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Title EFFECTS OF SUB-LETHAL LEVELS OF PENTACHLOROPHENOL ON THE GROWTH AND METABOLISM OF A CICHLID FISH.

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Abstract approved _____
(Major professor)

A study was conducted at the Pacific Cooperative Water Pollution and Fisheries Research Laboratories, Oregon State University, to determine the effects of sub-lethal levels of pentachlorophenol on the well-being of the fish Cichlasoma bimaculatum (Linnaeus). Experiments were performed from June, 1963, through February, 1964, on growth, food consumption, food conversion efficiencies and swimming ability of fish subjected to pentachlorophenol. Adenosine triphosphate, cytochrome oxidase and aldolase were also studied.

Potassium pentachlorophenate (KPCP) was used in all experiments. Bioassays were conducted to determine the concentrations of KPCP suitable for experimental investigation. The 36 hour median tolerance limits (TL_m) of solutions of KPCP in running water, and aerated and unaerated standing water, were 0.37, 0.47 and 0.27 mg of KPCP per liter respectively. In all subsequent experiments the concentrations of KPCP were 0.20 mg/l or less, and no fatalities were noted at any time with concentrations at 0.2 mg/l or lower

concentrations.

Three growth and three starvation experiments were performed. Most of the experiments were conducted in porcelainized steel troughs in a 25 C constant temperature room. One starvation experiment was conducted in troughs in which the fish could be forced to swim continuously. A constant exchange of water having a temperature of 25 C was maintained in all experiments, and the desired concentrations of KPCP were maintained by introducing KPCP from Mariotte bottles with small metering pumps.

Tubificid worms constituted the only food given the fish during the experiments. Unlimited food was given during two growth experiments, while the third experiment was performed under conditions of equal but restricted rations at all KPCP concentrations. Fish held in water having no KPCP were used as controls in each experiment.

Growth was found to be dependent primarily on food consumption, and food consumption differences could not be correlated with the concentrations of KPCP used. A KPCP concentration of 0.20 mg/l consistently lowered the efficiency of food conversion, while lower concentrations produced no consistent effects. At a KPCP concentration of 0.20 mg/l when unlimited food was available, the proportion of fat deposited by the fish was lower than in the controls. Under conditions of restricted food consumption, protein, fat and phospholipid depositions were all less than in controls.

Calorimetric data obtained from starving fish were used to

determine the energy requirements of fish in various concentrations of KPCP. KPCP increased the energy requirements of the fish, and 0.20 mg of KPCP per liter caused the greatest increase.

Fish which had been exposed to KPCP up to 2 weeks were tested for swimming ability. Initially those in KPCP did not swim as well as the controls; after 2 weeks exposure to KPCP concentrations of 0.06 and 0.20 mg/l the fish appeared to swim better than the control fish. After swimming, adenosine triphosphate was lower in fish which had been exposed to 0.20 mg of KPCP per liter than in fish not exposed to KPCP.

No lasting differences were noted in aldolase and cytochrome oxidase activities in liver homogenates of fish exposed to KPCP.

EFFECTS OF SUB-LETHAL LEVELS OF PENTACHLOROPHENOL ON THE GROWTH AND METABOLISM OF A CICHLID FISH

by

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EFFECTS OF SUB-LETHAL LEVELS OF PENTACHLOROPHENOL ON THE GROWTH AND METABOLISM OF A CICHLID FISH

INTRODUCTION

The effects of pentachlorophenol (PCP) on the growth and metabolism of a cichlid fish, Cichlasoma bimaculatum (Linnaeus), were studied in a series of experiments conducted from June, 1963, through February, 1964, at the Pacific Cooperative Water Pollution and Fisheries Research Laboratories, Oregon State University. The study was a part of a research program on the biochemistry and physiological ecology of poisoned fish, the objective of this program being to determine how toxic substances influence the well-being of fish in terms of growth, development, reproduction, movement and survival.

The objective of the study here reported was to determine the effects of sub-lethal levels of PCP on the growth, food consumption, food conversion efficiency, activity and metabolic rates of fish. The selection of PCP as the toxic agent to be used in the study was made because of its action in uncoupling the pathways of oxidative phosphorylation (11, p. 385) (20). Two previous studies on the effects of sub-lethal levels of PCP and cyanide had been conducted at this laboratory (4, p. 1-56) (14), and it is upon this research that much of the work presented here has been based. Brockway (4, p. 49-54) indicated several promising areas of research on the effects of chronic levels of PCP and cyanide.

Research concerning the effects of toxic substances on fish has

been concerned primarily with acute toxicity, and there have been only a few studies of chronic poisoning and its effects on the well-being of fish. Crandall and Goodnight (6) reported decreased growth rates in guppies (Lebistes reticulatus) reared in 0.5 ppm (parts per million) sodium pentachlorophenate for 90 days; the rate of growth, even in controls was quite low and a mortality of 44 percent of the fish held in the toxic solution occurred. They also found that the oxygen consumption of guppies which were exposed to 0.5 and 1.0 ppm of sodium pentachlorophenate was increased to 1.3 and 2.0 times that of control fish, respectively.

The general toxic effects of pentachlorophenol have been the subject of considerable research for the past 25 years. The effects of PCP on mammals were studied by Deichman et al. (9, p. 104-117) and by Kehoe, Deichman-Gruebler and Kitzmiller (13, p. 160-172), who reported increased respiration and pyrexia in rabbits, dogs, rats and guinea pigs.

Weinbach (21) postulated that part of the toxicity of PCP can be attributed to its property of uncoupling oxidative phosphorylation. Weinbach (23) and Weinbach and Bowen (24) reported that adenosinetriphosphatase activity was increased at low concentrations of PCP and greatly decreased at high concentrations of PCP. Weinbach and Nolan (25) found that snails (Australorbis glabratus) which were exposed for 24 hours to 7.5×10^{-6} M PCP (2 ppm) contained 2.5×10^{-4} M PCP in the tissue, and that pyruvate, lactate, acetate and inorganic phosphate levels increased. Weinbach (22, p. 129-143)

reported that PCP in low concentrations (2.5×10^{-5} M) completely prevented phosphate uptake associated with the oxidation of beta-hydroxy-butyrate in a preparation of albumen gland of the aquatic snail Lymnaea stagnalis.

Crandall and Goodnight (5) found PCP to be more toxic to fat-head minnows (Pimaphales promelas) at high temperatures and that PCP is much more toxic under acidic conditions (pH 6) than at neutrality or under alkaline conditions (pH 9). The physiological effect of PCP and other substituted phenols, as measured by chlorosis in the plant Lemna minor, and inhibition of growth of the mold Trichoderma viride were correlated with the degree of dissociation (2, p. 45-54) (3, p. 55-71).

The investigation reported in this thesis includes growth experiments for determining the effects of PCP on growth rate, food consumption and food conversion efficiency, and starvation experiments for determining the effect of PCP on the rate of utilization of body energy stores. The swimming performances of control fish and of fish exposed to various levels of PCP were measured as were the activities of liver aldolase and cytochrome oxidase.

There are presented in this thesis the methods and data from studies on the effects of sub-lethal levels of KPCP on the growth, metabolism and performance of a single species of fish. The results are discussed specifically with regard to the effects of PCP on the species of fish used, and they are discussed generally in an attempt to evaluate the techniques used in this study for use in further studies involving chronic poisoning of fish.

MATERIALS

Experimental Animals

The test animal used in this investigation was Cichlasoma bimaculatum (Linnaeus), a tropical aquarium fish of the family Cichlidae. These fish were progeny of a stock which had been maintained at this laboratory since January, 1962. This species has a high rate of reproduction in the laboratory, spawning at any time of the year, thus yielding fish of a desired age and size throughout the year. The fish are relatively disease resistant, can withstand considerable handling and grow rapidly to a size convenient for laboratory work. The behavior of cichlids has been quite thoroughly studied (1, p. 1-243).

Toxicant

The potassium pentachlorophenate (KPCP) used in this study was obtained from Dr. Shih-Dzung C. Lu, Department of Agricultural Chemistry, Oregon State University, who recrystallized the salt from technical grade KPCP. The product was found to be over 99 percent pure as determined by gas chromatography.

Food for Experimental Fish

The only food given to the fish during experiments was a small aquatic worm of the genus Tubifex. This worm was selected because of its relative availability, its ability to live in the experimental environment and its habit of forming an aggregate mass,

which facilitated quantitative recovery of uneaten portions of the ration. The worms were collected periodically from fish rearing ponds at the Oregon State Game Commission's Roaring River Trout Hatchery, near Scio, Oregon.

APPARATUS

Experimental Troughs

Most of the experiments were conducted in a series of rectangular 75 x 10 x 10 inch porcelainized steel troughs (Figures 1 and 2) which were kept in a 25 C constant temperature room. The room was lighted by six banks of fluorescent lights and had a constant photoperiod of 16 hours of light and 8 hours of dark. Water from a spring-fed stream was passed through a filter and brought into the room through polyethylene pipes.

The incoming water was passed through a heat exchange unit and then entered a constantly overflowing head jar equipped with a stainless steel heater. The water in the head jar was heated to 25 C, vigorously aerated and siphoned into a distribution jar. Lines of Tygon plastic tubing carried water from the distribution jar through ball-displacement flow meters into the troughs.

The water level in the troughs was fixed by adjusting the height of a standpipe located at one end of each trough. A small centrifugal pump was used to circulate the water continually in each trough, thus making the temperature and the concentration of the toxicant more uniform throughout the trough.

Each trough was partitioned into sections with porcelainized steel partitions, 9 2/3 inches square. The partitions were held in place by inserting sections of Tygon plastic tubing between the partitions and the sides of the trough. Each partition had a centrally located circular opening, 4 1/2 inches in diameter, which was covered

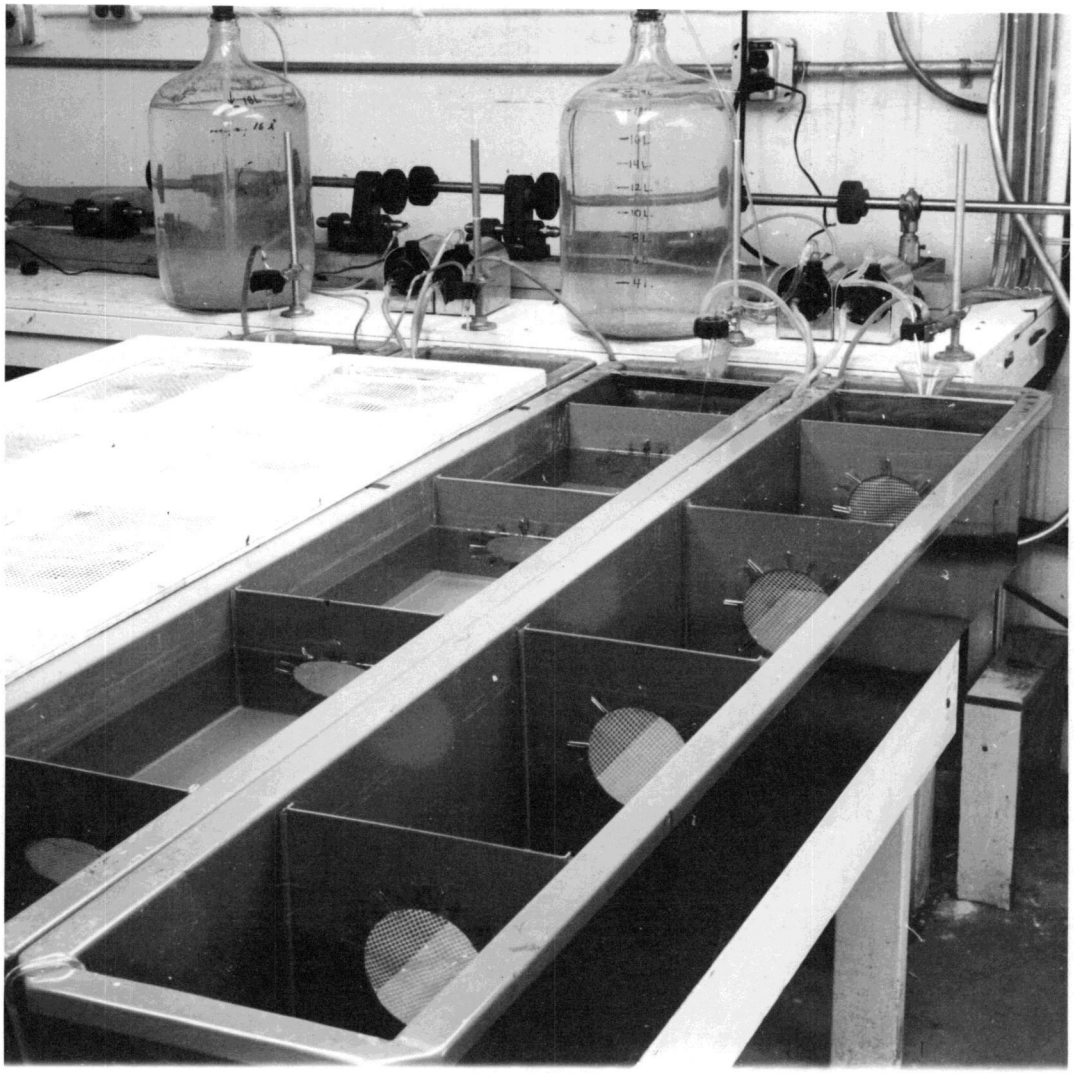

Figure 1. Porcelainized steel troughs in which the fish were held.

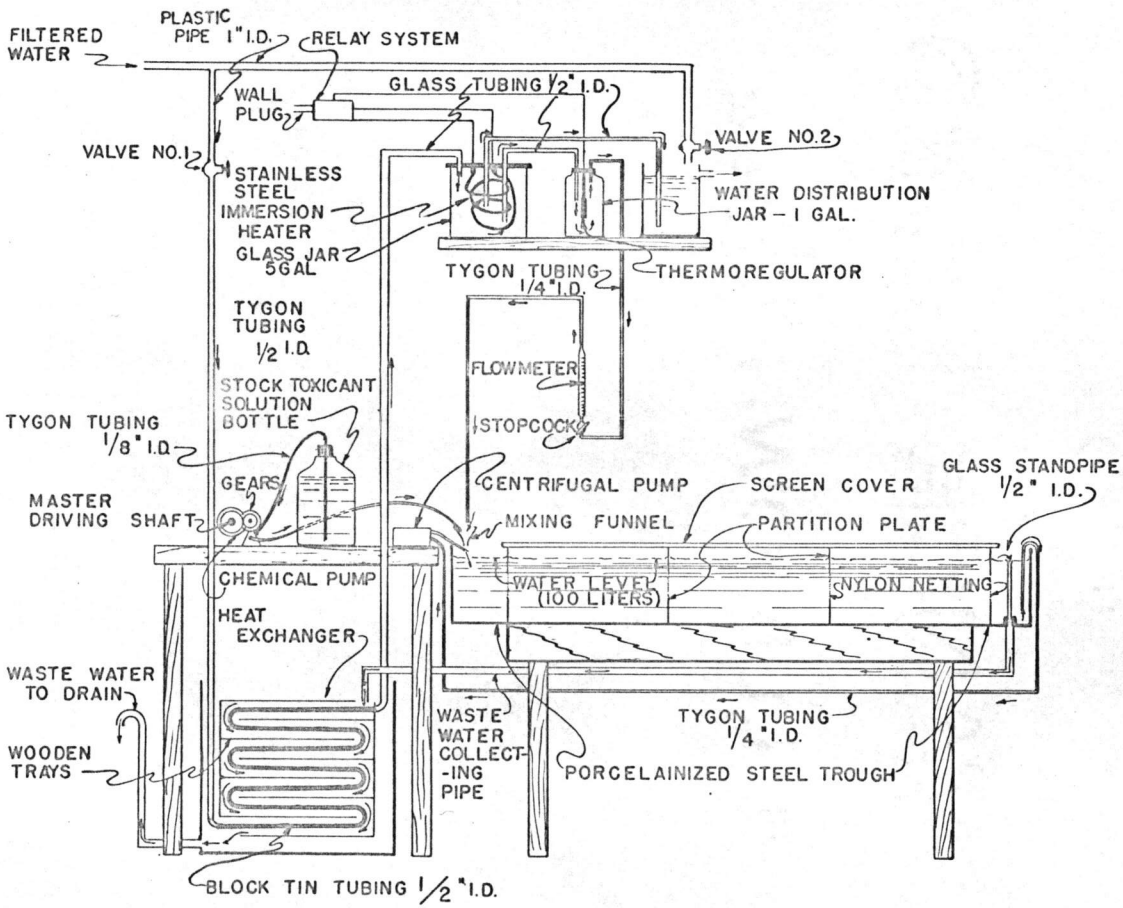

Figure 2. Flow diagram of water distribution system.

with a piece of 3/16 inch mesh nylon netting secured by paper clips. The troughs were placed on wooden frames approximately 2 feet high. Screens were placed over the troughs tc prevent fish from jumping out.

Exercise Troughs

One starvation experiment was conducted in a series of three exercise troughs (Figure 3) similar in design to that of Lemke and Mount (15, p. 372-378). These troughs were roughly oval, 42 inches long and 18 inches wide, with two parallel straight sections 22 inches long. The depth of the troughs was 5 inches and the width of the channel was 6 inches. The troughs were constructed of galvanized sheet metal and were painted, first with a primer and subsequently with several coats of white paint. A drain was present on the bottom near one end of each trough, and the water level was fixed by adjusting the height of a standpipe.

Aluminum paddlewheels, 12 inches in diameter, were used to maintain a flow of water around the troughs. The paddlewheels were placed on a steel shaft which was connected to a Graham variable speed drive. A paddlewheel was set into one of the straight sections of each trough. The straight section opposite the one containing the paddlewheel was partitioned from the remainder of the trough by inserting two screens having aluminum frames and 10 x 5 mm mesh, nylon monofilament screening. A piece of one-inch, quarter-round, wooden molding was placed along the bottom inside edge of the screened section of each trough. The molding was secured to the trough with paint. Its purpose was to deny fish the opportunity of occupying

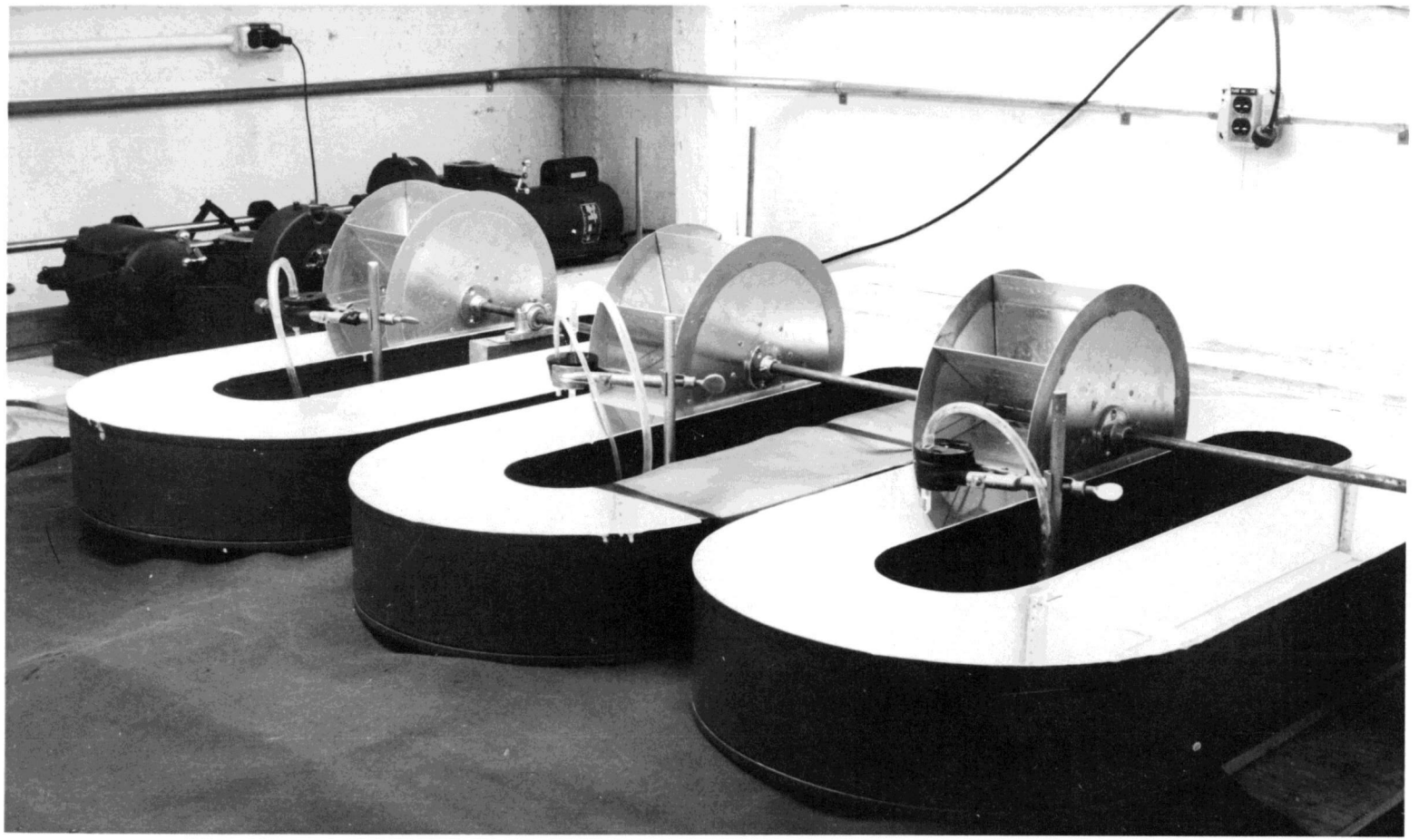

Figure 3. Exercise troughs used in starvation experiment three.

a part of the trough where low water velocities would have otherwise been present. These troughs were located in a 25 C constant temperature room. Screens were placed over the partitioned sections of the troughs to prevent fish from jumping out.

Toxicant Delivery System

Solutions of the toxicant, which were held in 5-gallon, narrow-mouth glass bottles, were dripped into the troughs. A delivery system, utilizing Zenith metering pumps controlled by a Graham variable speed drive, was used to introduce the toxicant. The delivery rates of the several pumps used were between 1.1 and 2.8 ml/min. Tygon plastic tubing, 1/8 inch in diameter, was used to carry the solutions from the bottles to the troughs. During the first several experiments the head in the solution bottles was allowed to drop as the toxicant was withdrawn. In later experiments, however, a constant head in the toxicant bottles was desired for more uniform toxicant flow rates. Therefore, constant-head Mariotte bottles were used in conjunction with the delivery system (Figure 4).

Swimming Performance Apparatus

Fish were tested for swimming ability in an apparatus originally described by Katz, Pritchard and Warren (12) and subsequently modified and redescribed by Davis et al. (8, p.114) and by Dahlberg (7, p. 9-13). The test chamber of this apparatus consisted of a Pyrex glass tube, 60 inches long and 4 inches in diameter. Water was recirculated through this tube by a centrifugal pump, and the velocity was regulated by a gate valve. Water temperature was

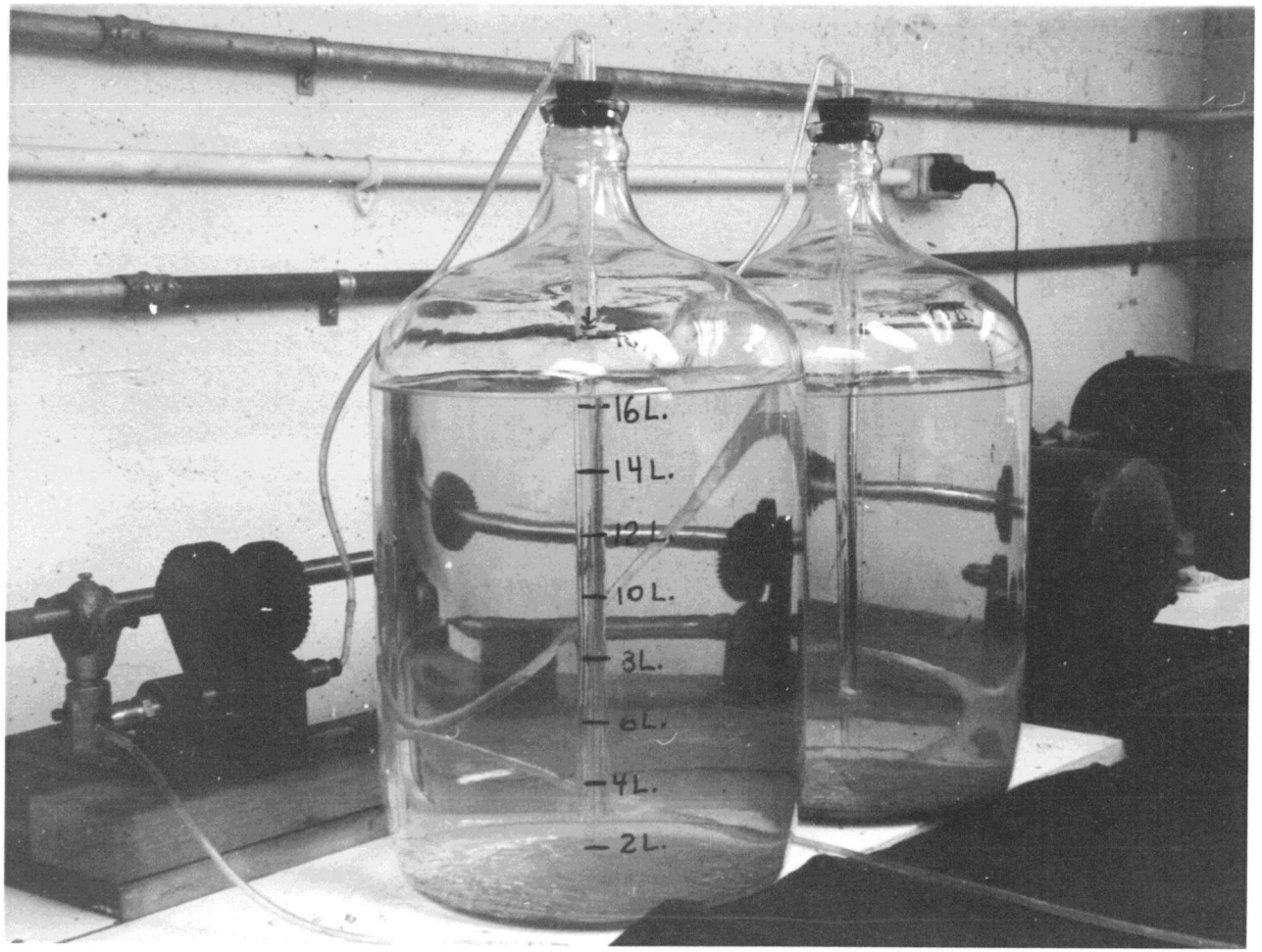

Figure 4. Mariotte bottles and Zenith metering pumps used for toxicant delivery.

controlled by a system containing heating and refrigerating units. An exchange flow of about one liter per minute of aerated, 25 C water was maintained.

METHODS

Experimental Animals

Brood fish, whose progeny were used in these experiments, were held in 50-gallon glass aquaria. When fry were hatched, they were left with the parent fish until they were about 15 mm in length, at which time they were separated from the parents and transferred into other aquaria or wooden rearing tanks. Fry were fed brine shrimp and dry food (Wardley's Vitafare); young fish were fed tubificid worms and dry food (Dina-fish); and adult fish were fed dry food (Dina-fish). All fish were held at 25 C in aerated aquaria or tanks having a constant exchange flow of water.

Toxicant

Stock solutions of KPCP were made with distilled water, usually so that the solute concentration was 10 mg/l. A 50 mg/l stock solution was used for the running water bioassays. Stock solutions were stored in 1-liter volumetric flasks which were covered with aluminum foil so that the contents were held in the dark. All toxicant concentrations were computed as milligrams of KPCP per liter.

Food for Experimental Fish

Tubificid worms were held outdoors at the laboratory in wooden troughs which contained gravel and were supplied with flowing water from a nearby stream. Dry fish food was distributed in the troughs periodically to provide the worms with food. The worms could be held for months under these conditions.

The worms, before being fed to the fish, were cleaned until they were free from silt and other detritus. Materials such as leaves, twigs and gravel were removed; and the worms were placed in a fine mesh net and washed in flowing water to remove most of the silt. The partially cleaned worms were placed on a 3 mm-mesh screen over a pan of water and a light placed directly over them. The worms passed through the screen and into the water in a few minutes, leaving behind most of the remaining detritus. The worms were then held in flowing, filtered water for 24 hours until their digestive tracts were empty. This procedure was considered adequate for cleaning the worms since additional holding in flowing water did not decrease the percent dry weight of the worms.

Bioassays

Standing water and running water bioassays were conducted to determine the lethal limits of KPCP for the experimental fish. The standing water tests were conducted in 5-gallon, wide-mouth, glass jars; and the running water tests were conducted in the porcelainized steel troughs. All bioassays were conducted at 25 C.

The initial standing water bioassay tested concentrations of KPCP from 0.25 to 0.50 mg/l in 15 liters of test solution. Ten fish, approximately 2 months of age and having a mean weight of about 1 gram, were placed in each test solution. The solutions were gently aerated. The dissolved oxygen concentrations of the solutions were checked periodically.

A second standing water bioassay was conducted in which the

toxicities of KPCP and Santobrite[1/], a technical grade sodium salt of PCP, were compared. Considerable data on the toxicity of Santobrite to this cichlid were available from previous work of this laboratory (4, p. 28-33). The concentrations tested ranged from 0.30 to 0.60 mg KPCP per liter and from 0.20 to 0.50 mg Santobrite per liter. Each jar contained 10 liters of solution and received eight fish, having an age of about 4 months and a mean weight of about 0.65 grams. Except for the solution in one duplicate test jar at 0.50 mg KPCP per liter, none of the solutions were aerated. The dissolved oxygen concentrations of the solutions were checked periodically.

A third standing water bioassay was conducted to determine what effect volatility of the toxicant might have on the toxicity of the solutions. The KPCP concentrations tested were 0.25, 0.40 and 0.55 mg/l. Two test solutions at each concentration were vigorously aerated for 36 hours prior to the bioassay. The solutions were placed away from direct light during this time to minimize possible photodecomposition of the PCP. Two additional series of test solutions were prepared just prior to the bioassay, thus giving four test solutions at each concentration. Eight fish, approximately 5 months of age having a mean weight of 1.9 grams, were placed in 10 liters of solution in each jar. Two of the series of test solutions were aerated during the bioassay, one series which had been preaerated

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1/ Reg. T. M. Monsanto Chemical Co.

and one series which contained fresh solutions. The two remaining series of test solutions were not aerated during the bioassay. Dissolved oxygen and pH determinations were made periodically.

A running water bioassay (which tested KPCP concentrations of 0.30, 0.45 and 0.60 ppm) was conducted at the same time as the second standing water bioassay. Eight fish, about 4 months of age having a mean weight of about 0.65 grams were placed in each trough; and the flow of toxicant was started. Fresh solution was added at one end of each trough at a rate of 300 ml/min and a 300 ml/min overflow occurred at the other end. A small pump provided additional mixing and circulation of the solution by removing water from the overflow end and reintroducing it at the intake end at a rate of 500 ml/min. The average rate of water flow through the trough was calculated to be about 2 cm/min.

Growth Experiments

Three separate growth experiments were conducted in the porcelainized steel troughs. The rate of water exchange in all three experiments was 300 ml/min. The circulation rate in each trough was about 2 l/min during growth experiments one and two, and about 500 ml/min during growth experiment three. The reduced circulation rate during the third growth experiment was due to the water reentering the trough through a constricted jet of glass tubing which was placed into the system in an effort to maintain the dissolved gases in the water near air-saturation values.

Paraffin was used to close all cracks and spaces around

partitions through which tubificid worms might pass from one section to another. Prior to the first growth experiment, the space between the bottom of each partition and the bottom of the trough was sealed with paraffin. Before the two subsequent growth experiments a paraffin coating was placed over the entire bottom and about 1 inch up the walls of each section of the troughs.

Experiment One. Fish were exposed to KPCP concentrations of 0.02, 0.04, 0.10 and 0.20 mg/l during the first growth experiment. The 0.10 mg/l concentration was discontinued midway through the experiment when all the fish in this trough died when the water flow into the trough stopped. Six troughs were used in this experiment, one for each of the four toxicant concentrations plus two control troughs which received no toxicant. Two hundred forty fish, about 2 months old and ranging from 0.61 to 1.36 grams were divided into six groups of 40 each, and each group subsequently placed into one of the six troughs. All troughs were partitioned into four sections; therefore, each group of 40 fish was further divided into four groups of ten. The mean weights of the fish in each section ranged from 0.94 to 0.99 grams. Fish were distributed so that each subgroup had similar size distributions of fish. Attempts were made to place groups of nearly equal weight in corresponding sections of each trough. The above principle for selection of fish was utilized in arranging for all growth and starvation experiments.

Before being placed into the troughs, the fish were blotted in a damp cotton cloth to remove excess water and were then measured

and weighed. Weights were obtained to the nearest 0.01 gram on a Mettler balance, Model K7T, having a top pan. An additional group of 15 fish, ranging in weight from 0.82 to 1.30 grams with a mean weight of 0.96 grams, was selected and used as an initial sample.

The initial sample and subsequent samples from the experiment were handled in the same manner. When a sample was removed, each fish was measured, and then was blotted in a cotton cloth to remove excess water. Fish were killed by severing the spinal chord, and the individual fish were weighed to the nearest 0.001 gram on a Mettler H15 balance. The sample fish from each trough were placed in a tared container and dried in a 70 C oven for 10 days. Dried samples were weighed and then ground to a coarse, meal-like consistency. The amount of crude fat in each sample was determined by weighing the material removed by refluxing diethyl ether through 1- or 2-gram samples of the dried material for 4 hours in a Goldfisch extraction apparatus. Portions of the dried samples weighing approximately 0.5 grams were ashed in a 600 C furnace for 6 hours and the percentage of ash determined.

Throughout the experiment the fish in each section were fed a weighed quantity of tubificids every 2 days. The worms were blotted on paper towels until they were free from excess moisture, and portions were then weighed to the nearest 0.01 gm on a Mettler balance having a top pan. Each time the fish were fed, a sample of worms of about 10 grams was weighed in a tared container, dried in a 70 C oven for 10 days and the percent dry weight determined.

Feeding levels were kept above food consumption levels in all troughs so that there were worms remaining at the end of each 2-day period. The supply of tubificids at the laboratory diminished to the point that it was not possible to feed an excess of worms from the twelfth through the fifteenth day of this experiment. On the days when no fresh food was introduced into the troughs, the remaining worms (which tended to group together) in each section were redistributed throughout that section.

At the end of each 2-day feeding period, the uneaten worms were recovered from each section, blotted on paper towels and weighed. The worms from all sections of each trough were then pooled and a sample of approximately 10 grams was weighed into a tared container, dried in a 70 C oven for 10 days and the percent dry weight determined.

Samples of fish were removed from each trough after 8, 14, 24 and 32 days. At each sample time, all fish were removed from a corresponding section of each trough. The fish in the sections to be sampled were not fed for 24 hours prior to their removal. During the first 8 days of this experiment, no toxicant was introduced into the troughs. The introduction of toxicant was started on the ninth day of the experiment, and the toxicant and water flow rates were checked daily thereafter.

Experiment Two. The second growth experiment was performed in a manner similar to the first. The number of troughs, the number of sections per trough, the concentrations of KPCP and the feeding procedures were identical with those employed in the first experiment.

Samples of tubificids and fish were removed and handled in the manner described for the first experiment, except that most fish samples were weighed only to the nearest 0.01 gm.

One hundred ninety-two fish, about 2 months of age and ranging in weight from 0.65 to 1.27 grams were divided into 24 groups of 8. Each trough received 32 fish, eight per section, and the mean weights of the fish in each section ranged from 0.92 to 0.93 grams. An additional 16 fish, ranging in weight from 0.61 to 1.15 grams with a mean weight of 0.93 grams, were selected as an initial sample. Toxicant was present in the troughs throughout the experiment, and samples of fish were removed after 11, 22, 32 and 42 days.

In order to obtain information on the uniformity of growth between sections of each trough, the fish from each section were weighed in aggregate the day before each sample was to be removed, i.e. after 10, 21 and 31 days. This procedure was not necessary during the last period as only one section of fish remained in each trough at this time. Daily checks were made of toxicant and water flow rates.

Experiment Three. The third growth experiment differed from the first two in several respects. The fish were fed daily a specific amount of food which was less than the amount they had the ability to consume. The KPCP concentrations used were 0.06, 0.10 and 0.20 mg/l, and only a single control trough was set up. Four troughs were used in this experiment, with only three sections per trough.

Ninety-six fish, about 2 months old and ranging from 0.83

to 1.40 grams, were divided into 12 groups of eight. Each trough received 24 fish, eight per section; and the mean weights of the fish in each section ranged from 1.06 to 1.10 grams. An additional 14 fish, ranging in weight from 0.82 to 1.39 grams with a mean weight of 1.07 grams, were selected as an initial sample. A total of six fish were found dead during the first day of the experiment and were replaced. Three of the fatalities occurred in 0.10 mg of KPCP per liter and one each in the control and 0.06 and 0.20 mg of KPCP per liter. No further mortality occurred. Water temperatures and water and toxicant flow rates were checked daily. Dissolved oxygen concentrations were determined periodically.

Samples of fish were removed after 10, 20 and 30 days as described for the other growth experiments. The fish in each sample were weighed, their livers removed for enzyme determinations and the fish reweighed. The fish samples were then handled in the manner described for the other growth experiments. Samples of the dried material were analyzed for methanol extractable materials as well as for their ash and crude fat contents. One- or 2-gram samples of dried material, which had already been subjected to ether extraction, were extracted with methanol for 10 hours in the Goldfisch extraction apparatus. A second methanol extraction of 4 hours was then performed on these samples and the weights of the two extracts combined. The methanol extraction was adopted in an effort to estimate the amount of phospholipid material in the samples.

An equal amount of food was fed to the fish in each section in

all troughs; this amount was kept constant throughout the ten day period following a sample time but was adjusted following the next sampling time. The feeding levels were based on the wet weights of those fish removed at each sample time and were computed to achieve a food consumption rate of 0.25 gm/gm/day (wet weights) at the beginning of each period. Although the amount of food fed to each section was the same during a given period, the specific food consumption rate (food per day per gram of fish) varied with growth differences between troughs. The fish were fed 2.00 gm/section/day during the first period, 3.25 gm/section/day during the second period and 5.25 gm/section/day during the final period.

Each day when the fish were fed, two samples of worms were weighed in tared containers. These samples were dried in a 70 C oven for 10 days and the percent dry weight determined. These two samples were the first and last samples weighed daily, and the percent dry weights of these samples were used to determine the change in percent dry weight taking place as a result of dessication occurring during the process of weighing the several portions of worms needed daily. Later in the experiment, when only one section of fish remained in each trough, a single sample of worms was taken each day for percent dry weight determinations; and this sample was removed about half-way through the daily food weighing procedure. In order to compensate for any change in percent dry weight of the worms occurring during the weighing period, the order in which the portions of food were fed was reversed daily, so that the section which received the first portion weighed on any given day was fed the last

portion weighed on the following day.

Deriving Data. The weight data obtained from fish removed from one section of each trough at a given sample time were utilized in deriving weight data for fish in the unsampled sections of the trough. The basis for the derivation of data was food conversion efficiency (weight increase/food consumption). Since food consumption was known for each section, it was possible to compute weight increase by assuming that the food conversion of fish in the sampled section and the unsampled sections of each trough were the same. The product of food conversion efficiency and the food consumption of the fish in a given section was used as the weight increase of the fish in the section.

During the second growth experiment, the fish in each section were weighed in aggregate one day prior to the removal of each sample, in order to determine if the efficiencies of food utilization for growth in all sections of a given trough were similar. By subsequently dividing the dry weight of the group of fish from the sampled section by the wet weight of the group one day before sampling, a ratio was obtained which was used for computing the dry weights of groups of fish in the other sections of the trough from their wet weights. Data on weight increase obtained by this method were found to be similar to those computed by efficiencies, although those based on efficiencies averaged 2.8 percent higher.

Starvation Experiments

Three starvation experiments were performed. The first and

second experiments were conducted in the porcelainized steel troughs. During the second experiment, heavy black canvas was used to cover the troughs to create a condition of darkness. A series of three exercise troughs were used in the third experiment so that the fish could be made to swim against a constant current throughout the experiment. The water exchange rate was 300 ml/min in all three experiments, and the water was circulated by pumps at a rate of about 500 ml/min in experiments one and two.

Fish were measured, weighed and dried; ash, crude fats and methanol soluble materials were determined. In addition, a Parr Model 1411 Bomb Calorimeter was used to determine heats of combustion of samples of approximately 0.12 grams of the dried material.

Experiment One. During the first starvation experiment, fish were exposed to KPCP concentrations of 0.06, 0.10 and 0.20 mg/l. One trough was used for each KPCP concentration, while a fourth trough received no toxicant and provided control conditions. Eighty-four fish, approximately 2 1/2 months of age rangine in weight from 1.70 to 3.93 grams, were divided into 12 groups of seven each. Twenty-one fish were placed in each trough, seven per section, and the mean weights of the fish in each section ranged from 2.72 to 3.05 grams. An additional seven fish, ranging from 2.15 to 3.37 grams and with a mean weight of 2.84 grams, were selected as an initial sample. Samples of fish were removed from the troughs after 10, 20 and 30 days. Water temperatures and water and toxican flow rates were checked daily. Dissolved oxygen concentrations were determined

periodically.

Experiment Two. The second starvation experiment utilized fish which had been fed at a controlled rate prior to the experiment. These fish were from the same group and were fed before the experiment at the same controlled rate as the fish in growth experiment three. The feeding period of these fish coincided with the first 15 days of growth experiment three. Following this feeding period the fish were deprived of food for 24 hours, after which they were weighed, measured and placed in the troughs for the starvation experiment. The KPCP concentrations used in this experiment were the same as those used in the previous experiment. One trough was used for each KPCP concentration, while one trough again provided control conditions.

Ninety-six fish, ranging in weight from 1.58 to 3.25 grams, were divided into 16 groups of six, and 24 fish were placed into each trough. The troughs were not divided into sections, but the fish to be placed into each trough were divided into four groups and the fish in each group marked by clipping the spines of certain fins. The mean weights of the fish in each group ranged from 2.05 to 2.20 grams. An additional 24 fish, ranging in weight from 1.72 to 2.91 grams, were divided into four groups of six and used as initial samples. The mean weights of fish in the initial samples ranged from 2.04 to 2.25 grams.

The troughs were kept dark by covering them with heavy black canvas. The troughs were checked for dead fish on the first and second days of the experiment; no mortality occurred. Two groups of

fish were removed from each trough at 10 days, and the remaining two groups at 20 days. Each group was treated in the manner previously described, except that the two groups removed from each trough at a given time were pooled after the dry weights were determined. The flow rates of toxicant and water were checked daily. Temperatures and dissolved oxygen concentrations were determined several times during the experiment.

Experiment Three. Fish in the third starvation experiment were held under conditions that forced them to swim. The concentrations of KPCP to which fish were exposed were 0.06 and 0.20 mg/l. One trough was used for each concentration, and one trough received no toxicant and provided control conditions. The water depth in the trough was 4 cm. The mean water velocity was about 13 cm/sec; however, the fish concentrated in areas where the velocity was lowest, about 8 to 9 cm/sec.

Thirty-six fish, about 5 months of age and ranging in weight from 1.50 to 2.42 grams, were divided into six groups of six. Two groups were placed into the swimming section of each trough. One of the groups in each trough was marked with a dorsal spine clip and the other group was unmarked. The mean weight of the fish in each group ranged from 1.86 to 1.99 grams. An additional six fish, ranging in weight from 1.67 to 2.24 grams with a mean weight of 1.92 grams, were selected as an initial sample. One group was removed from each trough after 10 days and the other after 20 days. Water temperature, water velocity and water and toxicant flow rates were checked daily.

Occasional pH determinations were made.

During the first several days of this experiment the fish were conditioned to avoid resting against the back screen with an electric shocking device and also by frightening the fish away with hand movements. During this period, one fish died in each of the two troughs containing KPCP. The deaths were attributed to electrical shock. One additional fish in the trough having a KPCP concentration of 0.20 mg/l passed through the screen and jumped out of the trough. All three fish which were lost were identified by length and weight data so that their loss caused no difficulty in treating the data for each group of fish.

Swimming Performance Tests

Two separate swimming experiments were conducted to compare the swimming ability of fish exposed to KPCP with that of control fish. In the first experiment, 30 fish, about 3 months of age and ranging in weight from 1.32 to 2.40 grams, were divided into three groups of ten, and each group placed in a trough. The mean weights of the fish in each trough ranged from 1.83 to 1.88 grams. Concentrations of 0.06 and 0.20 mg KPCP per liter were used in two of the troughs, while the third trough received no toxicant and served as a control. Water in each trough was exchanged at 300 ml/min and recirculated at 500 ml/min. Water temperatures and water and toxicant flow rates were checked daily; the dissolved oxygen concentrations were determined periodically. The fish were fed about 0.65 grams wet weight of tubificids per fish per day, but the amounts were controlled

in order to maintain similar growth of fish in all troughs. After 2 weeks the fish in each trough were separated into 2 comparable groups of five fish each. One group of five fish from each trough was tested in the swimming apparatus the day after separation while the remaining five fish in each trough were tested 2 days after separation. Both groups of fish were without food for 24 hours prior to their use. Only one group was tested in the swimming apparatus at any given time, and no toxicant was used in the apparatus at any time.

At the beginning of each test a group of five fish was placed into the tube of the swimming apparatus and allowed to acclimate at a velocity of 15 cm/sec for 1 hour. After the acclimation period, the velocity was increased to the test velocity of 38 cm/sec. Each fish was removed from the tube when it was swept back against the rear screen and would no longer swim. The times, in minutes, of individual failures were recorded, and the individual fish were either marked or kept separated following their removal from the tube. Spent fish from the first day's tests were placed on ice immediately following their removal from the tube. The chilled fish were weighed, measured and placed back on ice. A determination of adenosine triphosphate (ATP) in the tissue of each fish was made by a micro-fluorometric method correlating the amount of light evolved from luciferin in a standard fire-fly tail preparation with the amount of ATP present in an added aliquot of fish tissue preparation (16).

The second day's tests were run in an identical manner until the spent fish were removed from the tube. As each of the five fish was removed from the tube, it was fin-clipped to facilitate recognition of individual fish and then placed in a beaker receiving the outflow of exchange water from the swimming apparatus. The fish were left in the beaker for 1 hour and were then chilled rapidly by placing a large amount of crushed ice into the beaker. The fish were then weighed, measured and placed on ice. A determination of ATP was made on the tissue from each fish.

For the second swimming experiment, 20 fish, approximately 5 months of age with a mean weight of 1.80 grams, were placed into each of two troughs. One trough contained KPCP at a concentration of 0.20 mg/l, and the other trough contained no toxicant. Water was exchanged at 300 ml/min in each trough and recirculated at 500 ml/min. Water temperatures and water and toxicant flow rates were checked daily.

Ten fish were removed from each trough for testing after 1 day, and the remaining ten fish were removed for testing after 7 days. Seven fish from each group of ten were used in the swimming performance tests, while the remaining three were used for aldolase determinations. The fish were fed an excess of tubificids from the second through the sixth day of the experiment and thus were not fed for 24 hours before testing. After 7 days, the fish had a mean weight of about 2.25 grams. Procedure for the swimming performance tests was the same as that used in the first swimming performance experiment except that groups of seven fish were used in the tube.

When spent fish were removed from the tube, they were weighed, measured and discarded. No ATP determinations were performed on these fish.

Enzyme Determinations

Determinations were made of cytochrome oxidase and aldolase activities in liver homogenate preparations of control fish and fish exposed to KPCP. Livers were removed from the fish, weighed and hand homogenized in ice-cold phosphate buffer of pH 7.40. The activity of cytochrome oxidase was determined manometrically in a Bronwill Warburg Apparatus Model V by the method presented by Umbreit, Burris and Stauffer (19, p. 174). Either 3 or 6 mg of tissue were used in the flasks. Aldolase activity was measured spectrophotometrically using the Sigma Aldolase Kit No. 750 (17). The procedure was modified slightly in that 1 mg of tissue was used as a sample, and the incubation temperature was 30 C. Cytochrome oxidase determinations were made on fish from the third growth experiment; and aldolase determinations were made on fish from the third growth experiment and the second swimming performance experiment.

RESULTS

Bioassays

Bioassays to determine the lethal level of KPCP for the experimental fish were conducted in running water solutions and in aerated and unaerated standing water solutions. Figure 5 shows the 50 percent mortality curves for each of the three bioassays. The 36-hour median tolerance limits (calculated concentration that should kill 50 percent of the fish in 36 hours) for fish in the running water and in the aerated and unaerated standing water solutions were 0.37, 0.47 and 0.27 mg/l, respectively. At a KPCP concentration of 0.20 mg/l, in running water, no mortality was observed in 40 days during growth experiment two and in 30 days during starvation experiment one. The dissolved oxygen concentrations of the aerated solutions were always greater than 5 mg/l and those of the running water solutions were always greater than 7 mg/l. The dissolved oxygen concentrations of the unaerated solutions were about 5.6 mg/l after 12 hours and about 3.5 mg/l after 36 hours.

Preaeration of the KPCP standing water solutions for 36 hours appeared to have no consistent effect on the toxicity of the solutions (Table 1). However, in the unaerated bioassay, the preaerated solution at 0.4 mg of KPCP per liter appeared to be less toxic than the fresh solution; in the aerated bioassay the preaerated solution at 0.55 mg of KPCP per liter appeared to be more toxic than the fresh solution.

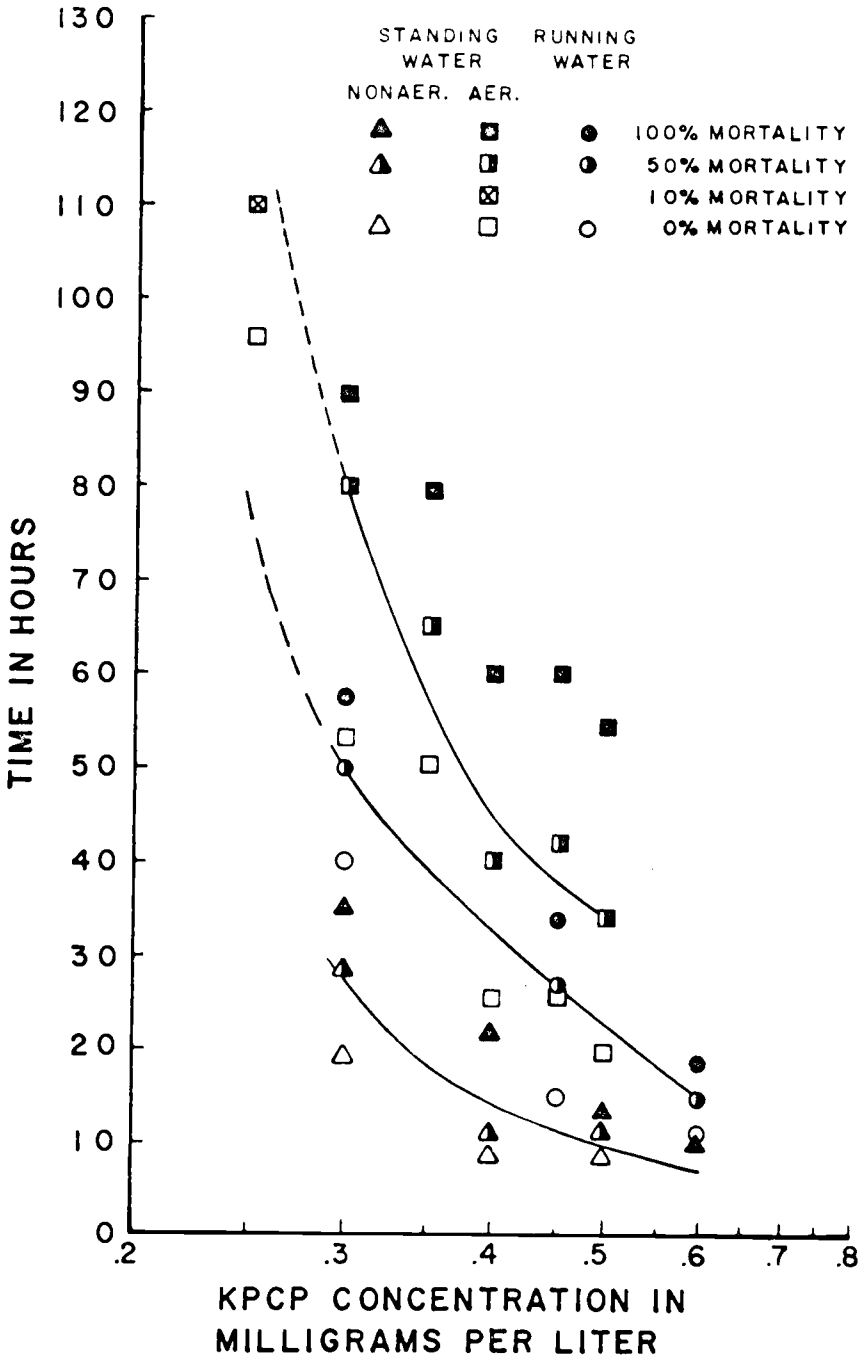

Figure 5. The relationship between the 50 percent mortality curves obtained by exposing cichlids to various concentrations of KPCP in running water and in aerated and unaerated standing water solutions.

Table 1. Hours required to produce 50 percent mortality in aerated and unaerated standing water solutions of KPCP which were either preaerated for 36 hours or were fresh solutions

	0.00 mg/l	0.25 mg/l	0.40 mg/l	0.55 mg/l
		Aerated		
preaerated	-	NM	NM	34
fresh	-	NM	NM	42
		Unaerated		
preaerated	-	25.5	15	8
fresh	NM	28	8	6.5

NM - No mortality

Growth Experiments

Three experiments were conducted to determine the effects of KPCP on the growth of the fish. Figure 6 shows, for the three experiments, the total growth at each sample time, expressed as mean dry weight increase per fish.

In experiment one, two control groups were used, and by the termination of the experiment there was a significant difference in weight between the two controls. The two control groups had similar growth for the first 24 days of the experiment; but at 32 days the average dry weights per fish were 2.12 and 3.33 grams. The data plotted in Figure 6 at each sample time for the controls are the means of the two control groups.

When compared with fish under control conditions, fish at all concentrations of KPCP exhibited less growth by 24 days (16 days in KPCP), with the effect being most marked at 0.20 mg/l. After 32 days

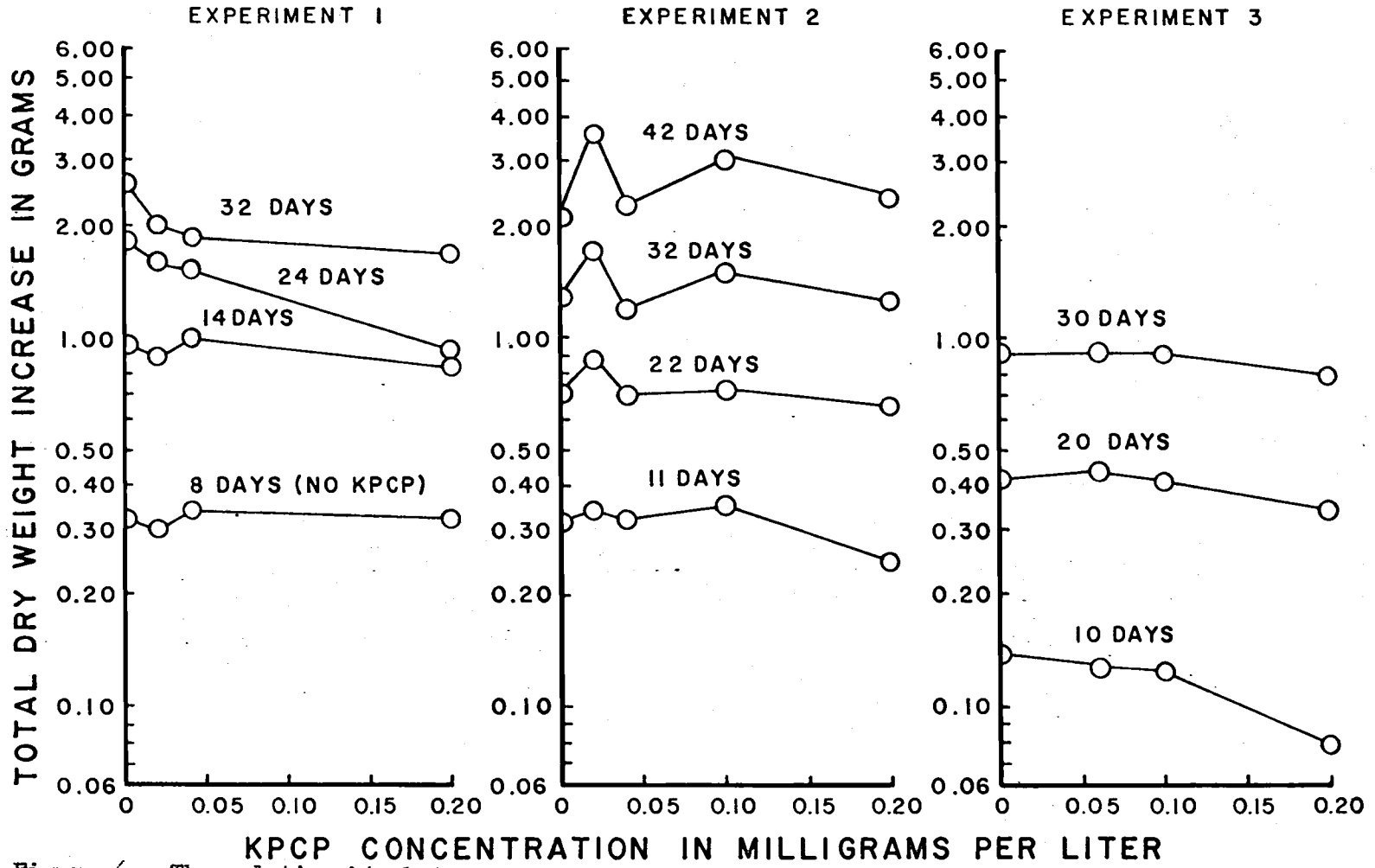

Figure 6. The relationship between the total dry weight increment per fish at each sample time and the KPCP concentration to which the fish were exposed. Increments indicated represent the total weight gain from the beginning of the experiment to the indicated sample time.

(24 days in KPCP) the differences in growth between the fish in KPCP and those in the faster growing control group were still quite large, while the growth of fish in the slower growing control group was only slightly greater than that of the fish held in KPCP. The fish held in 0.20 mg/l were apparently affected more than were those fish exposed to 0.02 and 0.04 mg/l on the basis of the growth attained by 24 days; however, by 32 days the growth of those fish in 0.20 mg/l was only slightly less than those in 0.02 and 0.04 mg/l.

In experiment two, which was conducted in nearly the same way as was experiment one, the two control groups and the fish exposed to KPCP concentrations of 0.04 and 0.20 mg/l showed only small differences in growth. Data at each sample time from the two control groups were averaged and plotted as a single point in Figure 6. The fish exposed to KPCP concentrations of 0.02 and 0.10 mg/l grew more rapidly than the control fish.

In experiment three, where the food consumption of fish in all concentrations of KPCP was regulated at the same level, the growth rate of control fish and of the fish exposed to 0.06 and 0.10 mg/l were very similar. The growth rate of the fish exposed to a KPCP concentration of 0.20 mg/l was less, at every sample time, than that of those exposed to the lower concentrations. This difference in growth observed at the KPCP concentration of 0.20 mg/l became progressively less on a percentage basis, but progressively greater on an absolute basis.

Food Consumption. Food consumption data presented in Figure

7 and the growth data in Figure 6 indicate an obvious relationship between food consumption and growth. The two control groups in experiment one had similar food consumption for the first 24 days of the experiment, but by 32 days the control group showing the more rapid gain in weight also had a much higher food consumption. Food consumption data from the two control groups of the second experiment were similar throughout the experiment, but these food consumption rates were only about one-half of those observed in the controls from experiment one.

In experiment one the food consumption of fish in KPCP was lower than that of control fish. The difference was small for fish in a KPCP concentration of 0.02 mg/l and was larger at KPCP concentrations of 0.04 and 0.20 mg/l. In experiment two, food consumption was higher in KPCP concentrations of 0.02, 0.10 and 0.20 mg/l than in the controls. In experiment three, where the food consumption was controlled, food consumption of the control fish and the fish in all concentrations of KPCP was the same.

Food Conversion Efficiency. Values for efficiency of conversion of food to tissue (total dry weight increase/total dry weight of food consumed) at each sample time are shown in Figure 8. These data show that the food conversion efficiencies of fish exposed to a KPCP concentration of 0.20 mg/l are consistently lower than those of fish held in lower concentrations or under control conditions. Over a 30 day period, efficiencies of food conversion for control fish were approximately 0.41, and efficiencies for fish exposed to a

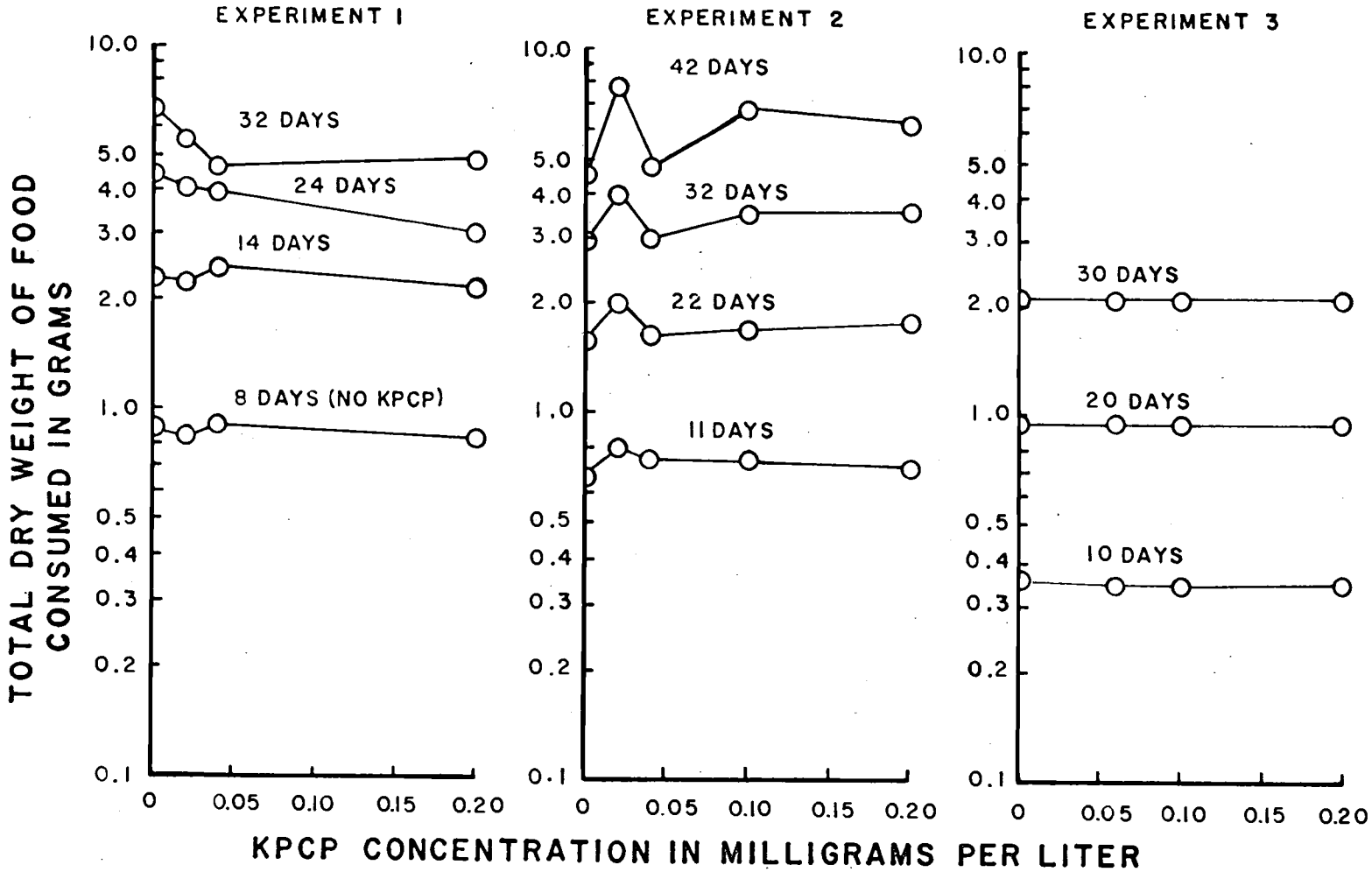

Figure 7. The relationship between the total dry weight of food consumed from the beginning of the experiment to the indicated sample time and the KPCP concentration to which the fish were exposed.

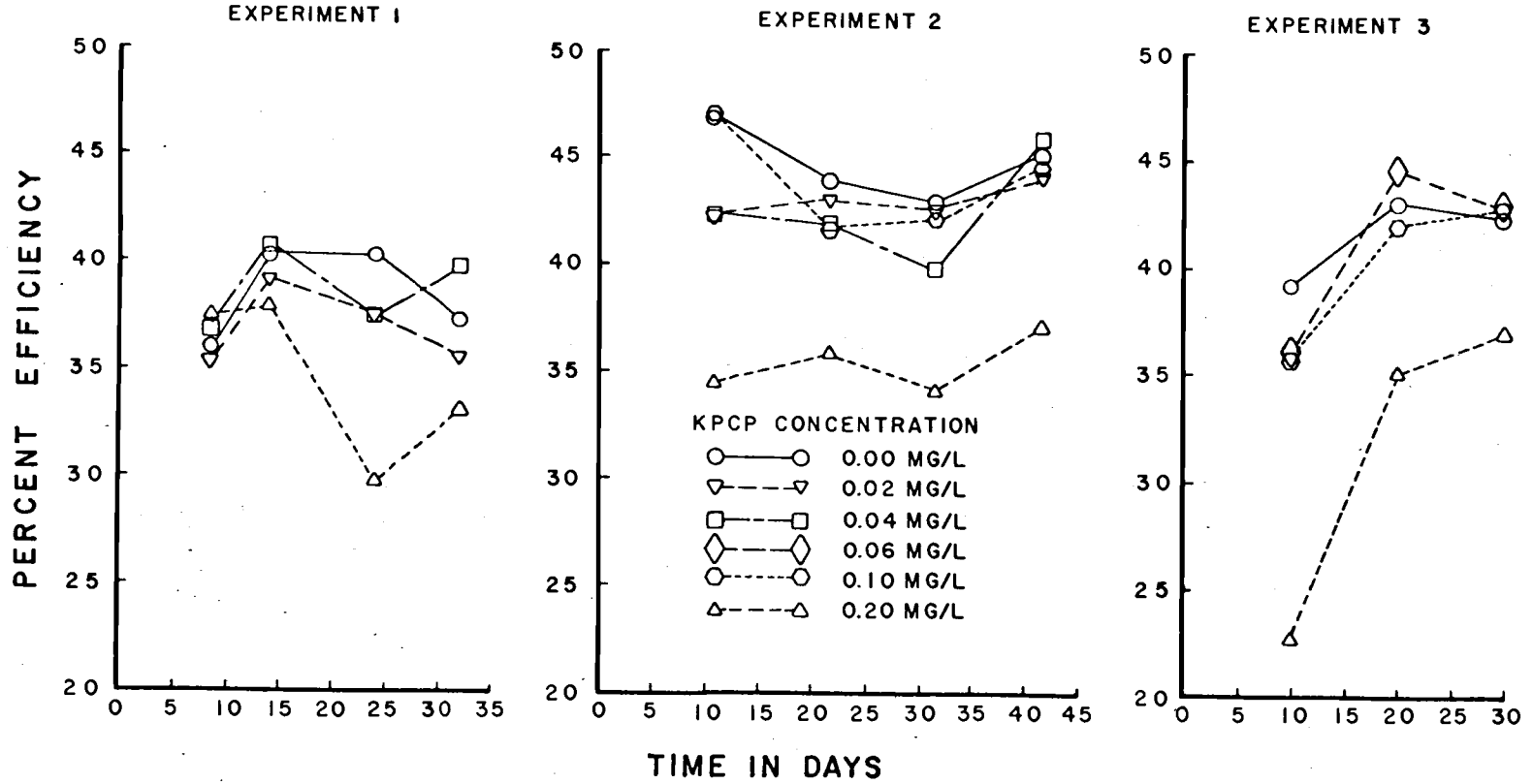

Figure 8. The dry weight conversion efficiencies (dry weight increment of fish/dry weight of food consumed) obtained at indicated sample times under exposure to various concentrations of KPCP. Food consumption and dry weight increment were calculated from the beginning of the experiment to the indicated sample time.

KPCP concentration of 0.20 mg/l were approximately 0.35.

Protein, Lipid and Ash. In order to determine if differences in dry weight increase were due primarily to differences in protein or in fat elaboration, fat and ash determinations were made. The fat- and ash-free dry weight was considered as crude protein; data for the third experiment included methanol extractable materials, probably phospholipids plus some methanol soluble non-lipids. Therefore, the protein fraction in experiments one and two includes all material termed phospholipid in experiment three. Data on the total increase per fish of these constituents at the termination of the three experiments are shown in Figure 9. A comparison between the increments of each tissue constituent plotted in Figure 9 and the final total dry weight increments as presented in Figure 6 indicates that the increment of each constituent closely parallels the total growth increment.

In experiment one KPCP reduced the deposition of crude fat and protein below the levels of deposition in the controls. The reductions in fat and protein deposition were about equal on an absolute basis; but since fat is present in a smaller fraction than protein, the fat deposition was reduced more on a percentage basis. The weights of the ash deposited in the presence of KPCP and under control conditions were not markedly different.

In experiment two, as stated previously, food consumption rates of fish in the presence of KPCP concentrations of 0.02 and 0.10 mg/l were higher than they were for the control fish. Fat, protein and

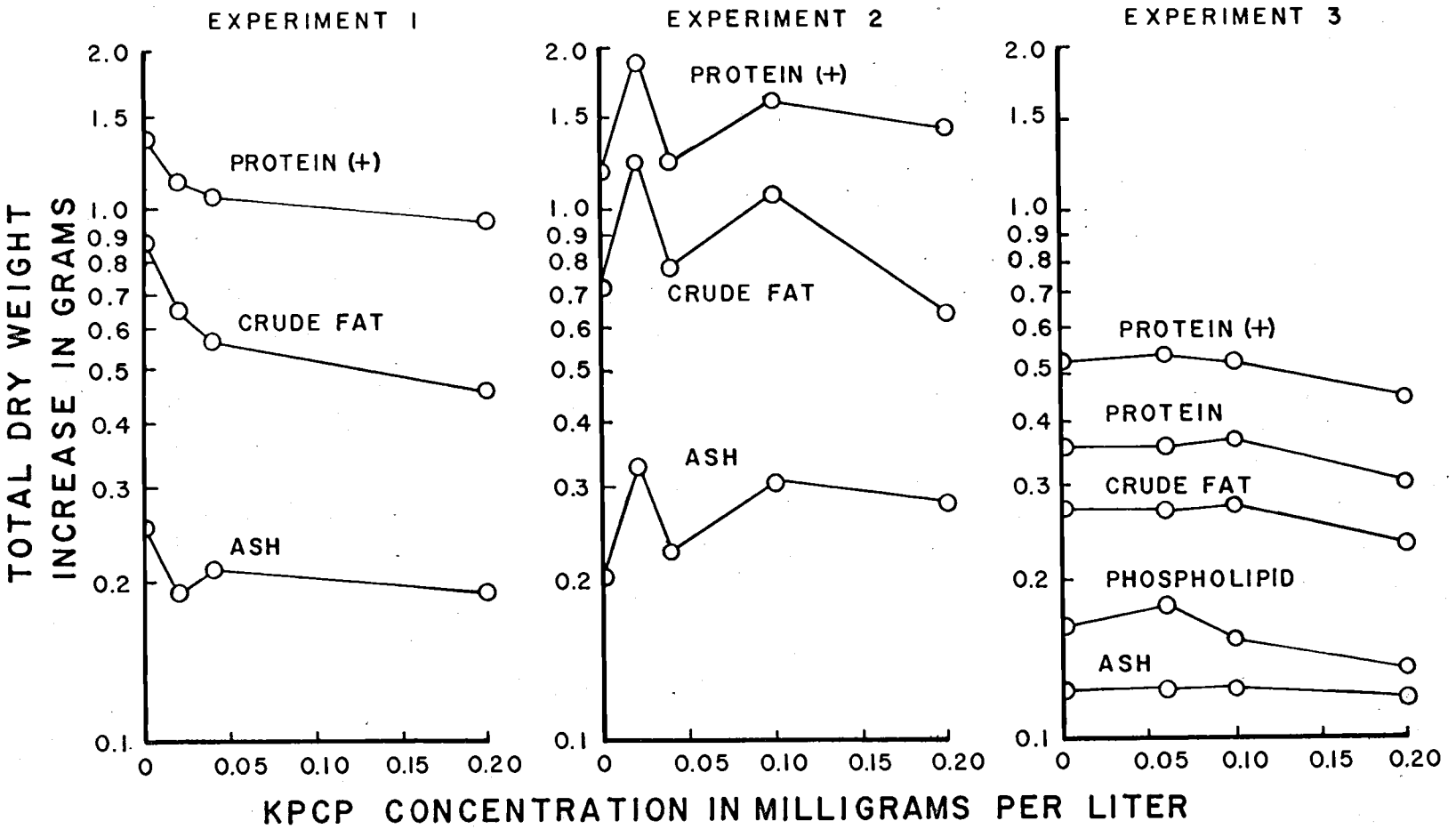

Figure 9. The final dry weight increments per fish of protein, fat, ash and phospholipid of fish exposed to various concentrations of KPCP.

ash deposition were high at KPCP concentrations of 0.02 and 0.10 mg/l. At a KPCP concentration of 0.20 mg/l, protein and ash deposition were higher but fat deposition was lower than in the control fish, even though food consumption was higher than in the control fish. At the end of experiment two, the ratio of protein to fat was similar in the two control groups and in the groups at KPCP concentrations of 0.02, 0.04 and 0.10 mg/l. The ratio was higher at the KPCP concentration of 0.20 mg/l.

In experiment three, where food consumption was somewhat restricted, the deposition of fat, phospholipid (methanol extractable materials) and protein were lower at a KPCP concentration of 0.20 mg/l, but not at concentrations of 0.06 and 0.10 mg/l as compared to the control groups. Protein and fat (ether extractable) were deposited in the same ratio in control groups and in groups at the 0.20 mg/l concentration, but less protein and less fat were deposited at this concentration. The ash content of control fish and fish in KPCP were almost identical. Data indicating the ratios of conversion of food to each of the body constituents were computed by dividing the total increment of each fraction by the total dry weight of food consumed. These data, presented in Figure 10, show that lower ratios at a KPCP concentration of 0.20 mg/l were most noticeable in the crude fat fraction in experiments one and two. In experiment three, where food consumption was lower, fat, phospholipid and protein efficiencies were lower in the presence of a concentration 0.20 mg/l than they were under control conditions.

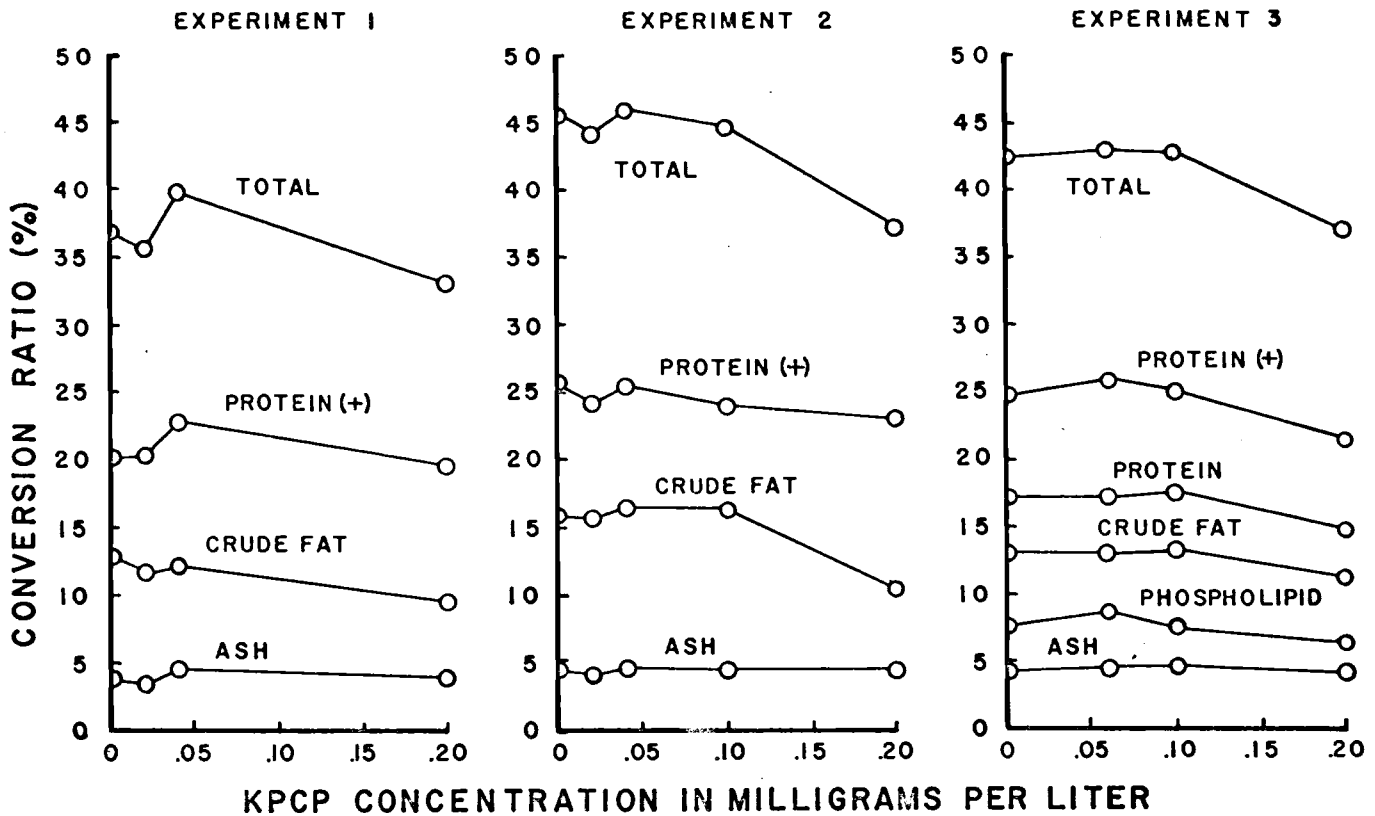

Figure 10. The final dry weight conversion ratios (dry weight gain/dry weight of food consumed) of total dry material, protein, fat, ash and phospholipid from fish exposed to various concentrations of KPCP. The dry weight gains and food consumption are overall figures from the beginning to the termination of the experiment.

Starvation Experiments

Three starvation experiments were conducted to determine the possible effects of KPCP on the rate of energy utilization of fish. Total energy utilization, expressed as calories used per mean kilocalorie of tissue present, for the three experiments is shown in Figure 11. The total energy utilized per gram of fish (dry weight) during the starvation period was calculated by subtracting from the heat of combustion per gram of fish on day zero, the heat of combustion per gram on the sampling day. The figures for mean kilocalories were determined by taking the logarithmic means between the total kilocalories initially present per gram of fish and the total kilocalories remaining per gram of fish at the time of sampling.

The energy utilization of the fish exposed to KPCP was greater than that of control fish, and generally the utilization was greater at the higher KPCP concentrations. At the 0.20 mg/l KPCP concentration a major portion of the increase in energy utilization apparent in experiments one and two occurred during the first ten days of exposure. There were no appreciable differences between the energy utilization of fish held in the light (experiment one) and in the dark (experiment two).

Data from experiment three, where the fish were exercised, show that the rate of energy utilization was increased so that the energy utilization by 14 days in the exercised fish was about the same as that at 30 days in the fish from experiment one. The energy utilizations in experiment three all appear to have a nearly

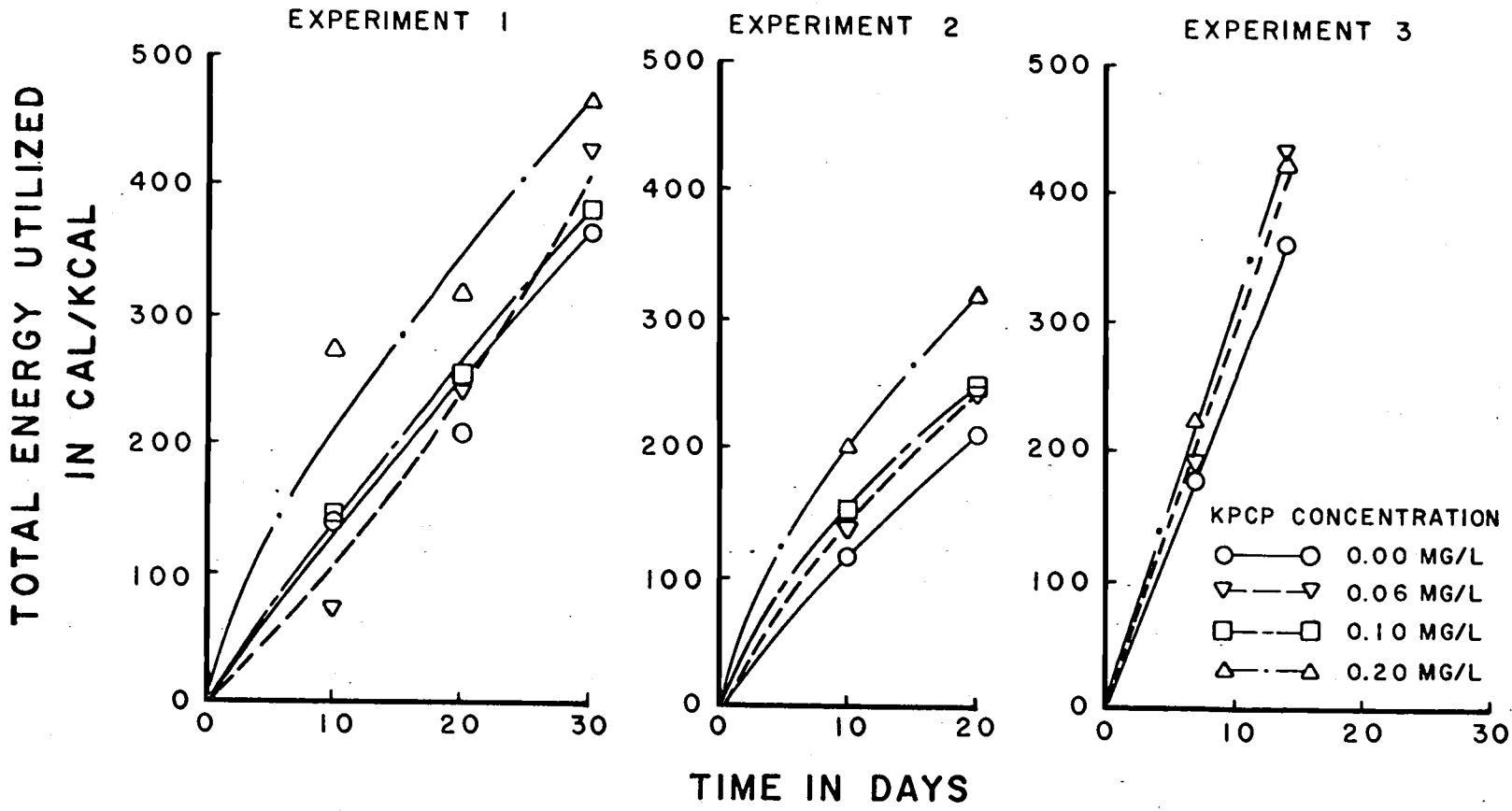

Figure 11. Total energy utilization (calories used per mean kilocalorie of tissue present) from the beginning of the experiment to the indicated sample times.

straight line relationship with time. Initially the differences in energy utilization in experiment three between control fish and fish exposed to KPCP are smaller than occur in experiments one and two, but after two weeks the differences observed in experiment three more closely approximate those found in the other two experiments. The differences in energy utilization by 14 days between control fish and fish exposed to the 0.06 mg/l concentration of KPCP appear to be larger in experiment three than in experiments one or two whereas the differences at the 0.20 mg/l concentration are smaller in experiment three.

Swimming Performance

The data in Table 2 show the length, weight, ATP content and time of failure to swim at a water velocity of 1.25 ft/sec. for fish exposed to KPCP concentrations of 0.00, 0.06 and 0.20 mg/l. The data indicate that exposure to a concentration of 0.20 mg/l of KPCP for one day may reduce swimming performance under the conditions tested, but that no differences between controls and fish held in KPCP could be established after 7 days exposure.

Fish which had been exposed to a KPCP concentration of 0.20 mg/l for 14-15 days tended to swim upstream to the front of the tube and locate themselves directly behind the screen, with their heads thrust as far through the mesh as possible. Control fish usually attempted to take advantage of reduced velocities in this area, but were frightened away by light, motion or noise. It appeared that the fish which had been exposed to the 0.20 mg/l concentration of KPCP

Table 2. The lengths, weights, times of failure to swim and ATP content of fish which were tested for swimming ability following 1, 7 and 14-15 days exposure to the indicated concentrations of KPCP.

1 Day Exposure

	0.00 mg/l			0.20 mg/l		
	Length cm	Weight gm	Time min	Length cm	Weight gm	Time min
	4.60	1.87	2.75	4.30	1.57	2.00
	4.40	1.68	3.00	4.10	1.43	2.00
	4.50	1.78	4.75	4.30	1.70	2.00
	4.20	1.63	4.75	4.60	2.00	2.00
	4.60	2.03	5.25	4.60	1.95	4.00
	4.50	1.81	5.25	4.50	1.93	5.75
	4.90	2.39	6.50	1/		
avg.	4.53	1.88	4.61	4.40	1.76	2.96

1/ One fish lost behind rear screen.

7 Days Exposure

	0.00 mg/l			0.20 mg/l		
	Length cm	Weight gm	Time min	Length cm	Weight gm	Time min
	4.50	1.96	3.25	4.50	1.74	3.25
	4.50	1.97	3.33	4.80	2.19	3.67
	4.60	2.11	4.33	4.80	2.36	3.67
	5.00	2.53	4.67	4.90	2.57	4.00
	5.00	2.51	4.92	4.50	2.00	5.67
	4.90	2.40	5.33	4.80	2.33	5.83
	4.50	1.78	5.58	5.00	2.60	7.42
avg.	4.71	2.18	4.49	4.76	2.26	4.79

Table 2. (Continued)

14 Days Exposure

	0.00 mg/l				0.06 mg/l				0.20 mg/l			
	Length cm	Weight gm	Time min	ATP ugm/gm	Length cm	Weight gm	Time min	ATP ugm/gm	Length cm	Weight gm	Time min	ATP ugm/gm
	5.30	3.20	3.50	16.3	5.00	2.43	5.83	14.7	5.00	2.70	7.50	14.3
	5.40	3.76	4.08	13.3	5.45	4.10	7.50	13.9	5.00	2.65	7.67	14.6
	5.40	3.32	4.50	15.5	5.15	3.70	8.75	12.3	6.50	6.59	11.33	8.9
	5.50	3.74	6.00	13.7	6.20	5.97	10.08	10.3	6.00	4.64	*	10.9
	6.10	5.22	9.50	11.4	5.90	4.78	10.33	13.2	5.40	3.31	*	10.6
avg.	5.54	3.85	5.52	14.0	5.54	4.20	8.50	12.9	5.58	3.98	8.83	11.9

15 Days Exposure

	0.00 mg/l				0.06 mg/l				0.20 mg/l			
	Length cm	Weight gm	Time min	ATP ugm/gm	Length cm	Weight gm	Time min	ATP ugm/gm	Length cm	Weight gm	Time min	ATP ugm/gm
	4.80	2.39	5.75	12.2	5.25	3.27	5.50	13.4	5.10	2.85	5.50	13.8
	5.50	3.94	5.75	16.7	5.50	3.87	6.67	12.8	5.20	3.12	7.00	12.9
	5.10	3.13	6.83	11.2	6.20	5.63	11.67	9.0	5.50	3.70	*	11.0
	5.40	3.75	7.00	12.5	5.70	4.32	12.50	11.6	6.00	4.91	*	10.1
	5.70	4.22	7.92	11.6	5.00	3.87	*	12.9	4.95	2.70	*	16.7
avg.	5.30	3.49	6.65	12.8	5.53	4.19	9.09	11.9	5.35	3.46	6.25	12.9

* Fish which stayed at the front screen and were forced off by increasing the velocity after all other fish had failed and been removed.

for 14-15 days were less susceptible to being frightened. Those individuals which managed to position themselves at the front screen invariably maintained this position until all other fish ceased to swim. The fish on the front (upstream) screen were then forced off by greatly increasing the water velocity. No data concerning time of failure were obtained for these fish. After two weeks exposure to a KPCP concentration of 0.06 fish appeared to be able to perform somewhat better than control fish. Data concerning swimming time for fish exposed to a KPCP concentration of 0.20 mg/l are incomplete, and observations on the effect of this concentration require the interpretation of the probable swimming stamina of those fish which stayed at the front of the tube. It is believed that these fish probably were the stronger swimmers, and therefore that the fish exposed to a KPCP concentration of 0.20 mg/l were able to perform better than control fish.

To amplify the data on swimming performance determinations of ATP were made on each fish which was tested for swimming ability after 14-15 days exposure to the test solutions. The data for the amounts of ATP, expressed as micrograms per gram of tissue in control fish and fish which had been exposed to 0.20 mg/l, are shown in Figure 12. Points for ATP content in the fish exposed to the 0.06 mg/l concentration of KPCP were not included in Figure 12 as they were widely scattered; however, the points generally fell nearer to the control curve than the 0.20 mg/l curve. It is obvious from Figure 12 that larger fish had comparatively less ATP

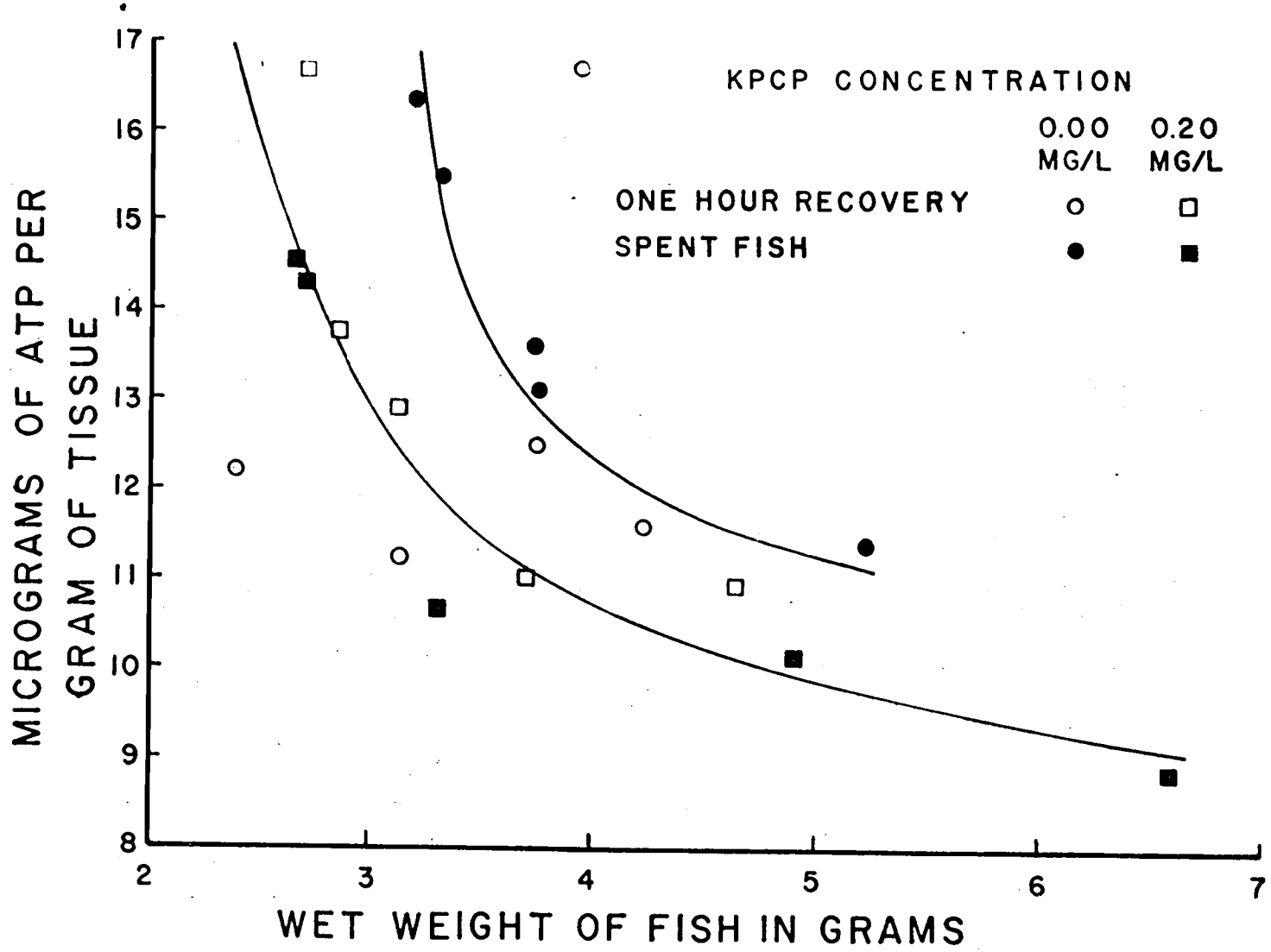

Figure 12. The ATP content of fish rapidly exercised to exhaustion following 14-15 days exposure to 0.00 and 0.20 mg of KPCP per liter.

per gram than did smaller fish.

The amount of ATP in those fish which had been held in a KPCP concentration of 0.20 mg/l before swimming was less than in control fish (Fig. 12). Control fish which were allowed to recover for one hour following swimming had ATP values which frequently fell far off the curve describing ATP concentration in spent control fish. There were no apparent differences in the amount of ATP in the fish from 0.20 mg/l which were allowed to recover for one hour following swimming and those taken immediately after failure to swim. Thus KPCP fish had lower ATP concentration than control fish, and the recovery process in KPCP fish apparently followed a different pattern than in the control fish.

Enzyme Determinations

The activities of cytochrome oxidase in homogenates of livers from the fish used in growth experiment three are presented in Figure 13. KPCP concentrations of 0.06, 0.10 and 0.20 mg/l had no appreciable effect on the activity of this enzyme.

Liver homogenates from fish exposed to various concentrations of KPCP were tested for aldolase activity; the results are shown in Figure 14. It appeared that a KPCP concentration of 0.20 mg/l produced a temporary decrease in aldolase activity which was most marked at about 10 days.

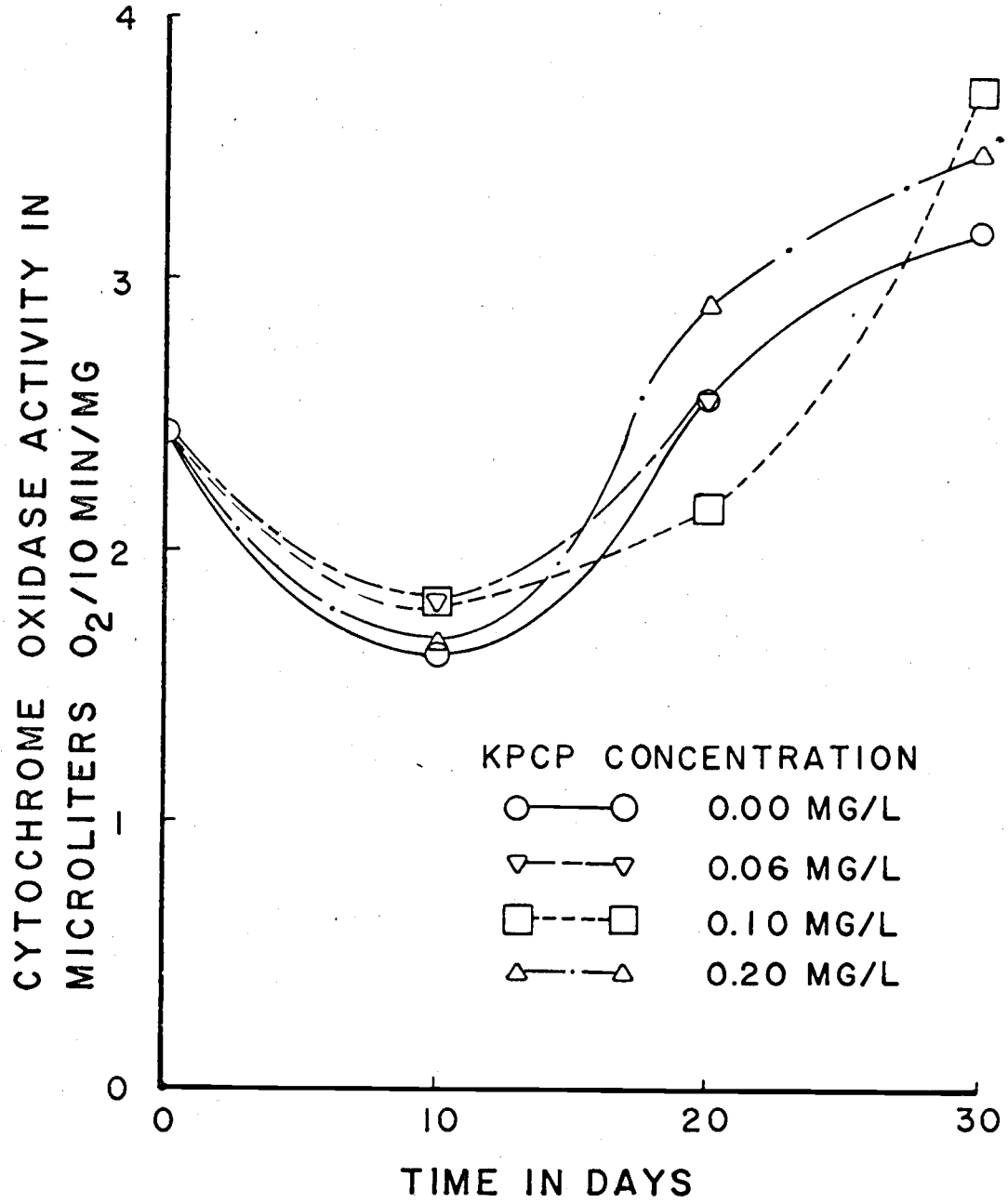

Figure 13. Cytochrome oxidase activities obtained in liver homogenates of fish exposed to the indicated concentrations of KPCP for 10, 20 and 30 days.

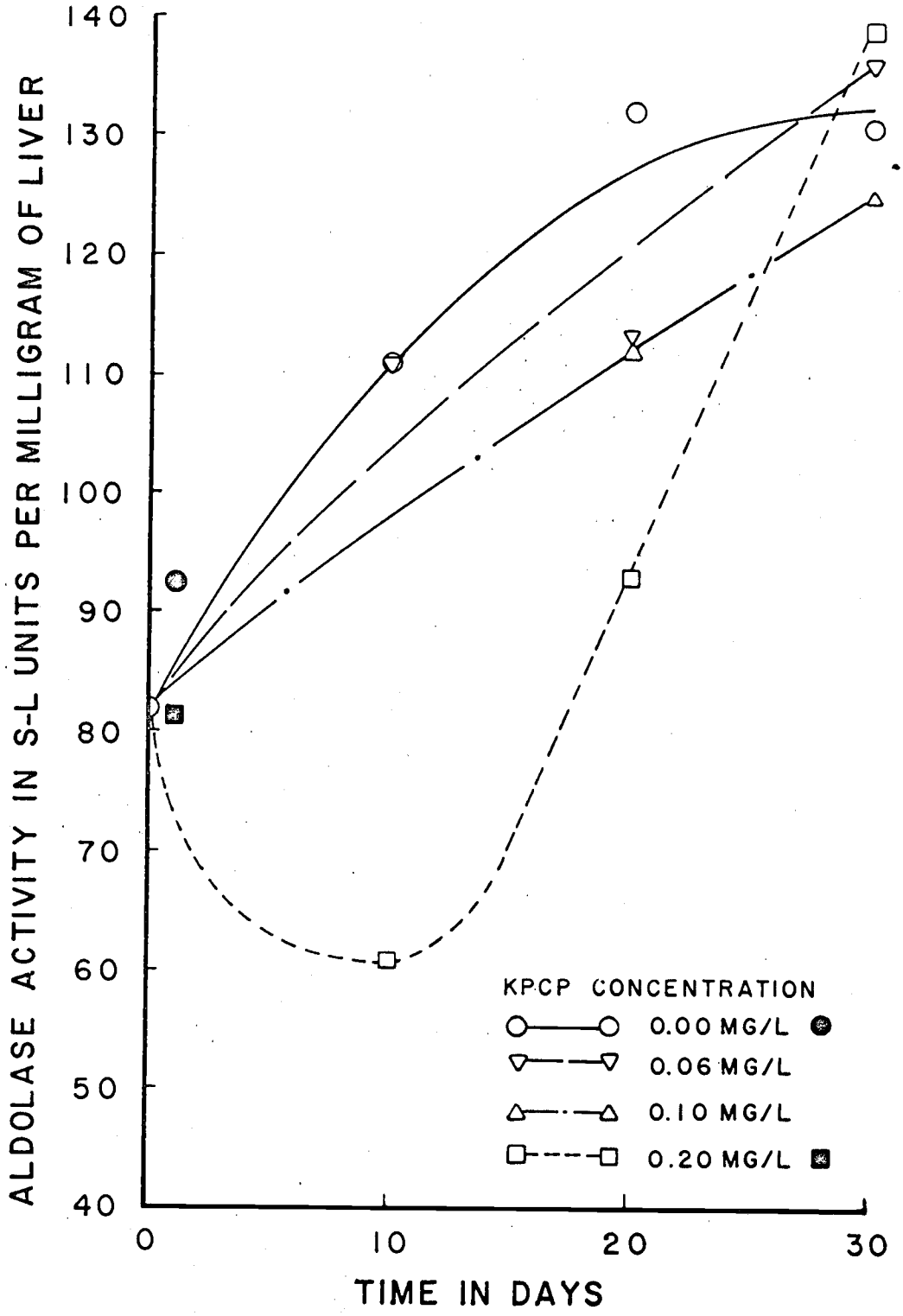

Figure 14. Aldolase activities obtained in liver homogenates of fish exposed to the indicated concentrations of KPCP for various times. The dark figures represent one day determinations made during a separate experiment.

DISCUSSION

A major difficulty encountered during this study was the inability to measure the low toxicant concentrations present in the troughs. It was therefore not possible to determine if the fish were being exposed to the desired concentration of KPCP. The solutions may have been partially detoxified by the action of bacteria and light. The rate of solution exchange was probably rapid enough to minimize the effects of such decomposition.

Other problems were encountered in the course of the growth experiments. Dominance by individual cichlids was obvious following feeding of the fish, even though the tubificid worms were distributed evenly throughout each section of the trough so that they would be more available to all fish. The worms would form one or more small balls in about an hour after feeding, and a dominant fish would guard each mass of worms, thus limiting the food consumption of other fish. This behavior undoubtedly tended to cause uneven food consumption and growth rates between fish in each section, even though the worms were redistributed daily.

Sealing the bottom of each section of the troughs with paraffin eliminated the problem of worms passing from section to another, but may have created several new problems. With the sealing of the spaces beneath the partitions between sections, the circulation of water was decreased; and as a result the water near the bottom of the trough was exchanged more slowly and probably had a lower

dissolved oxygen concentration than water in other areas. The paraffin may also have contributed to possible detoxification of the KPCP by providing a substrate for growth of microorganisms or by removing some toxicant from the solution.

The concentrations of KPCP used for this study were selected on the basis of bioassay data on the lethal levels of KPCP for Cichlasoma bimaculatum. The bioassays indicated that standing water solutions which were aerated were less rapidly toxic than those which were unaerated, and that flowing water solutions were intermediate in toxicity. Preaeration of standing water solutions for 36 hours prior to the bioassay resulted in no definite effect on the toxicity solutions; and it was concluded that volatility of the toxicant, while possibly a contributing factor, was not the major cause of the reduced toxicity of the aerated solutions. Those differences in toxicity noted following preaeration were inconsistent, and it was assumed that they mirrored other sources of experimental variation.

It is probable that the differences between the toxicities of aerated and unaerated solutions was due primarily to a greater toxicity of KPCP at lower dissolved oxygen concentrations. Cyanide has been shown to be more toxic to rainbow trout at lower dissolved oxygen concentrations (10), and it is probable that such a phenomenon is common with toxicants having relatively direct effects on the tissue respiration of fish.

The finding that running water solutions of KPCP had toxicities intermediate between aerated and unaerated standing water solutions

may also indicate an effect of oxygen concentration; thus, running water solutions having higher dissolved oxygen concentrations are less toxic than unaerated standing water solutions. It is suggested however that an additional factor may prove more important here than dissolved oxygen concentration. The running water solutions were constantly renewed so that the fish in these solutions were always in contact with the desired concentration of KPCP, whereas any detoxification of the standing water solutions diminished the quantity of KPCP in the solution accordingly. Other factors tending to reduce the toxicity of the solutions may have been photodecomposition and detoxification of the solutions by fish and bacteria.

As deaths were noted with concentrations of 0.25 mg/l and higher, 0.02, 0.04, 0.06, 0.10 and 0.20 mg/l were chosen as sub-lethal concentrations for the growth, starvation and exercise experiments.

A major objective of this study was to determine if exposure to sub-lethal levels of KPCP might affect the well-being of fish in terms of growth. The growth of fish is largely dependent upon the following factors: (1) the quality and quantity of food consumed, (2) the degree of absorption, and (3) the energy requirements of the fish. Experiments were conducted which measured or controlled all of these factors except absorption. Since the only food given to the fish during these experiments was tubificid worms, the quality of the food consumed was virtually constant under all experimental conditions. The tubificids fed to fish being exposed to KPCP could

have accumulated sufficient quantities of KPCP from the solutions so that the ingestion of the worms could be considered as a significant source of KPCP to the fish. It is doubtful, however, that exposure of the worms to KPCP for one or two days altered the nutritional value of the worms *per se*.

Food consumption can be determined with relative ease and accuracy, so the main problem was to evaluate the energy requirements and the degree of absorption. It is possible to assess the combined effect of absorption and energy requirements by computing gross efficiencies for the conversion of food to fish tissue. Different rates of food consumption could cause alterations in food conversion efficiency in the absence of any other variables; however, where food consumption is regulated so that it is the same in all treatments, efficiencies probably reflect energy requirements. If it is assumed that the effect of KPCP on absorption of food is small, then efficiencies vary primarily with energy requirements. Energy requirements can be measured quite accurately by using starvation experiments, where the energy utilization is unaffected by food consumption, digestion and growth costs.

It was apparent from the growth experiment data that the gain in weight and the amount of food consumed were directly related and that, in general, groups which consumed more food grew more rapidly. However, continued exposure to a KPCP concentration of 0.20 mg/l caused the growth of fish to be less in all three experiments than would have been predicted on the basis of the amount of

food consumed by these fish. This lower growth rate per gram of food intake in fish at 0.20 mg of KPCP per liter was reflected in a lower food conversion efficiency. With the exception of fish held at 0.20 mg of KPCP per liter differences in food consumption between groups accounted for most of the differences in growth.

The lower food conversion efficiency of fish in a KPCP concentration of 0.20 mg/l was thought to be due to one or more factors. The degree of utilization of the food consumed could have been altered by effects of KPCP on the digestion, absorption or assimilation of the consumed food. No attempt was made to determine if there had been any such differences in utilization of food consumed.

The action of KPCP could result in a lower P/O ratio (number of atoms of inorganic phosphate incorporated into organic phosphates/atom of oxygen consumed) due to a partial uncoupling of oxidative phosphorylation, thus requiring the utilization of more food material to produce a given amount of energy. A partial uncoupling of oxidative phosphorylation may also make increased demands on the respiratory mechanism of the fish to provide more oxygen as a compensation for the low P/O ratio.

The presence of KPCP in the environment may cause increased activity of the fish and therefore increased metabolism. The increased activity could be in response to irritation caused by the PCP or it might be due to some organs or tissues having specific increased metabolic demands. No differences in activity were noted between fish in KPCP and control fish; however, no regular observations

were made of the activity.

Exposure to KPCP produced no consistent results on total growth or food consumption of the fish. However, exposure to a concentration of 0.20 mg/l of KPCP consistently caused decreased food conversion efficiency. Food conversion efficiencies were thought to generally reflect energy requirements, so that lower efficiencies were taken to indicate increased metabolism. Exposure of fish to KPCP appeared to increase energy requirements, with the effect being especially evident at a KPCP concentration of 0.20 mg/l. Estimates of energy requirements obtained by starving fish also indicated that KPCP increased the energy requirements of the fish. An increase in energy requirements under starvation indicates increased metabolism and is independent of the effects of absorption. Food conversion efficiencies for those fish exposed to a KPCP concentration of 0.20 mg/l and energy utilization in fish starved at this concentration indicated that metabolism was increased. The energy requirements of fish starved at lower concentrations of KPCP also showed an increase over that of control fish, while the food conversion efficiencies of fish in low KPCP concentrations were not consistently lower than in controls. The finding that energy requirements of starving fish are increased in the presence of KPCP had also been obtained by Brockway (3, p. 23-47).

Low concentrations of KPCP increased the energy requirements of starving fish, although the food conversion efficiencies obtained in the growth experiments were not altered consistently.

While both total energy utilization in starving fish and food conversion efficiency in growing fish may generally reflect metabolic rate, comparisons between the two are subject to several limitations. Differences in food conversion efficiencies are not directly comparable with differences in metabolic rate because of differences in metabolic rate due to food consumption. Presumably where food consumption and food utilization are nearly the same at all treatments, the efficiency of food conversion would reflect total metabolic rate; however, comparisons between food conversion efficiencies in growing, actively feeding fish and rate of energy utilization of starving fish must be considered carefully since these are two distinctly different physiological states. In addition, the threshold effects of chemicals at low concentrations are often variable and difficult to evaluate; a given concentration of chemical may cause one effect with certain individuals or conditions and an opposite effect with other individuals or conditions.

The use of direct calorimetric data on energy utilization of starving fish is probably more dependable for the measurement of small differences in metabolic rate than a more gross and less direct method such as food conversion efficiency. The use of calorimetric methods in the study of metabolic rates appears to be of great value when comparing small differences in metabolism occurring over relatively extended periods of time and much may be gained from their use in future studies.

The swimming performance of the fish was not depressed to any large extent by KPCP under the conditions tested. It appeared that

KPCP might initially cause a slight adverse effect on swimming performance, but that after two week's exposure to KPCP fish were able to perform somewhat better than control fish. Those fish which had been exposed for 14-15 days may have developed KPCP detoxification mechanisms which enabled them to perform better in the swimming tests than those fish which had been exposed for only one or seven days. If the fish exposed to KPCP for 14-15 days are able to swim better than control fish, one explanation may be that the fish in KPCP, having an increased metabolic rate and perhaps increased activity, may be physiologically better adapted for swimming. Thus, acclimating C. bimaculatum to swimming at low velocities for periods up to 24 hours immediately prior to swimming performance tests significantly increased their ability to perform during the test (18).

After swimming, fish exposed to a KPCP concentration of 0.20 mg/l had lower ATP contents than the control fish; the lower ATP contents of the poisoned fish is due to a greater utilization of ATP during swimming and/or a lower ATP content before swimming. A greater disappearance of ATP could be caused by a slower rate of reformation of ATP during swimming. Fish exposed to a KPCP concentration of 0.20 mg/l (for 14-15 days) swam for a longer period of time than did the control fish; and this might be considered as the cause of differences in ATP; however, the fish exposed to a KPCP concentration of 0.06 mg/l swam about as long as those exposed to 0.20 mg/l while they contained about as much ATP after swimming as the control fish. It was thought that the lower ATP content in the fish exposed to 0.20 mg/l was a result of this exposure and was probably independent

of the swimming performance test.

The lower ATP concentrations in larger fish are probably related to the greater percentage of fat and skeletal material present in these larger fish.

Data on aldolase and cytochrome oxidase indicated that KPCP caused no lasting effect on the activities of these enzymes. The main effect observed was a temporary reduction of aldolase activity in fish exposed to a KPCP concentration 0.20 mg/l. Brockway (4, p.27-36) found a one-third reduction in aldolase activity and a doubling of cytochrome oxidase activity in the liver homogenates of cichlids exposed to 0.02 mg PCP per liter for 60 days.

The chronic effects of KPCP on C. bimaculatum are not particularly severe under the conditions studied. It is of interest to note that no indications of chronic toxicity of PCP were observed in rabbits (9, p. 116) (13, p. 171) and that significant tolerance to the chemical developed in rabbits (9, p. 112). There is the possibility that the increased metabolic requirements in the presence of KPCP may cause increasingly severe survival problems where food scarcity, inimical temperatures or low dissolved oxygen concentrations exist. Sub-lethal levels of KPCP or other toxicants, while not causing severe chronic poisoning, might cause greater harm to the well-being of the fish by disrupting or depleting the food supply of the fish or by accumulating in relatively high concentrations in food organisms.

Cichlasoma bimaculatum is an important experimental fish because

it is readily available and can be raised in the laboratory allowing control of environmental and genetic factors. It is desirable that more information be obtained concerning the variability and adaptability of this species.

Several questions are left unanswered by this investigation and may warrant further study. The effects of KPCP on food consumption are not known and may depend, in part, on the original condition of the fish. The exact cause of the increased metabolic requirements observed in fish exposed to KPCP is not understood. The significance and cause of differences in ATP between fish exposed to KPCP and control fish are not known. Current investigations are being conducted by several investigators to study more closely the effects of KPCP on ATP and selected enzymes in C. bimaculatum.

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APPENDICES

Appendix A. Ranges of KPCP concentration (based on flows), dissolved oxygen concentration and temperature observed in the growth experiments.

Desired levels of KPCP concentration (mg/l)	0.00	0.02	0.04	0.06	0.10	0.20
Growth exp. 1						
KPCP conc. (mg/l)	0.00	0.01-0.03	0.04	-	-	0.19-0.20
Growth exp. 2						
KPCP conc. (mg/l)	0.00	0.02	0.04	-	0.10-0.11	0.19-0.21
D.O.(mg/l)[1]	4.0 -5.0	4.0 -5.0	4.0 -5.0	-	4.0 -5.0	4.0 -5.0
Growth exp. 3						
KPCP conc. (mg/l)	0.00	-	-	0.06-0.07	0.10	0.19-0.20
D.O.(mg/l)	5.5 -7.1	-	-	5.4 -6.6	6.7- 7.7	5.5 -7.3
temp. (^{o}C)[2]	23.2 -25.2	-	-	23.1 -25.2	23.2 -25.1	23.1 -25.0

1/ D.O.s determined only during last few days of experiment.

2/ All temperatures down to 20 C on one day.

Appendix B. Ranges of KPCP concentration (based on flows), dissolved oxygen concentration, pH and temperature observed in the starvation experiments.

Desired levels of KPCP concentration (mg/l)	0.00	0.06	0.10	0.20
Starvation exp. 1				
KPCP conc. (mg/l)	0.00	0.04- 0.09	0.10	0.19- 0.20
D.O. (mg/l)	7.1 - 8.7	7.3 - 8.2	7.2 - 8.7	7.4 - 9.0
temp. (° C)	24.1 -25.0	24.1 -25.1	24.2 -25.0	24.0 -24.9
pH	7.7	-	7.8	7.8
Starvation exp. 2				
KPCP conc. (mg/l)	0.00	0.05- 0.06	0.10	0.20- 0.21
D.O. (mg/l)	8.1 - 8.4	8.2 - 8.6	8.2	8.0 - 8.3
Starvation exp. 3				
KPCP conc. (mg/l)	0.00	0.06 - 0.07	-	0.20- 0.22 1/
temp. (° C)	22.2 -25.5	-	-	-
pH	7.82- 7.85	7.82- 7.84	-	7.70- 7.80

1/ KPCP concentration 0.24 on one day.

Appendix C. Growth experiment 1; wet and dry weights, fat and ash expressed as percents of dry weight and dry weight food conversion efficiencies at indicated sample times. The weights for the initial sample are the weights of a 15 fish group and all other sample weights are for 10 fish groups.

Sample Time	KPCP Conc. (mg/l)	Initial Wet Weight (grams)	Final Wet Weight (grams)	Dry Weight (grams)	Percent Fat	Percent Ash	Food Conversion Efficiency
Day 0		14.44	14.44	3.142	9.3	16.7	
	0.00	9.80	20.27	5.011	17.9	13.5	.33
	0.00	9.79	21.92	5.520	21.6	12.9	.39
Day	0.02	9.53	20.59	5.141	20.1	13.3	.35
8	0.04	9.73	21.39	5.388	21.3	12.5	.37
	0.10	9.80	20.84	5.246	21.1	12.8	.36
	0.20	9.60	21.54	5.351	20.3	12.9	.37
	0.00	9.75	40.44	10.823	26.3	11.3	.39
	0.00	9.84	43.69	11.609	27.0	11.8	.41
Day	0.02	9.63	42.00	11.309	26.6	10.7	.39
14	0.04	9.72	46.92	12.497	26.7	11.4	.41
	0.10	9.65	41.19	11.251	25.7	11.8	.42
	0.20	9.62	43.44	11.446	27.3	12.0	.38
Day	0.00	9.81	75.82	21.392	28.8	11.6	.40
24	0.00	9.66	70.90	20.265	29.4	10.8	.41
	0.02	9.58	64.01	18.686	29.8	11.6	.38
	0.04	9.78	64.12	18.278	27.3	11.4	.37
	0.20	9.87	34.63	9.591	23.6	13.1	.30
	0.00	9.79	71.08	21.266	30.9	10.6	.33
	0.00	9.72	110.25	33.280	34.5	10.6	.40
Day	0.02	9.61	74.10	22.013	30.8	10.4	.36
32	0.04	9.72	70.89	20.529	28.6	12.0	.40
	0.20	9.71	64.51	18.405	26.5	12.4	.33

Appendix D. Growth experiment 1; food consumption in grams per 10 fish per indicated period.

Period		1st to 8th Days		9th to 14th Days		15th to 24th Days		25th to 32nd Days	
Sample Time	KPCP Conc. (mg/l)	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
	0.00	39.88	8.68	-	-	-	-	-	-
	0.00	40.32	8.66						
	0.02	40.46	8.68						
Day	0.04	40.95	8.87						
8	0.10	40.11	8.62						
	0.20	40.55	8.70						
	0.00	37.54	8.18	72.17	13.92				
	0.00	42.87	8.49	76.91	14.38				
	0.02	40.25	8.65	77.70	14.90				
Day	0.04	44.08	9.42	83.87	16.06				
14	0.10	41.12	7.80	72.21	14.16				
	0.20	41.35	8.44	79.50	16.27				
	0.00	51.06	10.96	72.03	13.86	131.88	23.91		
	0.00	39.36	8.43	74.16	13.83	121.98	22.01		
	0.02	41.18	8.82	71.53	13.74	103.85	21.65		
Day	0.04	42.55	9.16	77.64	15.32	102.02	18.68		
24	0.20	35.31	7.71	45.48	10.27	36.58	7.01		
	0.00	37.13	8.12	100.54	20.57	81.90	16.78	112.03	19.79
	0.00	42.00	9.02	80.46	15.37	129.11	23.25	158.55	29.92
	0.02	39.73	7.49	64.86	12.89	75.51	18.55	89.09	17.00
Day	0.04	43.47	8.73	69.51	13.41	72.26	13.59	61.24	10.51
32	0.20	40.23	8.64	63.60	13.03	73.22	13.88	70.35	13.68

Appendix E. Growth experiment 2; wet and dry weights, fat and ash expressed as percents of dry weight and dry weight food conversion efficiencies at indicated sample times. The weights for the initial sample are the weights of a 16 fish group and all other sample weights are for eight fish groups.

Sample Time	KPCP Conc. (mg/l)	Initial Wet Weight (grams)	Final Wet Weight (grams)	Final Dry Weight (grams)	Percent Fat	Percent Ash	Food Conversion Efficiency
Day 0	-	14.85	14.77	3.672	27.2	10.5	-
	0.00	7.44	13.74	4.01	32.0	9.4	.44
	0.00	7.38	16.22 1/	4.69	30.9	9.6	.48
	0.02	7.42	15.30	4.44	28.8	9.4	.42
Day	0.04	7.42	14.48	4.28	31.6	9.6	.42
11	0.10	7.38	15.20	4.40	30.9	9.7	.47
	0.20	7.40	12.73	3.75	27.5	10.4	.34
	0.00	7.45	22.93	6.724	30.0	9.9	.43
	0.00	7.42	27.45	8.035	31.1	9.6	.45
	0.02	7.40	26.38	7.751	31.9	9.6	.43
Day	0.04	7.47	24.90	7.233	30.1	10.0	.42
22	0.10	7.46	24.20	7.049	31.7	9.8	.42
	0.20	7.47	22.92	6.450	28.8	10.4	.36
	0.00	7.38	42.78	12.99	33.0	9.9	.42
	0.00	7.38	38.16	11.43	32.0	9.0	.44
	0.02	7.41	45.68	13.70	31.4	9.7	.43
Day	0.04	7.33	37.36	11.07	31.0	9.7	.40
32	0.10	7.42	41.81	12.44	31.0	9.8	.42
	0.20	7.36	37.89	10.64	28.7	11.5	.34
	0.00	7.37	53.88	17.66	32.8	9.8	.45
	0.00	7.44	58.61	19.07	33.4	10.0	.46
	0.02	7.34	89.83	29.44	35.1	9.5	.44
Day	0.04	7.45	56.50	19.44	33.4	10.2	.46
42	0.10	7.37	79.68	25.68	35.3	10.3	.45
	0.20	7.37	66.71	20.36	27.3	11.8	.37

1/ Seven fish sample with data adjusted to eight fish by multiplying by 8/7.

Appendix F. Growth experiment 2; food consumption in grams per eight fish per indicated period.

Sample Time	KPCP Conc. (mg/l)	1st to 11th Days Wet	1st to 11th Days Dry	12th to 22nd Days Wet	12th to 22nd Days Dry	23rd to 32nd Days Wet	23rd to 32nd Days Dry	33rd to 42nd Days Wet	33rd to 42nd Days Dry
	0.00	23.21 1/	4.94 1/						
	0.00	32.84 1/	5.94 1/						
	0.02	30.20	6.14						
Day	0.04	27.10	5.74						
11	0.10	26.24	5.40						
	0.20	25.00	5.51						
	0.00	22.77	4.97	36.83	6.39				
	0.00	33.76	5.95	46.13	7.82				
	0.02	31.41	6.36	42.35	7.40				
Day	0.04	29.52	5.97	37.21	6.93				
22	0.10	28.94	5.92	38.62	6.53				
	0.20	21.98	4.92	44.21	7.92				
	0.00	24.90	5.28	46.56	8.10	78.88	13.49		
	0.00	23.28	4.62	40.36	6.72	61.52	10.32		
	0.02	36.35	6.36	51.61	9.03	73.80	12.37		
Day	0.04	26.50	5.59	35.58	6.75	64.49	10.86		
32	0.10	28.37	5.73	42.88	7.49	68.54	12.02		
	0.20	24.43	5.26	43.78	7.87	70.91	12.64		
	0.00	26.82	5.62	39.24	7.27	57.07	9.74	74.42	12.89
	0.00	30.76	5.98	44.09	7.41	57.63	9.52	83.25	14.27
	0.02	38.30	6.60	67.41	11.91	100.17	17.05	153.66	26.93
Day	0.04	31.71	6.59	35.58	6.75	61.46	10.68	79.43	13.96
42	0.10	32.71	6.56	42.88	7.49	89.59	15.45	130.89	22.98
	0.20	24.43	5.26	43.78	7.87	84.71	14.54	104.64	18.57

1/ There were only seven fish in this section, therefore the data have been adjusted to eight fish by multiplying by 8/7.

Appendix G. Growth experiment 3; wet and dry weights, fat and ash expressed as percents of dry weight, and dry weight food conversion efficiencies at indicated sample times. The weights for the initial sample are the weights of a 14 fish group and all other sample weights are for eight fish groups.

Sample Time	KPCP Conc. (mg/l)	Initial Wet Weight (grams)	Sample Wet Weight (grams)	Sample Dry Weight (grams)	Per-cent Fat	Per-cent Phos. Lipid	Per-cent Ash	Food Conversion Efficiency
Day 0		14.93	14.63	3.621	25.9	16.3	11.6	
	0.00	8.46	12.92	3.242	27.0	21.1	10.7	.39
	0.06	8.78	13.18	3.259	27.0	17.5	11.2	.36
Day	0.10	8.76	13.31	3.218	27.8	17.6	10.9	.36
10	0.20	8.55	11.66	2.815	23.2	21.4	11.9	.23
	0.00	8.63	21.34	5.493	28.0	19.7	10.8	.43
	0.06	8.74	21.80	5.641	28.3	21.2	10.5	.45
Day	0.10	8.72	21.25	5.418	27.8	19.3	10.7	.42
20	0.20	8.59	19.53	4.884	26.3	20.2	11.4	.35
	0.00	8.48	35.37	9.236	29.7	18.0	10.7	.42
	0.06	8.70	35.07	9.395	29.0	19.1	10.6	.43
Day	0.10	8.73	34.69	9.372	29.7	17.2	10.8	.43
30	0.20	8.57	30.84	8.385	29.3	17.4	11.4	.37

Appendix H. Growth experiment 3; food consumption in grams per eight fish per indicated period.

Sample Time	KPCP Conc. (mg/l)	1st to 9th Days		10th to 18th Days		19th to 29th Days	
		Wet	Dry	Wet	Dry	Wet	Dry
	0.00	15.73	2.74	-	-	-	-
	0.06	15.98	2.79	-	-	-	-
Day	0.10	15.55	2.70	-	-	-	-
10	0.20	15.77	2.77	-	-	-	-
	0.00	16.02	2.79	29.25	4.84	-	-
	0.06	16.00	2.79	29.25	4.84	-	-
Day	0.10	15.84	2.76	29.25	4.84	-	-
20	0.20	16.03	2.80	29.25	4.84	-	-
	0.00	16.01	2.79	29.25	4.84	50.50	8.69
	0.06	15.75	2.74	29.25	4.84	50.50	8.69
Day	0.10	16.04	2.80	29.25	4.84	50.50	8.69
30	0.20	16.01	2.79	29.25	4.84	50.50	8.69

Appendix I. Starvation experiment 1; wet and dry weights, fat and ash expressed as percents of dry weight, and calories per gram of dry tissue. All sample weights are for seven fish groups.

Sample Time	KPCP Conc. (mg/l)	Initial Wet Weight (grams)	Final Wet Weight (grams)	Final Dry Weight (grams)	Per-cent Fat	Per-cent Phos. Lipid	Per-cent Ash	Cal./ Gram Dry Weight
Day 0	-	19.91	19.81	4.788	23.2	22.4	13.5	5,555
	0.00	20.15	18.93	4.697	21.9	17.2	15.1	5,283
	0.06	19.99	18.96	4.971	21.5	16.7	15.2	5,293
Day	0.10	21.34	20.02	4.878	21.3	17.8	15.7	5,360
10	0.20	20.84	19.24	4.326	20.4	16.9	15.7	5,193
	0.00	19.18	17.63	4.212	19.8	17.7	15.7	5,243
	0.06	19.20	17.97	4.192	19.3	20.6	16.5	5,097
Day	0.10	19.66	18.09	4.189	18.5	20.1	16.4	5,158
20	0.20	19.07	17.00	3.984	17.3	17.7	17.7	4,955
	0.00	20.09	17.89	3.990	15.9	18.3	17.9	4,962
	0.06	19.65	17.41	3.788	14.1	21.4	18.1	4,802
Day	0.10	19.88	17.88	3.964	14.7	19.1	18.1	4,863
30	0.20	20.34	17.68	3.843	13.9	19.8	18.9	4,727

Appendix J. Starvation experiment 2; wet and dry weights, fat and ash expressed as percents of dry weight and calories per gram of dry tissue. All sample weights are for six fish groups.

Sample Time	KPCP Conc. (mg/l)	Initial Wet Weight (grams)	Final Wet Weight (grams)	Final Dry Weight (grams)	Per-cent Fat	Per-cent Phos. Lipid	Per-cent Ash	Cal./ Gram Dry Weight
	-	13.48	13.48	3.601				
Day	-	12.96	12.99	3.499	27.2	20.6	10.4	5,802
0	-	12.62	12.62	3.299				
	-	12.19	12.22	3.278				
	0.00	12.74	12.24	3.083	26.8	19.5	11.8	5,675
	0.00	12.83	12.24	3.129				
	0.06	13.16	12.15	3.090	27.3	20.3	11.7	5,661
	0.06	13.02	11.99	3.133				
Day	0.10	12.29	11.72	2.899	25.9	20.7	11.9	5,609
10	0.10	12.43	11.94	2.947				
	0.20	13.13	12.20	2.993	26.2	20.8	12.6	5,564
	0.20	12.95	11.74	2.937				
	0.00	12.47	11.46	2.830	24.3	17.3	13.3	5,511
	0.00	13.04	12.03	2.972				
	0.06	13.21	11.87	2.971	24.8	16.2	13.3	5,464
	0.06	13.08	11.75	2.871				
Day	0.10	12.89	11.74	2.853	24.9	17.6	13.5	5,451
20	0.10	12.38	11.16	2.751				
	0.20	13.14	11.53	2.729	23.5	20.2	13.9	5,415
	0.20	11.36 1/	10.02	2.358				

1/ There were only five fish in this sample.

Appendix K. Starvation experiment 3; wet and dry weights, fat and ash expressed as percents of dry weight, and calories per gram of dry tissue. All sample weights represent six fish.

Sample Time	KPCP Conc. (mg/l)	Initial Wet Weight (grams)	Final Wet Weight (grams)	Final Dry Weight (grams)	Percent Fat	Percent Phos. Lipid	Percent Ash	Cal./ Gram Dry Weight
Day 0	-	11.54	11.47	2.808	22.5	22.4	13.5	5,329
	0.00	11.63	10.40	2.484	20.6	-	16.3	5,121
Day	0.06	9.63	8.50	2.002	21.4	23.1	15.9	5,167
7	0.20	9.60	8.45	1.985	20.1	21.6	17.3	5,045
	0.00	11.23	9.49	2.147	16.7	16.7	19.2	4,766
Day	0.06	11.46	9.54	2.083	16.1	-	19.2	4,678
14	0.20	10.19	8.40	1.854	15.3	-	19.8	4,709