THE INFLUENCE OF CHLORINATED HYDROCARBON RESIDUES AND REARING TEMPERATURE ON SURVIVAL AND GROWTH OF COHO SALMON AND RAINBOW TROUT SAC FRY

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ABSTRACT

THE INFLUENCE OF CHLORINATED HYDROCARBON RESIDUES AND REARING TEMPERATURE ON SURVIVAL AND GROWTH OF COHO SALMON AND RAINBOW TROUT SAC FRY

By

James Edward Johnson

Coho salmon fry from Lake Michigan parent stock incurred greater mortalities from hatching to the time they began feeding than did fry from Lake Huron or Oregon stocks ($p<.05$). Mortality among the Lake Michigan fry was less when reared at 13 C than at 5 C, 9 C or 17 C ($p<.05$). At colder temperatures development of Lake Michigan fry was delayed to a greater extent than was that of fry from the other sources. Pesticide analyses revealed higher levels of DDT residues in Lake Michigan eggs and fry than in the other salmon stocks. Significant amounts of other residues were also present in fry of this group.

Lake Michigan rainbow trout fry exposed to DDT at time of hatching displayed no greater mortality at any of the four rearing temperatures tested than did control fry.

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James Edward Johnson

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PART I

THE INFLUENCE OF CHLORINATED HYDROCARBON RESIDUES AND REARING TEMPERATURE ON SURVIVAL AND GROWTH OF COHO SALMON SAC FRY

INTRODUCTION

In recent years unusually large mortalities have been reported for fry of steelhead and coho salmon of Lake Michigan parent stock. The losses have been most severe during the sac fry stage shortly prior to the time of feeding. Neither disease nor adverse rearing conditions appear to be at the source of the losses and their cause remains without satisfactory explanation (Johnson and Pecor, 1969; Willford, 1969).

However, DDT has been shown to lead to similar losses of fry when present in sufficient quantities in the eggs (Macek, 1968; Allison et al., 1964; Burdick et al., 1964). Eggs from otherwise healthy adult females apparently may contain levels that can prove toxic to the fry. Most of the residues appear to be stored in the yolk material of the fry until late in the sac fry stage. When the last of the yolk material is absorbed by the embryo a large proportion of the residues is mobilized (Atchison, 1970). This release of DDT apparently may constitute a lethal dosage for heavily contaminated fry (Macek, 1968; Burdick, 1964).

Reports of DDT contamination of the Great Lakes have become a matter of serious concern. Hickey et al. (1966)

found evidence of DDT contamination at all trophic levels of western Lake Michigan. Reinert (1970) reported levels of DDT residues in flesh and eggs of Lake Michigan coho salmon that, on the basis of findings of other investigations, he suggested were approaching levels that could adversely affect reproduction. Johnson and Pecor (1969) reported that higher levels of DDT residues in eggs of Lake Michigan coho salmon were generally associated with greater losses of fry.

However, there is only circumstantial evidence to show that DDT levels presently in fish of the Great Lakes can in fact be damaging to their reproduction. Furthermore, residues of other substances similar in nature to DDT have been detected in Great Lakes fish (Armour and Burk, 1970; Veith, 1970). Mixtures of chlorinated hydrocarbons known collectively as polychlorinated biphenyls have been found in quantities equal to or exceeding those of DDT and its derivatives in fish of Lake Michigan. Although little is known of the long term effects of the polychlorinated biphenyls, it appears likely that they may pose a threat similar to that of DDT to the reproduction of fish and other wildlife (Gustafson, 1970).

The present study was undertaken to further evaluate the relationship between the level of pesticide residue contamination and losses of coho salmon fry. An investigation of the influence of rearing temperature on fry survival was

also conducted with the objective of discovering a range of rearing temperatures at which losses of Great Lakes salmon fry could be minimized.

MATERIALS AND METHODS

EGG COLLECTIONS

Eggs of three parent stocks of coho salmon were collected during the fall of 1970.

On 3 November, approximately 12,000 recently fertilized eggs of Lake Michigan parent stock were obtained from the Michigan Department of Natural Resources Platte River egg taking station. Fertilization had been carried out by Department personnel during routine egg taking operations. These eggs were dipped from a large container in which eggs of roughly 25 females were held.

On 17 November, approximately 10,000 eggs were collected from Lake Huron coho salmon taken by fishermen on the Thunder Bay River. The eggs were stripped from six female salmon while the fish were still alive. Milt was added to the eggs about 3 minutes prior to the addition of water. Two male salmon were used to fertilize the eggs of each female. A total of 12 male salmon were therefore employed.

Eggs of Oregon parent stock were shipped to this laboratory when at the eyed stage of development. These eggs were from Oregon's Fall Creek Hatchery, which lies in the Alsea River watershed. The estimated fertilization date was 17 November.

A fourth egg lot was to contain augmented levels of DDT. Up to 4 mg technical grade DDT (77% p-p' isomer) was injected into the body cavities of four ripe coho salmon. The DDT was administered in suspension with 9 ml Ringers Solution. The treated females were then held for 42 hours prior to egg taking. All salmon survived the holding period although one individual developed obvious signs of distress. Following fertilization, samples were taken for pesticide analysis, which revealed no measurable change in the DDT composition of these eggs from that of untreated Lake Michigan egg samples. The treatment procedure was therefore considered unsuccessful and the treated group of eggs was discarded from the investigation.

Following fertilization, eggs of both Great Lakes sources were transported to the laboratory in 1-quart glass jars filled with water and half-filled with eggs. The eggs were then placed on the bottoms of 5-gallon aquaria where they were incubated at 9 C with a flow-through water supply. About 250-300 eggs were placed in each tank. All egg transfers were performed under water. Following hatching, fry numbers were thinned to approximately 100 per tank.

Oregon eggs were reared in incubation trays and transferred to the 5-gallon test tanks shortly before hatching.

Fertility of the Lake Michigan eggs was poorer than expected, and by the time of hatching it appeared that insufficient numbers of fry remained to complete the experiment.

Immediately following hatching, therefore, all Lake Michigan fry reared in the aquaria were replaced with Lake Michigan fry that had been hatched in incubation trays. These fry were received as eyed eggs from the Platte River Hatchery. The estimated date of fertilization was 3 November, the same as for the fry they replaced. Survival data and pesticide levels for Lake Michigan eggs were taken from the initial group. All information pertaining to the fry, beginning with the time of hatching, was derived from the fry that were substituted for the initial group at hatching.

EXPERIMENTAL DESIGN

Four rearing temperatures were employed following the time of hatching. Two replicate tanks of fry from each of three egg lots were reared at each temperature. The three egg lots were from Lake Michigan, Lake Huron, and Oregon. Thus, 24 test tanks were utilized. The rearing temperatures employed were 5, 9, 13 and 17 C.

The experimental tanks were common 5-gallon glass aquaria equipped with adjustable standpipe drains. During the period of incubation prior to swimup the water in the tanks was kept at one-third volume to increase the water exchange rate near the bottom. During this period the bottoms of the tanks were lined with aquarium gravel which was removed at the time of swimup. It was hoped that the gravel, by providing a more porous substrate, would contribute to better aeration of the

eggs and sac fry. Flows for all tanks were maintained at 5 gallons (18.93 liters) per hour. This flow produced an exchange rate of approximately one hour per 95% exchange for the period prior to swimup and 2.5 hours per 95% exchange for the period following swimup when the drains were raised (determined from table supplied in Sprague, 1969).

The outsides of the aquaria were lined with lids and sides of "styrofoam" sheets to protect the fry from direct sunlight and to insulate the tanks during the rearing temperature study.

The water supply was chlorinated tap water, distributed throughout the laboratory in plastic (polyvinyl chloride) plumbing. Though passed through a charcoal filter, dechlorination was not complete. Although chlorine levels were not continually monitored, several samples of water collected from test tanks on 1 April, 1971, contained free chlorine, monochloramine and dichloramine. Their combined concentrations ranged from 0.04 to 0.06 ppm.

The water was well aerated in head tanks before distribution to the aquaria. Saturation levels for oxygen are a function of water temperature and varied from 12.8 ppm at 5 C to 9.7 ppm at 17 C. Periodic checks of dissolved oxygen revealed levels to be continually approaching saturation for all temperature regimes.

Rearing temperatures were not differentiated until all egg lots had been distributed to their designated aquaria

and had hatched or were reaching the point of hatching. The thermal acclimation period lasted four days during which time temperature changes did not exceed 2 C per day. Both the 17 C and 13 C temperatures were obtained by mixing ambient water with water from a glass-lined water heater. Ambient water, cooled in the main head tank with a 1/8 horsepower cooling unit, was used for the 9 C temperature regime. A 1/3 horsepower cooling unit was employed to maintain the 5 C temperature.

For each aquarium, daily records were kept of the number and date of occurrence of egg and fry mortalities. Eggs turning opaque were considered dead. Dead eggs containing no visible embryo when cleared in 10% acetic acid were considered infertile.

Subsamples of eggs and fry were taken for pesticide analysis at fertilization, hatching, swimup and at termination of the study. To minimize weight loss by evaporation during storage, samples were wrapped in aluminium foil and preserved at -20 C. Average egg and fry weights were recorded for each of these subsamples.

The fry were fed Ewos starter diet three times daily beginning shortly prior to swimup. Ewos is a dry diet developed by Aktiebolaget Ewos (Subsidiary of Astra Pharmaceutical Products, Inc.) of Södertälje, Sweden.

The study of each fry lot was considered to be completed when all fry were feeding well and appeared to be clearly no

longer likely to sustain losses that could be attributed to pesticide residues. The length of time for the fry to reach this stage varied with egg source and rearing temperature, ranging from 138 days or 780 Centigrade temperature units from hatching for Lake Michigan fry reared at 5 C, to 78 days or 1,231 temperature units for Lake Michigan fry reared at 17 C. (Temperature units are the products of the rearing temperature multiplied by the number of rearing days.)

METHODS OF PESTICIDE ANALYSIS

All samples were blotted to a consistent degree of dryness prior to extraction of pesticide residues. Approximately 5 g of eggs or fry were then ground in anhydrous sodium sulfate and extracted three times with ethyl ether-hexane (6:94). The extract was cleaned on activated florisil using the method of Mills et al. (1963). The solvent from the eluted fraction was evaporated to near dryness and transferred quantitatively to graduated centrifuge tubes. The extracts were then analyzed by gas chromatography. A Micro-tek 220 instrument was used, equipped with a nickel electron capture detector and a ¼ inch by 6 foot glass column packed with 3% SE 30 on 60-80 mesh Gas Chrom-Q. Temperatures of the inlet, column and detector were 204, 174 and 280 C respectively, with a nitrogen carrier gas flow of 75 ml/min.

Sample extracts and solutions of known concentration were analyzed alternately. Residue levels of samples were then measured with respect to heights of response peaks of the known solutions. Results were computed in parts per million (ppm) based on the wet weight of the sample at the time of extraction.

RESULTS AND DISCUSSION

EGG LOSSES

Losses from fertilization to hatching for Lake Michigan and Lake Huron eggs are presented in Table 1. The losses are for eggs incubated under uniform conditions at 9 C on aquarium bottoms. Because they were received only shortly prior to hatching, no mortality data were available for the Oregon eggs.

Table 1. Losses from fertilization to hatching for eggs of Lake Michigan and Lake Huron parent stock.

Egg Source	Initial Egg Number	Percent Infertile	Percent Embryos Lost	Percent Total Losses
Lake Michigan	1,971	58.95	2.68	61.64
Lake Huron	3,133	47.02	6.28	53.27

Losses among both egg lots exceeded 50%. The bulk of the dead eggs appeared to be infertile. Embryo mortality was light.

The fertility of the Lake Michigan eggs was poorer than expected. A similar low fertility was observed for other

Lake Michigan eggs incubated on trays in the same laboratory. Lower egg fertility may be an unavoidable result of massive egg taking operations such as that in Michigan, in which it is more likely that occasional females used may not be in peak spawning condition. However, gradual loss of fertility has been documented for succeeding generations of coho salmon adjusting to a landlocked condition (West, 1965; Beal, 1955), a circumstance which may be taking place presently among Great Lakes coho.

The fertility of Lake Huron eggs approximately parallels that of Lake Michigan. However, these eggs were taken from salmon that had been "snagged" by fishermen. Some had been hooked through the abdomen, possibly admitting enough water to partially harden some of the eggs prior to fertilization. Thus it is unclear whether the fertility of Lake Huron eggs of this study accurately reflects that of Lake Huron salmon in general.

FRY LOSSES WITH RESPECT TO EGG SOURCE

When the mortality data presented in Table 2 are treated as a complete randomized block design and the final mortality means for the three fry lots compared using Duncan's multiple range test (Steel and Torrie, 1960), the higher loss of Lake Michigan fry proves to be significant ($p<.05$).

Though losses of Lake Huron fry exceeded those of Oregon, this difference is not significant at the .05 level.

Table 2. Percent fry losses with respect to rearing temperature and egg source.*

Egg Source		Rearing Temperature (°C) 5	9	13	17	Mean Fry Loss for Egg Source
Lake Michigan	Rep. 1**	84.92	95.00	58.56	75.00	
	Rep. 2	84.67	91.80	62.10	88.89	
	X	84.79	93.39	60.43	81.97	80.36
Lake Huron	Rep. 1	24.51	52.29	26.44	41.32	
	Rep. 2	3.97	42.98	26.83	46.42	
	X	12.25	47.39	26.66	44.24	32.74
Oregon	Rep. 1	50.00	3.33	8.21	49.19	
	Rep. 2	19.00	2.20	7.69	52.99	
	X	34.02	2.76	7.97	51.05	25.49

*Final losses are for 600 temperature units from hatching for all 5 C tanks, 1000 temperature units for all 9 C tanks, 1100 temperature units for 13 C and 1200 temperature units for 17 C.

**Designates replicate rearing tank.

The difference might have been more substantial had not Oregon fry reared at 5 C and 17 C suffered unusually high losses owing to what appeared to be an infectious agent. Oregon losses at 9 C and 13 C were only 2.76% and 7.97% respectively. Others have reported losses of Oregon coho sac fry of less than 5% (Johnson and Pecor, 1969; Willford et al., 1969).

Cumulative losses and mortality rates are shown in Figures 1-3 and in the Appendix in Tables A1-A3 according to time intervals from hatching.

Symptoms of distress distinct from the behavior of Oregon fry were observed among the Lake Michigan and Lake Huron fry beginning at the time of swimup. Affected fry characteristically displayed bursts of convulsive swimming behavior and were often of a darker pigmentation. Some possessed distended abdomens and clouded eye lenses. These symptoms were observed among Lake Michigan and, to a lesser extent, Lake Huron fry until the time the fry were clearly taking food. A high incidence of affected fry was usually followed by numbers of moribund individuals laying either on the surface or on the tank bottoms.

Unusually large numbers of Lake Michigan fry apparently failed to begin feeding, which added considerably to the losses experienced by this group. Fish that failed to begin feeding developed into a starved "pinhead" condition prior to settling to the tank bottoms where they died. Although

Figure 1. Percent losses of Lake Michigan coho salmon fry, cumulative and incremental, according to time intervals from hatching.

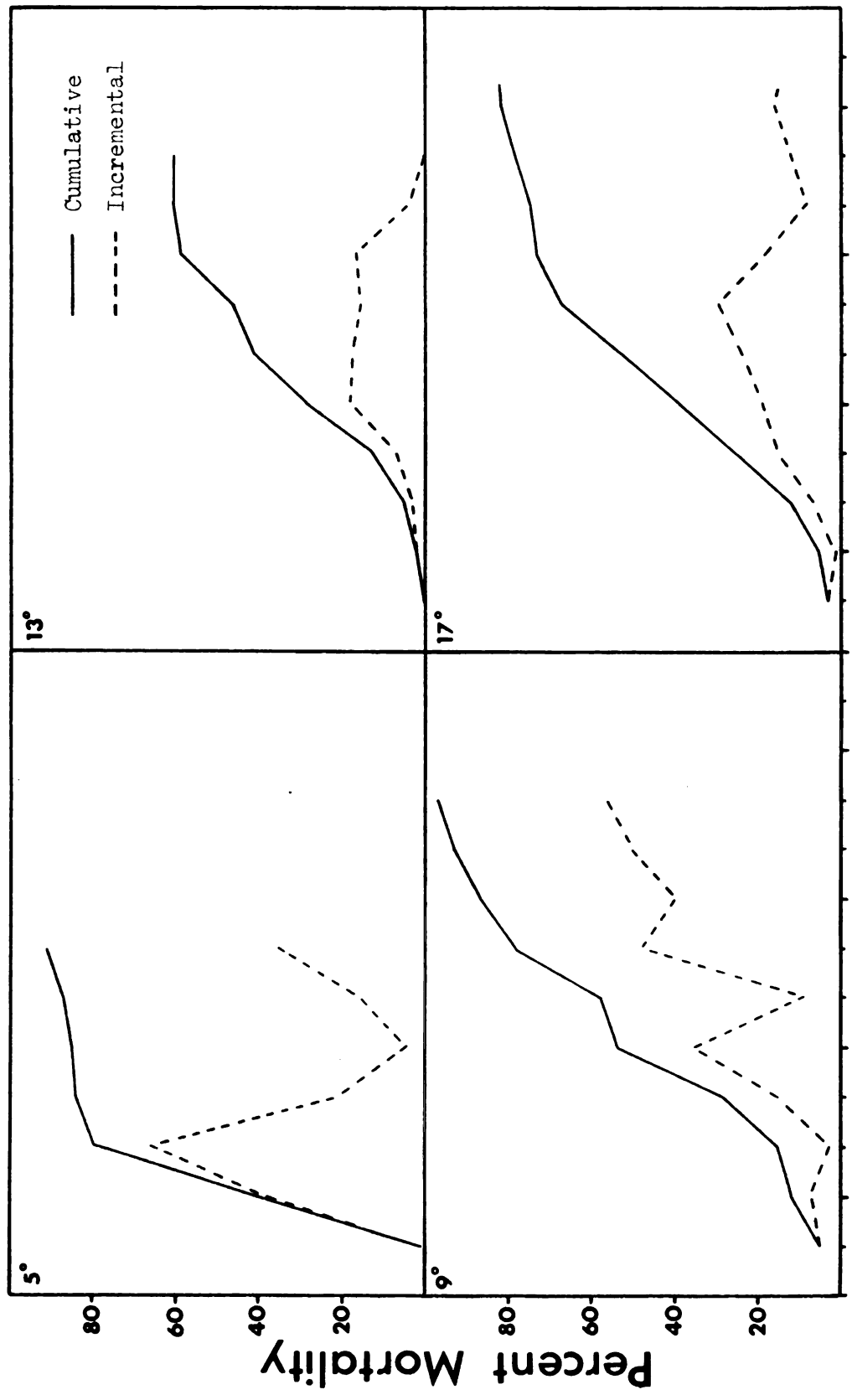
Cumulative
Incremental
5°
9°
13°
17°
Percent Mortality
20
40
60
80
100
300
600
900
1200
Temperature Units From Hatching

Figure 1.

Figure 2. Percent losses of Lake Huron coho salmon fry, cumulative and incremental, according to time intervals from hatching.

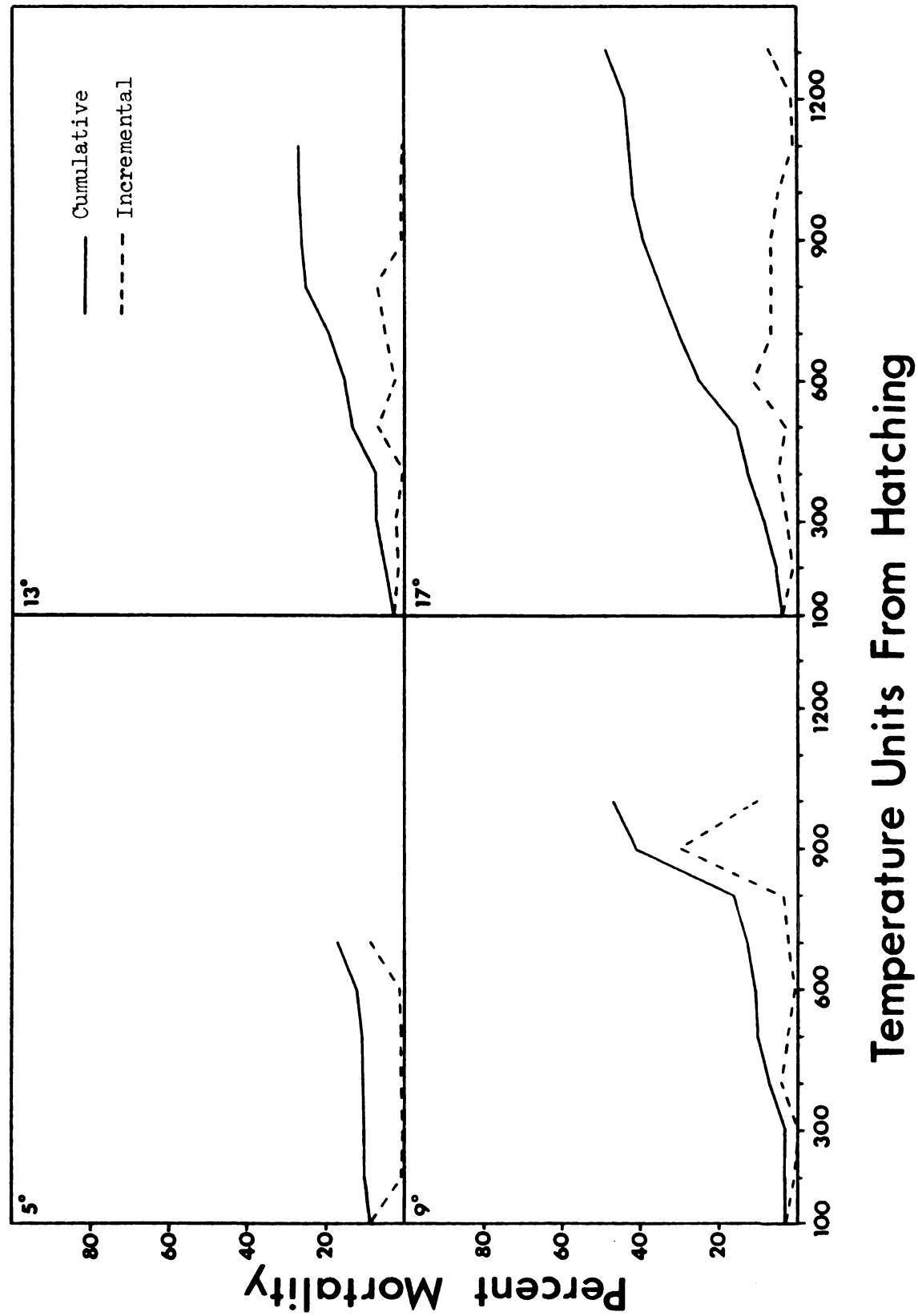
Cumulative
Incremental
5°
9°
13°
17°
Percent Mortality
20
40
60
80
100
300
600
900
1200
Temperature Units From Hatching

Figure 2.

Figure 3. Percent losses of Oregon coho salmon fry, cumulative and incremental, according to time intervals from hatching.

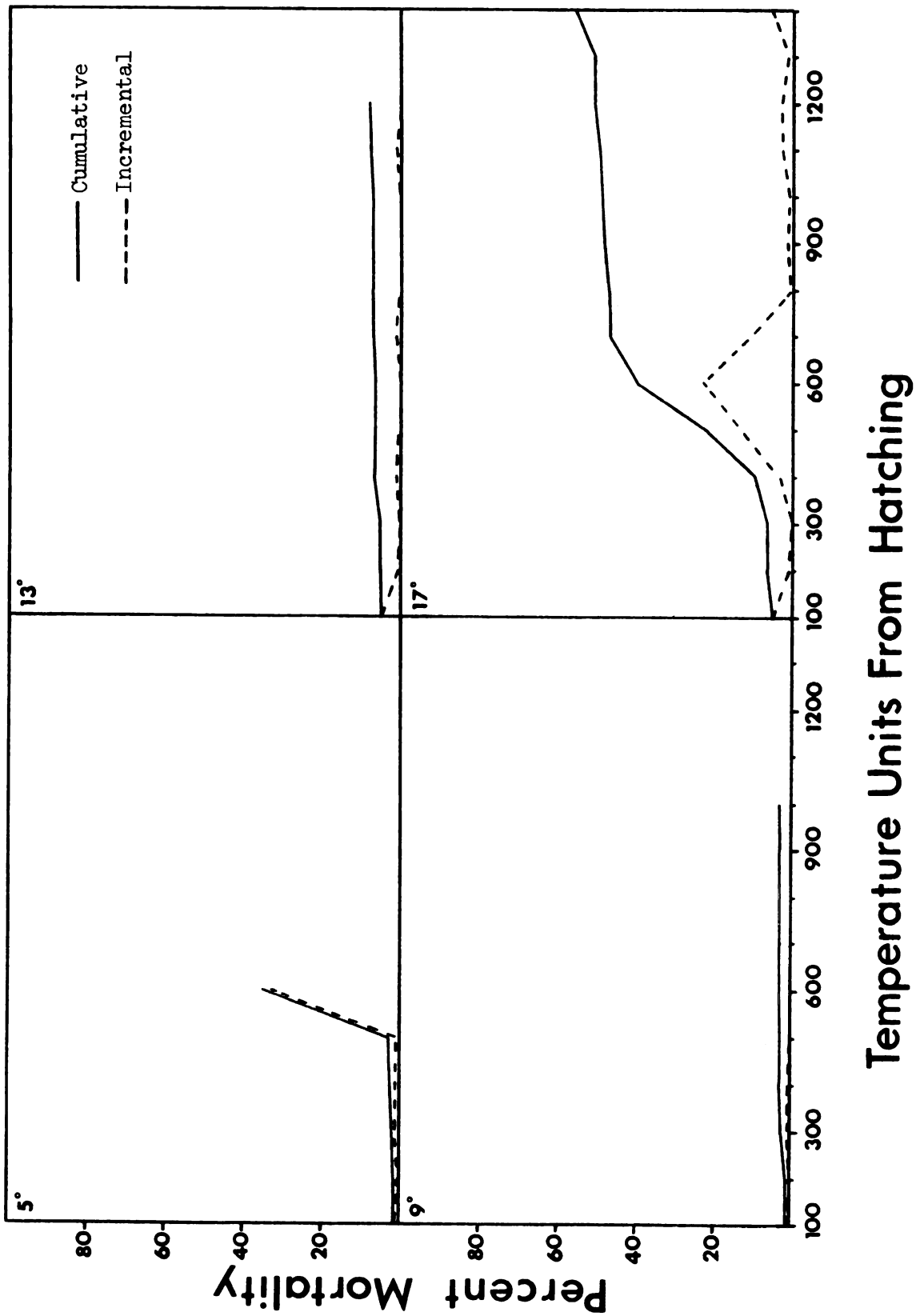
Cumulative
Incremental
5°
9°
13°
17°
Percent Mortality
Temperature Units From Hatching
20
40
60
80
100
300
600
900
1200

Figure 3.

starved fish were observed in all fry lots, including Oregon, they were particularly numerous in the Lake Michigan tanks.

Combined DDT residues (DDE, DDD, and DDT) approached 5 ppm in the eggs of the Lake Michigan salmon and exceeded 7 ppm in the hatching fry (Table 3). The increase was probably largely owing to chorion loss in hatching. Eggs of the Lake Huron lot contained 2.9 ppm total residues. Less than 0.1 ppm residues were found in eggs of the Oregon group.

Johnson and Pecor (1969) observed symptoms of stress and heavy losses among developing Lake Michigan coho salmon sac fry during 1968-1969 that were nearly identical to those observed during the present study. Burdick et al. (1964) also reported remarkably similar observations for lake trout sac fry that sustained large mortalities in New York. In both of these studies the eggs and fry were reported to be contaminated with residues of DDT.

DDT residue contamination could be a major source of losses of Lake Michigan fry of the present investigation. A threshold of 4.75 ppm combined DDD and DDT in the eggs is reported by Burdick et al. (1964) to be the lowest level that produced losses of lake trout sac fry. A characteristic mortality syndrome is described by Burdick for fry sustaining losses that were attributed to DDT. All fry lots containing 2.93 ppm or more combined DDD and DDT at the time of

Table 3. Residues of DDT and its derivatives and dieldrin in eggs and fry of Lake Michigan, Lake Huron and Oregon coho salmon.

Source of Eggs	Stage Sampled	Dieldrin (ppm)	DDT residues (ppm) DDE	DDD	DDT	Total
Lake Michigan	Eggs	-	3.25 (0.13)	0.46 (0.00)	1.26 (0.01)	4.97 (0.14)
	Hatching	0.079 (0.004)*	5.18 (0.36)	0.39 (0.04)	1.70 (0.15)	7.27 (0.54)
Lake Huron	Eggs	-	1.93 (0.36)	0.16 (0.03)	0.79 (0.08)	2.88 (0.36)
	Hatching	0.042 (0.007)	3.00 (0.07)	0.20 (0.03)	0.92 (0.09)	4.12 (0.18)
Oregon	Eggs	-	-	-	-	-
	Hatching	0.005 (0.000)	0.046 (0.003)	0.004 (0.000)	0.024 (0.003)	0.074 (0.004)

*Standard Error in parentheses (n = 2).

commencement of feeding were reported to develop the syndrome and experience mortalities, with some groups containing as little as 2.93 ppm sustaining a 100% occurrence of the syndrome.

Burdick's investigation differed from the present study with respect to fish species and analytical techniques, and caution must be exercised when comparing results of the two. Fry of lake trout and coho salmon may differ considerably with respect to their susceptibilities to DDT residues. The Schechter-Haller procedure, involving spectrophotometry, was used by Burdick for residue analysis rather than gas chromatography. Furthermore, DDT levels reported by Burdick for fry were only estimates of residues levels based on concentrations measured in the eggs.

However, the combined value of DDD and DDT in Lake Michigan salmon eggs of the present study was only 1.72 ppm, less than one-third of Burdick's threshold for lake trout eggs. Although, residue levels were not determined for fry at the time they were ready to feed, the concentration in fry at swimup was essentially the same as for the eggs and still considerably below the 2.93 ppm threshold given by Burdick for the fry stage.

Macek (1968a) reported losses of brook trout fry from parent stock treated with DDT. Although losses of fry from treated parents were roughly twice those from untreated parents, mortality was very low in both treatment groups

with 4% to 8% losses of treated fry. Losses of less than 15% are considered normal for the 60 days following hatching in some hatchery situations (Currier et al., 1967).

Total residue levels in eggs of Macek's treated trout approached those in Lake Michigan coho eggs of the present study. In contrast to Lake Michigan salmon, however, DDT was the primary constituent of the residues with only a very slight contribution of its metabolites. Also, Macek's observations are for the 15-week period following hatching but do not extend to the time of commencement of feeding.

Allison et al. (1963 and 1964) reported losses exceeding 90% among cutthroat trout fry from treated eggs containing approximately 10 to 20 ppm total DDT residues. Losses of fry containing residue levels ranging from 3 to 12 ppm were described as "discernibly higher" than for groups containing less DDT. However, no supporting data for fry survival were given. Although results of residue analyses were highly variable, pesticide levels reported by Allison were generally higher than those for Lake Michigan salmon eggs. As in Macek's investigation, the residues were primarily composed of DDT, which would seem to be a more potentially lethal condition than that of the present study, in which DDE predominated. Currier et al. (1967) reported unusual losses of rainbow, cutthroat and brook trout fry from eggs containing as little as 0.5 ppm combined DDT residues. Mortalities ranged from 30% to 90% among fry containing 0.5 to 1.3 ppm

total residues. The very low threshold concentration does not agree with evidence of other investigations or with the present one, and appears to complicate the question concerning the extent of the affect of DDT contamination on the reproductive success of salmonids.

It therefore appears that, although DDT residue contamination may be involved in the poor fry survival of the present study, it is as yet unclear whether residue levels such as those encountered here could alone account for the 60% to 90% losses of Lake Michigan fry.

Other sources may be involved. The fry were fed only three times daily. In the absence of automatic feeders it was impractical to attempt feeding them more often. Yet for optimal growth and survival of fry fed Ewos starter diet, the manufacturer's recommended feeding rate is three times hourly. Furthermore, chlorine removal from the water supply was incomplete. The extent to which these two factors influenced survival is uncertain. However, all three fry stocks were subjected to these stresses equally. Yet losses of Lake Michigan fry averaged 80.4% as compared to the 33.7% and 25.5% losses of the Lake Huron and Oregon groups respectively. This can at least in part be explained by the observations of other investigators that DDT residue contamination, besides its overt affects on survival, also appears to work indirectly, rendering fish more susceptible to the affects of various other stresses. One such stress in particular

was reported to be shortage of food (Macek, 1968b, Allison et al. 1963 and 1964).

However, environmental contaminants other than residues of DDT should be considered as possible sources of the higher Lake Michigan fry mortality. Figure 4 illistrates a gas chromatogram typical of Lake Michigan eggs and fry. The combined peak heights of DDE, DDD and DDT comprise only about 50% of the sample's chromatographic response. The remaining peaks recorded are unidentified residues which appeared in all samples of Lake Huron and Lake Michigan eggs and fry. These residues were retained by the fry throughout the study period, closely paralleling the persistence of the DDT residues. Furthermore, alkaline hydrolysis of Lake Michigan fry samples revealed the peak corresponding to the retention time of p-p' DDT to be composed of approximately 50% residue unresponsive to saponification (see discussion of pesticide analyses below). Therefore, the DDT levels reported in this study are probably twice the actual values owing to this interference. The extent, if any, to which these residues contribute to fry losses, either by themselves or in conjunction with DDT residues, can only be surmised.

Previous investigations have occasionally noted the presence of unidentified residues in flesh samples of Great Lakes wildlife and it has been widely suspected that these residues are composed of certain mixtures of chlorinated hydrocarbons known collectively as polychlorinated biphenyls

Figure 4. Gas chromatogram of a typical Lake Michigan coho salmon fry extract showing the presence of residues of DDT as well as of other, unidentified, substances.

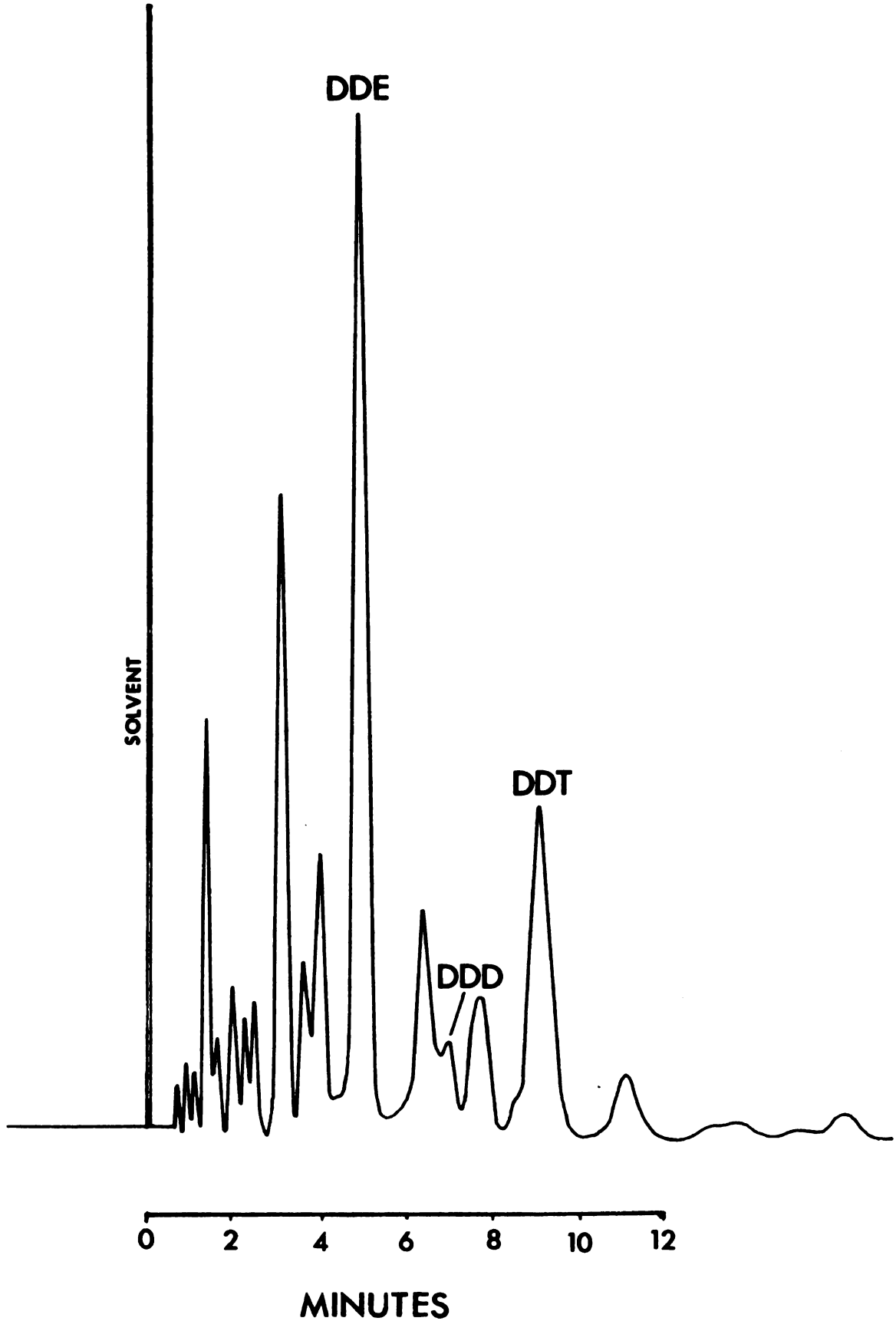

Figure 4.

(Veith, 1970). However, until quite recently there has been a paucity of explicit confirmation of their identity. This has largely been owing to poor success in completely separating these compounds from any pesticide residues in the sample and to a lack of suitable confirmatory techniques. Furthermore, there is the problem of selecting a suitable mixture of polychlorinated biphenyls with which to prepare standard solutions. It is against these solutions of known concentration that levels in the sample are measured (Veith, 1970).

Among the first to report levels of polychlorinated biphenyls in Great Lakes fish was the investigation of Armour and Burke (1970). Residues other than those of the DDT complex are reported in Lake Michigan coho salmon amounting to 14.6 ppm. These residues most closely resembled Aroclor 1254, a commercially prepared mixture of polychlorinated biphenyls. Veith (1970) reported identification of polychlorinated biphenyls in fish of western Lake Michigan. Concentrations in chinook salmon, coho salmon, rainbow trout and lake trout ranged from 18.4 to 26.3 ppm and again the residues most closely resembled the Aroclor 1254 mixture.

The polychlorinated biphenyls closely resemble DDT and its derivatives with respect to chemical structure and composition and behavior in the environment (Veith, 1970). They have widespread commercial usage as ingredients of lubricants, electrical insulating materials, plastic softeners and fire retardants. As a constituent of some forms of asphalt

and other construction materials they are susceptible to leaching into urban stormwater runoff and are commonly associated with waste water treatment discharges (Veith, 1970). It therefore appears likely that the distribution of polychlorinated biphenyl compounds may be widespread in the Great Lakes, particularly in Lake Michigan, and that they probably compose the bulk of the unidentified residues in Great Lakes fry of the present study.

The polychlorinated biphenyls have a relatively low acute toxicity compared to that of DDT (Gustafson, 1970). However, nearly all studies of these substances in animals indicate that it is chronic (long term) rather than acute toxicities in which polychlorinated biphenyls appear to be the most potentially harmful (Gustafson, 1970). The only evidence available to date on the influence of residues of these substances on reproduction of salmonids is a Swedish study in which only 1 to 2 ppm were reported to lead to 70% to 100% losses of Atlantic salmon eggs (Johansson, 1970). However, no unusual losses were reported for the fry and no information is given as to the identity of the particular polychlorinated biphenyl mixture encountered. The effect of these residues on the reproductive success of fish and other wildlife deserves further investigation.

FRY LOSSES WITH RESPECT TO REARING TEMPERATURE

Survival of Lake Michigan fry was significantly better at 13 C than at the other rearing temperatures ($p<.05$).

Survival of Lake Huron fry was also relatively high at 13 C, though somewhat better at 5 C. Lowest survival of both Lake Michigan and Lake Huron stocks was at 9 C but losses of Oregon fry at 9 C were very slight (Table 2).

The mortality curves of Figures 1 and 2 suggest that Great Lakes fry at 5 C might have sustained considerably higher losses had they been reared to at least 1,000 temperature units, as were fry at other temperature regimes. Survival data for the 5 C rearing temperature are for more than 120 days following hatching, which is equivalent to only 600 temperature units. Peak losses at the other three temperatures occurred between 600 and 1,000 temperature units and the 5 C fish were beginning to experience substantial losses at the time the study was discontinued. Consequently, the peak mortality of 5 C fry may not have yet occurred by the end of the investigation.

Losses of Lake Michigan and Lake Huron fry at 17 C were relatively high, approaching those sustained at 9 C. Possibly these fry were more adversely affected by the three-times-daily feeding rate than were fry of colder temperature regimes, owing to the increased metabolic rate imparted by the warmer temperature. However, the 17 C rearing temperature may have been excessively warm for optimal survival of coho fry of any source. The preferred temperature range for 5-month-old coho salmon has been reported to be 12-14 C (Brett, 1952).

The high loss of Oregon fry at 5 C and 17 C, as noted above, appeared to be of an infectious source.

Although the affect of temperature on the toxicity of polychlorinated biphenyl compounds apparently remains unexplored, numerous investigators have examined the influence of temperature on the toxicity of DDT. A negative temperature coefficient of activity has been well documented for DDT in insects (O'Brien, 1967) and is very likely the case in fish (Anderson, 1968; Macek, 1968b; Elson, 1967; Cope, 1965; Fisheries Research Board of Canada, 1961).

Currier et al. (1967), however, observed that brook and rainbow trout fry from eggs containing DDT incurred 90% losses when reared at 40 F (4.4 C) but only 15% losses when reared at hatcheries operating at 32 to 36 F (0 to 2.2 C). There are few other references concerning the affect of temperature on the toxicity of DDT to salmonid sac fry but it appears possible, in theory at least, that temperature relations in sac fry may not conform with those in older fish. Atchison (1970) reported that the greatest rate of uptake of DDT from the yolk material of brook trout alvins was during the final stage of yolk absorption. This release of DDT coincided with the time of peak phospholipid mobilization. It was suggested that a large part of the DDT residues contained in the yolk material are retained in the phospholipids until late in the sac fry stage. It appears likely, therefore, that rearing temperature, by its influence on the metabolic rate, could affect the rate of phospholipid mobilization and thus the rate of release of DDT residues.

Theoretically, higher rearing temperatures late in the sac fry stage would then be expected to lead to a greater rate of release of DDT, consequently increasing the lethality of any DDT residues contained in the yolk material.

The results of the present study do not reveal a definite formula for the affect of rearing temperature on sac fry survival. Nor is it clear which, if any, of the chlorinated hydrocarbon residues found in Great Lakes fry were actually at the source of their poor survival. Nevertheless, the relatively good survival of Great Lakes fry reared at 13 C suggests that the nature of the mortality of this investigation is not described by a positive temperature coeffient. More likely, losses are either maximized at negative temperatures or at both temperature extremes.

GROWTH AND DEVELOPMENT

The three salmon stocks differed somewhat with respect to fry size at hatching, with Oregon the largest and Lake Huron fry the smallest (Table 4). Rearing temperatures were essentially the same for all groups of eggs up to this time.

Average weight gains of 25, 16, and 4.5% occurred between hatching and swimup for the Lake Huron, Oregon and Lake Michigan fry respectively (Table 5). All treatment combinations gained weight with the exception of Lake Michigan 5 C fry. On a dry weight basis, sac fry usually lose weight during this period. Changes in wet weight are

Table 4. Weights of hatching coho salmon fry from three parent stocks.

Egg Source	Average Weight Per Fry (g)
Lake Michigan	0.28
Lake Huron	0.25
Oregon	0.33

Table 5. Weights of three stocks of coho salmon fry reared from hatching to swimup at four temperature regimes.

Source of Fry	Number Sampled	Average Weight Per Fry at Swimup (g)	% Weight Gain From Hatching
Lake Michigan			
5 C	25	0.25	-10.7
9 C	40	0.34	21.4
13 C	50	0.29	3.6
17 C	50	0.29	3.6
$\bar{X}$		0.29	4.5
Lake Huron			
5 C	135	0.34	36.0
9 C	50	0.31	24.0
13 C	50	0.31	24.0
17 C	50	0.29	16.0
$\bar{X}$		0.31	25.0
Oregon			
5 C	150	0.42	27.3
9 C	50	0.38	15.2
13 C	100	0.38	15.2
17 C	100	0.35	6.1
$\bar{X}$		0.38	15.9

probably attributable to increases in water content (Blaxter, 1969; Smith, 1957) and possibly to the affect of size selective mortalities. Lake Michigan fry reared at 5 C were the only group to sustain heavy losses prior to swimup (Figure 1, Table A1). This early loss of fry would explain their apparent loss of weight if larger fry were most severely affected.

The time of sampling at hatching and swimup was indicated by the appearance of distinct physical and behavioral changes. No such clear-cut developmental phase accompanied the final samples and their timings were unavoidably more subjective. The weight data given for fry at the end of the observation period (Table 6) therefore cannot be considered as meaningful as those for the two earlier samplings. However, it is of interest that again weight gains were greatest among the Lake Huron fry, with average weight gains of 337, 227, and 200% from hatching for Lake Huron, Oregon and Lake Michigan fry respectively. The smaller size of fry reared at 5 C is probably largely owing to their premature sampling.

Figure 5 illustrates the relative time from hatching to swimup for each salmon lot according to rearing temperature. The relative time to swimup was determined on the basis of days from hatching to swimup at 17 C. Thus the time to swimup at 5 C was nearly six times that at 17 C for Lake Michigan fry. Swimup at 5 C for Lake Huron salmon was

Table 6. Final weights and lengths of coho salmon fry reared from hatching at four temperature regimes.

Source of Fry	Temperature Units When Sampled	Average Length Per Fry (cm)	Average Weight Per Fry (g)	% Weight Gain From Hatching
Lake Michigan				
5 C	780	4.3 (0.07)*	0.60 (0.014)	114.3
9 C	1,089	4.7 (0.21)	0.90 (0.190)	221.4
13 C	1,078	4.5 (0.07)	0.84 (0.134)	200.0
17 C	1,231	4.7 (0.07)	1.02 (0.262)	264.3
$\bar{X}$	1,044	4.6	0.84	200.0
Lake Huron				
5 C	670	4.6 (0.14)	0.78 (0.060)	212.0
9 C	1,009	5.2 (0.00)	1.18 (0.042)	372.0
13 C	1,113	5.3 (0.07)	1.39 (0.099)	456.0
17 C	1,273	4.8 (0.00)	1.02 (0.014)	308.0
$\bar{X}$	1,016	5.0	1.09	337.0
Oregon				
5 C	590	4.8 (0.28)	0.87 (0.163)	163.6
9 C	1,012	5.4 (0.00)	1.31 (0.042)	297.0
13 C	1,166	5.2 (0.07)	1.26 (0.071)	281.8
17 C	1,350	4.5 (0.00)	0.87 (0.021)	163.6
$\bar{X}$	1,030	5.0	1.08	226.5

*Numbers in parentheses are standard errors based on the average values of two replicate tanks.

delayed by a factor of about three. Oregon fry took approximately twice as long to reach swimup at 5 C as at 17 C.

Figure 6 illustrates the time from hatching to swimup on the basis of Centigrade temperature units instead of days. Considerably more temperature units were required to rear Lake Michigan fry from hatching to swimup at colder than at higher temperatures. Lake Huron fry required essentially the same number of temperature units at all temperature regimes. Oregon fry exhibited nearly the reverse phenomenon shown by the Lake Michigan lot, requiring fewer temperature units to reach swimup at colder than at warmer temperatures.

The time of commencement of feeding could be roughly ascertained by the time of appearance of fecal material on the tank bottoms. Although there appeared to be no delay in commencement of feeding among Lake Michigan fry reared at the two warmer temperatures, fry of the colder Lake Michigan tanks took roughly two weeks longer to begin feeding than did Oregon and Lake Huron fry.

The difference between salmon stocks in delay of swimup at colder temperatures is considerable. The extent of delay is greater in stocks containing higher levels of DDT, indicating that the degree of residue contamination may have some bearing on the rate of development, particularly at lower rearing temperatures. However, residues of unidentified compounds were also present in the fry and may have influenced developmental rates. The possible influence of these

Figure 5. Delay in the time of swimup induced by colder temperatures relative to the time to swimup at 17 C for each of the three salmon lots.

Figure 6. Temperature units required to rear three different stocks of salmon fry from hatching to swimup at four rearing temperatures.

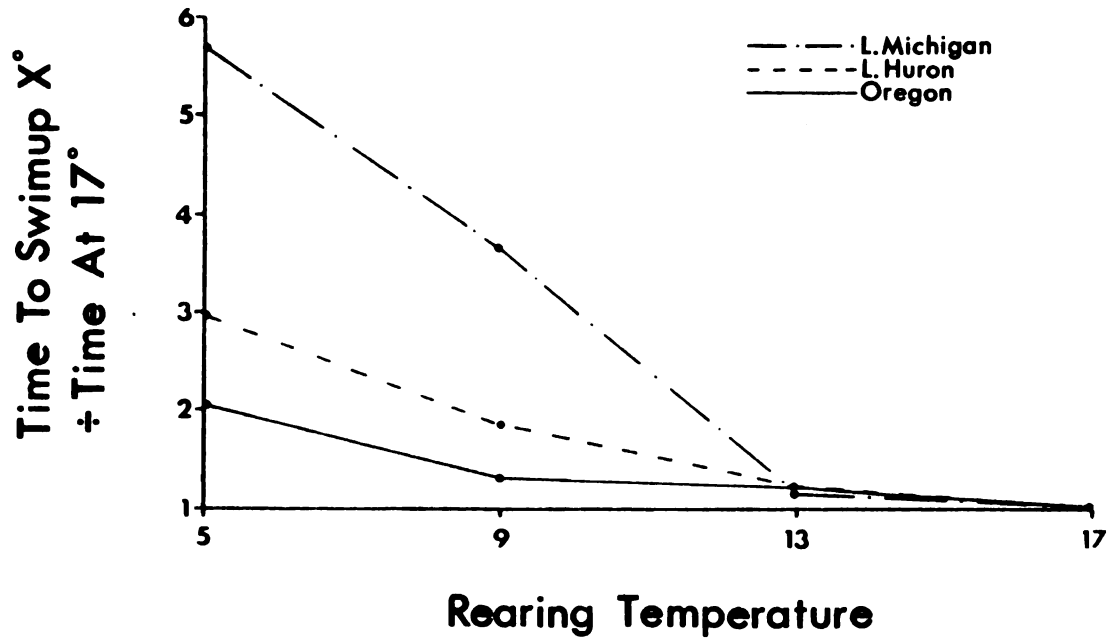

Figure 5.

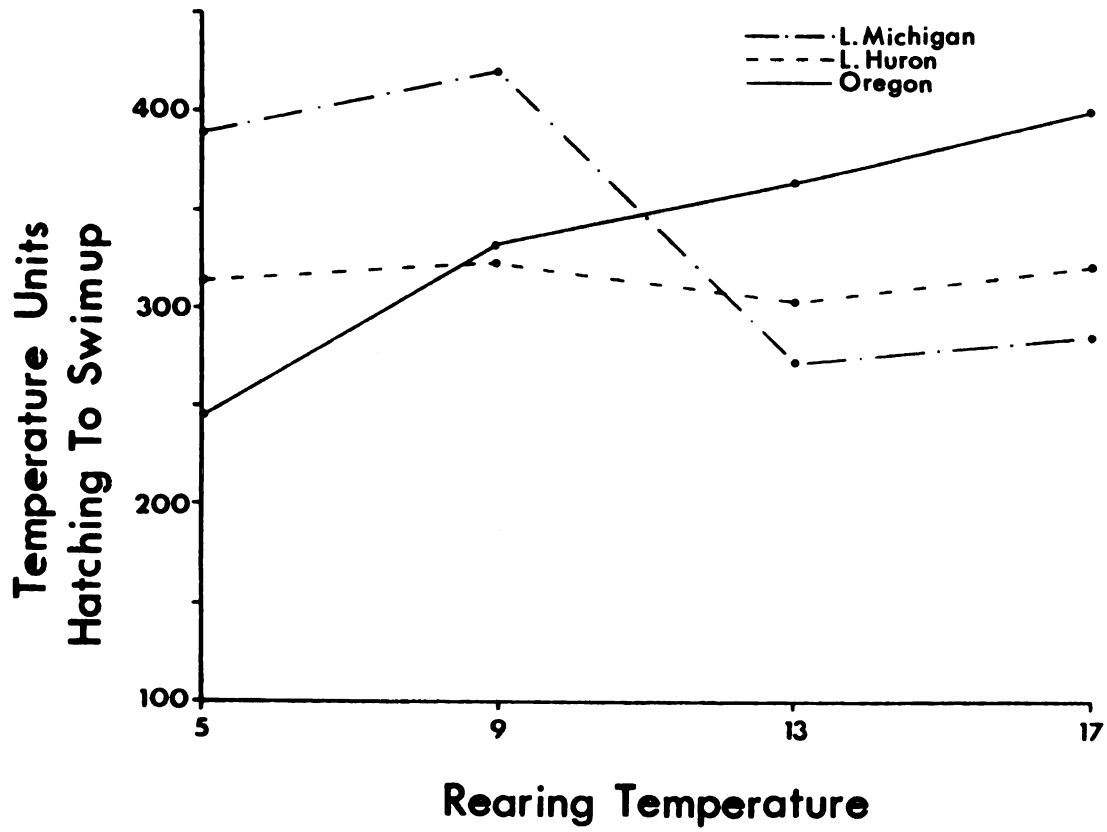

Figure 6.

residues, as well as of DDT, on development of sac fry deserves further investigation.

PESTICIDE ANALYSES

Except for dieldrin, residue concentrations given in Tables 3, 7 and 8 have been corrected for the percent recovery of the extraction and cleanup procedures. Recoveries of DDE, DDD, and DDT were 83.2, 74.8, and 74.3% respectively. Recoveries for the cleanup portion of the procedure approached 100%, indicating that the largest portion of the residue loss occurred during extraction.

Only the Lake Michigan group was analyzed for DDT residues for the period following hatching (Tables 7 and 8). These fry showed a decrease in DDT concentrations of more than 50% between the swimup and final sampling dates (Figure 7). Nevertheless, when these results are interpreted in terms of μg residue per individual (Figure 8), it becomes evident that most of the residue present in the eggs was retained by the fry throughout the study. The sharp decline in ppm residue (Figure 7) probably is largely owing to dilution of the original pesticide quantity by growth.

The higher pesticide levels in hatching fry than in the eggs is probably mostly due to chorion loss at hatching. However, the continued rise in apparent residue levels with later developmental stages appears to indicate that the residues were in a more readily extractable state in these

Table 7. Mean DDT residues (ppm) in Lake Michigan coho salmon eggs and fry analyzed at four developmental stages.

Sample	DDE	DDD	DDT	Total
Eggs	3.25 (0.13)*	0.46 (0.00)	1.26 (0.01)	4.97 (0.14)
Hatching	5.18 (0.36)	0.39 (0.04)	1.70 (0.15)	7.27 (0.54)
5 C Swimup**	5.58 -	0.38 -	1.58 -	7.54 -
5 C Final	2.80 (0.29)	trace***	0.50 (0.05)	3.29 (0.27)
9 C Swimup	-	-	-	-
9 C Final	1.93 (0.08)	trace	0.36 (0.02)	2.29 (0.10)
13 C Swimup	4.35 (0.42)	0.29 (0.01)	1.37 (0.07)	6.01 (0.50)
13 C Final	2.29 (0.20)	trace	0.45 (0.04)	2.74 (0.24)
17 C Swimup	4.72 (0.26)	0.28 (0.01)	1.54 (0.02)	6.54 (0.27)
17 C Final	2.10 (0.49)	trace	0.41 (0.10)	2.51 (0.58)

*Standard error (n = 2).

**Based on a single analysis.

***Less than 0.10 ppm.

Table 8. Mean DDT residues (μg/individual) in Lake Michigan coho salmon eggs and fry analyzed at four developmental stages.

Sample	DDE	DDD	DDT	Total
Eggs	0.91	0.15	0.41	1.63
Hatching	1.45	0.11	0.48	2.04
5 C Swimup	1.40	0.10	0.40	1.89
5 C Final	1.68	trace	0.30	1.97
9 C Swimup	-	-	-	-
9 C Final	1.73	trace	0.32	2.06
13 C Swimup	1.26	0.08	0.40	1.74
13 C Final	1.92	trace	0.37	2.30
17 C Swimup	1.36	0.08	0.45	1.90
17 C Final	2.14	trace	0.42	2.56

Figure 7. DDT residues (ppm) in Lake Michigan salmon eggs and fry at different stages following fertilization.

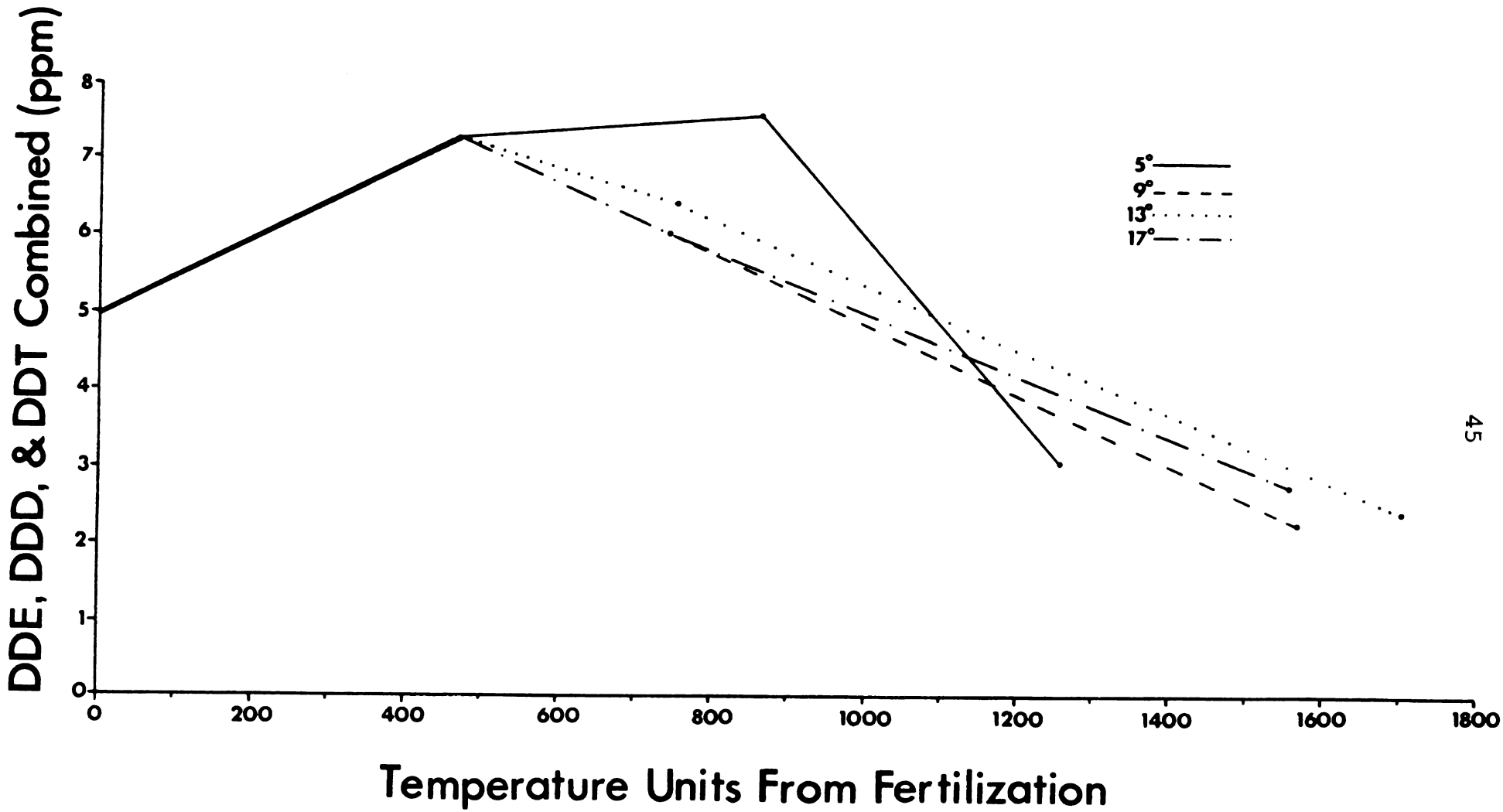
DDE, DDD, & DDT Combined (ppm)
0
1
2
3
4
5
6
7
8
0
200
400
600
800
1000
1200
1400
1600
1800
5°
9°
13°
17°
Temperature Units From Fertilization

Figure 7.

Figure 8. Micrograms DDT and micrograms DDT and derivities in coho salmon eggs and fry at different stages following fertilization.

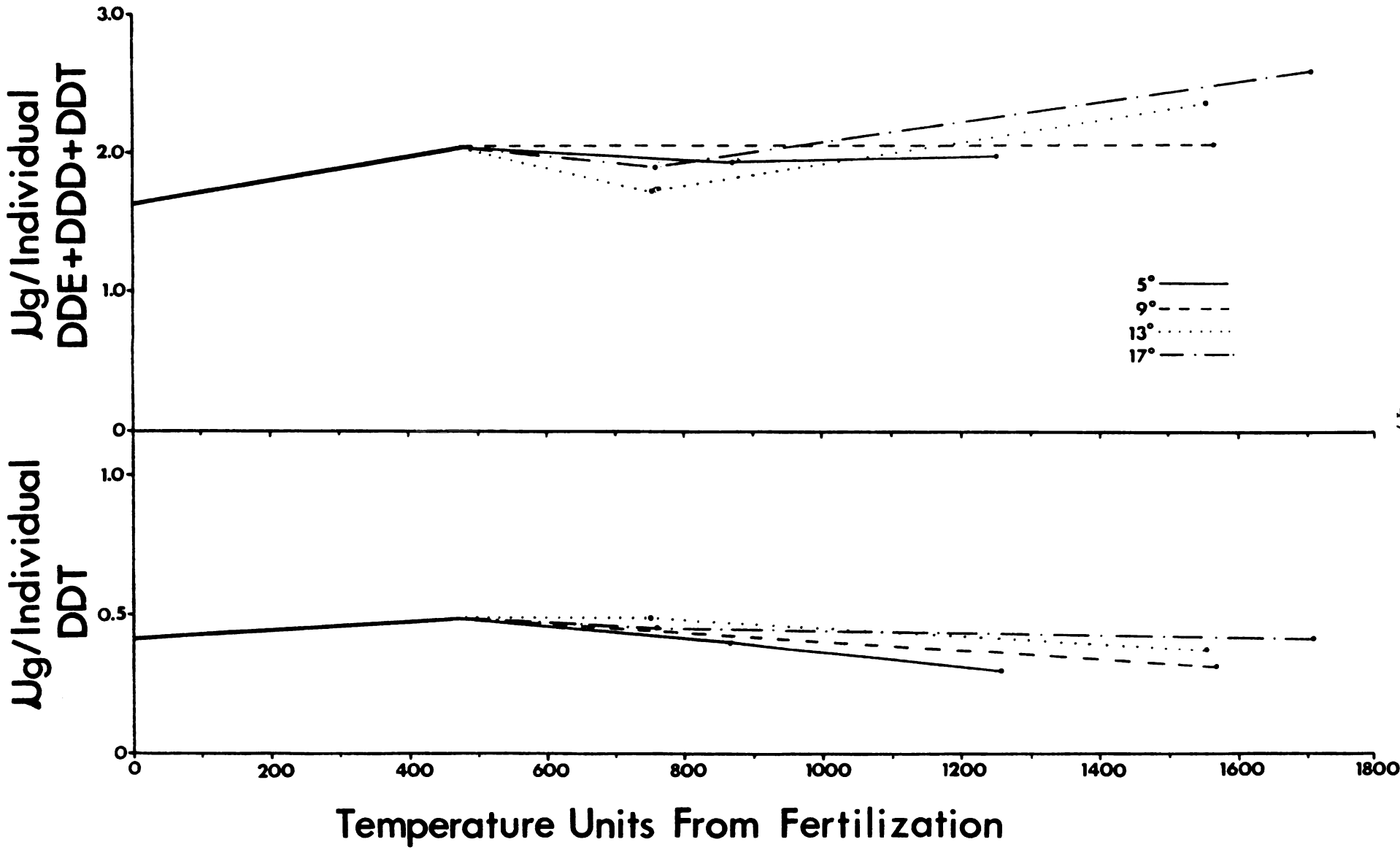
3.0
2.0
1.0
0
µg/Individual
DDE+DDD+DDT
5°
9°
13°
17°
1.0
0.5
0
µg/Individual
DDT
0
200
400
600
800
1000
1200
1400
1600
1800
Temperature Units From Fertilization

Figure 8.

samples than previously. Atchison (1970) noted a similar trend for DDT residues in developing brook trout eggs and fry. He also found that peak phospholipid mobilization did not occur until late in the swimup stage and pointed out that the ethyl ether-hexane extraction solvent removes triglycerides, only some phospholipids and no lipoproteins. He suggested that the apparent increase in residues at later developmental stages could be the result of metabolism of lipoproteins and phospholipids, thus allowing previously bound DDT to enter a more organosoluble state.

Evidently some DDT was being metabolized to DDE throughout the period of study (Figure 9). DDT composed 25.4% of the combined DDT residues (DDE, DDD, and DDT) in the eggs but constituted only about 16% of the final samples. This amounts to a 37% decline in the contribution of DDT to the residue complex. Most of this reduction took place between swimup and the final samples and was most substantial at lower temperatures (Table A4). A decline in the relative contribution of DDD to the residue complex is also evident in later samples.

Dehydrochlorinase activity has been conclusively demonstrated for the liver of fish (Grzenda et al., 1970; Greer and Paim, 1968; Wedemeyer, 1968; Premdas and Anderson, 1963) and is the most probable source of the DDT degradation observed here. Generally, the principle location of DDT degradation is the intestinal microflora (Buhler et al., 1969;

Figure 9. The approximate percentage composition of DDT residues in Lake Michigan salmon fry at different developmental stages averaged for the four rearing temperatures.

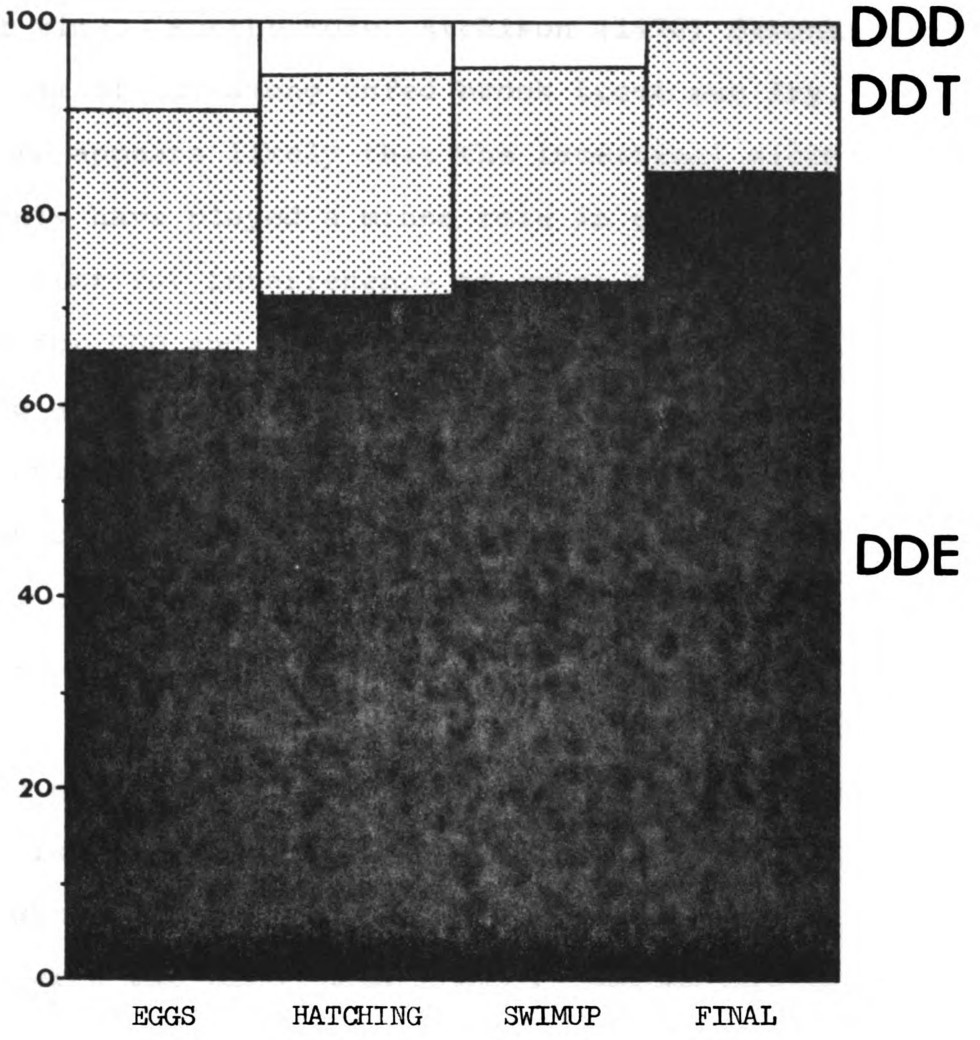

Figure 9.

Grzenda et al., 1970; Cherrington et al., 1969; Wedemeyer, 1968). However, fry in the present study were fed for only a short time. Furthermore, Atchison (1970) demonstrated metabolism of DDT among unfed brook trout sac fry. It therefore appears likely that the intestinal microflora could only have played a minor role in the present study.

To test for interference of other residues with the results for DDD and DDT, extracts of Lake Michigan fry at hatching and swimup were subjected to alkaline hydrolysis for a period of one hour. When analyzed by gas chromatography, the peak corresponding to the retention time of DDT persisted at 45.5% of its original height, a percentage that did not appear to vary substantially with sampling date. The identical procedure, when applied to samples prepared from standard solutions of DDE, DDD and DDT showed complete hydrolysis of DDD and DDT with a corresponding increase in the height of the DDE peak. It therefore appears that a residue with the same retention time as DDT but resistant to saponification was present in the Lake Michigan fry. The DDT results have not been corrected for this interference.

PART II

THE INFLUENCE OF DDT RESIDUES AND REARING TEMPERATURE ON SURVIVAL AND GROWTH OF RAINBOW TROUT SAC FRY

INTRODUCTION

The investigation of coho salmon fry survival reported in Part I suggested a possible relation between levels of DDT contamination and the extent of fry loss during the period between hatching and swimup. However, it was noted that, in addition to DDT, considerable amounts of other residues were also present in extracts of Lake Michigan and Lake Huron salmon fry. If residue contamination was indeed the source of the fry losses, it is unclear whether DDT residues or the accompanying extraneous residues were primarily responsible, or if both were somehow acting in conjunction.

The influence of rearing temperature on coho fry survival was also explored. Although survival appeared to be enhanced at certain temperatures, further information was necessary to more clearly illuminate the relation between rearing temperature and survival of residue contaminated fry.

A further study was therefore conducted in an attempt to clarify some of these issues, particularly with respect to the role of DDT. Rainbow trout fry from spring spawning Lake Michigan parent stock were used. One group of fry was exposed to an aqueous suspension of DDT shortly after hatching.

These fry, and a second untreated group, were then subjected to four temperature regimes and observed until well past that period of development for which most previous DDT related losses have been reported.

MATERIALS AND METHODS

Eggs from spring-spawning Lake Michigan rainbow trout were obtained from the Michigan Department of Natural Resources Platte River egg-taking station on 13 April, 1971 and incubated on trays in the laboratory.

At time of hatching approximately 1,200 sac fry were placed in a half-filled 20-gallon aquarium and exposed for 11 hours to an aqueous suspension of 30 ppb DDT. The DDT, in solution with 3 ml acetone, was introduced into a second 20-gallon aquarium serving as a reservoir for the treatment tank. Water was circulated between the two tanks with a submersible pump. Water temperature of the exposure apparatus was maintained at 11 C to 12 C by suspending the reservoir tank in a 200 gallon constant temperature water bath maintained at 11 C. Dissolved oxygen was maintained at 9 ppm or more by airstones in the reservoir. The reservoir and exposure tanks constituted a closed system with no water renewals during the exposure period.

An additional group of fry was treated in the same manner but for 27 hours. These fry were then reared in two 20-gallon aquaria at ambient temperature and observed until well beyong the sac fry stage of development.

The experimental design for the temperature study was essentially the same as that of the salmon study described in Part I but with a few modifications. Thirty-two test tanks and four temperatures were employed. Four replicate tanks were used for each treatment-temperature combination. The four rearing temperatures employed were 7, 10, 13, and 16 C. The fry were fed Oregon Moist starter diet beginning shortly prior to swimup. Well water was available, thus eliminating the problems of chlorine contamination encountered earlier in the investigation. Aeration of the water took place in the laboratory's main head tank and in a second head tank incorporated into the experimental design. Dissolved oxygen was always maintained at saturation.

Immediately after the 11-hour exposure, treated and untreated fry were transferred to the rearing tanks. Water temperature of the rearing tanks at time of transfer was within 2 C of that of the exposure tank and incubation trays. The four desired temperature regimes were then established over a period of two days.

Samples of fry were frozen at time of hatching, after treatment, and at the end of the study to be analyzed for pesticide content.

The study of each temperature regime was considered to be completed when all fry were feeding well and appeared no longer likely to sustain losses that could be attributed to pesticide residues. At that time, all fry of that temperature,

both treated and untreated, were weighed and packaged for later pesticide analysis. Owing to the affect of rearing temperature on developmental rate, the time of termination of the four temperature regimes varied from 38 days or 608 temperature units from hatching for fry reared at 16 C, to 63 days or 469 temperature units for fry at 7 C.

Methods of pesticide analysis have been described in Part I.

RESULTS AND DISCUSSION

LOSSES WITH RESPECT TO TREATMENT AND REARING TEMPERATURE

No accurate records were maintained for egg losses. However, there appeared to be no unusual incidence of infertility or embryo loss during the incubation period.

Losses of both the untreated and 11-hour treated groups of fry were relatively light and similar in nature (Figures 1 and 2, Table 1, and Tables A5 and A6 of the Appendix). Final mortalities, averaged over the four rearing temperatures, were 11.6 and 13.3%, treated and untreated respectively. This difference in mortality proved to be not significant ($p>.5$) when the data of Table 1 was treated statistically as a split plot design (Cochran and Cox, 1957).

A fouled water supply line stopped the flow of water to one of the replicate tanks of 13° C untreated fry, resulting in a complete loss of fry in this tank. For the purpose of statistical analysis, a value was supplied for this replicate using the missing value procedure for split plot designs given in Cochran and Cox (1957).

The additional group of fish that had been treated 27 hours at hatching were reared in two 20-gallon aquaria. Water temperatures of these tanks varied from 12 to 14 C.

Table 1. Percentage losses following hatching for untreated and DDT-treated Lake Michigan rainbow trout sac fry reared at four temperatures.*

Rearing Temperature	Treatment		Mean of Treatments
	Untreated	Treated	
7 C	9.05 (8.60)**	6.19 (4.30)	7.62
10 C	16.07 (9.36)	10.64 (5.54)	13.36
13 C	9.65 (1.15)	10.99 (5.31)	10.32
16 C	18.28 (3.59)	18.53 (6.92)	18.41
$\bar{X}$	11.59	13.26	

*Final losses are for 500 temperature units from hatching for all 5 C tanks and 600 temperature units from hatching for all 10, 13, and 16 C tanks.

**Standard error (n = 4).

Figure 1. Percent losses of untreated Lake Michigan rainbow trout fry, cumulative and incremental, according to time intervals from hatching.

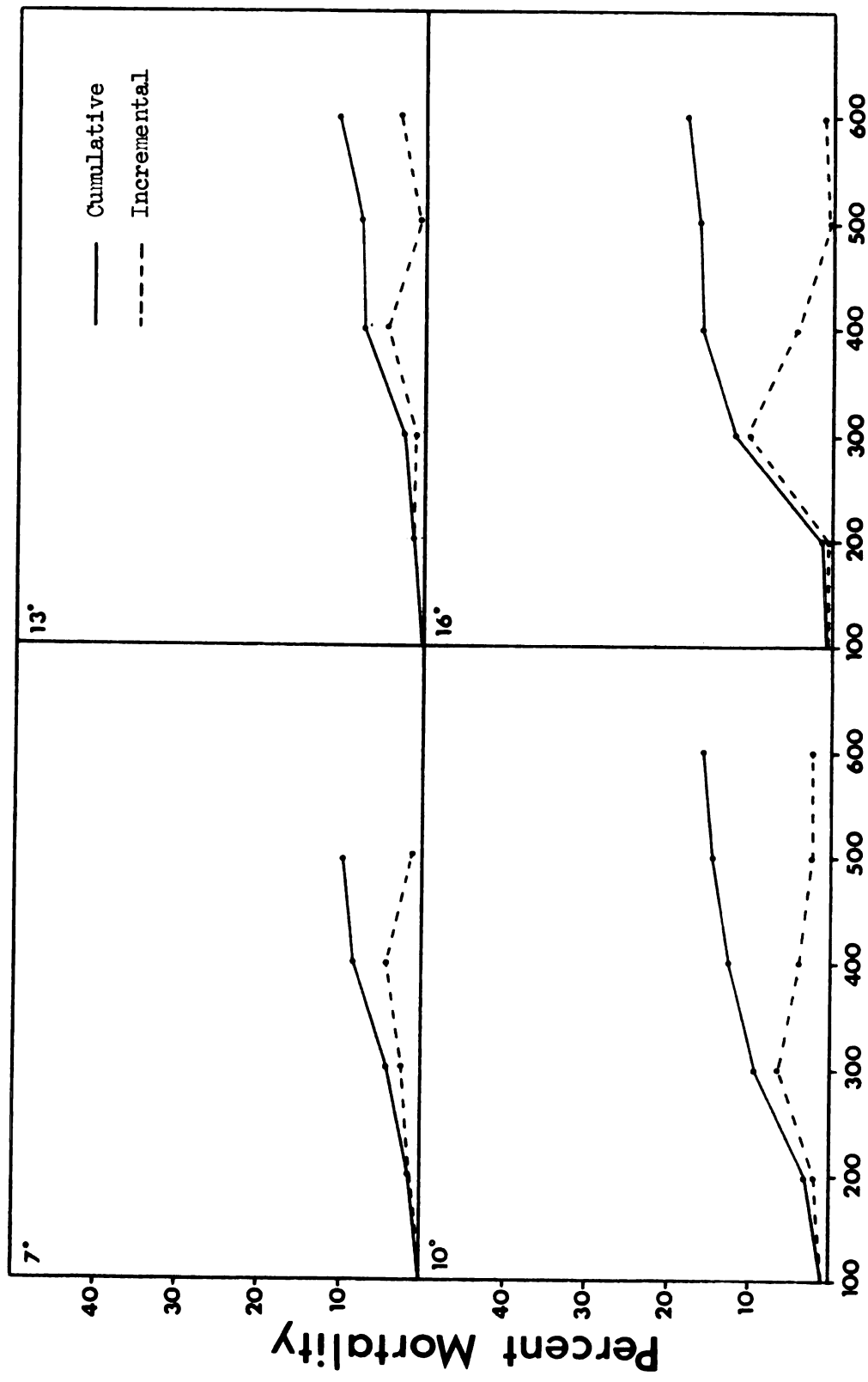
Cumulative
Incremental
7°
10°
13°
16°
Percent Mortality
Temperature Units From Hatching
10
20
30
40
100
200
300
400
500
600

Figure 1.

Figure 2. Percent losses of 11-hour treated Lake Michigan rainbow trout fry, cumulative and incremental, according to time intervals from hatching.

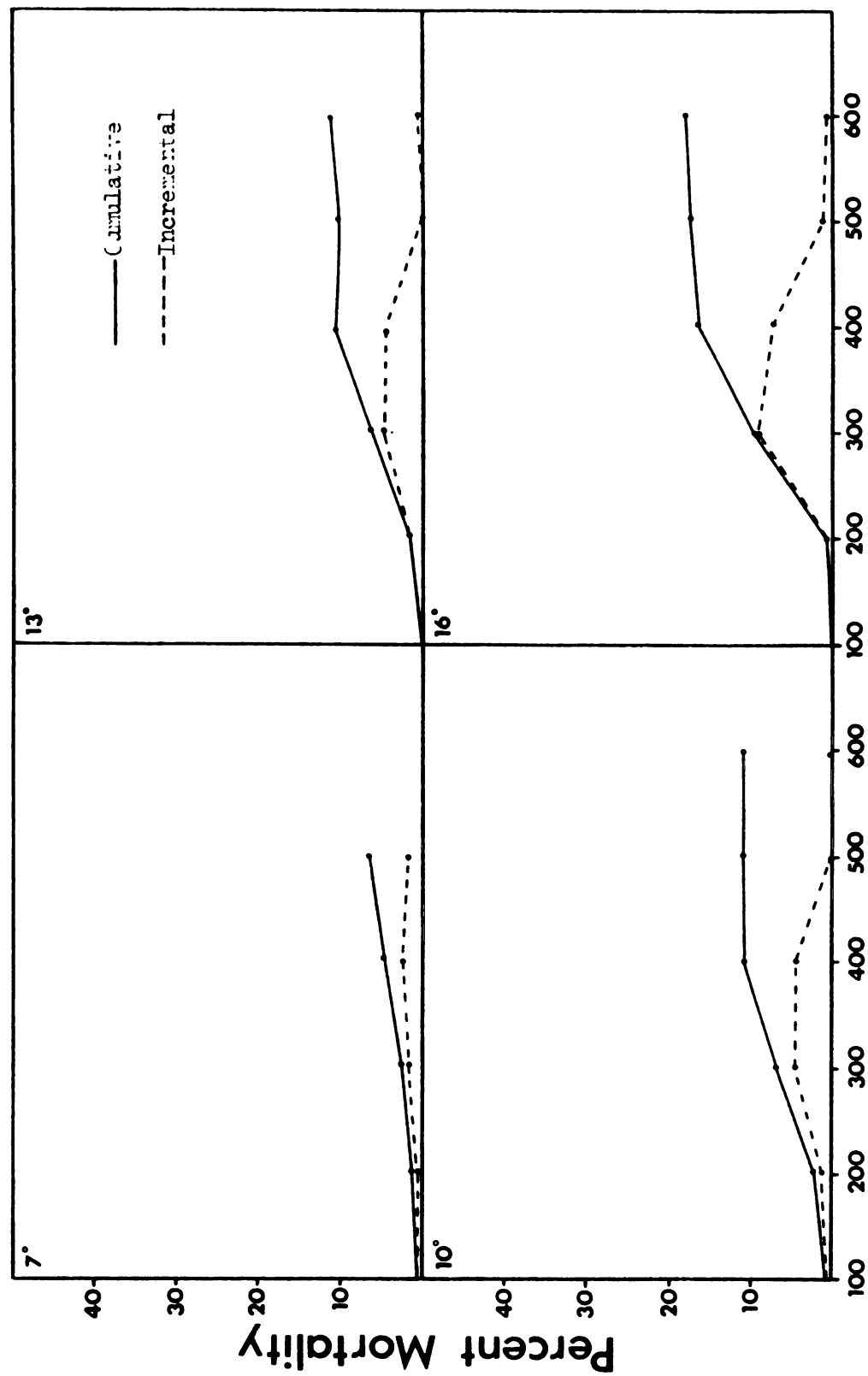
7°
10°
13°
16°
Cumulative
Incremental
Percent Mortality
10
20
30
40
100
200
300
400
500
600
Temperature Units From Hatching

Figure 2.

The two tanks contained 201 and 273 fry initially. Losses during the 38 days following hatching were 11.0 and 13.4%, with no further losses of any consequence observed thereafter. The average loss of the two tanks was 12.2%.

The mortality of the more heavily treated fry compares very closely with those of both treated and untreated fry used for the study of rearing temperatures. It therefore must be concluded that the amounts of DDT introduced to the treated groups of fry at hatching had no bearing on their later survival.

An effect of rearing temperature was somewhat more evident, but proved significant only at the 0.1 level. Although survival of both treatment groups was generally better at colder temperatures, no one temperature could be shown to produce a significant difference on fry survival.

Nearly all losses were among "pinhead" fry that apparently failed to begin feeding. Convulsive swimming activity and other symptoms of distress seen among Lake Michigan coho fry were seldom observed.

DDT levels of 1.12 ppm for untreated hatching rainbow trout (Table 3) were below the 1.70 ppm found in Lake Michigan coho fry. However, DDT levels in rainbow fry following the 11-hour treatment were more than twice those of untreated trout and exceeded those of coho fry by approximately 1 ppm. DDT levels in rainbow trout fry following the 27-hour treatment were more than 7 times those of the untreated group

and exceeded concentrations in the salmon fry by roughly 6.5 ppm.

The 8.34 ppm DDT in fry of the 27-hour treatment group exceeds thresholds previously reported to lead to losses of rainbow, cutthroat, brook and lake trout fry (Macek, 1968a; Currier et al., 1967; Burdick et al., 1964). However, it cannot be concluded, on the basis of the failure of either group of treated rainbow fry to develop increased mortalities, that the amounts of DDT residues in coho salmon fry of Part I were not sufficiently high to influence their reproductive success. Dissimilar specific tolerances to DDT residues may have contributed to the differences in survival observed between coho salmon and rainbow trout.

Furthermore, previous reports of DDT related fry losses have been for fry that had obtained DDT from residue contaminated parents. The effects of DDT when introduced to the fry at hatching may differ considerably from its effects if indigenous to the egg. Atchison (1970) hypothesized that the potential lethality of DDT residues in sac fry may depend on the site of deposition of DDT in the yolk material. Thus, if DDT is stored in different components of the yolk when the fry are treated at hatching than when residues are obtained from the female parent, the potential toxicities of residues obtained by the two means may not be the same.

GROWTH AND DEVELOPMENT

Eggs and hatching fry were of equal weight, averaging 0.102 g per individual. The effect of chorion loss at hatching on weight per individual was apparently compensated by gains of water content by the fry, resulting in no net change in weight. No fry weights were recorded at swimup.

Fry weights at termination of the study are presented in Table 2. Comparisons can be made between the weights of untreated and 11-hour treated fry since, for each temperature regime, both groups were sampled simultaneously. When the data of Table 2 are treated as a complete randomized block design the growth of treated fry proves to be significantly greater than that of the untreated group ($p<.025$). The mean weight of 27-hour treated fry when sampled for pesticide analysis 46 days after hatching (600 temperature units) was 0.34 g per fry. No statistical significance can be attached to this value since these fry were reared apart from the experimental design of the other two treatment groups.

There were no apparent differences between treatment groups within the same temperature regimes with respect to the timing of swimup and commencement of feeding.

Figure 3 illustrates the relationship between rearing temperature and the number of temperature units from hatching to swimup. For each temperature, swimup of untreated and 11-hour treated fry appeared to occur at essentially the same time. Therefore, Figure 3 is based on the pooled data of

Table 2. Weights of untreated and DDT-treated rainbow trout sac fry reared from hatching at four temperatures.*

Rearing Temperature	Fry Weights (g) Untreated	Treated	Mean of Treatments
7 C	0.268 (0.021)**	0.268 (0.023)	0.268
10 C	0.330 (0.087)	0.408 (0.022)	0.369
13 C	0.293 (0.040)	0.460 (0.008)	0.377
16 C	0.328 (0.117)	0.368 (0.034)	0.348
$\bar{X}$	0.305	0.401	

*Final weights are for 469 temperature units from hatching for all 5 C tanks, 550 temperature units for all 10 C tanks, 603 temperature units for 13 C and 608 temperature units for 16 C.

**Standard error (n = 4).

Figure 3. Temperature units required to rear both untreated and 11-hour treated rainbow fry from hatching to swimup.

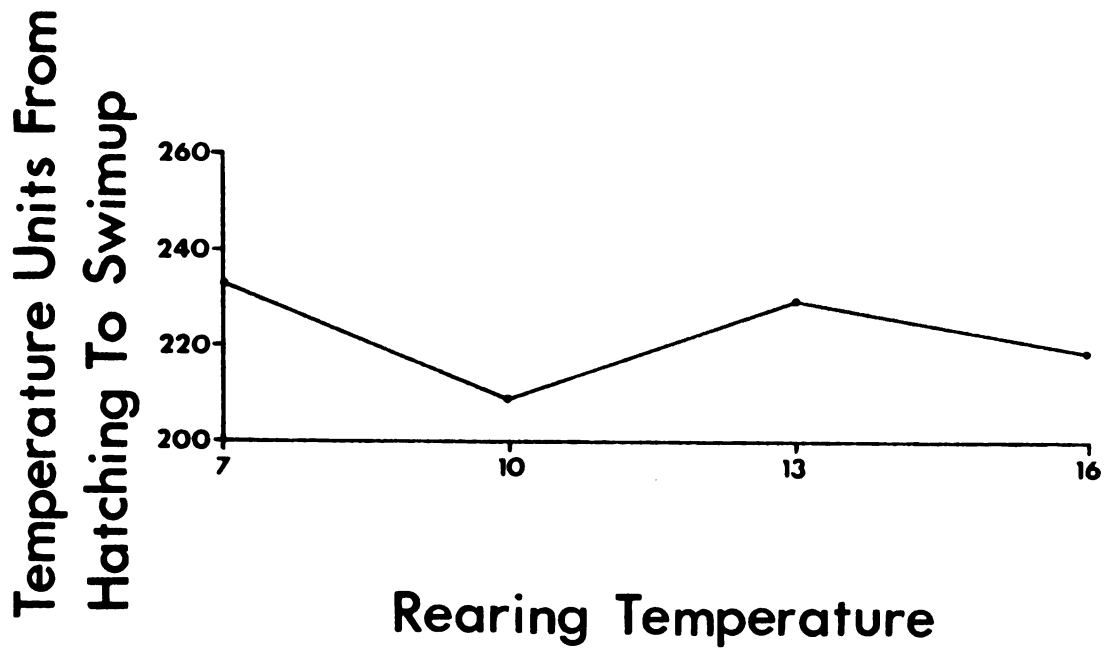
Temperature Units From
Hatching To Swimup
260
240
220
200
7
10
13
16
Rearing Temperature

Figure 3.

both of these groups. The number of temperature units at swimup was fairly uniform for the four temperatures, varying from 209 to 233.

The greater rate of growth of 11-hour treated rainbow fry contrasts with observations reported in Part I, in which Lake Michigan coho fry, the most heavily residue laden of the three salmon stocks studied, displayed the slowest rate of growth. Furthermore, the delay of development at colder temperatures observed in Great Lakes coho salmon fry (Part I) was not evident in treated rainbow fry.

However, the increased growth rate of treated rainbow fry appears to be in accord with observations reported by others. Allison (1964) noticed that cutthroat trout treated with DDT were generally larger than untreated individuals but attributed the difference to the effect of size selective mortalities. Mount (1962) reported an apparent increase in growth of bluntnose minnows (Pimephales notatus) exposed to endrin. Macek (1968a) noticed that the size of DDT treated male brook trout tended to increase with the level of treatment and that underyearling brook trout fed DDT grew faster than untreated fish (1968b).

The studies of coho salmon and rainbow trout differed with respect to sources of DDT residues in the fry. Furthermore, there may be substantial differences between the two species in growth characteristics and tolerances to DDT residues. It is therefore not valid to interpret the growth

of coho fry of Part I in terms of the growth rates observed for DDT treated rainbow fry.

PESTICIDE ANALYSES

The residue concentrations given in Tables 3 and 4 have been corrected for the recovery efficiency of the extraction and clean-up technique. Recoveries of DDE, DDD and DDT were 100.0, 88.8, and 87.0% respectively. Since extraction and clean-up procedures were essentially the same as for the coho fry, the increase in recovery from that of the previous study does not easily lend itself to explanation. The improvement may simply be owing to a gain of experience in the extraction technique.

Fry of all three treatment groups showed a decrease in concentrations of DDT and its analogs of approximately 70% between hatching and the end of the study (Table 3). When these results are interpreted in terms of μg residue per fry (Table 4), it becomes evident that, as was the case for coho fry of Part I, there was little or no net loss of residue following hatching. The sharp decline in residue (Table 3) is probably due to dilution of the pesticide quantity by growth. Any apparent differences between the three treatment groups with respect to net loss of residue are within the range of normal variability of the analysis technique.

Table 3. Mean DDT residues (ppm) in untreated and DDT/treated Lake Michigan rainbow trout eggs and fry.

Sample	Treatment	Residue Levels (ppm) DDE	DDD	DDT	Total
Eggs	None	3.58 (0.08)*	0.21 (0.02)	1.08 (0.05)	4.87 (0.09)
Hatching	None	3.40 (0.74)	trace**	1.12 (0.21)	4.52 (0.95)
Final					
7 C	None	1.65 (0.13)	trace	0.42 (0.01)	2.07 (0.14)
10 C	None	1.24 (0.10)	trace	0.33 (0.03)	1.57 (0.13)
13 C	None	1.15 (0.14)	trace	0.27 (0.01)	1.43 (0.13)
16 C	None	1.41 (0.23)	trace	0.35 (0.05)	1.76 (0.28)
Hatching	11-hour	3.90 (0.00)	0.61 (0.06)	2.75 (0.07)	7.25 (0.14)
Final					
7 C	11-hour	1.51 (0.08)	trace	0.66 (0.03)	2.17 (0.10)
10 C	11-hour	1.12 (0.40)	trace	0.36 (0.08)	1.48 (0.14)
13 C	11-hour	1.19 (0.19)	trace	0.31 (0.02)	1.50 (0.22)
16 C	11-hour	0.95 (0.05)	trace	0.45 (0.24)	1.41 (0.08)
Hatching	27-hour	4.01 (0.24)	1.77 (0.01)	8.34 (0.36)	14.12 (0.59)
Swimup***	27-hour	3.22 -	1.04 -	5.16 -	9.41 -
Final	27-hour	1.88 (0.63)	0.36 (0.08)	1.96 (0.35)	4.20 (1.06)

*Standard error (n = 2).

**Less than 0.10 ppm.

***Based on a single analysis.

Table 4. Mean DDT residues (μg per individual) in untreated and DDT-treated Lake Michigan rainbow trout eggs and fry.

Sample	Treatment	Weight Per Individual	Residue levels (μg/individual)			
			DDE	DDD	DDT	Total
Eggs	None	0.102	0.365	0.021	0.111	0.497
Hatching	None	0.102	0.347	trace	0.114	0.462
Final						
7 C	None	0.268	0.441	trace	0.114	0.555
10 C	None	0.330	0.408	trace	0.109	0.517
13 C	None	0.293	0.338	trace	0.803	0.418
16 C	None	0.328	0.462	trace	0.116	0.577
Hatching	11-hour	0.102	0.398	0.062	0.280	0.740
Final						
7 C	11-hour	0.268	0.405	trace	0.176	0.617
10 C	11-hour	0.408	0.455	trace	0.147	0.602
13 C	11-hour	0.460	0.548	trace	0.141	0.689
16 C	11-hour	0.368	0.350	trace	0.167	0.517
Hatching	27-hour	0.102	0.409	0.180	0.851	1.441
Final	27-hour	0.335	0.631	0.120	0.657	1.408

A decline in the proportion of DDT in the residue complex is evident for all three treatment groups for the period following hatching (Table 5). The decrease on the contribution of DDT to the complex between hatching and final samples amounted to 18.4, 28.4 and 21.3% for untreated, 11-hour treated and 27-hour treated fry respectively. The decline of DDT was accompanied by an increase in the proportion of DDE, indicating that DDT was being metabolized by the fry to DDE. The apparently greater rate of metabolism of DDT in treated fry may indicate that treatment with DDT acted to stimulate liver dehydrochlorinase activity. Evidence that DDT exposure induces dehydrochlorinase activity has also been reported for goldfish (Grzenda et al., 1970).

Table 5. Percentage composition of DDT residues in untreated and DDT-treated rainbow trout fry at different developmental stages.

Sample	Treatment	Percent composition ± 90% C.I. DDE	DDD	DDT
Eggs	None	73.53 ± 2.05	4.21 ± 0.56	22.26 ± 1.49
Hatching	None	75.22 ± 0.24	trace	24.78 ± 0.24
Final*	None	79.78 ± 0.63	trace	20.22 ± 0.63
Hatching	11-hour	53.76 ± 2.36	8.34 ± 1.57	37.89 ± 0.78
Final*	11-hour	72.87 ± 4.45	trace	27.12 ± 4.45
Hatching	27-hour	28.40 ± 1.10	12.51 ± 1.40	59.30 ± 0.41
Swimup**	27-hour	34.16 -	11.05 -	54.79 -
Final	27-hour	44.77 ± 6.69	8.54 ± 0.22	46.68 ± 6.61

*Averaged for the four temperature regimes.

**Based on a single analysis.

SUMMARY

Coho salmon fry from Lake Michigan, Lake Huron and Oregon parent stocks were subjected to four rearing temperatures beginning with the time of hatching. Losses of Lake Michigan fry exceeded those of Lake Huron and Oregon ($p<.05$) and growth and developmental rates of Lake Michigan fry appeared to be substantially retarded.

Contamination by chlorinated hydrocarbon residues was considered to be the most probable source of the losses. Pesticide analyses revealed higher levels of residues in Lake Michigan eggs and fry than in those of Lake Huron and Oregon parent stocks. The residues consisted of DDT and its analogs as well as unidentified substances believed to be largely polychlorinated biphenyl residues. The nature of the losses of Lake Michigan fry closely resembled previously reported fry losses that were attributed to DDT. However, because of the presence of substances other than DDT in the fry, it could not be shown conclusively that any one contaminant or combination of contaminants was responsible for the losses.

Although no precise formula was evident for the effect of rearing temperature on fry survival, losses of Lake Michigan fry were generally greater at colder temperatures.

To further evaluate the effect of DDT on salmonid reproduction, Lake Michigan rainbow trout fry were treated with DDT at the time of hatching and reared at four temperatures. Levels of p-p' DDT in treated rainbow trout fry were higher than levels encountered in Lake Michigan coho fry and exceeded lethal threshold concentrations reported by earlier investigations. However, no increase in mortality was established for treated fry and mean losses of neither treated nor untreated trout exceeded 20%. None of the four rearing temperatures tested had a significant effect on fry survival.

The failure of DDT treated rainbow fry to sustain increased losses was not considered to be sufficient evidence that DDT was not the source of losses of Lake Michigan coho salmon fry. Differing means by which DDT residues were attained and dissimilar specific tolerances were cited as possible sources of the apparent differences in survival between the two species.

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APPENDIX

Table A1. Percentage mortality, incremental (a) and cumulative (b) for Lake Michigan coho fry with rearing time.*

Temperature Units From Hatching	Rearing Temperature (°C) 5	9	13	17
a.				
100	-	-	-	-
200	1.14 (0.47)**	4.96 (4.73)	0.00 (0.00)	3.86 (5.43)
300	40.00 (5.45)	7.39 (1.39)	2.13 (0.77)	1.79 (2.44)
400	66.02 (3.95)	3.29 (2.14)	3.48 (4.00)	6.82 (6.90)
500	20.75 (1.00)	15.53 (0.30)	7.66 (9.33)	15.12 (5.56)
600	4.76 (0.33)	35.63 (0.93)	18.05 (3.57)	18.39 (2.11)
700	15.00 (15.25)	8.93 (2.26)	17.86 (3.49)	23.93 (1.36)
800	35.29 (9.09)	48.04 (13.82)	15.94 (0.25)	29.63 (8.54)
900		39.62 (9.36)	16.38 (8.67)	18.42 (14.21)
1000		50.00 (4.90)	4.12 (2.74)	8.08 (3.42)
1100		56.25 (7.07)	0.00 (0.00)	12.28 (9.72)
1200				16.00 (22.39)
b.				
100	-	-	-	-
200	1.14 (0.47)	4.96 (4.73)	0.00 (0.00)	3.86 (5.43)
300	40.68 (5.67)	11.98 (3.06)	2.13 (0.77)	5.58 (3.00)
400	79.85 (0.42)	14.88 (4.84)	5.53 (4.65)	12.12 (3.72)
500	84.03 (0.13)	28.10 (3.83)	12.77 (13.07)	25.32 (8.04)
600	84.79 (0.18)	53.72 (1.80)	28.51 (7.67)	39.06 (8.13)

continued

Table A1--continued

Temperature Units From Hatching	Rearing Temperature (°C) 5	9	13	17
700	87.07 (2.47)	57.85 (0.33)	41.28 (3.84)	53.65 (7.00)
800	91.63 (2.74)	78.10 (6.17)	46.38 (3.37)	67.38 (1.01)
900		86.78 (5.69)	58.52 (1.43)	73.40 (3.81)
1000		93.39 (2.26)	60.43 (2.50)	75.74 (4.39)
1100		97.11 (0.55)	60.43 (2.50)	78.54 (6.20)
1200				81.97 (9.82)

*Mortality increments expressed as percentage losses of fry surviving the immediately preceding time interval.

**Standard error (n = 2).

Table A2. Percentage mortality, incremental (a) and cumulative (b) for Lake Huron coho fry with rearing time.*

Temperature Units From Hatching	Rearing Temperature (°C) 5	9	13	17
a.				
100	9.48 (10.38)**	2.61 (0.19)	3.33 (0.14)	4.09 (0.06)
200	0.87 (1.70)	0.89 (1.34)	1.97 (1.93)	1.94 (1.39)
300	0.00 (0.00)	0.00 (0.00)	2.51 (0.05)	2.77 (2.46)
400	0.44 (0.87)	4.05 (4.12)	0.00 (0.00)	4.87 (2.96)
500	0.44 (0.87)	2.81 (0.13)	6.19 (3.19)	3.42 (0.12)
600	1.33 (0.95)	0.49 (0.71)	2.20 (2.57)	11.50 (2.12)
700	8.42 (2.96)	2.43 (0.55)	5.06 (1.03)	6.50 (2.90)
800		3.98 (3.83)	7.69 (0.53)	6.42 (2.59)
900		30.05 (6.09)	0.64 (0.77)	6.86 (0.28)
1000		10.29 (9.40)	0.65 (0.77)	4.91 (0.21)
1100			0.00 (0.00)	1.29 (0.07)
1200				1.96 (2.86)
1300				7.40 (0.51)
b.				
100	9.48 (10.83)	2.61 (0.19)	3.33 (0.14)	4.09 (0.06)
200	10.26 (12.22)	3.48 (1.49)	5.24 (2.00)	5.95 (1.27)
300	10.26 (12.22)	3.48 (1.49)	7.62 (1.99)	8.55 (3.55)
400	10.67 (12.91)	7.39 (2.54)	7.62 (1.99)	13.01 (6.10)
500	11.06 (13.60)	10.00 (2.34)	13.33 (4.72)	15.99 (5.67)
600	12.25 (14.52)	10.43 (1.70)	15.24 (2.42)	25.65 (3.22)

continued

Table A2--continued

Temperature Units From Hatching	Rearing Temperature (°C) 5	9	13	17
700	17.39 (15.85)	12.61 (2.15)	19.52 (1.41)	30.48 (5.18)
800		16.09 (5.59)	25.71 (0.88)	34.94 (6.68)
900		41.43 (1.20)	26.19 (0.29)	39.40 (6.03)
1000		47.39 (6.58)	26.66 (0.28)	42.37 (5.61)
1100			26.66 (0.28)	43.12 (5.50)
1200				44.24 (3.75)
1300				48.32 (3.22)

*Mortality increments expressed as the percentage losses of fry surviving the immediately preceding time interval.

**Standard error (n = 2).

Table A3. Percentage mortality, incremental (a) and cumulative (b) for Oregon coho fry with rearing time.*

Temperature Units From Hatching	Rearing Temperature (°C) 5	9	13	17
a.				
100	1.03 (0.04)**	1.66 (0.40)	4.78 (2.94)	5.81 (3.76)
200	0.00 (0.00)	0.00 (0.00)	0.84 (1.13)	0.88 (0.07)
300	0.52 (0.76)	0.56 (0.79	0.00 (0.00)	0.00 (0.00)
400	0.52 (0.76)	0.56 (0.81)	0.84 (1.23)	3.56 (0.29)
500	0.53 (0.73)	0.00 (0.00)	0.00 (0.00)	13.36 (0.82)
600	35.50 (22.92)	0.00 (0.00)	0.00 (0.00)	22.87 (2.04)
700		0.00 (0.00)	1.28 (1.90)	11.72 (2.35)
800		0.00 (0.00)	0.00 (0.00)	0.78 (1.09)
900		0.00 (0.00)	0.00 (0.00)	1.57 (2.24)
1000		0.00 (0.00)	0.00 (0.00)	0.80 (1.16)
1100			0.43 (0.65)	2.42 (3.54)
1200			0.00 (0.00)	2.48 (1.38)
1300				1.69 (0.16)
b.				
100	1.03 (0.04)	1.66 (0.40)	4.78 (2.94)	5.81 (3.76)
200	1.03 (0.04)	1.66 (0.40)	5.58 (4.00)	6.64 (3.80)
300	1.55 (0.80)	2.21 (0.00)	5.58 (4.00)	6.64 (3.80)
400	2.06 (0.09)	2.76 (0.40)	6.37 (2.79)	9.96 (3.93)
500	2.58 (0.62)	2.76 (0.40)	6.37 (2.79)	21.99 (2.67)
600	34.02 (21.92)	2.76 (0.40)	6.37 (2.79)	39.83 (0.24)

continued

Table A3--continued

Temperature Units From Hatching	Rearing Temperature (°C) 5	9	13	17
700		2.76 (0.40)	7.57 (0.97)	46.88 (1.01)
800		2.76 (0.40)	7.57 (0.97)	47.30 (1.58)
900		2.76 (0.40)	7.57 (0.97)	48.13 (0.37)
1000		2.76 (0.40)	7.57 (0.97)	48.55 (0.12)
1100			7.97 (0.37)	49.79 (2.04)
1200			7.97 (0.37)	51.04 (2.69)
1300				51.86 (2.72)

*Mortality increments expressed as percentage losses of fry surviving the immediately preceding time interval.

**Standard error (n = 2).

Table A4. Percentage composition of DDT residues in Lake Michigan coho salmon fry at different developmental stages.

Sample	Percent composition ± 90% C.I.		
	DDE	DDD	DDT
Eggs	65.39 ± 3.95	9.26 ± 1.21	25.35 ± 2.72
Hatching	71.25 ± 1.47	5.36 ± 0.36	25.38 ± 1.09
Swimup	72.80 ± 0.58	4.71 ± 0.22	22.46 ± 0.77
Final	84.12 ± 0.34	trace	15.88 ± 0.34

Table A5. Percent mortality, incremental (a) and cumulative (b) with rearing time from hatching for untreated Lake Michigan rainbow trout sac fry.*

Temperature Units From Hatching	Rearing Temperature (°C) 7	10	13	16
a.				
100	0.00 (0.00)**	1.03 (1.19)	0.00 (0.00)	0.67 (1.47)
200	1.52 (1.98)	2.07 (2.89)	1.59 (2.00)	0.68 (1.51)
300	2.58 (1.99)	6.35 (2.64)	1.08 (1.27)	10.88 (1.49)
400	4.23 (4.40)	3.95 (3.22)	4.89 (3.04)	4.54 (1.96)
500	1.66 (1.32)	2.35 (2.38)	0.57 (1.11)	0.80 (1.39)
600		2.41 (4.28)	2.39 (3.23)	1.61 (2.80)
b.				
100	0.00 (0.00)	1.03 (1.19)	0.00 (0.00)	0.67 (1.47)
200	1.52 (1.98)	3.08 (2.66)	1.59 (2.00)	1.34 (2.94)
300	4.06 (3.72)	9.23 (5.00)	2.65 (1.98)	12.08 (4.55)
400	8.12 (7.15)	12.82 (5.66)	7.41 (3.10)	16.11 (5.22)
500	9.64 (7.31)	14.87 (7.29)	7.94 (2.99)	16.78 (4.68)
600		16.92 (9.36)	10.58 (1.15)	18.12 (3.59)

*Mortality increments expressed as percentage losses of fry surviving the immediately preceding time interval.

**Standard error (n = 4).

Table A6. Percent mortality, incremental (a) and cumulative (b) with rearing time from hatching for 11-hour treated Lake Michigan rainbow trout sac fry.*

Temperature Units From Hatching	Rearing Temperature (°C) 7	10	13	16
a.				
100	0.52 (1.06)**	0.68 (2.38)	0.00 (0.00)	0.00 (0.00)
200	0.52 (1.08)	1.37 (1.24)	1.59 (2.42)	0.56 (1.11)
300	1.56 (1.06)	4.86 (1.44)	4.84 (5.02)	9.04 (3.86)
400	2.12 (4.00)	4.38 (4.10)	4.52 (1.70)	7.45 (4.15)
500	1.62 (1.80)	0.00 (0.00)	0.00 (0.00)	1.34 (1.47)
600		0.00 (0.00)	0.59 (1.43)	0.68 (1.22)
b.				
100	0.52 (1.06)	0.68 (2.38)	0.00 (0.00)	0.00 (0.00)
200	1.03 (1.24)	2.04 (1.95)	1.59 (2.42)	0.56 (1.11)
300	2.58 (2.08)	6.80 (2.86)	6.35 (4.89)	9.55 (4.34)
400	4.64 (2.49)	10.88 (5.54)	10.58 (5.17)	16.29 (7.41)
500	6.19 (4.30)	10.88 (5.54)	10.58 (5.17)	17.42 (7.18)
600		10.88 (5.54)	11.11 (5.31)	17.98 (6.92)

*Mortality increments expressed as percentage losses of fry surviving the immediately preceding time interval.

**Standard error (n = 4).