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Title: INDIVIDUAL AND COMBINED EFFECTS OF CYANIDE, PENTACHLOROPHENOL AND ZINC ON JUVENILE CHINOOK SALMON AND INVERTEBRATES IN MODEL STREAM COMMUNITIES

Abstract approved: Gerald E. Davis

The effects of sublethal concentrations of cyanide, pentachlorophenol and zinc individually and in combination on the growth and production of juvenile chinook salmon, Oncorhynchus tshawytscha (Walbaum), and aquatic invertebrates in model stream communities were studied in three experiments during 1971 and 1972 at the Oak Creek Fisheries Research Laboratory, Oregon State University. The individual toxicants were tested at concentrations of 0.1 toxic units (96 hour TL_m) in experiments I and II, while the tests with mixtures were carried out at values of 0.1 (low treatment) and 0.3 (high treatment) toxic units. All treatment concentrations were doubled in experiment III, which resulted in individual concentrations equivalent to 0.2 toxic units for cyanide, pentachlorophenol

and zinc. The three toxicants were tested in combination at concentrations equivalent to 0.2 toxic units (low treatment) and to 0.6 toxic units (high treatment).

The juvenile salmon were weighed biweekly during the course of each experiment. Benthic invertebrate samples were taken monthly and drift samples were generally taken every three weeks.

The individual toxicant concentrations of 0.1 toxic units of cyanide, pentachlorophenol and zinc did not result in any marked reductions of juvenile salmon growth or benthic densities during experiments I and II. Individual toxicant concentrations of 0.2 toxic units of cyanide, pentachlorophenol and zinc during experiment III adversely affected salmon growth and production as well as benthic densities. Zinc appeared to be the most deleterious of the individual treatments while cyanide was the least harmful of the three toxicants to salmon growth. Cyanide appeared to enhance salmon production at 0.1 toxic units (10 ppb). In both experiments I and III, reductions in salmon biomass were always greater in streams receiving mixtures of toxicants and reductions were always greater in the high treatments than in the low treatments.

Decreases in salmon production and biomass in the high and low combination treatments were not predictable based on the responses of salmon exposed to the individual toxicants, but were always greater than the decreases observed in any one individual

treatment. The results of experiments I and III indicated that levels of individual toxicants apparently safe for juvenile salmon were deleterious to salmon growth and production when combined at levels equivalent to those of the individual toxicants. The results, especially those of experiment I, indicate that mixtures of the three toxicants equivalent to 0. 1 toxic units impair salmonid growth, and suggest that even lower concentrations may be harmful to juvenile salmon.

Individual and Combined Effects of Cyanide, Pentachlorophenol and Zinc on Juvenile Chinook Salmon and Invertebrates in Model Stream Communities

by

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INTRODUCTION

Industrial and municipal waste discharges have resulted in increasingly complex mixtures of toxicants in our natural waterways. Existing water quality standards and water pollution research to date have primarily been based on the effects of acutely lethal levels of individual toxicants on fishes and other aquatic organisms. However, animals in an aquatic environment are often chronically exposed, not only to one toxicant, but to many different compounds concurrently, at sublethal rather than lethal concentrations. Studies of combinations or mixtures of pollutants have been few, and conducted mostly by European investigators.

Southgate (1932) studied mixtures of poisons by the simple addition of fractions of short-term lethal concentrations. Bergstrom and Vallin (1932), comparing the relative toxicities of pulp mill wastes, named the resultant units they derived giftenhet, or "toxic unit," which was later adopted by Sprague and Ramsay (1965) as the "incipient" lethal level or the concentration of a toxicant that would be lethal to 50 percent of the test organisms in a specified period of time. This concentration is often referred to as the LC_{50}

or TL_m (median tolerance limit).

Effects similar to those reported by Southgate (1932) and Bergstrom and Vallin (1932) were also observed by Bandt (1946), Cherkinsky (1957), Friedland and Rubleva (1958) and Bucksteeg (1955). Extensive work by the British at the Water Pollution Research Laboratory in Stevenage, U.K. has resulted in some papers dealing with the effects of mixtures of two and three toxicants in the laboratory and up to as many as five in field studies. Most notable among these papers are those of Lloyd (1961), Lloyd and Herbert (1962), Lloyd and Jordan (1964), Herbert and Shurben (1964), Herbert and VanDyke (1964), Brown (1968), Brown, Jordan and Tiller (1969), Brown and Dalton (1970) and Brown, Shurben and Shaw (1970). The empirical results of these studies suggest that the toxicity of mixtures of poisons may be adequately described by summation of the fractional toxicities of TL_m values of the individual toxicants within the mixture. Effects which are more than additive may also occur, as noted by Sprague and Ramsay (1965), at levels of two and five toxic units of copper and zinc.

If water quality standards are to protect aquatic communities, they must be based upon evidence of the chronic effects of mixtures of toxicants. The toxicants, cyanide, pentachlorophenol and zinc, were chosen for the research reported herein because they are: (1) representative of important classes of chemicals; (2) widespread

in their occurrence in waterways; and (3) diverse in their physiological actions. Cyanide is capable of blocking the oxygen-procuring cytochrome oxidase system (Jones, 1947). Pentachlorophenol uncouples oxidative phosphorylation (Weinbach, 1954; and Pasley, 1969). And zinc may damage gill epithelial tissue (Lloyd, 1960; Mount, 1965; and Skidmore, 1970), or accumulate internally causing extensive tissue damage (Cairns and Scheier, 1957; Goodman, 1951; Mount, 1964; and Crandall and Goodnight, 1962 and 1963).

Three experiments were conducted at the Oak Creek Laboratory, Department of Fisheries and Wildlife, Oregon State University, between February 21, 1971, and May 17, 1972, to evaluate the chronic effects of toxicants introduced individually and in combination on the growth of juvenile chinook salmon, Oncorhynchus tshawytscha (Walbaum), and on the levels of abundance of the invertebrate fauna in model stream communities. An important objective was to determine the extent to which toxicants introduced at "safe application" levels would combine in their effects and thereby produce changes in the aquatic communities. A supplementary goal, deriving from a demonstration that the toxicants influenced salmon growth, was an examination of the operational effects of the toxicants; whether their action was directly physiological or mediated through the food chain of the fish.

MATERIALS AND METHODOLOGY

Experimental Apparatus

Laboratory Streams

The principal facilities used in this study were six model streams, which were housed in a wooden building with a translucent fiberglass roof. Each stream consisted of a wooden box divided by a median partition into two channels 30.0 cm wide, 2.95 m long and 25 cm deep. Openings at each end of the partition permitted recirculation of the water, which was taken from a spring tributary near the laboratory and trickled through a sand filter before it was used in the streams. Water velocities of 24 cm per second were maintained in the riffles of each stream channel by a paddle wheel driven by a 1/10 horsepower, low-speed gearmotor. The stream water was exchanged at the rate of two liters per minute and a constant volume of 215 liters was maintained in each stream by a standpipe drain.

The area of each stream bottom was 1.60 square meters. The respective areas of riffles and pools were 0.95 m^2 and 0.65 m^2. The riffle substrate was composed of rubble 3.0 to 20.0 cm in diameter, while that of the pools was composed of gravel 0.5 to 2.0 cm in diameter. A more detailed and functional description

of the experimental streams may be seen in Ellis (1968), Seim (1970) and Lichatowich (1970).

Stream temperatures were recorded continuously in one of the streams with a spring-driven thermograph. Alkalinity and hardness were measured monthly according to procedures prescribed by American Public Health Association et al. (1971). Measurements of pH were made with a Corning Model 12 meter. Since the characteristics of the laboratory water supply have remained seasonally constant for many years, it was not deemed necessary to make more frequent determinations.

Toxicant Delivery System

Toxicant solutions were kept in acrylic plastic boxes (24.1 cm × 40.6 cm × 53.3 cm), which were air-tight and painted black to reduce photochemical decomposition. Three holes, spaced equidistantly along one side of the reservoir and 1.8 cm from the bottom, were fitted with stoppers having either one or two glass tubing outlets 2 mm in diameter. The toxicants flowed from these outlets through funnels to the diluent water supply before passing to the mixing chambers (Figure 1). The boxes operated on the Mariotte bottle principle, each having an air inlet tube permitting air to replace the toxicant as it dripped out, thus providing a constant head pressure. The rate of toxicant flow, which was dependent on the vertical

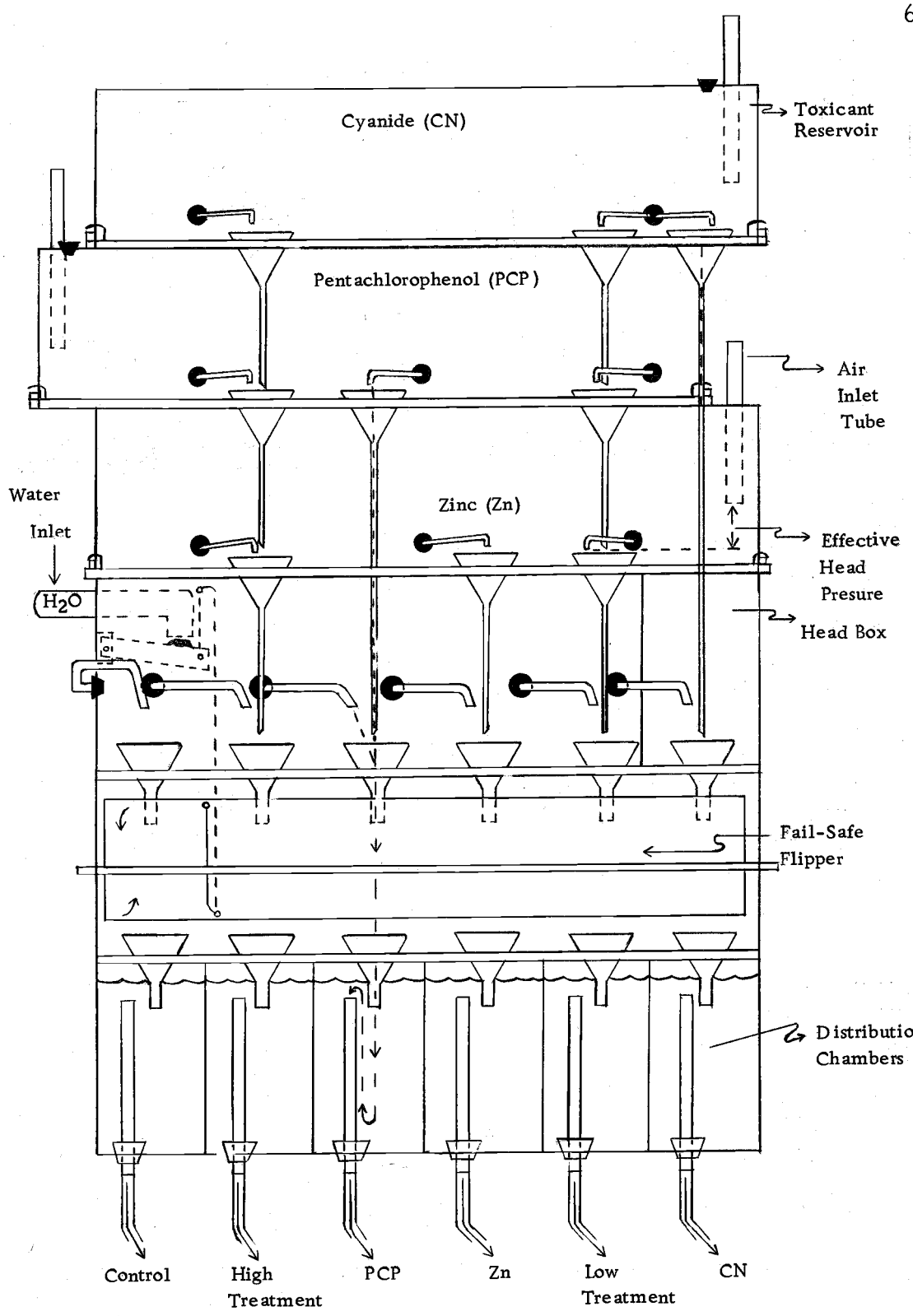

Figure 1. Diagram of toxicant reservoirs and dilution apparatus.

distance between the bottom of the air inlet and the toxicant outlet, was controlled by adjusting the level of the outflow tubes. The delivery system was limited to a minimum flow of 2-3 ml per minute because of its susceptibility to air-lock at very low flow rates. Flow rates were more stable during periods of constant temperature than during periods of temperature fluctuation because of pressure changes resulting from expansion and contraction of the plastic toxicant reservoirs.

The water diluter was constructed of plastic and consisted of two units; a head box (43.4 cm × 10.1 cm × 15.2 cm) and a mixing reservoir (53.3 cm × 10.1 cm × 15.2 cm) divided into six equal chambers (Figure 1). Water flow into the head box of the diluter was regulated by a float valve. The head box contained six outlets, one for each laboratory stream, and also one overflow tube. From these outlets water passed into individual funnels receiving the toxicants. These funnels carried the diluent water and toxicant directly to the respective mixing chambers. A flipper was installed immediately below these funnels to divert toxicant flows from the streams in case of failure of the water supply. From the mixing chambers the test solutions were conducted to each stream through black vinyl tubing 13 mm in diameter. The toxicants were discharged immediately downstream from the paddle wheels to minimize volatization of the chemicals, particularly cyanide. The toxicant delivery

system was in operation continuously during an experiment except for a period of 10-12 minutes every second day when the test solutions were renewed.

Toxicants

The 96 hour median tolerance limit (TL_m) values that were used to establish the test concentrations were based upon published results of acute toxicity bioassays with juvenile chinook salmon and other salmonids. The value for cyanide was selected from the data of Neil (1957), Herbert and Downing (1955), Herbert and Merkens (1952) and LeDuc (1966). The value for pentachlorophenol was taken from Chapman (1969) and Brockway (1963). The zinc value was selected from an analysis of the data of Sprague (1964), Sprague and Ramsay (1965), Goodman (1951), Lloyd (1960) and Anon. (1958). The respective TL_m values chosen for cyanide (CN), pentachlorophenol (PCP) and zinc (Zn) were 0.100, 0.200 and 1.500 milligrams per liter ($mg \cdot l^{-1}$) at 10° C.

During all experiments the toxicants were tested individually and in combination; the tests with mixtures being carried out at two levels of toxicity. During experiments I and II, cyanide, pentachlorophenol and zinc were introduced separately into three streams, each at a concentration equivalent to 0.1 toxic units (0.1 TL_m). Two other streams received mixtures of the three toxicants. In

one of these streams, all three toxicants were introduced each at a concentration equivalent to 0.1 of its TL_m value; the sum of the decimal fractions being equivalent to 0.3 toxic units, i.e., CN (0.1) + PCP (0.1) + Zn (0.1). This combination of toxicants will be referred to as "high treatment" in experiments I and II. In the other stream, the three toxicants were introduced each at a concentration equivalent to 0.033 of its TL_m value. When summed, the decimal fractions were proportional to 0.1 toxic units, i.e., CN (0.033) + PCP (0.033) + Zn (0.033) and thereby equivalent, in terms of fractions of a toxic unit, to the toxicity levels maintained in the streams receiving the individual toxicants. The combination of toxicants tested at this lower level will be referred to hereafter as "low treatment" in experiments I and II.

The toxicant concentrations were increased twofold during experiment III in order to produce more substantial changes in the stream communities than were observed during the previous experiments. Concentrations in streams receiving the individual toxicants were each equivalent to 0.2 toxic units. In the stream receiving "high treatment" the three toxicants were combined to give 0.6 toxic units, i.e., CN (0.2) + PCP (0.2) + Zn (0.2), whereas, in the "low treatment" each toxicant was introduced in concentrations equivalent to approximately 0.067 toxic units, i.e., CN (0.067) + PCP (0.067) + Zn (0.067), giving a combined value of 0.2 toxic units. The toxicity

of the high treatment mixtures established for experiment III was tested in a bioassay with very young chinook fry. The fish, which were kept at 5.0° C, did not survive longer than 72 hours at a concentration equivalent to 0.6 toxic units. Although the toxicity of this mixture was unaccountably high, the concentration was retained as a test treatment to evaluate the ability of the stream community to alter or modify the toxic substances and thereby ameliorate the effects of the toxicants on the salmon.

Toxicant Preparation, Dilution and Analyses

Well water, filtered through 116 μ nylon screen, was used in preparing all stock solutions. The well water was constant in quality throughout the year. The pH of zinc stock solutions was lowered by adding 10 ml of concentrated sulfuric acid to 40 liters of solution to prevent precipitation.

Cyanide, pentachlorophenol and zinc stock solutions were prepared with technical grade potassium cyanide (65.114 m.w.), sodium pentachlorophenate (289.344 m.w.) and hydrated zinc sulfate (287.560 m.w.), respectively. Individual stock solutions were freshly prepared every 40-48 hours in 60-liter, plastic buckets and immediately pumped to separate reservoirs with a small submersible pump.

Appropriate test concentrations were obtained by a 400:1 dilution

of the stock solutions. The individual and high treatment flow rates for all experiments were 5.0 ml per minute, with a corresponding diluent water flow of 1995 ml per minute. The toxicant flow rate for the low treatment was 2.0 ml per minute for each toxicant, with a water flow of 1998 ml per minute.

Analyses of water samples for zinc were performed by Mr. Herschel W. Pendell of the Agricultural Chemistry Department, Oregon State University, on an atomic absorption spectrophotometer. Cyanide concentrations were determined by Mr. Steven J. Broderius of the Department of Fisheries and Wildlife, Oregon State University. Pentachlorophenol was not chemically analyzed, but monitored only by flow rates.

Experimental Organisms and Procedures

Experimental Stream Procedures

Three experiments, each involving five treatment streams and one control, were conducted between February 21, 1971, and May 17, 1972. Algal and benthic invertebrate communities were allowed to develop in each stream from September, 1970, to February, 1971, before the introduction of salmon fry and the commencement of experiment I. Treatment conditions for each experiment, the duration of toxication and the respective periods of preliminary data

collection before toxication, may be seen in Table 1. Between experiments, treatments were re-randomized, and each stream flushed free of residual chemicals.

Juvenile Salmon

Juvenile spring chinook salmon were selected as the test fish because of their recreational and commercial value; their length of residence in fresh water systems and subsequent exposure to effluent discharges; and their sensitivity to pollutants. The fish used in the experiments were obtained from the Fish Commission of Oregon (South Santiam Hatchery) in January and November of 1971.

The initial stocking biomasses for each experiment may be seen in Table 2. All fish were individually branded at the beginning of each experiment, and again, when necessary, during the course of the experimental period, according to the method of Groves and Novotny (1965). The salmon were removed from each stream bi-weekly, blotted dry and individually weighed to the nearest milli-gram in a 100 ml cup. Weights of the fish were taken within 30 minutes after removal from the stream and therefore included any food which may have been present in the stomachs. If an individual escaped capture on any sampling day, but was captured on the next, production and growth rates for the previous sampling interval were adjusted accordingly. Production values for salmon in each stream

Table 1. Experimental randomization schedule and treatment concentrations.

	Experiment I (2/21-7/12/71) Treatment Exposure (4/19-7/12/71)		Experiment II (9/10-11/29/71) Treatment Exposure (10/4-11/29/71)		Experiment III (2/15-5/17/72) Treatment Exposure (3/14-5/17/72)	
Stream	Treatment	Toxic Units	Treatment	Toxic Units	Treatment	Toxic Units
1	C	--	CN	0.033	PCP	0.20
			PCP	0.033		
			Zn	0.033		
2	PCP	0.10	CN	0.10	Zn	0.20
			PCP	0.10		
			Zn	0.10		
3	CN	0.10	CN	0.10	C	--
	PCP	0.10				
	Zn	0.10				
4	CN	0.10	Zn	0.10	CN	0.067
					PCP	0.067
					Zn	0.067
5	CN	0.033	C	--	CN	0.20
	PCP	0.033			PCP	0.20
	Zn	0.033			Zn	0.20
6	Zn	0.10	PCP	0.10	CN	0.20

Where:

C - Control
CN - Cyanide
PCP - Pentachlorophenol
Zn - Zinc

And:

0.033 - one thirtieth of the 96 hour TL_m value
0.067 - one fifteenth of the 96 hour TL_m value
0.10 - one tenth of the 96 hour TL_m value
0.20 - two tenths of the 96 hour TL_m value

96 hour TL_m values at 10.0°C

Cyanide - 0.100 mg/l
Pentachlorophenol - 0.200 mg/l
Zinc - 1.500 mg/l

Table 2. Initial and terminal juvenile chinook salmon biomasses ($g \cdot m^{-2}$) for the various treatments in experiments I, II and III.

	Stream Number					
	1	2	3	4	5	6
Experiment I Treatment	C	PCP	High	CN	Low	Zn
Initial	2.53	2.53	2.53	2.53	2.52	2.53
Terminal	11.766	10.375	4.944	13.888	9.450	10.630
Experiment II Treatment	Low	High	CN	Zn	C	PCP
Initial	6.92	6.92	6.74	6.76	6.91	6.90
Terminal	3.96	4.16	4.75	4.49	4.14	3.89
Experiment III Treatment	PCP	Zn	C	Low	High	CN
Initial	2.60	2.60	2.59	2.60	2.60	2.60
Terminal	3.65	2.36	5.94	1.54	1.21	4.31

Where:

C - Control
CN - Cyanide
PCP - Pentachlorophenol
Zn - Zinc
Low - Low treatment (see text for explanation)
High - High treatment (see text for explanation)

were obtained by summing the individual values of weight change. The term production in this sense, is taken to mean the total quantity of tissue elaboration in a given period of time regardless of the fate of that tissue during that time (Warren, 1971). All values of biomass and production were expressed as grams per square meter of stream area ($g \cdot m^{-2}$) and milligrams per square meter of stream area per day ($mg \cdot m^{-2} \cdot day^{-1}$), respectively. Average relative growth rate was expressed in milligrams per gram per day ($mg \cdot g^{-1} \cdot day^{-1}$) and calculated according to:

$$\frac{\triangle W}{\overline{W} \cdot t}$$

Where:

$\triangle W$ = gain or loss in fish weight during a sampling interval in milligrams

$\overline{W}$ = average fish biomass during the sampling interval in grams

t = length of sampling interval in days

Individual dry weights were taken at the end of an experiment after drying each fish in an oven for five days at 70.0° C. Initial and terminal condition factors were calculated according to the following:

$$C.F. = \frac{\text{Weight (gm)} \times 100}{\text{Length}^3 \text{ (cm)}}$$

Where:

C. F. = the condition factor
Length = total length of fish

Invertebrate Fauna

Each laboratory stream was initially stocked with aquatic invertebrates (Ephemeroptera, Plecoptera, Trichoptera, Diptera and Coleoptera) from Oak Creek and a nearby spring-fed stream in September, 1970. Inoculations were continued monthly until February 21, 1971. No invertebrates were stocked during the experimental periods. Any recruitment to the stream communities during these periods, in the form of eggs and early larval instars, would have been through the water supply. Inoculations were again performed between experiments.

The benthic community was sampled prior to the introduction of the fish in any experimental period and monthly thereafter. Samples equivalent to an area of 324 cm^2 were taken from the riffles with a PVC pipe 12" long and 8" in diameter. The pipe was weighted with lead and its base was fitted with a sponge-rubber collar to insure a water-tight seal against the stream bottom. Rocks were removed from the sampler, placed in a bucket and the remaining slurry of organic material and aquatic invertebrates was siphoned from the pipe. After the rocks were scrubbed, the entire contents of the sample were strained through nylon screen (size 116 μ).

Drift samples were taken continuously for 168 hour periods at three-week intervals by passing the outflow water from each stream through a plankton net (size 116 μ). The organic material and insects from both the benthos and drift samples were preserved in 70% ethanol.

Aquatic invertebrates in the benthos and drift were picked with the aid of a binocular microscope, separated into 1 mm size intervals and identified according to the following references:

Ephemeroptera - Unpublished keys of Lemkuhl for the mayflies of Oak Creek

Plecoptera, Trichoptera, Diptera and Coleoptera - Pennak (1953) and Usinger (1963)

All insects were blotted dry and weighed to the nearest 0.01 milligrams. Those organisms greater than 15.0 mm in length were excluded in calculating total benthic and drift sample wet weights, since these organisms were considered too large to be utilized by the chinook fry.

Sunlight intensity was reduced approximately 80 percent during the summer by the suspension of Saran shading material five feet above each stream to prevent excessive algal growth. In the fall, allochthonous material, in the form of leaves, was added in an attempt to simulate natural conditions and to create an additional energy source for the streams.

Metabolic Rate Studies

The physiological effects of toxication on the metabolic activity of juvenile salmon were evaluated by measuring the rates of oxygen consumption of the fish in continuous-flow respirometers. Toxicant concentrations were identical to those used in experiment III. Oxygen values were recorded for a 40 hour period according to the schedule presented in Appendix VIII.

The experimental apparatus consisted of six Erlenmeyer flasks that were covered with aluminum foil in an effort to reduce the activity level of the fish. Each vessel was stoppered and contained two liters of water. An inlet tube 2 mm in diameter discharged the toxicant solutions near the bottom of the chamber. The effluent water flowed through a tube of similar size positioned near the water surface. Water flow rates were regulated by a screw clamp fastened to each outlet tube, and ranged from 50 to 100 ml per minute.

The juvenile salmon were starved for 24 hours before they were placed in the test vessels, and acclimated to test conditions for 44 hours before initial oxygen samples were taken. Each flask contained five fish, with total wet weights ranging from 6.749 to 6.793 g.

Oxygen consumption values (mg $O_2 \cdot kg^{-1}$(body weight)$\cdot$ $hour^{-1}$)

were calculated by the following formula:

$$\frac{(A)\,(B)\,(1000\ g)\,(60\ min)}{(C)\,(kg)\,(hour)}$$

Where:

A = oxygen inflow concentration ($mg \cdot l^{-1}$) minus oxygen outflow concentration ($mg \cdot l^{-1}$)

B = water flow rate through each test vessel ($l \cdot min^{-1}$)

C = total wet weight (g) of salmon tissue per vessel

RESULTS

The effects of the individual toxicants and combinations of toxicants on the model stream communities were evaluated on the basis of differences in the growth rates, biomasses and production rates of the salmon and in the quantities of invertebrate food organisms. Since there were seasonal changes and differences in the concentrations of toxicants (Table 1 and Appendix II), water temperatures (Figure 2) and water quality (Appendix I), during the course of the study, the results of each experiment will be presented separately.

Experiment I

Growth Rate, Biomass and Production of Juvenile Salmon

The relative growth rates of the salmon were not greatly different between streams during the pre-toxication period. The highest growth rates were reached during the first six weeks of the experiment, which suggests that the capacity of the streams to support salmon was limited even before toxicants were introduced (Appendix III). Two weeks after the toxicants were introduced, growth rates in all treatment streams had decreased to levels below those of controls. After four weeks, growth rates in the high

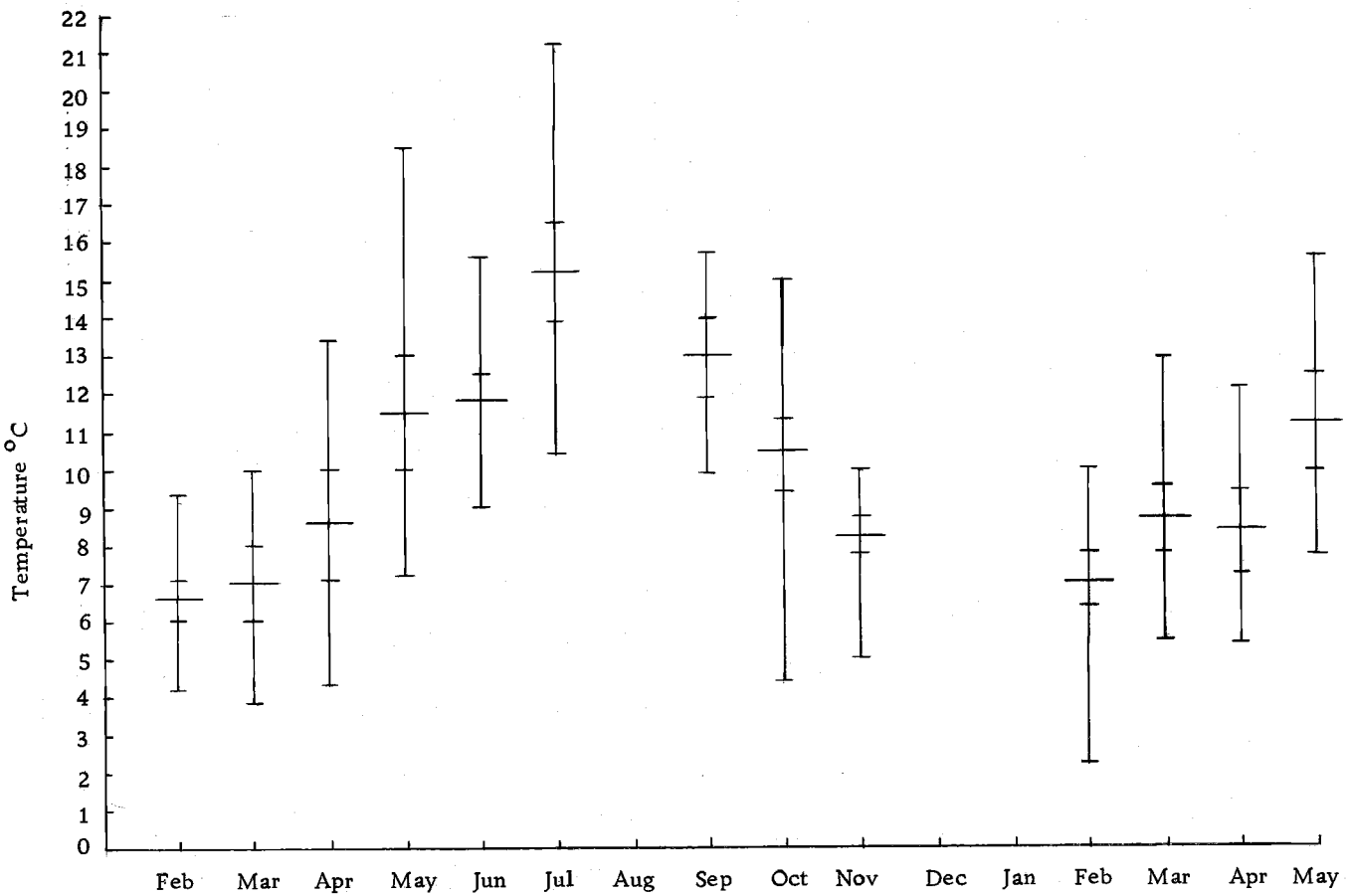

Figure 2. Monthly mean minimum and maximum temperatures, average temperatures and ranges of temperatures for the model streams from February 14, 1971, to May 17, 1972.

treatment had reached zero. The growth rates of salmon exposed to cyanide were greater than those of controls eight weeks after toxication, whereas those of fish in streams receiving pentachlorophenol, zinc and low treatment were reduced to levels intermediate between those of controls and the high treatment.

Curves of change of salmon biomass (Figure 3), plotted for each two-week interval of the experiment, also illustrate the effects of the different treatments. The biomass of salmon in the high treatment stream markedly declined during the last half of the experiment. This was attributable, in part, not only to negative values of growth rate but also to the death of three of the seven experimental fish. There were small changes of salmon biomass in the other streams, excepting the cyanide stream, during the treatment phase of the study. The small reductions that occurred were the result of the deaths of one fish each in the pentachlorophenol and low treatment streams. Only in the stream exposed to cyanide did salmon biomass continue to increase until the experiment was terminated. The biomasses of salmon that existed under the different experimental conditions at termination ranged from approximately 5 to 14 $g \cdot m^{-2}$ (Table 2).

Values of salmon production, like growth rate, increased in all streams during the first six weeks of the experiment and then declined markedly during the remainder of the study (Appendix III).

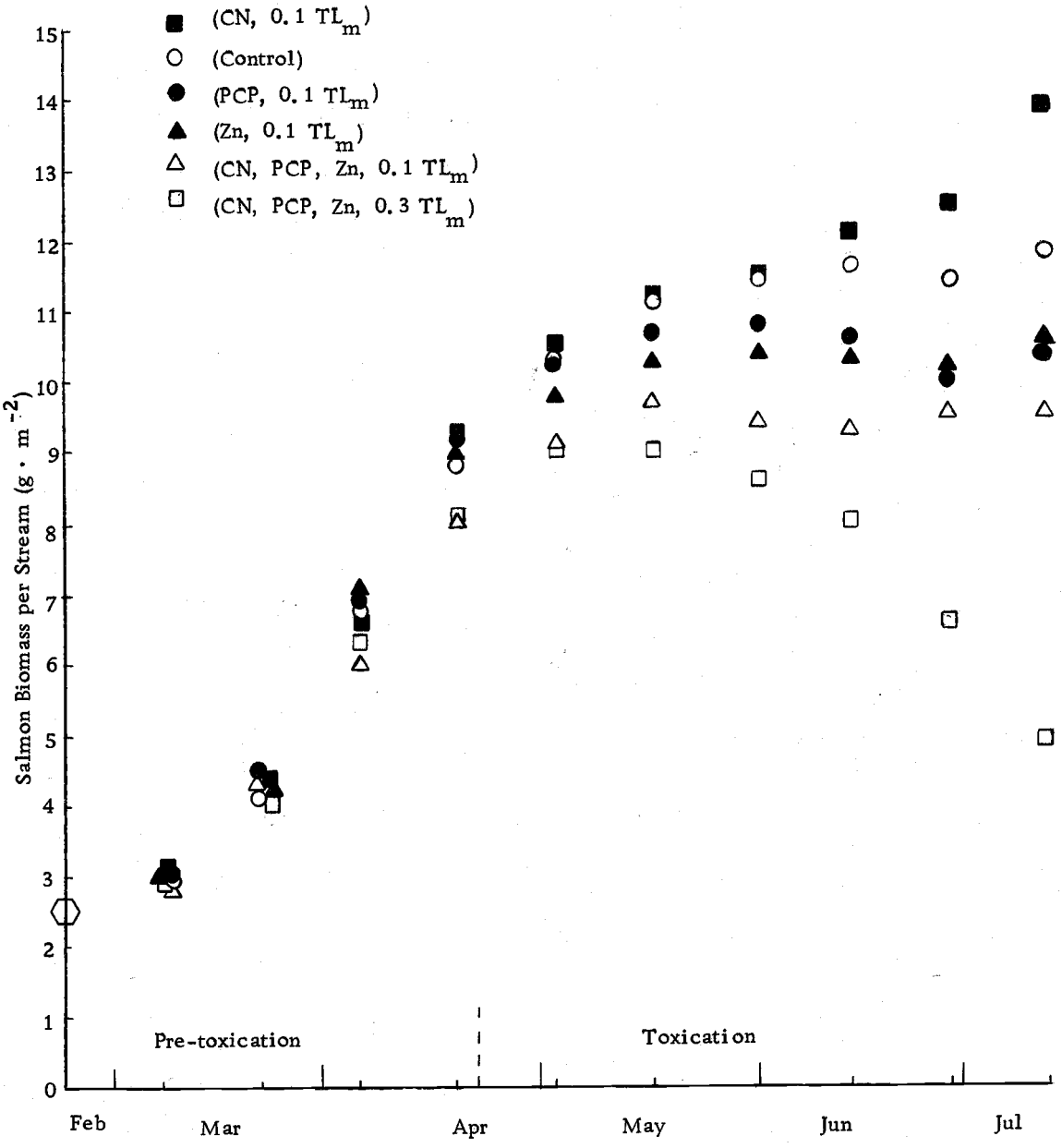

Figure 3. Biweekly changes of salmon biomass during the pre-toxication and toxication periods in experiment I.

For purposes of comparing the different treatments, production values for each two-week interval have been summed for both the pre-toxication and toxication periods (Table 3).

Relationships between salmon production and biomass (Figure 4) permit further comparison of the effects of the toxicants on salmon growth rates and indicate the additional effects of increases of salmon biomass on growth and, in turn, on the production values. Salmon production in each stream, excepting the stream receiving cyanide, declined to the zero level (zero growth rate) as salmon biomass increased during the course of the study. The decline of production with increases of salmon biomass was generally greater in treatment streams than in the control stream. A comparison of the salmon biomasses corresponding to values of zero production provides a measure of the maximum amount of salmon biomass that could be supported by the streams under the different treatment conditions.

Initial condition factors, on the average, were similar for all populations. Terminal condition factors were reduced 21-28 percent for all streams with the exception of cyanide, which was decreased 12 percent (Appendix IV). Percentages of dry weight determined for a preliminary sample of salmon indicated that fish in all treatments were approximately equal. There was, however, some variation with respect to terminal percentages of dry weights between test populations: high treatment resulted in a 20 percent

Table 3. Values of production ($mg \cdot m^{-2} \cdot day^{-1}$) during the pre-toxication and toxication periods for experiments I, II and III.

	Stream Number					
	1	2	3	4	5	6
Experiment I Treatment	C	PCP	High	CN	Low	Zn
Pre-toxication	447.0	474.0	396.0	490.0	394.0	461.0
Toxication	217.0	140.5	69.0	329.0	180.0	131.0
Experiment II Treatment	Low	High	CN	Zn	C	PCP
Pre-toxication	8.0	3.0	0.0	18.0	26.0	16.0
Toxication	-67.0	-77.0	-25.0	-80.0	-67.0	-117.0
Experiment III Treatment	PCP	Zn	C	Low	High	CN
Pre-toxication	124.0	143.0	118.0	92.0	153.0	103.0
Toxication	134.0	104.0	184.0	64.0	74.0	136.0

Where:

C - Control
CN - Cyanide
PCP - Pentachlorophenol
Zn - Zinc
Low - Low treatment (see text for explanation)
High - High treatment (see text for explanation)

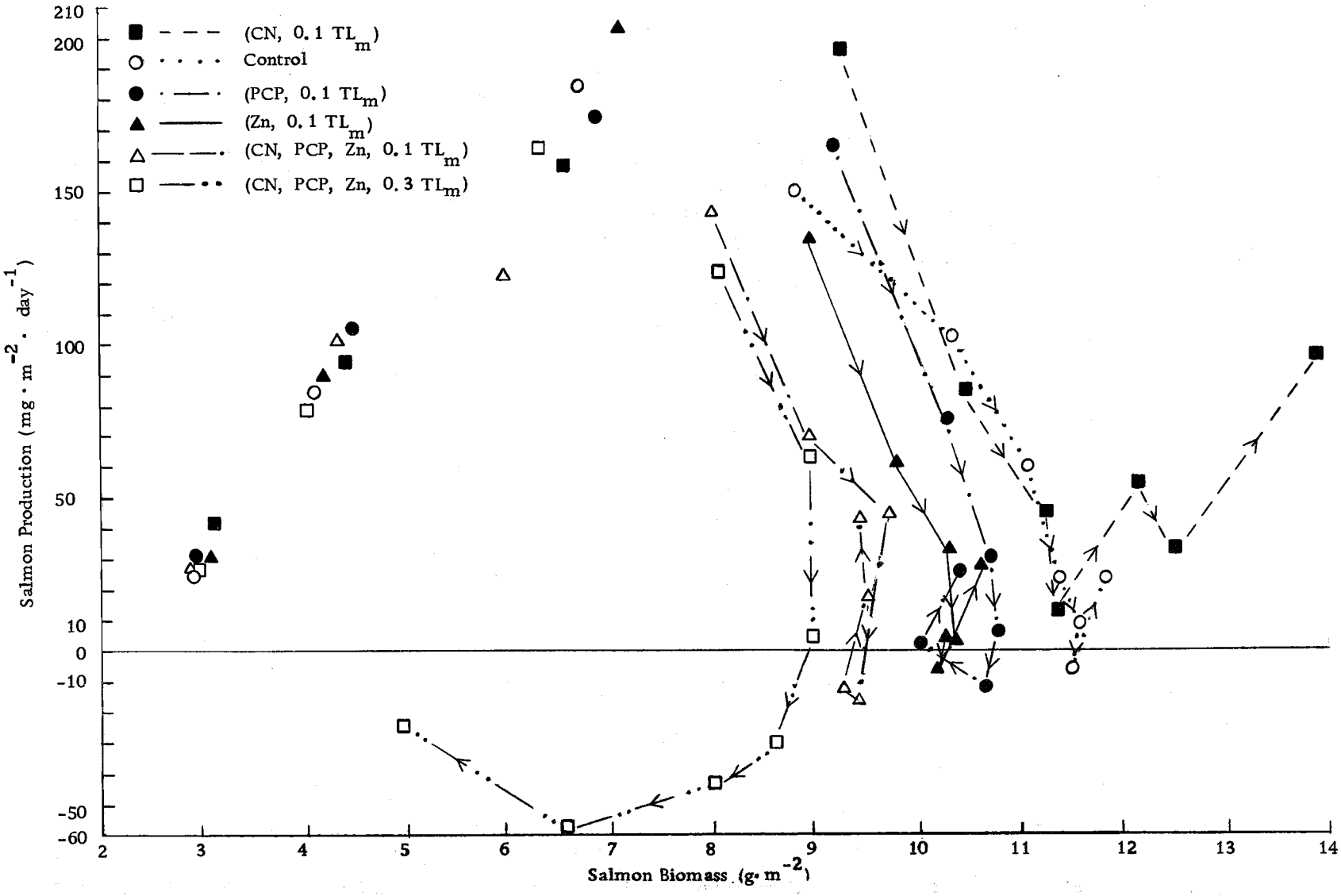

Figure 4. Relationship between salmon production and biomass during experiment I. Lines connect values for toxication period.

reduction between initial and terminal values; a slight reduction occurred in the control and zinc streams; pentachlorophenol and cyanide treatment led to a slight increase in terminal dry weight; and the low treatment remained virtually unchanged.

The Effects of Toxication on Benthic and Drifting Aquatic Invertebrates

Toxication appeared to have little effect on the densities of benthic invertebrates, except in the stream receiving zinc separately. Benthic densities in the streams scheduled to receive zinc, pentachlorophenol and cyanide were markedly reduced even before toxication (Appendix V). Benthic densities in the streams treated with pentachlorophenol and cyanide exhibited a marked recovery during the period of toxication, but benthic densities in the zinc system remained low throughout the treatment period.

Before toxicants were introduced into the model streams, the benthic organisms were represented mainly by the mayfly, Cinygmula, and several genera of the family, Chironomidae. The stream selected to receive high treatment was exceptional in that it contained only chironomids in significant numbers. After one month of toxication, the densities of these invertebrates in the streams exposed to high treatment, low treatment, cyanide and zinc, were substantially decreased. Cinygmula was the most abundant organism in the

pentachlorophenol and control streams, but its density was greatly reduced in the systems receiving zinc and cyanide, and it was entirely absent from the low and high treatment streams. After two months of toxication, Cinygmula was rare in the benthic samples of all treatments, having been replaced by the stonefly, Nemoura, in the control and pentachlorophenol streams. Chironomids were predominant in the other streams. Before the end of the experiment the mayfly, Baetis, had appeared in all systems except for the control and high treatment streams.

Excluding the streams receiving zinc, the toxicants did not appear to affect the densities of drifting aquatic invertebrates (Appendix VI). The average drift density in the stream receiving only zinc was less than one-third that of the control. Average densities in the streams receiving pentachlorophenol and the high treatment were reduced 20 percent, and drift density in the low treatment stream was reduced 50 percent from that of the control.

There was no well-defined relationship between values of drift and benthic density. This was probably related to differences in the life history stages of the benthic organisms. The emergence of insects may strongly influence drift density, but may not be reflected in measurable changes in their abundance in the benthos.

Interrelations between salmon growth rate, production and prey density were poorly defined, but generally good relationships

could be shown between values of salmon biomass and benthic density (Figure 5). These relationships, which are presented as phase diagrams, suggest that there were important differences between control and treatment streams. Benthic densities markedly declined with increases of salmon biomass in the treatment streams. The densities in streams receiving zinc (zinc, low treatment and high treatment) remained at generally low levels, whereas those in streams receiving cyanide and pentachlorophenol increased substantially during the latter half of the study. Levels of salmon biomass at the end of the experiment were lower in streams receiving zinc, especially in the high treatment, than in streams receiving pentachlorophenol or cyanide. The significance of these relationships in terms of predator-prey interactions will be considered further in the Discussion.

A comparison of values of drift density and salmon biomass between streams during the period of toxication suggests there were important differences in the response of the fish to this component of the food resource. Drift densities in streams exposed to cyanide, pentachlorophenol, zinc, low treatment and high treatment, respectively, were 108, 79, 31, 47 and 78 percent of the density in the control stream, whereas the respective percentages of control salmon biomass were 105, 93, 92, 83 and 69. It is notable that the low relative salmon biomass in the high treatment existed at

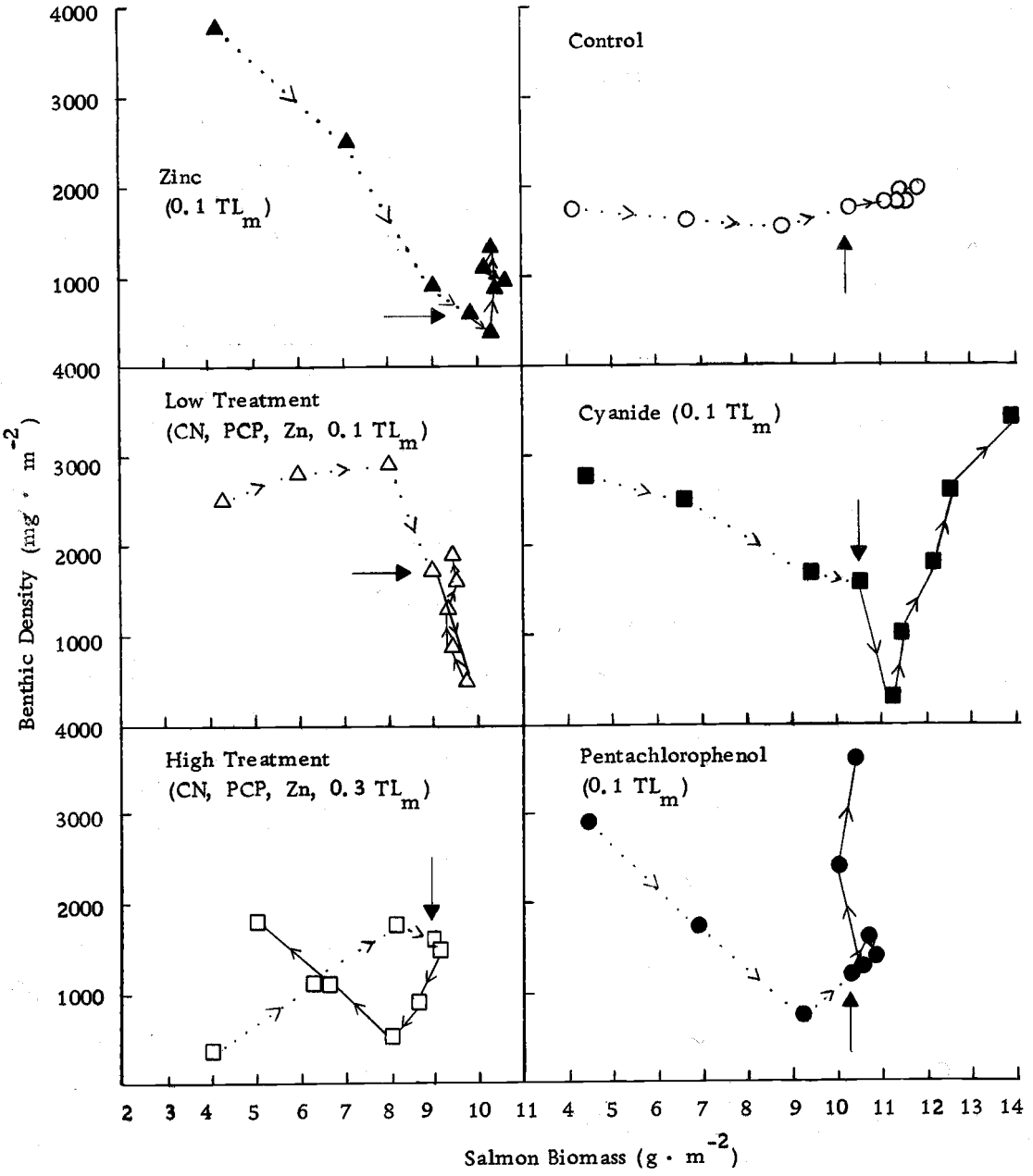

Figure 5. Phase diagrams relating benthic density and salmon biomass during experiment I. Solid lines connect values for toxication period. Arrows indicate time of toxicant introduction.

moderately high relative food densities, while a relatively high biomass of salmon was maintained by a relatively low mean drift density in the stream receiving zinc.

Experiment II

Growth Rate, Biomass and Production of Juvenile Salmon

Salmon growth rates followed similar patterns in all treatments, and were positive in only one two-week interval before toxication. During the treatment period, growth rates were negative for all streams. They were least negative in the cyanide stream, followed in decreasing rank by values for the control, high treatment, zinc, low treatment and pentachlorophenol streams (Appendix III).

Initial salmon biomasses were nearly equal in all streams (Table 2), but some differences in biomass had occurred by the time toxicants were introduced. The smallest and largest biomasses were recorded for streams scheduled to receive cyanide and pentachlorophenol, respectively. Salmon biomass decreased in each treatment throughout the period of toxication. At the end of the experimental period, values of salmon biomass were highest in the cyanide treatment followed in decreasing order by those in streams receiving zinc, high treatment, control, low treatment and pentachlorophenol (Table 2). Terminal biomasses were within 1.0 $g \cdot m^{-2}$ for all

treatments, suggesting that the effects of toxication were probably slight.

Production values reflected those of relative growth rate (Appendix III). Summation of production values for each two-week interval showed that production before toxication was highest in the control, followed in decreasing rank in those streams that were to receive zinc, pentachlorophenol, low treatment, high treatment and cyanide (Table 3). The greatest decreases in production during toxication occurred in the stream receiving pentachlorophenol. Reductions were intermediate for those streams exposed to zinc, high treatment, low treatment and control, and least for the cyanide treatment. There was no discernible relationship between salmon production and biomass, as salmon biomasses were not greatly different, and values of production were negative and similar.

Initial condition factors of the juvenile salmon ranged from 0.84 (pentachlorophenol) to 0.88 (control and low treatment). At termination, condition factors were lowest for the zinc treatment and highest for the control. Percentage changes between initial and terminal condition factors for all treatments were nearly equal (Appendix IV). Percentages of dry weight were also similar, excepting those of pentachlorophenol which were lower than the other treatment values (Appendix IV).

The Effects of Toxication on Benthic and Drifting Aquatic Invertebrates

Densities of benthic invertebrates in the control, low treatment and zinc streams exhibited considerable fluctuation during the treatment period. Densities in the stream exposed to cyanide markedly increased, whereas those in streams receiving pentachlorophenol and high treatment generally decreased. Changes in benthic densities in the various treatments could not be related to changes in the abundance of any particular kind of organism, as increases and decreases in any one species usually occurred concurrently in the control stream.

Drift densities, on the average, were lower for all streams before toxication than during the toxication period, except for the stream receiving zinc, which was lower during the period of toxication than before toxicants were introduced. Drift densities increased markedly in the pentachlorophenol and high treatment streams following the introduction of toxicants (Appendix VI). No relationship could be demonstrated between drift densities and benthic densities.

A relationship could be shown between benthic density and salmon biomass, when plotted in phase diagrams (Figure 6). As benthic densities decreased, salmon biomasses also decreased, except in the control and cyanide treatments, where salmon biomasses decreased with increases in benthic densities. Those

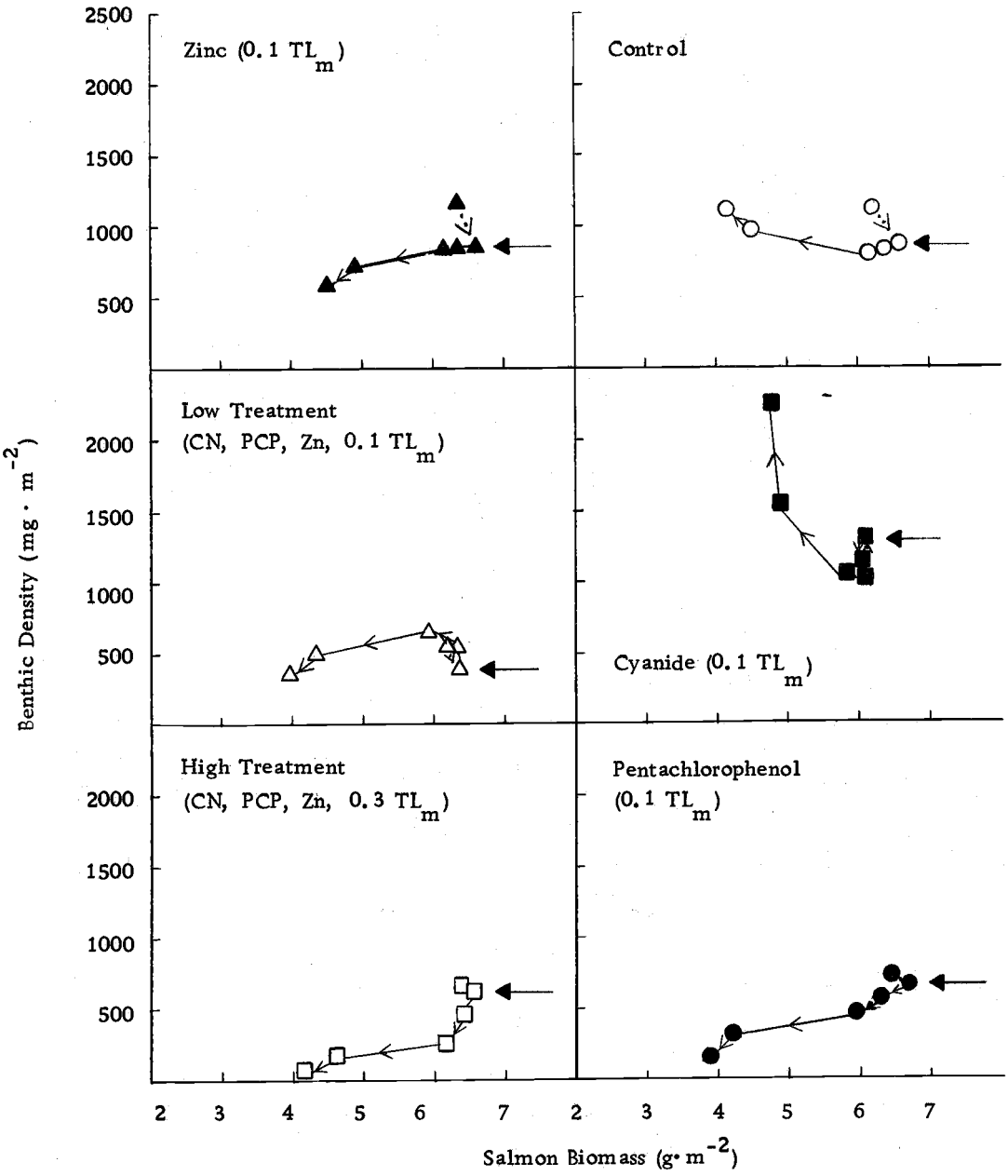

Figure 6. Phase diagrams relating benthic density and salmon biomass during experiment II. Solid lines connect values for toxication period. Arrows indicate time of toxicant introduction.

streams receiving zinc generally maintained lower levels of benthic invertebrate biomasses than did the other streams (Figure 6). The relationship shown for the control stream, and particularly the cyanide treatment, suggests that reductions in salmon biomass were ameliorated by increases in benthic densities. These relationships will be considered in the Discussion.

Experiment III

Growth Rate, Biomass and Production of Juvenile Salmon

Salmon growth rates increased in all streams during the pre-toxication period, but began to decline in the control stream and the streams receiving zinc and high treatment at about the time toxicants were introduced. Salmon in the other streams did not exhibit decreases in growth rate until two weeks after the toxicants were introduced (Appendix III). One month after toxication, salmon in the control stream had attained higher growth rates than those in the other treatment streams. These higher levels of growth rate were maintained in the control stream until the final week of the study. Growth in the low treatment was substantially reduced within the first month of toxication. Cyanide did not appear to enhance the growth of salmon as in the previous experiments. The general decline of growth rate of controls during the treatment period

suggests that the decreased growth rates of fish in the treatment streams could be attributed, in part, to costs associated with maintaining increased salmon biomasses.

Though salmon biomasses at the beginning of experiment III were similar for all treatments (Table 2), biomass in the control stream immediately before toxication was reduced by the deaths of two fish resulting from their capture in a drift net. Three deaths occurred in the zinc system and another in the low treatment four days after toxication. These fish were replaced on March 21, 1972, but within seven days those restocked in the zinc treatment had died and an additional fish, exhibiting abnormal behavior, was removed. The fish that had been stocked in the low treatment died within five days. In the high treatment, six salmon died between March 21 and March 28. One fish in the cyanide treatment also died during this period and was replaced. On March 28, additions of 6, 5, 1 and 1 fish were made in the high treatment, zinc, low treatment and cyanide streams, respectively. Continued mortality in the zinc and high treatments, suggested such events were not fortuitous, but probably attributable to zinc poisoning. Survivors in the zinc and high treatment apparently acclimated to the chemical stress, as most of those that survived the initial period of toxication, lived the duration of the experiment. During the period of toxication, salmon biomasses were markedly reduced in the zinc, high treatment

and low treatment (Figure 7). Percentage increases or decreases in salmon biomass from the control are presented in Appendix VII. The maximum percentage change in salmon biomass in all streams relative to the control was only 18 percent before toxication. This value was exceeded, however, after only two weeks of treatment, in the zinc and high treatments because of mortality. After one month, salmon biomass in the low treatment was reduced 25 percent from the control. The effects of the cyanide and pentachlorophenol treatments were not evident until after eight weeks of toxication, when values of salmon biomass were considerably lower than those of the control. The numbers of survivors at termination were 7, 6, 4, 3, 2 and 2 in the control, cyanide, pentachlorophenol, zinc, low treatment and high treatment, respectively.

Salmon production, like relative growth rate, increased in all streams during the pre-toxication period and continued to increase in the streams receiving cyanide, pentachlorophenol and low treatment, until two weeks after toxication (Appendix III). Except for the control and high treatment, production values generally declined within two weeks after toxication. Values for the control remained constant for four weeks after treatment, whereas the value for high treatment increased during one sampling interval two weeks after toxication. After four weeks of toxication, production decreased in all treatments until the final week of the study, when it increased

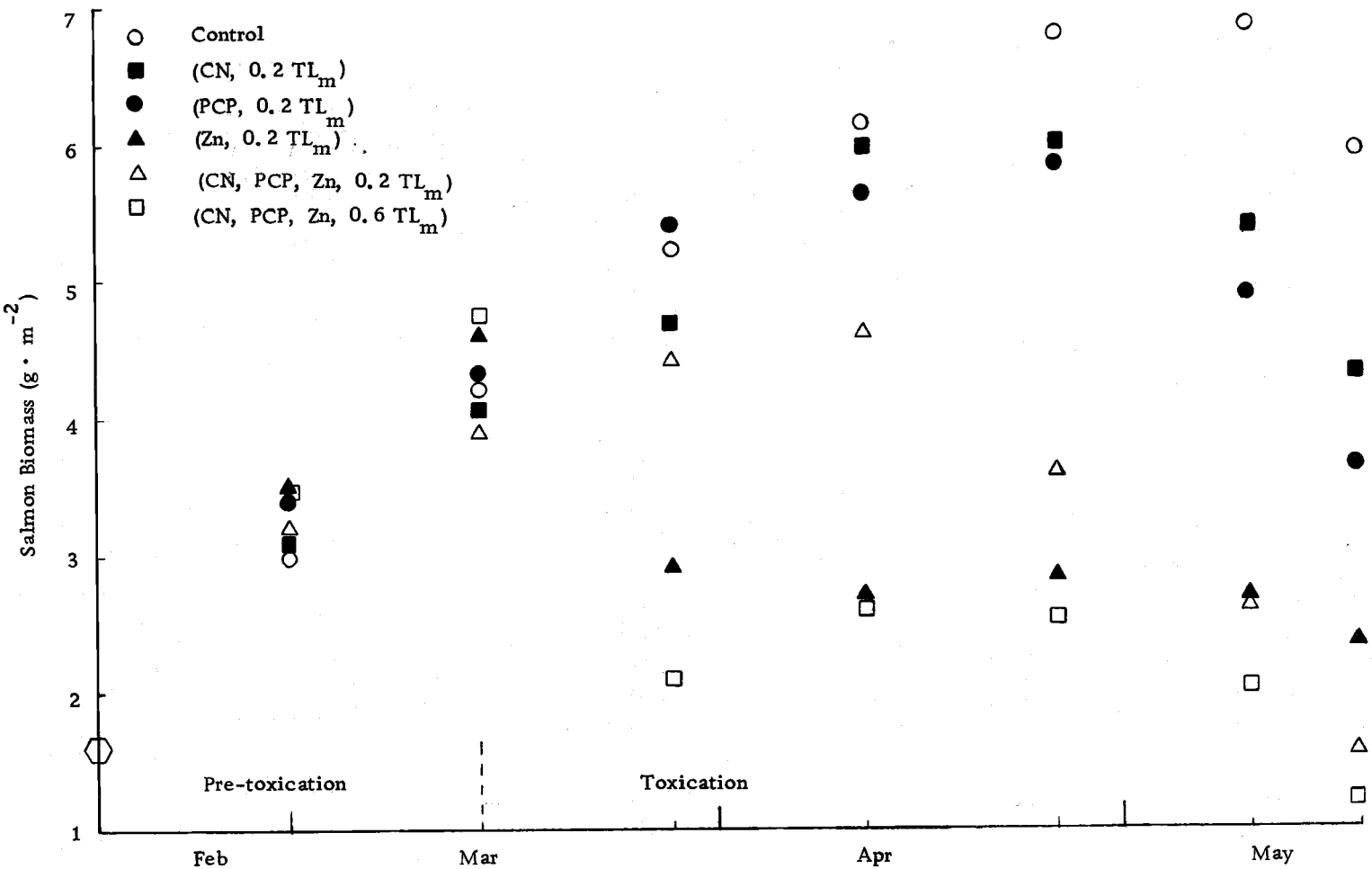

Figure 7. Biweekly changes of salmon biomass during the pre-toxication and toxication periods in experiment III.

slightly in the high treatment stream.

Streams scheduled for the different treatments had the following descending order of production values during the pre-toxication period: high treatment, zinc, pentachlorophenol, control, cyanide and low treatment. The rank order of production values during the toxication period was: control, cyanide, pentachlorophenol, zinc, high treatment and low treatment (Table 3).

The relationship between salmon production and salmon biomass further demonstrates the effects of toxicant stress (Figure 8). During the four weeks before the introduction of toxicants, salmon production and biomass increased in all streams. Two weeks after toxication, production and biomass had declined markedly in those streams receiving the high treatment and zinc, largely because of mortality. In the stream receiving low treatment, production and biomass continued to increase until two weeks after the introduction of toxicants, but then production declined sharply four weeks into the toxication period, while salmon biomass increased slightly. Small reductions in values of production continued in the low treatment stream, but salmon biomass decreased markedly until termination of the study. In the remaining streams, i.e., control, cyanide and pentachlorophenol, salmon production generally decreased and salmon biomass increased, until production became negative and both salmon biomass and production decreased. At those points corresponding to values

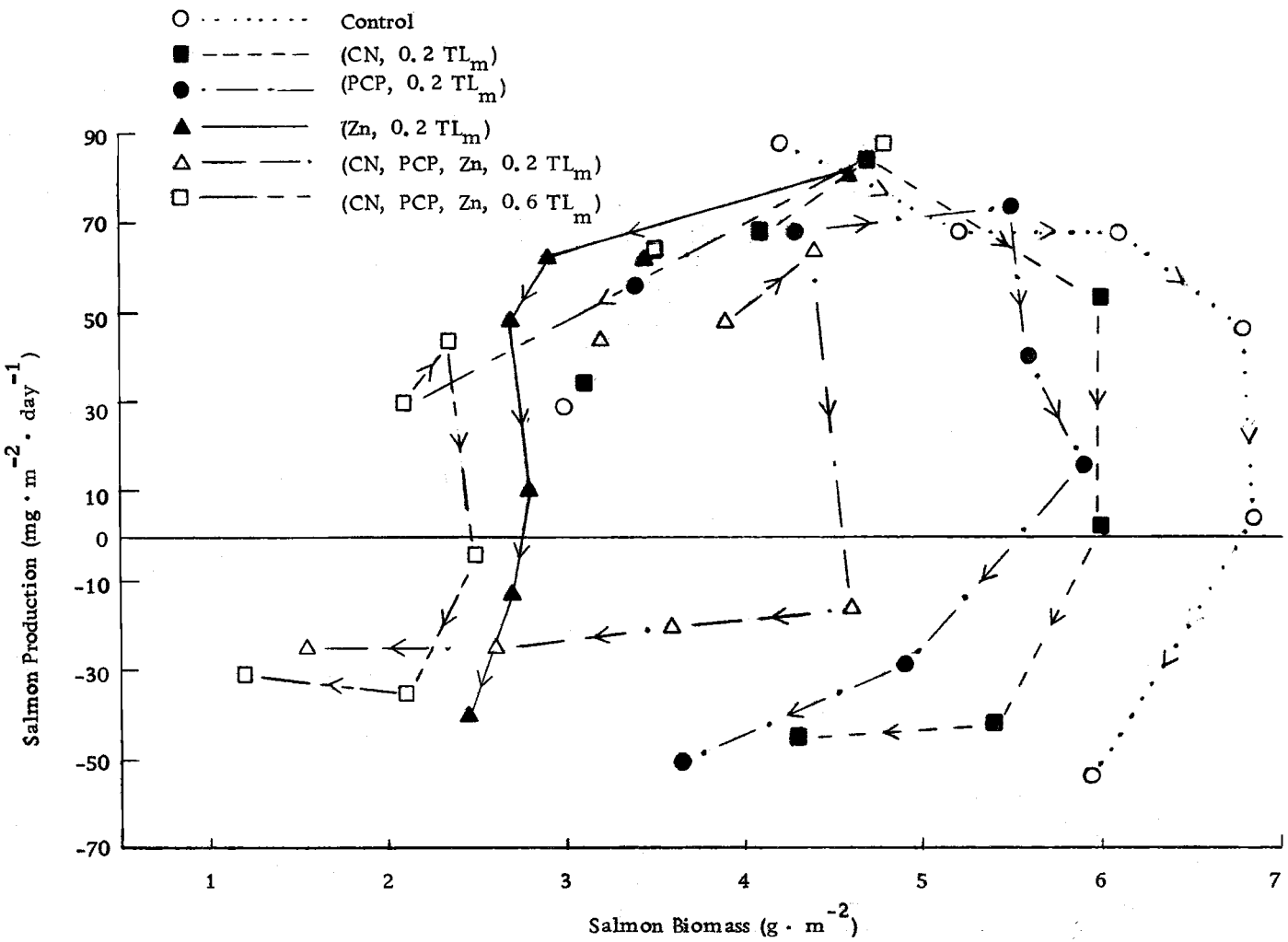

Figure 8. Relationship between salmon production and salmon biomass during experiment III. Lines connect values for toxication period.

of zero production, salmon biomasses ($g \cdot m^{-2}$) were 6.8, 5.96, 5.6, 4.6, 2.75 and 2.5 for the control, cyanide, pentachlorophenol, low treatment, zinc and high treatment, respectively. The biomasses of salmon in the cyanide and pentachlorophenol treatments were reduced to 87.6 and 82.4 percent of the control, while the low treatment, zinc and high treatment biomasses were reduced to 67.6, 40.4 and 37.6 percent, respectively, of the control value.

At termination, salmon biomass was substantially reduced in those streams receiving zinc, i.e., high treatment, low treatment and zinc. Salmon biomass was also reduced from the control in the pentachlorophenol and cyanide streams. Terminal values of salmon biomass were highest in the control, followed in order by those of cyanide, pentachlorophenol, zinc, low treatment and high treatment (Table 2).

The average condition factor at termination for salmon exposed to high treatment was 93 percent lower than that of the control, while the value for salmon exposed to pentachlorophenol was 33 percent greater than the control value. The condition factors of salmon in streams receiving zinc, cyanide and the low treatment were reduced 25, 27 and 7 percent, respectively, from those of the controls. Percentage reductions in dry weights, relative to the control were 250, 225, 50 and 38 percent for the low treatment, high treatment, cyanide and zinc, respectively. Dry weight changes of salmon

exposed to pentachlorophenol were 25 percent greater than those of salmon in the control stream (Appendix IV).

The Effects of Toxication on Benthic and Drifting Aquatic Invertebrates

During the pre-toxication period the densities of benthic invertebrates increased in the control stream and those streams that were to receive zinc, low treatment and high treatment, and decreased in those that were to receive cyanide and pentachlorophenol. After the introduction of toxicants, benthic biomasses declined markedly in the zinc, high treatment and low treatment streams, while increases in density occurred in the pentachlorophenol and cyanide systems. Benthic density in the control stream decreased slightly during the toxication period, while the low and high treatments experienced a steady decline in benthic density throughout the treatment period. Benthic biomass decreased considerably in the zinc stream but increased at termination. Values of benthic density in the pentachlorophenol and zinc systems fluctuated extensively.

Before toxicants were introduced, the most abundant aquatic insects present in all streams were chironomid larvae, followed in decreasing order of abundance by Baetis, Isoperla and Nemoura. The numbers and biomasses of chironomid larvae were significantly reduced in the zinc and low treatment streams during toxication.

The remaining systems were also apparently affected by toxication, as the numbers and kinds of organisms were substantially lower than those in the control stream.

Before toxication, drift densities in the treatment streams ranged from 167 to 234 percent higher than the control stream. Drift densities during the period of treatment were 103 to 240 percent greater than the control value for those streams other than zinc and low treatment, which maintained average densities lower than the controls. One week after the introduction of toxicants, drift densities declined in all streams, excepting those in the control and in the high treatment, which increased considerably (Appendix VI). Biomass values of drifting invertebrates at termination were lowest for the pentachlorophenol stream and highest in the high treatment and cyanide streams.

The effects of toxication may be further evaluated by examining the interrelationships between salmon and invertebrate biomasses. Plots of values of salmon biomass and benthic density (Figure 9) in phase diagrams, indicate there were important differences in these relationships between the control and treatment streams.

Decreases in salmon biomass after toxication began could generally be associated with reductions in benthic density (Figure 9). Reductions in values of salmon biomass, but not necessarily those of benthic density, were greatest in the streams receiving zinc.

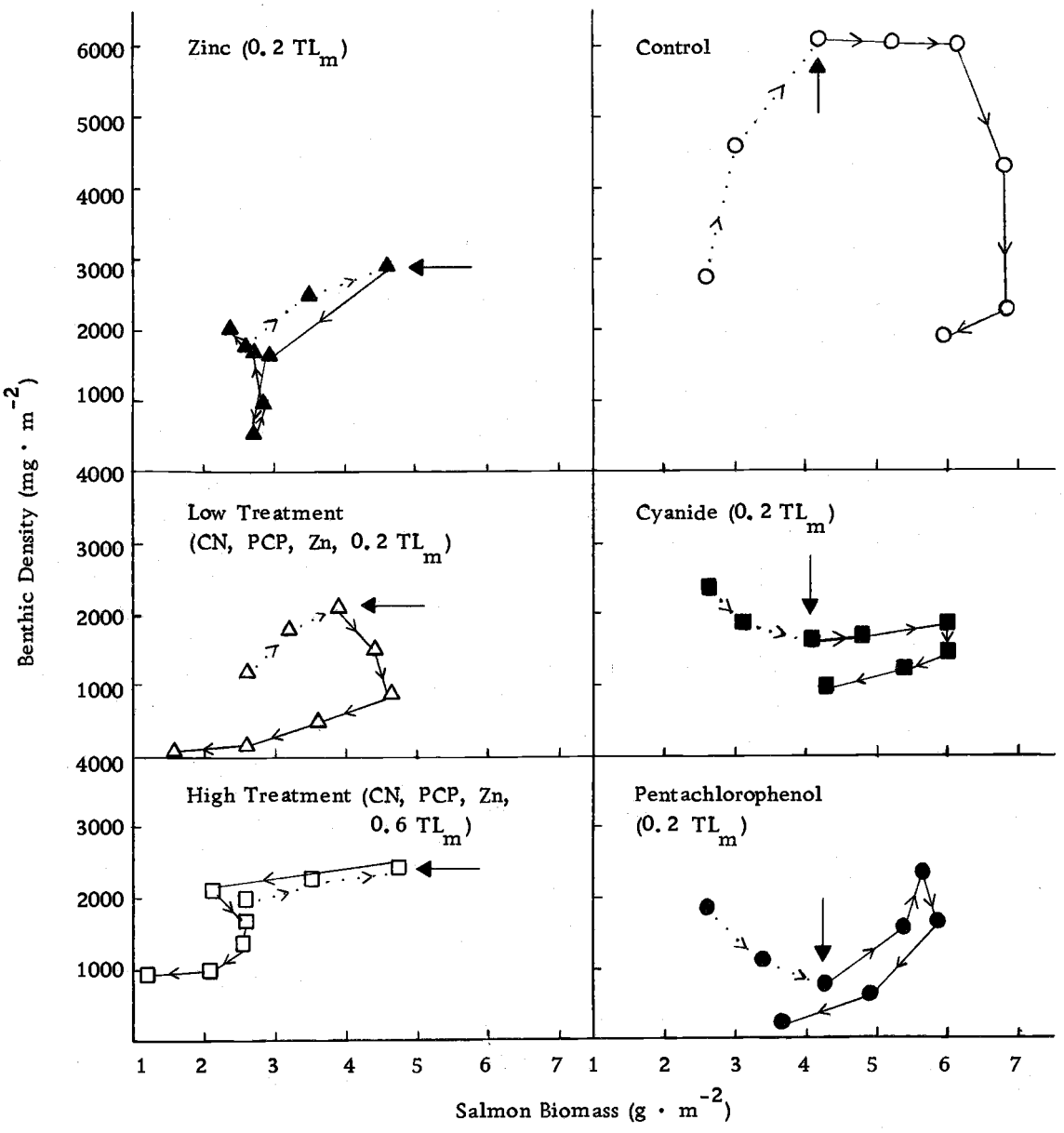

Figure 9. Phase diagrams relating benthic density and salmon biomass during experiment III. Solid lines connect values during toxication. Arrows indicate time of toxicant introduction.

Values of benthic biomass were much greater in the control than in treatment streams, however, increases in salmon biomass were not as large as might have been expected. This suggests that salmon in the control stream were either unable to influence benthic densities at levels greater than 3500-4000 mg · m^{-2}, or were limited by some other factor, e.g., space or territory. The significance of these relationships in terms of predator-prey interactions will be considered in the Discussion.

The Effects of Toxication on Metabolic Rate

The effects of toxication on the metabolic rate of juvenile salmon are indicated by the results of experiments on oxygen consumption. Mean rates of oxygen consumption were 45 percent higher and 55 percent lower in fish exposed to pentachlorophenol and high treatment, respectively, relative to the value for controls. In low treatment, mean values of oxygen consumption were only 0.7 percent below those of the control, whereas the individual treatments of zinc and cyanide resulted in percentage reductions of 14 and 31 percent, respectively, from the mean control value (Appendix VIII).

Mean values based upon five to seven observations of oxygen consumption by juvenile salmon in the treatment streams were not different from that of the control (student's t-test). Mean values of oxygen consumption for fish in high treatment, cyanide, and

pentachlorophenol were significant at probability levels of 0.92, 0.83 and 0.78, respectively, while those of fish exposed to low treatment and zinc could be attributed to random variation.

The high treatment caused extreme stress to the juvenile salmon, as 100 percent mortality occurred within 44 hours of exposure during acclimation of one test group. Acclimation of a second group of fish to high treatment for only 24 hours caused no mortality, but these fish died within 24 hours of further toxicant exposure.

The initial values of salmon biomass in each treatment were similar. Terminal dry weights expressed as percentages of wet weight were also comparable, excepting those of fish exposed to zinc, which were slightly lower than the others (Appendix IX).

DISCUSSION

Since growth is essentially an integrative function governed by a number of biochemical and physiological processes, it might be expected that the combination treatments of this study would be more deleterious to salmon growth and production than the individual treatments, because of the presence of higher levels of toxic units and the diversity of physiological action of each toxicant in these mixtures. An organism may be capable of continued growth and survival through internal compensatory mechanisms when exposed to only one chemical. However, when confronted with a number of toxic compounds, e.g., cyanide, pentachlorophenol and zinc, an organism may be unable to successfully shift to alternative physiological processes for energy to sustain life processes, because of the cumulative effects of the individual chemical stresses.

Histological effects observed by Crandall and Goodnight (1963) in guppies (Poecilia reticulata) exposed to sublethal levels of sodium pentachlorophenate for 180 days, showed striking kidney degeneration. Fish that were exposed to sodium pentachlorphenate for only 30 days exhibited a high degree of vascularization of the internal organs and heart. Brockway (1963) noted that cichlids (Cichlasoma bimaculatum) acclimated to pentachlorophenol were less tolerant than controls to a high lethal level of cyanide, but more tolerant than those previously

exposed to cyanide. Fish acclimated to cyanide were less resistant than control fish or fish acclimated to pentachlorophenol when exposed to a high lethal level of pentachlorophenol. He suggested there was probably an additive effect due to the uncoupling of oxidative phosphorylation and inhibition of cytochrome oxidase which decreased survival time of fish exposed to both cyanide and pentachlorophenol. Brockway also found that levels of pentachlorophenol and cyanide in which cichlids could survive indefinitely were only about 25 percent lower than the 48 hour TL_m values. In general, cichlids exposed to pentachlorophenol concentrations of 75 ppb and 150 ppb had higher rates of energy utilization at lower efficiencies than control fish. Jones (1947) and Skidmore (1964) noted a depression of oxygen consumption and decreased opercular rates in sticklebacks exposed to cyanide. They attributed these decreases to respiratory inhibition at the tissue level. LeDuc (1966) reported adverse effects on growth, food consumption, food conversion efficiency, the utilization of energy reserves during starvation and the swimming ability of cichlids and coho salmon exposed to chronic cyanide poisoning at concentrations ranging from 0.01 to 0.10 mg $\cdot$ l^{-1} HCN. Chronically toxic concentrations of zinc also subject fish to stress, inducing in essential organs, e.g., the gut and liver, adverse changes causing general enfeeblement and retardation of growth and maturation (Skidmore, 1964). Thus, chronic exposure to sublethal concentrations

of pentachlorophenol, cyanide and zinc in combination would appear to be potentially more harmful to the growth and production of juvenile salmon than the toxicants individually. The effects of such long-term exposure to sublethal concentrations were particularly evident in experiment III at the low treatment level, where production was greatly reduced through mortality and decreased growth of the survivors.

With respect to salmon biomasses, the low treatment of experiment I resulted in a 20 percent reduction in salmon biomass at termination relative to the control, while high treatment reduced biomass values nearly 60 percent from those of the control. Terminal values of salmon biomass in experiment III, in low treatment and high treatment, respectively, were 74 and 80 percent lower than values of biomass in the control stream. In experiment II, reductions of terminal values of salmon biomass from those of the control in all of the various treatments were too slight to be considered a significant result of the effects of toxication.

The individual treatments of pentachlorophenol and zinc at 0.1 toxic units did not cause any important changes in the biomasses of the salmon or benthic invertebrates in experiments I and II. Cyanide on the other hand, at 0.1 toxic units appeared to enhance the growth and production of salmon, as values of salmon biomass were higher than those of the control in both experiments. LeDuc

(1966) observed increased growth and food consumption by coho salmon at concentrations of 0.01 to 0.08 mg·l^{-1} HCN in aquarium studies and suggested three possible adaptive mechanisms to cyanide poisoning: (1) a behavioral alteration, i.e., reduction of activity in fish exposed to 0.08 mg·l^{-1} HCN; (2) a compensatory increase in activity of some enzymatic pathways which counteracts the deleterious action of cyanide; and (3) an increase in the rate of enzymatic detoxification of cyanide to non-toxic thiocyanate. I did not observe any particular behavioral changes in those salmon exposed to cyanide in the model streams. However, salmon in the stream receiving cyanide could have consumed more food than salmon in the control, since average benthic densities were as great if not greater than the average benthic densities in the control stream.

A twofold increase in toxicant concentrations in experiment III, resulted in reductions of salmon biomass of 27, 38 and 60 percent from control values in the individual treatments of cyanide, pentachlorophenol and zinc, respectively, suggesting that zinc was the most deleterious to salmon production of any of the individual toxicants. In both experiments I and III, reductions in salmon biomass were always greater in streams receiving the mixtures of toxicants and reductions were always greater in the high treatment than in the low treatment streams.

Terminal values of salmon biomass in each of the treatment

streams in experiments I and III were normalized to the control biomass values. Figure 10 provides a comparison between toxicant concentrations in toxic units and percentages of change in terminal salmon biomass. The relationship was not well defined. In part, this may have resulted from seasonal differences or differences in the periods of exposure to toxicants between the two experiments. The values plotted in Figure 10 do indicate that salmon biomass generally decreased with increases of toxicity. The combination treatments in each experiment resulted in greater reductions of biomass than did the individual treatments.

Changes in the densities of benthic and drifting aquatic invertebrates that were attributable to toxication were evident only in experiment III and were less pronounced than changes in salmon biomass. Thus, the effects of toxication on salmon, particularly in experiment I, were probably more direct and not necessarily mediated through the food chain, while the more marked reductions in salmon biomass in experiment III were probably due not only to the direct effects of toxication, but also to a decreased food supply.

Phase diagrams relating salmon biomass and insect biomass for the various treatments in experiments I, II and III, illustrate density-dependent relationships between the predator (salmon) and its food resource and important differences in these relationships between treatments. The range over which salmon and prey

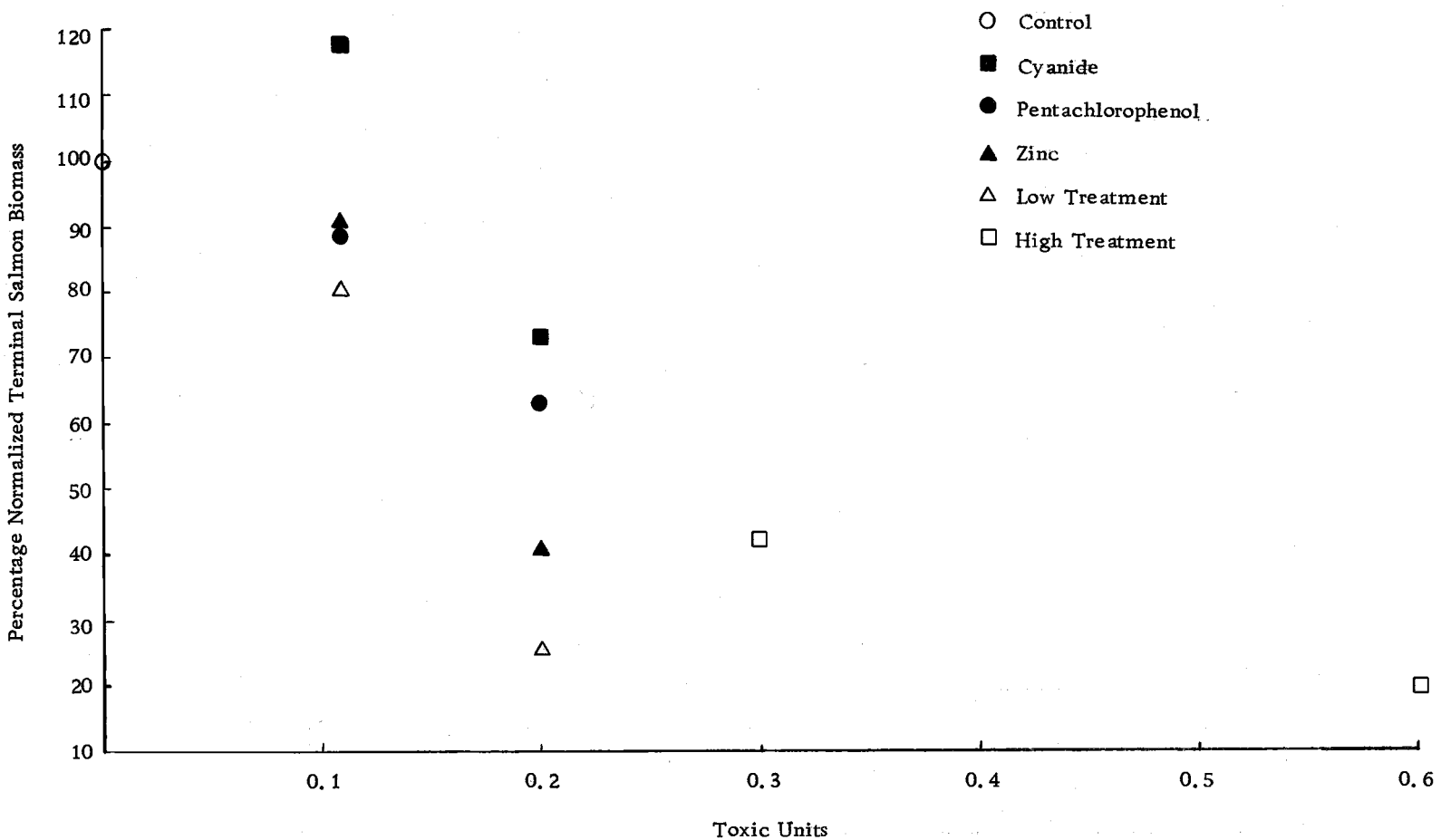

Figure 10. Relationship between percentage changes in normalized values of terminal salmon biomass and toxic units during experiments I, II and III.

biomasses increased and decreased was markedly greater in the control stream and the stream receiving cyanide, where the effects of toxication were either slight or non-existent, than in the other treatment streams. However, where the effects of toxication were evident, as indicated by considerable reductions of salmon biomass, the changes of predator and prey biomasses were confined to only narrow ranges. Where the effects of chemical stress were severe as in the high and low treatment of experiment III, the prey appeared unable to recover from initial reductions in biomass, which in turn strongly limited further increases of salmon biomass. Changes of predator biomass with changes of prey biomass in the control streams during experiments I and III were much less pronounced than were similar changes in treatment streams. In experiment II where the effects of toxication did not produce changes in salmon biomass greatly different from those of the control stream, the relationships between predator (salmon biomass) and prey (insect density) were similar in all streams including the control.

In view of the results of experiments I, II and III, this study indicated that toxicants when tested in combination were more harmful to salmon growth and production than when tested individually at equivalent levels of toxicity. The decreases in salmon production in the high and low treatments were not necessarily additive based on the responses of salmon exposed to the individual toxicants, but

were greater than the effects observed in any one individual treatment.

Considering the large reduction in salmon biomass in the zinc treatment during experiment III, the decreases in salmon biomass that occurred in the low and high treatments appeared to be largely the result of the effects of zinc. If zinc is indicative of the effects of toxic heavy metals, in general, they must be considered the most harmful to juvenile salmon of the three classes of compounds tested in the three experiments. The toxicity of zinc can be related to its chemical stability and resistance to degradation. Pentachlorophenol, on the other hand, rapidly photodecomposes in the presence of ultraviolet light, and the toxic form of cyanide, hydrogen cyanide, is very volatile.

Though attempts have been made to establish levels of toxic units which will permit the survival of aquatic life, safe fractions of toxic units have yet to be determined for mixtures of pollutants in nature. According to Herbert et al. (1965), if the concentrations of poisons in a river never exceeded 0.7 of the predicted 48 hour LC_{50}, then no more than an insignificant percentage (5 percent) of fish as sensitive as rainbow trout would be killed within a few days. Studies by Brown et al. (1970), which are in direct conflict with those of Herbert et al., indicate that half of a trout population was killed within 48 hours at 0.6 to 0.7 toxic units. Additional data,

collected in the field by Herbert et al., however indicated that a reasonable fishery could not be naturally maintained unless the toxicity was less than 0.2 to 0.4 toxic units based upon the 48 hour LC_{50}. Brown et al (1970) also challenged the field data adduced by Herbert et al., because it was not determined that the population was continuously and unavoidably exposed to 0.2 to 0.4 toxic units, nor was it determined whether a stable population was being maintained by emigration or by recruitment. Edwards and Brown (1966), according to Sprague (1970), concluded that fish populations could generally exist where soluble poisons did not exceed 0.3 to 0.4 toxic units and where concentrations of dissolved oxygen and suspended solids were satisfactory.

The results of the three experiments performed in this study suggest that individual toxicants, particularly the heavy metal zinc, at levels of 0.2 toxic units, would be harmful to the growth and production of juvenile chinook salmon as well as benthic invertebrates. However, an apparent "no effect" level of 0.1 toxic units of the individual toxicants in experiment I did in fact produce a deleterious effect when all three chemicals were combined, each at a concentration of 0.1 toxic units. Furthermore, the results of the low treatments at 0.1 and 0.2 toxic units in experiments I and III indicated an "effect level" of the individual toxicants at concentrations as low as 0.033 and 0.067 toxic units when in combination.

Consequently, when cyanide, pentachlorophenol and zinc are in combination, apparently safe levels would be lower than one third of the individual "no effect" levels of 0.1 toxic units for each toxicant. Thus, when evaluating the toxicity of a mixture of pollutants, we must not only consider the summed value of toxic units of the constituents in the mixture, but also the levels of toxic units of the individual compounds within the mixture. Furthermore, the summation of toxic units may not be valid for mixtures containing large numbers of toxicants, the individual concentrations of which might not prove harmful to aquatic life. The addition of small units of toxicity for large numbers of toxicants could result in a value that may be a gross overestimate of the toxicity of the mixture.

We must also be aware that chemical analysis of toxicant concentrations in the aquatic environment may not always be a valid measure of a toxicant's effective concentration or its availability to the organism. Levels of exposure of fish and other organisms to toxicants may be significantly reduced by chemical complexation, adsorption and/or biological degradation.

The study of multi-toxicity and its application to the formulation of water quality standards is relatively new and untested. Prediction of the effects of mixtures of toxicants has been shown to be valid only for as few as three to five toxicants and at only acutely lethal concentrations in the laboratory. In order to predict the

effects of multi-toxicity on the growth, production, reproduction and behavior of fishes and other aquatic organisms, we must understand the effects of complex mixtures of toxicants at sublethal concentrations on complex biological systems.

Model streams are intermediate in their biological complexity between laboratory aquaria and natural lotic systems. Laboratory aquaria studies are most often autecological in nature and permit us to examine physiological and bioenergetic phenomena in the absence of the influence of many abiotic and biotic variables. Model stream communities, on the other hand, are composed of interacting populations of organisms that are influenced by their physical and chemical environments. In these systems the juvenile chinook salmon were subject to many of the abiotic and biotic conditions of natural streams, and were forced to compete for space (territory) and food that was produced naturally within each stream. Despite the fact that natural systems are even more biologically diverse and larger than model streams, the results and relationships obtained for populations in model stream communities are more indicative of and realistically applicable to nature than are results based upon physiological studies in the laboratory at the individual level of organization.

BIBLIOGRAPHY

American Public Health Association, American Water Works Association and Water Pollution Control Federation. 1971. Standard methods for the examination of waste and wastewater. 13th ed. New York, N. Y. 874 p.

Anon. 1958. Water pollution research for 1957. Water Pollution Research Board, Dept. of Scientific and Ind. Res., H. M. Stationary Office, London.

Bandt, H. G. 1946. Ueber verstärkte Schadwirkungen auf Fische, insbesendere über erhöte Giftwirkung durch Kombination von Abwassergiften. Briträge Wasser, Abwasser-u. Fischereichemie. No. 1:15-23.

Bergström, H. and S. Vallin. 1937. Vattenförorening genom auloppsvattnet fran sulfatcellulosafabriker. (Water pollution due to waste from sulphate pulp factories.) Sweden, Kungl. Lantbruksstyrelsen, Meddelanded fran statens undersöknings-och försöksanstalt för sötvattensfisket. No. 13, 19 pp. (Fish. Res. Bd. Can., Trans. Series No. 582:10 p.)

Brockway, D. L. 1963. Some effects of sublethal levels of pentachlorophenol and cyanide on the physiology and behavior of a cichlid fish, Cichlasoma bimaculatum (Linnaeus). M. S. thesis. Corvallis, Oregon State University. 56 numb. leaves.

Brown, V. M. 1968. The calculation of the acute toxicity of mixtures of poisons to rainbow trout. Water Res. 2:723-733.

Brown, V. M. and R. A. Dalton. 1970. The acute lethal toxicity to rainbow trout of mixtures of copper, phenol, zinc and nickel. J. Fish. Biol. 2:211-217.

Brown, V. M., D. H. M. Jordan and B. A. Tiller. 1969. The acute toxicity to rainbow trout of fluctuating concentrations and mixtures of ammonia, phenol and zinc. J. Fish. Biol. 1:1-9.

Brown, V. M., D. G. Shurben and D. Shaw. 1970. Studies on water quality and the absence of fish from some polluted English rivers. Water Res. 4:363-383.

Bucksteeg, W., H. Thiele and K. Stöltzel. 1955. Die Beeinflussung von Fischen durch Giftstoffe aus Abwässern. Vom Wass. 22: 194-211.

Cairns, J., Jr. and A. Scheier. 1957. The effects of temperature and hardness of water upon the toxicity of zinc to the common bluegill. Notul. Nat. Acad. Phil. No. 299:12 p.

Chapman, G. A. 1969. Toxicity of pentachlorophenol to trout alevins. Ph. D. thesis. Corvallis, Oregon State University. 87 numb. leaves.

Cherkinsky, S. N. 1957. The theoretical basis of hygienic standardization of simultaneous pollution of water courses with several harmful substances. Gig Sanit. 22:3-9. English summary.

Crandall, C. A. and C. J. Goodnight. 1962. Effects of sublethal concentrations of several toxicants on growth of the common guppy. Limnol. Oceanog. 7:233-239.

_____ 1963. The effects of sublethal concentrations of several toxicants to the common guppy. Trans. Amer. Microscop. Soc. 82:59-73.

Edwards, R. W. and V. M. Brown. 1966. Pollution and fisheries: a progress report. Water Pollut. Control, Lond. (J. Proc. Inst. Sewage Purif.) 66:63-78.

Ellis, R. H. 1968. Effects of kraft mill effluents on the production and food relations of juvenile chinook salmon in laboratory streams. M. S. thesis. Corvallis, Oregon State University. 55 numb. leaves.

Friedland, S. A. and M. N. Rubleva. 1958. The problem of hygienic standards for water simultaneously polluted with several harmful substances. Gig. Sanit. 23:12-16. English summary.

Goodman, J. P. 1951. Toxicity of zinc for rainbow trout. Cal. Fish and Game. 37:191-194.

Groves, A. B. and A. J. Novotny. 1965. A thermal marking technique for juvenile salmonids. Trans. Amer. Fish. Soc. 94: 386-389.

Herbert, D. W. M. and K. Downing. 1955. A further study of the toxicity of KCN to rainbow trout. Ann. Appl. Biol. 43:237.

Herbert, D. W. M., D. H. M. Jordan and R. Lloyd. 1965. A study of some fishless rivers in the industrial Midlands. J. Proc. Inst. Sewage Purif. 569-582.

Herbert, D. W. M. and J. C. Merkens. 1952. The toxicity of potassium cyanide to trout. J. Exp. Biol. 29:632-650.

Herbert, D. W. M. and D. S. Shurben. 1964. The toxicity to fish of mixtures of poisons. I. Salts of ammonia and zinc. Ann. Appl. Biol. 53:33-41.

Herbert, D. W. M. and J. M. VanDyke. 1964. The toxicity to fish of mixtures of poisons. II. Copper-ammonia and zinc-phenol mixtures. Ann. Appl. Biol. 53:415-421.

Jones, J. R. E. 1947. The oxygen consumption of Gasterosteus aculeatus L. in toxic solutions. J. Exp. Biol. 23:298-311.

LeDuc, G. 1966. Some physiological and biochemical responses of fish to chronic poisoning by cyanide. Ph. D. thesis. Corvallis, Oregon State University. 146 numb. leaves.

Lichatowich, J. A. 1970. Influences of kraft mill effluents on the production of chinook salmon in laboratory stream communities. M. S. thesis. Corvallis, Oregon State University. 52 numb. leaves.

Lloyd, R. 1960. The toxicity of zinc sulphate to rainbow trout. Ann. Appl. Biol. 48:84-94.

Lloyd, R. 1961. The toxicity of mixtures of zinc and copper sulfates to rainbow trout (Salmo gairdnerii Richardson). Ann. Appl. Biol. 49:535-538.

Lloyd, R. and D. W. M. Herbert. 1962. The effect of the environment on the toxicity of poisons to fish. J. Instn. Public Health Engrs. 61:132-145.

Lloyd, R. and D. W. M. Jordan. 1964. Predicted and observed toxicities of several sewage effluents to rainbow trout: A further study. J. Proc. Inst. Sewage Purif. Pt. 2:183-186.

Mount, D. I. 1964. An autopsy technique for zinc-caused mortality. Trans. Amer. Fish. Soc. 93:174-182.

_____ 1965. The effect of total hardness and pH on acute toxicity of zinc to fish. Air Water Pollut. Int. J. 10:49-56.

Neil, J. H. 1957. Some effects of potassium cyanide on speckled trout. Fourth Ontario Industrial Waste Conf., Ontario Water Resources Comm. (1957).

Pasley, J. N. 1969. Some effects of thyroxine and PCP on the bioenergetics of cichlid fish. Ph. D. Thesis. Corvallis, Oregon State University. 118 numb. leaves.

Pennak, R. W. 1953. Fresh-water invertebrates of the United States. Ronald Press, New York, N. Y. 769 p.

Seim, W. K. 1970. Influence of biologically stabilized kraft mill effluent on the food relations and production of juvenile chinook salmon in laboratory streams. M. S. thesis. Corvallis, Oregon State University. 55 numb. leaves.

Skidmore, J. F. 1964. Toxicity of zinc compounds to aquatic animals, with special reference to fish. Quart. Rev. Biol. 39(3):227-248.

_____ 1970. Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. J. Exp. Biol. 52: 481-494.

Southgate, B. A. 1932. The toxicity of mixtures of poisons. Quart. J. Pharm. Pharmac. 5:639-648.

Sprague, J. B. 1964. Lethal concentrations of copper and zinc for young Atlantic salmon (Salmo salar salar). J. Fish. Res. Bd. Canada. 21:17-26.

Sprague, J. B. 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Res. 4:3-32.

Sprague, J. B. and A. B. Ramsay. 1965. Lethal levels of mixed copper-zinc solutions for juvenile salmon. J. Fish. Res. Bd. Canada. 22:425-432.

Usinger, R. L. 1956. Aquatic insects of California with keys to North American genera and California species. University of California Press, Berkeley, Calif. 508 p.

Warren, C. E. 1971. Biology and water pollution control. W. B. Saunders Co., Philadelphia, Penn. 434 p.

Weinbach, E. C. 1954. The effect of PCP on oxidative phosphorylation. Biol. Chem. 210:525.

APPENDICES

Appendix I. Summary of water quality characteristics for the model streams during experiments I, II and III.

Water Quality Criteria	4/30/71	7/27/71	8/24/71	9/24/71	10/8/71	11/12/71	12/7/71	2/16/72	3/17/72	4/20/72	5/17/72
Methyl Orange Alkalinity (as mg/l HCO_3^-)	84.2		125		115	89.13	59.42	60.0	59.0	71.9	76.9
Hardness (as mg/l $CaCO_3$)	75		105		101	70.	51.	61.3	56.0	66.5	74.3
pH	8.32	8.44	8.64	8.07	8.24	7.99	7.99	8.30	8.10	8.20	8.02

Appendix II. Stream numbers, treatments and concentrations of cyanide (CN) and zinc (Zn) in streams exposed to low treatment, high treatment, cyanide and zinc during experiments I, II and III, in milligrams per liter.

Date	Stream Number						Stock Solution	Chemical
	I	II	III	IV	V	VI	Concentrations	
Experiment I	C	PCP	High Treatment	CN	Low Treatment	Zn	($mg \cdot l^{-1}$)	
6/9/71			I = .0002	I = .0003	I = .0002		2.86	HCN
			O = .0004	O = .0002	O = .0003			
6/10/71				O = .0019				HCN
6/12/71							3.47	HCN
6/13/71							2.30	HCN
6/14/71							3.70	HCN
6/15/71			O = 0.0010					HCN
6/22/71			O = 0.0012					HCN
7/7/71			I = 0.003					HCN
7/20/71			I = 0.021					HCN

Date	I	II	III	IV	V	VI	Stock Solution	Chemical
Experiment II	Low Treatment	High Treatment	CN	Zn	C	PCP	Concentrations	
10/12/71							65.0	Zn
10/14/71		I = 0.100						
		O = 0.080						Zn
10/18/71							58.8	Zn
10/18/71			I = .016				7.3	CN^-
10/25/71		I = 0.180		I = 0.150			70.0	Zn
		O = 0.150		O = 0.100				
10/25/71		O = 0.009					7.14	CN^-
11/27/71	I = 0.070	I = 0.120		I = 0.120				Zn
	O = 0.080	O = 0.080		O = 0.100				Zn

Appendix II. Continued

Date	Stream Number						Stock Solution Concentrations	Chemical
	I	II	III	IV	V	VI		
Experiment III	PCP	Zn	C	Low Treatment	High Treatment	CN		
3/18/72		IS = 0.077			I = 0.230			Zn
4/4/72		I = 0.178		I = .037	IS = 0.161			Zn
		O = 0.060		O = .000	O = 0.094			
4/25/72		I = 0.196		I = .027	I = 0.196			Zn
		IS = 0.144		IS = .027	IS = 0.111			Zn
4/28/72				I = .094	I = 0.200			Zn
					IS = 0.163			
5/10/72				O = .005	O = .0072		10.38	CN^-
5/17/72		IS = 0.213		IS = 0.094	I = 0.247			Zn
					IS = 0.144			

Where: I = Inlet Sample, O = Outlet Sample and IS = Instream Sample

Appendix III. Values of salmon growth rate (mg · g^{-1} · day^{-1}), biomass (g · m^{-2}) and production (g · m^{-2} · day^{-1}) for all treatments during two-week intervals in experiments I, II and III.

Treatment	Control			Pentachlorophenol			High Treatment			Cyanide			Low Treatment			Zinc		
Experiment I Sampling Interval	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion
2/22/71		2.5			2.5			2.5			2.5			2.5			2.5	
3/8/71	69.1	2.9	0.027	78.0	3.0	0.031	72.4	2.9	0.028	100.9	3.1	0.041	68.7	2.9	0.027	79.0	3.0	0.031
3/22/71	168.7	4.1	0.085	196.8	4.5	0.104	158.3	4.0	0.078	172.9	4.4	0.093	195.9	4.3	0.101	175.4	4.2	0.090
4/5/71	238.9	6.7	0.185	211.8	6.9	0.174	222.9	6.3	0.165	202.2	6.6	0.159	166.8	6.0	0.123	253.1	7.1	0.204
4/19/71	135.3	8.8	0.150	143.4	9.2	0.165	121.3	8.1	0.125	170.7	9.3	0.197	142.4	8.0	0.143	118.4	9.0	0.136
Pre-toxic ation																		
Means	153.0	5.0		157.5	5.2		143.7	4.8		161.7	5.2		143.5	4.7		156.5	5.2	
Totals			0.447			0.474			0.396			0.490			0.394			0.461
5/3/71	75.6	10.3	0.103	54.9	10.3	0.076	52.4	9.0	0.064	60.0	10.5	0.085	57.6	9.0	0.070	45.6	9.8	0.061
5/17/71	38.5	11.1	0.059	13.1	10.7	0.030	0.1	9.0	0.005	26.9	11.2	0.046	34.3	9.7	0.047	20.7	10.3	0.033
6/1/71	10.9	11.4	0.023	-7.7	10.8	0.007	-33.8	8.6	-0.030	7.0	11.4	0.013	-19.4	9.4	-0.016	1.7	10.4	0.004
6/14/71	1.3	11.6	0.009	-23.9	10.7	-0.012	-51.8	8.0	-0.044	33.4	12.1	0.055	-19.5	9.3	-0.012	1.5	10.3	0.005
6/28/71	-11.2	11.4	-0.007	-7.8	10.00	0.001	-65.7	6.6	-0.058	19.0	12.5	0.033	-1.0	9.5	0.018	-10.5	10.2	-0.006
7/12/71	9.3	11.8	0.023	12.2	10.4	0.027	-26.5	4.9	-0.024	51.1	13.9	0.097	17.8	9.5	0.045	7.2	10.6	0.028
Toxication																		
Means	20.7	11.3		6.8	10.5		-20.9	7.7		32.9	11.9		11.6	9.4		11.0	10.3	
Totals			0.210			0.129			-0.087			0.329			0.152			0.125

Appendix III. Continued

Treatment	Low Treatment			High Treatment			Cyanide			Zinc			Control			Pentachlorophenol		
Experiment II Sampling Interval	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion
9/10/71		6.9			6.9			6.7			6.8			6.9			6.9	
9/21/71	-38.6	6.2	-0.064	-29.0	6.4	-0.048	-40.8	6.0	-0.066	-27.5	6.3	-0.042	-41.5	6.2	-0.067	-25.6	6.5	-0.043
10/4/71	5.2	6.3	0.008	2.3	6.5	0.003	-1.0	6.1	0.000	8.5	6.6	0.018	16.7	6.6	0.026	9.3	6.7	0.016
Pre-toxication																		
Means	-16.7	6.5		-13.4	6.6		-20.9	6.3		-9.5	6.6		-12.4	6.6		-8.2	6.7	
Totals			-0.056			-0.045			-0.066			-0.024			-0.041			-0.027
10/18/71	-2.6	6.3	-0.004	-3.9	6.4	-0.006	-1.2	6.0	-0.002	-12.8	6.3	-0.018	-8.8	6.4	-0.014	-17.7	6.3	-0.029
11/1/71	-14.4	6.0	-0.021	-11.1	6.2	-0.017	-8.6	5.9	-0.008	-7.2	6.2	-0.012	-10.3	6.1	-0.016	-15.2	6.0	-0.024
11/15/71	-16.0	4.4	-0.024	-13.0	4.6	-0.020	-1.5	4.9	-0.002	-12.8	4.9	-0.021	-8.2	4.5	-0.013	-19.9	4.2	-0.029
11/29/71	-20.4	4.0	-0.028	-22.4	4.2	-0.033	-7.6	4.8	-0.013	-18.3	4.5	-0.029	-16.4	4.1	-0.023	-26.6	3.9	-0.035
Toxication																		
Means	-13.4	5.2		-12.6	5.4		-4.7	5.4		-12.8	5.5		-10.9	5.3		-19.9	5.1	
Totals			-0.077			-0.076			-0.025			-0.080			-0.066			-0.117

Appendix III. Continued

Treatment	Pentachlorophenol			Zinc			Control			Low Treatment			High Treatment			Cyanide		
Experiment III Sampling Interval	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion	Relative Growth Rate	Bio-mass	Produc-tion
2/15/72		2.6			2.6			2.6			2.6			2.6			2.6	
2/29 72	147.5	3.4	0.056	160.2	3.5	0.062	79.2	3.0	0.029	120.2	3.2	0.044	167.6	3.5	0.064	94.4	3.1	0.034
3/14/72	139.0	4.3	0.068	158.7	4.6	0.081	193.1	4.2	0.088	103.6	3.9	0.048	169.4	4.8	0.089	150.9	4.1	0.069
Pre-toxication																		
Means	143.3	3.4		159.5	3.6		136.2	3.3		111.9	3.2		168.5	3.6		122.7	3.3	
Totals			0.124			0.143			0.117			0.092			0.153			0.103
3/28/72	108.9	5.4	0.078	98.7	2.9	0.062	94.2	5.2	0.067	94.7	4.4	0.064	47.5	2.1	0.030	136.0	4.7	0.084
4/11/72	44.0	5.6	0.040	39.3	2.7	0.032	82.7	6.1	0.067	-49.5	4.6	-0.016	55.8	2.6	0.044	66.8	6.0	0.049
4/25/72	0.3	5.9	0.016	11.2	2.9	0.010	56.6	6.8	0.046	-42.8	3.6	-0.020	-5.5	2.5	-0.004	-8.1	6.0	0.003
5/9/72	-43.5	4.9	-0.029	-13.9	2.7	-0.013	-13.0	6.9	0.004	-41.8	2.6	-0.025	-45.9	2.0	-0.035	-70.3	5.4	-0.042
5/17/72	-56.2	3.7	-0.050	-47.7	2.4	-0.040	-76.4	5.9	-0.054	-30.9	1.5	-0.025	-49.1	1.2	-0.031	-67.1	4.3	-0.045
Toxication																		
Means	10.7	5.1		17.5	2.7		28.8	6.2		14.1	3.3		0.6	2.1		11.5	5.3	
Totals			0.055			0.051			0.130			-0.022			0.004			0.049

Appendix IV. Initial and terminal condition factors and percentages of dry weight for fish in each treatment during experiments I, II and III.

	Condition Factors			Percent Dry Weight		
	Average Initial	Average Terminal	Percent Change	Average Initial	Average Terminal	Percent Change
Experiment I						
Control	0.916	0.658	-28	18.6	18.0	-3.5
Cyanide	0.905	0.790	-12	18.6	18.7	+0.4
Pentachlorophenol	0.905	0.718	-21	18.6	18.8	+0.9
Zinc	0.923	0.683	-26	18.6	18.1	-2.7
Low Treatment	0.901	0.655	-28	18.6	18.6	0.0
High Treatment	0.883	0.667	-25	18.6	14.9	-20
Experiment II						
Control	0.877	0.627	-29	--	17.7	--
Cyanide	0.866	0.621	-28	--	17.9	--
Pentachlorophenol	0.839	0.606	-28	--	15.9	--
Zinc	0.843	0.583	-31	--	17.2	--
Low Treatment	0.877	0.620	-31	--	17.2	--
High Treatment	0.860	0.607	-29	--	17.5	--
Experiment III						
Control	0.710	0.604	-15	17.6	16.9	-4.0
Cyanide	0.712	0.580	-19	17.6	16.6	-6.0
Pentachlorophenol	0.708	0.639	-10	17.6	17.1	-3.0
Zinc	0.725	0.591	-19	17.6	16.6	-5.5
Low Treatment	0.704	0.591	-16	17.6	15.2	-14.0
High Treatment	0.715	0.506	-29	17.6	15.3	-13.0

Appendix V. Biomasses of the most abundant benthic invertebrates in the control and treatment streams during experiments I, II and III in milligrams per square meter.

Experiment I			High		Low	
Treatment	C	PCP	Treatment	CN	Treatment	Zn
Sampling date and Taxon						
3/20/72						
Ephemeroptera						
Cinygmula	836	1406		1918	1693	2140
Diptera						
Chironomidae larvae	842	1705	237	1003	745	1949
Totals	1678	3111	237	2921	2438	4089
4/18/71						
Ephemeroptera						
Cinygmula	1317	361	1483	1249	2896	626
Diptera						
Chironomidae larvae	240	322	289	509	331	338
Totals	1557	683	1772	1758	3227	964
5/19/71						
Ephemeroptera						
Cinygmula	1782	1616		99		228
Cinygma					222	46
Plecoptera						
Isoperla			1356			
Diptera						
Chironomidae larvae	42	52	93	117	117	81
Totals	1824	1668	1449	216	339	355
6/12/71						
Ephemeroptera						
Cinygmula		123				
Cinygma		31				617
Plecoptera						
Isoperla	157				176	
Nemoura	330					
Diptera						
Chironomidae larvae	1325	1018	373	1688	1088	721
Totals	1812	1172	373	1688	1264	1338
7/13/71						
Ephemeroptera						
Baetis		629		681	163	46
Cinygma	1110	463		268		
Plecoptera						
Nemoura	31	783	675	302	413	34
Diptera						
Chironomidae larvae	816	1764	1141	2257	1350	824
Totals	1957	3639	1816	3508	1926	904
Pre-toxication mean	1618	1897	1005	2339	2832	2527
Toxication mean	1874	2160	1213	1804	1176	866

Appendix V. Continued

Experiment II Treatment / Sampling date and Taxon	Low Treatment	High Treatment	CN	Zn	C	PCP
9/8/71						
Ephemeroptera						
Baetis	302	210	373	552	179	56
Cinygma			46	93	46	
Plecoptera						
Nemoura	373	204	200	549	46	3
Diptera						
Chironomidae larvae	77	275	150	343	1154	771
Totals	752	689	769	1537	1425	830
10/2/71						
Ephemeroptera						
Baetis	108	237	339	56	173	62
Cinygma	102	324	802	385	509	185
Plecoptera						
Nemoura	59	43	46	228	49	188
Diptera						
Chironomidae larvae	104	76	125	204	128	219
Totals	373	680	1312	873	859	654
10/30/71						
Ephemeroptera						
Baetis		62	92			92
Cinygma			28	46		
Plecoptera						
Nemoura	561	126	694	660	484	333
Coleoptera				125		
Diptera						
Chironomidae larvae	114	70	182	39	295	65
Totals	675	258	996	870	779	490
11/30/71						
Plecoptera						
Nemoura	330	3	1079	370		
Isoperla			540		777	
Coleoptera			205	114		
Diptera						
Chironomidae larvae	14	83	424	93	369	134
Totals	344	86	2248	577	1146	134
Pre-toxication mean	563	685	1041	1205	1142	742
Toxication mean	510	172	1622	723	962	312

Appendix V. Continued

Experiment III Treatment Sampling date and Taxon	PCP	Zn	C	Low Treatment	High Treatment	CN
2/14/72						
Ephemeroptera						
Baetis	644	364				404
Cinygmula					228	
Cinygma		771				
Paraleptophlebia	372		600	429		
Iron						469
Plecoptera						
Isoperla		290	638	419		450
Nemoura	391				333	592
Diptera						
Chironomidae larvae	503	245	472	301	543	490
Trichoptera			916		823	
Totals	1910	1670	2626	1149	1927	2405
3/11/72						
Ephemeroptera						
Baetis						241
Ameletus		1203	1203	401	802	
Paraleptophlebia			850			
Rhithrogena		786	771	570		
Plecoptera						
Isoperla		897				
Paraperla			1332			379
Diptera						
Chironomidae larvae	572	284	1920	774	805	581
Trichoptera				478	493	376
Oligochaeta					462	
Totals	572	3170	6076	2223	2562	1577
4/13/72						
Ephemeroptera						
Ameletus	1295		2282			
Paraleptophlebia	284		743			
Plecoptera						
Nemoura	410		817			
Isoperla		200	1597	324		278
Diptera						
Chironomidae larvae	425	109	537	233	561	330
Chironomidae pupae				102	330	429
Trichoptera		143		200	743	776
Totals	2414	452	5976	859	1634	1813

Appendix V. Continued

Experiment III Treatment Sampling date and Taxon.	PCP	ZN	C	Low Treat-ment	High Treat-ment	CN
5/11/72						
Ephemeroptera						
Baetis						154
Paraleptophlebia			364			
Plecoptera						
Nemoura			271			185
Isoperla		1018	601			247
Acroneuria	385			2498	160	
Brachyptera			296			
Diptera						
Chironomidae larvae	145				148	111
Oligochaeta					225	
Megaloptera						
Sialis						447
Trichoptera		740	596		417	
Totals	530	1758	2128	2498	950	1144
Pre-toxication mean	1241	2420	4351	1686	2245	1991
Toxication mean	1472	1105	4052	1679	1292	1479

Appendix VI. Biomasses of the most abundant drifting invertebrates in the control and treatment streams during experiments I, II and III in milligrams per cubic meter.

Experiment I Treatment Sampling Interval and Taxon	C	PCP	High Treatment	CN	Low Treatment	Zn
3/29-4/5/71						
Ephemeroptera						
Baetis	1.24	0.53	0.02	1.42		0.45
Cinygmula	.61	0.64	0.09	0.02	0.31	0.48
Iron	.56	0.17			0.04	0.01
Plecoptera						
Isoperla	.07	0.12		.01		0.25
Diptera						
Simuliidae larvae	.43	0.11	0.03	0.13	0.45	0.18
Chironomidae larvae	.04	0.06	0.03	0.04	0.01	0.07
Totals	2.94	1.63	0.17	1.62	0.81	1.44
4/26-5/3/71						
Ephemeroptera						
Baetis	0.77	0.52	2.42	0.63	0.81	1.92
Cinygmula	0.80	0.40	2.03	0.52	0.59	0.37
Iron	0.58	0.59	1.91	0.23	0.18	0.40
Plecoptera						
Nemoura	0.47	0.14		0.07		
Diptera						
Simuliidae larvae						0.52
Chironomidae larvae	0.01		0.08	0.13	0.10	0.08
Dipteran adults		2.01	0.05	0.26	0.01	0.05
Totals	2.63	3.67	6.49	1.84	1.69	3.34
5/24-5/31/71						
Ephemeroptera						
Baetis	0.72	1.20	0.25	0.39	0.93	0.32
Cinygmula	1.86	1.14	0.88	0.16	1.88	0.27
Ironodes	1.58		1.59		0.02	
Iron	0.89	0.77				
Diptera						
Chironomidae larvae	0.06	0.08	0.05	0.10	0.06	0.07
Totals	5.11	3.19	2.77	0.65	2.90	0.66
6/21-6/28/71						
Ephemeroptera						
Baetis	0.17	0.45	0.26	0.48	0.44	0.24
Cinygma						0.30
Iron	0.50					
Plecoptera						
Nemoura	0.30	0.33	0.74			0.20
Diptera						
Chironomidae larvae	0.14	0.42	0.42	0.20	0.43	0.18
Copepoda	0.82	0.04	0.07	0.99	0.14	0.10
Cladocera	1.01			5.27	0.15	0.01
Totals	2.94	1.24	1.49	6.94	1.16	1.03

Appendix VI. Continued

Experiment I Treatment Sampling Interval and Taxon	C	PCP	High Treatment	CN	Low Treatment	Zn
7/19-7/26/71						
Ephemeroptera						
Baetis	0.88	0.87	3.98	5.29	1.99	0.82
Paraleptophlebia	0.41	0.14		1.49	0.06	0.20
Plecoptera						
Nemoura	0.07	0.39	0.30	0.16	0.23	0.23
Diptera						
Chironomidae larvae	1.23	1.33	1.91	2.81	1.71	0.57
Cladocera	8.51	6.13	0.35	4.22	0.26	
Copepoda	0.01	0.24	0.06	0.13	0.38	0.04
Totals	11.10	9.10	6.60	14.10	4.65	1.86
Pre-toxication mean	2.94	1.63	0.17	1.62	0.81	1.44
Toxication mean	5.45	4.30	4.34	5.88	2.60	1.72

Experiment II	Low Treatment	High Treatment	CN	Zn	C	PCP
9/13-9/20/71						
Ephemeroptera						
Baetis	0.39		1.07	0.30		0.03
Coleoptera	0.81	0.52	0.44	0.22	0.81	0.07
Diptera						
Chironomidae larvae	0.01		0.09		0.16	0.14
Copepoda				2.66		
Totals	1.21	0.52	1.61	3.18	0.97	0.24
10/11-10/18/71						
Ephemeroptera						
Baetis	0.51	0.44	0.14	0.49	0.22	
Cinygmula	0.49		0.34	0.35		
Ironodes		0.76	0.03	0.01		0.05
Plecoptera						
Nemoura		0.79	0.14		0.02	0.75
Coleoptera	0.73	0.73	0.56	1.06	0.51	0.99
Diptera						
Chironomidae larvae	0.02	0.02	0.02	0.14	0.01	0.05
Cladocera		1.90		2.29	0.75	0.89
Totals	1.75	4.66	1.24	4.34	1.51	2.73
11/8-11/15/71						
Ephemeroptera						
Baetis	0.30	0.01	0.29	0.18		
Paraleptophlebia	0.12	0.08	0.15	0.06	0.01	
Ironodes			0.40			

Appendix VI. Continued

Experiment II Treatment Sampling interval and Taxon	Low Treatment	High Treatment	CN	Zn	C	PCP
11/8-11/15/71						
Plecoptera						
Nemoura	0.57	0.46	0.20	0.31	0.06	0.35
Capnia	0.08				0.07	0.25
Isoperla	0.18	0.01		0.03	0.01	
Coleoptera	0.18	0.52	0.71	0.07	0.04	0.15
Diptera						
Chironomidae larvae	0.04	0.02	0.06	0.01	0.02	0.04
Cladocera	0.67	0.67	0.54	0.12	0.35	0.38
Totals	2.14	1.77	2.35	0.79	0.56	1.17
Pre-toxication mean	1.21	0.52	1.61	3.18	0.97	0.24
Toxication mean	1.95	3.22	1.80	2.57	1.04	1.95

Experiment III	PCP	Zn	C	Low Treatment	High Treatment	CN
2/22-2/29/72						
Ephemeroptera						
Baetis	2.38	2.02	1.51	2.14	3.44	1.30
Ameletus	5.06	1.98			3.18	
Cinygmula	4.03	5.96	3.96	5.01	3.98	8.64
Paraleptophlebia					1.92	
Plecoptera						
Brachyptera				1.40		
Diptera						
Simuliidae larvae		1.17		1.06		
Totals	11.46	11.13	5.47	9.61	12.52	9.94
3/7-3/14/72						
Ephemeroptera						
Baetis		2.45	1.78	2.85	3.00	1.59
Ameletus	13.63	6.22	0.96	3.98	3.82	2.73
Cinygmula		3.43	2.25	4.90	5.23	5.05
Cinygma			0.84			
Paraleptophlebia						1.30
Plecoptera						
Nemoura					1.47	
Isoperla	4.75		1.46			3.13
Totals	18.37	12.10	7.29	11.73	13.53	13.80

Appendix VI. Continued

Experiment III Treatment Sampling interval and Taxon	PCP	Zn	C	Low Treatment	High Treatment	CN
3/21-3/28/72						
Ephemeroptera						
Baetis		2.55	0.88	2.12	7.10	1.28
Ameletus	13.30	2.18	2.58	4.00	4.66	2.98
Cinygmula		1.36		1.61	4.75	2.67
Paraleptophlebia		1.57				
Iron					5.34	
Plecoptera						
Isoperla	3.62	1.17	2.65	1.71	6.20	3.78
Alloperla			1.31			
Totals	16.92	8.84	7.43	9.43	28.05	10.71
4/18-4/25/72						
Ephemeroptera						
Baetis		0.23		0.14	0.38	0.27
Ameletus		0.47			1.29	
Cinygmula				0.15		0.47
Cinygma	1.60					
Paraleptophlebia				0.31		
Plecoptera						
Isoperla				0.30		
Coleoptera	1.26	0.15	0.81	0.40	0.99	
Diptera						
Simuliidae pupae				0.17		
Trichoptera			1.17			
Totals	2.86	0.85	1.98	1.48	2.66	0.74
5/2-5/9/72						
Ephemeroptera						
Cinygmula						0.69
Ironodes		0.79		0.55		
Plecoptera						
Isoperla	0.40			0.97	0.66	
Brachyptera				0.25		
Coleoptera	0.26	0.88	1.80	0.40	2.39	0.73
Diptera						
Chironomidae larvae	0.36					
Diptera adults	0.16					
Trichoptera	0.13		2.83			1.61
Totals	1.31	1.67	4.63	2.17	3.05	3.04
Pre-toxication mean	14.92	11.62	6.38	10.67	13.03	11.87
Toxication mean	7.03	3.79	4.68	4.36	11.25	4.83

Appendix VII. Percentages of change of salmon biomass in treatment streams relative to the control during two-week intervals in experiments I and III.

Experiment I Treatment Sampling interval	C	PCP	High Treatment	CN	Low Treatment	Zn
3/8/71		+1.5	-0.7	+5.6	-1.6	+0.7
3/22/71		+7.9	-2.7	+6.4	+4.1	+2.3
4/5/71		+2.4	-6.0	-1.6	-10.5	+5.3
4/19/71		+4.1	-8.6	+5.8	-9.2	+1.7
5/3/71		-0.1	-12.8	+2.6	-12.4	-4.3
5/17/71		-3.8	-18.6	+0.7	-12.9	-7.2
6/1/71		-5.5	-25.0	-0.7	-17.6	-9.5
6/14/71		-7.7	-30.4	+4.4	-19.7	-10.6
6/28/71		-12.6	-42.0	+9.5	-16.8	-10.6
7/12/71		-11.8	-58.0	+18.0	-19.7	-9.7

Experiment III Treatment	PCP	Zn	C	Low Treatment	High Treatment	CN
2/29/72	+12.6	+15.3		+6.6	+16.3	+2.3
3/14/72	+2.8	+9.2		-7.8	+12.6	-4.0
3/28/72	+4.1	-44.1		-14.9	-59.7	-9.7
4/11/72	-8.3	-55.8		-24.8	-57.8	-2.7
4/25/72	-13.7	-58.0		-47.0	-62.6	-11.7
5/9/72	-28.3	-60.9		-61.7	-70.1	-21.1
5/17/72	-38.6	-60.3		-74.1	-79.6	-27.4

Appendix VIII. Oxygen consumption rates ($mg \cdot kg^{-1} \cdot hr^{-1}$) of juvenile chinook salmon under different treatment conditions in experiment III.

Treatment Date and Time	PCP	Zn	C	Low Treatment	High Treatment	CN
5/14/72						
1800	859.2	206.5	117.8	344.9	--	163.9
2230	297.9	202.4	151.4	345.7	--	89.9
5/15/72						
0100	380.4	291.1	218.7	271.3	--	218.5
1040	421.7	349.1	478.4	101.0	--	210.1
1750	222.8	290.2	426.1	244.0	150.1	182.0
2130	--	--	--	--	74.7	--
2330	231.0	137.0	199.3	235.6	74.7	200.8
5/16/72						
1100	--	--	--	--	211.7	--
1230	446.0	223.7	376.9	412.2	--	292.8
1430	--	--	--	--	000.0	--
Mean	408.4	242.8	281.2	279.2	127.8	194.0
Standard error of mean	82.2	26.9	54.2	38.2	33.1	23.2
Standard deviation	217.5	71.1	143.3	101.2	66.3	61.3

Appendix IX. Wet weights of juvenile chinook salmon employed in studies of the effects of toxicants on oxygen consumption rates.

Individual initial wet weights Treatment	PCP	Zn	C	Low Treatment	High Treatment	CN
	1.23	1.19	1.27	1.19	1.21	1.27
	1.24	1.29	1.30	1.31	1.26	1.30
	1.38	1.33	1.31	1.36	1.27	1.39
	1.44	1.44	1.37	1.38	1.50	1.39
	1.51	1.54	1.53	1.55	1.54	1.41
Total	6.80	6.79	6.78	6.79	6.78	6.76
Mean	1.36	1.36	1.36	1.36	1.36	1.35
Individual terminal wet weights						
	1.13	1.10	1.15	1.07	1.32*	1.16
	1.23	1.22	1.20	1.20	1.33*	1.18
	1.24	1.23	1.21	1.23	1.35*	1.28
	1.32	1.34	1.28	1.24	1.60*	1.28
	1.42	1.65*	1.39	1.44	1.68*	1.34
Total	6.34	6.54	6.23	6.18	7.28	6.24
Mean	1.27	1.31	1.25	1.24	1.46	1.25
Estimated wet weight for the actual experimental period used to calculate metabolic rates.	6.46	6.61	6.36	6.33	6.42	6.37
Dry weights						
Total	1.20	1.18	1.23	1.19	1.22	1.21
Mean	0.24	0.24	0.25	0.24	0.24	0.24

*Recovered dead at end of experimental period, therefore terminal wet weights may be misleading.