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In situ toxicity tests of fishes in acid waters

DAVID W. JOHNSON, HOWARD A. SIMONIN, JAMES R. COLQUHOUN and FRANK M. FLACK

New York State Department of Environmental Conservation, Bureau of Environmental Protection, Rome Field Station, Rome, New York 13440, and Albany, NY 12233, U.S.A.

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Abstract. Toxicity of waters within the North Branch of the Moose River to various life stages of lake trout (*Salvelinus namaycush*), brook trout (*Salvelinus fontinalis*), creek chub (*Semotilus atromaculatus*), and blacknose dace (*Rhinichthys atratulus*) were examined in situ. Study sites were selected that were expected to range from toxic to favourable water quality. For example, pH varied from 4.25 to 7.17, inorganic monomeric Al ranged from ND (< 0.01 mg/l) to 0.40 mg/l, and Ca, from 0.41 to 4.27 mg/l.

Toxicity tests were conducted at times when the life stages would naturally occur in these waters and were continued until a range of mortality was observed at the various sites. These experiments suggested that the extent of the drainage system that is toxic to fish increases during snowmelt and major runoff events. Summer base flow water quality was generally the least toxic.

Critical life stages were upon hatching and as early feeding fry. In general, young of the year fish were the most tolerant life stage tested. Yearling and adult fish, however, were very sensitive. Blacknose dace were the most sensitive fish of the four species tested, and brook trout were the most tolerant.

Hydrogen ion (H^+) concentration was the most toxic variable in the majority of tests. Inorganic monomeric Al was the most toxic in several, and the combination of H^+ and Al seemed to cause increased toxicity in many instances. A three-variable model employing hours of exposure, H^+ concentration, and inorganic monomeric Al predicted mortality quite well. A simple two-variable model using H^+ and color was nearly as good (R^2 from 0.49 to 0.94).

Documented differences in toxicity among sites and species agreed with observed patterns of fish distribution. These in situ results indicated that laboratory estimates of safe levels of pH and concentrations of Al can result in mortality of fishes in surface waters subject to acidification.

Introduction

Because native fish populations of dilute surface waters are endangered in regions impacted by acid precipitation (Oden and Ahl, 1970; Beamish and Harvey, 1972; Schofield, 1976; Baker, 1984), it is important to evaluate the sensitivity of specific fish species and life stages to water quality changes. Toxicity is believed to result primarily from low pH and increased Al concentrations and can be mitigated by Ca, Mg, and dissolved organic carbon (DOC).

Low pH has been frequently reported to limit fish distribution. Cooper and Wagner (1973) concluded that pH values less than 5.0 may limit distribution of brook trout (*Salvelinus fontinalis*) and that waters below 4.5 pH contained few fish species. Oden and Ahl (1970) reported that Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) disappeared from a number of rivers in Scandinavia and attributed this to acid rain. Beggs et al. (1985) similarly found that lake trout (*Salvelinus namaycush*) populations were reduced in Canadian lakes of pH $\leqslant$ 5.3.

Aluminum has been implicated as the primary toxicant in low-color, acidic surface waters (Baker, 1981; Baker and Schofield, 1982). Driscoll et al. (1980) reported that in natural waters Al is found in different chemical forms (and relative toxicities) depending on pH, organic content of the water, and the availability of other complexing agents.

Calcium, Mg, and DOC mitigate the toxicity of acidic waters. Schofield and Trojnar (1980) found that survival of stocked brook trout in 53 Adirondack lakes was higher in those with higher Ca, Mg, and lower total Al concentrations. Laboratory studies (Hunn et al., 1985; Ingersoll et al., 1985) showed that addition of Ca reduced the toxic effects of low pH and high Al concentrations on developing brook trout eggs and fry. Brown (1981) showed that Ca increased the survival of year-old brown trout exposed to acid waters.

Variation in the tolerance of fish species and life stages to low pH and high Al water may result in differential survival. Haines (1981) and Baker (1984) presented extensive reviews of the sensitivity of different fish species to acidic waters. Grande et al. (1978) reported that brook trout were most tolerant of low pH water in Norway, followed by brown trout, Atlantic salmon, and rainbow trout. They found that genetic variability, age of fish, physical condition, and water quality may influence the relative tolerance of fish species to acidic water. Johnson (1975) found significant differences in tolerance of low pH in various brook trout strains and life stages. Baker (1981) reported that Al toxicity in relation to pH varied with early life stages of brook trout and white suckers (*Catostomus commersoni*). Colquhoun et al. (1983) tested a number of fish species at fingerling life stage in situ in Adirondack waters and found significant differences in species tolerance of low pH and high Al concentrations.

Because of variation in surficial geology (Newton et al., 1987) and hydrologic influences (Peters et al. 1987), acidity and Al gradients exist in the North Branch of the Moose River (NBMR), (Driscoll et al., 1987). We found that stream and lake sites ranged in water quality from toxic to quite favourable for fish survival. The NBMR, therefore, provided a good study area to conduct in situ toxicity tests. These tests were designed with the following objectives.

— to test interspecific differences in survival of fish exposed to acidic waters;

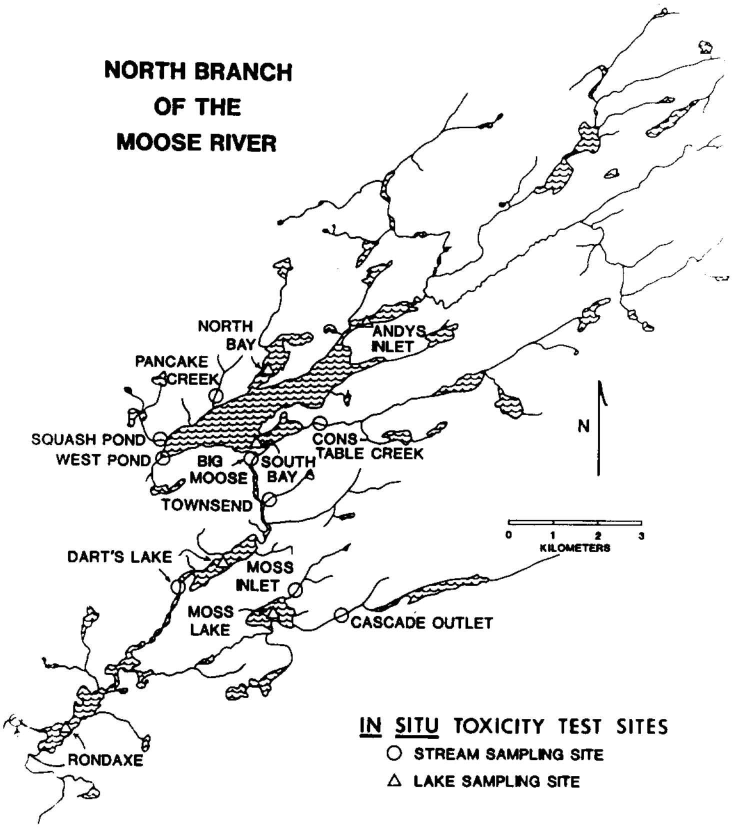

Figure 1. Location of stream and lake sites for *in situ* toxicity tests.

— to test differences in survival of the various life stages of fish;
— to determine the relative response of fish to a range of pH and Al; and
— to correlate the results of these tests to the fish distribution within the watershed.

Materials and methods

Test area

In situ toxicity tests were conducted within the NBMR at 10 stream sites and 6 lake sites (Figure 1, Table 1). Sites were selected based on water chemistry data available for the NBMR (Driscoll, unpublished data). For a description of the watershed see Driscoll et al. (1987); Peters et al. (1987); and Newton et al. (1987).

Test animals

Lake trout, brook trout, creek chub (*Semotilus atromaculatus*), and blacknose dace (*Rhinichthys atratulus*) were selected as experimental animals for

Table 1. Design of experiments in which four fish species were exposed *in situ* to a variety of water qualities

Test	(mm) Size Range	# Test sites	Test chamber	Total Fish/Site (# replications per site)	Starting & ending dates	Range Eq pH	IMAL Range (mg/l)	Ca Range (mg/l)	Mg Range (mg/l)	Source of Fish
Lake Trout (*S.namaycush*)										
egg to feeding fry	–	6	Plastic trays in 4-l containers	100(5)	10/18/84–05/02/85	4.75–7.08	0.00–0.25	1.19–3.38	0.24–0.71	Raquette Lake
sac fry	–	6	4-l containers	100(5)	01/17/84–04/30/84	4.43–6.63	0.01–0.27	1.60–3.44	0.26–0.59	Raised at RFS
feeding fry	–	6	4-l containers	100(4)	05/02/84–05/16/84	4.75–6.64	0.00–0.18	1.49–2.75	0.21–0.47	Raised at RFS
young of the year	40–80	6	4-l containers	30(3)	10/22/84–11/27/84	5.05–7.8	0.00–0.25	1.19–3.38	0.24–0.71	Raised at RFS
yearling	100–160	6	Floating nets	30(3)	10/22/84–11/27/84	5.05–7.08	0.00–0.25	1.19–3.38	0.24–0.71	Raised at RFS
Brook Trout (*S.fontinalis*)										
egg to sac fry	–	10	Plastic trays in 4-l containers	100(5)	10/26/84–05/03/85	4.25–7.08	0.00–0.40	0.41–3.52	0.15–0.68	Lost Pond
sac fry	–	10	4-l containers	100(4)	04/02/84–05/01/84	4.32–5.90	0.00–0.40	1.13–2.96	0.18–0.41	Raised at RFS
feeding fry	–	10	4-l containers	100(4)	05/02/84–05/16/84	4.53–6.36	0.01–0.25	1.08–2.27	0.16–0.44	Raised at RFS
young of the year	40–100	10	4-l containers	30(3)	09/28/84–12/17/84	4.37–7.17	0.00–0.40	0.41–4.27	0.10–0.76	Raised at RFS
yearling	161–270	7	Plastic minnow traps	30(3)	10/19/84–12/17/84	4.37–7.17	0.00–0.34	0.41–4.20	0.14–0.76	Rome Strain
Creek Chub (*S.atromaculatus*)										
egg to sac fry	–	10	Plexiglass chambers in 4-l containers	100(5)	05/25/84–06/08/84	4.39–6.58	001–0.26	0.95–3.05	0.14–0.54	Wild
sac fry to feeding fry	–	10	1-l containers	100(4)	06/11/84–06/25/84	5.02–6.86	0.00–0.17	1.29–3.26	0.23–0.62	Wild
young of the year	21–53	10	4-l containers	30(3)	09/28/84–10/26/84	4.50–7.17	0.00–0.20	0.75–4.27	0.10–0.76	Wild
adult	41–140	7	Plastic minnow traps	30(3)	10/19/84–12/17/84	4.37–7.17	0.00–0.34	0.41–4.20	0.14–0.76	Wild
Blacknose Dace (*R.atratulus*)										
egg to feeding fry	–	7	Plexiglass chambers in 4-l containers	40(2)	06/12/84–06/26/84	5.04–6.86	0.00–0.18	1.71–3.26	0.25–0.61	Wild
young of the year	21–45	10	4-l containers	30(2)	09/28/84–10/23/84	4.50–7.17	0.00–0.20	0.92–4.27	0.10–0.76	Wild
adult	40–87	10	4-l containers	30(3)	07/13/84–08/10/84	4.39–7.13	0.00–0.23	0.65–4.32	0.09–0.72	Wild

these toxicity tests. These species are native to the NBMR watershed but are not as widely distributed as during the early 1900's (Schofield and Driscoll, 1987). They include two important game species and two important forage species.

To obtain egg and early life stages of all species, ripe adult males and females were collected. Eggs were dry-fertilized in a basin, and mixed with a gentle rocking motion. Eggs were then pooled, counted into test lots, and hardened in test site water for $1\frac{1}{2}$ hours, following which they were transported to the stream or lake test site or back to the Rome Field Station (RFS) for rearing and subsequent testing at a later life stage.

Lake trout adults (avg. length = 600+ mm) were trap netted at Raquette Lake (pH ~6.5, alk. ~40 μeq/l). Yearling lake trout were from the same Raquette Lake strain and were reared at the Rome Field Station.

Brook trout adults (average length = 400+ mm) were trap netted at Lost Pond (pH ~6.4, alk. ~60 μeq/l) in October 1983 and 1984. The Lost Pond fish used in these tests were F_2 Temiscamie (Canadian wild) × domestic (New York State) strain. Yearling brook trout were Rome Lab, furunculosis resistant strain, which were used because they were available although this may have introduced variability in acid sensitivity due to strain differences (Johnson, 1975).

An additional brook trout toxicity test was conducted during April and May 1985 to compare seasonal effects and the effects of fish size on sensitivity to acid waters. Two sizes of young of the year and one group of yearling fish were used in these tests. These brook trout were all F_2 Temiscamie × domestic strain.

Native creek chub were collected by seine and electrofishing within the Adirondack Region during May 1984. Mortality of fish due to electrofishing was negligible and fish were held for at least a week following collection to eliminate variability due to delayed handling mortality. A number of ripe males and females were injected with carp pituitary extract (Ball and Bacon, 1954) to induce spawning. Creek chub reached spawning condition when the daytime high water temperature reached ~14 °C. Young of the year (yoy) and adult creek chub were collected in the same manner for direct use in tests.

Native blacknose dace were also collected by seine and electrofishing within the Adirondack Region during May and early June. Blacknose dace yoy and adults were collected on a later date for direct use in tests. Blacknose dace adults neared spawning readiness at ~20 °C daytime high water temperature, as Raney (1940) recorded. A sudden and prolonged spell of hot weather in late spring, 1984, seemed to disrupt normal spawning. Prolonged seining and electrofishing throughout the Adirondack Region yielded several ripe females with good eggs, the remainder appeared to be overripe and to have begun reabsorbing their eggs.

All test species and life stages were acclimated for one week at the control sites (Cascade Lake Outlet for stream tests, Moss Lake for lake experiments). Mortality during this acclimation for all tests was less than 1%. All tests were conducted without feeding because the feed might alter water quality and benefit test fish at favorable sites. Test durations were selected so that sufficient body reserves could provide adequate survival, and each test was set up to include a control (pH $\geqslant 6.0$) site within the NBMR. Experiments were terminated if mortality at control sites reached 10%.

Test containers

Because of size differences of the various species and life stages, a variety of test containers was used. Brook trout and lake trout eggs were placed individually (to prevent fungusing) in 20-chamber plastic trays covered by fiberglass screen (1.4-mm mesh). Blacknose dace and creek chub egg to sac fry were tested in 20-chamber plexiglass trays covered with 0.5-mm mesh Nitex® (Flack et al., 1987) modified after Hulsman et al. (1983). These trays were then placed in 4-liter polyethylene containers with two 7×12-cm openings screened with fiberglass (1.4-mm mesh). These 4-liter containers were also used as the test chamber for many other tests (Table 1).

A similarly constructed 1-liter bottle with 0.5-mm mesh Nitex® screening was used for the creek chub sac fry to feeding fry test because of their extremely small size (< 10 mm). Both types of containers were put into cages constructed of wood and wire mesh, both painted with black polyurethane paint. The cages held up to eight 4-liter container together during exposure in the streams.

The larger brook trout yearlings and creek chub adults were tested in plastic mesh minnow traps that had the large inlet (trap) holes plugged. All lake trout except yearlings were tested in the 4-liter containers. The yearling lake trout were tested in nylon floating nets. These containers were suspended at approximately 3 m depth in water with suitable temperature and oxygen.

Two to five replicate test containers were used at each exposure site. This was done to assess the variability in response resulting from handling, differences in water current within the containers, and natural genetic variability.

The survival of the test animals was determined by periodic checks at intervals which varied from daily to weekly, depending on the expected sensitivity of the fish being tested. Death was recorded for white or fungus-covered eggs for the egg tests, cessation of movement and heartbeat for sac fry, and no gill movement and no response to stimuli for 1 min for swim-up through adult life stages. Loss of equilibrium and other observed effects were also recorded. Because of the small size of the cyprinid sac fry, mortality was established in some cases by examination for heartbeat with a $10\times$ hand lens.

Water quality analysis

Physical and chemical analyses of water quality were generally conducted at weekly intervals during the conduct of each test. These analyses were conducted in accordance with Standard Methods (APHA et al., 1980), except as required for critical measurements by using techniques developed for dilute waters. Dissolved oxygen (D.O.), minimum–maximum temperatures, and initial pH (field) were recorded at stream sites; vertical temperature and oxygen profiles were recorded for the lake sites. Water samples from lake sites were collected at the depth being used for test exposures. All dissolved oxygen concentrations and temperature proved satisfactory for the four species.

Water samples were collected, stored at 4 °C, and analyzed for pH, alkalinity, color, and conductivity within one week. Past experience in our laboratory has shown little change in these parameters over this time period. Initial and air equilibrium pH were measured by using a Corning pH meter with dual electrodes. Alkalinity was determined by a modification of the Gran's plot titration (Schofield, 1978). Color was measured by using a Hach color comparator, and conductivity was determined by using a Beckman conductivity bridge.

Samples were also prepared within 24 hours for analysis of dissolved organic carbon (DOC), total monomeric Al (TMAL), organic monomeric Al (OMAL), and the cations Ca, Mg, Na, and K. Inorganic monomeric Al (IMAL), the toxic form (Baker, 1981), was determined as the difference between TMAL and OMAL. Samples for DOC analysis were filtered and ampulated for later determination by gas chromotography. Samples for monomeric Al were extracted by 8–hydroxyquinoline into methyl isobutyl ketone and later analyzed by atomic absorption spectrophotometry. Driscoll et al. (1986) discuss the chemical methodology in greater detail. The prepared samples were sent to Department of Civil Engineering at Syracuse University for actual analysis. Monthly water samples were collected by Syracuse University at our study sites for a separate project (Driscoll and Newton, 1985; Driscoll et al., 1987), and we were also able to utilize these data.

Data handling and statistical analysis

All toxicity tests data were recorded on field forms or laboratory report forms and tabulated. The Statistical Analysis System (SAS) was used for data storage and manipulation. Since mortality over time was strongly sigmoidal, cumulative mortality percentages were converted to probits (Fisher and Yates, 1957; Mather, 1965). Exploratory multiple regression analysis of the data was conducted using the following variables: hours, equilibrium pH, minimum pH, weighted mean pH, alkalinity, color, TMAL, IMAL, DOC, Ca, Mg, and NO_3.

In order to compare differences in species and life stage sensitivity to the test conditions, predictive equations using the variable of primary concern were developed. Water chemistry and fish mortality data from each of the individual toxicity tests were used to develop the multiple regression equations. The three variables considered most important for fish survival and used for the predictive equations were pH (expressed as μeq/l of H^+), IMAL (mg/l) and length of exposure (hours) (Baker, 1981, 1984; Driscoll et al., 1980). The equation used was in the form:

$$\text{PROBIT} = a + b(\text{Hours}) + c(H^+) + d(\text{IMAL})$$

Relative contributions to toxicity of H^+ and IMAL for each test were determined by examining partial regression leverage plots of the dependent variable (probit) and the selected regressor after they had been made orthogonal to the other regressors in the equation. Partial regression coefficients of H^+ and IMAL were also compared to evaluate their relative contributions to toxicity.

The regression equations which were developed were used to produce predicted mortality at a series of values for the independent variables (hours, H^+, and IMAL). These predicted values with the associated 95% confidence intervals were then used to compare interspecific and inter-life stage differences in fish sensitivity to these conditions.

A second predictive equation was also developed and tested for its value in predicting the observed experimental mortality. This equation used a (H^+) (Color) term as a replacement for the IMAL term in the previous equation. The (H^+) (Color) term was expected to be negative and to therefore reduce the predicted probit. This was based on the assumption that DOC can bind with and reduce the toxicity of IMAL (Driscoll et al., 1980) and the observation that colored streams are often less toxic to fish than clear water streams (Colquhoun et al., 1983). Color is an easily measured surrogate for measurements of DOC. Since the beneficial impact of DOC would theoretically be active only under low pH (high H^+) conditions, the product of H^+ and color would be greatest under these conditions and would therefore reduce the predicted probit by the largest amount. Time (hours) was not included as a separate variable in this equation since predictions were only made at specific exposure lengths. The resulting predictive equation was:

$$\text{PROBIT} = a + b(H^+) + c(H^+)(\text{Color})$$

Results

Seventeen in situ toxicity tests were conducted with the various life stages of the four test species (Table 1). The timing of the tests depended upon when the life stages naturally occurred in the Adirondack Region. Results

Table 2. Lake trout (*Salvelinus namaycush*) survival at various life stages and range of pH, IMAL, Ca, and DOC encountered at 6 lake sites

Site	Egg to sac fry (4704 hours)					Sac fry (720 hours)					Feeding fry (336 hours)					Young of the year (552 hours)					Yearling (552 hours)				
	% Survival (range of reps)	pH (6)*	IMAL (2)	Ca (2)	DOC (2)	% Survival (range of reps)	pH (2)	IMAL (1)	Ca (1)	DOC (1)	% Survival (range of reps)	pH (1)	IMAL (1)	Ca (1)	DOC (1)	% Survival (range of reps)	pH (3)	IMAL (2)	Ca (2)	DOC (2)	% Survival (range of reps)	pH (3)	IMAL (2)	Ca (2)	DOC (2)
Big Moose Lake Andy's Inlet	0	4.75–5.60	0.17–0.25	1.86–2.00	5.9–6.9	0	4.43–5.30	0.14	2.13	5.1	0	4.75	0.18	1.60	4.7	100	5.05–5.60	0.17–0.25	1.86–2.00	5.9–6.9	13 (0–20)	5.05–5.60	0.17–0.25	1.86–2.00	5.9–6.9
Big Moose Lake North Bay	0	4.94–5.94	0.01	1.45–2.38	2.9–3.2	86 (75–95)	5.12–5.14	0.13	2.01	4.0	12 (0–24)	5.06	0.18	1.49	3.1	97 (90–100)	5.72–5.94	0.01	1.45–2.38	2.9–3.2	33 (20–50)	5.72–5.94	0.01	1.45–2.38	2.9–3.2
Big Moose Lake South Bay	0	4.90–5.98	0.06–0.12	1.19–1.93	2.9–3.5	69 (32–90)	4.97–4.98	0.15	2.00	3.9	33 (24–40)	5.00	0.17	1.74	3.6	97 (90–100)	5.30–5.98	0.06–0.12	1.19–1.93	2.9–3.5	3 (0–10)	5.30–5.98	0.06–0.12	1.19–1.93	2.9–3.5
Dart Lake	0	4.97–5.70	0.05–0.09	1.23–2.04	3.0–3.3	63 (50–79)	4.82–4.89	0.18	1.97	5.2	70 (56–84)	5.07	0.16	1.82	3.5	97 (90–100)	5.52–5.70	0.05–0.09	1.23–2.04	3.0–3.3	20 (10–40)	5.52–5.70	0.05–0.09	1.23–2.04	3.0–3.3
Moss Lake	44 (10–80)	6.65–7.08	0.00	2.06–3.38	3.5–3.6	95 (90–100)	6.34–6.57	0.02	3.44	3.3	90 (84–92)	6.64	0.00	2.75	2.8	100	6.76–7.08	0.00	2.06–3.38	3.5–3.6	93 (90–100)	6.76–7.08	0.00	2.06–3.38	3.5–3.6
Rondaxe Lake	33 (10–60)	5.34–6.76	0.01	1.45–2.21	2.7–3.4	90 (84–100)	5.91–6.05	0.07	2.63	4.2	100	5.54	0.08	2.01	3.0	93 (90–100)	6.46–6.76	0.01	1.45–2.21	2.7–3.4	73 (60–80)	6.46–6.76	0.01	1.45–2.21	2.7–3.4

* Number of water chemistry measurements during the time period tested.

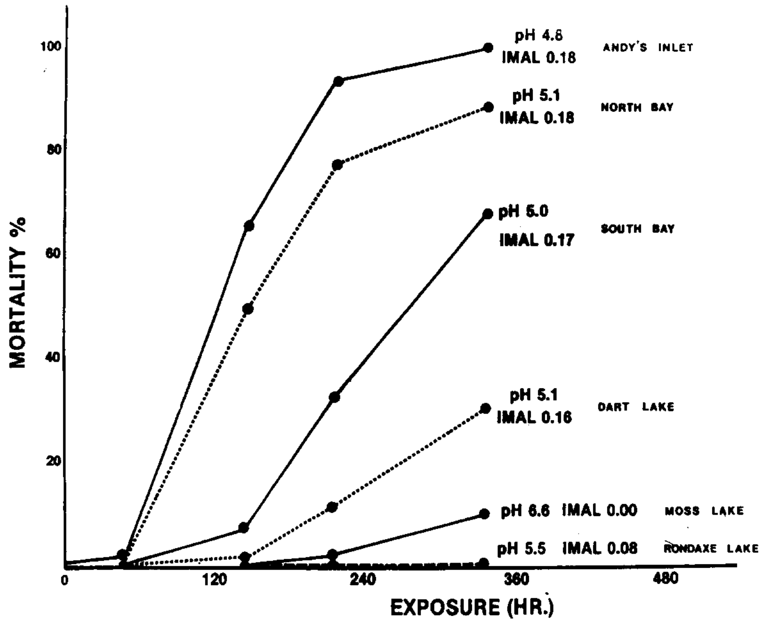

Figure 2. Observed mortality during lake trout feeding fry toxicity test conducted 5/02/84 to 5/16/84 at six different exposure sites. IMAL units are mg/l.

from the replicate test containers indicated that there were no significant differences between replicates. Survival data are therefore presented as combined replicates.

Lake trout tests

The lake trout egg to sac fry test was conducted from October 1984 until May 1985. The lowest pH values occurred during late winter and spring snowmelt, with May pH levels ranging from 4.75 to 6.65 (Table 2). IMAL levels over the period of the test ranged from ND (<0.01 mg/l) to 0.25 mg/l. Driscoll et al., (1987) discuss the water chemistry of these waters in greater detail. Survival of lake trout eggs to the sac fry stage was low overall, but 33% and 44% survived to reach the swim-up stage in Rondaxe and Moss Lakes, respectively. Many lake trout eggs that reached the hatching stage died at hatch (65.4%).

The lake trout sac fry test (720 h) resulted in a range in survival from 0% to 95% (Table 2). Water chemistry over this period similarly ranged from pH 4.43 at the most toxic site to pH 6.57 at the control site.

Feeding fry proved to be a very sensitive life stage, ranging from 0% to 33% survival for the three Big Moose Lake sites and 70% in Dart Lake after 336 hours. Figure 2 is a graphical example of observed mortality over time for the six lake sites. Water in Andy's Inlet (pH 4.75, IMAL

Table 3. Brook trout (*Salvelinus fontinalis*) survival at various life stages and range of pH, IMAL, Ca, and DOC encountered at 10 stream sites

Site	Egg to sac fry (2856 hours)					Sac fry (240 hours)					Feeding fry (336 hours)					Young of the year (1920 hours)					Yearling (672 hours)				
	% Survival (range of reps)	pH (14)*	IMAL (5)	Ca (5)	DOC (5)	% Survival (range of reps)	pH (3)	IMAL (2)	Ca (2)	DOC (2)	% Survival (range of reps)	pH (1)	IMAL (1)	Ca (1)	DOC (1)	% Survival (range of reps)	pH (13)	IMAL (5)	Ca (5)	DOC (5)	% Survival (range of reps)	pH (7)	IMAL (3)	Ca (3)	DOC (3)
Squash Pond Outlet	30 (20–45)	4.25–4.61	0.16–0.37	0.41–1.37	7.7–9.6	0	4.32–4.40	0.19–0.21	1.13–1.40	6.0–6.9	0	4.53	0.18	1.08	6.4	0	4.37–4.68	0.11–0.34	0.41–1.30	7.3–9.0	0	4.44–4.68	0.11–0.18	0.41–1.03	8.0–9.0
Pancake Hall	Flood Damage					1 (0–4)	4.68–4.75	0.27–0.35	1.61–1.64	3.1–3.8	0	4.87	0.25	1.54	3.8	77 (60–90)	4.85–6.16	0.03–0.26	0.73–1.93	4.0–8.4	Not tested at this site				
Constable Creek	48 (40–55)	4.57–5.35	0.04–0.25	1.15–2.29	4.1–5.7	22 (12–40)	4.55–4.63	0.21–0.29	1.77–1.85	4.0–4.9	0	4.76	0.22	1.68	4.5	70 (50–90)	4.65–5.42	0.04–0.24	1.15–2.12	4.1–4.9	80 (50–100)	4.82–5.42	0.04–0.05	1.15–2.06	4.4–4.8
West Pond Outlet	57 (50–70)	4.57–5.36	0.00–0.06	0.87–2.01	5.3–8.3	71 (60–80)	4.76–4.85	0.00–0.01	1.53–2.96	5.3–6.2	71 (64–84)	4.94	0.03	1.54	6.0	77 (70–80)	4.64–5.57	0.00–0.06	0.87–2.06	5.7–8.2	67 (40–100)	4.64–5.57	0.00–0.05	0.87–2.06	5.9–8.3
Big Moose Lake Outlet	Flood Damage					44 (24–6-)	4.72–4.90–	0.12–0.15	1.88–1.93	3.7–5.1	5 (0–16)	5.00	0.15	1.79	3.7	57 (50–100)	5.20–5.46	0.01–0.16	1.13–2.22	2.6–3.4	90 (80–100)	5.26–5.46	0.01–0.16	1.13–2.02	2.8–3.4
Dart Lake Outlet	47 (25–70)	4.73–5.64	0.00–0.27	1.25–1.93	2.8–5.7	31 (20–36)	4.66–4.88	0.13–0.22	1.93–1.96	4.1–4.7	13 (4–16)	5.04	0.16	1.79	3.5	87 (70–90)	5.25–5.64	0.00–0.27	1.25–2.34	2.6–3.2	77 (30–100)	5.48–5.64	0.00–0.27	1.25–2.28	2.6–3.2
Townsend Pond Outlet	64 (50–80)	4.68–6.46	0.01–0.40	1.43–1.90	3.1–3.5	36 (12–56)	4.73–4.82	0.31–0.40	1.56–1.64	2.9–3.4	3 (0–4)	5.03	0.21	1.71	2.3	90	4.90–6.52	0.01–0.40	1.43–2.74	3.1–3.5	Not tested at this site				
Moss Lake Inlet	50 (35–65)	5.10–6.90	0.09–0.14	1.88–2.39	3.1–3.3	75 (64–84)	5.07–5.33	0.05–0.16	2.20–2.23	2.9–3.2	30 (12–40)	5.66	0.06	2.15	2.7	100	5.27–6.91	0.00–0.14	1.88–3.00	3.1–3.8	67 (50–80)	5.56 6.91	0.09	1.88	3.3
Cascade Lake Outlet	82	5.77–7.08	0.00–0.05	2.96–3.52	3.2–4.6	100	5.51–5.90	0.01–0.03	2.47–2.58	3.1–4.1	82 (72–92)	6.36	0.01	2.27	2.9	97 (90–100)	6.34–7.17	0.00–0.05	2.96–4.27	3.2–5.0	100	6.44–7.17	0.00–0.01	3.25–4.19	3.5–5.0
Rondaxe Lake Outlet	57 (30–80)	5.20–6.81	0.00–0.06	2.09–2.63	2.6–5.2	100	5.23–5.50	0.07–0.18	2.15–2.18	3.5–4.0	99 (96–100)	5.63	0.09	2.06	3.6	80 (50–100)	6.02–6.81	0.00–0.04	2.09–2.94	2.5–3.7	Not tested at this site				

* Number of water chemistry measurements during the time period tested.

0.18 mg/l) proved to be the most toxic, while all fish survived in Rondaxe Lake (pH 5.54, IMAL 0.08 mg/l). Three sites (North Bay, South Bay, and Dart Lake) had similar water chemistry with survival ranging from 12% to 70%. This difference could have been due to undetected variability in water chemistry among the three sites.

Young of the year lake trout were relatively tolerant of the test conditions as compared to other life stages. This test was conducted during the fall of 1984 when pH levels ranged from 5.05 to 7.08 and IMAL from ND (< 0.01 mg/l) to 0.17 mg/l. Survival was good at all six sites over the 552 hour test (Table 2).

The yearling lake trout test was run concurrently with the young of the year test and demonstrated a greater sensitivity of the yearling fish as compared to the young of the year. Percent survival ranged from 3% to 33% at all Big Moose sites and Dart Lake, where pH values were less than 6.0. Survival was 93% in Moss Lake (pH 6.76–7.08).

Brook trout tests

A summary of the brook trout toxicity test results at the ten stream sites is presented in Table 3. The egg-to-sac fry test was started in October 1984 when water quality at most of the sites was favorable (all except Squash Pond Outlet had pH values over 5.0). Stream pH levels gradually fell throughout the winter, with lowest pH values occurring during high stream flow/snowmelt episodes. A storm in late December resulted in 14 cm of rainfall within a 30-hour period and melted most of the 60-cm snowpack. This event had a great impact on the stream sites, but relatively little effect on the lake sites. Test containers adequately protected eggs from the flood damage at eight of the ten sites. Survival of brook trout egg-to-sac fry ranged from 30% to 82% in the stream sites after 2,856 hours of exposure, which included the flood conditions of late December (Table 3). Egg development proceeded at slightly different rates at each stream site because of small differences in water temperature. Both toxic and favourable water quality sites were distributed throughout the range in temperatures. As in the lake trout tests, many brook trout eggs (70.4% overall) that reached the hatching stage died at hatch. After 4,500 hours of exposure, survival of sac fry was observed at only Rondaxe Lake Outlet (28% survival overall) and Cascade Lake Outlet (38% survival).

The brook trout sac fry tests lasted 240 hours and resulted in a range of survival from 0% to 100%. Squash Pond Outlet (pH 4.32–4.40) proved to be the most toxic site while survival was excellent in Cascade Lake Outlet (pH 5.51–5.90) and Rondaxe Lake Outlet (pH 5.23–5.50) (Table 3).

As with the lake trout, the brook trout feeding fry were relatively sensitive to toxic test conditions. Survial rates ranged from 0% to 99% at the end of the 336-hour test. The pH levels at all sites were higher for the feeding fry test than for the sac fry test, but still proved to be toxic (Table 3).

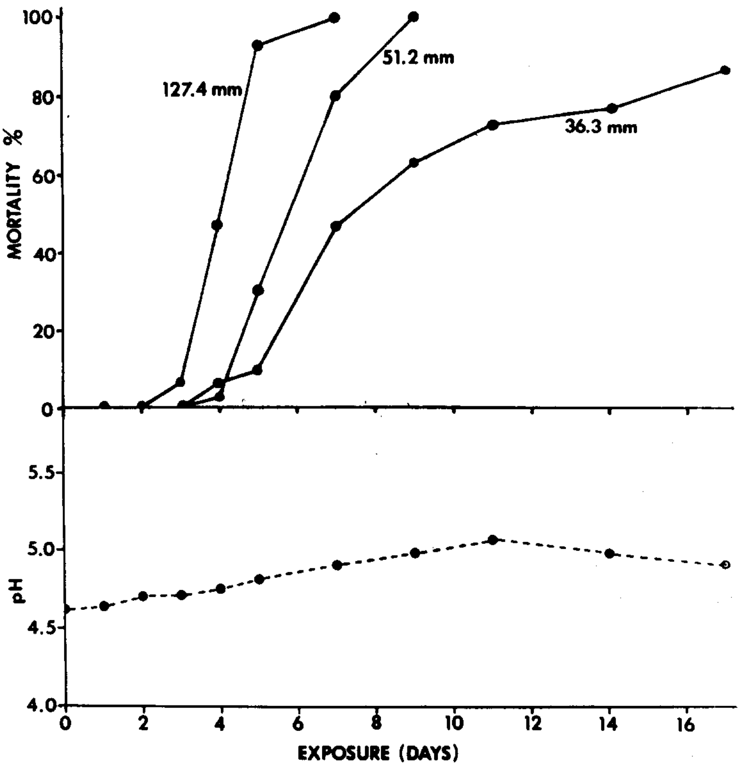

Figure 3. Observed mortality of three different sizes of brook trout tested in Pancake Hall Creek from 4/22/85 to 5/09/85. Lengths are means of 30 fish.

Young of the year brook trout proved highly tolerant of the test conditions with the exception of Squash Pond Outlet (5% survival) and Big Moose Outlet (57% survival). These toxicity tests were started in September 1984 when stream water pH was generally above 5.40. Conditions became more toxic as the tests progressed and stream flow increased. Pancake Hall Creek, for example, had a pH change from 6.10 early in the test to 4.85 at the end, and still had a 77% survival rate (Table 3).

Survival of yearling brook trout exposed at seven sites for 672 hours during the fall of 1984 ranged from 0% in Squash Pond Outlet (pH 4.44–4.68) to 100% in Cascade Lake Outlet (pH 6.44–7.17). As with the young of the year test which was conducted at the same time, the yearling brook trout at most sites had relatively high survival (Table 3). This was again the result of higher pH water conditions early in the test which gradually became more toxic. The pH in West Pond Outlet was 5.46 at the start of the test and 4.64 after 672 hours.

The additional toxicity test conducted in April and May 1985 showed that seasonal differences and fish size differences are important factors in determining sensitivity to acid waters. This test was conducted during spring snowmelt and resulted in higher young of the year mortality than the previous 1984 test. Mortality of yoy brook trout exposed in Pancake Hall Creek (pH 4.61–5.07) was 88% after 336 hours while mortality of fish in Cascade Lake Outlet (pH 6.09–6.78) was only 3%. Yearling brook trout were even more sensitive with 90% mortality at Pancake Hall Creek in only 120 hours. Figure 3 shows the mortality curves for the three sizes of brook trout which were tested concurrently. Small young of the year (mean size = 36.3 mm) were found to be more tolerant than the larger young of the year (mean size = 51.2 mm). Similarly, the yearling brook trout (mean size 127.4 mm) were the least tolerant of the three groups (Figure 3).

Creek chub tests

Early life stages of creek chub developed from fertilized egg to feeding fry stage in approximately 400 hours, compared to approximately 4,700 hours for lake and brook trout because of species and water temperature differences. For this reason the early life stages were tested as an egg-to-sac fry test and a sac fry-to-feeding fry test. The egg-to-sac fry stage was very sensitive to the test conditions with survival greater than 1% occurring only in Moss Lake Inlet (30%), Rondaxe Lake Outlet (41%), and Cascade Lake Outlet (66%). Sites with less than 1% survival generally had pH levels of 5.0 or below whereas the only site with greater than 50% survival (Cascade Lake Outlet) had pH levels above 6.00 (Table 4).

Sac fry-to-feeding fry were much more sensitive than the earlier life stage. This was in spite of the fact that there were higher pH levels at all of the test sites than for the egg-to-sac fry test (Table 4). After only 96 hours, survival at half of the sites was 7% or less. Only in Townsend Pond Outlet, Moss Lake Inlet, Cascade Lake Outlet, and Rondaxe Lake Outlet was there high survival (61–91%) after 336 hours.

Young of the year creek chub were tested during fall baseflow water quality conditions characterized by more moderate pH (generally above 5.40) and IMAL concentrations (less than 0.05-mg/l). This resulted in good survival at all sites except Squash Pond Outlet (0% survival, pH 4.50–4.68, IMAL 0.11–0.20 mg/l). There was intermediate survival (63%) at Constable Creek after the 672-hour test and marginal water quality (pH 5.03–5.42, IMAL 0.04 mg/l). Young of the year creek chub were tested concurrently with brook trout and blacknose dace yoy and exhibited intermediate tolerance to the test conditions.

Adult creek chub were tested in the fall of 1984, along with yearling brook trout, and were slightly more tolerant than the trout. There was greater survival of creek chub than of brook trout at five of the seven test

Table 4. Creek chub (*Semotilus atromaculatus*) survival at various life stages and range of pH, IMAL, Ca, and DOC encountered at 10 stream sites

Site	Egg to sac fry (336 hours)					Sac fry to feeding fry (96 hours)					Young of the year (672 hours)					Adult (672 hours)				
	% Survival (range of of reps)	pH (3)*	IMAL (1)	Ca (1)	DOC (1)	% Survival (range of of reps)	pH (1)	IMAL (1)	Ca (1)	DOC (1)	% Survival (range of of reps)	pH (5)	IMAL (2)	Ca (2)	DOC (2)	% Survival (range of of reps)	pH (7)	IMAL (3)	Ca (3)	DOC (3)
Squash Pond Outlet	0	4.39–4.45	0.20	0.95	7.3	3 (0–4)	5.87	0.13	2.49	4.1	0	4.50–4.68	0.11–0.20	1.03–1.21	7.3–8.8	0	4.44–4.68	0.11–0.18	0.41–1.03	8.0–9.0
Pancake Hall	10 (5–15)	4.71–4.98	0.24	1.37	5.0	7 (0–20)	5.29	0.03	1.60	5.7	100	5.74–6.10	0.03	1.77	8.4	Not tested at this site				
Constable Creek	0	4.63–4.94	0.19	1.59	5.1	0	5.29	0.10	1.99	4.1	63 (30–100)	5.03–5.42	0.04	2.06–2.12	4.8–4.9	87 (70–100)	4.82–5.42	0.04–0.05	1.15–2.06	4.4–4.8
West Pond Outlet	1 (0–5)	4.81–5.09	0.05	1.79	9.7	43 (16–92)	5.25	0.16	1.72	8.2	90 (70–100)	5.04–5.57	0.01–0.02	1.96–2.06	5.9–6.1	73 (60–90)	4.64–5.57	0.00–0.05	0.87–2.06	5.9–8.3
Big Moose Lake Outlet	1 (0–5)	4.95–4.97	0.16	1.70	4.1	0	5.05	0.17	1.91	3.1	87 (80–100)	5.24–5.46	0.04	2.02–2.22	2.6–2.8	100	5.26–5.46	0.01–0.16	1.13–2.02	2.8–3.4
Dart Lake Outlet	0	5.02–5.05	0.13	1.81	3.4	0	5.11	0.14	1.99	2.9	100	5.40–5.56	0.03	2.06–2.12	4.8–4.9	97 (90–100)	5.48–5.64	0.00–0.27	1.25–2.28	2.6–3.2
Townsend Pond Outlet	1 (0–5)	4.77–5.70	0.26	1.48	3.9	98 (96–100)	6.17	0.03	2.45	2.1	100	6.20–6.52	0.01	2.74	3.5	Not tested at this site				
Moss Lake Inlet	30 (10–40)	5.35–6.19	0.09	2.00	3.7	94 (92–96)	6.54	0.02	2.45	3.0	100	6.32–6.91	0.00	3.00	3.8	100	5.56 6.91	0.09	1.88	3.3
Cascade Lake Outlet	66 (50–85)	6.08–6.52	0.12	2.62	6.7	95 (92–96)	6.86	0.00	3.26	2.4	100	6.95–7.17	0.00–0.01	4.19–4.27	4.4–5.0	100	6.44–7.17	0.00–0.01	3.25–4.19	3.5–4.6
Rondaxe Lake Outlet	41 (30–65)	5.81–5.89	0.02	2.09	2.9	94 (92–96)	6.30	0.01	2.26	3.0	100	6.54–6.81	0.00–0.01	2.82–2.94	2.5–2.8	Not tested at this site				

* Number of water chemistry measurements during the time period tested.

Table 5. Blacknose dace (*Rhinichthys atratulus*) survival at 3 life stages and range of pH, IMAL, Ca, and DOC encountered at 10 stream sites

Site	Egg to feeding fry (168 Hours)					Young of the year (600 Hours)					Adult (144 Hours)				
	% Survival (range of reps)	pH (2)*	IMAL (2)	Ca (2)	DOC (2)	% Survival (range of reps)	pH (4)	IMAL (2)	Ca (2)	DOC (2)	% Survival (range of reps)	pH (1)	IMAL (1)	Ca (1)	DOC (1)
Squash Pond Outlet	25	5.84–5.87	0.13–0.25	0.82–2.49	4.1–6.7	0	4.50–4.68	0.11–0.20	1.03–1.21	7.3–8.8	0	4.83	0.18	1.40	5.4
Pancake Hall	Not tested at this site					100	5.74–6.10	0.03	1.77	8.4	83 (80–90)	5.80	0.10	1.36	10.2
Constable Creek	Not tested at this site					3 (0–7)	5.03–5.42	0.04	2.06–2.12	4.8–4.9	80 (60–100)	5.12	0.15	1.98	4.7
West Pond Outlet	20 (10–30)	5.25–5.71	0.01–0.16	1.72–2.05	8.2–11.2	3 (0–7)	5.14–5.57	0.01–0.02	1.96–2.06	5.9–6.1	30 (10–60)	4.96	0.02	1.61	7.5
Big Moose Lake Outlet	0	5.05–5.06	0.11–0.17	1.71–1.91	3.1–3.7	13 (0–27)	5.24–5.46	0.04	2.02–2.22	2.6–2.8	0	5.32	0.14	2.11	2.6
Dart Lake Outlet	0	5.11–5.14	0.07–0.14	1.75–1.99	2.9–3.0	47 (40–53)	5.40–5.54	0.03	2.28–2.34	2.6	60 (30–80)	5.27	0.12	2.11	2.4
Townsend Pond Outlet	Not tested at this site					100	6.20–6.52	0.01	2.74	3.5	90	5.83	0.06	2.11	2.0

Moss Lake Inlet	40	6.49–6.54	0.02–0.03	2.29–2.45	3.0–4.0	100	6.50–6.91	0.00	3.00	3.8	100	6.49	0.03	2.65	3.9
Cascade Lake Outlet	90 (85–95)	6.74–6.86	0.00–0.01	3.13–3.26	2.4–6.7	100	6.96–7.17	0.00–0.01	4.19–4.27	4.4–5.0	Potential copper toxicity—under investigation				
Rondaxe Lake Outlet	48 (30–65)	6.22–6.30	0.00–0.01	2.16–2.26	3.0–3.5	100	6.54–6.80	0.00–0.01	2.82–2.94	2.5–2.8	Dam repair, cage out of water				

* Number of water chemistry measurements during the time period tested.

Table 6. Multiple regression statistics for in situ toxicity tests where Probit = a + b(Hours) + c(H^+) + d(IMAL)

Test	Adj. R-Squared	a, Intercept		b, Hours		c, H^+ Conc.		d, IMAL	
		Parameter Estimate	Prob. > T	Parameter Estimate	Prob. > T	Parameter Estimate	Prob. > T	Parameter Estimate	Prob. > T
Lake Trout (*S.namaycush*)									
Sac Fry at 720 Hours	0.7915	2.1998	0.0001	0.0033	0.0001	0.1290	0.0001	−8.0445	0.0030
Feeding Fry at 336 Hours	0.6803	0.5649	0.2487	0.0101	0.0001	0.1837	0.0073	−1.6851	0.7512
Young of the Year at 552 Hours	0.1630	2.1164	0.0001	0.0016	0.0148	−0.0187	0.7787	−1.4648	0.4147
Yearling at 552 Hours	0.5695	2.0637	0.0001	0.0055	0.0001	0.2619	0.0443	3.6397	0.2845
Brook Trout (*S.fontinalis*)									
Sac Fry at 240 Hours	0.7232	0.1219	0.7217	0.0134	0.0001	0.0801	0.0001	2.8406	0.0544
Feeding Fry at 336 Hours	0.7123	0.3676	0.3738	0.0134	0.0001	0.0318	0.2395	12.2955	0.0001
Young of the Year at 1920 Hours	0.6973	1.7699	0.0001	0.0010	0.0001	0.0978	0.0001	−0.3650	0.7332
Yearling at 672 Hours	0.7691	1.3828	0.0001	0.0030	0.0001	0.0777	0.0001	7.3026	0.0006
Creek Chub (*S.atromaculatus*)									
Egg to Sac Fry at 336 Hours	0.7113	1.3171	0.0009	0.0144	0.0001	0.0624	0.0002	4.0248	0.0854
Sac Fry to Feeding Fry at 96 Hours	0.5961	0.2602	0.7209	0.0452	0.0001	0.4050	0.0039	2.0648	0.7732

Young of the Year at 672 Hours	0.7991	1.2251	0.0001	0.0023	0.0001	0.2601	0.0001	−10.9348	0.0035
Adult at 672 Hours	0.7767	1.2299	0.0001	0.0016	0.0003	0.1207	0.0001	1.6575	0.3954
Blacknose Dace (*R.atratulus*)									
Egg to Feeding Fry at 168 Hours	0.6638	2.1831	0.0001	0.0227	0.0001	0.1818	0.0711	−0.7034	0.8965
Young of the Year at 600 Hours	0.6533	1.5657	0.0001	0.0039	0.0001	0.2925	0.0001	−12.5878	0.1156
Adult at 144 Hours	0.4856	0.4529	0.5947	0.0228	0.0008	0.1300	0.1195	10.8882	0.1312

sites (Tables 3 and 4). Squash Pond Outlet was again the most toxic site with no survival after 672 hours.

Blacknose dace tests

The blacknose dace early life stages developed more quickly than the creek chub and were therefore combined into one egg-to-feeding fry test of 168 hours. There was no survival at two sites with pH levels of 5.05–5.14; 20–25% survival at two sites with pH values of 5.25–5.87; and 90% survival in Cascade Lake Outlet with pH levels of 6.74–6.86 (Table 5).

Young of the year survival ranged from 0% in Squash Pond Outlet to 100% in sites where pH $\geqslant 5.7$. There was 47% survival in Dart Lake Outlet (pH 5.40–5.54, IMAL 0.03 mg/l) at the end of the 600-hour test.

Adult blacknose dace were found to be very sensitive, resulting in a 144-hour toxicity test. As with the other fish species tested, the adult life stage proved to be less tolerant than young of the year. There was no survival in Squash Pond Outlet (pH 4.83, IMAL 0.18 mg/l) for the test, and, as expected, 90–100% survival in the higher pH sites. Lack of survival at the Big Moose Lake Outlet site, however, could not be explained since the site was intermediate in pH level (Table 5).

Predictive equations

Exploratory multiple regression analysis of each toxicity test indicated that pH was the variable best correlated with fish mortality. Converting pH to hydrogen ion concentration provided a better correlation in the majority of the tests. Hours of exposure and IMAL also were found to be well correlated with mortality in most of the tests.

Data from 15 of the toxicity tests were used to develop species and life stage specific multiple regression equations. Data from the lake trout and brook trout egg tests could not be treated in this manner because of the extreme length of these tests and the variability in water chemistry. The multiple regression equations were then used to compare differences in fish species and life stage sensitivity to specific water quality conditions.

Although imperfect, the equation "PROBIT = a + b(Hours) + c(H^+) + d(IMAL)" predicted mortality fairly well. Adjusted R^2 values (a more conservative R^2 value adjusted for degrees of freedom) were fairly high for all except the lake trout young of the year test (Table 6). Examination of the partial regression leverage plots and the partial regression coefficients showed that H^+ was consistently the important toxicant in the predictive equations. IMAL appeared to be more variable in its effect on the predicted probit as indicated in Table 6, where the sign for IMAL parameter estimate was negative in 7 of the 15 equations. This may indicate a non-linear relationship between IMAL and mortality.

Several combinations of independent variables were used to make comparisons between the predictive equations. Although some of the

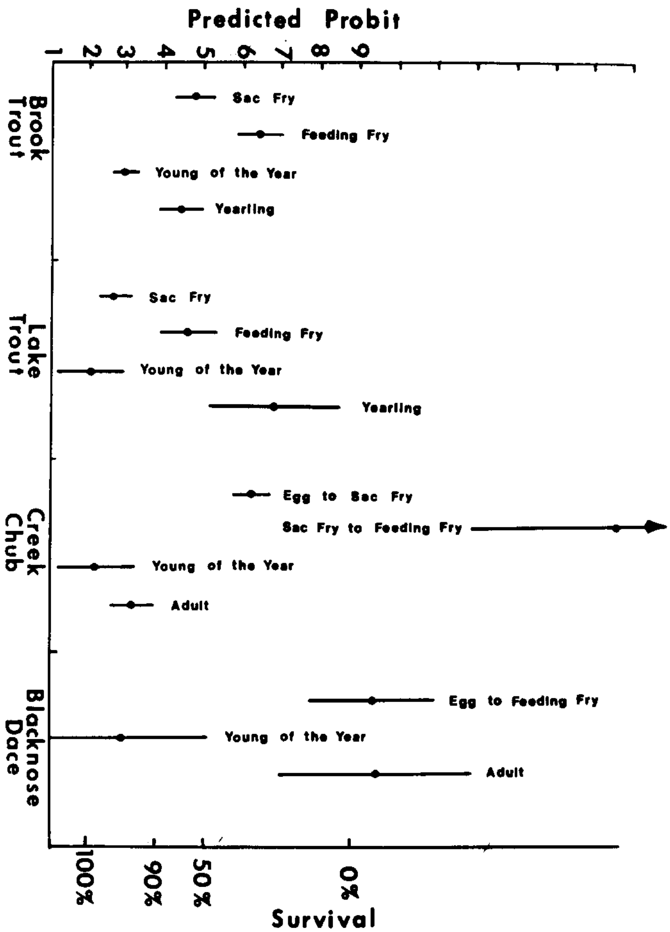

Figure 4. Predicted survival of various life stages of four fish species at pH 5.0 and 0.20 mg/l IMAL after 240 hours. The dots are point estimates, and vertical bars define the upper and lower 95% confidence interval. Predicted responses without overlapping confidence limits are significantly different ($P \leqslant 0.05$).

predicted values were beyond the bounds of the probit relationship (predicted mortality greater than 100%), these estimates were accompanied by confidence limits and allowed for comparisons among species and life stages, Figure 4 is a graphical presentation of the predicted values for a 240-hour exposure to pH 5.0 and an IMAL concentration of 0.20 mg/l.

Because of the rapid early development of the creek chub and blacknose dace, it was difficult to compare the sac fry life stage of these cyprinids to salmonid (brook trout or lake trout) sac fry. Yet, our predictions (Figure 4) indicated that early life stages of the cyprinids were more sensitive than the salmonid early life stages. Overlapping confidence limits for the young of the year life stage indicated no significant differences among species at this life stage. The predictions of adult or yearling mortality indicate that blacknose dace were the most sensitive followed by lake trout yearlings, brook trout yearlings, and finally creek chub adults.

The predicted mortality values (Figure 4) for the life stages within each species indicated that the young of the year life stage was the least sensitive to the given pH and IMAL levels. The feeding fry stage of brook trout and creek chub was the most sensitive stage for these species and was comparable to adult (yearling) sensitivity in lake trout and blacknose dace.

The second predictive equation developed was expressed as "PROBIT = a + b(H^+) + c(H^+) (Color)". With several exceptions, this simple equation explained variations in mortality very well (Table 7). Most tests resulted in a high positive partial correlation coefficient for H^+ and probit, with probit being negatively correlated with (H^+) (Color) in most cases. Organic constituents did not appear to indicate any mitigation of toxicity in four of the tests: brook trout yearling and young of the year, creek chub adult, and lake trout sac fry.

Discussion

These in situ toxicity tests have documented that there are many waters in the NBMR watershed that are toxic to fish. The degree and timing of this toxicity depends on the fish species, life stage, and season of the year. Our data compare favourably with those of Schofield and Driscoll (1987) who studied the present fish species distribution in the NBMR watershed. They found blacknose dace and creek chub, our most sensitive species, were restricted in their distribution because of the water quality of the region. Brook trout similarly were found by Schofield and Driscoll (1987) to successfully reproduce only in sites where summer pH levels were greater than 6, and only at these sites was there good egg-to-feeding fry survival in our tests. They reported that lake trout are no longer present in Dart and Big Moose Lakes, and we found that water at these sites is currently toxic to several life stages of lake trout.

Our tests in the NBMR suggest that the extent of the drainage that is toxic to fish increases during snowmelt and major runoff events. Moss Lake Inlet, for example, provides excellent brook trout habitat in the summer and fall, but becomes toxic to fish during the spring snowmelt period. Townsend Pond Outlet also had acceptable water quality and showed good fish survival in our summer and fall tests, but caused rapid fish lethality during spring tests.

Although our water chemistry data are limited, the results show a clear relationship between fish mortality and water quality. Variability in water chemistry undoubtedly occurred during the longer toxicity tests and continuous monitoring of all the chemical parameters would have been valuable in interpreting the results. The study sites, however, exhibited a range

Table 7. Relationship between H^+, color, and mortality for most toxicity tests; equation: Probit = a + b(H^+) + c(H^+) (color).

Test	Exposure (hours)	Correlation coefficients			Line equations		
		r Partial		Multiple R^2	*a*	*b*	*c*
		(H^+; Probit)	(H^+)(C); Probit)				
Lake Trout (*S.namaycush*)							
sac fry	720	−0.62	0.72	0.61	3.8950	−0.6241	+0.0278
feeding fry	336	0.36	−0.14	0.81	2.3330	0.5272	−0.0095
young of the year	552	0.68	−0.76	0.58	2.5552	0.3369	−0.0102
yearling	552	0.91	−0.34	0.88	4.1068	0.6573	−0.0030
Brook Trout (*S.fontinalis*)							
sac fry	240	0.86	−0.56	0.87	2.0248	0.2461	−0.0028
feeding fry	192	0.85	−0.75	0.75	2.8861	0.3941	−0.0046
young of the year	1920	0.42	−0.04	0.85	3.2782	0.1985	−0.0004
yearling	672	0.34	0.01	0.85	3.2125	0.1900	+0.0001
Creek Chub (*S.atromaculatus*)							
egg to sac fry	336	0.65	−0.40	0.49	5.5838	0.1872	−0.0019
sac fry to feeding fry	96	0.89	−0.42	0.81	3.5651	0.7153	−0.0033
young of the year	672	0.74	−0.35	0.94	1.8383	0.4008	−0.0031
adult	672	0.34	0.05	0.90	1.6172	0.2169	+0.0006
Blacknose Dace (*R.atratulus*)							
egg to feeding fry	168	0.96	−0.49	0.93	4.6052	0.5218	−0.0020
young of the year	600	0.77	−0.68	0.79	2.3013	1.0109	−0.0169
adult	144	0.70	−0.40	0.54	3.1706	0.4699	−0.0026

in water quality which resulted in a comparable range in observed mortality.

In general, the large lake outlets appeared to be more stable in water quality than the smaller stream sites. Big Moose Lake Outlet, Dart Lake Outlet, and Rondaxe Lake Outlet were large lake outlet sites and had relatively narrow ranges of water chemistry for each toxicity test. The smaller stream sites, such as Moss Lake Inlet and Townsend Pond Outlet, are affected the most by spring snowmelt and summer storms when there are large inputs of acid to surface waters.

Our work clearly indicated that there are three different sensitive life stages of all four species tested. Fry at hatching, feeding fry, and adults all experienced high mortality in our toxicity tests in acid waters. Other workers have similarly found that brook trout fry are very sensitive to low pH and high Al concentrations (Baker and Schofield, 1982; Hunn et al., 1985; Ingersoll et al., 1985). Kwain and Rose (1985) reported that at pH 5.0 and 4.5 brook trout fry died at hatch because of a failure to shed the egg capsule without irreversible damage to the yolk sac. Schofield and Driscoll (1987) reported a similar situation in yellow perch (*Perca flavescens*), where there was an increased rigidity of the chorionic layer of the egg when raised in acid waters. Mortality in our egg-to-fry toxicity tests was most likely due to similar causes since fry which died at hatch were often still partly enclosed in the egg capsule.

Adult sensitivity was shown in our tests by the greater survival of the young of the year life stage in each of our four test species. Other researchers have reported that tolerance of fish to low pH alone appears to increase with age and size (Lloyd and Jordan, 1964; Johnson, 1975; Jagoe et al., 1984). The activity of IMAL in our toxicity tests may be the factor which differentiates our tests from these published reports. Previous preliminary results from South Lake, NY (low pH, high Al), had also shown that sensitivity of post-juvenile brown trout and creek chub exposed to toxic conditions increased with size and age (Colquhoun et al., 1983). Based on the results of our studies, the stocking of adult salmonids in acid waters does not appear to be a viable option. The presence of refuge sites of favorable water quality within a stream or lake could, however, allow some fish to survive periods of toxic conditions. These factors are of critical importance to the survival of a fish species in a habitat subject to acidic water episodes.

Frenette and Dodson (1984) studied the population structure of brook trout in Lac Tantaré, Quebec and concluded that the smaller brook trout in a year class die at a higher rate than do the larger fish. They also observed the larger fish in the population to occupy the areas in the lake with the most favorable water quality (springs). Their conclusion that the smaller fish are being exposed to the more toxic areas of the lake and therefore suffering higher mortality rates does not necessarily conflict with our findings that the larger fish are more sensitive to low pH/high Al concentrations. It is important to consider behavioral interactions when applying toxicity data to native fish populations.

Many researchers have reported that Ca can mitigate the toxicity of acid waters to fish (Brown, 1981; Hunn et al.,1985; Ingersoll et al., 1985). In the waters we tested, there was a relatively narrow range of Ca (Tables 1–5). Multiple regression analysis of our data showed that a predictive equation including Ca was only slightly better than one without Ca (average R^2 values of 0.888 and 0.854 respectively). For this reason, Ca was not included as a variable in our predictive equations. Ca was also found to vary with pH in our study, and this limited the ability to adequately investigate its mitigative value in acid waters.

Because many water chemistry variables are naturally interrelated, it was difficult to separate the effects due to individual variables in our study. High IMAL concentrations, for example, occur in dilute acid waters also characterized by low Ca, Mg, Na, K, and alkalinity. In our predictive equations, we included IMAL as a linear variable relative to mortality, even though laboratory tests indicate that Al is most toxic to brook trout at pH levels of 5.0–5.5 (Baker and Schofield, 1982). This may explain why IMAL is not better correlated with mortality in the predictive equations. This was done because few similar lab tests have been conducted with lake trout, creek chub, and blacknose dace, and because we felt that our data set was inadequate to permit legitimate study of this issue. Our predictive equations also make the assumption that water chemistry is constant over the length of the exposure. This is frequently not the case.

Regardless of the deficiencies of our predictive equations, they are useful as a means of comparing life stage differences in our data and also as a means of estimating responses of fish in typical Adirondack surface waters. Predictions of survival of brook trout yoy at Cellar and Bradley Brooks agreed well with actual results reported by Colquhoun et al. (1983). Survival at Cellar Brook was 25% at 120 hours, compared to our prediction of 28% survival, and survival at Bradley Brook was 45% compared to a prediction of 49%. However, the brook trout yoy predictive equation underestimated the mortality of fish exposed during a spring 1985 test (75% predicted survival at 10 days compared to actual survival of 0% in 6–12 days). This may have been caused by the greater toxicity of surface waters at this time of the year.

Gunn and Keller (1984) observed 18% mortality in lake trout sac fry exposed during a 5-day episode to pH levels ranging from 4.5 to 5.0 and IMAL levels which reached a peak of 0.05 mg/l. Over a pH range of 4.5 to 5.0 our predictive equation for lake trout sac fry would estimate 6–79% mortality. Higher, more toxic IMAL levels were observed by Gunn and Keller (1984) in the interstitial waters where naturally spawned eggs would be incubated. They hypothesized that this more toxic environment may explain differences between laboratory bioassay results and observed responses in natural fish populations.

When our predictive equations were used to evaluate how well laboratory studies reflect field conditions, all of the lab studies that were looked at underestimated the mortality that we observed in acidic Adirondack waters. Baker and Schofield (1982) reported 90% survival of post-larval sac fry exposed for 336 hours to pH 4.5 and IMAL of 0.30, whereas we would predict 0% survival under these conditions. Hunn et al. (1985) found 61.4% survival in lab tests under similar conditions. An explanation of the laboratory vs. field discrepancy is difficult, but there appears to be greater toxicity observed in field conditions than would be predicted based solely on laboratory results. Laboratory approaches do not precisely mimic actual field conditions. Certain environmental factors in the field have increased toxicity, underscoring the value of in situ toxicity tests. Neither lab studies nor our toxicity tests have evaluated the importance of water quality fluctuations relative to toxicity. Although we could not include continuous water chemistry monitoring at our test sites, we know that fluctuations do occur and are one of the factors of importance to the survival of fish species.

The following additional research would help to answer unresolved questions about the mechanisms of toxicity in waters impacted by acidic deposition:

(1) The relative importance of fluctuating water chemistry and threshold values for toxic constituents needs to be determined.
(2) The ability of fish to seek, recognize, and use refugia may explain anomalies in fish distribution and needs to be investigated.
(3) Lab studies of the potential for Ca mitigation should be designed to test a range of values corresponding to those encountered in the acidified waters (generally between 1 and 4 mg/l Ca and pH from 4.5 to 5.5).
(4) The physiological basis for the greater sensitivities of larger, older fish compared to young of the year fish should be determined.

When considered along with the results of Schofield and Driscoll (1987), our studies provide convincing evidence that acidification of surface waters in the NBMR has had a major impact on fish distribution. Our project showed that there are definite differences in sensitivity to acid waters of different fish species and that certain life stages are more sensitive than others. Water at study sites with low pH and high IMAL levels was toxic to most fish.

These toxicity studies were conducted under naturally occurring stream and lake conditions, and the results are therefore more representative of native fish responses than are laboratory tests. This work provided evidence that laboratory predictions of mortality at certain pH levels and Al and Ca concentrations will underestimate the magnitude and timing of toxicity to fish in natural waters subjected to acidic deposition.

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