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Effects of DDT and PCB's on Survival of Lake Trout Eggs and Fry in a Hatchery and in Lake Michigan, 1973-1976

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EFFECTS OF DDT AND PCB'S ON SURVIVAL OF LAKE TROUT EGGS AND FRY IN A HATCHERY AND IN LAKE MICHIGAN, 1973-1976 [1]

By Thomas M. Stauffer

Abstract

In the early 1950's lake trout in Lake Michigan were near extinction. Beginning in 1965, 1 to 3 million yearlings were stocked annually to re-establish the lake trout. Survival to maturity was good and significant numbers of young were expected by 1970 or 1971. However, no natural reproduction has been found, despite extensive sampling. The role of DDT and PCB's in the apparent failure of lake trout reproduction was determined. Mortality of Lake Michigan eggs and fry that were relatively high in contaminants was compared with that of Marquette Hatchery eggs and fry, that ere very low in contaminants, under normal hatchery incubation, during incubation in enclosures on the bottom of Grand Traverse Bay, and under stress of starvation or limited food in a hatchery.

In normal hatchery incubation, lake trout were reared from the green egg stage to 6 weeks after swim-up. In 1973-1974, overall mortality (80% ± 16) of Lake Michigan eggs (2.7-5.2 ppm DDT and 5.3-9.9 ppm PCB's) and fry was about twice that (46% ± 14) of Marquette Hatchery eggs (not more than 0.2 ppm DDT and 0.3 ppm PCB's) and fry. However, in the similar 1974-1975 test, mortality (50% ± 9) of Lake Michigan eggs (2.6-4.0 ppm DDT and 4.8-8.3 ppm PCB's) and fry was the same as that (47% ± 9) of Marquette Hatchery eggs (0.2 ppm DDT and 0.3 ppm PCB's) and fry. In Grand Traverse Bay, for eggs placed in enclosures when "green" there was no significant difference between percentage mortality of Lake Michigan eggs (2.6-4.0 ppm DDT and 4.8-8.3 ppm PCB's) and fry and Marquette eggs (0.2 ppm DDT and 0.3 ppm PCB's) and fry at the eyed stage (41% ± 19 and 62% ± 22), swim-up (average, 63% and 66%) and 3 weeks after swim-up (66% ± 14 and 78% ± 17). For eggs placed at eye-up in Grand Traverse Bay, mortality (30% ± 13) of Lake Michigan eggs at swim-up was significantly greater than that (10% ± 5) of Marquette Hatchery eggs, but at 3 weeks after swim-up there was no significant difference (87% ± 20 and 92% ± 13). In starvation tests, mortality of unfed Lake Michigan fry was significantly less (65% ± 6) than that (89% ± 6) of Marquette fry after 11 weeks of starvation. Under stress of competition with similar-sized brown trout for a limited food supply for a period of 10 weeks after swim-up, mortality (53% ± 11) of Lake Michigan fry was not significantly different from that (61% ± 11) of Marquette Hatchery eggs and fry.

[1] Contribution from Dingell-Johnson Projects F-31-R and F-35-R, Michigan.

DDT and PCB's were not the cause of reproductive failure by lake trout in Lake Michigan. This conclusion was based primarily on the absence of greater mortality for Lake Michigan eggs and fry than for Marquette Hatchery eggs and fry when the two groups were reared in the hatchery in 1974-1975, when they were reared in Grand Traverse Bay (except for one instance), when they were starved and when they were subjected to stress of competition with brown trout for limited food. Also, for Lake Michigan eggs and fry incubated in the hatchery in 1973-1974 and 1974-1975, mortality was linearly correlated with DDT and PCB content in only one time period. Finally, in the hatchery experiments, no distress symptoms that were characteristic of DDT or PCB poisoning were observed.

Some data were not in accordance with the conclusion of little effect. First, in 1973-1974, mortality of Lake Michigan eggs and fry was significantly higher than that of Marquette Hatchery eggs and fry. However, difference in treatment of the eggs of the two groups before placement at the hatchery is suspected of causing the higher mortality (70-100%) of all lots of Lake Michigan eggs which obscured any possible effect of DDT and PCB's. Mortality from fertilization to eye-up in the 1974-1975 test in the hatchery was linearly correlated with amounts of DDT and PCB's which suggested an adverse effect of PCB's in this particular instance.

Introduction

Sea lamprey (Petromyzon marinus) predation, possibly combined with commercial fishing, led to the near extinction of lake trout (Salvelinus namaycush) in Lake Michigan in the early 1950's (Smith 1968). To re-establish the lake trout, state and federal agencies cooperated to implement successful sea lamprey control measures during the early 1960's, and to stock 1-3 million yearling lake trout per year beginning in 1965. These fish were expected to produce significant numbers of young by 1970 or 1971. However, no natural reproduction has been found, despite extensive sampling (Great Lakes Fishery Commission 1976). The large numbers of spawning lake trout concentrated at or near shore planting sites indicated that a significant number of the planted fish survive to maturity. One theory for this lack of natural reproduction is that mature, planted fish "home" to and deposit eggs at their shoreline planting site. These shoreline planting sites generally offer little suitable spawning substrate and are often subject to heavy scouring action by waves and ice. However, a few ripe and spent lake trout have been found on some traditional Lake Michigan lake trout spawning reefs during the spawning season, along with evidence of egg deposition. So, another possibility or theory is that lake trout eggs cannot survive incubation in Lake Michigan water, even though they are deposited on apparently suitable substrate.

Lake Michigan waters are now less than pristine. Beeton and Edmondson (1972) found that sulfate levels in 1965 were much higher (about 20 ppm) than in 1900 (about 8 ppm). Eutrophication has occurred, as demonstrated by changes in the benthos composition of Lake Michigan from 1931 (Eggleton 1937) to 1962-1964 (Robertson and Alley 1966; Cook et al. 1965, Cook and Risley 1963) as reported by Cook and Johnson (1974). In addition, pesticides such as DDT and dieldrin are now present in Lake Michigan, as well as contaminants such as PCB's and mercury (Hesse 1975). The effects of DDT and PCB's on egg survival may be severe and should not be overlooked, even though eggs taken from hatchery-reared trout that matured in Lake Michigan have hatched normally in hatcheries. Keller

(1971) reported a survival of 59% to swim-up fry for eggs taken from hatchery-reared lake trout on a spawning reef in Lake Michigan. On the other hand, Burdick et al. (1964) and Macek (1968a) have shown that DDT causes mortality of salmonid eggs and Jensen et al. (1970) and Hogan and Brauhn (1975) observed PCB-induced mortality of salmonid eggs.

Although eggs taken from planted lake trout in Lake Michigan and reared in hatcheries survive satisfactorily, such may not be the case for eggs that hatch in Lake Michigan for the following reasons. First, eggs incubating in Lake Michigan may absorb enough additional contaminants to cause death. Second, DDT lessens resistance to stress (Macek 1968b) so that eggs and fry containing DDT may not be able to cope with the more rigorous regime in Lake Michigan than in hatcheries where they survive. The objective of this report is to assess the effect of DDT and PCB's on early survival of lake trout.

Methods

The overall method of study was to compare mortality of Lake Michigan eggs and fry that were relatively high in contaminants with that of Marquette Hatchery eggs and fry that were very low in contaminants. Mortality of the two groups was compared (1) under normal hatchery incubation, (2) during incubation in enclosures on the bottom of Grand Traverse Bay, and (3) under conditions of stress in a hatchery.

Each fall in 1973-1975, eggs from four Lake Michigan and four Marquette Hatchery females were taken for use in the tests (Table 1). Lake Michigan females caught near Charlevoix were spawning for the second to fifth time and were of ages VI-VIII. They were 64-75 cm long, contained eggs 4.9-5.4 mm in diameter, and had been planted in Lake Michigan in 1968 or 1970 as yearling hatchery fish. Marquette females were spawning for the second to fifth time, were ages VIII-X, 47-68 cm long, had eggs 5.0-5.6 mm in diameter and had spent their entire life at the hatchery. All females were fertilized each year by milt from four males of the same respective groups.

Table 1.--Characteristics of female lake trout and the DDT and PCB content of their eggs (wet weight), 1973-1975.

Year, origin, and designated female	Females			Eggs [a]		
	Age	Length (cm)	Number of times spawned	Average diameter	DDT (ppm)	PCB's (ppm)
1973						
Lake Michigan						
LA	VI	72	2-3	5.1	4.44	7.76
LB	VI	73	2-3	5.1	4.98	9.13
LC	VI	71	2-3	5.3	5.24	9.90
LD	VI	72	2-3	5.1	2.74	5.33
Marquette Hatchery [b]						
HA	VIII	47	2-3	5.3		
HB	VIII	56	2-3	5.3		
HC	VIII	58	2-3	5.3		
HD	VIII	65	2-3	5.3		
1974						
Lake Michigan						
LA	VII	70	3-4	4.9	3.23	6.69
LB	VII	72	3-4	5.3	2.57	4.84
LC	VII	74	3-4	5.1	3.95	8.30
LD	VII	75	3-4	5.1	3.16	6.60
Marquette Hatchery						
HA	IX	61	3-4	5.3	0.16	0.28
HB	IX	66	3-4	5.6	0.17	0.26
HC	IX	65	3-4	5.6	0.20	0.28
HD	IX	68	3-4	5.4	0.19	0.28
1975						
Lake Michigan						
LA	VI	74	2-3	5.3	3.23	6.28
LB	VIII	75	4-5	5.4	3.55	6.33
LC	VI	64	2-3	4.9	1.41	3.16
LD	VI	73	2-3	4.9	1.82	3.80
Marquette Hatchery						
HA	X	59	4-5	5.3	0.05	0.23
HB	X	68	4-5	5.1	0.06	0.26
HC	X	61	4-5	5.0	0.05	0.20
HD	X	63	4-5	5.0	0.06	0.23

[a] After 1-2 hours of water hardening.

[b] A sample of abdominal wall flesh from 11 mature female trout in early 1973 averaged 0.73 ppm total DDT and 1.04 ppm total PCB's on a wet weight basis (Wu 1974).

Handling and placement of the eggs in the various experiments were as follows: Eggs were taken and fertilized at the Charlevoix Station (Lake Michigan eggs) and Marquette Hatchery on 31 October 1973, 28 October 1974, and 13 November 1975. After "water-hardening" for 1-2 hours, they were divided into the required number of lots and placed in water and oxygen-filled plastic bags; the bags were then put into coolers containing ice for transportation. All lots were transported by plane or car to Thompson Hatchery and Grand Traverse Bay and installed in the various experimental sites within 24 hours of fertilization. Placement of the lots (in separate containers) and sampling thereafter were done according to a stratified, random design. In the hatchery, the same lots from each female were used to assess mortality over the period of study. However, in Grand Traverse Bay, it was necessary to use several different lots from each female to assess mortality at different stages of development because the examination procedure caused total mortality.

A sample of newly fertilized eggs from each female was frozen for DDT and PCB analysis, as described by Reinert (1970). The DDT isomers (p, p', DDT + p, p' DDE) were quantified as p, p' DDE, and PCB's as 1254 (Table 1). The amount of DDT was highly correlated ($\underline{r} = 0.980$, $\underline{F} = 432$) with amounts of PCB's. This caused difficulty in separating the effects of the two contaminants, except by considering the time at which mortality occurred.

Mortality of the two groups was compared by use of mean mortality and 95% confidence limits. For the hatchery experiments, the 95% confidence limits usually were calculated from a pooled standard error. These were derived from two-way (lake × fish) nested analyses of variance (Scheffe' 1959) of total mortality during the experiments and from three-way (lake × fish × time) nested analyses of variance of mortality in different time periods of the experiments. Confidence limits for the period from fertilization to 6 weeks after swim-up in 1973-1974 and all other limits were calculated in the usual manner.

Linear regressions of percentage mortality on DDT and PCB content were calculated for Lake Michigan eggs incubated in the hatchery in 1973-1974 and 1974-1975 to test any direct effect of contaminants. Polynomial regressions were also calculated because there seemed to be some curvilinear relationships between percentage mortality and DDT and PCB content.

<u>1973-1974 tests</u>

Mortality of green eggs to 6 weeks after swim-up for Lake Michigan and Marquette Hatchery eggs and fry was compared at Thompson Hatchery where they were reared by standard hatchery methods. Four 300-egg lots from each of eight females were stacked in egg trays in two hatchery troughs and reared at a near constant 7 C. Mortality of each lot was counted at eye-up, hatching, and at 6 weeks after swim-up. Soon after hatching, the four lots from each female were combined into one lot. Fry were counted and a random sample of fry from each female was measured for total length (TL) at the end of the experiment.

Mortality of the two groups in enclosures in Lake Michigan was compared at New Mission Bay, a small bay on the west side of Grand Traverse Bay. The enclosures rested on platforms 0.3-0.6 m off the bottom, in 5-6 m of water over a sand bottom some 18 m offshore. Over the period of study, the water became very turbid at times due to wave action on the nearby shore. Water temperatures at the platforms were 11 C (1 November), 0 C (13 February), and 6 C (17 April). The egg enclosures consisted of 13-liter steel pails, 23 cm high, 30 cm in diameter, and open at the top. Each pail had a horizontal row of 3-mm diameter holes in its side, located midway between the top and bottom. Each was filled with layers of sand, gravel and rubble to simulate lake trout spawning habitat and a 13-mm mesh netting cover tied on the top to exclude large predators.

One portion of the test involved mortality of green eggs to middle sac fry stage in which four 50-egg lots from each of eight females were used. These 32 lots (in separate enclosures) were placed on a platform in New Mission Bay on 1 November. One lot from each female was removed and examined for survival at the following stages: early eye, middle eye, hatch, and early sac fry. A second test compared mortality of eyed eggs of the two groups to middle sac fry stage in pails in New Mission Bay. Again, four 50-egg lots from each of eight females were used. Eyed eggs for this experiment were taken from the respective lots (32) at Thompson on 13 December and put in enclosures on a platform in New Mission Bay on 14 December. Usually, one lot from each female was removed and examined at the following development stages: middle eye, hatch, early sac fry, and middle sac fry.

1974-1975 tests

In 1974-1975 the comparison of mortality of Lake Michigan and Marquette Hatchery eggs and fry reared under standard hatchery procedures was repeated. However, this test differed in that the lots were kept separate for the entire experiment and mortality was also assessed at swim-up.

The site of the tests in Lake Michigan was Bowers Harbor, a small bay on the east side of the west arm of Grand Traverse Bay. Enclosures were placed on platforms positioned about 30 cm off the bottom in 6-8 m of water on a rubble bottom some 0.5 km offshore. Underwater currents apparently were present because little siltation was seen by divers when samples were lifted. Water temperatures at the site were 11 C (29 October), 8 C (11 December), 2 C (7 May), 12 C (2 June), and 12 C (17 June); the oxygen level was near saturation.

Different types of egg enclosures were used. The bottoms of pails like those used in 1973-1974 were cut out and replaced with wire mesh (3-mm spacing, 18-gauge, double galvanized) and three horizontal rows of 3-mm diameter holes were drilled in the sides of the pails. The tops of the pails were provided with a solid metal lid. A second type of egg incubator used was a cylinder 24 cm in diameter and 31 cm high, with a solid wooden top and bottom, and wire mesh (as above) for sides. A larger type of incubator (fry cage) was cylindrical in shape with a diameter of 60 cm and a height of 38 cm. The top and bottom were solid wood while the cylinder wall again was composed of wire mesh. All three types of containers were sprayed with aluminum paint to prevent rusting. Prior to placement of the eggs for incubation, each container was filled with what I considered to be lake trout spawning substrate (gravel of 1-8 cm in diameter and rubble of 10-15 cm in diameter). Six platforms of steel and plywood supported the egg containers on the bottom of Bowers Harbor. The tops of the platforms that supported the 13-liter pails consisted of mesh made of reinforcing rods to allow water circulation through the bottom of the pails. The platform tops for the cylinders and fry cages were made of solid plywood.

Three comparisons of mortality of the two groups were made at Bowers Harbor. In the first test (Test 1), I assessed mortality of green eggs to 5 weeks after swim-up in pails which involved six 50-egg lots from each of the eight females. Forty-eight lots of green eggs in screen-bottomed pails were positioned on two mesh-topped platforms in Bowers Harbor on 29 October. Egg or fry samples of each female were removed for examination at eye-up (two lots), swim-up (three lots) and 5 weeks after swim-up (one lot). The pails (lids removed) containing the lots examined at 5 weeks after swim-up had been placed in fry cages at swim-up. The fry cages, with gravel and rubble on the bottom and open tops, had been placed on the platforms on 29 October. I hoped that a bottom fauna colony would develop in the cages and thus furnish a food supply for lake trout fry emerging from the pails. After the pails were placed, lids were fastened on the top of the fry cages to prevent escapement of lake trout fry. Test 2 was similar to Test 1, with some exceptions. Screen cylinders were used in Test 2 and only 32 lots (four from each female) were involved. Egg or fry samples from each female were removed for examination at swim-up (one lot), swim-up plus 3 weeks (two lots) and at swim-up plus 5 weeks (one lot). No lots were transferred to the fry cages, and the stomachs of the survivors at 3 and 5 weeks after swim-up were examined for evidence of feeding. In the third test, I compared survival of eyed eggs in pails to 3 weeks after swim-up for the two groups. The samples consisted of four 50-egg lots from each of eight females. Eyed eggs for this test were taken from the respective lots at Thompson Hatchery. The 32 lots were placed in separate, screen-bottomed pails on a mesh platform on 11 December. Egg or fry samples from each female were removed for examination at swim-up (three lots) and at 3 weeks after swim-up (one lot). The pails (lids removed) containing the lots examined at 3 weeks after swim-up had been placed in fry cages at swim-up as was done for Test 1. The stomachs of these survivors at 3 weeks after swim-up were examined for evidence of feeding.

1975-1976 tests

Lake Michigan and Marquette Hatchery eggs were incubated at Thompson Hatchery until the swim-up stage was reached for use in stress experiments. Before the stress experiments began, mortality of the two lots was somewhat different. Lake Michigan eggs experienced an average mortality of 52 ± 7% from fertilization to eye-up as compared to a mortality of 22 ± 7% for Marquette Hatchery eggs. From eye-up to swim-up there was no significant difference in average mortality in eggs and sac fry between the Lake Michigan group (15 ± 11%) and the Marquette Hatchery group (12 ± 5%). The high mortality of Lake Michigan eggs very likely occurred because the females were partially spent or overripe when the eggs were taken. I used the survivors of the two groups to determine if the relatively high DDT and PCB content of Lake Michigan fry reduced their resistance to starvation and ability to compete for food. Water temperatures during the experiments ranged from 6 to 8 C. Comparative resistance to starvation was assessed by placing 50 unfed fry from each lot (where there was sufficient survival) into separate compartments without food on 4 March. Mortality was determined by removal and counts of dead fish at intervals, and by counting live fry at the end of the experiments (19 May).

The two groups were compared with respect to their ability to compete, as measured by mortality and growth, with brown trout (Salmo trutta) fry for a limited amount of food. Eighteen test compartments were used (22 × 32 × 29 cm deep) which had screened sides and a gravel substrate. The compartments were suspended in a hatchery tank where water temperature was about 7 C. Twenty-five lake trout fry and 25 brown trout fry were installed in each compartment on 4 March. The average weight of both lake trout and brown trout fry was about 0.1 g. For lake trout, three lots from each of three Lake Michigan females and three lots from each of three Marquette Hatchery females were used in the test. Brown trout were of hatchery origin and were slightly older than the lake trout. Because of high mortality of the brown trout first installed, 10 more brown trout fry (average weight, about 0.1 g) were installed in each compartment on 31 March. Average number of brown trout per compartment was 20 (18 March), 18 (31 March), 12 (14 April), 8 (28 April), and 3 (12 May). Feeding was at the rate of one-half normal fry ration of "starter" Oregon moist pellets. Three times a day an ice cube in which food had been frozen was suspended in the surface layer of the water and allowed to melt, thus releasing food at a slow rate. The experimental fish were subjected to an unprogrammed stress which was the slow rate of water exchange between the compartments and the fresh water supply. This was evidenced by heavy fungal growth in the compartments and considerable mortality of the first brown trout introduced from gill disorders. Mortality was determined by removing dead trout at weekly intervals and by counting survivors at the end of the experiment (12 May).

Results

1973-1974

At Thompson Hatchery, mortality of Lake Michigan eggs usually was higher than that of Marquette Hatchery eggs (Table 2). Mortality of Lake Michigan eggs was significantly higher in the first two periods than mortality of Marquette Hatchery eggs. Thereafter, from hatching to 6 weeks after swim-up, there was no significant difference in mortality of sac fry and feeding fry. Overall, mortality of Lake Michigan eggs and fry (80%) was about twice that of Marquette Hatchery eggs and fry (46%).

There was little relationship between mortality and DDT or PCB content for Lake Michigan eggs. Linear regressions of mortality within periods on DDT or PCB's were far from significant. Polynomial regressions were significant only for the periods of hatching to 6 weeks after swim-up and fertilization to 6 weeks after swim-up. In both periods, mortality was

Table 2.--Average percentage mortality (with 95% confidence limits) within stages of lake trout eggs and fry from Lake Michigan and Marquette Hatchery raised at Thompson Hatchery, October 1973-April 1974.

Group	Female	Stage and inclusive dates			
		Fertilization to eye-up	Eye-up to hatching	Hatching to 6 weeks after swim-up[a]	Fertilization to 6 weeks after swim-up[a]
		31 Oct-12 Dec	13 Dec-21 Jan	22 Jan-8 Apr	31 Oct-8 Apr
Lake Michigan	A	72 ± 6	44 ± 9	52	93
	B	55 ± 6	46 ± 6	24	82
	C	48 ± 6	46 ± 6	16	73[b]
	D	34 ± 6	25 ± 6	21	70
	All fish	52 ± 4	40 ± 4	28 ± 26	80 ± 16
Marquette Hatchery	A	38 ± 6	5 ± 6	9	48
	B	20 ± 6	5 ± 6	18	39
	C	12 ± 6	14 ± 6	24	57[b]
	D	21 ± 6	6 ± 6	12	39
	All fish	23 ± 4	8 ± 4	16 ± 11	46 ± 14

[a] At the end of the second stage, the four lots from each female were combined. Hence, no limits could be set on the average mortality within females during the last two stages.

[b] A maximal percentage, because some live fry escaped from one lot.

significantly correlated with the content of DDT (r = 0.967, F = 78.4; r = 0.985, F = 209.9, respectively) and PCB's (r = 0.915, F = 29.8; r = 0.971, F = 107.7, respectively). The polynomial regressions were the shape of an upright "V" or "U" which meant that mortality was high at small and large contaminant levels and low at moderate levels.

At the end of the experiment there was little or no difference between total length of Marquette and Lake Michigan fry (Table 3).

Mortality was complete for Marquette green eggs in New Mission Bay (Table 4). Lake Michigan egg mortality was slightly less, but all had died by 13 February. The high mortality of both groups probably was caused by excessive siltation by fine sand suspended by severe wave action on the nearby beach and rust deposits from the unpainted pails. Predation by relatively large predators was not the cause of mortality because they were excluded by 13-mm mesh netting. Smaller potential predators (or consumers) were present such as mayflies, scuds, beetle larvae, small crayfish, sculpins (*Cottus* spp.) and sow bugs. However, I believe that few eggs were eaten by these organisms, because many dead eggs were whole when examined on 14 December and 7 January.

For eyed eggs, the mortality percentages in Table 4 show no evidence of superiority for either group. Mortality was moderate for the first month, but subsequently was very high and was associated with heavy siltation by fine sands.

1974-1975

At Thompson Hatchery, mortality of Lake Michigan eggs was not different at any stage from mortality of Marquette Hatchery eggs. This is illustrated by averages of mortality percentages and 95% confidence limits in Table 5. On the average, Marquette fry were significantly larger than Lake Michigan fry (Table 6). The apparent difference in fry size may have been related to the larger average size of Marquette eggs (5.5 mm diameter) as compared to Lake Michigan eggs (5.1 mm).

For Lake Michigan eggs alone, there was little direct relationship between mortality and DDT and PCB content. Within periods, only the linear

Table 3.--Average total length (in millimeters), with 95% confidence limits, of Lake Michigan and Marquette Hatchery lake trout fry in Thompson Hatchery at 6 weeks after swim-up, April 1974.

Female	Lake Michigan			Marquette Hatchery		
	Number	Total length	95% confidence limits	Number	Total length	95% confidence limits
A	17	33.6	1.1	72	33.5	0.4
B	57	34.8	0.4	50	32.6	0.5
C	53	33.1	0.8	56	34.0	0.4
D	52	33.5	0.7	80	33.8	0.4
Average		33.8	0.4		33.5	0.2

Table 4.--Average percentage [a] mortality (with 95% confidence limits) at various stages of green and eyed lake trout eggs incubated in New Mission Bay, Lake Michigan, October 1973-April 1974.

Installation stage, and group	Stage and date when examined				
	Early eye (14 Dec)	Middle eye (7 Jan)	Hatch (13 Feb)	Early sac fry (12 Mar)	Middle sac fry (17 Apr)
Green eggs [b]					
Lake Michigan	80 ± 46	97 ± 6	100 ± 0	100 ± 0	
Marquette Hatchery	100 ± 0	100 ± 0	100 ± 0	100 ± 0	
Eyed eggs [c]					
Lake Michigan		58 ± 40	98 ± 0	99 ± 2	100 ± 2
Marquette Hatchery		50 ± 30	94 ± 9	100 ± 2	100 ± 2

[a] One lot of 50 eggs from each of the eight females was examined at each stage except for Lake Michigan eyed eggs when only three lots were examined at hatch.

[b] Installed 1 November.

[c] Installed 14 December.

Table 5.--Average percentage mortality (with 95% confidence limits) within stages of lake trout eggs and fry from Lake Michigan and Marquette Hatchery lake trout raised at Thompson Hatchery, October 1974-April 1975.

Group, and female	Stage and date of examination				
	Fertilization to eye-up	Eye-up to hatching	Hatching to swim-up	Swim-up to 6 weeks after	Fertilization to 6 weeks after swim-up[a]
	28 Oct-8 Dec	9 Dec-19 Jan	20 Jan-24 Feb	25 Feb-8 Apr	8 Oct-8 Apr
Lake Michigan					
A	18 ± 6	10 ± 6	7 ± 6	10 ± 6	38 ± 49
B	12 ± 6	23 ± 6	18 ± 6	14 ± 6	50 ± 12
C	54 ± 6	18 ± 6	26 ± 6	13 ± 6	76 ± 12
D	13 ± 6	8 ± 6	5 ± 6	8 ± 6	30 ± 12
All fish	24 ± 4	15 ± 4	14 ± 4	11 ± 4	50 ± 9
Marquette Hatchery					
A	25 ± 6	12 ± 6	14 ± 6	8 ± 6	47 ± 12
B	38 ± 6	14 ± 6	2 ± 6	16 ± 6	56 ± 12
C	27 ± 6	6 ± 6	18 ± 6	7 ± 6	51 ± 49
D	14 ± 6	12 ± 6	3 ± 6	9 ± 6	34 ± 17
All fish	26 ± 4	11 ± 4	9 ± 4	10 ± 4	47 ± 9

[a] Within females, averages are based on four lots except for Lake Michigan female A (two lots), Marquette Hatchery female C (two lots) and D (three lots). For analysis, the missing values were estimated as the averages of the lots from the same female with the loss of one degree of freedom for each estimated value.

Table 6.--Average total length (in millimeters), with 95% confidence limits, of Lake Michigan and Marquette Hatchery lake trout fry in Thompson Hatchery at 6 weeks after swim-up, April 1975.

Female	Lake Michigan			Marquette Hatchery		
	Number	Total length	95% confidence limits	Number	Total length	95% confidence limits
A	133	33.5	0.4	148	37.0	0.5
B	132	34.7	0.5	138	39.4	0.5
C	134	34.5	0.4	148	38.9	0.5
D	158	35.8	0.4	141	37.8	0.5
Average		34.6	0.2		38.3	0.2

regression for the period of fertilization to eye-up was significant (for DDT, $r = 0.895$, $F = 48.3$ and for PCB's, $r = 0.850$, $F = 31.3$). On the other hand, polynomial regressions were significant for three time periods. These were: fertilization to eye-up ($r = 0.988$, $F = 232.6$ for DDT and $r = 0.988$, $F = 222.0$ for PCB's); swim-up to 6 weeks after ($r = 0.703$, $F = 5.4$ for DDT and $r = 0.704$, $F = 5.4$ for PCB's); and fertilization to 6 weeks after swim-up ($r = 0.871$, $F = 17.4$ for DDT and $r = 0.872$, $F = 17.4$ for PCB's). The regression for the first time period was in the shape of a length-weight curve which meant that mortality increased at an increasing rate with contaminant content. Regressions for the other two periods were in the form of upright "V" 's or "U" 's which meant that high mortality was associated with small and larger contaminant content and that intermediate mortality was associated with intermediate content.

In Test 1 in Bowers Harbor, comparison of averages and 95% confidence limits showed no difference in mortality between Lake Michigan and Marquette eggs at either the eyed or swim-up stages (Table 7). For the two groups, mortality was 41-62% at the eyed stage and 68% at swim-up. No live fry and very few dead fry were found in the cages and pails examined at 5 weeks after swim-up. In these fry cages and pails, only 36% of the eggs originally installed could be accounted for as compared to 60% for eggs in the cylinders during the same time. Thus, fry in the cages either escaped or were eaten by the 13 sculpins (40-69 mm) that entered the cages when the lids were off. The former seems most likely because there was no apparent relationship between number of unaccountable fry and numbers of sculpins (1-3) in the cages even though two fry were found in stomachs of sculpins in a cage for Test 3. The average percentage of dead eggs and fry remaining in the pails and cages of Test 1 at 5 weeks after swim-up was 39 ± 22 for the Lake Michigan lots and 32 ± 23 for the Marquette lots. Although the total mortalities are unknown, the similar known mortalities imply that there was no difference in mortality between the two groups at 5 weeks after swim-up.

In the similar Test 2, there was no significant difference between average mortality of Lake Michigan and Marquette fry (Table 7) at swim-up

Table 7.--Average percentage mortality (with 95% confidence limits) at various stages of Lake Michigan (L) and Marquette Hatchery (H) lake trout eggs and fry incubated in containers in Bowers Harbor, Grand Traverse Bay, October 1974-June 1975.

Development stage	Date examined	Experiment[a] and egg source					
		Test 1		Test 2		Test 3	
		L	H	L	H	L	H
Eyed	11 Dec						
Percent mortality		41	62				
± 95% confidence limits		19	22				
Number of lots		8	8				
Swim-up	7-12 May						
Percent mortality		68	68	58	63	30	10
± 95% confidence limits		11	11	44	34	13	5
Number of lots		12	12	4	4	12	12
Swim-up + 3 weeks	2 June						
Percent mortality				66	78	87	92
± 95% confidence limits				14	17	20	13
Number of lots				8	8	4	4
Swim-up + 5 weeks	17 June						
Percent mortality		100	100	98	98		
± 95% confidence limits		0	0	4	2		
Number of lots		4	4	4	4		

[a] Eggs for Tests 1 and 2 were installed on 29 October 1974, when "green" and eggs for Test 3 were installed on 11 December 1974 when in the eyed stage.

(58-63%) and at 3 weeks after swim-up (66-78%). By 5 weeks after swim-up, mortality was nearly complete, but again there was no difference between the two groups. Although fry mortality in the hatchery due to starvation did not occur until about 9 weeks after swim-up (see Table 8), starvation may have been involved in the high mortality of both groups in Bowers Harbor by 5 weeks after swim-up. In Bowers Harbor, water temperatures were higher (up to 12 C) than in the hatchery (6-8 C) resulting in a higher metabolic rate for Bowers Harbor fry. The absence of feeding by Bowers Harbor fry was confirmed by stomach analysis. Only 3 of 90 fry preserved at 3 weeks after swim-up contained food (a single *Daphnia* each) and 1 of the 7 fry preserved at 5 weeks after swim-up contained food (either a copepod or *Daphnia*).

In Test 3, comparison of simple averages (Table 7) suggested that mortality of Lake Michigan eggs and fry, to swim-up, was greater (30%) than that of Marquette eggs (10%), but that at 3 weeks after swim-up, there was no difference (87 and 92%). The cause of the apparently high mortality at 3 weeks after swim-up is uncertain. The stomachs of 42 fry contained only 3 organisms (*Daphnia* or copepod) which indicated that the fry were starving. Some may have escaped since only 48% of the original number installed were accounted for as compared to 72% for eggs in the cylinders during the same period of time. The 13 sculpins (40-57 mm) in the cages apparently did not have a significant effect on mortality because only two fry were found in their stomachs and because there was no relationship between number of unaccountable fry and number of sculpins (0-3) in the cages.

1975-1976

In the starvation test, average mortality of Lake Michigan fry was significantly less than that of Marquette fry after 7 weeks and for the period of study as a whole (Table 8).

In the test of ability to resist stress, there were no significant differences in mortality between the two groups in most time periods but in the second period, mortality of Marquette Hatchery eggs was significantly

Table 8.--Average percentage mortality (with 95% confidence limits), within periods, of starved Lake Michigan and Marquette Hatchery fry, 4 March-18 May 1976.

Group, and female	Number of lots	Period in which mortality occurred (weeks since swim-up in parentheses)			
		4 Mar-20 Apr (1-7)	21 Apr-5 May (8-9)	6 May-18 May (10-11)	4 Mar-18 May (1-11)
Lake Michigan					
A	3	7 ± 8	3 ± 8	36 ± 8	42 ± 12
B	3	9 ± 8	35 ± 8	74 ± 8	84 ± 12
C	3	16 ± 8	2 ± 8	60 ± 8	68 ± 12
All fish		11 ± 5	13 ± 5	57 ± 5	65 ± 6
Marquette Hatchery					
A	4	8 ± 6	15 ± 6	76 ± 6	80 ± 9
B	4	7 ± 6	18 ± 6	90 ± 6	90 ± 9
C	2	7 ± 25	35 ± 25	98 ± 25	98 ± 35
D	2	14 ± 25	38 ± 25	95 ± 25	96 ± 35
All fish [a]		8 ± 4	23 ± 4	87 ± 4	89 ± 6

[a] Means are not based on averages of individual females because the number of lots were not the same among females.

Table 9.--Average percentage mortality (with 95% confidence limits), within periods, of lake trout fry under stress of competition with brown trout fry for a limited food supply, 4 March-12 May 1976.

Group, and female[a]	Period in which mortality occurred					
	4-17 March	18-30 March	31 Mar-13 Apr	14-27 April	28 Apr-11 May	4 Mar-11 May
Lake Michigan						
A	2 ± 6	19 ± 6	2 ± 6	9 ± 6	30 ± 6	52 ± 21
B	3 ± 6	7 ± 6	14 ± 6	9 ± 6	26 ± 6	45 ± 21
C	5 ± 6	12 ± 6	4 ± 6	17 ± 6	40 ± 6	61 ± 21
All fish	3 ± 3	13 ± 3	7 ± 3	12 ± 3	32 ± 3	53 ± 11
Marquette Hatchery						
A	7 ± 6	52 ± 6	4 ± 6	4 ± 6	37 ± 6	76 ± 21
B	2 ± 6	10 ± 6	12 ± 6	17 ± 6	35 ± 6	54 ± 21
C	2 ± 6	15 ± 6	0 ± 6	8 ± 6	39 ± 6	53 ± 21
All fish	4 ± 3	26 ± 3	5 ± 3	10 ± 3	37 ± 3	61 ± 11

[a] Three lots of 25 fry for each female.

greater (Table 9). Also, at the end of the experiment, the average weight of Lake Michigan fry (0.130 ± 0.007 g) was not significantly different from that of Marquette Hatchery fry (0.137 ± 0.007 g). Average weight at the end of the experiment of brown trout (0.211 ± 0.022 g) confined with Lake Michigan fry was the same as that of brown trout confined with Marquette Hatchery fry (0.199 ± 0.021 g).

Discussion

The bulk of the evidence in my experiments indicated that DDT and PCB's either singly or in combination did not have a severe effect on early survival of eggs and fry produced by Lake Michigan trout. In the hatchery, mortality of Lake Michigan eggs (2.6-4.0 ppm DDT and 4.8-8.3 ppm PCB's) and fry to 6 weeks after swim-up was similar to that of Marquette eggs (0.2 ppm DDT and 0.3 ppm PCB's) and fry in 1974-1975 (Table 5). Also, for Lake Michigan eggs and fry incubated in the hatchery in 1973-1974 and 1974-1975, mortality was linearly correlated with DDT and PCB content in only one time period (fertilization to eye-up, 1974-1975). Finally, in the hatchery, I and my assistants observed no symptoms that were characteristic of DDT poisoning (Johnson and Pecor 1969) or PCB poisoning (Halter and Johnson 1974; Hogan and Brauhn 1975). In Grand Traverse Bay in 1974-1975, Lake Michigan and Marquette Hatchery eggs and fry, when reared in enclosures, had nearly identical mortality rates at eye-up, swim-up (except for Test 3) and 3 weeks after swim-up (Table 7). In tests of stress, the ability of Lake Michigan fry (from eggs with 1.4-3.6 ppm DDT and 3.2-6.3 ppm PCB's) to resist starvation (Table 8) and cope with a limited food ration (Table 9) was equal to, or greater than, the ability of Marquette Hatchery fry (from eggs with < 0.1 ppm DDT and 0.2-0.3 ppm PCB's).

My findings of little effect of DDT (1.4-5.2 ppm) and/or PCB's (3.2-9.9 ppm) are supported by certain other scientists and observations. Burdick et al. (1964) compared survival of lake trout eggs (total DDT content 2-19 ppm, wet weight) and observed considerable mortality above 5 ppm and negligible mortality below 5 ppm. Johnson and Pecor (1969) compared mortality of three groups of developing coho salmon (<u>Oncorhynchus</u>

kisutch) eggs and fry that had different amounts of DDT in the eggs. Mortality of fry attributed to DDT was 15-73% for the 6-7 ppm group, but was negligible for the 2 and < 1 ppm groups. Degurse et al. (1973) observed no mortality due to total DDT concentrations of 0.5-3.5 ppm plus PCB levels of 2.0-9.4 ppm in eggs (wet weight) of coho salmon. Finally, mature siscowet lake trout in Lake Superior had average DDT and PCB contents in fillets of 14.9 and 32.0 ppm (Great Lakes Environmental Contaminants Survey 1975), yet reproduced successfully (Great Lakes Fishery Commission Annual Meeting 1977).

There were some data and information that may not be in accordance with the evidence of little effect by DDT and PCB's on survival of lake trout eggs and fry. First, in 1973-1974, mortality of Lake Michigan eggs (2.7-5.2 ppm DDT, 5.3-9.9 ppm PCB's) and fry incubated in Thompson Hatchery usually was significantly higher than that of Marquette Hatchery eggs that had much lower DDT and PCB content (Table 2). DDT can be excluded as a cause of mortality because other scientists (cited above) found that DDT at the levels I encountered did not cause mortality and that mortality at higher levels of DDT occurred near swim-up. Most of the Lake Michigan egg and fry mortality occurred before hatching, which is when PCB-induced mortalities are believed to occur (Jensen et al. 1970). PCB's are also suspect because other scientists observed mortality at concentrations much less (see below) than that which occurred in Lake Michigan eggs. Jensen et al. (1970) examined mortality of Atlantic salmon (Salmo salar) from fertilization to the feeding stage. The eggs had PCB concentrations (wet weight) of 0.4-1.9 ppm and mortality ranged from 16-100% for eggs with various amounts of PCB's. I used Jensen's data to calculate that mortality was positively correlated with amounts of PCB's (n = 12, r = 0.877, F = 33.4). However, during the same period of development that was observed by Jensen et al. (1970), mortality of the Lake Michigan eggs and fry in 1974-1975 was not at all correlated (r = −0.490, F = 3.80) with amount of PCB's (4.8-8.3 ppm). Hogan and Brauhn (1975) observed that rainbow trout (Salmo gairdneri) fry from eggs with 2.7 ppm of Aroclor 1242 suffered a mortality of 75% within 30 days after hatching. In five other groups of less-contaminated eggs, 10-28% mortality was observed during the same period. Hansen et al. (1973) examined the effect of Aroclor 1254 on survival of embryos and fry of sheepshead minnows (Cyprinodon variegatus). At concentrations of PCB's (3.7-9.5 ppm) similar to that in these experiments, mortality to 4 weeks after hatching was 16-92%. In comparison, mortality below this level of PCB's was 4-40%.

On the other hand, the relatively high mortality of Lake Michigan eggs and fry may have been due to unrecognized differences in treatment of the parents, eggs, or sperm prior to the placement of the eggs at Thompson Hatchery. One recognized difference in treatment of the two groups was the mode of transportation of the fertilized eggs to Thompson. Lake Michigan eggs were flown 160 miles to Thompson on a day of extreme air turbulence while Marquette Hatchery eggs were transported 80 miles in a station wagon. Furthermore, the high mortality (70-100%) of all lots of Lake Michigan eggs, irregardless of PCB or DDT content, also suggests that there was an overriding mortality agent that obscured any possible effect of DDT and PCB's.

Second, certain regressions within time periods of mortality on contaminant amounts were significant and suggested a contaminant effect of some sort. Mortality in the time period of fertilization to eye-up in 1974-1975 was positively associated with DDT or PCB's in linear regressions which suggested an adverse effect by PCB's. Significant polynomial regressions of mortality on contaminant amount (either DDT or PCB's) occurred in the periods of fertilization to eye-up (1974-1975), hatching to 6 weeks after swim-up (1973-1974), swim-up to 6 weeks after (1974-1975) and fertilization to 6 weeks after swim-up in both years. The regressions were in the form of upright "U" 's or "V" 's, meaning that mortality was high at both small and large amounts of contaminants and low at intermediate amounts. Such a relationship cannot be satisfactorily explained. I was unable to locate information in the literature that would verify or disprove such a relationship.

In summary, it is conceivable that DDT and PCB's at the levels tested may have a slightly adverse effect on survival of young lake trout.

However, it appears unlikely that contaminant-induced mortality is severe enough to account for the apparent complete failure of reproduction in Lake Michigan.

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