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IE U.S. FISH AND WILDLIFE SERVICE

105. Chlorinated Hydrocarbons as a Factor in the Reproduction and Survival of Lake Trout (*Salvelinus namaycush*) in Lake Michigan

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

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Technical Papers

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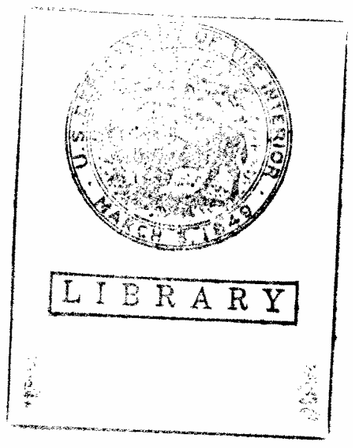
105. Chlorinated Hydrocarbons as a Factor in the Reproduction and Survival of Lake Trout (*Salvelinus namaycush*) in Lake Michigan

Great Lakes Fishery Laboratory,
Ann Arbor, Michigan



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Introduction and Summary[1]

by

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Abstract

Although lake trout (*Salvelinus namaycush*) were considered extinct in Lake Michigan by the mid 1950's, control of the parasitic sea lamprey (*Petromyzon marinus*) and extensive restocking resulted in an abundance of hatchery-produced lake trout in the lake by the early 1970's. However, no naturally produced yearling or older lake trout have been found in the lake during nearly a decade of assessment sampling. Among the numerous hypotheses proposed to account for this apparent reproductive failure of the planted lake trout, a frequently suggested cause is the well-documented contamination of the fish by toxic substances such as DDT and its metabolites, and polychlorinated biphenyls (PCB's) at concentrations reported as adversely affecting the hatching of eggs and survival of larval fish. However, manually stripped and fertilized eggs of Lake Michigan lake trout have hatched successfully and the fry have survived normally under a variety of hatchery conditions. This observation led to studies at the Great Lakes Fishery Laboratory on the performance and survival of fry hatched from eggs of Lake Michigan lake trout and exposed for 6 months to PCB's (Aroclor 1254) and DDE at concentrations similar to those present in offshore waters and zooplankton of Lake Michigan (10.0 ng/L PCB's and 1.0 ng/L DDE in water; 1.0 μg/g PCB's and 0.1 μg/g DDE in food), and at concentrations 5 and 25 times higher. Cumulative mortality of the fry exposed to simulated Lake Michigan levels of PCB's and DDE for 6 months was 40.7%—nearly twice that of unexposed (control) fry—and mortality at the highest exposure level was 46.5%. Evaluation of the growth, swimming performance, predator avoidance, temperature preference, and metabolism of the fry showed no significant effects attributable to exposure to PCB's and DDE, except for a lowering of preferred temperature at the highest (25×) exposures (the only concentration tested) to each contaminant and (additively) both contaminants combined. Although several factors have undoubtedly contributed to the lack of recruitment of naturally produced lake trout in Lake Michigan, the levels of PCB's and DDE present during the early to mid 1970's were sufficient to significantly reduce survival of any fry produced in the lake and thereby impede restoration of the lake trout population to self-sustainability. The added exposure of the fry to other toxic substances known to be present in the lake could have further reduced survival.

Introduction

During the past three decades, a coalition of State, Provincial, and Federal agencies, coordinated by the Great Lakes Fishery Commission, has been working toward the control of factors instrumental in the near extinction of lake trout (*Salvelinus namaycush*) in the Great Lakes and the restoration of naturally reproducing populations of this valued species. Although this effort has resulted in limited success in Lake Superior (Pycha and King 1975), it has been largely unsuccessful in Lake Michigan—i.e., the abundant stocks of lake trout in Lake Michigan are composed wholly of planted fish that have failed to produce detectable numbers of surviving offspring (Kutkuhn 1981). Several potential causes for this lack of recruitment have been proposed and are under investigation, not the least of which is the known contamination of the lake by toxic substances.

In this report, we briefly review the status of lake

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trout in Lake Michigan and summarize a number of studies conducted through 1977 at the Great Lakes Fishery Laboratory as part of an attempt to evaluate the potential role of toxic substances in the apparent reproductive failure of lake trout in the lake. These studies focused on the effects of PCB's (polychlorinated biphenyls as represented by Aroclor 1254) and DDE (1,1,-dichloro-2,2-bis [*p*-chlorophenyl] ethylene), the predominant metabolite of DDT, on the early life stages of lake trout. These particular compounds were selected for study (both singly and in combination) because of their prominence as contaminants of lake trout in the lake during the 1970's. The detailed descriptions and results of the studies are presented in the six reports that follow this summary.

History of the Fishery

From about 1890 until 1945, the lake trout was the most valuable and sought-after commercial species in Lake Michigan. The annual commercial catch averaged 3,700 metric tons (t) from 1890 to 1911, 3,200 t from 1912 to 1926, and 2,400 t from 1927 to 1939. The catch increased slightly to an annual average of 3,000 t during 1940 to 1944, but then began to decline precipitously in 1945; by 1949 it had fallen to only 155 t. In 1954 the catch was a mere 15 kg, and by 1956 the species was probably extinct in Lake Michigan (Wells and McLain 1973).

The gradual decline in the commercial harvest of lake trout from 1893 to 1938 is believed to have resulted from excessive exploitation (Van Oosten 1949; Wells and McLain 1973). Although the commercial harvest of lake trout continued into the early 1950's, the apparent extinction of the species in about 1956 is believed to have been caused directly by the parasitic sea lamprey (*Petromyzon marinus*), an exotic species that became firmly established in Lake Michigan in the decade following its first reported presence there in 1936 (Wells and McLain 1973).

Early attempts to control the sea lamprey consisted of installing electrical and mechanical barriers, which blocked the spawning runs of adults. Between 1953 and 1958, barriers were constructed across 65 tributaries flowing into Lake Michigan. At about the same time (in the late 1950's) a successful lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), was discovered and developed by scientists at the Hammond Bay Biological Station of the U.S. Fish and Wildlife Service. This compound was soon being used to kill larval sea lampreys (ammocetes) in tributary streams before they could metamorphose and migrate downstream into the lake. Most barrier operations were discontinued in 1960 in favor of TFM treatments, thus setting the stage for the highly successful sea lamprey control program that followed. This program and a lake trout restocking program, which began in 1965 in Lake Michigan with the planting of 1.3 million yearling lake trout, have been coordinated by the Great Lakes Fishery Commission. In 1965–78, an average of over 2 million fin-clipped lake trout per year were planted in the lake (data provided by the Great Lakes Fishery Commission) as part of an effort to restore lake trout stocks to self-sustainability.

By the early 1970's, lake trout were once again considered abundant in Lake Michigan (Wells and McLain 1973) and an excellent sport fishery developed. Although widespread spawning activity was observed as early as 1971 (Great Lakes Fishery Laboratory 1974), no naturally produced yearling or older lake trout (recognizable by their lack of clipped fins) were detected in the lake during nearly a decade of routine assessment sampling (Great Lakes Fishery Laboratory 1978; Kutkuhn 1981). Therefore little progress has been made toward the goal of rehabilitating self-sustaining stocks of lake trout, even though the lake contains a population of mature fish that should be capable of reproducing naturally.

Rehabilitation Problems

Following the reports of widespread spawning activity of lake trout in the early 1970's, concern deepened about the apparent failure of the fish to produce surviving progeny. The numerous hypotheses that have been proposed to account for this reproductive failure include the following:

- Contamination of the water and fish by toxic substances such as pesticides and industrial chemicals.
- Deterioration in bottom conditions on spawning reefs as a result of eutrophication and possibly increased sedimentation.
- "Homing" of planted trout as spawning adults to the sites where they were planted—generally shallow, inshore areas that offer little suitable spawning substrate and are vulnerable to sedimentation or scouring action by waves and ice.
- Predation on, or feeding competition with, young lake trout by the now abundant introduced species, rainbow smelt (*Osmerus mordax*) and alewife (*Alosa pseudoharengus*).
- Artificial selection, extensive inbreeding, or physiological and behavioral conditioning of hatchery fish, which somehow result in their inability to spawn successfully or to produce young that are capable of surviving in the wild.
- Insufficient size of spawning aggregations of mature lake trout to enable successful reproduction.

Various studies addressing these hypotheses were soon initiated by the Michigan Department of Natural Resources and the Great Lakes Fishery Laboratory

(Rybicki and Keller 1978). Of greatest concern initially was the problem of toxic substances. The fish were known to contain substantial residues of total DDT (DDT, DDD, and DDE) and of PCB's (Reinert 1970; Stalling and Mayer 1972). Concentrations of each of these contaminants exceeded 10 μg/g in adult lake trout (Willford 1975) and 4 μg/g in their eggs (Reinert and Bergman 1974). Published reports on the effects of DDT and its metabolites and of PCB's indicated that the concentrations of these contaminants in lake trout and their eggs were sufficient to interfere with reproduction. For example, Burdick et al. (1964) reported that concentrations of DDT in excess of 2.9 μg/g in the eggs of lake trout resulted in increased mortality of fry. This effect was later confirmed by Macek (1968), who studied brook trout (*Salvelinus fontinalis*) fed DDT. Unusually high mortality of fry of coho salmon (*Oncorhynchus kisutch*) hatched from eggs of Lake Michigan fish, and possible correlation of that mortality with elevated levels of DDT and other chlorinated hydrocarbons were also reported (Johnson and Pecor 1969; Willford et al. 1969). In addition, reduced hatchability of salmon eggs in Sweden was reported to be correlated with elevated PCB residues (Jensen et al. 1970). Nevertheless, hatchery records showed that when the heavily contaminated eggs of planted Lake Michigan lake trout were manually stripped, fertilized, and hatched, and the fry were reared in hatcheries, survival was "normal" or "satisfactory" (Stauffer 1979).

Hatchability of Eggs

In 1972–73, scientists at the Great Lakes Fishery Laboratory further investigated the hatchability of eggs from Lake Michigan lake trout under three sets of incubation conditions: normal hatchery conditions; a temperature regime similar to that of winter and spring in Lake Michigan; and the temperature and chemical conditions characteristic of water from the Hammond Bay Biological Station's intake in Lake Huron (Mac et al. 1981). Related studies were carried out by the Michigan Department of Natural Resources at the Marquette State Fish Hatchery, at the Thompson State Fish Hatchery, and at two locations (in egg-holding enclosures) in Lake Michigan's Grand Traverse Bay from 1973 to 1976 (Stauffer 1979). In all of these studies, the survival of contaminated eggs and fry from Lake Michigan lake trout was compared with that of relatively uncontaminated eggs and fry from hatchery brood stock. Although occasional differences in survival were noted between groups of eggs and fry reared under the various experimental conditions, no consistent relation between hatching success and the concentrations of PCB's or total DDT in the eggs was apparent. The conclusion reached in the studies performed at the several locations by the two agencies was that existing levels of PCB's and total DDT (predominantly DDE) in eggs of Lake Michigan lake trout did not significantly affect survival of eggs or of early stages of the fry.

The reproductive failure of lake trout in the lake was nevertheless still apparent in the mid 1970's. We then speculated that, although the eggs could hatch and the fry survive in a clean (hatchery or laboratory) environment, the additional chronic exposure to PCB's and DDE in the water and food organisms in Lake Michigan might reduce the stamina, strength, or wariness of the fry sufficiently to preclude their survival in the rigorous lake environment.

Survival of Fry

This hypothesis of posthatching toxicity was tested in a 6-month study begun in the winter of 1975–76 on the effects of chronic exposure of fry of Lake Michigan lake trout to PCB's and DDE (Berlin et al. 1981). In addition to making routine observations on growth and mortality of the fry, biologists also measured the swimming performance, predator avoidance, temperature preference, and metabolism of fry (Rottiers and Bergstedt 1981; Mac 1981; Mac and Bergstedt 1981; Passino 1981). For convenience in this summary and the reports that follow, the young lake trout are termed fry, although they would have been considered fingerlings by the end the study. All of the fry studied originated from a lot of about 27,000 eggs that were manually stripped and fertilized with milt from lake trout (about 10 females and 20 males) gillnetted in southeastern Lake Michigan near Saugatuck, Michigan, in fall 1975. Contaminant levels in adult lake trout from this area had been monitored for several years and the fish were known to contain average whole-body concentrations of about 22 μg/g total PCB's, 7.5 μg/g total DDT, and 0.3 μg/g dieldrin (Great Lakes Fishery Laboratory, unpublished data). Eyed eggs sampled from those collected for this study contained 7.6 μg/g total PCB's and 4.7 μg/g total DDT. Samples of 1-day-old sac fry hatched from these eggs and analyzed at the Columbia (Missouri) National Fisheries Research Laboratory of the U.S. Fish and Wildlife Service contained 3.8 μg/g PCB's (Aroclor 1254), 2.3 μg/g total DDT, 0.06 μg/g dieldrin, 0.12 μg/g *cis*-chlordane, and about 5.7 μg/g of a chemical resembling toxaphene. Later analyses showed that the toxaphene-like residue was composed of several chlorinated camphenes of undetermined origin.

The fry were then exposed for 6 months to 10.0 ng/L PCB's (Aroclor 1254) and 1.0 ng/L DDE in water, and 1.0 μg/g PCB's and 0.1 μg/g DDE in food. These values approximate the exposure received by fish in the lake (herein designated as 1×), as determined by analyses of water and plankton collected offshore in south-

eastern Lake Michigan. Concentrations 5 and 25 times these values (5× and 25×) were also tested to allow dose-effect interpretation and prediction of potential effects on fry hatched in the more heavily contaminated, nearshore areas of the lake (Lake Michigan Interstate Pesticides Committee 1972).

About a week after the eggs hatched, 650 fry were randomly placed into each of 30 tanks in a constant-flow bioassay system. Serial diluters supplied the appropriate concentrations (1×, 5×, 25×, and control) of the contaminants, singly or in combination, in well water at 9°C. The experimental design thus provided three replicates of 10 different treatments (including the controls). After 16 days of exposure, the fry began to exhibit feeding behavior and were fed the corresponding dosage of either or both contaminants, which was added to their food. Analyses of water during the study showed that the actual average exposures received by the fry corresponding to 1×, 5×, and 25× were 20.8, 64.7, and 327 ng/L PCB's and 1.8, 6.3, and 32.7 ng/L DDE. Analyses of the food showed that actual concentrations were all within 28% of agreement with nominal concentrations.

During the first 16 days of exposure to the three levels of PCB's, DDE, and PCB's + DDE in water, the percentages of fry that died (mean of three replicates per treatment) ranged from 1.9 to 3.7 among the different treatments. Mortalities of fry among the nine exposed groups were not significantly different from those among the controls. During the next 40 days (days 17–56), when exposed fry were receiving contaminants from their food as well as from the water, the mortality of fry remained relatively low (2.2 to 8.6%) in all exposure groups except those receiving 25× PCB's (24.2%). Mortality during this period was consistently higher in the controls (7.3%) than in the 1× and 5× exposures (2.2 to 5.9%). By the end of the second 40-day period (days 57–96), however, the mortality of fry increased significantly in all treatments. This increase was particularly pronounced in the nine exposed groups, in which mortality ranged from 19.0 to 35.4% compared with 11.2% in the controls. The rates of mortality then declined steadily during the next two 40-day periods (days 97–136 and 137–176), but nevertheless remained higher in the exposed groups than in the controls. The final cumulative mortalities on day 176 in the nine exposed groups averaged 30.5 to 46.5%, whereas that in the control group was 21.7%. In the combination exposures to PCB's + DDE, final mortalities were positively correlated with dose (i.e., mortalities increased as concentrations of PCB's + DDE increased), but in exposures involving only PCB's or DDE, mortalities were not correlated with dose although they were all significantly higher than in the controls.

Especially noteworthy was the final cumulative mortality of fry in the 1× combination of PCB's + DDE (simulated Lake Michigan exposure)—40.7%, or nearly double the final cumulative mortality of the controls. This result suggests that if lake trout in Lake Michigan spawned successfully in the mid 1970's and their eggs hatched, nearly twice as many of the resulting fry died within the first 6 months as would have died if these contaminants had not been present.

Physiology of Fry

In addition to observations on the survival of fry during the chronic exposure, observations were made periodically on the growth, swimming performance, predator avoidance, temperature preference, and metabolism of fry from the same lots of experimental fish. Contaminant analyses were also made to determine the concentrations of PCB's and DDE in the fry after the 6-month exposure. These analyses (Berlin et al. 1981) showed an unexpected decrease in the average concentration (μg/g) of PCB's and DDE in fry exposed to simulated Lake Michigan levels (1×). However, a net increase in the body burden of PCB's and DDE (μg/fish) occurred in the fry in all treatment groups during the 6-month exposure. Thus the fry accumulated additional PCB's and DDE, even though the average concentration (at the 1× exposure) declined in the fry. These seemingly anomalous results were apparently a function of the rapid growth of the fry and dilution of their initially elevated tissue levels of PCB's and DDE with new, less contaminated tissues during the study.

In a comparison of the effects of PCB's and DDE on the growth of lake trout fry in each of the 10 treatments, Berlin et al. (1981) subsampled and weighed surviving fry five times during the study: 16 days after the beginning of exposure to contaminants in the water (and just before the beginning of exposure in the food), and on days 56, 96, 136, and 176. The authors observed no significant differences among growth rates of fry in different exposure groups and replicates, neither within any of the four 40-day growth periods or all growth periods combined, nor between the mean weights from any exposure group within any of the four growth periods. There was some suggestion, however, that potential effects on growth were obscured by density-dependent factors in exposed groups where fry mortality was high and the population density of the survivors thus reduced, and by a size-specific mortality phenomenon in which mortality tended to be higher among the smaller fry in each exposure group.

The swimming performance of fry exposed to the three levels of PCB's, DDE, and PCB's + DDE was compared after about 50, 120, and 170 days (Rottiers and Bergstedt 1981) by forcing the fry to swim in a stamina channel during a series of gradually increasing velocities until the fish became impinged on the downstream screen. Although significant differences in critical swimming speeds were observed between a few test groups, no consistent relation was detected between the swimming performance of the fry and exposure to PCB's and DDE.

Predator avoidance by fry from each treatment group was measured by comparing their relative vulnerability to capture by a predator (Mac 1981). Vulnerability to predation, operationally defined as the ratio of the number of fry escapes to the number of attacks by the predator (a rainbow trout, *Salmo gairdneri*), was not significantly affected by either 90 or 165 days of exposure to the three levels or combinations of PCB's and DDE tested. In addition, no qualitative differences were apparent in the predator avoidance behavior between control and exposed fry. These results suggest that exposure to PCB's, DDE, and PCB's + DDE at the three levels tested did not affect vulnerability of young lake trout to predation.

Previous investigators have shown that exposure to organochlorine compounds may cause a shift in preferred temperature in some species of fish. To determine if lake trout in this study were similarly affected, Mac and Bergstedt (1981) tested the preferred temperatures of fry (only fry from the controls and 25× exposures were tested) in a vertical thermal gradient tank during the fourth month of exposure. Lake trout fry exposed to 25× PCB's, DDE, or PCB's + DDE had mean preferred temperatures that were 0.9°, 1.4°, and 2.5°C lower, respectively, than the preferred temperature of the control fry (11.2°C). Furthermore, these lowered preferred temperatures of fry in the 25× exposures appeared to be additive: when the results from exposure to PCB's and DDE were added, the sum (2.3°C) was nearly equal to the measured reduction of 2.5°C in fry exposed to PCB's + DDE. Although these results at the 25× exposures are not directly applicable to offshore conditions in Lake Michigan, they represent the lowest concentrations yet reported as altering the preferred temperature of fish. In the more contaminated nearshore areas of the lake, concentrations of PCB's and DDE could be sufficient to alter preferred temperature of the fry, and thus reduce their growth and survival as a result of impaired energetic efficiency. In addition to influencing biochemical processes in the fry, alterations in the preferred temperature could cause the fry to select a habitat that is inferior in types or amounts of available food.

Biochemical characteristics of the fry were initially evaluated in cooperation with scientists of the Columbia National Fisheries Research Laboratory, who compared the biochemical profile of 1-day-old sac fry hatched from eggs of Lake Michigan lake trout with that of fry hatched from eggs of relatively uncontaminated hatchery lake trout (Passino 1981). Total protein and ascorbic acid (vitamin C) were lower in the fry from Lake Michigan eggs than in fry from hatchery eggs. Also, the ratio of proline to hydroxyproline (amino acids related to collagen formation) was about 80% higher in the Lake Michigan fry. Similar differences have been shown in studies by other investigators to be associated with organochlorine contamination. In this study, however, dietary or genetic differences between the parental fish could also have been involved.

The respiration rates and lactate levels in fry used in the swimming performance tests of Rottiers and Bergstedt (1981) were measured as additional indicators of the stamina of the fry and their ability to recover from fatigue (Passino 1981). No significant differences were observed between treatment groups immediately before swimming tests for either weight-specific oxygen consumption rates at 84–94 days of exposure or for whole-body lactate concentrations at 85 days. Oxygen consumption rates and lactate concentrations in fry that had been exercised to exhaustion, however, indicated a reduction in stamina of fry exposed to the three levels of PCB's. This observation was not fully supported statistically, however, and more extensive work would be required to establish the relation between stamina and contaminant exposure.

In a related but independent study (Passino 1981), the activity of the enzyme allantoinase was measured in juvenile and adult lake trout as an indicator of sublethal effects of Great Lakes contaminants. Fifty percent inhibition of allantoinase occurred in vitro at 6.0 mg/L Cu^{++}, 6.7 mg/L Cd^{++}, 34 mg/L Hg^{++}, or 52 mg/L Pb^{++}, but no effects were observed during in vitro exposures to DDE or DDT up to 10 μg/g or PCB's up to 7 μg/g. Allantoinase was activated slightly, however, after in vivo exposure that resulted in residues of 2.6 μg/g PCB's in the whole fish. Allantoinase activity was negatively correlated with body length for fish from Lake Michigan but not for those from Lake Superior or from laboratory stocks. Mercury, PCB's, and DDT's, possibly acting in combination with each other and with additional contaminants, may be associated with the decreased allantoinase activity in large Lake Michigan lake trout.

Conclusions

On the basis of these several studies conducted at the Great Lakes Fishery Laboratory from 1972 to 1977, we conclude that the levels of PCB's and DDE present in the water and biota of Lake Michigan during the early to mid 1970's were sufficient to sig-

nificantly reduce the survival of lake trout fry that may have hatched in the lake. Laboratory simulations of PCB and DDE levels in Lake Michigan demonstrated that reduced survival of lake trout fry (which have inherited substantial contaminant residues from their parents) was attributable to the additional, chronic exposure of the fry to PCB's and DDE in their water and food—i.e., the inherited residues alone were insufficient to reduce survival of the fry. Whether these two contaminants are the sole cause for reproductive failure of the planted lake trout is not known. Several other factors potentially play a role—e.g., the presence of exotic species, the spawning behavior and physiological condition of planted fish, the condition of spawning grounds, and the adequacy of spawning aggregations of mature fish in the lake. Declines in the levels of total PCB's and DDT in fishes of Lake Michigan during the 1970's would seem to place even greater importance on these other factors in the future. The known presence, however, of additional chlorinated hydrocarbons such as dieldrin, chlordane, and chlorinated camphenes, as well as of several other organic and inorganic contaminants in the water and biota of the lake, raises serious questions about the potential additive or synergistic effects of these multiple contaminants. Regardless of the ultimate answer to these questions, the levels of PCB's and DDE in the lake in the 1970's appeared sufficient to impede the restoration of self-sustaining populations of lake trout in Lake Michigan.

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Comparative Hatchability of Lake Trout Eggs Differing in Contaminant Burden and Incubation Conditions[1]

by

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Abstract

In 1972, fertilized eggs of lake trout (*Salvelinus namaycush*) from the Marquette (Michigan) State Fish Hatchery (where levels of contaminants are relatively low) and eggs from lake trout collected in Michigan waters of Lake Michigan near Saugatuck and Charlevoix (where levels of PCB's and DDE are elevated) were incubated at hatchery temperatures (6°C) and at temperatures simulating the natural temperature cycle of Lake Michigan (1–8°C). Survival to yolk absorption of larvae from these three sources ranged from 40.3 to 65.5%, and no correlation was observed between survival and the levels of PCB's and DDE in the eggs. Additional studies in 1975 with lake trout eggs from the same three sources confirmed previous observations that the elevated levels of PCB's and DDE in eggs from Lake Michigan did not appear to affect the percent hatch of lake trout eggs or survival of the fry to the swim-up stage. When fry hatched from eggs with an elevated contaminant burden were starved for several weeks, we observed no abnormal increase in posthatching mortality during the period when the yolk stores were being consumed.

Introduction

Researchers have been concerned that chlorinated hydrocarbons present in adult lake trout (*Salvelinus namaycush*) are passed to their eggs, thereby reducing the viability of the eggs and of the fry hatched from them. Such a concern resulted in the study described here, in which we examined the survival of progeny of lake trout collected in different parts of the Great Lakes region where the concentrations of PCB's and DDE in the fish and eggs were known to differ. We tested the fry through the period of yolk absorption, a critical period in developing salmonids (Vladimirov 1975). We also conducted an experiment to determine whether the release of stored contaminants from the yolk of the larvae during starvation would result in mortalities that could be related to the contaminant burden.

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Materials and Methods

Eggs were taken from lake trout collected during the 1972 and 1975 spawning seasons at three locations: in southeastern Lake Michigan near Saugatuck, Michigan (hereinafter referred to as "Saugatuck" eggs); in northeastern Lake Michigan near Charlevoix, Michigan ("Charlevoix" eggs); and from brood stock lake trout at the Marquette (Michigan) State Fish Hatchery ("Marquette" eggs). Eggs were fertilized as soon as possible after collection and transported to the Great Lakes Fishery Laboratory, where they were incubated in processed Laboratory water (see Berlin et al. 1981), in hatchery trays from Heath Tecna Corporation.

Marquette, Saugatuck, and Charlevoix eggs were obtained in 1972 and incubated at a constant 6°C (hatchery temperature) and at a fluctuating temperature ranging from 1° to 8°C, similar to the range of natural temperatures measured in Lake Michigan (Great Lakes Fishery Laboratory 1970). About 2,600 Marquette, 6,300 Saugatuck, and 8,500 Charlevoix eggs were divided equally between the two temperature treatments. In addition, 13,000 Saugatuck eggs were incubated at the Laboratory's Hammond Bay Biological Station in Lake Huron water at ambient lake temperatures (1–8°C).

In fall 1975, 27,000 Saugatuck and 15,000 Charlevoix lake trout eggs were incubated and hatched at the Laboratory (at about 5°C). Because the Marquette eggs were received in the eyed stage, we were unable to complete comparisons of their survival with that in the other groups. We withheld food from 200 of the newly hatched fry from each source and recorded their mortality daily. Water temperature was held at 9.0°C.

Statistical evaluation of the effects of egg source, water temperature, and incubation location on egg and fry survival was made with chi-square tests. Contaminant concentrations in all eggs were based on saponified samples analyzed by gas chromatography (Reinert 1970). PCB's were quantified as Aroclor 1254 and the DDT as *p,p'*-DDE.

Results

The use of eggs from three sources allowed us to compare the effects of three levels of PCB's and DDE on survival. Levels of the two contaminants were low in Marquette eggs, intermediate in Charlevoix eggs, and high in Saugatuck eggs. This general relation between egg source and contaminant level for Charlevoix and Saugatuck eggs was consistent in both 1972 and 1975 (Tables 1 and 2).

At hatchery temperatures, survival to yolk absorption for eggs collected in 1972 was significantly higher ($P < 0.001$) for Marquette fry (68.5%) than for the fish from the two lake sources (44%; Table 1). However, at fluctuating temperatures, survival to yolk absorption was significantly higher ($P < 0.001$) for Saugatuck fish (59%) than for Charlevoix (40.3%) or Marquette fish (45.6%). In Saugatuck eggs held at the Hammond Bay Biological Station, survival was 46.3% to hatching and 44.6% to yolk absorption—both significantly ($P < 0.001$) lower than the percentages for Saugatuck eggs hatched at the Great Lakes Fishery Laboratory.

Survival of Saugatuck eggs collected in 1975 was 61.8%, or nearly double that of the 31.5% in the less heavily contaminated Charlevoix eggs (Table 2). Within the first few days of incubation, only 8% of the Saugatuck eggs and 16% of the Charlevoix eggs became opaque and thus were presumably unfertilized. This observation suggested that problems in collecting, holding, or transporting the Charlevoix eggs may have been responsible for their poor hatching success.

Starvation studies on fry from the three egg sources in 1975 revealed no relation between concentration of contaminants in the fry and time to 50% mortality (Fig. 1); this time was 73.5 days for Saugatuck fry (which contained the highest concentration of PCB's and DDE; Table 2), and 67 and 66 days for Charlevoix and Marquette fry. The shapes of the mortality curves during the period preceding the 50% mortality point differed for fry from the three egg sources. By day 50, less than 4% of the Marquette fry, but 15.5% of the Saugatuck and 48% of the Charlevoix fry, had died (Fig. 1). Uncontaminated Marquette fry had the lowest mortality up to the point where the rate changed sharply (Fig. 1). However, most of the Saugatuck fry, despite the higher burden of PCB's and DDE, withstood starvation longer than did the Charlevoix fry.

Table 1. *Contaminant levels and percent survival to various developmental stages for lake trout eggs collected in Lake Michigan and from brood stocks at the Marquette Hatchery and incubated at different temperature regimes in 1972.*

Egg source and contaminants (μg/g)			Incubation medium and temperature	
PCB's (Aroclor 1254)	DDT (saponified *p,p'*-DDE)	Stage[a]	Lake water, 1–8°C	Hatchery water, 6°C
Marquette				
0.50	0.27	Eyeing	71.5	83.4
		Hatching	49.4	71.5
		Yolk abs.	45.6	68.5
Charlevoix				
3.57	2.50	Eyeing	85.1	84.4
		Hatching	42.6	49.4
		Yolk abs.	40.3	44.3
Saugatuck				
11.08	7.15	Eyeing	85.7	71.3
		Hatching	61.2	49.5
		Yolk abs.	59.0	44.5
Saugatuck[b]				
11.08	7.15	Hatching	46.3	
		Yolk abs.	44.6	

[a]Yolk abs. = yolk absorption stage.
[b]Eggs hatched at the Hammond Bay Biological Station of the Great Lakes Fishery Laboratory.

Table 2. *Contaminant concentrations and percent survival to hatching for lake trout eggs collected in Lake Michigan near Charlevoix and Saugatuck in 1975.*

	Contaminants (μg/g)		
Egg source	PCB's (Aroclor 1254)	DDT (saponified *p,p'*-DDE)	Survival of eggs (%)
Charlevoix	5.4	3.2	31.5
Saugatuck	7.6	4.8	61.8

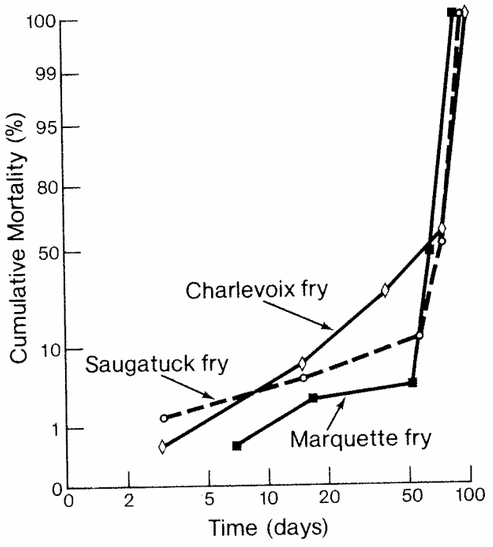

Fig. 1. Cumulative mortality of starved lake trout fry hatched from three sources: Marquette Hatchery (squares), Saugatuck (circles), and Charlevoix (diamonds). Mortality is plotted as probits over log time.

Differences in survival of Saugatuck eggs hatched at the Laboratory and at the Hammond Bay Biological Station (Table 1) suggest that water quality was a controlling factor; however, sample sizes were too small to rule out natural variation as the reason for these differences. In a similar study, Stauffer (1979) also observed a lower survival for lake trout eggs held in Lake Michigan water than for eggs held in hatchery water. Although these studies do not clearly indicate whether chlorinated hydrocarbons present in natural waters were responsible for the observed differences in survival, they cannot be ruled out as a possible contributing factor.

Even though Marquette fry had the highest percent survival up to the 50th day of starvation, no evidence suggests that contaminants influenced the time to 50% mortality in any group. In agreement with results of recent work by Atchison (1976), we found no evidence of a delayed surge in mortality that might have been expected if contaminants were suddenly released while embryonic yolk and lipid stores were being used up. Stauffer (1979) observed that lake trout fry from Charlevoix withstood starvation better than hatchery fry in a similar test, supporting our finding of no apparent relation between contaminant concentrations in fry and their ability to withstand starvation.

Our results and those of Stauffer (1979) strongly suggest that the presence of contaminants in Lake Michigan lake trout eggs did not substantially reduce their hatchability.

Discussion

We observed no significant relation between contaminant levels and percent survival for lake trout eggs hatched in either 1972 or 1975. Variation in survival of 1972 eggs appeared to be related to differences in incubation temperature rather than to differences in contaminant burden. Survival of both Marquette and Saugatuck eggs was higher when the eggs were incubated at temperatures similar to those experienced by their parents (Great Lakes Fishery Laboratory, unpublished data) than when Marquette eggs were incubated at lake temperatures and Saugatuck eggs at hatchery temperature. Hubbs and Bryan (1974) obtained similar results in incubation studies on the Mississippi silverside *Menidia audens*. However, Charlevoix eggs did not show this pattern (Table 1); survival to yolk absorption differed little between fry incubated at lake and hatchery temperatures (40.3 and 44.3%). Charlevoix eggs yielded the lowest survival percentages in both years, suggesting that other factors affected hatching success.

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Growth and Mortality of Fry of Lake Michigan Lake Trout During Chronic Exposure to PCB's and DDE[1]

by

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Abstract

Fry hatched from eggs of Lake Michigan lake trout (*Salvelinus namaycush*) were exposed (beginning about 1 week after hatching) to contaminant concentrations of PCB's and DDE similar to those in water and plankton in southeastern Lake Michigan (1× level), and to concentrations about 5 (5×) and 25 (25×) times greater. Body concentrations of contaminants in fry (μg/g) decreased at 1× levels of PCB's and at 1× and 5× levels of DDE, but generally increased at all other contaminant exposure levels. Uptake of PCB's and DDE was evident from increases in body burden (μg/fish) of the contaminants at all exposure levels and the controls. Growth was not significantly affected by any of the contaminant exposures. Mortalities of fry exposed to the lower concentrations (1× and 5×) were significantly less than those of control fry before day 56; however, between days 57 and 136, mortality rates increased dramatically and were significantly higher in all nine exposed groups than in the control group. For the last 40-day period (days 137–176), mortality was low and leveled off, but continued to be significantly higher in all exposed fry (except those in 1× and 5× PCB's) than in control fry. By the end of the 176-day study, the total cumulative mortality ranged from 30.5 to 46.5% in the exposed groups and was 21.7% in the control group.

Introduction

Although a variety of PCB and DDE dosage levels and durations of exposure have been tested on fish, apparently no attempt has been made to test the effects of simulated environmental levels over long periods. We exposed fry hatched from eggs of Lake Michigan lake trout (*Salvelinus namaycush*) to contaminant concentrations similar to those in water and plankton in southeastern Lake Michigan, to test our hypothesis that such contaminant concentrations were high enough to adversely affect growth and survival of fry in the lake. To assess the effect of elevated contaminant levels, we also exposed fry to about 5 and 25 times the concentration of PCB's and DDE found in Lake Michigan water and plankton.

[1]Contribution 563 of the Great Lakes Fishery Laboratory.

Materials and Methods

Treatment of Fry, Food, and Water

Fry Source and Treatment

Approximately 27,000 eggs were collected from lake trout captured during fall 1975 in southeastern Lake Michigan (near Saugatuck, Michigan) and incubated at the Great Lakes Fishery Laboratory at 5–7 °C in filtered well water (Table 1) in Heath Tecna Corporation incubators. The sac fry were transferred to fiberglass hatchery troughs soon after hatching, held for 4–10 days at 7–8 °C, and then transferred to 189-L fiberglass oval tanks (water volume, 116 L; flow rate, 2 L/min; temperature, 9 °C) for the duration of the study.

We randomly selected and hand-counted 650 fry, 4 to 10 days old, into each of 30 oval tanks. Fry with obvious abnormalities were discarded. Three tanks of fry

Table 1. *Chemical and physical characteristics of filtered well water, Great Lakes Fishery Laboratory.*

Characteristic[a]	Concentration (mg/L)
Total hardness	465
Calcium hardness	315
Magnesium hardness	150
Total alkalinity ($CaCO_3$)	330
Sodium	13.5
Calcium	114
Magnesium	31
Potassium	2.7
Iron	0.38
Sulfates	0.12
Chloride	38
Total phosphorus	0.02
Silica	15
Nitrate-nitrite	0.08
Ammonia as N	0.06

[a]Not tabulated are conductivity (500 μmhos/cm) and pH (7.2).

were used as controls (contaminant exposure was confined to background levels of PCB's and DDE in the food and water) and the remaining 27 tanks of fry were divided randomly into sets of three, each set receiving one of the nine contaminant exposures—1×, 5× and 25× each for PCB's (Aroclor 1254), DDE (*p,p'*-DDE), and combinations thereof (here termed PCB's + DDE) in food and water.

Tanks were cleaned three times each week. All detrital material was siphoned into a screen-walled container to prevent the loss of fry accidentally caught by the siphon. Any siphoned fry that were uninjured were returned to the tanks; fry killed or obviously injured were discarded, but not recorded as mortalities. The number, apparent condition, and disposal of siphoned fry were recorded to check for possible correlations with subsequent mortalities. Screen covers on the standpipes prevented fry loss.

Except during cleaning and maintenance procedures and observations of fry behavior, one half of each tank was covered with translucent fiberglass and the other half with Styrofoam sheets to reduce disturbance of the fry. Overhead fluorescent lighting provided the 12-h photoperiod (0700 to 1900) used throughout the study. Water temperatures, which were recorded daily, were regulated by portable chillers (Frigid Units, Inc., Toledo, Ohio) and thermo-regulated heater units in head reservoirs; they were held within the narrow range of 8.9 ± 0.01 to 9.2 ± 0.02 (mean ± SE) in the array of tanks. Water was delivered to the tanks from reservoirs by gravity flow.

Sampling of Fry

Fry were sampled for weight (growth) determinations beginning on day 17. Thereafter, samples were taken about every 40 days throughout the 176 days of contaminant exposure. Sample size changed during the period because fry size increased and the number of fry available was reduced by mortality. The ranges in number of fish from different treatment groups sampled for weight determinations were 94–104 at the time of first feeding (day 17), 49–70 on day 55, 30–31 on day 94, 25–26 on day 135, and 34–37 on day 176. Fry used for weight determinations were placed in a beaker of water containing a lethal dose of tricaine methanesulfonate (MS-222), poured onto a screen to remove excess water, and then weighed on a Mettler P 1200 balance in a tared beaker with water. Samples of fry were also removed for determinations of contaminant residue levels, as discussed later.

Analytical Procedures

Concentration of PCB's and DDE in Fish

Fry were sampled from treatment tanks for PCB and DDE analysis at the beginning (day 0) and end (day 176) of the study. The numbers and weight (about 10 g) of fry sampled from each tank per sampling time were similar. Fry collected on day 0 from all tanks sampled for each treatment group were composited into one sample for analysis, wrapped in aluminum foil, and frozen. Before analysis, the fry were thawed, homogenized, placed in polyethylene vials, and refrozen.

Fish samples were analyzed for PCB's and DDE by saponifying and then extracting them with hexane (Reinert 1970). After extraction, 1 mL of sample was added to a 5× 50-mm column of 2% deactivated Florisil. PCB's and DDE were eluted from the column into a Teflon-lined screw cap vial with 20% benzene until 10 mL of eluate had been collected. Exactly 5 μL of sample were injected into a gas chromatograph equipped with a ^{63}Ni EC detector and a glass column (2 × 2,000 mm) packed with 2% OV-101 coated on 80/100 mesh AW-DMCS chromosorb W. Gas chromatographic conditions were as follows: injector, 200°C; column, 170°C with 30 mL/min N_2 flow; and detector, 270°C.

PCB's were quantitated by summing the height of all major peaks except those with retention times similar to those of DDE; the concentration of PCB's was then determined by comparison with known standards. The concentration of DDE was similarly quantitated, except that it had only one peak. Whenever the samples were saponified, values for DDE included *p,p'*-DDT, because DDT was converted to DDE. Unless otherwise indicated, all PCB and DDE concentrations were calculated from the wet weight of the sample.

Concentration of PCB's and DDE in Water and Food

Water from control tanks, 25× exposure tanks, and the diluters was sampled for analysis of contaminant concentrations. The concentrations of contaminants in the water of the 1× and 5× exposure tanks were not determined directly because the concentrations were too low to measure accurately. Rather, the concentrations were estimated from concentrations measured in the diluter supply containers and the proportionate dilution in the test tanks.

Water samples of 500 mL were collected on Monday, Wednesday, and Friday of each week. Each water sample was extracted with two 50-mL portions of petroleum ether and the extracts were combined. Extracts of samples taken during a given week from a given tank were combined and stored in a glass-stoppered 300-mL Erlenmeyer flask. Each composited sample was concentrated to 5 mL and stored in a culture tube with a Teflon-lined screw cap. A petroleum ether blank and a water sample spiked with PCB's and DDE were similarly composited and used to determine background and percent recovery of contaminants from the water. Samples were quantified by gas chromatography as previously described.

We used the saponification method to analyze three samples for PCB's and DDE from each batch of Oregon Moist feed dosed with PCB's, DDE, or both.

Laboratory well water was measured for hardness and alkalinity (Table 1) by standard methods (American Public Health Association 1971). Sodium, calcium, magnesium, and potassium were measured with a Perkin-Elmer 403 atomic absorption spectrophotometer (Perkin-Elmer 1973).

Growth Measurements

All analyses of fry growth were based on the mean measurements of fry sampled from three replicate tanks for all groups of fry except one; all fry in one 25×-combination tank were accidentally killed on day 21. Growth of fry was determined as specific growth rate (SGR), i.e., percent weight change per day, by the following equation:

$$\mathrm{SGR} = 100\frac{ln\ wt_1 - ln\ wt_0}{t_1 - t_0}$$

where *ln* is the logarithm (base e), wt_1 and wt_0 are final and initial weights for a given time interval, and $t_1 - t_0$ is the time interval in days (Brown 1957). Specific growth rates of the fry were compared by analysis of variance—one-way and factorial design. Three separate analyses were made: (1) the entire test period (four growth periods), (2) between growth periods, and (3) between treatment groups for a given growth period. A Duncan *k*-ratio *t* test (Duncan 1975) was used to test the significance of differences between the mean specific growth rates for the four growth periods, hereafter designated as GP I, GP II, GP III, and GP IV. Mean weights of fry from each of the treatment groups at the end of each growth period were also compared by analysis of variance.

Mortality

Although mortality of lake trout fry was recorded daily throughout the 176 days of exposure, the data were difficult to interpret because different numbers of fry were removed from each tank for various tests. Cumulative percent mortality was determined by combining the mortality data for the first 16 days of the study with those of the 16 subsequent 10-day periods. For each period, a mortality rate was calculated by dividing the number of dead fry recovered by the number of fry assumed to be present in the tank at the beginning of that period. This mortality rate was applied to the cumulative number of fry sampled before the beginning of that period. This theoretical number of dead fry was subtracted from the number of fry sampled and added to the number of dead fry recovered from the tank. This adjusted cumulative number of dead fry for each tank was divided by 650, the initial population, to obtain cumulative percent dead at the end of each period and at the end of the 176-day study (final mortality). An example of such a calculation for a tank of control fry follows.

A = Period (days elapsed in parentheses)	1 (0–16)	2 (17–26)	3 (27–36)
B = No. of dead recovered in period	11	9	15
C = Cumulative no. of fry sampled prior to period	0	101	103
D = Mortality rate ΣB/650–(c+ΣB [prior period])	0.017	0.017 (9/538)	0.028 (15/527)
E = C–ΣF (prior periods)	0	101	101.3
F = Theoretical no. dead (D × E)	0	1.7	2.9
G = Adjusted no. dead (B + F)	11	10.7	17.9
H = Adjusted mortality rate (G/650)	0.017	0.016	0.028

Values reported are the totals for all the tanks for each treatment group. Mortality curves were constructed from the percent dead at the end of each period and a final cumulative percent mortality was determined. Mortality curves were compared by dividing mortalities into five time intervals: days 0–16, 17–56, 57–96, 97–136, and 137–176. Statistical comparisons of mortality through these intervals and of final mortality were made by chi-square analysis (Cochran and Cox 1957). Mortality rate is expressed as the percentage of the fry that died (and were recovered) of the total number of fry believed to be present at the beginning of an interval.

Monitoring Fry Populations

On day 120, a direct visual count of the fry in each tank was made by trapping a small number of fry at one end of a tank behind a movable partition and counting them, trapping another lot of fry in the space

adjacent to the first partition with a second partition and counting them, then lifting out the first partition and placing it outside the second partition and counting the fry between the two partitions, and so on, until all the fry in a given tank had been thus segregated into lots and counted. No other direct census of tank populations was made during the studies.

Addition of PCB's and *p,p'*-DDE to Food and Water

We chose a concentration ratio of total PCB's to DDE (the principal metabolite of DDT) of 10:1 for this study on the basis of the following considerations: (1) in 1972, the PCB:DDT concentration ratio in the water was 2:1; (2) from a 1975 analysis of zooplankton, we estimated a PCB:DDE concentration ratio of 25:1; (3) PCB concentrations remained constant after 1972 but DDT concentrations declined. We therefore arbitrarily selected the 10:1 ratio to simulate concentrations existing in Lake Michigan through the early 1970's. In Lake Michigan water, concentrations of PCB's and DDT averaged 12.4 and 5.4 ng/L, respectively (Lake Michigan Interstate Pesticides Committee 1972). Inasmuch as crustacean zooplankton may constitute up to 87% of the diet of lake trout fry and fingerlings (Eschmeyer 1956), we analyzed three samples, consisting primarily of zooplankton, collected offshore in Lake Michigan in 1975. Concentrations of PCB's and DDE (DDE + DDT) in these plankton samples (dry weight) averaged 1.50 and 0.06 μg/g, respectively.

To simulate contaminant concentrations in Lake Michigan and levels 5 and 25 times higher, we used two serial diluters—one for PCB's and the other for DDE—to maintain concentrations of 10, 50, or 250 ng/L of PCB's or 1, 5, or 25 ng/L of DDE, in the designated exposure tanks. Each diluter consisted of a vertical array of three 18-L stainless steel containers, each of which had an independently regulated water supply and an overflow tube 15 mm below the top rim. Water containing toxicant was supplied to designated tanks at 100 mL/min from each diluter. Diluter and tank flows (2 L/min) were checked daily and minor adjustments made to provide the required contaminant dilution. The carrier solvent used for PCB's and DDE during the first 4 weeks of the study was 95% ethanol; thereafter, contaminants were added by using a coated matrix column similar to that described by Veith and Comstock (1975).

Commercial Oregon Moist mash and pellets that averaged 0.8 and 1.2 mm in diameter were fed to the fry. To simulate contaminant concentrations in the plankton in Lake Michigan and at elevated levels, we dosed separate batches of food with 1.0, 5.0, and 25 μg/g of PCB's (Monsanto, St. Louis, Missouri); 0.1, 0.5, and 2.5 μg/g of 99+% *p,p'*-DDE (Analabs, Inc., North Haven, Connecticut); or both PCB's and DDE at rates (μg/g) of 1.0 PCB's + 0.1 of DDE, 5.0 PCB's + 0.5 DDE, and 25 PCB's + 2.5 DDE. Contaminants were dissolved in acetone which evaporated during the mixing process. Food fed to control fry was identical with the treated food, except that no acetone, PCB's, or DDE were added.

Results and Discussion

Contaminants in Fish, Water, and Food

Analyses conducted at the Great Lakes Fishery Laboratory revealed that eyed lake trout eggs from fish collected offshore in Lake Michigan near Saugatuck contained 7.6 μg/g PCB's and 4.7 μg/g DDE (DDE and DDT); analyses by the Columbia National Fisheries Research Laboratory showed that day-old fry hatched from these eggs contained 3.8 μg/g PCB's and 2.1 μg/g of DDE (DDE and DDT). Our analyses also indicated that concentrations of PCB's and DDE (μg/g) decreased from those in day-old fry in fish exposed for 176 days to 1× levels of PCB's, singly or in combination, and to 1× and 5× levels of DDE (Table 2). However, these decreases were apparently a result of growth of the fry—i.e., dilution by growth which masked the uptake of contaminants. This dilution is evidenced by the increase in body burdens from day 1 levels of 0.38 μg/fish PCB's and 0.21 μg/fish DDE to day 176 levels of 5.81 μg/fish PCB's and 0.99 μg/fish DDE in fry from 1× PCB's and 1× DDE, respectively. Similar conversions of data were given by Leib et al. (1974), who found that although PCB concentrations did not increase, total body burdens did, in rainbow trout (*Salmo gairdneri*) fed 15 μg/g of PCB's in their food for 32 weeks.

Table 2. *Concentrations and body burden of PCB's and DDE in samples of lake trout after 176 days of exposure. (Before exposure, samples of 1-day-old sac fry contained 3.8 μg/g [0.25 μg/fish] PCB's and 2.1 μg/g [0.14 μg/fish] DDE + DDT.)*

Contaminant and exposure group[a]	PCB's μg/g	PCB's μg/fish	DDE μg/g	DDE μg/fish	No. tanks of fish sampled
Control	0.30	0.90	0.19	0.57	1
PCB's					
1×	1.53	5.81			2
5×	5.06	19.5			3
25×	26.3	109			2
DDE					
1×			0.29	0.99	3
5×			0.67	2.40	3
25×			2.68	9.95	3
PCB's + DDE					
1×	1.13	5.30	0.43	2.02	2
5×	4.22	17.7	0.55	2.31	3
25×	20.9	107	2.37	12.1	2

[a]See Table 3 for exposure concentration of different groups.

Average concentrations of the contaminant in the water corresponding to 1×, 5×, and 25× exposure levels were 20.8, 64.7, and 327 ng/L for PCB's, and 1.8, 6.3, and 32.7 ng/L for DDE. Desired concentrations were 10, 50, and 250 ng/L for PCB's and 1, 5, and 25 ng/L for DDE. The concentrations of PCB's and DDE in the three combination exposure tanks were calculated by dividing the concentration of contaminant added to the tank by the water flow rate into the tank.

Samples of water from control tanks, concentrated 500-fold and analyzed by gas chromatography, frequently contained trace levels of PCB-like compounds. However, the peak ratios of extracts from control samples did not closely match those of the standards used to measure concentrations of PCB's and were generally below detection limits (10 ng/L) for the procedure used. The source of these PCB-like compounds is unknown.

Average concentrations of PCB's and DDE measured in spiked fish food used in the study (Table 3) were within 28% of the intended concentrations, except for food containing the lowest levels of contaminants. Background concentration of PCB's and DDE in the food accounted for most of this error.

Table 3. *Mean concentration (SE in parentheses) of PCB's and DDE in Oregon Moist food fed to lake trout fry.*[a]

Contaminant and dosing level[b]	Concentration (μg/g) Intended PCB's	Intended DDE	Actual PCB's	Actual DDE
Control	0	0	0.20 (0.009)	0.16 (0.062)
PCB's				
1×	1.00		1.05 (0.084)	
5×	5.00		4.77 (0.154)	
25×	25.00		22.63 (1.096)	
DDE				
1×		0.10		0.26 (0.052)
5×		0.50		0.62 (0.049)
25×		2.50		2.32 (0.131)
PCB's + DDE				
1×	1.00	0.10	1.19 (0.069)	0.28 (0.059)
5×	5.00	0.50	3.74 (0.900)	0.69 (0.039)
25×	25.00	2.50	23.41 (0.667)	3.02 (0.201)

[a]All determinations based on four analyses except for 5× DDE (five samples).
[b]Concentrations at the 1× dosing levels were similar to those of contaminants in water and plankton in Lake Michigan.

Growth of Fry

Mean specific growth rates for all four growth periods (GP I–IV) combined were lowest for control fry and highest for fry exposed to PCB's + DDE at all three dosage levels (Table 4). Growth rates of fry for GP I (days 18–55; Table 4) and mean fry weights for GP I and II (Table 5) suggested that contaminants initially suppressed growth. If so, however, subsequent rates and weights suggest a shift in contaminant effect on growth, from suppression to acceleration, although PCB's and DDE may have affected growth indirectly through size-selective mortality or concomitant changes in fish density in individual tanks. Whereas SGR's did not differ significantly during GP III, those of the control fry were lower than those of all except the 25× DDE group. For GP IV, the SGR and final mean weight of the control fry were lower than those of all exposed groups; the rate differences were generally larger than those for previous periods.

Analysis of variance indicated no significant differences at the 0.05 level in mean SGR's in the 10 treatments, either for a given growth period or for all growth periods combined (Fig. 1 and Table 4). Growth rates were higher in GP's I and II than in either GP III or IV. Analysis of variance showed significant differences (0.01 level) between SGR's for the growth periods, but did not indicate if more than two differed significantly; a Duncan *k*-ratio *t* test indicated that differences between mean SGR's were significant (0.05 level), except between GP's I and II.

At the end of GP I and GP II, mean weights for controls were slightly higher than those of all exposed groups. For GP III, mean weights were similar, but by the end of GP IV, the mean weight of the controls was 13 to 41% less than that of fry in the PCB, DDE, and PCB + DDE groups (Table 5). The 25× exposure groups for each of these three contaminant exposure categories had the largest weight increases—up to 181% for the 25× PCB + DDE treatment (from day 135 to 176). Although values for SGR and mean weight were generally highest for fry exposed to PCB's + DDE, intermediate for those fry exposed to PCB's, and lowest for fry exposed to DDE, analysis of variance showed no significant differences between mean weights of fry in any of the 10 treatments for any

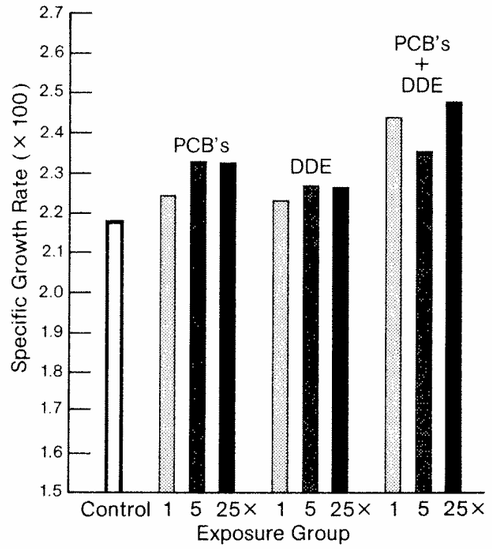

Fig. 1. Mean specific growth rates of lake trout fry of different exposure groups (including controls) for four growth periods (G–GIV, Table 4) combined.

growth period. Macek (1968) reported that, although no significant differences were indicated between the weight of brook trout (*Salvelinus fontinalis*) fed sublethal concentrations of DDT and the weight of controls, a definite trend was indicated, suggesting a direct relation between higher dosage and greater lengths attained by male brook trout. As expected, mean weight gains of the lake trout in different treatments for each growth period corresponded closely with their respective SGR's (Tables 4 and 5). On day 17, when feeding began, the mean weights were 0.093 g for control fry and 0.096 g for all exposed fry combined.

Comparisons of our results with those from other studies are difficult because of differences in the species studied, age of fish tested, chemical dosage levels, duration of testing, and the kind of PCB Aroclors used (as indicated by Stalling and Mayer [1972], the toxicity of PCB's to fish is inversely related to the percent chlorine present). Most studies on the effect of PCB's or DDE on fish growth have indicated either a reduction or no effect (Table 6). Grant and Swedberg (1972) reported reduced growth of juvenile lake trout fed a diet containing the PCB Aroclor 1248 for 3- and 6-month periods; however, the PCB's seemingly increased thyroid activity, and such an increase is likely to stimulate swimming and general activity, and result

Table 4. *Mean specific growth rates (SGR) for lake trout fry in different exposure groups for each period of about 40 days and all growth periods combined (SGR's are expressed as percentages; standard deviations are in parentheses).*

Exposure group	Growth periods (GP) and days of exposure at end of period[a]				
	GP I–55	GP II–94	GP III–135	GP IV–176	Periods combined[b]
Control	3.03 (.1996)	2.80 (.3715)	1.90 (.3539)	0.98 (.2326)	2.18 (.8826)
PCB's					
1×	2.66 (.0644)	2.57 (.7202)	2.63 (.3873)	1.14 (.9469)	2.25 (.8554)
5×	2.93 (.1800)	2.50 (.5284)	1.97 (.2751)	1.92 (.3325)	2.33 (.5273)
25×	2.61 (.1925)	2.76 (.4808)	2.16 (.1992)	1.81 (.0924)	2.33 (.4604)
DDE					
1×	2.88 (.3060)	2.30 (.6787)	2.35 (.9575)	1.40 (.7747)	2.23 (.8294)
5×	2.74 (.1593)	2.62 (.3064)	2.29 (.0484)	1.42 (.4077)	2.27 (.5843)
25×	2.74 (.1290)	2.80 (.1371)	1.74 (.7269)	1.80 (.6582)	2.27 (.6751)
PCB's + DDE					
1×	2.85 (.0158)	2.60 (.6927)	2.14 (.5878)	2.16 (.1868)	2.44 (.5059)
5×	2.63 (.1866)	2.88 (.3237)	2.40 (.5052)	1.55 (.5905)	2.36 (.6401)
25×	2.51 (.0375)	2.83 (.7046)	1.98 (.8345)	2.59 (.0663)	2.48 (.5319)
Mean	2.77 (.2079)	2.66 (.4623)	2.16 (.5160)	1.64 (.6181)	2.31 (.6493)

[a]For each exposure period, two fish samples were weighed for the groups exposed to 25× PCB's + DDE and three each for all others.
[b]The numbers of fish samples weighed were 8 for the group exposed to 25× PCB's + DDE and 12 each for all others.

Table 5. *Mean weights in grams (SD in parentheses) of lake trout fry by exposure group—i.e., mean of three replicate means[a]—at the time of first feeding (day 17) and succeeding growth periods of about 40 days each.*

Exposure group	Days of exposure				
	17	41	94	135	176
Controls	0.093 (.0015)	0.308 (.0195)	0.948 (.1281)	2.03 (.2492)	2.99 (.1909)
PCB's					
1×	0.097 (.0017)	0.281 (.0119)	0.806 (.2135)	2.26 (.2704)	3.80 (1.510)
5×	0.092 (.0038)	0.296 (.0095)	0.813 (.1429)	1.81 (.4426)	3.85 (.6776)
25×	0.099 (.0036)	0.282 (.0138)	0.855 (.1346)	2.01 (.1749)	4.15 (.3764)
DDE					
1×	0.095 (.0052)	0.301 (.0202)	0.768 (.1611)	1.96 (.3568)	3.43 (.7032)
5×	0.094 (.0035)	0.282 (.0275)	0.803 (.0642)	2.00 (.1208)	3.58 (.6630)
25×	0.098 (.0065)	0.292 (.0150)	0.898 (.0838)	1.84 (.4673)	3.71 (.4311)
PCB's + DDE					
1×	0.094 (.0038)	0.293 (.0127)	0.846 (.1921)	1.96 (.1768)	4.69 (.7485)
5×	0.095 (.0060)	0.271 (.0046)	0.862 (.1184)	2.24 (.2033)	4.20 (.7978)
25×	0.098 (.0035)	0.266 (.0057)	0.846 (.2524)	1.82 (.0559)	5.12 (.0212)

[a]One exception: two replicates for 25× PCB's + DDE.

Table 6. *Summary of previous studies in which effects of PCB's and DDT on fish growth have been observed.*

Contaminant	Species and life stages	Dosage (μg/g in diet [D] or μg/L in water [W])	Duration of study (days)	Effect on growth	Source
Aroclor 1248	Lake trout, juvenile	D, 1.2-12.0	90	Decreased 6-28%	Grant and Swedberg (1972)
			180	Decreased 50%	
Aroclor 1248	Fathead minnows, fry	W, 2.2-5.1	30	Decrease	Nebecker et al. (1974)
Aroclor 1254	Rainbow trout, 14-32 weeks old	D, 15	224	None	Leib et al. (1974)
Aroclor 1254	Rainbow trout	D, 1-100	330	None	Nestel and Budd (1975)
Aroclor 1254	Brook trout, alevin and adults	W, 0.01-0.94	90 and 497	None	Snarski and Puglisi (1976)
Aroclor 1254	Brook trout, fry	W, 0.43-13	128[a]	Decrease (48 days) None (118 days)	Mauck et al. (1978)
Aroclor 1254	Coho salmon, fingerling	D, 0.4-580	240	None	Stalling and Mayer (1972)
Aroclor 1254	Coho salmon, alevin	W, 15	42	Decrease	Halter and Johnson (1974)
Aroclor 1254, *p,p'*-DDT	Coho salmon, alevin	W, 32.2 PCB's and 3.2 DDT	7-14	None	Halter and Johnson (1974)
Chlorobiphenyls (isomers similar to Aroclor 1242, 1248, and 1254)	Coho salmon, juvenile	D, 10.2	165	Decrease	Gruger et al. (1975)
PCB (Clophen A_{50})	Brown trout, young (50 g)	D, 10 μg/g[b]	43	None	Johannson et al. (1972)
			203[c]	Increase	
DDT	Atlantic salmon, embryos and fry	W, 0.005-0.10	60[d]	Decrease	Dill and Saunders (1971)

[a]Ten days before and 118 days after hatching.
[b]Dosage of 5 μg/g body weight on days 1 and 5.
[c]The 203 days include the 43 days shown above this entry; after 43 days, fish were starved for 4 months and then again fed for 40 days.
[d]Exposure for 30 days as embryos (from gastrulation to hatching) and 30 days as fry.

Table 7. *Numbers of lake trout fry estimated to be alive at the beginning of different periods and percent that died during the period, among lake trout fry exposed to PCB's and DDE. (Values are the totals for three tanks except for the value for 25× PCB's + DDE, which is for two tanks.)*[a]

Exposure group	Period (days) 0-16 Total fish (no.)	0-16 Mortality (%)	17-56 Total fish (no.)	17-56 Mortality (%)	57-96 Total fish (no.)	57-96 Mortality (%)	97-136 Total fish (no.)	97-136 Mortality (%)	137-176 Total fish (no.)	137-176 Mortality (%)	Cumulative mortality (%) after 176 days Mean	Cumulative mortality (%) after 176 days Range
Control	1,950	2.9	1,591	7.3	1,198	11.2	900	1.3	746	0.8	21.7	19.3-26.3
PCB's												
1×	1,950	1.9	1,609	4.1*	1,326	35.4*	731	10.6*	531	1.2	46.4*	42.8-48.3
5×	1,950	3.6	1,583	5.9	1,271	20.8*	839	10.0*	650	1.5	36.3*	30.5-39.7
25×	1,950	3.7	1,573	24.2*	965	28.8*	550	13.4*	350	4.0*	56.8*	52.5-64.9
DDE												
1×	1,950	2.3	1,609	2.2*	1,350	22.2*	904	9.0*	717	3.1*	34.4*	26.5-46.2
5×	1,950	2.5	1,595	3.5*	1,313	19.0*	916	10.0*	740	3.2*	33.6*	29.4-35.8
25×	1,950	2.8	1,593	7.5	1,235	17.3*	903	4.5*	750	2.2*	30.5*	22.9-37.5
PCB's + DDE												
1×	1,950	2.2	1,608	3.9*	1,311	29.6*	797	8.7*	610	2.0*	40.7*	37.1-44.5
5×	1,950	2.9	1,596	5.1*	1,290	28.4*	787	11.2*	561	2.5*	42.9*	34.8-48.6
25×	1,300	2.2	1,071	8.6	815	29.3*	472	12.9*	291	2.9*	46.5*	37.8-55.2

[a]Percent dead is based on visible mortality (carcasses recovered).
*Denotes significant difference from controls ($P < 0.05$).

in a decrease in the amount of food available for growth (Brown 1957). Grant and Swedberg (1972) stated that "depressed growth rates and elevated thyroid activity imply metabolic inefficiency producing a 'loading stress' that can adversely affect viability and reproductive success." Stalling and Mayer (1972) showed that thyroid activity also increased in most coho salmon (*Oncorhynchus kisutch*) fed Aroclor 1254 for 240 days.

The results of most other investigations of the effects of PCB's and DDT (DDE) on fish growth thus did not agree with ours, unless a factor such as size-selective mortality was involved. The only previous observation that exposure to PCB's resulted in increased fish growth was reported for PCB-treated brown trout (*Salmo trutta*) that had been starved and then fed again (Johannson et al. 1972).

Mortality

Forty-day Periods

Mortality of lake trout during the first 16 days of exposure to contaminants (in water only) ranged from 1.9 to 3.7% among the different groups of exposed fry—not significantly different ($P > 0.05$) from that of control fry (Table 7).

After 56 days of exposure, fry in the 25× PCB concentration showed a highly significant ($P < 0.01$) increase in mortality—more than three times that of control fry (Fig. 2). Up to day 56, mortality of fry exposed to many of the lower concentrations (1× PCB's, 1× and 5× DDE, 1× and 5× PCB's + DDE) was significantly lower than that of control fry (Fig. 2). This phenomenon has been observed in other fry exposure studies (Defoe et al. 1978; Hansen et al. 1975) and may be caused by the slowing of metabolic processes by contaminants and thus the delaying of mortality. Low concentrations of similar organochlorines (DDT) have been shown to reduce standard metabolism in yearling Atlantic salmon (*Salmo salar*), whereas higher concentrations elevate it (Anderson 1971). Halter and Johnson (1974) observed a reduction in yolk-sac utilization in coho fry exposed to PCB's.

Mortalities increased significantly in all groups during the next 40 days (57-96) in comparison with mortalities during the preceding 40 days. Contaminant exposure was apparently beginning to affect survival, since mortality was significantly higher in all nine exposed groups than in control fry for this period (Table 7). Mortalities dropped significantly ($P < 0.01$) between days 97 and 136, compared with those in the previous 40 days. However, mortality was still significantly higher ($P < 0.01$) in all nine exposed groups than in control fry. During the final 40 days of exposure (137-176), mortality was low, ranging from 0.8% for control fry to 4.0% for fry exposed to 25× PCB's (Table 7). It continued to be significantly higher in all exposed fry (except those in 1× and 5× PCB's) than in control fry.

Cumulative Mortality to Day 176

Mortality in all nine groups of lake trout exposed to PCB's and DDE was significantly higher ($P < 0.01$) than that in control fry (Table 7). Cumulative mortalities were highest in fry exposed to the 25× PCB's (56.8%), 1× PCB's (46.4%), and 25× PCB's + DDE (46.5%). Mortality of fry exposed to 25× DDE was lower (30.5%) than that in all other treatments. Mortality was significantly higher ($P < 0.05$) in 5× DDE concentrations (33.6%) than in 25× DDE (30.5%), but no different from that in 1× DDE (34.4%). This lack of correlation between contaminant dose and mortality is unexplained; however, the lower mortality at higher concentrations might be caused by increased induction of detoxifying enzymes by the fish and greater ability to detoxify these contaminants (Chhabra and Fouts 1973; Grote et al. 1975; Sivarajah et al. 1978). Alternatively, our sample size may have been inadequate to detect a dose-response relationship that might have been expressed by a susceptible subpopulation of the test group (Plaa 1978).

In general, mortality was lower in fry exposed to DDE than in those exposed to PCB's. Mortality was significantly lower among fish exposed to any of the three DDE concentrations than among those exposed to PCB's at 1× (46.4%) or 25× (56.8%; $P < 0.01$; Table 7). The cumulative (176-day) mortality of fry exposed to 5× PCB's (36.3%) was not different from that of fry exposed to 1× and 5× DDE, but was significantly higher ($P < 0.01$) than that of fry exposed to 25× DDE. The fact that mortality of fry exposed to PCB's was higher than that of fry exposed to DDE should not be surprising, considering that PCB concentrations were 10 times that of DDE. However, in acute toxicity tests of short duration (5-14 days), DDT, which is similar in toxicity to DDE (Kouyoumjian and Uglow 1974) has been shown to be much more toxic than Aroclor 1254; Stalling and Mayer (1972), in 5-day tests on rainbow trout, reported LC50's of 2.26 μg/L for DDT and 156 μg/L for Aroclor 1254; and Halter and Johnson (1974) determined median survival times of only 175 h for coho salmon fry exposed to 0.8 μg/L DDT, but more than 336 h for fry exposed to 32.2 μg/L Aroclor 1254. Our results suggest that the toxicity of PCB's can be better evaluated from long-term exposures.

Effects of exposure to PCB's + DDE on the survival of lake trout did not appear to be additive and may have been antagonistic. Mortality of fry exposed to 25× PCB's + DDE (46.5%) was significantly lower ($P < 0.01$) than that of fry exposed to 25× PCB's

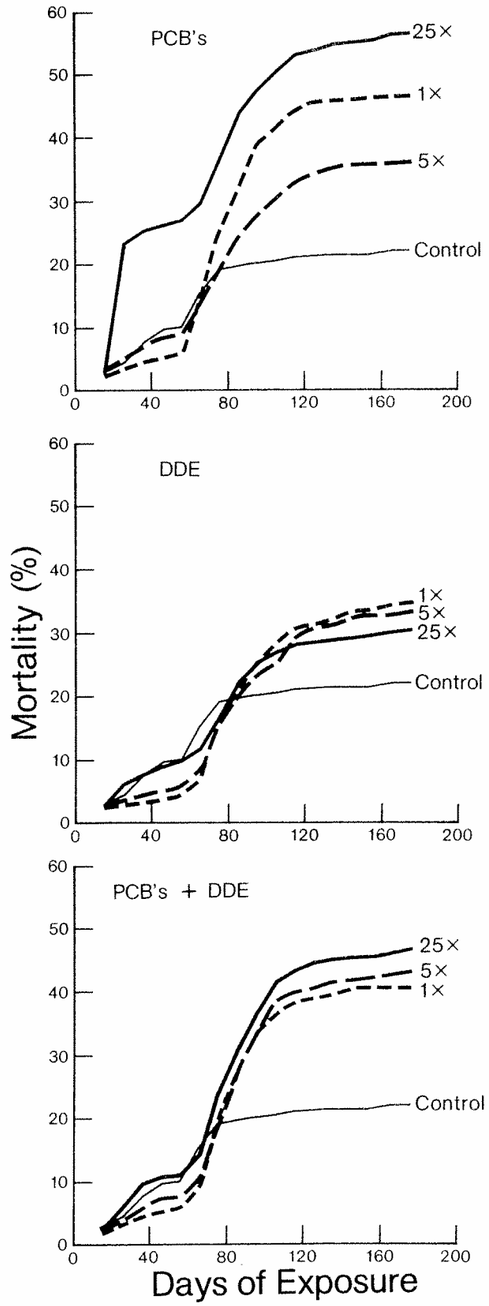

Fig. 2. Mortality curves for lake trout fry exposed to three concentrations each of PCB's (upper panel), DDE (middle), and PCB's + DDE (lower panel), and for control fry (identical for all treatments).

alone (56.8%). This relation was again evident in fry exposed to 1× PCB's + DDE, in which mortality (40.7%) was significantly lower ($P < 0.01$) than in fry exposed to 1× PCB's alone (46.4%). Although mortality in fry exposed to 5× PCB's + DDE (42.9%) was significantly higher ($P < 0.01$) than that in fry exposed to either 5× PCB's (36.3%) or DDE (30.5%), our results for the 1× and 25× comparisons agree with those of Halter and Johnson (1974) who found no additivity in toxicity of PCB's and DDT.

Mortality of lake trout exposed to 1× PCB's + DDE (contaminant doses similar to those found in Lake Michigan in both food and water) was 40.7%—almost double that of unexposed lake trout fry (21.7%).

Mortality Patterns

Mortality curves of fish in all treatments were somewhat similar (Fig. 2). A slight rise in mortality from day 0 to day 36 was followed by a plateau to day 56. The sharp rise in mortality that lasted until day 76 for control fry extended until day 126 for exposed fry. Little mortality occurred after day 126 in any treatment group. Mortality of fry exposed to 25× PCB's increased sharply between days 16 and 26 in comparison with that of fry exposed to 1× and 5× PCB's (Fig. 2).

The initial inflection in the mortality curves (up to day 36) of fry from several treatments coincides with the time of yolk sac absorption—a well-documented critical period in salmonids (Vladimirov 1975). However, mortality did not increase in all groups during this period. This observation suggests that the contaminants present in the egg may not be associated with this mortality, because all fry originated from the same lot of eggs. Fry exposed to 1× PCB's, 1× and 5× DDE, and 1× PCB's + DDE showed no increases in mortality, whereas those exposed to 25× PCB's showed the largest increase (25.4%) by day 36. Although Burdick et al. (1964) suggested that large amounts of contaminants are absorbed from the yolk sac just before the first feeding of the fry, our observations tend to agree with those of Atchison (1976), who found the rate of uptake of DDT from the yolk sac to be almost constant in developing fry. Our results support the work of Stauffer and Wagner (1976), who observed no unusually high mortality at swim-up for lake trout fry from eggs that contained 5.33 to 9.90 μg/g PCB's and 2.74 to 5.24 μg/g DDT.

A second critical period began at day 56 of contaminant exposure when the fry were about 63 days old. This period coincides with critical periods observed for Atlantic salmon 75–85 days after hatching (Privol'nev 1949), and for brown trout 20–40 days after yolk absorption or the beginning of active feeding (Le Cren 1965). The addition of PCB's and DDE in the water and diet thus increased the duration of this critical period from 20 days in the control fry to 60–70 days in exposed fry. This increase in the length of the critical period is apparently responsible for the significant differences in final mortality between exposed and control fry. After this critical period, little mortality was observed in any of the treatments (Fig. 2).

No mortality differences between control fry and fry in most of the exposure treatments were observed until after day 76 and the maximum difference was not observed until day 146 (Fig. 2). The duration of many toxicity tests is much shorter; our data indicate that the toxicity of organochlorine compounds may be underestimated in 30- and 90-day exposures.

Because fry exposed to the 1× PCB + DDE concentration received contaminant doses similar to those experienced by lake trout fry in Lake Michigan, the difference in final mortality between these fry (40.7%) and control fry (21.7%) is particularly important. Not only is it difficult to assess the effects of a near doubling of mortality of fry exposed to PCB's and DDE over the mortality of control fry, but other sources of mortality of young-of-the-year lake trout must also be considered. Other contaminants, both organic and inorganic, as well as impingement at power plant intakes, could add substantially to the mortality of young lake trout. Even if sufficient numbers of adult lake trout are present in Lake Michigan and these fish are capable of spawning successfully, the high mortality of the offspring in their first year could be sufficient to be responsible for total reproductive failure.

Associated Effects of Growth and Mortality

Growth effects and mortalities have not been associated, except in studies on the effects of PCB's on coho salmon (Stalling and Mayer 1972), brook trout (Mauck et al. 1978), and fathead minnows, *Pimephales promelas* (Nebecker et al. 1974). In studies with rainbow trout that were exposed to PCB levels similar to those we used over a period of 10 to 11 months, Nestel and Budd (1975) and Leib et al. (1974) attributed no mortalities to PCB's.

In the present study, the narrow range for mean specific growth rates (2.18 to 2.48) by treatment groups across all growth periods (Fig. 1), but marked differences in final (day 176) mean weights of fish in the different exposure groups (Table 5), suggested that size-selective mortality (possibly associated with cannibalism) may have completely obscured the effect of contaminants on growth. Furthermore, fish sample weights reflect a density-dependent effect of different mortality rates in different treatment groups. Such a relation was suggested by the final mean cumulative percent of dead fry and final mean weights of fry by treatment category, e.g., all DDE. The control mean

was based on three values, compared with eight or nine values for each of the others; for controls, PCB, DDE, and PCB + DDE exposures, visible mortality percentages were 21.7, 32.8, 46.5, and 43.4, and weights were 2.99, 3.57, 3.93 and 4.67 g, respectively.

Allison et al. (1964) suggested that an apparent size increase in cutthroat trout (*Salmo clarki lewisi*) exposed to DDT may have been an indirect effect of DDT, because of mortality of small diseased and slow-growing fish in DDT-exposed lots. In our study, greater ratios of gill surface to total body volume in smaller fish may have been a factor in contaminant uptake and subsequent mortalities of lake trout fry. In work with mosquitofish (*Gambusia affinis*), Murphy (1971) found that the smallest fish were the most efficient at taking up DDT from water and that this efficiency diminished rapidly as the fish increased in weight.

Visible Mortality, Covert Mortality, and Cannibalism

As stated earlier, we had assumed that every instance of fry mortality in the tanks would result in later sighting and recovering of a carcass, so that the number of dead fry recovered would represent an accurate record of actual mortality. On day 93, however, cannibalism among the fry was observed for the first time, and by day 119, eight additional instances had been noted. On day 120, a direct visual census of the number of surviving fry in each tank revealed a substantial difference between the actual and assumed number of fry in each tank (Table 8). These differences, which ranged from 15 to -110 (or about 5.7% to -39%), can be attributed to covert mortality, cannibalism, errors in the initial counting and distribution of fry among the 30 experimental tanks, or counting errors in the direct visual census itself.

If cannibalism had caused the bulk of the differences between actual and assumed numbers of fry, it could not have done so before substantial size differences had developed among fry in a given tank. We observed that the fry that had died in each exposure group were generally smaller than the survivors. For example, at and near the 94-day interval (midpoint of the growth period segment), the average weight of contaminant-exposed fry that died was less than half that of the survivors (0.346 vs. 0.833 g). Similarly marked differences were indicated when weights of live and dead fry were compared at later intervals for growth determinations. However, because most of the mortality took place before day 90, we believe that cannibalism had little effect during the most critical periods of mortality (Fig. 2).

If counting errors were mainly responsible for these population discrepancies, our adjusted mortality percentages (Table 7) underestimated actual mortalities; in all tanks except one, the number of fry counted was less than the number assumed to be present. Regression analysis showed no significant correlation between these differences (Table 8) and the final adjusted mortalities (Table 7). Therefore, no consistent bias was caused by these discrepancies. Because the source of the discrepancies could not be ascertained, the assumed number of fry in each tank

Table 8. *Number of lake trout fry assumed to be present in each exposure group on day 120, compared with the number counted by a direct census.*

Exposure group	Tank no.	Number of fry: Assumed to be present[a]	Counted	Difference
Control	7	288	233	-55
	8	263	278	15
	23	300	257	-45
PCB's				
1×	15	245	175	-70
	17	191	134	-67
	22	217	179	-38
5×	16	291	193	-98
	27	235	185	-50
	34	272	257	-15
25×	24	186	180	-6
	28	133	98	-35
	36	197	167	-30
DDE				
1×	9	320	284	-36
	13	288	249	-39
	21	250	226	-24
5×	26	285	225	-60
	31	328	285	-43
	33	273	240	-33
25×	25	253	217	-36
	30	301	230	-71
	35	353	304	-49
PCB's + DDE				
1×	10	220	196	-24
	12	279	169	-110
	19	259	213	-46
5×	18	278	198	-80
	20	210	144	-66
	32	239	206	-33
25×	11	174	123	-51
	29	239	179	-60
	14[b]	—	—	—

[a]Based on 650 fry initially placed in each tank, minus the numbers that died or were removed for tests and samples.
[b]All fry accidentally killed on day 21.

(650) at the start of the study and the visible mortality (number of carcasses recovered) were used in the adjusted mortality calculations.

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Swimming Performance of Young Lake Trout After Chronic Exposure to PCB's and DDE[1]

by

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Abstract

Swimming performance was measured in fry of lake trout (*Salvelinus namaycush*) exposed to PCB's, DDE, and a combination of these two contaminants in both food and water at concentrations equal to, and 5 and 25 times higher than, levels found in Lake Michigan water and plankton. Fry were tested after about 50, 110, and 165 days of exposure. We measured swimming performance by forcing the fry to swim through a continuous series of incrementally increased velocities until the fish were exhausted. Although we observed significant differences in swimming performance between a few test groups, we detected no relation between swimming performance of the fry and exposure to PCB's or DDE, or both, at the concentrations tested. Inasmuch as swimming performance apparently was not affected by the levels of contamination by PCB's and DDE in Lake Michigan, impairment of swimming by these contaminants cannot account for the failure of lake trout reproduction in Lake Michigan.

Introduction

The ability of fish to capture food and avoid predators or unfavorable conditions depends in part on their swimming ability. Young fish forced to swim during periods of rapid growth may place such heavy demands on their limited energy reserves that stored contaminants are released that disrupt vital metabolic processes. Webb and Brett (1973) pointed out that, although swimming performance is related directly to the metabolism of stored energy, the need for energy is immediate, and failure to swim can be detected by short-term testing. We used the critical swimming speed (CSS) method of Brett (1964) because it is a standardized procedure for measuring swimming speed that provides an index for comparing performance of various test groups. Brett (1964) found that CSS provided a close measure of the maximum sustained swimming speed derived from the more laborious fixed-velocity method used in earlier studies. The purpose of the present study was to measure the effects of PCB's and DDE at ambient Lake Michigan concentrations and above-ambient concentrations on the swimming performance of young lake trout (*Salvelinus namaycush*).

Materials and Methods

Lake trout hatched from eggs from Lake Michigan fish were exposed through food and water to PCB's (Aroclor 1254), DDE, and a combination of PCB's and DDE at concentrations equal to 1 (1×), 5 (5×), or 25 (25×) times greater than concentrations found in Lake Michigan water and plankton (for methods, see Berlin et al. 1981). All fish were acclimated to and tested at 9°C. Photoperiod was 12 h light:12 h darkness. Tests were conducted after 49–51, 100–118, and 163–170 days of exposure (experiments E1, E2, and E3, respectively).

The swimming speed test apparatus, a controlled velocity stream, was an oval channel similar to that used by Lemke and Mount (1963) and MacLeod (1967), except that the two paddle wheels were mounted near

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one end of the channel, one on each side of the major axis, and fish movement was restricted to the test chamber by screens at each end of the chamber. A funnel, fixed at the upstream end of the test chamber, served to increase the velocity of water flowing through the chamber, and baffles mounted in the funnel reduced pulsations and turbulence caused by the paddle wheels. We used a simple direct-reading flowmeter (a lead weight suspended 4 mm above the floor of the test chamber by a nylon thread) to measure flow rates in the test chamber without disturbing the fish. Deflections of the weighted thread were calibrated for direct reading of current velocities of 5 to 40 cm/s, at 5-cm/s increments, by dye injections, a Pygmy current meter, and a portable magnetic flowmeter. Current velocities could be reproduced by adjusting the speed (revolutions per minute) of the paddle wheels so that the weighted thread was deflected into alignment with the appropriate calibration marks.

Test fish were removed from exposure tanks between 0800 and 0830 on the test day, at least 16 h after the last feeding. Order of testing and selection of a replicate test group from one of three tanks at a particular exposure was determined by the throwing of dice. An experiment comprised 10 tests of 10 fish each. For each test, 10 fish were placed together in the test chamber while the drive unit was engaged, but with no flow in the test chamber. They were allowed to adjust to test conditions for 5–10 min. After they became quiet, the test was begun by increasing the velocity of water in the test chamber from 0 to 5 cm/s. In the first of these experiments (E1), the water velocity was increased in 5-cm/s increments every 5 min until all fish in a test group had failed; in the other two experiments (E2 and E3), the velocity was increased in 5-cm/s increments every 10 min. An individual within a given test group of fish was considered to have failed when it became impinged on the rear (downstream) screen for 10–15 s and did not swim when prodded. As each fish failed, we removed it and measured its total length.

We used different procedures to collect the samples for each of the three experiments performed. Fish for E1 were dipnetted as needed in lots of 10 from a group of fish herded into one end of the tank. For E2, fish of similar total lengths were selected to reduce variation in CSS due to size differences, and to simplify data analysis. Since CSS varied with length over even narrow ranges of length in some tests in E1 and E2, we collected fish for E3 that were more closely representative of the range of total lengths in each of the exposure groups. Samples for this experiment were collected as in E1, except that we tried to ensure that at least one large and one small fish were selected from each test population. The discovery of a significant linear relation between CSS and length of fish tested in E3 enabled us to compute a least squares regression for each exposure group and compare the regression lines by analysis of covariance. In E1 and E2, the narrow ranges of fish length in most groups tested precluded computation of significant regressions, so the groups were compared by using a one-way analysis of variance. Results of E2 were not analyzed statistically because samples were not chosen at random.

Prolonged swimming speeds are those between sustained and burst speeds that fish can maintain for 15 s to 200 min (Webb 1975). Standard techniques for measuring prolonged swimming of fish were developed by Brett (1964). To measure the prolonged swimming ability of lake trout and compute CSS, we used Brett's stepwise procedure, in which the water velocity is increased at regular intervals until the fish fails from fatigue. In these tests, the water velocity was increased by discrete intervals ΔU every t minutes. When the fish fails t_i min after a velocity increase from U to U + ΔU, then

$$CSS = U + \frac{\Delta U t_i}{t}$$

and CSS is called the t-min CSS (Webb 1975).

Because the 5-min test interval used in E1 was too short for the larger fish in E2 and E3, the length of the test interval was increased to 10 min. The 10-min interval was short enough to enable us to test all 10 groups within 1 week. Although some controversy exists over the requisite value for t, Dahlberg et al. (1968) considered a t as small as 10 min to be satisfactory when CSS alone is required and ΔU is not large.

Results of the three experiments were analyzed separately because different test and sampling procedures were used in each and, more importantly, because the fish substantially increased in length during the study. The concentrations of PCB's, DDE, and PCB's + DDE that approximate 1, 5, and 25 times ambient Lake Michigan levels are referred to as 1×, 5×, and 25×.

Results and Discussion

Within each experiment the specific swimming speed (SSS) was essentially independent of length. However, when the entire range of total lengths of fish used in the study (2.4–9.5 cm) was considered, SSS decreased with length (Table 1). Critical swimming speed for fish in most exposure groups increased linearly with length (Fig. 1), a trend that is commonly observed when the size range is great enough.

The SSS's of fish in two exposure groups in E1 were significantly different ($P < 0.05$) only from those of fish in certain other exposure groups (one-way analysis of variance, Duncan k-ratio t test; Table 2 and Fig. 2):

Table 1. *Mean length, critical swimming speed (CSS), and specific swimming speed (SSS; fish total lengths [TL] per second) of lake trout exposed for different periods to various concentrations and combinations of PCB's and DDE. (Numbers in parentheses in body of table are standard deviations.)*

Contaminant			Experiment number, and days of exposure[a]									
			Experiment 1 (49–51 days)				Experiment 2 (100–118 days)				Experiment 3 (163–170 days)			
Exposure group	Mean concentration: Food (μg/g)	Mean concentration: Water (ng/L)	No. of fish[b]	Average total length (cm)	CSS (cm/s)	SSS (TL/s)	No. of fish[b]	Average total length (cm)	CSS (cm/s)	SSS (TL/s)	No. of fish[b]	Average total length (cm)	CSS (cm/s)	SSS (TL/s)
Control	0.15 to 0.19	0	39	6.1 (0.76)	24.0 (3.48)	3.9 (0.31)	20	7.5 (0.72)	25.2 (2.27)	3.4 (0.20)				
PCB's														
1×		1.02	20	5.6 (0.88)	21.2 (5.98)	3.7 (0.65)	20	7.8 (1.13)	26.2 (4.96)	3.4 (0.32)				
5×		4.94	20	5.6 (0.64)	19.5 (4.56)	3.5 (0.56)	20	7.6 (0.86)	27.1 (6.17)	2.6 (0.47)				
25×		23.4	20	5.2 (1.01)	18.2 (6.69)	3.4 (0.74)	19	7.7 (1.01)	26.8 (4.24)	3.5 (0.32)				
DDE														
1×		0.24	10	5.1 (0.71)	19.7 (5.22)	3.8 (0.74)	19	7.4 (1.12)	25.9 (3.17)	3.5 (0.25)				
5×		0.60	10	4.8 (0.95)	21.1 (7.32)	4.3 (0.80)	19	7.6 (1.02)	24.2 (4.84)	3.2 (0.43)				
25×		2.39	10	5.1 (0.57)	20.2 (4.67)	3.9 (0.63)	19	7.4 (0.86)	25.7 (3.18)	3.5 (0.33)				
PCB's + DDE														
1×		1.11 + 0.25	20	5.7 (0.58)	21.2 (4.01)	2.7 (0.52)	20	7.8 (0.92)	26.1 (3.25)	3.3 (0.24)				
5×		4.98 + 0.71	20	5.0 (1.12)	16.1 (6.35)	3.1 (0.72)	19	7.8 (0.97)	27.1 (4.28)	3.5 (0.29)				
25×		24.19 + 3.28	20	5.7 (0.58)	20.7 (5.69)	3.6 (0.84)	20	7.5 (0.98)	25.7 (4.48)	3.4 (0.35)				
Total or mean			189	5.5 (0.66)	20.6 (5.66)	3.7 (0.68)	195	7.6 (0.95)	26.0 (4.08)	3.4 (0.34)				

Contaminant			Experiment 1 (49–51 days)				Experiment 2 (100–118 days)				Experiment 3 (163–170 days)			
Exposure group	Mean concentration: Food (μg/g)	Mean concentration: Water (ng/L)	No. of fish	Average total length (cm)	CSS (cm/s)	SSS (TL/s)	No. of fish	Average total length (cm)	CSS (cm/s)	SSS (TL/s)	No. of fish	Average total length (cm)	CSS (cm/s)	SSS (TL/s)
Control	0.15 to 0.19	0	20	2.9 (0.22)										
PCB's														
1×		1.02	10	2.9 (0.12)										
5×		4.94	10	2.9 (0.21)										
25×		23.4	10	2.9 (0.20)										
DDE														
1×		0.24	10	3.1 (0.20)										
5×		0.60	10	2.9 (0.24)										
25×		2.39	7[c]	3.0 (0.05)										
PCB's + DDE														
1×		1.11 + 0.25	10	3.0 (0.15)										
5×		4.98 + 0.71	10	2.9 (0.10)										
25×		24.19 + 3.28	10	3.0 (0.18)										
Total or mean			107	2.9 (0.19)										

[a] Days of exposure to contaminants in water; exposure to contaminants in food was 17 days less, since fry did not begin feeding until about day 17. A 5-min test interval was used in Experiment 1 and a 10-min interval in Experiments 2 and 3.
[b] In tests with 19 fish, data for 1 fish that failed to swim were not used in any calculations.
[c] Only seven fish tested because equipment failed.

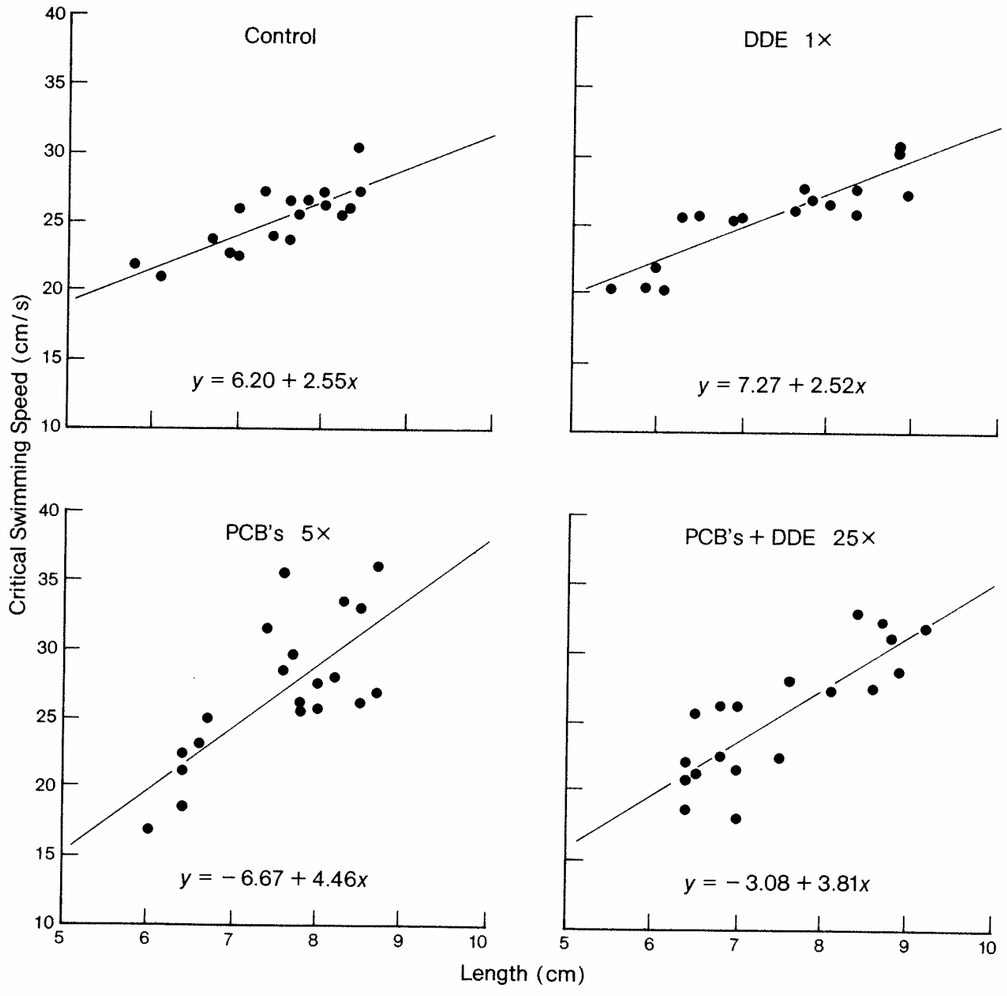

Fig. 1. Representative relation between critical swimming speed and length for lake trout exposed to PCB's and DDE for 163-170 days (experiment 3). Equations for exposure groups not illustrated follow: 5× DDE, $y = -3.92 + 3.69x$, and 25× DDE, $y = 6.69 + 2.56x$. 1× PCB's, $y = -4.85 + 4.00x$, and 25× PCB's, $y = 0.47 + 3.41x$. 1× PCB's + DDE, $y = 2.95 + 2.96x$, and 5× PCB's + DDE, $y = -3.40 + 3.93x$.

(1) Lake trout exposed to 5× PCB's + DDE had a significantly lower mean SSS (5.8) than did control fish (7.4), or fish exposed to 25× DDE or 25× PCB's + DDE; however, the SSS's of fish in the other six test groups were not significantly different from those exposed to 5× PCB's + DDE; (2) Fish exposed to 1× PCB's + DDE had the lowest CSS and SSS measured in E1, significantly lower than those for fish in seven other groups tested, but not significantly different from those exposed to 1× DDE and 5× PCB's + DDE.

Even though statistical comparisons were not made on data from E2, some differences were apparent. Fish exposed to 5× PCB's + DDE had the lowest CSS

Table 2. *Comparison of mean specific swimming speed (SSS; fish lengths/s) by Duncan k-ratio t test for experiments 1 and 3.*

Experiment and exposure	Mean SSS (TL/s)[a]
Experiment 1	
Control	7.4109
PCB's + DDE 25×	7.2526
DDE 25×	6.7990
DDE 5×	6.7472
PCB's 1×	6.7336
PCB's 5×	6.7245
PCB's 25×	6.6947
DDE 1×	6.6148
PCB's + DDE 5×	5.7719
PCB's + DDE 1×	5.6097
Experiment 3	
PCB's 5×	3.5681
DDE 1×	3.5283
PCB's + DDE 5×	3.4852
DDE 25×	3.4770
PCB's 25×	3.4681
PCB's + DDE 25×	3.3991
Control	3.3884
PCB's 1×	3.3601
PCB's + DDE 1×	3.3339
DDE 5×	3.1676

[a]TL/s = fish total lengths per second; individual vertical lines span nonsignificant differences in the means (test level, 0.05).

(16.1) and SSS (3.1) of any exposure group in either E2 or E3, whereas fish exposed to 5× DDE had the highest SSS (4.3) of any exposure group in either E2 or E3 (Table 1).

In E3, the SSS of fish in only one group was significantly different from those in fish of certain other exposure groups. Fish exposed to 5× DDE had a significantly lower SSS than did fish in all test groups

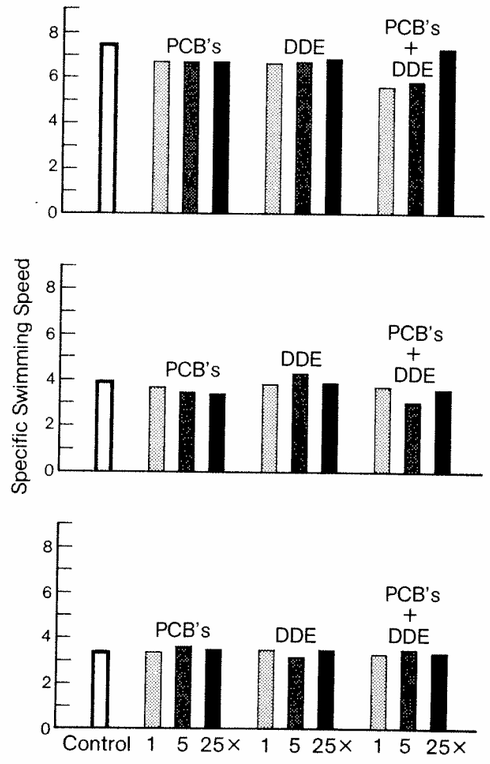

Fig. 2. Mean specific swimming speed (fish total lengths per second) for lake trout after three different periods of exposure to 1×, 5×, or 25× PCB's, DDE, or PCB's + DDE. Approximate exposure periods: 50 days (upper panel), 109 days (middle panel), and 166 days (lower panel).

Table 3. *The maximum and minimum fish total length, critical swimming speed (CSS), and specific swimming speed (SSS) for lake trout exposed to different concentrations of PCB's and DDE for different lengths of time.*

Experiment no., exposure (days) in parentheses, and extremity of range	Total length (cm)	CSS		SSS	
		cm/s	Exposure group	TL/s[a]	Exposure group
Experiment 1 (49-51)					
Maximum	3.4	28.0	Control	9.3	Control
Minimum	2.4	10.8	PCB's 25×	3.8	Control
Experiment 2 (100-118)					
Maximum	7.6	31.1	Control	6.1	DDE 5×
Minimum	3.2	10.0	PCB's + DDE 25×	2.7	PCB's + DDE 25×
Experiment 3 (163-170)					
Maximum	9.5	26.0	PCB's 5×	5.3	PCB's 5×
Minimum	5.5	14.1	DDE 5×	3.2	DDE 25×

[a]TL/s = fish total lengths per second.

except four: control fish, and fish exposed to 1× PCB's or to 1× or 25× PCB's + DDE. Comparison of lines describing the relation between CSS and total length for each exposure group by analysis of covariance showed no significant difference between the slope and adjusted means of control and test groups (Fig. 1).

Although some significant differences existed between exposure groups, we observed no consistent relation between swimming performance and exposure to either PCB's or DDE. Comparison of maximum and minimum values for CSS and SSS for individual fish supported this observation (Table 3). We estimated empirically that our methods in E3 were sensitive enough to detect a 10% difference in CSS.

Bengtsson (1980) used a rotary-flow apparatus to test swimming performance of minnows (*Phoxinus phoxinus*), which had been orally dosed for 40 days with food containing four levels of Clophen A50 (trade name for a mixture of more than 50 PCB-compounds): 0.09 (control), 25, 270, and 2,500 μg/g PCB's (dry weight). He found that subsequent swimming performance, at 94 to 114 days and at 149 to 166 days, was not related to the level of PCB dosage. Although control fish in the two sets of tests seemed to have higher performance values in comparison with groups given PCB's, the differences were not significant.

McNeish (1969) reported that the swimming performance of fingerling Atlantic salmon (*Salmo salar*) exposed to 0.01 and 0.02 mg/kg of DDT (in food) was not significantly different from that of controls. He concluded on the basis of his and other studies that exposure of fish to sublethal concentrations of DDT in food was not likely to measurably alter their swimming performance.

Inasmuch as exposure of young lake trout to PCB's and DDE did not measurably affect their swimming performance, and since the levels of contaminants accumulated by fish in some of the test groups exceeded levels expected to occur in Lake Michigan lake trout during their first year of life, we do not believe that contamination of the fish by PCB's and DDE in the lake would affect the ability of the fish to feed or avoid predation.

No eggs were exposed to contaminants during incubation in this study. However, lake trout eggs exposed to PCB's and DDE during incubation in the lake may accumulate a body burden of these contaminants high enough to reduce the ability of fry to swim at the swim-up stage. If these contaminants do reduce survival by impairing the fish's swimming ability, they would probably have the greatest adverse effect at this point in the life history. Critical and specific swimming speeds of lake trout at swim-up were not measured because we were unable to measure flow rates accurately enough, or develop uniform flow patterns in the test chamber at velocities low enough, to test the sac fry. Variations in yolk sac size and the inherently limited swimming ability of sac fry make the detection of any real differences in swimming performance of sac fry among the various test groups unlikely.

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Vulnerability of Young Lake Trout to Predation After Chronic Exposure to PCB's and DDE[1]

by

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Abstract

The vulnerability of fry of Lake Michigan lake trout (*Salvelinus namaycush*) to predation by rainbow trout (*Salmo gairdneri*) was tested after the fry had been exposed to PCB's, DDE, and a combination of these contaminants in both water and food at concentrations corresponding to ambient levels (1×) in water and plankton in Lake Michigan and at levels 5 and 25 times higher. Vulnerability of the fry, measured as the ratio of escapes to predator attacks, was not significantly increased by either 90 or 165 days of exposure to the contaminants at any of the concentrations tested, and no behavioral differences were observed between control and exposed fry in their reaction to predators. Exposure to PCB's and DDE at environmental and higher concentrations thus did not affect the vulnerability of lake trout fry to predation. This observation suggests that the failure of lake trout reproduction in Lake Michigan was not caused by contaminant-induced reductions in the ability of the fry to escape predators.

Introduction

Survival of young fish in the environment may be severely reduced if their ability to escape predators is impaired; however, few investigators have examined the effects of contaminants on the vulnerability of young salmonids to predation. Hatfield and Anderson (1972) reported that vulnerability of yearling Atlantic salmon (*Salmo salar*) to predation by brook trout (*Salvelinus fontinalis*) increased after 24-h exposure to 1.0 mg/L Sumithion, an organophosphate pesticide, but that exposure to 0.1 mg/L Sumithion or 0.07 mg/L DDT produced no effects. In studies on crustaceans, Tagatz (1976) showed that exposure to 0.025 μg/L mirex for 14 days increased the vulnerability of grass shrimp (*Palaemonetes vulgaris*) to predation by pinfish (*Lagodon rhomboides*); and Ward and Busch (1976) found that a concentration of less than 0.01 μg/g Temefos, an organophosphate, in marsh fiddler crabs (*Uca pugnax*) increased their vulnerability to predation. However, the effects on predator-prey interactions in fish after long-term exposures to chlorinated hydrocarbons at levels similar to those in Lake Michigan are not known.

The purpose of this study was to determine whether long-term exposure to PCB's and DDE at concentrations similar to those in Lake Michigan increases the vulnerability of lake trout (*Salvelinus namaycush*) fry to predation, thereby reducing survival. The experimental design was based on the premise that if PCB's and DDE increased vulnerability of Lake Michigan lake trout fry to predation, a measurable decrease in the ability of exposed fry to escape the attacks of a predator would be observed. Therefore, vulnerability to predation was expressed as a ratio of the number of escapes by contaminant-exposed and control lake trout fry to the number of attacks by an experimental predator (rainbow trout, *Salmo gairdneri*).

Materials and Methods

Lake trout fry exposed to different concentrations of PCB's and DDE, separately or combined (see Berlin et al. 1981), were subjected to predation in test troughs (sections of a fiberglass fish rearing trough, divided by

[1]Contribution 565 of the Great Lakes Fishery Laboratory.

screens) 240 cm long, 53 cm wide, and 30 cm deep. Water depth was 25 cm and temperature 9.0 ± 0.5°C throughout the experiments. The experimental area was surrounded by a black plastic curtain into which holes were cut that enabled an observer to watch the fish without distracting them. Two 100-W incandescent bulbs 3 m above the troughs provided illumination. Light intensity, controlled by a rheostat, was set at the lowest illumination that would allow adequate observation (as determined in pretesting) and held constant throughout the study.

Before an experiment began, fry were randomly netted by passing a small dip net through the exposure tank. From the large number of fry thus collected, the 10 needed for each test were dipped from the net into a beaker, then placed in a bottomless screen cylinder 15 cm in diameter and 30 cm tall standing inside the test trough. Each trough held one rainbow trout (about 24–30 cm long), which remained in the trough until all testing with that predator trout was completed. After a 30-min acclimation period, the cylinder was lifted from the trough, thus exposing the fry to the predator. The number of attacks made by the predator and the number of fry left in the trough at the end of the test period were recorded. Each lunge by the predator at a point in the water that had been occupied by a prey fish immediately before the lunge was defined as an attack. Movements of prey not initiated by such an attack, i.e., avoidances, were not considered escapes because these avoidances could not be differentiated from random movements.

Although juvenile rainbow trout are not natural lake trout predators, they were chosen as experimental predators because of their aggressive feeding behavior and their docility under observation. Rainbow trout were allowed to prey on unexposed lake trout fry under the experimental conditions for at least a week before the tests, to ensure that only successful predators were used and to reduce the likelihood of predators becoming more skilled with experience gained during the actual tests. Predators were presented with only one group of fry per day. On days when no tests were run, predator trout were presented with the same number of fry as on a test day. A predator was used until it had participated in tests with each of the 10 groups (9 exposed to contaminants and 1 control group).

Two experiments were carried out (Table 1): the first began on day 89 of exposure (73 days after exposure to contaminants in food began) and ended on day 107; the second began on day 164 and ended on day 174. In each experiment, three predators were tested.

New predators were used in the second experiment, and because of the increased size of the lake trout, 6 prey were used for each test instead of the 10 used in the first experiment. Predators were allowed 5 min of active feeding time for each test in the first experiment, and 15 min in the second. In addition, a portion of the test trough with a standpipe that had provided limited cover for the fry in the first experiment was screened off, shortening the test area to 220 cm. The presence of cover would necessitate a much longer observation period than feasible to obtain at least 50% predation. Open-water attacks by the predator provided a better test of fry awareness, acceleration, and maneuverability. Since no comparisons were made between the two experiments, these slight changes in procedure did not affect the conclusions.

Table 1. *Lengths and weights of predator rainbow trout, and mean number of attacks and mean number of lake trout fry caught. Mean values are results of tests with all 10 groups of fry (9 exposure and 1 control). Ten lake trout fry were offered as prey in experiment 1 and six in experiment 2. Standard deviations are shown in parentheses.*

Experiment and predator no.	Size of predator: Length (cm)	Size of predator: Weight (g)	Attacks (no.)	Lake trout fry (no.)
Experiment 1				
1	26.8	259	25.8 (5.45)	9.5 (0.71)
2	24.5	200	22.3 (6.62)	9.4 (0.97)
3	28.9	274	21.4 (4.60)	8.2 (0.79)
Experiment 2				
4	34.0	475	26.4 (8.68)	5.4 (1.07)
5	29.0	357	31.3 (6.99)	5.2 (0.79)
6	30.0	337	29.0 (7.06)	4.0 (1.05)

I analyzed the data by using a two-way analysis of variance on the number of prey caught, the number of attacks made by the predator, and the ratio of fry escapes to predator attacks (Yocom and Edsall 1974). Contaminant exposure levels and predators were regarded as treatments (Table 2).

Results

Contaminant treatment had no significant effect ($P > 0.05$) on any of the three variables measured (number of fry caught, number of attacks, or number of escapes/number of attacks) in either of the two experiments (Table 2). Values for the ratio of escapes to attacks for fry from each treatment (Table 3) showed that control fry had the highest ratio of escapes (0.692) in experiment 1, but this value was not significantly greater than that for any exposed group (Dunnett's test; $P > 0.05$). In experiment 2, lake trout fry escaped more often than in experiment 1, but again, the contaminants did not significantly affect prey vulnerability.

Table 2. *Statistical results: observed F values in an analysis of variance with predators and contaminants as treatments.*

Item	df	No. of attacks	Number of fry caught	No. escapes/ no. attacks
Experiment 1				
Predators	2	2.46	7.72[a]	1.71
Contaminants	9	2.30	1.03	1.45
Error	18			
Experiment 2				
Predators	2	1.22	7.81[a]	4.47[b]
Contaminants	9	1.53	1.94	1.10
Error	18			

[a]Significant at $P < 0.01$.
[b]Significant at $P < 0.05$.

Predator activity was consistent throughout any one experiment. The mean number of attacks did not differ significantly between predators in either experiment (Table 1). Highly significant ($P < 0.01$) differences existed between the mean number of fry caught by predators in both experiments (Table 2) and the escape:attack ratio was significantly different ($P < 0.05$) between predators in experiment 2 (Table 3). This statistical test indicated a difference between predators in their efficiencies of capture, but since fry from each treatment were tested with each predator, test results were not affected. The training of predators under experimental conditions before the tests began was apparently helpful, since no significant correlation was evident between predator efficiency and trial number (experience).

Discussion

The ability of lake trout fry to escape nearly 60% of the predator attacks in experiment 1 and 80% in experiment 2 is evidence that the fry were at least temporarily successful in eluding the predator, even though little or no cover was present. Extended exposure to the various levels of PCB's and DDE had no detectable effect on this ability, although these organochlorines are believed to impair the central nervous system (Bahr and Ball 1971; Davy et al. 1972; Weis and Weis 1974). In most studies of the effects of contaminants, the dosing period was 24 h or less and concentrations were much higher than those that fish might encounter in the aquatic environment. Long-term exposure (80–176 days) to environmental levels of contaminants could have effects that are more subtle and difficult to measure.

Table 3. *Mean ratio of the number of escapes by lake trout fry to the number of attacks by a rainbow trout, after the fry had been exposed to different concentrations of PCB's and DDE. Values are the mean of tests with three predators.*

Exposure	Experiment 1	Experiment 2
Control	0.692	0.836
PCB's		
1×	0.571	0.767
5×	0.646	0.760
25×	0.510	0.862
DDE		
1×	0.549	0.842
5×	0.544	0.842
25×	0.560	0.803
PCB's + DDE		
1×	0.560	0.858
5×	0.612	0.827
25×	0.661	0.804

Lake trout fry exposed to PCB's and DDE in this study suffered significantly higher mortality than did control fry (Berlin et al. 1981). Experiment 1 of the vulnerability tests extended through a period of high mortality (days 89–107), but experiment 2 (days 164–174) occurred during an interval when mortality in all groups of fish was low. Because of this difference in mortality, significant changes in escapability would have been more likely to occur in the fry in experiment 1. However, no apparent relation existed between prey vulnerability and mortality of fry stocks during a test period.

Contaminant-caused changes in behavior that could affect prey vulnerability have been reported. Cooke (1971) observed hyperactivity in tadpoles treated with 0.05 mg/L DDT for 5–19 h. This behavior evoked more attacks when the tadpoles were subjected to predation by newts, although no changes in vulnerability of the tadpoles were observed. In the present study, analysis of variance revealed no significant changes between exposure levels and the number of attacks. In addition, I observed no differences between groups of fry in their behavioral response to the presence of predators. Responses of fry to attack and modes of capture by predators were indistinguishable in all groups tested.

Inasmuch as concurrent swimming speed tests revealed no consistent relation between contaminant exposure and the critical swimming speed of lake trout fry (Rottiers and Bergstedt 1981), the results of the present study strongly support the inference that

exposure to PCB's and DDE at the levels tested does not increase the vulnerability of lake trout fry to predation.

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Temperature Selection by Young Lake Trout After Chronic Exposure to PCB's and DDE[1]

by

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Abstract

Temperature selection tests were conducted with fry of Lake Michigan lake trout (*Salvelinus namaycush*) exposed to PCB's, DDE, and a combination of these contaminants in food and water at levels 25 times the ambient levels in plankton and water in Lake Michigan. The observed effect of the contaminants was a lowering of the preferred temperature. After 98 days of exposure, mean preferred temperatures were 10.3°C for fry exposed to PCB's, 9.8°C for those exposed to DDE, and 8.7°C for those exposed to PCB's + DDE, as compared with 11.2°C for control fry. Frequency distributions of residence temperatures were significantly ($P < 0.01$) different among all treatments. Such a change in the preferred temperature caused by a contaminant could reduce the energetic efficiency of a fish and thereby reduce growth and survival.

Introduction

Temperature, one of the most important components of a cold-blooded animal's environment, affects growth and metabolism through its direct effect on the rates of metabolic processes and, more subtly, through changes in enzymes and metabolic pathways (Wieser 1973). Fish, being mobile, can select a temperature from a range of environmental temperatures usually available. Although many factors influence this selection, under a given set of conditions the preferred temperature of a species represents a thermoregulatory response evolved to maximize energetic efficiency (Crawshaw 1977). A deviation from the normal preferred temperature under that set of conditions could result in a decrease in energetic efficiency and therefore in growth and survival.

Several studies have shown that organochlorine compounds alter the temperature preference of fish (Ogilvie and Anderson 1965; Javaid 1972; Peterson 1973; Gardner 1973). However, these studies involved short exposure periods (24 h) and much higher concentrations (2 μg/L to 2 mg/L) than those occurring in the Great Lakes.

The purpose of the present study was to determine the effects of PCB and DDE exposure on the temperature selected by lake trout fry originating from Lake Michigan, and to ascertain whether these contaminants are capable of affecting the performance and survival of the fry.

Materials and Methods

We measured temperature preference in a vertical temperature gradient tank consisting of a modified 750-L circular fiberglass tank 70 cm deep and 123 cm in diameter, fitted with a window (62 × 70 cm) on one side. A black plastic enclosure outside the window excluded light from the observation area and reduced the possibility of disturbance of the fish by the observer. A temperature gradient was established by introducing chilled water (3-4°C) through a perforated tube on the bottom, while at the same time passing heated water down through a heat exchange tube coiled along the perimeter of the tank. The resulting gradient of 4-20°C was linear, except near the bottom and surface of the water column.

Temperature was measured continuously with two sets of 12 thermistor probes and a 24-channel recorder. Each set was attached to a meterstick and the probes

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were held at 6-cm intervals; one set was placed at the center of the tank and one near the edge. Depth was plotted against temperature for each test and the resulting curve was used to convert the observations from depth to residence temperature.

A possible problem with fish in a vertical temperature gradient is gas bubble disease, caused by supersaturation of gases as the cold water entering at the bottom is warmed (McCauley 1977). However, dissolved oxygen concentration in water samples taken at 12-cm depth intervals was a constant 7.0 mg/L and percent saturation ranged from 55 to 80. To investigate the possibility of N_2 supersaturation, we confined a group of yearling yellow perch (*Perca flavescens*) in the warmest surface layers. At the end of 1 week, they showed no sign of gas bubble disease.

The history and treatment of the test fry (lake trout, *Salvelinus namaycush*) were described by Berlin et al. (1981). Fry hatched from eggs collected from Lake Michigan lake trout were exposed to PCB's (327.0 ng/L), DDE (32.7 ng/L), or both, in water, and later fed spiked Oregon Moist pellets containing concentrations of 22.6 μg/g PCB's, 2.3 μg/g DDE, or a combination of 23.4 μg/g PCB's and 3.02 μg/g DDE. These concentrations were about 25 times ambient levels in water and plankton, respectively, in southeastern Lake Michigan.

Four lake trout fry from the control group and four fry from each of the three exposure groups (total of 16 fish) were tested after 90 to 120 days of exposure. In addition, we observed the vertical movements of four fry—one from each exposure group tested in the gradient tank in the absence of a thermal gradient.

Before each test, the gradient was destroyed by bubbling air from an airstone and the temperature was adjusted to within 0.5°C of the acclimation temperature (9°C). One fish was then removed from the exposure tank between 1400 and 1500 h on the day before observation and placed in the gradient tank. Fish chosen were seemingly healthy individuals of about average size. The airstone was removed and the temperature gradient allowed to form overnight. The acclimation temperature was therefore available to the fish at all times, reducing the probability of thermal shock. Fish were not fed while in the gradient tank.

Observations began at 0815 h. By this time the gradient was stabilized, and the fish had had 17–18 h in which to acclimate to the tank. We made an observation every 30 s over four 10-min periods spaced 1 h apart and a total of 80 observations of each fish's residence temperature. The 80 residence temperatures were averaged to obtain a mean preferred temperature for each fish and these values were averaged by treatment. We constructed frequency distributions from the 320 observations from each treatment and used chi-square tests to compare these distributions.

Results and Discussion

In the absence of a thermal gradient, the frequencies with which the fry were observed at each 1-cm depth interval appeared to be evenly distributed, except for an increase in frequency near the top and bottom of the water column (Fig. 1). This distribution did not resemble the unimodal distributions obtained in the preference tests. In the test environment, temperature was apparently the major factor controlling the distribution of the fry.

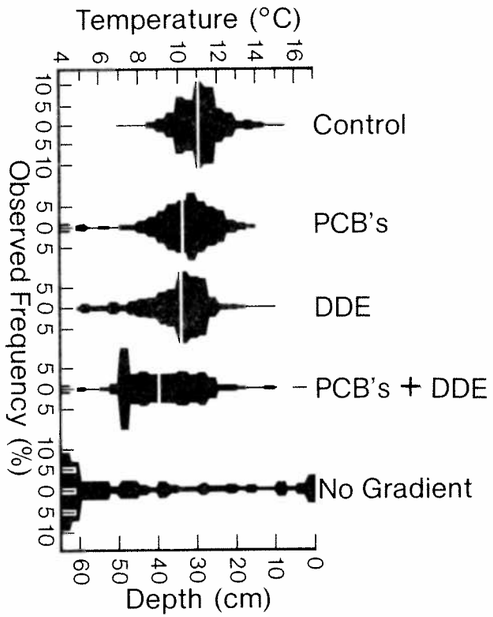

Fig. 1. Temperature selected by lake trout fry exposed to PCB's and DDE, alone or in combination. The width of the polygon represents the relative frequency of observations of the fish at the corresponding temperature. The horizontal line across each polygon represents the mean preferred temperature. Panel at right shows distribution of fry at 9°C in the absence of a thermal gradient.

The effect of the contaminants appeared to be a lowering of the preferred temperature. The mean preferred temperature was 11.2°C for control fry, 10.3°C for fry exposed to PCB's, 9.8°C for fry exposed to DDE, and 8.7°C for fry exposed to PCB's + DDE (Table 1). Chi-square tests revealed that all the frequency distributions differed significantly ($P < 0.01$) from one another (Fig. 1). The modes of these distributions are additional evidence suggesting that exposure

Table 1. *Mean preferred temperatures selected by lake trout fry exposed for 90-120 days to PCB's, DDE, or PCB's + DDE equal to 25 times ambient levels in water and plankton in Lake Michigan. Each value is a mean of four fish (standard deviations in parentheses).*

Exposure	Temp. (°C)
Control	11.2 (1.2)
PCB's	10.3 (1.8)
DDE	9.8 (1.8)
PCB's + DDE	8.7 (2.0)

to the contaminants causes a lowering of the preferred temperatures. Modal preferred temperatures were 11.0–11.5°C for control fry, 10.5–11.0°C for fry exposed to PCB's, 10.0–10.5°C for those exposed to DDE, and 7.0–7.5°C for those exposed to PCB's + DDE.

The effects of the two contaminants appeared to be additive in lowering the preferred temperature. The mean preferred temperature of fry exposed to PCB's was 0.9°C lower than that of control fry, and the mean preferred temperature of fry exposed to DDE was 1.4°C lower than that of control fry; the sum of these differences (2.3°C) nearly equals the difference in mean preferred temperature between control fry and fry exposed to PCB's + DDE (2.5°C).

The preferred temperature of control fry (mean = 11.2°C) agrees well with the 11.7°C mean preferred temperature observed by McCauley and Tait (1970) for yearling lake trout. The observed contaminant effect is similar to that observed by Gardner (1973), who found that brook trout (*Salvelinus fontinalis*) exposed to 20 μg/L *p,p'*-DDE for 24 hours preferred a temperature 1.5°C lower than that preferred by unexposed fish.

Judging by the existing literature on the effects of chlorinated hydrocarbons on preferred temperature, the shift of the preferred temperature caused by DDT and related compounds has been shown to be concentration dependent; lower concentrations appear to reduce the preferred temperature and high concentrations to raise it (Ogilvie and Anderson 1965; Javaid 1972). Our results tend to agree with those in the literature, as our concentrations were lower than any used by previous researchers and we observed a lowering of the preferred temperature. However, a comparison of published studies (24-h exposure at 2 to 2000 μg/L) with ours (90–120 day exposure at 25 to 250 ng/L) is difficult because the relation between contaminant effects caused by a short exposure to high concentrations and long exposure to low concentrations is not known.

Any change in the preferred temperature of a fish is significant because it is potentially detrimental to growth and survival. Behavioral thermoregulation and the resultant preferred temperature range of a species probably evolved to maximize overall energetic efficiency. The preferred temperature may not represent the temperature at which maximum growth could occur with unlimited rations (although this may be true in a laboratory situation), but under natural conditions of light, pressure, food availability, etc., temperatures are selected to minimize energy requirements for locomotion and food conversion. The adaptive value of selecting for energetic efficiency is the maximizing of both growth and survival (Priede 1977). A change in the preferred temperature caused by an unnatural factor (contaminant) could cause fry to select habitat inferior in types and amounts of food available and reduce the overall energetic efficiency of a fish, thereby reducing growth and survival.

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A possible problem with fish in a vertical temperature gradient is gas bubble disease, caused by supersaturation of gases as the cold water entering at the bottom is warmed (McCauley 1977). However, dissolved oxygen concentration in water samples taken at 12-cm depth intervals was a constant 7.0 mg/L and percent saturation ranged from 55 to 80. To investigate the possibility of N_2 supersaturation, we confined a group of yearling yellow perch (*Perca flavescens*) in the warmest surface layers. At the end of 1 week, they showed no sign of gas bubble disease.

The history and treatment of the test fry (lake trout, *Salvelinus namaycush*) were described by Berlin et al. (1981). Fry hatched from eggs collected from Lake Michigan lake trout were exposed to PCB's (327.0 ng/L), DDE (32.7 ng/L), or both, in water, and later fed spiked Oregon Moist pellets containing concentrations of 22.6 μg/g PCB's, 2.3 μg/g DDE, or a combination of 23.4 μg/g PCB's and 3.02 μg/g DDE. These concentrations were about 25 times ambient levels in water and plankton, respectively, in southeastern Lake Michigan.

Four lake trout fry from the control group and four fry from each of the three exposure groups (total of 16 fish) were tested after 90 to 120 days of exposure. In addition, we observed the vertical movements of four fry—one from each exposure group tested in the gradient tank in the absence of a thermal gradient.

Before each test, the gradient was destroyed by bubbling air from an airstone and the temperature was adjusted to within 0.5°C of the acclimation temperature (9°C). One fish was then removed from the exposure tank between 1400 and 1500 h on the day before observation and placed in the gradient tank. Fish chosen were seemingly healthy individuals of about average size. The airstone was removed and the temperature gradient allowed to form overnight. The acclimation temperature was therefore available to the fish at all times, reducing the probability of thermal shock. Fish were not fed while in the gradient tank.

Observations began at 0815 h. By this time the gradient was stabilized, and the fish had had 17–18 h in which to acclimate to the tank. We made an observation every 30 s over four 10-min periods spaced 1 h apart and a total of 80 observations of each fish's residence temperature. The 80 residence temperatures were averaged to obtain a mean preferred temperature for each fish and these values were averaged by treatment. We constructed frequency distributions from the 320 observations from each treatment and used chi-square tests to compare these distributions.

Results and Discussion

In the absence of a thermal gradient, the frequencies with which the fry were observed at each 1-cm depth interval appeared to be evenly distributed, except for an increase in frequency near the top and bottom of the water column (Fig. 1). This distribution did not resemble the unimodal distributions obtained in the preference tests. In the test environment, temperature was apparently the major factor controlling the distribution of the fry.

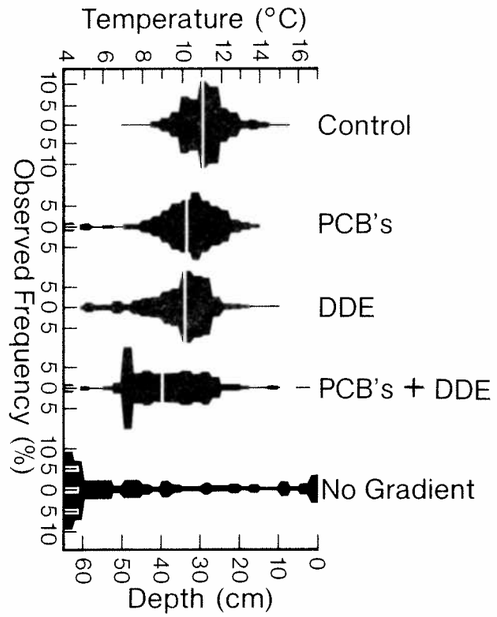

Fig. 1. Temperature selected by lake trout fry exposed to PCB's and DDE, alone or in combination. The width of the polygon represents the relative frequency of observations of the fish at the corresponding temperature. The horizontal line across each polygon represents the mean preferred temperature. Panel at right shows distribution of fry at 9°C in the absence of a thermal gradient.

The effect of the contaminants appeared to be a lowering of the preferred temperature. The mean preferred temperature was 11.2°C for control fry, 10.3°C for fry exposed to PCB's, 9.8°C for fry exposed to DDE, and 8.7°C for fry exposed to PCB's + DDE (Table 1). Chi-square tests revealed that all the frequency distributions differed significantly ($P < 0.01$) from one another (Fig. 1). The modes of these distributions are additional evidence suggesting that exposure

Table 1. *Mean preferred temperatures selected by lake trout fry exposed for 90-120 days to PCB's, DDE, or PCB's + DDE equal to 25 times ambient levels in water and plankton in Lake Michigan. Each value is a mean of four fish (standard deviations in parentheses).*

Exposure	Temp. (°C)
Control	11.2 (1.2)
PCB's	10.3 (1.8)
DDE	9.8 (1.8)
PCB's + DDE	8.7 (2.0)

to the contaminants causes a lowering of the preferred temperatures. Modal preferred temperatures were 11.0–11.5°C for control fry, 10.5–11.0°C for fry exposed to PCB's, 10.0–10.5°C for those exposed to DDE, and 7.0–7.5°C for those exposed to PCB's + DDE.

The effects of the two contaminants appeared to be additive in lowering the preferred temperature. The mean preferred temperature of fry exposed to PCB's was 0.9°C lower than that of control fry, and the mean preferred temperature of fry exposed to DDE was 1.4°C lower than that of control fry; the sum of these differences (2.3°C) nearly equals the difference in mean preferred temperature between control fry and fry exposed to PCB's + DDE (2.5°C).

The preferred temperature of control fry (mean = 11.2°C) agrees well with the 11.7°C mean preferred temperature observed by McCauley and Tait (1970) for yearling lake trout. The observed contaminant effect is similar to that observed by Gardner (1973), who found that brook trout (*Salvelinus fontinalis*) exposed to 20 μg/L *p,p'*-DDE for 24 hours preferred a temperature 1.5°C lower than that preferred by unexposed fish.

Judging by the existing literature on the effects of chlorinated hydrocarbons on preferred temperature, the shift of the preferred temperature caused by DDT and related compounds has been shown to be concentration dependent; lower concentrations appear to reduce the preferred temperature and high concentrations to raise it (Ogilvie and Anderson 1965; Javaid 1972). Our results tend to agree with those in the literature, as our concentrations were lower than any used by previous researchers and we observed a lowering of the preferred temperature. However, a comparison of published studies (24-h exposure at 2 to 2000 μg/L) with ours (90–120 day exposure at 25 to 250 ng/L) is difficult because the relation between contaminant effects caused by a short exposure to high concentrations and long exposure to low concentrations is not known.

Any change in the preferred temperature of a fish is significant because it is potentially detrimental to growth and survival. Behavioral thermoregulation and the resultant preferred temperature range of a species probably evolved to maximize overall energetic efficiency. The preferred temperature may not represent the temperature at which maximum growth could occur with unlimited rations (although this may be true in a laboratory situation), but under natural conditions of light, pressure, food availability, etc., temperatures are selected to minimize energy requirements for locomotion and food conversion. The adaptive value of selecting for energetic efficiency is the maximizing of both growth and survival (Priede 1977). A change in the preferred temperature caused by an unnatural factor (contaminant) could cause fry to select habitat inferior in types and amounts of food available and reduce the overall energetic efficiency of a fish, thereby reducing growth and survival.

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Biochemistry and Metabolism of Lake Trout: Laboratory and Field Studies on the Effects of Contaminants[1]

by

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Abstract

To evaluate the effects of ambient and higher concentrations of PCB's (Aroclor 1254) and DDE in food and water on fry of lake trout (*Salvelinus namaycush*) from Lake Michigan, I measured several biochemical indicators of stress in exposed and unexposed (control) fry. No differences between treatments were observed in oxygen consumption rates or lactate concentrations of unexercised fry, but apparent differences in specific swimming speed and lactate response in fry that swam to exhaustion suggested that exposed fry had lower stamina. Observed differences between biochemical profiles of 1-day-old sac fry reared from eggs originating from lake trout collected off Saugatuck and those originating from eggs collected from brood stock at the Marquette (Michigan) hatchery may have been caused by organochlorine contamination or by genetic and dietary differences between the parental stocks. Activity of the enzyme allantoinase was measured in juvenile and adult lake trout as an indicator of sublethal effects of Great Lakes contaminants. The 50% inhibition of allantoinase in vitro occurred at 6.0 mg/L Cu^{++}, 6.7 mg/L Cd^{++}, 34 mg/L Hg^{++}, and 52 mg/L Pb^{++}. Allantoinase was not affected by in vitro exposure to PCB's up to 7 μg/g, or DDE or DDT up to 10 μg/g; however, in vivo exposure resulting in 2.6 μg/g PCB's in the whole fish activated allantoinase slightly (10% significance level). Allantoinase activity was negatively correlated with total length for fish from Lake Michigan but not for fish from Lake Superior or from laboratory stocks. Mercury, PCB's, and DDT's, possibly acting in combination with each other and with additional contaminants, may be the cause of the negative correlation of allantoinase activity with size in Lake Michigan lake trout.

Introduction

To assess the effects of ambient and higher concentrations of toxic substances in the Great Lakes on lake trout (*Salvelinus namaycush*), one would need to observe the survival, growth, and reproduction of the fish during exposure to ambient levels for more than one generation. However, such an approach is impractical because lake trout rarely mature before they are 6 or 7 years old. As an alternative to chronic toxicity tests, toxicological tests may be performed either in vivo or in vitro on fish that are exposed for shorter periods. Enzymes are not only useful in standard toxicological tests, but they may sometimes serve as short-term indicators of sublethal toxicity when they are inhibited by a single chemical or class of chemicals and when inhibition can be related to survival of fish (Coppage and Matthews 1974).

In the study described here, I examined allantoinase, an enzyme of intermediary nitrogen metabolism, as a short-term indicator of sublethal toxicity. Concentrations of contaminants that produce inhibitory effects on enzyme preparations from lake trout liver were determined and related to contaminant concentrations in fish from Lakes Michigan and Superior. In addition, I made clinical observations to detect stress in chronically exposed fry. Even though fry—one of the most sensitive life stages of a fish—may be able to survive when exposed to PCB's and DDE under laboratory conditions, alterations of homeostatic mechanisms by contaminants may adversely affect their ability to survive in their natural environment.

[1]Contribution 567 of the Great Lakes Fishery Laboratory.

Part I. Metabolism of Chronically Exposed Lake Trout Fry

As part of an evaluation of the influence of ambient and higher concentrations of PCB's (Aroclor 1254) and DDE in food and water on the survival and performance of fry hatched from eggs of lake trout collected off Saugatuck, Michigan (Berlin et al. 1981), I measured certain metabolic and biochemical indicators of stress in exposed and unexposed fry. These tests included the determination of respiration (oxygen consumption rates) as a measure of overall energy use (Fry 1971) and the determination of respiration rates and lactate concentrations before and after swimming performance tests, to aid in interpreting differences in swimming endurance and ability to recover from fatigue (Driedzic and Kiceniuk 1976). In addition, biochemical profiles of 1-day-old fry were provided by the U.S. Fish and Wildlife Service Columbia (Missouri) National Fisheries Research Laboratory. Personnel at the Laboratory measured ascorbic acid (vitamin C) as an indication of disease resistance; detoxification ability; adrenocortical steroid production; the formation of collagen necessary for cartilage, bone, and wound repair; and the ratios of the amino acids proline to hydroxyproline, since this ratio is related to collagen formation. A functional deficiency in ascorbic acid can result in impaired hydroxylation of proline and lysine to hydroxyproline and hydroxylysine and subsequent spinal deformities (Mayer et al. 1978).

Methods

Newly hatched fry were exposed to PCB's (Aroclor 1254) and *p,p'*-DDE in water and food, as described by Berlin et al. (1981). The nominal exposure levels were 1×, 5×, and 25× ambient Lake Michigan levels, where 1× = 10 ng/L PCB's or 1 ng/L DDE, or both, in water; and 5 μg/g PCB's or 0.1 μg/g DDE, or both, in food. In addition, fertilized eggs obtained from the Marquette (Michigan) State Fish Hatchery were hatched and the resultant fry were compared with the Lake Michigan fry. One-day-old sac fry from Saugatuck eggs and Marquette eggs were frozen and later analyzed for ascorbic acid (Hubmann et al. 1969), total protein (Lowry et al. 1951), proline (Chinard 1952; Troll and Lindsley 1954), and hydroxyproline (Woessner 1961).

Five fry from each of the 30 tanks described by Berlin et al. (1981) were sampled for measurement of respiration at 21–35 days and 84–94 days after the beginning of contaminant exposure. In addition, lactate was analyzed in four or five fry per tank at 95 days. Fry were netted directly from the treatment tanks (deformed fry were returned to the tanks). For respiration measurements on fry that swam to failure (Rottiers and Bergstedt 1981), I selected three or four fry that failed at nearly the same time; hence, the interval between failure and the beginning of the respiration measurement was only about 15 min. For lactate analysis, fry were blotted quickly with tissue paper while still in the net and were immediately placed on dry ice until movement ceased—usually 5 to 20 s. Thus the fish were immobilized and frozen within the 3-min interval recommended by Chavin and Young (1970). I calculated specific swimming speeds (Brett 1964) of fish that I used for the respiration and lactate measurements. Of the fish tested by Rottiers and Bergstedt (1981) at 100–118 days, I used all the fry tested at 100–102 days, except for four fish that were lost.

To measure respiration of the fry, I used a closed respirometer with transparent sides and opaque top (modified from Kapoor and Griffiths 1975). After the fry had been sealed in the respirometer and submerged in a dimly lighted, constant temperature (9°C) bath, I waited one-half hour to allow the fry to recover from handling and to acclimate to the chamber before I measured their oxygen uptake for an hour. Their rate of oxygen uptake for the entire 1½-h period was used to detect any evidence of payment of oxygen debt. Observation of the fry when the respirometer was out of the bath showed that they were not orienting to water movement caused by the stirring bar. Their activity level was spontaneous or "routine" (Fry 1971). At the end of the test period, the fry were removed, blotted, and weighed. Dry weight was determined after they were dried at 70°C to constant weight (Jawed 1973).

Whole frozen fry were analyzed for lactate after 1 year of storage at -20°C. A 50-μL aliquot of a 1:3 homogenate in 5% trichloroacetic acid was analyzed for lactate enzymatically (Sigma Chemical Company 1976).

Results

Although the biochemical profile of 1-day-old sac fry should be considered tentative because the sample size was small, some differences between fry from Saugatuck eggs and from Marquette Hatchery eggs were suggested (Table 1). Total protein and ascorbic acid were lower in fry from Saugatuck eggs. Also, the ratio of the amino acids proline to hydroxyproline was higher in Saugatuck fry. Similar differences in the biochemical profile of young fish of other species have been shown to be associated with organochlorine contamination (Mayer et al. 1978); however, genetic or dietary differences in the parental stocks could also be involved.

Oxygen consumption rates, on a dry-weight basis, for Saugatuck fry at 21–35 days and 84–94 days were

Table 1. *Biochemical profile of 1-day-old lake trout sac fry. Values represent the mean of two composite samples of 10 sac fry each.*

Chemical and units	Location	
	Lake Michigan off Saugatuck	Marquette Hatchery
Protein (mg/g tissue)	104.5	113.5
Ascorbic acid (μg/g tissue)	26.5	42.4
Hydroxyproline (μg/g protein)	642	752
Proline (mg/g protein)	164	106
Proline/hydroxyproline	0.255	0.141

1.96 ± 0.056 (mean ± SE) and 1.92 ± 0.034 mg O_2/g•h, respectively. Analysis of variance of all respiration data for unexercised fry of both ages showed no significant differences ($P > 0.05$) between treatments (controls or exposed fry) or between ages. For fry at 21-35 days exposure, oxygen uptake rate was lowest in the 1× DDE exposure group and highest in the 5× DDE group. At 84-94 days, the rate was lowest in the 5× DDE exposure group and highest in the 25× DDE group. Although these differences were not significant, DDE tended to cause a greater perturbation of oxygen uptake rate than did PCB's.

In swimming performance studies after 100-102 days of contaminant exposure, weight-specific oxygen consumption rates of fry exercised to exhaustion were significantly different (Duncan Multiple Range Test [Bliss 1967]) from the rates in unexercised fish in only two treatment groups—25× DDE and 25× PCB's + DDE (Table 2). All exercised fish consumed less oxygen than did unexercised fish, except for the fish exposed to the 1× and 25× concentrations of PCB's alone.

Lactate concentrations of unexercised fry at 95 days showed no significant differences between treatments (control or exposed fry). The concentration of lactate was 110 ± 4.3 mg/100 g tissue (mean ± SE; n = 29). Differences in lactate concentrations between exercised and unexercised fry within the 10 treatments were not significant, but significant differences were apparent between treatments (Table 2). Control fry had the highest lactate concentration among the exercised fish from all treatments. Lactate decreased abnormally, after forced swimming, in fish exposed to PCB's and to 5× PCB's + DDE. This decrease in lactate implies lower stamina in fish of these groups (Black 1957).

In swimming performance tests, I used fry from a narrower time span (100-102 days of exposure) than did Rottiers and Bergstedt (1981). The specific swimming speeds (Table 2) of the controls and of the fry exposed to 5× or 25× DDE were significantly faster ($P < 0.05$; Duncan k-ratio t test [Duncan 1975]) than those of fry exposed to all three concentrations of PCB's and to 5× PCB's + DDE. Thus the fish exposed to PCB's and those exposed to the 5× concentrations of PCB's + DDE tended to have lower stamina, as indicated by both the lower specific swimming speeds (Brett 1964) and the abnormal lactate response to exertion. However, interpretation of the data on the exercised fry must be qualified because the fry were not sampled randomly across all size classes, but were selected to be near mean length. Further testing of exercised and unexercised fry would be necessary to establish a relationship between stamina and contaminant exposure.

Table 2. *Oxygen uptake rates and lactate concentrations in Lake Michigan lake trout fry exposed to different concentrations of PCB's and DDE, before and after forced swimming to failure, and specific swimming speeds of exposed fry.*

Treatment[a]	Oxygen uptake rate (mg O_2/g dry wt •h)			Lactate (mg/100 g)			Specific swimming speed[c,d]
	Unexercised[b]	Exercised[c]	O_2 uptake	Unexercised[b]	Exercised[c]	Lactate	
Control	2.05	1.66	−0.39	128	173	45	4.1
PCB's							
1×	1.54	2.01	0.47	143	93.9	−49	3.2
5×	1.92	1.90	−0.02	94.1	86.8	−7.3	3.2
25×	1.69	1.78	0.09	130	99.2	−31	3.0
DDE							
1×	1.83	1.74	−0.09	110	152	42	3.8
5×	1.77	1.38	−0.39	102	135	33	4.2
25×	2.31	1.78	−0.53	118	128	10	3.9
PCB's + DDE							
1×	1.94	1.50	−0.44	65.7	117	51	3.7
5×	1.99	1.88	−0.11	142	50.6	−91	2.7
25×	2.14	1.64	−0.50	90.6	124	33	3.7

[a]Contaminant concentrations shown are nominal: × = 10 ng/L PCB's or 1 ng/L DDE, or both, in water, and 5 μg/g PCB's or 0.1 μg/g DDE, or both, in food; see Berlin et al. (1981) for actual concentrations.
[b]Exposure, 84-94 days.
[c]Exposure, 100-102 days.
[d]Specific swimming speed = critical swimming speed/total length.

Discussion

In spite of higher mortality in most treatment groups of fry exposed to PCB's and DDE (Berlin et al. 1981), biochemical and metabolic tests did not show definitive corresponding subacute effects. I observed no significant differences in respiration rate for unexercised fry at 21-35 or 84-94 days. McLeay and Brown (1974) stated that an elevated metabolic response caused by many toxicants indicates some general form of stress. Respiration rate might be expected to reflect changes in overall metabolism or activity level. I observed no general differences in activity level between treatment groups of lake trout fry in this study.

Although I observed no differences in lactate concentration between treatment groups of unexercised fry, elevated lactate in muscle of fish has been used to document tissue hypoxia in rainbow trout, *Salmo gairdneri* (Hodson 1976). These rainbow trout had been exposed to lethal concentrations of zinc that caused gill damage. In clinical medicine, elevations in blood lactate are used to detect circulatory failure (Marbach and Weil 1967) or to determine the cause of metabolic acidosis (Field et al. 1966). My results indicated that the fry exposed to PCB's and DDE in the present study were undergoing normal aerobic metabolism.

The abnormal lactic acid response of exercised fish that had been previously exposed to PCB's and to 5× PCB's + DDE suggests that either these fish lacked adequate stored glycogen or that glycolysis was suppressed. Evidence has been found of suppression of glycolysis in fish that were exposed to endrin and dieldrin and then forced to swim (P. M. Mehrle, personal communication).

The apparent reduced swimming speeds of the fish exposed to three concentrations of PCB's and 5× PCB's + DDE (Table 2 and Experiment 2 of Rottiers and Bergstedt 1981) might be expected to decrease their ability to capture food or to escape from predators. However, Mac (1981), who tested the vulnerability of these fry to predation at 89 to 107 days, found no significant difference in ratios of escapes to attacks between unexposed fry and any of the groups of exposed fry.

Part II. Effects of PCB's, DDT, and Metals on the Enzyme Allantoinase in Juvenile and Adult Lake Trout

As a means of assessing the impact of PCB's, DDT's, mercury, and other metals on the health of lake trout, I evaluated the effects of these contaminants on allantoinase (EC 3.5.2.5), an enzyme of uric acid catalysis in the pathway of purine degradation. Inhibition of allantoinase by contaminants could result in the deposition of allantoin and in failure to excrete nitrogen from nucleic acids, proteins, and amino acids that are normally eliminated through purinolytic and uricolytic pathways. In this study, I investigated the following: (1) characteristics or properties of allantoinase in lake trout liver; (2) in vitro sensitivity of the enzyme to PCB's, DDT's, mercury, lead, cadmium, and copper; (3) allantoinase activities during in vitro and in vivo exposure to PCB's; (4) allantoinase activities in lake trout from Lakes Michigan and Superior; and (5) the relation between enzyme activity and contaminant residues in lake trout from the two lakes.

Methods

Lake trout fingerlings obtained from the Jordan River National Fish Hatchery, Elmira, Michigan, were reared at the Great Lakes Fishery Laboratory. Adult lake trout were gillnetted from Lake Michigan near Saugatuck and Grand Haven, Michigan, in October 1974 and from Lake Superior near Isle Royale in August 1974. After fish were killed by a blow to the head, the livers were removed and either assayed immediately or stored for several months at −20°C until analyzed.

Allantoinase assays were performed on centrifuged (2,500 *g*) liver homogenates of wild or laboratory trout or on extracts of an acetone powder prepared from livers of laboratory lake trout (Passino and Cotant 1979). Allantoinase activity is reported as units per milligram of protein, where a unit equals μmoles glyoxylate produced per minute. The method of in vitro exposure of allantoinase to contaminants was described by Passino and Cotant (1979).

Fingerling lake trout were exposed in vivo to PCB's (0.11 ± 0.018 μg/L Aroclor 1254; mean ± SE) in water for 28 weeks (R. J. Hesselberg and L. W. Nicholson, unpublished data). The diet of both controls and ex-

perimentals consisted of Oregon Moist Pellets, incidentally containing < 0.2 μg/g PCB's.

Fish livers were saponified (Reinert 1970) before analysis for PCB's (Willford et al. 1976) and DDT + DDE (Hesselberg and Scheer 1974). Samples from fish exposed in vivo were quantitated by comparison with standard Aroclor 1254. Standards consisting of a 1:1:1 mixture of Aroclors 1248, 1254, and 1260 were used for quantitation of samples of fish from Lakes Michigan and Superior. Livers and fillets of fish from these lakes were analyzed for total mercury by a combustion-amalgamation technique (Willford et al. 1973).

Allantoinase and Contaminants in Lake Trout Liver

Characteristics of allantoinase in livers of laboratory lake trout were described by Passino and Cotant (1979). The concentrations of inorganic metals (mg/L) at which 50% inhibition of allantoinase occurred during in vitro exposure were as follows: 6.0 Cu^{++}, 6.7 Cd^{++}, 34 Hg^{++}, and 52 Pb^{++}. On a molar concentration basis, allantoinase was most sensitive to cadmium. Allantoinase activity was not affected by 10-minute in vitro exposure to ≤ 10 μg/g DDT or DDE or ≤ 7 μg/g PCB's (Aroclor 1254). Preincubation of allantoinase with 7 μg/g PCB's for 1.5 h did not affect enzyme activity (Passino and Cotant 1979).

In 20 lake trout previously exposed in vivo for 28 weeks to low levels (0.11 μg/L) of PCB's in water, allantoinase activity in livers, which had been frozen, equaled 0.168 ± 0.021 unit/mg protein (mean ± SE) compared with 0.119 ± 0.016 in 20 control fish. This apparent activation of the enzyme by PCB's was significant at the 90% confidence level. Analysis of 10 whole fish after the 28 weeks of exposure showed 0.16 ± 0.01 μg/g wet weight PCB's (Aroclor 1254) in the controls and 2.6 ± 0.2 μg/g in the experimentals. The residues in the control fish were probably due to incidental PCB's in the food.

Allantoinase activity in lake trout from Lakes Michigan and Superior (Table 3) was reported by Passino and Cotant (1979). Enzyme activities were significantly higher ($P < 0.01$) in small than in large lake trout from Lake Michigan, but not in fish from Lake Superior or from Great Lakes Fishery Laboratory stocks—although enzyme activity of smaller fish was higher in all three lots. Not all sizes of fish were analyzed for contaminants, but only samples of the largest and smallest fish (Table 4).

Significance of Allantoinase and Contaminants in Lake Trout

Although short-term in vitro exposure of allantoinase to PCB's did not affect enzyme activity, the activity increased slightly after in vivo exposure for 28 weeks. These apparently different results may indicate that insufficient time occurred during the in vitro exposure for the PCB's to affect the enzyme (Jackim 1974). The localization of allantoinase in the soluble fraction of teleost liver cells (Goldenberg 1977) and the affinity of PCB's for this same subcellular fraction in environmentally exposed lake trout (Passino 1978) suggest that PCB's are more likely to affect allantoinase than enzymes associated with other fractions. PCB's may affect the level of allantoinase activity by inducing the synthesis of the enzyme from precursors or through conformational changes of existing molecules (Hill et al. 1976). Enzyme induction would require more time than our maximum in vitro exposure of 1.5 h (Lehninger 1975:993). The results of the in vivo exposure should be more reliable than those of the in vitro exposure as predictors of expected results in nature.

Although interpretation of data from field samples is complicated by many unknown variables (e.g., season, temperature, diet) that may be operating on enzyme systems in the fish, the trend exists for the enzyme activity to be inversely correlated with length of the fish (Table 3). This trend may be caused in part by the greater age of the fish and also by higher contaminant levels in older fish, especially those from Lake Michigan (Michigan Department of Agriculture 1974). Another factor that may contribute to the difference in enzyme activities between Lake Michigan and Lake Superior fish is the possible genetic differences between two strains of fish. The lake trout collected near Isle Royale were primarily of the "humper" strain (Rahrer 1965). Isozymes of allantoinase may differ in the two strains and respond unequally to contaminants.

Table 3. *Allantoinase activity of individual samples of frozen livers from wild lake trout collected in Lake Michigan off Saugatuck and Lake Superior off Isle Royale in 1974, and from Laboratory stocks of lake trout (± SE in parentheses). (From Passino and Cotant 1979.)*

Source, and number of fish	Total length (mm) Group	Total length (mm) Mean	Allantoinase specific activity (unit/mg protein)
Lake Michigan			
19	> 500	707 (14)	0.0701[a] (0.00755)
18	< 500	310 (26)	0.140[a] (0.0109)
Lake Superior			
18	> 500	592 (10)	0.0712 (0.0103)
32	< 500	350 (11)	0.0749 (0.00869)
Laboratory[b]			
3	> 500	564 (6)	0.109 (0.0127)
30	< 500	290 (14)	0.197 (0.0215)

[a]Difference between means of allantoinase activity within fish length group was highly significant ($P < 0.01$) by analysis of variance.
[b]Fish maintained at Great Lakes Fishery Laboratory.

Table 4. *Contaminant analysis of composite samples of livers and individual samples of fillets of lake trout collected off Saugatuck in Lake Michigan and off Isle Royale in Lake Superior in 1974. (From Passino 1981.)*

Source, and number of fish	Total length (mm) Group	Total length (mm) Mean ± SE	Livers PCB's (μg/g)[a]	Livers DDT + DDE (μg/g)[a]	Livers Total Hg (μg/g)[a,b]	Fillets Total Hg (μg/g)[a,c]
Lake Michigan						
10	> 500	717 ± 23	21.4	4.68	0.939	0.594
10	< 500	210 ± 14	< 0.6	0.141	0.479	0.0552
Lake Superior						
10	> 500	586 ± 14	4.55	1.86	0.690	0.581
8	< 500	284 ± 4	< 0.6	0.206	0.105	0.152

[a]μg/g wet weight.
[b]Average value of three (fish < 500 mm long) or four (> 500 mm) analyses per composite sample.
[c]Average value of two analyses per fillet for specified number of fish.

The difference between mercury levels in livers and fillets may be due in part to partitioning of various compounds of mercury between tissues. The higher liver-to-fillet ratio for mercury in Lake Michigan fish than in Lake Superior fish (Table 4) may indicate that the exposure of the Lake Michigan fish to mercury has been the more recent (Laarman et al. 1976).

The highest value for mercury (0.939 μg/g; Table 4) was far below the level (34 μg/g) at which 50% inhibition of allantoinase occurred during the 10-min in vitro exposure. However, the value for total mercury probably includes some methyl mercury (Bache et al. 1971), which may be more inhibitory to the enzyme than the inorganic mercury that was tested. The presence of 2.6 μg/g PCB's in whole fish after in vivo exposure tended to activate allantoinase, whereas 21.4 μg/g PCB's in large fish—a value nearly 10 times the in vivo level—did not; perhaps the PCB's acted in combination with mercury and other contaminants present in the fish to inhibit the enzyme. The Laboratory lake trout, which were comparatively uncontaminated, tended to have higher enzyme activities for a given size group than did fish from the lakes.

The cause of decreased allantoinase activity in large Lake Michigan lake trout may be the PCB's, DDT's, and mercury present, possibly acting in combination with each other and with additional contaminants. The significance of this reduction for survival of the fish in Lake Michigan is not known. In vitro assays provided some indication of the mode of action and relative toxicity of different metals (Passino and Cotant 1979). The results of in vitro and in vivo tests with PCB's were not in agreement. Consequently, I conclude that allantoinase is probably not a useful short-term indicator of the chronic effects of the common contaminants investigated in lake trout.

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