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Effects of cadmium on juvenile bluegill (*Lepomis macrochirus*) foraging behavior and growth

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Effects of cadmium on juvenile bluegill (*Lepomis macrochirus*) foraging behavior and growth

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GENERAL INTRODUCTION

Current assessment of a chemical's potential hazard to aquatic organisms relies primarily on standardized acute and chronic toxicity tests which measure physiological effects on growth, survival, and reproduction (Maki 1979; Sandheinrich and Atchison 1989). However, these standardized tests inadequately consider the histological (e.g., Fowler 1987; Hinton and Lauren 1990; Hinton et al. 1992), biochemical (e.g., Jacobson and Turner 1980; Reddy et al. 1989; Meyer et al. 1991; Stegeman et al. 1992), and behavioral (e.g., Marcucella and Abramson 1978; Olla et al. 1980; Westlake 1984; Finger et al. 1985; Rand 1985; Atchison et al. 1987; Little et al. 1985; Sandheinrich and Atchison 1989, 1990; Beitinger 1990; Henry and Atchison 1991) mechanisms responsible for observed effects. Hence, their utility in predicting direct and indirect effects of chemical exposure to organisms in complex ecosystems is limited.

Histological and biochemical studies yield insight into chemical mode of action at the lowest levels of biological organization. Homeostatic imbalances that render physiological mechanisms or biochemical pathways inoperable will likely affect higher levels of biological organization (Versteeg and Giesy 1986; Adams 1990; Meyer et al. 1991; Stegeman et al. 1992). Knowing only that a toxicant changes a biochemical or physiological parameter can be useful for assessing chemical exposure (Thomas 1990), but leaves many unanswered questions regarding the chemical's ecological effects on whole organisms and populations in the field (Mello 1975).

Toxicologists must seek measures that relate basic cellular and subcellular effects to whole animal responses which, in turn, affect population and community dynamics. Toxicant-altered biochemical parameters may have little ecological relevance unless they impede organisms' abilities to conduct activities, such as foraging, avoiding predation, and reproducing, which are essential to sustaining themselves and their populations. These important activities are intimately based upon numerous complex behaviors, which are the functional integration of physiological and biochemical systems (Warner et al. 1966; Rand 1985; Little 1990; Schreck 1990; Henry and Atchison 1991). Thus, subtle changes in an organism's homeostasis, as a result of sublethal toxicant exposure, will likely alter behavior. Modified behaviors,

therefore, can serve as relevant endpoints for assessing chemical-induced stress in whole organisms as they relate biochemical and physiological effects to ecological function.

Numerous studies have shown behavioral measures to be sensitive indicators of toxicant-induced stress in fish and have called for their use in the hazard assessment process (see reviews by Olla et al. 1980; Westlake 1984; Little et al. 1985; Rand 1985; Atchison et al. 1987; Beitinger 1990). In addition to being sensitive indicators of toxicant exposure, behavioral effects may result in toxicant-altered growth, survival, or reproduction in wild fishes (Olla et al. 1980; Henry and Atchison 1984; Little et al. 1985; Atchison et al. 1987; Brown et al. 1987; Beitinger 1990; Little 1990; Sandheinrich and Atchison 1990; Henry and Atchison 1991).

Foraging behavior tests are criticized, however, because they lack standardization and because clear connections between altered behaviors and reduced growth, survival, or reproduction have not been adequately established. Great uncertainty surrounds the extrapolation of behavioral effects observed in the laboratory to the field (Sandheinrich and Atchison 1989; Henry and Atchison 1991). These problems may be overcome by further refinement of behavioral studies using experimental designs that incorporate greater ecological realism. To do so requires a union of ecology and toxicology (Kendall and Lacher 1991). Understanding the behavioral mechanisms that facilitate critical ecological activities, such as foraging and growth, will lead to more sensitive and predictive toxicity tests. Environmental toxicologists must utilize the vast ecological literature available on test organisms and communities if they hope to interpret and extrapolate their findings beyond the laboratory.

Concentrating on behaviors leading to successful foraging and growth of juvenile bluegill (*Lepomis macrochirus*), the following experiments demonstrated how key behavioral endpoints can be used to predict and explain growth effects induced by toxicant exposure. In this dissertation, I interpret the association between altered foraging behavior and reduced growth in juvenile bluegill exposed to cadmium. Finally, I discuss the ecological ramifications of reduced young-of-the-year fish growth to populations in

natural systems based on our current understanding of fish ecology and population dynamics.

Explanation of the Dissertation Format

This dissertation includes two papers preceded by a general introduction and followed by a summary. Literature cited in the general introduction and summary follows the summary. The two papers are prepared in the accepted style for publication in the *Canadian Journal of Fisheries and Aquatic Sciences*. Data acquisition, statistical analyses, and preparation of the manuscripts were the responsibility of the candidate. Guidance and editorial assistance were supplied by Dr. Gary J. Atchison and Dr. Mark B. Sandheinrich. The papers constituting this dissertation will be submitted as co-authored publications to scientific journals with the candidate as first author.

PAPER 1. CADMIUM EFFECTS ON THE PLANKTIVOROUS FORAGING PERFORMANCE AND GROWTH OF JUVENILE BLUEGILL

ABSTRACT

Current standardized toxicity testing protocols for assessing chemical hazards to aquatic organisms are primarily designed to determine physiological effects on growth, survival, and reproduction. They inadequately consider behavioral effects of toxicants; yet, organisms behaving abnormally in the wild experience reduced growth, fitness, and higher mortality. Hence, the utility of standardized tests for predicting direct and indirect effects of chemical exposure on aquatic organisms in complex ecosystems is limited. This study determined the effects of cadmium (0, 30, 60, 120, and 240 μg/L) on juvenile bluegill (*Lepomis macrochirus*) foraging behavior in three 28-d functional response experiments, each using a different size *Daphnia* as prey. This study also examined how cadmium-altered behaviors contributed to reduced growth rates. With one exception, bluegill consumption rate increased with prey density. In all three experiments, cadmium-exposed fish attacked fewer prey per unit of time compared to control fish. The degree of change (i.e., trends over time) in the number of *Daphnia* attacked per 30 s was the most consistently sensitive behavioral measure of sublethal stress in exposed bluegill; the lowest observed effect concentration (LOEC) for this metric was 30 μg Cd/L. Effects on prey attack rates were inversely related to prey size; cadmium had a greater effect on fish foraging on small compared to large prey. Cadmium had no effect on prey capture efficiency or handling time. Reductions in the consumption of *Daphnia* may be the result of cadmium-altered prey search strategy and motivation to feed. Growth in bluegill length and weight was significantly reduced by all cadmium concentrations. Growth was a more sensitive endpoint than the foraging behaviors because growth is an integration of cadmium's behavioral, biochemical, and physiological effects. In managing environmental contaminants, we must limit exposure to concentrations that do not inhibit organisms' ability to carry-out their most basic and essential ecological activities (i.e., foraging, avoiding predation, and reproducing). Behavioral toxicity tests provide a powerful tool for achieving this goal. By understanding the effects of chemicals on fish foraging behavior, toxicologists can begin to utilize optimal foraging and bioenergetics models to predict toxicant-altered diets and growth in the field.

INTRODUCTION

One goal of aquatic toxicology is to assess the potential effects of contaminants on organisms, populations, and communities so that we may prevent excessive degradation of aquatic ecosystems. Toxicologists are united in this purpose, but are divided concerning the type and amount of testing and information required to achieve it.

Current assessment of a chemical's potential hazard to aquatic organisms relies primarily on standard acute and chronic laboratory toxicity tests which measure physiological effects on growth, survival, and reproduction (Maki 1979; Sandheinrich and Atchison 1989). It is questionable whether these tests adequately protect aquatic life in the wild, however, because they fail to consider many behavioral effects that are ecologically critical to the well-being of individual organisms, their populations, and communities (e.g., Marcucella and Abramson 1978; Olla et al. 1980; Henry and Atchison 1984; Westlake 1984; Little et al. 1985; Rand 1985; Atchison et al. 1987; Sandheinrich and Atchison 1989, 1990; Beitinger 1990; Henry and Atchison 1991).

Ecologically important behaviors are commonly affected at or below the lowest observed effect concentration (LOEC) determined from standardized tests (see reviews by Olla et al. 1980; Westlake 1984; Little et al. 1985; Rand 1985; Atchison et al. 1987; Sandheinrich and Atchison 1990). For example, in their reviews of the effects of metals on fish behavior, Atchison et al. (1987) and Sandheinrich and Atchison (1990) cited numerous studies where avoidance, cough and ventilation rates, social interactions, reproductive courtship, feeding, and predator avoidance behaviors were significantly altered by metal concentrations near or below the LOECs derived from standardized tests. Because of their sensitivity, Atchison et al. (1987) suggested that fish behavior tests be added to the existing set of standardized toxicity tests used for hazard assessment and the establishment of water quality criteria. Although clearly shown to be sensitive indicators of toxicant exposure, few behaviors have been sufficiently studied to identify their role in growth, survival, or reproduction (Little 1990; Sandheinrich and Atchison 1990). Additional research is needed to identify altered behaviors that impact individual organisms and community dynamics within exposed ecosystems. Such behavioral measures will

strengthen our current hazard assessment protocol by increasing our understanding of toxicant modes of action and by providing sensitive, ecologically relevant endpoints likely to be predictive of real-world effects (Sandheinrich and Atchison 1990).

Little information currently exists on cadmium-altered fish behavior. No study to date has taken a mechanistic approach to assessing cadmium effects on fish foraging behavior nor have the implications of such effects for fish growth been investigated. In this study I investigate the effects of cadmium on the planktivorous foraging performance of juvenile bluegill (*Lepomis macrochirus*). I compare the sensitivity of behavioral endpoints among themselves and to that of growth. Finally, I attempt to determine the behavioral mechanism(s) of action of cadmium which may contribute to growth effects. My objectives were: (1) to compare the sensitivity of traditional behavioral endpoints (e.g., capture efficiency and general motivation to feed) to critical mechanisms of the foraging sequence (e.g., search, attack and capture, and handling time) for assessing and interpreting the effects of cadmium on bluegill foraging; (2) to determine the effect of cadmium on bluegill growth; (3) to compare cadmium concentrations that alter foraging behavior with those that alter growth and to interpret the contribution of altered foraging behavior to the growth effect; and (4) to interpret these findings within an ecological context.

METHODS AND MATERIALS

Toxicant

Cadmium was selected as the test chemical for several reasons. First, cadmium is a common environmental contaminant (Eaton 1974; Kay 1985; Nriagu and Sprague 1987) and is very toxic to most aquatic organisms (USEPA 1984; Eisler 1985). Second, cadmium is extremely toxic to fish and alters several fish behaviors at or below the LOEC measured by standardized chronic tests (Atchison et al. 1987). Eaton (1974) reported a 96-h LC_{50} (20,400 µg/L), lowest observed effect concentration for growth and survival (80 µg/L), and a no adverse effect concentration (31 µg/L) for bluegill exposed to cadmium in hard water (200 mg/L as $CaCO_3$). Finally, much is known about the environmental chemistry and physiological effects of cadmium in aquatic systems (e.g., Gardiner 1974; Eisler 1985). I used American Chemical Society (ACS) certified cadmium chloride ($CdCl_2 \cdot 2^1/_2\ H_2O$) to establish desired cadmium concentrations in treatment tanks.

Test Organisms

The bluegill was chosen as the test species because it: (1) is biologically, recreationally, and economically important; (2) is commonly used in toxicity testing, and its foraging behavior has been well documented; and (3) has been extensively used in the development of optimal foraging and bioenergetics models. Bluegill were purchased from Osage Beach Catfishery, Osage Beach, Missouri or were collected from a 3-ha pond north of Ames, Iowa. Fish were allowed to acclimate to laboratory conditions for one week prior to stocking into diluter tanks. The mean (± SE; N = 180) total length of bluegill at the initiation of the experiments was 31.1 (± 0.1) mm.

Bluegill and daphnid prey (*Daphnia magna* and *D. pulex*) were reared in dechlorinated Ames, Iowa municipal tap water. Water used for toxicity experiments was also adjusted for pH and alkalinity by adding appropriate amounts of 5% hydrochloric acid (HCl) and 25 g/L sodium bicarbonate ($NaHCO_3$) solutions, respectively.

Functional Response Experiments

Three functional response experiments, each using a different size of *Daphnia* as prey, were conducted to assess the effects of cadmium on the zooplanktivorous foraging performance of juvenile bluegill. Bluegill were maintained in diluter tanks on a 16L:8D photoperiod. Test fish were allowed to acclimate to diluter tanks for a minimum of 3 d before testing began, during which time they were fed frozen brine shrimp (*Artemia salina*) *ad libitum* once each day.

In each experiment, 60 bluegill, 6 assigned randomly to each of ten 57-L flow-through tanks (two replicates of 5 treatments), were used in a proportional diluter system. Nominal exposure concentrations included a control (no added cadmium) and 30, 60, 120, and 240 μg Cd/L. All fish were cold-branded for identification purposes (Everest and Edmundson 1967) so that individuals could be presented with the same foraging opportunity throughout the experiment. Each tank received one L of water every 8.5 min throughout the experiment, resulting in a complete volume replacement every 10 h.

Each experiment consisted of a 5-d pretreatment (undosed) period, when the foraging ability of individual fish was characterized, followed by a treatment (dosed) period of 21-22 d when the effects of cadmium were assessed in three 2 to 3-d testing periods. Within an experiment, pretreatment data were pooled across days for each fish to define foraging capabilities of individuals prior to cadmium exposure. Cadmium exposure began on day 6 and foraging trials resumed when cadmium in diluter tanks reached target concentrations. Data collected on each fish during the first 3 post-treatment test days were also averaged to provide mean values for the interval. This initial post-treatment period constituted time period 2 of the experiment. The various foraging parameters assessed in these experiments were again measured for 2-3 consecutive days during the middle (time period 3) and end (time period 4) of the dosed portion of the experiment.

To conduct a foraging trial, an individual fish was placed into one of three aquaria containing 24 L of uncontaminated water and either a low (0.5), medium (2.5), or high (15 or 20 prey/L) density of prey. Fish were allowed to forage for 30 s from the time of first prey capture. The first experiment used large *Daphnia magna* (2.5 ± 0.3 mm) as prey with the highest density being 15

prey/L. The second and third experiments used *D. pulex* (1.5 ± 0.2 mm) and small *D. magna* (0.85 ± 0.15 mm) as prey, respectively, with the highest density being 20 prey/L. Data recorded from a foraging trial included the total number of prey attacked, captured, capture efficiency (prey captured divided by prey attacked), mean handling time per prey (recorded for trials in high prey density tanks only), and a general assessment of feeding motivation. Feeding motivation was assessed by ranking the duration of feeding activity between 0-4 (0 = fish did not feed; 1 = fed for < 25% of the allotted 30 s trial; 2 = fed for 25-50% of the trial; 3 = fed for 50-75% of the trial; 4 = fed for 75-100% of the trial).

In a similar study conducted by Sandheinrich and Atchison (1989), bluegill typically did not feed continuously during 30-s foraging trials. Thus, handling time per prey item was measured over shorter periods of continuous feeding. Handling time was assessed in high prey density tanks only (where search time was minimal) and was determined as the time required to capture three or more *Daphnia* consecutively divided by the number of prey captured minus one.

Following the completion of daily foraging trials, each treatment tank received 2.0 g of frozen brine shrimp and fish were allowed to feed for 8 min before all excess brine shrimp were siphoned from the tank. Bluegill were then starved for approximately 18 h before beginning foraging trials the following day. The order in which tanks were tested was randomized at the start of each day. Fish were fed once daily when foraging trials were not conducted. Each treatment tank received 2.5 g of frozen brine shrimp and fish were allowed to feed for 8 min before remaining food was siphoned from the tank.

Growth Assessments

In addition to determining the effects of cadmium on bluegill foraging behavior, I also determined whether growth was affected in the *D. pulex* and small *D. magna* experiments. Each fish was weighed to the nearest 0.1 mg and measured to the nearest 0.5 mm at test initiation and termination. Care was taken throughout the experiment to assure that all fish in the treatment tanks received an equal opportunity to feed daily (both during and following the foraging trials).

Statistical Analyses

Statistically, experiments were a split-plot, completely randomized design with treatments randomly assigned to 57-L tanks (experimental units). The linear statistical model contained cadmium as the whole-plot treatment effect. The subplot contained the effects of prey density and the interactions of cadmium with prey density. The error term used to test for the effect of cadmium (whole-plot) was the mean square error (MSE) for tanks nested within cadmium (F with 4,5 df). The subplot contained the effects of density (F with 2,10 df) and cadmium*density interaction (F with 8,10 df). The error term used to test for the effect of time (F with 3,15 df) and cadmium*time interaction (F with 12,15 df) was the MSE for tanks nested within cadmium*time. Final fish lengths and weights were used as covariates in these analyses. Cadmium effects on bluegill growth (length and weight) were also determined by analysis of variance (F with 4,5 df). For all tests of cadmium effects, the 4 treatment df were also subdivided into single df contrasts to individually compare the control mean to that of each cadmium treatment (F.with 1,5 df).

The experimental design and data collected are of a rather unique nature, and, therefore, warrant additional discussion concerning statistical analyses for cadmium effects. It was known *a priori* that all fish in these experiments would change their prey consumption rate over time due to learning and growth. It was also known that fish behave as individuals. Some fish feed rapidly and aggressively while others forage more slowly. Thus, the frequently used approach of analyzing the magnitude of the differences in dependent variables (e.g., the number of prey eaten per 30 s) separately for each time period would not be statistically appropriate for several reasons. First, such an approach would not allow for control of the experiment-wise error rate. Second, the magnitude of responses in the 4 time periods lack independence. Finally, this approach would not account for effects on response variable magnitude due to fish individuality, but rather combines these effects with those due to cadmium. The appropriate statistical question is how cadmium modifies fishes' changes in foraging behavior through time. The analysis must account for individual fish differences and remove this effect so that differences analyzed are due only to the effects of cadmium. This was achieved by conducting the analysis of variance tests on

the slopes of lines generated by regressing the dependent variables against time for each fish. By analyzing the trends over time (i.e., the rates of change in response variables) rather than the raw data values, I removed the variability in the magnitude of responses due to fish individuality. This approach allowed analysis of the initial effects of cadmium (i.e., differences in trends or rates of change in response variables between time periods 1 and 2) as well as effects resulting from longer exposures (i.e., through time periods 3, and 4).

Within time periods 2 and 4 of the experiments, regression analyses were performed on the mean number of prey attacked per 30 s, averaged over prey density. All analysis of variance and least-squares regression tests were done using the Statistical Analysis System (SAS) General Linear Models (GLM) and regression (REG) procedures, respectively (SAS Institute, Inc. 1985). Null hypotheses were rejected at $P \leq 0.05$.

Water Chemistry and Cadmium Analyses

All water chemistry analyses were conducted using standard procedures (APHA 1989; ASTM 1990). Temperature, pH, conductivity, and dissolved oxygen of diluter-tank water were measured daily with a Celsius thermometer, a Corning 150 pH/ion meter, a Cole Parmer Model 1481-50 conductivity meter, and a Yellow Springs Instrument (YSI) model 57 oxygen meter, respectively. The oxygen and conductivity meters were calibrated weekly, whereas the pH meter was calibrated daily using 3 commercially available buffer solutions of pH 4.0, 7.0, and 10.0. Total alkalinity and total hardness were measured 4 times weekly according to standard methods (APHA 1989). U.S. EPA quality control samples (1 and 2) for mineral analysis were analyzed along with each batch of water samples to verify the accuracy and precision of these procedures.

Concentrations of cadmium in treatment tanks were determined on three 50-ml aliquot samples collected from each tank on 2 or 3 occasions during the treatment period. Samples were acidified to a pH <2 with 70% Baker Instra-Analyzed nitric acid (HNO_3) (J.T. Baker Inc., Phillipsburg, NJ) immediately after collection and were analyzed the same day. Total cadmium concentrations in acidified water samples were determined by flame level

atomic absorption spectrophotometry using an Instrumentation Laboratories model 251 atomic absorption spectrophotometer according to standard methods (APHA 1989). The method detection limit (MDL) and the limit of quantification (LOQ) for this analytical procedure were 5.5 and 17.5 μg/L, respectively (Taylor 1987). Procedural blanks, calibration standards, spiked samples, and externally supplied quality-assurance samples (Environmental Resource Associates, Arvada, CO) were taken through analytical procedures for each batch of water samples. The certified quality assurance samples were analyzed to assess the accuracy of my procedures. Precision was assessed by analyzing samples in triplicate, while the recovery of cadmium from spiked samples (at least 10% of all samples analyzed) was used to further assure accuracy. All sample containers and glassware used for metal analysis were washed according to APHA guidelines (1989).

Mean water chemistry parameters for the three experiments conducted were as follows: temperature, 23.7 ± 0.3°C; pH, 8.0 ± 0.2; dissolved oxygen, 7.4 ± 0.4 mg/L; conductivity, 708 ± 33 μmohs/cm^2 (± SE; n = 26); total alkalinity, 141 ± 7 mg/L as $CaCO_3$; and total hardness, 174 ± 10 mg/L as $CaCO_3$ (± SE; n = 12). Measured mean cadmium concentrations (μg/L) for the experiments with large *D. magna*, *D. pulex*, and small *D. magna* were 3.3 ± 0.8 (control), 26.8 ± 1.5, 61.9 ± 0.8, 121.1 ± 1.7, and 242.6 ± 7.4 (± SE; n = 2); 2.8 ± 0.3, 37.3 ± 11.1, 64.4 ± 6.3, 130.2 ± 24.8, and 238.8 ± 5.6 (± SE; n = 3); and 3.1 ± 2.8, 53.1 ± 14.7, 60.8 ± 2.2, 109.2 ± 14.0, and 228.0 ± 3.9 (± SE; n = 2), respectively. Measured concentrations for the externally supplied cadmium quality assurance samples were always within the certified 95% confidence interval. Mean recovery (± SD) of cadmium from 14 spiked water samples was 103.4 ± 1.6% (range 100.4-106.2%). The relative standard deviation from analysis of triplicate water samples for cadmium averaged 2.4% (range 0.0-6.0%).

RESULTS

Behavioral Effects

Among the 5 foraging parameters assessed during the three experiments, the rate of change (i.e., trends over time) in the number of prey attacked per 30 s was the most consistently sensitive indicator of cadmium toxicity to bluegill. The LOECs for this behavioral endpoint were 61.9 (nominal concentration = 60), 130.2 (120), and 53.1 (30) μg/L for bluegill foraging on large *D. magna*, *D. pulex*, and small *D. magna*, respectively (Table 1).

The number of prey attacked per 30 s changed significantly with prey density (F-tests, 2,10 df, $P < 0.001$) and time (F-tests, 3,15 df, $P < 0.010$) in all three experiments. With the exception of the 240 μg/L treatment, bluegill in the large *D. magna* experiment increased their consumption of prey as prey density increased. In the *D. pulex* experiment, fish generally exhibited a typical type II functional response to increasing prey density (i.e., increased prey consumption with increased prey density until reaching an asymptote where prey consumption per unit of time was at a maximum). Similar trends were observed for bluegill in the small *D. magna* experiment; however, the asymptote of maximum consumption rate was not reached by bluegill in any treatment. In all three experiments, cadmium-exposed fish generally attacked fewer prey per unit of time compared to control fish (Figure 1).

When data were averaged over prey density, the mean number of prey attacked by control fish increased with time. Conversely, with the exception of the 30 μg/L treatment in the *D. pulex* experiment, fish exposed to cadmium initially showed no increase or even a decrease in the number of prey attacked per 30 s. In experiments using large *D. magna* and *D. pulex* as prey, these reduced prey attack rates were followed by dose-related partial or full recoveries (in prey attack rates) later in the experiment. Only fish exposed to 60 μg/L cadmium showed any recovery in prey attack rate when small *D. magna* were used as prey (Figure 2).

In all three experiments, dose-related trends in the number of prey captured per 30 s were nearly identical to those for prey attacked because cadmium did not significantly affect prey capture efficiency. The only exception was for the 240 μg/L treatment in the fourth time period of the small *D. magna* experiment ($F=10.69$, 1,5 df, $P=0.022$) (Figure 3). Although prey

Table 1. Summary of analysis of variance tests performed on the mean numbers of prey attacked by bluegill exposed to 5 cadmium concentrations. For each zooplankton taxon, the slopes of the lines generated by regressing the mean number of prey attacked per 30 s against time during the experiment were analyzed (i.e., trends over time). Results are reported for increasing durations of exposure during the 4-week experiments. Numbers reported represent probabilities of a greater F-value with 4,5 df for the main effect and 1,5 df for the contrasts.

	Time Periods		
	1-2	1-3	1-4
Large *Daphnia magna* (2.5 ± 0.3 mm) Experiment			
Main Effect			
Cadmium	0.747	0.036	0.801
Contrasts			
0[a] vs. 30 µg/L[b]	0.717	0.104	0.669
0 vs. 60 µg/L	0.261	0.006	0.686
0 vs. 120 µg/L	0.696	0.029	0.770
0 vs. 240 µg/L	0.887	0.018	0.494
***Daphnia Pulex* (1.5 ± 0.2 mm) Experiment**			
Main Effect			
Cadmium	0.293	0.223	0.036
Contrasts			
0 vs. 30 µg/L	0.335	0.490	0.688
0 vs. 60 µg/L	0.168	0.673	0.794
0 vs. 120 µg/L	0.081	0.054	0.012
0 vs. 240 µg/L	0.472	0.332	0.050
Small *Daphnia magna* (0.85 ± 0.15 mm) Experiment			
Main Effect			
Cadmium	0.154	0.007	0.008
Contrasts			
0 vs. 30 µg/L	0.043	0.001	0.001
0 vs. 60 µg/L	0.037	0.001	0.007
0 vs. 120 µg/L	0.031	0.003	0.002
0 vs. 240 µg/L	0.048	0.003	0.003

[a] Control treatment (no added cadmium).

[b] Nominal treatment concentrations. Actual measured concentrations for each experiment are provided in the text.

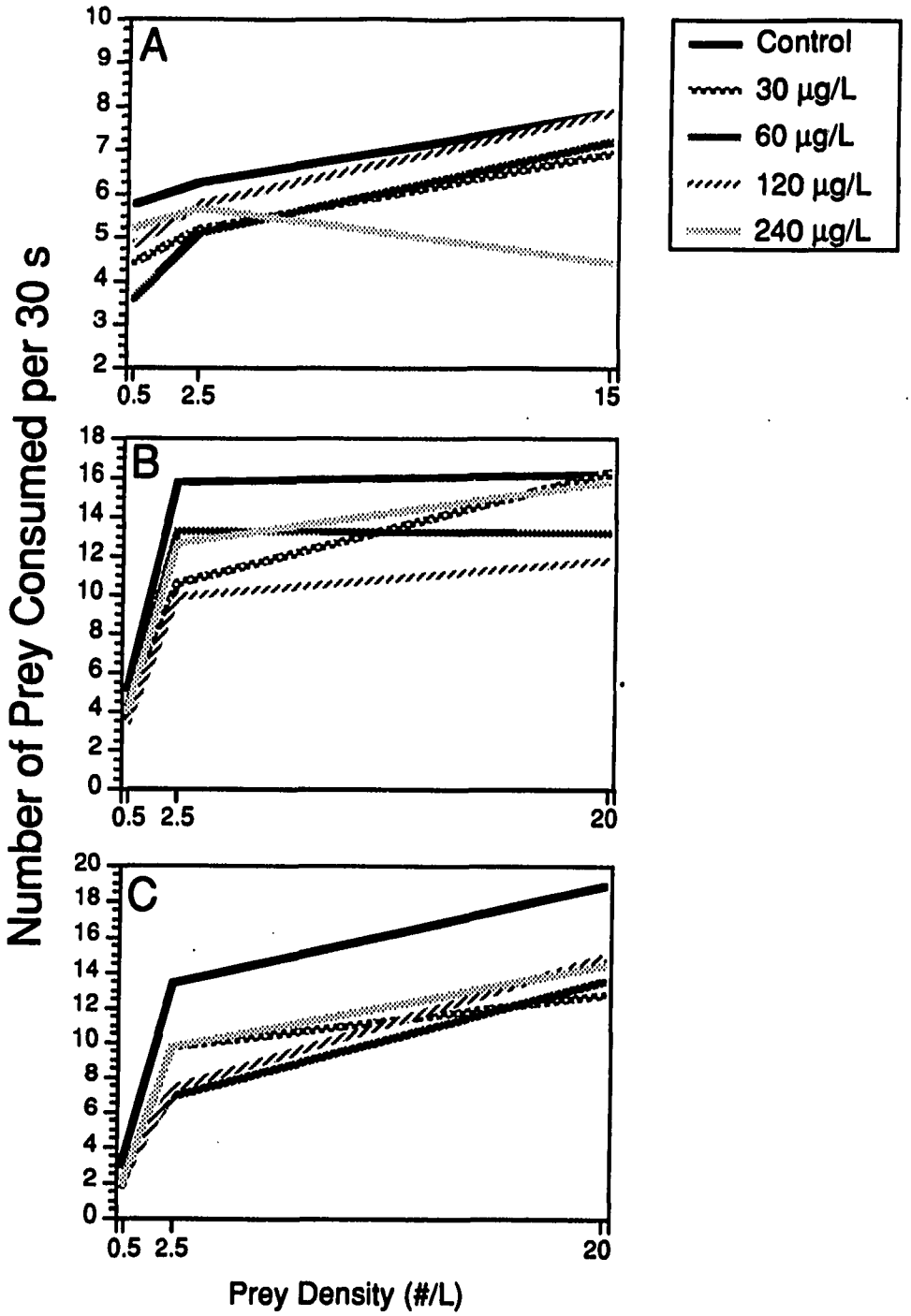

Figure 1. Cadmium effects on the functional response of juvenile bluegill foraging on (A) large *Daphnia magna* (2.5 ± 0.3 mm), (B) mid-sized *D. pulex* (1.5 ± 0.2 mm), and (C) small *D. magna* (0.85 ± 0.15 mm). Mean prey consumption rates of control and cadmium-exposed bluegill are shown at various prey densities

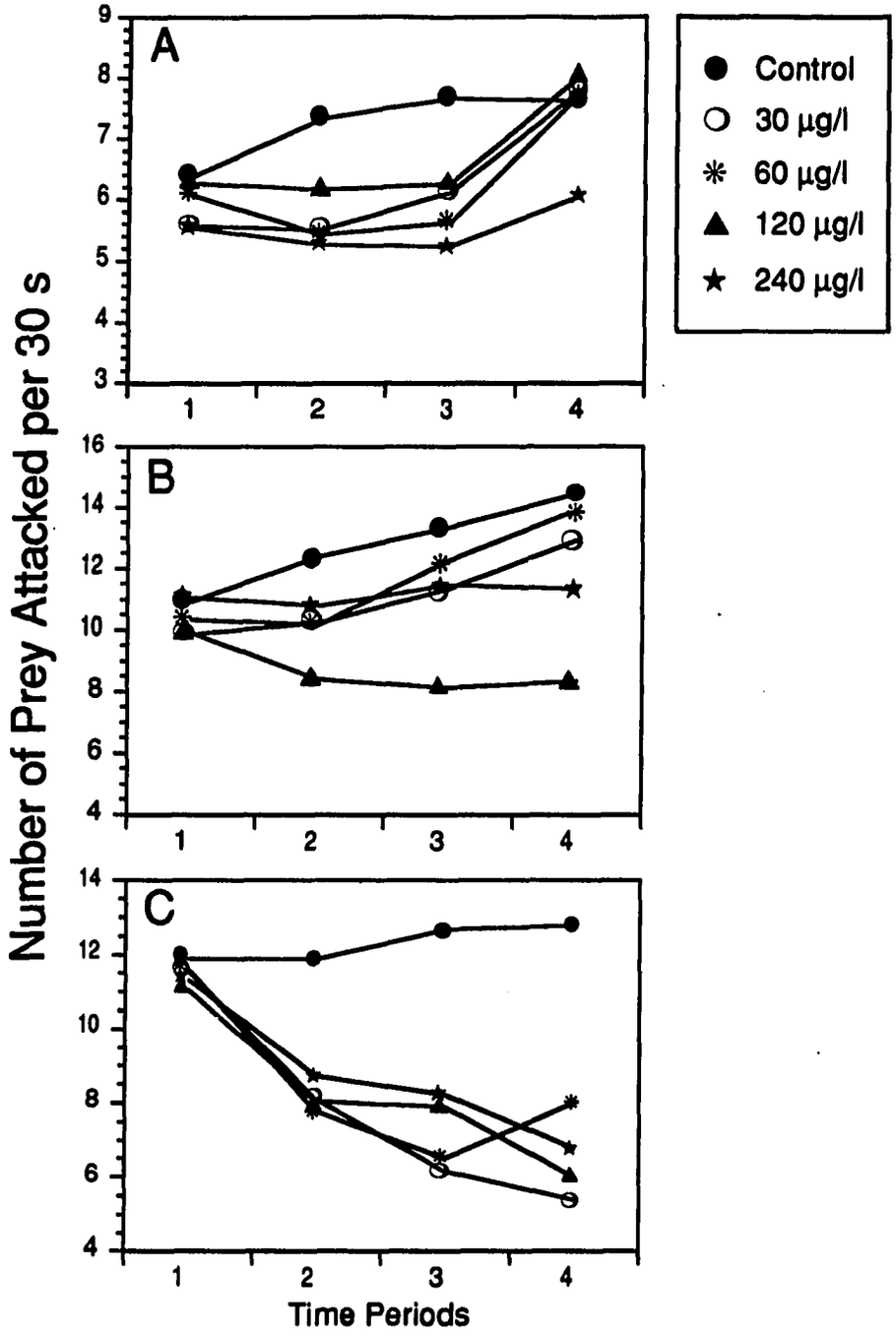

Figure 2. Cadmium effects on the mean number of (A) large *Daphnia magna* (2.5 ± 0.3 mm), (B) mid-sized *D. pulex* (1.5 ± 0.2 mm), and (C) small *D. magna* (0.85 ± 0.15 mm) attacked by juvenile bluegill during 30-s foraging trials conducted during four-week experiments. For means in time period 1, n=60, and for means in time periods 2-4, n=36

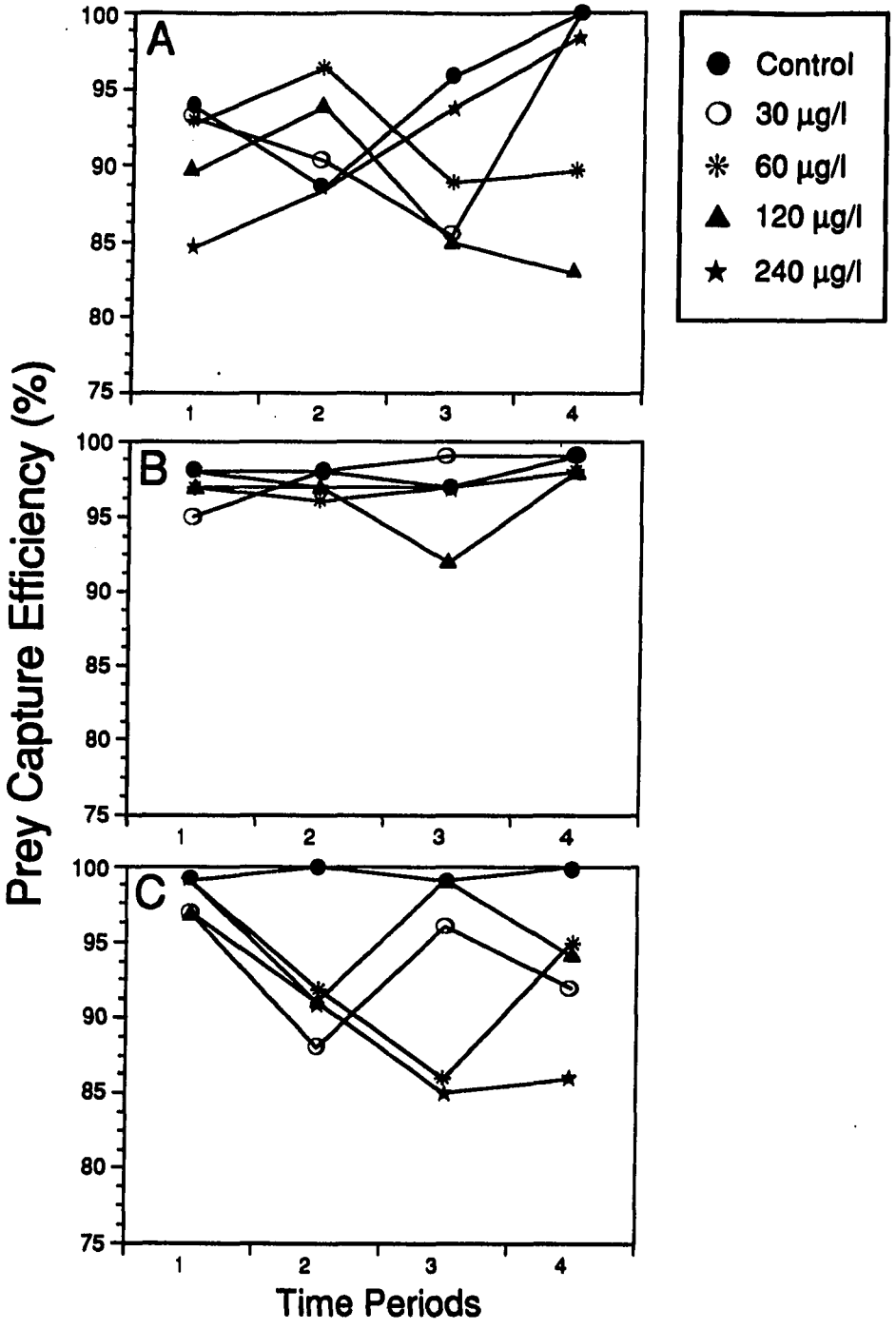

Figure 3. Effects of cadmium on juvenile bluegill prey capture efficiency when foraging on (A) large *Daphnia magna* (2.5 ± 0.3 mm), (B) *D. pulex* (1.5 ± 0.2 mm), and (C) small *D. magna* (0.85 ± 0.15 mm) during 30-s foraging trials conducted during four-week experiments. For means in time period 1, n=60, and for means in time periods 2-4, n=36

capture efficiency was not significantly reduced in a dose-dependent manner in any of the experiments, cadmium effects on this metric were related to prey size. The greatest and most consistent effect of cadmium on prey capture efficiency occurred when using the small *D. magna* as prey and the smallest effect when using the mid-sized *D. pulex* (Figure 3). Cadmium did not significantly affect mean handling time or general motivation to feed in the experiments where these metrics were assessed (Table 2).

Although exposure to cadmium reduced the number of prey attacked and captured by bluegill per unit of time, the magnitude of these reductions was not significantly related to cadmium concentrations initially (Figure 4) or after 3 weeks of exposure (Figure 5).

Growth Effects

In the *D. pulex* and small *D. magna* experiments where growth was assessed, bluegill length and weight were significantly reduced ($P < 0.02$) by all cadmium concentrations (Table 3). The LOEC could not be determined because there was a significant growth effect at the lowest cadmium concentration established in each experiment (37.3 and 53.1 μg/L, respectively).

Table 2. Effects of cadmium on bluegill foraging performance. Reported means were averaged over prey density and the three weeks of cadmium exposure. For the large *Daphnia magna* experiment, n=28 for handling time and n=84 for all other parameters. For the *D. pulex* experiment, n=40 for handling time and n=120 for all other parameters

Cadmium Concentration (µg/L)[a]	Attack Rate (#/30 s)		Capture Rate (#/30 s)		Capture Efficiency (%)		Handling Time (s)		Mot ation to ed (rank)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Large *Daphnia magna* (2.5 ± 0.3 mm) Experiment										
0[b]	7.2	0.5	7.2	0.7	94.8	5.8	2.3	0.4	2.9	0.2
30	6.3	1.3	6.1	1.5	91.9	7.4	2.7	0.7	2.7	0.3
60	6.0	1.2	5.8	1.2	91.9	3.5	1.8	0.4	2.6	0.4
120	6.1	0.5	5.9	0.5	87.2	5.8	2.4	0.6	2.6	0.2
240	5.4	0.5	5.2	0.7	93.5	5.1	3.8	1.2	2.6	0.1
***Daphnia pulex* (1.5 ± 0.2 mm) Experiment**										
0	13.4	1.0	13.3	1.2	97.9	1.1	1.3	0.1	--	--
30	11.5	1.4	11.3	1.4	98.7	0.7	1.3	0.1	--	--
60	12.1	1.9	11.8	2.1	97.1	1.2	1.4	0.3	--	--
120	8.3	0.1	8.1	0.1	95.7	3.4	1.7	0.3	--	--
240	11.2	0.3	10.8	0.3	97.4	0.9	1.4	0.1	--	--

[a] Nominal treatment concentrations.

[b] Control treatment (no added cadmium).

-- Parameter not measured.

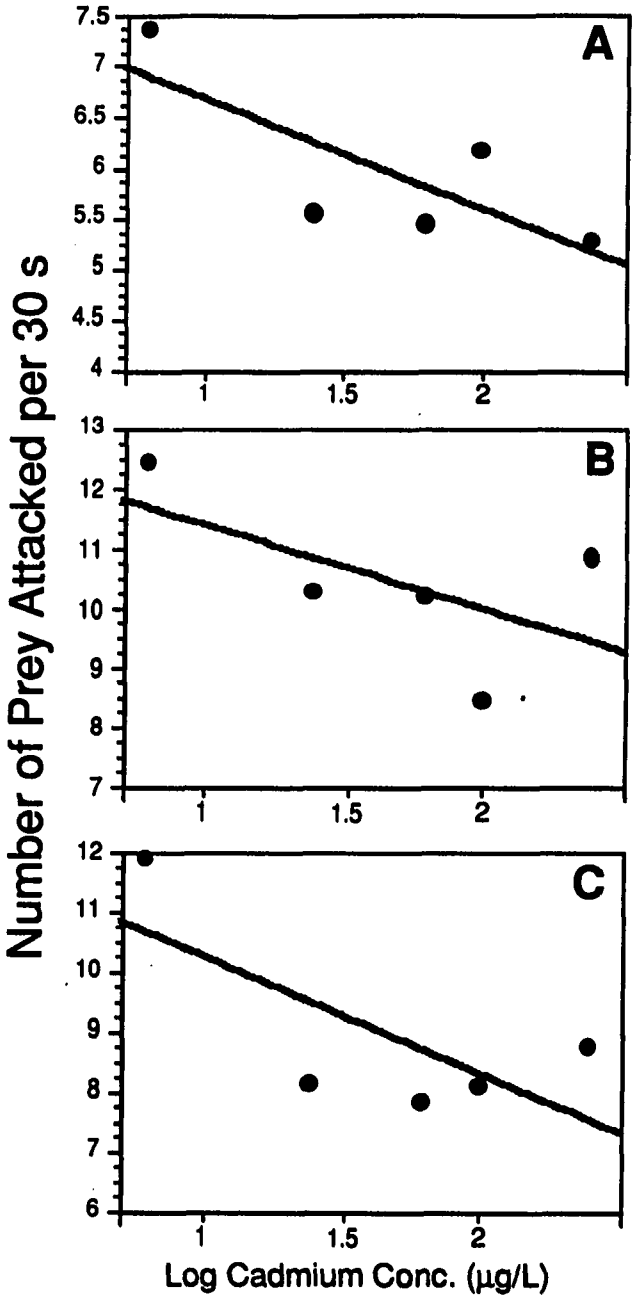

Figure 4. Relationship between cadmium concentration and bluegill attack rates for three sizes of *Daphnia* prey during time period 2. Number of attacks was averaged over prey density. Statistical results from regression analyses are as follows: A) large *Daphnia magna*: R^2= 0.76, P=0.054, SE=0.48; B) *D. pulex*: R^2=0.50, P=0.180, SE=1.2; and C) small *D. magna*: R^2=0.74, P=0.060, SE=0.99

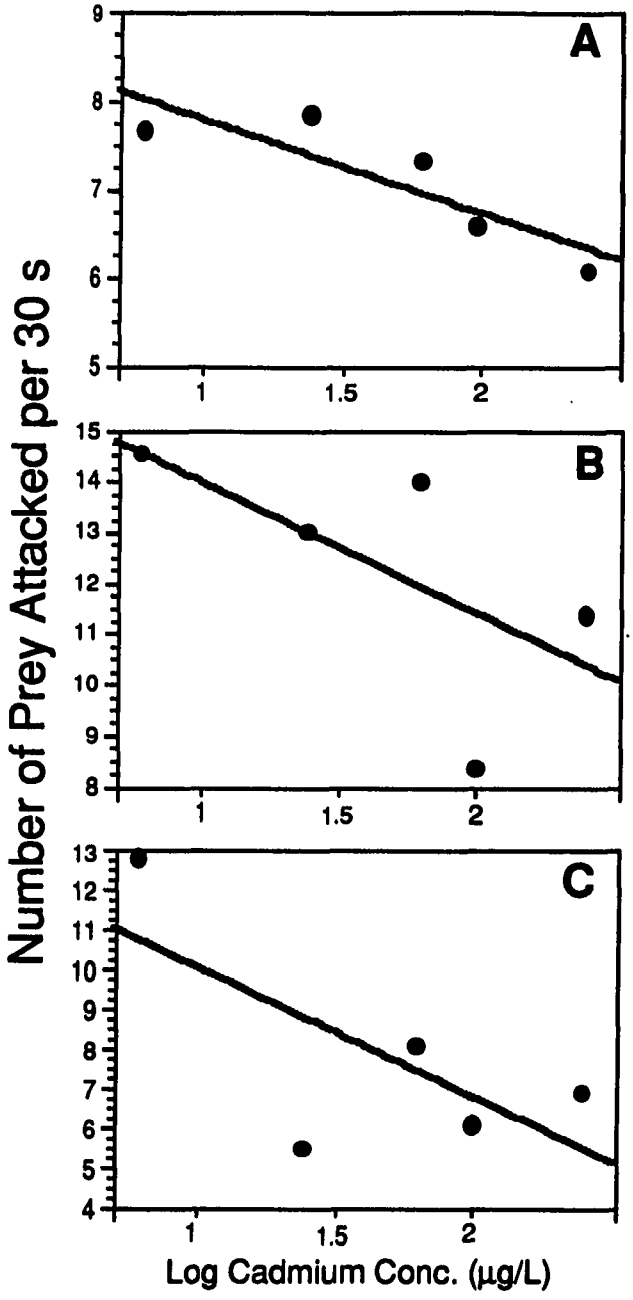

Figure 5. Relationships between cadmium concentrations and bluegill attack rates for three sizes of *Daphnia* prey during time period 4. Number of attacks was averaged over prey density. Statistical results from regression analyses are as follows: A) large *Daphnia magna*: R^2= 0.52, P=0.170, SE=0.60; B) *D. pulex*: R^2=0.39, P=0.258, SE=2.2; and C) small *D. magna*: R^2=0.71, P=0.071, SE=1.8

Table 3. Mean increase in total length and wet weight of juvenile bluegill during two 28-d experiments, both involving three weeks of cadmium exposure. Bluegill were fed a combination of *Daphnia* and frozen brine shrimp (*Artemia salina*). For all means, n=12

Cadmium Concentration (µg/L)[a]	Total Length (mm)		Weight (mg)	
	$\bar{x}$	SE	$\bar{x}$	SE
***Daphnia pulex* (1.5 ± 0.2 mm) Experiment**				
0[b]	11.7	0.6	0.627	0.044
30	10.6	0.4	0.546	0.029
60	10.5	0.4	0.539	0.037
120	8.7	0.5	0.431	0.029
240	8.2	0.3	0.397	0.030
Small *Daphnia magna* (0.85 ± 0.15 mm) Experiment				
0	9.9	0.8	0.502	0.061
30	8.0	0.5	0.360	0.032
60	7.2	0.4	0.296	0.024
120	7.3	0.4	0.322	0.029
240	7.0	0.3	0.298	0.024

[a] Nominal treatment concentrations.

[b] Control treatment (no added cadmium).

DISCUSSION

Cadmium-altered Foraging Behavior

Among the foraging behaviors studied, the rate of change (i.e., trends over time) in prey attack and capture rates were most consistently sensitive to cadmium-induced stress in bluegill. Relative to control fish, the prey consumption rate was initially reduced in fish exposed to all cadmium concentrations. Ability of bluegill to regain control-level consumption rates was dependent on prey size. Farmer et al. (1979), Drummond et al. (1973), and Lett et al. (1976) also reported initial reductions in food consumption or cessation of feeding by fish continuously exposed to zinc and copper, followed by partial or full recovery to control-level feeding within 20 d. Conversely, Collvin (1985) reported that perch (*Perca fluviatilis* L.) exposed to copper for 30 d never regained control-level prey consumption rates. Sandheinrich and Atchison (1989) reported that acute copper exposure reduced juvenile bluegill consumption of *Daphnia* and macroinvertebrate prey. However, because exposure lasted only 4 d in their experiments, these investigators were unable to address the ability of exposed bluegill to regain control-level feeding during chronic copper exposure.

The physiological and behavioral compensation mechanisms in fish that facilitate recovery in foraging performance during continuous exposure to sublethal concentrations of metals currently remain unclear; however, substantial evidence suggests that metallothionein is important (Kito et al. 1982; Benson and Birge 1985; Leland and Kuwabara 1985; Hamilton and Mehrle 1986; Fu et al. 1990; Roesijadi 1992). Because of such compensation mechanisms, the sensitivity of behavioral endpoints may be greatly influenced by the duration of exposure prior to assessment. Thus, in assessing the utility of behavioral assays for hazard assessment, it is important to determine if acute behavioral effects are predictive of chronic effects on growth, survival, or reproduction.

Both exposure concentration and prey size influenced recovery to control-level consumption rates in this study. Prey attack and capture rates returned to control levels for all treatments during the third week of exposure when using large *D. magna* as prey, only for the two lower cadmium concentrations when using the mid-sized *D. pulex*, and for no cadmium

concentration when using small *D. magna* as prey. Hence, the effects of cadmium on prey attack and capture rates were inversely related to prey size; cadmium had a greater effect on fish foraging on small than large prey. If reductions in these metrics were primarily due to increased prey handling time, the opposite should have occurred, with decreases in foraging rate being directly related to prey size. Consequently, these observations suggest that cadmium-altered search strategy, rather than increased prey handling time, is the probable cause of reduced prey consumption rates in these experiments. The lack of detectable effects on prey handling time from direct measures during these experiments further support this idea. Finally, preliminary evidence from a companion study (Bryan, unpublished data) indicates that cadmium does indeed affect bluegill search strategy by disrupting the normal pause-run or "saltatory" search process (see Janssen 1982; Evans and O'Brien 1988; O'Brien at al. 1986, 1989; and Ehlinger 1989, 1990 for descriptions of saltatory search strategies). An altered search strategy may reduce food intake by reducing prey encounter rates (Ehlinger 1989, 1990).

In addition to cadmium-altered search strategy, exposed bluegill spent less time engaged in foraging activity compared to control fish, suggesting reductions in feeding motivation. Motivation to feed, as defined and measured in this study, was a poor indicator of cadmium-induced stress in bluegill. However, by definition, the rank assigned to fish could not precisely quantify their motivation to feed, and was simply a general reflection of the time spent engaged in foraging activity during the 30 s trials. Thus, I can not dismiss feeding motivation as an affected foraging parameter in fish exposed to cadmium. Relative to controls, bluegill exposed to the two highest cadmium concentrations often did not begin feeding as quickly upon being placed into a foraging tank, nor did they feed for as long a period of time during the trial. Prey refusal was uncommon among control fish, but observed commonly among cadmium-exposed fish. The mechanism responsible for suppression of feeding motivation may involve elevations in the hormone cortisol (Donaldson 1981) which is a common response to stress. A review of the physiological control of feeding motivation or appetite in fish is given by Fletcher (1984).

To adequately assess feeding motivation, more sensitive and quantitative methods of assessment must be employed. Vinyard and O'Brien (1975)

attempted to quantitatively measure feeding motivation or interest in particular prey using a tilt-box technique. Their approach may be appropriate for quantitatively assessing toxicant effects on fish feeding motivation.

Sandheinrich and Atchison (1989) reported that copper-exposed bluegill (47.6 ± 1.5 mm) feeding on large *Daphnia magna* (2.4 ± 0.3 mm) and larger invertebrate prey (e.g., *Enallagma* sp. and *Hyalella azteca*) had significantly increased prey handling times compared to controls. It should be noted, however, that increased handling time was, in part, the result of repetitive spitting and recapturing of prey. Zebrafish (*Brachydanio rerio*) exposed to zinc (Cairns and Loos 1967) and lead (Nyman 1981) and flagfish (*Jordanella floridae*) exposed to alkyl benzene sulphonate detergent (ABS) (Foster et al. 1966) repetitively seized and ejected their prey while foraging. Repeated spitting or regurgitation and recapture of prey items was not observed in bluegill exposed to cadmium.

Capture efficiency of *Daphnia* was not affected by cadmium exposure, presumably due to the ease with which bluegill capture and ingest these non-evasive, easily handled prey (Eggers 1977; Drenner et al. 1978; Vinyard 1980; Ehlinger 1989). Studies reporting toxicant-altered capture efficiency (Finger et al. 1985; Mathers et al. 1985; Sandheinrich and Atchison 1989) involved larger and/or more evasive prey than those used in this study. Sandheinrich and Atchison (1989) concluded that the sensitivity of the capture-efficiency endpoint in toxicity testing may be related to the ratio of predator to prey size. I concur and add that prey evasiveness is also important in determining the sensitivity of this behavioral metric.

This study revealed some relationships between capture efficiency and prey size that suggest factors other than physical size and evasiveness of prey may influence capture efficiency of fish exposed to cadmium. Relative to controls, exposed bluegill had little difficulty capturing large- and medium-sized *Daphnia*, but initially experienced reduced capture efficiency at all cadmium concentrations when feeding on small *Daphnia*. This effect persisted and was statistically significant by the end of the third week of exposure for fish exposed to 240 µg/L. These results can not be explained by the generalization that capture efficiency decreases with increasing prey size and evasiveness. In fact, this generalization would predict results opposite from

those found in this study. I hypothesize that the rapidly developing visual acuity of juvenile bluegill (Breck and Gitter 1983; Li et al. 1985; Walton et al. 1992) may be affected by cadmium exposure either directly, or indirectly via cadmium effects on fish size. The visual resolution of juvenile bluegill increases rapidly as fish length increases (Li et al. 1985); thus, cadmium-reduced growth would also reduce visual acuity. Cadmium-altered visual acuity would have a greater effect on prey capture efficiency in bluegill feeding on small than large prey because small prey are inherently more difficult to locate and track (Confer and Blades 1975; Breck and Gitter 1983). Toxicant-induced reductions in visual acuity would, therefore, result in more miss-directed attacks at small than at large prey. Nyman (1981) reported that lead reduced the reaction distance of zebrafish (*Brachydanio rerio*) feeding on *Daphnia magna*. To my knowledge, no study has directly assessed the effect of cadmium on developing visual acuity in young fishes. Because visual acuity and reactive distance to prey increase rapidly as young fish grow (Breck and Gitter 1983; Li et al. 1985; Blaxter 1986), and because great visual resolution is essential for efficient foraging and growth in young planktivors such as bluegill, this area deserves attention in future toxicological foraging behavior studies.

Low replication and large variability in response of treated fish precluded the establishment of a significant relationship between the number of prey attacked and cadmium concentration. Different cadmium concentrations often produced behavioral responses in treated fish that differed substantially from control fish, but typically differed from one another (i.e., among cadmium treatments) by less than 25%. Sorting out treatment effects differing by 25% in magnitude when coefficients of variation ranged from 20 to 60% would require more than the two replicates logistically afforded in this study. Bluegill prey attack rate is probably related to cadmium concentration beyond some threshold level, below which bluegill foraging behavior does not differ from controls. Fish have been shown to tolerate exposure to low levels of cadmium and other toxic metal without suffering adverse biological effects (e.g., Leland and Kuwabara 1985). Certainly there are physiological and biochemical mechanisms, such as metallothionein, that can sequester small doses of metals in the body (George and Young 1986;

Leland and Kuwabara 1985; Hamilton and Mehrle 1986; Roesijadi 1992). To demonstrate what this threshold dose is for juvenile bluegill, or to statistically relate effects to dosage, however, would require more replication.

Cadmium-treated bluegill occasionally became hyperactive when approached in their treatment tanks or when captured for transfer to a foraging tank. Bouts of hyperactivity were typically short and normally followed by periods of reduced activity or inactivity, even if fish were presented with a foraging opportunity. Ellgaard et al. (1978) also reported hyperactivity in bluegill exposed to 250 µg Cd/L. Drummond and Russom (1990) stated that the hyperactivity syndrome is diagnostic of xenobiotics that cause metabolic dysfunction (i.e., uncouplers of oxidative phosphorylation). This syndrome is characterized by greatly accelerated locomotor activity, overreaction to stimuli, and increased ventilatory activity. Cadmium-altered activity levels would probably affect feeding motivation, search performance, and growth. Energy used for increased activity is not available for growth (Brett 1979).

Cadmium-altered Growth

Growth was significantly reduced at cadmium concentrations equal to or lower than the LOEC from behavioral tests. This suggested that reduced food intake was not the only factor contributing to reduced growth in bluegill exposed to cadmium. Elevated daily activity levels, higher resting metabolic rates, and/or less efficient food assimilation may also contribute to cadmium-altered growth rates. Waiwood and Beamish (1978) reported similar findings concerning copper-altered growth of rainbow trout (*Oncorhynchus mykiss*). Growth was reduced by copper exposure when appetite (i.e., food consumption) was normal because of lower gross assimilation efficiency and increased basal metabolic rates. Collvin (1985) and Borgmann and Ralph (1986) also reported that reduced food assimilation efficiency reduced growth of fish exposed to copper and cadmium, respectively. Borgmann and Ralph (1986) reported a 50% reduction in food assimilation efficiency in white sucker (*Catostomus commersoni*) larvae exposed to 150 µg Cd/L. Lett et al. (1976) reported depressed growth in rainbow trout exposed to copper; however, growth of these fish recovered to near the control-level with a recovery in food consumption after 40 d of exposure. Assimilation efficiency was unchanged, suggesting

that depressed growth was due to appetite suppression. Contradictions in the literature are to be expected because of differing methodologies, durations of exposure, sources and ages of test fish, etc. Nevertheless, these studies suggest that metals such as copper and cadmium probably affect growth by altering several important processes including prey consumption, food assimilation, metabolism, and activity levels. Recent experiments conducted by Atchison et al. (1993) demonstrated that the same cadmium concentrations used in this study significantly increased basal metabolism of juvenile bluegill. Energy used for increased metabolism is not available for growth (Collvin 1985).

Growth was the most sensitive indicator of chronic cadmium toxicity in bluegill. Results from this and other studies suggest that growth is sensitive to cadmium's behavioral, biochemical, and physiological effects. These various modes of action are not independent of each other. For example, physiological effects of cadmium that lead to increased basal metabolism and hyperactivity adversely affect foraging behavior and subsequently reduce foraging success (i.e., prey capture rates). In this study, cadmium-altered foraging behaviors contributed to reduced growth. In fact, the ecologically realistic manner in which fish were fed throughout these experiments may, in part, explain why the LOEC for growth derived from this study is lower than the LOEC of 80 μg Cd/L reported by Eaton (1974) for bluegill chronically exposed to cadmium in hard water (200 mg/L as $CaCO_3$). Fish in this study had to pursue and capture live prey while managing their energy expenditures relative to the amount of energy gained through limited foraging opportunities. Reduced food consumption, episodes of hyperactivity, and elevated basal metabolism probably all contributed to the reduced growth rates measured for all cadmium concentrations.

Choosing predator-prey species combinations that are ecologically realistic for laboratory study is, therefore, critical for accurate assessment and prediction of a toxicant's effect(s) on foraging and growth in the field. Standardized growth tests that use unnatural prey (e.g., trout pellets) remove important behaviors from the foraging process that are the basis and means of food acquisition in the wild. Growth assays conducted in this manner have been found to be less sensitive for determining LOECs compared to tests

assessing survival and reproduction in more than 70% of the studies reviewed (Woltering 1984). I concur with Sandheinrich and Atchison (1989) who stated that the insensitivity of growth assays is largely due to the manner in which they are conducted.

Because the LOECs derived from the behavioral and growth endpoints were similar, and because certain behavioral endpoints (e.g., prey attack rate) were typically affected immediately upon cadmium exposure, behavioral tests could be used as quick, inexpensive screening tools for assessing potential chronic growth effects of xenobiotics on fish. In a 6-d study, the LOEC for prey attack rate could be determined. It could then be predicted that growth would generally be chronically affected at concentrations below this LOEC because metals (and presumably many other toxicants) affect fish growth through their combined effects on behavior, biochemistry, and physiology. The simplistic and objective procedures involved with measuring prey attack rates could be readily standardized for this purpose.

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PAPER II. CADMIUM EFFECTS ON JUVENILE BLUEGILL FORAGING EFFICIENCY, PREY SELECTION, AND GROWTH

ABSTRACT

Behavior is the functional integration of many physiological and biochemical systems within organisms, and is often affected by toxicant exposure. Organisms behaving abnormally in the wild typically experience reduced growth, fitness, and higher mortality. Hence, when assessing a chemical's hazard to fish and fish populations, toxicant-altered behaviors should be considered. This study determined the effects of cadmium (0, 30, 60, and 120 μg/L) on the planktivorous foraging behavior and growth of 30-mm bluegill (*Lepomis macrochirus*) in a 24-d experiment. Initial rates of increase in large *Daphnia* consumption were depressed ($P \leq 0.002$) for all cadmium concentrations compared to the control, and were also affected ($P \leq 0.034$) by prey community structure. Throughout the 18-d exposure period, cadmium reduced the number of large *Daphnia* consumed per 45 s ($P = 0.009$) in a dose-dependent manner, which reduced the caloric gain of bluegill per unit time. Consumption of small *Daphnia* was not affected as severely, nor was the effect dose-dependent. Evidence suggests that cadmium-altered prey consumption was mediated primarily through an effect on search strategy. Motivation to feed and prey handling time may have also been affected. Cadmium effects on bluegill prey selection were dependent upon prey community structure. The ability of bluegill to maintain foraging efficiency, when switching between foraging arenas containing different prey community structure, was not affected by cadmium exposure. Reduced caloric gain, mediated primarily through cadmium-induced reductions in large prey consumption, was presumably a key factor contributing to reduced fish growth. The lowest observed effect concentration (LOEC) for growth was 60 μg Cd/L. The lower LOEC for changes in prey consumption suggests that behavioral metrics are sensitive indicators of sublethal toxicant stress in fish. Tests designed to determine toxicant-induced changes in prey consumption could be standardized and would bring ecological realism and increased sensitivity to early-tier hazard assessments. By understanding the effects of toxicants on fish foraging behavior, toxicologists can also begin to utilize optimal foraging and bioenergetics models to predict toxicant-altered diets and growth in the wild.

INTRODUCTION

Most toxicological studies on fish foraging and growth provided test fish with large quantities of easily handled prey (e.g., trout pellets) (see Atchison et al. 1987; Sandheinrich and Atchison 1990). Unlike wild fish, fish in these studies did not have to optimize their foraging strategy (i.e., search time and prey selection) in a multi-prey arena in order to maximize energy gain. Laboratory foraging tests that oversimplify complex predator-prey interactions may produce incomplete and inaccurate data concerning the effects of toxicants on important foraging behaviors and growth. Consequently, the predictive value of these studies is limited. Difficulty in predicting field-level responses from behavioral tests conducted in the laboratory has lead many toxicologists to question the utility of such tests (Maki 1979; Olla et al. 1980; Henry and Atchison 1991). Indeed, many important questions must be answered and experimental designs improved if behavioral tests are to play an important role in the hazard assessment process (Rand 1985; Little 1990).

Henry and Atchison (1991) stated that ecological realism and field validation lie at the heart of the experimental design problem of past fish behavioral toxicology. Behavioral effects are rarely studied in the field because field experimentation is complex, expensive, and laden with inherent variability, which often makes interpretation of results difficult (Little et al. 1985; Rand 1985; Little 1990). Hence, improving the ecological realism and complexity of laboratory micro- or mesocosm studies may be the best approach to studying and understanding the direct effects of toxicant-altered foraging behavior on fish and fish populations in the wild. By designing behavioral tests that provide the predator with a choice of natural prey in a realistic setting, important ecological questions, such as whether toxicants alter search strategy, prey selection, handling time, and rate of energetic gain, can be addressed. Understanding how xenobiotics affect fish foraging behavior will improve our ability to predict growth effects in the wild through the use of optimal foraging and bioenergetics models. By better understanding direct effects on fish, we also improve our ability to predict indirect and cascading effects (Kerfoot 1987; Mills et al. 1987) of toxicant exposure throughout aquatic communities.

If fish behavioral toxicology is to advance in this manner, toxicologists must make better use of the ecological literature already available on the mechanisms and strategies employed by foraging fishes (e.g., Werner 1974; Werner and Hall 1974; Vinyard 1980; Gardner 1981; Luecke and O' Brien 1981; Mittelbach 1981, 1983; Bartell 1982; Eggers 1982; Janssen 1982; Breck and Gitter 1983; Li et al. 1985; O' Brien et al. 1976, 1985, 1989, 1990; Wetterer and Bishop 1985; Evans and O' Brien 1988; Wanzenbock and Schiemer 1989; Walton et al. 1992). A clear understanding of normal fish foraging behavior, coupled with more ecologically realistic laboratory experimental designs, will provide the necessary framework within which toxicologists may identify toxicant effects on fish foraging behavior and growth.

Leland and Kuwabara (1985) called for studies correlating metal-induced physiological responses with impaired whole-animal responses critical to population survival. The ability of young fish to forage successfully is a key factor affecting year-class strength (Hjort 1926; May 1971, 1973; Werner and Blaxter 1980; Werner and Gilliam 1984; Werner and Hall 1988). In fact, body size, initially dictated by egg size and later by foraging success and subsequent growth, is the connective thread among factors affecting young fish survival (Miller et al. 1988). Hence, xenobiotics that adversely affect foraging success of larval and juvenile fishes affect fish population dynamics.

The goal of this study was to determine the effects of cadmium on the planktivourous foraging behavior and growth of juvenile bluegill (*Lepomis macrochirus*) in an ecologically realistic manner within the constraints of the laboratory. By limiting daily foraging opportunities and providing predators with two sizes of prey, bluegill had to minimize daily energy expenditures while maximizing caloric intake in order to maximize growth. My specific objectives were to: (1) determine if the rate of energetic gain is reduced in planktivorous juvenile bluegill exposed to cadmium when foraging in a mixed assemblage of two sizes of *Daphnia magna* ; (2) determine whether cadmium alters bluegill prey selection; (3) determine, through indirect methods associated with the two prey assemblages, the behavioral mechanisms responsible for observed changes in prey consumption rates and/or altered prey selection; (4) ascertain whether cadmium exposure affects the ability of bluegill to maintain high foraging efficiency when forced to switch between

environments containing different prey compositions (i.e., during patch switching events); and (5) determine if cadmium affects bluegill growth.

METHODS AND MATERIALS

Test Organisms

Juvenile bluegill were collected from a 3-ha pond north of Ames, Iowa. In the laboratory, bluegill and prey (*Daphnia magna*) were reared in dechlorinated Ames, Iowa municipal tap water. All fish were acclimated to laboratory conditions for one week prior to stocking into exposure tanks. The mean total length of bluegill at test initiation was 31.1 mm (SE = 0.3; n = 48). Water used for toxicity experiments was adjusted for pH and alkalinity by adding appropriate amounts of 5% hydrochloric acid (HCl) and sodium bicarbonate ($NaHCO_3$).

Experimental Approach

Forty-eight bluegill, six randomly assigned to each of eight 57-L flow-through tanks, were used in a proportional diluter system. Each diluter tank received one L of reconstituted water every 8.5 min throughout the experiment, resulting in a complete volume replacement every 10 h. Photoperiod was maintained at 16L:8D. Test fish were allowed to acclimate to diluter tank conditions for 3 d before testing began, during which time they were fed frozen brine shrimp (*Artemia salina*) *ad libitum* once each day.

The experiment consisted of a pretreatment (undosed) period of 6 d, when the foraging ability of individual fish was characterized, followed by a treatment (dosed) period of 18 d when the effects of cadmium were determined in four 3-d testing periods. Nominal exposure concentrations included a control (no added cadmium), and 30, 60, and 120 μg Cd/L. All treatments were replicated twice.

Two aquaria, each containing 15 L of uncontaminated water and two sizes of *D. magna* (1.2 ± 0.2 and 2.1 ± 0.2 mm) as prey, were used as foraging arenas. One aquarium contained 16 large and 126 small prey, which presented fish with equal perceived prey densities (6-7 of each prey size per reactive volume). The aquarium containing this prey assemblage is referred to as arena "S" because of the high proportion of small prey. The second aquarium (referred to as arena "L") contained 41 large and 101 small prey. In this arena, large prey were perceived by the bluegill to be 3-4 times more abundant than small prey. Numbers of perceived prey were calculated from

data on reaction distance and search volume reported by Li (1982) and Breck and Gitter (1983) for juvenile bluegill.

All fish were cold-branded (Everest and Edmundson 1967) for identification purposes. This facilitated dividing the six fish in each treatment tank into two groups of three fish each. Throughout the pretreatment period, group 1 fish were allowed to forage in prey arena S while group 2 fish foraged in arena L. The groups of fish switched arenas in the third, fourth, and fifth time periods (Figure 1).

To conduct a foraging trial, a fish was netted from its treatment tank and placed into the appropriate foraging aquarium and allowed to feed for 45 s from the time of first prey capture. During each 45-s foraging trial, the number of each prey size eaten and any misses or spits made by the fish were recorded and used to calculate capture efficiency. Mean dry weight was determined for both prey sizes by drying multiple samples containing several hundred individual *Daphnia* and dividing the total dry weight by the number of organisms in the sample. Total dry weight (mg) of *Daphnia* consumed during all foraging trials was calculated for each fish. I assumed caloric gain to be a direct function of the dry weight of *Daphnia* ingested (i.e., the product of total dry weight and a constant) (Cummins and Wuycheck 1971; Walton et al. 1992); thus, I used total dry weight ingested as an index of caloric gain.

Following the completion of daily foraging trials, each treatment tank received 2.0 g of frozen brine shrimp and fish were allowed to feed for 8 min before all excess brine shrimp were siphoned from the tank. Bluegill were then starved for approximately 18 h before beginning foraging trials the following day. The order in which tanks were tested was randomized at the start of each day.

Growth Assessments

Each fish was weighed to the nearest 0.1 mg and total length measured to the nearest 0.5 mm at test initiation and termination. Care was taken throughout the experiment to assure that all fish were presented with an equal opportunity to feed daily (both during and following the foraging trials).

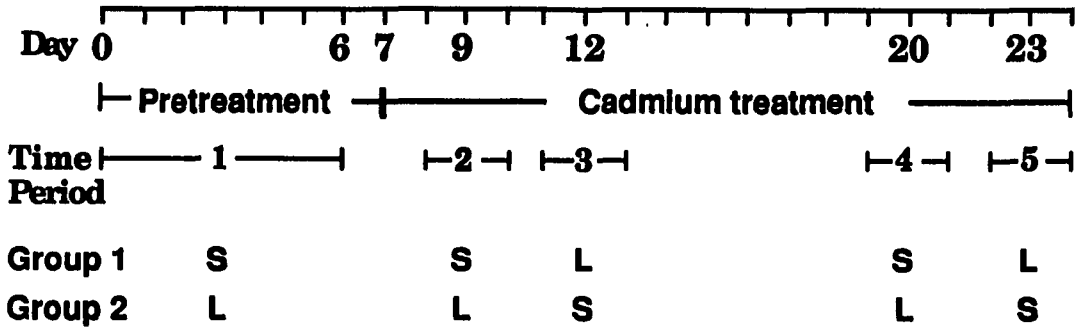

Figure 1. Experimental design used for assessing the effects of cadmium on the planktivorous foraging behavior of juvenile bluegill. Group 1 and 2 each contained half the fish within each treatment tank. Fish foraged in arenas with a preponderance of small (S) or large (L) prey during different time periods of the experiment

Statistical Analyses

Statistically, this experiment was a split-plot, completely randomized design where treatments were randomly assigned to 57-L tanks (experimental units). The linear statistical model contained cadmium as the whole-plot treatment effect. The subplot contained the effects of arena and the interactions of cadmium with arena. The error term used to test for the effect of cadmium (whole-plot) was the mean square error (MSE) for tanks nested within cadmium (F with 3,4 df). The subplot contained the effects of arena and cadmium*arena interaction. The error term used to test for arena effects (F with 1,4 df) and cadmium*arena interaction (F with 3,4 df) was the MSE for tanks nested within cadmium*arena. The error term used to test for the effect of time (F with 4,16 df) and cadmium*time interaction (F with 12,16 df) was the MSE for tanks nested within cadmium*time. Final fish lengths and weights were used as covariates in these analyses to remove effects on dependent variables due to fish size. Cadmium treatment effects on increased fish length and weight were also determined by analysis of variance (F with 3,4 df). For all tests of cadmium treatment effects, the 3 treatment df were also subdivided into single df contrasts to compare the control mean to that of each cadmium treatment individually (F with 1,4 df).

The experimental design and data collected are of a rather unique nature, and, therefore, warrant additional discussion concerning statistical analyses for cadmium effects. It was known *a priori* that all fish in this experiment would change their prey consumption rate over time due to learning and growth. It was also known that fish behave as individuals. Some fish feed rapidly and aggressively while others forage more slowly. Thus, the frequently used approach of analyzing the magnitude of the differences in dependent variables (e.g., the number of prey eaten per 45 s) separately for each time period would not be statistically appropriate for several reasons. First, such an approach would not allow for control of the experiment-wise error rate. Second, the magnitude of responses in the 5 time periods lack independence. Finally, this approach would not account for effects on response variable magnitude due to fish individuality, but rather combines these effects with those due to cadmium. The appropriate statistical question is how cadmium modifies fishes' changes in foraging behavior

through time. The analysis must account for individual fish differences and remove this effect so that differences analyzed are due only to the effects of cadmium. This was achieved by conducting the analysis of variance tests on the slopes of lines generated by regressing the dependent variables against time for each fish. By analyzing the trends over time (i.e., the rates of change in response variables) rather than the raw data values, I removed the variability in the magnitude of responses due to fish individuality. This approach allowed analysis of the initial effects of cadmium (i.e., differences in trends or rates of change in response variables between time periods 1 and 2) as well as effects resulting from longer exposures (i.e., through time periods 3, 4, and 5). All analysis of variance tests were performed using the Statistical Analysis System (SAS) General Linear Models (GLM) procedure (SAS Institute, Inc. 1985). Null hypotheses were rejected at $P \leq 0.05$.

In studies such as this one where variance among units treated alike is high (e.g., coefficients of variation between 20-60%), treatment effects can be largely masked by background variability (Daniel 1978). Variability among like units can be reduced by conducting the analysis of variance on the ranks of the raw data. If issues of data normality are also a concern, the Friedman nonparametric two-way analysis of variance by ranks test (X_r^2) (Daniel 1978) can be used. The null-hypothesis of this test states that treatments have no effect on measured responses. The alternative hypothesis states that at least one treatment tends to consistently yield larger values than at least one other treatment. This analysis is sensitive to even small treatment effects because it assesses whether or not a dose-related effect (of any magnitude) consistently occurs throughout experimental blocks. This analysis was used, therefore, to determine whether cadmium exposure had any consistent effect on bluegill foraging behavior (i.e., the size of response variables) throughout the experiment by treating time periods as blocks. This was possible because the correlations of response variables between time periods were not significant past the lag of one time period as shown by the covariance matrices.

I applied this test to data generated during the six consecutive days of pretreatment testing to determine whether any differences in foraging existed among the treatment groups of fish prior to cadmium exposure. No

significant differences among treatment groups existed regarding the number of large or small prey consumed per 45 s, capture efficiency, the percentage of the diet composed of large prey, or the total dry weight of *Daphnia* ingested during a foraging trial. This confirmed that all treatment groups of bluegill fed similarly prior to cadmium exposure.

Water Chemistry and Cadmium Analyses

All water chemistry analyses were conducted using standard procedures (APHA 1989; ASTM 1990). Temperature, pH, conductivity, and dissolved oxygen of test water were measured daily using a Celsius thermometer, a Corning 150 pH/ion meter, a Cole Parmer Model 1481-50 conductivity meter, and a Yellow Springs Instrument (YSI) model 57 oxygen meter, respectively. The oxygen and conductivity meters were calibrated weekly whereas the pH meter was calibrated daily using 3 commercially available buffer solutions of pH 4.0, 7.0, and 10.0. Total alkalinity and total hardness were measured 4 times weekly according to standard methods (ASTM 1989). U.S. EPA quality control samples (1 and 2) for mineral analysis were analyzed along with water samples to verify the accuracy and precision of these procedures.

Concentrations of cadmium in exposure tanks were determined on three 50-ml aliquot samples collected from each tank at the beginning, middle, and end of the treatment period. Samples were acidified to a pH <2 with Baker Instra-Analyzed 70% nitric acid (HNO_3) (J.T. Baker Inc., Phillipsburg, NJ) immediately after collection and were analyzed the same day. Total cadmium concentrations in acidified water samples were determined by flame level atomic absorption spectrophotometry using an Instrumentation Laboratories model 251 atomic absorption spectrophotometer according to standard methods (ASTM 1989). The method detection limit (MDL) and the limit of quantification (LOQ) for this analytical procedure were 5.5 and 17.5 μg/L, respectively (Taylor 1987). Procedural blanks, calibration standards, spiked samples, and externally supplied quality-assurance samples (Environmental Resource Associates, Arvada, CO) were taken through analytical procedures for each batch of tank samples. The certified quality assurance samples were analyzed to assess the accuracy of my procedures. Precision was assessed by analyzing

samples in triplicate, while the recovery of cadmium from spiked samples (at least 10% of all samples analyzed) was used to further assure accuracy. All sample containers and glassware used for metal analysis were washed according to APHA (1989) guidelines.

Means values for water chemistry parameters in experimental tanks were as follows: temperature, 23.9 ± 0.2°C; dissolved oxygen, 7.7 ± 0.2 mg/L; conductivity, 699 ± 22 μmohs/cm^2; and pH, 8.1 ± 0.1 (± SE, n = 24). Total alkalinity and total hardness averaged 132.9 ± 5.3 mg/L and 179 ± 9.1 mg/L as $CaCO_3$, respectively (± SE, n = 12). Measured mean cadmium concentrations in μg/L for each treatment were: 2.1 ± 0.1 (control), 31.7 ± 2.0, 62.2 ± 4.9, and 120.6 ± 2.2 (± SE; n = 3). Measured concentrations for the externally supplied cadmium quality assurance samples were always within the certified 95% confidence interval. Mean recovery (± SD) of cadmium from 6 spiked water samples was 101.9 ± 2.4% (range 99.2-105.4%). The relative standard deviation from analysis of triplicate water samples for cadmium averaged 2.3% (range 0.0-6.8%).

RESULTS AND DISCUSSION

Cadmium-induced Changes in Prey Consumption and Energetic Gain Over Time

Large Prey Consumption

When data were pooled across prey arenas, initial rates of increase in large prey consumption (between time periods 1 and 2) were significantly reduced (F-tests, 1,4 df, $P \leq 0.002$) for all cadmium treatments compared to the control (Figure 2a). Separate analyses were performed on the rates of change in large prey consumption for sequentially increasing durations of exposure (i.e., through time periods 3, 4, and 5). These analyses indicated significantly depressed (F-tests, 1,4 df, $P \leq 0.002$) rates of increase in large prey consumption for bluegill exposed to all cadmium treatments through time period 3, but not beyond (i.e., through time periods 4 or 5).

Throughout the experiment, cadmium reduced the number of large prey consumed per 45 s by bluegill in a dose-dependent manner ($X^2_r = 11.5$, 3 df, $P = 0.009$), with the 120 µg/L treatment being significantly reduced compared to the control ($X^2_r = 11.5$, 1 df, $P < 0.001$). Copper (Drummond et al. 1973; Lett et al. 1976; Waiwood and Beamish 1978; Collvin 1985; Sandheinrich and Atchison 1989) and zinc (Farmer et al. 1979) also have reduced feeding rates of exposed fishes, typically at concentrations less than or equal to the lowest observed effect concentrations (LOECs) derived from standard chronic endpoints (Sandheinrich and Atchison 1990).

Rates of change in large prey consumption between pretreatment means and those of each subsequent time period were also significantly affected (F-tests, 1,4 df, $P \leq 0.034$) by the arena in which bluegill foraged. Fish of all treatments consumed more large prey per unit of time and increased their consumption faster over time when foraging in prey arena L compared to arena S.

Analyzing data from each arena separately indicated that bluegill initially exposed to 120 µg Cd/L while foraging in arena S experienced a significant reduction ($F=7.77$, 1,4, df, $P = 0.049$) in their rate of change in large prey consumption relative to controls, whereas fish exposed to this

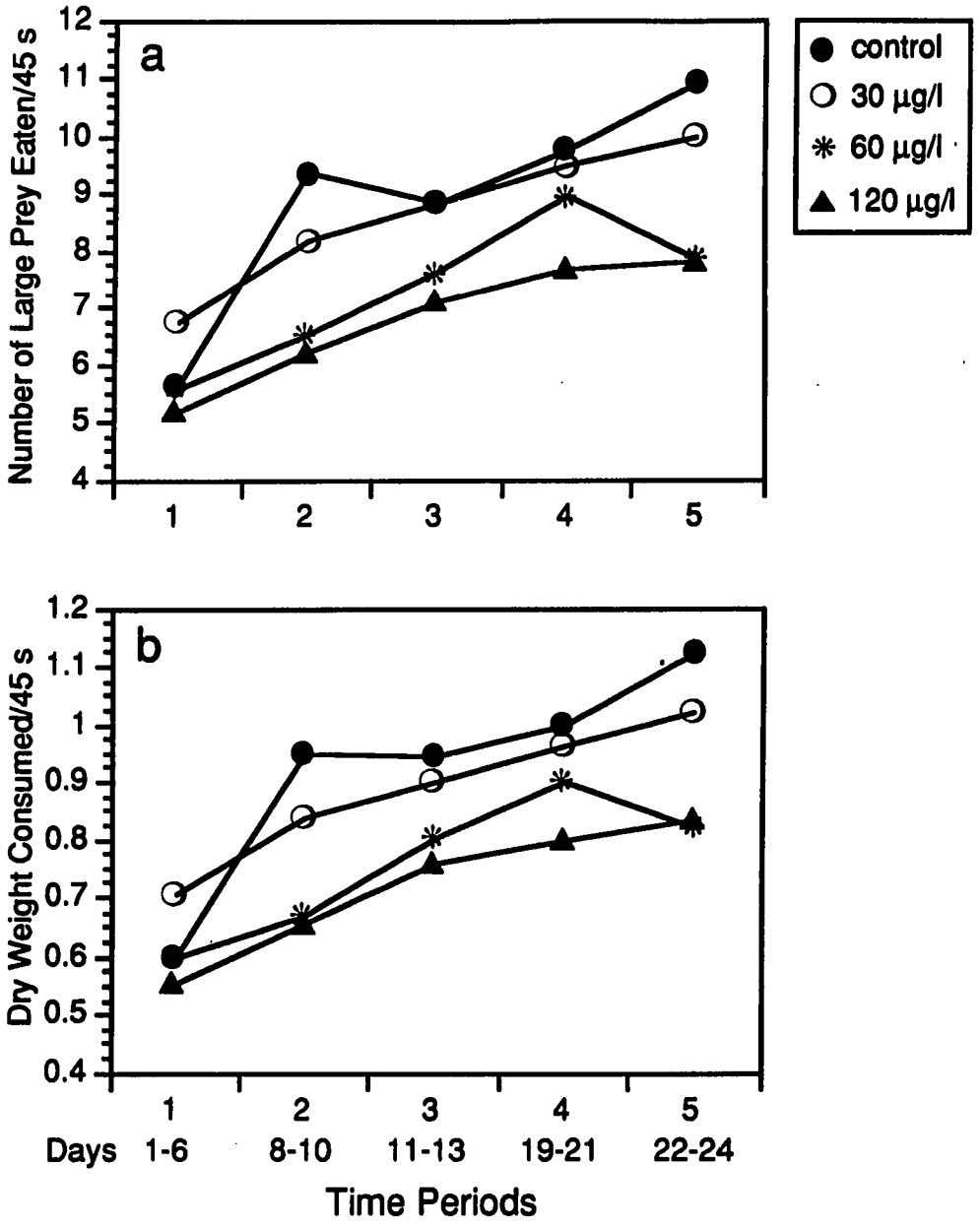

Figure 2. Effects of cadmium on juvenile bluegill consumption of large (2.1 ± 0.2 mm) *Daphnia* (a) and their total dry weight (mg) of *Daphnia* ingested (b) per unit time over 18 days of exposure. Plotted points represent means from all fish within a treatment. For means in time period 1, $n = 72$, and for means in time periods 2-5, $n = 36$

same concentration in arena L did not (F=1.19, 1,4, df, P = 0.337). This resulted in bluegill exposed to 120 μg Cd/L eating only 57.8% (SE = 8.6, n = 18) of what control fish consumed in arena S, but 73.1% (SE = 4.5, n = 18) of the control mean in arena L (Figure 3).

Results obtained from arena L were most useful for determining whether cadmium affected prey handling time. Because large prey density was high in this arena, the importance of prey search and pursuit in determining prey capture rates, and fishes' ability to increase these rates over time, was of lesser importance relative to handling time. If cadmium had caused a significant increase in prey handling time, treated fish should have increased their consumption of large prey more slowly than control fish. This did occur, although the depressed rate of increased large prey consumption observed for fish exposed to 120 μg Cd/L was not significantly different from control fish (Figure 3b). This suggests that cadmium may have affected prey handling time, but that this effect was small. Because initial cadmium-induced depressions in the rates of increased large prey consumption were greater in arena S than in arena L, I deduce that cadmium's effect on large prey consumption was not mediated primarily through increased prey handling time, but rather through changes elsewhere in the overall foraging process. These results, and those from similar experiments (Chapter 1; Bryan unpublished data), suggest that search strategy may be the key component of the foraging process affected by cadmium exposure. A reduced ability to locate prey would have the greatest effect on the rate of increase in large prey consumption where large prey density was lowest (arena S) and least where large prey density was highest (arena L), as was observed in this experiment. In all likelihood, observed effects on large prey consumption resulted from a combination of increased prey handling time and altered search strategy, with the latter presumably being the more important of the two.

Assessing the effects of cadmium on bluegill search strategy will not, however, be as simple as measuring cadmium effects on reaction distance to prey (e.g., Nyman 1981; Sandheinrich and Atchison 1989). Reaction distance is only a small part of the complex strategies that zooplanktivorous fishes employ while foraging (see Janssen 1982; Evans and O'Brien 1988; O' Brien

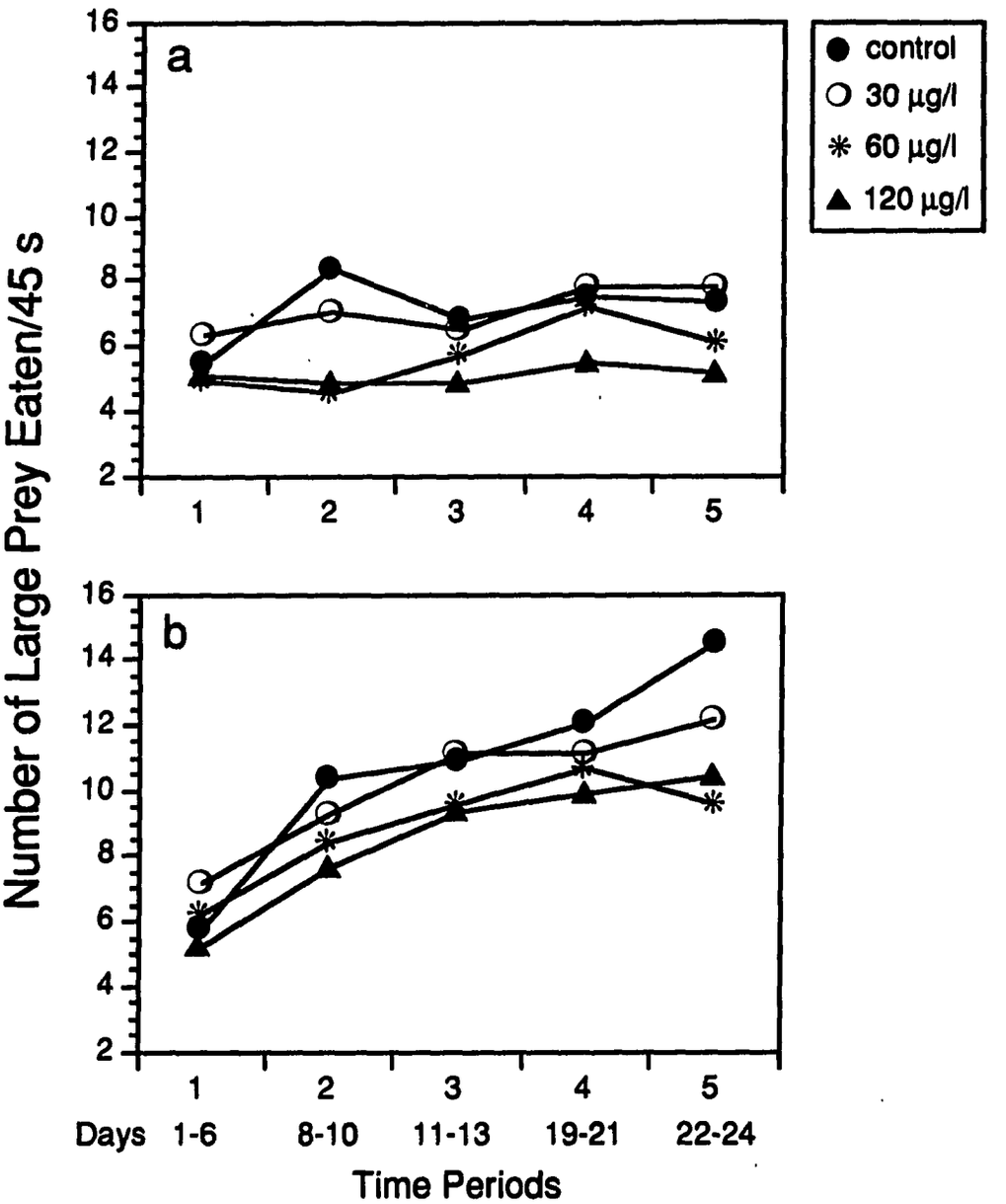

Figure 3. Effects of cadmium on juvenile bluegill consumption of large (2.1 ± 0.2 mm) *Daphnia* when foraging in arena S (a), containing 8.4 small and 1.1 large prey/L, and arena L (b), containing 6.7 small and 2.7 large prey/L, over 18 days of exposure. For means plotted in time period 1, n = 36, and for means plotted in time periods 2-5, n = 18

et al. 1976, 1985, 1989, 1990; Ehlinger 1989). For example, bluegill tailor the length of their search pauses and the distance of their repositioning movements to maximize the efficiency of their "saltatory" search for specific prey types and sizes (Janssen 1982) and for environments of variable complexity (Ehlinger 1989). Irrespective of effects on maximum reactive distance, cadmium may decrease search efficiency, and hence prey encounter rates, by altering the amount of time allocated to actual searching (i.e., pauses) and that devoted to moving to new unsearched areas (i.e., runs). Maximum reaction distance is of lesser importance compared to these other mechanisms that literally define optimal search strategy in bluegill. Preliminary evidence from a companion study (Bryan unpublished data) indicates that cadmium does affect bluegill search strategy by disrupting the normal pause-run or "saltatory" search process.

Small Prey Consumption

Similar analyses indicated that the rates of change in small prey consumption over time did not differ significantly (F-tests, 1,4 df, $P > 0.05$) among treatments (Figure 4). Other studies of bluegill foraging behavior and prey selection (e.g., Werner and Hall 1974; Li 1982; Li et al. 1985; Walton et al. 1992) predict that bluegill should select large prey over small in both arenas. The effects of cadmium on consumption should, therefore, be greater for the preferred large prey than for small prey, as was found in this study. Arena had little effect on the rate of increase in small prey consumption throughout the experiment; a significant effect ($F = 7.81$, 1,4 df, $P = 0.049$) occurred only when data were analyzed through time period 3. No arena effect was demonstrated initially (i.e., between time periods 1 and 2) nor did one persist throughout the experiment. Lack of an arena effect was likely due to the relatively high densities of small prey in both arenas, which differed by only 25%.

Throughout the experiment, cadmium altered ($X^2_r = 9.9$, 3 df, $P = 0.019$) the number of small prey consumed per 45 s, but not in a dose-dependent manner. The number of prey consumed by fish was significantly reduced by 60 µg Cd/L, but not by 120 µg Cd/L (Figure 4). Reasons for this apparent

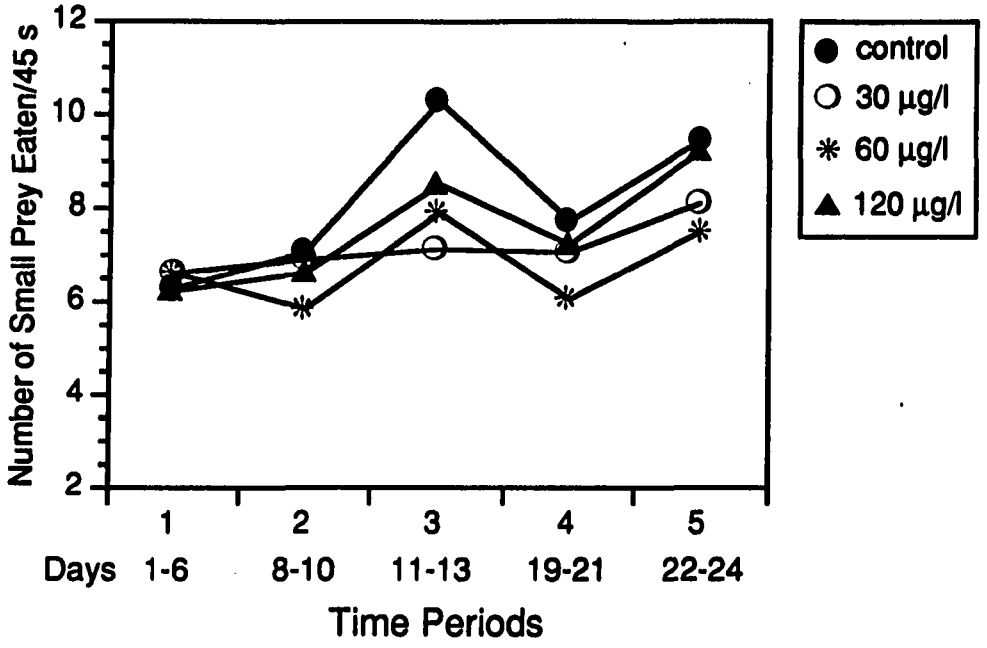

Figure 4. Effects of cadmium on juvenile bluegill consumption of small (1.2 ± 0.2 mm) *Daphnia* per unit time over 18 days of exposure. Plotted points represent means from all fish within a treatment. For means in time period 1, $n = 72$, and for means in time periods 2-5, $n = 36$

inconsistency will be discussed later under prey selection.

Total Dry Weight of Daphnia Consumed per Trial: An Index of Caloric Gain

Depressed rates of increase in large prey consumption of treated fish resulted in significantly depressed (F-tests, 1,4 df, $P \leq 0.012$) rates of increased caloric gain through time period 3 for fish exposed to all cadmium concentrations (Figure 2b). The caloric gain by bluegill was dependent upon the number of large prey consumed because large prey had a mean dry weight that was 7.05 times greater than small prey. Thus, as with changes in large prey consumption, cadmium-induced depressions in the rate of increased dry weight consumed were statistically significant initially upon cadmium exposure, but did not remain so throughout the experiment (i.e., through time periods 4 or 5).

However, cadmium-exposed bluegill did not recover to control levels of prey consumption and caloric gain as reported for fish continuously exposed to metals in other studies (Chapter 1; Drummond et al. 1973; Farmer et al. 1979; Lett et al. 1976). On the contrary, the consistent dose-related reductions in the number of large ($X^2_r = 11.5$, 3 df, $P = 0.009$) (Figure 2a) and small ($X^2_r = 9.9$, 3 df, $P = 0.019$) (Figure 4) prey consumed per foraging trial previously discussed, and the resulting dose-dependent reductions in caloric gain per unit of time ($X^2_r = 11.1$, 3 df, $P = 0.011$) (Figure 2b) that occurred throughout the experiment suggest just the opposite. Reduced caloric gain, mediated primarily through cadmium-induced reductions in large prey consumption, was presumably a key factor contributing to reduced fish growth.

Cadmium-induced Changes in Prey Selection and Capture Efficiency Over Time

Percentage of the Diet Composed of Large Prey

As all fish were presented with the same foraging opportunities throughout the experiment, it is informative to examine how the ratio of large to small prey consumed changed among treatments to evaluate whether cadmium significantly affected bluegill prey selection. Initial cadmium exposure caused a significant decline in the percentage of the diet composed of

large prey ($F = 7.67$, 1,4 df, $P = 0.050$) in fish exposed to 120 μg Cd/L foraging in arena S (Figure 5a), but not in fish exposed to this same concentration foraging in arena L ($F = 0.62$, 1,4 df, $P = 0.476$) (Figure 5b). Closer examination of fish group 1 over time (Figure 5a) revealed the following. Prior to cadmium exposure, group 1 bluegill assigned to the control and 120 μg/L treatments consumed nearly identical proportions of large and small prey. When these fish were allowed to forage in arena L during time periods 3 and 5, the percentage of the diet composed of large prey in fish exposed to 120 μg Cd/L averaged 1.3% higher (SE = 3.1, $n = 18$) and 5.4% lower (SE = 2.9, $n = 18$) than control fish, respectively. In other words, when preferred large prey were abundant, the proportion of large prey in the diets of undosed and high-dosed fish remained similar. However, during time period 4, when these same fish returned to foraging in arena S, the percentage of the diet composed of large prey in fish exposed to 120 μg Cd/L declined an average of 11.5% (SE = 3.3, $n = 18$) more than the control. Although this change in diet composition was not statistically significant, it suggests that cadmium effects on prey selection are dependent upon the arena in which fish were foraging.

An examination of bluegill foraging in arena S over time revealed that the percentage of the diet composed of large prey was lowest for fish exposed to 120 μg Cd/L in all 4 exposure periods ($X^2_r = 8.18$, 3 df, $P = 0.042$) (Figure 6a). Throughout the exposure periods of the experiment, the proportion of large prey in the diet of these fish was reduced by an average of 9.1% (SE = 1.9, $n = 72$) relative to control fish. Conversely, and perhaps to compensate for reduced large prey consumption, small prey consumption by fish exposed to 120 μg Cd/L was consistently higher than that of bluegill exposed to 30 and 60 μg Cd/L (Figure 4). This explains the apparent inconsistency concerning the dose-independent effect of cadmium on small prey consumption.

Cadmium had no consistent effect on the proportion of large prey consumed by bluegill foraging in arena L ($X^2_r = 6.00$, 3 df, $P = 0.112$) (Figure 6b).

I conclude that cadmium does not affect prey selection directly, but rather indirectly through its effects on prey search strategy. Results from

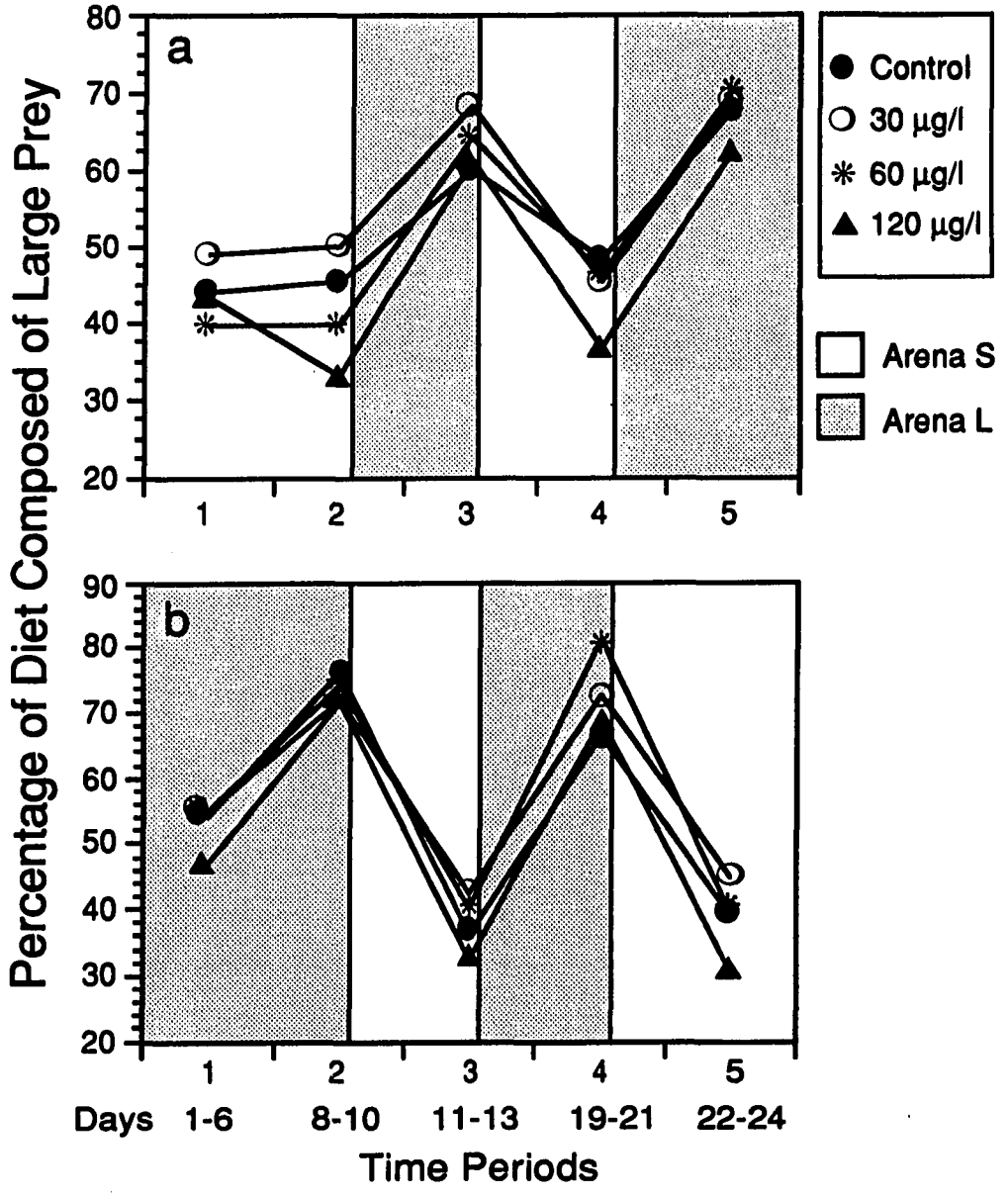

Figure 5. Cadmium effects on prey selection of group 1 (a) and 2 (b) bluegill foraging in different prey arenas offering different densities of large (2.1 ± 0.2 mm) and small (1.2 ± 0.2 mm) *Daphnia*. Selection was assessed by the numerical percentage of the diet composed of large prey. For means plotted in time period 1, $n = 36$, and for means plotted in time periods 2-5, $n = 18$

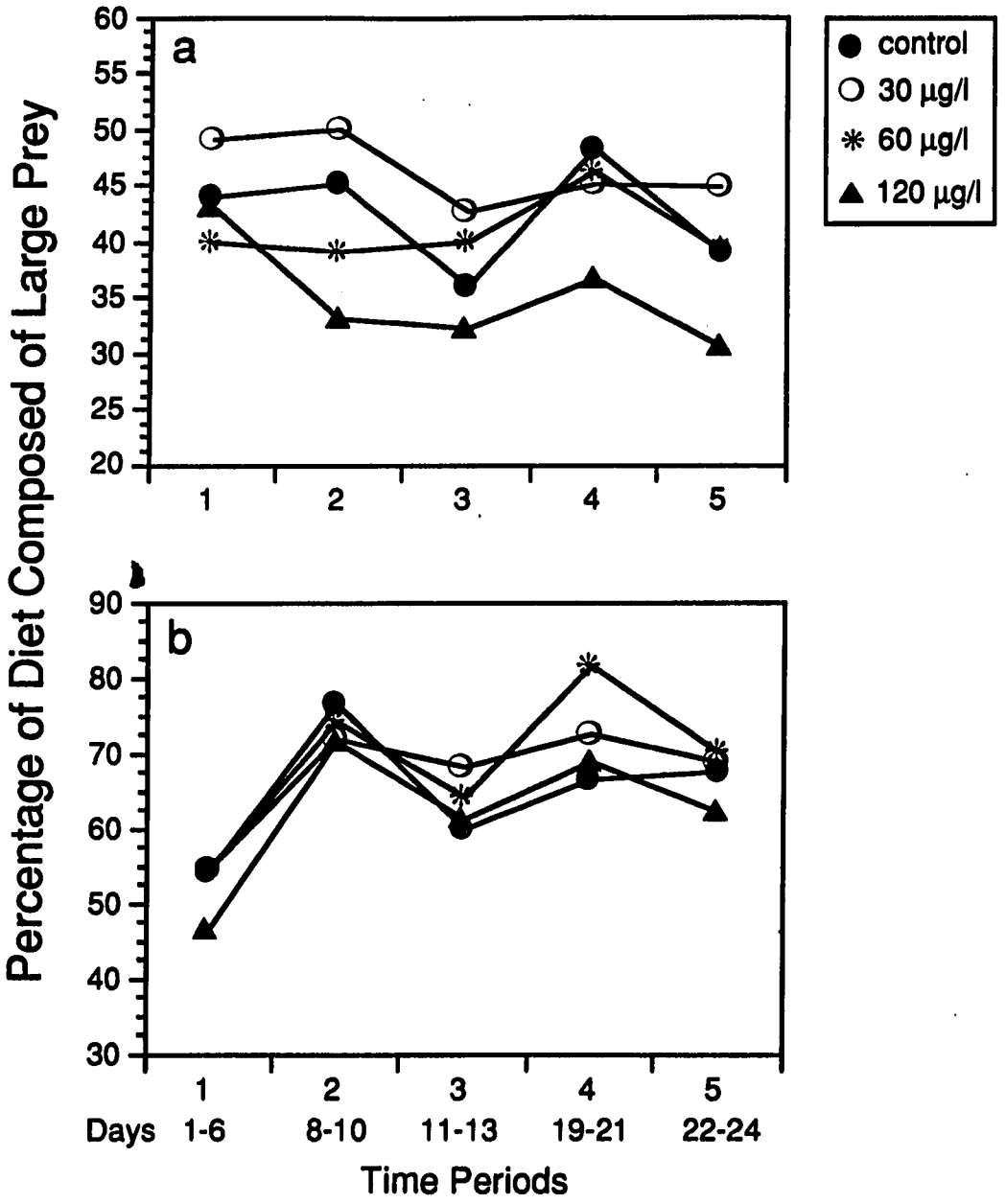

Figure 6. Effects of cadmium on bluegill prey selection while foraging in prey arenas S (a) and L (b). Prey selection was assessed by the numerical percentage of the diet composed of large prey. For means plotted in time period 1, n = 36, and for means plotted in time periods 2-5, n = 18

arena L indicated that cadmium-exposed bluegill did not reject large prey when encountered. Therefore, the reduced proportion of large prey in the diet of bluegill exposed to 120 μg Cd/L and foraging in arena S was the result of reduced encounters with these prey. Rather than actively searching for and consuming large prey, fish exposed to 120 μg Cd/L may have simply consumed prey as encountered while swimming aimlessly through the water column (i.e., not employing saltatory search). Based on the density of small and large prey in arena S, I would have predicted an equal proportion of the two prey sizes in the diet. However, this is assuming a uniform distribution of prey in the test tanks, which rarely occurred. Instead, large prey typically aggregated on or near the floor of test tanks with few in the water column. Small prey could be found on the tank bottom as well, but many were also distributed throughout the water column. Hence, if cadmium exposure altered or eliminated the normal saltatory search behavior of foraging bluegill, resulting in unstructured and undirected searching throughout the water column, fish would encounter and consume a greater proportion of small prey than predicted by the prey stocking ratios. Those fish actively searching for large prey (i.e., control fish) found them near the bottom, where they pursued and consumed them. This resulted in the proportion of large prey in their diets being greater than that of fish exposed to 120 μg Cd/L.

Prey Capture Efficiency

Cadmium exposure did not cause significant (F-tests, 1,4, df, $P > 0.05$) declines in prey capture efficiency over the course of the experiment. In fact, fish exposed to 120 μg Cd/L were more efficient after exposure (Figure 7). This is not surprising, however, as fish become more efficient in capturing and handling novel prey with increasing experience (Ehlinger 1989).

During the 6 d of pretreatment testing, no treatment-group consistently achieved greater overall prey capture efficiency than the others ($X_r^2 = 1.65$, 3 df, $P = 0.648$). However, over the course of the experiment, cadmium-treated fish consistently had lower prey capture efficiency compared to control fish, with bluegill exposed to 120 μg Cd/L having the

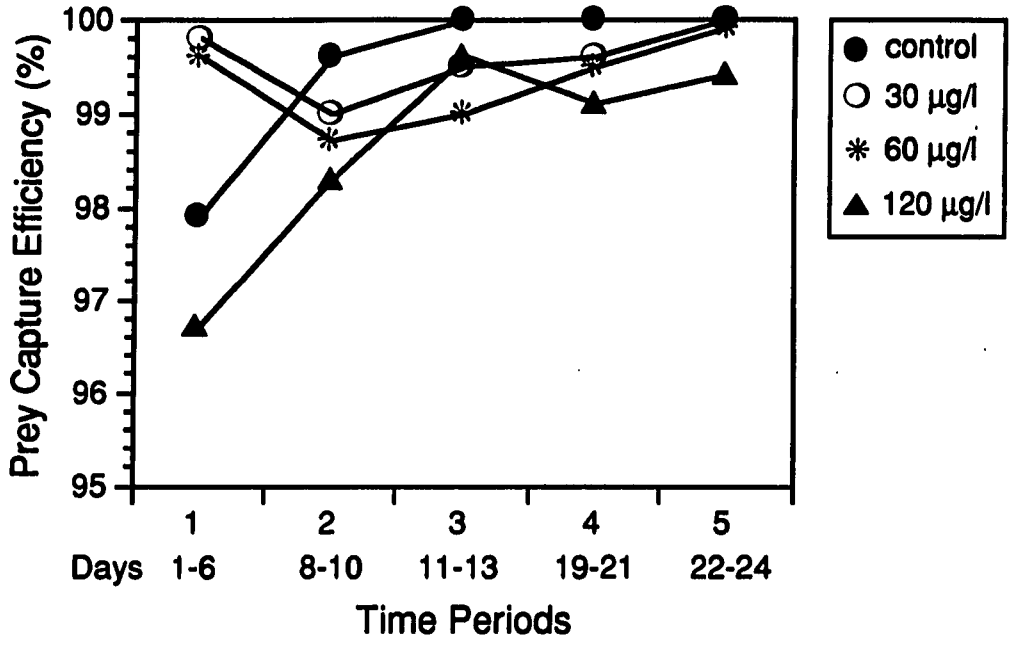

Figure 7. Effects of cadmium on overall prey capture efficiency of bluegill foraging on large (2.1 ± 0.2 mm) and small (1.2 ± 0.2 mm) *Daphnia magna* over 18 days of exposure. Plotted points represent means from all fish within a treatment. For means in time period 1, $n = 72$, and for means in time periods 2-5, $n = 36$

lowest capture efficiency during 3 of the 4 post-treatment time periods (X_r^2 = 8.63, 3 df, P = 0.035).

Although cadmium effects on prey capture efficiency were statistically significant, two points should be considered before interpreting these results. First, the magnitude of the effect due to cadmium exposure was only about half that due to inexperience of untreated bluegill during the pretreatment time period (Figure 7). Second, reductions in capture efficiency in treated fish, relative to control fish, never exceeded 2%, and were ≤ 1% during the latter 3 time periods (Figure 7). In other words, after bluegill became acclimated to feeding on *Daphnia*, mean capture efficiency was always 98% or greater, regardless of treatment. Cadmium-induced reductions in prey capture efficiency of less than 2%, although statistically significant, are probably ecologically insignificant.

Sandheinrich and Atchison (1989) found capture efficiency significantly reduced in copper-exposed juvenile bluegill (47.6 ± 1.5 mm) foraging on *Hyalella azteca* (3.2 ± 0.9 mm) and *Enallagma* sp. (8.2-13.6 ± 1.0 mm), but not when feeding on *Daphnia pulex* (1.6 ± 0.3 mm) or *D. magna* (2.4 ± 0.3). Capture efficiency of *Daphnia* is probably not affected by copper or cadmium due to the high efficiency with which bluegill capture and ingest these non-evasive, easily handled prey (e.g., Eggers 1977; Drenner et al. 1978; Vinyard 1980; Ehlinger 1989; 1990).

Sandheinrich and Atchison (1989) suggested that the sensitivity of the capture efficiency metric may be related to the ratio of predator size to prey size. I believe prey evasiveness is also important. The harder a particular prey is to capture and consume, the more likely toxicants may affect the predation event. More importantly, the ecological relevance of behavioral toxicity data is dependent upon choosing realistic predator-prey combinations for conducting foraging studies. Observed changes in prey capture efficiency, handling time, and other behavioral metrics can then, and only then, be meaningfully applied to optimal foraging and bioenergetics models (e.g., Werner and Hall 1974; Mittelbach 1981; 1983; Rice 1990; Wildhaber and Crowder 1990) to predict toxicant-altered diets and growth of fish in the wild.

Arena Switching Efficiency

Optimal foraging theory suggests that organisms attempt to maximize energy gain while foraging (e.g., Werner and Hall 1974; Mittelbach 1983; Stephens and Krebs 1986). Foraging efficiency was compared among treatments by measuring the total dry weight of *Daphnia* consumed per 45-s foraging trial as an index of caloric gain. Once again it was useful to analyze the two fish groups separately in order to determine how cadmium affected the ability of bluegill to maintain foraging efficiency when forced to switch between the 2 prey arenas (i.e., during patch switching events). Cadmium reduced the dry weight of *Daphnia* consumed by fish in groups 1 ($X^2_r = 9.9$, 3 df, $P = 0.019$) and 2 ($X^2_r = 11.1$, 3 df, $P = 0.011$) throughout the experiment. However, when these fish were switching between prey arenas during time periods 3, 4, and 5, the relationship between dry weight consumed and treatment was maintained. The similar slopes of lines between time periods indicated that cadmium exposure did not significantly affect patch switching efficiency for either fish group (Figure 8).

These results seem contradictory to those stated previously which suggested that cadmium effects on large prey consumption were influenced by arena. When bluegill switched from foraging in arena L to arena S, the 120 µg/L treatment fish typically consumed even fewer large prey than would have been expected from their performance in arena L (Figure 5). Again, this was probably due to an altered search strategy, which reduced large prey encounters in arena S. Nevertheless, these fish apparently compensated, at least in part, for decreased large prey consumption by eating more of the readily available small prey. This accounts for the high rate of small prey consumption by bluegill exposed to 120 µg Cd/L (Figure 4) and the lack of an effect on patch switching efficiency as measured by total dry weight consumed (Figure 8).

Growth in Length and Weight

Growth in length and weight was significantly reduced (F-tests, 1,4 df, $P < 0.05$) in fish exposed to 60 and 120 µg Cd/L (Table 1). Thus, the LOEC for growth was 60 µg Cd/L.

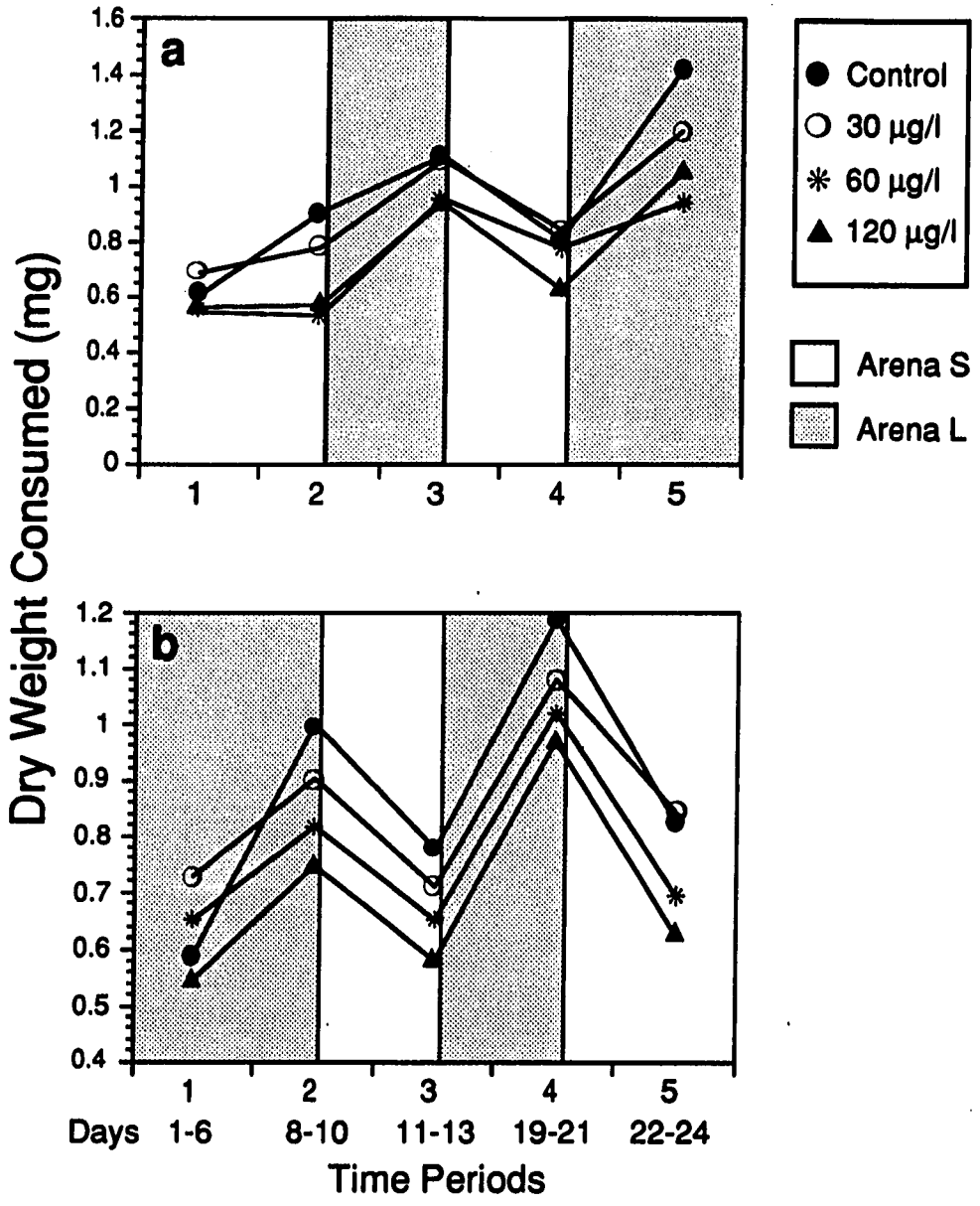

Figure 8. Cadmium effects on the ability of group 1 (a) and group 2 (b) bluegill to maximize their dry weight (mg) of *Daphnia* consumed when switching between prey arenas. For means plotted in time period 1, n = 36, and for means plotted in time periods 2-5, n = 18

Table 1. Mean increase in total length and wet weight of juvenile bluegill during a 24-d experiment, with 18 d of cadmium exposure. Bluegill were fed a combination of *Daphnia magna* and frozen brine shrimp (*Artemia salina*). For all means, $n=12$

Cadmium Concentration (µg/L)[a]	Total Length (mm)		Weight (mg)	
	$\bar{x}$	SE	$\bar{x}$	SE
0[b]	7.7	0.6	0.341	0.048
30	6.7	0.4	0.292	0.034
60	6.0	0.3	0.273	0.028
120	5.1	0.3	0.179	0.019

[a] Nominal treatment concentrations.
[b] Control treatment (no added cadmium).

In two similar experiments (see Chapter 1), growth was significantly reduced at 30 μg Cd/L. In this study, the 6 fish in one of the control tanks had lower mean growth and greater variability in individual growth than fish in the other tanks. This was probably due to the variability in fish size at the start of the experiment. Differences in fish size resulted in hierarchical interactions which affected individual-specific food consumption and growth (personal observations).

The LOEC for the growth endpoint from this experiment is similar to the LOEC of 80 μg/L reported by Eaton (1974) for bluegill exposed to cadmium in hard water (200 mg/L as $CaCO_3$). However, Eaton (1974) tested cadmium concentrations of 2.3 (control), 31, 80, 239, 757, and 2,140 μg/L. Thus, no information is available from his study for fish exposed to 60 μg Cd/L. Eaton (1974) also fed natural zooplankton, but provided this food twice daily, and presumably at higher densities and for longer periods of time compared to this study. To maximize the sensitivity of growth assays using juvenile bluegill, researchers should: (1) minimize the size differences in fish at test initiation and (2) impose short foraging opportunities on test fish to simulate the foraging time-predation risk trade-off in the wild (Sih 1980; Werner and Hall 1988). Excessively high feeding rates of even appropriate, live prey may mask growth effects due to toxicant exposure that would otherwise be apparent.

Conclusions

The rate of energetic gain in cadmium-exposed juvenile bluegill foraging on *Daphnia* was significantly depressed in a dose-dependent manner relative to control fish throughout 18 d of exposure. The proportion of the diet composed of large *Daphnia* (the preferred prey) in bluegill exposed to 120 μg Cd/L was significantly lower than control fish when foraging in an arena where large prey were relatively scarce, but did not differ significantly from control fish when large prey were abundant. I conclude, therefore, that the search component of the foraging sequence was more severely affected by cadmium exposure than was prey handling time. An altered search strategy, and perhaps reduced motivation to feed, may result in reduced consumption of preferred prey due to altered prey encounter rates. Cadmium had little effect on bluegill prey capture efficiency. Although cadmium reduced caloric gain

per unit of time, it did not alter the ability of bluegill to maintain foraging efficiency when switching between different foraging arenas (i.e., patch switching).

The LOEC for growth in juvenile bluegill chronically exposed to cadmium was 60 μg/L in this study, which is similar to the LOEC of 80 μg/L reported by Eaton (1974) for bluegill chronically exposed to cadmium in hard water. In designing growth studies, toxicologists should impose constraints and challenges on test fish similar to those encountered in the wild during foraging opportunities if the LOEC generated for growth is to be sensitive and predictive of effects in the field.

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SUMMARY

Among the foraging parameters used as endpoints in this study (i.e., changes in prey attack rate, capture rate, capture efficiency, handling time, general motivation to feed, rate of caloric gain, and prey selection), the rate of change in the number of prey attacked per unit time was the most consistently sensitive indicator of cadmium toxicity to bluegill. Initial exposure to cadmium concentrations as low as 30 μg/L caused a significant decrease in prey attack rates of 30-mm bluegill foraging on *Daphnia*. Rate of change in the number of prey captured per unit time was also a sensitive endpoint because cadmium had little effect on prey capture efficiency; hence, the number of prey captured was typically equal to the number attacked. Reduced caloric gain resulting from cadmium-reduced prey consumption rates was presumably a key factor contributing to reduced growth.

Cadmium exposure did not significantly affect bluegill prey handling time as measured in these experiments. Evidence acquired from this study suggests that cadmium-induced reductions in prey consumption rates were mediated primarily through an effect on bluegill search strategy and perhaps motivation to feed. Cadmium may interfere with the rapidly developing visual acuity of juvenile bluegill (Walton et al. 1992), thereby altering normal search capability and strategy and consequently altering prey encounter rates.

Prey selection was presumably affected through cadmium effects on bluegill search strategy. A cadmium-altered search strategy may have resulted in reduced prey encounter rates leading to the altered prey selection observed. In the final experiment, where foraging bluegill had a choice of two prey sizes, fish exposed to 120 μg Cd/L and foraging where preferred (large) prey were scarce altered their diet relative to control fish, whereas fish foraging where large prey were more abundant did not.

Although altering prey selection, cadmium did not affect the ability of bluegill to maintain foraging efficiency when switching between different foraging arenas (i.e., patch switching). I hypothesize that wild bluegill exposed to cadmium, in an attempt to maintain high rates of energy gain, may also alter their diet in order to compensate for cadmium effects on normal foraging activity and success. Altered predation pressures may alter

zooplankton community structure and composition (Kerfoot 1987; Mills et al. 1987).

The ability of cadmium-exposed bluegill to regain control-level foraging performance was dependent upon prey size. In both types of experiments conducted, cadmium had the greatest effect on prey consumption rates when fish were foraging in arenas dominated by small prey and reduced effects when arenas were dominated by large prey. Because bluegill demonstrated an ability to recover from initial cadmium effects on foraging performance, the sensitivities of behavioral endpoints are heavily influenced by the duration of exposure prior to the time of assessment. Thus, in assessing the utility of behavioral assays for hazard assessment, it will be important to determine if acute behavioral effects are predictive of chronic effects on growth, survival, or reproduction.

Because the LOECs derived from behavioral and growth measures in this study were numerically similar, and because certain behavioral endpoints (e.g., prey attack rate) were typically affected immediately upon cadmium exposure, I feel that behavioral tests which monitor prey attack rates could be used as quick, inexpensive screening tools for predicting chronic growth effects of xenobiotics on fish. Such a test could be easily standardized and would bring needed ecological realism to current hazard assessment protocols.

Growth was significantly reduced in bluegill exposed to nominal concentrations of 30 μg Cd/L in two of three experiments and at 60 μg Cd/L in the third experiment. Because significant growth effects were found at the lowest concentration tested in two of the three experiments assessing growth, an accurate LOEC for this endpoint can not be calculated. However, based on my findings I estimate the LOEC for growth in juvenile bluegill chronically exposed to cadmium in hard water (165-190 mg/L as $CaCO_3$) to be approximately 30 μg/L.

This study suggests that the growth endpoint is equally or more sensitive than the behavioral endpoints used. However, the sensitivity of the growth measure is, in part, dependent upon natural foraging behavior. Hence, these behaviors should not be overlooked when designing and conducting growth studies. The growth endpoint may be the most sensitive

indicator of chronic cadmium toxicity because growth is sensitive to cadmium's behavioral (e.g., food consumption rates), physiological (e.g., basal metabolic rate, food assimilation efficiency), and biochemical (e.g., metallothionein production and enzymatic function) effects.

Cadmium-altered foraging behaviors contributed to the observed growth effects in bluegill by reducing net energy gain per unit time. Choosing ecologically realistic predator-prey species combinations for laboratory testing is, therefore, critical for accurate and meaningful assessment and prediction of toxicant effects on foraging success and growth in the wild. Growth assay sensitivity can be further improved by minimizing fish size variability at test initiation and by limiting daily feeding to levels that allow control fish to grow at rates similar to fish in the wild. Excessively high feeding rates of even appropriate live prey may mask growth effects due to toxicant exposure that would otherwise be apparent.

The experiments conducted in this study defined the specific foraging behaviors affected by cadmium exposure. Such information is necessary if toxicologists are to appropriately apply the optimal foraging and bioenergetics models developed by ecologists to predict real-world effects of toxicants on diet and growth. For example, Borgmann and Ralph (1986) suggested that a toxicant that reduces growth by affecting food assimilation efficiency could be expected to have a cumulative effect up the trophic pyramid, resulting in large reductions in top predator production. Hence, it is important to understand not only whether growth is affected by toxicant exposure, but also the mechanisms responsible for growth effects. Understanding the behavioral, physiological, and biochemical effects of cadmium and other toxicants will provide important insight that can be used to guide field validation studies.

Finally, it is important for toxicologists and regulators to understand the ecological significance of their data so that inferences drawn are accurate and regulatory actions taken protective of the resource. Simply put, there are two main ecological concerns over toxicant-altered foraging behavior in young-of-the-year fishes: (1) altered predation pressure by young fishes can alter zooplankton community structure which may, in turn, cause cascading effects throughout aquatic food webs (Kerfoot 1987; Mills et al. 1987); and (2) the development of foraging abilities is crucial to the survival and subsequent

recruitment of young fishes (Houde 1987; Persson 1989; Miller et al. 1992). Hjort (1926) and May (1971, 1973) suggested that larval fish survival is primarily food-related, and that the strength of a year-class is largely determined by the ability of young fish to forage successfully on abundant and appropriate planktonic prey (Laurence 1974). If fish larvae do not successfully and frequently feed following complete absorption of their yolk-sac, mortality is certain beyond a "point of no return" (Brown 1985; Miller et al. 1988).

Relative to larvae, juvenile fishes are proficient foragers and have great diet breadths (Keast 1980, 1985). Thus, risk of starvation may be less important to survival compared to that of predation during the juvenile life stage (Hunter 1981). Reduced growth rates of juvenile fishes, however, will prolong the period that juveniles are vulnerable to various predators and will directly influence the probability of mortality due to predation (Werner and Hall 1974, 1988; Werner and Gilliam 1984; Post and Prankevicius 1987). In fact, body size, initially dictated by egg size and later by foraging success and subsequent growth, is the connective thread among factors affecting young fishes' survival (Miller et al. 1988). Hence, even small reductions in growth rates resulting from toxicant exposure may translate into substantial differences in survival and subsequent recruitment into the fishery (e.g., May 1971; Laurence 1974; Werner and Blaxter 1980; Leiby 1984; Adams and DeAngelis 1987; Miller et al 1988).

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