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Separating Starvation Losses from Other Early Feeding Fry Mortality in Steelhead Trout *Salmo gairdneri*, Chinook Salmon *Oncorhynchus tshawytscha*, and Lake Trout *Salvelinus namaycush*[1]

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The Field Toxicant Research Unit of the New York State Department of Environmental Conservation has conducted salmonid reproductive studies in various lakes throughout New York State since 1960. Burdick et al.(1964) summarizes early results of this work which correlates high lake trout (Salvelinus namaycush) feeding fry mortality with DDT in several New York lakes. Death of these fry was characterized by a specific syndrome which included distended air bladder, air in the intestinal tract, and erratic swimming. The syndrome appeared when the young fry left the bottom of the basket or trough, oriented into the current and appeared ready to accept food (swim-up). In these fish, swim-up occurred two to three weeks after hatch depending upon water temperature, or just prior to total yolk sac absorption. As a result of this study, the term syndrome has been used in New York State fish hatcheries to characterize mortality of disease-free salmonid fry from swim-up to 90 days post-feeding. Syndrome symptoms have also been expanded to include dark coloration, loss of equilibrium, hyper-irritability, and paralysis followed quickly by death.

Since 1964 syndrome has been observed in chinook salmon (Oncorhynchus tshawytscha) and steelhead trout (Salmo gairdneri) fry. Pesticide analysis of eggs and fry has shown intermediate levels of many pesticides but no correlations could be made between high fry loss and specific pesticide levels.

Other authors have reported fry losses in various species of salmonids to be related to pesticides (Willford 1980; Jensen et al. 1970; Macek 1978; Burdick et al. 1974). Lemm and Hendrix (1981) and Fowler (1980) on the other hand, have attributed early feeding fry losses in some species of salmonids to the nutritional quality of certain diets, citing variation in protein, fat and ash as a factor in suitability of diet.

In most of the studies conducted through 1978 by the New York State Field Toxicant Research Unit at Rome, New York, all fry mortality from swim-up through 90 days has been combined and recorded as total feeding fry loss. Prior to 1978 all experimental lots were fed whatever

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open-formula diet was purchased under New York State contract. In 1978 a decision was made to switch to a closed-formula salmon starter diet. With this change it became apparent that the progeny from some females had syndrome while others did not (Skea, unpublished data), and that there were different periods of mortality. This was true even if all females were collected from the same habitat at the same time. It was decided to conduct a series of studies in an attempt to separate syndrome mortality from other feeding fry loss and to determine how long fry would live if held unfed.

MATERIALS AND METHODS

During the period 1981-1983 eggs were spawned and fertilized from steelhead trout, lake trout, chinook salmon and rainbow trout from several bodies of water. Table 1 summarizes these collections. Eggs were collected from Lake Ontario steelhead trout so that there were two lots of 100 swim-up fry from each female. One of these lots was unfed while the other was fed a closed-formula salmon starter diet.

Table 1 also includes data from earlier work on lake trout from collections made during the period 1960-1962 in Lake George. (Burdick et al. 1964). With the exception of these early collections all eggs, whether from individual females or pooled egg lots, were incubated at the New York State Department of Environmental Conservation Field Station, located at Rome, New York. Separate subdivided hatching baskets (34x7x12 cm) were placed in troughs 126x36x14 cm deep. Water depth was held at 4.5 cm while flow was maintained between 12 and 20 l/min. The 1960-1962 lake trout were incubated at the New York State Fish Hatchery, Lake George, New York. Baskets and troughs were of the same type as those used at Rome. All lake trout were incubated at 6-9°C while all other species tested were held at 11-12°C. Water supplies used in these studies were within acceptable limits of quality as outlined by Piper et al. (1982). Each lot of eggs was treated with formalin at the rate of 1,660 ul/l (1:600) for 15 minutes three times a week until eye-up to prevent fungus.

Mortality and temperatures were recorded daily and centigrade temperature units (degree-days) were calculated from day of fertilization using the following:

$$\text{Temperature units (TU)} = \frac{\text{temperature (°C) x hours at temperature}}{24}$$

Mortality rate per temperature unit interval was calculated using the following equation:

$$\text{Mortality Rate} = \frac{\frac{N_1 - N_2}{N_1}}{\text{TUI}}$$

Where N_1 = Number of fish alive at first observation (O_1)

N_2 = Number of fish alive at second observation (O_2)

TUI = (Temperature Unit Interval) Temperature units accumulated from O_1 to O_2

Cumulative percent mortality was calculated by dividing the total number of fry that had died from swim-up to any age (expressed as temperature units) by the number alive at swim-up. Comparisons were made between the mortality rates and mortality periods as a function of temperature unit using single factor analysis of variance (Snedecor and Cochran 1973) and Duncan's new multiple-range test (Steel and Torrie 1960) for fed and unfed lots of fry.

Periodic examination of fry mortality was conducted by the New York State Department of Environmental Conservation Fish Disease Control Unit. Complete virological and bacterial disease assays as well as histopathological studies were conducted on all fry examined. In no case was any evidence of infectious disease detected.

Table 1. Summary of source of various lots of fry utilized in this study.

Source	Year	Species	# of Females	# of lots	Avg. # of fry per lot
L. Ontario	1983	Steelhead trout	16	16	100[1] Fed
L. Ontario	1983	Steelhead trout	16	16	100[1] Unfed
Cayuga L.	1982	Rainbow trout	10	10	184[1] Fed
L. Michigan	1982	Chinook salmon	?	10	200[2] Fed
L. Michigan	1982	Chinook salmon	?	1	100[2] Unfed
L. Ontario	1982	Chinook salmon	10	10	150[1] Fed
L. Ontario	1982	Chinook salmon	10	1	100[2] Unfed
L. George	1964	Lake trout	9	1	5,000[2] Fed
L. George	1981	Lake trout	10	1	100[2] Unfed
Raquette L.	1982	Lake trout	4	1	100[2] Unfed

(1) Lots obtained from individual females
(2) Lots obtained from pooled egg lots

RESULTS AND DISCUSSION

Syndrome and the mortality associated with it was found to be female dependent in steelhead ($p<0.05$). Six of the sixteen fish spawned produced fry that had high mortality prior to 705 TU. This was true whether the individual lots were fed or unfed. Table 2 summarizes cumulative percent mortality of six lots of 100 fry each from individual steelhead trout females that had syndrome, and cumulative percent mortality of 100 unfed fry from the same female. Mortality through 705 TU in these two groups of fry were found not significantly different

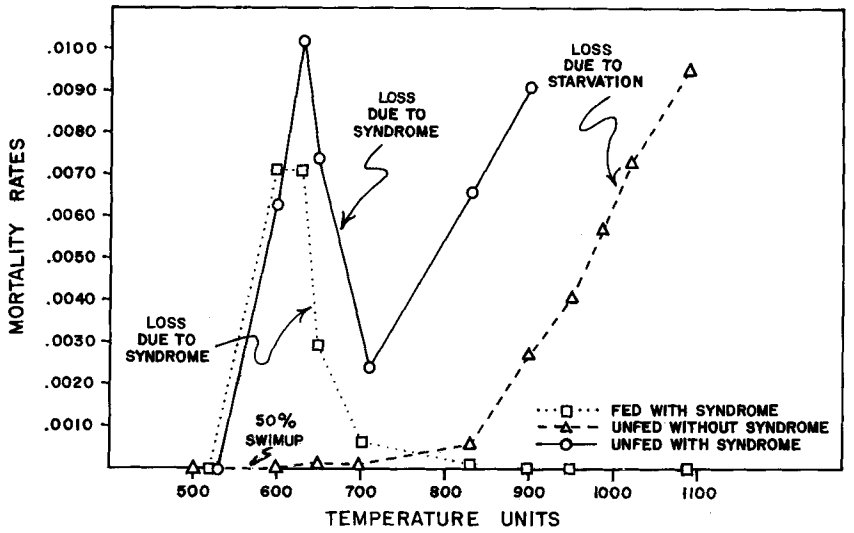

Figure 1. Comparison of rate of mortality per temperature unit for fed and unfed groups of Lake Ontario steelhead trout.

by single factor analysis of variance ($p<0.025$). The cumulative percent mortality in fed lots of fry from ten individual steelhead trout females that did not have syndrome and in unfed lots of fry from the same ten females were found not to be significantly different through 705 TU ($p<0.025$). Corresponding data for fry of ten Cayuga Lake rainbows that did not show syndrome and of Lake Ontario steelhead trout that did not show syndrome were found to be significantly different from those for the steelhead with syndrome but not different from each other ($p<0.05$) by Duncan's new multiple-range test. As can be seen in Table 2 and Figure 1, surges in mortality due to syndrome occurred shortly after swim-up and was over within about 150 TU. It is also apparent that feeding these fry prevented significant additional mortality after this point. Starvation in healthy disease-free fry started to appear at about 800 TU with total loss by 1200 TU. The appearance of fish that died during this time was characterized by a large head in relation to body size (pinhead). They appeared weak and lethargic. Indeed, these fish were so weak the water current in the baskets exhausted them and forced them against the discharge screen. Death and rigor mortis in these fish was a slow process, not at all similar in fish that died of syndrome.

Test data for 5000 feeding fry from earlier work on lake trout from Lake George (Burdick et al. 1964) were recalculated using the temperature unit concept (Table 3). Syndrome losses in this group were correlated with high levels of p'p' DDT. Mortality rate/TU in contaminated feeding fry occurred between 850 and 950 temperature units (Figure 2). Death due to

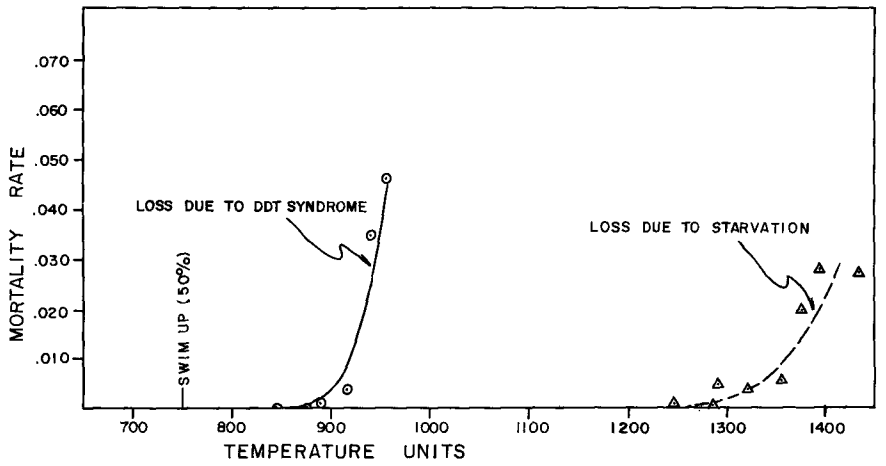

Figure 2. Comparison of rate of mortality per temperature unit for DDT contaminated lake trout feeding fry and an unfed control group.

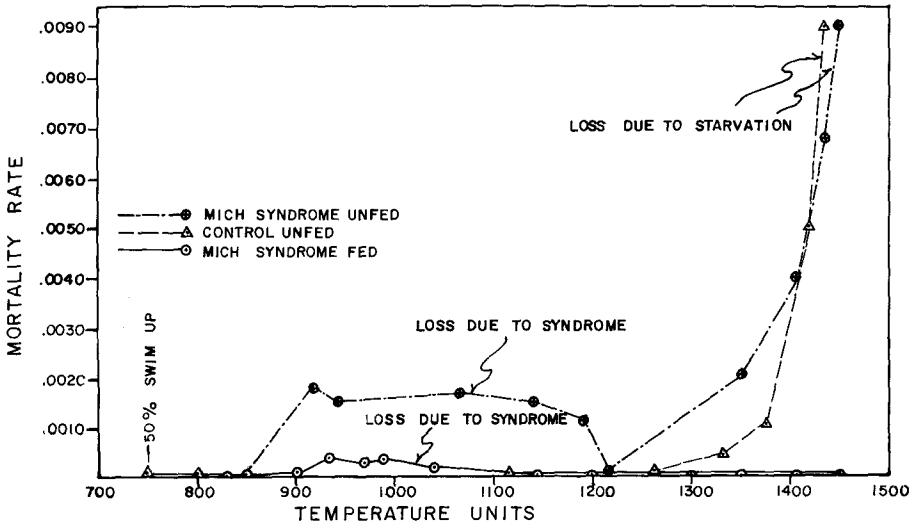

Figure 3. Comparison of rate of mortality per temperature unit for Lake Michigan fed and unfed chinook salmon and an unfed control group.

Table 2. Comparison of cumulative percent mortality at different ages (expressed in temperature units) for fed and unfed lots of Lake Ontario steelhead trout fry and a control group from Cayuga Lake.*

Temperature units from fertilization	Cumulative Percent Mortality				
	Lake Ontario fry with syndrome		Cayuga Lake fry without syndrome	Lake Ontario fry without syndrome	
	Fed	Unfed	Fed	Fed	Unfed
530 (swim-up)	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
550	23.8^{a}	23.1^{a}	0.0^{b}	0.0^{b}	0.0^{b}
600	50.0^{a}	44.0^{a}	0.2^{b}	0.5^{b}	0.1^{b}
630	60.7^{a}	63.2^{a}	0.7^{b}	1.4^{b}	0.4^{b}
685	63.5^{a}	70.0^{a}	1.3^{b}	1.6^{b}	0.6^{b}
705	64.7^{a}	73.7^{a}	2.5^{b}	1.9^{b}	1.2^{b}
835	64.7^{a}	96.3^{c}	4.1^{b}	3.5^{b}	8.6^{b}
900	64.7	98.5	5.8	4.7	26.8
950	64.7	98.5	6.7	5.0	41.7
985	64.7	98.5	7.4	5.0	53.3
1022	64.7	98.5	7.9	5.0	66.0
1090	64.7	98.8	8.2	5.0	88.0
1165	64.7	100.0	8.2	5.0	96.6
1200	64.7	100.0	8.2	5.0	100.0

*Means in the same row followed by the same letter are not significantly different ($p<0.05$). No statistical tests were made after 835 temperature units.

starvation in uncontaminated lake trout fry did not occur until 1300-1450 temperature units. In the lots of disease-free contaminated fish, all losses occurred between swim-up and 1300 temperature units and can be assumed to be the result of an unnatural cause such as high levels of p'p' DDT. Burdick et al. (1972) showed that lake trout fed DDT had high feeding fry losses. When their data from 1972 is expressed in temperature units, these losses occurred between 860 and 1000 temperature units and are related to high dietary DDT intake.

Lake Michigan chinook salmon showed a similar relationship between fed and unfed lots of fry (Table 4, Figure 3). As shown in Table 4, fed Lake Michigan lots had considerable mortality between 877 and 1040 temperature units. Comparing this group with unfed fry from the same pool of eggs, mortality in the unfed group is several times that of the fed fry. Figure 3 shows that the mortality surge in the unfed Lake Michigan fry begins at the same time as in the fed group but continues beyond 1040 temperature units. It does drop off, however, before deaths due to starvation begin. All fry which died in these two groups prior to 1200 temperature units exhibited the same symptoms that have been described for the steelhead trout. As shown in Table 4, fed and unfed groups of fry from Lake Ontario chinook salmon did not exhibit the syndrome and significant mortality did not occur until after 1200 temperature units.

Table 3. Relationship of cumulative mortality of Lake George lake trout fry caused by high DDT levels in the eggs and losses from starvation in fry hatched from uncontaminated eggs.

Temperature units from fertilization	Cumulative Percent Mortality	
	5000 Lake George DDT contaminated fry	200 Lake George uncontaminated fry
	Fed	Unfed
750 (swim-up)	0.6	0.0
806	0.8	0.0
845	1.1	0.0
875	1.8	0.0
890	3.2	0.0
915	11.3	0.0
935	76.8	0.0
955	100.0	0.0
1200		0.0
1290		0.0
1320		14.0
1355		33.0
1375		58.0
1395		80.0
1435		100.0

Table 4. Comparison of cumulative percent mortality at different ages (expressed in temperature units) for fed and unfed lots of Lake Michigan and Lake Ontario chinook salmon.*

Temperature units from fertilization	Cumulative Percent Mortality			
	1000 Lake Michigan fry with syndrome	100 Lake Michigan fry with syndrome	1500 Lake Ontario fry without syndrome	100 Lake Ontario fry without syndrome
	Fed	Unfed	Fed	Unfed
750 (swim-up)	0.0[a]	0.0[a]	0.5[a]	0.0[a]
795	0.0[a]	0.0[a]	0.6[a]	0.0[a]
877	1.9[a]	0.0[a]	1.0[a]	0.0[a]
900	2.9[a]	6.0[b]	1.3[a]	0.0[a]
955	4.8[a]	11.0[b]	1.4[c]	0.0[c]
1040	5.7[a]	20.0[b]	1.7[c]	0.0[c]
1160	5.8[a]	37.0[b]	1.7[c]	0.0[c]
1210	5.9[a]	39.0[b]	1.8[c]	0.0[c]
1250	6.1	41.0	1.8	3.0
1338	6.5	44.0	1.9	7.0
1370	6.5	47.0	1.9	16.0
1400	6.5	53.0	2.0	39.0
1420	6.6	59.0	2.0	60.0
1450	6.7	71.0	2.0	87.0
1475	6.7	81.0	2.0	91.0
1525	6.8	100.0	2.0	100.0

*Means in the same row followed by the same letter are not significantly different ($p<0.05$). No statistical tests were made after 1210 temperature units.

These data show that mortality due to syndrome occurred at a significantly different time than that due to starvation and mortality in unfed fry from Lake Michigan was also higher than that in fed fry from the same group. ($p<0.05$)

Syndrome losses occurred in all three species within approximately 300 temperature units from 50% swim-up regardless of the length of time to reach this stage of development. These losses were found to be female dependent in steelhead trout. Contaminant analysis of steelhead trout eggs and fry from Lake Ontario showed no correlation between mortality and levels of PCB (Aroclor 1254) in excess of 2.0 mg/kg; Mirex in excess of .08 mg/kg; and detectable levels of DDE, Dieldrin and hexachlorobenzene. There were, however, a large number of other contaminants present in this lake which were not analyzed for. Pesticides therefore cannot be ruled out as the cause of syndrome. What is interesting is that chinook salmon from Lake Ontario did not exhibit syndrome but steelhead trout did. The reason for this is not known. Significant feeding fry losses that occur in salmonids during the first four weeks after swim-up (at 10°C) can be considered abnormal. This may be a result of pesticide contamination or nutritional deficiency in the female. Mortality during this period should not be confused with losses from starvation due to fry being given an improper or inadequate diet (in which case death occurs at a different time). Stauffer (1979) has shown that death due to starvation in Lake Michigan lake trout occurred at about nine weeks after swim-up at 6-8°C. His data converted into temperature units indicates starvation would occur at approximately 450 temperature units from swim-up. This is in agreement with our work with Lake George lake trout which showed starvation at about 1300 temperature units from fertilization (500 temperature units from swim-up).

Schachte (unpublished data) demonstrated that early feeding fry loss in brook trout was related to the diet received by the broodstock. The conclusion can thus be made that losses due to starvation occur at a specific developmental time based on temperature units for each species. Losses from other sources occur prior to the starvation period and generally within the first 3-4 weeks after swim-up at 10°C. It has also been shown by Symula et al. (in press) that if early mortality due to syndrome is anticipated, proper manipulation of diets can prevent additional losses just prior to/and during the period of starvation.

We suggest that syndrome loss, which is female dependent, may be the result of pesticides or nutritional deficiencies during egg oogenesis and/or vitellogenesis (yolking of eggs). Mortality that occurs after this early period can be correlated with poor fry diet or other cultural causes.

Utilization of this method of separating early syndrome loss from starvation in chinook salmon has already been adopted in the New York State hatchery system. It has resulted in significant savings in production costs.

Finally, it is becoming apparent that researchers attempting to correlate pesticide levels with feeding fry losses should compare unfed lots from individual females up to the point of starvation. This would rule out the confounding effect of diet and its role in reducing or compounding feeding fry losses prior to the period when fry become vulnerable to death by starvation.

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