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Effects of Reduced pH on Three Life Stages of Sunapee Char *Salvelinus alpinus*

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The landlocked arctic char (*Salvelinus alpinus*) occurs in only 11 waters in Maine, although closely related populations are found throughout the northern portions of the northern hemisphere (Scott and Crossman 1973). Maine's landlocked char are subjectively separated into two groups, the blueback and the Sunapee (Kornfield et al. 1981). The Sunapee is native to only one body of water, which is subject to possible habitat degradation. Therefore, the Sunapee char was chosen for experimentation, with the establishment of new, self perpetrating populations as a major goal (Kircheis 1981). Since 1969, Sunapee char have been introduced into 10 lakes, as eggs, fingerlings, yearlings, or adults (sexually mature fish of various ages). Seven of the 10 introductions resulted in the establishment of self-sustaining populations. One of the remaining three was made too recently to enable full evaluation of the results. The other two are known to be failures. One was probably due to the presence of a large number of predatory fish in the lake prior to stocking. In the other, 5000 fingerlings and 2500 yearlings were introduced into Little Long Pond, Hancock County, during the period 1977-80, but only two mature fish have been recovered in annual sampling with gill nets.

Surface pH levels in the seven ponds successfully stocked ranged from 6.6 to 7.0. However, water samples collected from Little Long Pond during 1980-83 revealed a depressed pH (range 4.95-5.77 at the surface, 4.79-5.83 in the hypolimnion). The lake is located in an area identified as sensitive to acid precipitation, and has a low buffering capacity (National Atmospheric Deposition Program 1982). There are no disturbances such as roads or human habitation in the watershed. The water has little color, and sulfate is the major anion present (Haines and Akielaszek 1983). These factors suggest that the low pH is due to atmospheric deposition. The reason for the failure of introductions in Little Long Pond has not been determined. Levels of pH within the range encountered in Little Long Pond are known to adversely affect some salmonids (see review by Haines 1981). Therefore, we hypothesized that low pH had prevented the establishment of Sunapee char, and undertook this study to determine the effects of low pH on three life stages.

MATERIALS AND METHODS

Eggs from wild Sunapee char for stocking purposes are collected and fertilized at Floods Pond annually, and are hatched and reared at the Maine State Hatchery at Enfield. Eggs used in this study were collected at Floods Pond, and yearlings and fry were obtained from the hatchery. The three life stages were tested sequentially: yearlings first, sac fry second, and eggs last. Thus, all fish were drawn from the same population, and three year classes were represented in the tests.

Procedures necessarily varied somewhat depending on the particular life stage being tested. In each procedure however, all aquaria and glassware were washed with concentrated HCl and thoroughly rinsed with tap and distilled water before use. Bioassays were of the static water type, with daily water changes. Water for use in all experiments was prepared daily for immediate use by titrating distilled water to a specific conductance of 12-14 µS/cm with dechlorinated tap water. Results of analyses of the distilled and dechlorinated waters used, including determinations of metals by atomic absorption spectrophotometry are shown in Table 1. Aliquots of the daily water preparations were analyzed for pH (Fisher model 107 or Cole-Parmer Digisense meters), specific conductance (Markson model 10 meter), and alkalinity (Gran plot; Stumm and Morgan 1970). Means, standard errors, and ranges of these values for the water used during the course of each experiment are shown in Table 2. Test aquaria were filled with test water titrated to the appropriate experimental pH with 0.1N H_2SO_4. Controls consisted of test water without added acid. Temperature throughout the course of all experiments was 9±1°C. Each aquarium was continuously aerated through a glass tube. Observations were made at intervals of no more than 12 h and dead individuals were removed.

Table 1. Composition of the two sources of water used in acclimation and testing of three life stages of Sunapee char.

Factor	Distilled water	Dechlorinated tap water
pH	6.1	7.3
Alkalinity (µeq/L)	14.2	185.0
conductivity (µs/cm)	2.1	47
Color (pt units)	<10	<10
Mn (µg/L)	0	13
Al_{2+} (µg/L)	0	21
Ca^{2+} (mg/L)	0	-
Mg^{2+} (mg/L)	0	-
Fe_{+} (mg/L)	0	-
Na^{+} (mg/L)	0	12.84
K^{+} (mg/L)	0	1.14

- = not determined

Table 2. Characteristics of water used during bioassay of three life stages of Sunapee char. Numbers are expressed as mean ± one standard error; range is shown in parentheses.

Life stage	pH	Alkalinity (μeq/L)	Specific conductance (μS/cm)
Yearling	6.7 ± 0.6	37.9 ± 1.3	13 ± 2
	(6.5-6.9)	(27.8-48.2)	(10-15)
Sac fry	6.6 ± 1.4	34.7 ± 1.9	13 ± 0.3
	(6.6-6.8)	(27.8-42.4)	(12-14)
Eggs	6.7 ± 1.4	41.9 ± 1.2	13 ± 0.4
	(6.6-6.8)	(38.5-45.2)	(12-14)

Yearlings spawned in fall 1979 were obtained from the hatchery in January 1981. These were the pooled offspring of several crosses. To ensure freedom from disease and assess mortality during transport, they were held in aerated, flowing well water (pH 7.9, alkalinity 2087 μeq/L, specific conductance 490 μS/cm) for at least three weeks before undergoing acclimation to the test water. Mortality during this period was less than 1%. Four groups of 35-50 fish were acclimated to unacidified test water in a 600-L fiberglass tank for 9-15 days. Groups were done sequentially; that is, one group was acclimated and then used in a bioassay, then the next, and so on. Water in the acclimation tank was recirculated through a filter of glass wool and activated carbon and aerated. Due to the metabolic activity of the fish, alkalinity, pH and specific conductance generally increased during the acclimation period, and distilled water was added every few days as needed. In this manner, an alkalinity of 27-60 μeq/L and a pH of 6.6-6.9 was maintained in the tank. Fish were fed pelletized food *ad libitum* during this period. The entire group of fish was removed from the acclimation tank, and individuals were randomly assigned to one of the 20-L glass test aquaria. Numbers of fish tested, pH levels used and durations of the exposures are shown in Table 3. Fish were not fed during the experimental exposures. Criteria of death were cessation of opercular movement and lack of response to prodding with a glass rod.

Sac fry spawned in fall 1980 were obtained from the hatchery in March 1981. These fry were the pooled results of crosses of several adults. They were held in hatchery water overnight upon receipt, and fry that died due to transport and handling (about 8%) were removed. The remaining fry were transferred to a 60-L fiberglass tank containing aerated, nonacidified test water. Because the fry had visible yolk sacs, they were not fed. After 2 days of acclimation, during which mortality was less than 2%, fry were randomly assigned to the test aquaria.

Table 3. Design of experiments in which yearling Sunapee char were exposed to waters of differing pH. Control pH ranges are shown in parentheses.

Test Number	Duration (hours)	Number of fish exposed to a given pH level	Levels of pH tested
1	96	7 in each	3, 4, 5, 6, control (6.8-6.9)
2	192	6 in 3.5 7 in 4,4.5,5 5 in control	3.5, 4, 4.5, 5, control (6.5-6.8)
3	456	7 in each	3.5, 4, 4.5, 5, control (6.6-6.9)
4	192	5 in each	4, 4.5, 5, control (6.6-6.7)

Four experimental pH levels were tested: 4, 4.5, 5, and 5.5. Three replicates were tested concurrently at each pH level, with three controls. Twenty fry were assigned to each of the aquaria, which contained 4-L of water. As the fry were semi-transparent, heart activity could be observed. Criteria of death were cessation of movement and heartbeat.

To obtain eggs, ripe adult Sunapee char were collected with trap nets at Floods Pond in October 1981. Eggs from three females of approximately equal size were stripped into separate pans. Eggs in each pan were then dry fertilized using 2-5 males. Males were used for only one cross; thus, the three groups were genetically distinct. Immediately after fertilization, aliquots of eggs were rapidly transferred to perforated polyethylene cups, submerged in 4-L glass aquaria containing acidified or unacidified test water, and transported to the laboratory. Four experimental pH levels, 4, 4.5, 5, and 5.5 were used. Three replicates, representing the three females used, were tested simultaneously. Ten hours after fertilization, all dead or unfertilized eggs were removed, amd surviving eggs in each aquarium were counted. Non viable eggs developed an opaque, milky-white color that made them easily distinguishable from viable eggs. Mortalities during the initial 10 h period were assumed to be due to transport and handling as well as unfertilized eggs. Overall mortality during this period was about 14%. Eggs were observed, and dead eggs removed, at 24, 53, 78, 96, 126, 150, 174, and 198 h after fertilization.

The LC50s and associated 95% confidence intervals over various periods were calculated according to the method of Litchfield and Wilcoxson (1949). Indefinite LC50s were calculated according to the method of Green (1965).

RESULTS AND DISCUSSION

Water acidified to pH 3 killed all yearlings in less than 4 h. A pH of 3.5 was also invariably fatal, survival times ranging from 16.5 to 19 h. Fish exposed to each of these pH levels darted rapidly about the aquarium, ultimately sinking to the bottom, often on their sides or backs. Opercular movements continued for some time after the cessation of other motion. Gills were coated with brown mucus, and considerable mucus was also seen on body surfaces. A pH of 4 was generally lethal to yearlings, although there were survivors in one replicate: three of the seven fish survived this pH in the first experiment. All others in the remaining pH 4 replicates died after exposures ranging from 52.5 to 151.5 h. Shortly before death, fish exposed to pH 4 behaved much like those at pH 3 and 3.5, although the activity was less marked. Increased amounts of gill and body mucus were again noted. No fish held at the higher pH levels tested (4.5, 5, 5.5, 6, and controls) died during the experiment.

Sac fry exposed to pH 4 water rapidly became hyperactive. Length of the hyperactive period varied, but all fish became quiescent after several hours and settled to the bottom of the aquarium. One fry died within 1 h, and more than half were dead after a 17 h exposure. Exposure to this pH for 28 h was lethal to all fry in all replicates. At pH 4.5, mortalities were first observed after 117 h of exposure. More than half the fry exposed to pH 4.5, however, survived for the duration of the experiment (212 h). No mortality occurred at pH 5 or 5.5, or in the controls during the entire period.

Egg mortality during the first 10 h was not correlated to hydrogen ion concentration ($r = 0.47$, ns). This initial mortality was apparently not related to genetic factors, as each cross showed about the same percentage of dead eggs at the end of the period (11.6-17.1%). Therefore, the assumption that egg mortality during this period was due to infertility or damage during transport appears justified. By 24 h after fertilization, pH-related mortality had occurred. About 92% of all eggs exposed to pH 4.0 died during this period, whereas mortalities of only a few percent occurred at higher pH levels. All eggs exposed to water acidified to pH 4 were dead after 53 h. After 78 h of exposure of eggs to pH 4.5, all were dead in one aquarium, and 50-60% had died in the other two aquaria. A pH of 4.5 was lethal to all eggs within 96 h. By the end of the experiment (198 h), 40-60% of the eggs exposed to pH 5 had died, depending on the replicate. Average mortality of eggs in the aquaria with water at pH 5.5 and in the controls were 15% and 14%, respectively.

Calculated LC50s for exposures of 96 and 192 h, and for an indefinite period (Table 4) differed among life stages. There was little difference among the replicates for a given stage. Yearlings were the most resistant of the three groups to acid stress, and eggs the most sensitive. Encapsulated embryos have generally been found to be more resistant then fry to acid stress (Daye and Garside 1977; Milbrink and Johanssen 1975; Trojnar

1977a). In contrast, we found eggs of Sunapee char to be more sensitive than sac fry to low pH water. Part of the reason for this may lie in the experimental protocols used. Lee and Gerking (1980) noted that eggs are particularly sensitive to acid immediately after fertilization, and that this sensitivity decreases as the chorion's permeability changes during the first hours after fertilization. Inasmuch as eggs used in this experiment were dry fertilized and immediately placed in the test solutions, they would be expected to show higher sensitivity than eggs fertilized and water hardened at high pH (the procedure followed in some other studies).

Table 4. Calculated mean LC_{50}s, (with 95% confidence intervals in parentheses), for each life stage of Sunapee char for the indicated periods.

Life Stage and Replicate Number	96 hour	192 hour	Indefinite
Yearlings			
1	4.0 (3.7-4.4)	-	4.2
2	4.2 (4.0-4.5)	4.2 (4.0-4.5)	4.2
3	4.0 (3.8-4.2)	4.2 (4.0-4.5)	4.3
4	4.0 (3.8-4.2)	4.2 (4.0-4.5)	4.3
Sac fry			
1	4.3 (4.2-4.4)	4.5 (4.3-4.6)	4.5
2	4.3 (4.2-4.4)	4.3 (4.2-4.5)	4.4
3	4.3 (4.2-4.4)	4.4 (4.3-4.5)	4.4
Eggs			
1	5.0 (4.9-5.1)	5.1 (5.1-5.1)	5.1
2	4.6 (4.5-4.7)	4.9 (4.5-5.2)	5.2
3	4.8 (3.3-5.2)	4.9 (4.5-5.1)	4.9

Comparisons of bioassay data for a particular toxicant among studies involving different species are generally difficult, due to variations in methods, water sources, temperatures, life stages and methods of calculating results, and other factors. Under such circumstances, an idea of relative susceptability of differing species to a toxicant is usually the best that can be achieved. This is still of considerable use, for example, in predicting which species will be most sensitive in a given situation. The effect of acid stress on various salmonids has been assessed in several studies (Lloyd and Jordan 1964; Kwain 1975; Daye and Garside 1975, 1977; Graham and Wood 1981). Spry et al. (1981) summarized and tabulated results from these and other studies, including non-salmonid fishes. By comparison, Sunapee char appear to be more sensitive than brook trout (*Salvelinus fontinalis*) to

acid stress and less sensitive than rainbow trout (*Salmo gairdneri*). Lake trout (*Salvelinus namaycush*) has been ranked less sensitive than rainbow trout, but more sensitive than brook trout to acid stress (Grande et al. 1978). Beamish (1976) noted that populations of lake trout ceased reproduction at pH 5.5 and disappeared at pH 5. Daye and Garside (1975) reported a lethal limit of about pH 4 for alevins of Atlantic salmon (*Salmo salar*) during a 7-day exposure. Grande and Andersen (1981) reported 100% mortality of sac fry and fingerlings of Atlantic salmon at about pH 4.5 over a period of 11-18 days. Thus, it appears that Sunapee char are roughly equivalent to Atlantic salmon in sensitivity to acid stress. Kornfield et al. (1981) suggested that Sunapee char be considered a subspecies of Arctic char. While little information is available on the relative tolerance of Arctic char to acid water, Edwards and Hjeldnes (1977) noted that when different salmonids were exposed to lethally low pH, survival was highest in brown trout (*Salmo trutta*) intermediate in Arctic char and lowest in rainbow trout. These findings support the relative ranking offered above.

Extrapolation of these results to natural situations is also difficult, but some conclusions may be drawn. Sunapee char do not construct redds, nor do they bury or cover their eggs (Kircheis 1980). Therefore, under natural conditions, eggs and newly emergent fry could not be protected from transient low pH conditions by buffered microenvironments in the substrate as has been suggested to occur for some other species (Trojnar 1977b; Gunn and Keller 1981). Other potential toxicants, particularly aluminum, may be important causes of fish mortality in acidified environments, especially in the pH range 4.5-5.5 (Schofield 1977). Aluminum concentrations in the range 47-100 ppb at the surface and 36-120 ppb in the hypolimnion have been measured in Little Long Pond (S. Norton and S. Kahl, unpublished data). Aluminum concentrations near the high end of this range adversely affect various fish species (Haines 1981). In the present study, we considered only the effects of hydrogen ion, but clearly the aluminum present in a natural system in this pH range could increase mortality. Although the results indicate that the pH levels in Little Long Pond were apparently not low enough to kill older Sunapee char, the combination of low pH and elevated aluminum concentrations could have caused the observed stocking failures. Finally, effects such as alterations in sperm mobility (European Inland Fisheries Advisory Commission 1969), or the failure of females to produce viable ova (Beamish 1976) were not considered. Consideration of these factors suggests that pH levels below about 5.5 would preclude successful establishment of self-sustaining Sunapee char populations.

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