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Reproductive Failure of Landlocked Atlantic Salmon from New York's Finger Lakes: Investigations into the Etiology and Epidemiology of the "Cayuga Syndrome"

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Abstract.—We describe a disease syndrome that afflicts larval, landlocked Atlantic salmon *Salmo salar* from Cayuga Lake, one of central New York's Finger Lakes. Mortality associated with the "Cayuga syndrome" is 98–100%. Death usually occurs between 650 and 850 centigrade degree-days after fertilization, approximately 2–4 weeks before yolk resorption is complete. Although there is minor temporal variation in the onset of the Cayuga syndrome in progeny from individual females, all sac fry eventually succumb. Incubation of embryos and sac fry under constant, ambient, or reduced temperature regimens slightly alters the degree-day timing of syndrome onset, but does not improve survival. Based on mortality rate, manifestation of the Cayuga syndrome has not changed in the past 10 years, even though incubation waters of varying chemistry and temperature have been used. Mortality of the negative control stocks used for these studies never exceeded 10% from hatching to first feeding. Findings from reciprocal crossbreeding experiments indicate the problem is associated with ova only. A noninfectious etiology is indicated by the lack of consistently identifiable fish pathogens from syndrome-afflicted sac fry and by the failure to

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transmit the condition horizontally. Suspect contaminants were eliminated as potential causative factors. Epidemiological studies on the viability of other Finger Lakes stocks indicate that Atlantic salmon from Keuka and Seneca lakes are also afflicted (100% mortality), yet those from Skaneateles Lake are not. The cause of this syndrome appears to be nutritional.

Mortality of embryonic and larval fishes has been linked to improper husbandry (Leon and Bonney 1979), poor water quality (MacKinnon 1969; Rosenthal and Alderdice 1976), infectious agents (Symula et al. 1990), and contaminant exposure (Von Westernhagen 1988; Hall 1991). However, the etiologies of many early life stage diseases are not so clearly identifiable. For example, early-mortality syndromes of Great Lakes lake trout *Salvelinus namaycush* (Mac et al. 1985), steelhead *Oncorhynchus mykiss* (Skea et al. 1985), coho salmon *Oncorhynchus kisutch* (Johnson and Pecor 1969; Smith et al. 1994), and chinook salmon *Oncorhynchus tshawytscha* (Giesy et al. 1986; Smith et al. 1994) continue to perplex researchers. Husbandry conditions and infectious agents are not responsible for these syndromes, and correlations with contaminant burdens have generally proved inconclusive (Williams and Giesy 1992; Mac et al. 1993; Smith et al. 1994).

Here we describe an early life stage disease afflicting progeny from wild-caught landlocked Atlantic salmon *Salmo salar* from Cayuga Lake. Cayuga Lake is a large (length, 61.4 km; maximum width, 5.8 km; maximum depth, 133 m), well-buffered (pH 7.5–8.0), glacially carved, mesotrophic basin in the Finger Lakes chain of central New York (Oglesby 1978). The New York Department of Environmental Conservation (NYDEC) maintains the Atlantic salmon fishery in Cayuga Lake by annually stocking yearling juveniles originating from Little Clear Pond in the Adirondack Mountains. Locks on the Seneca River prevent these salmon from outmigrating to Lake Ontario (Figure 1). Atlantic salmon from Little Clear Pond mature in 2–3 years within Cayuga Lake and are captured during fall spawning migrations. Cayuga Lake also supports productive sport fisheries for lake trout, rainbow trout and brown trout *Salmo trutta*; however, we have not identified reproductive disfunction in these salmonids.

The "Cayuga syndrome" of landlocked Atlantic salmon was first observed in 1974 at the NYDEC hatchery in Rome. In this initial attempt to rear Cayuga Lake spawn, all sac fry died, despite the use of proven husbandry practices. Subsequent efforts to rear progeny from Cayuga Atlantic salmon in 1978, 1980–1984, and 1987 also were completely unsuccessful.

In this initial report, we detail efforts to characterize the Cayuga syndrome and elucidate its etiology. These efforts consisted of four phases: (1) a multiyear evaluation of the effects of various incubation conditions on syndrome manifestation (i.e., time to death); (2) bacteria, parasite, and virus isolation studies; (3) attempts to effect vertical and horizontal transmission; and (4) epidemiological studies to determine the viability of Atlantic salmon in other Finger Lakes. The consistent stage specificity and epizootiology of the Cayuga syndrome indicate that it is a unique early life stage disease heretofore undescribed in the Salmonidae.

Methods

Broodstock Sources

Cayuga Lake Atlantic salmon were captured during their spawning migrations at a weir on Cayuga Inlet at the lake's south end or by electroshocking adults in other tributaries. Females were gravid between the first and third week of November, when water temperatures had declined to 9°C or below. Females were manually stripped, and their eggs were fertilized dry with the sperm of two or three males. Eggs were subsequently water-hardened with Cayuga Inlet water (unless otherwise stated) for at least 30 min before they were moved to incubation facilities at Cornell University or to NYDEC hatcheries in Bath or Rome.

Eggs used as the negative control stock for all studies were from Little Clear Pond broodstock. Each fall landlocked Atlantic salmon are trapped from this natural pond and spawned. Their progeny are cultured in the adjacent NYDEC Adirondack hatchery (near Saranac Lake). The Little Clear Pond broodstock subsist on a natural diet of rainbow smelt *Osmerus mordax*. Since 1984, this stock has been the sole source of landlocked Atlantic salmon stocked throughout New York; Atlantic salmon now in Cayuga Lake are of this genetic origin. Between 1973 and 1984, Cayuga Lake had been stocked with Sebago, Gullspang, Penobscot, and Lake George strains of Atlantic salmon when Little Clear Pond fish were unavailable. Spawning of Little Clear Pond salmon generally precedes Cayuga Lake spawning by 7–10 d.

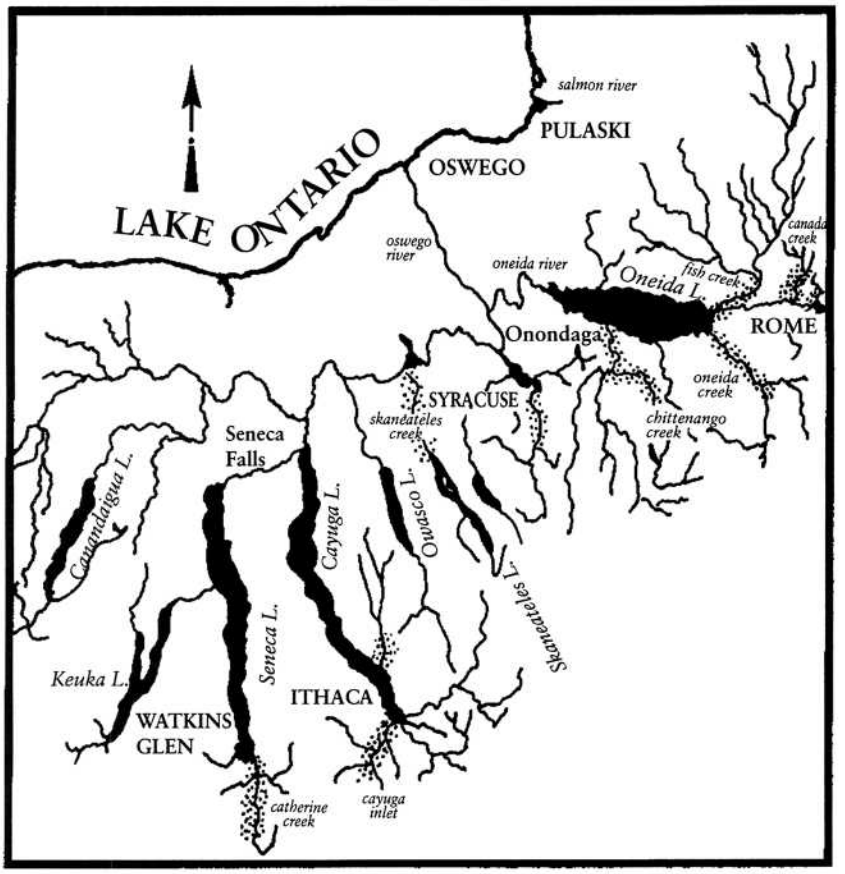

FIGURE 1.—Cayuga Lake and the Finger Lakes watershed in central New York. Stippled areas represent historical Atlantic salmon spawning areas prior to the erection of mill dams in the 1800s (modified from Webster 1982; courtesy of *New York Fish and Game Journal*).

Effect of Incubation Conditions on the Cayuga Syndrome

To determine whether incubation conditions altered the incidence or the timing of syndrome-related mortality, we examined data generated for the 4 years from which maternally specific survival records were available. Over these seasons (1983–1984, 1984–1985, 1992–1993, 1993–1994) the eggs and larvae were water-hardened or incubated (or both) under a variety of water quality conditions and temperature regimens (Table 1).

In 1983, progeny of 10 Cayuga Lake females were incubated in vertical, flow-through Heath™ trays (Heath-Techna, Inc., Kent, Washington) at the NYDEC facility in Rome; either a constant (8°C) or a reduced temperature regimen was used. For the reduced temperature regimen, the incubation temperature was decreased to 2–4°C from eye-up through hatch and then raised again to 8°C through first feeding. Survival of 200 eyed eggs from each Cayuga female was monitored for each treatment (i.e., constant or depressed temperature). The survival of eggs and sac fry from 10 Little Clear Pond females was monitored under identical conditions; however, these negative-control eggs were water-hardened with Little Clear Pond water and incubated at the Adirondack hatchery until eye-up. After eye-up, these eggs were transferred to the Rome hatchery for rearing alongside the Cayuga Lake embryos, but in a separate series of Heath incubators.

The procedure used in 1984–1985 was similar to that of 1983–1984 with three exceptions: (1) the Cayuga and Little Clear (negative control) eggs were water-hardened in hatchery lake water (Delta Reservoir on the Mohawk River) at the NYDEC Field Station in Rome; (2) the negative control egg source was from Cedar Springs, a now-defunct hatchery stock; and (3) all eggs were incubated at the reduced temperature regimen.

TABLE 1.—Chemical profiles of hardening and incubation waters used for husbandry of Atlantic salmon from Cayuga Lake. Empty cells indicate no data.

Variable	Cayuga Inlet[a]	Cayuga Lake[b]	Fall Creek[c]	Little Clear Pond[d]	Rome hatchery[e]
pH	8.0	8.2–8.3	7.0–7.9	6.49	7.6–7.9
Hardness (mg/L)[f]	231–233	152–164	157–258	22.0	52–80
Alkalinity (mg/L)[f]		104–113	174[g]	21.46	43–76
Conductivity (μS/cm)		365–422		41.3	150–197
Chloride (mg/L)	29–30	38–48	17–29	0.36	

[a] Ranges of replicate samples taken on November 20, 1992, and analyzed with Hach™ water quality test kits (Hach Inc., Loveland, Ohio).
[b] Ranges of seven samples taken February 5–May 11, 1994 (P. Tunison, Southern Cayuga Water Management District, personal communication).
[c] Ranges of 26 samples, 1992–1993 season, analyzed with Hach kits. Fall Creek is a southern tributary of Cayuga Lake.
[d] Single October sample taken by NYDEC Engineering (E. Grant, Adirondack hatchery, personal communication).
[e] Ranges of recorded values, fall and winter 1984 (H. Simonin, NYDEC, personal communication).
[f] As $CaCO_3$.
[g] Single sample.

During the 1992–1993 season, the survival of progeny from five Cayuga and five Little Clear females was evaluated in aquaculture facilities at the Cornell Veterinary College. After water hardening, eggs from both stocks were incubated in ambient-temperature, filtered, dechlorinated tap water from Fall Creek, the largest tributary of Cayuga Lake. This water supply is delivered from a water tower that cools rapidly in the fall and heats quickly in the spring. The ambient water temperatures throughout the 1992–1993 season averaged 7.3°C (SD, 1.17) in November, 3.9°C (1.08) in December, 3.6°C (0.41) in January, 2.6°C (0.40) in February, 2.5°C (0.27) in March, 6.5°C (2.01) in April, and 12.8°C (1.89) from May 1 to May 11, when the experiment was concluded.

In 1993–1994, survival was monitored for progeny of six Cayuga Lake females whose eggs were water-hardened and incubated at a different Cornell facility that uses ambient-temperature, filtered, dechlorinated tap water taken from Cayuga Lake. The inlet pipe for this water source is at a depth of 21 m in the lake; hence, the temperature of the incubation water changes very gradually. Water temperatures during the 1993–1994 season averaged 9.5°C (SD, 0.48) during November 10–30, 7.5°C (1.07) in December, 4.8°C (0.69) in January, 3.3°C (0.38) in February, 2.9°C (0.17) in March, 4.8°C (1.09) in April, and 7.4°C (0.36) from May 1 to May 20, when the experiment was concluded. Eyed eggs from 10 Little Clear Pond females were transferred to the Cornell facility from the Adirondack hatchery to serve as negative controls for this study.

During both the 1992–1993 and 1993–1994 seasons, the eggs from the Cayuga and Little Clear stocks were separated by female and incubated in screened, 7.6-cm-diameter polyvinyl chloride cups (75 eggs/cup, 1 cup/female). The cups were suspended 8–10 cm below the water surface in a single 280-L, rectangular fiberglass tank (1992–1993), or a 90-L glass aquarium (1993–1994). Flow rates to the incubation tanks averaged approximately 2 L/min, and supplemental aeration was provided. At eye-up, all infertile eggs in each cup were removed and the numbers were normalized back to 75/cup with eggs from surplus.

For all four seasons, the syndrome-related mortality rate was used as the dependent variable to evaluate whether incubation conditions altered the syndrome. Mortality rate was based on the percentage dead of the total number of Cayuga Lake larvae that hatched, minus any live specimens removed for other studies. Analysis of variance (ANOVA) was used to evaluate times to 75% death (TTD75) for Cayuga sac fry (Zar 1974). The ANOVA evaluations included (1) the year of study, (2) the source of hardening water, (3) the source of incubation water, and (4) the incubation temperature regimens.

Vertical and Horizontal Transmissibility Studies

A crossbreeding experiment was performed in 1988–1989 to determine if the Cayuga syndrome in Atlantic salmon from Cayuga Lake was associated with the eggs or the sperm, or with both. Eggs from 10 Cayuga and 10 Little Clear females were pooled by lake source and divided into four replicate lots of 500 (i.e., eight total). The following crosses were then performed with the pooled sperm of 10 Little Clear or 10 Cayuga males: (1) Little Clear ova × Little Clear sperm, (2) Cayuga

ova × Little Clear sperm, (3) Little Clear ova × Cayuga sperm, and (4) Cayuga ova × Cayuga sperm. Eggs from crosses (1) and (3) were incubated in vertical, flow-through Heath trays at the Adirondack hatchery until eye-up, when they were transferred to the Bath hatchery. The progeny from crosses (2) and (4) were incubated at Bath throughout the experiment, also in vertical Heath egg incubators. When appropriate, survival frequency was compared by chi-square analysis ($\alpha = 0.05$).

The potential to horizontally transmit the causal agent of the Cayuga syndrome to healthy Little Clear Pond larvae was evaluated in both the 1992–1993 and 1993–1994 seasons by culturing the control Little Clear larvae in the same aquaria as the affected Cayuga stocks; the husbandry conditions just described were used. To further support these findings, a second negative control stock was included, which was donated by the U.S. Fish and Wildlife Service's Tunison Laboratory of Fish Nutrition in Cortland, New York. The Tunison Atlantic salmon strain originated from sea-run stocks acquired principally from the Penobscot River in Maine in the 1970s (Dale Heddrick, U.S. Fish and Wildlife Service, personal communication). During the 1992–1993 season, survival of progeny from five Tunison females (75 eyed eggs/female) was monitored. In 1993–1994, aliquots of eggs from five Tunison females were pooled (79 eyed eggs at start).

Microbiological and Virological Studies

Surplus eggs and sac fry from the 1988–1989 reciprocal crossbreeding experiment were processed in an attempt to isolate bacterial, parasitic, or viral pathogens responsible for the Cayuga syndrome. Eggs or sac fry from the Cayuga and Little Clear stocks were sampled at eye-up, at hatch, and at 2-week intervals thereafter until all Cayuga sac fry had expired. For bacteriology, five eggs or sac fry from each source were dipped in a 1% Betadine solution in sterile distilled water for 4 min, then rinsed thrice in sterile distilled water. An additional five eggs or sac fry from each source were disinfected with 1% Roccal. Fluid was aspirated from the brain and yolk sac of the sterilized fish with sterile 1-mL syringes. One drop of each of these fluids was then streaked onto sterile selective agars (cytophaga, brain–heart infusion [BHI], blood, and kidney disease medium-2); these agars were prepared as described by Austin and Austin (1989). The surface sterility of the individuals was checked by dipping each into a sterile tube of fluid thioglycollate broth (FTG). Finally, eggs or sac fry were cut open aseptically and immersed in a tube of FTG. Culture plates were sealed in plastic bags, and plates and tubes were incubated at 15°C for at least 1 month. Two weeks after inoculation, a sterile loop was used to streak samples from each FTG tube onto both blood and BHI agar plates. These cultures were examined for an additional month. To look specifically for *Cytophaga* sp., an additional five sac fry from each source were bacteriologically processed without surface decontamination 1 week after onset of the Cayuga syndrome. The Minitek™ Numerical Identification System (BBL Microbiology Systems, Cockeysville, Maryland) was used to determine the genus and species (where possible) of bacterial isolates.

For virus isolations, replicate groups of 25 eggs or sac fry were processed from each source at each sampling time. Homogenates (1:10 weight : volume in sterile physiological buffered saline with 100 units penicillin/mL, 100 μg streptomycin/mL, and 25 μg fungizone/mL) were prepared in sterile stainless steel blenders. Homogenates were centrifuged 5 min at 1,000 × gravity. The supernatant, with fat removed, was filtered through a low-binding 0.45-μm filter. Dilutions of the filtered homogenate (0.1 and 1.0%) were prepared in minimal essential medium with Hank's balanced salt solution (Gibco BRL, Grand Island, New York) containing 4.5 mM sodium bicarbonate and 5% fetal calf serum (FM4-5%). Using separate cell monolayers of rainbow trout gonad, chinook salmon embryo, epithelioma papillosum cyprini, and Atlantic salmon heart, grown in 24-well plates (2 cm^2 per well), we inoculated three wells each with 0.1 mL of 1:10, 1:100, and 1:1,000 dilutions of each fish homogenate. Triplicate uninoculated control and positive control (infectious pancreatic necrosis virus) wells were prepared on each tissue culture plate. Homogenate aliquots were allowed to adsorb to monolayers for 1 h at 15°C; then an additional 1 mL of FM4-5% was added to each well. Plates were sealed in plastic bags and incubated at 15°C for 1 month. At this time, plates were frozen at −80°C and thawed; then 0.1-mL aliquots of each well were used to inoculate fresh monolayers for a second passage, after which aliquots were incubated for 1 month at 15°C.

One week after onset of the Cayuga syndrome, one of the replicate groups of sac fry used in virus isolation was processed as usual and the other replicate was used to check for the presence of highly cell-associated viruses. Single-cell suspensions were prepared from whole sac fry by teasing the fry through a 60-μm-mesh, autoclavable screen

(Tetko, Elmsford, New York). Suspensions of these cells were prepared in FM4-5% and cell concentrations were determined with a hemocytometer. Cell viability was assessed by trypan blue exclusion. Monolayers of the four cell lines were inoculated with 10^4, 10^5, or 10^6 live cells. Cultures were incubated for 1 month at 15°C. Second passages of these cultures were prepared as for the conventional virus isolations.

At sampling times in which sac fry were examined, wet mounts of skin scrapings and gill tissues were taken from 10 sac fry from each source. Impression smears of blood, anterior kidney, and skin stained with Diff-Quick (Dade Diagnostics, Aguada, Puerto Rico) were also examined from these fish.

Epidemiological Investigations

Given the lethal condition exhibited by Cayuga Lake sac fry, we began efforts to determine the viability of stocks from Owasco, Keuka, Seneca, and Skaneateles lakes–four of the five other Finger Lakes periodically stocked with the landlocked Atlantic salmon. In the fall of 1989, five males and eight females were electroshocked from Grout Brook, a tributary of Skaneateles Lake. These Skaneateles fish were tagged and held at the weir on Cayuga Inlet along with the returning Cayuga fish. After spawning, dry fertilization, and water hardening in Cayuga Inlet water (≈9.0°C), the eggs were transported to the Bath hatchery. Eggs from the five Cayuga and eight Skaneateles females were pooled by lake source and incubated in vertical Heath trays served with spring water (7.8–9.7°C). At eye-up, the Cayuga and Skaneateles eggs were divided into triplicate lots at a density of 600/tray; a fourth tray was used to hold the surplus eggs from each stock (Cayuga, 4,184; Skaneateles, 7,167). Also at eye-up, 3,000 eggs from five Little Clear Pond females were pooled and transported from the Adirondack hatchery to the Bath facility. At the Bath hatchery, the Little Clear eggs were split into triplicate Heath incubators as described (600/tray).

The Little Clear, Skaneateles, and Cayuga stocks were maintained in the same vertical series of incubators, with the Little Clear stock on top and the Cayuga stocks at the bottom. Mortality was monitored at least every 2 d beginning within 0 d (Little Clear) or 3 d (Cayuga and Skaneateles) of 50% hatch; development had proceeded for approximately 430 or 515 degree-days (cumulative average daily temperatures above 0°C) for the Cayuga and Skaneateles and the Little Clear stocks, respectively. The replicates from each stock were pooled on January 30, 1990, to clear space for new stocks. By this time, the Cayuga and Skaneateles stocks had accumulated 657 degree-days and the Little Clear stock had developed for 742 degree-days. When appropriate, chi-square statistics were calculated to compare survival frequencies ($\alpha = 0.05$).

Obtaining mature Atlantic salmon from the other Finger Lakes has been difficult because of the small populations in these systems. Nonetheless, one female was captured from Cold Brook, the largest tributary to Keuka Lake, in the fall of 1992. Eggs from that fish were fertilized with sperm from two Cayuga Lake males because no Keuka males were obtained and because (as reported herein) fertilization with Cayuga sperm does not result in Cayuga syndrome. The eggs were cultured at ambient temperature in the same tank previously described for the horizontal transmission studies. A second female from Cold Brook was obtained in the fall of 1993, as were eight female Atlantic salmon from the mouth of Katherine Creek, the principal inlet of Seneca Lake (Figure 1). These eggs were fertilized with sperm from two or three Seneca Lake males. Survival of the offspring from these 1993–1994 broodstock collections was compared against survival of progeny from six females from Cayuga Lake and 10 females from Little Clear Pond, as previously described.

Results

Effects of Incubation Conditions on Cayuga Syndrome Manifestation

Survival was not improved by decreasing incubation temperatures from eye-up through hatch. In 1983–1984, three sac fry from one Cayuga Lake female (1.5%) survived under the constant-temperature culture regimen. Many progeny from a second female survived up to first feeding (which began at about 950 degree-days) when cultured at both the reduced temperatures (15% survival) and constant temperature (69% survival); however, all of these first-feeding fry eventually died. A third female's progeny cultured at constant temperature exhibited low survival up to the feeding stage (6.5% survival), but died shortly thereafter. None of the progeny cultured at depressed temperature survived to first feeding in the 1983–1984 or 1984–1985 seasons. Survival of control sac fry from hatch to first feeding (950 degree-days) averaged 94.96% (SD, 4.52) in 1983–1984, and 92.49% (5.56) in 1984–1985.

Temperature reduction significantly accelerated

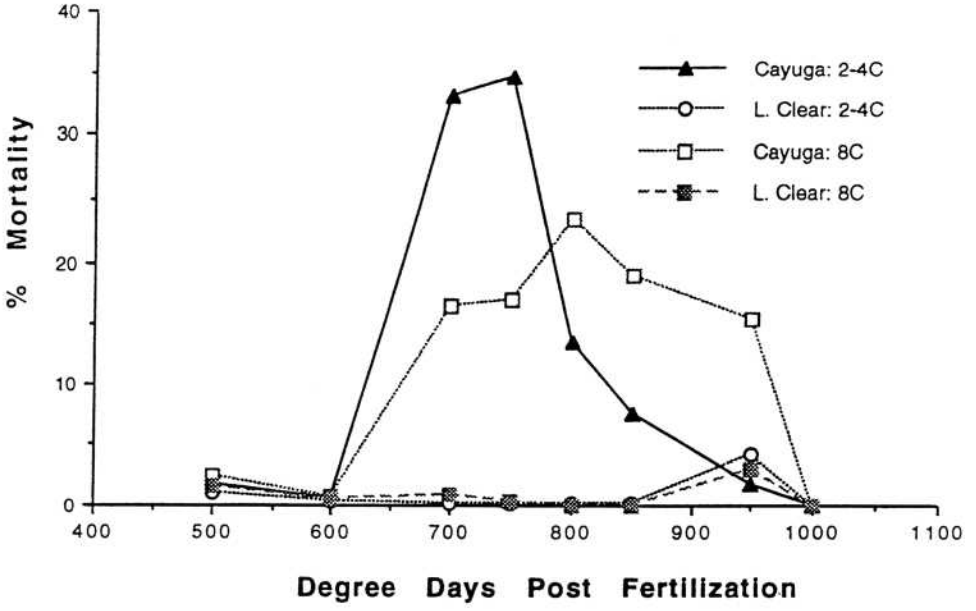

FIGURE 2.—Mortality of Atlantic salmon sac fry from Cayuga Lake and Little Clear Pond (control) incubated at a constant 8°C or with a temperature reduction (from 8°C to 2–4°C) from eye-up through hatching. Data are average percentages of total progeny mortality. Ten females from each of the two sources were used; 200 progeny per female were subjected to each temperature regimen.

the degree-day timing of the Cayuga syndrome (Figure 2) ($F = 10.985$, df = 1, $P = 0.009$; for females, $F = 6.817$, df = 9, $P = 0.0043$, 1983–1984 data only). Embryonic mortality of Cayuga Lake progeny was not affected by temperature manipulation, averaging 3.0% (SD, 3.1) at decreased temperature, and 2.4% (1.6) at constant temperature (exclusive of an outlier datum from one female's progeny that exhibited 37% egg mortality).

Culture of Cayuga progeny under ambient temperatures did not improve survival in either 1992–1993 or 1993–1994 (Figure 3). As observed in the

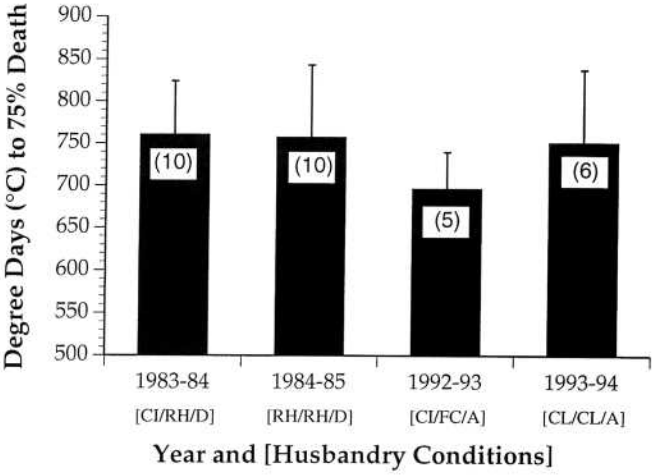

FIGURE 3.—Average degree-days to 75% death (error bars indicate SD) for Cayuga Lake sac fry incubated under a variety of conditions. Number of females represented are shown in parentheses. The bracketed husbandry codes signify hardening water/incubation water/temperature regimen. Code terms are CI = Cayuga Inlet, FC = Fall Creek (filtered and dechlorinated), RH = Rome hatchery lake water (Delta Reservoir, Mohawk River), CL = Cayuga Lake (filtered and dechlorinated); D and A = depressed and ambient temperature, respectively.

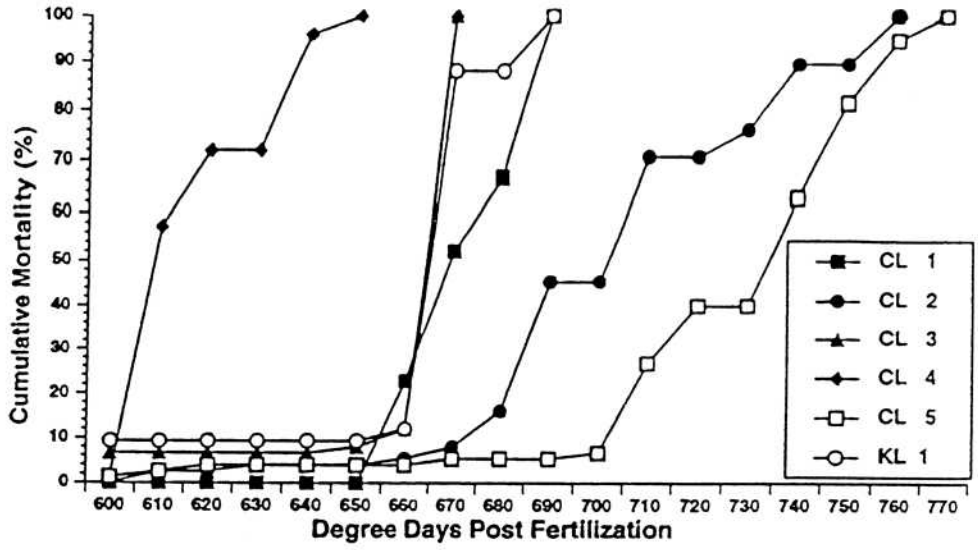

FIGURE 4.—Cumulative percent mortality of Cayuga Lake (CL) and Keuka Lake (KL) landlocked Atlantic salmon progeny separated by female (numbers in the key). Data represent starting numbers of about 75 eyed eggs/female during the 1992–1993 season.

temperature reduction study, the onset of mortality occurred between 600 and 750 degree-days in both seasons.

Despite widely varied water chemistries (Table 1) and thermal regimens, there was no significant difference in the TTD75 of Cayuga sac fry over the past 10 years ($F = 0.9325$, df = 3, $P = 0.439$; Figure 3) or when the hardening and incubation waters were considered together in a two-way ANOVA calculation (for hardening, $F = 1.302$, df = 2, $P = 0.286$; for incubation, $F = 3.82$, df = 1, $P = 0.060$). Nonetheless, there was obvious variation in the onset and progression of the Cayuga syndrome among progeny from individual females within a single season. To illustrate this point, we present the cumulative mortality curves for the progeny from the 1992–1993 broodstock only (Figure 4). A one-way ANOVA of the 1992–1993 data from five females highlighted a significant difference in the mortality rate (i.e., total number of larvae dead after x degree days) among progenies ($F = 363.84$, $P \leq 0.0001$). Scheffé's multiple comparison indicated that the progeny death rate in 1992–1993 was significantly different ($P \leq 0.05$) for all comparisons of Cayuga dams except the one between Cayuga females 1 and 3 (Figure 4).

Transmissibility Studies

Crosses with Cayuga Lake ova resulted in 100% mortality of the sac fry regardless of sperm source, whereas Cayuga sperm had no deleterious effect on larval survival (Table 2). The survival from hatching to first feeding did not differ significantly between the negative control cross (Little Clear eggs × sperm) and the cross of Little Clear ova × Cayuga sperm ($\chi^2 = 6.048$, $P > 0.10$). Furthermore, there was no unusual difference in mortality rate between sac fry from the cross of Cayuga ova × Little Clear sperm and those from the positive control cross (Cayuga ova × sperm); all progeny from these crosses died between 650 and 850 degree-days, as observed in the other studies. Replicates of each treatment were pooled for analysis of prehatch survival; there was a significant difference between crosses from fertilization to eye-up ($\chi^2 = 224.3$, $P < 0.001$) and from eye-up to hatching ($\chi^2 = 22.1$, $P < 0.001$).

There was no evidence that a causal agent for the Cayuga syndrome could be horizontally transmitted to progeny from the Little Clear or Tunison control stocks. During the 1992–1993 season, the average cumulative mortality observed among Little Clear progeny from eye-up to first feeding was 2.93% (range, 0–5.3%), and that observed among Tunison progeny was 9.85% (4–21.3%). During 1993–1994, the cumulative egg mortality of the Little Clear stock averaged 0.39% (range, 0–1.33%); the Tunison stock egg mortality was 46.84%. The survival from hatch to first feeding was excellent for both control stocks (Little Clear average, 99.7%; Tunison, 100%).

TABLE 2.—Survival data from a reciprocal cross-breeding experiment with Cayuga Lake (CL) and Little Clear Pond (LCP) landlocked Atlantic salmon, 1988–1989 season; f denotes females and m denotes males. Empty cells indicate no data.

Stage	CL(f) × CL(m)		CL(f) × LCP(m)		LCP(f) × LCP(m)		LCP(f) × CL(m)	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
	Number of eggs or sac fry							
At start	500	500	500	500			500	500
Eyed eggs[a]	478	482	363	349	500	500	415	420
Eggs hatched[b]	469	472	353	337	482	477	412	414
First feeding	0	0	0	0	460	438	388	395
	Percent survival							
From eggs to hatching	98.1	97.9	97.3	96.6	95.4	91.8	99.3	98.6
From hatching to first feeding	0	0	0	0	95.4	91.8	94.2	95.4

[a] Significantly different between-cross survival from fertilization to eye-up (χ^2, $P < 0.001$).
[b] Significantly different between-cross survival from eye-up to hatching (χ^2, $P < 0.001$).

Microbiological and Virological Investigations

A variety of gram-positive and gram-negative bacteria were isolated, primarily from FTG-enriched preparations. No consistent genera were isolated nor was heavy growth of bacteria observed on primary isolates from fish (Table 3). Neither parasites nor microbial pathogens were observed in the preparations of skin scrapings, gill tissues, or impression smears. Finally, no cytopathic effects were observed in any of the cell cultures in first or second passages. A less-extensive effort in the 1989–1990 season also failed to isolate any microbial pathogens associated with the Cayuga syndrome (unpublished data).

Epidemiological Investigations

1989–1990 study.—Survival was significantly different between the Skaneateles and Little Clear stocks from the start of the experiment (eye-up) until the replicates were pooled ($\chi^2 = 47.99$, $P < 0.001$). By the time of pooling, the cumulative mortality in the Cayuga replicates averaged 77.4% (range, 66.8–85.8); all the remaining Cayuga sac fry died within 4 d thereafter (Table 4). After the replicates were pooled, there was no significant difference in survival between the Little Clear and Skaneateles stocks through first feeding ($\chi^2 = 0.3226$, $P > 0.50$).

1992–1994 studies.—All progeny from the single, wild-caught, Keuka Lake female, obtained during the fall of 1992, died as sac fry between 650 and 690 degree-days after fertilization (Figure 4). Although Scheffe's multiple comparison indicated that the mortality rate of Keuka Lake progeny differed significantly ($P \leq 0.05$) from that of all Cayuga Lake sac fry obtained during the same

TABLE 3.—Genera and species (when identifiable) of bacterial isolates from Little Clear Pond (control) and Cayuga Lake (syndrome-afflicted) sac fry of Atlantic salmons. All cultures were isolated from internal milieu in fluid thioglycollate (FTG) broth, during 1988–1989 studies. If greater than one, the number of isolates is given in parentheses.

Little Clear Pond		Cayuga Lake	
Eggs	Sac fry	Eggs	Sac fry
Inside egg	*Acinebacter*	Inside egg	*Aeromonas hydrophila*
Bacillus spp. (2)	*Acinebacter calcoaceticus*	*Bacillus* spp. (3)	*Aeromonas salmonicida*
Corynebacterium spp. (2)	*Bacillus* sp.	*Corynebacterium* sp.	*Bacillus* sp.
Staphylococcus spp. (2)	*Klebsiella pneumoniae*	*Pseudomonas* spp. (2)	*Cardiobacterium* sp.
	Micrococcus sp.	*Staphylococcus* sp.	*Corynebacterium aquaticum*
	Moraxella sp.		*Chromobacterium* sp.
Outside egg	*Pasteurella* sp.	Outside egg	*Eikenella* sp.
Pseudomonas vesicularis	*Pasteurella haemolytica*	*Pseudomonas* spp. (3)	*Klebsiella* sp.
Moraxella sp.	*Plesiomonas vibrio*	*Pseudomonas piketti*	*Pasteurella* spp. (2)
	Pseudomonas sp.	*Pseudomonas putida*	*Pseudomonas* spp. (10)
	Pseudomonas cepacia	*Enterobacter* sp.	*Pseudomonas vesicularis*
	Pseudomonas picketti		*Yersinia* sp.
			Acinetobacter calcoaceticus[a]
			Aeromonas hydrophila[a]

[a] Surface was not disinfected.

TABLE 4.—Survival of young Atlantic salmon in the 1989–1990 epidemiology investigations into the Cayuga syndrome. Ranges are in parentheses.

Lake source of eggs	Number of eyed eggs at start, per replicate	Number alive by pooling date (Jan 30, 1990)	% alive:		
			Eye-up to pooling	Pooling to first feeding	Cumulative
Skaneateles	600	574 (568–578)[a]	95.6 (94.7–96.3)	97.9	97.0
Little Clear	600	540 (530–555)	89.4 (88.3–92.5)	97.6	90.7
Cayuga	600	135 (85–199)	22.6 (14.2–33.2)	0	0

[a] Significantly better survival than Little Clear Pond (control) progenitor stock ($P < 0.001$).

season—except those from Cayuga female 1—the general pattern of mortality and the pathological lesions exhibited by the Keuka sac fry were very similar to those observed among the Cayuga Lake fish (Fisher et al., in press).

All progeny derived from Cayuga, Seneca, and Keuka lake broodstocks during the 1993–1994 season succumbed to the syndrome, and their clinical signs were consistent with previous observations of Cayuga sac fry (Fisher et al., in press). There was no significant difference in the mortality rate (TTD75) of progeny from these three Finger Lakes ($F = 1.1508$, df = 2, $P = 0.3398$; Figure 5). Progeny from one Seneca female exhibited no signs of the syndrome, and survival of her progeny was 100% from hatch to first feeding. From genetic analysis it was determined that this female was a misidentified brown trout (D. Perkins, Cornell University, personal communication).

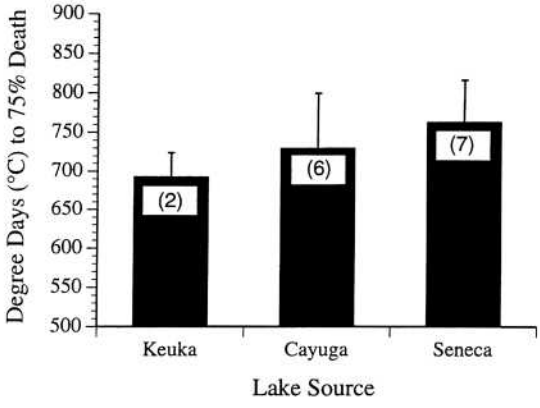

FIGURE 5.—Average centigrade degree-days to 75% death (error bars indicate SD) of sac fry from three syndrome-affected Finger Lakes. Averages are for the 1993–1994 season except the one for Keuka Lake, which includes data from the single female obtained in the 1992–1993 season. Numbers of females represented are shown in parentheses; averages represent about 60 sac fry/female.

Discussion

The results of these studies indicate that prolonged residence within Cayuga Lake results in complete reproductive failure for the female landlocked Atlantic salmon. Over the course of these studies, the viability of progeny from 46 Cayuga Lake salmon was examined, yielding effectively identical results: 100% sac fry mortality. Thus, we conclude that the lack of survival of syndrome-afflicted Atlantic salmon in the Finger Lakes is not female specific.

The results from the reciprocal crossbreeding studies presented here conclusively demonstrate that the Cayuga syndrome is vertically transmitted via the ova (Table 3). That the progeny of Little Clear ova × Little Clear sperm and Little Clear ova × Cayuga sperm crosses were cultured at the Adirondack hatchery until eye-up, rather than at the Bath hatchery throughout incubation, does not invalidate these results because we have shown herein that neither the water-hardening nor incubation waters determine whether progeny will succumb to the syndrome. Numerous fish pathogens such as infectious pancreatic necrosis virus (Bullock et al. 1976), infectious hematopoetic necrosis virus (Wolf et al. 1973), and *Renibacterium salmoninarum* (Evelyn et al. 1984) are known to be transmitted vertically through the ova, and all can cause high mortality among offspring. With all of the aforementioned disease agents, horizontal transmission via infected water can be demonstrated (Post 1987). We were unable to horizontally transmit the Cayuga syndrome, nor were we able to consistently isolate bacterial, parasitic, or viral fish pathogens. Therefore, we conclude that the Cayuga syndrome has a noninfectious etiology.

One of the clinical signs seen in approximately 30% of syndrome-afflicted sac fry is a mild subcutaneous edema between the yolk sac and vitelline membrane (Fisher et al., in press). In severe cases, this edematous condition is known as "blue-sac" (Spitsbergen et al. 1991). Temperature de-

pression significantly reduced the incidence of blue-sac among lake trout and greatly increased survival (Symula et al. 1990), but temperature reduction was without effect on Cayuga Lake Atlantic salmon. The apparent acceleration of the Cayuga syndrome under the reduced temperature regimen probably reflects a more advanced development state of these sac fry relative to that of fry held at constant temperature (Figure 2). The progeny held at reduced temperature generally hatched in fewer degree-days than the constant-temperature stocks; this direct relationship between temperature and degree-days to hatch has been established for numerous other salmonid species (Leitritz and Lewis 1976). Culture of Cayuga Lake progeny at ambient temperatures provided a natural environmental thermal regime for incubation, yet still did not improve survival.

One of the most unusual characteristics of the Cayuga syndrome is its consistent life stage specificity. We have shown here that the stage specificity has not changed notably over the past 10 years, despite the variety of incubation conditions under which Cayuga salmon were reared. Such discrete timing of the Cayuga syndrome is typical of a contaminant-mediated disease (Spitsbergen et al. 1991); in such a model, the variation in the time of syndrome onset might reflect a dose–response effect. Yet, investigations into contaminant etiologies revealed (1) maximum PCB concentrations in Cayuga eggs of only 0.4 μg/g wet weight as Aroclors 1254 and 1260 (Fisher 1995), (2) a background level in egg extracts of only 2 pg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents per gram of egg (D. Tillitt, National Biological Service, personal communication), (3) an inability to reproduce the syndrome by experimentally burdening Little Clear Pond embryos with up to 18 μg/g wet weight of a 1:1:1:1 mixture of Aroclors 1016, 1221, 1254, and 1260 (Fisher et al. 1993), (4) an inability to reproduce the syndrome by microinjecting hexane extracts of Cayuga Lake eggs into healthy Little Clear Pond embryos or sac fry (Fisher 1995), and (5) levels of heavy metals in both eggs and sac fry of Cayuga Atlantic salmon below toxicity thresholds (Fisher 1995). Further evidence against a contaminant etiology is the lack of similar reproductive problems in the other salmonid species inhabiting the syndrome-affected Finger Lakes. This latter point is highlighted by the excellent survival of the progeny from the misidentified Seneca Lake brown trout during 1993–1994.

The Cayuga syndrome differs from mortality syndromes reported in Great Lakes salmonids on three key points: (1) it occurs well before first feeding (i.e., swim-up), when approximately 30–80% of yolk reserves remain (Fisher et al., in press); (2) mortality is virtually always 100%; and (3) the maternal source of eggs affects only the time of syndrome onset during development, not the ultimate survival of the sac fry. The syndromes documented for lake trout and Pacific salmonids from the Great Lakes generally occur at swim-up, during first-feeding (Mac et al. 1985; Skea et al. 1985); mortality usually does not exceed 40% (Mac and Edsall 1991), and the viability of progeny from hatch to first feeding is female specific (Skea et al. 1985; Mac et al. 1993). Efforts to obtain Atlantic salmon from Lake Ontario have thus far been unsuccessful, so it is possible that the Cayuga syndrome simply reflects a species difference in the syndrome(s) manifest in Great Lakes salmonids.

It was shown that the Cayuga syndrome also affects salmon from Keuka and Seneca lakes (Figure 5), revealing that this condition is more widespread than initially thought. However, the excellent survival of progeny from Skaneateles salmon (Table 4) indicates that maturation within at least one of the Finger Lakes will not result in syndrome-related mortality. Based upon reported nutrient profiles, Skaneateles Lake is considered oligotrophic, Keuka Lake varies between an oligotrophic and mesotrophic status, and Cayuga and Seneca lakes can be described as mesotrophic (Oglesby et al. 1975). The major ion concentrations also vary substantially between the Finger Lakes. For example, Cayuga and Seneca are dominated by excessive concentrations of Na^+ due to underlying salt deposits (Figure 6). We consider it unlikely that the differing chemistries of these waters play a role in the perpetuation of the Cayuga syndrome for two reasons. Firstly, all of the Finger Lakes with Atlantic salmon populations are extremely well-buffered waters due to the calcareous shale, siltstone, and Tully limestone common to the area (Oglesby et al. 1975). Hardness and pH "biases" are thus introduced (Szumski and Barton 1983), so that the toxicities of many pollutants known to affect fishes, such as acid rain and aluminum (DeLonay et al. 1993) and cadmium, copper, and lead (Schubauer-Berigan et al. 1993), are greatly reduced. Secondly, it was not possible to reproduce the Cayuga syndrome in situ by rearing control Little Clear and Tunison stocks from fertilization to swim-up in Cayuga Lake or Cayuga

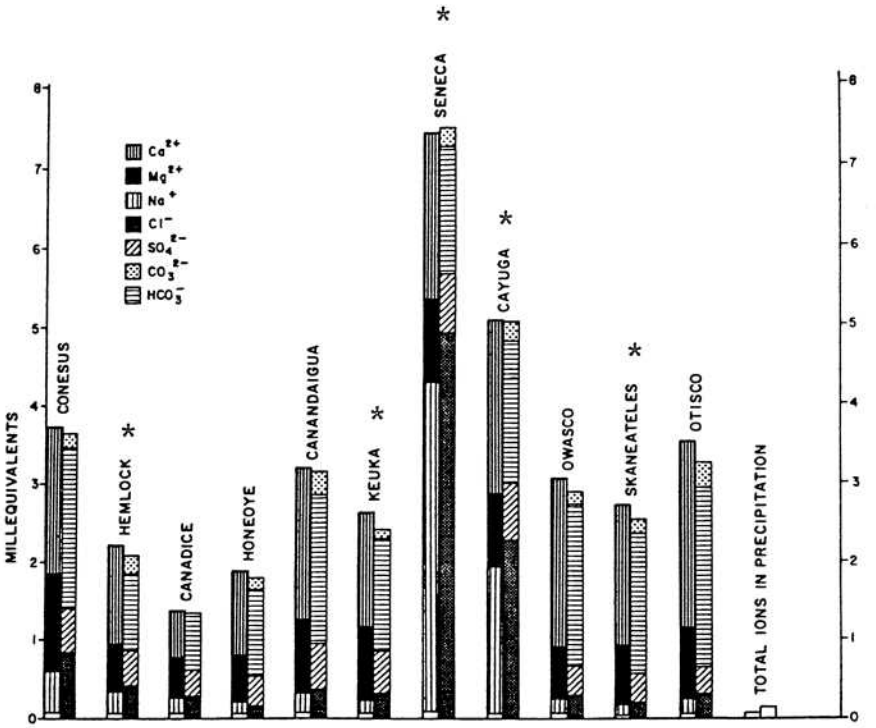

FIGURE 6.—Milliequivalent balances for Finger Lake waters. Asterisks indicate systems with ongoing Atlantic salmon stocking programs (modified from Oglesby et al. 1975, with the author's permission).

Inlet water that was not filtered or previously chlorinated (Fisher 1995).

Perhaps more noteworthy than the water quality differences between the Finger Lakes is that Cayuga, Seneca, and Keuka lakes have similar forage bases, principally alewife *Alosa psuedoharengus* and rainbow smelt. In contrast, Skaneateles Lake has no smelt or alewives, and the negative control salmon from Little Clear Pond subsist principally on rainbow smelt, because there is no alewife forage there. Reports of high thiaminase in alewives (Gnaedinger 1964) suggest that a naturally occurring thiamin deficiency may be involved. Indeed, the ataxia, paralysis, cardiovascular lesions, and edema exhibited by moribund Cayuga Lake sac fry (Fisher et al., in press) are consistent with the behavioral and pathological manifestations of thiamin deficiencies reported in other animals (Green and Evans 1940; Marcus and Coulston 1990).

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