PESTICIDE RESIDUES IN COHO SALMON EGGS AND THEIR RELATIONSHIP TO EGG AND FRY MORTALITY

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
CHARLES HENRY PECOR
1972

DCN 7 72-564

202

DEC 0 5 1999

DEC 0 5 1999

ABSTRACT

PESTICIDE RESIDUES IN COHO SALMON EGGS
AND THEIR RELATIONSHIP TO EGG AND
FRY MORTALITY

By

Charles Henry Pecor

During the fall and winter of 1968-69, the pesticide residues in eggs of coho salmon and the mortality of eggs and fry were investigated. Fertilized egg samples were collected from 104 individual female salmon from four Lake Michigan streams, two Lake Superior streams and one Oregon stream. An additional 96 egg samples were obtained for pesticide analysis only.

Four major pesticide residues were identified and quantified in the salmon eggs: p,p'-DDT, p,p'-DDD, p,p'-DDE and dieldrin. The total concentration of these four pesticide residues in Lake Michigan eggs was approximately 6 times higher than those in Lake Superior eggs and approximately 55 times higher than in the eggs from Oregon.

Among the three systems, the mortality of fry in Lake Michigan groups was higher than in Lake Superior and Oregon groups. The Lake Michigan fry mortality was

charact

and pro

The sym

final s

Superio

did not

eight

correl

the eg

group.

among

sample

pesti

of fa

corre

fry m

evide

sugge

May b

characterized by loss of equilibrium, erratic swimming and prolonged convulsions in response to a disturbance. The symptoms and mortality appeared abruptly during the final stage of yolk-sac absorption. Mortality among Lake Superior and Oregon fry occurred at an earlier age and did not show these symptoms.

Statistical analysis of the mortality data on the eight fry groups within Lake Michigan did not show a correlation between pesticide residue concentration in the eggs and mortality of fry with the exception of one group. However, pesticide residue content and mortality among Lake Michigan fry were both significantly lower in samples collected later in the spawning run.

No statistical relationship was found between pesticide residue concentration in the eggs and amount of fat in the eggs, parent fish length or egg mortality.

Although the data did not show a statistical correlation between pesticide residue concentrations and fry mortality, it did show substantial circumstantial evidence to support the relationship and the results also suggested that other toxic materials or unknown factors may be involved in the fry mortality.

PESTICIDE RESIDUES IN COHO SALMON EGGS AND THEIR RELATIONSHIP TO EGG AND FRY MORTALITY

By

Charles Henry Pecor

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1972

Dr. H.
study.
especi
tribut

visors
Natura
for th
eggs f

vided
ation
The M
and De
WP-10

ACKNOWLEDGMENTS

I would like to express my special thanks to Dr. H. Johnson for his advice and guidance during this study. I am also grateful to the graduate students, especially Jerry Hamelink and Ronald Waybrant, who contributed to this study by discussions and suggestions.

Recognition and thanks is given to the supervisors and personnel of the Michigan Department of Natural Resource's hatcheries and egg-taking stations for their efforts and assistance in the collecting of eggs from coho salmon.

The financial support for this study was provided by: The William A. Angell Foundation in cooperation with the Sport Fisheries Research Foundation, The Michigan Department of Natural Resources, Research and Development Division and FWPCA Training Grant - 5T1-WP-109.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

General

Effects of pesticides on reproduction in fish through chronic exposure to sublethal concentrations have been shown to occur in nature. Burdick et al. (1964) concluded the mortality of the lake trout fry (Salvelinus namaycush) in hatcheries from several New York State lakes occurred when the ether extract of eggs contained 2.9 ppm or more DDT based on the wet weight of the fry. Cuerrier et al. (1967) found that when levels of DDT and metabolites exceeded 400 ppb in trout eggs, fry mortality ranged from 30 to 90 percent in the 60-day period following the swim-up stage. Kleinert (1967) indicated a possible association between DDT levels in the eggs and the mortality of walleye (Stizostedion vitreum) eggs from several Wisconsin lakes, and Johnson and Pecor (1969) reported DDT residues were a possible cause of high mortalities of coho salmon fry in Michigan hatcheries during 1968. Anderson and Everhart (1966) also reported high DDT residues in landlocked salmon (Salmo salar) in Lake Sebago, Maine and a failure of recruitment, but did not observe an abnormal mortality of hatchery reared fry.

of pest

in long

found t

where t

Macek (

doses o

eight w

abnorma

embryos

or grea

(vivipo

cides

in con

ductio

found

many w

Boyd (

cause

critic

Mortal

1964;

Macek,

temper

Several laboratory studies have documented effects of pesticides on fish reproduction. Allison et al. (1964) in long-term tests with cutthroat trout (Salmo clarki) found the mortality of developing fry increased in lots where the females were exposed to higher doses of DDT. Macek (1968) reported fry from brook trout fed higher doses of DDT suffered a greater mortality during the eight weeks following hatching. Johnson (1967) found abnormalities developed in medaka (Oryzias latipes) embryos when the females were exposed to 0.03 ppb endrin or greater.

Other laboratory studies, with live bearers (viviporous species), have also shown effects of pesticides on reproduction. Mount (1962) stated that endrin in concentrations as low as 0.5 ppb curtailed reproduction in guppies (Lebistes reticulata). King (1962) found DDT did not prevent reproduction in guppies but many were born dead or died within several hours. Boyd (1964) also found different insecticides may cause mosquitofish (Gambusia affinis) to abort.

A number of workers have reported there is a critical period during the development of the fry when mortality occurs (Allison et al., 1962; Burdick et al., 1964; Currier et al., 1967; Johnson, 1967; Kleinert, 1967; Macek, 1968). The critical stage varies with species, temperature and possibly other parameters not yet

determined but the mechanism is assumed to be the same. It has been hypothesized that DDT and other persistent chlorinated insecticides, because of their high solubility in lipids, are concentrated in the conspicuous oil globules of fish eggs. It is further hypothesized that organochlorine pesticides are released from the yolk to the fry during the last stages of development. Smith (1957) reported that the triglyceride lipids are metabolized at this stage of development. It is during a similar critical period or stage of development that the coho fry from Lake Michigan experienced a high mortality (Johnson and Pecor, 1969).

History of Michigan Salmon

In the fall of 1964 the Michigan Department of Natural Resources received its first shipment of eyed coho salmon (Oncorhynchus kisutch) eggs collected from the Columbia River at the Bonneville Dam, Oregon. The fry were reared in Michigan hatcheries until the spring of 1966 when 650,000 were released in two streams (Platte River, Benzie Co. and Bear Creek, Manistee Co.) in the Lake Michigan drainage and 200,000 in the Big Huron River (Baraga Co.) in the Lake Superior drainage. In the spring of 1967 2.2 million smolts reared from coho eggs collected at the Cascade River, Oregon and the Toutle River, Washington, were released in four Lake Michigan tributaries (Thompson Creek, Schoolcraft

Co.; P

Creek,

(Big H

spawni

imatel

the pl

taking

Michig

19 Lak

4 Lake

eight

second

Michig

Juveni

are ap

in le

Michig

two to

Pestic

the Pe

large

resid

Great

Co.; Platte River, Benzie Co.; Little Manistee and Bear Creek, Manistee Co.) and one Lake Superior tributary (Big Huron River, Baraga Co.).

The fall of 1967 produced the first successful spawning run of mature coho salmon in Michigan. Approximately eight million fertilized eggs were collected at the Platte River, Bear Creek and Big Huron River egg-taking stations and distributed to hatcheries throughout Michigan. In 1968, 1.95 million smolts were released in 19 Lake Michigan streams, 8 Lake Superior streams and 4 Lake Huron streams. In the fall of 1968 approximately eight million fertilized eggs were collected from the second mature run of coho salmon and distributed to Michigan hatcheries.

The coho salmon has a three-year life cycle. Juvenile coho salmon are released as smolts when they are approximately 18 months old and four to six inches in length. Mature adult salmon after 18 months in Lake Michigan ranged in size from seven to eleven pounds and two to four pounds in Lake Superior.

In 1969 attention was focused on the existing pesticide residue levels in Lake Michigan fishes when the Federal Food and Drug Administration confiscated a large shipment of coho salmon because of high total DDT residues. Recent studies in selected areas of the Great Lakes have shown the presence of pesticide

resid

sampl

Pollu

Hughe

resid

fishe

two t

lakes

have

3.5 t

(John

Reine

chlor

orga

the

Reyn

iden

fere

nate

with

resi

demo

with

show

from

residues in all vertebrate, invertebrate and sediment samples tested (Hickey et al., 1966; Federal Water Pollution Control Administration, 1968; Brown and Hughes, 1969). Carr and Reinert (1968) found DDT residues in the flesh of all species of Great Lakes fishes with the residue levels in Lake Michigan fish two to four times higher than those from the other lakes. The eggs of coho salmon from Lake Michigan have total p,p'-DDT, DDD and DDE residues ranging from 3.5 to 7.3 ppm and p,p'-DDT residues of 1.0 to 2.5 ppm (Johnson and Pecor, 1969; Carr and Reinert, 1968; Reinert, 1969).

The recent discovery of the presence of polychlorinated biphenyls (PCB's) in tissues of aquatic organisms and wildlife from various locations around the world (Koeman et al., 1969; Holmes et al., 1967; Reynolds, 1969) has led to some confusion regarding the identification of pesticide residues. PCB's may interfere with gas-liquid chromatographic analysis of chlorinated hydrocarbon pesticides by producing residue peaks with retention times exactly the same as pesticide residue peaks. Lichtenstein et al. (1969) has also demonstrated the potential toxic interaction of PCB's with DDT and dieldrin in insects. Veith (1970) has shown that chlorophenyl compounds are present in fish from the Milwaukee River and Lake Michigan with

concentrations as high as 405 µg/gm body weight. Studies are currently underway at Michigan State University, Pesticide Research Center, to develop methods of analyzing for the compounds and to evaluate the effects of these compounds in the environment.

In 1967 the Michigan Department of Natural Resources reported an abnormally high mortality of coho salmon fry in their hatcheries. The mortality was restricted to fry from Lake Michigan sources. Similar mortalities were reported in other states where Lake Michigan salmon eggs were reared. Total losses in Michigan during this period accounted for 680,000 fry or approximately eleven percent of the original number of eggs collected (Michigan Department of Natural Resources, 1968).

The fry mortality occurred one to four weeks after absorption of the yolk-sac, depending upon the rearing temperature (Michigan Department of Natural Resources, 1968). The mortality commenced during the fifth week after hatching, increased to peak numbers during the sixth and seventh weeks and decreased by the end of the eighth week. In each case the mortalities followed the same pattern relative to the development stage of the fry. The mortalities occurred during the period when the fry were undergoing a transition from dependence on yolk nutrition to hatchery diet.

The fry mortalities were characterized by erratic swimming, loss of equilibrium, hypersensitivity and cessation of feeding. Many of the affected fish turned dark but this was not considered to be a specific symptom because many light-colored fish were also affected. Death usually followed one ro five days after the onset of symptoms. The affected fry gradually weakened, sank to the bottom, many in peculiar flexed positions, and died. There were no external or internal lesions observed although some did show degeneration of liver and kidney tissues but with no apparent correlation with fry mortality.

Fry reared from Oregon and Lake Superior eggs did not suffer unusual mortalities and no evidence of symptoms were observed in these groups even when they were reared in the same hatcheries with Lake Michigan fry.

Samples of affected and non-affected fry were examined by pathologists at the U.S. Fish and Wildlife Service, Eastern Fish Disease Laboratory in Leetown, West Virginia. No evidence of an infectious disease was found in the samples examined (Dr. Kenneth Wolf, personal communication). Additional tests by fish pathologists of the Michigan Department of Natural Resources failed to show any specific pathogen associated with the mortality. The absence of any apparent

bact

inse

fry

was

the

firs

pos

spr

stu

egg

res

reg

stu

Jul

bacterial or viral diseases led to speculation that insecticide contamination was a possible cause of the fry mortality.

Objectives

During the fall and winter of 1967-68 a study was undertaken at Michigan State University to examine the pesticide residues accumulated in the eggs of the first mature run of coho salmon in Michigan and the possible effects these residues might have on the offspring (Johnson and Pecor, 1969). Results from this study showed the presence of pesticide residues in all egg samples and a possible correlation between pesticide residues and the mortality of the fry. This study was regarded as preliminary and exploratory. The present study was initiated in June 1968 and continued through July 1969. The major objectives of this research were:

(1) Determine the identity and concentration of specific pesticide residues in coho salmon eggs from Lake Michigan, Lake Superior and Oregon stocks;

(2) Determine the relationship of pesticide residues in the eggs of individual coho salmon to:

(a) home stream or location sampled

(b) size and age of parent fish

(c) sampling date

(d) mortality of eggs and fry

MATERIALS AND METHODS

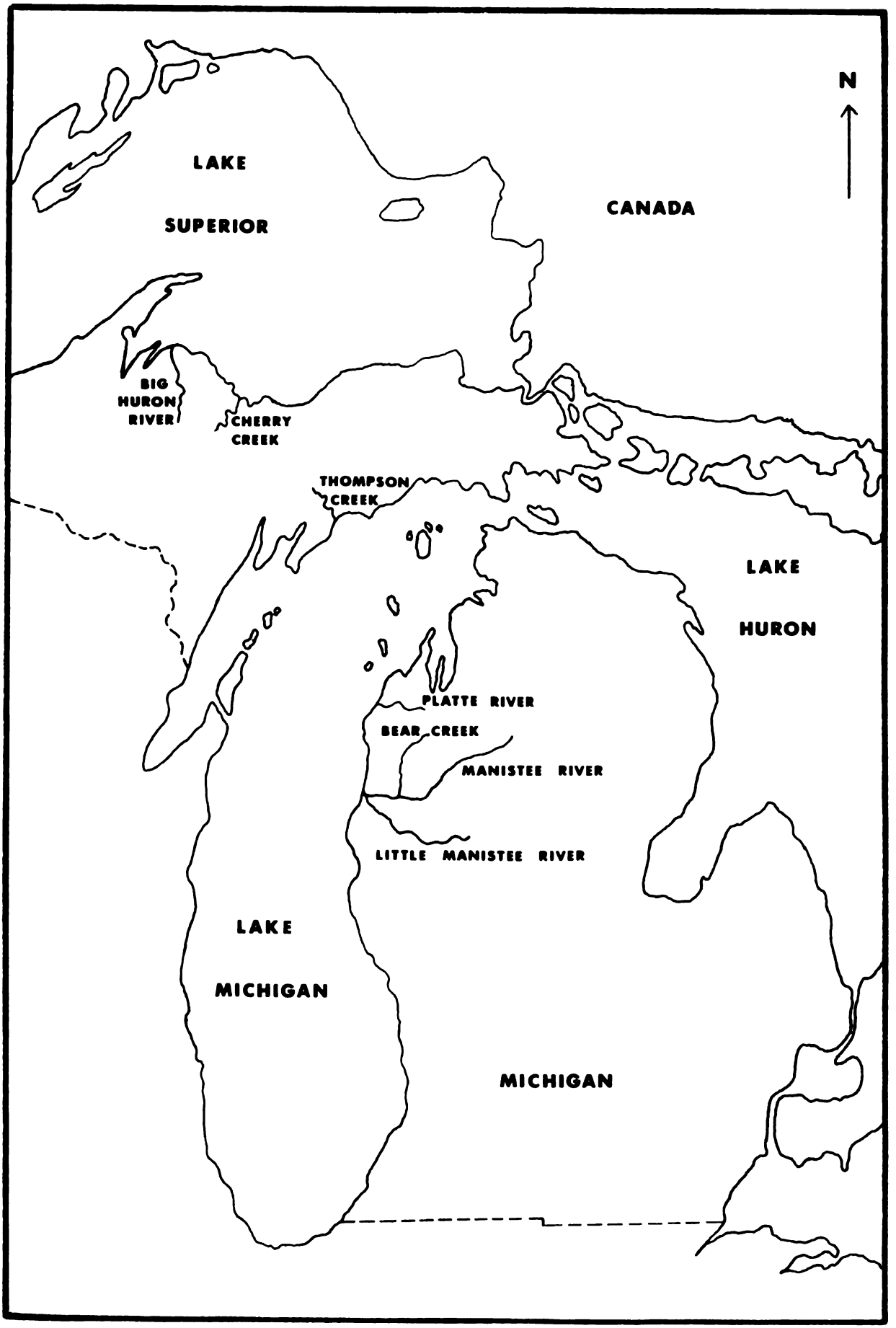
Field Collections

Sampling locations and schedules

This study was based on egg samples collected from coho salmon in Michigan during the fall and winter of 1968-69. Samples of coho salmon eggs were obtained from all major streams tributary to Lake Michigan and Lake Superior in which mature spawning runs were expected. This included the Platte River, Bear Creek, Little Manistee River and Thompson Creek in the Lake Michigan watershed; and the Big Huron River and Cherry Creek in the Lake Superior watershed (Figure 1). A shipment of coho salmon eggs from the state of Oregon was received by the Michigan Department of Natural Resources to supplement Michigan plants of salmon. Samples of these eggs were obtained from the Oden State Hatchery at Oden, Michigan to serve as controls for this study.

Egg samples were collected at hatchery or egg-taking stations on each of the streams in conjunction with routine spawning operations by state hatchery personnel. With the exception of the eggs from the

Figure 1. A map of Michigan showing the location of all the streams sampled during the 1968 spawning migration of coho salmon in Michigan.


N
LAKE
SUPERIOR
CANADA
BIG
HURON
RIVER
CHERRY
CREEK
THOMPSON
CREEK
LAKE
HURON
PLATTE RIVER
BEAR CREEK
MANISTEE RIVER
LITTLE MANISTEE RIVER
LAKE
MICHIGAN
MICHIGAN

Big
fer
of
sub
500
Sam
add
Hur
sam
lar
sub
fro
wer

the
Hor
su
beg
the
wer

Riv
sam
co
to

Big Huron River and Oregon, all the samples consisted of fertilized eggs from individual females for comparison of hatching success and fry survival with corresponding subsamples taken for pesticide analysis. Approximately 500 fertilized eggs were obtained from each female. Samples of unfertilized eggs were also collected from additional females for pesticide analysis. The Big Huron River and Oregon samples consisted of single samples of approximately 2,000 eyed eggs each from a large number of individual females (Table 1). Four subsamples of approximately 50 eyed eggs were collected from each for pesticide analysis and the remaining eggs were reared in the laboratory.

An attempt was made to sample each stream during the early, peak and late periods of the spawning runs. However, the unpredictable timings of the runs prevented such a sampling schedule. Samples were obtained at the beginning of the runs and again every 3 to 5 weeks until the spawning migrations were complete. The dates samples were collected are shown in Table 1.

Additional samples were collected from the Platte River for pesticide analysis only. Unfertilized egg samples were obtained weekly for the duration of the coho salmon run in that river, extending from September 19 to November 14, 1968 (Table 1).

LAKE MICHIGAN Thompson Creek		
Date sampled	9-17-68	10-13
Samples Collected	*	*

TABLE 1. Sampling dates and locations with the number and type of samples collected during the 1968 run of coho salmon.

LAKE MICHIGAN										
Thompson Creek										
Date sampled	9-17-68	10-13								
Samples collected	10*,15	10*,11								
Little Manistee R.										
Date sampled	9-7-68	9-20	10-24	11-7	12-13					
Samples collected	13	6	3*,7	9*,9	10*,10					
Bear Creek										
Date sampled	10-3-68	10-24	12-13							
Samples collected	12	10*,12	10*,10							
Platte River										
Date sampled	9-19-68	9-25	10-4	10-10	10-17	10-24	11-5	11-12	11-14	1-4-69
Samples collected	6**	6**	7**	5**	10*,10	6**	8**	8**	10*,10	10*,10
LAKE SUPERIOR										
Cherry Creek and Big Huron River										
Date sampled	11-1-68	1-20-69								
Samples collected	10*,10	composite sample*								
OREGON										
Oden Hatchery										
Date sampled	1-3-69									
Samples collected	composite sample*									

*Fertilized samples (Samples not asterisked were for pesticide analysis only)

**Samples collected by Michigan Department of Natural Resources

Sampling procedures

Eggs from individual "ripe" females were gently stripped into a porcelain pan. A subsample of eggs for pesticide analysis was taken at this time. The remaining eggs were fertilized with milt from two to three males, rinsed several times and placed into two-quart glass jars filled with river water. The jars were then packed in insulated boxes for transit to the laboratory. In each case, samples from individual females were maintained separately.

Eggs fertilized in the field from streams in the lower peninsula of Michigan were placed in the incubator within 6 hours after fertilization and those from the upper peninsula were placed in the incubator approximately 12 hours after fertilization.

The samples for pesticide analysis consisted of 100 to 150 unfertilized eggs from each female. The sample was procured from the posterior end of one of the two ovaries if the eggs were "green" (eggs attached to ovary) or by a random sample of both ovaries if the eggs were "ripe" (eggs lying free in body cavity). The eggs were placed in sealed polyethylene bags with caution to avoid water and ovarian fluid, and immediately frozen over dry ice. Samples were maintained in frozen condition until analysis.

The data recorded for each female included its fork length and its total weight. A scale sample from each female was also obtained from the region between the anterior edge of the dorsal fin and the lateral line. The age of each female fish samples was determined from the seasonal annuli formation of the scales (Rounsefell and Everhart, 1953). Four or five scales from each fish were washed and mounted between two microscope slides. The scales were then projected using a micro-projector and a 43X objective.

Hatchery Procedures

Incubation of eggs and early fry

The samples of fertilized eggs collected in the field were reared in the laboratory at Michigan State University in a 16-tray salmon egg incubator. Each tray was subdivided into four equal compartments with a fiberglass screen material. Thus the incubator had a capacity to contain egg samples from 64 individual females.

The incubator, supported over an insulated 715-liter capacity reservoir, comprised a semi-closed recycling water system. Two submersible pumps delivered water to the incubators at approximately 7.6 liters/minute. The water entered the top of the incubators traversed down through all the trays and emptied back into the

reservoir. The recycled water in the reservoir was continuously recharged with fresh water at a rate of approximately 26.5 liter/hour.

The water supplied to the incubator system was treated East Lansing municipal well water which was passed through an activated charcoal filter to remove residual chlorine and sediments. The water in the incubator system was maintained at 10.2 $\pm$ 0.1°C by a portable refrigeration unit.

Fertilized egg samples received at the laboratory were acclimated to the 10.2°C water temperature of the incubator at a maximum rate of 2.0°C/half-hour. After acclimatization to the water temperature, individual samples were gently poured into separate compartments in the incubators. The number of eggs in the samples usually covered the bottom of the compartments with one layer of eggs.

The incubator was in continuous operation from early September 1968 to early April 1969 when the last of the sac-fry were transferred to rearing tanks. The dissolved oxygen content of the water for this period ranged from 8.8 to 10.8 ppm as determined by weekly oxygen analysis. There was no measurable difference between the dissolved oxygen content of the water entering at the top of the incubator and the water leaving the incubator at the bottom. Analysis of a water sample

collected
pH of 7.9
was not de
R
were main
57 days o
first 16
sensitive
appearanc
ized eggs
developm
swim-up

the comp
because
hatching
bation w

Fry rear

sisted o
tank was
baskets.
was made
were sus
width of

collected from the reservoir in February 1969 showed a pH of 7.95 and a total alkalinity of 320 ppm. Chlorine was not detectable (Table 2).

Records of mortality for each of the egg samples were maintained on a biweekly schedule throughout the 57 days of development in the incubator, except for the first 16 days after fertilization when the eggs were too sensitive to examine. Dead eggs were recognized by the appearance of white coagulated yolk material. Unfertilized eggs were characterized by a lack of embryonic development. The mortalities after hatching and before swim-up were recorded as dead sac-fry.

Early mortality records were not available for the composite samples from Oregon and the Big Huron River because the eggs were received only a few days before hatching. Complete records of mortality during incubation were obtained for all other samples collected.

Fry rearing

The facilities for rearing coho salmon fry consisted of four 2.5m X 0.6m X 0.6m insulated tanks. Each tank was subdivided into 20 chambers by three rows of baskets. Each basket measured 25cm X 18cm X 30cm and was made of 0.32mm mesh nylon netting. The baskets were suspended in the tanks by rods traversing the width of the tanks. Rectangular glass-rod frames were

TABLE 2. Water analysis data for the carbon filter discharge (August 1968) and reservoir water (February 1969).

	Concentration*	
	Filter	Reservoir
pH	7.5	7.9
Total alkalinity	310	320
Hardness	362	380
Redox potential	-50	---
Dissolved oxygen	1.0	8.8-10.8
Free CO_2	18.0	7.0
Cl	ND < 40 ppb	ND < 40 ppb
PO_4 total	0.9	1.0
Temperature	11.5	10.2

*All units in ppm with exception of pH, redox potential (mv at 20°C) and temperature (°C).

ND = Not detected < 40 ppb

placed

desire

as tha

chlori

water

of wa

flow

throu

tent

0.5 p

after

rando

incub

time

feed

daily

ing.

Orego

fine

with

the

bask

were

placed in the bottom of the baskets to maintain the desired position and shape.

The water supplied to the tanks was the same as that supplied to the incubators (Table 2). Polyvinyl chloride (PVC) plastic pipe was used to construct a water distribution system that allowed a controlled flow of water to be jeted into each of the baskets. A total flow of approximately 6 liters/min. was maintained through each of the tanks. The dissolved oxygen content of the water remained nearly constant at 6.5 ppm $\pm$ 0.5 ppm and the temperature ranged from 11.5°C to 12.5°C.

Two weeks after hatching, approximately 57 days after fertilization, subsamples of 100 fry selected at random from each sample were transferred from the incubator to the rearing baskets. The fry at this time had a visible yolk-sac and had not yet started to feed. The fry were offered ground beef liver 3 times daily for the first week until they were actively feeding. At this time the liver diet was replaced with an Oregon Moist pellet (3/32 in.) which was ground to a fine consistency. The Oregon Moist diet was supplemented with ground liver one to three times weekly throughout the rearing period.

The condition and behavior of the fry in each basket were observed every two days and the mortalities were recorded. The fry were reared under uniform

conditions so peculiar behavior or mortality of the fry could be identified. All dead and dying fry were preserved in 10 percent formalin. Fry remaining at the end of the experiment also were preserved.

All eggs and fry were handled similarly and exposed to the same hatchery procedures and water temperature regime. The single exception to these conditions was the first ten samples of Thompson Creek fry. These fry were reared in ten gallon aquaria divided by a plastic screen so that two groups could be accommodated in one aquarium. The water temperature of these groups ranged from 13°C to 15°C. This difference from the other rearing groups was reduced by reporting mortality on the basis of "degree day" (degree day or temperature unit = [degrees F - 32] x days).

Analytical Methods

Extraction and cleanup of pesticide residues in salmon eggs

All organic solvents used in the extraction, cleanup and analysis were redistilled to remove interfering artifacts (Appendix I). Glassware was washed in hot water and detergent, rinsed once with distilled water and twice with acetone.

The egg samples were thawed and a subsample of approximately 20 unbroken eggs was removed, blotted dry

and weighed to the nearest 0.0001 gram. The eggs were mixed with approximately 20 grams of anhydrous sodium sulfate and 2 grams of clean ignited sand, and ground to a dry powder with a mortar and pestle. After thorough grinding the sample was extracted with four 20 ml portions of 94:6 petroleum ether:diethyl ether and the fractions were collected in a 125 ml erlenmeyer flask.

A standard Florisil column (Mills, 1959) was used in the clean-up procedure. The Florisil was reactivated at 150°C for at least 24 hours prior to use. Approximately 6 grams of activated Florisil was poured into a 25 mm diameter chromatograph column followed by 3 grams of anhydrous sodium sulfate. The column was prewetted and rinsed with 50 ml of petroleum ether which was discarded.

The total extract was added to the Florisil column. The flask which contained the extract was rinsed twice with 10 ml portions of petroleum ether which were added to the column. The column was eluted with 100 ml of 94:6 petroleum ether:diethyl ether and the eluate (total approximately 200 ml) was collected in a round bottom flask as the first fraction. The column was then eluted with 300 ml of 85:15 petroleum ether:diethyl ether (the diethyl ether contained 2 percent ethyl alcohol) and this second fraction was collected in a separate round bottom flask. The flow

throug

6 ml/m

3 or 4

transf

eluate

cide r

suitab

furthe

which

suitab

second

(95 pe

ether

was ad

conter

45 mi

125 m

water

for o

separa

separa

and e

each

50 ml

was d

through the column was maintained at approximately 6 ml/minute. Each fraction was then evaporated to 3 or 4 ml on a rotary evaporator and quantitatively transferred to graduated centrifuge tubes. The first eluate (6 percent EE/PE), which contained all the pesticide residues except dieldrin, aldrin and endrin, was suitable for gas-liquid chromatography (GLC) without further clean-up.

The second eluate fraction (15 percent EE/PE) which contained the remaining pesticide residues was not suitable for GLC without additional clean-up. The second fraction was saponified with 20 percent alcoholic (95 percent EtOH) KOH and then partitioned with petroleum ether (Mills, 1961). Approximately 5 ml of alcoholic KOH was added to the sample and then the centrifuge tube and contents were placed in a hot water bath (80°C) for about 45 minutes. The contents were then transferred to a 125 ml separatory funnel containing 50 ml of distilled water and 20 ml of petroleum ether, and gently shaken for one minute and allowed to stand until the two phases separated. The aqueous layer was drained into a second separatory funnel containing 20 ml of petroleum ether and extracted a second time. The petroleum ether from each extraction was combined and washed three times with 50 ml portions of 50 percent alcohol. The ether layer was dried by passing it through a filter funnel containing

anhydrc

quanti

tube.

this c

proced

98 to

dield

taken

fry f

6.19

unfor

effi

p,p'

for

resi

rec

ova

mat

Gas

by

a

wer

the

anhydrous sodium sulfate, evaporated to 3 or 4 ml and quantitatively transferred to a graduated centrifuge tube. The sample was suitable for GLC analysis after this clean-up procedure.

The efficiency of the extraction and clean-up procedure using fortified reagent blanks ranged from 98 to 101 percent for p,p'-DDT, p,p'-DDD, p,p'-DDE and dieldrin. A second group of efficiency tests were undertaken using approximately 3.5 grams of Oregon coho salmon fry fortified with 2.47 µg of DDT, 0.93 µg of DDD, 6.19 µg of DDE and 2.95 µg of dieldrin. Analyses of unfortified fry samples showed only a trace of DDE. The efficiencies for these tests were 89 ± 2 percent for p,p'-DDT, 91 ± 3 percent for p,p'-DDD, 93 ± 3 percent for p,p'-DDE and 94 ± 4 percent for dieldrin. Pesticide residue concentrations reported in this study are uncorrected for these percentages.

Analysis of three random subsamples from single ovaries showed a coefficient of variability of approximately 5 percent.

Gas chromatographic methods

All samples for pesticide analysis were analyzed by gas-liquid chromatography. Two gas chromatographs, a Wilkens Aerograph Model 660 and a Micro-Tek Model MT-220 were used to identify and quantify pesticide residues in the samples.

The Aerograph instrument was equipped with a 183 cm X 0.32 cm coiled glass column packed with 3 percent QF-1 on 60/80 mesh Gas Chrom Q. The electron capture detector was of concentric tube design with a tritium foil source. The operating temperatures used throughout the study were: column 186°C, detector 188°C and injection port 200°C. The carrier gas was commercial purified nitrogen adjusted to a flow of 35 ml/minute. The instrument developed 1,150 theoretical plates for dieldrin.

The MT-220 gas chromatograph was equipped with a 183 cm X 0.64 cm U-shaped glass column packed with 3 percent SE-30 on 60/80 mesh Gas Chrom Q. The column was connected to a parallel-plate electron capture detector with a tritium source. The detector utilized a 50 volt pulse mode power supply, which had a pulse rate of 100 and a width of one microsecond. A purge gas of 95%-argon-5% methane was maintained at 6 ml/minute through the detector. Commercial purified nitrogen served as a carrier gas at a flow of 70 ml/minute. The operating temperatures were 180°C for the column and detector and 220°C for the injection port. The chromatograph developed over 3,200 theoretical plates for p,p'-DDT.

Samples were made up to volume in graduated cylinders that would permit sample injections between

1 and 3 µl. Serial dilutions of pesticide standards were run each day to determine linearity curves and a basis for quantitative calculations. Care was taken to insure that the injected samples were within the linearity range of the detectors. Results were calculated from a linear regression of peak height (mm) versus picograms of insecticide. If the 95 percent confidence interval about the $\bar{Y}$ of the standard regression for a set of analyses exceeded ± 10 percent, the results were deemed unacceptable and the samples reanalyzed by GLC. Results were reported on the basis of µg/sample, µg/egg, ppm wet weight, ppm dry weight and ppm lipid weight (ether extractable lipids).

Proximate determination for dry and fat weights

A subsample of eggs from each sample was used to determine dry weights and percent fat. These values were then used to calculate the concentration of the pesticides in the original sample. A subsample of from 50 to 100 unbroken eggs was counted, blotted dry and weighed to the nearest 0.0001 grams. The eggs were placed in an oven at 50°C for 24 hours and then transferred to a desiccator for 48 hours. After this period the sample was weighed and the amount of the residue remaining was calculated as the dry weight. The lipid weight was determined by continuous extraction of the

prev

with

ethe

was

of t

Proc
resi

Lake

chro

iden

resi

stan

was

exch

cide

puri

Pest

iden

samp

(196

ated

of s

trac

previously dried sample in a Soxhlet extraction apparatus with 100 percent diethyl ether for 7 to 9 hours. The ether extractable weight or lipid weight of the sample was the difference between the dry weight and the weight of the residue after ether extraction.

Procedures for pesticide residue identification

Pesticide residues in the coho salmon eggs from Lake Michigan were identified by GLC and thin layer chromatography (TLC). GLC was used to tentatively identify the pesticide residues by comparison of sample residue retention times with those of authenic pesticide standards. Confirmation of the pesticide residue identity was made by separate analyses on a second column and by exchange of samples with two other laboratories. Pesticide standards were made up in benzene solutions from purified standards (98 percent plus) obtained from the Pesticide Repository, Perrine, Florida.

Thin layer chromatography was used to verify the identity of the pesticide residues present in the samples. The procedure used was described by Kovacs (1963) as useful for the chromatography of all chlorinated hydrocarbon pesticides. Additional clean-up of samples was necessary after the initial extraction and clean-up before the samples could be

spotted on TLC plates. The samples were partitioned twice with acetonitrile saturated with petroleum ether and then passed through two magnesium oxide-celite columns. The eluate was evaporated to 0.25 ml and spotted with appropriate standards on 20 cm square glass plates coated with aluminum oxide or silica gel G absorbents. The spotted plates were developed in 100 ml of either n-heptane or 20 percent ethyl ether in n-hexane for 20 to 30 minutes, air dried and sprayed with either 0.05 percent silver nitrate or Rhodamine B. The plates were placed under ultraviolet light for approximately 15 minutes to develop the spots and R_f values were calculated.

The identity of the pesticide residues in the samples were further verified by removing the TLC absorbent containing the spots, extracting the residue with petroleum ether and injecting the sample into a gas chromatograph. Retention times were compared to those of pesticide standards.

To evaluate the possible interference of other compounds, random subsamples of three to six egg samples from each stream were tested. The samples, after extraction for pesticide residues, were divided in half. One subsample was saponified with 20 percent alcoholic KOH and partitioned into an ether solution for injection into the gas chromatograph. The other

subsample was not saponified. The peaks which remained in the saponified portion of the samples with retention times equal to DDT and DDD were considered to be interfering residues. The percentage error due to the interfering compounds in the original analysis was determined by comparing the peak heights in the saponified and unsaponified portions of the samples.

Statistical methods

The analysis of variance formula for testing the hypothesis of no difference between means as presented by Sokal and Rohlf (1969) was used to test for differences in mean pesticide residues and mean fry mortalities between years, between sampling locations and between sampling dates. If ANOVA indicated significant differences within a group of means, then an "a posteriori" least significant range test (Sokal and Rohlf, 1969) was used to determine which means were significantly different. An arcsine transformation was applied to all percentage data before analysis.

Correlation coefficients were calculated for relationships between: all possible pairs of the four pesticide residues; DDT residues expressed as ppm wet weight and ppm dry weight, ppm wet weight and ppm lipid weight and ppm dry weight and ppm lipid weight; DDT residues and percentage lipid in the eggs; DDT residues and length of parent fish; pesticide residues and

sampling date; egg mortality and DDT, dieldrin and total DDT, DDD and DDE; and fry mortality and DDT, dieldrin and total DDT, DDD and DDE residues. The statistical probability of occurrence of the specific correlation coefficients was determined using tables from Snedecor (1956). Correlation coefficient values having a probability of occurrence of 0.05 or less were accepted as indicating statistical relationship between the two variables under study.

RESULTS AND DISCUSSION

Mortality Study

Egg and sac-fry mortality

The mortalities of the eggs and sac-fry for each of the groups of samples from individual streams were determined for each of four developmental periods (Table 3): start of incubation (6-18 hours), eye-up of the eggs (19 days or 360 temp. units), hatch (45 days or 810 temp. units) and swim-up of the sac-fry (57 days of 1,026 temp. units).

The mortalities during the first developmental period, from fertilization to start of incubation (6-18 hours), consisted of both fertilized and unfertilized eggs. The mortality during this period was considered an indication of the success of the sampling and transportation procedures. The low mortality, averaging 3.1 percent among all groups and ranging from 0.6 to 7.2 percent, suggested that the sampling procedures were adequate. The mortalities were independent of the sampling sites and there were no abnormalities observed during this early period.

TABLE 3. Average percentage cumulative egg and sac-fry mortalities salmon for the dates each of the streams were sampled.

Source	Sample Date	Incubator Mortality			
		Initial 0 TU*	Eye-up 360 TU	Hatch 810 TU	Transfer 1026 TU
		0 days	19 days	45 days	57 days
LAKE MICHIGAN					
Thompson Creek	9-17-68	2.9	21.4	25.3	28.6
	10-13-68	6.7	35.5	43.1	49.1
Bear Creek	10-24-68	7.2	26.0	41.9	42.1
	12-13-68	1.5	9.4	32.9	33.6
Little Manistee River	11-7-68**	1.8	33.0	65.5	71.8
	12-13-68	0.6	11.2	65.4	66.5
Platte River	10-17-68	1.8	32.3	43.6	44.8
	11-14-68	1.5	20.9	60.2	---
	1-4-69***	12.5	85.0	96.8	97.5
Average		3.1	24.5	47.4	48.0
LAKE SUPERIOR					
Cherry Creek	11-1-68	0.9	11.4	38.0	41.4

*TU = Temperature units - [(degrees F - 32) x days]

**Include the result of two samples collected 10-24-68

***Not included in average

During the second period of development, from start of incubation to eye-up of the eggs (visible eyes within eggs) there was an average mortality of 21.4 percent among all groups. The average cumulative mortality of 24.5 percent to eye-up was within the range of cumulative mortalities (7 to 30 percent) reported in Michigan hatcheries for the same period (Michigan Department of Natural Resources, 1968). The mortality consisted of both fertilized and unfertilized eggs. Also included in the mortality for this period was a relatively large number of unfertilized "blank" eggs that were removed after the eye-up stage. A little over half (11.1 percent) of the mortality for this period was accounted for by unfertilized "blank" eggs. There is no way of knowing what percentage of the dead eggs removed before eye-up were unfertilized eggs because an initial estimate of the percent fertilization was not obtained. However, a maximum of 88.9 percent fertilization was realized if the unfertilized "blank" eggs removed after eye-up are assumed to be the only unfertilized eggs. On the other hand, a minimum of 75.5 percent fertilization was achieved if the entire mortality up to eye-up, including the unfertilized "blank" eggs, is considered to be unfertilized eggs. The Michigan Department of Natural Resources (1968) reported a range of 86 to 89 percent fertilization for the eggs from the spawning run of coho salmon.

The eggs during the third period of development, from eye-up to hatch (26 days or 450 temp. units) sustained an average mortality of 22.9 percent. The average cumulative mortality up to the end of this period was 47 percent. The relatively high mortality of embryonated eggs was associated with a breakdown of the chorion just prior to hatch. The chorion in many eggs became very thin approximately 30 days after fertilization and ruptured with resulting protrusions of yolk material. Some of the embryos hatched early and survived but the condition was fatal to a large percentage of the embryos. The condition was first observed in the second group of samples collected at Thompson Creek. This group of samples was the second group to be placed in the incubator and every subsequent group, whether from Lake Michigan or Lake Superior streams exhibited chorion breakdown. There were no reports of this condition exhisting in Michigan hatcheries during 1968-69.

The fourth period of development, from hatch to transfer (12 days or 216 temp. units), accounted for less than one percent of the total cumulative incubator mortality. The mortality consisted mainly of deformed embryos. The Michigan Department of Natural Resources (1968) reported losses ranging from 0.6 to 3.0 percent for the same period of development and a total cumulative mortality ranging from 30 to 45 percent. The average

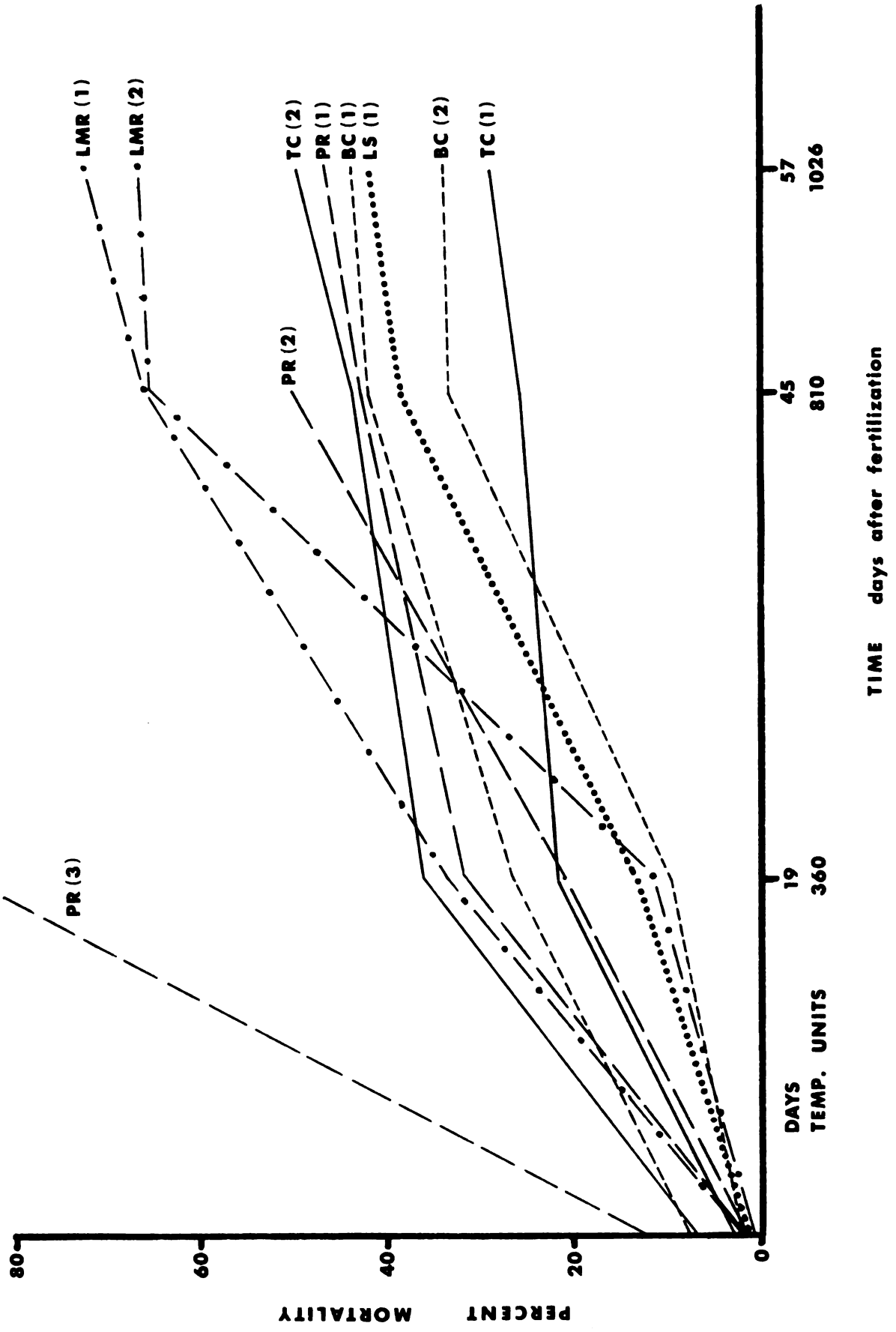
cumulative incubator mortality of eggs and sac-fry at the end of the fourth mortality period in this study was 48 percent. The majority of the losses can be attributed to premature hatching and chorion breakdown of the eggs.

The pattern of the egg and sac-fry mortalities were very similar with respect to developmental periods for both Lake Michigan and Lake Superior streams (Figure 2). The mortalities during the first and fourth developmental periods were very low but the second and third periods mortalities ranged between 8 and 54.2 percent.

The last group of samples from the Platte River did not adhere to the general mortality pattern. These samples sustained the highest average cumulative mortality of all samples collected with an 85 percent loss by the end of the second development period. The egg quality of these late groups was considered poor because of low fertilization and low survival to hatching. The Michigan Department of Natural Resources (1968) also reported a higher than average mortality for eggs collected at the same time from the Platte River. This group of samples was not considered representative and was excluded from the analysis of egg mortality data.

The average cumulative mortality for samples from the Little Manistee River was significantly greater than the mortalities of samples from other streams

Figure 2. Average cumulative mortalities of eggs and sac-fry, from fertilization to swim-up, of coho salmon collected at the various streams tributary to Lake Michigan and Lake Superior. T.C., Thompson Creek, (1) 9-17-68, (2) 10-13-68; B.C., Bear Creek, (1) 10-24-68, (2) 12-13-68; L.M., Little Manistee River, (1) 11-7-68, (2) 12-13-68; P.L. Platte River, (1) 10-17-68, (2) 11-14-68, (3) 1-4-69; and L.S., Lake Superior, (1) 11-1-68.


LMR (1)
LMR (2)
PR (3)
TC (2)
PR (1)
BC (1)
LS (1)
PR (2)
BC (2)
TC (1)
80
60
40
20
0
PERCENT MORTALITY
DAYS
TEMP. UNITS
19
360
45
810
57
1026
TIME days after fertilization

sampled in the Lake Michigan and Lake Superior watersheds. The differences in the average cumulative mortalities of samples from Bear Creek, Platte River, Thompson Creek (Lake Michigan) and Cherry Creek (Lake Superior) were not significantly different ($p < 0.05$).

The egg and sac-fry mortalities observed in this laboratory study were similar to the egg and sac-fry mortalities in Michigan hatcheries during 1968. The mortalities in each case were considered high when compared to that in West Coast hatcheries (Michigan Department of Natural Resources, 1968).

Fry mortality

Observations of fry mortality were initiated at the time the sac-fry were transferred from the incubators to the rearing tanks, approximately 57 days (1,036 temp. units) after fertilization, and continued for 63 days (1,371 temp. units) after transfer. The average cumulative mortalities of fry from each of the streams sampled are shown in Table 4.

Mortalities of fry from individual females showed a wide variation, ranging from 0 to as high as 90 percent. In recording the daily mortality in each group the symptoms of the dying fry were carefully noted. Specific symptoms which were common to a large percentage of the dying fry were: loss of equilibrium, erratic swimming, spasmodic and convulsive movements when disturbed, long

TABLE 4. Average percentage mortalities of coho salmon fry for the date each of the streams were sampled.

Source	Sampling Date	No. of Groups	Fry Mortality 1371 TU (63 days)	
			Total	With symptoms*
LAKE MICHIGAN				
Thompson Creek	9-17-68	10	23.4	18.3
	10-3-68	8	44.5	39.1
Bear Creek	10-24-68	10	37.6	31.6
	12-13-68	8	2.5	< 1.0
Lt. Manistee River	11-7-68	9	22.0	18.6
	12-13-68	7	10.0	7.0
Platte River	10-17-68	10	27.4	21.6
	11-14-68	1	41.0	38.0
	1-4-69	4	32.7	26.4
LAKE SUPERIOR				
Cherry Creek	11-1-68	10	55.6	< 1.0
Big Huron River	1-20-69	14	6.1	0.0
OREGON				
Oregon	1-4-69	10	11.3	0.0

*Symptoms described in text

periods of inactivity while laying on the bottom and cessation of feeding. The affected fry gradually weakened and died within three to six days after noticeable symptoms. This syndrome was characteristic of a major percentage of the mortalities in the samples from Lake Michigan. A very low percentage of the fry from Lake Superior and none of the fry from Oregon showed these symptoms (Table 4). Identical symptoms of dying fry were observed in Michigan hatcheries during 1968 (Michigan Department of Natural Resources, 1968) and in a preliminary study on salmon development (Johnson and Pecor, 1969).

Examination of the affected fry showed no external lesions. A small percentage had a clouding of the lens on one or both eyes. Microscopic examination of tissues from healthy and sick fry showed no major differences between the two with the exception of the frequency of the presence of food in the gut. Food, in most cases, was absent from the stomachs of affected fry. A clear yellowish mucus was present in the stomach and intestine of affected fry whether food was observed in the stomach or not. A clear oily liquid was also observed in the body cavity of many fry, both affected and nonaffected.

Average cumulative fry losses through the ninth week after transfer from the incubator ranged from 2.5 to

44.5 percent in individual sample groups from Lake Michigan streams. The average cumulative mortalities associated with the specific mortality syndrome described above ranged from less than one percent to 39.1 percent for the same groups of samples. There was no significant difference in the average cumulative mortalities of fry from eggs taken at different times from the same streams with the exception of Bear Creek samples. The second group of samples from Bear Creek (12-13-68) had significantly lower mortality ($p < 0.05$) than those from the earlier sample from this stream. Less than one percent of these mortalities were associated with the specific symptoms.

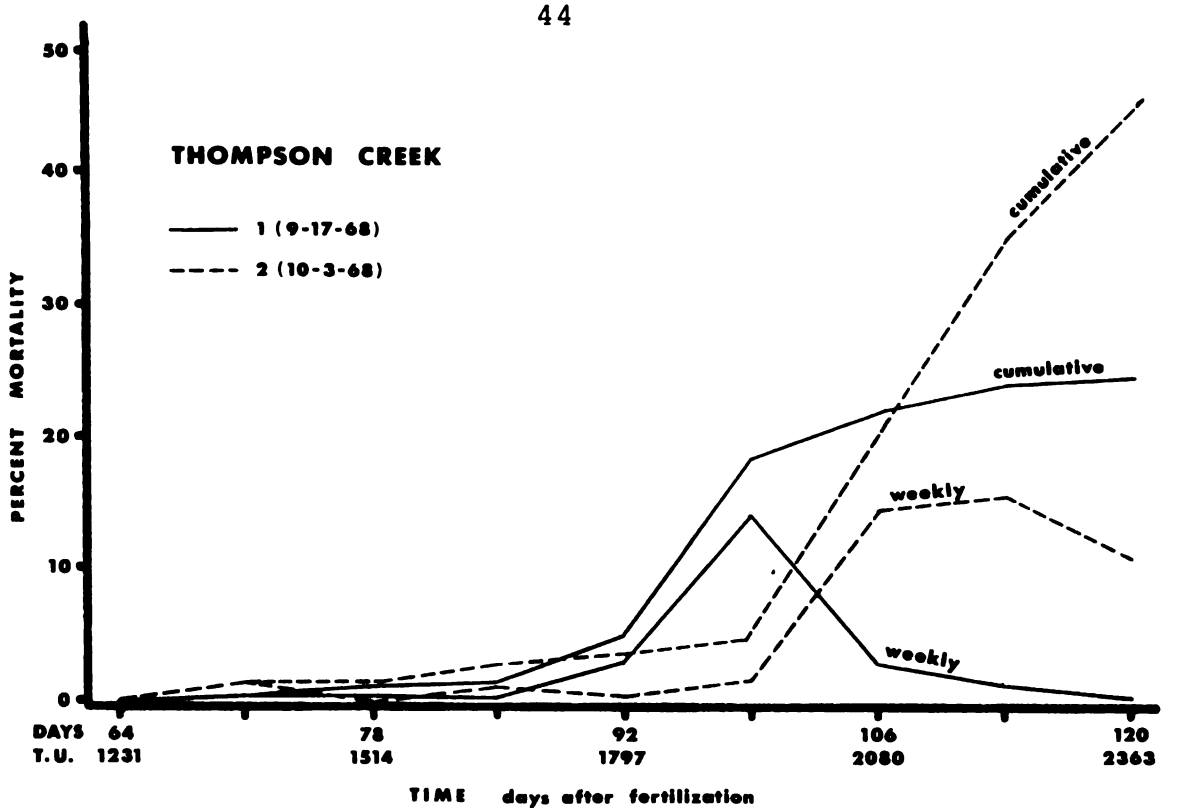
The mortalities of the Lake Michigan fry exhibiting the mortality syndrome were very specific with respect to time. The mortality generally increased and peaked abruptly during the sixth week (1,938 temp. units) after transfer from the incubator, although some fry with symptoms were observed during the fifth week and some mortality of fry with symptoms extended into the seventh week. The peak mortality occurred 99 days after fertilization during early feeding and final yolk absorption stage. The mortality of fry between the fifth week (1,797 temp. units) and the eighth week (2,221 temp. units) after transfer accounted for 81 percent of the total mortality observed in the Lake Michigan fry

samples. The Michigan Department of Natural Resources (1968) reported a similar mortality pattern in Michigan hatcheries, although the time period to initiation and peak mortalities with symptoms was different for hatcheries with different water temperatures.

The period of mortality for each of the groups of samples collected from each of the four Lake Michigan streams, Thompson Creek, Bear Creek, Little Manistee River and Platte River, were almost identical to the general mortality pattern previously discussed (Figures 3, 4, 5 and 6). Only minor differences existed within streams and between streams. The second group of samples from Thompson Creek exhibited a low mortality during the sixth week but a high mortality occurred for the next two consecutive weeks, the seventh (2,080 temp. units) and eighth (2,221 temp. units) weeks after transfer (Figure 3). The second group of samples from Bear Creek sustained the lowest mortality of all sample groups and specific symptoms were virtually absent (Figure 4). The peak period of mortality in the first group of samples from the Platte River occurred during the seventh week (2,080 temp. units) but in the second group of samples from this stream the mortality began to increase during the fifth week (1,797 temp. units) and peaked during the sixth (Figure 6).

Figure 3. The average weekly and cumulative fry mortality during the nine-week study for the two groups of Thompson Creek samples.

Figure 4. The average weekly and cumulative fry mortality during the nine-week study for the two groups of Bear Creek samples.


THOMPSON CREEK
1 (9-17-68)
2 (10-3-68)
PERCENT MORTALITY
50
40
30
20
10
0
cumulative
cumulative
weekly
weekly
DAYS 64 78 92 106 120
T.U. 1231 1514 1797 2080 2363
TIME days after fertilization

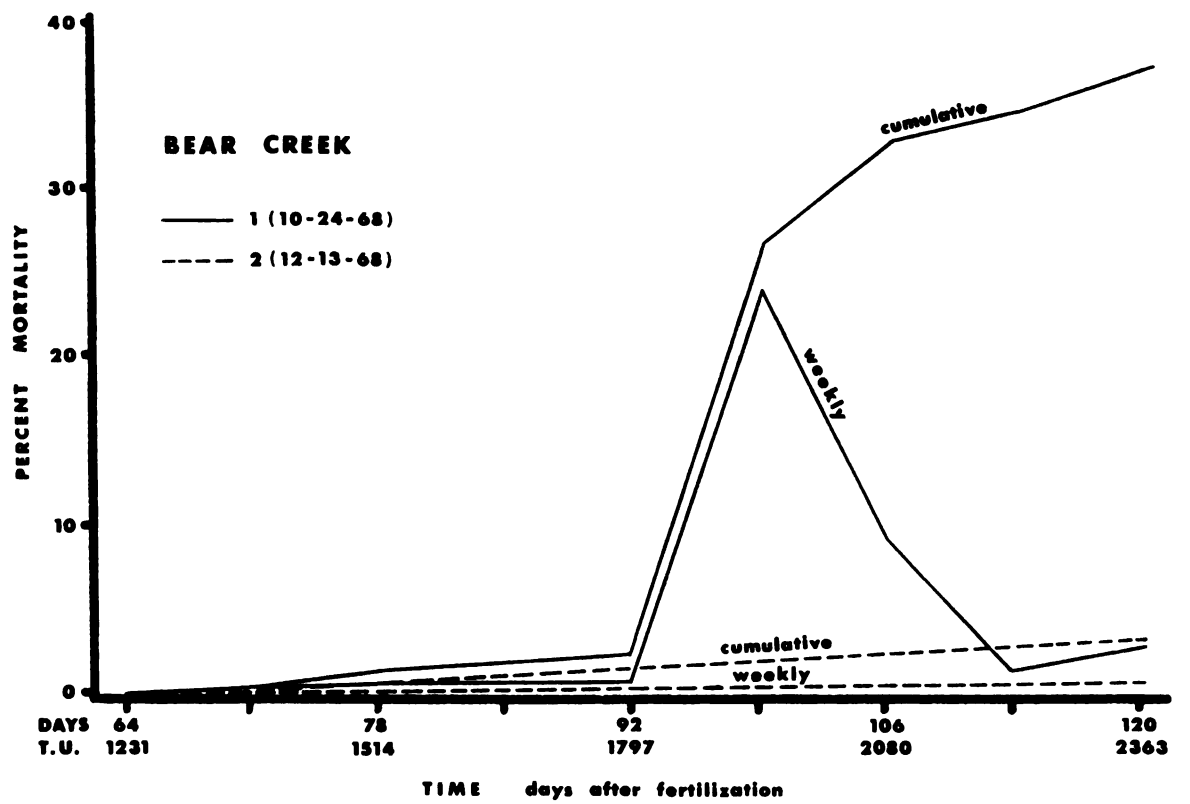
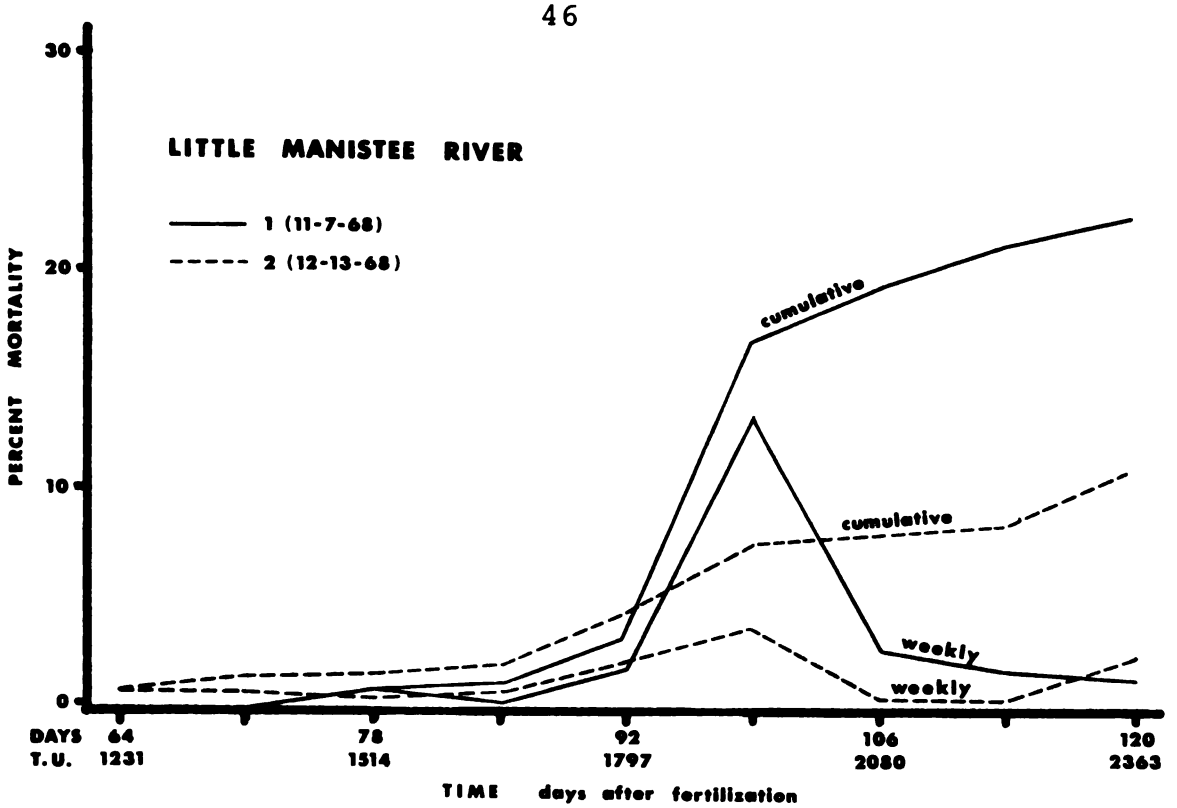
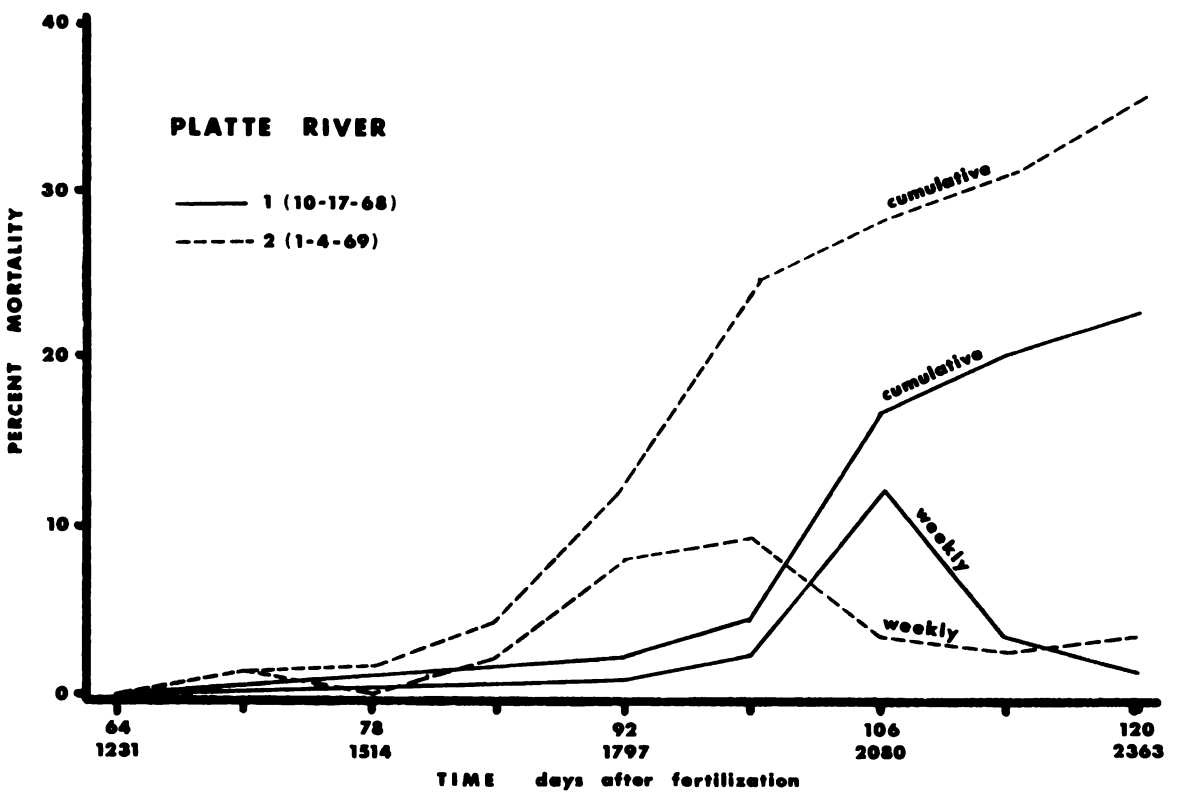

BEAR CREEK
1 (10-24-68)
2 (12-13-68)
PERCENT MORTALITY
40
30
20
10
0
cumulative
weekly
cumulative
weekly
DAYS 64 78 92 106 120
T.U. 1231 1514 1797 2080 2363
TIME days after fertilization

Figure 5. The average weekly and cumulative fry mortality during the nine-week study for the two groups of Little Manistee River samples.

Figure 6. The average weekly and cumulative fry mortality during the nine-week study for the two groups of Platte River samples.


LITTLE MANISTEE RIVER
1 (11-7-68)
2 (12-13-68)
PERCENT MORTALITY
30
20
10
0
cumulative
cumulative
weekly
weekly
DAYS 64 78 92 106 120
T.U. 1231 1514 1797 2080 2363
TIME days after fertilization


PLATTE RIVER
1 (10-17-68)
2 (1-4-69)
PERCENT MORTALITY
40
30
20
10
0
cumulative
cumulative
weekly
weekly
64 78 92 106 120
1231 1514 1797 2080 2363
TIME days after fertilization

The extent and pattern of mortality among fry from Lake Superior and Oregon was distinctly different from that observed in the Lake Michigan groups. The losses occurred at an earlier age and were not characterized by the symptoms observed in Lake Michigan samples. The Michigan Department of Natural Resources (1968) also reported that symptoms associated with the Lake Michigan fry mortality were not observed in the fry from Lake Superior sources.

The average cumulative mortality of fry from Big Huron River, Lake Superior was 6.1 percent, which was generally lower than those from Lake Michigan. This group of fry sustained a low constant mortality up to the ninth week when a slight increase in mortality was recorded (Figure 7). No specific symptoms were observed.

A very high loss of fry in the Cherry Creek samples (average cumulative mortality of 55.6 percent) was believed to be due to starvation shortly after yolk-sac absorption. The fry at swim-up averaged only 0.29 grams/fry as compared with 0.69 grams/fry for those from Lake Michigan. The small size probably contributed to their rapid emaciation and failure to feed. The mortality occurred one to two weeks earlier than was typically observed in Lake Michigan samples (Figure 7). Most of the fry had developed a "pinhead" condition when death occurred.

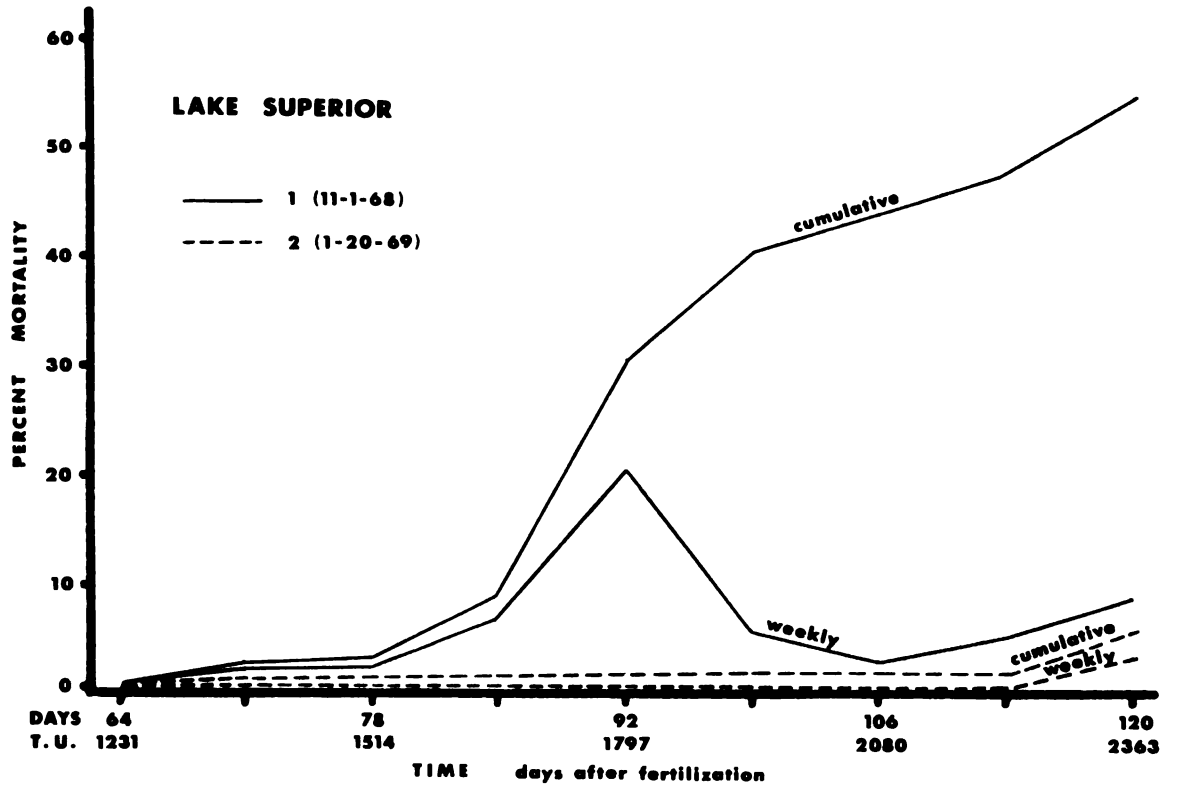
The cumulative losses of Oregon fry averaged 11.3 percent during the nine-week period. Lesions were observed on most of the dead and dying fry. The moribund fry showed discoloration and gradual necrosis of the skin and flesh around the dorsal fin and caudal peduncle. The losses appeared to be due to a systemic bacterial infection of low virulence which was fatal during the first three weeks in the rearing tanks (from 1,231 temp. units to 1,514 temp. units), but then gradually disappeared (Figure 8).

The average weekly mortality of the fry from each of the three major systems, Lake Michigan, Lake Superior and Oregon, showed a different pattern (Figure 9). Oregon fry samples suffered a peak mortality during the first three weeks after transfer from the incubator and a loss of less than one percent during the rest of the nine-week period (Figure 9). Lake Superior fry experienced a peak mortality during the fifth week after transfer and a second during the ninth week (Figure 9). The Lake Michigan fry samples experienced the highest average total cumulative mortality and the highest peak mortality which occurred during the sixth week (Figure 9).

The absence of the Lake Michigan mortality syndrome in Lake Superior and Oregon fry reared in the same tanks with Lake Michigan fry indicated the cause of death was not the same. There was no evidence of disease being a factor in the Lake Michigan fry mortalities.

Figure 7. The average weekly and cumulative fry mortality during the nine-week study for the two groups of Lake Superior samples.

Figure 8. The average weekly and cumulative fry mortality during the nine-week study for the single group of Oregon samples.


LAKE SUPERIOR
1 (11-1-68)
2 (1-20-69)
cumulative
weekly
cumulative
weekly
PERCENT MORTALITY
60
50
40
30
20
10
0
DAYS 64 78 92 106 120
T.U. 1231 1514 1797 2080 2363
TIME days after fertilization

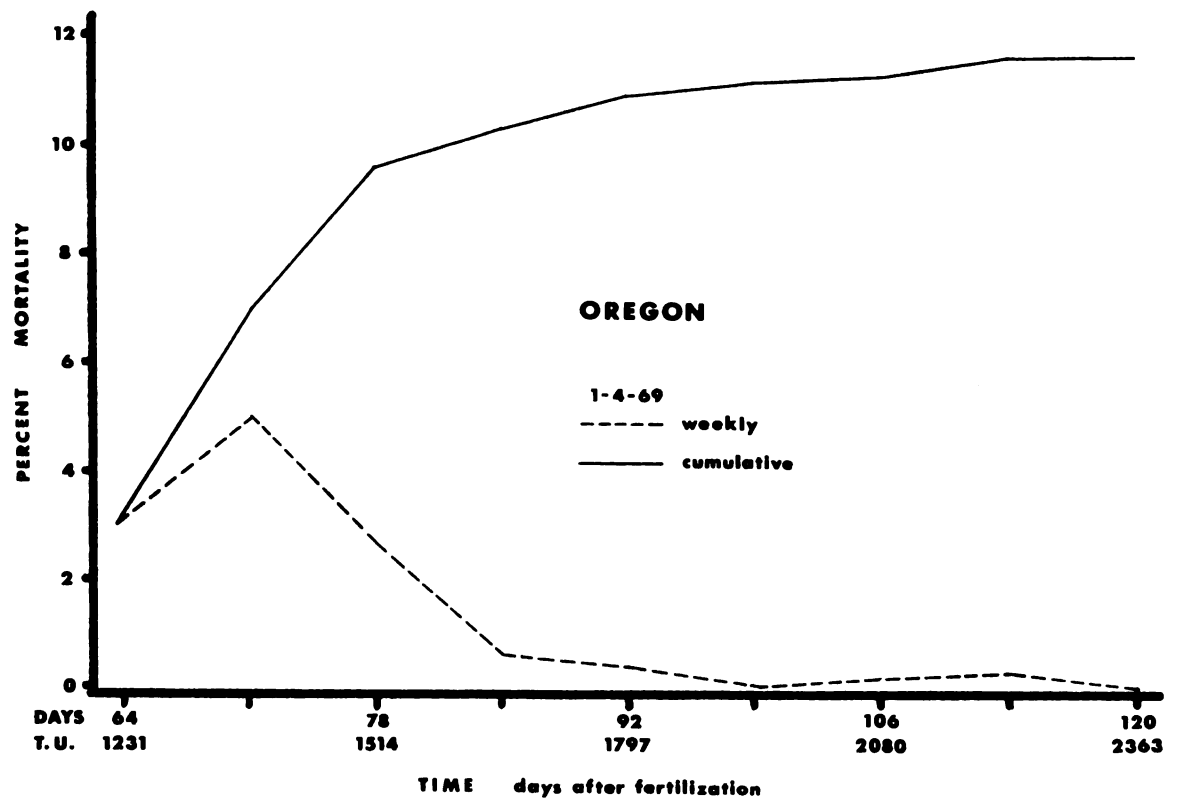
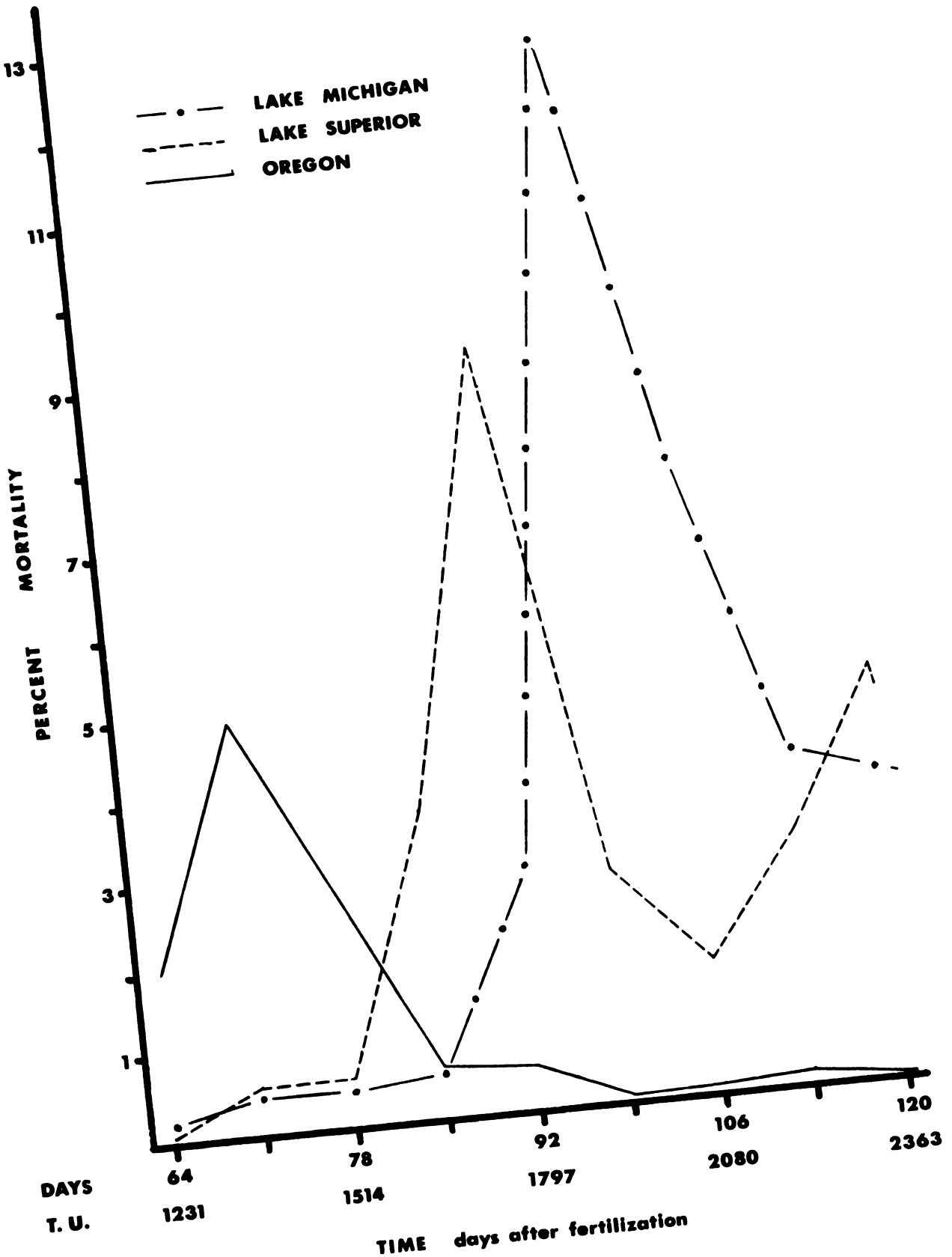

OREGON
1-4-69
weekly
cumulative
PERCENT MORTALITY
12
10
8
6
4
2
0
DAYS 64 78 92 106 120
T.U. 1231 1514 1797 2080 2363
TIME days after fertilization

Figure 9. The average weekly fry mortality during the nine-week study for Lake Michigan, Lake Superior and Oregon samples.


LAKE MICHIGAN
LAKE SUPERIOR
OREGON
PERCENT MORTALITY
13
11
9
7
5
3
1
DAYS
64
78
92
106
120
T. U.
1231
1514
1797
2080
2363
TIME days after fertilization

Residue Identification

Gas chromatography

Gas chromatographic analysis of the ether extract of coho salmon eggs showed 14 to 17 residue peaks in every egg sample. Six of these peaks were identified as pesticide residues, two were artifacts from the clean-up procedure and the identity of the remaining peaks was not determined. Five of the pesticide residues were DDT and related isomers and one was dieldrin, a chlorinated cyclodiene.

Analysis of the first elution fraction of the egg extracts with the Micro-Tek 220 gas chromatograph usually showed 13 residue peaks (Figure 10). The retention times of these 13 peaks, relative to p,p'-DDE (Table 5), were compared with retention times of pesticide standards for initial identification. The retention times of endrin, aldrin, methoxychlor, heptachlor and lindane pesticide standards were not related to any of the 13 peaks in the samples. One peak was identical to an anhydrous sodium sulfate residue and was apparently the result of the extraction and clean-up procedure.

Samples of polychlorinated biphenyl mixtures (Arochlors 1221, 1232, 1242, 1253 and 1260--Monsanto) were injected into the gas chromatograph and the resulting chromatograms compared to those of typical egg extracts. The polychlorinated biphenyls (PCB's)

Figure 10. A typical chromatogram of the first eluate fraction of coho salmon eggs using the Micro Tek 220 gas chromatograph. Peaks numbering 5, 7, 8 and 10 correspond to the pesticides p,p'-DDE, p,p'-DDD, o,p'-DDT and p,p'-DDT, respectively. Peak number 4 corresponds to both MDE and o,p'-DDE.

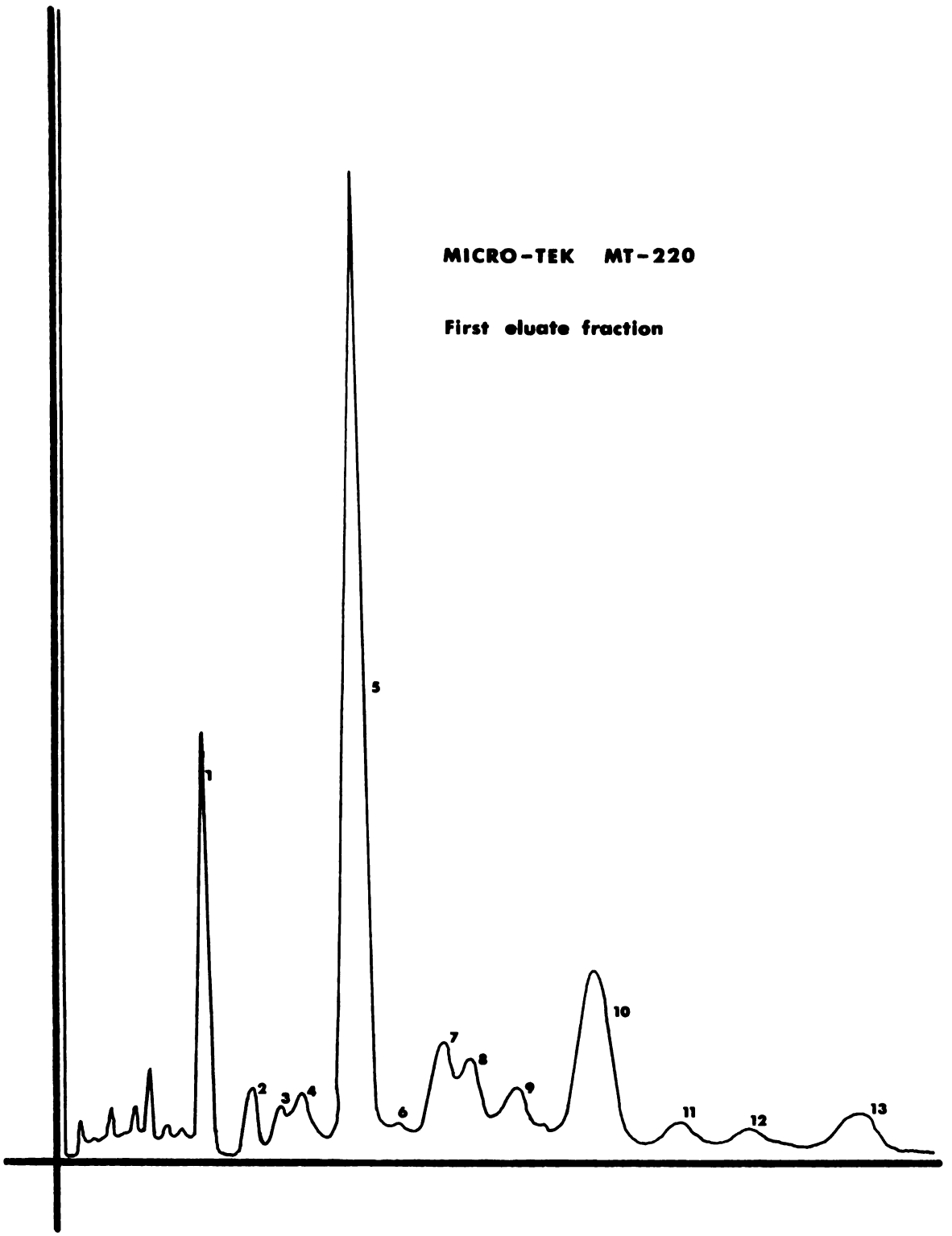

MICRO-TEK MT-220
First eluate fraction
1
2
3
4
5
6
7
8
9
10
11
12
13

TABLE 5. The relative retention times (p,p'-DDE = 1.0) and identification of residue peaks present in the ether extract of coho salmon eggs.

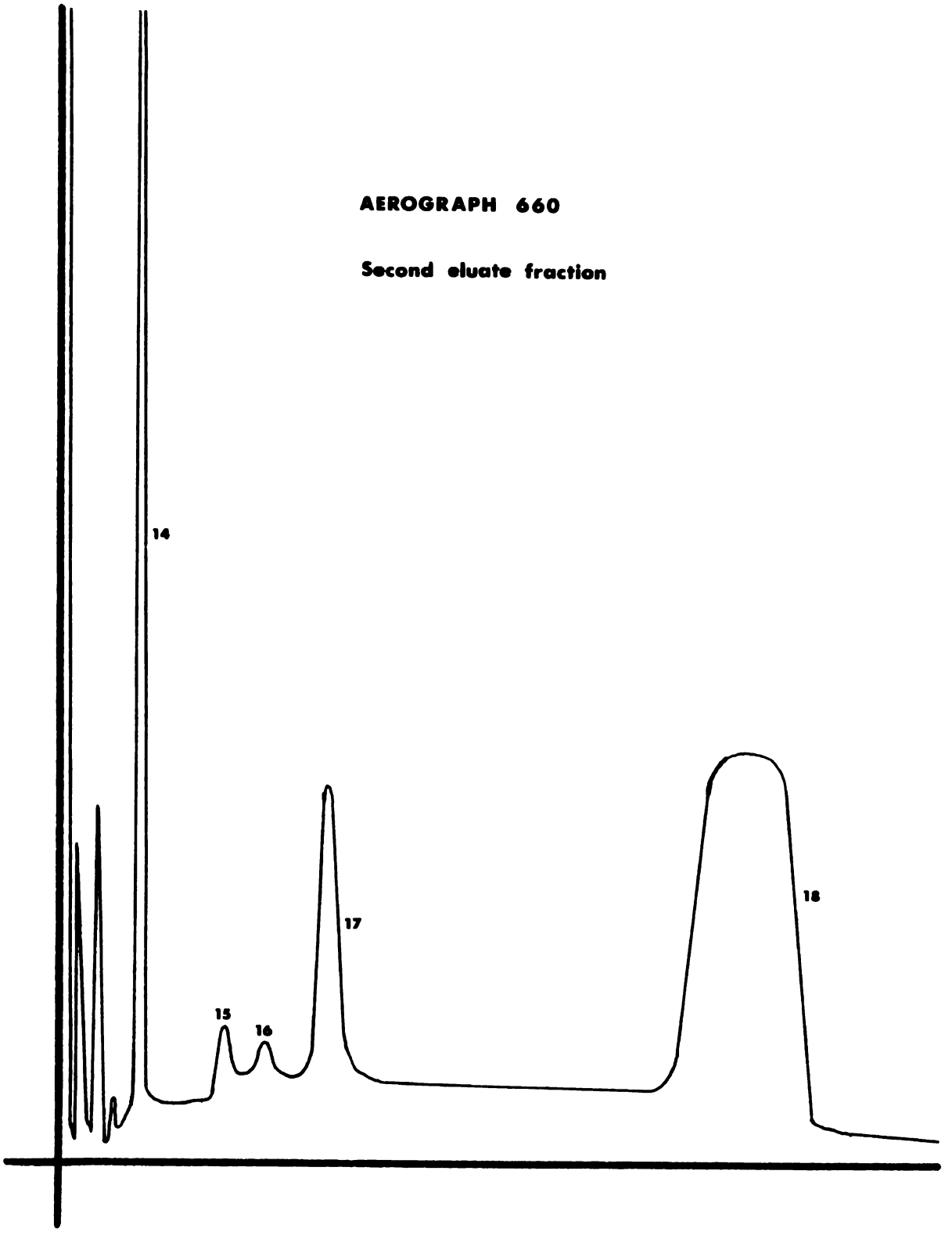
Chromatograph	Peak No.	Retention Time	Residue
Micro-Tek 220	1	0.49	$NaSO_4$
	2	0.64	unidentified
	3	0.74	unidentified
	4	0.81	o,p'-DDE MDE
	5	1.00	p,p'-DDE
	6	1.11	unidentified
	7	1.27	p,p'-DDD
	8	1.35	o,p'-DDT
	9	1.50	unidentified
	10	1.77	p,p'-DDT
	11	2.11	unidentified
	12	2.59	unidentified
	13	3.13	unidentified
Aerograph 660			
	14	0.32	unidentified
	15	0.79	unidentified
	16	1.00	p,p'-DDE
	17	1.32	dieldrin
	18	4.04	Florisil artifact

produced from 30 to 35 residue peaks of which at least one peak matched almost every peak in the egg sample extracts. The saponification of PCB's and pesticide standards showed that DDT and DDD were destroyed but the PCB mixture was not affected. Comparison of saponified and unsaponified portions of individual egg extracts indicated that an average of 19.8 percent (n = 41, standard deviation = ±5.95) of the DDT residue was not degraded upon saponification, indicating an inferfering residue was present.

There appeared to be no difference in the percentage of the interfering residue present in the samples from the four Lake Michigan streams or between Lake Michigan and Lake Superior streams. Inferferences of this type were not present or they were not identified for DDD and DDE although PCB's have been shown to interfere with both DDD and DDE (Koeman et al., 1968). No positive identification was made of the interfering residue and all DDT residue concentrations reported in this study are uncorrected for the average 19.8 percent interfering residue.

Analysis of the second elution fraction of the egg extracts with the Wilkens Aerograph 660 gas chromatograph usually showed the presence of five peaks in every egg sample (Figure 11). A trace of p,p'-DDE was also present in this fraction. The retention times of

Figure 11. A typical chromatogram of the second eluate fraction of coho salmon eggs using the Aerograph 660 gas chromatograph. Peaks numbered 16 and 17 correspond to DDE and dieldrin, respectively.


AEROGRAPH 660
Second eluate fraction
14
15
16
17
18

the peaks are recorded in Table 5. Two peaks were identified as p,p'-DDE and dieldrin, and one peak was identified as an artifact from the Florisil used in the clean-up procedure. Two peaks were not identified by comparison to pesticide standards, although the retention time of one was very close to that of lindane. Tests with PCB standards showed these were not found in the second elution fraction and thus were not potential inferfering compounds in this fraction.

Thin layer chromatography

Thin layer chromatography confirmed the presence of p,p'-DDT, p,p'-DDD, p,p'-DDE and dieldrin in the egg samples. These four pesticides were considered to be the most significant with respect to the quantity present and their toxic properties. Samples spotted on both aluminum oxide and silica gel G plates produced spots with R_f values which corresponded to R_f values of respective pesticide standards. The spots were scraped into vials, extracted with petroleum ether and an aliquot reinjected into a gas chromatograph. Single peaks were usually detected with retention times analogous to those of the appropriate pesticide standards.

PCB standards (Arochlor 1221, 1232, 1242, 1254, 1260--Monsanto) were also spotted on thin layer plates along with sample extracts and pesticide standards. The PCB standards moved with the solvent front and had an

R_f value similar only to DDE. The spots associated with DDE were extracted and injected into a gas chromatograph. The chromatograms showed a series of peaks with retention times similar to those in a chromatogram of the whole egg extract. These data are further evidence that PCB compounds may be present in the salmon eggs.

Pesticide Residue Concentrations in Coho Salmon Eggs

General

The concentrations of p,p'-DDT, p,p'-DDD, p,p'-DDE and dieldrin were determined for 200 samples of coho salmon eggs collected during 1968 from Lake Michigan, Lake Superior and Oregon. The average concentrations of pesticide residues for each of the samples are tabulated in Appendix II according to sampling date on the basis of ppm (parts per million) wet weight, dry weight, fat weight and in Appendix III on the basis of µg/egg.

In the salmon eggs from Lake Michigan and Lake Superior, DDT, DDD and DDE constituted approximately 27.1, 5.4 and 67.5 percent, respectively, of the total DDT complex and dieldrin accounted for approximately one percent of the total residues quantified. In the eggs from Oregon DDT, DDD and DDE constituted 17.9, 15.4 and 66.7 percent, respectively, of the total DDT complex while dieldrin accounted for approximately four

percent of the total residues quantified. Statistically there was not a significant difference ($p < 0.05$) between the percentages of the individual residues in Lake Michigan and Lake Superior eggs or between the percentages of DDE in Lake Michigan, Lake Superior and Oregon eggs. The percentages of DDT, DDD and dieldrin in Oregon eggs were significantly different ($p < 0.05$) from the respective percentages in Lake Michigan and Lake Superior eggs. The percentages of the four residues relative to the total were very uniform within the three major "lake" systems.

Of the four pesticide residues which were quantified in the study, DDT and dieldrin are generally considered to be the most toxic (Henderson, Pickering and Tarzwell, 1959; Katz, 1961). DDD is considered less toxic to vertebrates than DDT (O'Brien, 1967) but the effects of DDD on fish have not been adequately studied. DDE, generally, has a much lower toxicity to fish than DDT or DDD. Burdick (1964) found no relationship between DDE concentrations in lake trout eggs and mortality of the fry.

The concentration of DDT in the coho salmon eggs from each sampling site was significantly correlated ($p < 0.05$) with the concentration of DDD and DDE (Table 6). In considering the relationship of these compounds to the parameters evaluated in this study,

DDD and DDE were not considered separately. In the following discussion only p,p'-DDT values are noted except in some instances where the total DDT residues (DDT, DDD and DDE) are considered.

Dieldrin is approximately 2 to 3 times more toxic to fish than DDT (Henderson, Pickering and Tarzwell, 1959; Katz, 1961). Dieldrin was not significantly correlated ($p < 0.05$) with DDT, but there was a significant positive correlation with DDD and DDE although a very low percentage of the variation was common to dieldrin and DDD or DDE. Dieldrin residues were considered separately in the analysis and interpretation of the data.

Some discussion has occurred as to which expression of the pesticide residue concentration, ppm wet weight, ppm dry weight or ppm fat weight, presents the most useful and meaningful information for comparisons. It is my opinion that for discussion purposes it is adequate to express the pesticide residues as ppm wet weight in the salmon eggs in this study. Wet weight showed a significant positive correlation with both dry weight and fat weight as did dry weight with fat weight (Table 7). Expressing the pesticide residues as ppm wet weight accounts for a maximum of variation between the three expressions of pesticide residue concentration (Table 7).

TABLE 6. Correlation coefficient (r) data for the relationships between the four pesticide residues quantified.

Residues	n	r	r^2 X 100
DDT vs DDD	182	.753*	56.70
DDT vs DDE	182	.679*	46.10
DDD vs DDE	180	.563*	31.70
dieldrin vs DDT	115	.125	
dieldrin vs DDD	115	.264*	6.97
dieldrin vs DDE	115	.375*	14.06

*Significant at the 0.01 level of significance

TABLE 7. Correlation coefficient (r) data for the relationships between pesticide residue concentrations based on wet weight, dry weight and fat weight.

Weights	n	r	r^2 X 100
Wet vs Dry	182	.977*	95.48
Wet vs Fat	167	.811*	65.77
Dry vs Fat	167	.770*	59.29

*Significant at the 0.01 level of significance

Reinert (1969) stated concentrations based upon fat weight were the most useful in comparisons between fish species in the Great Lakes because chlorinated hydrocarbon pesticide residues were correlated with the amount of fat present. In cases where fat weight and dry weight are both highly variable or significantly different for different species or locations it would be more meaningful to express the residues as ppm fat weight and/or ppm dry weight. But in the situation where the percentage of fat and water are relatively constant, as in this study where water in the eggs averaged 56.72 percent (S.E. ±0.121, n = 158) and fat averaged 10.6 percent (S.E. ±0.126, n = 178) based on the dry weight of the eggs, there appeared to be no advantage in using either dry weight or fat weight over wet weight. On the basis of the points mentioned in the above discussion, residue concentration based upon wet weight was used for the interpretation of the results of this study.

Relationship of pesticide residues to percent fat in the eggs

The relationship between the total DDT, DDD and DDE as µg/egg and percentage ether extractable fat based on the dry wet of the eggs was inconsistent. The samples from the Little Manistee River were the only ones in which a significant positive correlation ($p < 0.05$)

between percent fat and pesticide residue was found (Table 8). The samples from the remaining four streams did not show a significant correlation ($p < 0.05$) between fat and pesticide residue concentration (Table 8). The results of this study indicate the general lack of correlation between pesticide residue concentration and amount of fat in the eggs.

Reinert (1969) reported a direct relationship between the percent fat and the amount of pesticides present in lake trout from Lake Michigan and Lake Superior. Assuming the quantity of fats present and the exposure of the fish are major factors in the concentration of pesticides, all species and sizes of fish exposed to similar pesticide residues should have the same concentration of pesticides in their fats. Reinert (1969) found this relationship existed in three out of four different species of fish from Lake Michigan. Although there were distinct species differences in the amount of fat, the concentration of DDT residues fell within the narrow range of 34 to 38 ppm. The coho salmon eggs from Lake Michigan in this study averaged 36 ppm DDT in the fat, although the coefficient of varibility (CV) ranged from 19 to 40 percent for the four Lake Michigan streams.

A second important factor in the relationship between fats and pesticide residues is the concentration

TABLE 8. The average percentage fat based on dry weight and correlation coefficients for percent fat and total DDT (DDT, DDD and DDE) in µg/egg.

River	n	Average percent fat	Correlation coefficient
Platte River	78	10.2	-0.017
Little Manistee	44	10.9	0.589*
Thompson Creek	10	12.1	-0.028
Bear Creek	34	11.0	0.300
Cherry Creek (Lake Superior)	10	10.9	0.403

*Significant at the 0.05 level of significance

of pesticides in the environment. The rather uniform concentrations of pesticides found in the fat of different fish species indicates an equilibrium with environmental concentrations. This equilibrium is considerably lower than saturation level. If maximum concentrations are assumed to be an equilibrium and not a saturation, then lower pesticide residues in the environment would result in lower concentrations in the fat of fish tissues. The lower pesticide residue concentrations in the eggs of coho salmon from Lake Superior and Oregon (Pacific Ocean) probably reflect the lower concentration of the residues in these environments. The percent fat in the eggs from all three systems were similar (Table 8).

Comparison of pesticide residue levels in coho salmon eggs collected during 1967 and 1968 from Lake Michigan, Lake Superior and Oregon

There were no significant differences ($p < 0.05$) in the average DDT concentrations (ppm wet weight) in salmon egg samples from corresponding streams during 1967 and 1968 (Table 9). The DDT residue concentration in samples from Oregon, Lake Superior and Bear Creek (Lake Michigan) were very similar for 1967 and 1968 (Table 9). The Platte River samples showed the greatest difference between years (Table 9). One possible explanation for this difference is that the coho salmon stocked in this stream were from two different sources.

TABLE 9. Average pesticide residues in coho salmon eggs collected during 1967 and 1968 (1967 data taken from Johnson and Pecor, 1969).

Year	Location	N	DDT	DDD	DDE	Total
1968	LAKE MICHIGAN					
	Thompson Creek	25	1.903 ± 0.125	0.417 ± 0.031	4.517 ± 0.259	6.829 ± 0.352
	Bear Creek	34	1.634 ± 0.050	0.310 ± 0.011	4.243 ± 0.142	6.186 ± 0.198
	Little Manistee R.	44	1.467 ± 0.056	0.323 ± 0.016	3.715 ± 0.149	5.506 ± 0.192
	Platte River	78	1.498 ± 0.039	0.282 ± 0.009	3.729 ± 0.108	5.521 ± 0.146
1967	Bear Creek	10	1.71 ± 0.14	-	-	-
	Platte River	10	1.77 ± 0.16	-	-	-
1968	LAKE SUPERIOR					
	Big Huron River	2	0.220 ± 0.007	0.033 ± 0.007	0.550 ± 0.014	0.802 ± 0.021
	Cherry Creek	10	0.278 ± 0.033	0.069 ± 0.014	0.789 ± 0.105	1.136 ± 0.152
1967	Big Huron River	6	0.270 ± 0.120	-	-	-
1968	OREGON	4	0.019 ± 0.000	0.017 ± 0.000	0.073 ± 0.017	0.109 ± 0.013
1967	OREGON	1	0.01			

The salmon which returned to the Platte River in 1967 were an Oregon strain averaging ten to twelve pounds as adults, but in 1968 a Washington strain returned which averaged seven to ten pounds. The same strains of fish were stocked in Bear Creek and Lake Superior both years.

Dieldrin residues were not quantified for the small group of samples collected in 1967, but Reinert (1969) reported an average dieldrin residue level of 0.16 ppm in the eggs from four coho salmon collected at the Platte River during 1967. The average concentration of dieldrin residues determined in this study ranged between 0.086 to 0.095 ppm in the eggs from Lake Michigan tributaries during 1968. The difference between these values and Reinert's probably reflect different analytical methods although it is possible that higher concentrations were found in 1967 than in 1968.

The concentration of pesticide residues in coho salmon eggs from each of the three major systems, Lake Michigan, Lake Superior and Oregon, were significantly different ($p < 0.01$). Lake Michigan samples had the highest average total pesticide residue of 5.83 ppm, including DDT, DDD, DDE and dieldrin. The average total pesticide residue in Lake Superior samples was 0.97 ppm, which was approximately one-sixth the level in the Lake Michigan samples. Oregon samples had the lowest average total pesticide residue content of 0.11 ppm, approximately

55 times lower than the Lake Michigan samples. Reinert (1969) reported that identical species of fish from Lake Superior had 4 to 7 times less DDT and 2 to 7 times less dieldrin than those from Lake Michigan.

Differences in pesticide residue levels in salmon eggs collected from various streams tributary to Lake Michigan during 1968

There were distinct differences in the pesticide residue content of egg samples from the four streams tributary to Lake Michigan (Thompson Creek, Bear Creek, Platte River and Little Manistee River) (Appendix II). The Thompson Creek samples had significantly higher average DDT concentrations ($p < 0.05$) than the samples from the other Lake Michigan streams. The Bear Creek samples showed the next highest average DDT concentrations although they were not significantly different ($p < 0.05$) from the average DDT residues in the Platte River and Little Manistee River samples. Platte River and Little Manistee samples had almost identical average DDT concentrations.

There was no apparent relationship between the average DDT residues present in the egg samples and the north-south location of the parent streams. Little is known about the migrations of salmon in Lake Michigan but it is expected that they move extensively throughout the lake. It is possible that the different strains,

such as those stocked in Thompson Creek, follow a different migration pattern and are thus exposed to different pesticide levels during their growth. Reinert (1969) reported higher pesticide residues in fish from southern Lake Michigan with relatively lower residues in fish from the northern portions of the lake. It is known that numerous salmon are located in southern Lake Michigan during the spring and summer prior to their spawning runs. If salmon from different streams spend different periods of time in southern Lake Michigan or other areas of different pesticide levels, then they may accumulate different pesticide concentrations.

There is some evidence to show a relationship between the genetic strains of salmon sampled and the average DDT concentrations found in the eggs. Thompson Creek fish were reared from an Alaskan strain, Bear Creek fish were reared from an Oregon strain and Platte River and Little Manistee fish were reared from a Washington strain of coho salmon. The three different strains of salmon all showed different average DDT concentrations. However, the samples from the two streams which had a single genetic strain of salmon had very similar average DDT concentrations. Thompson Creek and Bear Creek samples exhibited higher but different concentrations and the Platte River and Little Manistee samples had lower but almost identical residue

concentrations. The differences in the pesticide residues of the three strains of coho salmon indicates a possible physiological difference in the accumulation of pesticide residues between genetic strains or a different migration routes. This could be related to differences in growth rates.

Dieldrin residues were determined for samples from the Platte River, Bear Creek and Little Manistee River (Appendix II). Dieldrin residues were not determined for Thompson Creek samples because early attempts to analyze for dieldrin were inadequate and resulted in the loss of the residue during the clean-up procedure. There was no pattern in the residue levels for the two strains analyzed. The lack of dieldrin values for Thompson Creek limits the evaluation of these data.

Relationship between fish length and pesticide residues in the eggs

The relationship between the fork length of the adult fish and DDT residue levels in their eggs was inconsistent for the different streams sampled. There was a significant positive correlation ($p < 0.05$) between fish length and DDT residue levels in the eggs of the Platte River samples, while the same comparison for Little Manistee River samples showed a significant negative correlation ($p < 0.05$) (Table 10). The fish length and DDT residues in the eggs of the other streams

sampled, Thompson Creek, Bear Creek and Cherry Creek (Lake Superior) were not significantly correlated (Table 10).

The lack of a significant relationship between fish length and DDT residues in three groups of samples and the opposing significant correlations for the other two groups of samples, suggests there was no relationship between fish length and DDT residue concentration in the eggs. Although it was not statistically significant at the 5 percent level, there was indicated a trend for a decrease in DDT residues with increased fish length.

Other studies have shown both agreement and apparent conflict with the results concerning the relationship between female length and pesticide residue levels in the eggs. Kleinert (1967) stated pesticide residues in the eggs of walleye from Wisconsin lakes did not appear to be related to the length of the females. Reinert (1969) reported DDT and dieldrin residues in body tissues were directly related to the length of lake trout and walleyes collected from Lake Michigan. However, it is likely that ovarian tissues are subject to different physiological constraints than body tissues. The fish analyzed by Reinert also varied over a large size range incorporating several year classes as opposed to the restricted size range and single year class of the present study.

Comparison of the pesticide residue levels in the eggs of residual stream fish and lake run fish

One of the original objectives of this study was to compare the pesticide residue levels of eggs from salmon of different ages. However, scale analysis indicated that all mature females were three years old. A group of small fish collected at the Platte River, averaging two to five pounds, exhibited two annuli indicating a three-year-old fish. A frequency distribution of the lengths of the fish collected at the Platte River was distinctly bimodal, indicating that two independent constraints were acting on the growth rates. One explanation proposed before age determination was that the small fish were precocious females, but the fact that all the fish were three years old eliminated this possibility. A second proposal, which is most compatible with the data, is that the small fish were residual stream fish (remaining in the stream).

A comparison of the average pesticide residues in the large and small fish lends support to the residual stream theory. The group of small fish had higher average DDT residues (1.61 ppm) than the group of larger fish (1.48 ppm), but the difference was not significant ($p < 0.05$). One would expect that fish living in the river for all of their lives would be exposed to higher

pesticide levels and more restricted conditions for growth than the fish in the Great Lakes.

Relationship between pesticide residues in coho salmon eggs and sampling dates

DDT residues in coho salmon eggs, considering the samples from all the Lake Michigan streams together, were significantly correlated ($p < 0.05$) with sampling dates of the 1968 salmon spawning migrations (Table 11). The general trend was for DDT concentrations to decrease with later sampling periods (Figure 12). Samples were collected over a period of approximately 120 days covering the entire spawning run. Dieldrin residues expressed as ppm were not significantly correlated with sampling dates (Table 11), possibly because dieldrin residues were not determined for the early samples.

DDT residue concentrations in the eggs collected weekly at the Platte River for the duration of the run were not significantly correlated ($p < 0.05$) with sampling date (Table 11). However, the samples did not necessarily represent fish that returned to the stream during the week the samples were collected. The movement of the salmon into the Platte River and up to the egg-taking station at Honor, Michigan was controlled by a harvest weir at the mouth of the river. Large numbers of salmon were allowed past the harvest weir only two or three times during the run, so the samples

TABLE 10. The average fork length of the fish sampled and correlation coefficients (r) for fish length and average DDT concentration in their eggs.

River	N	Average length (Cm)	Correlation Coefficient (r)
Platte River	66	66.4	-0.317*
Little Manistee River	45	67.7	0.416*
Thompson Creek	26	58.9	-0.209
Bear Creek	34	67.8	-0.048
Cherry Creek (L.S.)	10	49.7	-0.521

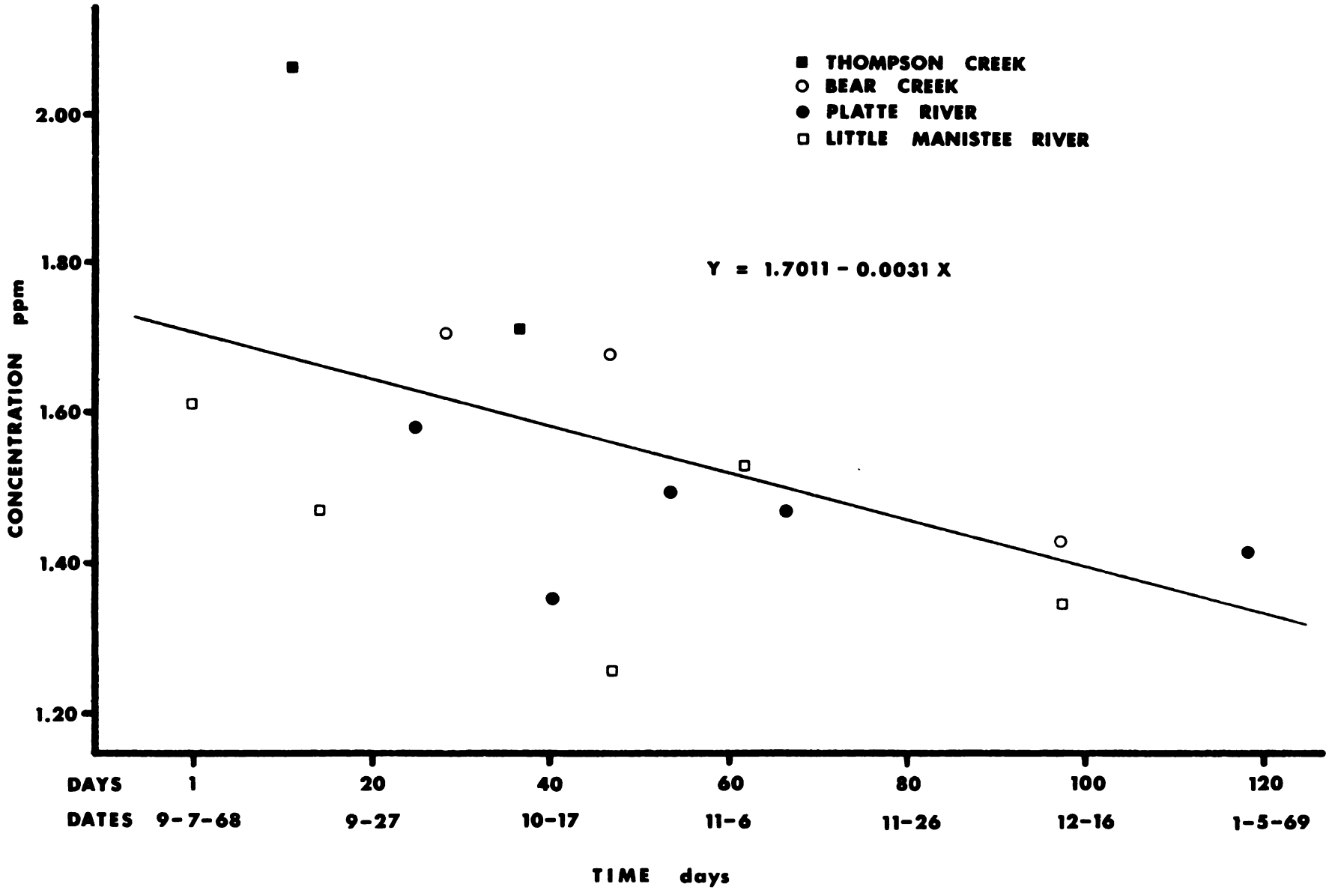
*Significant at the 0.05 level of significance

TABLE 11. Correlation coefficients (r) for the relationship of pesticide residues with sampling dates.

Residue	N	r
All Lake Michigan Samples Together		
DDT vs. sampling date	168	-0.267*
DDD vs. sampling date	168	-0.291
DDE vs. sampling date	168	-0.161*
Dieldrin vs. sampling date	106	-0.150
Platte River Samples Only		
DDT vs. sampling date	63	-0.196

*Significant ($p < 0.05$)

Figure 12. Scatter diagram and slope of regression line for the average DDT residue concentrations in coho salmon eggs from individual streams compared with sampling dates.


THOMPSON CREEK
BEAR CREEK
PLATTE RIVER
LITTLE MANISTEE RIVER
Y = 1.7011 - 0.0031 X
CONCENTRATION ppm
2.00
1.80
1.60
1.40
1.20
DAYS 1 20 40 60 80 100 120
DATES 9-7-68 9-27 10-17 11-6 11-26 12-16 1-5-69
TIME days

collected at the egg-taking station were representative of only two or three groups which had been held in the river for varying amounts of time.

Fish length and percentage fat were not correlated with sampling date, so the relationship between DDT residues and sampling date was not a secondary function of either. A possible biological explanation for the inverse relationship between DDT residues and sampling date is that the later fish remained in the streams where pesticide levels are probably lower during this time of year and have ceased feeding for a longer period of time. The decrease or elimination of the two major sources of pesticides would allow a decrease in the total body burden of pesticides.

Relationship of pesticide residues in coho salmon eggs to the egg and fry mortalities

Egg mortalities did not appear to be related to DDT or dieldrin residue concentrations (ppm wet weight). Correlation coefficients for DDT (-0.128) and dieldrin (-0.225) compared to egg mortalities of all the samples from Lake Michigan streams were not significant ($p < 0.05$). Ten samples from Lake Superior were the controls available during this portion of the study and the mortality of these eggs was similar to the lots of Lake Michigan eggs. No unusual mortality occurred that could not be accounted for. Allison *et al.* (1964) reported that egg lots from

females dosed with various concentrations of DDT had similar survival rates. However, Macek (1968) indicated eggs from females dosed with DDT had a higher mortality than eggs from control fish. The lack of adequate controls in this present study hindered the interpretation of the results.

To test the hypothesis that higher mortalities of coho fry are the result of higher pesticide residue concentrations in the eggs, a correlation analysis of DDT concentrations (ppm wet weight) and fry mortality was calculated (Table 12). No significant correlations ($p < 0.05$) were found between DDT concentrations and fry mortalities in the samples from each of the streams, with the exception of the second group of samples from Thompson Creek. Higher mortalities of these fry were associated with higher pesticide residues in the eggs. Lake Superior fry mortalities also were not significantly correlated to DDT residues (ppm wet weight).

A comparison of the fry mortalities of all sample groups from Lake Michigan to DDT, total DDT (DDT, DDD and DDE) and dieldrin residue concentrations (ppm wet weight) in the eggs showed negative correlations that were not significant ($p < 0.05$) (Table 13). The same comparisons on the basis of μg of DDT, dieldrin and total DDT (DDT, DDD and DDE) in the eggs also showed no significant correlations (Table 13).

TABLE 12. Correlation coefficient (r) data for the relationship between DDT residues (ppm wet weight) in the eggs and fry mortality in samples from each stream.

Source	Sample date	N	r
Thompson Creek	9-17-68	10	-0.492
	10-13-68	8	0.692*
Bear Creek	10-24-68	10	-0.128
	12-13-68	8	-0.223
Little Manistee River	11-7-68	9	-0.211
	12-13-68	7	0.559
Platte River	10-17-68	10	-0.254
	1-4-69	4	-0.844
Lake Superior	11-1-68	10	0.550

*Significant at the 0.05 level of significance

TABLE 13. Correlation coefficient (r) data for the relationship between DDT, dieldrin and total DDT (DDT, DDD and DDE) expressed as ppm wet weight and µg/egg and the total fry mortalities of groups from Lake Michigan.

	n	r*
ppm DDT vs. fry mortality	67	-0.148
µg/egg DDT vs. fry mortality	57	0.056
ppm total DDT vs. fry mortality	67	-0.010
µg/egg total DDT vs. fry mortality	57	0.142
ppm dieldrin vs. fry mortality	45	-0.154
µg/egg dieldrin vs. fry mortality	45	-0.068

*No significant correlations ($p < 0.05$)

On the basis of these correlation analyses there does not appear to be a direct relationship between DDT, dieldrin or total DDT (DDT, DDD and DDE) residues in the eggs and the mortality of the fry. However, these analyses alone are not adequate to assess the relationship between pesticide residues and fry mortality. Further consideration must be given to the interaction between specific pesticide residues in the eggs and to the remaining unidentified residues which were found in the eggs. It would also be interesting to compare the effects of pesticides on specific genetic strains of coho salmon reared under similar conditions.

There are many factors which may influence the effect of pesticide residues on the development of fry. Burdick et al. (1964) in a study of lake trout fry mortality from a number of lakes determined that a mortality syndrome occurred in fry groups reared from eggs which had a total DDT concentration of 4.75 ppm wet weight (combined ether and methanol extracts) or higher based on the Schechter Haller method. The combined DDT and DDD residue concentrations (ether extract) in the eggs of the present study are generally less than half this critical value, which would suggest no mortality resulting from pesticide contamination. Cuerrier et al. (1967) reported DDT and metabolites (p,p'-DDT, o,p'-DDT, DDD and DDE) concentrations of

grea
mort
valu
eve
the
cal
The
cid
on
qua
cri
stu

ci
of
pe
de
re
of
di
ci
bl
th
st

Cu

greater than 400 ppb wet weight in eggs resulted in fry mortalities ranging from 30 to 90 percent. The critical value of 400 ppb is below the total DDT concentrations of even Lake Superior eggs which had approximately one-sixth the residue concentrations of Lake Michigan eggs. Critical values are probably not reliable as a general rule. The number of parameters affecting the toxicity of pesticide residues, such as fish species, temperature, stress on the fish, resistance to pesticides and general water quality, are too variable in individual systems for critical values to be determined and applied to separate studies.

The lack of a direct relationship between pesticide residues and fry mortality suggest that the majority of Lake Michigan coho salmon have not exceeded a critical pesticide residue level, but based on circumstantial evidence some of the fry mortalities are probably the result of pesticide residues. The timing and symptoms of the fry mortality are of particular interest. Burdick et al. (1964) reported fry mortalities due to pesticide contamination were characterized by distended air bladders and air in the intestinal tract. Although these symptoms were not major characteristic in this study, they were present.

Burdick et al. (1964), Allison et al. (1964), Cuerrier et al. (1967) and Macek (1968) reported that

fry mortalities occurred during final stages of yolk absorption at a time when the fry were just starting to feed. The mortalities in this study occurred at a similar time in development. At the time of this mortality, little or no external evidence of yolk-sac was visible in the fry, but yolk and lipid material were still present within the gut. Separate analyses of composite samples of eviscerated fry and gut tissues showed that gut tissues had approximately 6 to 12 times higher concentrations of DDT than the eviscerated fry. It was hypothesized that pesticide residues are stored in the triglyceride lipids which are not utilized by the fry until the final yolk absorption (Smith, 1957). The metabolism of these lipids could result in toxic levels of pesticides entering the circulatory system. This would explain the abrupt onset of mortality just prior to the initial feeding stage.

The results of this study do not provide a satisfactory explanation for the cause of mortality among the Lake Michigan coho salmon fry. A comparison of pesticide residue concentrations in eggs of salmon from Lake Michigan, Lake Superior and Oregon indicates the mortality is associated with the higher pesticide residues, but the relationship does not hold for samples taken within the Lake Michigan system alone. After assessing the data collected in this study it is apparent that the factor(s)

causing the mortality is more than a simple critical threshold level of one specific pesticide. Thus the interaction of pesticide residues, possible PCB residues or other unidentified toxic materials may be important.

SUMMARY

1. Samples of coho salmon eggs were collected during 1967 and 1968 from four Lake Michigan streams, Bear Creek, Little Manistee River, Platte River and Thompson Creek; two Lake Superior streams, Big Huron River and Cherry Creek and one Oregon source.

2. Pesticide residues in coho salmon eggs were determined and tested for their relationship to the length of parent fish, sampling dates, location of sampling sites, percentage fat in the eggs and egg and fry mortality.

3. Four pesticide residues were identified and quantified in each sample: p,p'-DDT, p,p'-DDD, p,p'-DDE and dieldrin.

4. Compounds were found that interferred with the analysis of DDT in samples from all three sources. The interference resulted an average error of 19.8 percent in the quantification of DDT residues. These compounds were believed to be polychlorinated biphenyls (PCB's).

5. Lake Michigan coho eggs had the highest average total pesticide concentration of 5.83 ppm. The average total pesticide concentration in Lake Superior eggs was 0.97 ppm, which was approximately one-sixth the level in Lake Michigan eggs. Oregon coho eggs had the lowest average total pesticide residue content of 0.11 ppm, approximately 55 times lower than Lake Michigan eggs.

6. Among the Lake Michigan streams, the Little Manistee River and Platte River egg samples had almost identical concentrations of pesticide residues, Bear Creek samples were slightly higher and Thompson Creek samples had the highest pesticide residue concentrations.

7. The type and concentration of pesticide residues found in 1968 samples were very similar to those found in the 1967 samples.

8. There was no relationship between the length of the parent fish or the amount of fat present in the eggs and the pesticide residue concentration in the eggs.

9. Egg mortality was similar for both Lake Michigan and Lake Superior, and no relationship with pesticide residues was found.

10. The mortality of the fry from Lake Michigan, Lake Superior and Oregon, in actual numbers, was similar; but the symptoms, timing and causative agent of the mortalities were different. One group of samples from Thompson Creek, Lake Michigan, was the only group to show a significant positive relationship between fry mortality and pesticide residues. Circumstantial evidence, including the timing of the mortality, specific symptoms associated with the mortality of the Lake Michigan fry and the higher concentration of pesticide residues in the Lake Michigan coho eggs, indicates the mortality may be associated with pesticide toxicity.

LITERATURE CITED

LITERATURE CITED

Allison, D., B. J. Kallman, O. B. Cope and C. C. Van Valen. 1964. Some chronic effects of DDT on cutthroat trout. U.S. Bureau Sport. Fish and Wildl., Res. Rept. 64 30p.

Anderson, R. B. and W. H. Everhart. 1966. Concentrations of DDT in landlocked salmon (Salmo salar) at Sebago Lake, Maine. Trans. Am. Fish Soc. 95:160-164.

Boyd, C. E. 1964. Insecticides cause mosquitofish to abort. Progve. Fish. Cult. 26, 138.

Brown, J. R. and H. Hughes. 1969. Pesticide levels in wildlife located in the region of the Great Lakes. 12th Conf. on Great Lakes Res. (Abstracts) Ann Arbor, Michigan.

Burdick, G. E., E. J. Harris, H. J. Dean, T. M. Walker, J. Skea and D. Colby. 1964. The accumulation of DDT in lake trout and the effect on reproduction. Trans. Am. Fish. Soc. 93(2):127-136.

Carr, John F. and Robert E. Reinert. 1968. DDT and dieldrin in Great Lakes fish. 11th. Conf. on Great Lakes Res. Milwaukee, Wisconsin.

Currier, J. P., J. A. Keith, and E. Stone. 1967. Problems with DDT in fish culture operations. Le Naturaliste Canadian, Vol. 94:315-320.

Federal Water Pollution Control Administration, Great Lakes Region. 1968. Water pollution problems of Lake Michigan and tributaries, Report of Conference (Jan. 1968) 74pp.

Henderson, Croswell, Q. H. Pickering, and C. M. Tarywell. 1959. The toxicity of organic phosphorus and chlorinated hydrocarbon insecticides to fish. Trans. 2nd Seminar on Biol. Prob. in Water Poll. U.S. Public Health Serv., Robert A. Taft Sanitary Engineering Center. Cincinnati 26, Ohio.

Hickey, J. J., J. A. Keith and F. B. Coon. 1966. An exploration of pesticides in a Lake Michigan ecosyslem. J. Appl. Ecol. 3:141-154.

Holmes, D. C., J. H. Simmons, J. O'G. Tatton. 1967. Chlorinated hydrocarbons in British Wildlife. Nature. 216:227-229.

Johnson, H. E. 1967. Effects of endrin on reproduction in a freshwater fish (Oryzias latipes). Ph.D. Thesis. Univ. of Wash. Seattle, Washington. 149p.

Johnson, H. E. and C. Pecor. 1969. Coho salmon mortality and DDT in Lake Michigan. Proc. 34th N. Am. Wildl. and Nat. Res. Conf. 8p.

Katz, Max. 1961. Acute toxicity of some organic insecticide to three species of salmonids and to the threespine stickleback. Trans. Am. Fish. Soc. 90:264-268.

King, G. T. 1962. Some effects of DDT on the guppy and the brown trout. Bur. Sport. Fish and Wildl. Spec. Sci. Rept., Fish. No. 399.

Kleinert, Stanton J. 1967. Survival of walleyes from eggs of known DDT and dieldrin residues in three southeastern Wisconsin lakes. Wis. Dept. of Nat. Res., Tech. Bull. No. 21. 9p.

Koeman, J. H., M. C. TenNoever DeBruw, R. H. DeVos. 1969. Chlorinated biphenyls in fish, mussels and birds from the River Rhine and the Netherlands coastal areas. Nature Vol. 221.

Kovacs, M. F. Jr. 1963. Thin-layer chromatography for chlorinated pesticide residue analysis. J.A.O.A.C. 46:884.

Lichtenstein, E. P., K. R. Schultz, T. W. Fuhremann, and T. T. Liang. 1969. Biological interaction between plasticizers and insecticides. J. of Econ. Ent. 62(4):761-765.

Macek, K. J. 1968. Reproduction in brook trout (Salvelinus fontinalis) fed. sublethal concentrations of DDT. J. Fish. Res. Bd., Can. 25(9):1787-1796.

Michigan Department of Natural Resources. 1968. Interoffice communications. Unpublished Hatchery Reports.

Mills, P. A. 1959. Detection and Semiquantitative estimation of chlorinated organic pesticide residues in foods by paper chromatography. J.A.O.A.C. 42:734-739.

Mills, P. A. 1961. Collaborative study of certain chlorinated organic pesticides in dairy products. J.A.O.A.C. 44:171.

Mount, Donald I. 1962. Chronic effects of endrin on bluntnose minnows and guppies. Fish. Wildl. Serv., Bur. Sport Fish. Wildl., Res. Rept. 58:1-38.

O'Brien, R. D. 1967. Insecticides--Action and Metabolism. Acad. Press. New York, N.Y. 332pp.

Reinert, R. E. 1969. Summary of available information from the Bureau of Commercial Fisheries, Ann Arbor Biological Laboratory on pesticide levels in Great Lakes fish. Unpublished manuscript.

Reynolds, Lincoln M. 1969. Polychlorobiphenyls (PCB's) and their interference with pesticide residue analysis. Bulletin of Environmental Contamination and Toxicology. 4(3):128-143.

Rounsefell, George A. and W. R. Everhart. 1953. Fishery Science, Its Methods and Application. John Wiley and Son Inc., New York. 444pp.

Smith, S. 1957. Early development and hatching. In Brown, M. E. (ed.). The Physiology of Fishes, Vol. I, p. 323-360. Acad. Press, New York, N.Y.

Snedecor, George W. 1956. Statistical Methods. Iowa State College Press, Ames, Iowa. 534pp.

Sokal, Robert R. and F. James Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco, Calif. 776pp.

Veith, G. D. 1970. Environmental chemistry of the chlorobiphenyls in the Milwaukee River. Ph.D. Thesis. Univ. of Wis., Madison, Wis. 180p.

APPENDICES

APPENDIX I

Reagent Purification Procedures:

1. Petroleum ether

   Fisher brand, reagent grade, 30-60°C boiling range petroleum ether was used throughout most of the study. Several other brands were tried, but they frequently smelled foul and contained artifacts which could not be removed. The petroleum ether was purified by refluxing 3 liters of the solvent with 10 grams of sodium-lead granules (Dri-Na) for 3 to 4 hours. The solvent was slowly distilled in an all glass system, discarding the first 50 ml of distillate and collecting the remaining fractions up to 50°C.

2. Ethyl ether

   Fisher brand, reagent grade ethyl ether was twice redistilled in an all glass system prior to use. It was observed that the addition of 2 percent by volume ethyl alcohol greatly facilitated the elution of dieldrin from Florisil columns.

3. Benzene

   Fisher brand, reagent grade benzene was refluxed over freshly cut sodium, decanted and slowly distilled at 80°C in an all glass system.

4. Acetonitrile

   Fisher brand, reagent grade acetonitrile was purified weekly as required. The solvent was purified by refluxing for 4 hours with 85 percent H_3PO_4 and P_2O_5, added in the proportions of 1 ml acid and 30 grams pentoxide to 4 liters of acetonitrile slowly distilling and collecting the fraction distilling at 81 to 82°C.

APPENDIX II. The p,p'-DDT, DDD, DDE, total DDT (DDT, DDD, DDE) and dieldrin residue concentrations in the eggs of coho salmon expressed as ppm wet weight, ppm dry weight, and ppm lipid weight for the dates each of the streams were sampled.

Location	Date Sampled	DDT Residue Concentration ($\bar{Y}$ + SE)				
		N	Wet weight	Dry weight	(N)	Fat weight
LAKE MICHIGAN						
Thompson Creek	9-17-68	14	2.052 ± 0.167	5.243 ± 0.433	(5)	43.790 ± 4.492
	10-13-68	11	1.713 ± 0.181	4.369 ± 0.475		38.072 ± 5.385
	Average	25	1.903 ± 0.125	4.859 ± 0.326	(16)	39.859 ± 3.929
Bear Creek	10-3-68	12	1.703 ± 0.097	3.830 ± 0.215		33.493 ± 2.016
	10-24-68	12	1.696 ± 0.073	3.957 ± 0.156		36.482 ± 2.004
	12-13-68	10	1.475 ± 0.075	3.436 ± 0.177		32.731 ± 1.568
	Average	34	1.634 ± 0.050	3.750 ± 0.110		34.324 ± 1.108
Little Manistee River	9-7-68	12	1.641 ± 0.149	3.830 ± 0.329	(6)	28.443 ± 2.932
	9-20-68	6	1.470 ± 0.120	3.333 ± 0.236		34.187 ± 2.365
	10-24-68	7	1.266 ± 0.094	2.865 ± 0.228		32.701 ± 2.684
	11-7-68	9	1.513 ± 0.124	3.469 ± 0.292		32.322 ± 1.812
	12-13-68	10	1.340 ± 0.050	3.181 ± 0.133		31.094 ± 1.519
	Average	44	1.467 ± 0.056	3.388 ± 0.127	(38)	31.750 ± 0.057
Platte River	9-19 to 10-10-68	23	1.584 ± 0.085	3.618 ± 0.178		38.007 ± 1.981
	10-17-68	10	1.331 ± 0.111	3.154 ± 0.253		41.020 ± 4.163
	10-24 to 11-8-68	12	1.469 ± 0.093	3.408 ± 0.203		31.125 ± 2.336
	11-14-68	10	1.451 ± 0.103	3.380 ± 0.236		32.396 ± 1.827
	1-4-69	10	1.417 ± 0.049	3.292 ± 0.117		30.783 ± 1.196
	11-14-68*	13	1.617 ± 0.088	3.781 ± 0.228		33.971 ± 2.091
	Average	78	1.498 ± 0.039	3.474 ± 0.087		34.840 ± 1.053
LAKE SUPERIOR						
Cherry Creek	11-1-68	10	0.278 ± 0.033	0.726 ± 0.081		7.099 ± 0.819
Big Huron River	1-20-69	2**	0.220 ± 0.007	0.633 ± 0.023		7.066 ± 0.267
OREGON	1-4-69	4**	0.019 ± 0.000	0.065 ± 0.000		0.544 ± 0.038

APPENDIX II. (Cont.)

Location	Date Sampled	DDD Residue Concentration ($\bar{Y}$ ± SE)				
		N	Wet weight	Dry weight	(N)	Fat weight
LAKE MICHIGAN						
Thompson Creek	9-17-68	14	0.327 ± 0.021	0.870 ± 0.061	(5)	7.712 ± 0.991
	10-13-68	11	0.532 ± 0.048	1.359 ± 0.128		11.876 ± 1.511
	Average	25	0.417 ± 0.031	1.085 ± 0.081		10.575 ± 1.173
Bear Creek	10-3-68	12	0.296 ± 0.018	0.666 ± 0.038		5.836 ± 0.366
	10-24-68	12	0.334 ± 0.021	0.776 ± 0.048		7.217 ± 0.634
	12-13-68	10	0.299 ± 0.018	0.695 ± 0.043		6.607 ± 0.382
	Average	34	0.310 ± 0.011	0.713 ± 0.026		6.550 ± 0.291
Little Manistee River	9-7-68	12	0.440 ± 0.032	1.027 ± 0.069		7.858 ± 0.693
	9-20-68	6	0.300 ± 0.026	0.676 ± 0.059		6.880 ± 0.457
	10-24-68	7	0.258 ± 0.027	0.584 ± 0.065		6.549 ± 0.555
	11-7-68	9	0.269 ± 0.031	0.617 ± 0.071		5.812 ± 0.626
	12-13-68	10	0.293 ± 0.013	0.692 ± 0.032		6.762 ± 0.354
	Average		0.323 ± 0.016	0.749 ± 0.038	(38)	6.689 ± 0.251
Platte River	9-19 to 10-10-68	23	0.297 ± 0.018	0.677 ± 0.037		7.125 ± 0.445
	10-17-68	10	0.254 ± 0.021	0.605 ± 0.050		8.145 ± 0.885
	10-24 to 11-8-69	12	0.276 ± 0.017	0.642 ± 0.040		5.923 ± 0.502
	11-14-68	10	0.223 ± 0.032	0.519 ± 0.073		4.921 ± 0.624
	1-4-69	10	0.258 ± 0.006	0.598 ± 0.015		5.603 ± 0.199
	11-14-68*	13	0.347 ± 0.019	0.809 ± 0.049		7.255 ± 0.414
	Average	78	0.282 ± 0.009	0.654 ± 0.021		6.600 ± 0.244
LAKE SUPERIOR						
Cherry Creek	11-1-68	10	0.069 ± 0.014	0.179 ± 0.038		1.706 ± 0.390
Big Huron River	1-20-69	2**	0.033 ± 0.007	0.094 ± 0.007		1.048 ± 0.070
OREGON	1-4-69	4**	0.017 ± 0.000	0.055 ± 0.000		0.449 ± 0.046

APPENDIX II. (Cont.)

Location	Date Sampled	DDE Residue Concentration ($\bar{Y}$ ± SE)				
		N	Wet weight	Dry weight	(N)	Fat weight
LAKE MICHIGAN						
Thompson Creek	9-17-68	14	4.062 ± 0.216	10.358 ± 0.535	(5)	98.515 ± 12.022
	10-13-68	11	5.087 ± 0.479	12.960 ± 1.248		111.847 ± 12.823
	Average	25	4.513 ± 0.259	11.503 ± 0.665	(16)	107.681 ± 9.484
Bear Creek	10-3-68	12	4.416 ± 0.267	9.923 ± 0.592		87.073 ± 5.787
	10-24-68	12	4.496 ± 0.218	10.447 ± 0.494		96.417 ± 6.044
	12-13-68	10	3.730 ± 0.185	8.687 ± 0.435		82.634 ± 3.907
	Average	34	4.243 ± 0.142	9.745 ± 0.317		89.065 ± 3.233
Little Manistee River	9-7-68	12	3.789 ± 0.405	8.847 ± 0.918		72.534 ± 11.644
	9-20-68	6	3.927 ± 0.307	8.858 ± 0.676		90.784 ± 6.507
	10-24-68	7	3.203 ± 0.273	7.202 ± 0.640		81.042 ± 4.857
	11-7-68	9	4.079 ± 0.363	9.357 ± 0.835		87.360 ± 6.570
	12-13-68	10	3.530 ± 0.120	8.283 ± 0.315		81.280 ± 3.853
	Average	44	3.715 ± 0.149	8.563 ± 0.343	(38)	82.796 ± 2.924
Platte River	9-19 to 10-10-68	23	3.990 ± 0.249	9.136 ± 0.548		96.623 ± 6.435
	10-17-68	10	3.477 ± 0.253	8.241 ± 0.573		106.991 ± 9.549
	10-24 to 11-8-68	12	3.976 ± 0.283	9.207 ± 0.632		84.150 ± 6.901
	11-14-68	10	3.706 ± 0.229	8.672 ± 0.511		83.261 ± 3.977
	1-4-69 *	10	3.521 ± 0.144	8.186 ± 0.349		76.859 ± 4.221
	11-14-68*	13	3.463 ± 0.259	8.093 ± 0.644		72.173 ± 5.201
	Average	78	3.729 ± 0.108	8.651 ± 0.243		87.198 ± 3.011
LAKE SUPERIOR						
Cherry Creek	11-1-68	10	0.789 ± 0.105	2.059 ± 0.265		19.289 ± 2.793
Big Huron River	1-20-69	2**	0.550 ± 0.014	1.583 ± 0.041		17.677 ± 0.466
OREGON	1-4-69	4**	0.073 ± 0.017	0.243 ± 0.022		1.963 ± 0.113

APPENDIX II (Cont.)

Location	Date Sampled	Total DDT DDD and DDE Residue Concentration ($\bar{Y}$ ± SE)				
		N	Wet weight	Dry weight	(N)	Fat weight
LAKE MICHIGAN						
Thompson Creek	9-17-68	14	6.441 ± 0.340	16.014 ± 1.047	(5)	150.018 ± 16.039
	10-13-68	11	7.324 ± 0.665	18.688 ± 1.751		161.773 ± 18.927
	Average	25	6.829 ± 0.352	17.191 ± 0.984	(16)	158.101 ± 13.697
Bear Creek	10-3-68	12	6.416 ± 0.373	14.420 ± 0.825		126.402 ± 8.007
	10-24-68	12	6.526 ± 0.304	15.181 ± 0.675		140.283 ± 8.526
	12-13-68	10	5.503 ± 0.272	12.817 ± 0.640		121.971 ± 5.701
	Average	34	6.186 ± 0.198	14.217 ± 0.441		129.998 ± 4.533
Little Manistee River	9-7-68	12	5.896 ± 0.468	13.703 ± 1.034	(6)	108.835 ± 12.291
	9-20-68	6	5.707 ± 0.442	12.867 ± 0.949		131.851 ± 9.071
	10-24-68	7	4.728 ± 0.383	10.651 ± 0.905		120.292 ± 7.634
	11-7-68	9	5.812 ± 0.498	13.444 ± 1.155		125.383 ± 8.624
	12-13-68	10	5.173 ± 0.182	12.156 ± 0.469		119.136 ± 5.635
	Average	44	5.506 ± 0.192	12.699 ± 0.441	(38)	121.210 ± 3.721
Platte River	9-19 to 10-10-68	23	5.871 ± 0.339	13.431 ± 0.733		141.755 ± 8.637
	10-17-68	10	5.062 ± 0.382	11.608 ± 1.062		156.156 ± 14.412
	10-24 to 11-8-68	12	5.721 ± 0.384	12.974 ± 0.876		121.198 ± 9.518
	11-14-68	10	5.381 ± 0.336	12.571 ± 0.755		120.578 ± 5.603
	1-4-69	10	5.196 ± 0.168	12.592 ± 0.670		113.244 ± 5.211
	11-14-68*	13	5.427 ± 0.341	12.682 ± 0.865		113.398 ± 7.063
	Average	78	5.521 ± 0.146	12.784 ± 0.343		129.342 ± 4.073
LAKE SUPERIOR						
Cherry Creek	11-1-68	10	1.136 ± 0.152	2.965 ± 0.379		28.093 ± 3.907
Big Huron River	1-20-69	2**	0.802 ± 0.021	2.309 ± 0.065		25.791 ± 0.727
OREGON	1-4-69	4**	0.109 ± 0.013	0.364 ± 0.017		2.955 ± 0.084

APPENDIX II. (Cont.)

Location	Date Sampled	N	Dieldrin Residue Concentration ($\bar{Y}$ ± SE) Wet weight	Dry weight	Fat weight
LAKE MICHIGAN					
Thompson Creek		25	Present but not quantified		
Bear Creek	10-24-68	12	0.097 ± 0.006	0.224 ± 0.013	2.062 ± 0.123
	12-13-68	10	0.088 ± 0.005	0.205 ± 0.013	1.937 ± 0.120
	Average	22	0.093 ± 0.004	0.215 ± 0.009	2.005 ± 0.085
Little Manistee River	10-24-68	7	0.074 ± 0.007	0.168 ± 0.016	1.952 ± 0.261
	11-7-68	5	0.089 ± 0.012	0.201 ± 0.029	2.104 ± 0.189
	12-13-68	10	0.094 ± 0.009	0.210 ± 0.017	2.005 ± 0.156
	Average	22	0.086 ± 0.005	0.195 ± 0.011	2.010 ± 0.112
Platte River	9-19 to 10-10-68	21	0.118 ± 0.010	0.264 ± 0.023	2.765 ± 0.202
	10-17-68	10	0.085 ± 0.008	0.202 ± 0.020	2.592 ± 0.281
	10-24 to 11-8-68	11	0.084 ± 0.004	0.193 ± 0.010	1.739 ± 0.126
	11-14-68	10	0.075 ± 0.004	0.175 ± 0.013	1.693 ± 0.129
	1-4-69	10	0.096 ± 0.005	0.222 ± 0.014	2.078 ± 0.141
	11-14-68*	8	0.079 ± 0.007	0.178 ± 0.018	1.716 ± 0.238
	Average	70	0.095 ± 0.004	0.215 ± 0.009	2.208 ± 0.099
LAKE SUPERIOR					
Cherry Creek	11-1-68	10	0.023 ± 0.003	0.058 ± 0.011	0.526 ± 0.114
Big Huron River	1-20-69	2**	0.009 ± 0.000	0.027 ± 0.000	0.301 ± 0.000
OREGON	1-4-69	4**	0.004 ± 0.000	0.013 ± 0.002	0.111 ± 0.020

*Group of residual stream fish

**Analyses of one composit sample

APPENDIX III. The p,p' DDT, DDD, DDE, total DDT (DDT, DDD, DDE) and dieldrin residue concentrations in the eggs of coho salmon expressed as µg/egg for the dates each of the streams were sampled.

Location	Date Sampled	Residue Concentrations (X ± SE)			
		N	DDT	DDD	DDE
LAKE MICHIGAN					
Thompson Creek	9-17-68	4	0.350 ± 0.024	0.059 ± 0.001	0.638 ± 0.076
	10-13-68	11	0.334 ± 0.045	0.103 ± 0.011	0.979 ± 0.106
	Average	15	0.338 ± 0.033	0.091 ± 0.009	0.888 ± 0.088
Bear Creek	10-3-68	12	0.321 ± 0.019	0.056 ± 0.001	0.834 ± 0.055
	10-24-68	12	0.326 ± 0.019	0.063 ± 0.003	0.857 ± 0.053
	12-13-68	10	0.303 ± 0.019	0.062 ± 0.003	0.779 ± 0.042
	Average	34	0.317 ± 0.011	0.060 ± 0.002	0.826 ± 0.030
Little Manistee River	9-20-68	6	0.270 ± 0.024	0.054 ± 0.001	0.716 ± 0.066
	10-24-68	7	0.261 ± 0.028	0.054 ± 0.007	0.675 ± 0.085
	11-7-68	9	0.308 ± 0.022	0.055 ± 0.005	0.831 ± 0.071
	12-13-68	10	0.296 ± 0.014	0.065 ± 0.001	0.774 ± 0.037
	Average	32	0.287 ± 0.011	0.058 ± 0.002	0.757 ± 0.032
Platte River	9-19 to 10-10-68	23	0.278 ± 0.014	0.052 ± 0.002	0.710 ± 0.045
	10-17-68	10	0.288 ± 0.019	0.055 ± 0.001	0.755 ± 0.041
	10-24 to 11-8-68	12	0.303 ± 0.019	0.057 ± 0.003	0.824 ± 0.064
	11-14-68	10	0.297 ± 0.011	0.045 ± 0.004	0.767 ± 0.036
	1-4-69	10	0.291 ± 0.017	0.055 ± 0.001	0.753 ± 0.044
	11-14-68*	13	0.245 ± 0.019	0.052 ± 0.003	0.519 ± 0.055
	Average	78	0.283 ± 0.007	0.053 ± 0.001	0.717 ± 0.023
LAKE SUPERIOR					
Cherry Creek	11-1-68	10	0.037 ± 0.001	0.009 ± 0.0	0.103 ± 0.008
Big Huron River	1-20-69	2**	0.033 ± 0.000	0.005 ± 0.0	0.081 ± 0.001
OREGON	1-4-69	4**	0.004 ± 0.0	0.004 ± 0.0	0.017 ± 0.0

APPENDIX III. (Cont.)

Location	Date Sampled	Residue Concentrations (X ± SE)			
		N	Total	(N)	Dieldrin
LAKE MICHIGAN					
Thompson Creek	9-17-68	4	1.047 ± 0.071		-
	10-13-68	11	1.417 ± 0.157		-
	Average	15	1.318 ± 0.123		-
Bear Creek	10-3-68	12	1.212 ± 0.077		-
	10-24-68	12	1.247 ± 0.076		0.019 ± 0.001
	12-13-68	10	1.149 ± 0.062		0.018 ± 0.001
	Average	34	1.205 ± 0.041		0.019 ± 0.001
Little Manistee River	9-20-68	6	1.039 ± 0.093		-
	10-24-68	7	0.991 ± 0.119		0.015 ± 0.001
	11-7-68	9	1.194 ± 0.095	(5)	0.018 ± 0.001
	12-13-68	10	1.135 ± 0.054		0.019 ± 0.001
	Average	32	1.102 ± 0.045	(22)	0.018 ± 0.001
Platte River	9-19 to 10-10-68	23	1.040 ± 0.061	(21)	0.020 ± 0.002
	10-17-68	10	1.098 ± 0.063		0.018 ± 0.001
	10-24 to 11-8-68	12	1.184 ± 0.085	(11)	0.017 ± 0.001
	11-14-68	10	1.110 ± 0.046		0.015 ± 0.001
	1-4-69	10	1.109 ± 0.058		0.020 ± 0.001
	11-14-68*	13	0.830 ± 0.075	(8)	0.011 ± 0.001
	Average	78	1.053 ± 0.030	(70)	0.018 ± 0.001
LAKE SUPERIOR					
Cherry Creek	11-1-68	10	0.149 ± 0.012		0.003 ± 0.000
Big Huron River	1-20-69	2**	0.119 ± 0.000		0.001 ± 0.000
OREGON	1-4-69	4**	0.025 ± 0.001		0.001 ± 0.000

*Group of residual streamfish

**Analyses of one composite sample