Avoidance of Low pH and Elevated Al Concentrations by Brook Charr *(Salvelinus fontinalis)* Alevins in Laboratory Tests

John M. Gunn and David L.G. Noakes

Department of Zoology, University of Guelph,
Guelph, Ontario, Canada N1G 2W1

ABSTRACT. Laboratory studies were conducted to test the ability of brook charr (Salvelinus fontinalis) alevins, the earliest free-swimming life stage of the species, to detect and avoid toxic levels of H^+ and inorganic Al. Alevins were tested in steep gradient choice tanks using a range of H^+ (pH 4.0 to 5.5) and Al (0 to 500 µg L^{-1}) concentrations in low Ca (2.0 mg L^{-1}) water. The young brook charr actively avoided acidic water with a pH < 5.0. Aluminum additions of 500 µg L^{-1} increased the avoidance response. The observed behavioral response of alevins to low pH and elevated levels of Al, may be of significant adaptive advantage in systems undergoing acidification.

1. INTRODUCTION

Near the end of the yolk absorption period, brook charr (Salvelinus fontinalis) emerge from the gravel of the redd, fill their gas bladder and commence exogenous feeding. This shift from embryonic to juvenile life style not only involves morphological and physiological changes in the fish, but potentially exposes alevins to abrupt physiochemical changes in the water as they move from the interstitial to the surface environment. In areas receiving acidic precipitation, the surface waters are often far more acidic than the upwelling groundwater that percolates through the redd (Trojnar, 1977; Gunn, 1985). These chemical differences may be further exaggerated if emergence coincides with the pH depressions in surface waters that often accompany snowmelt.

While the sensitivity of the early life stages of fish to acidic water is well known (Haines, 1981) and many recognize the potential damage caused by episodic pH depressions (Jeffries et al., 1979), there is little information on the role that avoidance reactions may play in protecting fish from the deleterious chemical conditions typical of acid stressed systems. It has been shown that fish can sense minute concentrations of many other toxic chemicals and will often avoid contaminated waters at levels well below lethal conditions (Giattina and Garton, 1983).

In this study we tested the ability of brook charr alevins to

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avoid toxic concentrations of H+ and inorganic Al, the factors generally considered responsible for fish mortality in acidified lakes and streams (Haines, 1981; Baker, 1982). Tests were conducted using prepared solutions as well as water collected from an acidic lake.

2. MATERIAL AND METHODS

Brook charr eggs were obtained from 1) brook stock at Ontario Ministry of Natural Resources Fisheries Laboratory at Maple, Ontario, and 2) from wild fish in Dickson Lake (45°47'N, 78°12'W), Ontario. Shortly after hatching, embryos were acclimated to the control conditions that were used throughout this experiment: temperature 4.8 (± 0.2)°C ($\bar{X}$(± 95%CI)), pH 6.9(± 0.1), specific conductance @ 25 C 23.0(± 1.2) uS cm^{-1}, hardness 7.2(± 0.5) mg L^{-1} as $CaCO_3$, Ca^{++} 2.0(± 0.1) mg L^{-1}, Na^{++} 1.4(± .03) mg L^{-1}, and inorganic Al <0.001 mg L^{-1}. The avoidance experiments were run using fish from the start of swim-up to the end of yolk absorption, a period of approximately 3 weeks. The mean total length and dry weight of alevins used in the avoidance tests were 23.6 ± 0.3 mm and 13.4 ± 0.2 mg respectively.

Avoidance reactions were tested in steep gradient choice tanks (Figure 1) with a flow rate of 400 ml min^{-1} per side. Dye tests confirmed that sharp separation of the test waters occurred at the center drain in these tanks.

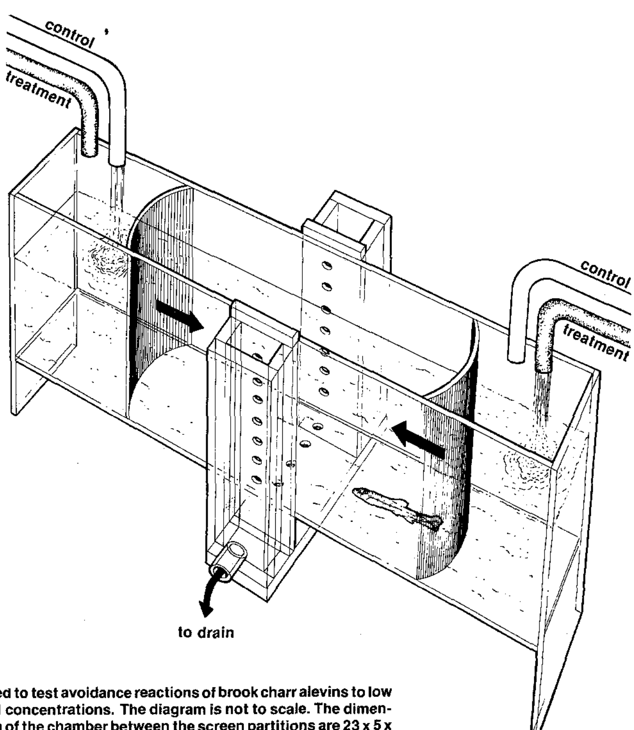

Fig. 1. Chamber used to test avoidance reactions of brook charr alevins to low pH and elevated Al concentrations. The diagram is not to scale. The dimensions of the portion of the chamber between the screen partitions are 23 x 5 x 10 cm. Alevins averaged 2.4 cm in total length.

A single alevin was placed in the tank while control water (pH 6.9, 0 Al) flowed from both sides. After an acclimation period of 5 min, the control water on one side was replaced by treatment water and the time spent on the left and right side of the center drain was recorded as the fish swam back and forth for 15 min. The trip durations were recorded manually by an observer using a Esterline Angus event recorder. Three chambers, each separated by opaque barriers were run simultaneously with the fish being observed using an overhead mirror. The start of a test run was defined as the point of first entry of the alevin into the treatment side water. There were approximately 12 fish used per treatment, with each fish used only once. Only the data from actively swimming fish (min of 2 return trips) were used in later analysis. The side used for the treatment water was reversed between each run to eliminate chamber effects. Control runs were conducted during which control water was maintained on both sides throughout the test period. All runs were conducted in dim light (1 mE m^{-2} s^{-1} at the water surface).

The avoidance reactions of alevins were tested under the following conditions:

1) a range of pH levels (4.0 to 5.5)
2) low pH (4.5, 5.0) + Al (0 to 500 µg L^{-1})
3) in "natural" water collected from an acidic lake

Synthetic test solutions were prepared in 200 L polyethylene barrels by adding $Al_2(SO_4)_3$ and H_2SO_4 to the control water mixture of deionized and dechlorinated tap water (98/2 mixture). Vigorous aeration (to drive off CO_2) and repeated titrations with 0.02N H_2SO_4 were used to produce stable pH conditions. Solutions were cooled to 5 C (using plastic emersion coils attached to a heat exchanger), aerated, pumped to insulated headtanks, and passed through calibrated flow valves to the avoidance chambers. Solutions were in contact with only the plastic components of the apparatus.

Water was collected from Ruth-Roy Lake (46°06' N, 81°14' W), an acid lake near Sudbury, Ontario to test the response of alevins to chemical conditions in "natural" water. In preliminary tests, using static bioassay procedures, the Ruth-Roy Lake water (pH 4.6, cond. 33 uS cm^{-1}, Ca 1.3 mg L^{-1}, Na 0.5 mg L^{-1}, inorg. Al 496 µg L^{-1}, Zn 26 µg L^{-1}, Cu 2 µg L^{-1}, Ni 17 µg L^{-1}) proved to be lethal to brook charr alevins in < 48 hr. For the avoidance tests, the lake water was handled in the laboratory with the same procedures outlined above.

Duplicate water samples were collected from the barrels for analysis of pH (Radiometer PM82), specific conductance (Radiometer CDM3), and hardness (EDTA titration). Samples were submitted to the Ontario Ministry of Environment (MOE) laboratory for major ion and metal analysis by procedures outlined in MOE (1981). Inorganic monomeric aluminum was analyzed using dialysis separation followed by graphite furnace atomic absorption spectroscopy. (On average the measured total and inorganic Al for each of the 4 nominal additions were: 50 - 38, 33 µg L^{-1}; 100 - 105, 72 µg L^{-1}; 200 - 205, 149 µg L^{-1}; 500 - 508, 389 µg L^{-1}). Samples were collected directly from the water flowing into the avoidance chambers for analysis of DO and free CO_2 (calculated from acid titration curve). In the first set of runs (using the Maple stock of fish) temperature was measured in the barrels at the start of a run. In the second set (Dickson L. fish) temperatures of the control and treatment solutions were measured continuously within the avoidance chambers using a YSI thermister calibrated against a NBS standard thermometer (accurate to 0.1°C).

The duration of time spent in the treatment and control ends of the tank were compared using nonparametric tests (Kruskal-Wallis, Wilcoxon, $p<.05$) after logit (deMarch and Scherer, 1980) and arcsine transformations of the data.

3. RESULTS AND DISCUSSION

Brook charr alevins avoided acidic water in the pH range of 4.0-5.0 (Figure 2), indicating that this early life stage can detect and will attempt to avoid the pH levels that other investigators (Baker and Schofield, 1982; Ingersoll et al., 1985) have found to be lethal to brook charr alevins in similar low conductivity, low Ca^{++} waters. The upper pH limit for the avoidance reaction was not well defined in this experiment, but most alevins appeared unaffected by pH > 5.0.

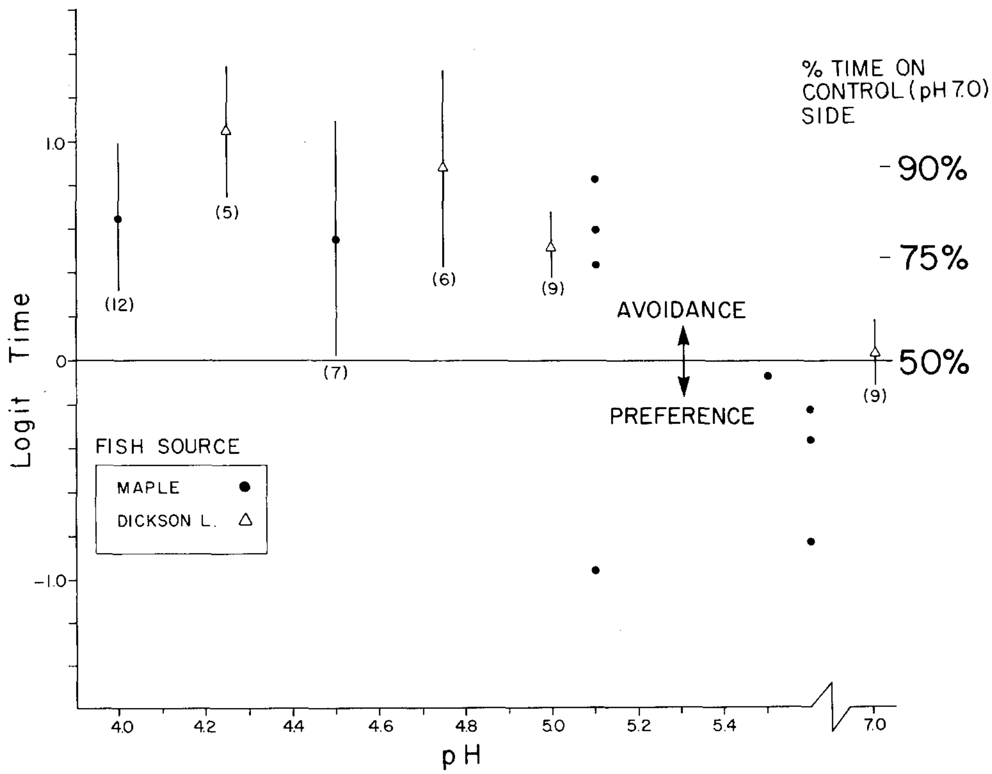

Fig. 2. Results from avoidance tests using brook charr alevins exposed to various low pH levels. Mean, 95% conf. interval, and number of runs are indicated. Data for individual fish are shown for pH levels for which there were too few values to construct confidence intervals. The location of some equivalent values for % time on the control side are indicated.

The procedures were designed to test the avoidance of H^+ ions alone. The use of soft water and excessive aeration kept free CO_2 to less than 5.0 mg L^{-1}. Hoglund and Hardig (1969) have shown that free CO_2, evolved through the acidification of test solutions, is more strongly avoided than the H^+, a fact that limits the usefulness of data on avoidance reactions to low pH in water with a high carbonate concentrations (Wells, 1915; Bishai, 1962; Ishio, 1965). The reagent additions increased the concentration of sulfate ions in the test solutions, but these were not expected to produce significant behavioral reactions (Maciorowski et al., 1977).

There were no observed differences between the response of alevins to acid lake water, and the prepared solutions with similar pH and Al concentrations (Figure 3). Both were strongly avoided.

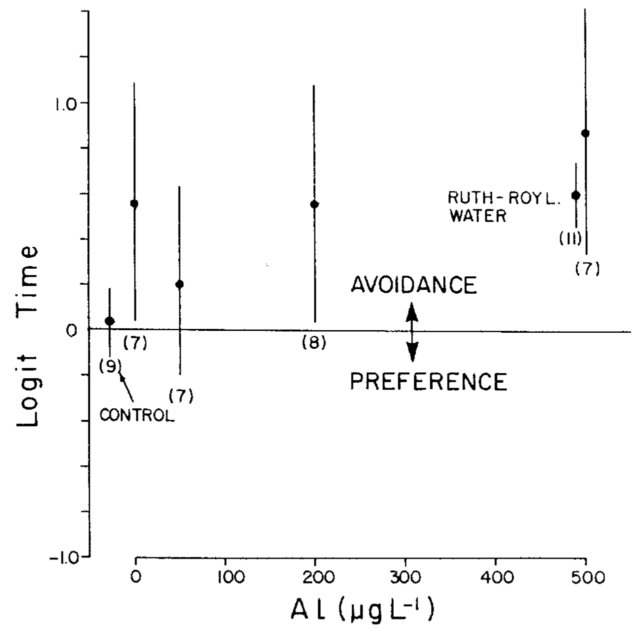

Fig. 3. Avoidance reaction to low pH (in all cases pH is 4.5) plus increasing concentrations of Al. Mean, 95% C.I., and number of runs are indicated. Aluminum levels are nominal values (see text for measured values).

There was an increase in the avoidance reaction (i.e. greater time spent on the control side) with the addition of Al to low pH (4.5) water (Figure 3), however, only with the highest Al concentration (500 μg L^{-1}) was the difference statistically significant ($P < .01$). Similarly, when alevins were confronted with low pH on both sides of the chamber, they avoided the side with the Al (Table I), but, again the enhanced avoidance reaction occurred only with the largest Al additions (500 μg L^{-1}). Van Coillie et al. (1983) conducted similar studies with adult brook charr (age 2+) and reported a threshold of approximately 100 μg L^{-1} of total AL to produce avoidance at pH 5.6.

Table I

Combined effects of low pH and aluminium on the avoidance reaction of brook charr alevins

	Control Side		Treatment Side		% Time on Control Side	Logit[a] Time
	pH	Al (μL^{-1})	pH	Al (μL^{-1})	x (95% C.I.)n	x (95% C.I.)n
Control						
	7.0	0	7.0	0	47.0(15.7) 9	.036 (.146) 9
Treatment						
	4.5	0	4.5	200	50.8(27.3) 6	.013 (.550) 6
	4.5	200	4.5	500	55.2(24.2) 7	.188 (.618) 7
	4.5	0	4.5	500	72.9(12.8) 7	.473 (.285) 7
	5.0	0	5.0	100	62.8(15.5) 9	.318 (.396) 9
	5.0	0	5.0	500	80.7(13.1) 8*	.720 (.356) 8*

[a] $\log\left(\frac{\text{time spent on control side}}{\text{time spent on treatment side}}\right)$

*significantly different ($p \leq 0.05$) than control

Avoidance responses have been inferred from the distribution of fish relative to areas of low pH (Muniz and Leivestad, 1980), but to date, there are no detailed in situ studies of avoidance reactions to acidic water for any fish species. Fish respond to a variety of abiotic and biotic factors in their natural environment. It is probably difficult to predict the response to a single factor, such as pH, in isolation, but these laboratory findings suggest that avoidance reactions may be of significant adaptive advantage to even the earliest free-swimming life stage of brook charr.

4. ACKNOWLEDGEMENT

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