AN ABSTRACT OF THE THESIS OF

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Title: TOXICITY OF PENTACHLOROPHENOL TO TROUT ALEVINS

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A study was conducted at the Pacific Cooperative Water Pollution and Fisheries Research Laboratories, Oregon State University, to determine the effects of pentachlorophenol (PCP) on the early developmental stages of the steelhead trout (Salmo gairdneri). Experiments were performed from May, 1965, through May, 1968, on the survival, growth, and bioenergetics of embryos, alevins, and juveniles exposed to sodium pentachlorophenate (NaPCP).

A technical grade sodium salt of PCP was used in all experiments. Preliminary static bioassays were conducted to determine the concentrations of NaPCP which were lethal to embryos and alevins. In an experiment where embryos were exposed to NaPCP from fertilization to hatching, 100% mortality occurred within 1 week after fertilization at concentrations down to 300 ppb; within 24 hours post-hatch, 100% mortality occurred down to 50 ppb of NaPCP. Alevin dry weight at hatch was decreased by exposure to NaPCP, and

hatching was delayed. In 5-day bioassays, alevins usually died within 24 hours at concentrations down to 200 ppb; but little mortality occurred at lower concentrations.

Seven experiments were subsequently conducted with alevins (20-41 days duration), one with juveniles (21 days), and one with embryos and alevins (92 days). In all but one experiment, alevins were denied exogenous food and grew to a maximum weight before starvation occurred when the yolk supply was exhausted.

Holding alevins in 30 to 100 ppb of NaPCP throughout the alevin stage, retarded growth, increased yolk catabolism, and increased mortality. Little mortality occurred during the first week; but after 3 to 4 weeks, mortality was nearly complete at 70 and 100 ppb. An NaPCP concentration of 45 ppb caused little mortality and no mortality occurred at 30 ppb or in controls (no NaPCP). Maximum dry weight gain of alevins reared in NaPCP was decreased approximately 6% for each 10 ppb increase in NaPCP concentration.

Intermittent exposure of alevins to NaPCP indicated that recovery from toxic effects occurred within several days after removal from NaPCP.

In the only experiment where alevins were given an exogenous food, the dry weight gain 57 days post-hatch of alevins in 40 ppb NaPCP was reduced about 75% from that of controls (33 mg vs 120 mg). Controls began feeding after 20 days post-hatch, while in NaPCP,

feeding began about 40 days post-hatch.

Juvenile steelhead held in 30 and 70 ppb NaPCP for 3 weeks showed retardation of growth but little mortality, indicating a greater tolerance to NaPCP than alevins or embryos. Continuous exposure to NaPCP from fertilization to complete yolk absorption produced 100% mortality at 40 ppb of NaPCP but little mortality at 20 or 10 ppb. However, at 5 mg/liter dissolved oxygen concentration, 20 ppb was 100% lethal and at 3 mg/liter, 10 ppb was 100% lethal. Little mortality occurred at these oxygen levels in the absence of NaPCP. Oxygen consumption rates of alevins in 40 ppb of NaPCP were higher than those of control alevins at 10 and 5 mg/liter of dissolved oxygen but not at 3 mg/liter.

While determinations of oxygen consumption, growth, and yolk utilization efficiency were too gross to produce definitive information as to the mechanism of action of PCP, the bioenergetic data obtained in this study were consistent with the concept that PCP disrupts energy metabolism. Alevins exposed to NaPCP grew less rapidly than controls due to higher rates of yolk catabolism and resultant lower efficiencies of yolk utilization. Congruently, NaPCP reared alevins also had higher rates of oxygen consumption.

Toxicity of Pentachlorophenol to Trout Alevins

by

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INTRODUCTION

Generally, investigations concerning the effects of toxic substances on fish have been confined to short-term bioassays. Such bioassays are the logical first step in most investigations of toxicity and they probably yield the most information for the time and effort involved. The species and life stages used in bioassays are usually selected on the basis of availability, economic importance, and ease of handling. Short-term bioassays do not yield sufficient information upon which to base criteria for allowable environmental levels of the toxic substance being considered. However, on the basis of information obtained from short-term bioassays, studies can be conducted which broaden the field of investigation to include other species and life stages, to include a multifactorial analysis of the effect of the toxic substance under various water quality conditions, and to determine the sub-lethal effects of the toxic substance. This study was undertaken to broaden the knowledge of the effects of the toxicant pentachlorophenol (PCP) on fish and to discuss the findings with respect to previous work with PCP and to water quality.

The establishment of water quality criteria for the protection of fish and other aquatic animals is dependent upon adequate information

as to the environmental requirements of the species involved and their tolerance to changes in environment. The nature of the problems in establishing water quality criteria has been summarized in the introduction of Section III of the Report of the National Technical Advisory Committee on Water Quality Criteria (U. S. F. W. P. C. A., 1968); portions of the introduction of the report are directly pertinent to the rationale behind the study reported in this paper and are quoted:

> ... The determination of water quality requirements is a very complicated task. The problem is further complicated by the fact that different species and different developmental or life stages of the same or different species may differ widely in their sensitivity or tolerance to different materials, to ranges in environmental conditions, and to the cumulative synergistic and antagonistic effects of toxicants... It is essential, also to realize that requirements must be maintained throughout periods of low water, maximum discharge, maximum temperature, minimum dissolved oxygen, variations in pH, turbidity, salinity, etc. Further, it should be understood that: (1) unfavorable conditions which may be resisted for long periods by adults may be entirely unfavorable for the survival of the species; (2) conditions need to be unfavorable for only a few hours to eliminate a population or group of a species; and (3) levels of environmental factors and concentrations of toxicants that appear to cause no harm during a few hours of exposure may be intolerable for extended periods or for recurring short-term exposure.

Some data, to be discussed later, have been reported with respect to the effects of environmental conditions on PCP toxicity to fish, on the toxicity to numerous species of fish and a few life stages, and on the long-term toxicity of PCP. These data are generally scant and have never been compiled in a single study. The investigation reported in

this paper was conducted in such a manner as to combine in a single study the effects of PCP on a susceptible, developmental stage, exposed to PCP for relatively long periods and at several concentrations of dissolved oxygen.

PCP: Uses and Properties

Pentachlorophenol and its salts, primarily sodium pentachlorophenate (NaPCP), have a wide spectrum of industrial and agricultural applications. A review of the uses, properties, toxicology, and analysis of PCP has been compiled and can be referred to for additional information (Bevenue and Beckman, 1967). The United States' production of PCP and its salts was nearly 40 million pounds in 1965 (U. S. D. A. A. S. C. S., 1966). Over half (56%) of the annual production was used in preservation of poles, timbers, fiberboards, plywood, etc., 9% as a slimicide, 3% as a herbicide, and the remainder in a number of other categories. With such an array of uses, it seems inevitable that some portion of the PCP would find its way into streams.

It has been reported that NaPCP is decomposed within hours by light in the ultraviolet range when it is applied to clear shallow water areas, but will persist longer in deeper and turbid waters (Hiatt, Haskins, and Olivier, 1960). Sodium pentachlorophenate appears to be very resistant to biological degradation by activated sludge (Ingols,

Gaffney, and Stevenson, 1966).

PCP: Enzyme Studies

Detailed investigations into the effects of PCP on oxidative phosphorylation, ATPase, and mitochondria have been conducted by Weinbach and co-workers. They found that PCP decreased or eliminated the uptake of inorganic phosphate associated with the oxidation of α-ketoglutarate and β-hydroxybutyrate (Weinbach and Garbus, 1954; Weinbach and Garbus, 1965). At low concentrations (10^{-5} M), PCP increased the phosphate released from mitochondria, but higher concentrations decreased the amount of phosphate released (Weinbach, 1956b; Weinbach and Bowen, 1958). Pentachlorophenol apparently binds to protein moieties of mitochondria (Weinbach and Garbus, 1964) producing swelling and dissolution of the mitochondria (Weinbach, Sheffield, and Garbus, 1963). The addition of bovine serum albumin (BSA) and ATP to mitochondria swollen by PCP restores mitochondrial morphology at low (less than equimolar) concentrations of BSA while equimolar concentrations of BSA also restore normal oxidative phosphorylation (Weinbach and Garbus, 1966a; 1966b). The BSA apparently acts by removing PCP from the mitochondria and binding the PCP to the albumin molecule.

PCP: Toxicity to Fish

The toxicity of NaPCP to 19 species of fish was studied by Goodnight (1942) who reported that exposure to solutions of NaPCP for 3 days was fatal to the more sensitive species of fish at a concentration of 0. 4 ppm, although hardier species survived at 0. 4 or 0. 6 ppm. The most sensitive fish studied was the silverjaw minnow (Ericymba buccata) and the least susceptible was the blackstripe top minnow (Fundulus notatus).

Other workers have since investigated the toxicity of NaPCP to fish and have reported minimum lethal concentrations between 0. 17 and 0. 75 ppm (Van Horn, 1943; Turnbull, DeMann, and Weston, 1954; Ferster, 1956; Alabaster, 1958; Mann, 1957; Bandt and Nehring, 1962; Brockway, 1963; Ozaki, 1963; and Chapman, 1965). A study of the long-term effects of NaPCP on fish was reported by Crandall and Goodnight (1962) who found that 0. 5 ppm caused retarded growth, increased mortality, and delayed sexual maturity in guppies (Lebistes reticulatus) over a 90-day period.

Goodnight (1942) also studied the effect of PCP on the eggs and young of lake trout (Salvelinus namaycush) and reported:

> Eggs of lake trout were found to be extremely resistant to pentachlorophenol. The newly hatched young in the yolk sac stage were found to be more sensitive than either the eggs or the more mature fish. Even in this sensitive stage, however, the young trout were hardier than

silver-mouthed minnows,[1] having longer survival times at fatal concentrations. The observations at different periods of growth indicated that sensitivity decreased as the trout advanced in age.

Salmonid Developmental Stages

The terminology applied to life history stages of salmonids has been used inconsistently in the past. For this reason, definitions of the terms used in this paper are given below:

embryo--developmental stages to the moment of hatching

alevin--developmental stages from hatching until all yolk has been absorbed

juvenile--young fish following complete absorption of yolk.

The embryonic and alevin stages of salmonids may take as long as 4 or 5 months to complete. The duration of these developmental stages depends on a number of factors; the primary factors being the size of the egg, the species involved, and the incubation temperature. The embryonic and alevin stages each comprise roughly one-half of the developmental time. Approximately halfway through the alevin period, the alevin attains a stage called the "swim-up". Prior to this stage, the steelhead alevins swim freely in troughs only for short bursts near the bottom of the troughs and usually rest on the bottom. The pre-swim-up alevins react negatively to light. Once

[1] Silverjaw minnow.

the swim-up stage is attained, as the name implies, the alevins swim freely about in the water and also the negative photo response ceases. At this stage, in nature, the alevins presumably emerge from the gravel and are ready to feed.

The total reliance upon yolk for nutrition lasts throughout the embryonic stages and through the alevin period up to the swim-up stage. The swim-up alevin can utilize a dual source of nutrition, yolk and exogenous food.

A number of laboratory studies of the growth and bioenergetics of embryos and alevins of salmonids have been conducted. Most notable are the studies by Smith (1947, 1952, and 1958), Gray (1926 and 1928), and the series of experiments summarized by Hayes (1949). Almost all investigations of this type have taken advantage of the closed nutritive system afforded by the yolk supply of these fish and have routinely denied the swim-up alevins a supply of exogenous food. Such an approach simplifies the analysis of growth data by eliminating the need for determination of food consumption and assimilation efficiencies.

The growth of salmonid alevins denied exogenous food is limited by the amount of yolk present and the relative proportions of the yolk which are utilized for tissue elaboration, maintenance, and activity. Thousands of eggs can be obtained from a single fish and fertilized at the same time, giving a population of embryos with

essentially uniform size, age, and genetic composition. With such a population, the amount of yolk is rather constant between eggs; and therefore the growth potential of each embryo is the same. Growth and bioenergetic parameters of embryos and alevins from such a population can be measured under a variety of conditions with relatively little variation within a given sample, thus allowing the detection of rather small changes in these parameters. This approach has been used to determine the effect of dissolved oxygen, water velocity, light, and temperature on the growth of salmonids (Gray, 1928; Wood, 1932; Hayes and Pelluet, 1945; Silver, Warren, and Doudoroff, 1963; Shumway, Warren, and Doudoroff, 1964; Brannon, 1965; and Marr, 1965; 1966).

The use of salmonid alevins and embryos in relatively long-term bioassays with chemicals has received little attention. Vernidub (1962) investigated the poisoning of Baltic salmon alevins by non-volatile phenols. The toxicity of tetrachloro- and dichlorocatechol to sockeye salmon alevins (O. nerka) and pink salmon alevins (O. gorbuscha) was determined by Servizi, Gordon, and Martens (1968). On the other hand, the toxic effect of chemicals on salmonid embryos has been little investigated, probably because eggs are considered to be essentially impermeable to many materials. With the potential of PCP as a toxicant and because of the economic importance of salmonids and of the advantages in working with salmonid embryos and

alevins, a program was developed to follow growth, development, oxygen consumption, and bioenergetics of steelhead trout (Salmo gairdneri) embryos and alevins held at various levels of NaPCP.

MATERIALS AND METHODS

Experimental Animals

Eggs of the steelhead trout, a sea-run form of the rainbow trout, were obtained from trout hatcheries in Oregon and transported to the laboratory within 2 hours of fertilization. Some batches of eggs were immediately used in experiments while others were held in running water in 2-liter separatory funnels. Just prior to hatching, the eggs held in the funnels were transferred to shallow troughs. The newly hatched alevins were held in the troughs until used in the experiments. Aerated 10° C water was continuously supplied to the troughs and separatory funnels at rates of about 500 ml/min.

Pentachlorophenol

A single lot of Santobrite,[2] a technical grade sodium salt of PCP, was used in all experiments. Santobrite has a minimum purity of 90% sodium pentachlorophenate (NaPCP) (Monsanto, 1966). All concentrations of NaPCP were calculated on the basis that Santobrite had a purity of 100% NaPCP.

[2] Trade Mark, Monsanto Chemical Company.

Static Bioassays

Static bioassays (as opposed to flowing water bioassays) were conducted to determine the concentrations of NaPCP which were lethal to embryos and alevins of steelhead trout and to select a range of NaPCP concentrations suitable for long-term studies. Bioassays with embryos and alevins were conducted in shallow glass vessels, 25 x 25 x 5 cm. The embryo and alevin bioassays were performed in 10 and 15° C constant temperature rooms respectively.

Embryos

An embryo bioassay, lasting about 1 month, was begun 15 minutes after the eggs were fertilized and was terminated shortly after hatching occurred. A series of NaPCP solutions was prepared with concentrations ranging from 0. 01 to 1 ppm; each vessel contained 1 liter of solution and approximately 100 eggs. The solutions were constantly aerated and were renewed every other day. When solutions were renewed, a fresh solution was prepared in a clean vessel and the eggs carefully removed from the old solution and lowered into the new one. To facilitate the transfer and to prevent injury to the embryos, especially during the sensitive pre-eye stages, the eggs in each vessel were held in a shallow wire-screen basket; within the basket was woven a network of nylon monofillament line

with a mesh size of about 1 cm square. Each egg lay inside a section of the mesh; the mesh restricted horizontal movement of the eggs during the transfer from one solution to another. Unfertilized eggs and eggs containing dead embryos were removed when the solutions were changed. Water temperatures during the bioassay ranged between 9. 0 and 10. 2° C.

Alevins

A series of 5-day bioassays was conducted using alevins hatched from the eggs of a single female. Bioassays were started when alevins were 3, 8, 14, and 22 days old (age from hatch). A second series was similarly performed using 4-, 10-, and 18-day old alevins from another female. A series of NaPCP solutions ranging in concentration from 0.1 to 60 ppm was prepared in the test vessels; each vessel received 1 liter of solution into which 20 alevins were placed. Alevins were transferred to fresh solutions once or twice daily, depending on the experiment; this procedure was adopted to minimize the effects of detoxification and deoxygenation of the solutions by the alevins. Water temperatures ranged from 12 to 15° C, with most of the variation caused by the renewal solutions occasionally being colder than room temperature. Observations were made of the number of live and dead alevins in each vessel, and dead alevins were removed when the solutions were renewed. The

cessation of respiratory movements was used as the criterion of death. Several bioassays were similarly performed using alevins of chinook salmon (Oncorhynchus tshawytscha) and coho salmon (O. kisutch).

Flowing Water Experiments

Experiments were conducted to determine the effects of: (1) continued exposure to NaPCP on the growth and survival of alevins; (2) continued exposure to NaPCP on the growth and survival of juveniles; (3) intermittent exposure to NaPCP on the growth and survival of alevins; (4) continued exposure to NaPCP on the growth and survival of alevins given an external food source; and (5) continued exposure to dissolved oxygen concentrations of 3, 5, and 10 mg/liter on the toxicity of NaPCP to embryos and alevins.

Apparatus

Experiments in which the dissolved oxygen concentrations were near saturation were run in open troughs (Figure 1). The experiments which included reduced dissolved oxygen concentrations were conducted using specially designed respirometers (Figure 2).

In all experiments, filtered stream water with a pH of about 7.8 was brought into a constant temperature room through polyethylene pipe and entered a head box via a float valve. The water in the head box was aerated with compressed air and heated to the desired

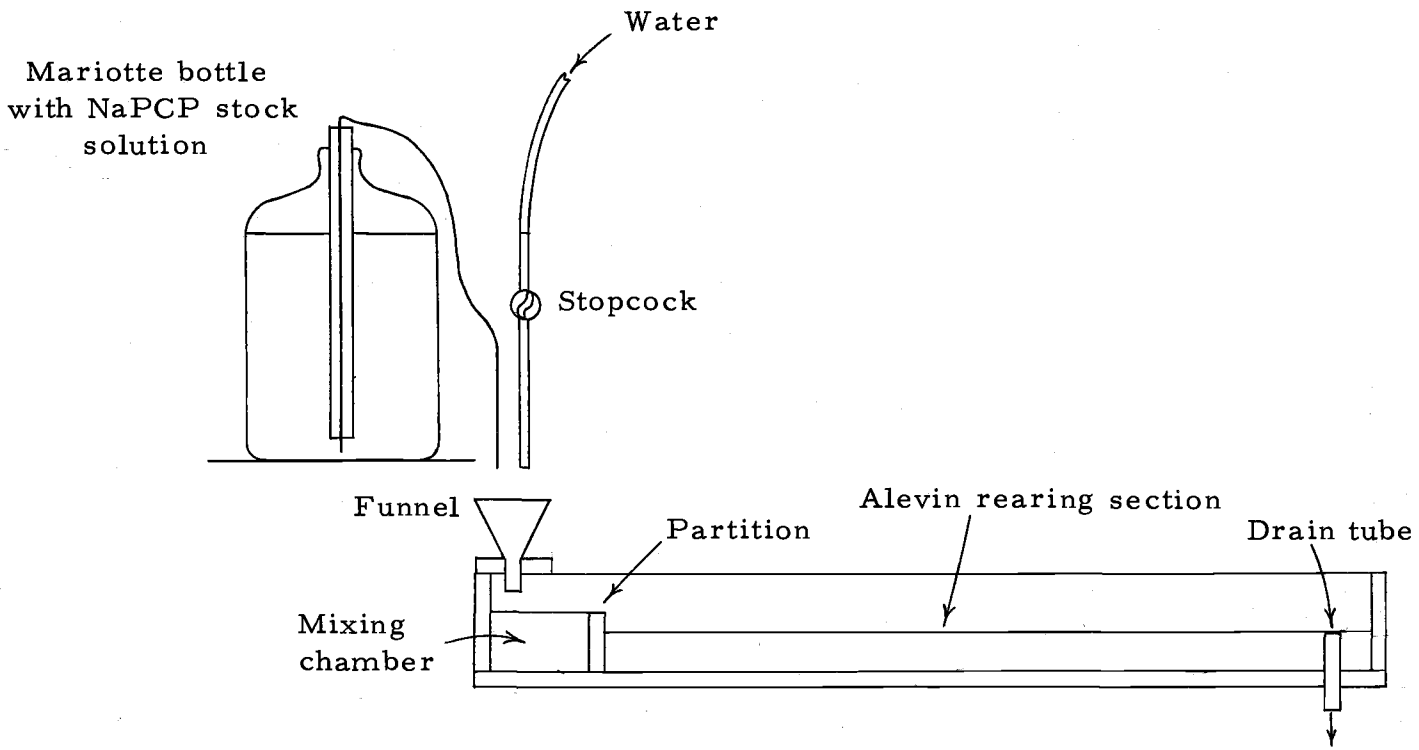

Figure 1. Side view of one of the troughs used for rearing alevins in a constantly renewed solution of NaPCP.

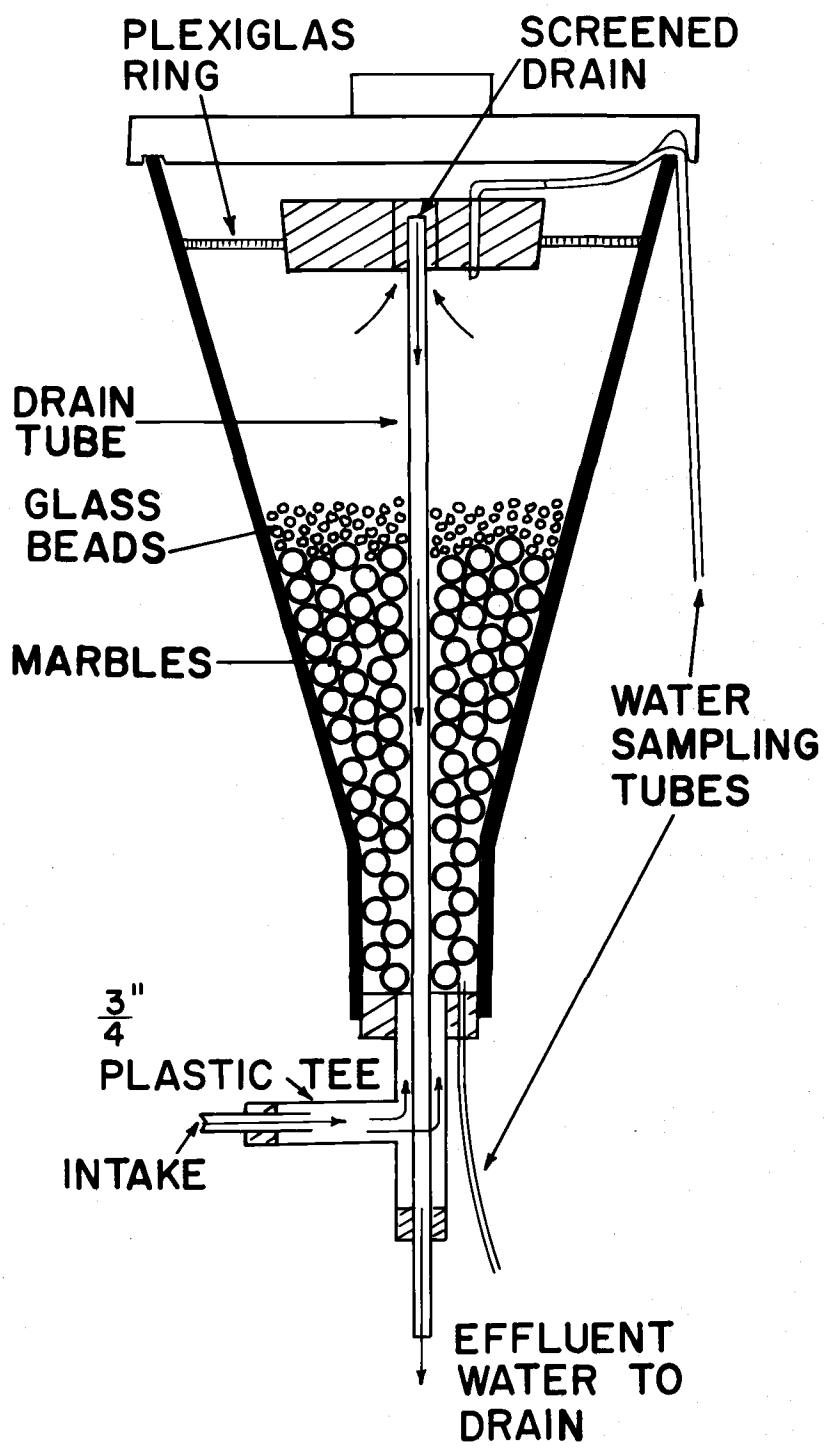

Figure 2. Cut-away view of the respirometer-rearing chamber used for holding steelhead trout embryos and alevins at constant levels of dissolved oxygen and NaPCP.

temperature by a thermostatically controlled stainless steel immersion heater.

Troughs

The water from the head box flowed to each trough through Tygon tubing and the rate of flow was controlled with a stopcock. The troughs were constructed from plywood, painted with several coats of white paint, and were approximately 100 cm long, 12 cm wide, and 12 cm deep. Each trough was divided into two sections by a 7 cm high partition located near the head of the trough. Water entering each trough flowed into the shorter section, spilled over the partition into the longer section, and was discharged at the opposite end through a screened outlet. Water was supplied to each trough at a rate of 250 ml/min. Sodium pentachlorophenate solutions of appropriate concentrations were introduced into the troughs at rates of from 1 to 3 ml/min from 18 liter, constant-head Mariotte bottles. Solutions in the bottles were replenished as required; at normal flow rates, the Mariotte bottles were emptied within 48 hours. Incoming water and NaPCP solutions entered the short section of the trough after flowing together through a glass funnel to ensure adequate mixing. The water in each trough was continuously aerated about midway along the trough. Water depth was maintained at 4 cm in the longer section of the trough by adjusting the height of the outlet tube.

Respirometer

The apparatus (Figure 3) used in experiments with NaPCP at various dissolved oxygen concentrations consisted of a series of components integrated into a system to deliver water with desired dissolved oxygen and NaPCP concentrations to respirometers designed for rearing salmonid embryos and alevins. Water was carried through Tygon tubing from the head box to each succeeding component in the system.

Water from the head box, heated to 10° C and aerated, flowed to glass cylinders. Water was introduced near the top of the cylinders and withdrawn from the bottom and air or nitrogen gas was introduced at the bottom, resulting in a counter-current flow of water and gas bubbles. Nitrogen was used to lower the dissolved oxygen concentration of the water while air was used to ensure that the oxygen concentration of the water was at air saturation. Each glass cylinder which received nitrogen was supplied by an individual tank of compressed nitrogen and the rate of gas flow was controlled with a two-stage reduction valve. The rate of nitrogen flow was adjusted to yield the desired concentration of dissolved oxygen in the water leaving each cylinder.

The water then flowed to the mixing chambers into which NaPCP solutions of appropriate concentrations were introduced from Mariotte

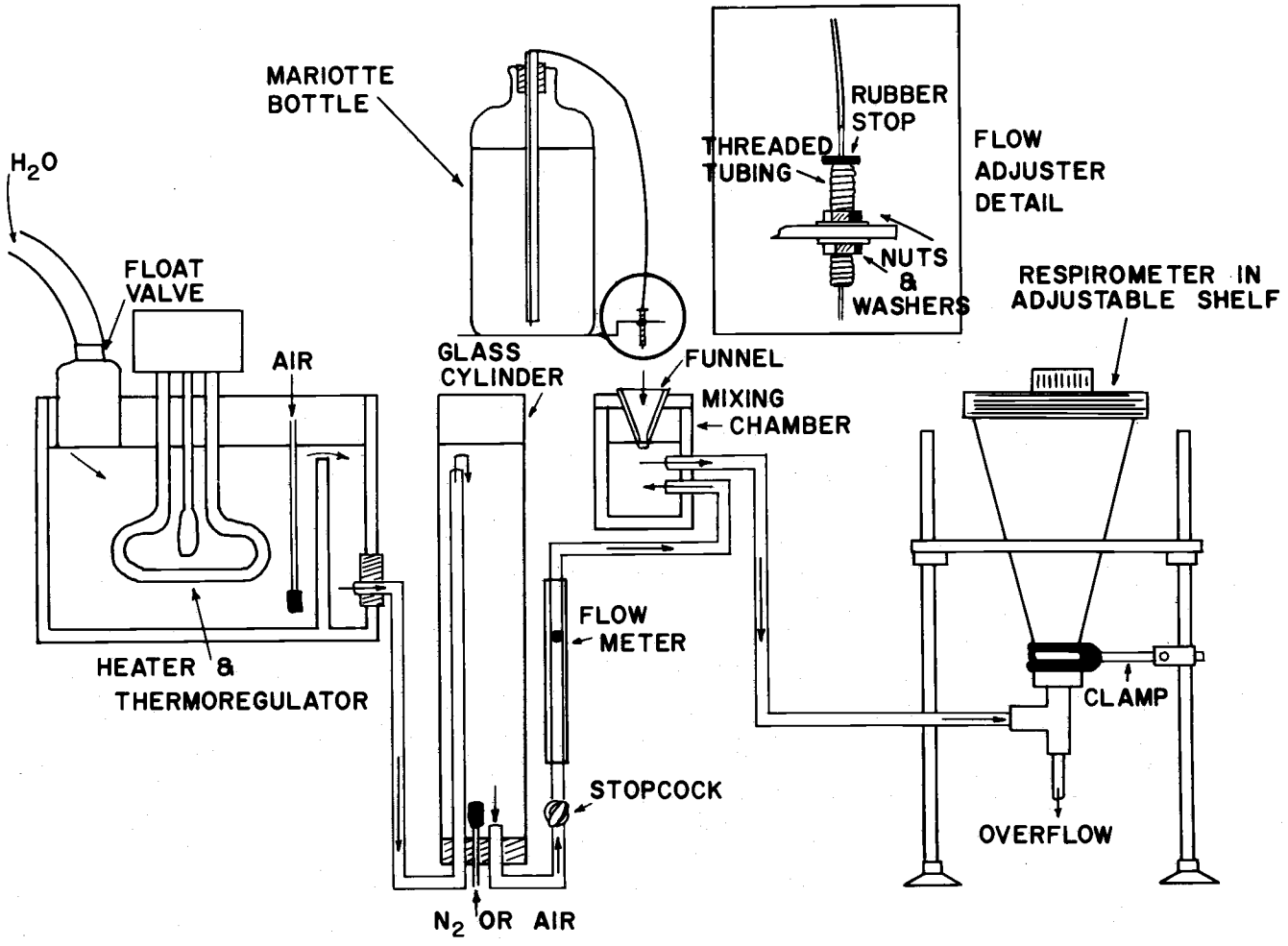

Figure 3. Diagram of the apparatus used in studies of the effect of dissolved oxygen concentration on the toxicity of NaPCP.

bottles. The rate of flow of water from the glass cylinders to the mixing chambers was controlled by means of stopcocks and flowmeters. The mixing chambers were designed to minimize contact between the water and the outside air. The water level in each chamber was maintained just above the level of the bottom of the funnel through which the NaPCP solutions were introduced. Each funnel was cemented into a circular opening in the top of the chamber; thus, the only effective interface for redistribution of gases between the water and the air was the small opening at the bottom of the funnel. The mixing chambers were placed on an adjustable height shelf so that the water level in the chambers could be adjusted. The water in the mixing chambers, having the desired NaPCP concentrations, then flowed to the respirometers.

The respirometers were constructed from 4-liter Erlenmeyer flasks, the bottoms of which had been removed to form large funnels. The respirometers rested in circular openings on a shelf which could be raised or lowered to obtain desired water flows. The respirometers were painted black and fitted with black styrofoam covers to exclude light.

Water entered at the bottom of each respirometer, was dispersed through a layer of 14 mm glass marbles and then through a layer 5 mm glass beads upon which the eggs or alevins were placed. Based on the rate of water flow (ml/min) and the cross-sectional area at

the level in the funnel occupied by the glass beads, the apparent water velocity was about 100 cm/hr. The water continued to flow upward and discharged from the respirometer via a vertical glass tube which passed downward concentrically through the larger inlet tube at the bottom of the respirometer. The chamber of the respirometer was essentially sealed from the atmosphere at the top by cementing a plexiglass ring inside the funnel about 4 cm below the top. A large rubber stopper, with a small central hole into which the drain tube extended, was fitted into the plexiglass ring. The head level in the respirometer (i.e., the top of the drain tube) was above the plexiglass ring but below the top of the rubber stopper so that the effective air-water interface was limited to the small area at the top of the drain tube, inside the hole in the stopper. Since water at the interface was immediately drained from the chamber, reoxygenation that occurred at this point was irrelevant. The drain tube and the hole at the top of the stopper were screened to prevent escape of the alevins. Water samples for determination of dissolved oxygen concentrations were withdrawn through small diameter glass tubes which were inserted through the rubber stoppers at the top and bottom of the respirometers.

Experimental Procedure

At the beginning of an experiment, approximately equal

numbers of alevins (or eggs) were placed into each trough or respirometer (Table 1). In addition, a sample of about 200 alevins was taken to determine the mean weight of the alevins and the mean weight of yolk present at the start of each experiment. Dead alevins were usually removed and counted daily. Thirty to 250 alevins, depending on the number remaining in each trough, their weight, and degree of development, were removed from each trough or respirometer at intervals spaced so that changes in alevin and yolk weights could be adequately defined. Samples of alevins were periodically removed until the alevins depleted their yolk to the extent that they were losing weight or until the supply of alevins was exhausted by the combined effects of sampling and mortality.

The alevins in each sample were divided into two sub-samples and killed in a solution of MS-222. The alevins in one sub-sample were blotted on paper towels, counted, and weighed in aggregate. Alevins in the other sub-sample were handled in the same manner except that their yolk was removed prior to weighing. The removal of yolk from young alevins was easily accomplished by excising the entire yolk sac. However, as the amount of yolk diminished and significant dermal overgrowth of the yolk sac occurred, it became necessary to remove the yolk by making a mid-ventral slit along the body cavity in order to ensure complete removal of yolk and prevent the excision of significant quantities of non-yolk tissue such as skin and

Table 1. The age, source, approximate number of individuals per trough (or respirometer) and initial dry weight data for each experiment.

Experiment number	Age in days from hatch	Source	Number per trough	Mean Dry Weight (mg)		
				Alevin	Yolk	Total
1[1]	9	Alsea River	600	9.6	48.1	57.7
2[1]	4	N. Santiam River	1,100	6.0	28.2	34.2
3	7	Big Creek	550	7.9	51.6	59.5
4[1]	2	N. Santiam River	850	4.2	28.3	32.5
5	22	N. Santiam River	110	19.3	----	19.3
6	4	Alsea River	525	4.7	51.6	56.3
7	1	Alsea River	265	4.0	44.3	48.3
8	1	Alsea River	550	4.0	41.9	45.9
9	Eggs	Alsea River	225	---	----	61.0

[1]Experiments 1, 2, and 5 were conducted at 15° C, all other experiments at 10° C.

viscera. After being weighed, the samples were dried at 75 to 80° C for 5 days, cooled in a dessicator, and weighed again. The amount of yolk present at a given time was determined by taking the difference between the weight of alevins with yolk and the weight of alevins from which the yolk had been excised. Where weights of embryos or alevins are given in this paper, they are dry weights and do not include the weight of the yolk unless so stated.

Bomb Calorimetry

In several experiments the caloric content of alevin and yolk tissue was routinely determined. Dried samples were finely ground and 100 to 200 mg portions combusted in a Parr Model 1411 bomb calorimeter. At least two determinations were performed on each sample, and duplicate samples usually yielded results within 1%. Caloric values for yolk were calculated from results obtained on samples of alevins with yolk and of alevins from which the yolk had been excised.

Oxygen Consumption

Measurements of the oxygen consumption of the alevins were made by determining the difference in oxygen content of water entering and leaving the respirometers. Oxygen determinations were made by using the azide modification of the Winkler method. Water

samples were withdrawn from the respirometers through small diameter glass tubes inserted through the rubber stoppers at the top and bottom of the respirometers. Determinations of oxygen consumption were normally made twice a day, once in the morning and once in the late afternoon. Blanks run before and after the experiments indicated there was no significant change in the dissolved oxygen concentration of the water passing through the empty respirometers. Oxygen consumption rates were calculated as follows;

$$Q_{O_2} \text{ mg/g alevin dry weight/hr} = \frac{(O_a - O_e) \cdot F}{Wn}$$

O = dissolved oxygen concentration (mg/l)

F = rate of water flow through respirometer (liters/hr)

W = alevin dry weight (grams)

n = number of alevins

a = afferent or in water sample

e = efferent or out water sample

The alevin dry weight (W) on each day was taken from curves of alevin weight vs. time, constructed with data obtained from the periodic samples of alevins. The number of alevins in a given respirometer on each day was back-calculated from sampling and mortality data. Rates of water flow were usually read from the flowmeters, but were occasionally measured directly. All oxygen consumption

rates are expressed as milligrams oxygen per gram alevin dry weight per hour.

Feeding

In experiments where exogenous food was used, the fish were provided an unrestricted ration of chopped tubificid worms. No attempt was made to determine the amount of food consumed in either experiment. In one experiment, food was removed from the troughs 6 to 8 hours before a sample of juveniles was to be taken. The food was replaced following sample removal. In the other experiment, each sample of alevins was removed from the trough the day before weighing and held in an aerated beaker of water for 24 hours without food.

RESULTS

Static Toxicity Bioassays

Embryos

Concentrations of NaPCP as low as 50 ppb produced 100% mortality of steelhead trout embryos in a bioassay in which exposure lasted from 15 minutes after fertilization of the eggs to shortly after hatching (Table 2). In all eggs held in NaPCP concentrations of 300 ppb or greater, a small, distinct white spot appeared at the animal pole during the first week of development. The spot was smallest and occurred earliest at the highest concentrations of NaPCP. The white spot was apparently produced as the embryo was dying and the blastodisc or blastoderm became opaque. The white spot was used as a criterion of death only after it became evident that no further embryonic development occurred.

Sodium pentachlorophenate concentrations of 50, 80, and 100 ppb caused 100% mortality, with most of the mortality occurring after hatching at 50 and 80 ppb and before hatching at 180 ppb. The alevins which hatched at NaPCP concentrations of 50 ppb and greater were very small, with tiny eyes, gaping mouths, white opaque appearance, dorsal curvature of the posterior half of the body, and essentially total paralysis. The only vital signs were a heartbeat and

some slight movement of the pectoral fins. These extremely weak alevins all died within a day of hatching, many still within the cleaved sections of their chorions. At the time of hatching, some eggs at 80 and 180 ppb of NaPCP appeared to have incompletely cleaved chorions with the embryos dead inside the chorion and part of the yolk protruding through the hole in the chorion. Control embryos and embryos from 10 and 30 ppb of NaPCP showed some early mortality (some or all may have been nonviable eggs) but no additional mortality occurred subsequently.

Table 2. Mortality of steelhead trout continuously exposed to NaPCP from fertilization to one day post-hatch.

	Percent mortality at various stages of development					
NaPCP concentration (ppb)	Pre-eyed embryos	Eyed embryos	Hatch	24 hours post-hatch	Total	Mean alevin dry weight at hatch (mg)
1,000	100	---	---	---	100	---
800	100	---	---	---	100	---
500	100	---	---	---	100	---
300	100	---	---	---	100	---
180	50	10	18	22	100	---
80	26	0	14	60	100	---
50	25	2	3	70	100	1.8
30	11	0	0	0	11	2.7
10	24	0	0	0	24	3.3
0	9	0	0	0	9	3.8

On the 37th day after fertilization, the eggs held under control conditions and at 30 ppb of NaPCP were 100% hatched; those at 10 ppb were about 70% hatched; a few eggs at 50 ppb were hatched; and no

eggs at 80 or 180 ppb were hatched. Hatching was complete at 10 ppb on day 38 and nearly complete at 50, 80 and 180 ppb on day 41. The dry weight at hatch of alevins reared in NaPCP was markedly less than that of control alevins (Table 2).

Alevins

During the 5-day static bioassays with alevins, little mortality occurred beyond 24 hours. Concentrations of NaPCP higher than 300 ppb always caused 100% mortality within 12 hours. The lowest concentration which produced 50% mortality in any of the bioassays was 150 ppb; the highest concentration which failed to produce 50% mortality was 250 ppb (Table 3). Younger alevins appeared to be susceptible to lower concentrations than older alevins; although, younger alevins survived slightly longer than older alevins at concentrations over 300 ppb.

Table 3. Percent mortality of steelhead trout alevins exposed to NaPCP for 5 days. The age indicated is days post-hatch at the start of each bioassay.

Age of alevins (days)	NaPCP Concentration (ppb)											
	400	350	300	250	200	150	100	90	60	30	10	Control
3	100	---	100	100	100	100	25	--	--	0	10	0
8	100	---	---	---	100	25	---	0	0	--	--	0
14	100	---	---	40	5	0	---	5	5	--	--	0
22	100	100	90	45	0	0	0	--	--	--	--	0
4	---	---	100	---	100	---	---	--	0	0	--	0
10	100	---	100	100	90	0	0	--	--	--	--	0
18	100	100	100	100	95	0	0	--	--	--	--	0

The relationship between NaPCP concentration in the static bioassays and the time required to produce 50% mortality of steelhead trout alevins is shown in Figure 4. Data from similar bioassays with chinook salmon alevins and coho salmon alevins are included for comparison. Alevins of steelhead trout, coho salmon, and chinook salmon appeared to be similar in their response to NaPCP, although chinook alevins survived somewhat longer at most concentrations.

Flowing Water Assays

Mortality

Significant mortality of alevins was observed during flowing water experiments at NaPCP concentrations between 45 and 100 ppb (Figure 5), although at concentrations below 100 ppb one or more weeks elapsed before any mortality appeared. Generally, no mortality of alevins was noted in controls or at 30 ppb. Mortalities of 6% to 25% were noted at 45 ppb. Concentrations of 70 and 100 ppb almost always caused mortality in excess of 75%. In experiment 1, 50% mortality occurred within 5 days at 140 ppb.

Growth

Alevins reared under control conditions showed rapid, steady weight gain until the yolk supply neared exhaustion, at which time the

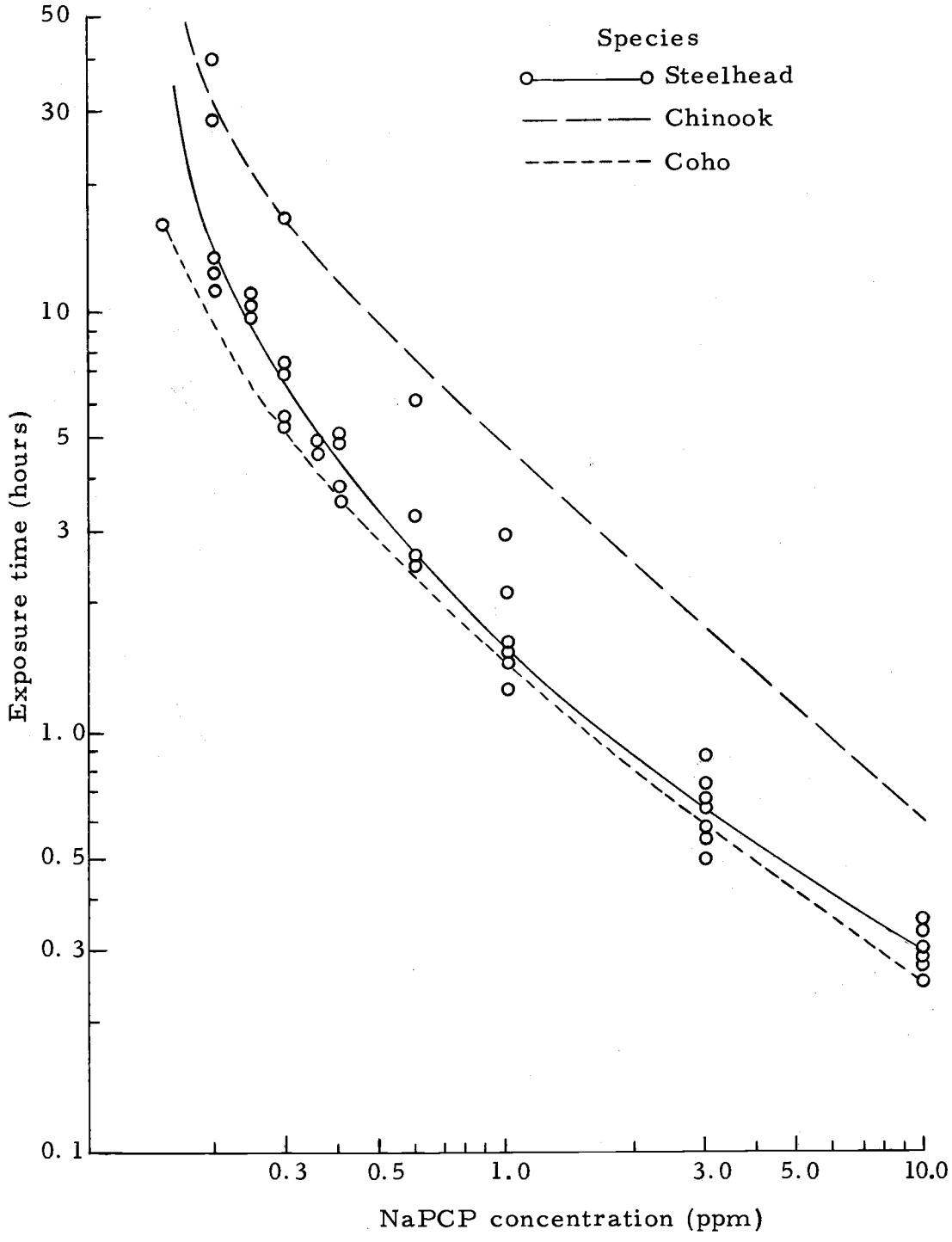

Figure 4. The time required to produce 50% mortality of alevins of three species of salmonids at the indicated concentrations of NaPCP. All data points are for steelhead trout. Chinook and coho salmon curves were fitted by eye to data from 5 chinook bioassays and one coho bioassay.

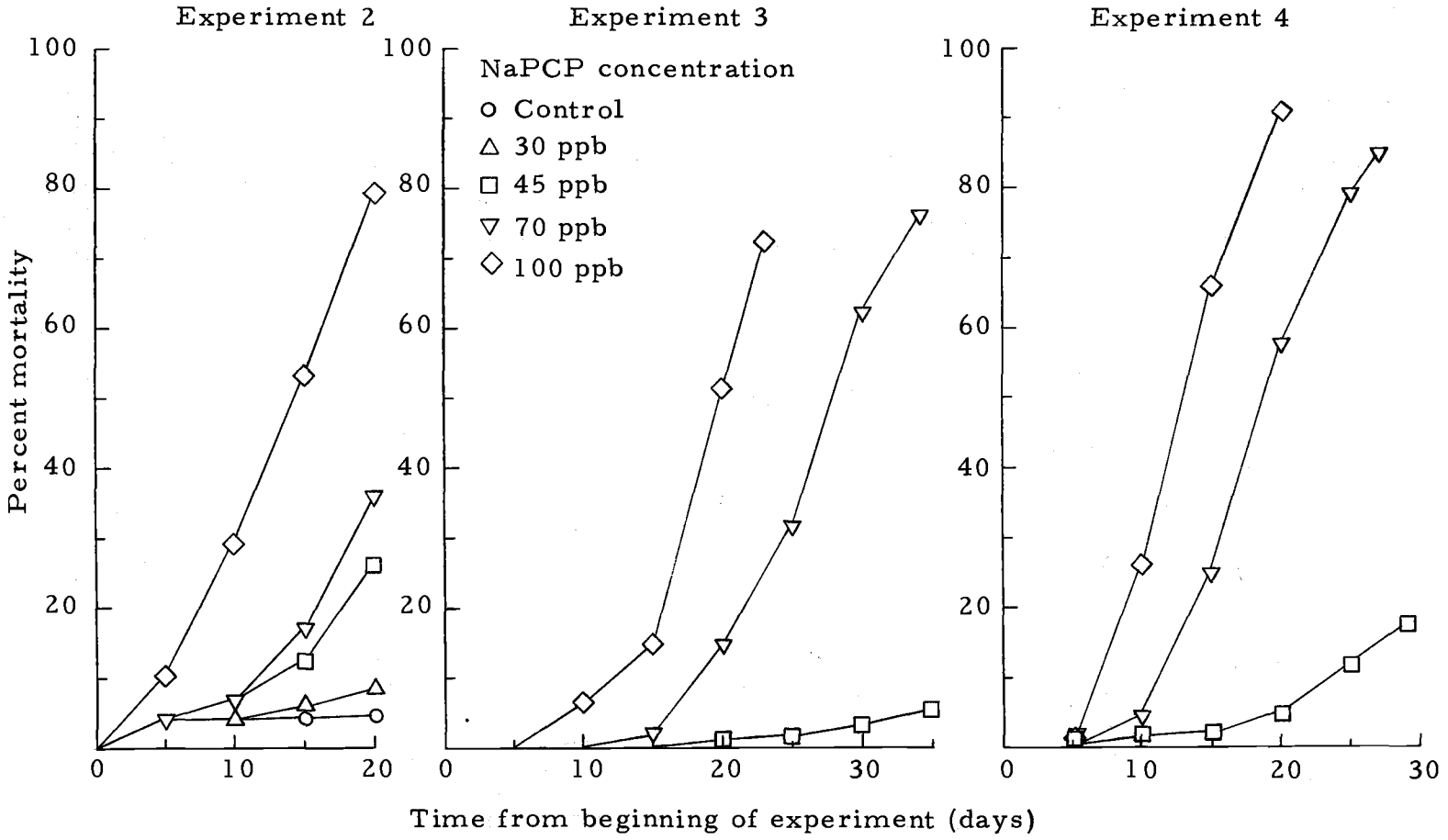

Figure 5. The cumulative percent mortality of steelhead trout alevins in experiments in which alevins were exposed to continually renewed solutions of NaPCP.

weight reached a maximum and started to decline as the last vestiges of yolk slowly disappeared (Figure 6 and Table 4). When compared to controls, alevins exposed to NaPCP had a lower rate of weight gain, a lower maximum weight, and required longer to attain maximum weight. As a rule of thumb, the dry weight gain of alevins (the maximum alevin weight less the initial alevin weight) exposed to NaPCP was reduced approximately 6% from that of controls for each 10 ppb increase in NaPCP concentration (Table 5).

Yolk Utilization Efficiency

The alevins were dependent upon their supply of yolk for material to be utilized for growth and for the production of energy. Because other food sources were not present, the maximum weight attained by the alevins was limited by the amount of yolk present and the efficiency in utilizing yolk for growth. Since all alevins had utilized essentially an equal amount of yolk by the end of each experiment, the reduction in maximum dry weight gain observed in alevins exposed to NaPCP indicated that their efficiency of yolk utilization for growth was lower than that of control alevins.

Although the pattern of yolk utilization efficiency was somewhat variable between experiments, generally within experiments the efficiency remained relatively constant during the period that the alevins were gaining weight (Table 6). Efficiencies decreased slightly during

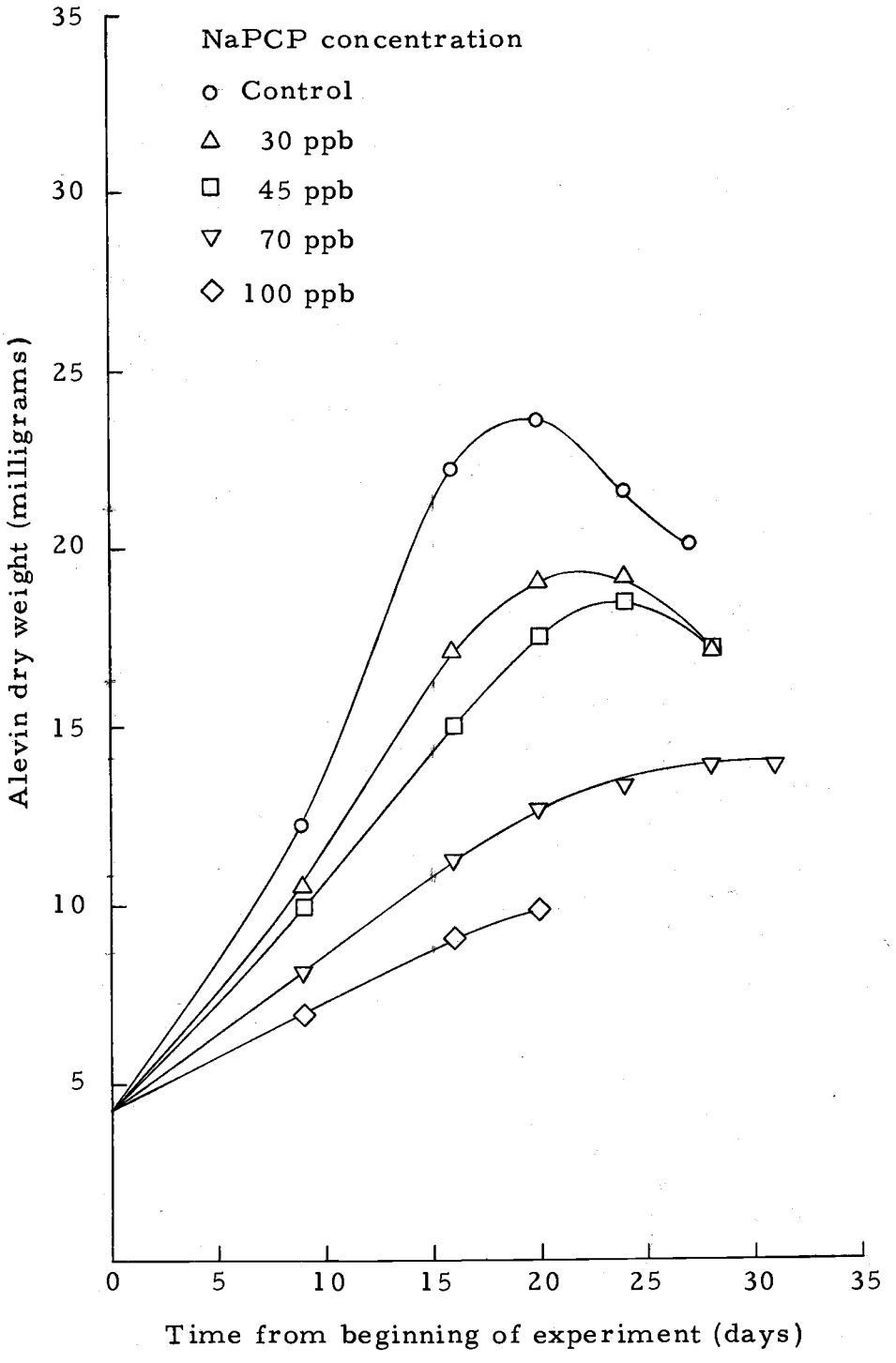

Figure 6. Alevin growth curves (dry weight) of steelhead trout exposed to continually renewed solutions of NaPCP (Experiment no. 4).

Table 4. The relationship between mean alevin dry weight and mean dry weight of yolk remaining at various times during experiments in which steelhead trout alevins were reared at various concentrations of NaPCP. Weights are indicated as follows: alevin/yolk.

		NaPCP concentration			
Day	Controls	30 ppb	60 ppb	100 ppb	140 ppb
Experiment 1					
0	9.6/48.1	-----	-----	-----	-----
5	16.1/39.5	14.1/42.8	13.8/39.9	11.1/41.7	10.9/43.2
10	26.2/26.5	24.1/27.6	19.0/31.6	15.8/32.8	
15	33.8/16.5	29.3/14.9	26.0/18.2	21.0/23.6	
20	36.4/ 4.9	33.4/ 7.1	29.1/ 9.4		
		NaPCP concentration			
		30 ppb	45 ppb	70 ppb	100 ppb
Experiment 2					
0	6.0/28.2	-----	-----	-----	-----
5	12.1/20.6	11.0/21.1	10.3/22.2	9.6/22.3	7.8/23.2
10	20.3/ 9.0	17.2/11.6	14.1/14.0	13.4/15.0	10.8/16.7
15	22.8/ 2.0	19.8/ 4.8	18.1/ 6.2	16.6/ 7.7	13.6/ 9.9
20	19.3/ 0	17.7/ 0	18.2/ 2.8	17.0/ 2.8	12.7/?
25			15.7/ 0	14.6/ 0	
Experiment 3					
0	7.9/51.6	-----	-----	-----	-----
10	20.2/34.5	17.6/37.2	16.0/39.3	14.1/39.9	11.4/42.0
17	37.0/14.9	29.3/20.6	26.3/23.0	19.2/28.2	15.0/31.2
21	41.1/ 9.4	-----	-----	-----	15.2/27.6
24	42.7/ 4.5	34.5/11.7	32.1/11.6	22.3/19.7	15.9/24.2
28	41.6/ 1.3	36.5/ 6.0	32.4/ 8.1	22.6/15.5	
31	40.5/ 0	35.9/ 4.0	32.4/ 6.1	23.5/11.8	
35	37.1/ 0	35.0/ 1.9	32.0/ 3.6	23.6/ 8.8	
38		33.1/ 0.6	31.4/ 2.0		
Experiment 4					
0	4.2/28.3	-----	-----	-----	-----
9	12.2/18.0	10.5/19.2	9.8/19.5	8.0/20.9	6.9/21.7
16	22.1/ 5.5	17.1/ 9.2	14.9/11.0	11.1/14.2	9.0/15.8
20	23.5/ 2.0	19.0/ 4.8	17.4/ 6.2	12.5/10.9	9.8/12.5
24	21.5/ 1.2	19.1/ 2.3	18.4/ 3.2	13.2/ 7.9	
27	20.0/ 0.5	-----	-----	-----	
28		17.1/ 1.8	17.0/ 1.9	13.8/ 4.8	
31				13.8/ 3.2	

Table 5. Percent reduction in maximum dry weight gain at indicated concentrations of NaPCP.

NaPCP (ppb)	Experiment number 1	2	3	4	Average
30	11	18	18	12	15
45	--	28	30	26	28
70	--	35	55	49	46
100	58	55	77	70	65

Table 6. The effect of NaPCP on the efficiency of yolk utilization for growth. *

Day	Controls	NaPCP concentration (ppb) 30	45	70	100
0	--	--	--	--	--
9	78	70	63	51	41
16	79(78)	65(67)	61(62)	47(49)	35(38)
20	40(73)	49(64)	56(60)	43(48)	26(36)
24	--(64)	3(57)	30(57)	22(44)	
27	--(57)	---	---	---	
28		--(49)	--(48)	20(41)	
31				--(38)	

* Figures in parentheses are cumulative percent efficiencies from the beginning of the experiment. The other figures represent efficiencies over the period since the preceding data, i. e., the control efficiency of 40% at day 20 represents 40% efficiency during the period between days 16 and 20; the 73% figure is the efficiency between day 0 and day 20.

the period of growth, and then a very marked reduction in efficiency occurred when the alevins approached their maximum weight. The efficiency of yolk utilization in control alevins varied between 70 and 80% during the period of alevin growth, while that of alevins exposed to NaPCP was usually between 40 and 70%, with the lower efficiencies occurring at the higher concentrations. The data in Table 7 indicate that the efficiency of yolk utilization from the beginning of each experiment to the time of maximum alevin weight was always lower at progressively higher NaPCP concentrations.

In general, the weight gain of alevins equals the mass of yolk absorbed by the alevin less that portion catabolized for energy; therefore, when efficiencies were low, there may have been an increase in the rate of yolk catabolism (mg catabolized per gram alevin per day) or there may have been a decrease in the rate of yolk absorption by the alevin (mg absorbed per gram alevin per day).

Yolk Uptake Rates

The rate of yolk absorption (mg/day) probably depends on the weight of the alevin, but it also depends on the weight of the yolk. Thus during the initial phases of alevin growth, the yolk availability exceeds the demands of the alevin, and alevin weight, in effect, limits the rate of yolk uptake. As the alevins grow, the rate of yolk uptake increases and the amount of yolk available decreases until the

Table 7. The effect of NaPCP on the efficiency of yolk utilization for growth from the beginning of the experiments to the time of maximum alevin weight.

NaPCP concentration (ppb)	Period (days)	Total[1] weight loss (mg)	Alevin weight gain (mg)	Efficiency of yolk utilization (%)
Experiment 1				
Control	1-20	16.4	26.8	62.0
30	1-20	17.2	23.8	58.1
60	1-20	19.2	19.5	50.5
100	1-15	13.1	11.4	46.5
Experiment 2				
Control	1-15	9.4	16.8	64.1
30	1-15	9.6	13.8	59.0
45	1-20	13.2	12.2	48.0
70	1-20	14.4	11.0	43.3
100	1-15	10.7	7.6	41.5
Experiment 3				
Control	1-24	12.3	34.9	73.9
30	1-28	16.9	28.5	58.3
45	1-31	21.0	24.5	53.8
70	1-35	27.0	15.6	36.8
100	1-24	19.4	8.0	29.3
Experiment 4				
Control	1-20	7.0	19.2	73.3
30	1-24	11.1	14.8	57.4
45	1-24	10.9	14.2	56.8
70	1-28	14.0	9.5	40.9
100	1-20	10.2	5.6	35.7

[1] Weight loss of alevin with yolk.

demands of the alevins for material for growth and maintenance exceeds the ability of the yolk supplying mechanisms to deliver. Eventually, the supply of yolk is depleted to the extent that the alevin cannot meet maintenance requirements and begins to lose weight.

In comparing yolk uptake between control alevins and alevins reared in NaPCP, resulting differences in alevin weight have to be considered. Initially the weight and yolk supply of all alevins were similar; however, the controls grew more rapidly than alevins exposed to NaPCP, thus comparisons on a rate basis (mg of yolk absorbed/day) soon became difficult to interpret. Yolk uptake rates per unit weight of the alevin (mg yolk absorbed per gram of alevin per day) provided comparisons which were easier to interpret, at least during the first part of the experiments when all alevins had sufficient yolk that the quantity of yolk presumably had little effect on yolk uptake rate. Once the control alevins approached maximum weight and depleted their yolk supply, which usually occurred while the poisoned alevins still had significant yolk remaining, the uptake rates of the poisoned alevins naturally exceeded that of controls.

The rate of yolk uptake per unit weight of alevin for alevins reared in NaPCP was equal to or slightly lower than that of control alevins, at least during the period while the alevins were rapidly increasing in weight (Table 8 and Figure 6). Rates of yolk catabolism, computed on the basis of mg of yolk/gram of alevin/day, were much

higher in alevins reared in NaPCP, with the highest rates associated with the highest NaPCP concentrations (Table 8).

Table 8. The effect of NaPCP on the mean rates of yolk uptake and yolk catabolism of steelhead trout alevins for the indicated periods.

NaPCP concentration (ppb)	Yolk uptake rate (mg/g/day)[1] Experiment number 2	3	4	Yolk catabolism rate (mg/g/day)[1] Experiment number 2	3	4
Control	154	108	120	39	22	26
30	150	106	119	49	33	39
45	138	107	118	59	38	44
70	138	103	116	60	53	59
100	139	108	116	81	70	72
Period (days)	1-10	1-17	1-16	1-10	1-17	1-16

[1]Milligrams yolk (dry weight)/mean grams alevin (dry weight)/day.

Caloric Data

Analysis of the caloric data from Experiment 2 indicated that the effect of NaPCP on the increment in calories per alevin closely followed the pattern seen in mg per embryo (Table 9). Alevins exposed to NaPCP attained a maximum caloric content considerably less than the calories per control alevin, with increasing NaPCP concentrations yielding alevins with progressively lower maximum calories per alevin. At each concentration of NaPCP, the percent reduction in maximum calories per alevin was almost identical to the percent reduction in maximum dry weight attained by alevins. The reduction

in calories and in dry weight was about 5% for each 10 ppb increase in NaPCP concentration.

Table 9. The effect of exposure to NaPCP on the growth and growth efficiency (dry weight and caloric) of steelhead trout alevins.

NaPCP concentration (ppb)	Period (days)	Maximum gain (mg) wt	Maximum gain cal	Percent reduction wt	Percent reduction cal	Efficiency wt (%)	Efficiency cal (%)	cal/wt[1]
Control	1-15	16.8	84.6	--	--	64.1	53.2	.83
30	1-15	13.8	71.8	18	15	59.0	51.4	.87
45	1-20	12.2	60.5	28	28	48.0	39.9	.83
70	1-20	11.0	56.1	35	34	43.3	36.6	.85
100	1-15	7.6	39.6	55	53	41.5	36.5	.88

[1]The ratio between caloric efficiency and weight efficiency.

Comparison of efficiencies of yolk utilization for growth with respect to both weight and calories indicated that at all concentrations of NaPCP and in controls, efficiencies were always higher on a weight basis than on a caloric basis. The ratios of caloric efficiencies to weight efficiencies were 0.85 ± 0.03, with no clear correlation between these ratios and the NaPCP concentrations. Caloric efficiencies lower than corresponding dry weight efficiencies indicated that the alevins were catabolizing material from the yolk with a relatively high heat of combustion and incorporating material from the yolk with a relatively low heat of combustion.

Intermittent Exposure

In order to determine if the sensitivity to NaPCP changed appreciably during the development of the alevin, an experiment was conducted in which alevins were exposed to NaPCP at 50 ppb for specific periods of time. The experiment was divided into three periods, days 1-8, 9-18, and 19-termination (32-36 days). Alevins were exposed to 50 ppb NaPCP for all possible combinations of one or more periods (Table 10). In addition, one group of alevins was exposed continuously to 25 ppb of NaPCP and one group served as controls.

After 8 days virtually no mortality was observed in any group of alevins. However, after 18 days appreciable mortality had occurred in the two groups of alevins which had been exposed to 50 ppb of NaPCP continuously since the beginning of the experiment. During the third period, the mortality increased rapidly in the groups of alevins reared in 50 ppb throughout the experiment. The alevins which were reared in clean water for the last period after being reared in 50 ppb for the first 18 days also showed high mortality on days 19 and 20 (the first 2 days in clean water); however the mortality rate then decreased rapidly and no additional alevins died after 1 week in clean water. Other groups showing mortality, all of which occurred during the last period, were the group exposed throughout the experiment to 25 ppb of NaPCP, the group exposed to

50 ppb for the first and third periods, and the group exposed to 50 ppb for the second and third periods.

Table 10. The effect of various periods of exposure of steelhead trout alevins to 50 ppb of NaPCP on maximum dry weight gain and yolk utilization efficiency.

Treatment[1] during period I-II-III	Dry weight gain (mg)	Reduction in dry weight increase (%)	Efficiency of yolk utilization (%)	Yolk uptake while in NaPCP (mg)	Days in NaPCP
O-O-O	34.2	0.0	72.0	0.0	0
X-O-O	34.0	0.6	71.2	3.2	8
O-X-O	31.6	7.0	69.6	17.8	10
X-X-O[2]	28.5(31.0)	16.1(8.5)	67.0(65.3)	19.1	18
O-O-X	31.0	8.8	66.4	31.5	18
X-O-X	29.7	12.6	65.6	32.3	26
O-X-X	28.4	16.5	64.5	42.2	28
X-X-X	20.2	40.5	55.6	51.6	32
$\frac{1}{2}$X-$\frac{1}{2}$X-$\frac{1}{2}$X[3]	31.3	8.0	65.2	51.6	36

[1] X represents the presence of 50 ppb of NaPCP; O represents control conditions (No NaPCP).

[2] These alevins had not reached maximum weight when the experiment was terminated. The last time the amount of yolk in the alevins (X-X-O) was measured (day 29), there were 15.5 mg yolk remaining and the alevins weighed 28.9 mg. Controls reached maximum weight with 4.1 mg of yolk remaining; thus group X-X-O would have utilized 11.4 mg of yolk during the time between day 29 and the time of maximum alevin weight. If this yolk had been used for growth at about 60% efficiency, the weight gain would have been 11.4 x 0.6 or 6.8 mg. The maximum weight would then have been 28.9 plus 6.8 or 35.7 and the weight gain would have been 35.7 minus the initial weight, 4.7, or 31.0.

[3] Continuous exposure to 25 ppb.

The growth of alevins in all groups exposed to NaPCP was less than the growth of control alevins (Figures 7 and 8). Maximum dry

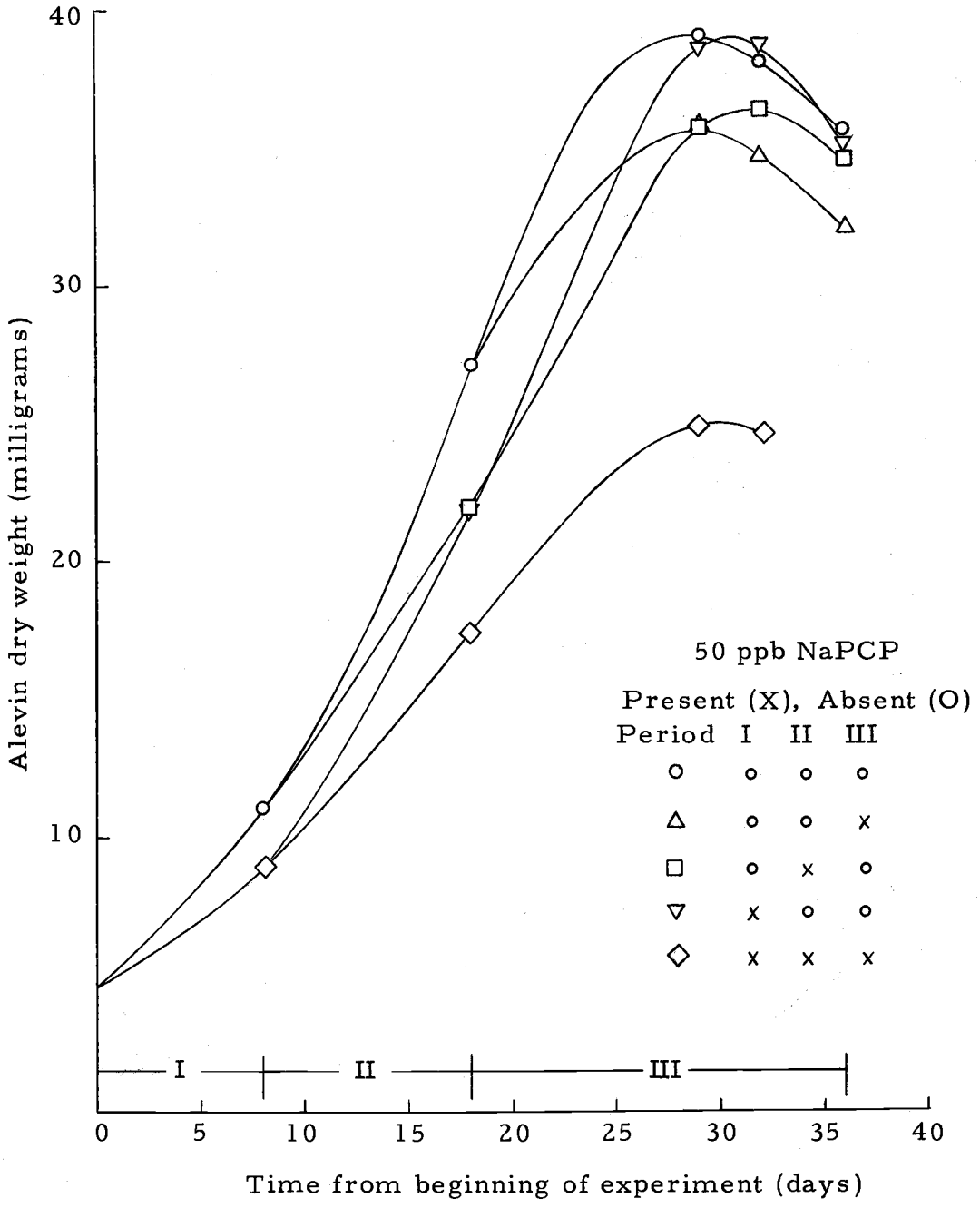

Figure 7. Dry weight of steelhead trout alevins reared at 50 ppb or under control conditions for the indicated periods.

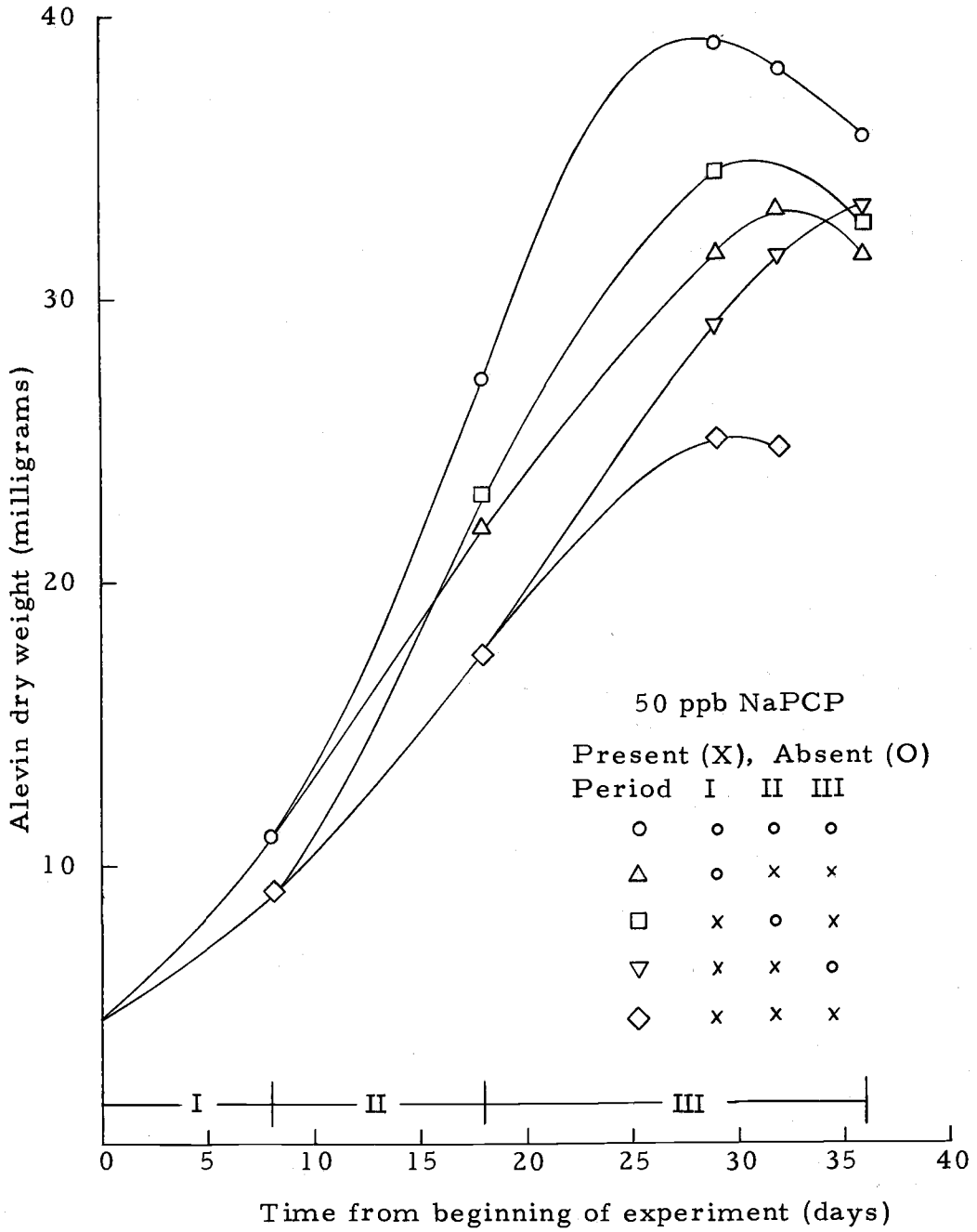

Figure 8. Dry weight of steelhead trout alevins reared at 50 ppb or under control conditions for the indicated periods.

weight increase of poisoned alevins was less than control maximum dry weight increase by as little as 0.6% in the group exposed to 50 ppb NaPCP for the first period only and by as much as 40.5% in the group exposed to 50 ppb throughout the experiment. The effect of NaPCP on maximum weight attained and the efficiency of yolk utilization by alevins was intimately related to the total duration of exposure to NaPCP and to the amount of yolk used while exposed to NaPCP (Table 10). The growth rates of alevins removed from NaPCP and placed in clean water increased dramatically, and conversely, alevins transferred from clean water to NaPCP showed marked reductions in growth rate.

Alevin Feeding Experiment

Most experiments were conducted so that yolk was the only food supply available to the alevins; this is obviously an unnatural situation since alevins are able to begin active feeding before the yolk is completely absorbed. An experiment was conducted to determine when alevins started to feed, how much more rapidly fed alevins grew than unfed alevins, if the yolk utilization of fed alevins differed from that of unfed alevins, and what effect NaPCP had on feeding and growth.

Unfed control alevins grew from an initial dry weight of 4 mg to a maximum weight of about 33 mg, while unfed alevins reared in 40 ppb of NaPCP grew from 4 to 25 mg (Figure 9). The reduction in

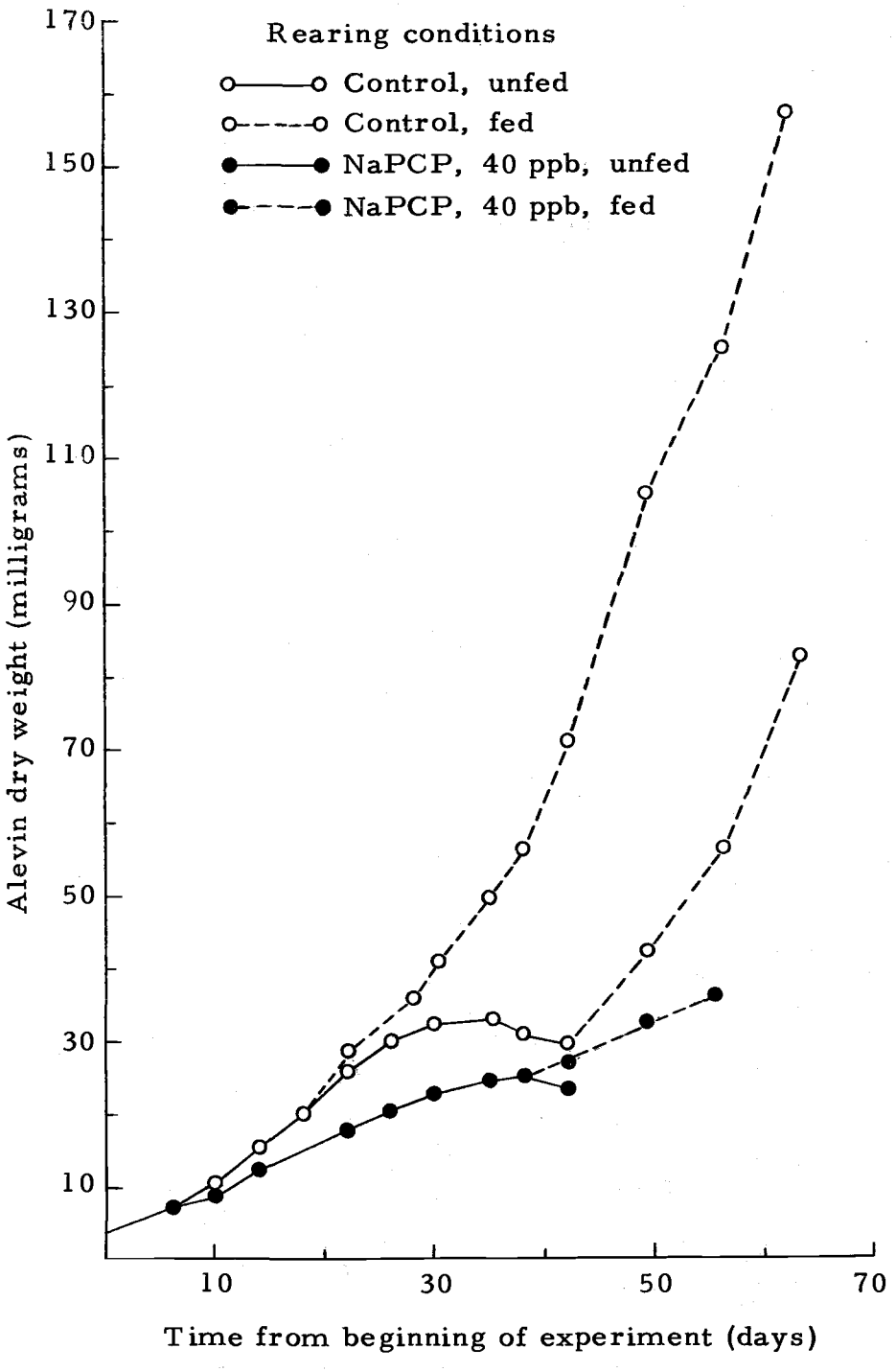

Figure 9. Growth curves (dry weight) of fed and unfed alevins reared under control conditions or at 40 ppb of NaPCP.

dry weight gain at 40 ppb was 27. 5%. This percentage closely agrees with the mean reduction at 45 ppb in Experiments 2, 3, and 4 (28%) (Table 5).

When alevins were given an external source of food (an excess of chopped tubificid worms), control alevins were observed feeding on the 20th day of the experiment, 2 days after the swim-up stage was reached. Alevins reared in 40 ppb of NaPCP were not observed to feed until the 40th day of the experiment, at which time yolk absorption was complete. No definitive development of a swim-up stage was ever noted in alevins reared in 40 ppb NaPCP.

The alevins reared in 40 ppb of NaPCP were observed to feed on the 40th day of the experiment at a dry weight of about 25 mg; but at the same time, the fed control alevins had attained a weight of about 63 mg. This amounts to a reduction in weight gain in the poisoned alevins of 64. 4%. After 8 weeks, fed control alevins weighed 124 mg and fed NaPCP exposed alevins weighed 37 mg, a reduction in weight gain of 72. 5%. Control alevins utilized yolk more rapidly than NaPCP poisoned alevins. Fed alevins utilized yolk at nearly the same rate as the unfed alevins with the possible exception of a temporary increase of yolk utilization of fed controls about the time that feeding began (Table 11).

When the unfed control alevins had absorbed all of their yolk, they were then provided an exogenous food supply (chopped tubificid

worms) for the remainder of the experiment (21 days). These fish grew at a rate similar to that observed earlier at the onset of feeding in fed control alevins (Figure 9).

Table 11. The mean dry weight of yolk remaining at various times in fed and unfed alevins reared under control conditions or at 40 ppb of NaPCP.

Day	Dry weight of yolk remaining (mg)			
	Control		40 ppb of NaPCP	
	(Unfed)	(Fed)	(Unfed)	(Fed)
0	44.3	44.3	44.3	44.3
6	38.7	39.6	40.3	38.3
10	36.0	34.1	37.3	35.7
14	29.4	26.7	28.9	31.1
18	24.5	23.3	----	----
22	16.4	10.4	21.7	21.6
26	10.8	12.4	17.6	15.9
28	----	8.8	----	----
30	3.2	5.0	11.9	11.7
35	3.1	3.2	8.0	6.4
38	1.8		0.9	6.6
42				0.8

Comparing the growth rates (from day 42 to day 49) of the fed alevins reared in 40 ppb of NaPCP and the controls fed from day 42 on, indicates that the controls grew at a rate of 47 mg/g/day while the poisoned alevins grew at a rate of 27 mg/g/day.

Dissolved Oxygen and NaPCP Toxicity

The mortality rate of alevins exposed to 40 ppb of NaPCP was highest at a dissolved oxygen concentration of 3 mg/liter, intermediate

at 5 mg/liter, and lowest at 10 mg/liter (Figure 10). Although no mortality was seen after 8 days of exposure to 40 ppb of NaPCP at 3, 5, or 10 mg of oxygen per liter, by 24 days alevins reared at 3 mg per liter had suffered 76% mortality compared to only 20% at the 5 mg oxygen level and 7% at the 10 mg level. After 41 days, mortality at 40 ppb of NaPCP was 79% at 5 mg and 24% at 10 mg per liter. The dissolved oxygen concentrations were of themselves non-lethal, since only slight and essentially equal mortality was observed in control alevins at 3, 5, and 10 mg of dissolved oxygen per liter.

Low dissolved oxygen concentrations had a small effect on the growth of control alevins; the maximum weight gain of controls reared at 10, 5, and 3 mg per liter was 32, 30, and 29 mg respectively (Figure 11). The time required to attain maximum weight was prolonged from 30 days at 10 mg/liter of dissolved oxygen to 32 days at 5 mg and to 38 days at 3 mg per liter.

The maximum dry weight gain of alevins reared at 40 ppb of NaPCP at the high dissolved oxygen concentration was 22.5% lower than that attained by control alevins. Alevins reared in 40 ppb of NaPCP at 5 and 3 mg per liter of dissolved oxygen never attained maximum weight before the last surviving alevins were removed; however, the nature of the growth curve of the surviving alevins reared at 5 mg per liter suggests that the final weight noted was near the maximum. The reduction in maximum weight of alevins reared in 40 ppb

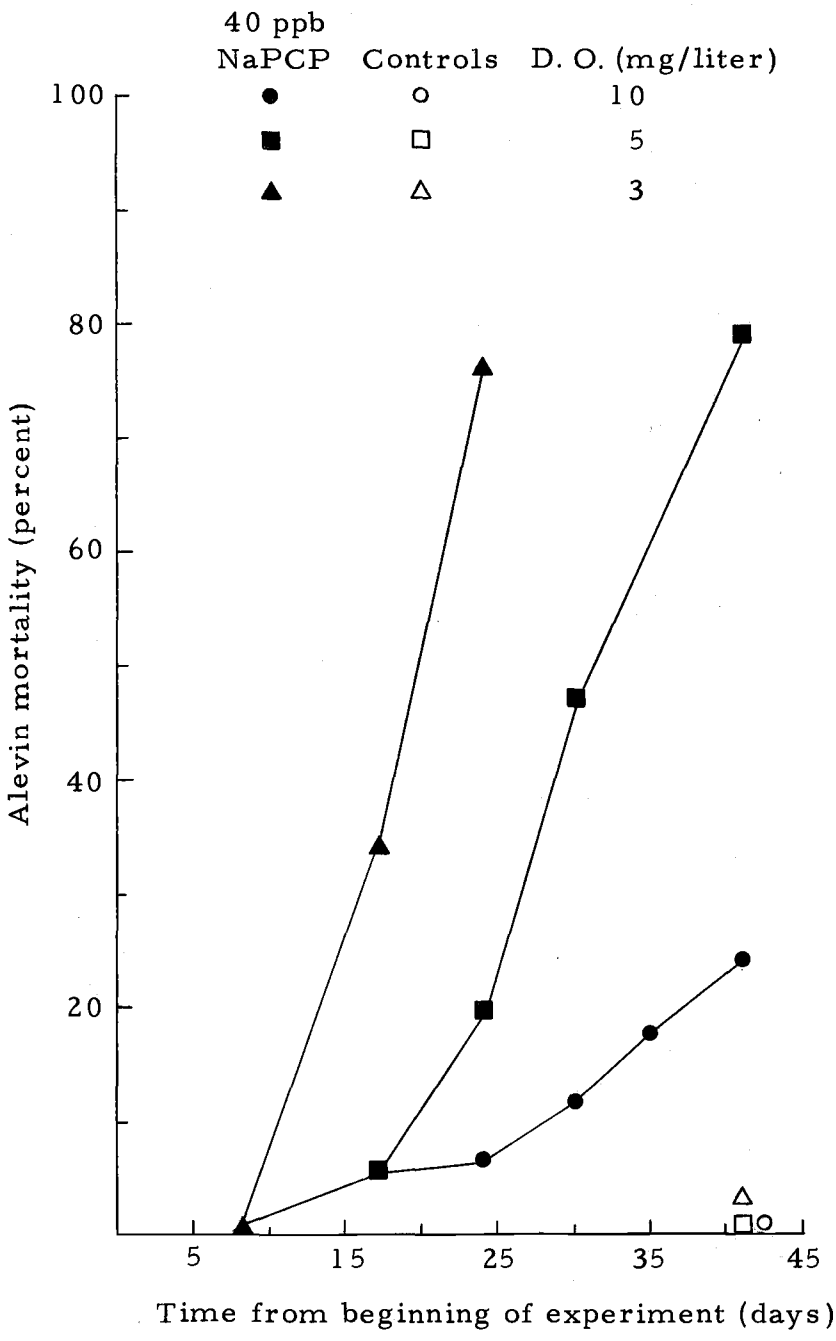

Figure 10. The effect of dissolved oxygen concentration on the mortality of steelhead trout alevins reared in 40 ppb of NaPCP.

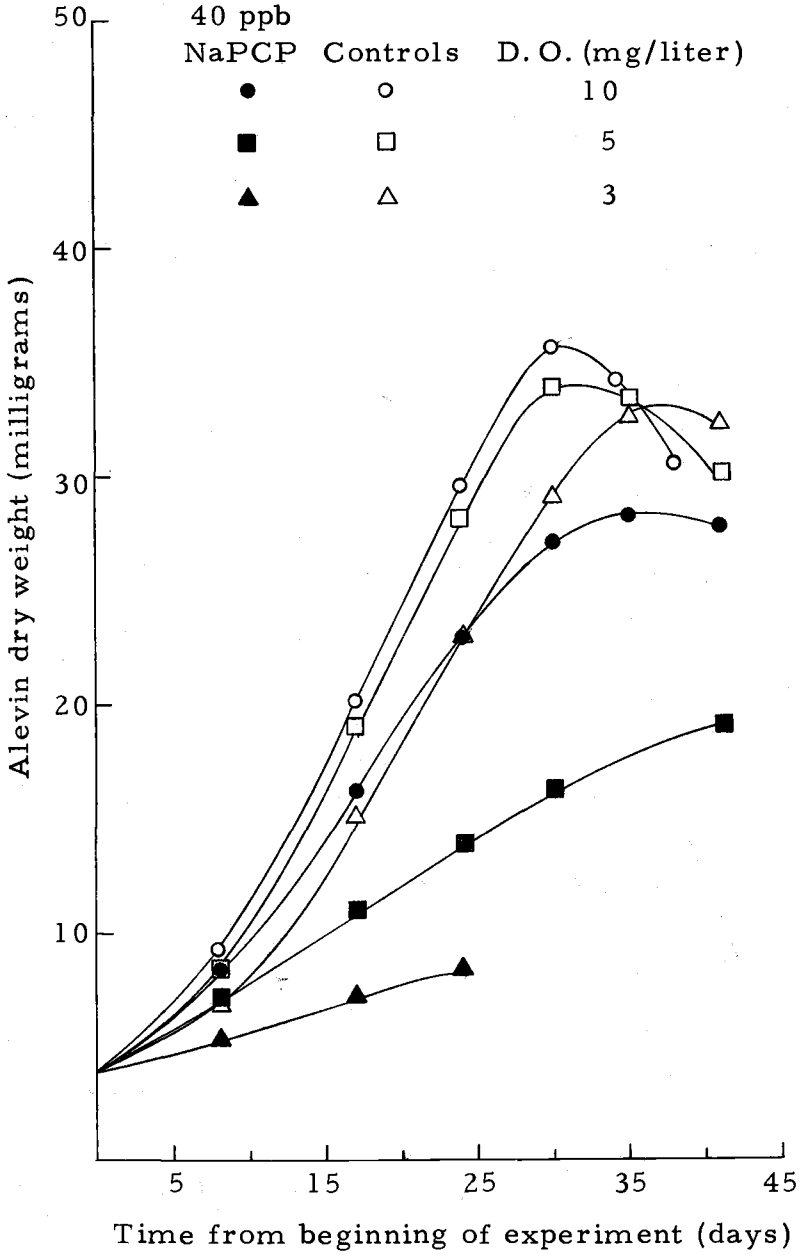

Figure 11. The effect of dissolved oxygen concentration on the growth (dry weight) of steelhead trout alevins reared under control conditions or at 40 ppb of NaPCP.

of NaPCP at 5 milligrams per liter would probably have been about 45%.

The effects of dissolved oxygen concentration and 40 ppb of NaPCP, both singly and in combination, on the efficiency of yolk utilization for growth are shown in Table 12. The efficiency reduction at 40 ppb NaPCP was obviously greater at low oxygen concentrations, while low dissolved oxygen concentrations alone only slightly reduced the efficiency of yolk utilization.

Table 12. The effect of dissolved oxygen concentration on the efficiency of yolk utilization of steelhead trout alevins reared under control conditions or at 40 ppb of NaPCP.

Dissolved oxygen (mg/liter)	Yolk utilization efficiency (%)	
	Controls	40 ppb NaPCP
10	82.9	65.0
5	80.3	55.2
3	78.6	38.5

The relative contributions of increased yolk catabolism rates and decreased yolk uptake rates to the reduced efficiencies of yolk utilization seen under conditions of exposure to 40 ppb of NaPCP at various dissolved oxygen concentrations are indicated in Table 13. Only at 3 mg per liter was an increased rate of yolk catabolism clearly the primary factor in decreasing the yolk utilization efficiency. A decrease in yolk uptake rate was apparent at 5 mg per liter of dissolved oxygen and was responsible for much of the observed reduction in efficiency. At a dissolved oxygen concentration

of 10 mg per liter the contribution of increased catabolism rates and decreased uptake rates to the lowering of efficiency were about equal. In the absence of NaPCP, dissolved oxygen concentrations of 5 and 3 mg per liter had no clear-cut effect on the rates of yolk uptake or yolk catabolism.

Table 13. The effect of dissolved oxygen concentration on the mean rate of yolk uptake and mean rate of yolk catabolism of steelhead trout alevins during the first 24 days of an experiment in which steelhead trout alevins were reared under control conditions or at 40 ppb of NaPCP.

Dissolved oxygen concentration (mg/liter)	Yolk uptake rate (mg yolk/g alevin/day)	
	Control	40 ppb NaPCP
10	85	82
5	89	71
3	84	73
	Yolk catabolism rate (mg yolk/g alevin/day)	
	Control	40 ppb NaPCP
10	16	20
5	20	26
3	15	45

Exposure of alevins to water of reduced oxygen concentration in the presence or absence of 40 ppb of NaPCP caused the caloric increments of the alevins to be lower than that of control alevins reared at 10 mg of dissolved oxygen per liter. The magnitudes of the reductions in caloric increment were similar to those produced in dry weight increment with slight reductions occurring in control alevins

at oxygen concentrations of 5 and 3 mg per liter and greater reductions in alevins exposed to 40 ppb of NaPCP, especially at lower oxygen concentrations (Table 14).

Table 14. The effect of dissolved oxygen concentration on the growth and growth efficiency (dry weight and caloric) of steelhead trout alevins reared under control conditions or at 40 ppb of NaPCP.

	Dissolved oxygen concentration mg/liter	Maximum gain (wt) mg	Maximum gain cal	Percent reduction wt	Percent reduction cal	Efficiency wt (%)	Efficiency cal (%)	Efficiency cal/wt[1]
Controls	10	31.5	168.9	----	----	82.9	75.2	0.91
	5	29.7	155.2	5.5	8.0	80.3	69.8	0.87
	3	28.6	154.5	9.1	8.5	78.6	70.0	0.89
50 ppb NaPCP	10	24.0	125.6	23.8	25.6	65.0	59.1	0.87
	5	14.9	77.2	52.7	54.3	55.2	48.6	0.88
	3	4.2	21.7	85.3	87.2	38.5	30.1	0.78

[1]The ratio between caloric efficiency and weight efficiency.

The efficiency of yolk utilization for growth was lower on a caloric basis than on a dry weight basis. The ratios between caloric efficiencies and dry weight efficiencies were 0.89 ± 0.02 under all rearing conditions except 40 ppb of NaPCP at 3 mg of dissolved oxygen per liter where the ratio was 0.78.

Oxygen Consumption

The oxygen consumption per gram of control and NaPCP reared alevins, at all concentrations of dissolved oxygen, was highest during the first week after hatching, declined steadily throughout the period

of alevin growth, and reached a minimum rate about 1 day before the alevins attained maximum dry weight (Figure 12). Once alevins started to lose weight, the oxygen consumption rates increased sharply to levels generally intermediate between the maximum and minimum rates. The oxygen consumption data are presented as mean rates for each period between removal of alevin samples for the determination of growth (Figure 11). Thus the first set of points (Figure 12) represent the mean oxygen consumption rates over the first 8 days of the experiment; the second set of points represent the oxygen consumption rates of alevins during the period between day 8 and day 17, etc.

The oxygen consumption rates of control alevins at 5 and 10 mg of dissolved oxygen per liter were similar and were higher during the first week after hatching than the oxygen consumption rate of alevins reared at 3 mg per liter (about 3 mg oxygen per gram per hour vs. about 2.4 mg per gram per hour). After the first week, the oxygen consumption rates of control alevins at 5 and 10 mg per liter had decreased to the level of the control alevins at 3 mg per liter; and thereafter the three rates followed very closely until the alevins reached maximum dry weight. The oxygen consumption rates, after maximum weight was attained, were similar at 5 and 10 mg per liter; but the rate of control alevins at 3 mg per liter was again below the rates of other controls.

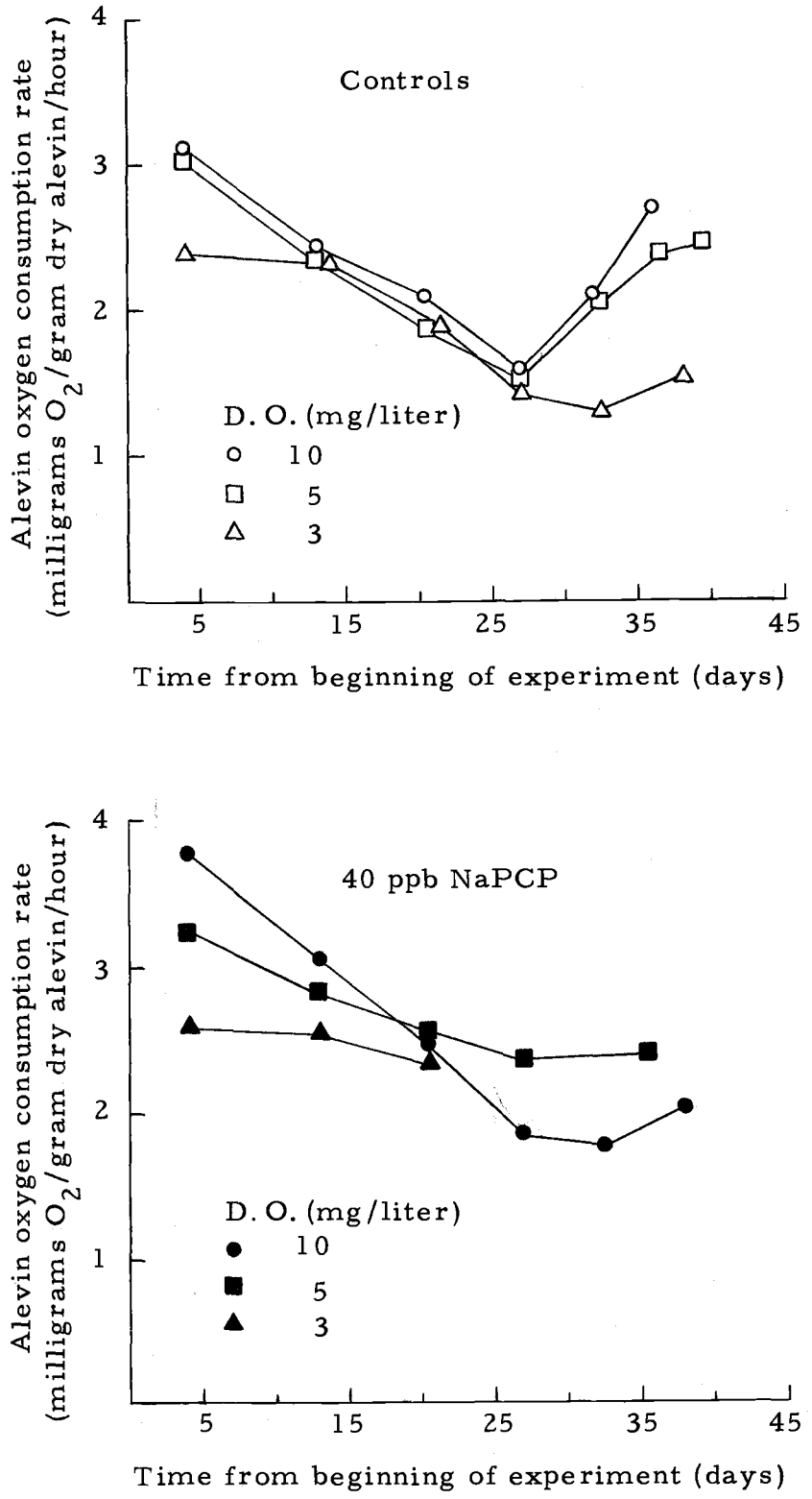

Figure 12. The relationship between time (days) after hatch and oxygen consumption rates of steelhead trout alevins reared under control conditions or at 40 ppb of NaPCP.

At 10 and 5 mg per liter dissolved oxygen concentrations, the oxygen consumption rates of alevins exposed to 40 ppb of NaPCP were higher than the rates of control alevins from the second day of the experiment until maximum alevin weight was attained (Figure 12). No such effect was seen at 3 mg of dissolved oxygen per liter where the oxygen consumption rates of NaPCP poisoned alevins and control alevins were similar.

The alevins steadily gained weight for about a month following hatching and the oxygen consumption per gram of alevin decreased steadily for about a month after hatching occurred, with the result that oxygen consumption per gram declined with increasing alevin weight. The oxygen consumption rates decreased from maximum values at about 5 mg dry weight (hatch) to minimum values which occurred at about the time of maximum alevin weight (Figure 13). During the first week following hatch the oxygen consumption rates of control alevins were again similar at 10 and 5 mg of oxygen per liter and somewhat lower at 3 mg per liter. At alevin weights up to 32 mg, there appeared to be a general correlation between oxygen consumption rate and dissolved oxygen concentration; thus, the oxygen consumption rate was highest at 10 mg per liter, intermediate at 5 mg per liter, and lowest at 3 mg of oxygen per liter.

When compared on the basis of alevin weight rather than age, the differences between oxygen consumption rates of NaPCP poisoned

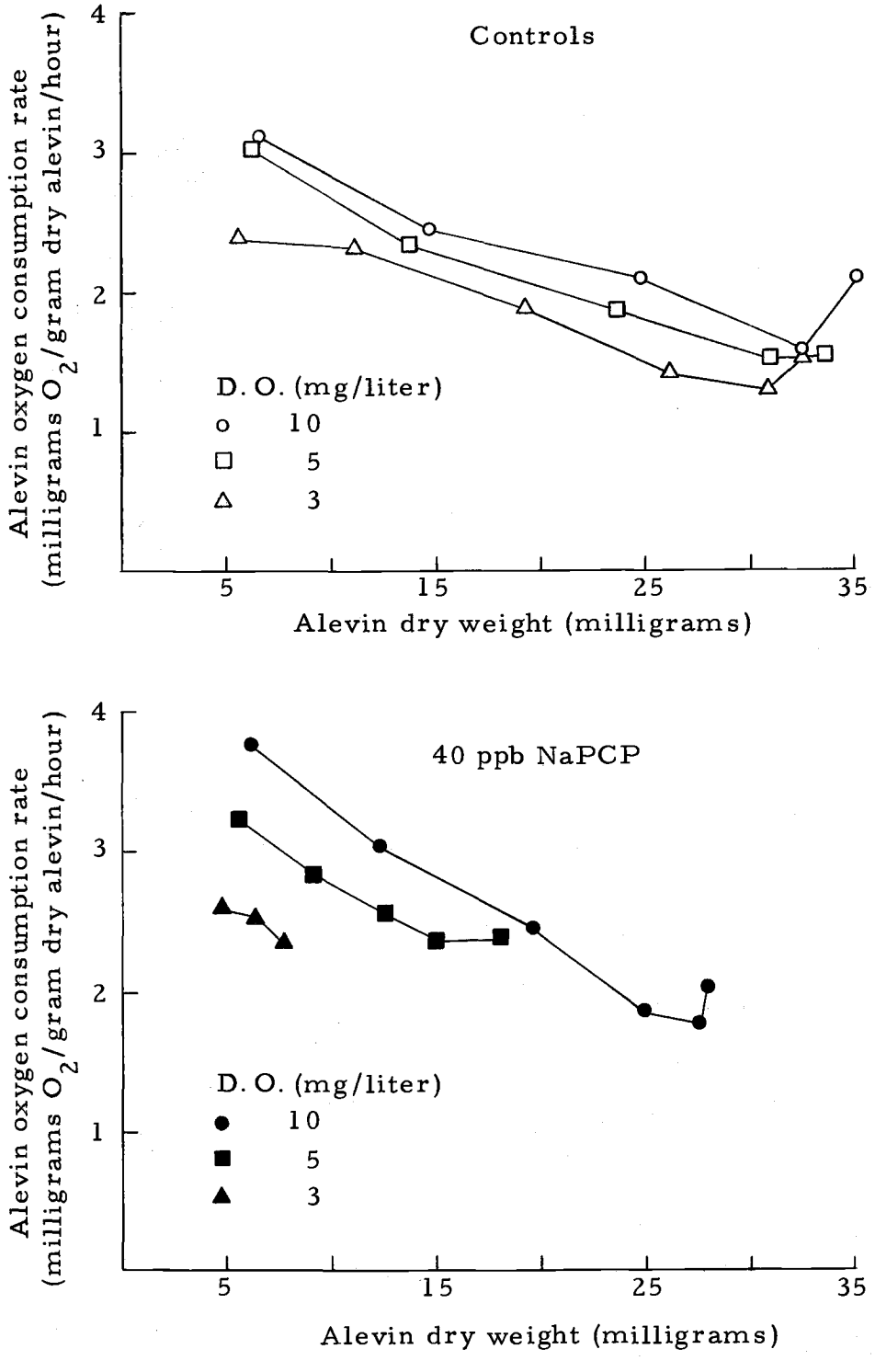

Figure 13. The relationship between alevin dry weight and oxygen consumption rates of steelhead trout alevins reared under control conditions or at 40 ppb of NaPCP.

alevins and control alevins were smaller. The oxygen consumption rate of NaPCP reared alevins at 10 mg of oxygen per liter was higher than that of controls at alevin weights up to about 20 mg. At 5 mg per liter, the difference in oxygen consumption rates between controls and NaPCP reared alevins was generally small, but NaPCP reared alevins had generally higher rates until they attained a weight of about 12 mg. There was again no essential difference between the oxygen consumption rates of control and NaPCP poisoned alevins reared at 3 mg per liter. Alevins reared in NaPCP at 5 and 10 mg of dissolved oxygen per liter attained dry weights of 12 and 20 mg respectively about 3 weeks after the start of the experiment.

The data on oxygen consumption rates also showed a correlation with growth rate (mg growth per gram dry alevin weight per day; Figure 14). The highest oxygen consumption rates corresponded with the highest growth rates and declined to minimum oxygen consumption rates just before the alevins started losing weight. Throughout the entire period of growth, the oxygen consumption rates of alevins exposed to 40 ppb of NaPCP were always higher at all levels of dissolved oxygen than were the oxygen consumption rates of control alevins.

The effects of 40 ppb of NaPCP and various concentrations of dissolved oxygen on the relationship between alevin growth and the cumulative quantity of oxygen consumed per alevin are shown in

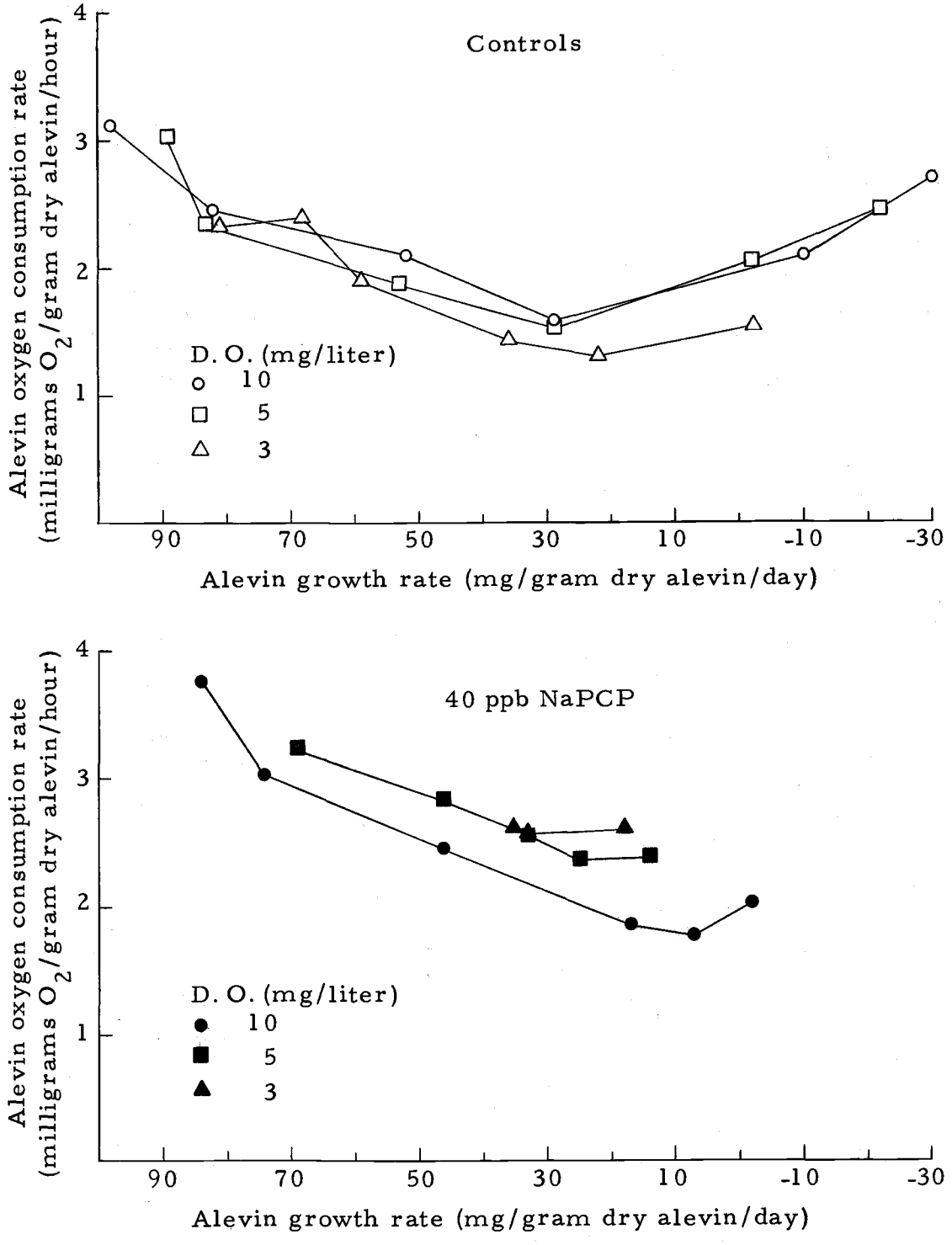

Figure 14. The relationship between alevin growth rate (mg growth/mean g alevin/day) and oxygen consumption rates of steelhead trout alevins reared under control conditions or at 40 ppb of NaPCP.

Figure 15. Control alevins reared at 10, 5, and 3 mg per liter of dissolved oxygen utilized nearly identical quantities of oxygen in the attainment of a given weight. Alevins reared at 40 ppb of NaPCP consumed much greater quantities of oxygen in growing to a given weight than did control alevins. The lower the dissolved oxygen concentration, the greater the amount of oxygen consumed by NaPCP reared alevins in attaining a given weight.

In the experiment conducted to determine the effect of exposure to NaPCP from fertilization to the time of completed yolk utilization, eggs and alevins were continuously exposed to NaPCP in water with a selected dissolved oxygen concentration. Alevins were held under control conditions or at NaPCP concentrations of 10, 20, and 40 ppb, each at 10, 5, and 3 mg of dissolved oxygen per liter.

No mortality of eggs was noted until after the eggs were eyed and had been shocked. The eggs were shocked by transferring them to another vessel via a siphon, and this caused sufficient disturbance that dead and nonviable eggs took up water and became opaque. Shocking was carried out after the embryos were eyed, and hence past the fragile stage. After the dead eggs were removed, the remaining eggs were placed back in the respirometers.

Control embryos at all oxygen concentrations and embryos reared in NaPCP (10, 20 and 40 ppb) at oxygen levels of 10 and 5 mg/liter had about 90% survival at the eyed stage. Approximately

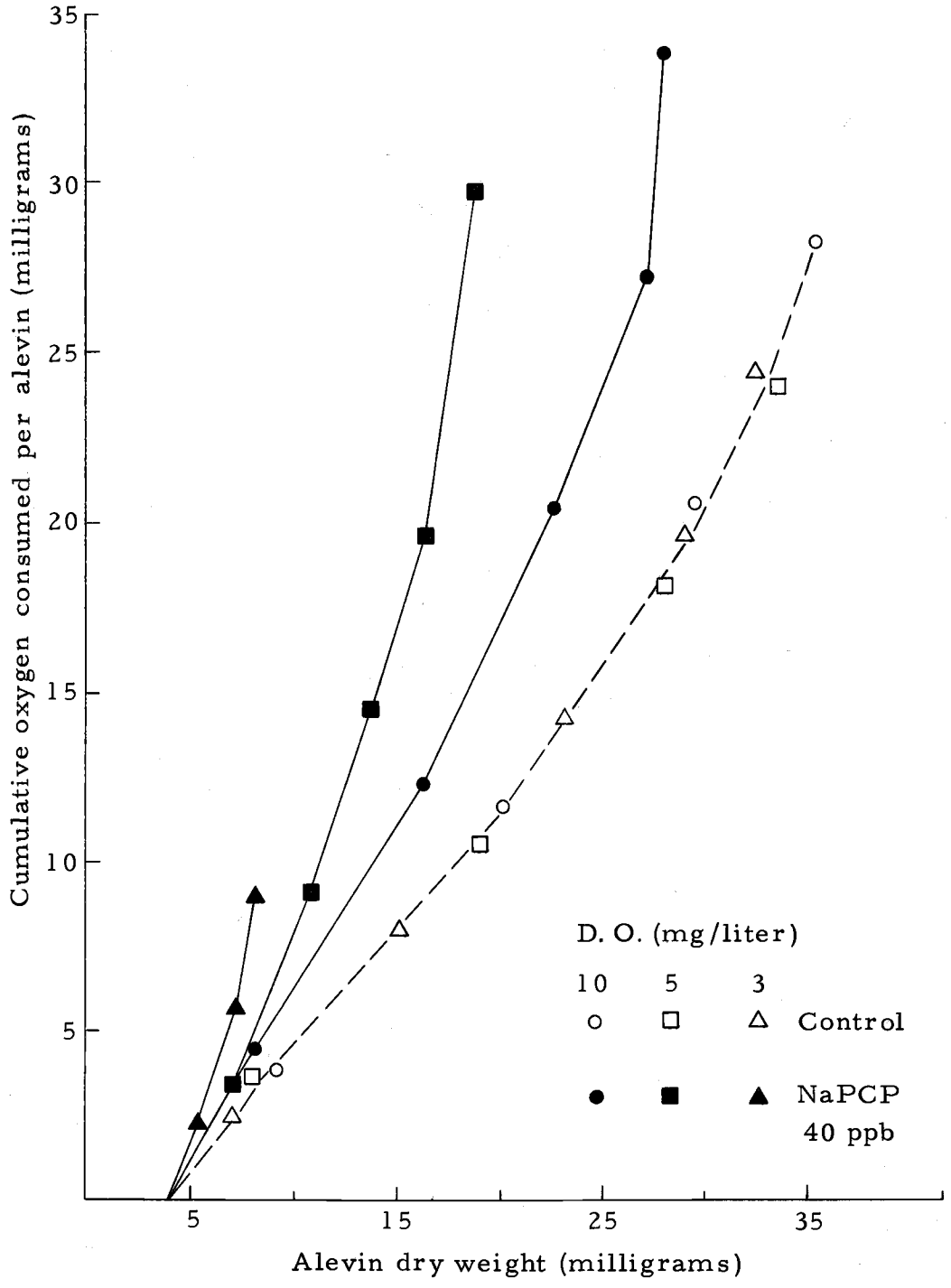

Figure 15. The effect of 40 ppb of NaPCP and various dissolved oxygen levels on the relationship between the amount of oxygen consumed and the dry weight attained by steelhead trout alevins.

one-third of the embryos in each group exposed to NaPCP at 3 mg of oxygen per liter were dead at the eyed stage. Mortality prior to hatching exceeded 50% only at 20 and 40 ppb of NaPCP at 3 mg of oxygen per liter (Table 15). The mean dry weight of newly hatched alevins was lower as the NaPCP concentration was increased; however dry weight at hatch was more affected by the prevailing dissolved oxygen concentration (Table 16). Hatching of control eggs was delayed by 1 day at 5 mg/liter of dissolved oxygen and by 3 days at 3 mg/liter. Alevins exposed to NaPCP were delayed in hatching by 2 days at 5 mg/liter of dissolved oxygen and by 5 days at 3 mg/liter dissolved oxygen.

Table 15. Effect of exposure to NaPCP and low dissolved oxygen concentrations on the percent mortality of steelhead embryos and on total mortality occurring from fertilization through the end of the alevin stage.

NaPCP concentration ppb	Percent mortality		
	Dissolved oxygen concentration mg/liter		
	10	5	3
	embryo-total	embryo-total	embryo-total
Controls	9.3- 9.3	14.0- 14.0	12.8- 13.3
10	6.5- 9.2	14.5- 27.4	36.0-100.0
20	13.8- 23.3	15.6- 90.8	60.4-100.0
40	11.5-100.0	24.4-100.0	99.6-100.0

Control alevins were capable of bursts of swimming activity shortly after hatching and when at rest, continuous rythmic

movements of mouth, opercles, and pectoral fins could be seen. The alevins which hatched at 40 ppb of NaPCP and 5 mg/liter of dissolved oxygen and at 20 ppb of NaPCP and 3 mg/liter of dissolved oxygen were moribund; many had only a heartbeat as a vital sign. One-hundred percent mortality of alevins eventually occurred at all concentrations of dissolved oxygen at 40 ppb of NaPCP and at oxygen concentrations of 3 mg/liter at 20 and 10 ppb of NaPCP (Table 15). Mortality of 90% occurred at 20 ppb of NaPCP and 5 mg/liter of dissolved oxygen. About 25% mortality was observed at 20 ppb and 10 mg/liter of dissolved oxygen and at 10 ppb of NaPCP and 5 mg/liter of dissolved oxygen. Little or no alevin mortality was noted in any control groups or in 10 ppb of NaPCP and 10 mg/liter of dissolved oxygen.

Table 16. Effect of exposure throughout the embryo stage to various concentrations of NaPCP and dissolved oxygen on mean dry weight at hatch of alevins.

NaPCP concentration (ppb)	Dissolved oxygen concentration (mg/liter)		
	10	5	3
	(Alevin dry weight - milligrams)		
Controls	4.6	2.9	1.7
10	4.3	---	1.7
20	4.1	2.4	1.3
40	3.4	1.9	---

Maximum dry weights attained were similar in control alevins at 10 and 5 mg/liter of dissolved oxygen and in 10 ppb of NaPCP at 10 mg/liter of dissolved oxygen (Table 17). About a 10% reduction in maximum dry weight was seen in controls at 3 mg/liter of dissolved oxygen, at 10 ppb of NaPCP and 5 mg/liter of dissolved oxygen, and at 20 ppb of NaPCP and 10 mg/liter of dissolved oxygen. The few alevins surviving in 20 ppb of NaPCP at 5 mg/liter dissolved oxygen were still gaining weight at the close of the experiment; however, it appeared that they would attain a maximum weight significantly lower than that of other surviving alevins, probably with a reduction of about 30% in maximum dry weight attained.

Table 17. Maximum dry weights (milligrams) attained by alevins of steelhead trout reared from fertilization through the alevin stage at various concentrations of dissolved oxygen and NaPCP. Figures in parentheses indicate the number of days from fertilization required to attain the maximum weight.

Dissolved oxygen concentration mg/liter	NaPCP concentration		
	Control	10 ppb	20 ppb
10	41.5 (66)	40.5 (66)	37.7 (71)
5	41.8 (76)	37.4 (86)	26.6 (92)
3	37.9 (81)	----	----

Data on mortality and growth at 10 ppb of NaPCP and 5 mg/liter of dissolved oxygen are perhaps questionable since due to a gear

failure which occurred at the time of hatching, the alevins were exposed to apparently very low dissolved oxygen concentrations for perhaps as long as 12 hours. Approximately one-half of the alevins died as a result of this low oxygen concentration; however, the survivors appeared in good condition the following day and little additional mortality occurred. Whether the growth of survivors was affected by the period of exposure to low dissolved oxygen concentrations is not known; probably the effect was minimal. Whether the exposure to low oxygen caused the death of the weakest alevins, leaving only the strongest alevins alive is also not known.

Juvenile Experiment

When alevins had absorbed all available yolk material and entered the juvenile stage, they appeared to be more resistant to the lethal effects of NaPCP. During a 3-week experiment with juvenile steelhead, concentrations of NaPCP of 30 and 70 ppb produced zero mortality and 8% mortality respectively. (Alevins exposed to 30 and 70 ppb for 20 days showed 8% and 35% mortality respectively.) However, the growth of juveniles in 30 and 70 ppb of NaPCP was considerably less than that observed in control juveniles (Table 18).

Table 18. The effect of a 3-week exposure to NaPCP on the growth (dry weight) and growth rate of juvenile steelhead trout. Growth rates were calculated separately for each week.

	Dry weight milligrams			Rate of dry weight gain mg gain/g weight/day		
	NaPCP concentration (ppb)			NaPCP concentration (ppb)		
Day	Control	30	70	Control	30	70
0	19.3	----	----	--	--	--
7	34.0	31.9	29.5	79	70	60
14	57.0	53.5	48.4	72	72	69
21	97.8	83.0	70.1	75	62	52

DISCUSSION

Long-term static bioassays with steelhead trout embryos showed that concentrations of NaPCP as low as 300 ppb were 100% lethal within 1 week of fertilization and that concentrations down to 50 ppb were 100% lethal by 1 day after hatching. These results appear to be contradictory to the more general observation by Goodnight (1942) that the eggs of lake trout were extremely resistant to PCP. The apparent discrepancy between my results and those of Goodnight probably was due to the duration of exposure of the eggs to PCP. Most of Goodnight's fish bioassays were of 3-day duration and the egg bioassays were probably not appreciably longer, although the duration was not reported. Also, the stage of development of the embryos used by Goodnight was not mentioned in his paper. Since salmonid eggs are extremely fragile between the second day after fertilization and the time of eyeing, it is probable that Goodnight used eyed eggs, although he could possibly have used newly fertilized eggs.

In experiments not included in this paper, I found that exposure of both newly fertilized eggs and eyed embryos to NaPCP produced obvious mortality within 3 days only at very high concentrations (100 and 10 ppm but not at 1 ppm). Therefore, embryos exposed to NaPCP for several days appeared to be able to survive concentrations of NaPCP at least as high as 1,000 ppb while alevins were rapidly killed

by concentrations as low as 200 ppb. On the other hand, prolonged exposure to NaPCP concentrations down to 50 ppb was essentially 100% lethal to embryos and concentrations of 70 ppb were essentially 100% lethal to alevins. The implication of these results is that the uptake of PCP by the alevin is much more rapid than the uptake by the embryo, but that long-term exposure to lower concentrations of NaPCP eventually results in the accumulation of lethal tissue concentrations in both alevins and embryos.

Since no measure of the concentration of PCP in embryo or alevin tissues was made, it was not possible to determine if the amount of PCP absorbed at the time of death at a given concentration of NaPCP was the same in embryos and alevins. If it is assumed that the tissue concentrations were essentially the same, then it is probable that the uptake of PCP by embryos was indeed slower than the uptake by alevins. The general permeability of the egg appears to decrease markedly soon after fertilization. A brief period of water uptake by the egg after release from the coelom of the female into water (hypotonic) is associated with water hardening or activation. The water uptake is completed within 1 hour (Hayes, 1949; Prescott, 1955). Following this period of water uptake, the chorion or outer egg membrane is permeable to water and salts but is impermeable to vital stains (Hayes, 1930). The permeability of the plasma membrane to water and salts is very limited, although Prescott (1955)

showed that some permeability to water was apparently retained. The permeability of the chorion and plasma membrane of eyed eggs of chinook salmon to zinc has been investigated by Wedemeyer (1968). Wedemeyer found that over periods of 30 to 45 minutes, the chorion was permeable to zinc; but the plasma membrane allowed very little zinc to pass into the embryo and yolk. Large amounts appeared to be bound to the chorion. Certain chemicals altered the permeability of the egg to zinc; thus, iodoacetate caused a great decrease in zinc bound to the chorion with a resultant increase in levels in the perivitelline fluid. Iodoacetate, however, had no effect on the permeability of the plasma membrane. Malachite green, on the other hand, increased zinc uptake into the yolk markedly.

The chorion is apparently sufficiently permeable to permit passage of many dissolved materials into the perivitelline fluid. Plasma membrane permeability, however, is quite limited but capable of being modified by chemical agents. Pentachlorophenol probably enters the perivitelline fluid but the uptake through the plasma membrane is very slow. Pentachlorophenol in the perivitelline fluid could bind with protein moieties of the plasma membrane in a manner analogous to that described by Weinbach and Garbus (1964) for mitochondria and produce direct toxic action without entering the yolk or embryo. The binding of PCP to the plasma membrane may also alter membrane permeability and permit more rapid passage of PCP into the embryo.

Goodnight (1942) reported that the alevins of lake trout were more susceptible to PCP than the eggs or more mature fish; he also concluded that lake trout alevins were less sensitive than silverjaw minnows (Ericymba bucatta), a species which was not killed by exposure to 200 ppb of NaPCP in 3 days. The acute toxicity results of the current investigation therefore differ from those of Goodnight since I found that 200 ppb was usually lethal to steelhead trout alevins in 24 hours. The pH of the water in this investigation (7.8) was similar to that used by Goodnight (7.6); however, Goodnight's experiment used PCP rather than NaPCP. Whether the difference in chemicals or species used or variability in other bioassay conditions caused the different results is not clear. The similarity of susceptibility to NaPCP of the alevins of the three salmonid species I tested would suggest that species differences may not account for the acute toxicity differences between this study and that of Goodnight.

While some quantitative discrepancy exists between the results reported by Goodnight and those which I have reported, the qualitative aspects of relative susceptibility between salmonid life stages in short-term exposure to NaPCP remained as reported by Goodnight. Hence, with short-term exposure, steelhead embryos appeared to be much more resistant to NaPCP than were alevins; only when exposure extended over the entire period of embryo or alevin development were the lethal levels of embryos and alevins essentially the same.

While there appeared to be little difference in the levels of NaPCP producing lethal effects with long-term exposure of embryos and alevins, it appeared that juveniles were more tolerant of NaPCP than embryos and alevins. Juvenile steelhead reared for 3 weeks (15° C) at NaPCP concentrations as high as 70 ppb showed only negligible mortality (8% with no mortality during the last week) while alevins held at 70 ppb for 20 days (15° C) had suffered 35% mortality and the mortality rate was increasing rapidly. Three- to twelve-month old rainbow and brown trout were killed by NaPCP concentrations of 170 ppb in 48 hours in 18° C flowing water bioassays (Alabaster, 1958), and I found 250 ppb to be the lowest NaPCP concentration lethal to yearling steelhead trout (50% mortality in about 60 hours) in a 10° C static 5-day bioassay. It appears that the lowest concentration of NaPCP that would be lethal to juvenile salmonids would be between 70 and 170 ppb.

When comparing bioassay results with fish, using PCP or NaPCP as the toxic agent, the pH of the water is a very important factor. The effect of changes in pH over the ranges occurring in natural waters greatly alters the toxicity of PCP and its salts. Thus, Crandall and Goodnight (1959) reported that the lower the pH, the more toxic NaPCP was to fathead minnows (Pimaphales promelas). One ppm of NaPCP produced 50% mortality in 30 minutes at pH 6, in 83 minutes at pH 7. 6, and no mortality occurred at pH 9.

Since PCP is an acid, it is ionized more and more with increasing pH. Uncharged molecules generally pass through biological membranes much more rapidly than ions. Where uptake of a chemical is requisite for toxic action, the pH of the medium and the pK of the chemical are extremely important. Pentachlorophenol has a pK of about 4. 8 (Blackman, Parke, and Garton, 1955); thus at the pH of most natural waters, PCP exists largely in the ionic form. The implications of changes in pH in this range are great. Returning to the data of Crandall and Goodnight, at pH 6. 0 about 6% of the PCP was in molecular form, at pH 7. 6 about 0. 2% was molecular, and at pH 9. 0 only 0. 01% was present in the molecular form. It appears that while the concentration of ionized PCP in the water may contribute to the total toxicity of a given level of PCP, the molecular concentration is the major factor.

The pH of the water could greatly alter the short-term toxicity of rather high concentrations of PCP, presumably by varying the proportion of molecular and ionized PCP. What effect would pH changes have on the long-term toxicity of NaPCP? If the long-term toxic effects were directly proportional to the level of molecular PCP present, then a small change in pH could appreciably alter the concentration of NaPCP which produced toxic manifestations. For example, at pH 7. 8, I have shown that 40 ppb can be 100% lethal to steelhead trout. At pH 7. 8, NaPCP was 99. 9% ionized so that the concentration of

molecular PCP was 0. 04 ppb. Considering 0. 04 ppb of molecular PCP to be lethal, then at pH 8. 8 (99. 99% ionized) the NaPCP concentration necessary to produce lethality would be much greater (400 ppb) and at pH 6. 8 (99% ionized), the NaPCP concentration necessary would be much less (4 ppb).

The effects of continuous exposure of alevins to low concentrations of NaPCP can obviously be very severe. It is clear, however, that intermittent exposure produces much less severe results. Apparently the alevins rather rapidly decrease the PCP content of their tissues or otherwise are able to overcome the toxic effects once they are placed in clean water. Uptake of PCP can be quite rapid as illustrated by the results of Weinbach and Nolan (1956); snails (Australorbis glabratus) exposed to 7.5×10^{-6} M NaPCP (2 ppm) accumulated 2.5×10^{-4} M PCP in tissues in 24 hours (a 30-fold concentration) and tissue levels were 4.4×10^{-4} M after 30 hours (a 60-fold concentration). Based on data presented by Tsuda and Kariya (1963), I have calculated that rainbow trout (Salmo gairdneri) killed in 20 hours at 3.8×10^{-7} M NaPCP (100 ppb) accumulated tissue levels of 1.2×10^{-4} M (a 300-fold concentration). In in vitro studies, Weinbach and Garbus (1965) found that mitochondrially bound PCP was not removed by repeated washing with 0. 25 M sucrose; but when bovine serum albumin was included in the washing medium, a single washing removed all the bound PCP. Whether tissue concentrations of PCP

decreased markedly or PCP was only debound from its primary sites of action, recovery from the effects of PCP poisoning appeared to be rapid in steelhead trout alevins placed in clean water.

Where dissolved oxygen concentrations were high, steelhead exposed to NaPCP throughout both the embryo and alevins stages could not survive continuous exposure to NaPCP at concentrations of 40 ppb or greater. Where dissolved oxygen levels fall to 5 mg/liter or less, levels of NaPCP down to 10 ppb would compromise the chances of good survival and growth. Under more natural conditions, where alevins were allowed to feed on an exogenous food supply, a level of NaPCP which caused no greater than 25% mortality (40 ppb) produced profound retardation in growth and development. Under conditions where exposure to 40 ppb of NaPCP was 100% lethal, i.e., exposure from fertilization through the alevin stage, a retardation of the growth of feeding alevins might occur at levels of NaPCP below 40 ppb. Once feeding started in NaPCP reared alevins or in juveniles, the ingestion of considerable quantities of PCP probably occurred due to uptake of PCP by the organisms serving as food. Determining whether this ingestion led to higher tissue levels of PCP than under conditions of exposure to NaPCP in the water alone would require analysis of PCP levels in the tissues.

Embryos and alevins reared in NaPCP at low oxygen concentrations were more severely affected than those at high oxygen levels.

This intensification of the effects of toxic materials has been reported before with ammonia, prussic acid, cyanide, and p-cresol (Wuhrmann, 1952). This phenomenon is usually ascribed to one of two causes. The first is that at low levels of dissolved oxygen the fish is required to pass more water over the gills in order to obtain a sufficient quantity of oxygen, and as a result, absorbs more toxicant via the gills than at higher oxygen levels and lower ventilation volumes. A second explanation is that some chemicals (e. g., prussic acid) have a mechanism of action which produces lesions in gill tissue, and this interferes with normal gas exchange. At low oxygen levels, a partial loss of effective gill area would be more severe than at high oxygen levels.

The mechanism of action of PCP is generally regarded to be one of uncoupling oxidative phosphorylation. Uncouplers dissociate electron transfer from phosphorylation, permitting oxidation to occur but inhibiting the formation of adenosine triphosphate (ATP) via the phosphorylation of adenosine diphosphate (Fruton and Simmonds, 1960). Current data could also be interpreted as indicating a more rapid breakdown of ATP once formed. Since PCP has been shown to bind to all cell fractions (Weinbach and Garbus, 1965), it is likely that the toxic action of PCP is attributable, at least in part, to other causes than uncoupling of oxidative phosphorylation or increasing the rate of ATP breakdown. This seems especially likely in long-term

experiments where secondary effects and interactions could come into full play. Regardless of the possible occurrence of other modes of action than uncoupling of oxidative phosphorylation, in the light of present knowledge of the effects of PCP, the primary consideration should probably remain with the action of PCP on ATP formation or breakdown.

The response to partial uncoupling of oxidative phosphorylation or to a more rapid decomposition of ATP would seem to require an increase in oxygen consumption correlated with an increase in substrate oxidation and electron transport. Thus, to obtain a given quantity of ATP, if oxidative phosphorylation were uncoupled to the extent that P:0 ratios fell to 2 (normal 3), would require the consumption of 50% more oxygen and the catabolism of 50% more substrate. (On the assumption that substrate level phosphorylation would be unable to contribute significant quantities of ATP for prolonged periods of time.) In fish exposed to PCP, the synergistic effect of low dissolved oxygen concentrations becomes obvious where increased oxygen consumption rates are required to maintain necessary quantities of ATP. Furthermore, the seemingly requisite increase in ventilation volume necessary for a fish to obtain greater amounts of oxygen would also produce the previously mentioned effect of increasing toxicant uptake.

While determinations of oxygen consumption, growth, and yolk utilization efficiency are obviously too gross to produce definitive

information as to the mechanism of action of PCP, the bioenergetic data obtained in this study are consistent with the concept that PCP disrupts energy metabolism. Alevins exposed to NaPCP grew less rapidly than controls due to higher rates of yolk catabolism and resultant lower efficiencies of yolk utilization. Congruently, NaPCP reared alevins also had higher rates of oxygen consumption.

The concept of increased ventilation rates at low oxygen concentrations causing the apparently synergistic effect of NaPCP and low dissolved oxygen is difficult to extend to the case of embryos since the oxygen uptake of embryos would seem to be passive and dependent on the oxygen concentration gradients between the water, perivitelline fluid and the embryo. With the development of the embryonic vascular system and hemoglobin, compensations for living in an environment with low levels of oxygen might conceivably occur. Most compensations which would actually or effectively increase contact between the embryo and the perivitelline fluid would presumably increase the uptake of PCP. Regardless of the mechanism of synergism, there is no doubt that embryos as well as alevins are less able to cope with the stress of NaPCP when the dissolved oxygen concentration is low.

Previously published research regarding the effect of dissolved oxygen concentration on the toxicity of NaPCP are limited and offer no support to my findings. Goodnight (1942) reported that oxygen

levels above 2 mg/liter had no effect on the survival times of the fish in NaPCP. Weber (1965) exposed guppies to water containing 5 and 10 ppm of NaPCP and various levels of oxygen. Under the conditions of his experiments, survival time was markedly increased at low oxygen concentrations and he reported that NaPCP was more toxic at high dissolved oxygen concentrations. However, I believe that Weber misinterpreted the results of his experiments. Due to the method (boiling) which he used to produce water with a low oxygen concentration, Weber markedly raised the pH of the partially deoxygenated water. For example, his results show that at oxygen levels of 8.2, 6.6, and 5.9 mg/liter, 5 ppm of NaPCP was most toxic at 8.2 mg/liter (pH 7.6), less toxic at 6.6 mg oxygen per liter (pH 8.7), and least toxic at 5.9 mg/liter (pH > 9). The effect of pH differences obscured whatever more subtle effect might have been produced by the variations in dissolved oxygen.

Allowable concentrations of PCP in water should apparently be well below the generally accepted 200 ppb lethal limit found in static bioassays. Based on bioassays with bluegills (Lepomis macrochirus), Turnbull et al. (1954) listed 100 ppb as the safe concentration for Santobrite (NaPCP); this concentration is a lethal level for embryos and alevins of steelhead trout. Concentrations of NaPCP down to 20 ppb are decidedly harmful to early life stages of salmonids; and allowable levels of PCP, at least in waters where salmonids

spawn, should be below this concentration. Obviously, such factors as the pH and dissolved oxygen concentration of the water can alter the toxicity of a given level of PCP and hence the allowable level. Whether a specified level of NaPCP (or any other toxic material) is safe or harmful depends to a great extent on the length of time that concentration of NaPCP is present in the environment. Obviously, the effect of fluctuating levels of NaPCP would be a more complex toxicological problem involving such factors as periodicity and maximum and minimum concentrations. Ultimately the decision must be made as to what degree of change in the organism involved should be regarded as harmful.

SUMMARY

1. Continuous exposure to NaPCP was essentially 100% lethal to steelhead trout embryos at a concentration of 50 ppb and to alevins at a concentration of 70 ppb.
2. Exposure to 40 ppb was 100% lethal if exposure was continuous throughout both the embryo and alevin stages.
3. Alevin growth (maximum dry weight) was decreased by about 6% for each 10 ppb increase in NaPCP concentration.
4. Intermittent exposure of alevins to NaPCP indicated that recovery from and onset of toxicity occurred rapidly.
5. When exposure lasted throughout the embryo and alevin stages at a dissolved oxygen concentration of 3 mg/liter, 10 ppb of NaPCP was 100% lethal and at 5 mg/liter, 20 ppb was 100% lethal.
6. Exposure to NaPCP caused lower growth rates, higher rates of yolk catabolism, higher rates of oxygen consumption, and lower efficiencies of yolk utilization for growth.
7. Juveniles were less susceptible than embryos or alevins, surviving at NaPCP concentrations of 30 and 70 ppb, although growth rates were lower than in controls.

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