex (references in Swain, 1978). It was beyond the scope of this

study to probe in detail the actual mechanism of the toxicity. However, several experiments performed suggest the importance of certain factors in the toxicity.

All work done on bog water toxicity during the course of the amphibians and reptiles peatland research program is summarized here; some of this work has appeared in previous progress reports.

2.0. PEATLAND WATER CHEMISTRY

Discussions of the general ecology of the boreal peatlands of northern Minnesota can be found in Heinselman (1970, 1963) and Hofstetter (1969). It is important to note that the bog habitats in this study are not of the familiar lake basin, floating Sphagnum mat type. The peatlands of northern Minnesota are extensive landscape features covering thousands of hectares. These environments began to form relatively recently (approximately 3000 years ago) and are the result of a complex interaction of hydrology, topography, climate and vegetation.

The water chemistry of a given peatland area strongly influences the vegetation and vice versa (Heinselman, 1963). Peatland water chemistry depends on the nature of the local water source, the amount of water received from that source and the distance the water has traveled through the peatland. It is generally agreed that there are two major categories of peatland based on water source: minerotrophic and ombrotrophic. Fen and bog are the terms used to describe the vegetation associated with minerotrophic and ombrotrophic peatlands respectively. These terms will be used in the remainder of the paper. The following descriptions are from Boelter and Verry (1977) and Heinselman (1963).

Fen (minerotrophic peatland). The water in fens is derived from mineral ion rich groundwater. The water is characterized by relatively high pH (> 5.8);

high calcium content (> 10 ppm); and high specific conductivity (corrected for hydrogen ions, > 75 μMhos). The predominant anion is bicarbonate and the predominant cation is calcium. The water may or may not be darkly colored (due to humic substances). The vegetation is dominated by sedges, grasses or reeds; Sphagnum moss is not a dominant component of the substrate. Fens may be open or forested. Compared to bogs, fens are nutrient rich with greater productivity and floristic diversity. The peat in fens is more decomposed than in bogs.

Bogs (ombrotrophic peatland). The water in bogs is derived from precipitation and hence is ion-poor. The water is characterized by low pH (< 4.3); low calcium content (< 2.8 ppm); and low specific conductivity (corrected for hydrogen ions, < 10 μMhos). The predominate anion is sulfate and the predominate cation is hydrogen. The water is darkly colored due to high concentrations of humic substances. The substrate is dominated by Sphagnum moss. Bogs may be open or forested. The vegetation is dominated by leatherleaf (Chamaedaphne calyculata), cottongrass (Eriophorum spp.), laurel (Kalmia polifolia), and cranberry (Vaccinium oxycoccos). Compared to fens, bogs are nutrient-poor and low in productivity and species diversity. The peat in bogs is relatively undecomposed.

The unusual water chemistry of bogs is due in large part to Sphagnum growth and accumulation which may eventually isolate the living bog growth from the local mineral ground water. This isolation results in the bog vegetation substantially controlling the local water chemistry and dependence on precipitation for nutrient input.

The low pH at bog water sites has important ecological consequences. There are several important factors which interact to produce acid conditions in bogs: 1) precipitation; 2) activity of sulfur metabolizing bacteria;

3) secretion by live *Sphagnum* plants of whole organic acid molecules; 4) cation exchange in the walls of *Sphagnum* plants. These four factors interact to produce the conditions seen at any given site (Clymo, 1964).

Humic substances are another important bog water constituent; they are polyphenolic organic compounds consisting of degraded tannin-protein-lignin-polysaccharide complexes of plant origin found in the peat soil and leached into the local water table. These substances are chemically very diverse and exceedingly resistant to microbial decomposition (Swain, 1978). Humic substances are by no means unique to bog habitats but are present in high concentrations due to the abundance of secondary plant compounds found in bog vegetation (Janzen, 1974).

The bog/fen descriptions presented above represent two ends of a spectrum; there is, of course, gradation between these extremes. Heinselman (1963) notes an intermediate category, weakly minerotrophic waters or poor fens. Table 1 presents measurements of several important water quality factors from bog and fen sites. Calcium and pH are particularly good indicators; Heinselman (1970) found calcium to be an especially valuable indicator of the trophic status of a site.

It is important to note that in a given peatland area a mosaic of water quality is found. Over a distance of meters there are often dramatic changes in water chemistry due to the complex interaction of topography, hydrology, and vegetation.

Figure 1 is a schematic map of my study area in northern Minnesota. Porter Ridge is a former beach of glacial lake Agassiz, formed as the lake slowly retreated at the end of the last Ice Age. In the poorly drained regions adjacent to the ridge typical water saturated peatland habitats have evolved over the last 3000 years. Water flow is to the north and the ridge acts as a dam creating particularly wet habitat to the south. Large bog forests have developed here and control the local water chemistry. Water percolating through the bog drains

Site type	pH	Conductivity (20°C, µmhos)	Ca (ppm)	Mg (ppm)	Na (ppm)	K (ppm)	Cl (ppm)	SO_4 (ppm)	Color (absorbance at 350nm)
Bog	4.1	15.4	1.39	0.52	0.09	2.43	1.13	2.7	0.394
Poor Fen[a]	5.5	27.0	2.72	0.9	0.13	6.56	0.8	1.8	0.245
Poor Fen[b]	6.7	52.2	8.44	3.42	1.61	4.94	0.7	1.2	0.196
Fen	6.5	125.0	16.6	2.88	2.0	1.1	0.4	6.0	--

Table 1. Water quality parameters at peatland sites. See section 2.0. for discussion. Location of sites: Bog-Porter Ridge bog drain; site 4 on Fig. 1. This was the site of bog water used in all egg and larval experiments. Poor Fen[a]-Porter Ridge Fen; site 1 on Fig. 1. This was a ridge associated fen, site of fen water used in egg experiments. Poor Fen[b]-Porter Ridge Fen; site 5 on Fig. 1. Fen-fen watershed, Marcell Experimental Forest, Itasca County (Boelter and Verry, 1977).

Fig. 1. The Porter Ridge Bog study area map. Outlined areas delineate the upland sand soil Porter Ridge; other major landscape units are set off with dotted lines and/or labeled. Circled numbers identify sites where water used in experiments was obtained. The following list indicates the general character of the sites.

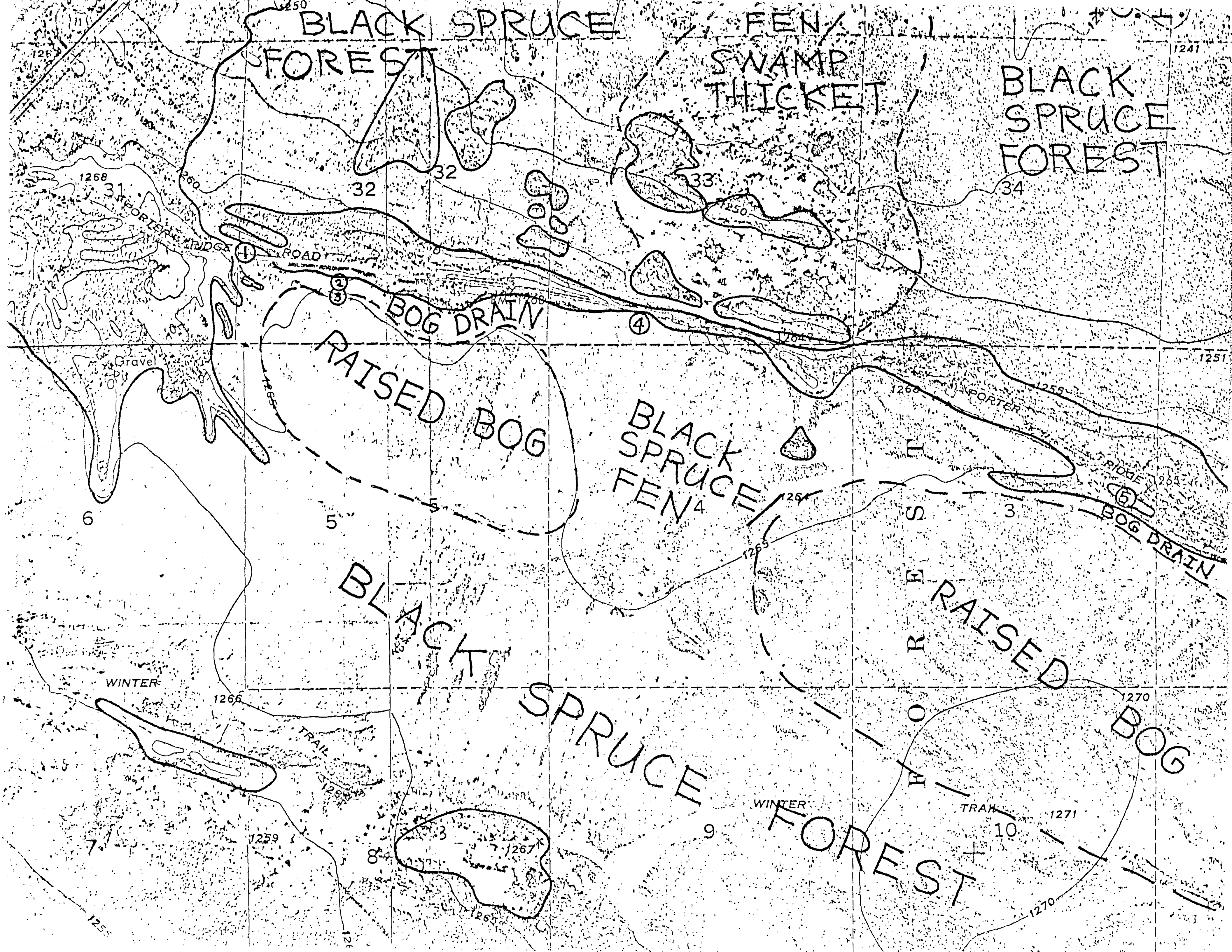
1 - fen

2 - bog drain

3 - bog drain

4 - bog drain

5 - fen



BLACK SPRUCE FOREST
FEN/ SWAMP THICKET
BLACK SPRUCE FOREST
PORTER RIDGE
ROAD
BOG DRAIN
RAISED BOG
BLACK SPRUCE FEN
BLACK SPRUCE FOREST
RAISED BOG
FOREST
PORTER RIDGE
BOG DRAIN
Gravel
WINTER TRAIL
WINTER TRAIL

into a moat-like channel (bog drain) which separates the bog forest from the upland forest of the sandy ridge. The particular water chemistry at a given site along the ridge depends on the degree to which the bog vegetation dominates the site. In the bog drain proper the pH is around 4.2 with typical bog water characteristics. At other sites the ridge chemistry dominates the near ridge environment, which results in poor fen to fen water conditions that abruptly give way to bog water conditions over distances of less than 100 meters. For example, pH dropped $\sim$ 3 units (7.0 to 4.2), calcium content dropped 20-fold (23.4 to 1.1 ppm) and conductivity 100-fold (105.1 to 10.3 μMhos) from a near ridge fen to the adjacent bog drain over a distance of $\sim$ 30 meters. Considering the toxic properties of bog water such changes are of great ecological significance for all organisms that interact with the aquatic environment along the ridge.

How constant are these water quality parameters? As Heinselman (1970) points out, there will be diurnal, seasonal and weather-induced changes in water quality. Using pH as an indicator of water quality, measurements taken in 1978, 1979 and 1980 indicate very stable water quality conditions once the spring thaw has occurred.

3.0. MATERIALS AND METHODS

3.1. Study Area

Porter Ridge bog described above is located in central Koochiching County, Minnesota, approximately seven miles south of the town of Big Falls (see Karns, 1979, for further description). This area was the site for all field work described in this study. Laboratory work was performed at the Big Falls Forestry Station. The majority of laboratory and field work was performed during the spring breeding seasons of 1978, 1979, and 1980. Some additional laboratory work was done at the University of Minnesota.

3.2. Water Chemistry

Water samples were collected in 2-ounce or 16-ounce polyethylene bottles. All bottles were rinsed with dilute nitric acid and then distilled water. Bottles were rinsed in the field three times with water from the site to be sampled. Samples were taken from standing water.

pH (index of hydrogen ions, measure of acidity) was measured in the laboratory from bottled samples. Measurement was usually made within several hours of collection. Laboratory measurement allowed all samples to be measured at the same temperature. A radiometer PHM29 portable pH meter with combined glass-calomel electrode was used.

For the following measurements water samples were stored at 3.5^{o}C and processed at the University of Minnesota at the earliest possible date after collection:

Specific conductivity data (index of total ionic concentration) were standardized to 20^{o}C; a correction factor was applied to eliminate the effect of increased hydrogen ions at low pH (Sjörs, 1950). Measurements were made with a Radiometer CDM2D conductivity meter with platinum electrode.

Water color (index of stagnation and total organic carbon) measured as light extinction at 350 nonometers in a 1-cm cell with corex filter using a Beckman Model 24 Spectrophotometer.

Ca, Mg, Na, K, Cl and SO_4 ion concentrations were measured by ICP emission spectroscopy. Analysis was performed by the Research Analytical Laboratory, Soil Science Department, University of Minnesota.

3.3. Bog water toxicity experiments: Eggs

3.3.1. Egg procurement. The majority of eggs utilized in experiments were obtained from freshly laid eggs found in the field. Eggs showing development beyond stage 8, mid-cleavage, (Gosner, 1960) at the time of experiment initiation were not used. Eggs were also obtained from pairs mated in the laboratory. Rana pipiens eggs for one fertilization experiment were obtained by artificial induction of ovulation and subsequent artificial insemination using standard procedures (Nace et al., 1974).

3.3.2. Fertilization. Data from three species were obtained using slightly different techniques: 1) Freshly laid Rana sylvatica eggs from a number of different matings were obtained from bog and fen sites. These eggs were examined in the lab; fertilization was determined by the presence of egg cleavage. 2) One experiment was done with Bufo americanus eggs; a mated pair was placed in five gallons of control (non-bog) water. After a quantity of eggs had been laid the egg string was cut and the still amplected pair was removed, rinsed in distilled water, and placed in a container with five gallons of bog water and allowed to complete egg deposition. A subsample of eggs from the two treatments was examined for fertilization. 3) Rana pipiens eggs from four different matings were obtained by standard laboratory induction techniques. The unfertilized eggs obtained from each cross were subdivided and placed in bog and control water treatments. These eggs were then artificially inseminated and examined for fertilization. Also, sperm from macerated R. pipiens testes was placed in bog and control water and qualitatively observed using a phase-contrast microscope.

3.3.3. General procedure. Each toxicity experiment consisted of a series of treatments of different water quality. Amphibian eggs were placed in these treatments and hatching success monitored. For each treatment series a freshly laid egg mass was carefully teased apart and small batches of eggs of approximately equal number were placed in the desired treatments. Each egg mass was the product of a different mating and therefore relatively genetically homogeneous. As many egg masses were tested in a given treatment series as logistical feasibility and availability of eggs allowed. All egg batches were scanned under a binocular microscope prior to initiation of treatment and obviously defective eggs removed; eggs that died during the course of the treatment were not removed.

The number of eggs and the volume of treatment water in a test chamber were important variables. 16 ounce glass jars filled with 300 ml of test solution were used. Preliminary experiments indicated the number of eggs that did not appreciably affect this volume of water as measured by pH over a 24 hour period; the number varied with the species. Water was changed in the test chambers every day to insure maintenance of the desired treatment effect and pH was monitored throughout the test period. Unless otherwise stated the temperature at which the tests were run was 15.5^{o}C. Test chambers were kept under conditions of semi-darkness during the test period.

A test was continued until it was apparent that no further hatching would occur (usually 5-6 days). Tests were scored by counting the number of hatched larvae and unhatched eggs in a test chamber. The larvae were further scored as normal (qualitatively normal morphology and swimming response to stimulus) or abnormal (qualitatively abnormal morphology, e.g. arched back or swollen abdomen, and consequent abnormality or lack of swimming response). Unhatched eggs were further scored as truncated (early termination of development, prior to stage 17) or coiled (relatively late termination of development, after stage 17). Embryos in the coil condition were usually past the stage (around stage 20 depending on the species) at which normal hatching occurs and were in an abnormal arched position often with head and tail touching (i.e. coiled).

The quality of the water used in the experiments was standardized as much as possible. Bog and fen water were collected in 20 liter nalgene containers; this water was filtered through fine mesh prior to use to remove macro-debris.

Water was always collected from the same site; bog water from site 2 on Fig. 1 and fen water from site 1. A standard control water was prepared from distilled water and reagent-grade chemicals ($NaHCO_3$, $CaSO_4.2H_2O$, $MgSO_4$ and KCl) following U.S. government guidelines for preparation of reconstituted soft freshwater for use in toxicity testing (Stephan, 1975). This solution had a pH of around 7.5. Preliminary tests indicated it was an excellent medium for development and hatching of amphibian eggs.

Brief descriptions of the various laboratory experiments performed are included in the Results section.

3.4. Egg field experiments and observations

Eggs of several amphibian species (especially R. sylvatica) were periodically collected from bog and fen sites and examined in the lab providing information on fertilization and development under field conditions. Hatching success of field eggs was quantified by carefully cutting off sections of egg masses which were near hatching. These samples were brought to the lab and maintained in water from the site of origin; they were allowed to hatch and the sample scored in the manner described above.

3.5. Bog water toxicity experiments: Larvae and young of the year

3.5.1. Source of larvae. All larvae used in the experiments were obtained from eggs hatched in the laboratory in either control or bog water. All larvae used were newly hatched (Stage 24-26). Prior to experimental use all larvae of a given species were mixed and subsamples chosen randomly for the various experimental treatments.

3.5.2. 96-hour survivorship test. Larvae of six species (Rana sylvatica, Bufo americanus, Hyla crucifer, Rana pipiens, Ambystoma laterale, and Pseudacris triseriata) were subjected to a series of treatments of bog water diluted with control water. This dilution altered both the pH and concentration of bog water.

One gallon glass jars filled with approximately three liters of test solution were used as test chambers; this volume of water maintained original pH levels for the entire test period and was not changed; pH was monitored throughout the test period. No more than 20 larvae (usually 15) were used per test chamber. Two replicates were run for each treatment. A standard 96-hour acute toxicity test format was followed (Stephan, 1975). Test chambers were checked for dead larvae at 12, 24, 36, 48, 72 and 96 hours, and dead larvae were removed.

3.5.3. Larval growth. A comparison of short-term growth of *R. sylvatica* larvae maintained in bog water-bog vegetation diet treatments versus fen water-fen vegetation diet treatments was made. Four replicates, each with 10 larvae were used for both treatments. All larvae were initially at the same stage of development. One gallon jars with approximately three liters of bog or fen water were used as test chambers. Water and vegetation was changed regularly and any dead larvae removed. An attempt was made to keep the number of larvae per test chamber equal since the number of larvae per volume of water is known to affect growth rate. The experiment was run for one month and terminated. Larvae were preserved and later measured.

3.5.4. Metamorphosis. Sixty *R. sylvatica* larvae hatched in bog water were maintained in bog water on a bog vegetation diet to determine if successful development and growth through metamorphosis was possible. A smaller number (20) of larvae were reared in control water on fen vegetation diets. Water and vegetation were changed regularly. Aerated 2-1/2 gallon fish bowls were used as test chambers.

3.5.5. Larval field census. Bog and fen sites were sampled in 1979 and 1980 to determine if larvae were surviving under field conditions. A 23 x 23 x 6 cm plastic tray was used to sample sites where breeding activity was known to have occurred. The tray was plunged into the water and the amphibian larvae caught were retained and preserved. Each fen site was sampled thirty times; each bog site 100 times. Sampling was done on June 9-12 and July 7-9 in 1979 and on May 20-22 in 1980. Nets were not an effective sampling technique in dense aquatic vegetation; the tray technique proved to be an effective sampling method.

3.5.6. Young of the year census. The number of metamorphosed larvae (young of the year) leaving natal bog and fen breeding sites were also sampled. Trapping fences (15 m x 50 cm strips of aluminum flashing buried 10 cm deep, with five 35 cm deep pitfall traps located on each side) were placed immediately adjacent and parallel to bog and fen breeding areas in 1979 and 1980. Emerging young of the year were caught in these traps.

4.0. RESULTS

4.1. Fertilization

The ability of anuran eggs to be fertilized in bog water was investigated experimentally utilizing the eggs from three species. Table 2 shows the percentage of eggs successfully fertilized in bog water treatments compared to fen (control) water treatments. There were no significant differences between the two treatments for any of the species tested (G-Test). In addition qualitative observations made at bog water breeding sites with *R. sylvatica*, *B. americanus*, and *H. crucifer* eggs showed a high percentage of fertilization at these sites as indicated by egg cleavage.

Sperm from the macerated testes of *R. pipiens* were placed in bog water and examined microscopically. Qualitatively these sperm showed no ill effects due to bog water immersion. They were mobile and appeared identical to sperm examined in control water.

These experiments and observations indicate that bog water is a perfectly viable medium for the fertilization of amphibian eggs.

Species Tested	% Fertilization BOG	CONTROL
Rana sylvatica (Wood Frog)	96.5 677(13)[1]	96.4 659(11)
Bufo americanus (American Toad)	99.0 100(1)	96.0 100(1)
Rana pipiens (N. Leopard Frog)	97.9 578(4)	98.9 466(4)

Table 2. Fertilization of amphibian eggs in bog water compared to control (fen) water. See section 4.1. for discussion.

[1]The number beneath percentage fertilization equals the total number of eggs sampled; number in parentheses equals the total number of different egg masses (matings) sampled.

4.2. Egg Development and Hatching

The experiments and observations dealing with the development and hatching of amphibian eggs in bog water fall into two main categories: 1) Descriptive-laboratory and field experiments and observations utilizing chemically unaltered bog water; 2) Mechanistic-laboratory experiments utilizing chemically altered bog water concerned primarily with the role of acidity in bog water toxicity. The first group is intended to describe the phenomenon of bog water toxicity and elucidate environmental factors important in its manifestation. The second group deals more with the mechanism of the toxicity.

4.3. Descriptive experiments

4.3.1. Bog water tolerance. The suitability of bog water as a medium for the development and hatching of amphibian eggs was investigated under laboratory conditions; eggs of six of the seven amphibian species common in the study area were tested (*H. versicolor* was not tested). Eggs from each egg mass sampled were placed in bog and control water treatments. Table 3 presents the results and clearly shows that bog water is an extremely deleterious medium for amphibian egg development and hatching. Egg mortality (i.e. no hatching) is extremely high in the bog water treatments. The difference in mortality between bog and control treatments is highly significantly different for all species tested (G-Test, $p<0.005$). Only *R. sylvatica* exhibits any ability to hatch in bog water (12.7% hatching success).

Another aspect of bog water toxicity is indicated by the percentage coil column. The coil condition indicates that eggs achieved an advanced state of development ($\geq$ stage 18) but did not hatch. Two of the six species tested (*R. sylvatica* and *A. laterale*) show a significant degree of the coil condition (75.2 and 58.5% respectively). The coil condition is virtually nonexistent in the control treatments for all other species. The coil condition is another indicator of the physiological tolerance of eggs to bog water as a developmental medium. The only amphibian which shows an ability to hatch in bog water,

	Treatment					
	BOG (pH 4.1-4.3)			CONTROL (pH 7.1-7.6)		
Species Tested	% Mortality	% Coil	n[1]	% Mortality	% Coil	n[1]
Rana sylvatica (Wood Frog)	87.3	75.2	1396(42)	3.1	0.1	1013(27)
Ambystoma laterale (Blue-spotted Salamander)	100.0	58.5	164(4)	2.6	0.0	142(4)
Bufo americanus (American Toad)	100.0	14.6	2575(49)	9.0	0.01	1373(24)
Rana pipiens (N. Leopard Frog)	100.0	0.7	435(12)	1.5	0.0	987(8)
Pseudacris triseriata (Chorus Frog)	100.0	0.0	285(8)	44.4	0.0	232(7)
Hyla crucifer (N. Spring Peeper)	100.0	0.0	325(7)	32.0	0.2	419(8)

Table 3. Mortality of amphibian eggs reared in bog water compared to control water. See section 4.3.1. for discussion.

[1]First number equals the total number of eggs sampled; number in parentheses equals the total number of egg masses (matings) sampled.

R. sylvatica, also shows the highest degree of the coil condition. Based on these results, R. sylvatica and A. laterale would have the greatest potential for successfully breeding in bog water areas since they are capable of advanced development in the medium. In the four other species development is truncated at an early stage of development.

4.3.2. Geographic variation in toxicity. As noted in the section on peatland water chemistry, the study area was a mosaic of patches of varying size of differing water quality. It was of interest to test water from several sites to determine the variability of its toxic properties. The primary area of interest was the large bog drain (Fig. 1) where amphibian breeding was known to occur. Water was taken from three sites in the bog drain and one fen site. (See caption Table 4 for location of sites). Eggs of the most tolerant species, R. sylvatica, were used for this test. Table 4 indicates that water from all three bog drain sites was extremely toxic relative to fen and control sites (G-Test, <0.005). The coil condition was associated only with the bog sites. There was significant variation in toxicity among bog drain sites. Of special interest are bog sites I and II; these sites are only 100 meters apart. Bog I is located on the black spruce bog forest side of the drain and bog II on the ridge side of the drain. Mortality drops by 44% between the two sides of the drain. There is a slight rise in pH between the two sides presumably due to the minerotrophic influence of the ridge.

4.3.3. Temperature. The relationship between bog water toxicity and temperature was investigated using eggs of R. sylvatica and B. americanus. Eggs from each of four different egg masses were placed in two bog and two control water treatments. One bog and one control were kept at 3.5^{o}C (cold treatment) and one bog and one control were kept at 15.5^{o}C (warm treatment). Results are shown in Table 5. Temperature does not affect mortality or

Site	% Mortality	% Coil	n[1]
Bog Drain I (pH 4.1)	90.8	55.3	76(3)
Bog Drain II (pH 4.5-4.6)	46.8	37.8	111(3)
Bog Drain III (pH 4.1-4.2)	90.3	75.3	93(3)
Fen (pH 5.5)	3.8	0.6	110(3)
Control (pH 7.5)	4.1	0.0	98(3)

Table 4. Geographic variation in bog water toxicity. Eggs of R. sylvatica were tested. Water for these experiments was taken from sites along Porter Ridge shown on Fig. 1: Bog Drain I = site 3; Bog Drain II = site 2; Bog Drain III = site 4; Fen = site 1. See section 4.3.2. for discussion

[1] First number equals the total number of eggs sampled; number in parentheses equals the total number of egg masses (matings) sampled.

Species tested and water treatment		COLD (3.5°C)			WARM (15.5°C)		
		% Mortality	% Coil	n[1]	% Mortality	% Coil	n[1]
Rana sylvatica (Wood Frog)	BOG	100.0	0.0	140(4)	94.6	85.9	185(4)
	CONTROL	1.4	0.0	146(4)	2.1	0.0	143(4)
Bufo americanus (American Toad)	BOG	100.0	0.0	219(4)	100.0	12.0	191(4)
	CONTROL	5.5	0.0	237(4)	4.0	0.0	199(4)

Table 5. Effect of temperature on the toxicity of bog water as measured by the egg hatching success of *R. sylvatica* and *B. americanus*. See section 4.3.3. for discussion.

[1] First number equals the total number of eggs sampled; number in parentheses equals the total number of egg masses (matings) sampled.

occurrence of the coil condition in the control water treatments for either species (G-Test, $p<0.05$). Low temperature alone did not increase mortality. Temperature is an important factor in the bog water treatments. B. americanus is the less bog water tolerant of the two species tested; cold or warm bog water treatment resulted in 100% mortality. There is a relatively small but significant increase (G-Test, $p<0.05$) in the occurrence of the coil condition between the cold (0.0%) and warm (12%) treatments. With R. sylvatica there was a low but significant level of hatching (5.4%) in the warm bog treatment compared to 0.0% in the cold bog (G-Test $p<0.05$). There is a dramatic difference in the occurrence of the coil condition between the two treatments for R. sylvatica. In the cold bog all eggs truncated at an early stage of development, while in the warm bog treatment 91.3% of the eggs reached an advanced stage (5.4% hatch, 85.9% coil). These differences are highly significantly different (G-test, $p<0.005$). These results indicate that the temperature of the bog water is an important factor in the manifestation of the toxicity of the water. The cold treatment eliminates the possibility of achieving advanced development and potential hatching. At higher temperatures the bog water is relatively more benign; eggs reach an advanced stage of development and some may hatch. As might be expected, the temperature effect is more pronounced with R. sylvatica, the more bog water tolerant species.

4.3.4. Field observations and experiments. It was important to determine how eggs responded to bog and fen water when exposed to the complexity of natural conditions. Eggs of the easily obtainable R. sylvatica were the main source of information. R. sylvatica was also of great interest ecologically since the laboratory experiments indicated it was the most bog water tolerant species. As described above, experiments indicated that water temperature was an important variable in the toxicity. A natural temperature experiment was performed during the two breeding seasons in which egg survivorship was sampled. The 1979 breeding season was extremely wet and cold with many overcast days compared to the dry, hot sunny 1980 season. Table 6 shows the water temperature as recorded in the bog drain during the R. sylvatica breeding period of each year. These temperatures shown are typical of non-shaded breeding sites, bog and fen, in the study area both years. There is no indication that water quality varied in an important manner between the two years. pH is a good general indicator of bog water quality; the range of pH values taken at sites of egg mass deposition were the same both years. The R. sylvatica laboratory temperature experiments predict that egg mortality in 1980, the warm year, should decrease. The R. sylvatica laboratory experiments also indicate variation in toxicity among sites within the generally toxic bog drain apparently due to variation in water quality. Field observations did show variation among bog sites in toxicity but this was confounded by another factor not studied in laboratory experiments, the number of egg masses present at an egg mass deposition site. R. sylvatica is an explosive breeder; large numbers of frogs breed at a particular site over a period of a few days. The result of this breeding is usually the formation of a "communal" site of egg mass deposition with large numbers of egg masses (up to 1,000) in close contact. Table 7 shows the results of a census of R. sylvatica eggs sampled from the bog and fen sites. The percentage of eggs reaching an advanced stage of development ($\geq$ stage 18, coil plus hatch) is given and grouped by year (large temperature differences), water quality (bog versus fen), and size of egg mass cluster. In the bog drain there is a significant difference in developmental success between large and small egg mass clusters (G-Test, $p<0.005$) in both years. Eggs do better when associated with large egg mass clusters. There is a significant difference between bog and fen egg development for both the small and large bog egg mass clusters in 1979 (G-Test, $p<0.005$). In 1980 the difference is significant between the fen and small bog

		1979 (21 April-15 May)	1980 (18 April-19 May)
Temperature (^{o}C)	$\overline{X}$ Max	14.0	26.6
	$\overline{X}$ Min	3.0	4.6
	Range	(0.0-18.9)	(0.0-32.2)
pH	Range	4.1-4.3	4.1-4.3

Table 6. Water temperature and pH values recorded in Porter Ridge bog drain in spring 1979 and 1980. Location of temperature measurement station was between sites 2 and 3 on Fig. 1. See section 4.3.4. for discussion.

Source of eggs	% Eggs in advanced development (coil and hatch, ≥ stage 18)			
BOG	1979 (cold year)		1980 (warm year)	
Large cluster of egg masses (65-110 masses)	47.3	n[1] = 2575(56)	95.4	n = 461(11)
Small cluster of egg masses (1-10 masses)	9.9	n = 1257(27)	72.4	n = 370(10)
FEN				
Large cluster of egg masses (> 250 masses)	93.5	n = 1396(40)	97.3	n = 778(12)

Table 7. Developmental success of *R. sylvatica* eggs sampled from bog and fen sites along Porter Ridge in 1979 and 1980. See section 4.3.4. for discussion.

[1]First number equals the total number of eggs sampled; number in parentheses equals the total number of egg masses (matings) sampled.

egg mass cluster only (G-Test, $p<0.05$). As predicted, many more bog eggs reach an advanced developmental stage in 1980, the warm year, as opposed to 1979. The difference is highly significant for both large and small egg mass clusters (G-Test, $p<0.005$). Note the very dramatic seven-fold increase with small egg mass clusters. Advanced development is high at fen sites in both years; there is a 3.8% increase in 1980 but this difference is not significant (G-Test). In 1980 the level of developmental success is equivalent between the fen and large egg mass cluster bog sites.

These results indicate that for R. sylvatica large egg mass cluster size and/or warm water temperatures can act as a "buffer" against the toxic effect of bog water, at least in terms of development.

The data presented above support the contention that bog water is a toxic medium for the development and hatching of amphibian eggs and that the exact site of egg deposition, size of egg mass cluster and temperature are important variables in the manifestation of the toxicity. However most of this data is for R. sylvatica, the most bog water tolerant species. The laboratory tolerance tests (Table 3) suggest that bog water is lethal (in terms of hatching success) to the other local species; the available field data support this. The best information is available for B. americanus. Eggs sampled from bog drain sites showed 0.0% hatch and 0.0% advanced development (n = 1388 eggs from six different egg masses). All eggs examined had truncated development early and many showed gross abnormalities. Hyla crucifer eggs (n = 500) found in the bog drain showed 0.0% hatch and 0.0% advanced development. Unfortunately no bog drain eggs of A. laterale, the only species besides R. sylvatica to show significant ability for advanced development in bog water were found.

4.5. Mechanistic Experiments

Role of acidity in bog water toxicity

The toxicity of acid media to the eggs, juveniles and adults of a variety of aquatic organisms is well known (references in EPA, 1980). The acid nature of bog water is one of its most conspicuous characteristics and acidity is immediately suspect as a primary agent in the observed toxicity of bog water. The role of acidity, as measured by pH, in the manifestation of bog water toxicity was investigated in a series of laboratory experiments.

4.5.1. Neutralized bog water. Preliminary experiments with *B. americanus* and *H. crucifer* indicated that toxic bog water (pH 4.2) could be transformed into a non-toxic developmental and hatching medium when neutralized to a pH of approximately seven with sodium hydroxide (NaOH). (*B americanus*: Hatch success at pH 4.2 = 0.0% (n = 185 eggs); at pH 7.0 = 97.2% (n = 178 eggs); tests are significantly different (G-Test, $p<0.005$). *H. crucifer*: Hatch success at pH 4.2 = 0.0% (n = 77 eggs); at pH 7.0 = 84.6% (n = 52 eggs); tests are significantly different (G-Test, $p<0.005$)). Gosner and Black (1957) and Saber and Dunson (1978) obtained similar results using eggs from a number of anuran species and NaOH as the neutralizing agent. Further experiments on the neutralization of bog water were done using calcium carbonate ($CaCO_3$) as the neutralizing agent; $CaCO_3$ is a very weak base and mimics the natural neutralization of bog water (which occurs when bog water comes into contact with minerotrophic chemical influences) to a greater degree than the very strong base, NaOH (Gorham, personal communication). Experiments were done with the bog water tolerant *R. sylvatica* and intolerant *B. americanus*. Test batches of eggs from each of four egg masses sampled were placed in five treatments for each species. The treatment series raised the pH in ∿0.5 pH unit increments from normal bog water (pH 4.1) to bog water of pH 5.5. Results are shown in Table 8.

Elevation of pH has a dramatic effect on hatching success; for both species the small 0.3-0.4 rise in pH from normal bog to pH 4.5 bog water results in a highly significant increase in hatching success (G-Test, $p<0.005$). *R.*

Treatment	R. sylvatica (Wood Frog) % Hatch	% Coil	n[1]	B. americanus (American Toad) % Hatch	% Coil	n[1]
Normal Bog pH 4.1-4.2	1.5	96.2	130(4)	0.0	2.5	201(4)
Treated Bog pH 4.5	63.6	31.8	132(4)	21.0	72.7	205(4)
Treated Bog pH 5.0	90.0	4.6	130(4)	97.5	1.0	198(4)
Treated Bog pH 5.5	94.5	0.7	136(4)	96.8	1.6	189(4)
Control ph 7.5	89.1	0.0	138(4)	97.3	1.4	219(4)

Table 8. Effect of elevation of pH on the toxicity of bog water as measured by the egg hatching success of R. sylvatica and B. americanus. See section 4.5.1. for discussion.

[1]First number equals the total number of eggs sampled; number in parentheses equals the total number of egg masses (matings) sampled.

sylvatica hatches significantly more at pH 4.5 than B. americanus (G-Test, $p<0.05$) as might be expected from the tolerance tests. At pH 5.0 and 5.5 hatching is not significantly different from the control for both species (G-Test).

The differential bog water tolerance of the two species is clearly demonstrated by the coil condition. In normal bog water, 97.7% of the R. sylvatica reach an advanced developmental stage of which 1.5% hatch; with B. americanus only 2.5% of the eggs reach an advanced stage and 0.0% hatch. In pH 4.5 bog water 95.4% of the R. sylvatica eggs are advanced and 63.6% hatch; a comparable proportion (93.5%) of B. americanus reach an advanced stage but only 21.0% hatch. At pH 5.0 and above the coil condition virtually disappears and hatching is high in both species.

In both species the pH of the bog water "controls" the toxicity; at pH values $\geq$ 5.0 the toxicity essentially disappears for both species.

4.5.2. Acidified fen water. The water source of the non-toxic fen sites along the ridge is the bog to the south; the minerotrophic chemistry of the ridge has in some manner interacted with the bog water to eliminate its toxic properties. If pH is a controlling factor in the toxicity, lowering the pH of the fen water might "restore" its toxic properties.

This experiment was done using R. sylvatica eggs. Eggs from each of four different egg masses were placed in four treatments: normal fen water (pH 5.5), acidified fen (pH 4.5 and 4.0) and control. The fen water was acidified by titration with H_2SO_4. Results are shown in Table 9 and agree closely with the bog neutralization experiments.

Hatching success declines with decreasing pH and occurrence of the coil condition increases; pH 4.5-5.5 is a "threshold" level above which normal hatching is restored. Normal fen and control are significantly different from pH 4.5 and 4.0 for both hatching success and coil (G-Test, $p<0.005$). The

Treatment	% Hatch	% Coil	n[1]
Acid Fen pH 4.0	2.3	97.7	133(4)
Acid Fen pH 4.5	73.8	11.9	126(4)
Normal Fen pH 5.5	96.2	0.6	158(4)
Control pH 7.5	96.6	0.7	148(4)

Table 9. Effect of acidification of fen water on hatching success of *R. sylvatica* eggs. See section 4.5.2. for discussion.

[1]First number equals the total number of eggs sampled; number in parentheses equals the total number of egg masses (matings) sampled.

difference between pH 4.0 and 4.5 treatments is significantly different for both hatching success and coil (G-Test, $p<0.005$).

4.5.3. Acidified control water. The neutralized bog and acidified fen water experiments show a strong correlation between acidity and toxicity and suggest that acidity may be a primary cause of the toxicity. If acidity *per se* is a main causal factor then acidified control water (non-bog, non-fen water) should produce a comparable degree of toxicity both quantitatively and qualitatively. Different acids are known to produce different toxic effects in aquatic organisms (e.g. Ellis, 1937). Sulfuric acid (H_2SO_4) was used as an acidifying agent because it is the dominant free acid found in northern Minnesotan peatland waters (Gorham, personal communication).

The experiment consisted of a series of acidified control water treatments ranging from pH 3.0 to 5.0 in 0.5 pH unit increments plus a pH 6.0 and control treatment. *R. sylvatica* and *B. americanus* were the test species; eggs from each of four egg masses sampled were placed in the seven treatments for both species. Results are presented in Table 10.

The acid series shows important differences between the bog and fen water experiments already presented in terms of both developmental and hatching success for both species.

For *R. sylvatica* there is a dramatic threshold of acid tolerance between pH 3.5 and 4.0. At pH values $\geq$ 4.0 hatching success is high; the differences are significant (G-Test, $p<0.005$). The pH 3.0 and 3.5 treatments were lethal; eggs showed no development past the stage they were in at the time of immersion. At the pH levels tested the coil condition is uncommon; it is most prominent at pH 4.0 (3.5%) but this is not significantly different from higher pH values (G-Test). Of special importance is the pH 4.0 treatment, which is close to the pH of the bog water tested. Experiments with unaltered bog water (pH 4.1-4.2)

R. sylvatica (Wood Frog)				*B. americanus* (American Toad)			
pH (range)	% Hatch	% Coil	n[1]	pH (range)	% Hatch	% Coil	n[1]
3.0 (3.0)	0.0	0.0	128(4)	3.0 (3.0)	0.0	0.0	209(4)
3.5 (3.5-3.6)	0.0	0.0	154(4)	3.5 (3.4-3.6)	0.0	0.0	208(4)
4.0 (3.9-4.0)	93.8	3.5	142(4)	4.0 (4.0-4.1)	2.3	40.5	262(4)
4.5 (4.5-4.7)	96.8	1.6	126(4)	4.5 (4.5-4.6)	97.8	0.0	276(4)
5.0 (5.0-5.2)	99.2	0.0	129(4)	5.0 (5.0-5.2)	83.6	0.0	220(4)
6.0 (6.0-6.5)	96.9	0.0	129(4)	6.0 (6.0-6.4)	98.1	1.9	215(4)
Control (7.4-7.6)	96.8	0.5	189(4)	Control (7.3-7.6)	94.8	0.0	251(4)

Table 10. Effect of acidification of control water on hatching success of *R. sylvatica* and *B. americanus* eggs. See section 4.5.3. for discussion.

[1]First number equals the total number of eggs sampled; number in parentheses equals the total number of egg masses (matings) sampled.

and acidified fen water (pH 4.0) (Tables 3,4,5,8, and 9) produced very low overall hatching success (<13.0%) and a very high occurrence of the coil condition (>75%). The pH 4.0 acid water experiments (Table 10) produced high hatching success (>93.0%) and low occurrences of the coil condition (<4%). These differences are highly significant (G-Test, p<0.005). There are also significant differences at pH 4.5 among the neutralized bog, acidified fen and acid treatments. In the first two treatments hatching success is lower and the coil condition is more prominent than in the acid treatment. At pH values of 5.0 or greater results are similar (high hatch, little or no coil condition seen).

With *B. americanus* the pattern is similar to *R. sylvatica*, however, the tolerance threshold is about 0.5 pH units higher; a dramatic increase in hatching success occurs between pH 4.0 and 4.5 compared to 3.5 and 4.0 for *R. sylvatica*. As with *R. sylvatica* the pH 3.0 and 3.5 treatments were lethal. The pH 4.0 treatment shows very low hatching while treatments >pH 4.5 produce high hatching. The differences are significant (G-Test, p<0.005). Only the pH 4.0 treatment produces a high number of coil eggs. Again pH 4.0 and 4.5 treatments, close to the pH of bog water, are of particular interest. In unaltered bog water (pH 4.1-4.2) *B. americanus* never hatched and few eggs reached an advanced developmental stage (<15.0%); many of the truncated eggs exhibited gross developmental abnormalities (Table 3,5, and 8). In acid water at pH 4.0 there was some hatching, many eggs reached an advanced developmental stage and few gross abnormalities were seen. The differences are significant (G-Test, pH<0.005). In neutralized bog water at pH 4.5 (Table 8) there was a moderate hatch (21.0%) and high occurrence of the coil condition (72.7%) hence a large proportion (93.7%) of eggs reached an advanced developmental stage but relatively few hatched. In pH 4.5 acid water the hatch is very high and no coil condition was seen. The differences are significant (G-Test, $p<0.005$). At pH values $\geq$ 5.0 similar results are seen in both neutralized bog and acid water treatments.

The results of the acid water experiment indicate that 1) the two species tested differ in their acid tolerance; R. sylvatica being the more tolerant. 2) Acid water treatments were lethal but only at pH values (3.0, 3.5) below those found in the bog water sites studied. 3) The acid treatments are less toxic (in terms of developmental and hatching success) than bog water treatments of equivalent pH. This strongly suggests that acidity, although an important factor, is not sufficient to explain the observed toxicity of bog water; these observations suggest the presence of another toxic factor(s) in the bog water interacting with low pH to produce the observed results. If acidity alone were the primary cause of toxicity, bog water and acid treatments of equivalent pH should produce similar results; they do not.

4.5.4. Dilution experiments. The relationship between acidity and other toxic factors in bog water was further investigated in a series of dilution experiments with eggs of R. sylvatica and B. americanus. Two types of dilution series were employed; normal and acid. In the normal series bog water was diluted with pH 7.5 control in a series of steps; this resulted in 1) a lowering of the concentration of bog water and any toxic factors in the bog water 2) a gradual increase in pH due to the neutralizing influence of the control water. In the acid dilution treatment series bog water is again diluted with control water but the elevated pH of the mixture is then lowered with H_2SO_4 to a pH level in which high hatching success would occur if the solution were 100% control water (as determined by the acid water experiment), pH 4.0 for R. sylvatica and pH 4.5 for B. americanus. Note that undiluted bog water at these pH values would be toxic for the two species. Eggs from each of the four or five egg

NORMAL DILUTION				ACID DILUTION			
% Bog Water	% Hatch	% Coil	n[1]	% Bog Water	% Hatch	% Coil	n[1]
100.0 (pH 4.2)	12.1	72.6	124(4)	100.0 (pH 4.1-4.2)	1.3	87.5	160(5)
90.0 (pH 4.4-4.7)	92.5	3.4	134(4)	90.0 (pH 4.0-4.1)	0.0	95.8	167(5)
80.0 (pH 5.3-5.5)	95.5	0.0	132(4)	80.0 (pH 4.0-4.1)	0.6	94.1	171(5)
70.0 (pH 6.2)	98.4	0.0	128(4)	70.0 (pH 4.0-4.1)	1.1	93.3	179(5)
60.0 (pH 6.6)	100.0	0.0	117(4)	60.0 (pH 4.0-4.1)	1.7	95.9	172(5)
40.0 (pH 6.8-7.0)	99.3	0.0	139(4)	40.0 (pH 4.0-4.1)	2.9	91.8	170(5)
20.0 (pH 7.1-7.2)	98.0	0.0	150(4)	20.0 (pH 4.0-4.1)	33.7	65.1	166(5)
Control (pH 7.4-7.6)	97.7	0.0	171(4)	Control (pH 7.5)	98.7	0.0	157(5)
				Control (pH 4.0)	94.4	1.3	160(5)

Table 11. *R. sylvatica* bog water dilution experiment. See section 4.5.4. for discussion.

[1] First number equals the total number of eggs sampled; number in parentheses equals the total number of egg masses (matings) sampled.

masses sampled were placed in the seven treatments for each experiment for both species.

The R. sylvatica normal dilution experiment (Table 11) shows that slight dilution of bog water (10%) with the concomitant pH elevation virtually eliminates the toxic properties of the bog water. The differences between 100% and 90% bog water are significant (G-Test $p<0.005$). This amelioration of the toxicity is expected, based on previous experiments (Table 8), due to the elevation of the pH alone. The very high hatching success in the 90% and above dilutions may have been aided by dilution and consequent reduction in the concentration of possible additional toxic factors in bog water but the pH elevation precludes any assessment of the effect.

The R. sylvatica acid dilution experiment (Table 11) reveals dramatic differences. All dilution treatments are significantly different than equivalent dilutions in the normal series (G-Test, $p<0.005$). The acidified diluted bog water is highly toxic in terms of both developmental and hatching success; a significant amelioration of toxicity is not seen until the bog water is diluted by 80%. The embryos observed in the 90-40% treatments were very tightly coiled, more so than 100% bog embryos. It was not until the 20% treatment that less tightly coiled embryos were seen. It is important to stress that 100% control water at pH 4.0 exhibits normal development and hatching while the bog/control water dilutions of similar pH do not.

The B. americanus experiments show the same general pattern (Table 12). In the normal dilution series relatively slight dilution eliminates the toxicity. B. americanus is less acid/bog water tolerant than R. sylvatica; there is no hatching and low percentage coil in 100% bog water. In 90% bog water (pH 4.5) there is significantly more hatching than 100% but significantly less than in greater dilutions with higher pH values (G-Test, $p<0.05$). These results agree well with previous experiments (Tables 3 and 8).

NORMAL DILUTION				ACID DILUTION			
% Bog Water	% Hatch	% Coil	n[1]	% Bog Water	% Hatch	% Coil	n[1]
100.0 (pH 4.2)	0.0	10.9	192(4)	100.0 (pH 4.1)	0.0	24.8	262(5)
90.0 (pH 4.5)	14.8	54.5	189(4)	90.0 (pH 4.5-4.6)	15.8	76.8	259(5)
80.0 (pH 5.1-5.4)	98.2	1.4	217(4)	80.0 (pH 4.5-4.6)	10.8	86.5	251(5)
70.0 (pH 5.9-6.5)	98.3	0.4	229(4)	70.0 (pH 4.5-4.6)	27.6	70.4	250(5)
60.0 (pH 6.5-7.0)	97.3	0.0	218(4)	60.0 (pH 4.5-4.6)	18.6	79.4	247(5)
40.0 (pH 6.9-7.0)	95.1	0.0	224(4)	40.0 (pH 4.5-4.6)	23.0	70.9	244(5)
20.0 (pH 7.1)	95.5	0.0	201(4)	20.0 (pH 4.5-4.6)	82.9	15.9	251(5)
Control (pH 7.5)	96.0	0.0	199(4)	Control (pH 7.5)	98.8	0.0	329(5)
				Control (pH 4.5)	98.9	0.0	269(5)

Table 12. *B. americanus* bog water dilution experiment. See section 4.5.4. for discussion.

[1]First number equals the total number of eggs sampled; number in parentheses equals the total number of egg masses (matings) sampled.

In the B. americanus acid dilution experiment (Table 12) there were significant differences between all equivalent dilutions of the acid and normal series except the 90% bog treatment (G=Test, $p<0.005$). There is a relatively low level of hatching success and high level of the coil condition observed until the major increase in hatching success of the 20% bog treatment. The hatching success in the 90-40% treatments are rather variable. In the 90-70% treatments the embryos observed were tightly coiled; the tight coiling became less prominent in the higher dilutions and the embryos were more typically elongate in the later stages of development. In the diluted treatments the gross abnormalities of early stage embryos associated with 100% bog water were rarely seen.

It is important to note that the pH level used in the B. americanus acid dilution series was 0.5 pH units higher than the R. sylvatica series to take into account the differences in bog/acid tolerance between the two species; this is the reason a moderate amount of hatching is seen in the less tolerant B. americanus acid dilution series compared to the more tolerant R.sylvatica.

The normal dilution series supports evidence from other experiments that pH is a dominant factor in bog water toxicity; elevated pH "masks" the effects of the bog water dilutions but provides a comparison with the acid dilution series. Acidification of dilutions "restores" the toxic properties of the bog water, however the pH levels used (pH 4.0 for R. sylvatica and pH 4.5 for B. americanus) are not toxic in control solutions. This strongly suggests that there are other toxic factors in bog water which pH interacts with in some manner; these other factors are apparently benign unless the pH of the solution is sufficiently low. If the pH environment is conducive, bog water is a potent toxin at low concentrations. In both species there is low hatching success at bog water concentrations $\geq$ 40% bog water. This indicates that 40% bog water is an effective dosage of bog water "toxin(s)" at the respective pH values of the test series. The low level of hatching success at higher bog water concentrations (40-90%) seen in the B. americanus series could presumably be eradicated by a slight lowering of pH.

4.5.5. Other toxic factors. The humic substances found in bog water are suspected toxic agents (Janzen 1974; Karns 1979), however, due to the biochemical complexity of bog water they are difficult to deal with experimentally. I had originally planned to investigate the role of humic substances as an important bog water toxin using commercially produced tannic acid (tannins are an important class of humic substances found in bog water). These experiments were terminated; it became apparent that it would be misleading to extrapolate from laboratory produced tannic acid to the role of naturally occurring humic substances; there were too many technical problems to make the experiments feasible.

Another possible class of potential toxins in bog water are heavy metals. At low pH values heavy metals in solution will precipitate out becoming available to biological systems. The toxicity of heavy metals to amphibian eggs is well documented (Porter and Hakansan, 1976). However, in spite of the low pH the availability of heavy metals in bog water systems is open to question due to the well established ability of peat to adsorb metals and metal ions (Crawford, 1978). This question deserves further investigation.

4.6. Larvae and young of the year

The experiments and observations described above have shown that bog water is a deleterious medium for the development and hatching of amphibian eggs; only R. sylvatica exhibited any significant tolerance to bog water. The experiments also indicated the complexity of the phenomenon; temperature, exact site of egg deposition, slight pH changes, etc. are all important. R. sylvatica eggs can hatch in large numbers at bog sites and given the complexity of the toxicity other less tolerant species, under various conditions, may also hatch at bog sites. Laboratory experiments and field observations investigated the ability of larvae to tolerate bog water.

4.6.1. 96-hour survivorship test. A standard 96-hour tolerance test (Stephan, 1976) utilizing a series of bog water dilutions was run with newly hatched larvae of six amphibian species (Table 13). Note that the dilutions elevate pH levels. With the exception of *R. sylvatica* these were all direct transfer tests; larvae were taken from fen water (pH 5.5), rinsed in distilled water, and placed directly into the bog water test chamber. For *R. sylvatica* fen and bog water hatched larvae were available and were tested separately. For all species tested, except *R. sylvatica*, 100% bog water was virtually lethal (two *H. crucifer* larvae did survive). As observed in the egg experiments mild dilution (10%), and the accompanying slight rise in pH eliminated the toxic effect. For all species 100% bog water was significantly different than 90% or higher dilution bog water (G-Test, $p<0.005$). Only *P. triseriata* shows a significant difference in mortality between the 90% bog water treatment and the remainder of the series (G-Test, $p<0.005$). *R. sylvatica* is the only species to tolerate 100% bog water; bog hatched larval survivorship is significantly greater than fen hatched (G-Test, $p<0.05$) and also different than the remainder of the treatment series (G-Test, $p<0.05$). In none of the 100% bog water tests did larvae exhibit any obvious behavioral reaction to the medium.

These experiments indicate that bog water is not a benign medium for young amphibian larvae and provides further evidence for the ability of *R. sylvatica* to tolerate bog water sites. Direct transfer tests between different media may be traumatic to the organism as suggested by the difference in survivorship between the bog and fen hatched *R. sylvatica* larvae. The high survivorship in the 90% bog dilution with its slight pH increase argues against a prominent traumatic transfer effect.

Species Tested: % Survivorship after 96 hours	% Bog Water						
	100.0 (pH 4.2-4.3)	90.0 (pH 4.4-4.5)	80.0 (pH 5.1-5.2)	60.0 (pH 6.5)	40.0 (pH 7.0)	20.0 (pH 7.2)	Control (pH 7.5)
Rana sylvatica (Wood Frog)	31.7[1] 83.3 (60)[2]	100.0 (30)	100.0 (30)	96.7 (30)	100.0 (30)	96.7 (30)	96.7 (60)
Bufo americanus (American Toad)	0.0 (60)	90.0 (60)	100.0 (30)	96.7 (30)	93.3 (30)	100.0 (30)	100.0 (40)
Pseuadcris triseriata (Chorus Frog)	0.0 (40)	15.0 (40)	100.0 (20)	100.0 (20)	95.0 (20)	100.0 (20)	100.0 (40)
Hyla c. crucifer (N. Spring Peeper)	6.7 (30)	100.0 (30)	100.0 (30)	100.0 (30)	96.7 (30)	100.0 (30)	100.0 (30)
Rana pipiens (N. Leopard Frog)	0.0 (60)	96.7 (30)	100.0 (30)	100.0 (30)	96.7 (30)	96.7 (30)	97.5 (40)
Ambystoma laterale (Blue-spotted Salamander)	0.0 (20)	100.0 (20)	100.0 (20)	100.0 (20)	100.0 (20)	100.0 (20)	100.0 (20)

Table 13. Survivorship of amphibian larvae after 96 hours of exposure to bog water treatmentsof varying concentrations. See section 4.6.1. for discussion.

[1] For the Wood Frog alone larvae were available that had been hatched in control water and bog water. In the 100% bog water test the upper number is survivorship of control hatched larvae and the lower number is survivorship of bog hatched larvae.

[2] The number in parentheses below each survivorship figure is the total sample size (n) for each treatment.

4.6.2. Larval growth. Even if an amphibian larva hatches and can tolerate a bog water medium it must be able to feed and grow through metamorphosis. The environment of a bog water pool is extremely different from that of a fen pool in terms of food resources. Bog plants, especially *Sphagnum* moss which is a dominant component of bog pools, are low in nutrients and rich in secondary plant compounds (Janzen, 1974). Feeding experiments were run using bog tolerant *R. sylvatica* larvae. Bog hatched larvae raised in bog water on bog vegetation for a month exhibited a mean body length of 3.6 mm (standard deviation [SD] = 0.55, range = 2.5-4.3 mm, n = 20) and mean total length of 9.4 mm (SD = 1.26, range = 7.0-11.2 mm, n = 20). Fen hatched larvae raised in fen water for a month exhibited a mean body length of 5.6 mm (SD = 0.60, range = 4.5-7.2 mm, n = 20) and mean total length of 14.4 mm (SD = 1.08, range = 13.0-16.3 mm, n = 20). The differences are highly significant for both head and body length (T-Test, $p<0.001$). The bog larvae because of the bog vegetation diet and/or water grew more slowly than fen larvae; this suggests that the time required for development through metamorphosis would be longer in bog sites with subsequent ecological consequences.

4.6.3. Metamorphosis. The larval growth experiments were of relatively short duration; a long term growth experiment was also initiated. A group of sixty bog hatched *R. sylvatica* larvae were kept in bog water/bog vegetation tanks in an attempt to raise them through metamorphosis. The larvae used were hatched the first week of May, 1979; by 6 June only one remained. This one larva did survive and metamorphosed in late July. The bog froglet was of normal appearance and size (13.5 mm, head-body length). Hence, under laboratory conditions it was possible for a *R. sylvatica* larva to survive through metamorphosis in a simulated bog drain environment but the overall success rate was extremely low.

4.6.4. Larval field surveys. The egg and larvae experiments indicated that *R. sylvatica* was capable of hatching and surviving through metamorphosis in bog water, although bog water is a deleterious medium. Field observations indicate R. sylvatica does lay eggs at bog water sites and hatching in large numbers can occur. Do these larvae survive in the field? The results of larval surveys taken in 1979 at bog and fen sites are shown in Table 14. There were virtually no larvae found in the bog drain in either year. It should be remembered that 1980 was a particularly warm year and a large hatch of R. sylvatica eggs was noted. All larvae found in the bog drain were R. sylvatica. In addition to the systematic sampling of sites bog sites were continually being checked on a casual basis; no larvae were seen. No advanced (> stage 30) larvae were found at bog sites.

Larvae were abundant at fen sites. Larvae of all species breeding in the fens were found. The drop in larval abundance between June and July of 1979 is to be expected due to mortality and dispersal away from hatching sites. Larval abundance in early spring was much greater in 1980 and 1979. In part this was probably due to the time of sampling; in 1980 sites were sampled rather early in the spring compared to 1979. The result was that in 1980 large numbers of R. sylvatica larvae were still in the immediate vicinity of the natal egg masses and were easily sampled in large numbers while in 1979 more had already dispersed. Also in 1980 many fen sites dried up early in the breeding season and those that remained shrunk in volume thus confining larvae to a smaller area.

The evidence from the field survey indicates that larvae do not survive in the bog drain in any substantial numbers; if present they are rare and were not detected by the sampling methods employed.

4.6.5. Emerging young. Another check on larval survivorship is the emergence of recently metamorphosed amphibians from breeding areas. Trapping fences used to check movement and activity of amphibians and reptiles in the study area provided a means to census emerging young at bog and fen sites

	FEN		BOG	
	No. samples, No. sites	No. Larvae/ sample	No. samples, No. sites	No. Larvae/ sample
1979				
June	210, 7	8.2	600, 6	.002(1/600)
July	210, 7	1.0	600, 6	0.0
1980				
May	90, 3	58.0	600, 6	.01(7/600)

Table 14. Census of amphibian larvae from bog and fen sites along Porter Ridge in 1979 and 1980. See section 4.6.5. for discussion.

Species	Number of Young of the Year Captured			
	1979 (July-15 Sept.)		1980 (August)	
	BOG (3 Fences)	FEN (6 Fences)	BOG (3 Fences)	FEN (3 Fences)
R. sylvatica (Wood Frog)	0	115	0	0
B. americanus (American Toad)	0	11	0	0
P. triseriata (Chorus Frog)	0	34	0	0
H. crucifer (N. Spring Peeper)	0	92	0	0
A. laterale (Blue-spotted Salamander)	0	58	0	0
Total Captured	0	310	0	0
$\overline{X}$ No./Fence	0.0	51.7	0.0	0.0

Table 15. Census of the emergence of recently metamorphosed (young of the year) amphibians from bog and fen breeding areas in 1979 and 1980. See section 4.6.5. for discussion.

(Table 15). In 1979 no young of the year were caught at sites adjacent to bog water compared to substantial numbers at fen sites. The difference is significant (G-Test, $p<0.005$).

Unfortunately 1980, the warm year when R. sylvatica hatching was extremely high, was also very dry and the breeding pools associated with both fen and bog sites virtually disappeared apparently destroying most, if not all, larvae at all sites. The fences failed to detect any emerging young of the year at either bog or fen sites that year. Note that trapping effort was less in 1980 than in 1979, but it is doubtful that more trapping effort would have produced different results.

5.0. SUMMARY

The main conclusions of this study are as follows:

1) Bog water (as defined in the peatland water chemistry section) does not affect the fertilization of amphibian eggs in the three species tested.

2) Bog water does deleteriously affect egg development, hatching and larval survivorship of all amphibian species tested. Only R. sylvatica showed any ability to tolerate bog water; it was the only species found capable of significant hatching, prolonged survivorship, and metamorphosis in the laboratory.

3) No evidence of advanced larval survivorship or metamorphosis was found under natural conditions, even for the bog tolerant R. sylvatica.

4) The toxicity of bog water is a complex phenomenon; temperature, pH, size of egg mass cluster (for R. sylvatica), and local variation in bog water quality were all found to be important factors in the manifestation of the toxicity.

5) pH was found to be a particularly important factor in bog water toxicity. Although pH is a necessary component of the toxicity it is not sufficient, by itself, to explain the observed toxic properties of bog water. pH seems to interact in some manner with other toxic factor(s) in bog water to produce the

observed effects. Humic substances and/or heavy metals are possible suspects as other important toxic factors.

6) Whatever the actual identity of these other toxic factors, they can be deleterious at relatively low concentrations given the proper pH environment.

This work is admittedly narrow in scope, involving only the effects of bog water on amphibian reproduction. However, several important points emerge from this work which have implications for other organisms concerning the potential danger of bog water toxicity if peatland development proceeds.

Of particular importance are the dilution series experiments. This work indicates that bog water can be toxic at low concentrations given the proper pH environment. For amphibians this pH is relatively low (pH 4.0-5.0 for the species tested). Such low pH values are relatively rare in the natural environment, hence even if large quantities of bog water were added to a local drainage system amphibians would probably not be directly affected as long as the pH was around 5.0 or greater. For other organisms this might not be the case; bog water might be toxic to many organisms at low concentrations in circumneutral environments more typical of the landscape. Janzen (1974) and Saber and Dunson (1978) cite examples of the toxicity of bog or blackwaters under circumneutral conditions.

There is very little known about the response of aquatic organisms to bog water under various conditions. The available information suggests there might be adverse effects if large quantities of waste bog water are released into local drainages in the wake of peat development operations. The quantity and quality of the waste bog water and the nature of the receiving waters are of obvious importance in this regard. This study dealt with natural bog water; its unusual characteristics were due to a complex interaction of peatland hydrology, topography and vegetation. There will probably be differences between the bog water utilized in this study and the waste bog water resulting from a peat mining operation. The results of this and other studies on natural bog water toxicity strongly suggest that waste bog water should be studied and a variety of organisms tested for toxic reactions when a pilot peat operation is set up and waste bog water is available.

It is also recommended that further toxicological studies be undertaken to pinpoint the mechanism of the toxicity. This study established the importance of acidity in the observed toxicity of natural bog water to amphibian embryos and larvae but did not isolate other factor(s) which low pH apparently interacts with, although humic substances and/or heavy metals are suspect. Other combinations of factors might be involved in the toxicity of peat development waste water to other organisms.

In conjunction with such studies, it would be advisable to monitor the impact of peat development waste water on local aquatic systems when a pilot peat operation is actually initiated. Such a study would, of course, involve before and after monitoring of local drainage systems affected by the peat operation. Changes in water quality and the abundance and diversity of aquatic organisms would be of special interest.

A combination of laboratory studies and field monitoring of the effects of bog water on aquatic organisms in association with a pilot peat operation should allow assessment of the risk of bog water toxicity before major peatland development is initiated. This report plus the reports of bog water toxicity cited in the introduction certainly indicate a thorough toxicological evaluation of the problem is called for.

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