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Fertilization of Eggs of Lake Michigan Lake Trout *Salvelinus namaycush* in Lake Water: Effect of PCBs (Aroclor 1254)

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Over 79 million lake trout (*Salvelinus namaycush*) were planted in Lake Michigan from 1965 through 1993 (Mark Ebener; Chippewa/Ottawa Treaty Fishery Management Authority; Sault Ste. Marie, Michigan, pers. comm. 1994) and some of these fish are known to have spawned (Brown et al. 1981, Holey et al. 1995). Nevertheless, intensive interagency sampling efforts have only found evidence of lake produced yearlings or older offspring in a few places including Grand Traverse Bay (13 % and 7 % of the 1976 and 1981 year classes) and Platte Bay (4% of the 1983 year class) during 1983-1989 (Rybicki 1991, for review: Holey et al. 1995). This failure of planted lake trout to develop self-sustaining populations has raised concerns about effects of chlorinated hydrocarbons, known to be present in this and other Great Lakes, on viability of gametes and early life stages. Polychlorinated biphenyls (PCBs) have been detected in lake trout eggs in the Great Lakes (Mac et al. 1985, Smith et al. 1990). A study by Stauffer (1979) found no evidence that viability of fertilized eggs of Lake Michigan lake trout was measurably impaired by PCBs and DDE present in the eggs. Nevertheless, other studies have proposed that the presence of PCBs, polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) in feral lake trout eggs may help explain mortality during early life stages (Berlin et al.1981; Mac et al. 1985, 1993, and Symula et al. 1990). Spitsbergen et al. (1991) and Walker et al. (1991) found that early life stages of lake trout are at higher risk of mortality than are adults from PCBs and related contaminants. Injections of PCBs and related congeners into rainbow trout (*Oncorhynchus mykiss*) eggs caused a dose-related increase in mortality from hatching to swim-up (Walker and Peterson 1991). However, the effect of PCBs present in the water during egg fertilization has not been evaluated experimentally (Zint et al. 1995).

We wondered if gamete viability (defined herein as the ability of gametes to form a recognizable zygote) might be reduced by contaminants present in the water during spawning. In the fall and winter of 1978-79, we tested the effect of added PCBs (Aroclor 1254) on fertilization of Lake Michigan lake trout eggs

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in lake water. To eliminate the possibly confounding effect of background levels of organochlorines present in the lake water used for these tests, the lake water was carbon-filtered before known amounts of PCBs were added. Because we used acetone as a carrier solvent for PCBs, we also added equal amounts of acetone alone to carbon-filtered and unfiltered lake water to examine acetone's effect *per se* on fertilization.

MATERIALS AND METHODS

We used the Great Lakes Science Center (GLSC) vessel base at Saugatuck, Michigan, as the site for the fertilization tests. Subsequent egg incubation was done at the GLSC. On November 7, 1978, lake water was collected and filtered through 12" of activated carbon in a 24" x 2" glass column to remove hydrocarbons from the water (Cheremisinoff and Ellerbusch 1978). We held all containers of water used for the tests in a constant temperature bath at 10.5° C, well within the range of spawning temperatures (5-16.7° C) for lake trout given by Martin and Olver (1980). The PCB stock solution (269 mg/L as Aroclor 1254--a concentration as high as ever could be expected to occur in the most contaminated nearshore waters) had been prepared earlier at the Great Lakes Science Center (Berlin et al. 1981). Four treatment media were prepared: carbon-filtered plus acetone (FA), carbon-filtered plus 269 ng/L of PCBs (Aroclor 1254) dissolved in acetone (FA+PCB), unfiltered lake water (UF), unfiltered plus acetone (UFA). The test concentration of PCBs to which the unfertilized eggs and milt were briefly exposed during actual fertilization, 269 ng/L, was more than 20 times ambient concentrations reported for Lake Michigan water: 12.4 ng/L (Lake Michigan Interstate Pesticides Committee 1972) and 9.1 ng/L (Durfee et al., 1976).

On November 8, about 32,500 eggs and 50 mL of milt were stripped from 11 female (total length 691-834 mm) and 12 male lake trout (total length 566-844 mm) gillnetted near Saugatuck. All eggs and milt were pooled to offset individual variation in gamete viability. We placed 35 mL of eggs (340-423 eggs) and 1 mL of milt (8 replicates for each treatment) simultaneously into 100 mL of a given treatment medium. The contents were stirred for 10 sec, then allowed to stand for 1 min, during which fertilization occurred. After 1 minute (trout sperm generally becomes inactive by then, according to Piper et al. 1982), we ended the actual fertilization test by decanting the water-milt mixture and rinsing the surplus milt from the eggs by adding 100 mL of iron-filtered well water from the laboratory, then immediately stirring, decanting, and adding another 100 mL of iron-filtered well water, after which the beaker was returned to the constant temperature bath. The eggs were then water-hardened for at least 1 h before transport and acclimated to a temperature of about 6 C en route to the Center. Each egg lot was randomly assigned to 1 of 32 compartments (4 in each of 8 drawers) in standard Heath incubator drawers, where they were incubated in iron-filtered well water (physical and chemical characteristics of water given by Berlin et a1.1981) at a mean temperature of 7.7°C (range, 5.4 to 9.5°), a

total hardness of 465 mg, and a flow rate of 2-3 L/min (12-18 exchanges of water per hour in each of the 10-L incubator drawers). We checked water temperature and flow rates daily, adjusted them as necessary, removed partially or completely opaque (dead) eggs every other day, and cleared such eggs in a 1% NaCl solution (Leitritz and Lewis 1976) to determine whether they had been fertilized (i.e., showed a recognizable cleavage stage or embryo).

We tested eight replicate lots of eggs within each of the four treatments using a randomized block design during testing and subsequent incubation of the eggs. The randomized block design enabled us to account for (1) effects of temporal changes in experimental conditions (e.g., test-to-test time lag) on gamete viability, and (2) a slight vertical thermal gradient effect (0.14°C maximum difference in mean temperature) of incubator drawer location on the mortality of eggs in a given replicate lot--one lot for each treatment was incubated at the GLSC in each of eight vertically stacked drawers. The actual number of eggs in each lot was determined by counting the eggs during subsequent observations that we recorded on their development in the incubator drawers. We used the Kruskal-Wallis Test (SAS NPAR1WAY Procedure), χ^2 approximation, to test for significant differences. We set the experimentwise error rate at α=0.05, so that the probability of making any type I error during the six comparisons made among the four treatment means did not exceed α, as recommended by Sokal and Rohlf (1981).

Analysis of PCBs (quantified as Aroclor 1254) in samples of unfertilized eggs indicated a wet weight concentration of 4.20 ± 0.38 µg/g (L. Nicholson, Great Lakes Science Center, pers. comm.). Samples of unfertilized eggs and milt from lake trout gillnetted in Lake Michigan near Saugatuck in 1977, one year prior to our beginning these tests, contained 7.12-11.40 µg/g of PCBs (eggs) and 0.04-0.05 µg/g PCBs (milt) (L. Nicholson, Great Lakes Science Center).

RESULTS AND DISCUSSION

Out of 10,600 eggs used in the tests, fertilization occurred in 7,800 (73.6%). Egg incubation (time from fertilization to 50% hatch) was 72 days at a mean temperature of 7.7 °C. The mean percentage fertilized, defined as the percentage, ± one standard error (SE), that developed recognizable cleavage stages or embryos varied from 71.4±2.1 to 76.2±1.0 (Table 1). Defective partitions between the four egg lots in one of the eight incubator drawers precluded use of egg fertilization and mortality data from that drawer, reducing the number of replicates for each treatment to seven.

We detected no significant differences among the mean percentage of eggs fertilized, indicating that the presence of acetone in unfiltered or carbon-filtered lake water or in combination with PCBs in carbon-filtered lake water had no detectable effect on fertilization (Table 1).

Table 1. Mean and standard error (SE) of the percentage of eggs fertilized and the percentage of fertilized eggs of the total number fertilized that died before hatching for four treatments (fertilization environments), with the 2 values from the Kruskal-Wallis tests that compared the means of the results of various treatments[1].

Treatment	% Fertilized	χ^2 Value			
	MEAN∓SE	FA	FA+PCB	UF	UFA
FA	76.2∓1.0	----	2.7653	2.5510	0.0041
FA+ PCB	71.9∓1.8	2.7653	----	0.0367	1.8000
UF	71.4∓2.1	2.5510	0.0367	----	1.4735
UFA	75.5∓2.3	0.0041	1.8000	1.4735	----
Treatment	% Dead Embryos	χ^2 Value			
	MEAN∓SE	FA	FA+PCB	UF	UFA
FA	7.3∓0.8	----	3.4327	4.4449	0.2004
FA+PCB	9.4∓0.8	3.4327	----	0.0367	2.5566
UF	8.0∓0.6	4.4449	0.0367	----	1.4767
UFA	8.5∓0.4	0.2004	2.5566	1.4767	----

[1]None of the statistics was greater than the critical level for χ^2 at an experimentwise error rate of 0.05.

The percentage (± SE) of fertilized eggs in which the embryos died before hatching ranged from 7.3±0.8 to 9.4±0.8 (Table 1). We detected no significant differences among the mean percentages of fertilized eggs in which such embryo mortality occurred (Table 1).

In studies by Mac et al. (1981) and Stauffer (1979), development of lake trout embryos from Lake Michigan and hatchery broodstock was monitored from earliest cleavage through hatching to yolk absorption and showed no correlation between the percent survival and the levels of residual PCBs and DDE in the eggs. Hansen et al. (1974) found fertilization success was not affected by PCB (Aroclor 1254) concentrations as high as 201 µ g/g in the eggs of the sheepshead minnow (*Cyprinodon variegatus*). We note that fertilized lake trout eggs in the FA + PCB treatment had the highest mortality (9.4%).

Fertilization of killifish (*Fundulus heteroclitus*) eggs was reduced when gametes were mixed in media containing 1 and 10 mg/L DDT (Crawford and Guarino

1976). The presence of acetone during fertilization had no apparent effect on subsequent egg mortality. No data were found in the literature on the effect of small concentrations of acetone (e.g., 0.001 mL/L) on fertilization and survival of fish eggs.

As previously noted, various studies indicate that PCBs appear to have an adverse effect on the viability of fertilized eggs and subsequent early life stages of lake trout and related species. Our tests detected no impairment of fertilization of lake trout eggs in PCB-dosed lake water. The concentration of PCBs in the fertilization medium that we used was more than 20 times as high as estimated ambient levels in southeastern Lake Michigan and it appears unlikely that ambient levels of PCBs in the water at fertilization would contribute significantly to the apparent widespread reproductive failure of lake trout there.

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REFERENCES

Berlin WH, Hesselberg RJ, Mac MJ (1981) Growth and mortality of fry of Lake Michigan lake trout during chronic exposure to PCB's and DDE. In: Chlorinated Hydrocarbons as a Factor in the Reproduction and Survival of Lake Trout (*Salvelinus namaycush*) in Lake Michigan. US Fish and Wildl Serv Tech Pap 105 : 11-22

Brown EH, Eck GW, Foster NR, Horrall RM, and Coberly CE (1981) Historical evidence for discrete stocks of lake trout (*Salvelinus namaycush*) in Lake Michigan. Can J Fish Aquatic Sci 38: 1747-1758

Cheremisinoff PN, and Ellerbusch F, eds (1978) Carbon adsorption handbook. Ann Arbor Sci Pub, Ann Arbor, MI

Crawford RB, and Guarino AM (1976) Effects of DDT in Fundulus: studies on toxicity, fate, and reproduction. Arch Environ Contam Toxicol 4: 334-348

Durfee RL, Contos G, Whitmore FC, Barden JD, Hackmann BB, Westin RA (1976) PCBs in the United States industrial use and environmental distributions. US Environ Prot Agency, EPA-560/6-76-005, Washington, DC

Hansen DJ, Schimmel SC, and Forester J (1974) Aroclor 1254 in eggs of sheepshead minnows: effect on fertilization success and survival of embryos and fry. Proc 27th Ann Conf S.E. Assoc Game and Fish Comm, October 14-17, 1973, Hot Springs, AR, pp 420-423

Holey ME, Rybicki RW, Eck GW, Brown EH Jr, Marsden JE, Lavis DS, Toneys ML, Trudeau TN, Horrall RM (1995) Progress toward lake trout restoration in Lake Michigan. J Great Lakes Res 21 (Supplement 1): 128-151

Lake Michigan Interstate Pesticides Committee at the Lake Michigan Enforcement Conference: An evaluation of DDT and dieldrin in Lake Michigan. (1972) Ecol Res Series, EPA-RE-72-003, US Environ Prot Agency, Washington, DC

Leitritz E, Lewis RC (1976) Trout and salmon culture. Calif Dept Fish and Game Fish Bull 164

Mac MJ, Berlin WH, Rottiers DV (1981) Comparative hatchability of lake trout eggs differing in contaminant burden and incubation conditions. In: Chlorinated hydrocarbons as a factor in the reproduction and survival of lake trout (*Salvelinus namaycush*) in Lake Michigan. US Fish Wildl Serv Tech Pap 105: 8-10

Mac MJ, Edsall CC, Seelye JG (1985) Survival of lake trout eggs and fry reared in water from the upper Great Lakes. J Great Lakes Res 11: 520-529

Mac MJ, Schwartz TR, Edsall CC, Frank AM (1993) Polychlorinated biphenyls in Great Lakes lake trout and their eggs: relations to survival and congener composition 1979-1988. J. Great Lakes Res 19: 752-765

Martin NV, Olver CH (1980) The lake charr, *Salvelinus namaycush.* Page 205 In: Balon, EK, (ed) Charrs: Salmonid fishes of the genus *Salvelinus.* Dr. W. Junk Pub, The Hague

Piper RG, McElwain IB, Orme LE, McCraren JP, Fowler LG, Leonard JR (1982) Fish Hatchery Management. US Dept Interior, Fish Wildl Serv, Washington DC

Rybicki RW (1991) Growth, mortality, recruitment and management of lake trout in eastern Lake Michigan. Dept Nat Resour Fish Res Rep 1979, Ann Arbor, MI

Smith LM, Schwartz TR, Feltz K, Kubiak TJ (1990) Determination and occurrence of AHH-active polychlorinated biphenyls, 2,3,7,8-tetrachlorodibenzo- *p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran in Lake Michigan sediment and biota. The question of their relative toxicological significance. Chemosphere 21: 1063-1085

Sokal RR, Rohlf FJ (1981) Biometry. Second Edition. W. H. Freeman & Co., New York, NY

Spitsbergen JM, Walker MK, Olson JR, Peterson RE (1991) Pathologic alterations in early life stages of lake trout, *Salvelinus namaycush*, exposed to 2,3,7,8-tetrachlorodibenzo- *p*-dioxin as fertilized eggs. Aquat Toxicol 19: 41-72

Stauffer TM (1979) Effect of DDT and PCBs on survival of lake trout eggs and fry in a hatchery and in Lake Michigan, 1973-1976. Trans Amer Fish Soc 108: 178-186

Symula J, Meade J, Skea JC, Cummings L, Colquhoun JR, Dean HJ, Miccoli J (1990) Blue-sac disease in Lake Ontario lake trout. J Great Lakes Res 16: 41-52

Walker MK, Spitsbergen JM, Olson JR, Peterson RE (1991) 2,3,7,8-Tetrachlorodibenzo- *p*-dioxin (TCDD) toxicity during early life stage development of lake trout (*Salvelinus namaycush*). Can J Fish Aquat Sci 48: 875-883

Walker MK, and Peterson RE (1991) Potencies of polychlorinated dibenzo-p-dioxin, dibenzofuran, and biphenyl congeners, relative to 2,3,7,8-tetra-chlorodibenzo-p-dioxin, for producing early life stage mortality in rainbow trout (*Oncorhynchus mykiss*). Aquat Toxicol 21:219-238

Zint MT, Taylor WW, Carl L, Edsall CC, Heinrich J, Sippel A, Lavis D, Schaner T (1995) Do toxic substances pose a threat to rehabilitation of lake trout in the Great Lakes? A review of the literature. J Great Lakes Res 21 (Suppl.1):530-546