JOURNAL OF AQUATIC ANIMAL HEALTH

Pathological and Behavioral Manifestations of the "Cayuga Syndrome," a Thiamine Deficiency in Larval Landlocked Atlantic Salmon

JEFFREY P. FISHER,[1,2] JAN M. SPITSBERGEN,[1,3] AND TINA IAMONTE

*Department of Avian and Aquatic Animal Medicine, College of Veterinary Medicine
Cornell University, Ithaca, New York 14853-6401, USA*

EDWARD E. LITTLE AND AARON DELONAY

*Midwestern Science Center, National Biological Service
Route 2, 4200 New Haven Road, Columbia, Missouri 65201, USA*

Abstract.—The "Cayuga syndrome" is a maternally transmitted, naturally occurring thiamine deficiency that causes 100% mortality of larval landlocked Atlantic salmon *Salmo salar* in several of New York's Finger Lakes. Results of multiyear studies to qualify and quantify the neurobehavioral and gross pathological signs of this condition are described. Affected sac fry became weak and ataxic and responded atypically to stimuli 1–2 weeks before death. Quantitative assays of stimulus-provoked swimming revealed a significant neuropathy whereby the sac fry exhibited abnormal thigmotactic and phototactic behaviors. Gross lesions observed in Cayuga sac fry included yolk-sac opacities, subcutaneous edema, vitelline hemorrhage or congestion, pericardial edema, retrobulbar edema, branchial congestion, foreshortened maxillae, hydrocephalus, and occasional caudal fin deformities. Lesion frequency in progeny differed significantly between dam source. Yolk conversion efficiency was decreased at least 1 week before death, suggesting that the bioenergetics of the fish was compromised and thereby supporting the thiamine residue and treatment data reported elsewhere. Comparisons with coagulated-yolk, blue-sac and swim-up syndromes are presented. The pathological signs of the Cayuga syndrome represent a unique departure from the lesions induced by toxicants or pathogens in other piscine models, and for the first time profile the profound effects of thiamine deficiency on cardiovascular and neurological systems of larval fish.

A variety of infectious and noninfectious agents can cause mortality of larval fishes with pathological signs lacking etiologic specificity. Thus, the diseases of larval salmonids known as coagulated-yolk and blue-sac share considerable pathological features. Coagulated-yolk disease, also known as white-spot, is characterized by the development of opaque, off-white concentrations of yolk in the normally translucent yolk sac of an embryo or eleutheroembryo (Mazuranich and Nielson 1959). Coagulated-yolk has been attributed to bacterial infection (Von Betegh 1912; Guberlet et al. 1931), rough handling (Dietrich 1939;

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[1] Address reprint requests to the Department of Avian and Aquatic Animal Medicine, Cornell University.

[2] Present address: Division of Environmental Disorders, Wadsworth Center for Laboratories and Research, Post Office Box 509, Albany, New York 12201, USA.

[3] Present address: Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331-6602, USA.

Davis 1953), incubation without substrate (Emadi 1973; Murray and Beacham 1986), gas-bubble disease (Wood 1968), exposure to zinc-galvanized metals (Wolf 1970), decreased oxygen concentrations (Agersborg 1933), soft incubation water (MacKinnon 1969), and repeated treatments of dilute malachite green (1:200,000 weight/volume) through hatching (Mazuranich and Nielson 1959). Recently, Norrgren et al. (1993) reported that coagulated-yolk was associated with the lethal M-74 syndrome of larval Baltic Atlantic salmon and suggested that lipophilic contaminants were responsible. Coagulated-yolk has not been reported with the swim-up syndromes of older, first-feeding lake trout *Salvelinus namaycush* in the Great Lakes basin (Mac et al. 1985; Mac and Edsall 1991; Fitzsimons et al., in press).

Coagulated-yolk disease may progress to the point that a clear to pale-blue fluid accumulates between the yolk sac and the perivitelline membrane. The diseased condition characterized by this subcutaneous edema is also known as blue-sac, hydrocoele embryonalis, or yolk-sac dropsy (Von Betegh 1912; Guberlet et al. 1931; Dietrich 1939). Like coagulated-yolk disease, blue-sac has been attributed to bacterial infection (Von Betegh 1912; Guberlet et al. 1931; Symula et al. 1990), but it has also been attributed to halogenated aromatic hydrocarbon (HAH) poisoning (Spitsbergen et al. 1991), ammonia and urea toxicity (Wolf 1957), and excessive incubation temperatures (Symula et al. 1990). Notably, blue-sac disease may occur without evidence of coagulated yolk (Wolf 1957; Symula et al. 1990; Spitsbergen et al. 1991). Likewise, coagulated-yolk disease has been observed when subcutaneous edema (i.e., blue-sac) was not apparent (Mazuranich and Nielson 1959; MacKinnon 1969; Rucker et al. 1970). Whether they occur independently or together, both blue-sac and coagulated-yolk disease are usually lethal conditions whose etiologies and pathogenesis have not been fully resolved.

In the present study, we describe the gross and neurobehavioral pathologies observed in larval landlocked Atlantic salmon *Salmo salar* afflicted with the "Cayuga syndrome." The Cayuga syndrome is a maternally transmitted, noninfectious disease that was first observed in landlocked salmon sac fry (alevins) from Cayuga Lake in central New York State and was recently found to afflict other Finger Lake stocks (Fisher et al. 1995). The Cayuga syndrome causes 98–100% sac fry mortality approximately 650 to 850 degree-days (°C) after fertilization. Coagulated-yolk and subcuta-

neous edema are also seen in sac fry afflicted with the Cayuga syndrome; however, the expression of these pathologies differs from that observed in previously reported early life stage diseases.

The landlocked Atlantic salmon in Cayuga Lake originate from Little Clear Pond stock, a captive, naturally fortified Adirondack broodstock. The Little Clear Atlantic salmon is the sole strain presently planted in New York waters, and it supports numerous fisheries throughout the state where the Cayuga syndrome is not observed. In the Finger Lakes, the stocking of the Little Clear strain has created highly desirable sport fisheries of significant economic and social value. Presently, fish are stocked as yearlings in 5 of the 11 Finger Lakes (Skaneateles, Cayuga, Seneca, Keuka and Hemlock), at rates of 1.2 to 5 fish/ha (T. Chiotti, New York State Department of Environmental Conservation [NYSDEC], personal communication). Survival to maturity in Cayuga Lake, albeit marginal, is by far the best of any of the Atlantic salmon fisheries in the Finger Lakes, and the NYSDEC would like to use the mature Cayuga Atlantic salmon as an additional broodstock. Because of the complete reproductive failure exhibited by these fish, such a management plan is not feasible, nor is it possible to establish self-sustaining populations. Consequently, the fishery is maintained only through the costly stocking program.

We have recently confirmed the hypothesis presented earlier (Fisher et al. 1995) that the Cayuga syndrome is a manifestation of a maternally transmitted thiamine deficiency. Our data continue to indicate that this deficiency arises because the salmon feed on thiaminase-rich alewives *Alosa pseudoharengus* (Fisher et al., in press). The gross and neurobehavioral signs of thiamine deficiency in the larval Atlantic salmon are reported here for the first time. The evidence of cardiovascular and neurological impairment associated with the Cayuga syndrome appears consistent with signs of thiamine deficiency reported in a variety of piscine and mammalian species (Harrington 1954; Irle and Markowitsch 1982; Okada et al. 1987).

Methods

Source of fish.—Progeny from the single Cayuga female obtained in the fall of 1990, a second captured in 1991, and five more taken in 1992 were examined in these studies. Sac fry from the single female captured from Keuka Lake in the fall of 1992 were also examined; this latter Finger Lake stock was evaluated as part of ongoing epidemiological investigations into the Cayuga syndrome

(Fisher et al. 1995). To serve as controls, eggs were pooled from five to eight Little Clear Pond females during the 1990–1991 and 1991–1992 seasons. In the 1992–1993 season, the Little Clear progeny were separated by dam ($N = 5$). Gross lesions exhibited by control Little Clear larvae were evaluated in the 1990–1991 and 1992–1993 seasons only.

The use of the Little Clear salmon as the control stock was warranted for these studies, because this captive, yet natural population is the progenitor for all landlocked salmon stocked by the NYSDEC (Fisher et al. 1995). In addition, eggs and sac fry from the Little Clear stock are routinely cultured without syndrome-related mortality at the adjacent NYSDEC Adirondack Hatchery near Saranac Lake, New York.

Husbandry.—All sac fry examined for lesions were from surplus stocks incubated in screened polyvinyl chloride cups at Cornell aquaculture facilities. These cups were housed in either a 20-L aquarium (1990–1991, 1991–1992), or in a 280-L fiberglass tank (1992–1993). Flow of dechlorinated tap water to the incubation aquaria was approximately 2 L/min. Densities in each culture cup ($N = 60$–140) decreased over time because of mortality and removal of fish for other studies. During the 1990–1991 season, the embryos and larvae were cultured at ambient temperatures until February 27, 1991, when a heating system was activated; average temperature was 8.9°C (SD, 1.04) during November, 6.2°C (0.91) during December, 3.6°C (0.48) during January, 3.5°C (1.06) during February, 6.9°C (1.06) during March, and 10°C (2.39) during April. During the 1991–1992 season, the fish were maintained at a constant 8.5°C ± 1°C. The ambient water temperatures used during 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 (2.01) from May 1 to May 11, when the studies were concluded. The water chemistry of the incubation waters was previously described (Fisher et al. 1995).

Gross lesion study.—During 1989–1990, a preliminary list of lesions associated with the Cayuga syndrome was established to quantify gross lesions for the present observations (Fisher and Spitsbergen, 1991 NYSDEC Biannual report, unpublished). Random samples of 18–22 moribund sac fry from each Cayuga dam were examined 3–7 d after behavioral signs of the syndrome were observed. Sac fry were anesthetized with a 100-mg/

L solution of tricaine methanesulfonate (MS-222; Sigma Chemical Co., St. Louis, Missouri) and examined with a dissecting microscope for the incidence of yolk capacity, subcutaneous edema, vitelline hemorrhage or congestion, pericardial edema, branchial hemorrhage or congestion, foreshortened maxilla, hydrocephalus, caudal congestion or atrophy, and spinal curvature. Because the sac fry were euthanized after examination and stocks were limited, only 10 progeny from each Little Clear female were examined to determine baseline (i.e., control) lesion frequencies. Where appropriate, contingency tables (χ^2) were used to evaluate the statistical significance of lesion frequencies between progeny from separate Cayuga females. Statistical analyses of between-female differences were not performed when the lesion frequency was 0 or 100%, because of bias in the chi-square statistic (Zar 1974).

Behavioral manifestations.—Assays of stimulus-provoked swimming behavior were performed on progeny from the 1992–1993 Cayuga and Little Clear broodstocks to assess the neurological impairment associated with the Cayuga syndrome. Single fish from each of the Cayuga and Little Clear females were placed in an 18.4-cm-diameter glass crystallizing bowl filled with 1 L of dechlorinated tap water (2.5 cm deep) at the ambient incubation temperature of 8°C ± 0.5°C. Each fish was videotaped in this open field for 4 min. The first 2 min were considered an acclimation period; the latter 2 min were considered the observation period. The water was changed completely after each assay. The glass bowl was placed inside a 23-L, semitransparent, Nalgene® (Nalgene, Inc., Rochester, New York) bucket, suspended approximately 30 cm above ground. A 200-W white lightbulb, projecting upward 10 cm beneath the Nalgene bucket, provided the light stimulus for swimming. The bucket diffused the light source evenly over the glass bowl and prevented the heating of the water during a trial. Water temperature did not change measureably during a trial.

All Little Clear and Cayuga sac fry were observed on the same date, and the order in which the fish were assayed was randomized. Sac fry from all five Little Clear females and from Cayuga (CL) females 1-92, 2-92, 4-92, and 5-92 were assayed. Sac fry from CL 3-92 died before the assay date. Five sac fry per female were evaluated, with the exception of CL 4-92, for which only the two larvae that remained alive were assayed. Little Clear progeny had developed for 694 degree-days (dd) and those from CL 1-92 and CL 2-92 for 686

dd, whereas those from CL 4-92 and CL 5-92 were 641 dd old. Progeny from CL 5-92 were observed a second time 9 d later (747 dd) to document how the syndrome-associated behavior changed over time.

The videos were displayed on a Panasonic™ CT-1382 S-VHS monitor (Panasonic, Inc., Seacaucus, New Jersey), and the images of sac fry swimming behavior were analyzed with a 486 PC-based real-time digital image processing system (Motion Analysis™ VP110, Motion Analysis Corp., Santa Rosa, California) (DeLonay et al. 1993). The distance traveled (D) by each fish was calculated, and we determined the average swimming speed (SS) by dividing the total distance swum by the time spent swimming; swimming time (T) was determined by stopwatch to the nearest second. For each 2-min acclimation and observation period, there were 110 s of video analyzed.

Data for distance, time, and swimming speed were grouped by lake source, because within-female variation compromised normality and variance-heterogeneity assumptions (Zar 1974). Data from CL 5-92 at 641 dd were excluded from the pooled analysis, as the sac fry had not yet exhibited obvious neurological signs of the syndrome. The Mann–Whitney U-test was used to compare medians, because data outliers (e.g., fish that did not swim) greatly altered the mean (Zar 1974). Separate analyses were performed for both the acclimation and observation periods. To determine whether any of the variables of interest differed between the Cayuga and Little Clear stocks between the acclimation and observation periods, two-sample t-tests for unequal variance were performed (Data Desk, Data Description, Inc., Ithaca, New York). For the t-tests, the ratio of the median acclimation time value to the median observation time value for each variable (i.e., D, T, and SS) was calculated for progeny from individual females, and these data were pooled by stock. Because this latter analysis was principally concerned with the rate of change between periods, the data from CL 5-92 at 641 dd were included (total df, 4).

Growth studies.—Yolk conversion efficiency was determined during the 1992–1993 season only. Sac fry were euthanized in MS-222 and fixed for at least 1 week in 10% neutral buffered formalin. Yolk sacs were dissected away from the body and dried separately on aluminum foil overnight at 38°C. Dissected yolk and body were weighed separately to the nearest 0.1 mg with a Mettler AE 160 (Mettler Instruments, Inc., Hights-

town, New Jersey) balance, and the ratio of yolk weight to total body weight (i.e., yolk weight/yolk + body weight) was calculated.

The sac fry assayed for stimulus-provoked swimming represented the "early" samples for this study (N = 5/female from CL 1-92, CL 2-92, CL 5-92, and each Little Clear dam). The "late" sample of sac fry from these same females (N = 10/dam) was taken at 748 dd (± 2 dd). Ten sac fry from CL 3-92 and CL 4-92 were sampled at 678 and 625 dd, respectively; subsequent mortality prevented a second sampling of progeny from these dams.

Possible differences in the yolk conversion were evaluated by an analysis of covariance to compare slopes (β) of yolk weight/total weight versus age (Zar 1974). Findings of significance (alpha = 0.05) were followed by Scheffé's multiple comparisons.

Results

Gross Pathology

The lesions exhibited by sac fry progeny from control Little Clear Atlantic salmon were primarily vascular in origin (e.g., intramuscular or vitelline petechia). The rarity of control lesions (Table 1), their mild severity, and their highly localized distribution suggested handling trauma from the examination. Development and vigor of the Little Clear sac fry were normal for the three seasons represented in these studies (Figure 1A, B). The maximum mortality of Little Clear sac fry used for these studies was only 3.4% from hatching through first feeding.

The frequency of most types of lesions exhibited by Cayuga and Keuka sac fry was highly female-dependent (Table 1); however, nearly all afflicted sac fry exhibited yolk opacities preceding death, regardless of maternal origin (Table 1; Figure 1B, C). In some cases, this was the only gross lesion observed (e.g., CL 5-92). These opacities were either granular (<1 mm diameter) and diffusely distributed throughout the yolk sac or large (1–3 mm) and irregular (Figure 1C). The larger opacities were commonly associated with the oil globule, the liver, or both (Figure 1D). Coagulated yolk was also observed forming perivascular plaques (Figure 1E).

Vascular congestion was commonly observed as the dilation of segmental vessels (Figure 1F); to a lesser extent it was also observed in the branchial region (Figure 1G) and around the caudal peduncle. Occasionally, hemorrhage caused pooling of blood on the distal aspect of the yolk sac (Figure

TABLE 1.—Gross lesion frequency in landlocked Atlantic salmon sac fry from Little Clear Pond (LCP), and syndrome-afflicted Cayuga Lake (CL) and Keuka Lake (KL). Results designated by female number (if cultured separately) are followed by year-class. Blank cells indicate that lesion frequency was not recorded. Standard errors are in parentheses.

Lesion or stage	Female number									
	LCP 90[a]	LCP 92[b]	CL 1-90[c]	CL 1-91	CL 1-92	CL 2-92	CL 3-92	CL 4-92	CL 5-92	KL 1-92
	Lesion frequency (number of sacfry with lesions/number examined or percent)									
YSO[d]	2/40	0.0 (0)	32/40	9/18	15/22	19/21	22/22	20/20	21/21	19/20
SCE[e]	1/40	2.0 (2.2)	10/40	6/18	6/22	1/21	10/22	14/20	0/21	19/20
PCE[f]		0.0 (0)			4/22	0/21	6/22	8/20	0/21	16/20
RBE[g]	0/40	0.0 (0)	1/40	2/18	0/22	2/21	4/22	2/20	0/21	6/20
VCHM[h]	3/40	1.4 (7.6)	3/40	6/18	7/22	7/21	9/22	8/20	1/21	13/20
BCHM[i]	0/40	0.0 (0)	4/40	6/18	7/22	3/21	8/22	5/20	0/21	11/20
HC[j]	0/40	0.0 (0)	0/40	1/18	0/22	0/21	0/22	0/20	0/21	3/20
FM[k]	0/40	0.0 (0)	0/40	0/18	0/22	0/21	1/22	1/20	0/21	5/20
CC-CD[l]	0/40	0.0 (0)	1/40	1/18	7/22	0/21	4/22	15/20	0/21	12/20
SC[m]	1/40	0.0 (0)	0/40	0/18	0/22	1/21	0/22	0/20	0/21	0/20
	Development reached (centigrade degree-days)									
DDays[n]	805, 834	678	750, 779	682	678	765	670	625	775	638
TTDO[o]	p	p	728 (11)	666	629 (24)	661	612	542 (0)	657 (35)	671 (12)
TTDC[q]	p	p	818 (11)	738	691 (6)	765	670	641 (0)	821 (0)	696 (0)

[a] Based on a pool of progeny from 8 females.
[b] Percent average of 10 progeny from each of five dams.
[c] Sum of two groups of 20 sac fry examined 4 d apart.
[d] Yolk-sac opacities.
[e] Subcutaneous edema.
[f] Pericardial edema.
[g] Retrobulbar edema.
[h] Vitelline congestion or hemorrhage.
[i] Branchial congestion or hemorrhage.
[j] Hydrocephalus.
[k] Foreshortened maxilla.
[l] Caudal congestion or deformity.
[m] Spinal curvature.
[n] Degree-days of development at time of examination.
[o] Degree-days reached at onset of syndrome.
[p] Not applicable; control progeny mortality from hatch to first feeding was 3.2% in 1990 and 2.9% (SE, 1.9) in 1992.
[q] Degree-days reached at conclusion of syndrome-related mortality.

1H). The frequency of vitelline hemorrhage or congestion in sac fry from CL 5-92 and CL 1-90 was significantly less than that in progeny from all other syndrome-affected females ($\chi^2 = 20.679$, $P < 0.005$).

Subcutaneous edema was the second most common lesion observed (Table 1). When present, however, it was not associated with complete vascular failure because blood circulated through the yolk sac until death (Figure 1C, I). The frequency of subcutaneous edema between progeny from the affected females was also significantly different ($\chi^2 = 62.279$, $P < 0.001$). An analysis of residuals indicated the frequency of subcutaneous edema in progeny from KL 1-92 and CL 4-92 was significantly greater than that of progeny from CL 1-90, CL 1-91, CL 1-92, and CL 3-92 whereas that of CL 5-92 progeny was significantly less ($P < 0.05$).

Pericardial and retrobulbar edema, foreshortened maxilla, hydrocephalus, and caudal deformities occurred at lower frequencies (Figure 1B,

J), and these lesions were absent in progeny from several females (Table 1). Commonly observed but not specifically quantified aberrations included bradycardia, periocular hemorrhage (Figure 1J), and fine tremors.

Behavioral Manifestations

Stimulated by light, the control Little Clear sac fry swam almost constantly by "ploughing" (Dill 1977) along the edge of the observation vessel during assay. When fatigued, Little Clear sac fry stopped swimming and drifted slowly onto their side. These rests usually lasted only a few seconds, whereupon they began swimming again, usually in the same direction as before.

Sac fry afflicted with the Cayuga syndrome exhibited a characteristic spastic behavior, whereby short bursts of uncoordinated swimming were followed by variable periods of stasis. In general, Cayuga sac fry lay on their sides until stimulated to swim, at which time they jerked themselves into
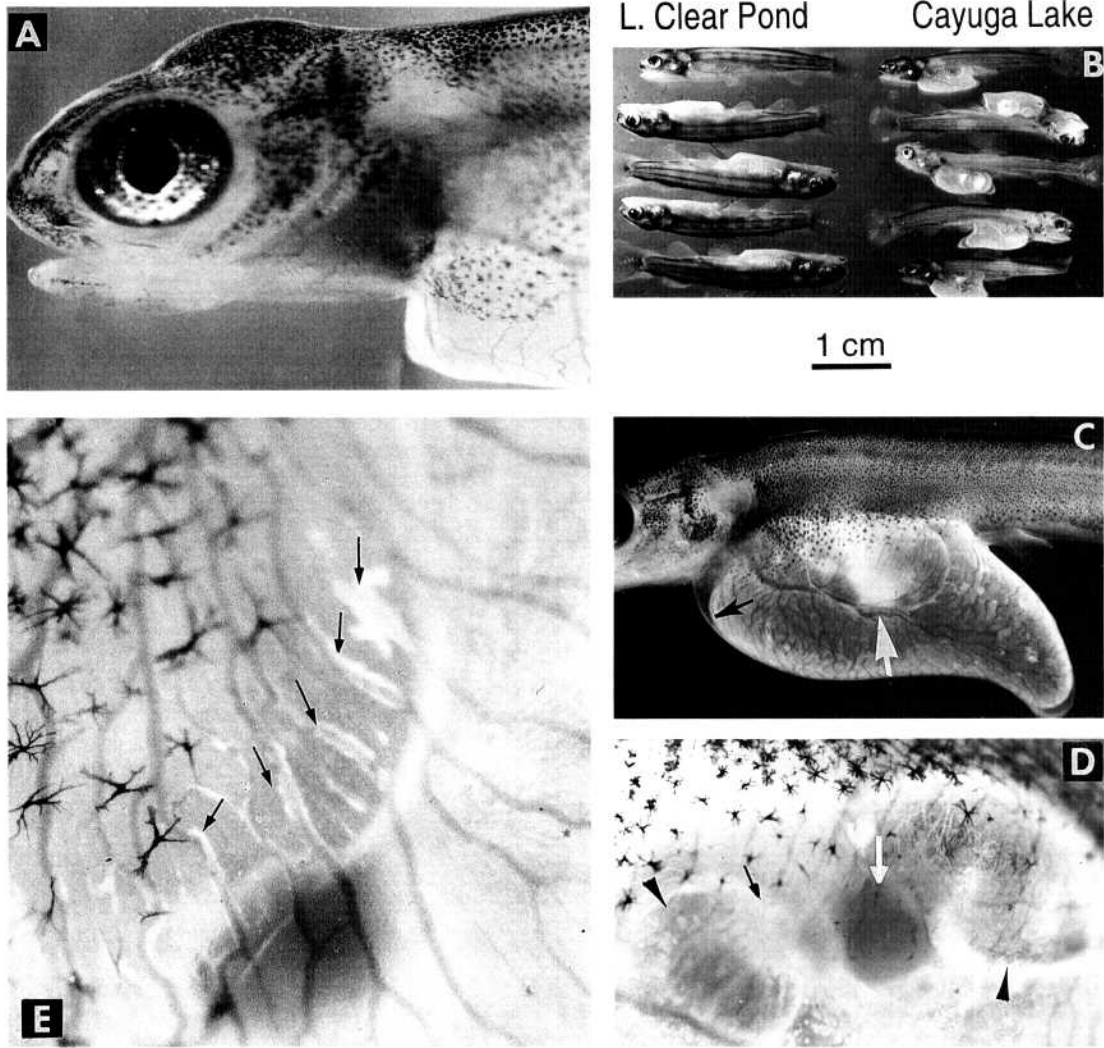
FIGURE 1.—Gross pathology of moribund (live) Cayuga Lake (CL) sac fry and healthy, control sac fry from Little Clear Pond (LCP). **(A)** An LCP sac fry at 746 centigrade degree-days shows normal cranial development and absence of branchial and circulatory lesions. **(B)** Identically aged LCP (left) and CL (right) sac fry show the smaller yolk sac and lack of opacities in LCP fry, whereas the CL fry show yolk opacities, a heterocercal tail (bottom fish), and small, malformed eyes and craniums (top and bottom CL fish). **C–J** Cayuga sac fry showing **(C)** large yolk opacities (white arrow) and mild subcutaneous edema (black arrow); **(D)** yolk opacities (thin black arrow) spatially associated with the liver (white arrow) and oil globule (black arrowheads); **(E)** yolk opacities forming plaques along blood vessels (black arrows); **(F)** congestion of segmental blood vessels in the yolk-sac (black arrows); **(G)** hemorrhage of branchial vasculature (white arrow); **(H)** hemorrhage causing pooling of blood in distal aspect of the yolk sac (white arrow); **(I)** severe subcutaneous edema (white arrow), although circulation in the yolk sac continues (black arrowhead) despite segmental vessel congestion (black arrows); **(J)** retrobulbar edema (thick black arrow), periocular hemorrhage (thin black arrow), and foreshortened maxilla (white arrowhead).

the vertical plough position, briefly swam in a non-directional pattern, stopped, and then drifted back onto their side. Reflecting this punctuated swimming behavior, the distance swum and the time spent swimming by Cayuga sac fry were signifi-

cantly less than that observed for control Little Clear sac fry for the acclimation period (median D: Cayuga = 98.8 cm, Little Clear = 623 cm, U = 51, P = 0.0001; median T: Cayuga = 15 s, Little Clear = 80 s, U = 36.5, $P \leq 0.0001$), and
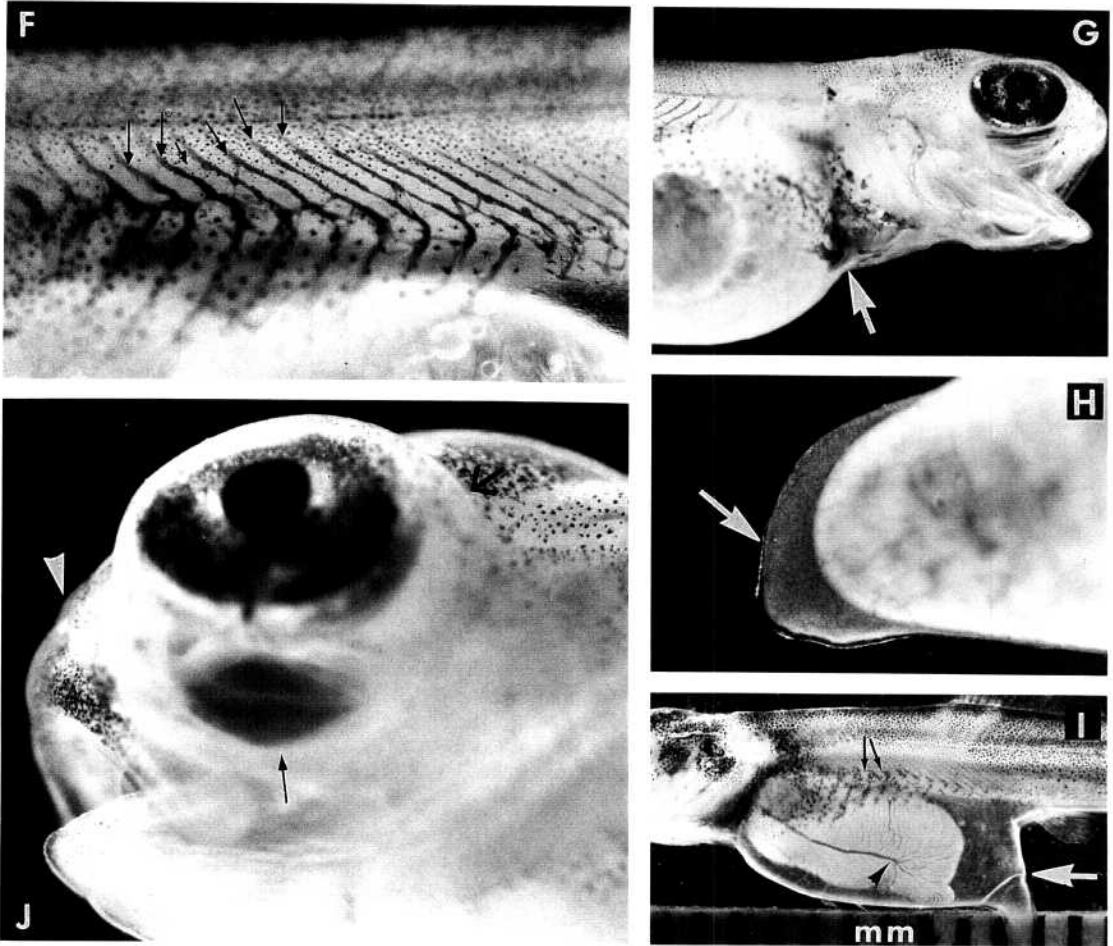
FIGURE 1.—Continued.

for the observation period (median *D*: Cayuga = 32.9 cm, Little Clear = 409 cm, *U* = 42, *P* ≤ 0.0001; median *T*: Cayuga = 4 s, Little Clear = 76 s, *U* = 50, *P* ≤ 0.0001) (Figure 2). Notably, the swimming speed (SS) of the Cayuga sac fry was greater than that of the Little Clear sac fry during both the acclimation period (median SS: Cayuga = 8.3 cm/s, Little Clear = 7.68) and the observation period (median SS: Cayuga = 9.67 cm/s, Little Clear = 6.04), although these speeds were not significantly different.

Although both the Cayuga and Little Clear sac fry moved more during the acclimation period than during the observation period (Figure 2), *t*-tests did not indicate any significant differences in swimming distance, duration, or speed between periods for either stock. The distance swum by progeny from Cayuga 5-92 declined during the later stages of the syndrome (747 dd) from that of younger fish (641 dd, when neurological signs of the syndrome were not obvious; Figure 2), but progeny from the other Cayuga females died before a later assay could be performed.

Swimming patterns differed markedly between Cayuga and Little Clear progeny (Figure 3). The Little Clear sac fry exhibited pronounced thigmotaxis by swimming along the edge of the observation bowl, as previously described. When a Little Clear sac fry deviated from this pattern, it swam in a nearly straight line across the open field until the perimeter of the vessel was again encountered, thereby initiating further thigmotactic behavior. In contrast, sac fry from Cayuga females rarely exhibited such a taxis. The failure of Cayuga sac fry to exhibit thigmotaxis was obvious, even in those individuals whose total distances and
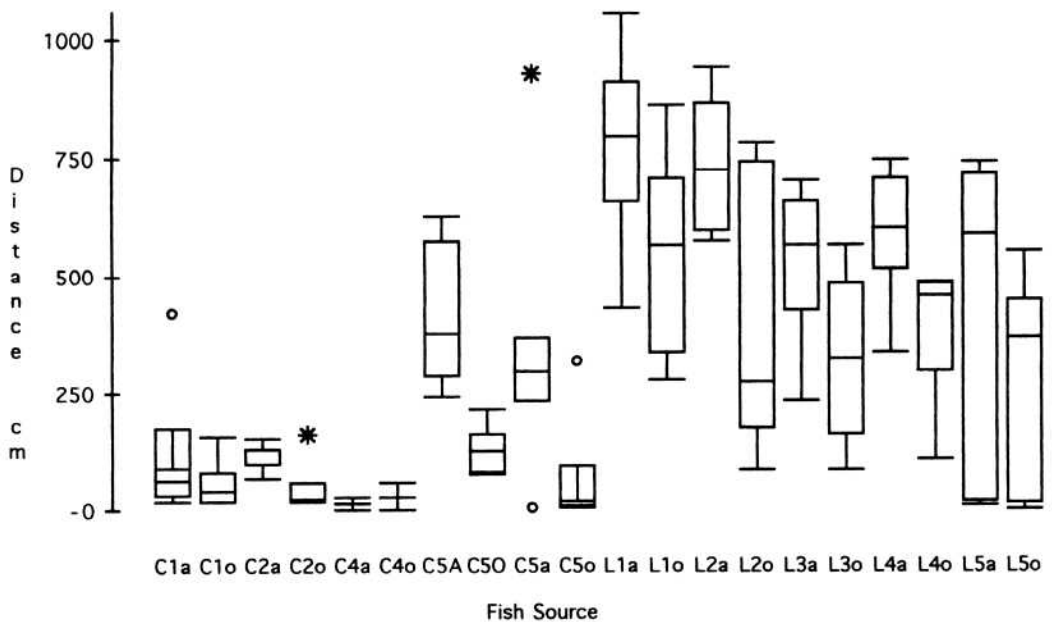
FIGURE 2.—Box plots of distance swum by Atlantic salmon sac fry from Cayuga Lake (C) and Little Clear Pond (L) during 110-s acclimation (a) and observation (o) periods, separated by individual female (number in the fish code). For Cayuga Lake 5-92 progeny, C5A and C5O assays were performed at 641 degree-days (dd), C5a and C5o at 747 dd. Each box depicts the data between the 25th and 75th percentiles; the horizontal line represents the median. Whiskers extending from the box embrace all data not considered outliers. Open circles indicate outliers (exceeding 1.5 times the vertical box dimension); asterisks depict extreme outliers (exceeding 3.0 times the vertical box dimension; Hoaglin and Velleman 1981).

times swum were similar to those of the Little Clear progeny (Figure 3: CL-1c1 and CL-5h2). The Cayuga sac fry spent the majority of the time near the center of the bowl, sometimes exhibiting abrupt changes in direction (Figure 3: CL-2a2 and CL-5h2) or swimming in semicircular whorls (Figure 3: CL-1c1 and CL-5h2). In extreme cases, Cayuga sac fry could not adopt the plough position and swam on their side using their pectoral fins only; the distance swum by such individuals was characteristically reduced (Figure 3: CL-2e1).

Further evidence of the pathological swimming pattern of Cayuga sac fry is depicted in the linearized plots of fish movement (Figure 3). The digitized pattern of control Little Clear sac fry

showed a high-frequency sinusoidal wave pattern of tight, regular oscillations. Such a pattern resulted because Little Clear sac fry always moved in either a vertical or horizontal direction within the circular apparatus. In contrast, the rapid changes of direction exhibited by Cayuga sac fry showed wavelengths of highly irregular amplitude and frequency (Figure 3). When plotted, the punctuated swimming of Cayuga sac fry showed frequent breaks in the sinusoidal pattern (Figure 3, arrows), followed by relatively long periods of stasis.

Growth Responses

Yolk absorption was impeded preceding death in progeny of Cayuga females 1-92 and 2-92 rel-

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FIGURE 3.—Light-provoked swimming behavior of representative control Little Clear Pond (LCP) and syndrome-afflicted Cayuga Lake (CL) sac fry from 1992 broodstock. **Left:** plots of actual digitized swimming patterns of individual sac fry. **Right:** plots of the horizontal movement of the same sac fry; vertical units (in cm) indicate actual position in the circular open field; arrows designate breaks in swimming; horizontal portions of the plots (marked by dots) indicate periods of stasis. Individual fish are identified by code; in LCP-1c2, for example, the 1 denotes progeny from Little Clear female 1-92, the c denotes the third fish assayed from this female, and the 2 identifies the observation period (the acclimation period is denoted 1 in this position). Exception: CL-5h denotes the third sac fry assayed from female Cayuga 5-92 at 747 degree-days. The horizontal axis encompasses 2 min in each of the right-hand plots.
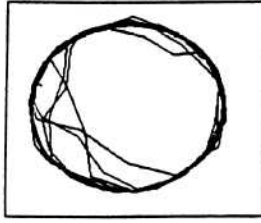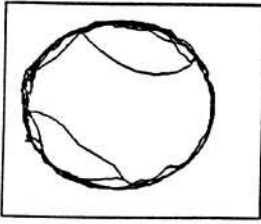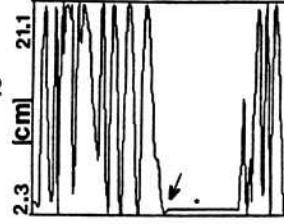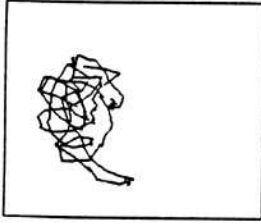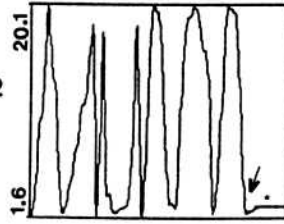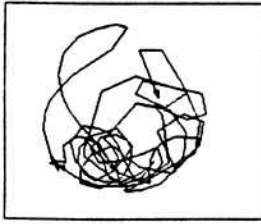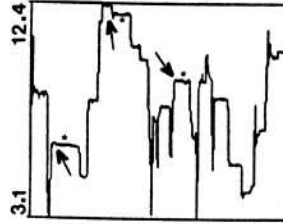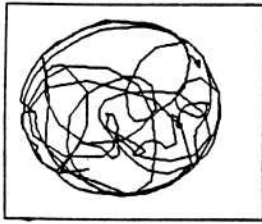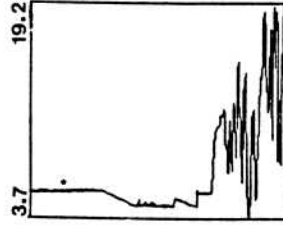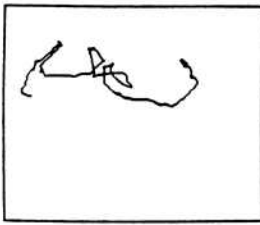
FIGURE 4.—Regressions of yolk weight/total weight versus developmental stage (centigrade degree-days) for progeny from syndrome-afflicted Cayuga Lake (CL) and control Little Clear Pond (LCP) females. Whiskers d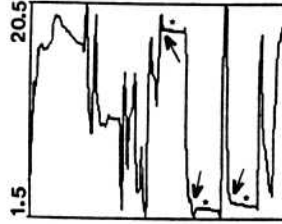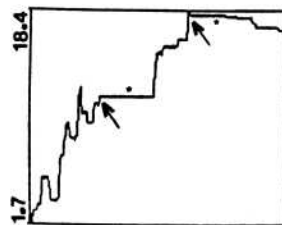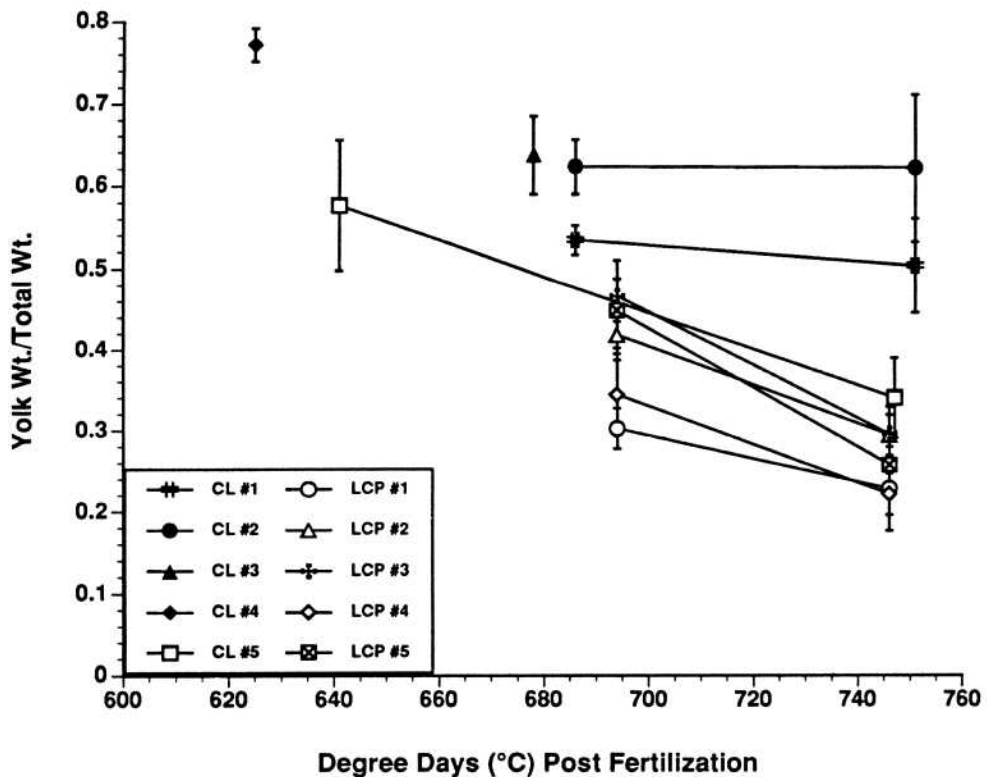enote standard deviations. Equations are given in Table 2. All regressions are for progeny of 1992 broodstocks; numbers after CL or LCP symbols refer to progeny of particular females. The CL progeny were syndrome-affected; LCP progeny were not.

ative to progeny of Little Clear females and those of Cayuga 5-92 females (Figure 4). The large difference in yolk reserves between Cayuga and Little Clear sac fry of the same age was grossly observable (Figure 1B). Yolk conversion was significantly different between progeny from individual females ($F = 72.03$, $P \leq 0.0001$; Table 2). A statistical evaluation of the rate of yolk absorption in progeny from Cayuga 3-92 and Cayuga 4-92 was not possible because growth data were obtained on only one sample date (Figure 4). Data for these progeny are shown only to highlight the excessive reserve of yolk remaining in these sac fry shortly before death.

Discussion

Through epizootiological studies, the Cayuga syndrome manifested by Atlantic salmon sac fry was recently correlated with a parental diet of the nonindigenous alewife (Fisher et al. 1995). The thiaminase associated with this clupeid (Gnaedinger 1964) rapidly hydrolyzes thiamine to its py-

rimidine and thiazole constituents (Kramptitz and Woolley 1944). We suspect that insufficient thiamine is absorbed by adult Atlantic salmon for transovarian deposition to satisfy the requirements of sac fry before first feeding. This schema is supported by the severely depressed whole-body thiamine concentrations detected in Cayuga sac fry and by the resolution of the syndrome with thiamine therapy (Fisher et al., in press). In light of these results, the Cayuga syndrome represents a unique natural model with which to examine the pathological correlates of thiamine deficiency in a lower vertebrate species.

Results from the present study indicate that the cardiovascular system is a major target for thiamine deficiency in larval Atlantic salmon. In mammalian species, thiamine deficiency reduces cardiac contractility, heart rate, and blood pressure (Cappelli et al. 1990; Onodera et al. 1991), thereby increasing susceptibility to cardiomegaly and congestive heart failure (Caster and Meadows 1980). Thus, the congestion, hemorrhage, and bradycar-

TABLE 2.—Regression equations describing efficiency of yolk absorption by Atlantic salmon sac fry from Cayuga Lake (CL) or Little Clear Pond (LCP).

Dam[a]	Yolk conversion regression[b]	Significant differences in slope[c] with:
CL 1-92	$Y = (-0.00050)X + 0.877$	CL 2-92, CL 5-92, all LCP progeny
CL 2-92	$Y = (-0.00004)X + 0.649$	CL 1-92, CL 5-92, all LCP progeny
CL 5-92	$Y = (-0.00216)X + 1.958$	CL 1-92, CL 2-92, LCP 1-92, LCP 4-92
LCP 1-92	$Y = (-0.00143)X + 1.293$	CL 1-92, CL 2-92, CL 5-92, LCP 2-92, LCP 3-92
LCP 2-92	$Y = (-0.00238)X + 2.071$	CL 1-92, CL 2-92, LCP 1-92
LCP 3-92	$Y = (-0.00328)X + 2.740$	CL 1-92, CL 2-92, LCP 1-92, LCP 4-92
LCP 4-92	$Y = (-0.00236)X + 1.983$	CL 1-92, CL 2-92, CL 5-92, LCP 3-92
LCP 5-92	$Y = (-0.00367)X + 2.991$	CL 1-92, CL 2-92

[a] Maternal source of eggs for growth study, captured and spawned in fall 1992.
[b] Y is yolk weight/total body weight; X is centigrade degree-days of development.
[c] Analysis of covariance and Sheffé's multiple-comparison tests ($P \leq 0.05$).

dia observed grossly in Cayuga syndrome-afflicted sac fry are consistent with cardiovascular signs of thiamine deficiency in mammals.

The edema associated with the Cayuga syndrome is presumably the result of congestion that, in turn, increases hydrostatic pressure, causing concomitant plasma leakage. Subcutaneous edema of the yolk sac, hydrocephalus, and retrobulbar edema reflect the susceptibility of the fine capillaries of these regions to congestion, yet blood circulated in the yolk sac until death, even when subcutaneous edema was severe (Figure 1I). Thus, the subcutaneous edema seen with the Cayuga syndrome differs substantially from the blue-sac disease caused by halogenated aromatic hydrocarbon (HAH) poisoning, in which LD100 doses (doses causing death of 100% of test fish) cause complete cessation of blood flow through the yolk sac well before death (Spitsbergen et al. 1991; Walker and Peterson 1991). Although the cited studies were performed with lake trout or rainbow trout *Oncorhynchus mykiss*, recent work in our laboratory indicates that all Atlantic salmon sac fry exposed to lethal doses of HAH also exhibit blue-sac preceding death (unpublished observations). With the Cayuga syndrome, subcutaneous edema is not prerequisite to death; fewer than 36% of the 184 sac fry examined in these studies exhibited this lesion.

The consistent finding of yolk opacities in nearly all afflicted sac fry is especially characteristic of the Cayuga syndrome (Figure 1C, D, E). This lesion is consistent with coagulated-yolk disease. However, the demonstrated causes of coagulated-yolk disease (bacterial infection, decreased oxygen concentration, incubation in soft water, rough handling, elevated ammonia) have been thoroughly investigated, and none are responsible for Cayuga syndrome (Fisher et al. 1995).

Thiamine deficiency causes acidosis resulting from increased plasma concentrations of pyruvate and lactate (Combs 1992). These alpha-keto acids accumulate because of the inhibition of thiamine pyrophosphate-dependent pyruvate dehydrogenase action (Molina et al. 1994). We suspect that the edematous fluid of syndrome-afflicted sac fry has been similarly acidified. It is therefore likely that the prominent yolk-sac opacities observed in moribund Cayuga sac fry (Figure 1) represent yolk proteins that have been denatured by the accumulation of lactate and pyruvate in the yolk milieu. The perivascular plaques observed in this study clearly support this schema (Figure 1E).

The relatively rare finding of caudal deformity represents further evidence of circulatory problems involved with the Cayuga syndrome. The heterocercal and scoliotic caudal fin development seen in some Cayuga sac fry (Figure 1B) has also been observed in first-feeding fry of chinook salmon *Oncorhynchus tschawytscha* that were briefly deprived of oxygen during embryonic development (Rucker 1975). Caudal deformities associated with the Cayuga syndrome suggest that reduced circulation through the peduncle region also causes oxygen deprivation, resulting in abnormal development of the caudal fin.

One of the most perplexing findings of this study was the great variation in gross lesion frequency between sac fry from different females. Some sac fry did not exhibit any gross lesions before death (e.g., 10% of the CL 1-92 sac fry), and death was preceded only by behavioral anomalies. Other sac fry such as those from KL 1-92, CL 3-92, and CL 4-92 exhibited multiple, severe lesions; notably, these progeny died earlier than those from other Cayuga females examined during the 1992–1993 season (Fisher et al. 1995). Sac fry from CL 5-92 exhibited only yolk-sac opacities and behavioral impairment before death, yet, like the progeny of all other Cayuga females, their mortality was 100%. The variation in lesion frequency might re-

flect slight differences in whole-body thiamine concentrations. Slightly greater thiamine reserves would prolong survival to a stage at which the larvae are more developed and perhaps less sensitive to the cardiovascular lesions induced by thiamine deficiency. However, whole-body thiamine concentrations varied little in moribund sac fry from 1993 broodstock. Levels were at or only slightly above the 100 ng/g detection limit of the thiochrome method, as compared to an average of 615 ng/g detected in control Little Clear sac fry (Fisher et al., in press). Ongoing studies will enable us to correlate lesion incidence with levels of thiamine in the eggs and dam via more sensitive high-performance liquid chromatography methods.

Although many of the gross features of the Cayuga syndrome are inconsistently expressed, behavioral anomalies are consistent. Under illumination, the normally photonegative salmonid sac fry attempts to avoid light by burying itself in substrate, positioning itself in the interstices of the substrate with highly developed thigmotactic responses (Fast et al. 1981; Fisher et al. 1993). Without substrate, the light stimulates sac fry swimming until the animal meets resistance from the boundaries of its containment vessel. In a rectangular apparatus without substrate, this inevitably results in sac fry positioning themselves in a corner. In a circular vessel, sac fry meet no resistance and continue to swim in circles along the edge of the container until fatigued. In the present studies, all Cayuga sac fry exhibited abnormal phototactic and thigmotropic behaviors, as evidenced by the pronounced lack of this "edging" effect. That this response was observed with the CL 5-92 progeny, even though the total distance and time swum by some of them was greater than shown by many of the Little Clear sac fry, highlights the unusual nature of the neuropathy associated with the Cayuga syndrome.

The upside-down and "corkscrew" swimming characteristic of Great Lakes salmonids exhibiting swim-up syndromes (Burdick et al. 1964, 1972; Mac et al. 1985; Fitzsimons et al., in press) has also been observed in Lake Ontario lake trout fry cultured in our laboratory, yet this symptom is rarely seen in Atlantic salmon sac fry with Cayuga syndrome. Rather, afflicted Cayuga sac fry exhibit convulsive, punctuated, burst-swimming, as emphasized by their increased swimming speed relative to that of Little Clear sac fry (Figure 3). As thiamine has recently been shown to remedy the swim-up syndromes of Lake Ontario lake trout

(Fitzsimons, in press), the behavioral differences between the Cayuga and swim-up syndromes probably reflect species-specific behavior.

Unlike Pacific salmonids, Atlantic salmon remain geopositive for several months after yolk resorption (i.e., emergence; Dill 1977). In addition, the Cayuga syndrome manifests itself in sac fry. Thus, the cumbersome yolk sac likely prevents the Cayuga sac fry from swimming upside-down, and their geopositive behavior probably thwarts the "corkscrew" swimming seen in other salmonid species with the swim-up syndrome.

The consistent behavioral anomalies associated with the Cayuga syndrome provide good evidence for involvement of the nervous system. In addition, the growth data presented here indicate that, for at least some progeny, yolk absorption is impeded before death. It is therefore possible that the neurobehavioral anomalies exhibited by the Cayuga sac fry simply reflect bioenergetic interferences. However, the progeny from Cayuga 5-92 apparently had no problem in using yolk nutrients (Figure 4), yet still exhibited characteristic neurological impairment and succumbed to the syndrome. Further study is ongoing to characterize the histopathological alterations in the nervous system that result from the Cayuga syndrome; however, preliminary examination of Cayuga sac fry in 1990 did not reveal substantial morphological changes relative to age-matched controls (Fisher and Spitsbergen, unpublished). In that study, highly localized and mild necrotic changes were observed in the dorsoposterior granular layer of the mesencephalon in 17 of 38 Cayuga sac fry; the same changes were observed in only 1 of 32 control sac fry. The significance of this "lesion" remains to be determined, because it was not observed in an earlier examination of 1989 specimens. Furthermore, there was no clear evidence of diencephalomalacia, cortical necrosis, phagocytic infiltration or hemorrhage, as observed in thiamine deficiencies of several mammalian species (Irle and Markowitsch 1982; Horita et al. 1983; Kril and Homewood 1993; Lonkar and Prasad 1994).

Few published reports regarding early life stage mortality specific to the Atlantic salmon are available for comparison with the Cayuga syndrome. With the Baltic M-74 syndrome mentioned earlier, the parents of afflicted offspring exhibited elevated cytochrome P450 enzymes and hypertrophy of hepatic smooth endoplasmic reticulum (Norrgren et al. 1993). Mac and Edsall (1991) cited this condition as "identical" to the swim-up syndrome observed in Great Lakes salmonids. However, the

mortality associated with M-74 occurs principally at the sac fry stage, and yolk opacities and hemorrhage are common, characteristics similar to those of the Cayuga syndrome. In contrast to the Cayuga syndrome, the M-74 sac fry exhibited decreased glycogen deposits and increased hepatocyte vacuolation, whereas histopathological examinations of Cayuga sac fry revealed neither these changes nor immunohistochemical evidence of cytochrome P450 induction (Fisher 1995).

It is not remarkable that mature salmon from the Baltic Sea would exhibit chemically induced physiological and histopathological changes, because xenobiotic contamination is widespread there. The Finger Lakes, however, remain relatively pristine. We have been unable to perform histological examinations of mature Atlantic salmon from Cayuga Lake because they have been released after spawning to accommodate sport fishermen. Nonetheless, the spawning adults are fecund and in robust condition, with no visible external lesions except an occasional scar caused by sea lamprey *Petromyzon marinus*. The thyroid hyperplasia seen in Great Lakes salmonids (Leatherland 1993) has not been observed in adult Cayuga Atlantic salmon.

The role of thiamine in the etiology of M-74 is presently under consideration. Given that the Baltic salmon feed predominantly on herring *Clupea harengus* and sprat *Sprattus sprattus,* forage species that exhibit high thiaminase activity (Deutsch and Hasler 1943; Nielands 1947; Anglesea and Jackson 1985), a thiamine deficiency is not inconceivable. Indeed, with the method developed initially for the Cayuga sac fry (Fisher et al., in press), thiamine has recently been shown to reduce M-74 mortality from 60% (untreated) to 2% (Bylund and Lerche 1995).

Whole-body thiamine concentrations can be chelated by heavy metal pollutants such as lead (Ghazaly 1991) and mercury (Siegel et al. 1991). In addition, deficiencies of thiamine have been demonstrated to accelerate xenobiotic-induced peroxidation of microsomal lipids (Lychko et al. 1987). An evaluation of these toxicant interactions would seem an especially fruitful avenue of research for syndromes where a vitamin B_1 deficiency is suspected in the absence of a clear dietary association.

Acknowledgments

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