Survival of walleye eggs and fry of known DDT residue levels from ten Wisconsin waters in 1967. Report 37 1968

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~~for fish~~

SURVIVAL OF WALLEYE EGGS AND FRY OF KNOWN DDT RESIDUE LEVELS FROM TEN WISCONSIN WATERS IN 1967

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INTRODUCTION

Studies of pesticide residues in Wisconsin fish show DDT to be universally present, while dieldrin has been present in over half of the fish examined (Kleinert et al., 1967). The concentration of pesticides in Wisconsin fish varies according to the location sampled. Residue values of the DDT complex (includes DDT, DDE, and DDD) have ranged from .02 to 16.2 ppm in whole fish samples. Dieldrin has been found in whole fish samples in amounts as great as 4.18 ppm; most dieldrin containing samples, however, held less than .05 ppm.

The significance of these pesticide residues in Wisconsin fish is largely unknown. Studies elsewhere, however, have indicated pesticide residues may present a serious threat to fisheries by interfering with fish reproduction. Burdick et al. (1964) showed that a DDT concentration in the ether extract of lake trout fry equivalent to 2.9 ppm or above in the weight of the fry resulted in mortality. The mortality syndrome appeared after absorption of the yolk sac when the fry were about ready to feed. Allison et al. (1964) found a critical period shortly after hatching when mortality was higher in the offspring of cutthroat trout exposed to high DDT concentrations.

In 1966 the Department of Natural Resources selected the walleye as the subject for the study of the association of pesticide residues with reproductive failures. The objectives of the study were to follow the survival of walleye eggs and fry from a number of Wisconsin waters to see if pesticide residues occurring in the eggs or fry were correlated with mortality. The walleye was selected due to its importance as a game fish, the extensive experience Wisconsin has had in culturing this species in hatcheries, and most importantly because fisheries researchers had detected a significant DDT content in walleyes from two lakes (Lake La Belle, Waukesha County, and Muskellunge Lake, Vilas County) where walleye reproduction had been poor.

In April and May of 1966 the survival of 16 lots of walleye eggs and fry from three southeastern Wisconsin lakes was followed at the Delafield Hatchery. Samples of the eggs were analyzed for DDT and dieldrin. A possible association between DDT levels in the eggs and the mortality of six lots of eggs just prior to hatching was indicated (Kleinert, 1967). Dieldrin was not believed to be associated with mortality and could not be detected in measurable amounts in most egg samples. A strong positive correlation was observed between the DDT content of walleye females and their eggs. The 1966 studies were regarded as both preliminary and exploratory. It was concluded a second study should be undertaken in 1967 using improved hatchery facilities, better hatchery water, and many more lots of walleye eggs having a wide range of DDT residue values from several areas of the state.

DESCRIPTION OF THE STUDY AREAS

Study Waters

Ten Wisconsin waters were selected for taking walleye spawn in 1967. These waters were selected on the basis of three criteria: (1) a wide range of DDT residue levels in fish as determined by previous survey information should be represented; (2) waters sustaining good walleye reproduction as well as those showing poor walleye reproduction should be included; and (3) experienced personnel should be available for taking walleye spawn in these waters in the spring of 1967.

Muskellunge and Escanaba Lakes in Vilas County, Green Lake in Green Lake County, the Wolf River in Outagamie County, Pike Lake in Washington County, and Lakes LaBelle, Golden, Pine, Upper Nemahbin, and Nagawicka in Waukesha County were subsequently selected for taking walleye eggs in 1967. Previous survey information describing the DDT and dieldrin levels found in walleyes in these waters and the status of walleye reproduction are presented in Table 1. The location of these waters in Wisconsin is illustrated in Figure 1.

Studies of walleyes from these ten waters prior to 1967 showed that DDT and its analogs in whole fish ranged from .082 ppm for a sample of Escanaba Lake walleyes to 7.62 for one of six walleye samples from Lake LaBelle. Dieldrin found in walleye samples from the ten waters prior to 1967 did not exceed .025 ppm in whole fish samples.

Natural walleye reproduction in Escanaba and Pike Lakes and the Wolf River has yielded year classes in most years while most of the other waters have failed to produce year classes.

Geographically, these waters represent southeastern and northeastern Wisconsin. Golden Lake, the smallest of the ten, covers 250 acres; Green Lake, the largest, covers 7,325 acres. The Wolf River is one of Wisconsin's major rivers. It joins the Fox River to flow into 137,708 acre Lake Winnebago. Water quality of the ten varies from the lakes of the northeast which are relatively soft, low in dissolved solids and total alkalinity, to the more fertile waters of the southeast which are moderately hard and much higher in dissolved solids and total alkalinity.

Comprehensive records of the amounts of pesticides used in Wisconsin do not exist. Neither are figures available on the amounts of pesticides sold in Wisconsin. DDT is used in Wisconsin to control household, lawn, agricultural, orchard and forest insects. DDT has been extensively used to attempt to control elm bark beetles, the carriers of the Dutch elm disease. The waters in the present study vary in terms

of the human population density of the watersheds and the known or suspected use of DDT in the watershed. Muskellunge and Escanaba Lakes are located in a forested region. Escanaba Lake has no history of DDT spraying and is completely undeveloped with the exception of a boat landing and a fisheries research station operated by the Department of Natural Resources. Muskellunge Lake is chiefly undeveloped with a public campground occupying part of the shoreline which has been treated with DDT for mosquito control. The Wolf River occupies a large watershed for which a past history of DDT use has not been determined. All of the other lakes in the study have shorelines partially or completely developed into summer and permanent homes and resorts. DDT use around certain of these lakes for either mosquito control or Dutch elm disease control is known. In general, where DDT use is known or suspected higher levels of DDT have been observed in fish samples.

In addition to these waters, walleye eggs were taken at Trout Lake in Vilas County for use in DDT exposure studies at Westfield. Trout Lake is an oligotrophic drainage lake of 3,870 acres having slightly alkaline water of high transparency. Trout Lake is only moderately developed as 96 percent of the shoreline is in public ownership.

Westfield Hatchery

Facilities at the Westfield Hatchery, located at Westfield, Wisconsin, were used for hatching the walleye eggs and for holding the fry in observation aquaria in 1967. The Westfield Hatchery was ideal for this work due to its central location and excellent water supply furnished by Artesian wells. The water has a nearly constant temperature of 50° F. and is free of silt and algae which could foul the fine screening required to retain walleye fry. Analysis of a sample of Westfield Hatchery water taken in April 1967 exhibited a pH of 7.6, a total alkalinity of 156 ppm, total phosphorus of .043 ppm and specific conductance of 300 micromhos (Table 2).

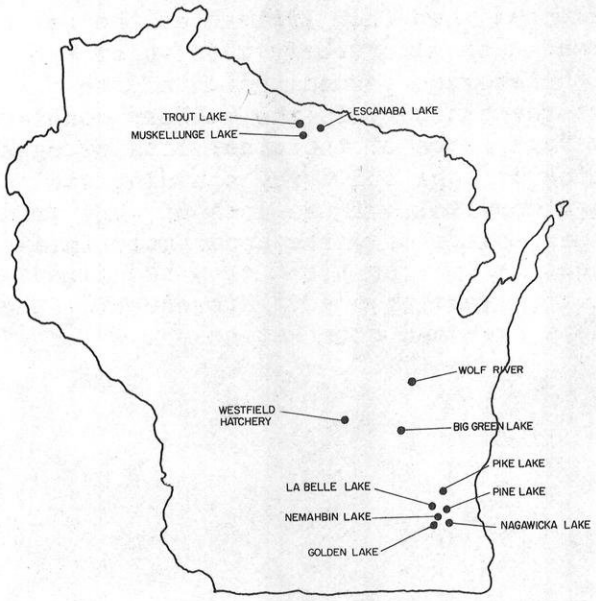


FIGURE 1.
Location of the Westfield Hatchery and Waters Where Walleye Spawn Was Taken in 1967.

MATERIALS AND METHODS

Field Work

Fyke nets were set in the nine lakes within two weeks of the spring ice melt to sample the walleye spawning run. Walleyes in the Wolf River were collected with an alternating current boom shocker. Walleye spawn was taken between April 8 and May 1, 1967. The southernmost waters in the study warmed earliest in the spring enabling nets to be set, and ripe walleye to be spawned ahead of the other waters in the study. Three to eight females from each water were spawned for a total of 53 lots of eggs. Four different netting crews and one electro-fishing crew participated in this study.

Taking Spawn

Instructions for fertilizing and handling walleye eggs were given to field personnel to insure that all lots of eggs were similarly treated. Five different fisheries workers took spawn in this study. Walleye eggs were fertilized by the wet method. Milt from the male was extruded into a pan containing less than three inches of water by gently compressing the sides of the fish. Eggs from a ripe female were immediately added to the pan by firmly stroking the female from head to vent. Milt from a second male was then added to the pan to insure fertilization. The eggs from a single female, milt and water were mixed by hand and added to a one gallon glass jar containing water and bentonite. The jar was then marked with a label and placed in a cardboard box for transport to the hatchery. The eggs were delivered to the hatchery the same day, in most cases within six hours of spawning.

Hatchery Methods

Some lots of eggs were badly clumped when they arrived at the hatchery. In certain cases the eggs formed a single rubbery mass which had to be broken apart by hand. However, the average percent hatch of the twelve lots of eggs which arrived at the hatchery in the rubbery condition was nearly identical to the average hatch of the other lots of eggs in this study. Consequently, clumping of eggs following spawning was not believed to be detrimental to egg survival. Three lots of eggs from Muskellunge Lake contained a large percentage of white, apparently immature eggs when they arrived at the hatchery. The field crew had immediately noted the white eggs when the fish were spawned. Subsequently these three lots of muskellunge lake walleye eggs had poor hatches.

After any clumps present had been broken apart by hand and the eggs separated, egg lots were placed in the hatching jars. Water flow was periodically adjusted to insure a gentle roll of the eggs. The flow required in each jar depended upon the quantity of eggs present, varying from 2.2 liters per minute for a 40 mililiter lot of eggs, to 7.1 liters per minute for a 1,065 mililiter lot of eggs.

The eggs in each jar were counted within 24 hours of placement in the jars and again when the eyed stage was reached. Egg counts were made by extrapolation from the number of eggs in three 1 cc. samples. To determine the percent hatch, the eyed egg count was divided by the count of eggs originally placed in the jars.

Four to five days after the eggs were placed in the jars a sample of each lot was examined under low power magnification in order to detect infertile eggs. We used methods developed by Olson (1966) for the detection of unfertilized eggs. By this time developing eggs had reached either the late blastula, epiboly, or embryonic strip stages. Infertile eggs could be recognized by an irregular white spot or opaque area on the egg yolk. In contrast, viable eggs were transparent and cell development was easily seen.

The infertile or dead eggs tended to congregate near the surface of the egg mass in the hatching jars by the eighth day. At that time, some dead eggs could be siphoned and removed. Dead eggs became progressively more bouyant and easier to remove as time passed. By the time the viable eggs were eyed and ready to hatch, almost all dead eggs had been removed. We believe that the dead eggs removed were eggs which failed to develop initially; no sudden egg mortalitites were noted in any of the lots of eggs after placement in the jars.

Water from each hatching jar flowed into its own 20 gallon plastic can. Water passed out of each plastic can via a windowed 32 ounce plastic bottle covered with a nylon stocking to retain the walleye fry.

Counts were made of the ratio of live to dead fry in the plastic cans over the period of hatching. As soon as hatching was well underway, samples of 200 fry were removed from each can and stocked in separate 20 gallon aquaria, without food. The aquaria were aerated and set in a waterbath of artesian water which maintained the aquarium temperature at a constant 51° F. Fry stocked in the aquaria were observed each day. As dead fry were found they were counted and removed.

All eggs and fry were handled similarly and exposed to the same hatchery procedures and water temperatures regime. Under these uniform conditions the peculiar behavior or mortality of any of the egg and fry lots could be identified. All fry would ultimately die in the aquaria from starvation since no food was available. We theorized, however, that eggs or fry of higher DDT levels would die earlier in the developmental period, or display a characteristic mortality syndrome and thus implicate DDT with mortality.

Sample Preparation and Laboratory Analysis

When each lot of eggs arrived at the hatchery, a 50 ml sample was removed for DDT analysis. The sample was placed in a 100 ml glass bottle. The water was separated from the eggs by stretching fine nylon over the mouth of the bottle and inverting the bottle. When the water was drained off, each sample bottle was covered with aluminum foil and frozen. Fry samples were taken when each lot of eggs had nearly finished hatching. Water was separated from the fry by stretching fine nylon mesh over the 100 ml sample bottle containing the fry and inverting the bottle on blotting paper. When the water had been removed from the fry sample, the sample bottle was covered with aluminum foil and frozen. We had hoped that 50 ml of fry could be collected from each lot of eggs; however, in most lots a lesser quantity of fry was available for analysis.

The egg and fry samples were kept frozen and delivered to the Nevin Laboratory freezer to await analysis.

All egg and fry samples were analyzed at the Department of Natural Resources' Nevin Laboratory. Identical laboratory methods were used to process both the egg and fry samples. Each sample was ground in a blender, mixed, and reground; aliquots of each sample were selected and stored in capped sample bottles at -20° F. until analysis. Throughout sample preparation, the samples were kept in a frozen, or near frozen condition.

Moisture determinations were made by drying the prepared samples for 8 to 12 hours in a forced-air oven at 102° C, with weighing before and after. Fat determinations were made on the dried samples by continuous extraction with ethyl ether for 8 to 10 hours.

Ten grams of ground, frozen sample were prepared for pesticide analysis according to procedures described for animal tissues in the U. S. Department of Health, Education and Welfare's Pesticide Analytical Manual (1965). This procedure was modified by excluding acetronitrile partitioning. Thus the concentrated extracts were placed directly on deactivated florisil columns and eluted with six percent ethyl ether and 94 percent redistilled hexane elutant. The deactivated florisil columns passed DDT and its analogs on the first elution.

The elutant from the florisil column was concentrated to 10 ml, one ml aliquot of which was passed through a sweep codistillation apparatus (Kontes Glass Company). The glass tubes of this apparatus were packed with glass wool. Four .5 ml injections of hexane were made at five minute intervals following the injection of the sample. The effluent from the sweep codistillation apparatus was made up to 10 ml with hexane; one microliter of sample was then injected into the gas chromatograph. Samples were diluted or concentrated as needed to insure the final concentrations for analysis were held to near 0.1 ppm of pesticide in the injection solutions.

Samples of trout fat low in pesticides were "spiked" with DDT, DDT analogs, and dieldrin and taken through the above procedure; recoveries of pesticides ranged from 90 to 100 percent.

DDT, DDD, and DDE residue levels were determined by electron capture gas chromatography (Beckman Model GC-5), utilizing a mixed bed column, 2 mm interior diameter by 6 feet glass, packed with 9 parts 10 percent DC200 and 5 parts 10 percent QF1 on gas chrom Q60-80 mesh. The column temperature was 210° C., and the flow rate was 26 ml helium per minute. The detector temperature was 250° C. The injector temperature 220° C.

The laboratory reported residues of DDT, DDD, and DDE as parts per million of the whole sample ("whole egg basis or whole fry basis").

Statistical Methods

Correlation coefficients were calculated for the fat percentage of eggs and fry in each lot, the DDT content of eggs and fry in each lot, the hatching success of egg lots of various fat percentages, the median days of life for fry lots of various fat percentages, the hatching success and median days of life for fry from egg lots of various DDT levels, and the median days of life for fry lots of various DDT levels. Correlation coefficients and linear regression relationships were calculated using the formulas and procedures described by Dixon and Massey (1957). The statistical probability of occurrence of the correlation coefficients calculated were determined by using tables from Simpson, Roe and Lewontin (1960). Correlation coefficient values having a probability of occurrence of .01 or less were accepted as indicating significant statistical relationship between the two variables under study.

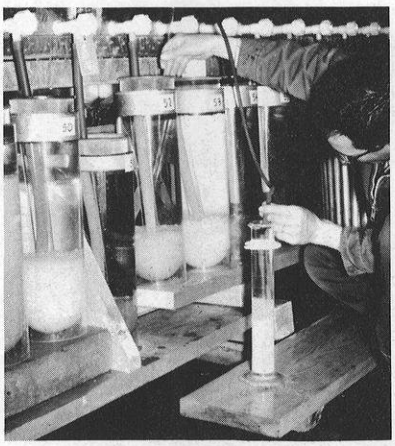
DDT Exposure Experiment

In addition to the study just described, an investigation was conducted at the Westfield Hatchery to see if high levels of DDT could be concentrated in walleye eggs by exposing the eggs to DDT. The objectives of the DDT exposure experiment were to: (1) determine if walleye eggs would take up DDT from the water; (2) determine if the eggs would take up DDT in amounts proportionate to its concentration in the water and time span of exposure; (3) determine if DDT was a causative agent affecting the survival of eggs and fry; and (4) determine the level of DDT that would affect the survival of eggs or fry. Samples of walleye eggs from Trout Lake, Vilas County, were used for this experiment. The hatchery culture methods used in this experiment were identical to the methods used with eggs and fry from the other study lakes.

Fertilized lots of eggs from several Trout Lake walleyes were mixed together in a plastic pail and then divided into 600 ml lots among six hatching jars. Five gallon muriote bottles filled with water and 500 ml of acetone containing three different concentration of technical grade DDT powder were fed into three hatching jars to give concentrations of .1, .01, and .001 DDT. Three control jars were also used, one containing water and 500 ml of acetone without DDT, the other two jars receiving only hatchery water. Number 18 gauge hypodermic needles were used to drip the solutions into the hatching jars. All needles were set at similar rates of flow. The exposure experiment was conducted for 72 hours during the egg developmental period. Egg samples were removed from the jars at 0, .5, 3, 9, 27 and 72 hour intervals. At each of these intervals, each sample was divided, with half of the sample being placed in a clean water hatching jar for the remainder of the hatching period and half of the sample being frozen in preparation for DDT analysis. When hatching occurred, fry coming from eggs from each experimental and control group were analyzed for DDT. Samples of 100 fry from each experimental and control condition were also held in aquaria without food until all fry had died to detect differences in the life span of the fry.

Setting the nets just after the ice went out in a walleye spawning area.

Taking walleye spawn in the field.



Checking and sampling walleye eggs from the hatching jars.

Making observations of walleye survival as the eggs hatch and the fry swim into plastic cans.

Removing fry sample from the plastic cans.

Recording observations of walleye fry in aquaria.

Microscopic examination of developing fry revealed a progressive reduction in yolk sac and oil droplet, coupled with the development of the mouth and appearance of teeth. In one lot of fry extensively studied, teeth and mouth appeared to be fully functional by the 19th day after hatching, which was the 38th day after spawning. The oil drop was no longer evident and the yolk was greatly reduced by the 42nd day. By the 53rd day only a vestige of the yolk remained and the fry were emaciated in appearance. Mortality increased at the time the oil drop disappeared and continued for many days until only a vestige of the yolk remained. By this point all fry had died.

Fat Content of Eggs and Fry

The fat content of walleye eggs varied from .7 percent to 5.8 percent and averaged 3.4 percent. The fat content of walleye fry varied from 5.2 percent to 11.3 percent and averaged 7.1 percent (Table 4). A correlation coefficient of .11 (35 df) was calculated for the fat content of eggs and fry for the various lots, indicating little or no relationship between the fat content of the eggs and their fry. These inconsistent data are difficult to explain and may suggest error in the fat analysis procedure for either the eggs or the fry.

DDT Content of Eggs and Fry

The sum of DDT and its analogs in the egg samples ranged from .067 to 9.380 ppm (Table 4). For all the egg samples combined DDE constituted 40.1 percent, DDD 23.7 percent and DDT 36.2 percent of the DDT complex. There was considerable variability in the percentage occurrence of the analogs in the various egg lots.

The sum of DDT and its analogs in fry samples ranged from .077 to 9.980 ppm (Table 4). For all of the fry samples combined DDE constituted 47.5 percent, DDD 25.4 percent and DDT 27.1 percent of the DDT complex. Again, there was considerable variability in the percentage occurrence of the analogs in the various fry lots. The average percentage of DDE, DDD, and DDT making up the DDT complex changed somewhat between the egg and fry stages revealing an increase in DDE and a decrease in DDT. This suggests some conversion to lower analogs between the egg and fry stages.

There was a strong positive correlation between the pesticide content of the eggs and fry from each lot (Table 5). The DDT content of eggs and fry from each lot yielded a correlation coefficient of .58 (.001 significance with 48 df). The combined DDT and DDE content of eggs and fry from each lot yielded a correlation coefficient of .76 (.001 significance with 48 df). The combined DDT, DDD, and DDE content of eggs and fry from each lot yielded a correlation coefficient of .51 (.001 significance with 48 df).

Pesticide residues in the eggs and fry showed characteristic magnitudes of concentration for each of the ten waters sampled. The lowest pesticide levels were observed in the Escanaba Lake samples where eggs and fry averaged .152 and .179 ppm DDT and its analogs respectively. The highest levels were observed in the Pine Lake samples where eggs and fry averaged 4.124 and 5.161 ppm DDT and its analogs respectively. The remaining waters sampled held eggs and fry of characteristic magnitudes ranging between these two extremes.

Evaluation of Factors Associated with Egg and Fry Mortality

Survival of Egg and Fry Lots of Various Fat Percentages: There was no statistically significant correlation between the percentage of fat in a lot of eggs and their hatching percentage (Table 6). Neither was there significant correlation between the percentage of fat in the eggs and the median life span of the fry. However, a positive correlation coefficient of .72 (.001 significance with 42 df) was obtained between the fat level of the fry and the median life span of the fry, indicating fry of higher fat levels tended to live longer. This finding was not surprising, as fatter fry would be expected to live longer under the starvation conditions imposed in the aquaria. The regression equation for this relationship was calculated to be $Y = 45.14 + 1.8 (X - 7.06)$ where Y is the median life span of walleye fry from each lot and X is the fat percentage of the walleye fry comprising each lot (Fig. 2).

Hatching Success and Fry Survival of Egg Lots with Various DDT Levels: The data indicated no relationship between the pesticide residue levels in the eggs and their hatching success. Correlation coefficient values for hatching success and DDT, combined DDT and DDD, and combined DDT, DDD, and DDE of the egg lots did not approach statistical significance (Table 7).

The data indicated no relationship between the pesticide levels in the eggs and the median life span of the fry. Correlation coefficient values for DDT, combined DDT and DDD, and combined DDT, DDD, and DDE residues in the egg lots compared with the median life span of the fry were not statistically significant (Table 7).

Survival of Fry Lots of Various DDT Levels: The data indicated no relationship between the pesticide levels in the fry and the median life span of the fry. Correlation coefficient values for DDT, combined DDT and DDD, and combined DDT, DDD, and DDE residues in the fry compared with the median life span of the fry were not statistically significant (Table 7).

DDT Exposure Experiment: The DDT exposure experiment did not work out as we had hoped. The DDT powder formed flakes of microscopic size in solution. These flakes tended to plug the hypodermic needles used to drip the DDT solutions into the hatching jars. The hypodermic needles had to be cleaned or replaced several times each hour in the .1 ppm DDT system to insure operation. Proportionately less needle cleaning was required in the .01 and .001 ppm DDT systems because of the smaller amounts of DDT present.

Analysis revealed that eggs exposed to DDT showed about the same ranges of DDT as eggs not exposed to DDT (Table 8). Analysis failed to conclusively prove that DDT residues increased in eggs with increasing time of exposure to DDT. These data suggest DDT passed through the hatching jars and was not taken up by the eggs. Observations of fry survival revealed no major differences in the life span of fry coming from eggs of control and experimental groups (Table 9).

Had the investigators been successful in getting the walleye eggs to absorb DDT these studies might have revealed residue levels at which pathology takes place. This knowledge might then have been useful in evaluating the significance of DDT residues found in walleye eggs and fry. It is interesting to note recent studies of the uptake of endrin by fertilized and unfertilized steelhead trout eggs reported by Kimura et al. at the University of Washington, Seattle (1967). Kimura showed that fertilized eggs exposed to endrin in constantly flowing stream water did take up endrin, but the amount taken up was so small that it seemed unlikely to affect the hatched fish. However, during hatching the concentration of endrin increased seventyfold in the yolk sac fry, indicating the fry can readily take up endrin.

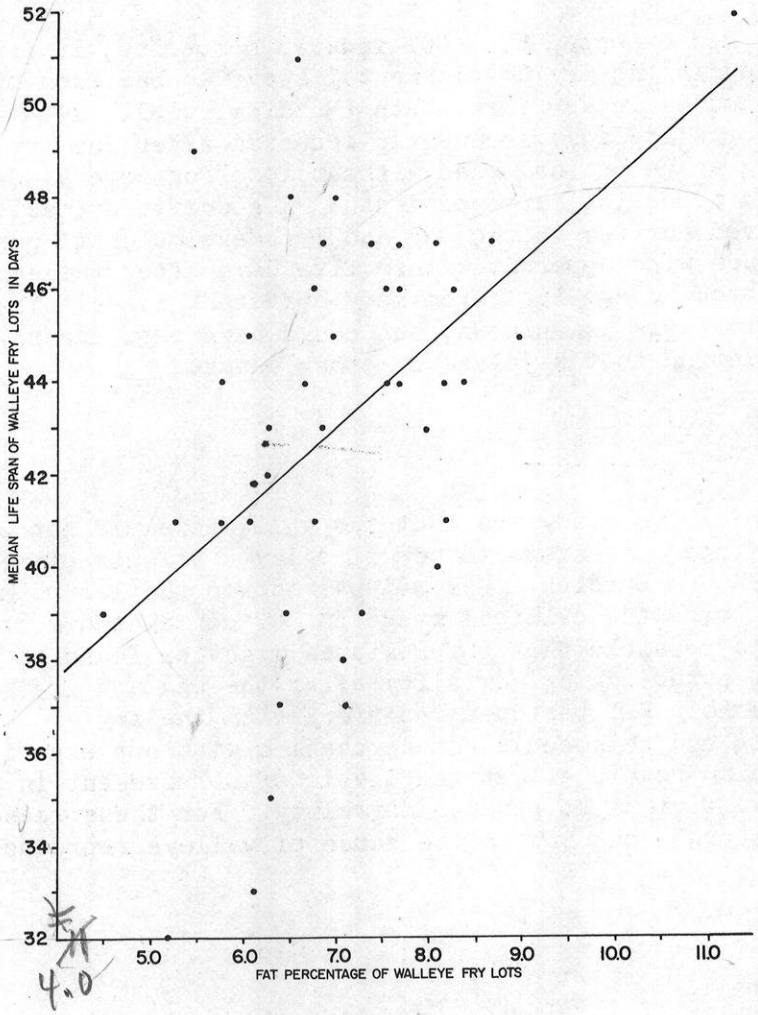


FIGURE 2.
Scatter Diagram and Regression Line for the Fat Percentage of Walleye Fry Lots Compared With the Median Life Span of the Fry Lots.

CONCLUSION

Summary of Findings

This study demonstrated eggs and fry of walleyes from ten different waters in Wisconsin contained DDT residues. The sum of DDT and its analogs in the egg samples ranged from .067 to 9.380 ppm. The sum of DDT and its analogs in fry samples ranged from .077 to 9.980 ppm. A strong positive correlation was demonstrated between the DDT content of the eggs and the fry. The magnitude of the DDT residues encountered, however, varied between the waters sampled. The sum of DDT and its analogs in the eggs and fry was greatest in samples from Pine Lake in Waukesha County and lowest in samples from Escanaba Lake in Vilas County.

The presence of DDT in the walleye eggs was not associated with the success of the hatch. Neither was the median life span of the fry associated with the DDT content of the eggs or the fry. The fat content of the fry, however, was positively correlated with the median life span of the fry; the fatter fry lived longer. Within the range of residue levels encountered, these studies failed to show a relationship between the DDT content and survival of walleye eggs and fry.

The investigators had expected that DDT-induced mortality, if present, would be expressed in the fry developmental stage as had been observed in lake trout studies in New York state (Burdick et al., 1964). In the present study mortality most frequently occurred after the fry had reached the feeding stage and had been without food for several days. Starvation was believed to be the cause of death. A specific mortality syndrome was not observed earlier in the egg and fry developmental periods. The greatest losses were apparent within five days after the eggs were taken and were common to egg lots from many different lakes. The cause of the death of the eggs is unknown, but could have been due to the failure of fertilization to take place or other causes.

Limitations

A major limitation of the study was that the walleyes could not be fed and held into the fingerling stage to see if delayed effects of residues might appear. Although Burdick el al. (1964) showed the last stages of yolk sac absorption to be the critical stage in DDT correlated mortality in lake trout, it is possible that DDT residues might be found to be associated with walleye pathology or mortality after the egg and fry stages are passed. Possibly DDT residues interfere with the fry's ability to begin feeding and this could not be checked with our experimental design. It is also possible that the levels of DDT present in the eggs and fry were not sufficient to produce mortality. For these reasons the present study cannot rule out DDT as the cause of walleye reproductive failures.

We would have liked to carry the study into the fingerling stage. Limitations of facilities, manpower, and the nature of the test animal itself, however, prohibited an extension of the study. Previous work at Delafield in 1966 had shown walleyes could not be raised in significant quantities in the laboratory. Olson and Scidmore (1964) observed that despite an abundance of natural food of adequate size, most newly hatched walleye fry would not feed when confined in tanks. Walleye fry can be reared in ponds, but multiple pond facilities sufficient to allow the separate stocking of over 50 lots of walleyes were not available in the spring of 1967.

Comparison of 1966 and 1967 Studies

Some lots of walleye eggs died just before hatching at the Delafield Hatchery in 1966. These lots of walleye eggs, from three southeastern Wisconsin lakes, contained from .362 to 3.32 ppm of DDT and its analogs. Eggs of similar DDT levels, taken from these same waters hatched normally at Westfield in 1967. These inconsistent data suggest factors other than the presence of DDT were responsible for the mortality of walleye eggs observed at Delafield in 1966.

The Westfield Hatchery studies were carried out under constant temperature and clean water conditions. The Delafield Hatchery studies conducted in 1966 were carried out utilizing a lake water source characterized by water of fluctuating temperature and frequently clouded by algae and silt. Factors of fluctuating water temperatures and poor water quality may have been associated with the mortality of walleye eggs observed at the Delafield Hatchery in 1966.

Discussion and Recommendations

Wherever pesticides are suspected to be related to fish reproductive failures, investigations should be undertaken. The documentation of poor walleye reproduction in certain Wisconsin lakes coupled with the discovery of significant levels of DDT in walleyes from these lakes cast suspicion upon DDT as a cause of walleye reproductive failures, prompting the present study. Similar situations in Wisconsin which suggest pesticides to be associated with fish reproductive failures may occur but are difficult to uncover without extensive fishery investigations.

Future studies designed to evaluate the occurrence of pesticide residues in eggs and fry with fish reproductive failures should follow the fish through all developmental stages including the fingerling stage, to uncover symptoms or mortality which may be expressed in later life stages. The present walleye study presents an impasse. We do not have the facilities or technology to repeat the walleye study holding all lots of walleyes into the fingerling stage. Until a reliable method is worked out, additional conventional attempts to rear walleye in aquaria may meet with failure. There is a possibility that walleye fry could be reared in large outdoor tanks, such as plastic swimming pools, but this method would also have to be perfected before it could be relied upon.

Perhaps the best means of studying the effects of pesticide residues on fish reproduction could be achieved by holding fish in ponds to establish various residue levels in the fish and their eggs and then evaluating reproduction. Such a study would require a large investment in facilities, time and manpower, but would provide information not obtainable by other methods. Modification of this approach which exposed cutthroat trout to different levels of DDT in bath and in food over a 20 month period involving one reproductive cycle was employed by Allison et al. (1964).

The failure of this study to induce walleye eggs to take up DDT from solution should not deter further investigations. New attempts should be made to artificially establish pesticide residues in fish eggs and fry through exposure to pesticide solutions. Bioassay of exposed eggs and fry could reveal the concentrations of pesticides required to cause pathology and mortality.

At this writing the Department of Natural Resources has no immediate plans for continuing investigations into the effects of DDT residues on fish reproduction. The Department is conducting a pesticide studies program to detect sources and indices of pesticide pollution in the Milwaukee River Watershed in southeastern Wisconsin, however. The Department is also monitoring pesticide residue levels in the state's fish and wildlife. DDT is still extensively used in Wisconsin, although newer pesticides are replacing DDT for certain uses. Without question, investigations into the ecological effects of various pollutants including pesticides upon fish populations will become increasingly important in the future, requiring similar investigations to be made. We hope this study has provided a frame of reference to guide similar investigations in the future.

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TABLE 1

DDT and Dieldrin Levels in Walleyes and the Status of Walleye Reproduction in Ten Waters Chosen for Studies of Walleye Spawning in 1967

Water	Acres	Month and Year Sampled	Number Fish in Sample	Pesticides in Whole Fish Samples (ppm) DDE	DDD	DDT	Total	Dieldrin	Status of Walleye Reproduction**
Escanaba	288	5/65	5	.042	.012	.037	.091	.003	Excellent
Escanaba	288	5/65	5	.039	.011	.032	.082	.002	Excellent
Muskellunge	266	5/65	1	1.25	0.21	0.88	2.34	.008	Poor
Muskellunge	266	5/65	1	.64	.094	.31	1.04	.016	Poor
Wolf*	--	5/65	5	.083	.042	.061	.186	.009	Good
Wolf*	--	5/65	5	.11	.055	.070	.235	.013	Good
Wolf*	--	5/65	5	.120	.106	.157	.383	--	Good
Green	7,325	--	-	--	--	--	--	--	Unknown
Pike	522	4/66	1	.24	.31	.39	.94	.017	Good
Pike	522	4/66	1	.16	.09	.16	.41	.014	Good
Pike	522	4/66	1	.15	.07	.18	.40	.009	Good
Pike	522	4/66	1	.21	.12	.19	.52	.013	Good
Pike	522	4/66	1	.20	.13	.23	.56	.009	Good
Pike	522	4/66	1	.21	.10	.21	.52	.016	Good
Pike	522	4/66	1	.22	.13	.24	.59	.015	Good
Pike	522	4/66	1	.17	.10	.20	.47	.013	Good
Nagawicka	917	10/66	3	.068	.034	.024	.126	.025	Poor
Nemahbin	283	8/66	4	1.14	.66	.83	2.63	T	Poor
Golden	250	4/66	1	.53	.34	.42	1.29	.002	Poor
Golden	250	4/66	1	.28	.13	.20	.61	.001	Poor
Golden	250	4/66	1	1.04	.53	.85	2.42	.021	Poor
Golden	250	4/66	1	.02	.04	.08	.14	T	Poor
LaBelle	1,117	4/66	1	3.02	1.25	1.21	5.48	.024	Poor
LaBelle	1,117	4/66	1	2.30	1.54	2.28	6.12	.017	Poor
LaBelle	1,117	4/66	1	2.65	1.74	3.12	7.51	.009	Poor
LaBelle	1,117	4/66	1	3.06	1.65	2.91	7.62	.008	Poor
LaBelle	1,117	6/66	6	2.6	1.82	2.24	6.66	T	Poor
LaBelle	1,117	6/66	17	2.1	.81	.47	3.38	T	Poor
Pine	703	6/66	2	5.00	4.35	2.14	11.49	--	Poor

* Samples were taken from Lake Winnebago. The Wolf River flows through Lake Winnebago. Many Winnebago walleyes migrate up the Wolf River to spawn.

** Status of walleye reproduction determined from Department of Natural Resources survey reports. Excellent and good reproduction indicates self-reproducing population with fish of several year classes represented. Poor reproduction indicates that one age group predominates in a small population and stocking may be the only source of adult fish.

TABLE 2

Water Analysis Data for Westfield
Hatchery Water, April 1967

Parameter	Concentration*
pH	7.6
Total alkalinity	156
Specific conductance	300
Dissolved oxygen	9.1
CL-	1.42
PO_4---(D)	0.006
PO_4---(T)	0.043
NO_3-	0.516
Na +	2.24
K +	0.88
Mg ++	21.70
Ca ++	22.50

* Units per ppm with an exception of specific conductance (micromhos at 20° C.) and pH.

TABLE 3

Survival of Walleye Eggs

Water	Date Eggs Taken	Lot Number	Female's Length In Inches	Egg Survival Estimates: Number Eggs Taken	Percent Viable Eggs	Number Eggs Reaching Eyed Stage	Percent Hatch	Percent Viable Fry	Days* of Life: 50% Fry Dead	Days* of Life: All Dead
Wolf	4/11	31	19.4	23,320	76.9	30,240	90.8	98.0	48	53
Wolf	4/11	32	21.6	27,930	57.7	10,440	37.4	98.0	32	34
Wolf	4/11	33	17.7	37,440	87.4	31,500	84.1	100.0	47	53
Wolf	4/11	34	20.2	39,240	81.4	32,595	83.1	99.6	45	51
Escanaba	4/26	4	23.0	41,870	66.1	31,320	74.3	97.6	47	49
Escanaba	4/26	5	24.4	88,245	70.9	73,530	83.3	98.2	35	42
Escanaba	4/26	6	23.7	76,035	84.0	--	--	97.6	49	53
Escanaba	4/26	7	23.8	59,055	84.4	30,940	52.4	99.5	48	55
Escanaba	4/26	8	24.0	45,375	79.8	29,500	65.0	99.0	43	44
Escanaba	4/26	9	24.4	53,340	45.2	35,520	66.6	99.8	48	51
Pike	4/11	20	18.3	31,040	90.1	25,575	82.4	97.9	50	53
Pike	4/11	21	20.4	17,205	84.1	13,230	76.9	98.1	40	47
Pike	4/11	22	18.4	31,860	79.4	28,500	89.4	98.9	38	40
Pike	4/11	23	20.1	32,480	87.9	27,495	84.6	99.6	41	45
Pike	4/11	24	16.2	35,030	74.0	26,160	74.7	96.5	48	54
Pike	4/11	25	17.5	15,820	93.9	13,440	85.0	98.6	34	40
Nagawicka	4/08	14	20.9	44,550	86.9	35,640	80.0	96.8	43	57
Nagawicka	4/08	15	21.0	22,560	80.5	18,400	81.6	98.5	46	54
Nagawicka	4/09	17	22.2	42,680	59.1	18,705	43.8	96.2	43	53
Nagawicka	4/09	18	19.2	15,035	77.0	11,845	78.8	97.9	46	56
Nagawicka	4/11	26	22.0	41,070	65.2	22,575	55.0	96.7	48	50
Nagawicka	4/11	27	23.1	76,720	78.4	69,930	91.1	98.3	46	50
Nagawicka	4/11	28	22.0	23,100	58.3	11,500	49.8	98.6	44	45
Nagawicka	4/11	29	20.5	48,880	47.8	11,475	23.5	99.4	49	54
Green	4/19	1	22.2	74,120	61.9	19,995	27.0	100.0	47	49
Green	4/19	2	24.7	15,260	43.1	6,930	45.4	86.9	55	62
Green	4/19	3	20.2	54,210	81.1	43,660	80.5	95.8	53	58
Nemahbin	4/11	37	24.5	110,450	81.4	93,720	84.8	97.8	49	52
Nemahbin	4/11	38	27.0	126,175	88.0	102,120	80.9	93.4	50	54
Nemahbin	4/11	39	22.2	58,300	72.5	54,390	93.3	94.8	37	48

TABLE 3 (cont.)

Survival of Walleye Eggs

Water	Date Eggs Taken	Lot Number	Female's Length In Inches	Egg Survival Estimates: Number Eggs Taken	Percent Viable Eggs	Number Eggs Reaching Eyed Stage	Percent Hatch	Percent Viable Fry	Days* of Life: 50% Fry Dead	Days* of Life: All Dead
Nemahbin	4/11	40	21.3	81,855	90.2	77,760	95.0	97.9	51	55
Muskellunge	5/01	50	28.3	110,970	20.1	5,760	5.2	95.9	--	--
Muskellunge	5/01	51	30.2	157,950	77.9	102,300	64.8	99.4	46	49
Muskellunge	5/01	52	30.8	131,325	71.2	85,000	64.7	99.4	49	51
Muskellunge	5/01	53	28.9	89,600	31.3	15,770	17.6	93.3	46	51
Muskellunge	5/01	54	27.7	103,170	85.3	70,555	68.4	99.2	45	48
Muskellunge	5/01	55	29.7	44,200	29.5	5,175	11.7	97.6	39	47
Golden	4/08	11	21.1	53,110	95.0	45,000	84.7	95.2	45	48
Golden	4/08	12	22.4	21,115	88.0	18,690	88.5	89.4	41	43
Golden	4/08	13	19.8	35,625	58.1	13,020	36.5	96.8	43	50
Golden	4/09	16	19.4	9,990	34.6	2,775	27.8	99.3	43	40
LaBelle	4/09	19	19.9	16,200	78.7	13,875	85.6	92.2	39	45
LaBelle	4/11	35	15.2	7,620	90.0	8,190	100.0	96.3	41	53
LaBelle	4/11	36	19.3	24,990	78.1	21,000	84.0	91.7	46	49
LaBelle	4/12	43	15.0	14,040	87.0	9,450	67.3	97.3	46	49
LaBelle	4/12	44	16.1	4,960	84.5	3,570	72.0	97.5	43	45
LaBelle	4/13	46	16.9	21,645	95.3	19,040	88.0	96.0	39	41
Pine	4/12	41	26.0	178,155	59.7	142,800	80.1	99.4	43	44
Pine	4/12	42	22.5	64,800	67.9	32,760	50.6	94.1	42	43
Pine	4/13	45	21.6	70,625	57.5	43,875	62.1	97.8	54	56
Pine	4/15	47	22.1	39,550	48.4	20,855	52.7	99.6	49	53
Pine	4/15	48	22.8	21,630	78.2	17,325	80.1	100.0	48	52
Pine	4/15	49	23.0	31,720	87.9	29,500	93.0	97.8	46	48
TOTAL			1,164.8	2,743,240	3,818.8	1,754,915	3,526.4	5,151.10	2,333	2,566
AVERAGE			21.9	51,759	72.1	33,748	67.8	97.2	44	49

* Days of life includes the number of days passed from the time the eggs were fertilized and covers both the egg and fry developmental periods.

TABLE 4

Fat Levels and DDT Residues in Walleye Eggs and Fry

Water	Lot Number	Egg Content					Fry Content				
		Pesticide (ppm)				Percent Fat	Pesticide (ppm)				Percent Fat
		DDE	DDD	DDT	Total		DDE	DDD	DDT	Total	
Wolf	31	.067	.060	.838	.965	4.3	.062	.026	.038	.126	7.6
Wolf	32	.050	.022	.040	.112	3.8	.088	.051	.049	.188	--
Wolf	33	.060	.041	.072	.173	4.6	.080	.039	.048	.167	6.1
Wolf	34	.028	.014	.025	.067	4.9	.039	.017	.021	.077	8.0
Escanaba	4	.097	.020	.037	.154	1.2	.152	.032	.028	.212	7.0
Escanaba	5	.049	.017	.040	.106	2.9	.094	.031	.043	.168	6.1
Escanaba	6	.077	.019	.037	.133	2.6	.073	.021	.030	.124	8.1
Escanaba	7	.083	.018	.037	.138	2.8	.091	.021	.044	.156	8.3
Escanaba	8	.126	.025	.080	.231	2.3	.135	.029	.075	.239	5.8
Escanaba	9	--	--	--	--	2.6	.094	.023	.057	.174	6.8
Pike	20	.105	.028	.063	.196	--	.136	.075	.064	.275	7.0
Pike	21	.066	.025	.069	.160	--	.161	.096	.100	.357	7.1
Pike	22	.067	.026	.038	.131	--	.207	.079	.106	.392	--
Pike	23	.112	.066	.132	.310	4.2	.150	.075	.063	.288	6.5
Pike	24	.085	.046	.134	.265	3.5	--	--	--	--	--
Pike	25	.123	.069	.104	.296	3.6	.114	.056	.043	.213	5.2
Nagawicka	14	.111	.038	.067	.216	3.9	.241	.089	.169	.499	6.1
Nagawicka	15	.087	.022	.048	.157	4.2	.359	.142	.251	.752	9.2
Nagawicka	17	.117	.031	.055	.203	--	.235	.072	.064	.371	5.3
Nagawicka	18	.105	.025	.045	.175	--	.304	.093	.121	.518	6.7
Nagawicka	26	.296	.097	.270	.663	3.7	.264	.084	.083	.431	7.7
Nagawicka	27	.110	.064	.124	.298	4.1	.420	.132	.261	.813	5.8
Nagawicka	28	.284	.070	.165	.519	4.1	.324	.107	.105	.536	6.3
Nagawicka	29	.140	.029	.055	.224	4.4	.342	.110	.136	.588	7.4
Green	1	.110	.038	.087	.235	2.8	.222	.083	.075	.380	9.0
Green	2	.600	.125	.202	.927	.7	.983	.253	.194	1.430	--
Green	3	.101	.032	.056	.189	3.4	.354	.099	.077	.530	6.6
Nemahbin	37	.271	.122	.211	.604	3.8	.399	.168	.304	.871	7.7
Nemahbin	38	.293	.133	.215	.641	5.3	.349	.147	.261	.757	6.5
Nemahbin	39	.364	.126	.216	.706	3.7	.340	.282	.271	.893	6.3
Nemahbin	40	.264	.145	.268	.677	--	.487	.161	.201	.849	5.5
Muskellunge	50	.499	.179	.268	.946	2.6	.642	.149	.439	1.230	--
Muskellunge	51	.444	.166	.260	.870	1.7	.665	.253	.046	.964	5.8
Muskellunge	52	.510	.163	.185	.858	2.9	.775	.293	.029	1.097	8.7

TABLE 4 (cont.)

Fat Levels and DDT Residues in Walleye Eggs and Fry

Water	Lot Number	Egg Content					Fry Content				
		Pesticide (ppm)				Percent Fat	Pesticide (ppm)				Percent Fat
		DDE	DDD	DDT	Total		DDE	DDD	DDT	Total	
Muskellunge	53	.554	.194	.575	1.323	3.4	.651	.277	.032	.960	--
Muskellunge	54	.562	.202	.509	1.273	2.2	.800	.275	.076	1.151	6.3
Muskellunge	55	.341	.100	.147	.588	3.1	.433	.119	.066	.618	--
Golden	11	--	--	--	--	1.5	1.37	.302	.571	2.243	6.9
Golden	12	.409	.114	.270	.793	3.3	.695	.285	.368	1.348	4.5
Golden	13	.520	.196	.319	1.035	3.0	.717	.276	.455	1.448	8.2
Golden	16	.544	.162	.394	1.100	4.8	1.09	.463	.968	2.521	--
LaBelle	19	1.00	.30	.58	1.88	--	2.28	1.39	1.90	5.570	6.4
LaBelle	35	1.82	.851	.814	3.485	2.9	1.37	.805	.789	2.964	7.3
LaBelle	36	1.19	.974	1.50	3.664	--	2.21	1.51	2.62	6.340	7.6
LaBelle	43	2.24	1.20	1.90	5.340	4.8	3.82	2.67	3.12	9.610	8.4
LaBelle	44	.818	.588	.634	2.040	4.4	2.31	1.27	1.33	4.910	--
LaBelle	46	1.36	1.01	1.15	3.520	5.8	2.95	1.96	2.37	7.280	7.1
Pine	41	2.01	2.19	4.30	8.500	4.1	5.20	2.95	1.83	9.980	6.8
Pine	42	3.33	2.30	3.75	9.380	3.3	2.96	2.03	1.45	6.440	8.1
Pine	45	.60	.17	.31	1.08	4.3	1.51	1.43	.518	3.458	11.3
Pine	47	.618	.678	.151	1.447	3.6	1.04	.978	.477	2.495	6.9
Pine	48	.960	.858	.596	2.414	1.6	2.30	.161	.089	2.550	--
Pine	49	1.03	1.02	.847	2.897	5.0	1.82	1.51	2.71	6.040	7.7
TOTAL		25.807	15.208	23.289	64.304	155.7	44.907	24.049	25.635	94.591	303.7
AVERAGE		.506	.298	.457	1.261	3.5	.864	.462	.493	1.819	7.1

TABLE 5

Correlation Coefficient Values for Pesticide Residues Expressed as ppm in Walleye Eggs and the Resulting Fry

Pesticide Content of Fry	Pesticide Content of Eggs		
	DDT	DDD & DDT	DDE, DDD & DDT
DDT	.58	--	--
	48 df	--	--
	$P \leq .001$	--	--
DDD & DDT	--	.76	--
	--	48 df	--
	--	$P \leq .001$	--
DDE, DDD & DDT	--	--	.51
	--	--	48 df
	--	--	$P \leq .001$

df = degrees of freedom
$P \leq$ indicates a probability of occurrence less than the figure given
$P \geq$ indicates a probability of occurrence greater than the figure given

TABLE 6

Correlation Coefficient Values for Fat Percentages of Walleye Eggs and Fry Compared with Egg and Fry Survival Data

Survival Data	Percent Fat	
	Eggs	Fry
Percent hatch of eggs	.14	--
	43 df	--
	$P \geq .1$	--
*Median life span of fry in days	-.03	.72
	42 df	42 df
	$P \geq .1$	$P \leq .001$

df = degrees of freedom

$P \leq$ indicates a probability of occurrence less than the figure given

$P \geq$ indicates a probability of occurrence greater than the figure given

* The median life span of the fry includes the total number of days passed from the time the eggs were fertilized.

TABLE 7

Correlation Coefficient Values for Pesticide Residues Expressed as ppm in Walleye Eggs and the Resulting Fry Compared With Egg and Fry Survival Data

Survival Data	Pesticide Content					
	DDT		DDD & DDT		DDE, DDD & DDT	
	Eggs	Fry	Eggs	Fry	Eggs	Fry
Percent hatch of eggs	.06	--	.01	--	.16	--
	48 df	--	48 df	--	48 df	--
	$P \geq .1$	--	$P \geq .1$	--	$P \geq .1$	--
*Median life span of fry in days	-.06	-.09	.03	-.06	.06	-.04
	48 df	49 df	48 df	49 df	48 df	49 df
	$P \geq .1$	$P \geq .1$	$P \geq .1$	$P \geq .1$	$P \geq .1$	$P \geq .1$

df = degrees of freedom

$P \geq$ indicates a probability of occurrence greater than the figure given

* The median life span of the fry includes the total number of days passed from the time the eggs were fertilized.

TABLE 8

DDT Levels in Walleye Eggs and Fry Following Exposure of the Eggs to Various Concentrations of DDT

EGGS

Hours Eggs Exposed to DDT	DDT Flowing Through Hatching Jars (ppm)												Control Hatching Jars											
	.1				.01				.001				Acetone & Water				Water Only-1				Water Only-2			
	DDE	DDD	DDT	Total	DDE	DDD	DDT	Total	DDE	DDD	DDT	Total	DDE	DDD	DDT	Total	DDE	DDD	DDT	Total	DDE	DDD	DDT	Total
0	.353	.130	.292	.775	.222	.078	.219	.519	.150	.069	.147	.366	.174	.066	.182	.422	.222	.086	.198	.506	.271	.120	.117	.508
.5	.204	.084	.195	.483	.212	.106	.192	.510	.206	.083	.248	.537	.304	.129	.353	.786	.270	.097	.205	.572	.291	.165	.340	.796
3.0	.305	.164	.270	.739	.238	.106	.062	.406	.215	.092	.143	.450	.269	.127	.197	.593	.371	.192	.336	.894	.347	.140	.271	.758
9.0	.400	.226	.476	1.102	.359	.166	.383	.908	.336	.160	.113	.609	.254	.138	.208	.600	.356	.160	.178	.694	.221	.124	.122	.467
27.0	.252	.206	.390	.848	.262	.124	.201	.587	.258	.117	.303	.678	.218	.107	.142	.467	.289	.122	.258	.669	.315	.126	.300	.741
72.0	.365	.504	1.700	2.569	.224	.164	.456	.844	.264	.144	.428	.836	.268	.113	.284	.665	.260	.120	.268	.648	.248	.106	.269	.623

FRY

0	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
.5	.355	.127	.349	.831	.727	.266	.617	1.610	.520	.208	.515	1.243	.848	.394	.824	2.006	.360	.110	.283	.753	.162	.078	.142	.382
3.0	.428	.192	.404	1.024	.504	.229	.500	1.233	.556	.250	.524	1.330	.532	.190	.480	1.202	.486	.212	.462	1.160	.438	.182	.391	1.011
9.0	.263	.120	.324	.707	.272	.115	.328	.715	.464	.172	.460	1.096	.448	.183	.192	.823	.412	.175	.336	.923	.272	.086	.213	.571
27.0	.528	.460	.520	1.508	.505	.245	.550	1.300	.472	.201	.464	1.137	.520	.217	.310	1.047	.267	.189	.184	.640	.630	.223	.624	1.477
72.0	.645	.450	.221	1.316	.344	.262	.046	.652	.412	.220	.035	.667	.440	.153	.338	.931	.497	.137	.302	.936	.440	.124	.259	.823

TABLE 9

Survival of Fry From Egg Lots Exposed to DDT Solutions

Egg Exposure Medium	Hours of Egg Exposure	Percent Viable Fry	Days of Life*	
			50 Percent Fry Dead	All Fry Dead
0.1 ppm DDT in acetone and water	0	--	--	--
	1/2	97.4	53	56
	3	99.1	54	56
	9	99.5	53	56
	27	98.7	51	52
	72	92.5	51	54
0.01 ppm DDT in acetone and water	0	--	--	--
	1/2	97.0	54	56
	3	97.9	46	56
	9	96.3	49	52
	27	97.5	45	54
	72	97.3	50	54
0.001 ppm DDT in acetone and water	0	--	--	--
	1/2	98.9	53	56
	3	97.6	52	55
	9	97.8	52	55
	27	99.4	52	56
	72	97.9	48	54
Acetone and water	0	--	--	--
	1/2	98.9	54	56
	3	96.5	51	55
	9	99.1	50	56
	27	99.2	53	56
	72	97.5	48	54
Water only	0	--	--	--
	1/2	95.6	54	55
	3	98.9	54	56
	9	97.2	54	56
	27	99.1	51	56
	72	98.8	47	54
Water only	0	--	--	--
	1/2	98.1	53	56
	3	99.6	50	55
	9	98.8	52	56
	27	99.6	53	56
	72	97.8	28	53

* Days of life includes the number of days passed since the eggs were fertilized.