AN ABSTRACT OF THE THESIS OF

Christina M. Rohm for the degree of Master of Science in the Department of Fisheries and Wildlife presented on July 12, 1985

Title: The Effects of Copper on Predator-Prey Interactions of Fathead Minnows (*Pimephales promelas*) and *Daphnia pulex*

Abstract approved: Signature redacted for privacy.
Richard A. Tubb

Total copper concentrations of 10 μg/l to 100 μg/l appeared to alter the predator-prey interactions of fathead minnows (*Pimephales promelas*) and *Daphnia pulex*. The effect of copper concentration on the total amounts of *D. pulex* consumed over a 12-h period varied with the density of prey stocked hourly in the aquaria. Four prey levels of 5, 10, 20 and 30 *D. pulex* per 20 l aquarium were used and control consumption rates ranged from 0.9% to 4.3% of dry minnow body weight. The functional consumption response to prey density shifted from an hyperbolic-Type II toward a sigmoidal-Type III shape and the predators became more efficient at obtaining prey at lower densities as copper concentration increased. Consumption was slightly depressed at intermediate levels of copper and prey density, it was greatly enhanced at high levels of both. Mechanistic components of the functional response, total time spent searching and average time spent in pursuit of a prey item, showed an opposite pattern of response. At intermediate levels of copper concentration and prey density the total

time spent searching and average time spent in pursuit increased while capture success and prey consumption fell slightly relative to controls; at high levels of copper and prey the total searching time and average time spent in pursuit decreased while capture success and prey consumption increased. Significant ($P < 0.05$) increases were observed in predator reactive distance with increased copper dose and prey density held constant. An assay of prey activity levels showed at least a 50% reduction in gross swimming activity of _D. pulex_ at all dose levels of copper. The effects of copper on the mechanistic components could not be used to predict the effect of copper on the functional response but indicated the shift in functional response was due to the differential susceptibility of predator and prey to copper and effects of copper on fish appetite.

THE EFFECTS OF COPPER ON PREDATOR-PREY INTERACTIONS OF FATHEAD MINNOWS (PIMEPHALES PROMELAS) AND DAPHNIA PULEX

by

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INTRODUCTION

The escalating development, production and disposal of potentially toxic substances poses important problems to aquatic toxicologists. To assess the risk posed to environmental health these substances must be identified with respect to their potentials for and modes of action in altering natural systems (Sanders, 1976; Maciorowski *et al.*, 1981). The traditional approach to defining "safe" levels of these substances in the aquatic environment has been to conduct single species bioassays and describe the effect of graded concentrations on organismal responses such as survival, reproduction or development. More recently tests have been developed to measure such physiological responses as oxygen consumption, swimming performance, activity, avoidance, cough reflex and rheotaxis in fishes (Morgan, 1977).

Single species assays represent a beginning toward a basis for hazard assessment (Cairns, 1983, 1984), but the individual organism is only one part of a complex functioning community in nature. A toxicant may affect "higher order" processes such as competition and predation that have a vital role in determing the species diversity and trophic structure, and the inherent stability and resiliancy of communities through time (NAS, 1981). The testing of responses of multispecies assemblages has been an important development in detecting the potentials of various substances for altering these higher order processes, if not to predict their direct effects in the natural environment (Hammons, 1981).

The importance of fish predation on zooplankton to the structure of aquatic communities (Brooks and Dodson, 1965; Hall *et al.*, 1976), the suitability of both types of organisms for laboratory culture and assay and the sensitivity of the behavioral measurements involved have made fish-zooplankton predation a good candidate for multispecies assays (Giddings, 1981). Most studies have been designed to either describe effects on specific mechanisms in the interaction or the effect on the overall ability of the fish predator to obtain prey. Studies on mechanisms such as prey searching (Ware, 1972), reactive distance (Ware, 1973; Lueke and O'Brien, 1981) and handling time (Werner, 1974) require extensive observation and replication but yield precise repeatable measures. Assays involving counting the numbers of prey consumed after exposing the prey (Baker and Modde, 1977; Farr, 1977; Goodyear, 1972; Sullivan *et al.*, 1978) or predator and prey (Tagatz, 1976; Farr, 1978) generally require less intense observation and focus instead on the total cumulation of effects on undefined mechanisms in the interactions.

Predator-prey studies have helped to identify tools that may be used to screen for potential effects of substances on natural communities. A major shortcoming of many existing predator-prey bioassays is the inability to use the results to generalize and predict specific effects in natural systems (Giddings, 1981; Geckler *et al.*, 1976). Designing an assay compatible with current models of predation in natural systems may bring results one step closer to this goal and aid in understanding the mode of action of a toxicant. An example of such an effort is Woltering's (1976) study of effects of ammonia on growth and predation rates at various levels of prey density. He evaluated effects on these rates as a function of prey density in the context of the "functional response" model, as coined by Solomon (1949). Examining toxicant induced changes in these rates as a function of prey density elucidated sub-lethal behavioral effects that would relate and perhaps lead to different results in natural systems depending on their trophic structure.

This study was designed to examine the effects of graded concentrations of a toxicant on predator-prey interactions in the context of the "functional response" model. The objectives were to not only determine the effects on the predator's overall effectiveness in obtaining prey, as measured by consumption rates, but to quantify effects on some specific mechanistic components in the model as theorized by Holling (1965): predator search time, reactive distance, pursuit time, handling time, and activity levels of predator and prey. Analysis of measurements on the cumulative total of all effects on undefined behaviors of predator and prey at various prey densities (functional response) as well as specifically defined behaviors (mechanistic response) should lead to a better understanding of the effect of copper on this particular interaction. The sensitivity of the measures should yield an indication of the potential of the toxicant to affect a natural system and whether those effects might differ depending on the availability of prey.

The fathead minnow (Pimephales promelas) and Daphnia pulex were chosen as the predator and prey organisms. Both species are easily cultured in the laboratory and are routinely used to assess both acute and chronic toxicity of compounds (APHA *et al.*, 1980; EPA Bioassay Committee, 1971). In addition, correlation between lethal levels of many toxicants to fathead minnows and *D. pulex* suggests that when tested together there will exist a fairly broad range of sublethal concentrations where toxic effects may be seen in both species (Maki, 1979). That *D. pulex* is frequently a principal prey item of naturally occurring fathead minnows (Held and Peterka, 1974; Lynch, 1979) leant realistic appeal to the choice of this species pair for testing.

Copper was chosen as the toxicant in this study both because of its prevalence as an environmental toxicant and its ease of handling and analysis in the laboratory. Copper occurs in toxic concentrations in the aquatic environment both from natural sources (Hutchinson, 1957) and as a product of agricultural (Owen, 1981), mining (Hallowell *et al.*, 1973) and industrial fabrication (Dean *et al.*, 1972) activities. Copper toxicity is influenced by the metals and anions associated with it (Doudoroff and Katz, 1953) and the hardness of the receiving water (Stiff, 1971; McCrady and Chapman, 1979). Its effect on survival and reproduction on *Daphnia* species (Biesinger and Christensen, 1972; Winner and Farrell, 1976) and *P. promelas* (Mount and Stephan, 1969; Mount, 1968; Pickering *et al.*, 1977) has been well documented. Many sublethal behavioral and physiological effects have also been reported (Sprague, 1964; Kleerekoper *et al.*, 1972; Waiwood and Beamish, 1978; Sellers *et al.*, 1975).

MATERIALS AND METHODS

The 3- to 7-month-old minnows used in this study were obtained from a culture facility on the premises (USEPA Western Fish Toxicology Station, Corvallis, OR). They were cultured in well water in flow-through tanks at 20°C and fed brine shrimp and *Daphnia pulex*. The 4-day-old *Daphnia pulex* were also obtained from an on-site facility where they were cultured at 20°C in 3-liter glass jugs containing well water adjusted to 100 μg/l $CaCO_3$-hardness (Appendix I). The culture water was changed 3 times weekly and the *Daphnia* were fed *Selanastrum capricornutum* at a rate of 2.5 g dry weight daily per jug (Goulden *et al*., 1982). The algae were cultured in a Woods Hole MBL medium (Nichols, 1973) which was slightly modified and supplmented with a double strength vitamin mixture for cladoceran nutrition (L. Provasoli *in* Goulden *et al*., 1982; S. Dominguez, personal communication; Appendices II-III).

Four-day-old *D. pulex* were obtained by culturing neonates isolated through a selective straining process (Goulden *et al*., 1982). Pure adult reproductive organisms were separated from mixed age(size)-class cultures by pouring the contents through a 1.0-mm mesh basket nested inside a 0.2-mm mesh basket. Adults cultured from the first basket were again strained 24 h later leaving their neonate offspring of 0- to 24-h-age. This enabled the use of prey items of equal size, a factor shown to be important in prey selection (Ware, 1972; Werner, 1974). The 4-day-old age maximized the size and ease of visibility while precluding reproductive capability. Work with *Daphnia magna* of this age has indicated sensitivity similar to that of neonates (<24-h old) exposed to various metals (Nebeker et al., in prep).

All consumption data were corrected for differences due to fish size and are reported as percent of dry body weight consumed (after Woltering, 1976). Wet to dry weight conversion factors were develoepd for the fish and Daphnia dry weights were determined for this purpose. Thirty-three minnows were used to develop the regression equation: $Y_{\text{dry weight in mg}} = 0.0007 + [0.1953 \times (x = \text{wet weight in mg})]$ the associated r^2 value was equal to 0.89. A total of 5,360 Daphnia in 15 samples were desiccated and yielded an average dry weight of 1.08×10^{-5} mg per individual with a standard deviation of 0.24×10^{-5}.

All predator-prey interactions were studied in eight all-glass 20-l aquaria. Each aquarium was surrounded with black plastic sheeting and drapes to eliminate visual disturbances. Aquaria were fitted with 2-way-mirrored plastic film on one end to facilitate observation of the fish without introducing exterior movement, to which the fish responded. The film did not reflect images distinctly nor did the fish appear to be disturbed by reflections during pilot experiments. Each aquarium was further modified to facilitate the quantification of observations. Sixteen 1.25-l quadrants measuring 12.5 cm wide by 10 cm deep and high, were delineated in each tank by applying dark-grey plastic tape 0.2 cm wide to the outside of each tank. This provided a double layer of 2 cells by 4 cells that was used in some of the mechanistic measurements (Figure 1).

The water for all tests was drawn from the well in 303-l batches and stored in a covered Nalgene tank. The quality of this well water

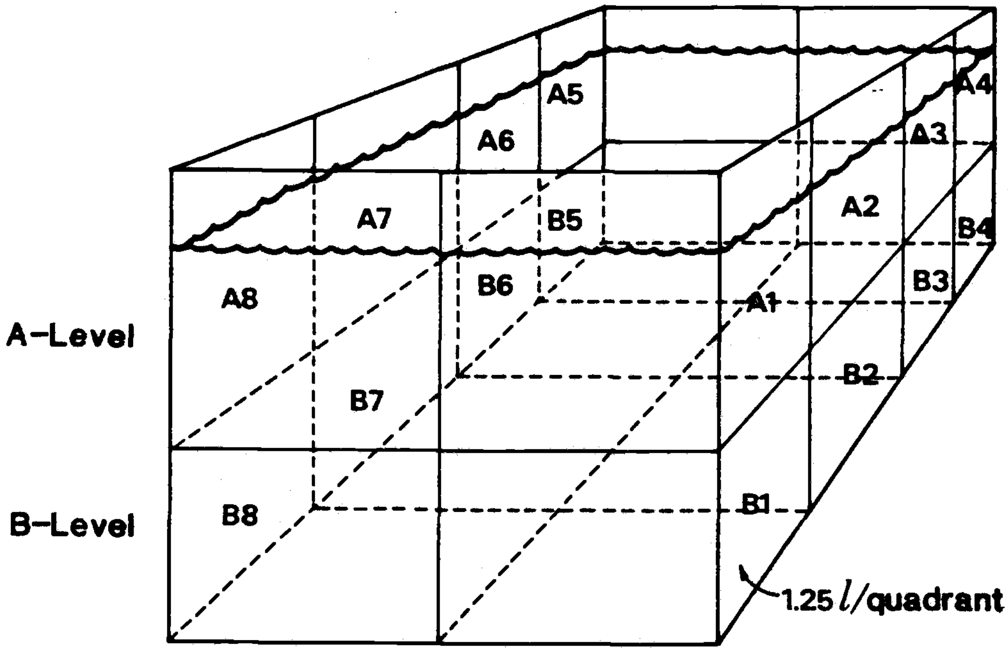


Figure 1. Partitioning and quadrant designation of aquaria (16 quadrants).

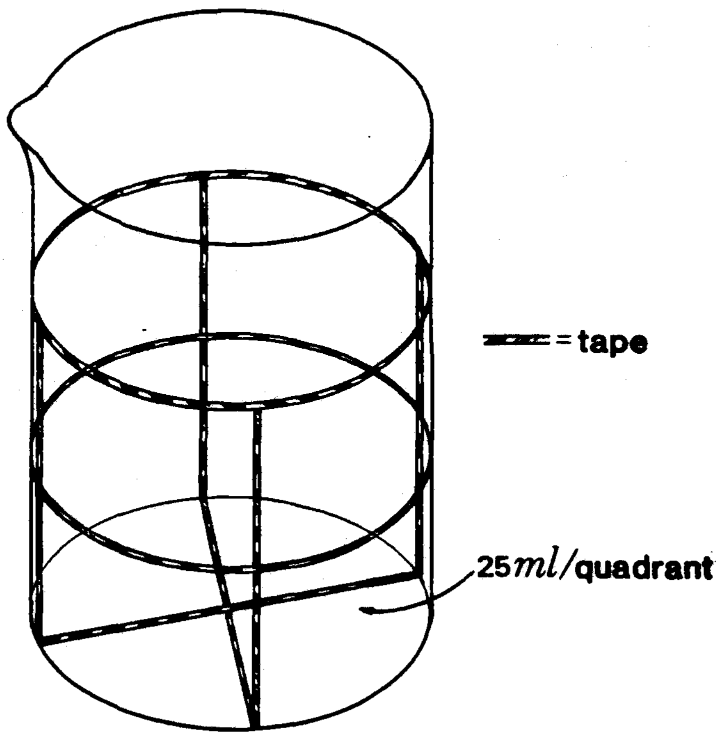


Figure 2. Partitioning of test chambers for Daphnia activity level experiments (8 quadrants).

was frequently monitored and specific characteristics can be found elsewhere (Samuelson, 1971; Chapman 1978). Each 303-l batch was analyzed for hardness by EDTA titrimetric method (APHA et al., 1980). Reagent grade $CaSO_4 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, $NaHCO_3$ and $KHCO_3$ were added as prescribed by G.A. Chapman based on results of Marking and Dawson (1973) to achieve a hardness of 75.0 mg/l as $CaCO_3$ for each batch (Appendix I). All water used was aerated and maintained at 20° ± 1°C. The photoperiod was regulated at 16 h-light and 8 h-dark, the cycle used by the minnow culture facility throughout the duration of the study. Illumination consisted of two 34-watt GE F40LW-RSWMII fluorescent bulbs mounted end-to-end one meter above the aquaria and provided an intensity of 50 ± 5 ft-c at the water surface.

All tests performed were static and copper was added and mixed into the aquaria by hand. Appropriate volumes of a 1.0-mg/l copper spike solution were added to each tank by disposable plastic pipette and stirred slowly for 20 sec. The spike solution was prepared from reagent grade $CuCl_2 \cdot H_2O$ and distilled water and acidified by the addition of 0.1 ml of concentrated nitric acid per liter of stock solution (Chapman, 1978). Ten-ml samples for verification of copper concentration in each tank were drawn at the termination of each experiment, placed in 10-ml capped plastic culture tubes and acidified with 10 µl of concentrated nitric acid. Metal levels were measured by carbon furnace atomic absorption spectrophotometry (U.S. Environmental Protection Agency, 1979).

The predatory behavior of 56 fathead minnows was studied to determine the effect of copper on "functional response", the consumption of prey as a function of prey density. Four prey density levels were used: 5, 10, 20 and 30 D. pulex per 20-l aquarium. Preliminary control experiments indicated that at densities of between 5 and 10 D. pulex per tank a fish could locate and consume all individuals in one hour. At densities of 30 per tank, many Daphnia were left after one hour of feeding; 30 per tank was also determined as the maximum number that could be effectively counted with certainty. Four dose-levels of copper were used: 0, 10, 32 and 100 μg/l. This sequence follows a logarithmic progression from the control level to a maximum level approximately 67% of the estimated 96-h LC50 of the fathead and extending slightly above the 48-h LC50 of Daphnia pulex.

Eight tanks were observed per time period. The procedure was repeated 7 times and treatments were randomly assigned to tanks with the following constraints: that there exist a minimum of 3 replicates at each prey density/copper concentration combination and that one particular combination of these be included each time the procedure was repeated as an "internal monitor" (after Woltering, 1976). The treatment of 10 D. pulex/tank and 32 μg/l of copper was chosen as the internal monitor and observations from these tanks indicated that no trends in responses occurred as a function of the calender date of the trial.

Three-hundred-ml water samples were withdrawn by siphon from each of the test tanks and placed in B.O.D. bottles just prior to the start and when each trial was terminated. They were analyzed directly after collection for: dissolved oxygen content, pH, alkalinity, and hardness according to standard procedures (APHA et al., 1980). Temperature in each tank was also measured at these times.

Fish were transferred from the culture facility to a static holding tank and fed ad libitum on 2- to 5-day-old D. pulex for 48 h prior to the start to facilitate accomodation and initiation of prey searching behavior. In the evening 12 h prior to the start of the experiment fish were placed in individual test tanks with approximately 30 prey items to continue eliciting searching behavior. Preliminary experiments indicated that starving fish prior to observations (Ware, 1972) produced extremely variable results in eating behavior in P. promelas, often causing cessation of feeding for long periods in some individuals, particularly at low prey densities.

At 0800 the following morning the experiment began. At this time 4-day-old Daphnia were introduced to each aquarium at the pre-assigned densities with a blunted plastic-tipped pipette randomly over the water surface. Every hour thereafter for 12 h the numbers of Daphnia remaining were counted, recorded and supplemented to achieve assigned density levels. This constituted the control portion of the experiment.

At the end of this trial the fish were again fed ad libitum for 36 h before the treatment phase of the test began. After 24 h (and 12 h prior to resumption of the test) the copper was introduced at the pre-assigned levels. The observations resumed at 0800 the following morning; the counting and supplementing proceeded for the next 12 h to complete the treatment phase. Fish were starved for the next 20 h and then wet weights and total lengths were measured. The dry weight of each fish was calculated, using the conversion function, as well as the dry weight of Daphnia consumed by that fish for each hour. All consumption data were then expressed as the dry weight of Daphnia consumed as a percent of the final dry body weight of the minnow for each 1-h observation period and for each 12-h phase.

Another 56 fathead minnows were studied to determine the effects of prey density and copper concentration on search time, pursuit time, handling time, resting time and fish activity levels. Search time was defined by Werner and Hall (1974) as "how long it takes to find [the given]...prey whether they are eaten or not" and was measured as all of the time spent swimming that did not fall into pursuit or handling categories. Pursuit time was measured as that time after the predator located the prey item and while it darted toward it culminating in either a successful or unsuccessful capture (Ware, 1972). Handling time was defined as "the time delay in searching once a prey is captured" and was measured as "the time from seizure until the prey [is] swallowed" (Werner, 1974). Resting time was measured as all the time the fish spent in a stationary non-swimming (not moving more than 1/2 a body length in a second) state. Activity level measurements consisted of: average time spent swimming and resting, the total number of quadrant changes made and swimming speed (number of quadrant changes/total time spent swimming).

Prey density levels and copper concentrations were the same as those used in the functional response experiments. Treatments were also randomly assigned to the tanks in the same way. The experimental procedure was repeated 7 times to yield a minimum of 3 replicates and an internal monitor. Holding, feeding and accomodating procedures were the same as those outlined in the functional response experiments. Fish were fed *ad libitum* until 0930 when prey levels were adjusted to assigned treatment levels and observations began. The same standard water quality measurements were made 1 h prior to this time and at the termination of the experiment.

Beginning at 0930 each of the tanks was observed for 15.0 min. The observed behavior of each fish, as divided into the previously defined categories, was recorded by speaking into a portable cassette voice recorder. These tapes were subsequently transcribed to recording paper with the times associated with various behaviors, measured with a stop clock, recorded. This procedure was repeated for each tank again at 1330.

Following each block of categorized behavior observations, at 1130 and 1530, each tank was observed for 5 min to note the general activity levels of each fish. The quadrant position of the fish and whether it was swimming or resting was noted on the recording tape. The 16 sectors marked out with grey tape were given the designations A1-A8 and B1-B8 for this purpose (Figure 1). The observations were also recorded on voice-tape and transcribed to the notebook with the associated measured durations.

Feeding, copper dosing, sample extraction for copper concentration verification and water quality sampling all took place as described previously. Observations were repeated in the above manner 48 h after the start of the control phase of the experiment and 12 h after the copper dosing. Fish were starved 20 h after the termination of the test and wet weights and lengths were measured.

Thirty-two minnows were used to study the effect of copper concentration on reactive distance. Reactive distance was defined and measured as "the distance between predator and prey when the [fish] finally darted toward the food object" (Ware, 1972). Prey density was held constant at approximately 10 Daphnia/tank. Treatments for each experiment were assigned randomly to the eight tanks and consisted of 2 replicates at each of the same four copper treatment levels: 0, 10, 32 and 100 μg/l. The experiment was repeated 4 times yielding a total of 8 replicates per copper treatment.

Much preliminary investigation revealed that confining fish to small troughs or partitioning them in small spaces, as in Werner and Hall (1974), resulted in inhibition of normal searching and feeding behavior regardless of the amount of time they were left to habituate or the degree to which they were starved. It was found that normal searching and feeding behavior occurred when the dimension of the interaction was reduced vertically by lowering the water depth in the tanks to 5 cm and allowing the fish to habituate to this situation for 10 days. Reactive distance was gauged after this period using a cm grid underneath each tank. A similar procedure was performed by Ware (1972). Minnows were fed juvenile Daphnia during the habituation period and the water was changed every 2 days to keep loading factors within acceptable limits (Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975).

The dosing and water sampling procedures were the same as described in previous sections. Reactive distance observations began at 0930 and lasted 15 min per tank, they resumed again at 1330, again for 15 min per tank and the results were pooled. Forty-eight h after the start of control observations treatment observations were made in the same manner. Wet weights and lengths were measured 20 h after the termination of the observations.

Eighty D. pulex were used to determine the effect of copper on prey activity levels. The design consisted of 5 levels of copper (0, 10, 32, 100 and 320 μg/l) with 4 replicates at each treatment concentration. The treatments were assigned randomly to the test beakers for each of the 4 times the experiment was repeated yielding a grand total of 16 replicates per treatment. The procedures followed were those developed by G.A. Chapman, S. Ota and F. Recht (MS) in accordance with the recommendations of the Committee on Methods for Toxicity Test with Aquatic Organisms (1975) for acute static 48-h tests with D. magna. Exceptions were: that only one organism was used in every test chamber to facilitate behavioral observations, and that organisms were 4-day-old D. pulex rather than 1-day-old D. magna.

Test chambers consisted of 250-ml glass beakers marked into eight quadrants of equal volume by the application of grey plastic tape to the outside of the beakers (Figure 2). One liter of each concentration of test solution was prepared and 200 ml poured into each test chamber. Daphnia were introduced with a blunt-tipped pipette into beakers arranged in random order with respect to treatments and beakers were covered with a glass plate to exclude foreign material. Water preparation, light regime and intensity, temperature and copper concentration verification were the same as in the previous sections. The control and 320 µg/l treatment samples were analyzed for D.O., pH, alkalinity, hardness and temperature. At no time were there substantial differences between the two extreme treatment samples so no other treatments were sampled.

Behavior observations took place immediately after the test was assembled at 0930 and at 1330. The daphnid in each beaker was observed for 5 min and the number of quadrant changes it made was tabulated. Beakers were observed in the order in which they occurred on the laboratory bench.

The mean values of the routine measures of dissolved oxygen, hardness, pH, alkalinity, temperature and total copper in the test chambers appear in Table 1. In all tests dissolved oxygen levels were within 80% of saturation. The average precision for all copper samples analyzed was 7.65%, expressed as relative standard deviation. The average spike recovery was 97.3%; values reported were not adjusted for spike recovery.

Table 1. Dissolved oxygen, hardness, pH, alkalinity, temperature and total copper concentration for each set of experiments. Non-copper values are means of all test chambers measured twice daily in both control and treatment phases. Copper values are means of all test chambers measured at the termination of each test. Parentheses enclose one standard deviation.

Experiment	Dissolved oxygen (mg/l	Hardness (mg/l)	pH	Alkalinity (mg/l)	Temperature (°C)	Nominal Copper concentration (μg/l)			
						10	32	100	320
Functional Response	8.82±(0.64)	73.8±(2.8)	7.54±(0.40)	60.5±(2.2)	18.0±(0.5)	8.1±(2.4)	25.5±(9.3)	86.3±(28.0)	
Mechanistic Response	8.74±(0.20)	74.7±(0.7)	8.00±(0.26)	62.3±(3.6)	19.0±(0.3)	11.1±(2.4)	33.9±(3.1)	155.8±(80.6)	
Reactive Distance	9.07±(0.40)	74.7±(1.2)	7.96±(0.09)	65.5±(4.1)	19.9±(0.3)	7.4±(1.4)	24.1±(3.2)	84.1±(21.9)	
Daphnia Activity	8.72±(0.08)	75.0±(0.6)	8.09±(0.18)	60.7±(1.5)	20.5±(0.2)	10.4±(3.5)	39.0±(5.7)	102.9±(17.2)	303.8±(37.8)

RESULTS

Mean cumulative consumption after 12 h ranged from 0.9% of the dry weight of the predator at the lowest (5/tank/h) prey density to 4.3% at the highest (30/tank/h) density in the control phase. The plot of the consmption functions over these prey densities illustrates a clear linear response, or constant consumption rate, with time (Figure 3). The consumption functions for 10/tank/h and 20/tank/h prey densities also showed the same constant rates and mean cumulative consumption values after 12 h were 2.0% and 4.1% of dry body weight respectively (Appendix IV). Actual feeding rates remained relatively constant for the duration of the control phase at each prey level. Feeding rates increased with increased prey density and variability between individual responses within each prey level group increased significantly ($P < 0.05$) as prey density increased.

The mean amount of prey consumed, plotted as a function of the prey density, the "functional response" as described by Solomon (1949), shows hyperbolic "Type II" form (Figure 4). This is similar to fish feeding responses observed by other investigators (summarized in Holling, 1965; Ware, 1972; Peterman and Gatto, 1978).

After the systems were dosed with copper the mean cumulative consumption functions remained linear but the slopes were altered. There appeared to be a slight decline in feeding rate at the 10 µg/l copper and 10, 20 and 30 prey density levels and at the 32 µg/l copper and 30 prey level relative to the control phase rates for those prey

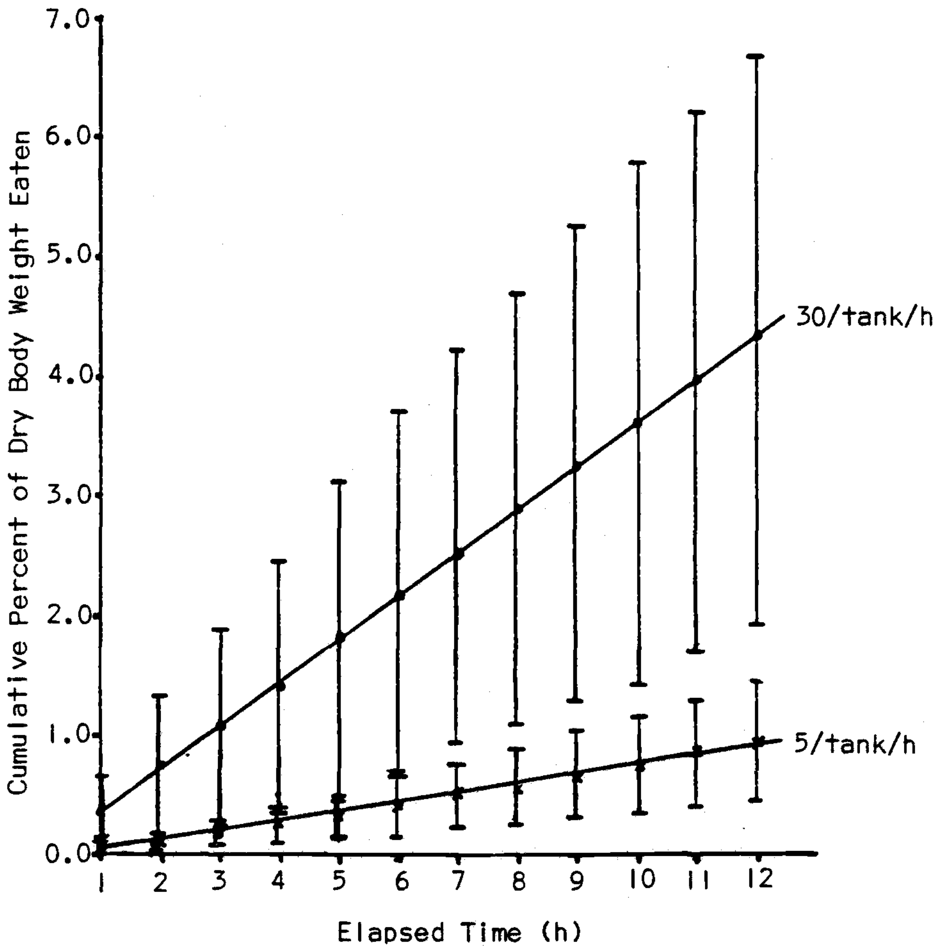


Figure 3. Cumulative percent of dry body weight eaten as a function of elapsed time (5/tank/h and 30/tank/h) for the control phase. Values represent mean responses of all fish at each hour at the lowest and highest prey densities and bars span the 95% confidence intervals of each of the means; lines were fitted by hand.

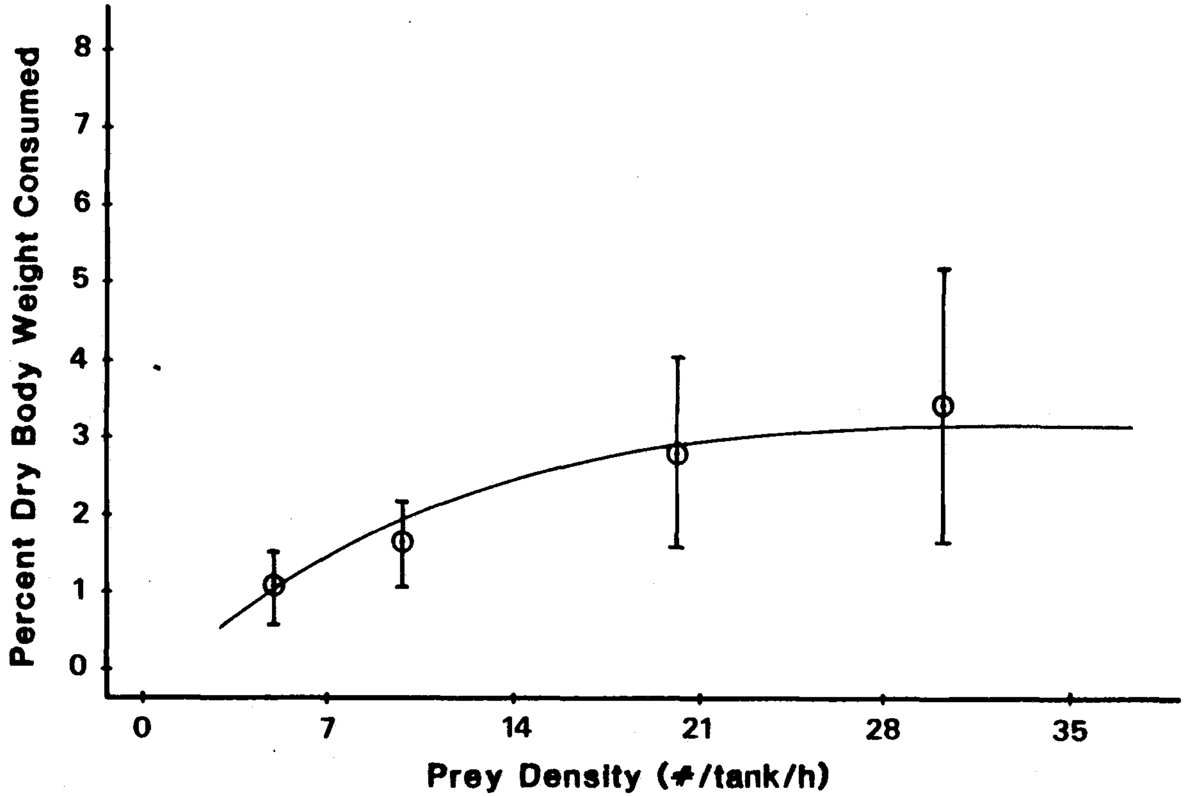


Figure 4. Functional response of P. promelas preying on D. pulex (control phase). Values represent mean response of all fish at each prey density level and bars span the 95% confidence intervals of the means; curve was fitted by hand.

levels (Appendix V). Feeding rates appeared to be increased at the 100 µg/l copper and densest prey level combinations.

Changes in feeding rates were manifest in the overall mean percent of dry body weight eaten for each treatment level during the 12-h treatment phase (Figure 5). There appeared to be depressed consumption at intermediate levels of copper concentration and prey density and much greater consumption relative to controls at high levels of both factors; there was no change at the 5/tank/h prey density level no matter how much copper was added. The effects of copper on predation were different in direction and magnitude and dependent on the dose level of copper and density of prey. Paired t-tests, using the control and treatment phase responses of each individual fish were performed for each prey density and copper dose combination and found to be non-significant ($P < 0.05$). This appeared to result from the variability in consumption between days, even in control fish, and the relatively low number of replicates. The variability in consumption was highest at high prey densities in both control and treatment phases (where a significant result might have been expected).

In order to show how the effects of copper changed the functional response at each dose the functional responses curves were converted to a family of lines using Real's (1979) disc equation. Drawing on the similarities between predatory functional responses and the kinetics of enzyme reactions Real (1977) derived a new formulation for the functional response that incorporated the concepts that: 1) "changing the ecological setting" may shift the response from Type II to Type III and 2) all responses may be portrayed as linear functions

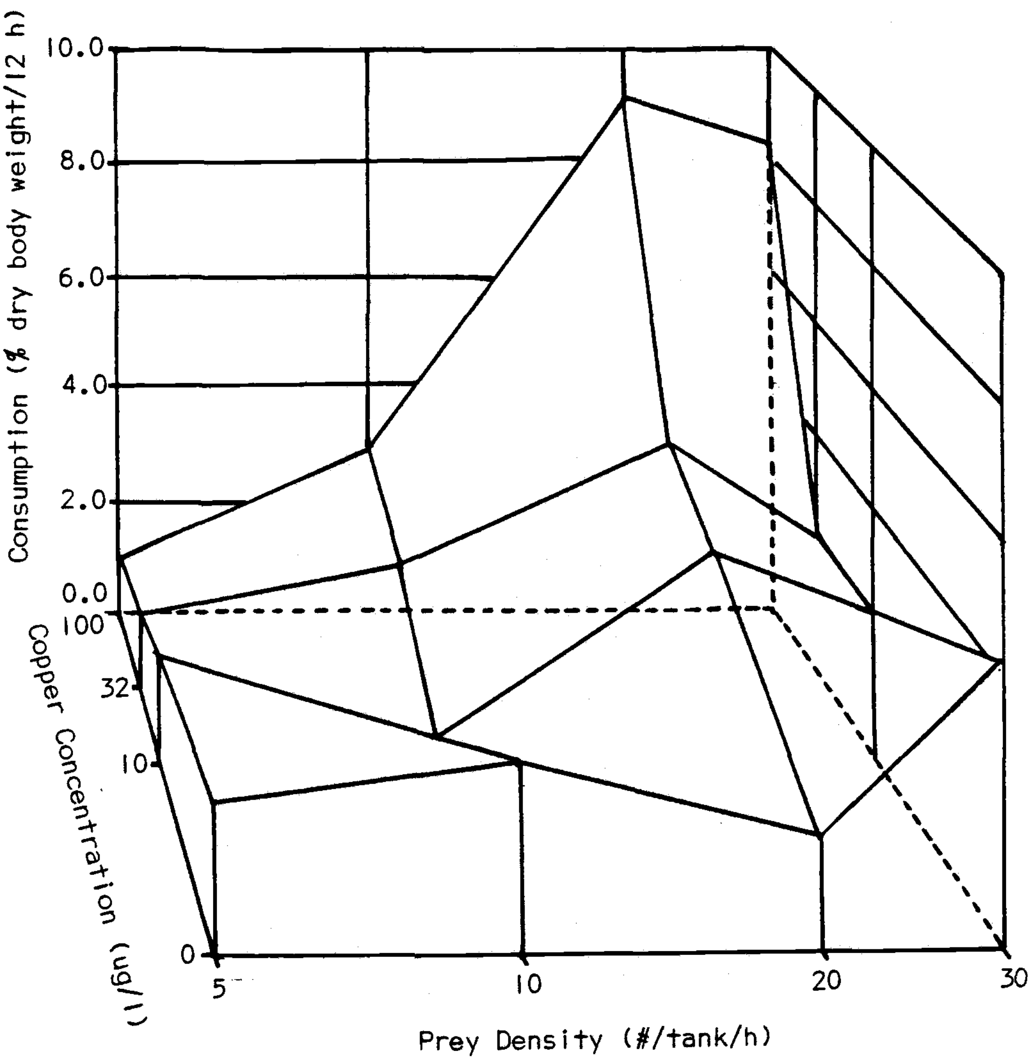


Figure 5. The effect of prey density and copper exposure on consumption. Prey density and copper concentration appear on $\log_{10}$ scales. Values represent copper consumption at each prey density-copper concentration combination.

and the degree to which a single element in the equations is different expresses the magnitude of the shift. Real's disc equation is:

$f = (kA^n)/(\chi + A)$ where f = feeding rate

k = maximal feeding rate

A = density of food items

χ = density of food items that generate half maximal feeding rates

n = parameter associated with the amount of increase in the rate of detection of a food item with increase in food density

As the parameter "n" is increased from 1 to 3 the functional response shifts from an hyperbolic-Type II to a sigmoidal-Type III characteristic of "learning" predators. The equation can be log-log transformed to always yield a straight line regardless of the underlying shape of the functional response:

$$\log [f/(k - f)] = \log (1/\chi) + n\log A$$

The functional response of the control phase was redrafted using the disc equation (Figure 6). The $\log_{10}$ of (f/k-f) was plotted versus the $\log_{10}$ of the prey density. The values for f and A came directly from the experimental results; k, the maximal feeding rate, was estimated as the asymptote on the hyperbolic curve (Figure 4). A least-squares regression yielded the equation as:

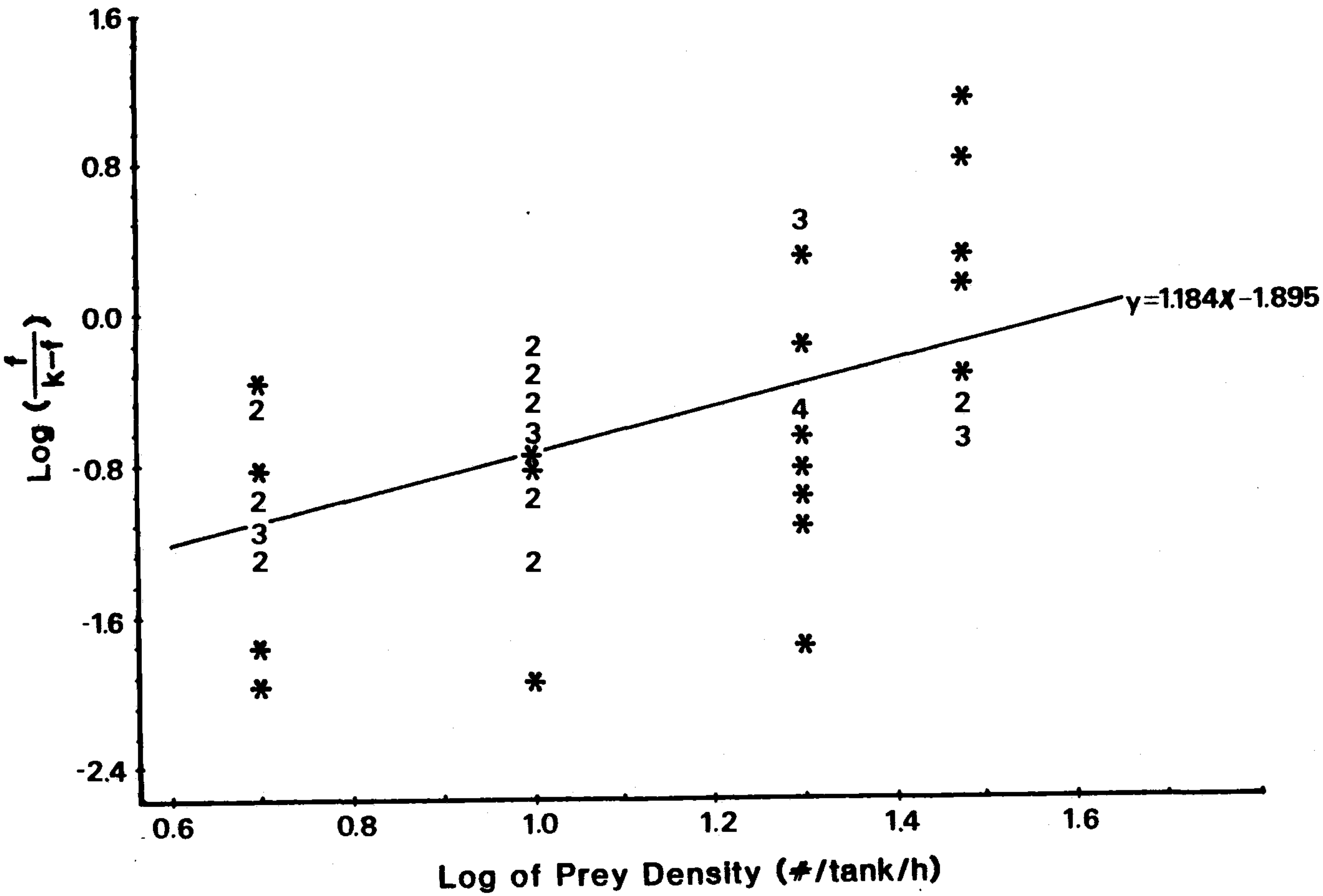


Figure 6. Fit of Real's (1979) disc equation to the functional response (control phase), k = 0.080, χ = 39.9, and n = 1.184.

$$\log [f/(k-f)] = 1.184 * \log (A) - 1.895$$

The slope of the line, 1.184, equals "n". The density of food items that generated the half maximal feeding rate, χ, was estimated by setting log (f/k-f) equal to zero and obtaining A. The χ-value, the most stimulatory density, for the control phase was 39.9 and can be thought of as an index of the "efficiency of the predator". In some ways the disc equation is like the logistic equation, where the greatest rate in population growth occurs at (k/2).

All four functional response disc equations, corresponding to each of the four dose levels of copper used in the treatment phase, were calculated and plotted (Figure 7). The lines tended to shift upward with increasing copper concentration. The slope, n, decreased slightly relative to controls in the 10-µg/l treatment and then increased with increasing copper concentration. At the 100 µg/l treatment n equaled 1.281 indicating a shift toward a Type III response. The χ-values showed a consistant decline, or "increase in efficiency" with increased copper dose.

An increase in n causing a shift in the functional response is generally thought to indicate some type of learning on the part of the predator which makes it more efficient (Real 1977) and evidence for a learning curve was sought in the mechanistic measures. Plots of time between successive pursuits, pursuit times and handling times as functions of successive encounters for each fish for both control and treatment phases showed no pattern or decrease that would tend to indicate previous encounter experience was affecting those behaviors.

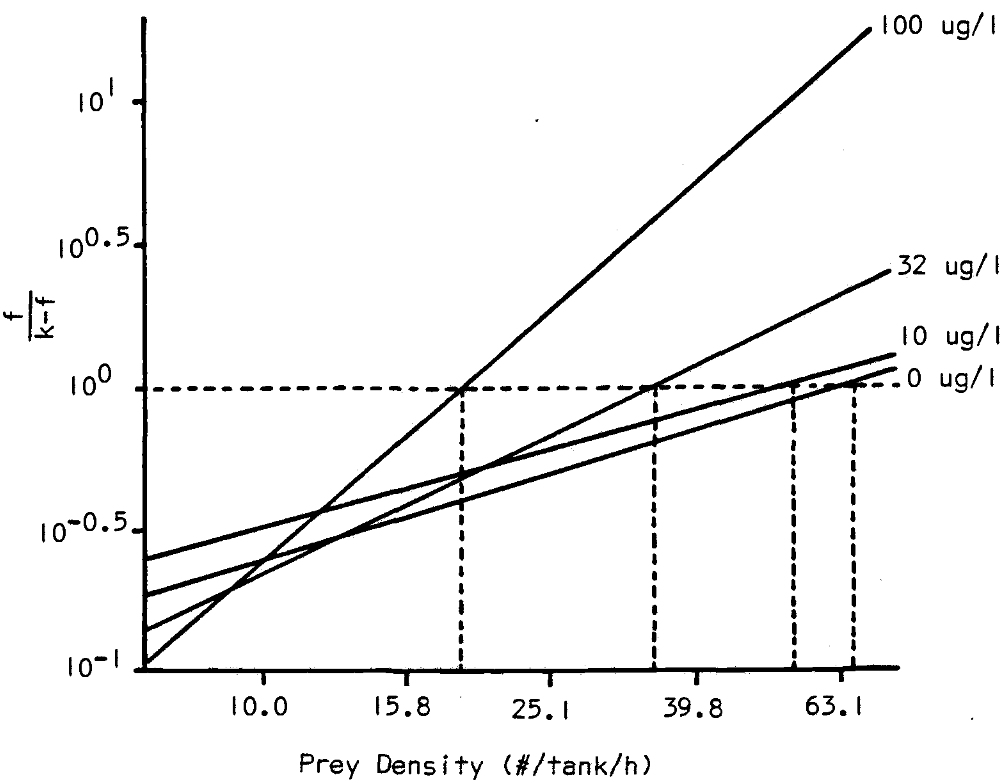


Figure 7. Fit of Real's (1979) disc equation to the functional response at 4 levels of copper exposure (treatment phase). Both axes are on $\log_{10}$ scales. Horizontal dashed line represents half maximal feeding rate; vertical dashed lines indicate prey density eliciting half the maximal rate (χ). Controls generated parameter values of k = 0.080, χ = 67.5, and n = 0.712. Ten μg/l generated parameter values of k = 0.060, χ = 56.0, and n = 0.662. Thirty-two μg/l generated parameter values of k = 0.120, χ = 35.6, and n = 1.192. One-hundred μg/l generated parameter values of k = 0.125, χ = 19.1, and n = 1.281.

Utilization of t-tests showed there were no significant differences between the AM and PM mechanistic and activity measurements in either control or treatment phases ($P < 0.05$). In order to best characterize fish behavior during the course of the 12-h phases, mechanistic and activity measurements from AM and PM observations were pooled. One-way analyses of variance were used to test for the effect of prey density on each of the mechanistic and activity parameters during the control phase and only the percentage of available prey eaten showed a statistically significant ($P < 0.05$) effect (Table 2; Figure 8a). Three interesting but non-significant trends were the decrease in average time spent searching and swimming with increased prey density in mechanistic and activity analyses respectively, and the increased average pursuit time with increased prey density (Figures 8b-8d).

Paired t-tests showed no significant differences between control and each treatment phase testing for the effects of copper at each prey level on the mechanistic and activity parameters of each fish. Five of the eight mechanistic measurements showed effects of copper concentration with probabilities of occurrence by chance of less than 0.18 in two-way analyses of variance for the treatment phase: total time spent searching, total and average times spent in pursuit, total time spent handling, and percent of available prey eaten (Table 3). Two of the 5 activity parameters measured also showed copper effects at the 0.18 probability level. The number of quadrant changes was significant at the $P < 0.05$ level; total time spent swimming also showed an effect (Table 3). Mean values of each of the 7 variables

Table 2. Mean values of each of the mechanistic and activity parameters measured for each prey level during the control phase. AM and PM measures were pooled before averaging. Parentheses enclose one standard deviation.

	Prey density level (#/tank/h)			
Parameter measured:	5	10	20	30
Mechanistic Parameters				
Total time spent searching (s)	585.20 (473.0)	449.1 (391.0)	292.1 (368.0)	449.7 (466.0)
Average time spent searching (s)	60.0 (54.9)	36.5 (30.5)	23.8 (25.9)	25.9 (26.6)
Total time in pursuit (s)	5.58 (7.72)	8.87 (15.50)	5.40 (10.00)	11.12 (16.80)
Average time in pursuit (s)	0.62 (0.56)	0.89 (0.61)	0.71 (0.87)	1.16 (1.92)
Total time spent handling (s)	6.28 (7.77)	5.24 (9.13)	4.65 (9.67)	5.59 (7.32)
Averate time spent resting (s)	195. (175.)	293. (293.)	437. (351.)	253. (287.)
Percentage of prey* eaten (%)	65.0 (72.9)	39.4 (46.3)	13.4 (31.1)	17.6 (20.0)
Percentage of successful encounters (%)	73.0 (32.6)	67.0 (29.0)	64.2 (48.0)	66.4 (29.8)
Activity Parameters				
Total time spent swimming (s)	149.2 (214.6)	167.6 (165.1)	111.7 (174.8)	121.3 (159.2)
Average time spent swimming (s)	59.3 (79.9)	38.7 (37.8)	36.9 (51.4)	30.5 (46.2)
Total # quadrant changes	43.7 (79.9)	63.2 (70.5)	47.6 (76.6)	52.1 (78.4)
Average time spent resting (s)	163.4 (103.7)	131.3 (95.5)	193.7 (106.8)	168.5 (120.3)
Swimming speed (quadrants/s)	0.16 (0.18)	0.31 (0.23)	0.28 (0.25)	0.24 (0.26)

* Statistically significant one-way ANOVA ($P < 0.05$)

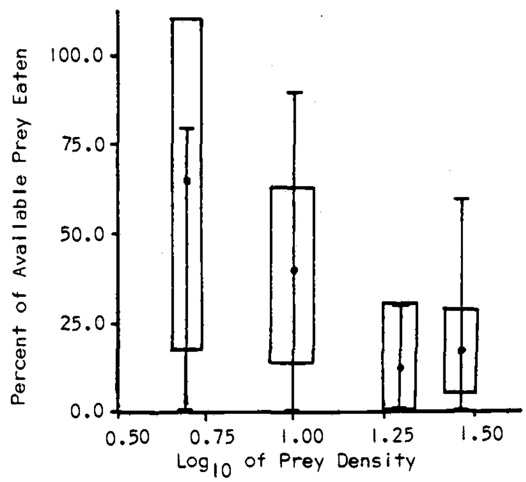


Figure 8a. Mean percent of available prey eaten as a function of the $\log_{10}$ of the prey density provided (5, 10, 20 and 30 *Daphnia*/tank/h) for the control phase. Bars represent total range and boxes span the 95% confidence intervals vertically and the sample size horizontally ($12 < n < 16$).

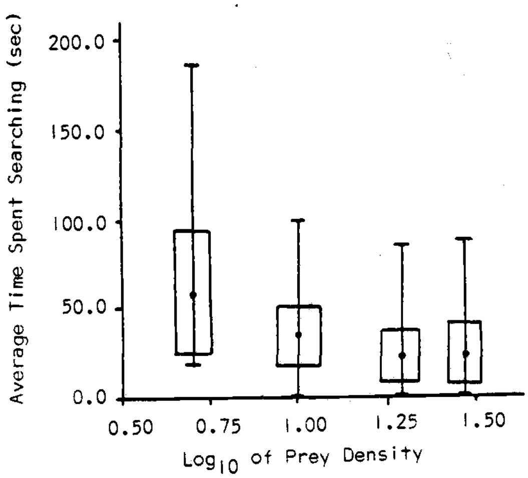


Figure 8b. Mean of the average time spent searching as a function of the $\log_{10}$ of the prey density provided (5, 10, 20 and 30 *Daphnia*/tank/h) for the control phase. Bars represent total range and boxes span the 95% confidence intervals vertically and the sample size horizontally ($12 < n < 16$).

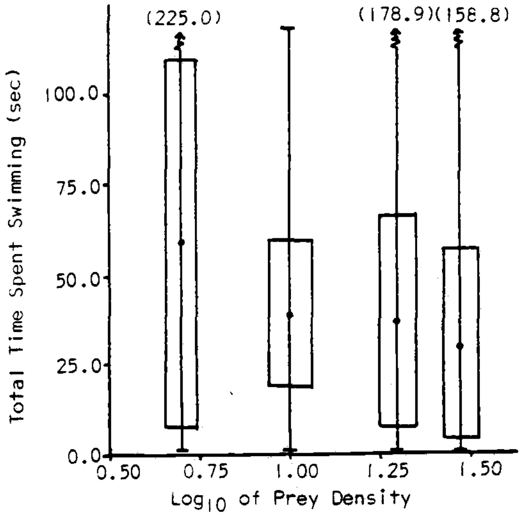


Figure 8c. Mean percent of the total time spent swimming as a function of the $\log_{10}$ of the prey density provided (5, 10, 20 and 30 Daphnia/tank/h) for the control phase. Bars represent total range and boxes span the 95% confidence intervals vertically and the sample size horizontally ($12 < n < 16$).

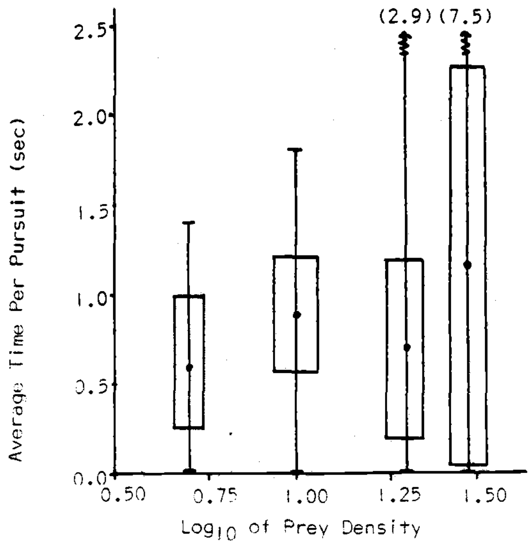


Figure 8d. Mean of the average time spent per pursuit as a function of the $\log_{10}$ of the prey density provided (5, 10, 20 and 30 Daphnia/tank/h) for the control phase. Bars represent total range and boxes span the 95% confidence intervals vertically and the sample size horizontally ($12 < n < 16$).

Table 3. P-values from two-way analyses of variance testing for effects of copper, prey density and copper-prey-density-interaction on the 13 mechanistic and activity variables measured.

Parameters	Copper	Prey density	Copper/ density interaction
Mechanistic Parameters			
Total time spent searching	0.1793^{o}	0.4895	0.5153
Average time spent searching	0.9037	0.5324	0.5543
Total time in pursuit	0.1771^{o}	0.6305	0.5642
Average time in pursuit	0.0994^{o}	0.7603	0.9072
Total time spent handling	0.1662^{o}	0.4801	0.6650
Average time spent resting	0.8289	0.7712	0.9585
Percentage of prey eaten	0.1482^{o}	0.1736^{o}	0.9420
Percentage of successful encounters	0.4779	0.4317	0.4481
Activity Parameters			
Total time spent swimming	0.1413^{o}	0.8376	0.9156
Average time spent swimming	0.2473	0.9422	0.9505
Total # quadrant changes	0.0185^{o}	0.7551	0.6961
Average time spent resting	0.3765	0.7150	0.8444
Swimming speed (quadrants/s)	0.2132	0.5383	0.9520

^{o} = $P < 0.18$

showing copper effects ($P < 0.18$), and the percentage of successful encounters, at each treatment combination, were analyzed (Tables 4a-4g, 5).

Total time spent searching (Table 4a, Fig. 9a) and average time in pursuit (Table 4c, Fig. 9b) showed patterns consistant with but inverse to the effects of copper on the functional response (Figure 5). At the intermediate levels of prey density and copper dose, where consumption is depressed, there appeared a slight increase in average pursuit time and total time spent searching. Where consumption was stimulated, at high levels of copper and prey density, average pursuit time and total time spent searching were depressed relative to controls. Similar trends can be seen in the total times spent in pursuit and swimming and numbers of quadrant changes made in the activity observations (Tables 4b, 4f, and 4g). It appeared that the fish were generally more active at intermediate levels but were also less effective at capturing prey relative to controls. Evidence of this was the depressed consumption and slightly elevated pursuit times. At the higher levels the fish were much less active yet prey capture was enhanced (reduced pursuit times and increased consumption). The percent of successful prey captures, while statistically non-significant, also showed this same pattern of decrease at intermediate treatment levels and increase at higher levels (Figure 9c).

Prey density was held constant to test for effects of copper on reactive distance. The mean reactive distance during the control phase of the experiment was 0.52 cm, [0.02 (SE), n = 32]. No

Table 4. Mean values of each of the 7 variables showing copper effects ($P < 0.18$) for each copper concentration/prey-level combination during the treatment phase. Parentheses enclose one standard deviation.

Copper Concentration	Prey density 5/h	10/h	20/h	30/h
a. Total Time Spent Searching				
0 µg/l	692. (702.)	640. (463.)	701. (820.)	301. (437.)
10 µg/l	548. (491.)	901. (532.)	545. (665.)	1083. (306.)
32 µg/l	1042. (446.)	508. (543.)	704. (875.)	220. (209.)
100 µg/l	672. (156.)	274. (174.)	294. (472.)	127. (72.)
b. Total Time in Pursuit				
0 µg/l	3.40 (5.63)	7.53 (8.22)	9.25 (16.90)	5.83 (6.64)
10 µg/l	7.00 (8.59)	14.55 (10.10)	6.67 (7.92)	43.60 (70.2)
32 µg/l	5.10 (5.20)	7.87 (15.30)	0.85 (1.20)	1.20 (1.90)
100 µg/l	0.67 (1.15)	3.75 (6.10)	5.44 (8.87)	0.55 (0.69)
c. Average Time in Pursuit				
0 µg/l	0.67 (0.91)	1.13 (1.06)	0.60 (0.49)	1.13 (0.61)
10 µg/l	1.40 (0.95)	1.00 (0.28)	1.07 (0.21)	2.13 (2.49)
32 µg/l	0.77 (0.68)	0.64 (0.82)	0.45 (0.64)	0.38 (0.44)
100 µg/l	0.33 (0.58)	0.95 (1.00)	0.54 (0.76)	0.45 (0.66)

Table 4. Continued.

Copper Concentration	Prey density 5/h	10/h	20/h	30/h
d. Total Time Spent Handling				
0 µg/l	4.7 (4.2)	4.5 (7.8)	0.0 (0.0)	3.7 (3.6)
10 µg/l	3.2 (2.9)	4.1 (5.7)	8.9 (15.4)	20.6 (28.0)
32 µg/l	3.8 (3.5)	4.5 (7.7)	0.0 (0.0)	6.1 (10.9)
100 µg/l	1.4 (2.4)	0.6 (1.2)	2.6 (5.9)	0.0 (0.0)
e. Percent of Available Prey Eaten				
0 µg/l	46.7 (64.3)	46.7 (56.9)	40.0 (73.4)	17.8 (21.9)
10 µg/l	60.0 (72.1)	75.0 (21.2)	31.7 (37.5)	35.7 (31.9)
32 µg/l	73.3 (64.3)	28.6 (47.8)	5.0 (7.1)	5.8 (9.6)
100 µg/l	13.3 (23.1)	20.0 (27.1)	12.0 (16.8)	1.8 (3.5)
f. Activity-Total Time Spent Swimming				
0 µg/l	205.9 (341.4)	257.2 (17.4)	272.6 (281.8)	164.1 (108.1)
10 µg/l	221.1 (202.1)	298.8 (330.4)	117.5 (104.2)	304.3 (190.3)
32 µg/l	267.6 (206.9)	174.0 (247.2)	137.8 (173.7)	70.0 (66.0)
100 µg/l	109.0 (97.1)	66.1 (78.5)	98.0 (178.2)	48.1 (63.1)

Table 4. Continued.

Copper Concentration	Prey density 5/h	10/h	20/h	30/h
g. Activity-Total Number of Quadrant Changes				
0 μg/l	61.0 (103.9)	101.3 (102.0)	110.0 (102.9)	45.0 (39.1)
10 μg/l	75.0 (81.1)	134.0 (162.6)	27.0 (23.4)	143.7 (105.0)
32 μg/l	54.7 (32.7)	55.4 (86.1)	41.0 (56.6)	38.0 (56.1)
100 μg/l	13.7 (12.7)	11.0 (15.6)	10.6 (13.7)	5.3 (9.2)

Table 5. Mean values of capture success (percent) at each combination of prey-level and copper concentration during the treatment phase. Parentheses enclose one standard deviation.

Copper Concentration	Prey density 5/tank/h	10/tank/h	20/tank/h	30/tank/h
Capture Success (percent)				
0 μg/l	100.0 (0.0)	92.5 (10.6)	78.7 (25.8)	100.0 (0.0)
10 μg/l	57.3 (51.6)	66.5 (47.4)	98.0 (3.5)	94.2 (10.0)
32 μg/l	100.0 (0.0)	73.3 (23.3)	100.0 (0.0)	100.0 (0.0)
100 μg/l	100.0 (0.0)	87.5 (17.7)	60.5 (31.8)	100.0 (0.0)

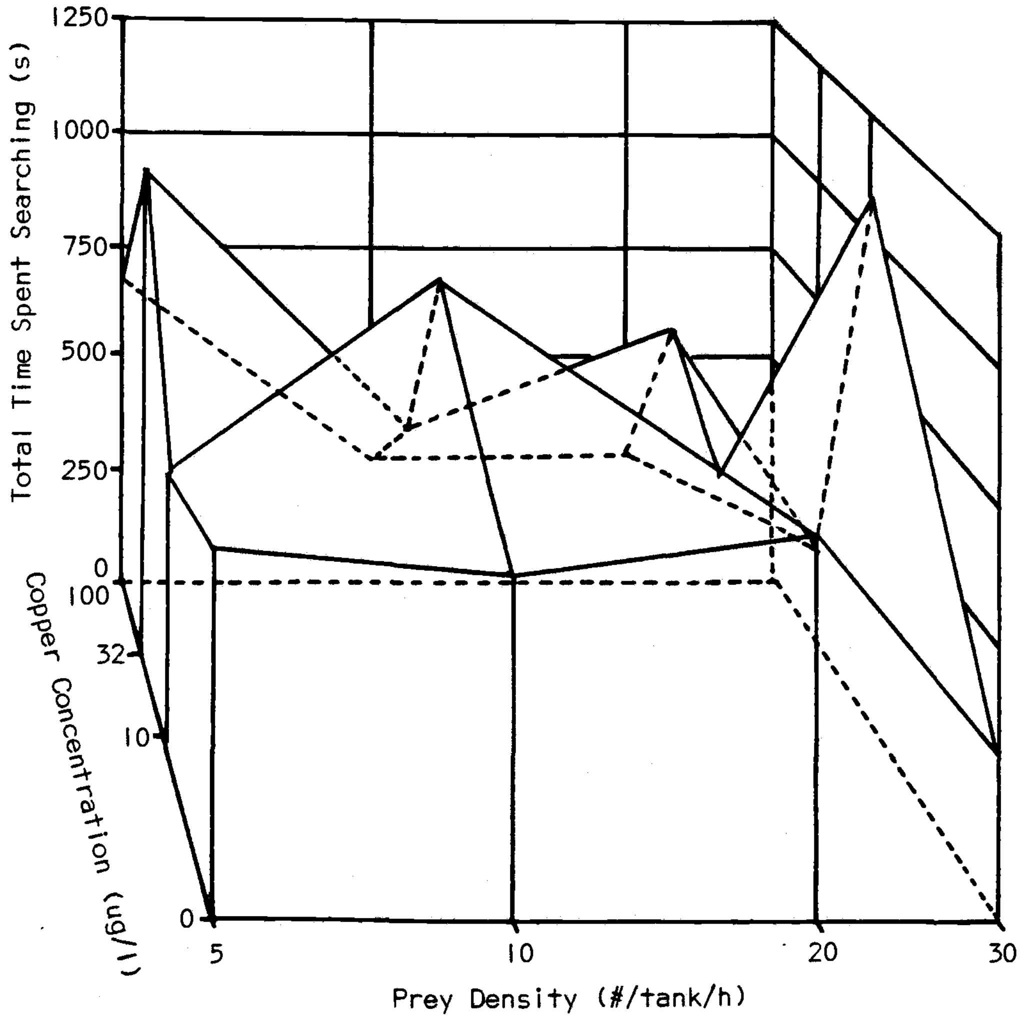


Figure 9a. The effect of prey density and copper exposure on total time spent searching. Prey density and copper concentration appear on $\log_{10}$ scales.

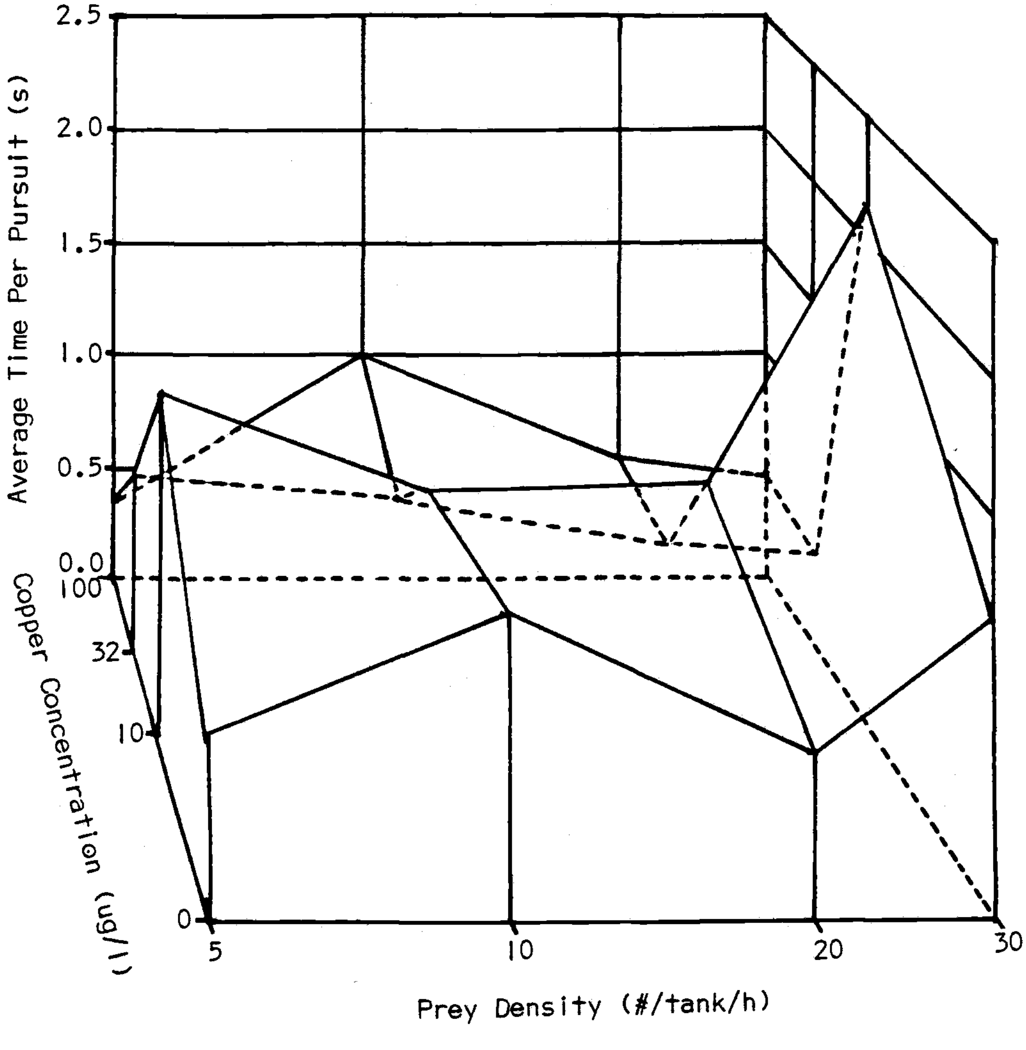


Figure 9b. The effect of prey density and copper exposure on average time per pursuit. Prey density and copper concentration appear on $\log_{10}$ scales.

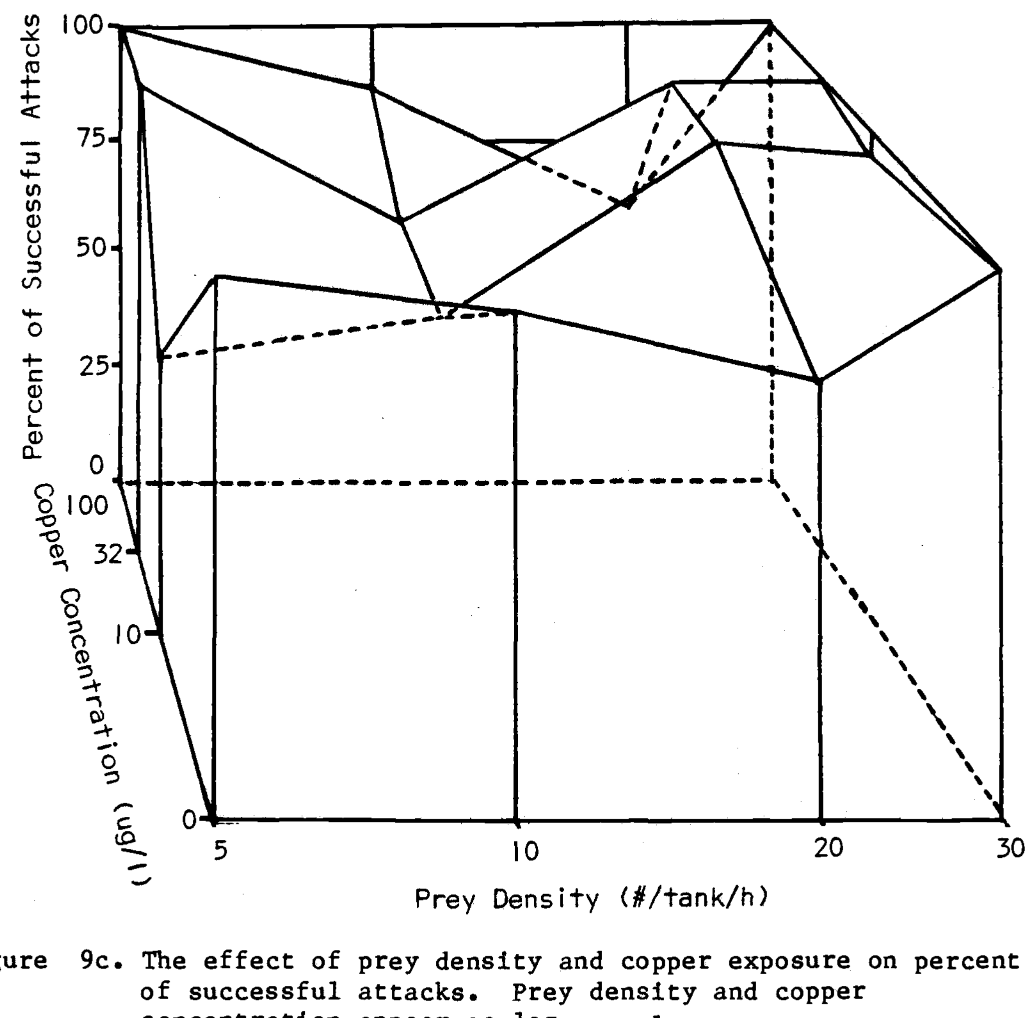


Figure 9c. The effect of prey density and copper exposure on percent of successful attacks. Prey density and copper concentration appear on $\log_{10}$ scales.

significant difference was found between the morning and afternoon phases of the experiment and results were pooled. A one-way analysis of variance to test for the effect of copper on reactive distance in the treatment phase revealed a distinct but non-significant pattern of increase in reactive distance with increased copper concentration (Figure 10). Paired t-tests comparing control and treatment observations for each individual fish yielded a significant treatment effect ($P < 0.01$) only at the highest (100 μg/l) copper treatment level. Again, for each individual fish reactive distances were generally longer during the treatment phase (except for controls) and this magnitude of increase was greater at the higher concentrations.

The assay of *Daphnia* activity levels was designed to test whether crude behavioral alterations could be detected and quantified in the prey species. Analysis of the impact of copper on *Daphnia* activity levels, measured in numbers of quadrants crossed, revealed a reduction in activity with increased exposure duration as well as significant ($P < 0.01$) reductions with increased copper concentration (Figure 11). The copper concentration causing 50% inhibition of the activity, measured as quadrant changes, relative to controls, was calculated for morning, afternoon and pooled observation periods and ranged from 2.66 μg to 5.65 μg copper /l. This corresponds to all dose levels of copper in the functional, mechanistic and activity experiments with both predator and prey.

The differential sensitivity of the minnows and *Daphnia* is illustrated by the comparison of the zooplankton activity level and χ, the half saturation prey density, at each copper concentration

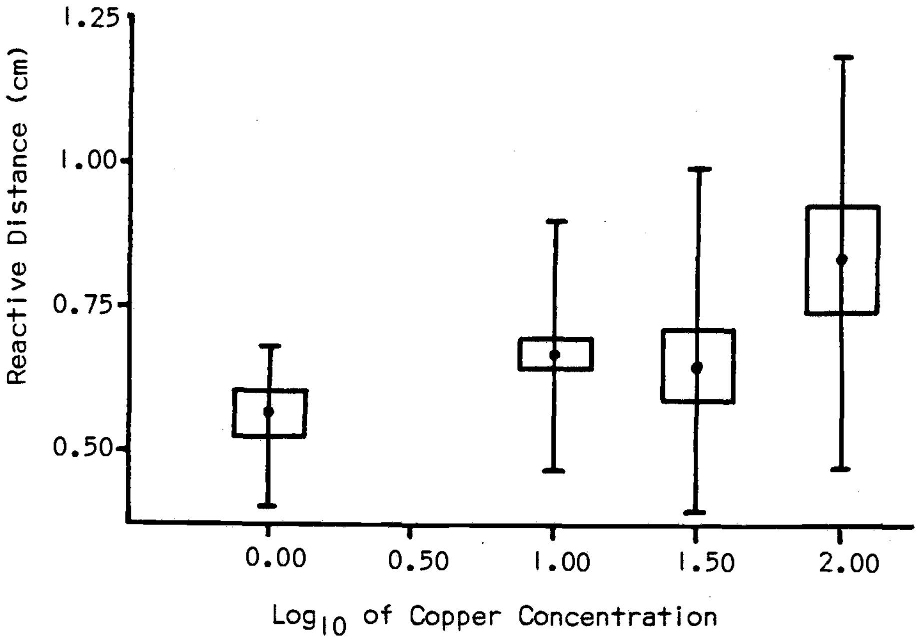


Figure 10. Mean reactive distance of predator to prey as a function of the $\log_{10}$ of the copper concentration (0, 10, 32 and 100 µg/l). Bars represent total range and boxes span the 95% confidence intervals vertically and the sample size horizontally (n = 8).

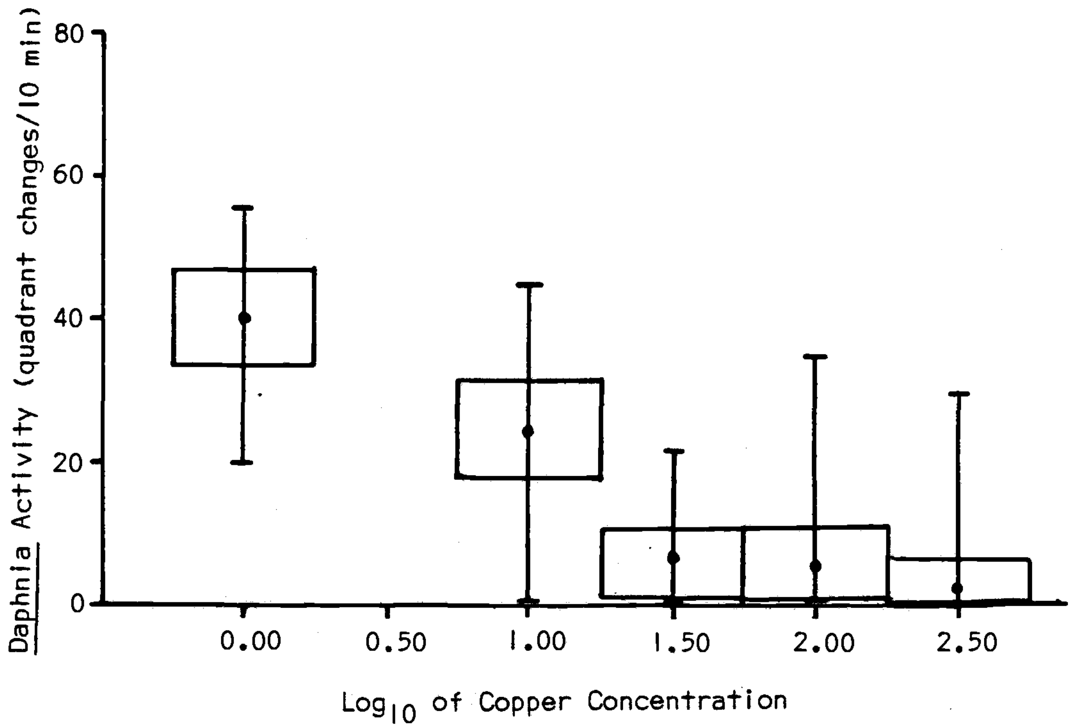


Figure 11. Mean Daphnia activity level as a function of the $\log_{10}$ of the copper concentration (0, 10, 32 and 100 µg/l). Bars represent total range and boxes span the 95% confidence intervals vertically and the same size horizontally (n = 16).

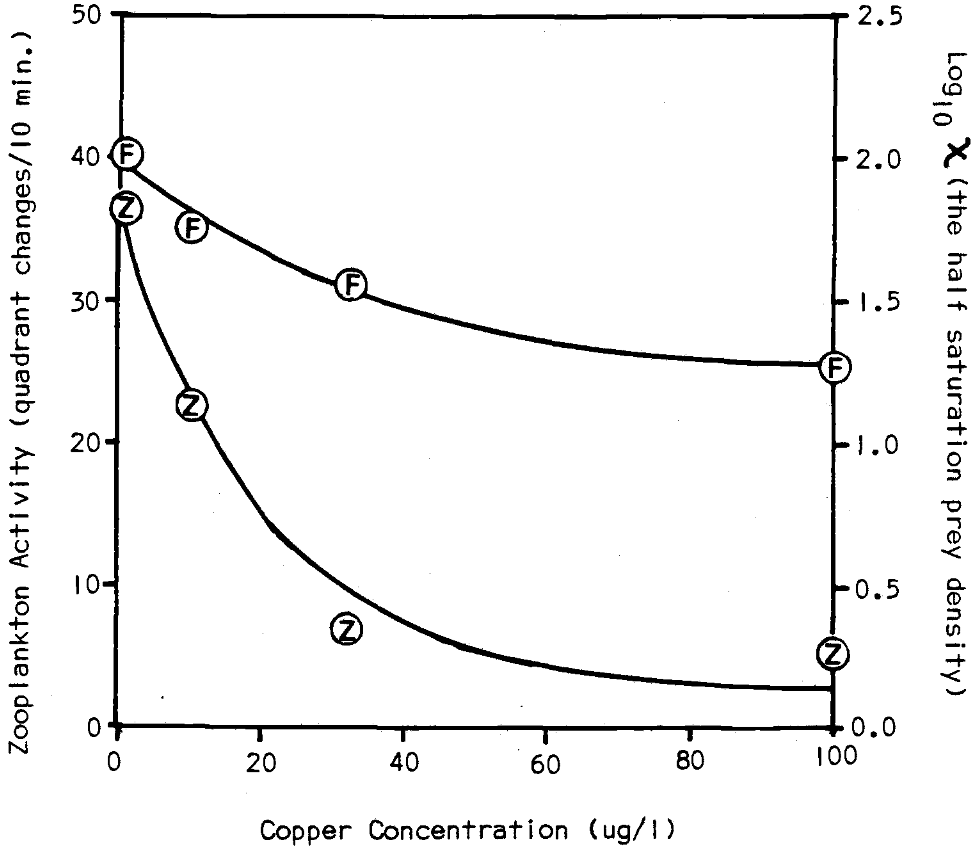


Figure 12. Zooplankton activity (symbol "Z", scale at left) and $\log_{10} \chi$ (symbol "F", scale at right) as functions of copper concentration.

level (Figure 12). Daphnia activity dropped off rapidly as copper concentration was increased and there was little additional decrease in activity in organisms exposed to over 32 µg/l of copper. The "inefficiency of the predator", as measured by χ, actually declined with increased copper concentration but the decline was not as rapid as that observed in Daphnia activity levels; inefficiency continued to decline slightly in fish exposed to over 32 µg/l of copper.

DISCUSSION

The results of the functional response experiments indicated that the addition of copper altered the functional response of minnows preying on D. pulex. As copper concentrations were increased the shapes of the responses tended to shift toward sigmoidal-Type III form. This shift was accompanied by a substantial decrease in χ indicating that the predators became more efficient in obtaining prey at lower densities as copper concentration increased. Analysis of the mechanistic and activity components did not completely explain the shift in form but suggested that it was not due to an increase in learning, as is generally proposed to be the reason for such a shift. The component responses indicated that the shift was due rather to the differential sensitivity of the predator and prey to copper and results were consistent with an impact of copper on fish appetite.

Consumption was slightly depressed at intermediate levels of copper and prey density but consumption increased at the highest levels of copper concentration and prey density. The validity of the trends in consumption was further substantiated by observing similar patterns of consumption during the mechanistic experiments. The use of a 2-factor design was valuable because it established that constant levels of copper affected predation differently depending on prey density.

It also seems clear that the effects of copper on the functional response could not have been predicted based only on the results of the impact of copper on the mechanistic components. Had effects on searching or handling time been used to predict consumption rates faulty inverse or no-effect conclusions could have been indicated. The measurement of the mechanistic components of the functional response did yield insight on how copper acted to change the functional response. No evidence for a learning curve was found but characterizing the mechanistic and activity behavior at the intermediate and high levels of copper concentration and prey density clarified possible explanations. It became clear that the fish were capitalizing on the relatively more impaired ability of the *Daphnia* to function, and escape, at increased copper concentration. Also there seemed to be evidence that copper was affecting the fish appetite.

The behavior of the fish exposed to intermediate levels of copper concentration and prey density, associated with depressed consumption, indicated that their ability to locate prey did not seem to be impaired. The total area they searched and the distance from which the fish reacted to the prey were increased relative to controls. The prey activity was substantially inhibited presumably making it easier to capture and handle, yet the effectiveness of predation dropped. The fish spent more time in pursuit of a single prey item, were less likely to be successful at capturing it and overall consmption rates fell.

At high levels of prey density and copper concentration the total time spent searching and the number of quadrants changed declined substantially but the ability to locate prey was probably enhanced by their increased density. Increased reactive distance measures indicated that the high concentrations of copper substantially increased the "visibility" of the prey to the predator even though prey activity was not additionally impaired (Figure 12). Once located, it seemed the prey were also more easily consumed as evidenced by the decreased pursuit times and high rates of capture success.

The most plausible explanation for the two different types of responses to copper is that copper was affecting the hunger levels or thresholds of the fish. The ineffectiveness of predatory activity at the intermediate levels of both factors, increased pursuit times and decreased capture success and consumption rates while prey activity was impaired, is consistant with the behavior of sated fish (Ware 1972). Several experimenters (Mount and Stephan, 1969; McKim and Benoit, 1971) have reported decreased growth rates for *Pimphales promelas* and brook trout exposed to sublethal, 0.10–0.40 times the 96-h LC50, levels of copper; these growth reductions were generally explained by observations that appetite was reduced. While carefully controlling ration size and locomoter activity Lett *et al*. (1976) initially observed a cessation of feeding activity in rainbow trout at levels of 0- to 100% of the 96-h LC50. They observed the return of consumption rates to control levels was inversely related to the ration level and copper dose. It is quite probable that a reduction in appetite was the mechanism that was responsible for the predatory behavior of the fish at the intermediate levels.

McLeay and Brown (1974), conversely, observed increased growth in juvenile salmon exposed to sublethal levels of toxicant. It was suggested that this may have resulted from an increase in appetite level triggered by the increase in metabolic load in compensating for the effects of the toxicant (Lett *et al.*, 1976). In experiments with copper and critical swimming performance Waiwood and Beamish (1978) concluded that copper acted as both "limiting" and "loading" factors. As these terms were defined by Brett (1958) copper reduced active metabolism as measured by reduced maximum oxygen consumption during critical swimming (limiting), and increased standard metabolic rate (loading) as measured by increased oxygen consumption relative to controls at a given speed. There is also evidence that copper acts as a loading factor by impairing osmotic balance (Courtois, 1976) and possibly nervous integration (Bengtsson, 1974) at lethal levels. The fish in this experiment may have responded to the loading nature of the copper at higher concentrations by reducing activity levels and increasing consumption at high prey densities where search and capture costs were low due to availability and impaired escape ability of the prey.

If copper affected appetite as suggested, it remains to be explained why the fish at intermediate levels of prey density and copper concentration were so active. The stimulation of swimming at the lower copper levels is probably explained by the specific toxic action of copper. Exposure to copper at levels as low as 0.25 times the 96-h LC50 has been shown to result in a buildup of mucus on the gills to which fish respond by increasing opercular activity (Morgan and Kuhn, 1974). The increased opercular activity had no effect on arterial oxygen pressure and eventually copper caused permanent damage to the gill epithelia (Sellers *et al.*, 1974). The increased swimming activity observed in this study may represent an attempt to flush the gill surfaces of the mucus and probably should not be interpreted as food searching behavior. At the higher copper concentrations, the "loading" nature of copper probably prevailed, making the metabolic cost of this swimming activity too great.

Waiwood and Beamish (1978) suggested that the ability of fish to "recover" from low levels of copper, as measured by swimming ability, indicates some physiological balancing mechanism. They offered as evidence several studies where recoveries from copper-induced alteration in blood characteristics occurred (McKim et al., 1970; Christensen et al., 1972). The recovery of initially depressed consumption and growth rates induced by copper and observed by Lett et al. (1976) and McKim and Benoit (1971) support this hypothesis. Although not in the scope of this study, it would be of interest to incorporate a time element to determine if recovery in consumption rates and return to more normal activity levels would occur at the intermediate copper levels.

The results clearly indicate that sublethal levels of copper affect the functional response and activity levels of P. promelas and D. pulex and the effects could not have been predicted using only one level of prey density. This conclusion is of special significance since natural systems are often food limited and food searching behavior comprises a major portion of the animal's energy budget. The effects of copper on the functional response also could not have been predicted based on its effects on the various mechanistic components. Many of the component measures seem to be suited to making many repetitions and achieving the high level of precision typical for traditional bioassay responses. This study has shown, however, that erroneous predictions might result from such an assay. In the same vein, more study on the functional response in a more complex context seems indicated. A future tier of investigations might examine the response with the addition of competing predators or alternate prey items.

BIBLIOGRAPHY

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1980. Standard Methods for the examination of water and waste water. 15th ed. Washington, DC.

Baker, J.A., and T. Modde. 1977. Susceptibility to predation of Blacktail shiners stained with Bismarck Brown-Y. Trans. Am. Fish. Soc. 106:334-338.

Bengtsson, B.E. 1974. The effect of zinc on the ability of the minnow Phoxinus phoxinus L. to compensate for torgue in a rotating water-current. Bull. Environ. Contam. Toxicol. 12:654-658.

Biesinger, K.E., and G.M. Christensen. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of Daphnia magna. J. Fish. Res. Board Can. 29:1691-1700.

Brett, J.R. 1958. Implications and assessments of environmental stress. Pages 69-93 in P.A. Larkin, editor. The investigation of fish-power problems. Inst. Fish., Univ. B.C.

Brooks, J.L., and S.I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150:28-35.

Cairns, J., Jr. 1983. Are single species toxicity tests alone adequate for estimating environmental hazard? Hydrobiol. 100:47-57.

_____. 1984. Multispecies toxicity testing. Environmental Toxicology and Chemistry 3:1-3.

Chapman, G.A. (personal communication). Western fish Toxicology Station, Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR.

_____. 1978. Toxicities of cadmium, copper, and zinc to four juvenile stages of chinook salmon and steelhead. Trans. Am. Fish. Soc. 107(6):841-847.

_____, S. Ota and F. Recht. 1979. Effects of water hardness on the toxicity of metals to Daphnia magna. (Manuscript). Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR.

Christensen, G.M., J.M. McKim, W.A. Brungs, and E.P. Hunt. 1972. Changes in the blood of the brown bullhead [Ictalurus nebulosis (Lesueur)] following short-term exposure to copper. Toxicol. Appl. Pharmacol. 23:417-427.

Committee on Methods for Toxicity Tests with Aquatic Organisms, The. 1975. Methods for acute toxicity tests with fish, macro-invertebrates, and amphibians. Ecol. Res. Ser. EPA-600/3-75-009. National Water Quality Laboratory, Duluth, MN.

Courtois, L.A. 1976. Copper intoxication - a possible biochemical homologue. Bull. Environ. Contam. Toxicol. 15:205-210.

Dean, J.G., F.L. Bosqui, and V.H. Lanouette. 1972. Removing heavy metals from wastewater. Environ. Sci. Technol. 6:518-522.

Dominguez, S. (personal communication) Western Fish Toxicology Station, Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR.

Doudoroff, P., and M. Katz. 1953. Critical review of literature on the toxicity of industrial wastes and their components to fish. II. The metals, as salts. Sewage ind. Wastes 25:802-839.

EPA Bioassy Committee. 1971. Recommended bioassay procedures for fathead minnow (<u>Pimeaphales promelas</u>, Rafinesque) chronic tests. National Water Quality Laboratory, Duluth, MN.

Farr, J.A. 1977. Impairment of antipredator behavior in <u>Palaemonetes pugio</u> by exposure to sublethal doses of parathion. Trans. Am. Fish. Soc. 106:287-290.

_____. 1978. The effect of methyl parathion on predator choice of two estuarine prey species. Trans. Am. Fish. Soc. 107:87-91.

Geckler, J.R., W.B. Horning, T.M. Neiheisel, Q.H. Pickering, and E.L. Robinson. 1976. Validity of laboratory tests for predicting copper toxicity in streams. EPA-600/3-76-116. Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN.

Giddings, J.M. 1981. Laboratory tests for chemical effects on aquatic population interactions and ecosystem properties. Pages 23-91 <u>in</u> A.S. Hammons, editor. Methods for ecological toxicology a critical review of laboratory multispecies tests. ORNL-5708 EPA-560/11-80-026. Office of Toxic Substances, U.S. Enviornmental Protection Agency, Washington, DC.

Goodyear, C.P. 1972. A simple technique for detecting effects of toxicants or other stresses on predator-prey interaction. Trans. Am. Fish. Soc. 11:367-370.

Goulden, C.E., R.M. Comotto, J.A. Hendrickson, Jr., L.L. Hornig, and K.L. Johnson. 1982. Procedures and recommendations for the culture and use of <u>Daphnia</u> in bioassay studies. Pages 139-160 <u>in</u> J.G. Pearson, R.B. Foster and W.E. Bishop, editors. Aquatic Toxicology and Hazard Assessment: 5th Conference, ASTM STP 766. American Society for Testing and Materials, Philadelphia, PA.

Hall, D.J., S.T. Threlkeld, C.W. Burns, and P.H. Crowley. 1976. The size efficiency hypothesis and the size structure of zooplankton communities. Ann. Rev. Ecol. System. 7:177-208.

Hallowell, J.B., J.F. Shea, G.R. Smithson, Jr., A.B. Tripler and B.W. Gosner. 1973. Water-pollution control in the primary non-ferrous metals industry. Volume I. Copper, zinc and lead industries. Environmental Monitoring Series EPA-R2-73-247a. U.S. Environmental Protection Agency, Washington, DC.

Hammons, A.S., editor. 1981. Methods for ecological toxicology a critical review of laboratory multispecies tests. ORNL-5708 EPA-560/11-80-026. Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, DC.

Held, J.W., and J.J. Peterka. 1974. Age, growth and food habits of the fathead minnow, Pimephales promelas, in North Dakota saline lakes. Trans. Am. Fish. Soc. 103:743-756.

Holling, C.S. 1965. The functional response of predators to prey density and its role in mimicry and population regulation. Mem. Ent. Soc. Can. 45:1-60.

Hutchinson, G.E. 1957. A treatise on limnology. John Wiley and Sons, Inc., NY.

Kleerekoper, H., G.F. Westlake, J.H. Matis, and P.J. Gensler. 1972. Orientation of goldfish (Carasius auratus) in response to a shallow gradient of sublethal concentration of copper in an open field. J. Fish. Res. Board Can. 29:45-54.

Lett, P.F., G.J. Farmer, and F.W.H. Beamish. 1976. Effect of copper on some aspects of the bioenergetics of rainbow trout (Salmo gairdneri). J. Fish. Res. Board Can. 33:1335-1342.

Lueke, C., and W.J. O'Brien. 1981. Prey location volume of a planktivorous fish: a new measure of prey vulnerability. Can. J. Fish. Aquat. Sci. 38:1264-1270.

Lynch, M. 1979. Predation, competition, and zooplankton community structure: an experimental study. Limnol. Oceanogr. 24(2):253-272.

Maciorowski, A.F., J.L. Sims, L.W. Little, and E.D. Gerrard. 1981. Bioassay-procedures and results. J. Water Pollut. Control Fed. 53(6):974-993.

Maki, A.W. 1979. Correlations between Daphnia magna and fathead minnow (Pimephales promelas) chronic toxicity values for several classes of test substances. J. Fish. Res. Board Can. 36:411-421.

Marking, L.L, and V.K. Dawson. 1973. Toxicity of quinaldine sulphate to fish. Invest. Fish Control No. 48. U.S. Fish. Wildl. Serv., Washington, DC.

McCrady, J.K., and G.A. Chapman. 1979. Determination of copper complexing capacity of natural river water, well water and artificially reconstituted water. Water Res. 13:143-150.

McKim, J.M, and D.A. Benoit. 1971. Effects of long-term exposure to copper on survival, growth, and reproduction of brook trout, Salvelinus fontinalis. J. Fish. Res. Board Can. 28:655-662.

McLeay, D.J., and D.A. Brown. 1974. Growth stimulation and biochemical changes in juvenile coho salmon (Oncorhynchus kisutch) exposed to bleached kraft pulpmill effluent for 200 days. J. Fish. Res. Board Can. 26:2449-2457.

Morgan, W.S.G. 1977. Biomonitoring with fish: an aid to industrial effluent and surface water quality control. Prog. Wat. Technol. 9:703-711.

_____, and P.C. Kuhn. 1974. A method to monitor the effects of toxicants upon breathing rate of largemouth bass (Micropterus salmoides Lacepede). Water Res. 8:67-77.

Mount, D.I. 1968. Chronic toxicity of copper to fathead minnows (Pimephales promelas, Rafinesque). Water Res. 2:215-223.

_____, and C.E. Stephan. 1969. Chronic toxicity of copper to the fathead minnow (Pimephales promelas) in soft water. J. Fish. Res. Board Can. 26:2449-2457.

National Academy of Sciences. 1981. Testing for effects of chemicals on ecosystems. A report by the committee to review methods for ecotoxicology.

Nebeker, A.V., M.A. Cairns, S.T. Onjukka, and R.H. Titus. (submitted) Effect of age on sensitivity of Daphnia magna to cadmium, copper and cyanizine. Arch. Environm. Contam. Toxciol.

Nichols, H.W. 1973. I: Growth media-freshwater. Pages 7-25 in J.T. Stein, editor. Handbook of phycological culture methods and growth measurements. Cambridge Press.

Owen, C.A. 1981. Biological aspects of copper. Noyes Publications. Park Ridge, NJ.

Peterman, R.M., and M. Gatto. 1978. Estimation of functional responses of predators on juvenile salmon. J. Fish. Res. Board Can. 35:797-808.

Pickering, Q., W. Brungs, and M. Gast. 1977. Effect of exposure time and copper concentration on reproduction of the fathead minnow (Pimephales promelas). Water Res. 11:1079-1083.

Real, L.A. 1977. The kinetics of functional response. Am. Nat. 111:289-300.

_____. 1979. Ecological determinants of functional response. Ecol. 60:481-485.

Samuelson, D.F. 1976. Water quality: Western Fish Toxicology Station and western Oregon rivers. Ecol. Res. Ser. EPA-600/3-76-077. U.S. Environmental Protection Agency, Duluth, MN.

Sanders, W.M. 1976. EPA needs in micocosm research. Intern. J. Environmental Studies. 10:3-5.

Sellers, C.M., Jr., A.G. Heath, and M.L. Bass. 1975. The effect of sublethal concentrations of copper and zinc on ventilatory activity, blood oxygen and pH in rainbow trout (Salmo gairdneri). Water Res. 9:401-408.

Solomon, M.E. 1949. The natural control of animal populations. J. Animal Ecology 18:1-35.

Sprague, J.B. 1964. Avoidance of copper-zinc solutions by young salmon in the laboratory. J. Water Pollut. Control Fed. 36:990-1004.

Stiff, M.J. 1971. Copper/bicarbonate equilibria in solutions of bicarbonate ion at concentrations similar to those found in natural water. Water Res. 5:171-176.

Sullivan, J.F., G.J. Atchison, D.J. Kolar, and A.W. McIntosh. 1978. Changes in the predator-prey behavior of fathead minnow (Pimephales promelas) and largmouth bass (Micropterus salmonides) caused by cadmium. J. Fish. Res. Board Can. 35:446-451.

Tagatz, M.E. 1976. Effect of Mirex on predator-prey interaction in an experimental estaurine ecosystem. Trans. Am. Fish. Soc. 105:546-549.

U.S. Environmental Protection Agency. 1979. Methods for chemical analysis of water and wastes. EPA-600/4-79-020. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.

Waiwood, K.G., and F.W.H. Beamish. 1978. Effects of copper, pH and hardness on the critical swimming performance of rainbow trout (Salmo gairdneri, Richardson). Water Res. 12:611-619.

Ware, D.M. 1972. Predation by rainbow trout (Salmo gairdneri): the influence of hunger, prey density and prey size. J. Fish. Res. Board Can. 29:1193-1201.

_____. 1973. Risk of epibenthic prey to predation by rainbow trout (Salmo gairdneri). J. Fish. Res. Board Can. 30:387-797.

Werner, E.E. 1974. The fish size, prey size, handing time relation in several sunfishes and some implications. J. Fish. Res. Board Can. 31:1531-1536.

_____, and D.J. Hall. 1974. Optimal foraging and the size selection of prey by the bluegill sunfish (Leopmis macrochirus). Ecol. 55:1042-1052.

Winner, R.W., and M.P. Farrell. 1976. Acute and chronic toxicity of copper to four species of Daphnia. J. Fish. Res. Board Can. 33:1685-1691.

Woltering, D.M. 1976. Predator-prey interactions under the influence of ammonia. M.S. Thesis. Oregon State University, Corvallis.

APPENDICES

Appendix I. Salts added to regulate water hardness.*

For each unit (mg/l) increase in hardness desired add the following mg of reagent:

$CaSO_4 \cdot 2H_2O$	1.219 mg
$MgCl_2 \cdot 6H_2O$	0.593 mg
$NaHCO_3$**	1.205 mg
$KHCO_3$**	0.204 mg

* prescribed by G.A. Chapman based on Marking and Dawson (1973)

** interpolated from Chapman tables

Appendix II. Modified Woods Hole MBL Medium.*

To make up medium, add 1.0 ml each of stocks A-G, and 2.0 ml of stock H to each liter of distilled water.

Stock	Salt	Amount (g)	Vol (ml)
A	$MgSO_4 \cdot 7H_2O$	18.49	500
B	$NaHCO_3$	6.3	500
C	K_2HPO_4	4.36	500
D	$Na_2SIO_3 \cdot 9H_2O$	14.21	500
E	$CuSO_4 \cdot 7H_2O$	0.005	500
F	$ZnSO_4 \cdot 7H_2$	0.011	500
G	$CaCl_2 \cdot 2H_2O$	36.76	1000
	$NaNO_3$	85.01	
	H_3BO_3**	2.47	
	EDTA**	none	
	$FeCl_3 \cdot 6H_2O$	3.15	
	$CoCl_2 \cdot 6H_2O$	0.010	
	$MnCl_2 \cdot 4H_2O$	0.180	
	$Na_2MoO_4 \cdot 2H_2O$	0.006	
H	Tris buffer Adjust pH to 7.2 with HCl	250.0	1000

* from Nichols (1973) modified by S. Dominguez (personal communication)

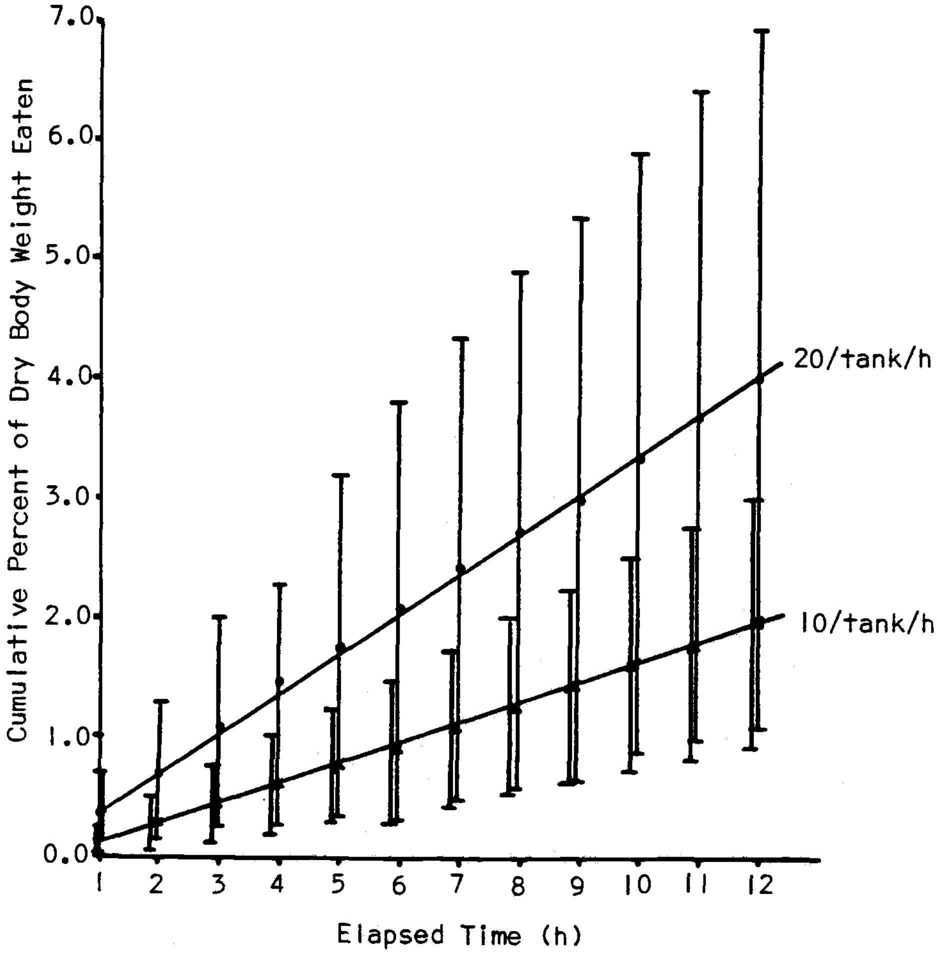
** represent departures from Woods Hole BML

Appendix III. Double strength *Daphnia* vitamin mixture.*

Concentration of growth factors in 1000 ml of medium:

Biotin	1.0 µg/l
Thiamine	200
Pyridoxine	200
Pyriodoxamine	6
Ca Pantothenate	50
B_{12}	1
Nicotinamide	100
Nicotinic acid	100
Folic acid	40
Riboflavin	6
Inositol	180

* modified and doubled by S. Dominguez (personal communication), original concentrations from L. Provasoli *in* Goulden *et al.* (1982).



Appendix IV. Cumulative percent of dry body weight eaten as a function of elapsed time (10/tank/h and 20/tank/h) for the control phase. Values represent mean response of all fish at each hour for the control phase at the medium prey densities and bars span the 95% confidence intervals of each of the means; lines were fitted by hand.

Appendix V. Changes in the slopes of the lines describing mean cumulative consumption over the 12-h treatment phase relative to the slopes during the control phase for each of the 4 prey levels.

	Prey level			
	5/tank/h	10/tank/h	20/tank/h	30/tank/h
Slope for each of the control phase consumption functions	0.0008	0.0017	0.0033	0.0036
Change in slope relative to control phase slope				
Copper Level				
0 μg/l	+0.0003	+0.0009	-0.0018	+0.0000
10 μg/l	+0.0009	-0.0013	-0.0003	-0.0016
32 μg/l	+0.0003	+0.0000	+0.0003	-0.0017
100 μg/l	+0.0000	+0.0008	+0.0044	+0.0039