SUBLETHAL EFFECTS OF AMMONIA AND CADMIUM ON GROWTH OF GREEN SUNFISH

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
David John Jude
1973

This is to certify that the

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SUBLETHAL EFFECTS OF AMMONIA AND

CADMIUM ON GROWTH OF GREEN SUNFISH

presented by

David John Jude

has been accepted towards fulfillment
of the requirements for

Ph.D degree in Fisheries & Wildlife

Niles R. Kevern
Major professor

Date September 5, 1973

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David John Jude

AN ABSTRACT OF A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Fisheries and Wildlife

1973

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ABSTRACT

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Nine experiments were initiated to evaluate effects of ammonia and cadmium on growth and food consumption of green and pumpkinseed sunfish. RNA-DNA ratios were determined for fish from selected experiments and the cadmium content of the whole body, gills and liver of all fish exposed to cadmium was also obtained. Comparative LC_{50} values also were determined. In all experiments eight fish per treatment were exposed to toxicant in individual cells within an aquarium using a flow-through system. Food (Gambusia) was available continuously to all fish and amounts eaten were monitored daily.

Results of the 40-day experiment exposing green sunfish to 6 ppm ammonia as N at three different temperatures showed that decreased food consumption and growth of exposed fish was directly proportional to the temperature.

Pumpkinseeds and green sunfish showed an initial decline in feeding and growth when exposed to concentrations of ammonia greater than 2 ppm at one temperature. Fish exposed to 2 ppm grew considerably larger than controls and exhibited the highest RNA-DNA ratio. The magnitude and long-term detrimental effects on growth increased and were

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of greater duration the higher the concentration of ammonia. Fish exposed to higher concentrations exhibited an acclimation phenomenon whereby feeding eventually was re-established at a rate comparable to that of controls.

An LC_{50} value for green sunfish exposed to ammonia was 33 ppm as N, while for pumpkinseeds the LC_{50} was 9.4 ppm as N. For green sunfish exposed to cadmium the LC_{50} value was 20.5 ppm Cd.

Green sunfish exposed to 3, 7 and 15 ppm Cd exhibited reduced food intake and growth. Sunfish exposed to lower concentrations of cadmium (0.23-2.48 ppm) also grew at rates lower than control fish. Growth of fish exposed to 0.05 ppm, however, exceeded that of control fish. Fish exposed for 20 days to 1 ppm Cd at three different temperatures appeared to be unaffected at cold and medium temperatures when contrasted with controls. Fish exposed to 30 C and 1 ppm Cd exhibited decreased food intake and growth as well as highest mortality.

Short-term exposure of sunfish to high concentrations of cadmium (5-50 ppm) and subsequent growth in uncontaminated water indicated that cadmium was detrimental to growth of exposed fish. Food conversion ratios were variable and no consistent trends were apparent.

Whole-body and gill cadmium content on a wet-weight basis for control fish was about 1 ppm. Whole-body

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measurements were the most consistent values obtained among whole body, gill and liver, while liver usually contained the highest concentration. Dead fish accumulated more cadmium than corresponding live fish at the same concentration. Uptake by fish exposed to 3, 7 and 15 ppm Cd was proportionally greater at 15 ppm. A threshold concentration in whole-body cadmium content above which fish died in 12 days was found at about 20 ppm. Fish exposed to 0.05 ppm Cd accumulated in 20 days as much cadmium as fish exposed to almost 2 ppm. Effects of temperature on cadmium uptake did not appear dramatic as fish exposed to 1 ppm Cd accumulated similar levels at all temperatures. Cadmium elimination after exposure to high concentrations for short periods was complete by 60 days.

An STC (Stimulation Threshold Concentration) is proposed as a more reliable and useful approach for replacing LC_{50} data and in some cases for preliminary determination of MATC (Maximum Acceptable Toxicant Concentration) values. The STC is defined as that concentration of a compound which over a long period of time promotes growth greater than controls. The STC value was used to calculate an application factor for green sunfish exposed to cadmium which agreed well with literature values.

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ACKNOWLEDGMENTS

One of the most difficult tasks in the completion of this work is the accurate portrayal of the way in which the suggestions, help and support of my friends affected the shape and direction of my research. I owe a debt of thanks to all these people for the time and effort expended in my behalf which has ultimately culminated in these pages.

I first want to thank Suzann Pyzik for the sacrifices and time spent in helping me with the capture and feeding of fish as well as recording of the large amounts of data connected with each experiment. She also helped with some of the water chemistries, did key punching, edited the thesis and typed many of the rough drafts and all of the tables. For belief in my ideas and the many other intangibles extended during times of stress, I can only remain indebted.

To Joseph Ervin, I want to extend my gratitude, for the many times he solved problems I could not, for the many times he helped me collect fish on short notice, for the many times he assisted in recording data, for times he acted as a listening ear, and for the times he gave me encouragement.

I want to acknowledge the academic stimulation, the guidance and many suggestions given me by Dr. H. E. Johnson who first started me thinking about the effects of stress on

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animals and who introduced me to the "Stress of Life". He was instrumental along with my major professor in obtaining a National Institute of Health Biomedical Sciences Support grant for which I am grateful. He critically read the thesis a number of times in its earlier versions and gave many invaluable criticisms.

My major professor, Dr. N. R. Kevern, was always available for discussing problems and shouldered the difficult task of reading the first and later drafts of the thesis. I want particularly to thank him for the financial support he provided me, both in obtaining the National Defense Education Act Fellowship and in finding means to support my unplanned equipment needs and computer time. For financial assistance I also want to acknowledge support from the Michigan State University Agricultural Experiment Station.

I want to thank the other members of my committee for their time in trying to make this a better thesis. My appreciation goes to Dr. R. E. Monroe for his understanding, to Dr. J. R. Hoffert for his physiological acumen and to Dr. P. O. Fromm for his critical review of my thesis and for the wisdom of his comments.

I wish to also express my gratitude to Jake Eckenrode for help with seining, for the pesticide analyses and for the very helpful suggestions in the design of the flow-through apparatus. Hugh Wright also assisted in the design and building of that apparatus. Other people who helped me

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with the seining and sometimes recording of data were Art Talsma, Frank Tesar, Tim Ervin, Jim Waybrandt, Robert Hite and Johnathan Livingston Janssen. Dean Eyman and Al McIntosh gave many valuable suggestions, helped with seining and tutored me in the use of the atomic absorption unit. I also received help with the spectrophotometer from Charles Annett and Dennis Tierney.

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INTRODUCTION

One of the central themes in pollution biology is the determination of "no-effect" levels of various compounds, important for setting "safe" water quality standards for aquatic life. To answer this question, scientists have used the traditional bioassay approach of subjecting a number of organisms to various levels of a substance, using death as a criterion of effect. The TL_m (medium tolerance limit) or more currently LC_{50} value (median lethal concentration--that concentration of a substance just killing 50% of the test organisms) is used to evaluate the toxicity of the substance with some safety or application factor applied to arrive at a "safe" level. Other techniques for detecting a "no-effect" level have focused on cellular damage to gills (Mount and Stephan, 1967), uptake and threshold concentrations of toxicants in the whole body, gills and blood (Eisler, 1971; Eisler, et al., 1972; Hogan and Roelofs, 1971; Mount, 1964; Eisler and Weinstein, 1967; Lane and Livingston, 1970), organ-body ratios (Robinson, et al., 1960), reproductive success (McKim and Benoit, 1971; Pickering and Gast, 1972), effects on liver mitochondria (Hiltibran, 1971), on urine composition

(Hunn, 1969), on low mobility serum proteins (Bouck and Ball, 1965; Fujiya, 1961), on cell strains (Rachlin and Perlmutter, 1968), and on osmolality (Lewis and Lewis, 1971). Goodyear (1972) has suggested evaluation of a toxicant by recording the behavior of prey fish exposed to the toxicant and placed in a situation with predators where any reduced ability in their avoidance response is fatal. The concept of an animal's "scope for activity" as outlined by Fry (1947) is also a useful principle for evaluating the metabolic cost of a stressor on an organism. Brown (1967) suggested that fish populations can generally exist where the sum of the fraction of the 48-hour LC_{50} of all soluble poisons does not exceed 0.3-0.4. Use of LC_{10} or LC_{90} is also proposed (Sprague, 1969).

In my research, I was interested in "stress" and its effect on fish, particularly as it was related to temperature--of concern because of the increasing use of thermal nuclear power and the need it creates for disposal of waste heat. My intent in this research was to help give a more refined answer to the overall question of "safe" levels and to specifically gain more information on the growth response of sunfish to ammonia and cadmium. Thus my objectives were: 1) to develop a faster and more accurate method for determining a "no-effect" concentration; 2) to evaluate cadmium uptake in the whole body, gills and liver of fish as a valid biopsy technique; and 3) to obtain information on rate and levels of uptake under different temperature and cadmium regimes.

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To accomplish these objectives nine experiments were conducted, five involving ammonia and four involving cadmium as the toxicant. Of the five ammonia experiments three involved green sunfish and two involved pumpkinseeds. An LC_{50} value and growth response of pumpkinseeds and green sunfish under ammonia stress accounted for four of the experiments. The other ammonia experiment was a temperature (3) by stress (2) factorial allowing evaluation of the effect of 6 ppm ammonia on growth of green sunfish at three different temperatures. The four experiments with cadmium used green sunfish, all of which were analyzed for cadmium content. These experiments established an LC_{50} value, furnished information about effects of low levels of cadmium on fish growth, and evaluated the effect of 1 ppm Cd on growth of green sunfish at three different temperatures. One static bioassay was designed to determine post-treatment effects on green sunfish exposed continuously to a series of cadmium concentrations for three different lengths of time.

Growth of exposed fish was used to determine if a substance had a detrimental effect since growth is one of the best indications of health in an animal and is easily measured. In bioassays, I devised a method to insure that feeding conditions would be as natural as possible and would provide for measurement of amount of food eaten over a given interval. This was accomplished by placing Gambusia, a readily available and uniform source of food,

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in aquaria with predator fish (sunfish). Food consumption, growth and food conversion efficiency of sunfish exposed to various toxicants and temperature regimes was then determined. "Safe" levels and chronic effects of the toxicants were then judged on the basis of these studies.

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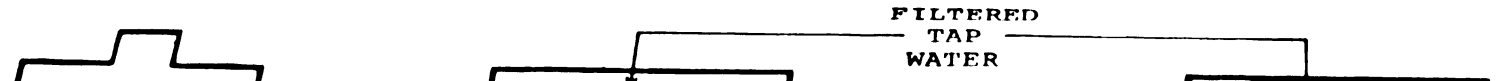
MATERIALS AND METHODS

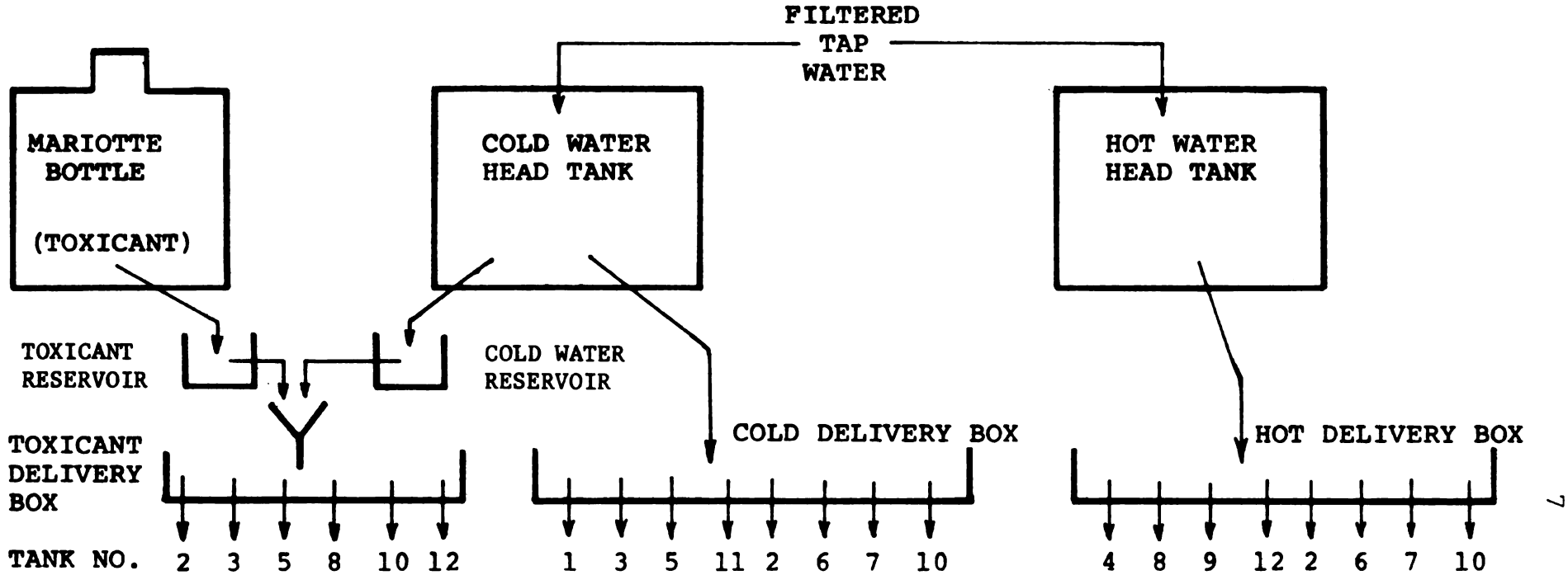
Bioassay Apparatus

Bioassays were conducted in a flow-through system similar to that of Chadwick and Brocksen (1969) which provided for three different water temperatures and controlled delivery of toxicants (Figure 1). One variation utilizing only seven aquaria and no temperature control was employed to give seven treatments at one temperature. When needed, water was heated by stainless-steel immersion heaters and controlled by thermoregulators placed in the hot-water head tank. Water flowed from head tanks to mixing boxes where toxicants were introduced from a Mariotte bottle and mixed with dilution water. Flow rates to the test tanks were controlled by varying the angles of glass drip tubes inserted in the delivery boxes.

Test tanks were twelve 70-l (20 gal) glass aquaria equipped with overflow tubes, plexiglass covers, and aerated by pumps and air stones. Each tank was partitioned into eight chambers (about 12 x 12 cm) with fiberglass screen to provide individual fish chambers. Two-in sections of 2-in diameter polyvinyl chloride (PVC) pipe were placed in each chamber to provide cover for fish. Water used (see Appendix Table A) was East Lansing tap water entering through iron pipes and then passing through

Figure 1. Schematic diagram showing the main features of the bioassay apparatus used to deliver continuous-flow concentrations of toxicants to 12 large aquaria containing individual cells for eight fish per aquarium.





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a carbon filter which was periodically backwashed. Water was then fed through PVC pipes to the two head tanks. Flow rate to aquaria for ammonia and cadmium experiments was 300 ml/min and 200 ml/min respectively, with 90% replacement times (Sprague, 1969) of 5 and 7.2 hours.

Light was provided by overhead mercury-vapor lamps. Photoperiod was manually controlled with approximately 8 hours of darkness and 16 hours of light daily.

The static bioassay apparatus consisted of seventeen 20-l (10 gal) aquaria each fitted with glass covers and aerated. Aquaria were located on both shelves of a two-shelf structure and treatments were randomly assigned to aquaria. Aquaria on the top shelf were consistently warmer by about 1-2 C. A number of 2-in sections of 1½-in diameter PVC pipe as well as empty brown jars were placed in each tank for cover. About 22 l of carbon-filtered tap water were used with 10 fish per tank. Recommended fish weight to water ratios of 1 g/l were sometimes exceeded (American Public Health Association, 1965). Lighting was similar to that of the flow-through system.

Fish

Green sunfish (Lepomis cyanellus) were used in all experiments except experiment 7-F and 8-F in which pumpkinseed sunfish (Lepomis gibbosus) were used (see Appendix Table B, C). Fish were collected from the Lansing area using seines and electroshocking. Once at the lab, fish

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were held in rectangular (860 l) or circular (1,680 l) tanks for a minimum of 2 weeks but usually much longer before they were used in an experiment. Depending on the experiment, stock fish were fed with either ground-up fish or small-sized trout chow, which fish readily accepted after a few days acclimation. Problems with Trichodina, a protozoan parasite, and some "popeye" were noted. Fish with Trichodina were eliminated and additional aeration alleviated "popeye" problems. For the two temperature experiments three groups of randomly selected green sunfish were acclimated to test temperatures for 10 days. Temperatures were raised from a holding-tank temperature of about 12 C (cold temperature) to around 20 C (medium temperature) and 28 C (hot temperature) at the rate of around 2 C per day in separate holding tanks (240 cm x 60 cm) equipped with thermoregulators.

After anesthetization weights of fish were determined to the nearest 0.01 g using MS-222 and a top-loading balance. Fish were anesthetized only to the degree that minimal movement still occurred and no ill effects due to anesthetization were noticed. Fish were randomly assigned to treatments from a group of fish previously selected for size and health.

LC_{50} values were obtained using graphical interpolation and methodology of the American Public Health Association (1965). Criteria for death were cessation of movement and no response after stimulation with a glass rod.

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The prey species used in all experiments as food for sunfish was the mosquito fish, Gambusia affinis, obtained from ponds (Appendix Table D) in the Lansing area. Gambusia were sorted according to size with average weight obtained by weighing a number of anesthetized fish after blotting. Gambusia were placed into each cell of the flow-through bioassay system which contained green sunfish. Each fish cell was usually checked daily and the number dead, regurgitated and live Gambusia remaining was recorded.

Fish Tissue Digestions

Fish exposed to cadmium were analyzed separately to determine rate and amount of cadmium absorbed. Fish were rinsed with tap water and then gill arches and liver were removed, weighed and placed into separate beakers. The remaining carcass, sometimes minus a strip of muscle from the upper right dorsal area (for RNA-DNA analysis), was also placed into a beaker. A boiling stone and nitric acid were then added. In the case of gills and liver, 10-20 ml of 4 N nitric acid was added and for the carcass, about 5 ml of concentrated nitric acid per g of fish was used. These beakers, covered with watch glasses, were then placed on a hot plate and allowed to boil until almost dry. If the fluid was reasonably clear 5 ml of 4 N nitric acid followed by distilled water was added and the contents then filtered through number 3 Whatman filter paper. Final volume of fluid used for cadmium analysis for gills and liver was 25 or 50 ml and for the carcass 50 or 100 ml was used.

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A number of these Whatman filters were analyzed for cadmium content. No cadmium was found on seven filters when the concentration of cadmium in the fish was less than 0.10 ppm Cd. When concentrations of cadmium in the fish were greater then 0.10 ppm it was found that an average of 3 ± 1.29 percent of the cadmium found in the fish was present on the filter. No adjustment was made for this finding. Unused filters were digested and no cadmium was ever detected in these samples.

Twenty samples of ground fish and beef heart of about 10 g each were "spiked" with 0.0025 mg Cd (8 samples), 0.010 mg Cd (8 samples), and 0.050 mg Cd (4 samples). A control sample was determined concurrently with each group and background levels subtracted from the amount found in spiked samples. The mean percent recovery was 100.25 ± 7.23.

RNA/DNA Procedures

The procedure for determining RNA-DNA ratios was that of Bulow (1970). A description of technique and necessary steps for analysis also appears in Haines (1969). Fish were removed from the freezer and then a longitudinal muscle strip along and just below the dorsal fin was excised and the skin removed. This flesh was then defatted using a chloroform ethanol-mixture and a 50 mg sample weighed. To calculate mg of DNA and RNA, the ratio of dry fat-free weight to wet weight was needed, which for flesh samples (0.72 to 3.32 g) from ten green sunfish was

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0.1749 ± 0.002. Flesh samples were then digested with trichloracetic acid and treated after immersion in a hot-water bath with orcinol for RNA and diphenylamine for DNA. Final concentrations are reported as mg of RNA or DNA per g of DFFT (dry fat-free tissue). RNA-DNA ratios also were calculated. Final concentration of the color reaction was measured using a Beckman DK2A spectrophotometer. Fish were frozen whole and stored sometimes up to 6 months before analysis.

Chemicals and Chemical Methods

Routine water chemistry tests (hardness, alkalinity, dissolved oxygen and nitrates) were determined according to Standard Methods (American Public Health Association, 1965). A pH meter was used for measuring pH, and temperature was determined with a glass mercury thermometer.

Ammonia was added to bioassay containers as ammonium chloride and analyzed using direct nesslerization (American Public Health Association, 1965) with a Klett-Summerson colorimeter. Concentrations of duplicate samples were similar using direct nesslerization and distillation with nesslerization. Concentrations of ammonia are reported ppm as N (Nitrogen). In the text ammonia refers to all forms of ammonia (NH_3, NH_4^+), while un-ionized ammonia (NH_3) will be specified when NH_3 is discussed. Concentrations of un-ionized ammonia (NH_3) were calculated using Table 14 in Spotte (1970).

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Cadmium was added as cadmium chloride and measured on a Jarrell-Ash Atomic Absorption spectrophotometer Model 800. Samples were run at 2360 Angstroms in the range 2 ppm to 0.02 ppm, the maximum sensitivity range. Concentrations were recorded as ppm Cd on a wet-weight basis. A number of 1 ppm cadmium standards of different acidities all had similar peak heights when measured on the spectrophotometer. Standards were compared with those from the Crops and Soils Science Department, Michigan State University and found in good agreement.

Water samples for chemical analyses were removed from the closest, right-most cell of the aquarium after temperature, ammonia and dissolved oxygen checks from the front, middle and back revealed complete mixing occurred in the entire aquarium. Samples for water chemistry were collected periodically when time for analysis was available. Samples for toxicant determinations were taken arbitrarily and attempts were made to get a sample at least once per day and sometimes more often. Ammonia samples were untreated but refrigerated immediately (3.5C). Samples in cold storage analyzed after a period of time for ammonia were not different from samples analyzed immediately. Cadmium water samples were treated with 3 ml nitric acid per 1 of water. In cadmium experiments glassware and bottles were washed between uses in dichromate-sulfuric acid cleaning solution (American Public Health Association, 1965).

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Computational and Statistical Procedures

The CDC 3600 and 6500 computers were used for data analysis. Special programs were written for some calculations, while the statistical Stat Pac from the Michigan State Computer Library was used for analysis of variance and linear regression. When analysis of variance was calculated, missing data, because of dead fish, were replaced with the mean of that particular cell. One degree of freedom was subtracted from error degrees of freedom for each time this was done. Attempts were made to verify at least some data on cards with the original data from each calculation.

Mean growth, food consumption and food conversion ratios were determined over an interval of time, usually 4 days for weight, and about 1 day for amount of food consumed. If a fish died over half-way through one of these intervals, the convention was used that its contribution would remain for that interval, but for none of the succeeding ones. If a fish died before it was half-way through an interval, its contribution to the mean for that interval was dropped. In all graphs depicting amount of Gambusia eaten and food conversion ratios, points were plotted midway through the interval over which the particular parameter was calculated.

The food conversion ratio for each fish was calculated by dividing the net weight gain for a given time interval by the weight of Gambusia eaten over that same interval.

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The mean for the entire group was then obtained. Ratios rather than efficiency values were calculated to avoid some of the statistical problems associated with percentages. In cases where one fish in the group in a given interval did not eat, its ratio was excluded from calculation of the mean. Any fish which registered a negative weight gain was given a food conversion ratio of zero, since extreme variation in mean food conversion ratios occurred if it was not treated as zero.

Standard error (S.E.) was calculated as the square root of the variance (s^2) divided by N. In some cases, a mean standard error was calculated which is the average of all standard errors for each of the means shown for one line on a graph. This method was used only when standard errors were small and uniform across time. If standard errors were not small and uniform but correlated with the mean as occurred with mean weight of fish, standard errors were shown for each mean using vertical, half bars (to save space) or individual standard errors were placed in the appendix.

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RESULTS AND DISCUSSION

Experiment 6-F

Experiment 6-F was designed as a 3 x 2 x 4 factorial to determine the RNA-DNA ratio and growth response of green sunfish continuously exposed to three different temperatures and concentrations of ammonia over four 10-day periods. The experiment lasted 40 days from November 10 to December 21, 1970. Four fish from each of three temperatures were sampled initially for RNA-DNA ratios. Thereafter, every 10 days four fish from each of the six treatment combinations were randomly selected from the two replicate aquaria. Three sets of four aquaria were maintained at mean temperatures of 13 C (cold), 22 C (medium) and 28 C (hot) (Table 1A, 1B, 1C). It was desired to maintain one concentration of ammonia in all aquaria receiving toxicant for each of the three temperatures so chronic effects of temperatures on ammonia toxicity could be evaluated. From pilot experiments, 6 ppm ammonia as N was selected as a nominal concentration which would not kill any fish. However, because ammonia was oxidized to nitrates proportionately more with increasing temperatures (Table 1A, 1B, 1C) a uniform nominal concentration of ammonia was not maintained over the three test temperatures. At the cold temperature 5.5 ppm ammonia as N was found,

Table 1A.

Treatment	NH_4 ppm as N	Un-ionized ppm as N	pH	Temperatur (C)	Dissolved Oxygen (pp	Alkalinity ppm as CaC	Hardness ppm as CaC	NO_3 (ppm a

Table 1A. Chemical characteristics of water used in the continuous flow experiment 6-F. (N is the number of samples used in determinations; $\bar{X}$ is the mean with one standard error enclosed in parentheses; C = Cold; M = Medium; H = Hot; S = Stressor; t = less than 0.01 ppm).

		Aquarium Number			
		1	11	3	5
Treatment		C	C	CS	CS
NH_4 ppm as N	N	3	3	17	19
	$\bar{X}$	0.13 (0.06)	0.12 (0.04)	5.48 (0.15)	5.53 (0.19)
Un-ionized NH_3 ppm as N		t	t	0.08	0.08
pH	N	6	6	6	7
	Range	7.68-7.82	7.73-7.89	7.58-7.79	7.52-7.80
Temperature (C)	N	30	30	30	30
	$\bar{X}$	12.8 (0.1)	13.4 (0.2)	13.1 (0.2)	13.3 (0.2)
Dissolved Oxygen (ppm)	N	5	5	5	5
	$\bar{X}$	8.1 (0.1)	8.2 (0.3)	6.4 (0.5)	6.6 (0.4)
Alkalinity ppm as $CaCO_3$	N	4	4	4	4
	$\bar{X}$	342 (2)	346 (1)	345 (3)	342 (3)
Hardness ppm as $CaCO_3$	N	3	3	3	3
	$\bar{X}$	346 (5)	347 (7)	346 (9)	349 (8)
NO_3 (ppm as N)	N	-	-	1	1
	$\bar{X}$			0.8	1.0

Table 1

Treatme												
NH_4 ppm as												
Un-ioni ppm as												
pH												
Tempera (C)												
Dissol Oxygen												
Alkali ppm as												
Hardne ppm as												
NO_3 (p												

Table 1B. Chemical characteristics of water used in the continuous flow experiment 6-F. (N is the number of samples used in determinations; $\bar{X}$ is the mean with one standard error enclosed in parentheses; C = Cold; M = Medium; H = Hot; S = Stressor; t = less than 0.01 ppm).

		Aquarium Number			
		6	7	2	10
Treatment		M	M	MS	MS
NH_4 ppm as N	N	2	3	18	19
	$\bar{X}$	0.15 (0.05)	0.12 (0.04)	4.04 (0.20)	4.20 (0.17)
Un-ionized NH_3 ppm as N		t	t	0.16	0.16
pH	N	6	6	6	6
	Range	7.95-8.06	7.75-8.00	7.62-8.12	7.59-7.95
Temperature (C)	N	30	30	30	30
	$\bar{X}$	20.8 (0.2)	21.5 (0.2)	20.0 (0.2)	21.3 (0.2)
Dissolved Oxygen (ppm)	N	5	5	6	5
	$\bar{X}$	7.3 (0.2)	7.1 (0.3)	5.2 (0.6)	5.3 (0.7)
Alkalinity ppm as $CaCO_3$	N	4	3	4	3
	$\bar{X}$	345 (1)	349 (4)	333 (3)	326 (1)
Hardness ppm as $CaCO_3$	N	3	3	3	3
	$\bar{X}$	352 (4)	343 (10)	347 (5)	246 (5)
NO_3 (ppm as N)	N	-	-	1	1
	$\bar{X}$			1.0	1.6

Table 1C.

Treatment	
NH_4 ppm as N	
Un-ionize ppm as N	
pH	
Temperatu (C)	
Dissolved Oxygen (p	
Alkalinit ppm as Ca	
Hardness ppm as Ca	
NO_3 (ppm	

Table 1C. Chemical characteristics of water used in the continuous flow experiment 6-F. (N is the number of samples used in determinations; $\bar{X}$ is the mean with one standard error enclosed in parentheses; C = Cold; M = Medium; H = Hot; S = Stressor; t = less than 0.01 ppm).

		Aquarium Number			
		4	9	8	12
Treatment		H	H	HS	HS
NH_4 ppm as N	N	2	2	18	19
	$\bar{X}$	0.08 (0.08)	0.09 (0.01)	1.49 (0.13)	1.69 (0.16)
Un-ionized NH_3 ppm as N		0.01	0.01	0.11	0.12
pH	N	7	6	7	6
	Range	7.80-8.22	8.05-8.20	7.56-8.20	7.63-8.25
Temperature (C)	N	30	30	30	30
	$\bar{X}$	28.0 (0.2)	28.7 (0.2)	27.9 (0.1)	27.5 (0.2)
Dissolved Oxygen (ppm)	N	5	5	5	6
	$\bar{X}$	6.1 (0.4)	6.5 (0.3)	4.7 (0.7)	4.2 (0.6)
Alkalinity ppm as $CaCO_3$	N	4	3	3	3
	$\bar{X}$	347 (4)	350 (3)	314 (4)	307 (3)
Hardness ppm as $CaCO_3$	N	3	3	3	3
	$\bar{X}$	352 (6)	344 (6)	347 (5)	348 (6)
NO_3 (ppm as N)	N	-	-	1	1
	$\bar{X}$			3.0	4.0

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while 4 and 1.5 ppm ammonia as N were found at medium and hot temperatures respectively. In addition, temperature was responsible for the reduction of dissolved oxygen in the controls from 8 ppm at cold temperature to 7 and 6 ppm for medium and hot temperatures respectively, while dissolved oxygen in all aquaria receiving toxicant at each temperature was about 2 ppm less than corresponding controls, 6, 5, and 4 ppm for cold, medium and hot temperatures, respectively. Effects of ammonia across temperature, therefore, may be partially confounded by dissolved oxygen concentration.

The 108 green sunfish used in this experiment ranged in weight from 3.97 to 15.79 grams (see Appendix Table B, Collection 4, 5 and 6). Fish were not fed during the three weeks before the start of the experiment so RNA-DNA ratios would be at a minimum.

Growth of green sunfish, shown as percent increase over the initial weight (because of the large variability in size of fish used in the experiment), was depressed at cold temperatures (13.2 C) and almost linear at medium temperatures (20.9 C) (Figure 2). At high temperatures (28 C) the greatest increase in growth occurred with a gradual tapering-off after day 20 to values comparable to those exhibited by fish exposed at medium temperatures. The analysis of variance (Table 2) showed that all main effects, temperature (3 levels--13, 20, 28 C), stress (2 levels--nominal concentrations of 0 and 6 ppm ammonia

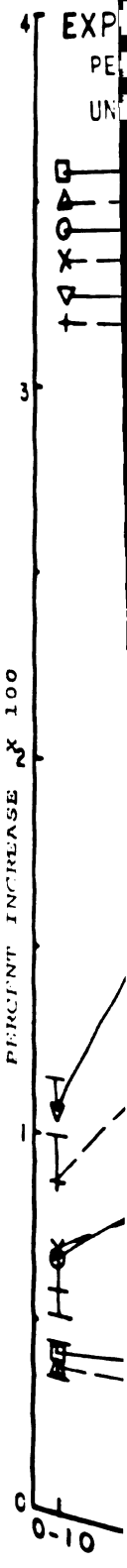

Figure 2. The ratio of the initial weight of green sunfish to **final weight under three** different temperatures and concentrations of ammonia. Each point represents the mean of four observations except as noted by a number in parentheses. One standard error is given as a dark vertical bar on only one side of the point for clarity.

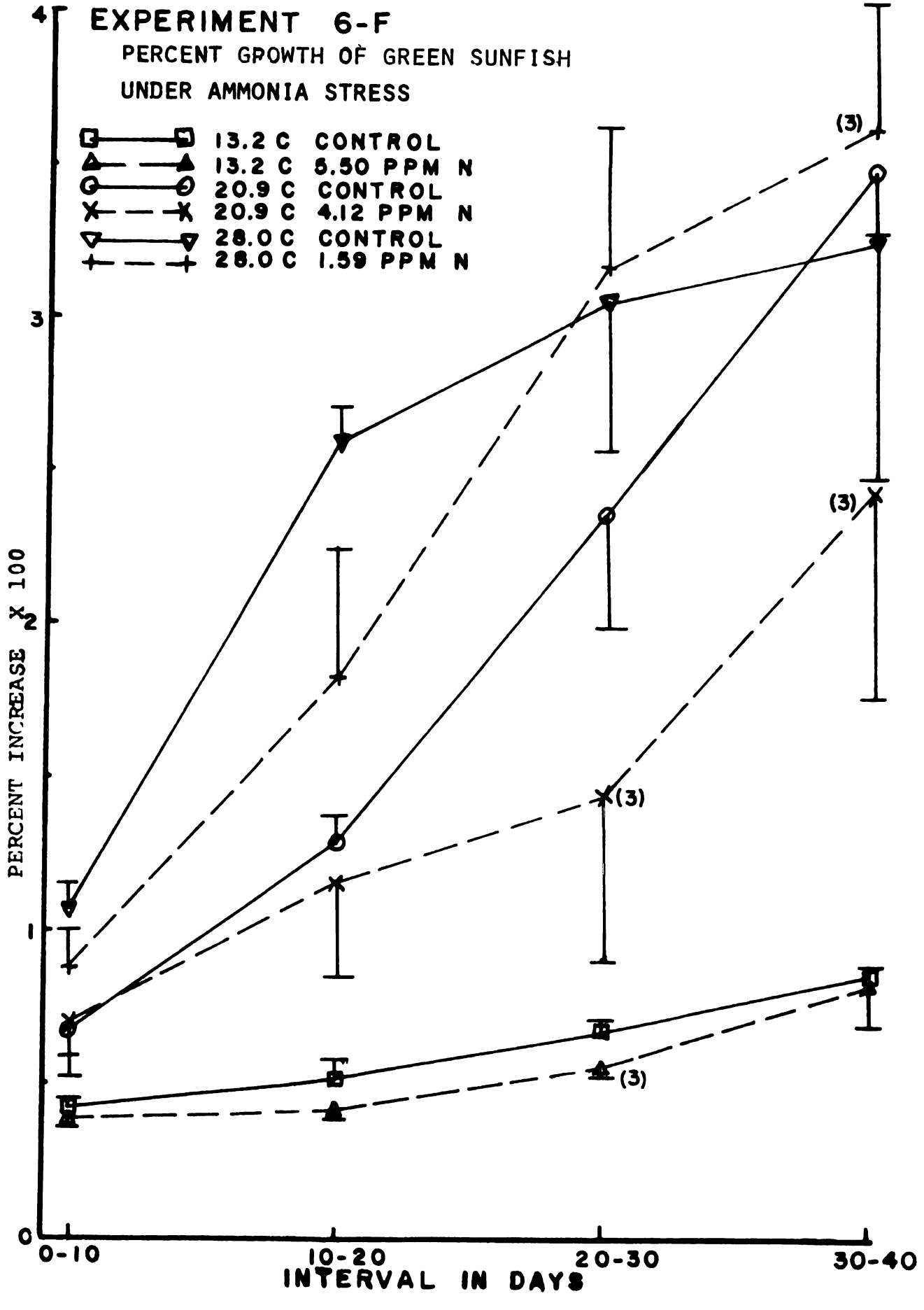
EXPERIMENT 6-F
PERCENT GROWTH OF GREEN SUNFISH
UNDER AMMONIA STRESS
13.2 C CONTROL
13.2 C 5.50 PPM N
20.9 C CONTROL
20.9 C 4.12 PPM N
28.0 C CONTROL
28.0 C 1.59 PPM N
PERCENT INCREASE X 100
0
1
3
4
0-10
10-20
20-30
30-40
INTERVAL IN DAYS
(3)
(3)
(3)
(3)

Table 2.

Source	
Temperatu	
Stress	
T x S	
Period	
T x P	
S x P	
T x S x P	
Error	
Total	

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Table 2. The analysis of variance table for the effects of three temperatures and two levels of ammonia (stress) over four periods on the percent weight gain of green sunfish. A log (percent weight gain + 20) transformation was used.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Temperature	4.95	2	2.48	54.23**
Stress	0.84	1	0.84	18.38**
T x S	0.22	2	0.11	2.43
Period	3.53	3	1.18	25.80**
T x P	0.29	6	0.05	1.07
S x P	0.13	3	0.04	0.97
T x S x P	0.29	6	0.05	1.04
Error	3.29	72	0.05	
Total	13.55	95		

**0.01 significance level.

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as N), and period (four periods of 10 days each) were significant. Since standard errors were postively correlated with mean percent increase, a log (percent increase plus 20) transformation was used to better satisfy the underlying assumption of homogeneity of variances. Thus these data indicate among other things, that ammonia had an average, additive detrimental effect on exposed fish across temperatures and time when compared with control fish. In all cases except for hot temperature, fish exposed to ammonia exhibited growth less than controls at the same temperature. Some evidence of acclimation at the highest temperatures existed, since after day 20, growth increase of ammonia-exposed fish was at least equal to or higher than that of controls.

Fish exposed to ammonia were more excitable than control fish, and those that died followed a consistent behavior pattern. Usually they stopped feeding, became listless and would, in cases where the aerator stream of bubbles was in their cell, maintain themselves in that stream. Prey Gambusia harassed moribund sunfish by nipping and biting at fin rays. In an effort to escape their cell, some ammonia-exposed sunfish (and a few controls) experienced very severe abrasions of their lower mandibles. The extent to which this affected their resistance to ammonia is unknown. Many fish that died in stressor tanks, however, possessed split lower mandibles. No control fish died, while six in the stressor tanks succumbed (Table 3).

Tabl

Fish No.
80
60
77
89
21
13

Table 3. Time and weight response of green sunfish that died after continuous exposure to three different temperatures and ammonia concentrations.

Fish No.	Time of Death (hrs)	Type of Stress	Initial Weight (g)	Final Weight (g)
80	249	medium stressor	9.63	8.98
60	364	hot stressor	4.82	4.90
77	393	medium stressor	7.15	7.70
89	460	hot stressor	11.21	9.94
21	496	cold stressor	7.72	7.63
13	678	medium stressor	8.69	11.29

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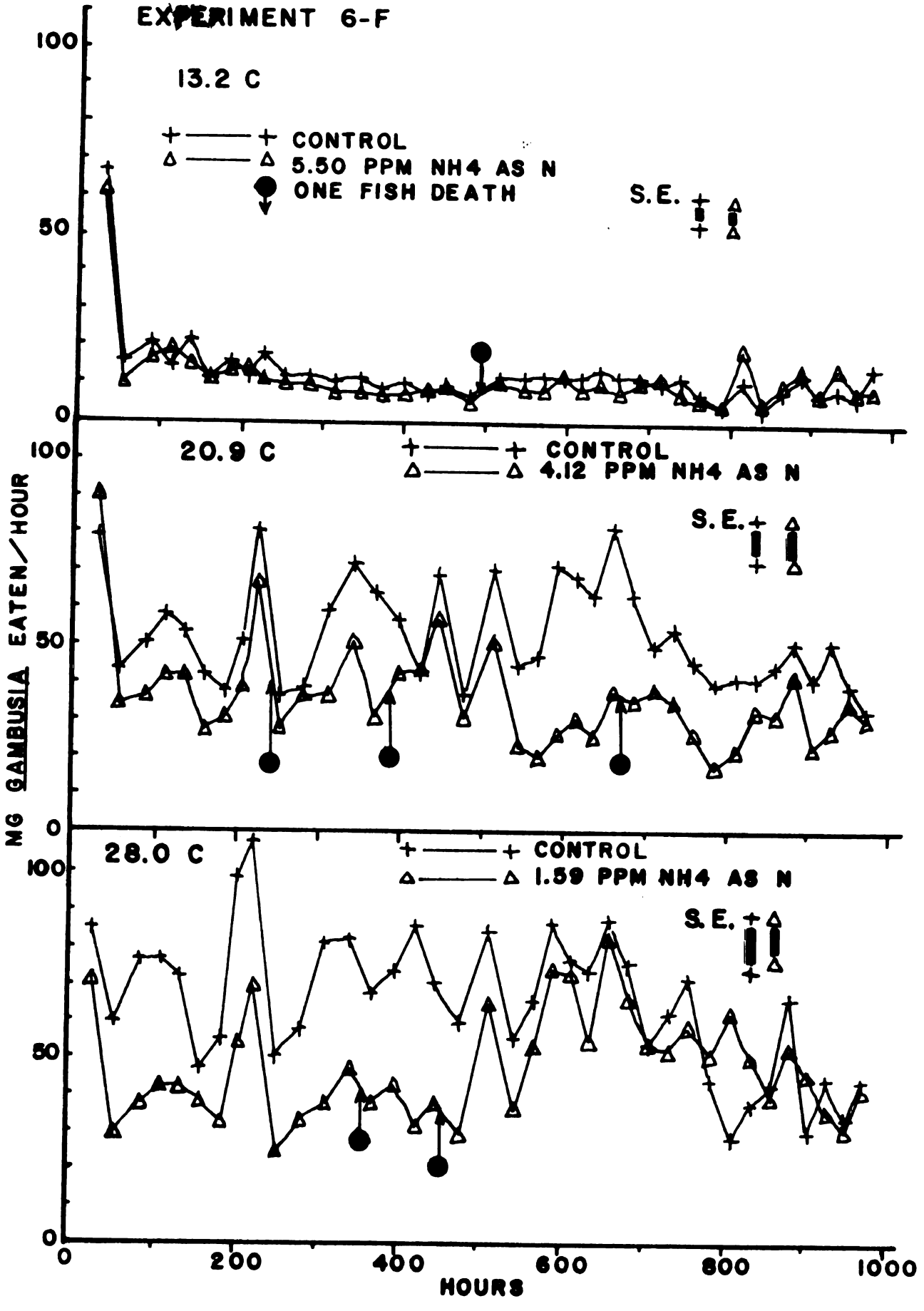
One fish died at cold temperature, three at medium temperature and two died at the highest temperature.

The depressing effect of ammonia on food consumption under different temperatures was also shown by computing the mg of Gambusia eaten on an hourly basis (Figure 3). The depressing effect of ammonia on food consumption was minimal at cold temperature, but larger differences were found at higher temperature. These trends were highly significant, as borne out by the analysis of variance (Table 4) which showed that all main effects, temperature, stress and period were significant. Amount of food consumed was directly proportional to temperature. The effect of stress in depressing food consumption was greatest at highest temperature. At the low temperature a small but consistently lower amount of food was eaten by exposed fish when compared with controls. The significant effect of time is related to growing fish consuming more and more food the larger they become.

Food conversion ratios, net weight gain divided by weight of food eaten, offered no consistent trends (Figure 4). In all cases except one, however, ratios for stressed fish were lower than values found for control fish. The analysis of variance showed that ammonia stress did not have a significant effect on food conversion ratios of all stressed fish when compared with all control fish over temperature and time (Table 5). However, there was a significant effect of temperature when averaged over time

Figure 3. Rate of consumption of Gambusia in mg per hr for green sunfish exposed continuously to three different temperatures and concentrations of ammonia. Each point represents from sixteen to four fish depending on deaths. Mean standard error is given for each concentration.


EXPERIMENT 6-F
13.2 C
CONTROL
5.50 PPM NH4 AS N
ONE FISH DEATH
S.E.
20.9 C
CONTROL
4.12 PPM NH4 AS N
S.E.
28.0 C
CONTROL
1.59 PPM NH4 AS N
S.E.
MG GAMBUSIA EATEN/HOUR
HOURS
0
50
100
200
400
600
800
1000

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T x P
S x P
T x S
Error
Total

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Table 4. The analysis of variance table for the effects of three temperatures and two levels of ammonia (stress) over four periods on the consumption of *Gambusia* by green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Temperature	1793.99	2	896.99	65.61**
Stress	180.62	1	180.62	13.18**
T x S	83.11	2	41.56	3.04
Period	190.26	3	63.42	4.63**
T x P	27.06	6	4.51	0.33
S x P	49.35	3	16.45	1.20
T x S x P	87.30	6	14.55	1.06
Error	931.06	[a]68	13.69	
Total	3342.77	95		

**0.01 significance level.
[a]4 degrees of freedom were subtracted for dead fish.

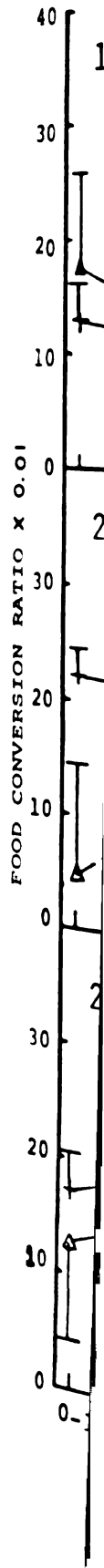

Figure 4. Food conversion ratios of fish exposed continuously to three different temperatures and concentrations of ammonia. Each point represents the mean of four fish except where otherwise noted by a number in parentheses. One standard error is shown by a vertical bar.

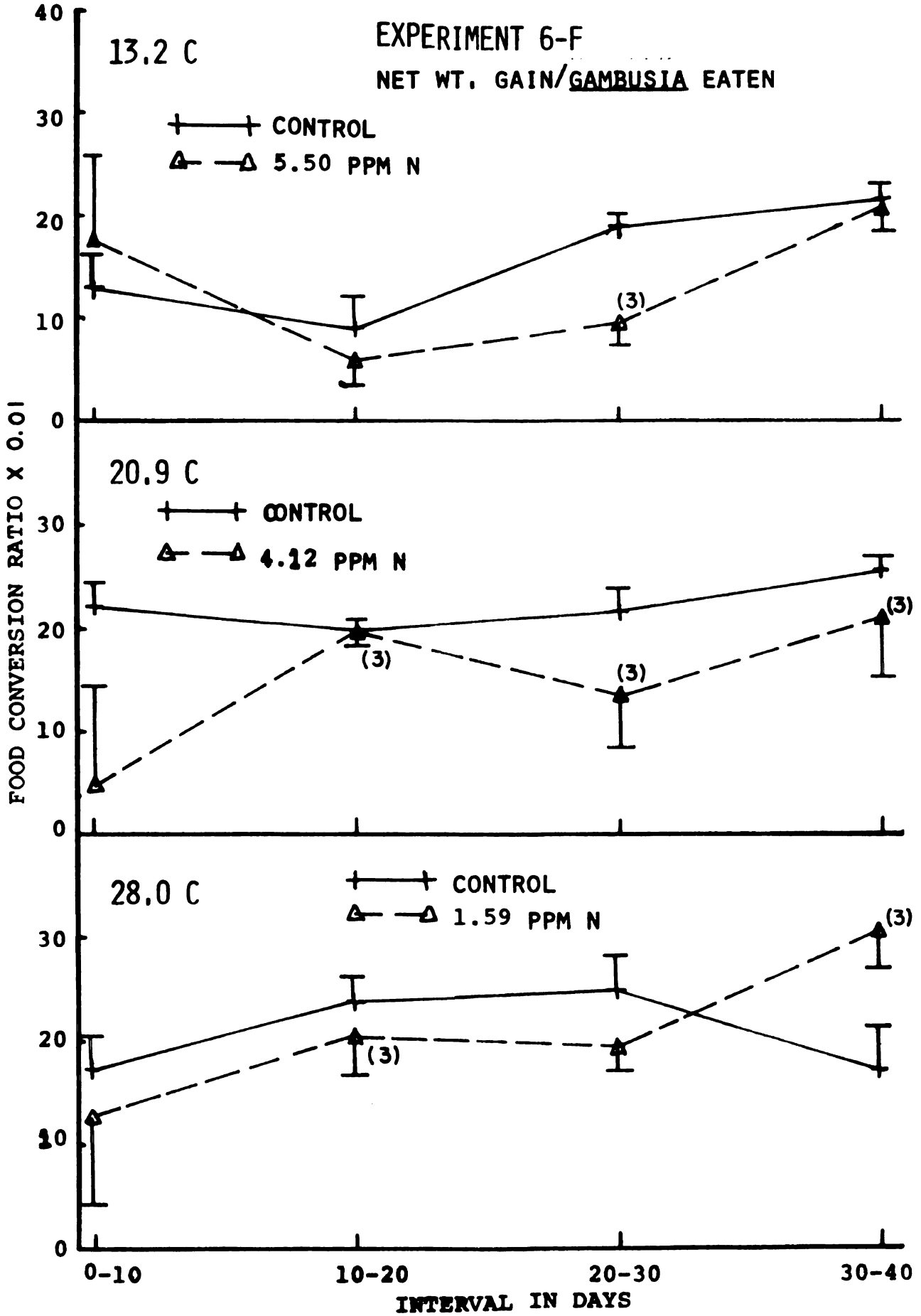
EXPERIMENT 6-F
NET WT. GAIN/GAMBUSIA EATEN
13.2 C
CONTROL
5.50 PPM N
20.9 C
CONTROL
4.12 PPM N
28.0 C
CONTROL
1.59 PPM N
FOOD CONVERSION RATIO X 0.01
INTERVAL IN DAYS
0-10
10-20
20-30
30-40
0
10
20
30
40
(3)

Table 5.

Source	
Temperat	
Stress	
T x S	
Period	
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Table 5. The analysis of variance table for the effects of three temperatures and two levels of ammonia (stress) over four periods on the food conversion ratios of green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Temperature	0.065988	2	0.032994	5.30**
Stress	0.023937	1	0.023937	3.84
T x S	0.024409	2	0.012204	1.96
Period	0.090232	3	0.030077	4.83**
T x P	0.064991	6	0.010832	1.74
S x P	0.037245	3	0.012415	1.99
T x S x P	0.063560	6	0.010593	1.70
Error	0.411670	[a]66	0.006231	
Total	0.782032	95		

**0.01 significance level.
[a]6 degrees of freedom were subtracted for dead and non-feeding fish.

and stress. The respective means were 0.34, 0.38 and 0.40 for fish at cold, medium and hot temperatures respectively, indicating that fish became progressively more efficient in their food conversion with increasing temperature. In addition, there was an unexpected significant effect of time on food conversion ratios as fish became progressively more efficient in their utilization of Gambusia. The means for the first through fourth 10-day period were 0.35, 0.36, 0.39 and 0.43.

RNA-DNA ratios of fish at all temperatures started low (around 10) then increased to levels between 20 and 30 for the remaining 40 days (Figure 5, Appendix Table E). The analysis of variance revealed that temperature, stress and period were all significant (Table 6). The means pooled over temperature indicated that fish at the cold temperature had a higher mean RNA-DNA ratio (27.21) than at either medium temperature (19.14) or hot temperature (23.14). The effect of ammonia stress considered across time and temperature showed a depressing effect on RNA-DNA ratios when the control value (25.62) was contrasted with that of ammonia-exposed fish (20.71). This reduction in RNA-DNA ratios indicates that, by some mechanism, RNA synthesis by stressed fish was depressed. Considering mean RNA-DNA ratios at each temperature over time indicated the difference between stressed and control fish was greatest for fish at cold temperatures (around 8), small for fish at medium temperatures (1.6) and somewhat larger for fish at high temperatures.

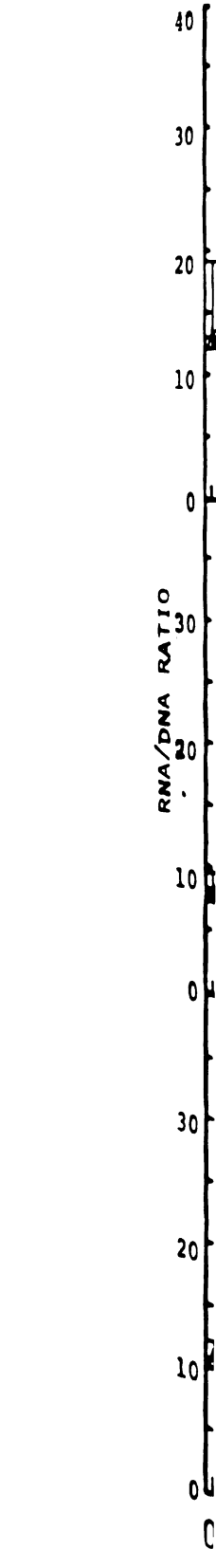
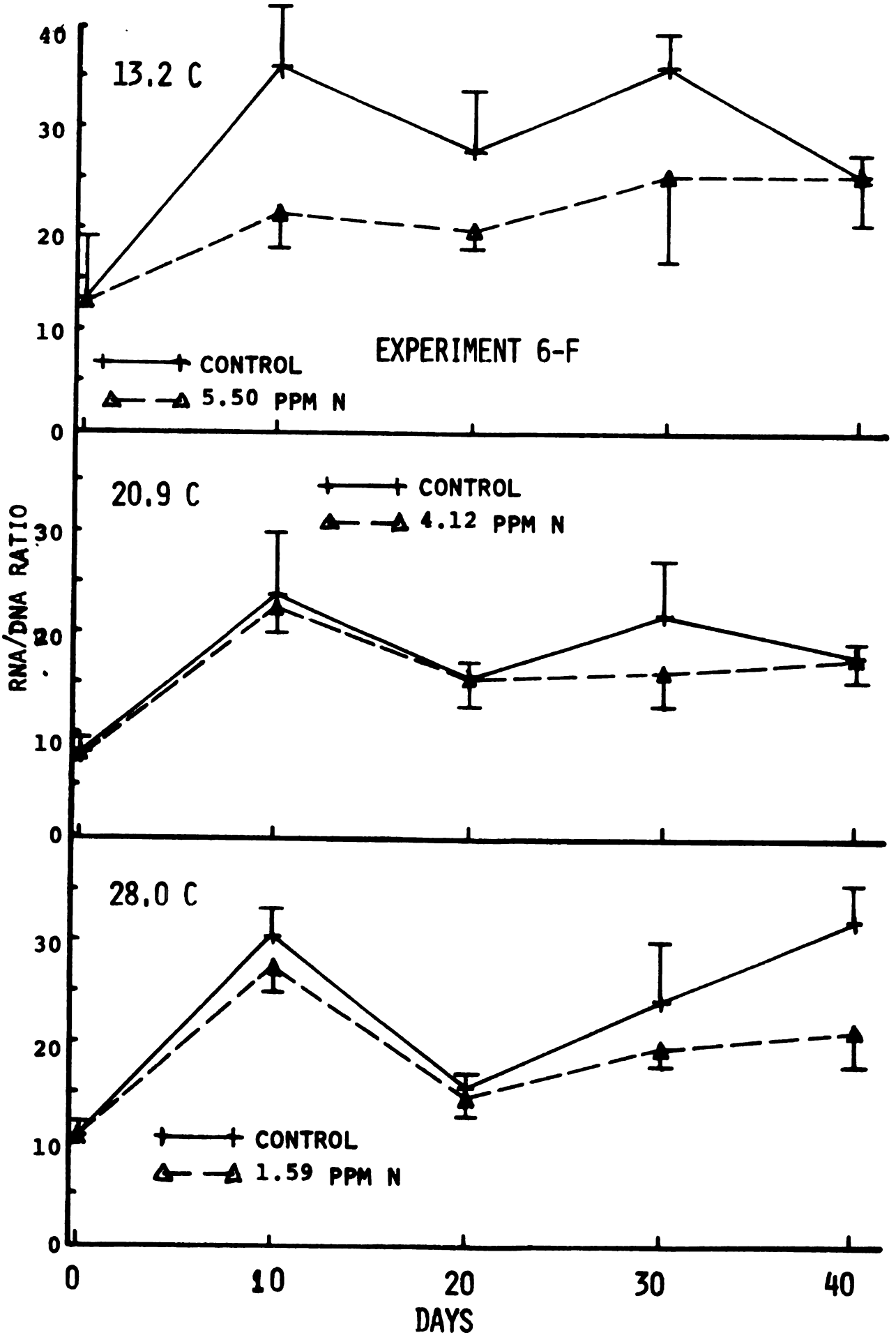


Figure 5. Average RNA-DNA ratio of green sunfish exposed continuously to three different temperatures and concentrations of ammonia. Each point represents the mean of four fish with one standard error given as a dark vertical bar on only one side of the point for clarity.


13.2 C
CONTROL
5.50 PPM N
EXPERIMENT 6-F
20.9 C
CONTROL
4.12 PPM N
28.0 C
CONTROL
1.59 PPM N
RNA/DNA RATIO
DAYS
0
10
20
30
40

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Source	
Temperature	
Stress	
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Period	
T x P	
S x P	
T x S x P	
Error	
Total	

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Table 6. The analysis of variance table for the effects of three temperatures and two levels of ammonia (stress) over four periods on the RNA-DNA ratios of green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Temperature	1040.97	2	520.48	9.19**
Stress	577.02	1	577.02	10.19**
S x T	169.98	2	84.99	1.50
Period	949.26	3	316.42	5.59**
T x P	419.32	6	69.89	1.23
S x P	67.98	3	22.66	0.39
T x S x P	311.03	6	51.84	0.92
Error	4078.78	72	56.65	
Total	7614.34	95		

**0.01 significance level.

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(around 5). A consideration of the effect of time on the RNA-DNA ratios across temperatures and stress revealed that ratios were highest after the first 10 days (27), declined considerably in the second 10 days (18), then leveled off for the remaining periods at around 23.

Experiment 7-F

Pumpkinseed sunfish were used in experiment 7-F which was a 30-day experiment conducted from March 11 to April 11, 1971. The experiment was done to evaluate the effect of ammonia on growth of a different species of sunfish so that results could be compared with those obtained using green sunfish. Unfortunately, as learned later, 13 of the "pumpkinseeds" were green x pumpkinseed hybrids, randomly distributed among tanks. Data showed these hybrids consumed more food than pumpkinseeds. Fish were seined from a pond (Appendix Table B, Collection 4, 5, 6) and ranged in weight from 4.13 to 9.22 g. Fish were exposed for 24 days to a temperature of 10.1 C (Table 7) and seven nominal concentrations of ammonia (0, 2, 6, 8, 10, 12, 14 ppm as N) at a flow rate of 300 ml/min per tank. Pumpkinseeds were given a 6-day acclimation period in the seven tanks, the first day without Gambusia present, and from the second day on with food present.

Sixty-four fish were used in this experiment; eight were sacrificed on the first day of the experiment for initial RNA-DNA ratios. Fish were weighed at the beginning and end of the experiment. Thus initial growth response

Table 7. Chemical characteristics of water used in the continuous-flow experiment 7-F. (N is the number of samples used in determinations; $\bar{X}$ is the mean with one standard error enclosed in parentheses; t = less than 0.01 ppm).

	Aquarium Number

Table 7. Chemical characteristics of water used in the continuous-flow experiment 7-F. (N is the number of samples used in determinations; $\bar{X}$ is the mean with one standard error enclosed in parentheses; t = less than 0.01 ppm).

		Aquarium Number						
		1	8	3	10	5	2	12
NH_4 ppm as N	N	18	19	19	19	19	19	19
	$\bar{X}$	0.10 (0.01)	2.13 (0.05)	5.73 (0.10)	7.88 (0.21)	9.16 (0.12)	11.27 (0.15)	13.60 (0.16)
Un-ionized NH_3 ppm as N		t	0.06	0.21	0.23	0.27	0.26	0.31
pH	N	10	10	10	10	10	10	10
	Range	7.70-7.89	7.91-8.03	7.93-8.10	7.80-8.12	7.91-8.09	7.78-7.90	7.78-7.93
Temperature (C)	N	22	22	22	22	22	22	22
	$\bar{X}$	9.8 (0.1)	10.3 (0.1)	10.0 (0.1)	10.4 (0.1)	10.1 (0.1)	10.1 (0.1)	10.3 (0.1)
Dissolved Oxygen (ppm)	N	6	6	6	6	6	6	6
	$\bar{X}$	337 (0.2)	341 (0.2)	342 (0.2)	342 (0.2)	341 (0.1)	343 (0.1)	343 (0.1)
Alkalinity ppm as $CaCO_3$	N	6	6	6	6	6	6	6
	$\bar{X}$	337 (3)	341 (2)	342 (2)	342 (2)	341 (2)	343 (2)	343 (2)
Hardness ppm as $CaCO_3$	N	6	6	6	6	6	6	6
	$\bar{X}$	338	352	347	349	347	345	346

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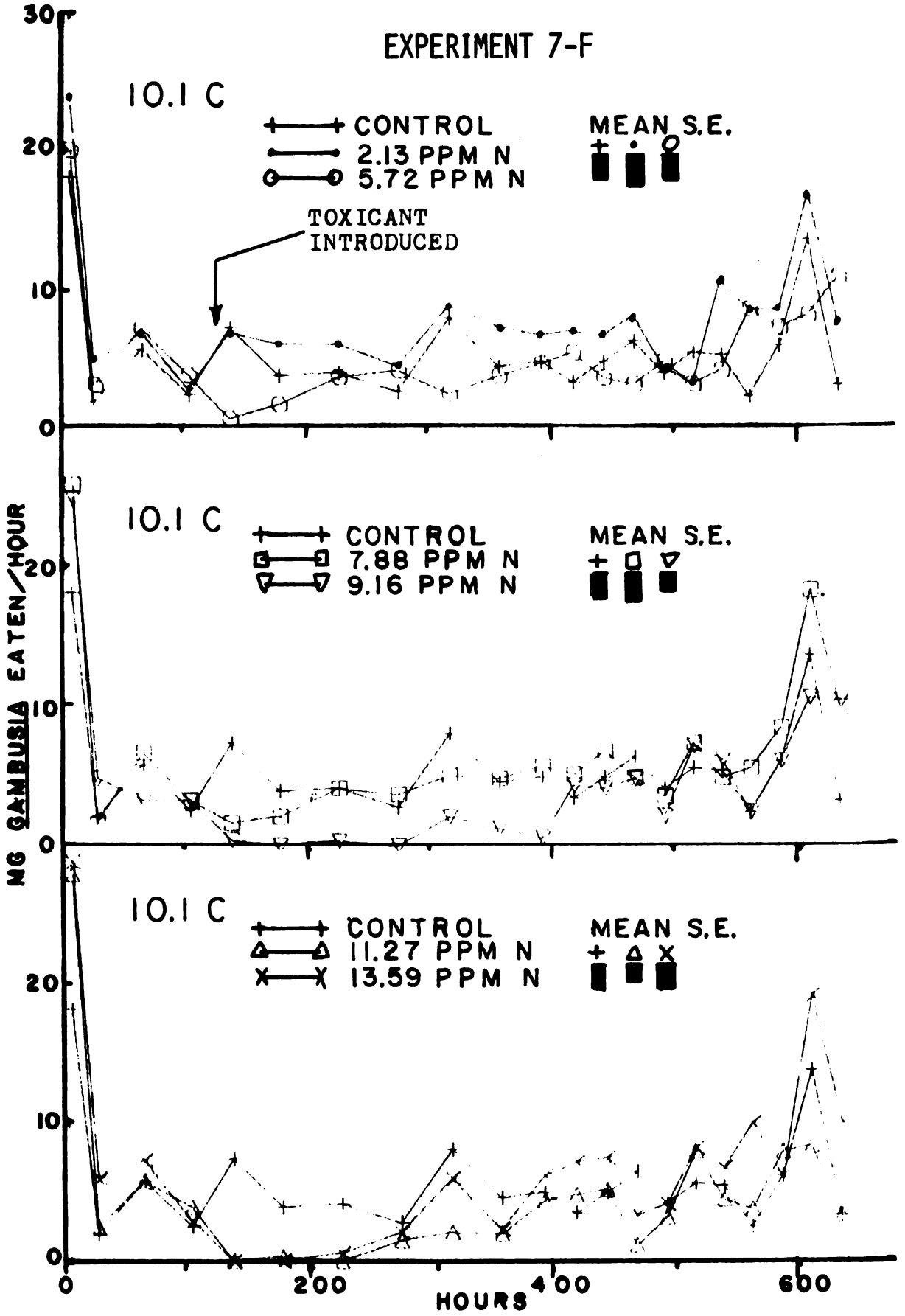
and any acclimation trends may be masked by not having measured weights during the experiment. The standard 96-hr LC_{50} determination was also performed on a group of these fish (Experiment 8-F). This was done so that: 1) the prior standard method results could be compared with results from my chronic tests and 2) so that application factors could be calculated. This 96-hr LC_{50} value was found for pumpkinseeds (no hybrids) to be 9.4 ppm as N (Appendix Table F).

Patterns of food consumption (Figure 6) indicated that all concentrations of ammonia above 5.72 ppm caused fish to stop feeding when the toxicant was introduced. Some regurgitation of previously eaten food also occurred. The higher the concentration of ammonia the less the amount of food that was eaten and the longer the time before increased consumption occurred. A one-way analysis of variance showed there was a significant difference among treatments in the amount of Gambusia eaten (calculated F = 2.45, tabular F, 0.05 level (6, 56) = 2.34). However, Dunnett's 2-tailed test (Steel and Torrie, 1960) to compare the control with all treatments failed to show any differences. This is undoubtedly related to the great variability in the growth and feeding response of sunfish among treatments which decreased the precision of these measurements. Food consumption by fish exposed to 2.13 ppm ammonia as N was similar to that of controls in the early part of the experiment, but throughout the remainder of the experiment amounts

Figure 6. Rate of consumption of Gambusia in mg per hr by pumpkinseeds exposed continuously to seven concentrations of ammonia. Each point represents the mean of eight fish. Mean standard error is given for each concentration.


EXPERIMENT 7-F
10.1 C
CONTROL
2.13 PPM N
5.72 PPM N
MEAN S.E.
TOXICANT INTRODUCED
10.1 C
CONTROL
7.88 PPM N
9.16 PPM N
MEAN S.E.
10.1 C
CONTROL
11.27 PPM N
13.59 PPM N
MEAN S.E.
MG GAMBUSIA EATEN/HOUR
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20
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eaten were greater than those eaten by control fish. In addition fish exposed to the other ammonia concentrations eventually ate amounts of Gambusia comparable to amounts eaten by controls.

Mean weight gain (initial weight divided by final weight) of exposed fish was a variable measurement that suggested fish exposed to the three highest concentrations of ammonia (9, 11 and 14 ppm) gained considerably less weight than controls (Table 8). Mean weight gain for the control (0.14), however, was not statistically different (calculated F = 0.83, tabular F, 0.05 level (6, 56) = 2.34) from weight gains made by fish exposed to other concentrations of ammonia. Weight gain by fish exposed to 2 ppm, although not significantly higher than that of control fish, was the highest mean weight gain of all groups of fish.

Food conversion ratios (net weight gain of a fish divided by weight of Gambusia eaten) ranged from 0.18 to 0.25, the control value being highest (Table 8). No significant difference was found among treatments (calculated F = 0.12, tabular F, 0.05 level (6, 56) = 2.34), which indicated that food conversion efficiency was unaffected by ammonia stress. However, variation and measurement of weights only at the beginning and end of the experiment would tend to mask any trends which occurred during the initial exposure and acclimation process.

The RNA-DNA ratios (Table 9) more closely reflected trends established by the food consumption curves (Figure 4).

Table 8. Me
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Aquarium No.	Co
1	
8	
3	
10	
5	
2	
12	

[a]Negative

Table 8. Mean weight gain and food conversion ratio of fish exposed to ammonia. (Standard error is given in parentheses after the mean).

Aquarium No.	Conc. of NH_4 (ppm as N)	No. of Fish	Mean Weight Gain	[a]Mean Food Conversion Ratio
1	Control	8	0.1453(0.0565)	0.2514(0.0858)
8	2.13	8	0.1638(0.0369)	0.2092(0.0354)
3	5.72	8	0.1532(0.0304)	0.2318(0.0353)
10	7.88	8	0.1562(0.0532)	0.2193(0.0631)
5	9.16	8	0.0864(0.0354)	0.2087(0.0773)
2	11.27	8	0.0536(0.0277)	0.1871(0.0899)
12	13.59	8	0.1229(0.0339)	0.2211(0.0550)

[a]Negative weight gains were treated as 0.

Table 9. Mean concentrations of RNA, DNA and the RNA-DNA ratio of pumpkinseed sunfish before and after 30 days exposure to ammonia. (DFFT is dry fat-free tissue from the dorsal muscle excluding skin. Standard error is enclosed in parentheses).

Aquarium	Conc. of NH	No. of	ug RNA per	ug DNA per	RNA-DNA

Table 9. Mean concentrations of RNA, DNA and the RNA-DNA ratio of pumpkinseed sunfish before and after 30 days exposure to ammonia. (DFFT is dry fat-free tissue from the dorsal muscle excluding skin. Standard error is enclosed in parentheses).

Aquarium No.	Conc. of NH_4 (ppm as N)	No. of Samples	µg RNA per 100 mg DFFT	µg DNA per 100 mg DFFT	RNA-DNA Ratio
Initial	—	8	176.2 (13.5)	37.8 (4.2)	5.26 (0.92)
1	Control	8	212.7 (9.6)	21.0 (2.7)	11.50 (1.44)
8	2.13	8	296.3 (24.7)	18.8 (2.6)	17.82 (2.12)
3	5.72	8	241.5 (19.2)	22.8 (3.4)	12.01 (1.57)
10	7.88	8	234.2 (18.0)	20.2 (1.5)	12.09 (1.06)
5	9.16	8	217.9 (14.0)	23.3 (3.4)	11.00 (2.04)
2	11.27	8	235.2 (26.7)	23.3 (1.9)	10.32 (1.09)
12	13.59	8	272.2 (30.3)	24.1 (1.8)	11.33 (0.99)

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Initially the RNA-DNA ratio was low (5.26), while after 30 days control fish had a mean ratio of 11.50. An analysis of variance (calculated F = 2.68, tabular F, 0.05 level (6, 56) = 2.34) showed a significant difference among the RNA-DNA ratios of fish from the various treatments. Dunnett's test (Steel and Torrie, 1960) showed that fish exposed to 2 ppm ammonia as N possessed a significantly higher RNA-DNA ratio (17.82) when contrasted with the control fish ratio (11.50). It is clear that fish exposed to 2 ppm ammonia were definitely stimulated to eat more and produce correspondingly more RNA.

Experiment 10-F

Experiment 10-F was performed to determine the growth response of green sunfish exposed to seven concentrations of ammonia. Also investigated was the post-treatment growth of fish in toxicant-free water. The experiment was conducted from May 12 to June 17, 1971 using seven tanks, 56 green sunfish (Appendix Table B, Collection 8) and nominal ammonia concentrations of 0, 2, 5, 10, 15, 20 and 25 ppm ammonia as N (Table 10). In this experiment all sunfish were placed in aquaria and fed Gambusia for 4 days before introduction of toxicant. Ammonia was then introduced for 20 days and then removed. Fish were then fed for 8 additional days in toxicant-free water. Fish were weighed at the beginning and every 4 days during the experiment to monitor changes in weight and food conversion

Table 10. *Chemical characteristics of water used in the continuous-flow experiment 10-F. (N is the number of samples used in determinations; $\bar{X}$ is the mean with one standard error enclosed in parentheses).*

	Aquarium Number

Table 10. Chemical characteristics of water used in the continuous-flow experiment 10-F. (N is the number of samples used in determinations; $\bar{X}$ is the mean with one standard error enclosed in parentheses).

		Aquarium Number						
		1	8	3	2	5	10	12
NH_4 ppm as N	N	22	24	25	24	24	46	46
	$\bar{X}$	0.15 (0.02)	1.93 (0.10)	4.84 (0.21)	8.67 (0.21)	13.23 (0.42)	20.02 (0.41)	24.03 (0.34)
Un-ionized NH_3 ppm as N		0.01	0.05	0.14	0.21	0.29	0.56	0.53
pH	N	9	9	9	9	9	9	9
	Range	7.83-8.01	7.60-8.13	7.57-8.03	7.59-8.12	7.50-8.09	7.55-8.10	7.52-7.95
Temperature (C)	N	24	24	24	24	24	24	24
	$\bar{X}$	15.6 (0.2)	15.9 (0.3)	15.6 (0.2)	15.6 (0.2)	15.7 (0.2)	16.0 (0.3)	16.0 (0.2)
Dissolved Oxygen (ppm)	N	7	8	8	7	7	7	8
	$\bar{X}$	8.2 (0.4)	5.5 (0.5)	5.6 (0.7)	5.6 (0.6)	6.1 (0.7)	5.9 (0.6)	5.0 (0.6)
Alkalinity ppm as $CaCO_3$	N	4	4	4	4	4	4	4
	$\bar{X}$	332 (6)	330 (7)	326 (10)	330 (8)	324 (10)	322 (12)	326 (8)
Hardness ppm as $CaCO_3$	N	4	4	4	4	4	4	4
	$\bar{X}$	328 (3)	330 (5)	332 (2)	338 (4)	335 (4)	332 (4)	333 (7)

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ratios. Mean water temperature was 15.8 C and flow rate to each tank was 300 ml/min.

To compare chronic results with the standard LC_{50} value and with the LC_{50} value obtained for pumpkinseeds, one was determined for green sunfish. In addition this value was needed for calculation of an application factor. The LC_{50} value was determined under continuous-flow conditions and found to be 33 ppm of ammonia as N (Appendix Table G). Other values reported by McKee and Wolf (1963) for bluegills included a 48-hr LC_{50} of 15 ppm as N at 20 C using tap water and a static system and 18.5 ppm as N for a static system using reoxygenated water. Cairns and Scheier (1959) gave a 96-hr LC_{50} value for bluegills of 6.0 ppm as N for small fish and 7.7 ppm ammonia as N for large fish (14 cm) under static test conditions.

The number of Gambusia eaten by fish exposed to various concentrations of ammonia declined when the toxicant was first introduced (Figure 7). The decline was most pronounced and lasted longer at the highest concentrations of ammonia tested, while fish at 1.93 ppm showed no initial response and those at 4.84 ppm exhibited a short-term decrease in feeding. Thereafter, fish exposed to 1.93 ppm ammonia consumed on the average consistently more food than control fish. The analysis of variance (Table 11) reflected the variable nature of the response as main effects and the ammonia stress x period interaction were highly significant. The significant interaction indicates

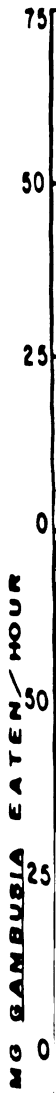

Figure 7. Rate of consumption of Gambusia in mg per hr by green sunfish before and after continuous exposure to different concentrations of ammonia. Each point represents the mean of eight fish unless deaths reduced that number. Mean standard error for each concentration is given as a dark vertical bar. Period of stress is indicated by the stippled area.

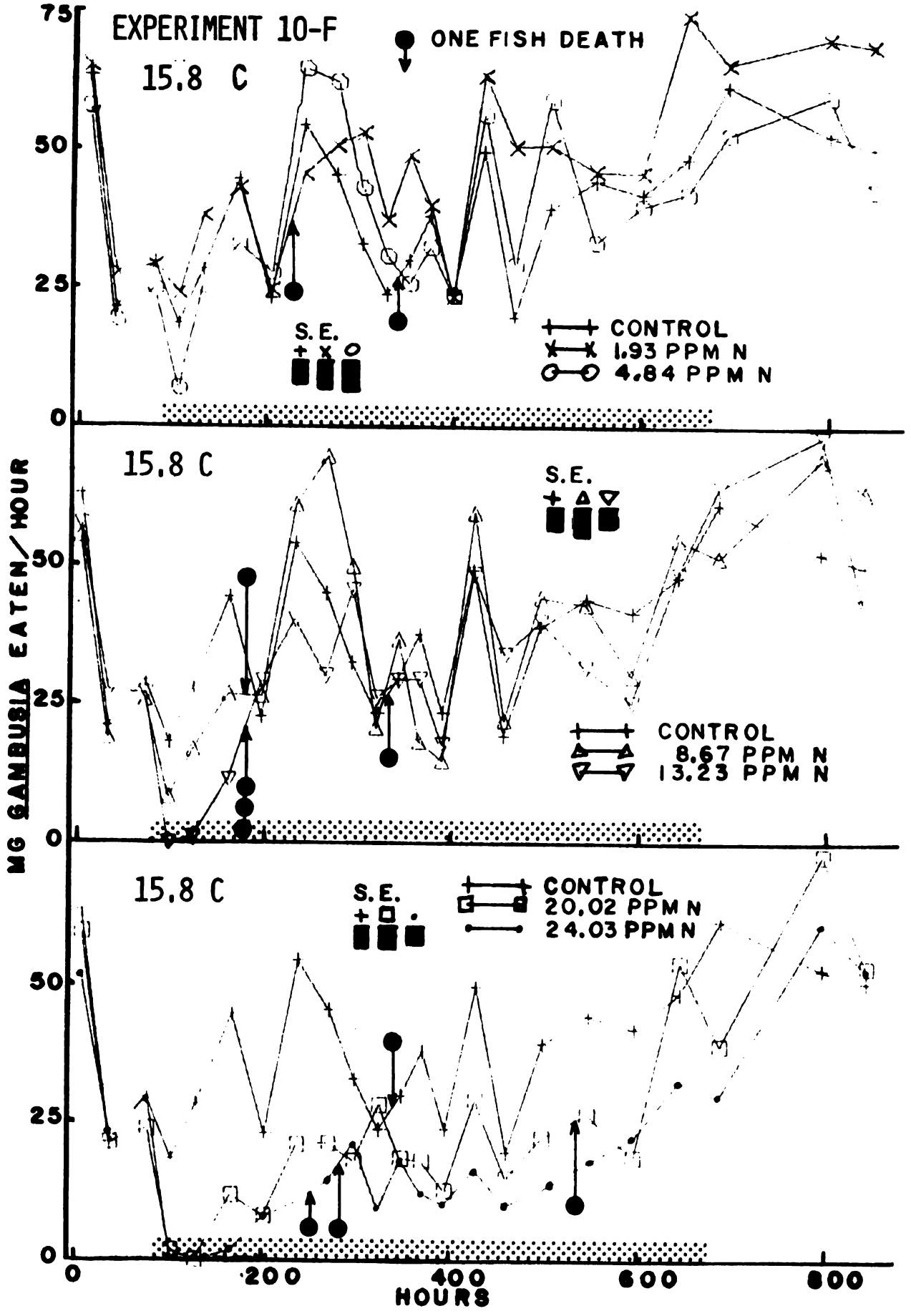
EXPERIMENT 10-F
ONE FISH DEATH
15.8 C
S.E.
CONTROL
1.93 PPM N
4.84 PPM N
15.8 C
S.E.
CONTROL
8.67 PPM N
13.23 PPM N
15.8 C
S.E.
CONTROL
20.02 PPM N
24.03 PPM N
MG GAMBUSIA EATEN/HOUR
HOURS
0
25
50
75
200
400
600
800

Table 11.

Source	
Stress	
Period	
S x P	
Error	
Total	

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Table 11. The analysis of variance table for the effects of seven concentrations of ammonia (stress) over six periods on the amount of Gambusia consumed by green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Stress	347.23	6	57.87	40.69**
Period	158.22	5	31.64	22.24**
S x P	73.09	30	2.44	1.71*
Error	359.82	[a]253	1.42	
Total	938.36	335		

**0.01 significance level.
[a]41 degrees of freedom were subtracted for dead fish.

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that the feeding response caused by the toxicant was not consistent over time, supporting the view (Figure 7) that fish were initially stressed to the point that a considerable decline in feeding occurred. Later these fish apparently acclimated to the ammonia stress as feeding rate became similar to that of control fish. Food consumption appeared to increase after toxicant removal especially in the two highest concentrations, where amount of food eaten was comparable to that eaten by controls.

The lowest concentration of ammonia (1.93 ppm as N) was apparently stimulatory to fish growth (Figure 8). After about 12 days, fish exposed to this concentration had mean weights greater than control fish. This difference became greater as time progressed. Fish exposed to 4.84 ppm of ammonia exhibited growth slightly lower than controls. At concentrations of ammonia greater than 4.84 ppm ammonia, growth was much less than for controls, particularly for 20 and 24 ppm ammonia as N. The analysis of variance (Table 12) showed a significant effect of time, but more importantly that ammonia stress had a significant effect on the growth of these fish. Some caution in interpretation must be exercised, because of the large number of dead fish, but it is still clear that stress adversely affected sunfish growth. Dunnett's procedure was applied to the mean weight averaged over time and it was found that growth of fish exposed to 20

Figure 8. Mean weight of green sunfish before, during (stippled area) and after continuous exposure to ammonia. Each point represents the mean of eight fish except where deaths decreased this number. See Appendix Table H for standard errors.

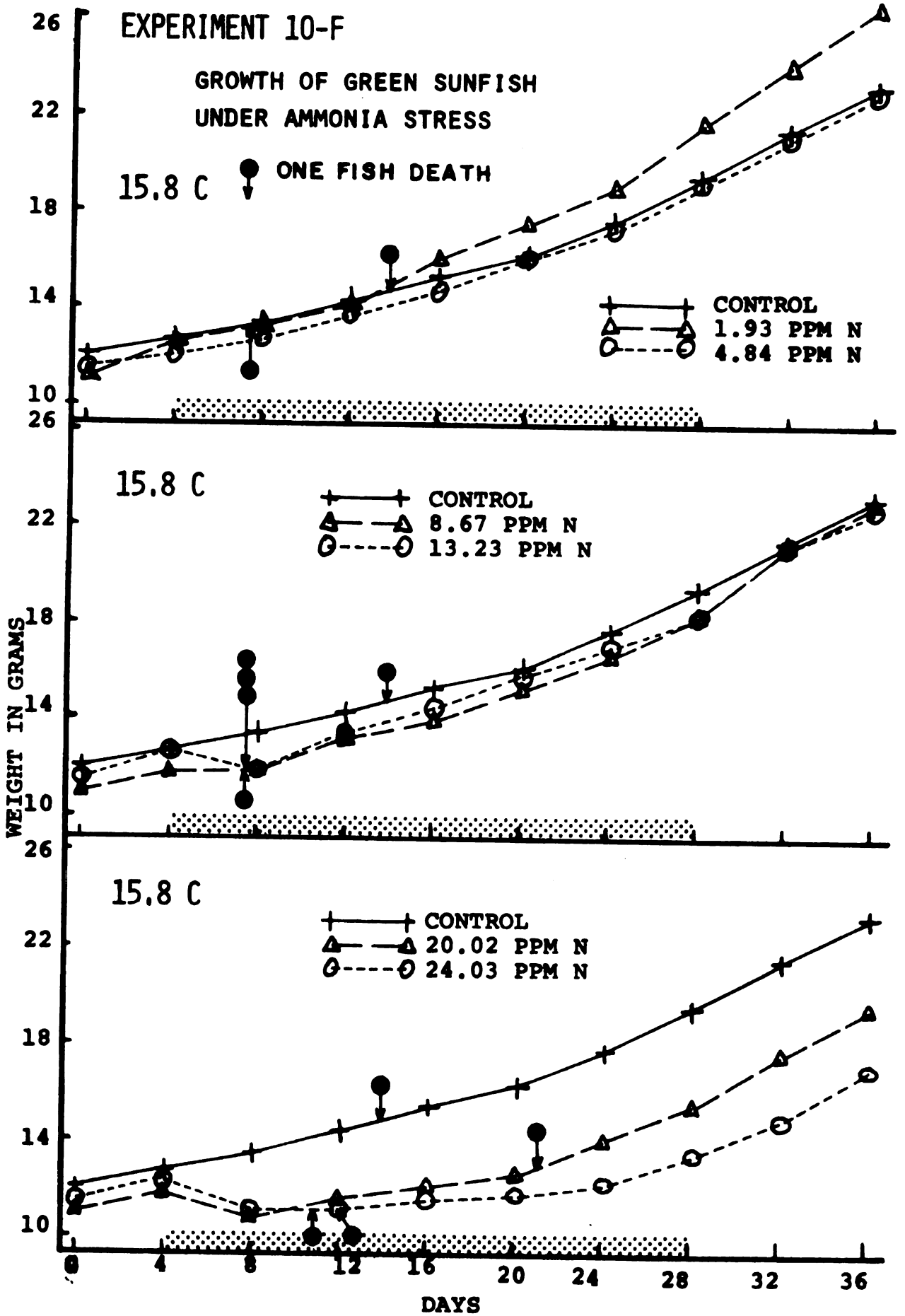
EXPERIMENT 10-F
GROWTH OF GREEN SUNFISH
UNDER AMMONIA STRESS
15.8 C
ONE FISH DEATH
CONTROL
1.93 PPM N
4.84 PPM N
15.8 C
CONTROL
8.67 PPM N
13.23 PPM N
15.8 C
CONTROL
20.02 PPM N
24.03 PPM N
WEIGHT IN GRAMS
DAYS
26
22
18
14
10
0
4
8
12
16
20
24
28
32
36

Table 12.

Source	
Stress	
Period	
S x P	
Error	
Total	

**0.01 sig
a40 degree

Table 12. The analysis of variance table for the effects of seven concentrations of ammonia (stress) over six periods on the growth of green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Stress	979.52	6	163.25	32.33**
Period	1269.35	5	253.87	50.77**
S x P	123.89	30	4.13	0.82
Error	1281.67	[a]254	5.05	
Total	3654.43	335		

**0.01 significance level.
[a]40 degrees of freedom were subtracted for dead fish.

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and 24 ppm of ammonia as N (12.80 and 11.81 g respectively) was significantly lower than growth of controls (16.06 g).

More deaths occurred at higher concentrations, although one control fish and one at 1.93 ppm died (Table 13). Three succumbed at 13.23 ppm early in the experiment (190 hrs); then later two died at 20 and one at 24 ppm as N. Fish usually lost weight in other experiments before death, while only four of nine did in this experiment. Fish exposed to higher concentrations of ammonia also had a greater incidence of mandible abrasions than fish at lower concentrations.

Removal of toxicant appeared to have no great effect on fish growth, as most fish continued to grow at about the same rate and exhibited the same trends of growth observed during exposure (Figure 8). Fish at 8.67 and 13.23 ppm appeared to be somewhat of an exception, as they grew at rates comparable to controls in the 8 days after removal of toxicant.

Some acclimation by fish under ammonia stress was indicated by growth data (Figure 8). For those treatments greater than 4.84 ppm as N, mean fish weight declined considerably after introduction of the toxicant, indicating in some cases a mean weight loss of about 1 g per fish over the first 4 days after toxicant introduction. Thereafter fish exposed to 8 and 13 ppm ammonia grew at an increasing rate while growth of fish exposed to 20 and 24

Table 13.

Fish No.
34
35
37
10
59
89
94
1
75

Table 13. Death and weight response of fish exposed to various concentrations of ammonia at 15.8 C.

Fish No.	Time of Death (hrs)	Type of Stress (ppm N)	Initial Weight (g)	Final Weight (g)
34	190	13.23	11.50	11.79
35	190	13.23	10.24	10.53
37	190	13.23	11.99	11.49
10	190	8.67	12.00	11.10
59	191	1.93	10.95	12.04
89	264	24.03	10.24	9.69
94	288	24.03	13.38	12.81
1	356	Control	12.31	14.75
75	524	20.02	10.02	10.59

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ppm remained the same for 12 days before greater increases in growth occurred.

Food conversion ratios showed an initial decline the first 4 days after toxicant introduction by fish from concentrations greater than 4.84 ppm (Figure 9). During the initial 4-day period, non-feeding and weight loss for these fish occurred. Apparently stressed fish (those exposed to ammonia) utilized energy for the "stress reaction" (Selye, 1956), thereby resulting in less tissue elaboration (growth) and consequently, lower food conversion ratios. After the first 4 days, the food conversion ratio of all stressed fish became essentially indistinguishable from control fish values (0.30-0.40). The analysis of variance for these data indicated that the stress x period interaction was significant (Table 14), which shows that the effect of ammonia on food conversion was not consistent over time. The reason for this interaction, the initial decline in food conversion after introduction of the toxicant, has been discussed.

Experiment 11-F

Experiment 11-F, performed between August 18 and September 7, 1971, was the first experiment using cadmium as the stressor. Objectives of the experiment were: 1) to record the growth response of green sunfish exposed to relatively high concentrations of cadmium; 2) to determine at what concentrations sub-lethal tests should be conducted; 3) to obtain an LC_{50} value for green sunfish; 4) to

Figure 9. Food conversion ratio is shown before (day 0-4), during (day 4-28, stippled area) and after (day 28-36) continuous exposure to ammonia concentrations. Each point represents the mean of eight fish, except where deaths decreased this number. Mean standard error is shown with dark vertical bars.

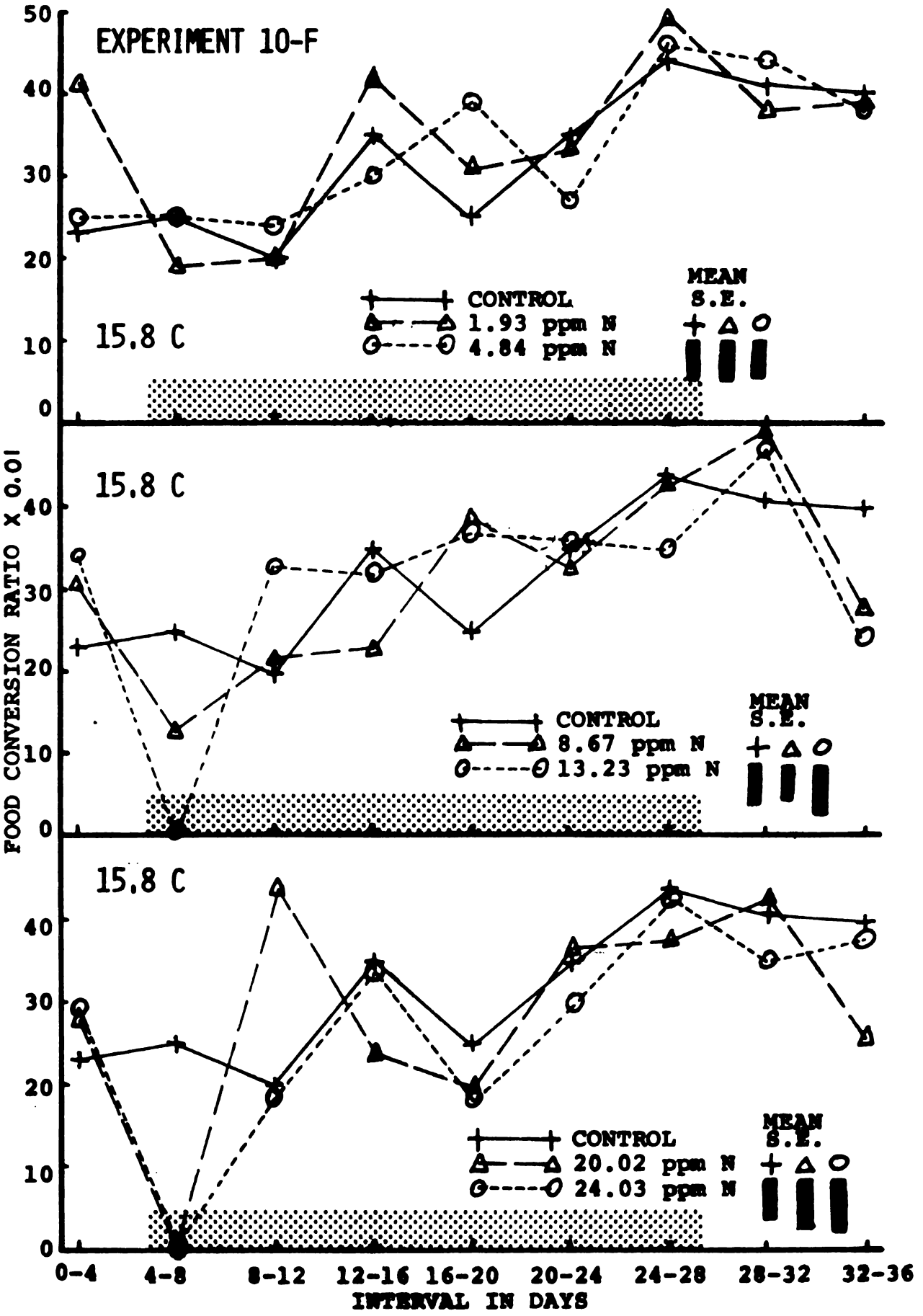
EXPERIMENT 10-F
15.8 C
CONTROL
1.93 ppm N
4.84 ppm N
MEAN
S.E.
15.8 C
CONTROL
8.67 ppm N
13.23 ppm N
MEAN
S.E.
15.8 C
CONTROL
20.02 ppm N
24.03 ppm N
MEAN
S.E.
FOOD CONVERSION RATIO X 0.01
0
10
20
30
40
50
0-4
4-8
8-12
12-16
16-20
20-24
24-28
28-32
32-36
INTERVAL IN DAYS

Table 14.

Source	
Stress	
Period	
S x P	
Error	
Total	

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Table 14. The analysis of variance table for the effects of seven concentrations of ammonia (stress) over six periods on the food conversion ratios of green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Stress	0.0772	6	0.0129	0.79
Period	0.8299	4	0.2075	12.16**
S x P	1.0191	24	0.0425	2.49**
Error	4.0964	[a]241	0.0170	
Total	6.0226	279		

**0.01 significance level.
[a]44 degrees of freedom were subtracted for dead fish.

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determine the rates of cadmium uptake in the gills, liver and whole body; and 5) to evaluate the use of chemical biopsy techniques. Fish used in this study ranged in weight from 7.20 to 10.81 g (see Appendix Table B, Collection 9, 10). They were subjected to seven nominal concentrations of cadmium (0, 5, 10, 20, 30, 40 and 50 ppm--see Table 15) for 16 days after a pre-stress period of 4 days. Flow rate to each tank was 200 ml/min and mean water temperature was 19.9 C. Sunfish were weighed at the beginning of the experiment and every 4 days thereafter. Gambusia were present at all times. Fish exposed to the higher concentrations of cadmium were very excitable, and handling stress during weighing undoubtedly was more significant for these fish than for controls. Fish affected by cadmium remained quietly at the surface of the water for long periods of time, or darted around trying to escape their respective cells. Many fish had severely-abrased lower mandibles and mucus secretions over their entire body, particularly on the eyes, mouth and fins.

The LC_{50} value derived for green sunfish under continuous-flow conditions of this experiment was 20.5 ppm Cd. Pickering and Henderson (1966) found a considerably higher LC_{50} value, 66 ppm, which was obtained under similar water chemistry conditions but using a static system.

Food consumption by fish at all levels of cadmium exposure was lower than that of controls; the higher the cadmium concentration, the lower the amount of food

Table 15. Chemical characteristics of water used in the continuous flow experiment 11-F.

Table 15. Chemical characteristics of water used in the continuous flow experiment 11-F. (N is the number of samples used in determinations; $\bar{X}$ is the mean with one standard error enclosed in parentheses; N.D. means non-detectable, less than 0.01 ppm).

		Aquarium Number						
		1	8	3	2	10	5	12
Cadmium ppm as Cd	N	14	14	15	13	15	15	15
	$\bar{X}$	N.D.	3.83 (0.18)	7.95 (0.23)	15.44 (0.39)	27.63 (0.99)	35.92 (0.79)	51.51 (2.26)
pH	N	3	3	3	3	3	3	3
	Range	7.82-8.00	7.85-8.04	7.78-8.09	8.06-8.14	7.88-8.05	7.73-7.96	7.55-7.93
Temperature (C)	N	12	12	12	12	12	12	12
	$\bar{X}$	19.8 (0.1)	19.7 (0.1)	19.8 (0.1)	19.8 (0.1)	20.1 (0.1)	19.7 (0.1)	20.4 (0.1)
Dissolved Oxygen (ppm)	N	2	3	3	3	3	3	3
	$\bar{X}$	7.8 (0.3)	7.8 (0.3)	7.6 (0.3)	7.9 (0.1)	7.9 (0.2)	7.8 (0.2)	7.6 (0.3)
Alkalinity ppm as $CaCO_3$	N	2	3	3	3	3	3	3
	$\bar{X}$	321 (1)	321 (10)	321 (9)	325 (7)	321 (13)	327 (17)	324 (20)
Hardness ppm as $CaCO_3$	N	2	3	3	3	3	3	3
	$\bar{X}$	322 (0)	333 (1)	333 (4)	337 (5)	333 (5)	340 (9)	345 (9)

consumed (Figure 10). Fish exposed to the two lowest concentrations of cadmium (3 and 7 ppm) showed an initial decline in feeding rate from about 30 mg Gambusia/hr to values around 5 mg/hr when the toxicant was first introduced. Feeding resumed at values around 30 mg/hr in the next 20-30 hrs and remained at this level for the remainder of the test, while control fish consumed food at a rate of 40-50 mg/hr. The analysis of variance confirmed the detrimental effect of the toxicant as the main effects, both stress and period, were highly significant (Table 16). Dunnett's test (Steel and Torrie, 1960) showed that the average rate of Gambusia consumption by control fish over all periods (4.18 mg/hr) was significantly different from consumption rates of 2.11, 1.77 and 0.79 mg/hr exhibited by fish at 3, 7 and 15 ppm Cd respectively.

Cadmium had a detrimental effect on growth rate of green sunfish at all concentrations (Figure 11). Amount of growth depression as well as the pattern of death was dose-dependent. At 51 ppm Cd all fish died in less than 24 hrs, while all fish at 35 ppm and 27 ppm Cd were dead in 4 days. Two died at 15 ppm, while none died in the control or at 7 and 3 ppm Cd during the 16-day period. Regression equations were calculated for controls and fish at 3, 7 and 15 ppm Cd relating mean growth and time. Only control fish exhibited a significant linear trend ($F = 46.36$, 0.05 level, 30 d.f.) with the regression equation being: Y (weight) $= 0.30\ X$ (time) $+ 8.47$. The

Figure 10. Rate of consumption of Gambusia in mg per hr by green sunfish 96 hrs before and during 384 hrs of continuous exposure to concentrations of cadmium. Each point represents eight fish except where deaths reduced this number. Mean standard error is given for each concentration as a dark vertical bar.

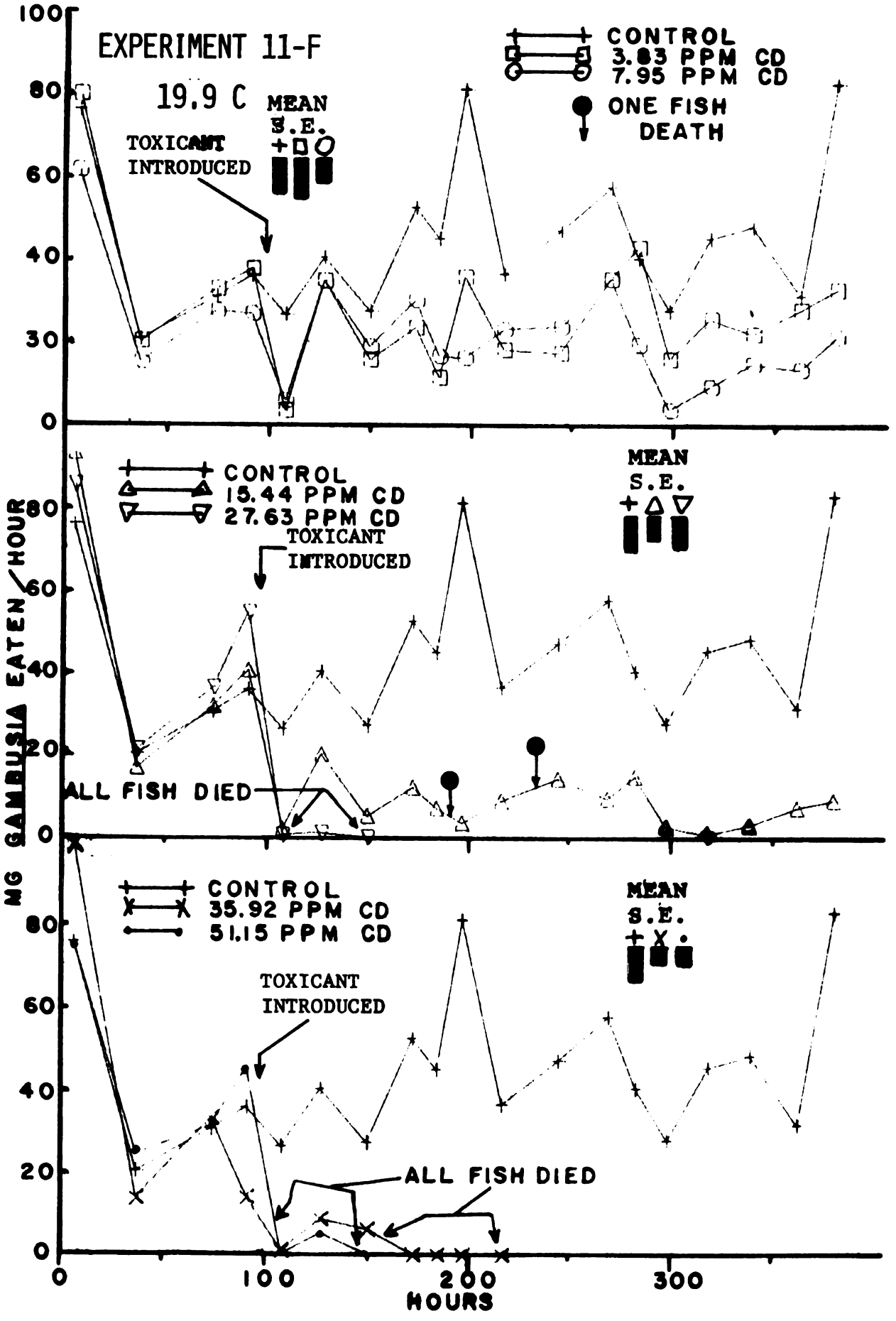
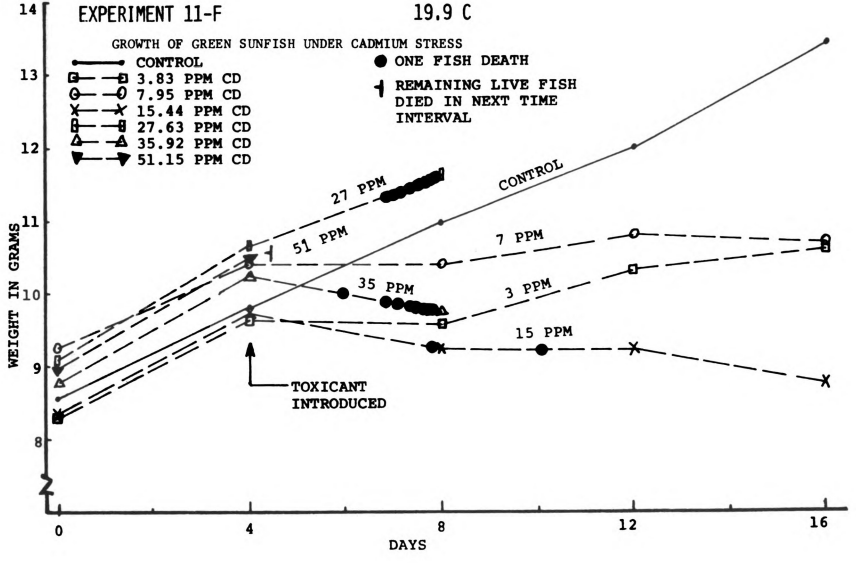

EXPERIMENT 11-F
19.9 C
MEAN
S.E.
TOXICANT
INTRODUCED
CONTROL
3.83 PPM CD
7.95 PPM CD
ONE FISH
DEATH
CONTROL
15.44 PPM CD
27.63 PPM CD
TOXICANT
INTRODUCED
MEAN
S.E.
ALL FISH DIED
CONTROL
35.92 PPM CD
51.15 PPM CD
TOXICANT
INTRODUCED
MEAN
S.E.
ALL FISH DIED
MG GAMBUSIA EATEN / HOUR
HOURS
0
20
40
60
80
100
200
300

Table 16. The analysis of variance table for the effects of four concentrations of cadmium (stress) over three periods on the amount of Gambusia consumed by green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Stress	145.95	3	48.65	29.85**
Period	14.70	2	7.35	4.51*
S x P	8.86	6	1.48	0.90
Error	130.37	[a]80	1.63	
Total	299.87	95		

*0.05 significance level.
**0.01 significance level.
[a]4 degrees of freedom were subtracted for dead fish.

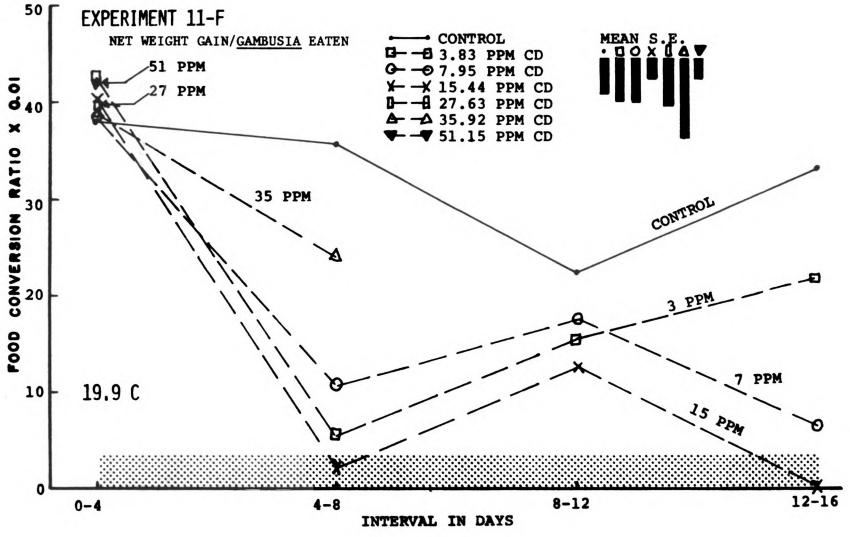
Figure 11. Mean weight of green sunfish 4 days before and during 16 days of continuous exposure to seven different concentrations of cadmium. Each point represents the mean of eight fish except where deaths reduced that number. See Appendix Table I for standard errors.


EXPERIMENT 11-F
19.9 C
GROWTH OF GREEN SUNFISH UNDER CADMIUM STRESS
CONTROL
3.83 PPM CD
7.95 PPM CD
15.44 PPM CD
27.63 PPM CD
35.92 PPM CD
51.15 PPM CD
ONE FISH DEATH
REMAINING LIVE FISH DIED IN NEXT TIME INTERVAL
WEIGHT IN GRAMS
DAYS
TOXICANT INTRODUCED
CONTROL
27 PPM
51 PPM
7 PPM
35 PPM
3 PPM
15 PPM

equation explained 61% of the variation relating growth of the control fish to time. The regression coefficient of the control was compared with the regression coefficient of all stressed fish. Because of the extreme variability in fish weights, no significant differences were detected between the control and any of the three treatments. This is a good example of the type of phenomenon which occurs in nature and in many laboratory tests. The stressor significantly affects a fairly large number of fish which soon die and others grow very little or lose weight. Others, which are genetically suited, are able to resist the stressor and even grow well. This leads to a large difference between individuals among stressed fish. Control fish, not subjected to such a drastic stress, are not selectively pushed to their genetic limits. When these factors are operating, statistical tests using an average variance to test effects can be inaccurate.

During the first 4-day pre-stress period fish possessed food conversion ratios very close to 0.40 (Figure 12). After introduction of toxicant, conversion ratios for fish under cadmium stress, when contrasted with controls, were considerably lower for the three remaining intervals. It appears that all concentrations of cadmium between 3 and 35 ppm were detrimental to fish since energy which normally would go into elaboration of tissue for new growth was diverted elsewhere or some detrimental effect on biochemical systems was occurring. This judgment was confirmed

Figure 12. Food conversion ratios of fish exposed for 16 days (after a 4-day acclimation period) to various high concentrations of cadmium. Each point represents the mean of eight fish except where deaths reduced that number. Mean standard error for each concentration is shown by a dark vertical bar.


EXPERIMENT 11-F
NET WEIGHT GAIN/GAMBUSIA EATEN
CONTROL
3.83 PPM CD
7.95 PPM CD
15.44 PPM CD
27.63 PPM CD
35.92 PPM CD
51.15 PPM CD
MEAN S.E.
51 PPM
27 PPM
35 PPM
CONTROL
3 PPM
7 PPM
15 PPM
19.9 C
FOOD CONVERSION RATIO X 0.01
INTERVAL IN DAYS
0-4
4-8
8-12
12-16
0
10
20
30
40
50

by the analysis of variance (Table 17) which showed a significant main effect ($F = 24.75$), and a significant interaction of stress with period ($F = 4.56$). The interaction effect was probably due to the initial decline in food conversion ratios by exposed fish while control conversion ratios remained high. The increase exhibited in the last period by fish exposed to 3 ppm Cd was also a factor.

Eight fish were randomly selected from the 72 fish used in this experiment and analyzed for cadmium at the beginning of the experiment and eight were analyzed at the end of 4 days of no stress (two control tanks were maintained for this reason). Then all 56 experimental fish were analyzed at the end of the experiment. Fish that died at 15 ppm Cd contained 5-8 times the amount of cadmium found in live fish at that concentration (Table 18A). Therefore dead fish were not pooled with live fish, but tabulated separately. Initially fish contained whole-body burdens of 0.68 ppm Cd and after 4 days of feeding on Gambusia in toxicant-free water, whole-body burdens increased to 0.98 ppm. The only reason which can be advanced for this increase is that Gambusia eaten contained 0.50 ± 0.18 ppm Cd (wet-weight basis), and that a considerable amount of this food was eaten, up to 2.5 g. The cadmium concentration in Gambusia was obtained from determinations on 3 separate samples from different times of the year weighing 16, 18 and 4 g respectively. After 16 days live fish

Table 17. The analysis of variance table for the effects of four concentrations of cadmium (stress) over three periods on the food conversion ratios of green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Stress	0.8081	3	0.2694	24.75**
Period	0.0150	2	0.0075	0.69
S x P	0.2979	6	0.0496	4.56**
Error	0.8706	[a]80	0.0109	
Total	1.9900	95		

**0.01 significance level.
[a]4 degrees of freedom were subtracted for dead fish.

Table 18A. Whole-body cadmium concentration on a wet-weight basis of green sunfish from experiment 11-F. (N is the number of fish used in analysis; one standard error is given in parentheses after the mean).

Treatment		Live Fish		Dead Fish	
Day	Ppm Cd	N	Ppm Cd	N	Ppm Cd
0	Control	8	0.68(0.07)	-	-
4	Control	8	0.98(0.13)	-	-
16	Control	8	0.82(0.09)	-	-
16	3.83(0.18)	8	3.99(0.30)	-	-
16	7.95(0.23)	8	4.77(0.38)	-	-
16	15.44(0.39)	6	21.52(3.75)	2	114.07(0.93)
16	27.63(0.99)	-	-	8	74.43(9.36)
16	35.92(0.70)	-	-	8	104.74(14.86)
16	51.51(2.26)	-	-	8	148.78(9.35)

exposed to 0, 3, 7 and 15 ppm Cd exhibited a linear relationship when the log of the cadmium concentration in the fish plus one (Y) was regressed against the cadmium concentration in the water (X). The equation describing this relationship (Y = 0.065 X + 0.316) explained 89% of the variation involved in measuring these variables. Fish exposed to 15 ppm accumulated proportionately more cadmium than fish at 3 and 7 ppm Cd. Apparently mechanisms that prevent excessive cadmium uptake such as mucus secretions, metaloproteins (Wisniewska, et al., 1970) and other unknown means become saturated. Saturation permits cadmium build-up which apparently reaches a lethal limit in green sunfish exposed for 16 days to 15 ppm, since two fish at this level and all fish exposed to higher concentrations died. The higher accumulation of cadmium by dead fish when compared with live fish at the same concentration cannot be attributed wholly to surface adsorbtion on mucus since all fish were washed thoroughly under tap water. Dead fish exposed to higher concentrations of cadmium generally accumulated increasing amounts of cadmium, the higher the concentration to which they were exposed. It would have been informative to have analyzed fish from these higher concentrations of cadmium before they died.

A similar pattern of cadmium uptake was exhibited by the gills from these fish, although gill cadmium content was more variable (Table 18B). Cadmium content in the gills increased from 0.46 on the first day of the experiment to

Table 18B. Cadmium concentration on a wet-weight basis in gills of green sunfish from experiment 11-F. (N is the number of fish used in analysis; one standard error is given in parentheses after the mean).

Treatment		Live Fish		Dead Fish	
Day	Ppm Cd	N	Ppm Cd	N	Ppm Cd
0	Control	8	0.46(0.15)	-	-
4	Control	8	0.99(0.28)	-	-
16	Control	8	0.62(0.16)	-	-
16	3.83(0.18)	8	3.22(0.44)	-	-
16	7.95(0.23)	8	3.40(0.68)	-	-
16	15.44(0.39)	6	21.44(4.44)	2	168.79(4.05)
16	27.63(0.99)	-	-	8	108.86(14.52)
16	35.92(0.79)	-	-	8	170.27(22.70)
16	51.51(2.26)	-	-	8	242.18(18.02)

0.99 ppm Cd on the fourth day. After 16 days of exposure a similar, though less exact pattern of cadmium uptake was shown by the gills as was seen in whole-body uptake. The equation explaining 74% of the variation observed in measuring cadmium uptake by the gills was: Y (log of the cadmium content in the gills + 1) = 0.066 X (cadmium content in the water) + 0.222. The concentration of cadmium in the gills of dead fish was higher than concentrations found in the whole body of these fish.

Cadmium content measurements of fish livers were the most variable among structures measured (Table 18C). Cadmium levels also increased in the liver from 0.35 ppm on day 0 to 1.12 ppm on day 4. After 16 days exposure the same type of relationship between cadmium in water and in tissue was observed. Only 60% of the variation was explained by the regression equation: Y = 0.086 X + 0.153. There was a very high accumulation by livers of live fish exposed to 15 ppm, twice as high (42 ppm) as concentrations found in livers of dead fish at higher concentrations.

After fish exposed to 50 ppm Cd had succumbed, eight large green sunfish (71.40 $\pm$ 9.16 g) were added to this tank. Most fish died within 24 hrs, a little sooner than did smaller fish at that concentration. Gills were removed from the large green sunfish after death and analyzed for cadmium content. Concentration of cadmium in these fish gills was 182.19 $\pm$ 20.95 ppm Cd which was less than that in gills of smaller fish (242.18 $\pm$ 18.02 ppm Cd--Table 18B) exposed to 50 ppm Cd.

Table 18C. Cadmium concentration on a wet-weight basis in the liver of green sunfish from experiment 11-F. (N is the number of fish used in analysis; one standard error is given in parentheses after the mean).

Treatment		Live Fish		Dead Fish	
Day	Ppm Cd	N	Ppm Cd	N	Ppm Cd
0	Control	8	0.35(0.35)	-	-
4	Control	8	1.12(0.35)	-	-
16	Control	8	0.41(0.20)	-	-
16	3.83(0.18)	8	4.27(0.66)	-	-
16	7.95(0.23)	8	7.24(3.00)	-	-
16	15.44(0.39)	6	42.81(9.09)	2	60.48(11.40)
16	27.63(0.99)	-	-	8	33.53(3.05)
16	35.92(0.70)	-	-	8	37.17(4.01)
16	51.51(2.26)	-	-	8	39.03(1.78)

Experiment 12-F

In this experiment, an attempt was made to maintain green sunfish at low concentrations of cadmium which are more likely to be found in waters as a result of man's activities. Growth, food consumption and RNA-DNA ratios were monitored under the low cadmium levels as was whole body, gill and liver cadmium uptake. The 72 green sunfish used ranged in weight from 5.67 to 7.47 g (Appendix Table B, Collection 9, 10). From this group of fish, eight were randomly assigned to each of nine different treatments, the first group of eight being sacrificed on day 0 to determine initial cadmium levels and RNA-DNA ratios. The second group was one of two control groups which was sacrificed on day 4 to determine what effect the 4-day acclimation period had on cadmium uptake and RNA-DNA ratios. The remaining seven groups made up the experimental groups of one control and six treatments exposed to 0.05, 0.23, 0.32, 1.31, 1.93 and 2.48 ppm Cd (Table 19). The experiment was started on September 10, 1971 and continued for 24 days including a 4-day period of acclimation to experimental conditions, then 20 days of stress with cadmium introduction. Food (Gambusia) was unlimited for sunfish throughout the experiment. Fish were exposed continuously at a flow rate of 200 ml/min and a mean temperature of 18.6 C and were weighed every 4 days to monitor growth.

Except for the 0.05 ppm treatment, fish at all concentrations of cadmium ate amounts of Gambusia comparable to

Table 19. Chemical characteristics of water used in the continuous flow experiment 12-F. (N is the number of samples used in determinations; $\bar{X}$ is the mean with one standard error enclosed in parentheses; N.D. means non-detectable, less than 0.01 ppm).

		Aquarium Number						
		1	12	3	8	2	5	10
Cadmium ppm as Cd	N	5	19	19	19	19	19	19
	$\bar{X}$	N.D.	0.05 (0)	0.23 (0.01)	0.32 (0.01)	1.31 (0.04)	1.93 (0.05)	2.48 (0.08)
pH	N	2	2	2	2	2	2	2
	Range	7.82-7.86	7.77-7.99	7.74-7.94	7.79-7.88	7.76-7.94	7.84-7.82	7.80-7.98
Temperature (C)	N	12	12	12	12	12	12	12
	$\bar{X}$	18.4 (0.2)	18.9 (0.3)	18.2 (0.3)	18.6 (0.3)	18.5 (0.2)	18.6 (0.2)	18.7 (0.2)
Dissolved Oxygen (ppm)	N	2	2	2	2	2	2	2
	$\bar{X}$	8.4 (0.1)	8.6 (0)	8.4 (0.1)	8.2 (0)	8.2 (0.2)	8.4 (0.1)	8.5 (0.1)
Alkalinity ppm as $CaCO_3$	N	2	2	2	2	2	2	2
	$\bar{X}$	351 (3)	344 (0)	349 (1)	350 (2)	344 (0)	349 (3)	351 (3)
Hardness ppm as $CaCO_3$	N	2	2	2	2	2	2	2
	$\bar{X}$	328 (8)	333 (7)	330 (10)	333 (7)	332 (12)	332 (12)	335 (9)

or less than that eaten by controls (Figure 13). Greatest depression of feeding and highest mortality occurred at 1.31 ppm Cd where three fish died toward the latter part of the experiment. One fish succumbed at 1.93 ppm Cd while none died at 2.48 ppm. Analysis of variance showed that cadmium stress and period had a significant effect on the amount of Gambusia eaten by these fish (Table 20). To sort out differences among treatments Dunnett's test (Steel and Torrie, 1960) was used, but it failed to show any treatments different from the control. It was noted, however, that fish exposed to 0.05 ppm Cd consumed the greatest amount of Gambusia (a mean of 1.67 g) as compared with the control value of 1.48 g. Fish exposed to higher concentrations of cadmium consumed amounts ranging from 0.87 to 1.21 g.

After about 200 hrs exposure to 0.05 ppm Cd fish began to eat at an increasingly higher rate when compared with control fish. It appeared that this concentration definitely stimulated growth and may be an important threshold value of significance in setting safe water quality standards. Some signs of acclimation toward the end of the experiment were shown by fish exposed to higher concentrations. A longer study would have better documented this phase of the chronic toxicity response.

Growth of green sunfish was depressed at all concentrations of cadmium, except at 0.05 ppm Cd (Figure 14). The analysis of variance (Table 21) showed that both main effects (cadmium stress and period) had a significant effect on

Figure 13. Rate of consumption of Gambusia in mg per hr 96 hrs before and during 480 hrs of continuous exposure to seven different concentrations of cadmium. Each point represents eight fish except where deaths reduced this number. Mean standard error is given for each concentration.

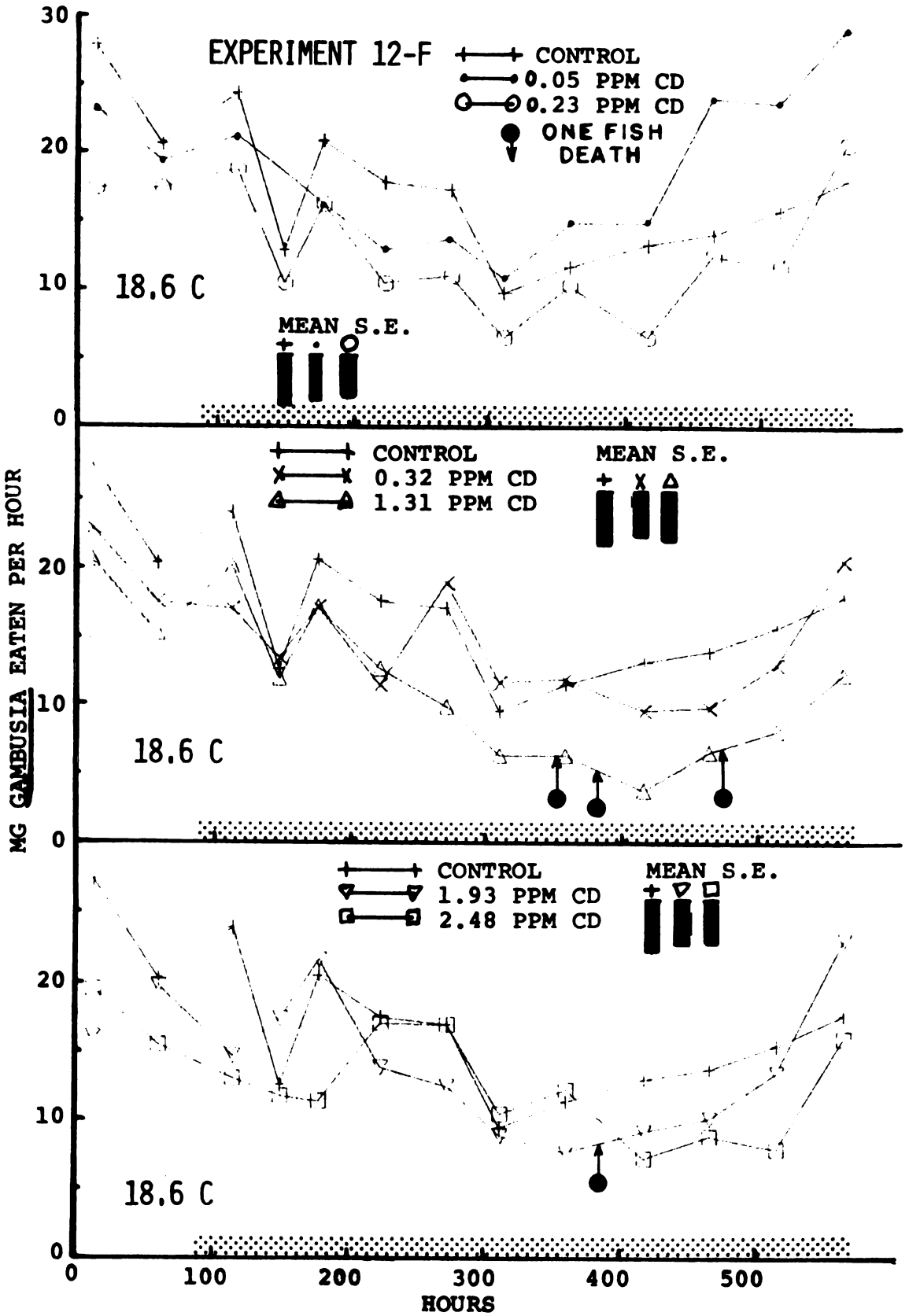

EXPERIMENT 12-F
CONTROL
0.05 PPM CD
0.23 PPM CD
ONE FISH DEATH
18.6 C
MEAN S.E.
CONTROL
0.32 PPM CD
1.31 PPM CD
MEAN S.E.
18.6 C
CONTROL
1.93 PPM CD
2.48 PPM CD
MEAN S.E.
18.6 C
MG GAMBUSIA EATEN PER HOUR
HOURS
0
10
20
30
100
200
300
400
500

Table 20. The analysis of variance table for the effects of seven concentrations of cadmium (stress) over five periods on the amount of Gambusia consumed by green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Stress	16.65	6	2.77	4.86**
Period	16.99	4	4.25	7.44**
S x P	14.00	24	0.58	
Error	135.81	[a]238	0.57	
Total	183.45	279		

**0.01 significance level.
[a]7 degrees of freedom were subtracted for dead fish.

Figure 14. Mean weight of green sunfish 4 days before and during 20 days of continuous exposure to various concentrations of cadmium. Each point represents the mean of eight fish except where deaths decreased this number. See Appendix Table J for standard errors.

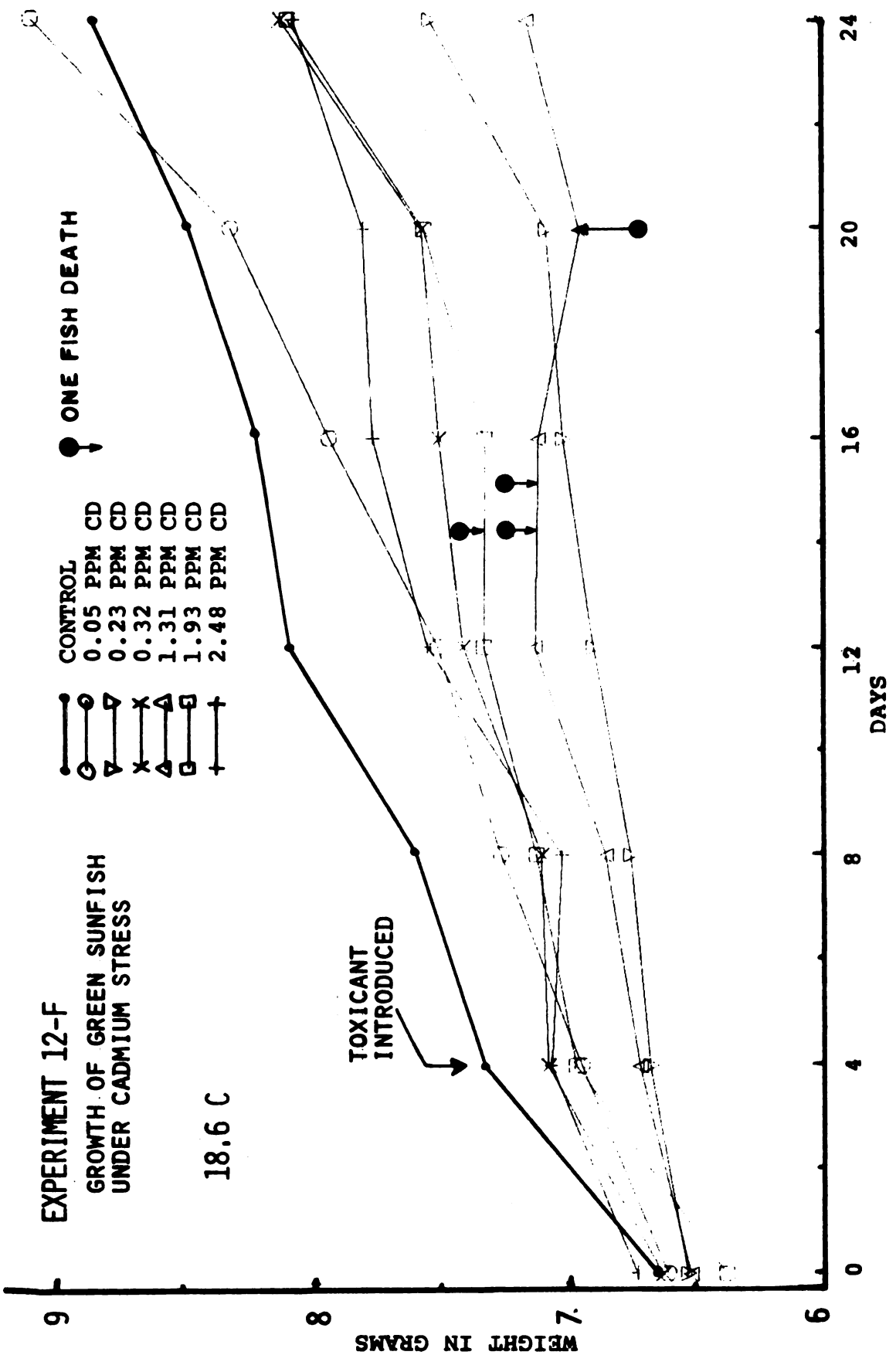

EXPERIMENT 12-F
GROWTH OF GREEN SUNFISH
UNDER CADMIUM STRESS
18.6 C
CONTROL
0.05 PPM CD
0.23 PPM CD
0.32 PPM CD
1.31 PPM CD
1.93 PPM CD
2.48 PPM CD
ONE FISH DEATH
TOXICANT
INTRODUCED
WEIGHT IN GRAMS
DAYS
9
8
7
6
0
4
8
12
16
20
24

Table 21. The analysis of variance table for the effects of seven concentrations of cadmium (stress) over five periods on the mean weight gains of green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Stress	32.71	6	5.45	7.68**
Period	19.28	4	4.82	6.79**
S x P	7.64	24	0.32	0.45
Error	168.88	238	0.71	
Total	228.51	279		

**0.01 significance level.
[a]7 degrees of freedom were subtracted for dead fish.

growth of these fish. Dunnett's test (Steel and Torrie, 1960) showed that fish exposed to 0.23 and 1.31 ppm Cd possessed mean weights significantly less than the control. Fish exposed to 0.05 ppm Cd had the highest mean weight, but it was not statistically higher than the control weight. The stimulatory effect of 0.05 ppm Cd clearly shown by food consumption (Figure 13) appeared somewhat later in the growth response (Figure 14). The greater rate of growth of these fish, when compared with controls, started after 3 days exposure. By the end of the experiment mean weight of these fish was greater than that of controls.

Food conversion ratios (Figure 15) were extremely variable, but the analysis of variance showed that both stress and period had a significant effect (Table 22). The magnitude of the F value showed time to be the greater of the two effects. Examination of the tables of means averaged over time showed that fish at 0.05 ppm had the highest food conversion ratio (0.25), while values from the other cadmium-exposed fish except one, were lower than the control value (0.20). None were significantly different (Dunnett's test) from control values. Fish exposed to 0.05 ppm Cd having the highest food conversion ratio suggests that the greater growth exhibited by these fish is due both to greater intake of food and a better efficiency of conversion when compared with controls. There was no consistent effect of period on the mean ratio considered over all treatments.

Figure 15. Food conversion ratios of green sunfish before and during continuous exposure to different concentrations of cadmium. Each point represents the mean of eight fish except where deaths reduced this number. Mean standard error for each concentration is shown by a dark vertical bar.

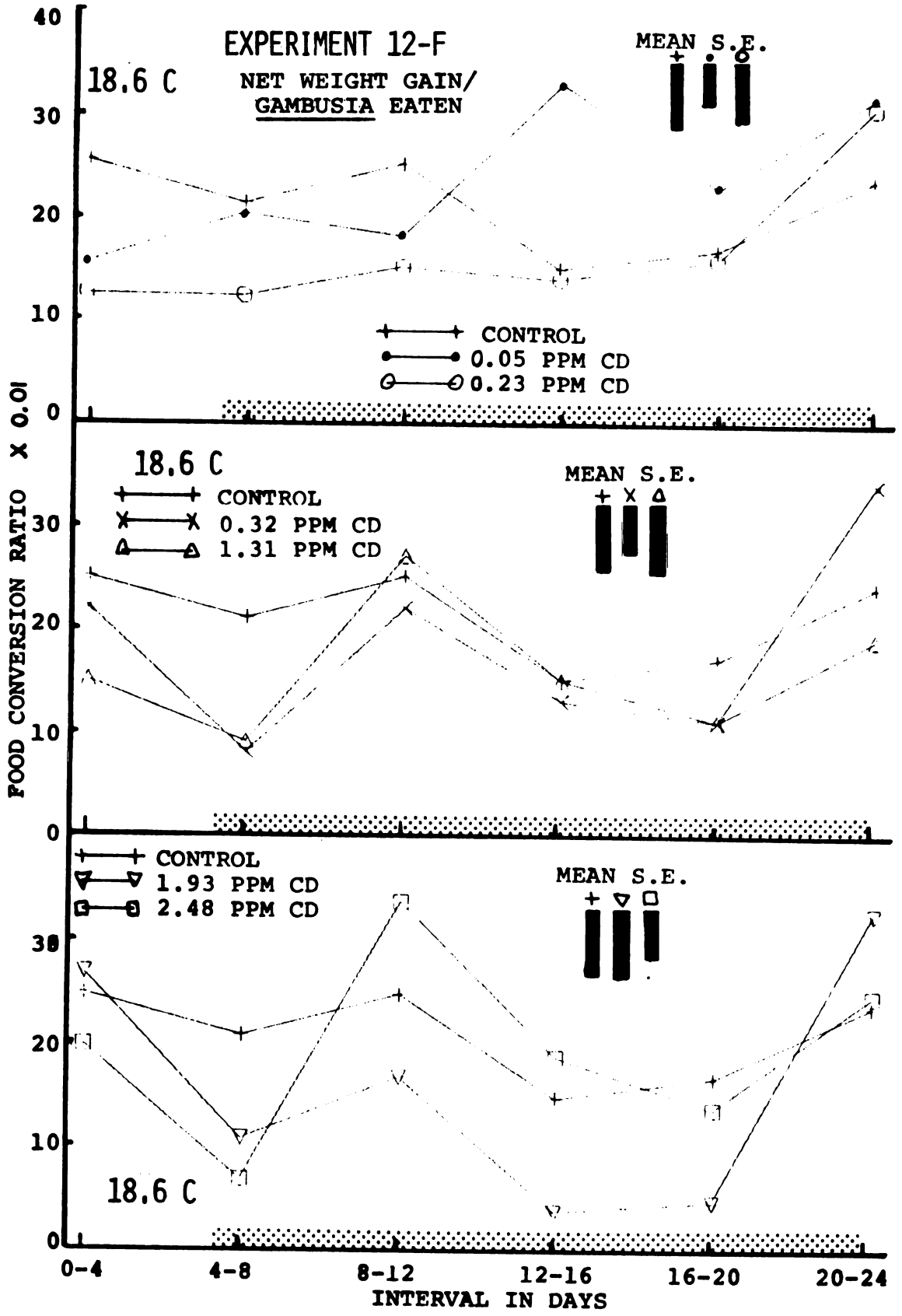

EXPERIMENT 12-F
18.6 C
NET WEIGHT GAIN/
GAMBUSIA EATEN
MEAN S.E.
CONTROL
0.05 PPM CD
0.23 PPM CD
18.6 C
CONTROL
0.32 PPM CD
1.31 PPM CD
MEAN S.E.
CONTROL
1.93 PPM CD
2.48 PPM CD
MEAN S.E.
18.6 C
FOOD CONVERSION RATIO X 0.01
0
10
20
30
40
0-4
4-8
8-12
12-16
16-20
20-24
INTERVAL IN DAYS

Table 22. The analysis of variance table for the effects of seven concentrations of cadmium (stress) over five periods on the food conversion ratios of green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Stress	0.2944	6	0.0491	2.27*
Period	1.1238	4	0.2810	13.01**
S x P	0.6674	24	0.0278	1.29
Error	5.0338	[a]233	0.0216	
Total	7.1195	279		

*0.05 significance level.
**0.01 significance level.
[a]12 degrees of freedom were subtracted for dead fish.

The mean RNA-DNA ratio for this group of fish started at 16.97 and increased to 19.28 at the end of the first 4 days before cadmium introduction (Table 23). After the next 20 days fish from the control tank possessed a mean RNA-DNA ratio of 22.54. Cadmium-stressed fish had ratios ranging from 21.87 (1.31 ppm Cd) to 27.51 (1.93 ppm Cd) with the 0.05 ppm-exposed fish having a ratio of 26.29. There was no significant difference among treatment means (calculated F = 0.34, tabular F, 0.05 level, (6, 49) = 2.37) after 20 days. Thus the RNA-DNA data were unable to demonstrate some of the results already shown. For example, one would have expected the 0.05 ppm-treated group to have a considerably higher ratio. This might have been shown if samples could have been taken midway through the experiment.

Whole-body burdens were measured initially at 0.95 ppm Cd and then decreased to 0.55 ppm after 4 days of feeding before cadmium was introduced (Table 24A). Apparently differences in background levels and perhaps water characteristics were responsible for this loss. In experiment 11-F this change was just the opposite. Control fish in experiment 12-F after 20 more days contained 0.65 ppm Cd, comparable to levels found after 4 days (0.55 ppm), so apparently equilibrium was reached at least by 4 days and maintained near that for the next 20 days. Fish exposed to 0.05 ppm Cd contained three times as much cadmium (1.84 ppm) as control fish (0.65 ppm), and even greater levels than found in fish exposed to 0.23 and 0.32 ppm. It is because of this unique behavior of

Table 23. Mean concentrations of RNA, DNA and the RNA-DNA ratios of green sunfish from experiment 12-F before and during continuous exposure for 20 days to various concentrations of cadmium. (DFFT is dry fat-free tissue from the dorsal muscle excluding skin; standard error is enclosed in parentheses; N.D. means non-detectable, less than 0.01 ppm).

Aquarium No.	Day No.	Ppm Cd	No. of Samples	µg DNA per 100 mg DFFT	µg RNA per 100 mg DFFT	RNA-DNA Ratio
Initial	0	-	8	24.4(1.2)	412.3(73.0)	16.97(2.54)
4	4	-	8	23.9(1.3)	445.0(56.9)	19.28(3.00)
1	24	N.D.	8	24.9(2.5)	518.2(58.2)	22.54(3.48)
12	24	0.05(0)	8	22.3(2.6)	572.5(51.3)	26.29(1.65)
3	24	0.23(0.01)	8	22.8(1.4)	500.4(61.0)	22.87(3.61)
8	24	0.32(0.01)	8	22.6(2.3)	527.9(35.6)	25.12(3.13)
2	24	1.31(0.04)	8	20.5(1.2)	435.9(41.2)	21.87(2.65)
5	24	1.93(0.05)	8	20.7(1.5)	519.8(66.1)	27.51(4.52)
10	24	2.48(0.08)	8	21.8(2.2)	458.4(72.3)	22.74(5.49)

Table 24A. Whole-body cadmium concentration on a wet-weight basis of green sunfish from experiment 12-F. (N is the number of fish used in analysis; one standard error is given in parentheses after the mean).

Treatment		Live Fish		Dead Fish	
Day	Ppm Cd	N	Ppm Cd	N	Ppm Cd
0	Control	8	0.95(0.16)	–	–
4	Control	8	0.55(0.08)	–	–
24	Control	8	0.65(0.07)	–	–
24	0.05	8	1.84(0.21)	–	–
24	0.23	8	1.21(0.15)	–	–
24	0.32	8	1.28(0.10)	–	–
24	1.31	5	2.12(0.23)	3	5.34(2.47)
24	1.93	7	2.14(0.27)	1	3.73
24	2.48	8	2.82(0.36)	–	–

fish exposed to 0.05 ppm in accumulating cadmium that regression equations relating cadmium in the water to cadmium uptake by fish failed to explain large amounts of the variability, even though significant linear trends were shown by analysis of variance (significant effect due to regression). The linear trend was highly significant for whole-body burdens when the amount of cadmium in the fish and that to which they were exposed was examined (calculated $F = 40.24$, tabular F, 0.05 level, $(6, 45) = 2.37$). This trend was also significant for the cadmium in gills and liver. The regression equation for whole-body concentration relating Y (log of the cadmium concentration in the body + 1) to X (cadmium concentration in the water) was $Y = 0.097 X + 0.325$ with an r^2 (coefficient of determination) equal to 0.46, while for gill and liver the r^2 was 0.10 and 0.38 respectively. Since green sunfish exposed to 0.05 ppm Cd were stimulated to eat more Gambusia and grow more they may have ingested more cadmium via this route than by water alone. Chadwick and Brocksen (1969) found that fish exposed to 0.5 ppb dieldrin and fed worms containing known amounts of dieldrin, did not accumulate more dieldrin than fish exposed to the same concentration but fed uncontaminated worms. Undoubtedly Gambusia eaten by sunfish will contain different amounts of cadmium depending on the length of exposure and concentration of cadmium in the experimental aquarium. Future experiments should expose fish with and without food at the same concentration of cadmium to clarify which route of heavy metal uptake is most important.

The pattern of cadmium uptake by green sunfish gills paralleled that of whole-body burdens (Table 24B). Initially fish gills contained a mean concentration of 1.42 ppm which decreased to 0.23 ppm after 4 days. Control fish contained 0.49 ppm Cd after the next 20 days at the end of the experiment. Gills of fish exposed to 0.05 ppm accumulated almost as much (2.08 ppm) as gills of fish exposed to 1.31 ppm Cd (2.38 ppm). Livers were the most variable of the structures measured but the same trends as found in the whole body and gills were apparent (Table 24C).

Experiment 13-F

This experiment was designed to evaluate effects of 1 ppm Cd on fish exposed to three different temperatures, cold (17.5 C), medium (23.9 C) and hot (30.0 C). The experiment was conducted for 24 days from October 20 to November 13, 1971. Four fish from each of the six treatments were sampled 2, 6, 12 and 20 days after toxicant introduction to evaluate changes in growth, food consumption, RNA-DNA ratios and cadmium uptake in the whole body, gills and liver. Two duplicate aquaria containing eight fish each were maintained at each of the six treatment combinations (3 temperatures and 2 levels of stress). No undesirable water chemistry changes were experienced (Table 25A, 25B, 25C) among treatments or between treatments and the control, as was a factor in experiments with ammonia. The 108 fish used in this experiment (see Appendix Table B, Collection 12, 13) ranged in weight from 5.17 to 9.10 g and were assigned randomly to

Table 24B. Cadmium concentration on a wet-weight basis in gills of green sunfish from experiment 12-F. (N is the number of fish used in analysis; one standard error is given in parentheses after the mean).

Treatment		Live Fish		Dead Fish	
Day	Ppm Cd	N	Ppm Cd	N	Ppm Cd
0	Control	8	1.42(0.50)	-	-
4	Control	8	0.23(0.16)	-	-
24	Control	8	0.49(0.15)	-	-
24	0.05	8	2.08(0.34)	-	-
24	0.23	8	1.64(0.49)	-	-
24	0.32	8	1.96(0.63)	-	-
24	1.31	5	2.38(0.86)	3	1.07(0.58)
24	1.93	7	1.93(0.49)	1	2.75
24	2.48	8	2.55(0.21)	-	-

Table 24C. Cadmium concentration on a wet-weight basis in the liver of green sunfish from experiment 12-F. (N is the number of fish used in analysis; one standard error is given in parentheses after the mean).

Treatment		Live Fish		Dead Fish	
Day	Ppm Cd	N	Ppm Cd	N	Ppm Cd
0	Control	8	0.98(0.98)	-	-
4	Control	8	1.17(0.80)	-	-
24	Control	8	0.51(0.34)	-	-
24	0.05	8	2.41(0.84)	-	-
24	0.23	8	1.57(0.85)	-	-
24	0.32	8	0.31(0.31)	-	-
24	1.31	5	1.63(1.63)	3	9.84(5.22)
24	1.93	7	3.87(1.09)	1	3.89
24	2.48	8	5.08(2.12)	-	-

Table 25A. Chemical characteristics of water used in the continuous-flow experiment 13-F. ($\underline{N}$ is the number of samples used in determinations; $\bar{X}$ is the mean with one standard error enclosed in parentheses; C = Cold; M = Medium; H = Hot; S = Stressor; N.D. = non-detectable, less than 0.01 ppm).

		Aquarium Number			
		1	11	3	5
Treatment		C	C	CS	CS
Cadmium ppm as Cd	$\underline{N}$	4	4	16	16
	$\bar{X}$	N.D.	N.D.	0.99 (0.02)	0.94 (0.02)
pH	N	2	2	2	2
	Range	7.77-7.81	7.74-7.93	7.75-7.88	7.78-7.90
Temperature (C)	$\underline{N}$	20	20	20	20
	$\bar{X}$	17.3 (0.2)	17.8 (0.2)	17.4 (0.2)	17.6 (0.2)
Dissolved Oxygen (ppm)	$\underline{N}$	2	2	2	2
	$\bar{X}$	8.4 (0.6)	7.4 (0)	7.8 (0.1)	8.4 (0.1)
Alkalinity ppm as $CaCO_3$	$\underline{N}$	2	2	2	2
	$\bar{X}$	342 (0)	343 (3)	344 (0)	338 (4)
Hardness ppm as $CaCO_3$	$\underline{N}$	2	2	2	2
	$\bar{X}$	325 (5)	332 (0)	332 (4)	330 (2)

Table 25B. Chemical characteristics of water used in the continuous-flow experiment 13-F. ($\underline{N}$ is the number of samples used in determinations; $\bar{X}$ is the mean with one standard error enclosed in parentheses; C = Cold; M = Medium; H = Hot; S = Stressor; N.D. = non-detectable, less than 0.01 ppm).

		Aquarium Number			
		6	7	2	10
Treatment		M	M	MS	MS
Cadmium ppm as Cd	$\underline{N}$	4	4	16	16
	$\bar{X}$	N.D.	N.D.	0.92 (0.02)	0.98 (0.02)
pH	N	2	2	2	2
	Range	7.92-8.00	7.98-8.20	7.74-7.91	7.81-7.91
Temperature (C)	$\underline{N}$	20	20	20	20
	$\bar{X}$	23.9 (0.2)	24.0 (0.2)	23.4 (0.1)	24.4 (0.1)
Dissolved Oxygen (ppm)	$\underline{N}$	2	2	2	2
	$\bar{X}$	7.6 (0)	7.6 (0.1)	7.1 (0)	7.4 (0.2)
Alkalinity ppm as $CaCO_3$	$\underline{N}$	2	2	2	2
	$\bar{X}$	343 (1)	336 (2)	339 (9)	344 (0)
Hardness ppm as $CaCO_3$	$\underline{N}$	2	2	2	2
	$\bar{X}$	331 (1)	327 (1)	329 (3)	330 (2)

Table 25C. Chemical characteristics of water used in the continuous-flow experiment 13-F. ($\underline{N}$ is the number of samples used in determinations; $\bar{X}$ is the mean with one standard error enclosed in parentheses; C = Cold; M = Medium; H = Hot; S = Stressor; N.D. = non-detectable, less than 0.01 ppm).

		Aquarium Number			
		4	9	8	12
Treatment		H	H	HS	HS
Cadmium ppm as Cd	$\underline{N}$	4	4	16	16
	$\bar{X}$	N.D.	N.D.	1.00 (0.02)	1.01 (0.02)
pH	N	2	2	2	2
	Range	8.01-8.22	8.01-8.11	8.13-8.24	8.02-8.09
Temperature (C)	$\underline{N}$	20	20	20	20
	$\bar{X}$	29.6 (0.1)	29.6 (0.1)	30.4 (0.1)	29.4 (0.1)
Dissolved Oxygen (ppm)	$\underline{N}$	2	2	2	2
	$\bar{X}$	7.2 (0)	6.8 (0)	7.0 (0.1)	6.6 (0.1)
Alkalinity ppm as $CaCO_3$	$\underline{N}$	2	2	2	2
	$\bar{X}$	338 (2)	344 (2)	344 (2)	346 (2)
Hardness ppm as $CaCO_3$	$\underline{N}$	2	2	2	2
	$\bar{X}$	327 (1)	332 (0)	332 (0)	331 (1)

the 12 aquaria of the experimental design. Before beginning the experiment 12 green sunfish were initially sacrificed, four from each temperature, to provide initial cadmium levels and RNA-DNA ratios. Fish were placed in the tanks and allowed to feed for 4 days prior to toxicant introduction. The first fish samples taken on day 2 after toxicant introduction represent accumulated changes from the first 4 days of pre-stress as well as the first 2 days of toxicant introduction. Each treatment combination started intially with 16 fish, and decreased by four at each sampling date so values for growth, food consumption and food conversion ratios were determined with reduced sample size as time progressed.

Examination of the amount of Gambusia eaten (Figure 16) revealed that 1 ppm Cd depressed amounts eaten by fish at all temperatures except 23.9 C, when contrasted with respective controls. Decreased consumption appeared greatest at the highest temperature where the only mortality in the experiment was recorded. The analysis of variance (Table 26) indicated that all main effects and interactions except the stress x period interaction were highly significant. Considering the magnitude of the F values and the table of means, it is probable that most of the interaction comes from two considerations. Stressed fish at medium temperatures consumed on the average more Gambusia (5.28 g) than controls (4.67 g) at that temperature, whereas cadmium-exposed fish at other temperatures consistently consumed less than controls. The other consideration is that the effect of temperature (averaged

Figure 16. Rate of consumption of Gambusia in mg per hr for green sunfish 96 hrs before and during 480 hrs of continuous exposure to 1 ppm Cd at three different temperatures. Each point represents eight fish except where deaths reduced this number. Mean standard error is given for each temperature and cadmium concentration.

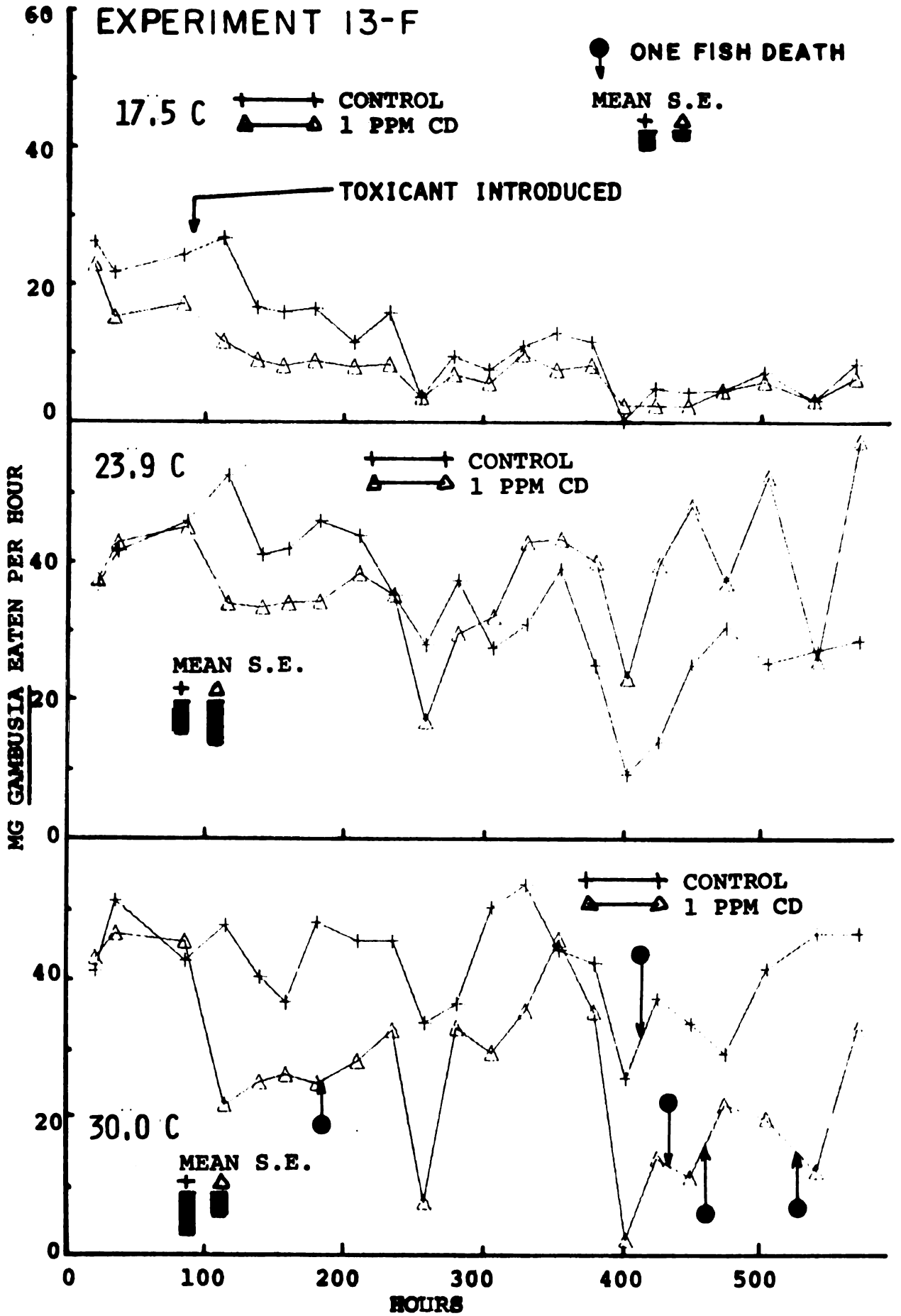

EXPERIMENT 13-F
ONE FISH DEATH
17.5 C
CONTROL
1 PPM CD
MEAN S.E.
TOXICANT INTRODUCED
23.9 C
CONTROL
1 PPM CD
MEAN S.E.
30.0 C
CONTROL
1 PPM CD
MEAN S.E.
MG GAMBUSIA EATEN PER HOUR
HOURS
0
20
40
60
100
200
300
400
500

Table 26. The analysis of variance table for the effects of three temperatures and two levels of cadmium (stress) over four periods on the consumption of Gambusia by green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Temperature	241.61	2	120.81	62.89**
Stress	17.47	1	17.47	9.09**
T x S	48.55	2	24.27	12.63**
Period	42.03	3	14.01	7.29**
T x P	45.19	6	7.53	3.92**
S x P	1.20	3	0.40	0.21
T x S x P	50.42	6	8.40	4.37**
Error	134.46	[a]70	1.92	
Total	580.93	95		

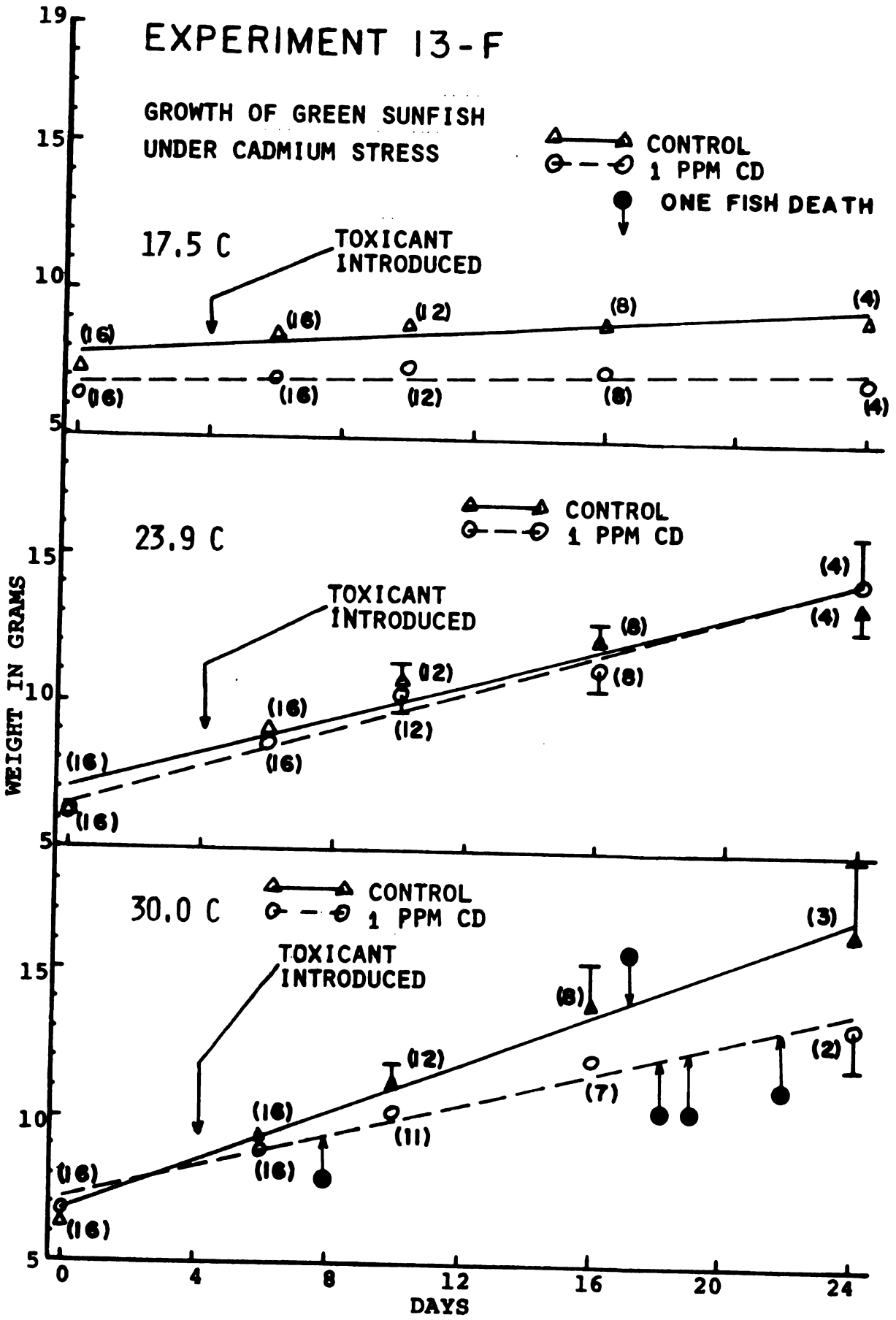
**0.01 significance level.
[a]2 degrees of freedom were subtracted for dead fish.

over stress) on Gambusia consumption was not consistent over each of the four periods. Fish at cold temperatures ate less and less over time, while consumption at medium and hot temperatures fluctuated randomly between periods.

Four deaths were recorded among fish exposed to cadmium at high temperatures. These deaths can probably be attributed to an interaction of cadmium with temperature. However, one of the 16 control fish at hot temperatures also died toward the end of the experiment.

Fish grew least at 17.5 C (Figure 17). Growth of fish at 23.9 C was greater than that observed at cold temperatures, while fish at 30 C grew slightly larger than fish at 23.9 C. Regression equations for control and stressed fish at each temperature were calculated and regression coefficients compared to determine if cadmium stress had a significant effect on growth. No significant differences between control and stressed fish were found at any temperature, the calculated F value being very low in every case. Fish exposed at 30 C and 1 ppm Cd were detrimentally affected since four of the 16 fish died during the 20-day period. Thus only two fish were available for the regression analyses for two sampling dates. This small sample size as well as the greater number of samples toward the beginning of the experiment were sufficient to prevent the regression equations from showing a significant difference in growth between control and exposed fish at hot temperatures.

Figure 17. Mean weight of green sunfish (the points) and the regression line of the mean weight against time for three different temperatures and two levels of cadmium stress. The number of fish comprising each mean is shown with a dark vertical bar on only one side of the point for clarity; standard errors less than 0.5 are not shown.


EXPERIMENT 13-F
GROWTH OF GREEN SUNFISH
UNDER CADMIUM STRESS
CONTROL
1 PPM CD
ONE FISH DEATH
17.5 C
TOXICANT INTRODUCED
23.9 C
30.0 C
WEIGHT IN GRAMS
DAYS
0
4
8
12
16
20
24
5
10
15
19

Food conversion ratios (Figure 18) reflected the same trends as those found for growth (Figure 17). The analysis of variance again showed a significant temperature x stress interaction (Table 27), due mainly to fish exposed to cadmium at medium temperatures possessing average food conversion ratios of 0.38 compared with the control value of 0.32. This trend is opposite to that exhibited by fish at cold and hot temperatures, where cadmium-exposed fish had consistently lower food conversion ratios than control fish. Food conversion ratios over time (the significant effect of period) declined consistently from 0.40 to 0.24. It was concluded that cadmium had an adverse effect on energy utilization at cold and probably hot temperatures, but energy utilization was not affected at medium temperatures.

RNA-DNA ratios were variable among individuals, and no obvious differences were apparent between control and cadmium-exposed fish, with the exception of fish at high temperatures (Table 28). Ratios appeared to increase to a maxima in fish at the three temperatures, the time to reach it being inversely related to temperature. Maxima were reached on day 16 for fish at cold temperature, on day 10 for fish at medium temperature and on day 6 for fish at hot temperature. Analysis of these data (Table 29) showed that stress did not have a significant effect on RNA-DNA ratios. Temperature and a temperature x period interaction were found to be significant. The temperature x period interaction is related to the previously discussed fact that RNA-DNA ratios appeared to

Figure 18. Food conversion ratios of fish exposed (after a 4-day acclimation period) for 20 days (stippled area) to 1 ppm Cd at three different temperatures. Number of fish used is given in parentheses. One standard error is shown with a dark vertical bar on only one side of the point for clarity.

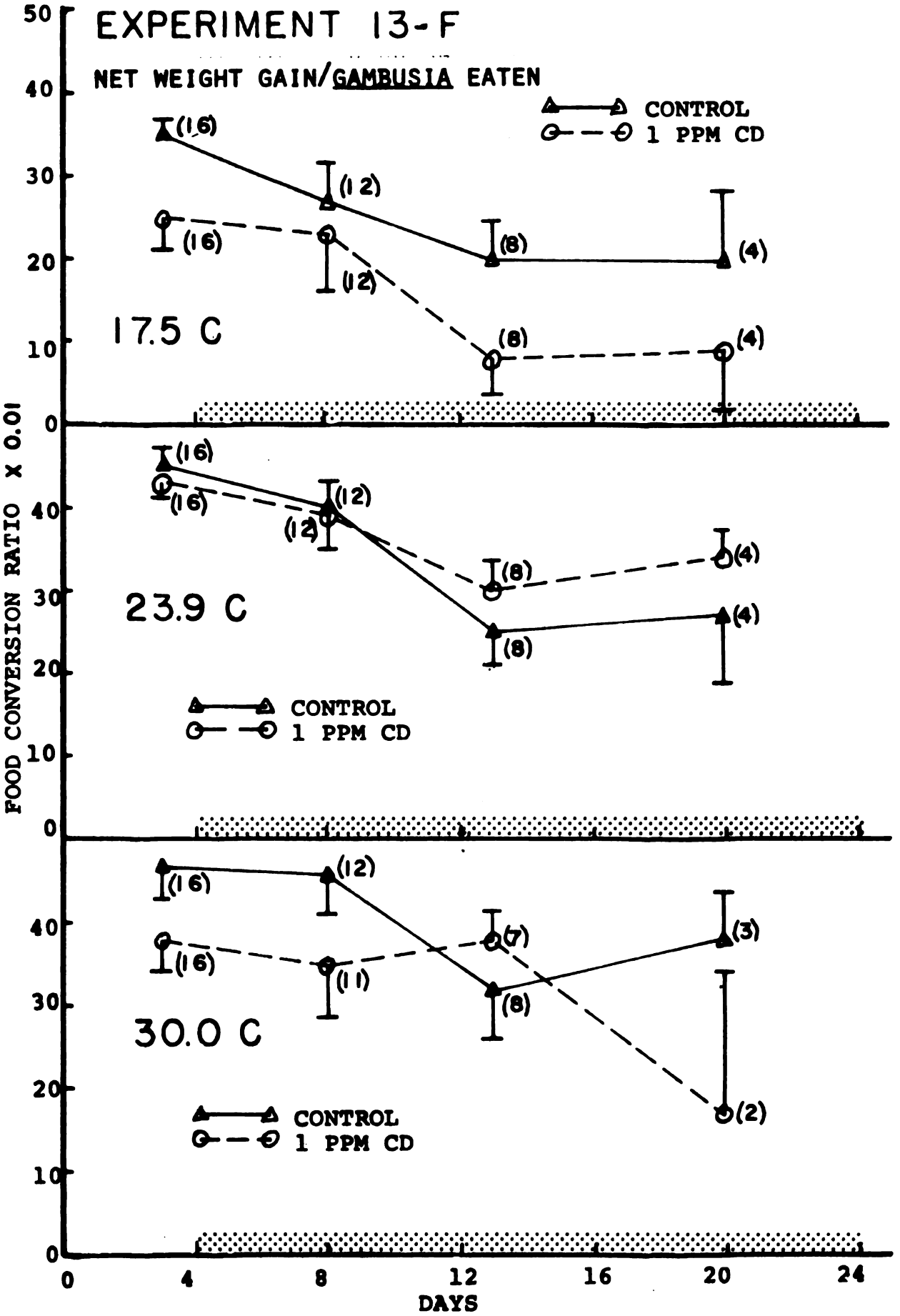

EXPERIMENT 13-F
NET WEIGHT GAIN/GAMBUSIA EATEN
CONTROL
1 PPM CD
17.5 C
23.9 C
30.0 C
FOOD CONVERSION RATIO X 0.01
DAYS
0
4
8
12
16
20
24
10
20
30
40
50

Table 27. The analysis of variance table for the effects of three temperatures and two levels of cadmium (stress) over four periods on the food conversion ratios of green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Temperature	0.3054	2	0.1527	9.15**
Stress	0.0667	1	0.0667	4.00*
T x S	0.1352	2	0.0676	4.05*
Period	0.4556	3	0.1519	9.10**
T x P	0.0646	6	0.0108	0.64
S x P	0.0425	3	0.0142	0.85
T x S x P	0.0892	6	0.0149	0.89
Error	1.1351	[a]68	0.01669	
Total	2.2943	95		

*0.05 significance level.
**0.01 significance level.
[a]4 degrees of freedom were subtracted for dead fish.

Table 28. Mean concentrations of RNA, DNA and the RNA-DNA ratios of green sunfish from experiment 13-F before and during continuous exposure to 1 ppm cadmium at three different temperatures for 20 days. DFFT is dry fat-free tissue from the dorsal muscle excluding skin. Standard error is enclosed in parentheses.

	Day	µg DNA per 100 mg DFFT	µg RNA per 100 mg DFFT	RNA-DNA Ratio
Cold	0	19.4(1.6)	302.1(29.2)	16.00(2.58)
	6	19.9(2.2)	502.5(71.2)	26.62(4.68)
	10	23.6(2.8)	506.7(35.5)	22.34(3.60)
	16	17.3(2.3)	570.1(44.6)	28.84(1.48)
	24	17.3(1.0)	506.7(37.9)	28.84(1.48)
Cold	0	-	-	-
Stressor	6	23.6(2.5)	481.0(48.4)	21.09(3.59)
	10	17.8(3.9)	411.2(65.2)	27.65(9.43)
	16	19.4(2.0)	655.1(32.5)	34.28(1.75)
	24	18.8(2.1)	473.1(34.6)	26.14(3.35)
Medium	0	25.2(2.3)	316.3(52.0)	12.88(2.52)
	6	26.2(5.0)	581.1(85.9)	25.78(7.35)
	10	22.0(3.6)	760.0(169.5)	38.82(11.30)
	16	22.6(1.8)	485.2(21.2)	22.36(2.62)
	24	25.2(2.3)	509.3(45.1)	21.18(2.27)
Medium	0	-	-	-
Stressor	6	24.7(2.9)	562.8(163.0)	21.94(3.87)
	10	26.2(2.6)	760.0(202.0)	31.80(10.50)
	16	18.9(1.9)	434.8(40.1)	23.28(1.56)
	24	15.7(1.4)	388.1(41.2)	25.82(4.98)
Hot	0	22.0(1.0)	317.3(10.2)	14.44(.69)
	6	22.0(2.2)	688.1(90.6)	33.44(7.04)
	10	24.7(1.8)	360.9(34.5)	14.59(.73)
	16	21.5(2.3)	461.6(30.6)	23.00(4.09)
	24	19.4(3.7)	366.1(62.9)	22.18(6.86)
Hot	0	-	-	-
Stressor	6	23.6(3.5)	503.5(49.3)	21.56(1.11)
	10	23.1(1.5)	448.4(45.3)	20.05(1.73)
	16	24.1(2.8)	421.2(54.2)	17.94(3.07)
	24	15.7(3.1)	263.3(101.1)	17.31(4.87)

Table 29. The analysis of variance table for the effects of three temperatures and two levels of cadmium (stress) over four periods on the RNA-DNA ratios of green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Temperature	729.18	2	364.59	3.31*
Stress	98.48	1	98.48	0.89
T x S	52.49	2	26.24	0.24
Period	82.02	3	27.34	0.25
T x P	1745.60	6	290.93	2.64*
S x P	227.99	3	75.99	0.69
T x S x P	367.74	6	61.29	0.56
Error	7933.54	72	110.19	
Total	11237.03	95		

**0.05 significance level.

increase to a maxima in fish, the time to reach it being dependent on temperature.

Whole-body cadmium uptake by green sunfish was not as greatly affected by temperature as expected (Figure 19). This might be partially related to the fact that dissolved oxygen concentrations (Table 25) were similar in all tanks, so that decreased oxygen at higher temperatures and concomitant cadmium uptake due to increased ventilation rates did not occur to any significant degree. Such a correlation between respiration and uptake was found by Murphy and Murphy (1971). Lloyd (1961) also demonstrated that the toxicity of many compounds to fish increased almost equally when oxygen was reduced by the same amount. He concluded that increased toxicity was due to increased respiratory irrigation bringing more of the toxicants to gill surfaces. After 20 days exposure control fish at all temperatures contained about 1 ppm Cd. Cadmium-exposed fish at cold temperatures accumulated 2 ppm or doubled their concentration over control levels after 2 days exposure to 1 ppm Cd and then remained at about 1.5 ppm for the remaining times. Exposed fish at medium and hot temperatures accumulated increasing amounts of cadmium the longer they were exposed to the stressor with no equilibrium values reached after 20 days. Analysis of variance for these data showed a highly significant effect of cadmium stress ($F = 156.54$) on cadmium uptake by these fish (Table 30). However, there was a significant temperature x stress as well as a temperature x period

Figure 19. Whole-body concentration of cadmium on a wet-weight basis in surviving green sunfish before and during continuous exposure to 1 ppm Cd at three different temperatures. Each point represents four fish except as otherwise noted by the number in parentheses. One standard error is given by a dark vertical bar on only one side of the point for clarity.

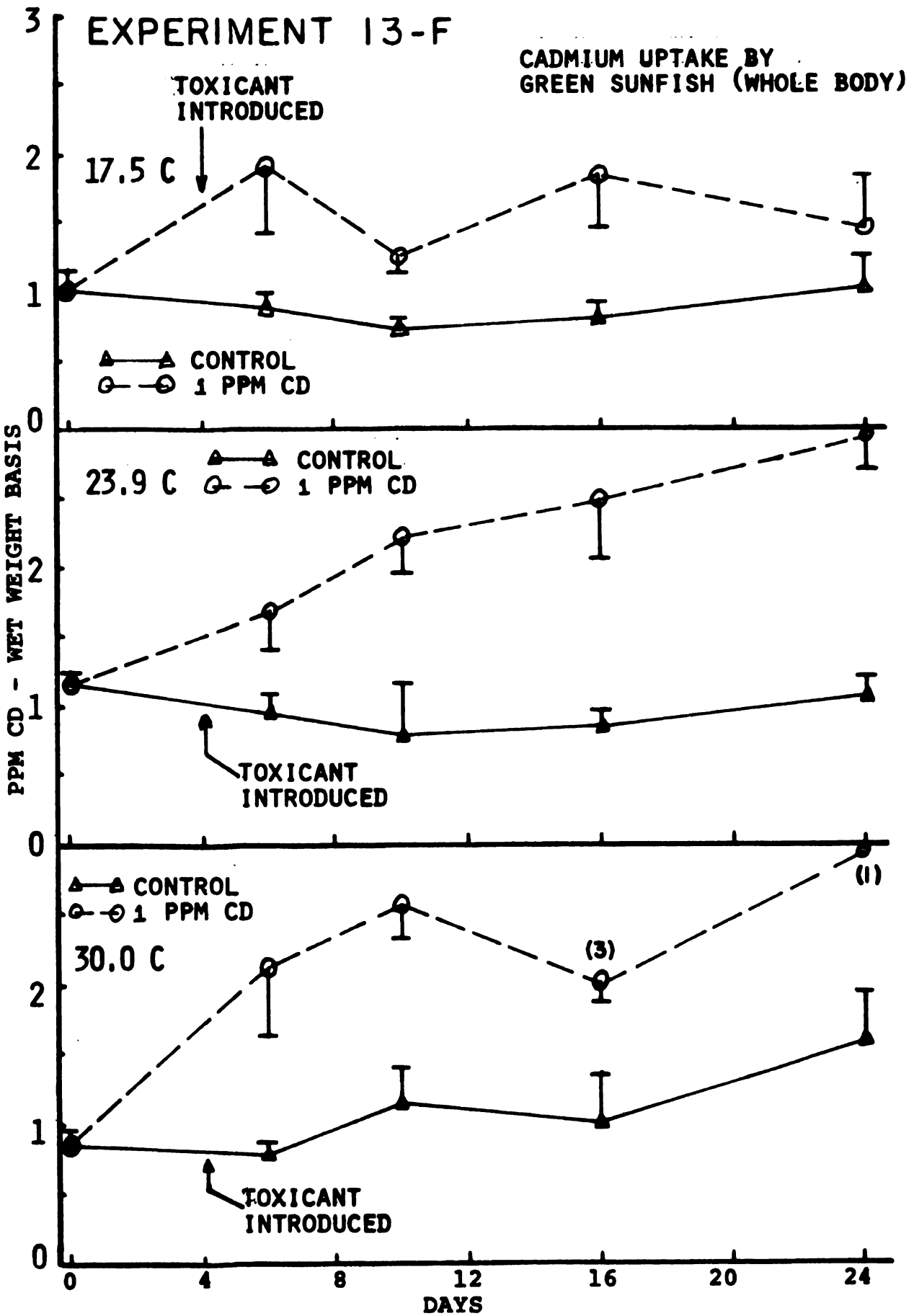

EXPERIMENT 13-F
CADMIUM UPTAKE BY
GREEN SUNFISH (WHOLE BODY)
TOXICANT
INTRODUCED
17.5 C
CONTROL
1 PPM CD
23.9 C
CONTROL
1 PPM CD
TOXICANT
INTRODUCED
30.0 C
CONTROL
1 PPM CD
(1)
(3)
TOXICANT
INTRODUCED
PPM CD - WET WEIGHT BASIS
0
1
2
3
0
4
8
12
16
20
24
DAYS

Table 30. The analysis of variance table for the effects of three temperatures and two levels of cadmium (stress) over four periods on the whole body burden of cadmium in green sunfish.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Temperature	5.06	2	2.53	11.48**
Stress	34.45	1	34.45	156.54**
T x S	2.41	2	1.20	5.47**
Period	2.63	3	0.88	3.98*
T x P	3.12	6	0.52	2.36*
S x P	0.37	3	0.12	0.56
T x S x P	2.42	6	0.40	1.83
Error	14.97	[a]68	0.22	
Total	65.41	95		

*0.05 significance level.
**0.01 significance level.
[a]4 degrees of freedom were subtracted for dead fish.

interaction. Examination of the table of means for the effect of stress (averaged over period) at each of the temperatures showed a trend toward increasing amounts of cadmium in both control and stressed fish the higher the temperature. Some overlap at the highest temperature was observed, however, undoubtedly contributing to the significant interactive effect. The other interaction of temperature x period averaged over stress showed that fish at cold temperatures initially accumulated the highest levels (1.40 ppm Cd) but thereafter lost cadmium. This trend was reversed for fish at the higher temperatures, thus giving a significant interaction.

Patterns of accumulation in the gills of green sunfish at these three temperatures were similar, although more variable, than trends established for whole-body burdens (Figure 20). The analysis of variance (Table 31) indicated that only stress had a significant effect on cadmium uptake. Since there was no significant effect of time, it can be concluded that the concentration of cadmium in fish gills reached an equilibrium level at least over the first 20 days of exposure to cadmium. Data of Mount and Stephan (1967a), however, showed that bluegill whole-body levels reached an equilibrium concentration only after 30 to 60 days, and Eaton (personal communication) reported values in bluegills exposed at lower concentrations for 11 months that were much higher than I observed. In addition, the analysis of variance indicated that accumulation of cadmium by gills was the same at all temperatures.

Figure 20. Cadmium uptake on a wet-weight basis by the gills of surviving green sunfish before and during continuous exposure to 1 ppm Cd at three different temperatures. Each point represents four fish except as otherwise noted by the number in parentheses. One standard error is given by a dark vertical bar on only one side of the point for clarity.

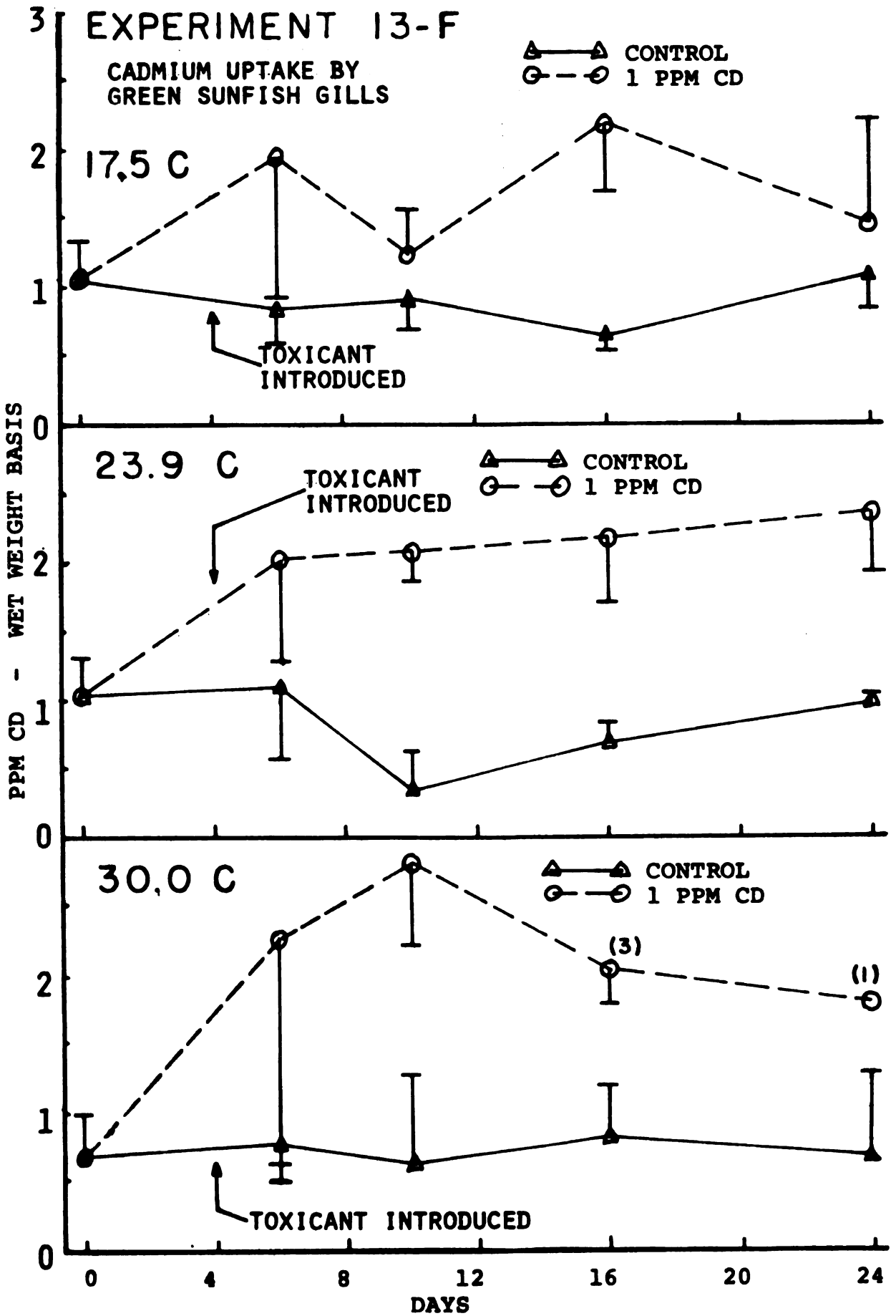

EXPERIMENT 13-F
CADMIUM UPTAKE BY
GREEN SUNFISH GILLS
CONTROL
1 PPM CD
17.5 C
TOXICANT
INTRODUCED
23.9 C
TOXICANT
INTRODUCED
CONTROL
1 PPM CD
30.0 C
CONTROL
1 PPM CD
(3)
(1)
TOXICANT INTRODUCED
PPM CD - WET WEIGHT BASIS
0
1
2
3
0
4
8
12
16
20
24
DAYS

Table 31. The analysis of variance table for the effects of three temperatures and two levels of cadmium (stress) over four periods on green sunfish cadmium uptake in the gill.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Temperature	1.78	2	0.89	0.71
Stress	40.35	1	40.35	32.04**
T x S	2.90	2	1.45	1.15
Period	1.16	3	0.39	0.31
T x P	2.21	6	0.37	0.29
S x P	1.38	3	0.46	0.37
T x S x P	4.55	6	0.76	0.60
Error	85.62	[a]68	1.25	
Total	139.96	95		

**0.01 significance level.
[a]4 degrees of freedom were subtracted for dead fish.

Cadmium accumulation in the livers of fish followed the same trends established for whole-body and gill cadmium uptake (Table 32). All main effects and a stress x period interaction were significant (analysis of variance--Table 33). For the liver, cadmium uptake apparently was affected by temperature. The stress x period interaction showed that the effect of cadmium stress on uptake by the liver was not consistent across time.

Experiment 10-S

The possibility of fish being exposed to high concentrations of cadmium for short periods of time in a river situation prompted an investigation of the effects such a temporary exposure would have on fish. An experiment was designed involving 17 static aquaria with 10 green sunfish per aquarium. Three sets of fish were exposed in the flow-through apparatus. The first set of fish was exposed for 24 hrs and included the control and 10 fish each at 5, 10, 20 and 30 ppm Cd (Table 34), as higher concentrations were shown to kill all fish in 24 hrs. The second set of fish was exposed for 1 hr to 5, 10, 20, 30, 40 and 50 ppm Cd (Table 34), and the third set of fish was exposed for 15 min to the same six concentrations as above. These fish ranged from 4.51 to 5.50 g (see Appendix Table B, Collection 12, 13) and were exposed in order: 24 hrs, then 1 hr and lastly 15 min. Immediately after exposure fish were weighed and transferred to the 17 static aquaria containing around 22 l (10 gal) of water and an unlimited supply of Gambusia. Fish were placed in the

Table 32. Concentration of cadmium on a wet-weight basis in the livers of fish exposed continuously to 1 ppm Cd at three temperatures for 20 days. (Sample size was four live fish unless otherwise indicated; standard error is enclosed in parentheses).

Treatment	Initial	Days 4	10	16	24
Cold	1.45(1.03)	1.42(0.49)	0(0)	.68(0.68)	.97(0.97)
Cold Stressor	——	0(0)	2.20(0.90)	.68(0.68)	3.04(1.17)
Medium	4.79(0.53)	2.23(0.64)	3.23(2.72)	1.12(0.70)	1.31(0.80)
Medium Stressor	——	4.13(2.14)	3.10(0.98)	3.30(0.74)	4.66(0.94)
Hot	2.15(2.15)	1.24(0.72)	1.35(1.35)	.46(0.46)	[a]1.99(0.85)
Hot Stressor	——	0(0)	4.49(2.67)	[b]3.69(1.43)	[c]1.81

[a]Includes one dead fish.
[b]Sample size was three. The fourth fish, which died, contained 3.56 ppm Cd in the liver.
[c]Sample size was one. The third fish which died contained a mean of 7.02(1.72) ppm Cd in the liver.

Table 33. The analysis of variance table for the effects of three temperatures and two levels of cadmium (stress) over four periods on green sunfish cadmium uptake in the liver.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic
Temperature	61.11	2	30.55	5.56**
Stress	71.14	1	71.14	12.94**
T x S	15.82	2	7.91	1.44
Period	60.59	3	20.20	3.67*
T x P	55.10	6	9.18	1.67
S x P	46.91	3	15.63	2.84*
T x S x P	33.27	6	5.55	1.01
Error	373.81	[a]68	5.49	
Total	717.73	95		

*0.05 significance level.
**0.01 significance level.
[a]4 degrees of freedom were subtracted for dead fish.

Table 34. The mean and standard error of chemical parameters, except pH, found for the water used in experiment 10-S. (Concentrations of cadmium given are for the continuous-flow portion of the experiment; water chemistry data are for the static part).

Aquarium	Treatment (ppm as Cd)	Temperature (C)	Dissolved Oxygen (ppm)	pH (Range)	Alkalinity (ppm as $CaCO_3$)	Hardness (ppm as $CaCO_3$)
	15 Min					
11	2.97(0.07)	20.9(0.7)	7.9(0.2)	8.10-8.30	338(2)	336(8)
6	5.52(0.24)	20.5(0.6)	7.9(0.5)	-	-	-
12	13.15(0.24)	21.1(0.7)	7.4(0.3)	-	-	-
15	27.34(0.91)	21.5(0.7)	6.7(0.7)	8.03-8.11	340(2)	326(6)
4	36.40(2.41)	20.7(0.7)	7.5(0.5)	-	-	-
7	47.31(3.86)	20.8(0.6)	8.1(0.6)	8.08-8.30	340(0)	332(4)
	1 Hr					
17	2.97(0.07)	22.0(0.7)	6.9(0.5)	-	-	-
9	5.52(0.24)	20.8(0.7)	7.7(0.5)	8.10-8.16	337(5)	332(8)
16	13.15(0.24)	21.7(0.6)	7.2(0.2)	-	-	-
10	27.34(0.91)	20.9(0.7)	7.3(0.5)	-	-	-
5	36.40(2.41)	20.6(0.6)	7.6(0.3)	8.01-8.13	327(7)	330(6)
14	47.31(3.86)	21.4(0.7)	7.1(0.5)	-	-	-
	24 Hrs					
13	Control	21.5(0.6)	7.5(0.5)	8.05-8.14	332(14)	331(4)
3	2.97(0.07)	20.7(0.6)	7.3(0.4)	7.99-8.04	297(29)	327(4)
1	5.52(0.24)	20.9(0.7)	8.0(0.4)	8.03-8.07	328(14)	328(5)
8	13.15(0.24)	20.8(0.6)	6.6(0.9)	-	-	-
2	27.34(0.91)	20.8(0.7)	8.4(0.1)	-	-	-
Number of Determinations	4	5	3	2	3	3

static aquaria and fed for 3 days prior to cadmium exposure in the flow-through apparatus. The experiment was conducted for 63 days, including the first 3 days prior to toxicant treatment, and 60 days of post-treatment feeding in the 17 cadmium-free aquaria. The experiment extended from September 4 to November 4, 1971. Water in the static aquaria was changed every 4 days for the first 28 days, then changed every 8 days for the remaining time. Mean water temperature for these aquaria varied between 20.5 and 21.7 C while mean dissolved oxygen concentrations ranged from 6.6 to 8.4 ppm (Table 34).

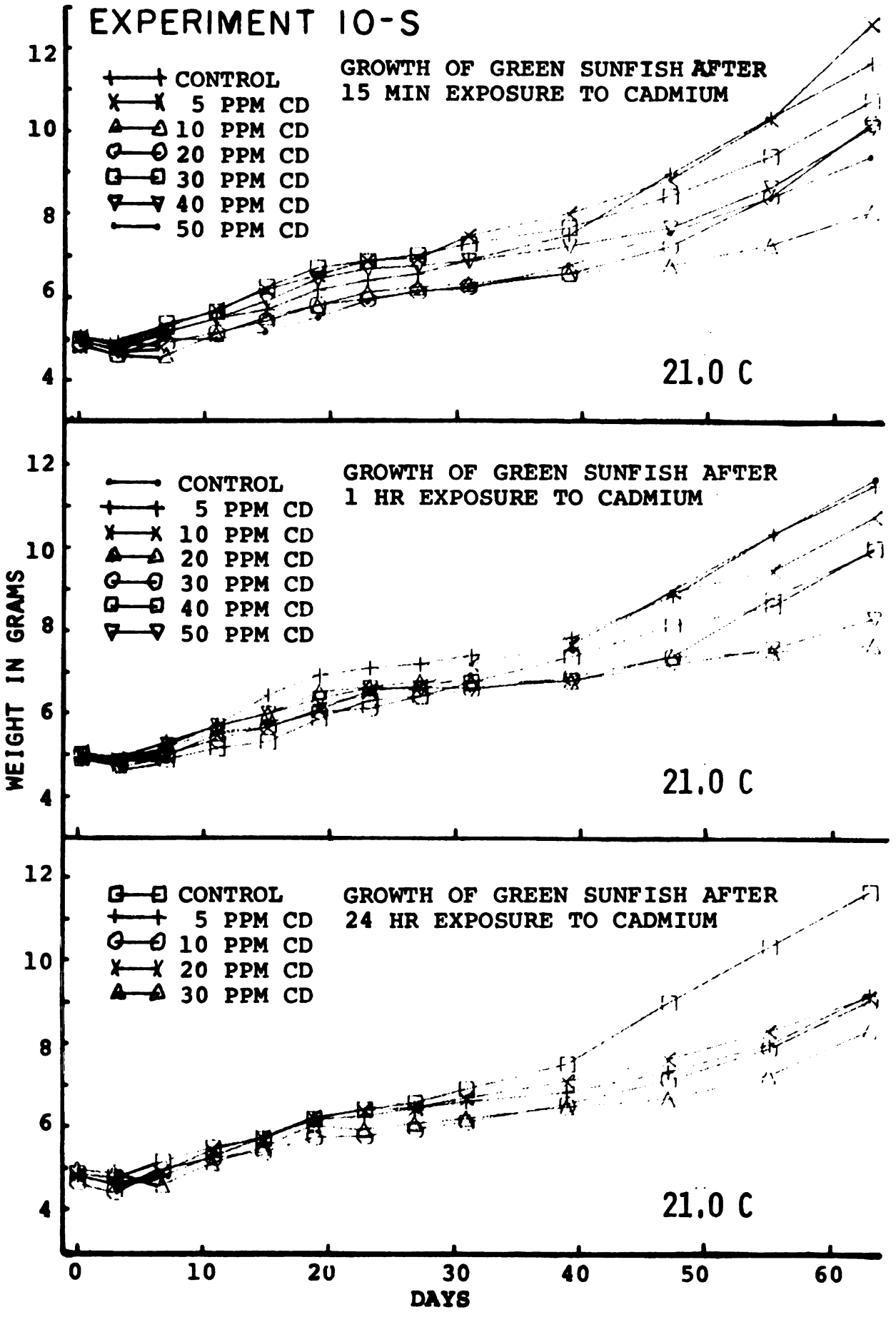
Mortality during exposure to cadmium was only recorded at the highest concentration during the 24 hr exposure, when seven of ten individuals exposed to 30 ppm Cd died (Table 35). Post-exposure mortality followed no consistent trends, although six of the ten deaths were from exposure at the three highest concentrations of 30, 40 and 50 ppm Cd (see Table 35).

Growth of fish from all treatments was essentially indistinguishable from the control through day 40 (Figure 21). Thereafter some differences became apparent. Fish exposed for 15 min to the six concentrations of cadmium exhibited growth curves which were all lower than the control, except fish exposed to 5 ppm Cd. The difference in mean weight between the control and stressed fish from the 15 min exposure ranged from 1 to 5 g on day 63. Fish exposed to 5 ppm Cd in both the 15 min exposure and 1 hr exposure grew at rates similar to or greater than control rates. For the 1

Table 35. Time of death and final weight of fish exposed for short periods of time to various concentrations of cadmium in experiment 10-S.

Aquarium No.	Time of Death (Days)	Type of Stress: Exposure Time	Type of Stress: (ppm Cd)	Final Weight (g)
2	1	24 hr	30	4.67
	1	24 hr	30	5.07
	1	24 hr	30	5.10
	1	24 hr	30	4.14
	1	24 hr	30	4.21
	1	24 hr	30	4.66
	1	24 hr	30	5.37
4	12	15 min	40	5.90
6	18	15 min	10	5.44
7	18	15 min	50	4.18
	18	15 min	50	5.11
1	22	24 hr	10	5.13
10	24	1 hr	30	5.23
11	25	15 min	5	4.66
1	31	24 hr	10	5.23
7	48	15 min	50	6.17
4	52	15 min	40	4.31

Figure 21. Mean weight of green sunfish before (3 days) and 60 days after 15 min, 1 hr and 24 hrs of continuous exposure to various concentrations of cadmium. Each point represents the mean of 10 fish unless deaths decreased this number. Standard errors are found in Appendix Table K, L, M.


EXPERIMENT 10-S
GROWTH OF GREEN SUNFISH AFTER
15 MIN EXPOSURE TO CADMIUM
CONTROL
5 PPM CD
10 PPM CD
20 PPM CD
30 PPM CD
40 PPM CD
50 PPM CD
21.0 C
GROWTH OF GREEN SUNFISH AFTER
1 HR EXPOSURE TO CADMIUM
CONTROL
5 PPM CD
10 PPM CD
20 PPM CD
30 PPM CD
40 PPM CD
50 PPM CD
21.0 C
GROWTH OF GREEN SUNFISH AFTER
24 HR EXPOSURE TO CADMIUM
CONTROL
5 PPM CD
10 PPM CD
20 PPM CD
30 PPM CD
21.0 C
WEIGHT IN GRAMS
4
6
8
10
12
0
10
20
30
40
50
60
DAYS

hr exposure growth curves were similar to those observed for fish exposed for 15 min. All concentrations of cadmium except 5 ppm inhibited growth to some degree when contrasted with the control. Fish exposed for 24 hrs exhibited a clearly interpretable relationship. Cadmium at 5, 10, 20 and 30 ppm decreased growth when compared with the control. This became apparent after day 40 when control fish grew at a greater rate than any treated fish. This judgment was not confirmed by statistical determinations. Variability as discussed previously was operating as the range in fish weights became extreme with time, this same type of phenomenon being observed by Brown (1946) and commented on extensively in Brown (1957, p. 372) as the hierarchy effect. She suggested the size-hierarchy effect was related to the order of dominance, with the largest, dominant fish growing fastest. Carline (1968) as cited in Warren (1972) also found a hierarchial effect among coho salmon. Although food was unlimited to all fish, dominant Group I fish prevented fish in subordinate groups from obtaining all the food they would otherwise have consumed. This effect was greatest with this static test (10-S) since individual fish were not separated as in flow-through tests.

Food conversion ratios calculated for all treatments followed an irregular, but similar pattern (Figures 22, 23, 24). Initially, because fish were not fed the 24 hrs during treatment of the first group, and because of the stress associated with exposure to cadmium, most fish recorded a

Figure 22. Food conversion ratio of green sunfish over 4 or 8-day intervals. Fish were exposed for 15 min to various concentrations of cadmium. Each point represents the mean of 10 fish except where deaths reduced this number.

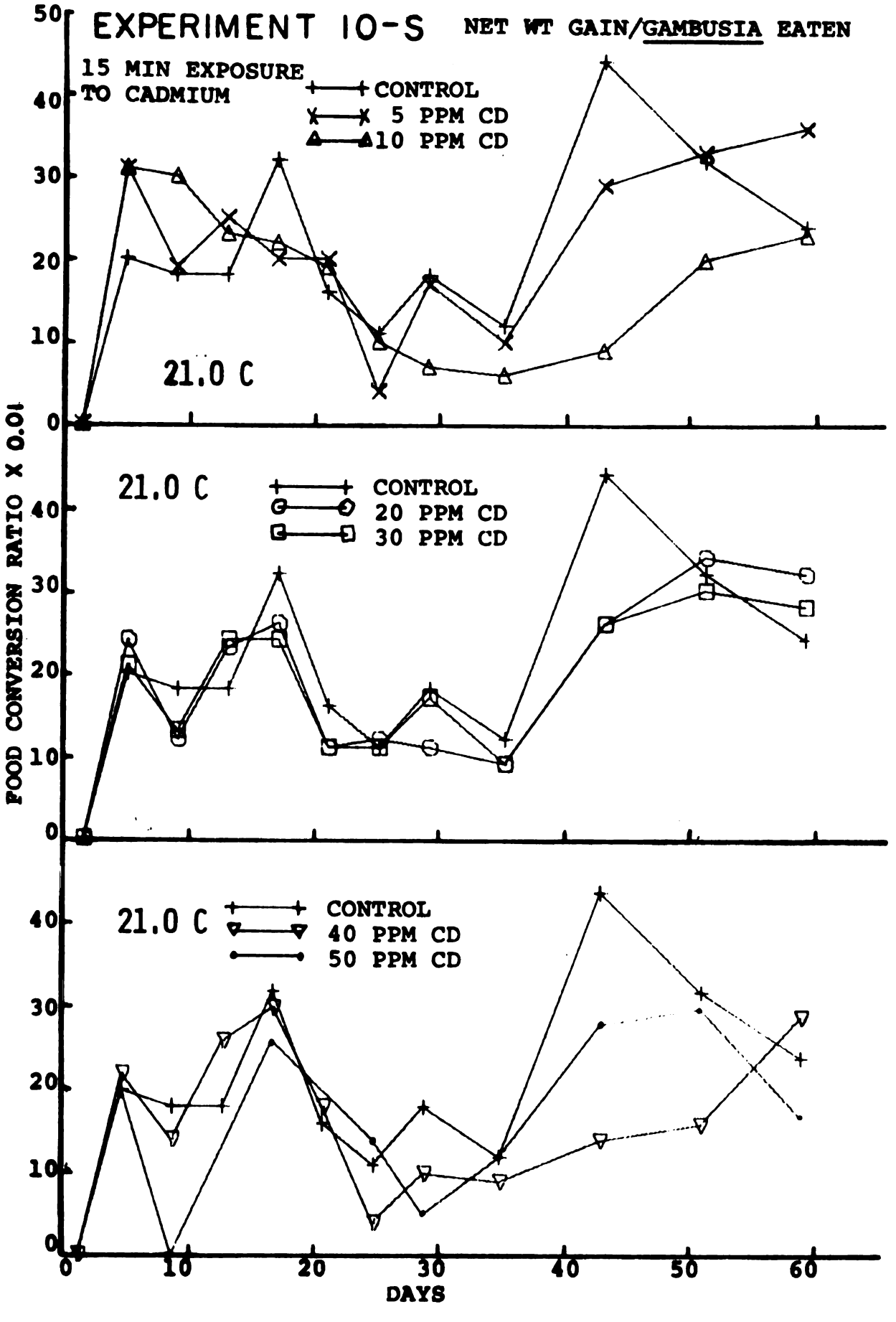

EXPERIMENT 10-S
NET WT GAIN/GAMBUSIA EATEN
15 MIN EXPOSURE
TO CADMIUM
CONTROL
5 PPM CD
10 PPM CD
21.0 C
21.0 C
CONTROL
20 PPM CD
30 PPM CD
21.0 C
CONTROL
40 PPM CD
50 PPM CD
FOOD CONVERSION RATIO X 0.01
DAYS
0
10
20
30
40
50
60

Figure 23. Food conversion ratio of green sunfish over 4 or 8-day intervals. Fish were exposed for 1 hr to various concentrations of cadmium. Each point represents the mean of 10 fish except where deaths reduced this number.

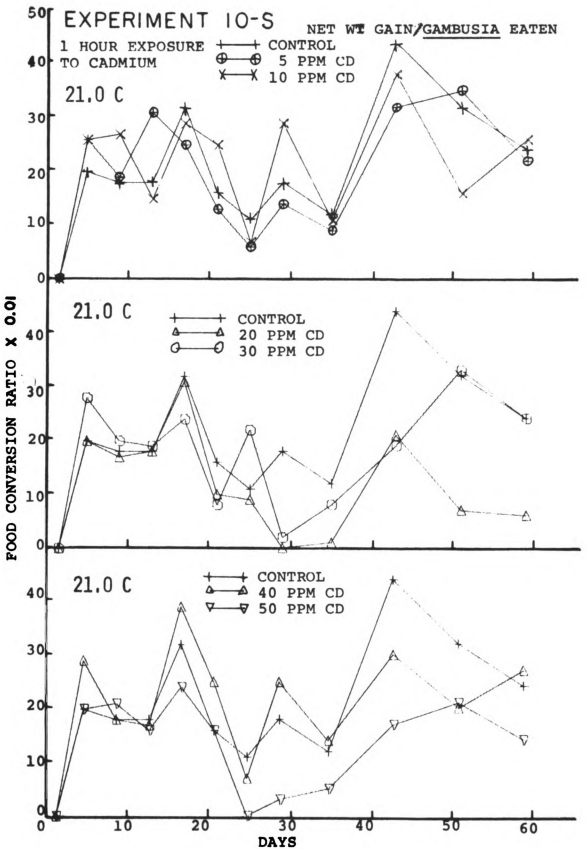
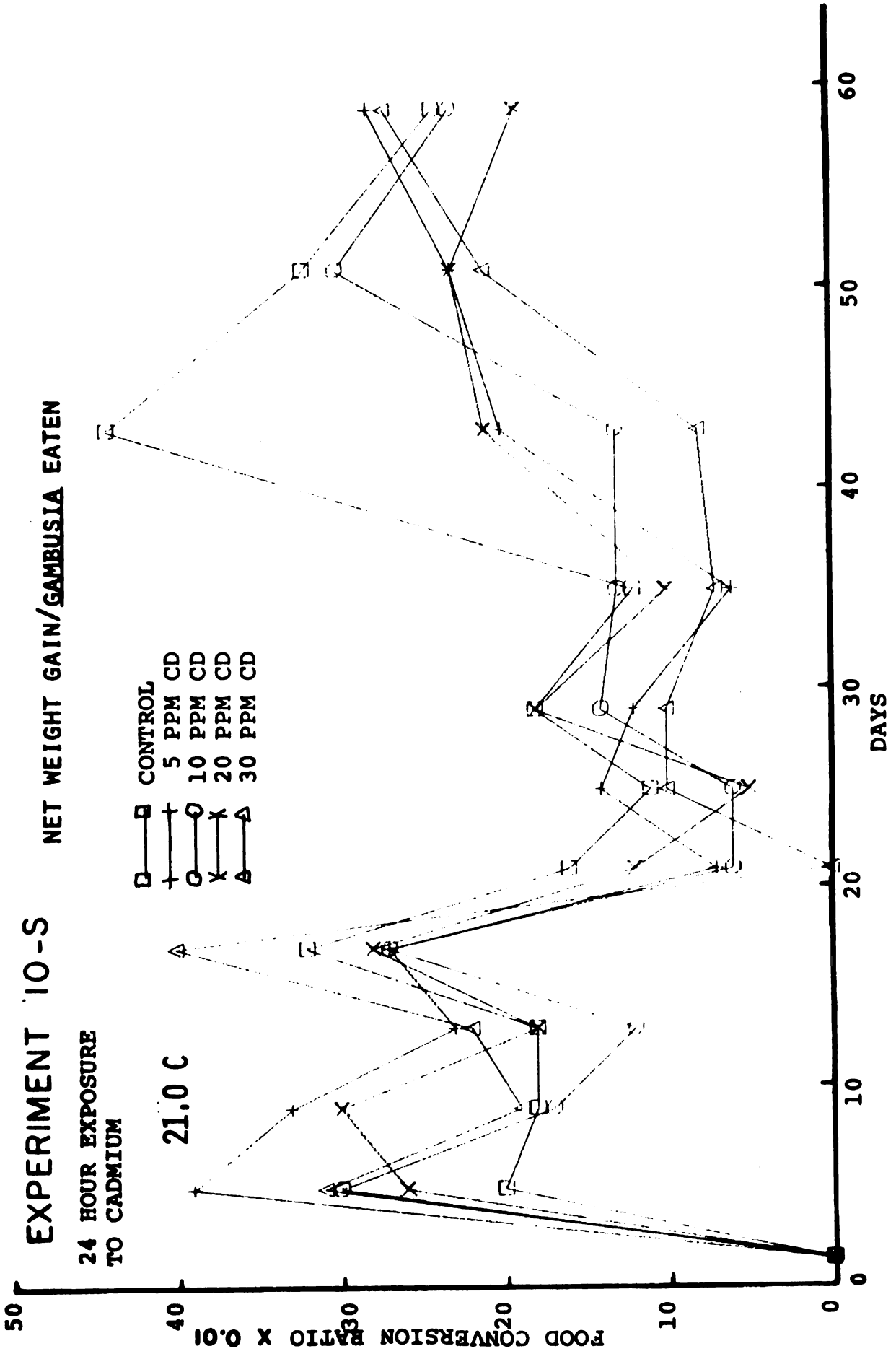

EXPERIMENT 10-S
NET WT GAIN/GAMBUSIA EATEN
1 HOUR EXPOSURE TO CADMIUM
CONTROL
5 PPM CD
10 PPM CD
21.0 C
CONTROL
20 PPM CD
30 PPM CD
21.0 C
CONTROL
40 PPM CD
50 PPM CD
21.0 C
FOOD CONVERSION RATIO X 0.01
DAYS
0
10
20
30
40
50
60

Figure 24. Food conversion ratic of green sunfish over 4 or 8-day intervals. Fish were exposed for 24 hrs to various concentrations of cadmium. Each point represents the mean of 10 fish except where deaths reduced this number.


EXPERIMENT 10-S
24 HOUR EXPOSURE
TO CADMIUM
21.0 C
NET WEIGHT GAIN/GAMBUSIA EATEN
CONTROL
5 PPM CD
10 PPM CD
20 PPM CD
30 PPM CD
FOOD CONVERSION RATIO X 0.01
DAYS
0
10
20
30
40
50
60

weight loss even though considerable amounts of Gambusia were eaten prior to that time. Thus all values for food conversion ratio were zero. Thereafter considerable variation was found in the ratios, but no large differences or trends were noted between control values and those of the 16 treatments. Thus short-term exposures to cadmium apparently did not affect food conversion efficiency in the days after exposure.

To determine if fish retained cadmium after exposure, cadmium whole-body burdens were determined for a pooled sample of all live fish remaining in each treatment tank after the experiment was ended (Table 36A). It was not known how much cadmium was taken up by fish from each treatment during exposure to cadmium and before introduction to clean water, but it was suspected in some cases to be considerable. For example, live fish from experiment 11-F exposed to 15 ppm Cd for 12 days contained 22 ppm (Table 18). Dead fish exposed to 15-50 ppm Cd contained from 74 to 148 ppm Cd in their bodies. Results of experiment 10-S indicated there was no significant long-term storage of cadmium by these fish as all values (Table 36A) were less than 1 ppm which was the amount found in control fish from all previous experiments. Comparative data also support this contention (Table 36B).

In conjunction with this experiment, I became interested in cadmium uptake in natural populations which may be exposed to cadmium. Therefore 12 green sunfish (14.65 $\pm$ 4.15 g) were

Table 36A. Final concentration (wet-weight basis) of cadmium in pooled samples of fish 60 days after short exposures to various continuous-flow concentrations of cadmium. (Standard errors are enclosed in parentheses). Comparative data are given in Table 36B.

Aquarium No.	Conc. of Cd in Water (ppm)	Time of Exposure	No. of Live Fish Analyzed	Final Whole Body Conc. of Cd (ppm)
11	2.97(0.07)	15 min	9	0.27
6	5.52(0.24)	15 min	9	0.26
12	13.15(0.24)	15 min	10	0.24
15	27.34(0.91)	15 min	10	0.71
4	36.40(2.41)	15 min	8	0.42
7	47.31(3.86)	15 min	7	0.38
17	2.97(0.07)	1 hr	10	0.33
9	5.52(0.24)	1 hr	10	0.37
16	13.15(0.24)	1 hr	10	0.62
10	27.34(0.91)	1 hr	9	0.25
5	36.40(2.41)	1 hr	10	0.29
14	47.31(3.86)	1 hr	10	0.27
13	Control	24 hrs	10	0.26
3	2.97(0.07)	24 hrs	10	0.32
1	5.52(0.24)	24 hrs	8	0.42
8	13.15(0.24)	24 hrs	10	0.47
2	27.34(0.91)	24 hrs	3	0.64

Table 36B. Some comparative data on whole-body burdens of cadmium in various fishes from the present study and from other sources. (N.D. means less than 0.01 ppm).

Type of Fish	Part of Fish Used	ppm Cd (Wet-Weight Basis)	Source
Green Sunfish	whole fish	0.68 ± 0.07	Controls--Table 18
Green Sunfish	whole fish	0.82 ± 0.09	Controls--Table 18
Green Sunfish	whole fish	0.95 ± 0.16	Controls--Table 24
Green Sunfish	whole fish	0.65 ± 0.07	Controls--Table 24
Green Sunfish	whole fish	1.02 ± 0.11	Controls--Table 30
Green Sunfish	whole fish	1.02 ± 0.16	Controls--Table 30
Green Sunfish	whole fish	1.07 ± 0.12	Controls--Table 30
Green Sunfish	whole fish	1.60 ± 0.30	Controls--Table 30
Carnivorous Illinois River	dorsal muscle	0.02	Mathis and Cummings (1971)
Non-carnivorous Illinois River	dorsal muscle	0.03	Mathis and Cummings (1971)
Various Great Lakes fishes	whole fish	0.06-1.4	Lucas, et. al. (1970)
Various Michigan fishes	whole fish	N.D.-0.5	Hesse and Evans (1972)
Green Sunfish Wintergreen Lake, Mich.	dorsal muscle	0.04-0.64	Mathis (personal communication)
Smelt, Perch, N. Pike	composite dressed fish	0.05-0.06	Uthe and Bligh (1971)

collected on June 15, 1972 below the East Lansing Waste-Water Treatment Plant outfall assuming that this area was most likely to be contaminated by cadmium. No analysis of the water was made. The mean whole-body cadmium content of collected fish was low, 0.16 $\pm$ 0.06 ppm Cd on a wet-weight basis, which was the lowest concentration of cadmium found among the sets of control fish collected from ponds.

SUMMATION DISCUSSION

Common Responses to Stress

In almost all experiments conducted a number of recurring salient features became obvious. When first exposed to higher concentrations of the toxicant, fish ceased feeding and even regurgitated food. Decreased feeding resulted in decreased growth of fish in succeeding periods. With ammonia as the toxicant, fish eventually adapted and after a period of time began to feed at a rate similar to that of controls. Even if fish adapted to the stress, as in the case of ammonia, their initial loss of weight was slowly if ever regained during the course of the experiment. Lloyd and Orr (1969) have also documented an acclimation response by rainbow trout exposed to ammonia. For cadmium at high concentrations, an initial decline in feeding by exposed fish was observed, but recovery and adaptation were small or nonexistent.

Among low concentrations of cadmium and ammonia, one concentration stimulated food consumption so that growth of these fish exceeded that of controls. Smyth (1967) discussed this phenomenon at length, referring to it as the "peck-of-dirt maxim" and as "sufficient but not overwhelming challenge." He discussed radiation data which showed that mice exposed to low levels of x-rays lived longer than controls. The sufficient challenge phenomenon in the medical field is named the

Arndt-Schulz law which is the basis of all chemotherapy. Smyth maintained that small, nonspecific responses measured in chronic toxicity studies are readjustments or adaptations to a non-lethal stress and show the well-being of an animal which is healthy enough to maintain homeostasis. He also stated that among animals exposed to various concentrations of a compound some responses will be indistinguishable from control responses. Between these and higher concentrations will be a narrow range in which exposed animals will perform better than controls, while concentrations above those in this range will cause dose-related injuries. This phenomenon was recorded in experiments 10-F and 7-F (ammonia) and in experiment 12-F (cadmium). A number of toxological studies in the literature also have noted such a response, but either failed to recognize it as a beneficial effect or merely alluded to that possibility. McKim and Benoit (1971) for example, found that low concentrations of copper caused greater growth in brook trout when compared with controls. Pickering and Gast (1972) found that egg production by fathead minnows under some concentrations of cadmium was twice that of controls, and suggest this may be an example of "sufficient challenge". Pickering (1968) observed that low concentrations of zinc promoted growth of bluegills, while Cope, et al. (1970) found that growth of bluegills exposed to 2,4-D when compared with controls was highest at the lowest concentrations. Cairns, et al. (1967), in another example, found that guppies grew better than controls when exposed to low levels of dieldrin.

I was consistently able to show a Stimulation Threshold Concentration (STC), defined as that concentration of a compound which over a long period of time promotes growth greater than controls. For ammonia this level was 2 ppm as N, while for cadmium it was 0.05 ppm Cd. This concept could be important in establishing water quality criteria for fish. Mount and Stephan (1967b) have proposed use of an LFPI (Laboratory Fish Production Index) which involves continuous exposure of fish (usually fatheads) to various concentrations of toxicant through at least one generation. A "no-effect" level is then obtained by comparing one or more of the toxicant levels with the control and determining by mortality, survival and growth of eggs, larvae and adults, which concentrations are "safe". My experiments are more complicated than EPA bioassays in one respect, since they require procurement and care of a large number of prey. However these experiments were considerably shorter than the 1 year or 1 life cycle required for some of the Environmental Protection Laboratory assays (McKim and Benoit, 1971; Pickering and Gast, 1972; Brungs, 1971; Mount and Stephan, 1967b). Ammonia (2 ppm) stimulated growth of pumpkinseeds after 4 days and growth of green sunfish after 10 days, while 0.05 ppm Cd stimulated Gambusia consumption by green sunfish after 10 days exposure. With some further confirmatory experiments, the STC might be used as a preliminary "safe" level in setting water quality criteria. Although it requires more time, the STC also would be a more appropriate method for

determination of the toxicity of many compounds now being judged solely with LC_{50} data using death as the criterion. Preliminary data for bluegills exposed to cadmium (John Eaton, personal communication) are available to compare with results of my experiments on green sunfish. Eaton exposed groups of 18 fish for 11 months to 0.03, 0.08, 0.24, 0.74 and 2.14 ppm Cd. All fish died in the 0.74 and 2.14 ppm tanks, while 16, 9 and 0 fish died at 0.24, 0.08 and 0.03 ppm, respectively. He estimated a "safe" concentration to be 0.03 ppm Cd on the basis of the above mortality data, spawning success and survival of larvae. Schweiger (1957), as cited in McKee and Wolf (1963), found that 0.03 ppm Cd was not harmful to one and two-year old tench, carp, rainbow trout and char, nor to the crustacea, worms and insect larvae on which they fed. Thus, depending on how the STC is regarded, a "safe" level for green sunfish in my experiments could be set at 0.05 ppm Cd if the stimulation of growth is viewed as beneficial, or at less than 0.05 ppm Cd if the effect is regarded as abnormal. For ammonia, I would set a "safe" level around 2 ppm as N for green and pumpkinseed sunfish.

McKim, et al. (1970) have also advocated use of a short-cut method to estimate the "safe" concentration. They found that after exposure of brook trout to copper for 6, 21 and 337 days, blood characteristics indicated fish were under stress during the first 21 days of exposure, but that an accommodation or adaptation seemed to occur subsequently for all blood factors except one. This same pattern was

established for bluegills and perch. After comparing the concentration at which short-term "transient" effects (6-37 days) occurred with the concentration of copper at which long-term effects after 337 days were noted, they concluded that blood measurements made after short exposure to a toxicant can be indicative of harmful concentrations which would occur after long-term exposure. Pickering and Gast (1972) advocated use of acute toxicity studies on the most sensitive life stage as a good indication of the chronic "safe" concentration. For fathead minnows, this was the developing embryo.

I believe that the STC is not a harmful concentration but that it definitely should not be exceeded, as Eaton found a small difference between a "safe" cadmium level (0.03 ppm) and one that can be injurious (0.08 ppm). Another point to consider with the STC is that uptake of cadmium by fish exposed to low concentrations is usually considerably higher than that found in controls. For my experiments, fish exposed to 0.05 ppm accumulated 1.84 ppm Cd after 20 days of active feeding, while control fish contained 0.65 ppm Cd. In conflict with this belief is Eisler (1971), who required that cadmium levels of fish from his "no-effect" concentration be similar to that found in controls. He found 0.1 ppm Cd was a "safe" concentration for mummichogs using that criterion. However he did not feed his fish during this exposure, a somewhat unrealistic procedure since wild fish would probably feed if not under severe stress and

if food was available. The heavy metal accumulation problem should be checked by monitoring fish for cadmium uptake over a longer time period to see that accumulation under low levels of exposure does not increase above lethal levels.

RNA-DNA Data

Use of RNA-DNA data for detecting sublethal effects of toxicants was found to have limited worth from responses I measured. Closer monitoring of daily changes due to the toxicant were more clearly shown with food consumption curves and to some extent by growth curves. In addition, the technique was time-consuming and variability in populations of fish was so extreme that only large differences were detected. Ratios were determined on the fish from two ammonia experiments and two cadmium experiments. In only one experiment, 7-F (ammonia), was there a significant difference shown with the RNA-DNA data but not with other data, such as growth or food consumption. In the other ammonia experiment, there was a significant depressant effect of ammonia on ratios of exposed fish when compared with controls. No significant differences were found among the RNA-DNA ratios of fish from either of the cadmium experiments (12-F and 13-F) despite the fact that food consumption, growth and mortality data indicated a detrimental effect of the toxicant. It appears that RNA-DNA data would be useful in experiments where either a greater number of samples were used or where less

variability existed in experimental fish. RNA-DNA ratios may be useful in detecting a short-term response before growth has occurred, since in the early days of toxicant introduction the ratios did monitor growth fairly well. My data as well as those of Bulow (1970) have shown that values tended to reach asymptotic levels which then changed little over time. This asymptotic level was reached earlier the higher the temperature in experiment 13-F (Table 28). Another advantage of this technique which may have application to toxicity research is that measurement of ratios in a field situation could be used to assess growth rate occurring at a given time once base levels were established. Measurement of the ratios from a number of fish from the field could then be compared with the levels established for slow-growing and fast-growing fish, or fish from above and below a point of toxicant introduction could be compared.

Cadmium Uptake Data

Dead fish accumulated considerably more cadmium than live fish at the same concentration so that dead fish were always kept separate from live fish in data analysis. This effect was also noted by Eisler (1971), while Mount and Stephan (1967a) proposed this difference as a means for detecting cadmium poisoning in fish. Higher concentrations of cadmium in dead fish when compared with live fish at the same concentration were probably due to absorption from the test medium and breakdown of detoxification mechanisms which

would mask any threshold concentration these fish may have reached before death. Gills of dead fish usually had the highest concentrations of cadmium among all structures studied, presumably because gills secrete large amounts of mucus, which complexes cadmium. It has also been shown that fish increase their respiratory ventilation rate during stress (Chiszare, et al., 1972), which should also increase cadmium uptake. Eisler (1971) devised an experiment to determine how much cadmium dead and live fish could take up and eliminate. He found that dead mummichogs accumulated 53 times as much cadmium in 24 hrs as live fish and 89 times as much as live fish exposed for 48 hrs. He found average loss of whole-body cadmium 24 hrs after treatment by fish surviving exposure for 24 and 48 hrs was 40 and 12%, respectively, while dead fish lost 48 and 36% after transfer to clean water. His data seemed to indicate that a considerable amount of passive diffusion was involved in uptake and elimination processes.

To judge if abnormal accumulation of cadmium has occurred in fish obtained from a fish kill or a field monitoring situation, background levels are necessary. For green sunfish, concentrations in the whole body of control fish were consistently the least variable of the three structures measured. Control fish contained about 1 ppm Cd or less on a wet-weight basis for the 19 sets of determinations of fish (sample size from 4 to 8). For the gills this value was slightly more variable, but still about 1 ppm Cd. In the

liver mean values were the most variable, between 0 and 4.79 ppm Cd. Data from experiment 10-S and those of Lucas, et al. (1970); Hesse and Evans (1972); Mathis and Cummings (1971); Uthe and Bligh (1971) and Lovett, et al. (1970) seem to indicate that exposure of fish to abnormal concentrations of cadmium should be suspected if whole-body burdens exceed much over 2 ppm Cd.

Among structures measured, both in control and exposed fish, liver cadmium concentrations were usually higher than those in the gill and whole body. This result was also reported by Mount and Stephan (1967a), who analyzed a number of other structures (bone, muscle, gut, spleen and kidney) and found that only gill and liver showed dose-related uptake. Buildup of cadmium in the liver of bluegills exposed for 11 months to 0.03 to 0.24 ppm was between 218 and 524 ppm Cd on a wet-weight basis (data of John Eaton, personal communication). The highest levels of cadmium I found in fish exposed to low levels of cadmium for 20 days (Table 24C) was 5 ppm. Fish exposed to 15 ppm for 16 days accumulated an average of 42 ppm in the liver (Table 18). Mount and Stephan found that the liver took up negligible quantities of cadmium when fish were exposed to lethal concentrations for short periods. However fish that died in this study which were exposed at 30-50 ppm Cd had livers with mean cadmium levels ranging from 33 to 39 ppm.

Good agreement was found among experiments in the concentration of cadmium found in fish exposed to comparable

concentrations. For example, whole-body burden of cadmium for fish in experiment 11-F exposed to 3.83 ppm Cd at 19.9 C was 3.99 ppm Cd. Gills contained 3.22, while liver contained 4.27 ppm Cd. These values compared well with fish exposed in experiment 12-F at a cadmium concentration of 2.48 ppm in the water and a temperature of 18.6 C. Whole-body burdens were 2.82 ppm, gill levels were 2.55 ppm and liver values were 5.08 ppm Cd.

It is postulated that uptake under exposure to high concentrations of cadmium reached a threshold concentration, above which fish died (Table 18). For experiment 11-F this value was about 20 ppm for accumulated cadmium in whole body and gills; for the liver the value was 40 ppm. Thus it can be stated that any fish which has accumulated levels of cadmium comparable to threshold levels (20 ppm in the whole body) was probably exposed to high concentrations of cadmium and is in danger of death. Mount and Stephan (1967a) reported that an equilibrium concentration of cadmium was found in bluegill gill and liver samples between 30 and 60 days. They also suggested a threshold value was apparently reached for cadmium in the gill and that death occurs when this value is exceeded. Eisler (1971) found a threshold concentration of cadmium in the body of mummichogs in excess of 86 mg Cd/kg ash (dry-weight basis).

Fish in all my experiments were given an unlimited supply of food. While this approximates natural environments in that fish must actively seek their prey, in some cases this

approach does not reflect the natural situation, since prey are hardly ever unlimited in nature. Thus fish exposed to toxicants in my experiments were able to resist adverse effects of the toxicant more vigorously than would fish fed on a limited diet. This type of experiment has been documented by Chapman (1965), as cited in Warren (1971). Chapman found that cichlids exposed to potassium pentachlorophenate in low concentrations and given unlimited food behaved exactly as did some fish in my experiments. Exposed fish initially consumed less, but then began to consume considerably more until their growth was equal to or surpassed growth of controls. A group of these same fish exposed at the same concentrations, but given a limited food supply, were not able to grow as fast as control fish. This was explained by the mechanism of toxic action of the chemical, which uncouples oxidative phosphorylation leading to a decrease in the efficiency with which energy is utilized to maintain life processes. Thus exposed fish, by consuming more food, compensated for decreased efficiency of energy utilization and attained the size of those not poisoned, while stressed fish fed limited amounts of food could not compensate. In my studies on ammonia (experiment 6-F) food conversion efficiency was generally lower for exposed fish at all three temperatures when compared with controls. No difference was found among food conversion ratios in experiment 7-F, while in 10-F only an initially low value was recorded, but thereafter exposed fish exhibited the same food conversion efficiency as

controls. For cadmium, fish exposed to 3, 7 and 15 ppm in 11-F possessed conversion efficiencies less than control values, while in 12-F there was little difference between the control and exposed fish. In the factorial experiment 13-F, there was a definite depression of food conversion efficiency of the exposed fish at cold and hot temperatures when compared with controls. Thus it appears that the mechanism discussed above (Chapman, 1965) was not occurring with my fish, since fish exhibiting low efficiencies seldom consumed enough to grow as well as controls. Conversely, those fish that were stimulated to consume more than controls possessed food conversion efficiencies comparable to or **greater than** control fish efficiencies.

Temperature did not have a significant effect on cadmium uptake (Figure 18, 19, Table 32) even though it was definitely established that fish at higher temperatures ate and grew more than fish at lower temperatures. Failure to observe increased cadmium uptake in fish at higher temperatures could be related to the higher rate of metabolism causing detoxification and elimination mechanisms to be considerably greater and thus off-set increased uptake. In addition dissolved oxygen levels were comparable among treatments which would also tend to equalize uptake rates, since ventilation rates would not be increased because of lower dissolved oxygen at higher temperatures. In view of the increased possibilities of additional heating of our waters, the interaction of temperature with uptake relationships of heavy metals certainly deserves more investigation.

One aspect investigated was whether short exposures of fish to high concentrations of cadmium exerted an effect on fish at some time after exposure (experiment 10-S). Gardner and Yevich (1970) expressed concern over post-exposure effects and Bonnell, et al. (1960) noted that cadmium-caused anomalies became progressively greater even though exposure had ceased. In another study Knoll and Fromm (1960) found rainbow trout exposed to chromium were found after return to non-toxic water to have selectively retained chromium in the kidney and spleen while other major sites such as gills and liver showed a rapid decline. In experiment 10-S, approximately 6 percent of the fish surviving the initial exposures died during the 60 days following exposure to various concentrations of cadmium. Whole-body cadmium content of these fish (about 0.6 ppm Cd) after 60 days was comparable to that of control fish in my other experiments and to concentrations found in fish from a number of surveys in Michigan, the Great Lakes, New York and Canada. Thus cadmium was eliminated by fish that were transferred to uncontaminated water. In a study similar to mine, Eisler (1971) found that mummichogs experienced post-treatment mortality which was a direct function of initial cadmium concentration and exposure period. Final cadmium concentrations in the groups of fish were still proportional to initial exposures although considerably lower.

Discussion of the Toxic Action of Ammonia and Cadmium

The main equations governing the behavior of ammonia in water are: $NH_3 + H_2O \rightleftharpoons NH_4OH \rightleftharpoons NH_4^+ + OH^-$ Thus ammonia (NH_3) upon dissolution in water forms ammonium hydroxide which in turn dissociates to form an ammonium ion plus a hydroxide ion. The toxic component (NH_3) is thus a function of pH, the higher the pH the more toxic the solution. Downing and Merkens (1955) stated that reduction in pH of water from 8.0 to 7.0 resulted in a tenfold decrease in the concentration of un-ionized ammonia. Spotte (1970) reported that only NH_3 can cross tissue barriers, which means that diffusion of ammonia will occur in the direction of lower pH (greater number of hydrogen ions). Fromm and Gillette (1968) stated that the free base (NH_3) is able to diffuse across all membranes easily because of its lipid solubility and lack of charge, whereas the ammonium ion (NH_4^+) penetrates membranes less readily because it is hydrated, charged and has low-lipid solubility. Brockway (1950) found that ammonia excretion increased with increased activity, with a rise in water temperature and after feeding. He noted that fish lost the ability to use oxygen when the concentration of ammonia in water increased, since blood carbon dioxide increased about 15% causing hemoglobin to take up considerably less oxygen. Lloyd and Herbert (1960) have shown for salmonids that the concentration of ammonia at the gill surface is the critical factor affecting ammonia toxicity. Carbon dioxide excreted by fish causes a decrease in pH at the gill surface which

reduces the amount of un-ionized ammonia at the gill. Thus depression of the amount of un-ionized ammonia at the gill is greatest when the carbon dioxide content of the water is lowest. They also stated that increased toxicity of NH_3 would occur under low dissolved oxygen conditions because decreased oxygen results in decreased carbon dioxide excretion at the gills. With decreased carbon dioxide, pH would increase at the gills, resulting in an increase in the toxicity of ammonia present, because more would be in the un-ionized state. This was documented by Downing and Merkens (1955), who showed increased ammonia toxicity under low dissolved oxygen tensions.

Fromm and Gillette (1968) found a direct, linear correlation between water NH_3 and blood NH_3, the blood NH_3 being higher than water NH_3. Since blood NH_3 concentration always exceeded the water NH_3 level, the increases in blood NH_3 were attributed to inhibition of NH_3 excretion rather than inward transfer of NH_3 against a concentration gradient. They also found that with an increase in water NH_3 total nitrogen (and ammonia) excretion decreased. They further suggested that increased toxicity of ammonia at higher temperatures is due to increased metabolism which causes greater production of internal ammonia (NH_3).

Burrows (1964) found that concentrations of un-ionized ammonia (NH_3) as low as 0.006 ppm as N could cause extensive hyperplasia in the gill epithelium of continuously-exposed chinook salmon fingerlings. These fingerlings could tolerate

levels of ammonium hydroxide as great as 0.7 ppm for 1 hr per day without apparent effect, while exposure periods greater than 12 hrs per day at levels of 0.1 ppm or greater caused reduced growth rate. Hyperplasia was thought to cause salmonids to become susceptible to gill disease. Continuous exposure to 0.1 ppm caused reduced stamina and disease resistance. Kawamoto (1961) showed a reduced growth rate of carp exposed to 0.3 ppm ammonium chloride for 3 months. Spotte (1970) reported that another study exposing fish to sublethal concentrations of ammonia found gill hyperplasia, congestion of mucus cells in the skin, abnormal concentration of blood corpuscles in the epidermis, congested blood vessels and inflammation of the liver. Ball (1967) found the lethal threshold (0.3-0.4 ppm as N--un-ionized ammonia) did not differ between trout and coarse fish, but more trout than coarse fish died in the early part of the test.

The toxic action of ammonia can be understood by considering the role of ammonia in fish metabolism. Nitrogen waste is usually eliminated as ammonia from blood at the gills by passive diffusion. Under "normal" conditions urea production is a minor component in nitrogen waste removal. Fromm and Gillette (1968) showed that ammonia concentration in trout blood almost doubled when fish were exposed to high ambient concentrations of ammonia. At some critical level of blood ammonia, they noted, a fish must either decrease its sensitivity to ammonia or convert ammonia to a less toxic compound. Further work of Olson and Fromm (1971) showed that

trout exposed to high external ammonia levels decreased ammonia excretion and total nitrogen excretion. Urea excretion increased slightly for trout, while goldfish significantly increased urea production in a very short time. The route of urea production was suggested to be from ammonia through purine synthesis and catabolism. It is thus suggested that green sunfish probably adapted to external ammonia levels by a gradual shift to urea production. Green sunfish did not succumb at relatively high concentrations of ammonia (25 ppm as N or about 0.5 ppm un-ionized ammonia). In addition green sunfish grew and consumed food at rates near those of controls after a certain acclimation period which suggests this sunfish may have the capacity to rapidly initiate mechanisms for ammonia detoxification and urea production.

The mechanism of toxic action of cadmium is not known and may well be different for different conditions of exposure. Eisler (1971), for example, reported that with brief exposure to high concentrations of cadmium, the gill appeared to be the primary site of damage, but prolonged exposure to low concentrations of cadmium affected the intestine, kidney and possibly other tissues not analyzed. This relation was generally borne out in literature surveys. Eaton (personal communication) found highest accumulation in the liver of bluegills (218-524 ppm Cd on a wet-weight basis) exposed continuously for 11 months to low concentrations of cadmium (0.03-0.24 ppm). High levels were also found in kidney, liver and caecum. Mount and Stephan (1967a) reported

that bluegills contained more cadmium in the liver (500 ug/g of tissue--dry weight) than gills (130 ug/g of tissue--dry weight) of fish exposed over 30, 60 and 90 days. Acute exposures that resulted in death of fish showed **gills to** contain high concentrations of cadmium while the liver contained minimal levels of cadmium.

The fact that gills are severely affected at high concentrations was certainly confirmed by the response of fish in my study. Fish exposed to 30-50 ppm Cd prior to and after death were covered with excessive mucus secretion, and considerable fusing of gill lamellae was noted. Gardner and Yevich (1970) also noted hypertrophy of gill filaments and hyperplasia of the epithelial surface of the respiratory lamellae in fish exposed to 50 ppm Cd. Schweiger (1957), as cited in Eisler (1971), noted that high concentrations of cadmium were found to cauterize gill lamellae of several freshwater fishes. Eisler (1971) found the same high rate of accumulation in gills of adult tautog, a marine fish.

Another finding by Lewis and Lewis (1971) may also be related to the toxic action of heavy metals. They found that fish exposed to copper and zinc exhibited a reduction in blood-serum osmolality which could result in mortality. The osmotic drop was principally related to damage of the head and gill region. They found sodium chloride added to water containing copper and zinc prevented distress symptoms and mortality usually associated with exposure to these heavy metals. Thus they concluded that heavy metals affected

the gill area and caused a drop in the salt concentration of the blood. Another important mechanism explaining the toxic action of heavy metals is thought to be poisoning of enzyme systems (McKee and Wolf, 1963). Metals are readily chelated by organic molecules and cadmium is listed as one of the metals which could combine with the cell membrane and affect permeability. Thus transport of sodium, potassium, chloride ions or organic molecules could be affected. Rupture of these membranes was also listed as a possible effect. Hiltibran (1971) investigated effects of cadmium and zinc on energy production as indicated by changes in oxygen and phosphorus metabolism in bluegill liver mitochondria. He found that cadmium and zinc can disrupt energy production through inhibition of oxygen uptake within the cells and that this disruption can occur at relatively low levels of cadmium and be of such severity as to cause death of fishes, particularly bluegills.

Application Factors

The concept of the LFPI (Laboratory Fish Production Index) proposed by Mount and Stephan (1967b) involves determination of a "safe" level or MATC (Maximum Acceptable Toxicant Concentration). The MATC is the concentration of toxicant which is judged safe by considering the mortality and growth of the adults, and eggs and fry derived from them after long-term exposure (a year or one life cycle) to the toxicant. The application factor is then derived by dividing

the MATC by the 96-hr LC_{50}. These values have been derived for a number of studies, mainly by workers from the Environmental Protection Agency (Table 37). Two things should be noted with these data. First, Pickering and Gast (1972) also gave the lethal threshold concentration value, which is another value usually equal to the 96-hr LC_{50} value. It is defined as that concentration of toxicant found when the toxicity curve becomes essentially parallel to the time axis. Using this value in deriving the application factor gives a value of 0.08-0.13, which is different by a factor of 10 from the tabular value of the application factor (Table 37). Secondly, for my data the STC (Stimulation Threshold Concentration) was used in determining the MATC. Considering that the four studies involving cadmium reported here involved such obvious differences in water conditions as freshwater versus saltwater, of different species of fish, and of different water chemistry parameters, agreement among these four values is very good.

For the ammonia experiments the MATC for both pumpkinseeds and green sunfish was judged to be 2 ppm as N. The LC_{50} value for pumpkinseeds was 9.4 ppm as N at a temperature of 12.0 C (see Appendix Table F); for green sunfish the LC_{50} value was 33 ppm ammonia as N at a temperature of 12.5 C (see Appendix Table G). Thus application factors for pumpkinseeds and green sunfish are 0.21 and 0.06 respectively. Lloyd and Orr (1969) gave an application factor of 0.12 for trout. The difference between the larger application factors

Table 37. A summary of studies showing some values of application factors (Mount and Stephan, 1967) as well as corresponding MATC (Maximum Acceptable Toxicant Concentration) and the 96-hr LC_{50} values.

Fish	Stressor or Toxicant	MATC	96-hour LC50	Application Factor	Source
Fatheads	carbaryl	0.21-0.68 ppm	9 ppm	0.023-0.075	Carlson (1972)
Fatheads	Zn	0.03-0.18 ppm	9.2 ppm	0.003-0.02	Brungs (1969)
Brook Trout	Cu	0.017-0.009 ppm	0.1 ppm	0.17-0.10	McKim and Benoit (1971)
Fatheads	Cu	0.033-0.14 ppm	0.43 ppm	0.08-0.03 (hard water) 0.13-0.22 (soft water)	Mount and Stephan (1969) Mount (1968)
Fatheads	Cd	0.057-0.037 ppm	7.2 ppm	0.005-0.008	Pickering and Gast (1972)
Mummichog (Marine Fish)	Cd	0.1 ppm	55 ppm	0.0018	Eisler (1971)
Bluegill Sunfish	Cd	0.03 ppm	20 ppm	0.0015	Eaton (personal communication)
Green Sunfish	Cd	0.05 ppm	20.5 ppm	0.0024	Present Study

for ammonia as compared with cadmium derives from the apparent reduced toxicity of ammonia on a relative scale compared with cadmium. Fish appeared to acclimate readily to ammonia, while no such acclimation to cadmium was observed. In addition, cadmium-exposed fish experienced a depression in feeding rate which remained throughout exposure.

These data and comparative literature data give considerably more support for advocating the STC (Stimulation Threshold Concentration) as a useful parameter in toxicity research. The STC, derived using food consumption and growth as the criteria, is suggested as a more meaningful parameter from which accurate water quality standards can be judged and should provide a more productive approach for evaluating potentially toxic substances.

SUMMARY

Experiment 6-F

Experiment 6-F, performed to evaluate the interaction of three temperatures with one level of ammonia, was not completed as designed, because decreased levels of ammonia and dissolved oxygen were observed at higher temperatures and confounded results. Temperature exerted a major effect on fish, the higher the temperature the greater the growth. In all cases except the latter 10 days at hot temperature, growth of ammonia-exposed fish was less than that of corresponding controls. The greatest difference occurred at medium temperature. Amount of Gambusia eaten reflected the same trends as observed with growth data. Food conversion ratios were variable and in all cases except one were lower than control values. RNA-DNA ratios, like efficiencies were generally lower for stressed fish, particularly for fish at cold temperature, when contrasted with corresponding control values. A tendency for values to level off was noted for all treatments.

Experiment 7-F

Pumpkinseed sunfish exposed to concentrations of ammonia 5.72 ppm and greater were stressed to the point that feeding was temporarily halted, which affected their growth and possibly their conversion efficiency.

When compared with controls, fish exposed to 2 ppm ammonia as N were stimulated to eat more food which resulted in these fish possessing a significantly higher RNA-DNA ratio. Fish at all other concentrations exhibited an acclimation phenomenon whereby after the initial decrease in food consumption (and also growth), feeding eventually was established at a rate comparable to controls. The recovery was sufficient enough so that values for mean weight gain and food conversion efficiency, although undoubtedly lower after introduction of toxicant, were not distinguishable from control values at the end of the experiment.

Experiment 10-F

Fish exposed to ammonia concentrations greater than 1.93 ppm reacted initially by regurgitation of previously eaten food and cessation of feeding. Fish exposed to 1.93 ppm ammonia as N were stimulated to eat and grow more than control fish. The long-term detrimental effects on growth increased and were of greater duration the higher the concentration of ammonia. Acclimation was observed only in the fast recovery of exposed fish (increased feeding and growth after a severe decline) in the second and succeeding 4-day periods after initial introduction of toxicant. Food conversion ratios of fish exposed to 8 ppm ammonia as N and greater were lower than those of controls during the first 4-day period. Thereafter ratios of all groups of fish were similar.

Experiment 11-F

Results of experiment 11-F showed that concentrations of cadmium greater than 27 ppm killed all fish within 4 days (LC_{50} value was 20.5 ppm Cd). At concentrations of 3, 7 and 15 ppm Cd, growth, food consumption and food conversion ratios were detrimentally affected, since feeding continued, but little weight increase occurred. These concentrations of cadmium are to be considered unfavorable for green sunfish growth, and perhaps eventually would cause death of the fish.

Experiment 12-F

Green sunfish exposed to low concentrations of cadmium (0.23 to 2.48 ppm) consumed lesser amounts of Gambusia and grew at a rate lower than controls. Fish exposed to 0.05 ppm, however, were stimulated by this lower level of cadmium, as amounts of Gambusia eaten increased over that eaten by controls, and mean weight consistently increased until it was greater than that of controls at the last sampling period. Food conversion ratios were slightly depressed in the first 4 days after toxicant introduction, but thereafter were indistinguishable from control values. RNA-DNA ratios increased from initial levels, but no significant differences were found among treatments after 20 days of exposure.

Experiment 13-F

Evaluation of the effect of 1 ppm Cd on growth and survival of green sunfish at three different temperatures

was based on growth, feeding, food conversion ratios and RNA-DNA ratios of these fish. At the cold temperature 1 ppm Cd appeared to have little effect on growth and food consumption. Efficiency of food conversion was depressed when contrasted with control efficiencies. Cadmium uptake was double that of controls; however no deaths at cold temperatures occurred during the duration of the study (20 days).

At medium temperatures growth, food consumption and efficiency of stressed fish were indistinguishable from respective values of control fish. Cadmium uptake was twice that of control fish.

Fish exposed at hot temperatures were detrimentally affected by the combination of cadmium and high temperature. Food consumption of stressed fish was considerably lower than amounts eaten by control fish, as was growth rate. In addition four of the 16 fish exposed at 1 ppm Cd and hot temperature died, as did one control fish.

Experiment 10-S

This experiment demonstrated that short-term exposure to various high concentrations of cadmium was detrimental to subsequent growth of cadmium-exposed fish when compared with control fish. Post-treatment growth of fish exposed to six concentrations of cadmium for 15 min and for 1 hr was less, but not significantly, than growth of controls for almost all treatment combinations. Growth of fish

after 24 hrs exposure to 5, 10, 20 and 30 ppm Cd was severely depressed over that of control fish. Food conversion efficiencies were extremely variable and differences among treatments were not consistent.

Cadmium Uptake Data

1. An equation ($Y = 0.065 X + 0.316$) with an $r^2 = 0.89$ adequately described the uptake curve relating the independent variable X (cadmium concentration in the water up to 15 ppm Cd) to the dependent variable Y (log of the cadmium concentration in the fish + 1).

2. Fish exposed to 3, 7 and 15 ppm Cd accumulated cadmium in a geometric manner, with highest water concentrations causing proportionately greater uptake than the two lower water concentrations of cadmium. Concentrations of cadmium greater than 15 ppm killed all fish in 16 days.

3. Whole body, gills and livers of dead fish contained considerably more cadmium than corresponding live fish at the same concentrations.

4. Livers of live fish consistently contained highest levels of cadmium, while for dead fish gills usually contained the highest concentration of cadmium among whole body, gills and liver.

5. Whole-body burdens of control fish contained a mean of about 1 ppm Cd on a wet-weight basis.

6. Among structures measured for cadmium content, whole-body burdens were the most stable and least variable.

7. A threshold concentration in whole-body cadmium content above which green sunfish died was found at about 20 ppm.

8. Fish exposed to the STC (Stimulation Threshold Concentration) of cadmium (0.05 ppm) for 20 days contained as much cadmium as fish exposed to almost 2 ppm for the same time. Higher uptake by fish exposed at 0.05 ppm is thought to be due to greater food consumption by these fish.

9. Fish exposed at three different temperatures (17.5, 23.9, 30.0 C) to 1 ppm Cd contained similar levels of cadmium about twice that of control fish.

10. Cadmium elimination after exposure to high concentrations for short periods was complete after 60 days and probably much sooner.

Stimulation of growth was observed for one low concentration of ammonia (2 ppm) and one low concentration of cadmium (0.05 ppm). This response, termed the STC (Stimulation Threshold Concentration), is proposed as a more accurate method than use of LC_{50} data and as a shorter method for obtaining tentative MATC (Maximum Acceptable Threshold Concentration) values. Application factors for this and three other studies were compared and found in good agreement.

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APPENDIX

Table A. A summary of water quality characteristics of filtered tap water from the Limnological Research Building. Samples were collected on June 2, 1970 and June 1, 1971.

Parameter	Concentration
Alkalinity ppm as $CaCO_3$	292
Hardness ppm as $CaCO_3$	328
pH	7.8
Ammonia ppm as N	0.03
Chloride ppm as Cl	5.2
Nitrate ppm as N	0.01
Phosphorus (total) ppm as P	0.85
Sulfate ppm as SO_4	6.0
Cadmium ppm as Cd	<0.01
Zinc ppm as Zn	<0.015
Copper ppm as Cu	<0.01
Iron ppm as Fe	<0.10
Lead ppm as Pb	<0.10
Conductivity (mho)	682

Table B. A summary of pertinent information on the method and location of capture and health of sunfish collected for bioassay purposes.

Collection	Date	Method of Capture	Location	Feeding Schedule	Mortality	Diseases or Parasites
1	5/15/70	6 ft seine	small gravel pit now non-existent near Burke Lake, in the Rose Lake Wildlife Area	periodically fed with ground fish and trout chow	less than 1%	some fish contracted pop-eye during holding
2	7/5/70	6 ft seine	Large gravel pit pond in Williamston	same as 1	less than 1%	same as 1
3	8/14/70	6 ft seine	same as 1	same as 1	less than 1%	same as 1
4	10/23/70	40 ft seine	same as 2	fed sparingly with trout chow during winter and starved prior to testing	greens 1% pumpkin-seeds 5%	same as 1
5	10/27/70	shocking	same as 2	same as 4	less than 5%	same as 1

Table B. continued

Collection	Date	Method of Capture	Location	Feeding Schedule	Mortality	Diseases or Parasites
6	10/30/70	40 ft seine	same as 2	same as 4	less than 5%	same as 1
7	4/12/71	100 ft seine	same as 2	fed daily ground fish or trout chow	less than 10%	Tricodina infestation in another batch from the same area treated with formalin. Eventual loss of the whole catch occurred because of delayed mortality. None in this batch appeared affected, although occasional fish deaths from unknown causes before the experiment did occur.
8	4/16/71	100 ft seine	same as 2	same as 7	less than 10%	same as 7

Table B. continued

Collection	Date	Method of Capture	Location	Feeding Schedule	Mortality	Diseases or Parasites
9	5/31/71	100 ft seine	gravel pit pond south and east of the intersection of US 27 and Jolly Road near Holt	same as 7	0%	no apparent diseases
10	6/14/71	shocking	same as 9	same as 7	20% initially before taken to lab due to high temp. and poor handling. 0% in the lab.	no apparent diseases
11	7/24/71	100 ft seine	same as 2	same as 7	less than 10%	some mortality, cause unknown

Table B. continued

Collection	Date	Method of Capture	Location	Feeding Schedule	Mortality	Diseases or Parasites
12	8/17/71	6 ft seine	small pond east of Burcham Rd.--Lake Lansing Rd. intersection in East Lansing	same as 7	less than 1%	no apparent diseases
13	8/26/71	6 ft seine	same as 11	same as 7	less than 1%	no apparent diseases

Table C. Pesticide residue analysis for five green sunfish collected for bioassay purposes from a Williamston pond. Fish from this collection were used in experiment 6-F.

		Concentration in ppb			
Fish weight (g)	Sex	DDE	DDD	DDT	Dieldrin
6.8000 (composite sample of three fish)	M M F	51.18	11.86	34.11	4.07
9.4024	M	52.63	12.69	23.63	2.57
9.3385	M	43.57	9.84	25.00	2.07

Table D. *Gambusia* pond locations.

Collection Site	Location
1	4 ponds located on the Michigan State campus north of the Grand Trunk Railroad and east of Farm Lane
2	1 pond at the north-east corner of Waverly Golf Course, intersection of Waverly Road and Saginaw in Lansing
3	Ponds in Grand Woods Park in Lansing
4	1 pond located north and east of the intersection of Hulett Road and Jolly Road in Meridian Township
5	3 large concrete ponds located at the Water Research Laboratory on campus

Table E. Mean concentrations of RNA, DNA and the RNA-DNA ratios of green sunfish from experiment 6-F before and during continuous exposure to various concentrations of ammonia at three different temperatures for 40 days. DFFT is dry fat-free tissue from the dorsal muscle excluding skin. Standard error is enclosed in parentheses.

	Day	µg DNA per 100 mg DFFT	µg RNA per 100 mg DFFT	RNA-DNA Ratio
Cold	0	22.6(4.8)	223.4(42.4)	12.69(5.14)
	10	15.7(2.6)	530.8(47.6)	35.97(5.31)
	20	20.5(1.0)	539.7(84.0)	27.74(5.15)
	30	15.7(2.5)	565.9(56.5)	36.09(3.68)
	40	16.8(1.5)	414.9(14.0)	25.40(2.13)
Cold	0	-	-	-
Stressor	10	15.7(1.8)	335.2(29.1)	21.62(2.88)
	20	19.9(3.3)	378.7(22.7)	19.87(2.04)
	30	17.8(1.8)	402.3(100.1)	25.37(8.13)
	40	17.3(2.9)	415.4(53.3)	25.58(4.86)
Medium	0	24.7(2.9)	186.2(15.0)	8.10(1.64)
	10	24.1(5.5)	509.3(91.7)	23.76(5.68)
	20	23.6(2.2)	367.2(19.4)	15.84(1.65)
	30	18.9(4.1)	358.2(23.0)	22.12(5.04)
	40	16.3(1.3)	287.4(10.7)	18.19(1.87)
Medium	0	-	-	-
Stressor	10	20.5(2.2)	472.6(77.7)	22.68(2.46)
	20	19.4(1.3)	312.1(60.4)	15.81(2.51)
	30	21.5(4.2)	331.0(54.9)	16.60(3.40)
	40	15.7(3.3)	265.4(11.4)	18.12(2.16)
Hot	0	19.9(2.0)	216.6(32.3)	10.66(.95)
	10	16.8(3.1)	490.4(65.7)	30.45(2.54)
	20	19.4(1.6)	295.8(5.1)	15.58(1.05)
	30	16.3(6.5)	285.8(19.2)	24.10(5.26)
	40	8.4(1.5)	260.1(17.0)	32.13(3.83)
Hot	0	-	-	-
Stressor	10	17.9(1.4)	481.5(23.8)	27.47(2.28)
	20	16.8(.9)	246.0(33.4)	14.64(2.01)
	30	13.1(1.8)	248.6(23.1)	19.47(1.98)
	40	11.0(2.0)	226.6(10.0)	21.30(2.99)

Table F. A summary of pertinent data for the LC_{50} determination on pumpkinseeds (4.46 ± 0.31 g) in experiment 8-F. Sample size for ammonia was four, and for all other determinations was one. (Standard error is enclosed in parentheses; t = less than 0.01 ppm).

	Aquarium Number						
	1	8	3	10	5	2	12
% survival after 96 hrs	100	100	40	20	0	0	0
Ammonia (ppm as N)	0.08 (0.03)	4.02 (0.14)	11.34 (0.21)	14.23 (0.21)	18.07 (0.58)	24.95 (1.53)	28.10 (0.82)
Un-ionized NH_3 (ppm as N)	t	0.10	0.35	0.21	0.56	0.60	0.53
Dissolved Oxygen (ppm)	8.7	8.4	8.3	8.3	8.0	8.5	8.3
pH	7.76	7.88	7.94	7.55	7.90	7.83	7.70
Temperature (C)	11.7	12.1	11.9	12.3	12.0	12.0	12.2

Table G. A summary of pertinent data for the LC_{50} determination on green sunfish (8.39 ± 1.37 g) in experiment 9-F. Sample size for ammonia was seven, for dissolved oxygen and pH two, and for alkalinity and hardness one. Standard error is enclosed in parentheses; range is given for pH. (t = less than 0.01 ppm).

	Aquarium Number						
	1	8	3	10	5	2	12
% survival after 96 hrs	100	100	80	80	0	0	0
Ammonia (ppm as N)	0.11 (0.01)	8.81 (0.26)	26.69 (0.38)	30.89 (0.82)	35.84 (1.62)	47.30 (2.35)	54.40 (2.00)
Un-ionized NH_3 (ppm as N)	t	0.21	0.82	0.74	1.36	1.14	0.96
Dissolved Oxygen (ppm)	8.6 (0.2)	8.0 (0.2)	8.7 (0.4)	8.0 (0.3)	8.4 (0.2)	8.1 (0.3)	8.5 (0.3)
pH	7.82-7.84	7.80-7.90	7.93-8.00	7.76-7.79	7.95-7.97	7.76-7.79	7.72-7.76
Temperature (C)	12.0 (0.1)	12.4 (0.2)	12.2 (0.1)	12.6 (0.2)	12.2 (0.2)	12.2 (0.1)	12.5 (0.3)
Alkalinity ppm as $CaCO_3$	336	328	336	336	332	338	326
Hardness ppm as $CaCO_3$	340	336	340	332	340	336	336

Table H. Standard errors for the mean weight changes of green sunfish exposed to various concentrations of ammonia in experiment 10-F.

Time	Treatment (ppm NH_4 as N)						
(Days)	0.15	1.93	4.84	8.67	13.23	20.02	24.03
0	0.37	0.38	0.40	0.47	0.44	0.26	0.53
4	0.48	0.60	0.48	0.57	0.53	0.38	0.52
8	0.43	0.66	0.60	0.60	0.53	0.34	0.49
12	0.52	0.67	0.66	0.84	0.83	0.43	0.44
16	0.56	0.87	0.87	0.89	1.08	0.57	0.37
20	0.78	0.95	0.99	1.03	1.18	0.79	0.39
24	0.94	1.02	1.15	1.10	1.38	1.10	0.42
28	1.10	1.12	1.36	1.21	1.21	1.18	0.53
32	1.10	1.30	1.47	1.38	1.57	1.62	0.74
36	1.17	1.65	1.64	1.50	1.37	2.05	1.02

Table I. Standard errors for the mean weight changes of green sunfish exposed to various concentrations of cadmium in experiment 11-F. (N.D. means less than 0.01 ppm Cd).

Time	Treatment (ppm Cd)						
(Days)	N.D.	3.83	7.95	15.44	27.63	35.92	51.15
0	0.29	0.35	0.40	0.26	0.41	0.43	0.48
4	0.31	0.48	0.41	0.39	0.49	0.45	0.58
8	0.42	0.56	0.47	0.33	0.64	0.42	—
12	0.45	0.74	0.51	0.25	—	—	—
16	0.46	0.83	0.50	0.37	—	—	—

Table J. Standard errors for the mean weight changes of green sunfish exposed to various concentrations of cadmium in experiment 12-F. (N.D. means less than 0.01 ppm Cd).

Time	Treatment (ppm Cd)						
(Days)	N.D.	0.05	0.23	0.32	1.31	1.93	2.48
0	0.17	0.16	0.17	0.12	0.17	0.12	0.12
4	0.32	0.17	0.20	0.35	0.31	0.12	0.24
8	0.24	0.18	0.25	0.38	0.33	0.13	0.26
12	0.29	0.20	0.32	0.39	0.33	0.14	0.33
16	0.31	0.21	0.33	0.40	0.32	0.15	0.37
20	0.37	0.26	0.32	0.42	0.31	0.18	0.38
24	0.48	0.36	0.36	0.52	0.46	0.29	0.43

Table K. Standard errors for the mean weight changes of green sunfish exposed for 15 min to various concentrations of cadmium in experiment 10-S. (N.D. means less than 0.01 ppm).

Time	Ppm Cd - 15 Min Exposure						
(Days)	N.D.	5	10	20	30	40	50
0	0.10	0.11	0.05	0.08	0.09	0.10	0.09
3	0.10	0.10	0.06	0.09	0.09	0.11	0.09
7	0.17	0.18	0.10	0.14	0.20	0.17	0.12
11	0.16	0.32	0.20	0.16	0.26	0.25	0.21
15	0.19	0.39	0.24	0.19	0.33	0.28	0.33
19	0.31	0.44	0.29	0.24	0.41	0.45	0.42
23	0.36	0.57	0.34	0.28	0.46	0.49	0.53
27	0.40	0.57	0.37	0.29	0.50	0.52	0.56
31	0.46	0.67	0.43	0.33	0.56	0.58	0.60
39	0.61	0.81	0.49	0.37	0.66	0.68	0.80
47	0.94	1.10	0.53	0.51	0.88	0.83	1.12
55	1.28	1.52	0.61	0.73	1.18	1.07	1.49
63	1.58	2.14	0.78	1.04	1.44	1.49	1.89

Table L. Standard errors for the mean weight changes of green sunfish exposed for 1 hr to various concentrations of cadmium in experiment 10-S. (N.D. means less than 0.01 ppm).

Time	Ppm Cd - 1 Hr Exposure						
(Days)	N.D.	5	10	20	30	40	50
0	0.10	0.06	0.09	0.10	0.07	0.09	0.09
3	0.10	0.06	0.08	0.08	0.07	0.05	0.08
7	0.17	0.16	0.11	0.09	0.17	0.12	0.16
11	0.16	0.24	0.19	0.17	0.23	0.17	0.22
15	0.19	0.28	0.22	0.24	0.34	0.17	0.21
19	0.31	0.34	0.37	0.29	0.43	0.31	0.24
23	0.36	0.38	0.43	0.32	0.48	0.39	0.31
27	0.40	0.44	0.46	0.36	0.54	0.47	0.33
31	0.46	0.50	0.51	0.36	0.63	0.62	0.37
39	0.61	0.63	0.64	0.40	0.70	0.80	0.42
47	0.94	0.79	0.88	0.53	0.89	1.09	0.52
55	1.28	1.09	1.09	0.62	1.30	1.26	0.64
63	1.58	1.35	1.36	0.66	1.61	1.68	0.75

Table M. Standard errors for the mean weight changes of green sunfish exposed for 24 hrs to various concentrations of cadmium in experiment 10-S. (N.D. means less than 0.01 ppm).

Time	Ppm Cd - 24 Hr Exposure				
(Days)	N.D.	5	10	20	30
0	0.10	0.09	0.07	0.11	0.11
3	0.10	0.11	0.06	0.11	0.17
7	0.17	0.16	0.11	0.18	0.77
11	0.16	0.21	0.16	0.30	1.03
15	0.19	0.26	0.19	0.43	1.25
19	0.31	0.30	0.27	0.59	1.51
23	0.36	0.34	0.27	0.67	1.65
27	0.40	0.41	0.33	0.72	1.71
31	0.46	0.43	0.41	0.81	1.78
39	0.61	0.51	0.47	0.99	2.01
47	0.94	0.63	0.65	1.23	2.11
55	1.28	0.87	0.93	1.60	2.52
63	1.58	1.21	1.24	1.77	3.11