

Effects of Hexavalent Chromium on the Growth and  
Reproduction of  
Chironomus riparius (Diptera)

by

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submitted to the Faculty of the  
Virginia Polytechnic Institute and State University  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Zoology

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August, 1983  
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## ACKNOWLEDGEMENTS

I would like to thank the members of my committee, Dr. Art Buikema, Jr., Dr. Ernest F. Benfield, and Dr. Albert C. Hendricks for their help and suggestions throughout my research and the writing of this thesis. I give very special thanks to Sylvia Sanford for many diverting lunch hours and to my husband Stephen Golladay for his advise, support, and encouragement.

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## INTRODUCTION

The ability to predict sublethal effects of chronic exposure to toxicants is necessary to insure survival of aquatic communities. Many investigators studying sublethal effects have measured the molecular (e.g., Larson 1975, Gould 1975, Ivanovici 1980) and the cellular impact of toxicants (e.g., Dawson 1979, Bodammer 1979). However, gross physiological parameters integrate many molecular and cellular processes and are more easily interpreted in terms of population survival. The primary basis for population survival is the growth and reproduction by individuals (Kersting 1975). The measurement of growth and reproduction should provide an understanding of toxicant effects on population survival. Prediction of toxicant effects may be difficult because of complicating factors in the natural environment.

Many investigators have reported significant changes in growth rates of aquatic organisms exposed to sublethal concentrations of toxicants. Macek et al. (1976a, 1976b) found that the biocides Atrazine and Lindane retarded development and delayed pupation and emergence of Chironomus tentans. Daphnia pulex has also exhibited reduced growth when exposed

to Atrazine (Schober and Lampert 1977). Wentzel et al. (1977), using naturally contaminated sediments containing Zn, Cd, and Cr, found the growth of Chironomus tentans was slightly retarded. Growth of the parthenogenetic midge, Paratanytarsus parthenogeneticus was reduced by exposure to sublethal concentrations of copper (Hatakeyama and Yasuno 1981).

In some cases, sublethal concentrations of trace metals have a stimulatory effect on growth when organisms are tested in a metal-limited environment. This effect has been demonstrated in various organisms ranging from bacteria to mice (Luckey 1975). It is assumed that the function of essential trace metals in metabolism is the means by which growth is stimulated. At low levels, non-essential compounds such as pesticides have stimulated reproduction (Lehmkuhl 1979).

Many authors report reproductive impairment as the result of sublethal toxicant stress. In Chironomus tentans egg hatching was reduced by DDE (Derr and Zabick 1972) and Atrazine (Macek et al. 1976a); the latter appeared to be a cumulative effect on hatching after exposure for a complete life cycle. Schober and Lampert (1977) demonstrated a reduction in offspring produced by a population of Daphnia pulex exposed to Atrazine. Production of offspring in

Daphnia magna was reduced during chronic exposure to the compound Diuron (Kersting 1975).

Multigeneration tests which measure changes in population size over time are less common in the literature. Daniels and Allan (1981) reported that Dieldrin, a chlorinated hydrocarbon insecticide, significantly reduced the intrinsic growth rate of a population of Eurytemora affinis (Copepoda). Winner and Farrell (1976) used the same approach to detect chronic toxicity of copper to four species of Daphnia. Reish and Carr (1978) found that population size was significantly smaller for Ophryotroch diadema (Polychaeta) after 21 days of exposure to hexavalent chromium when compared to controls. These experiments, utilizing small, rapidly reproducing organisms, are perhaps the best way of integrating individual survival, growth, and reproduction to predict the long-term effects of a toxicant on a population.

Any alteration in growth may effect other features of an organism's energy balance. Reproduction is one portion of the energy balance that requires large inputs of assimilated energy in the later stages of an insect's life. In the case of aquatic insects, adults are often nonfeeding. The nutritive base for the completion of egg development in the female must be acquired in the immature stages (Chapman 1971). Energy stores are built up prior to emergence as

lipids and glycogen (Chapman 1971). Thus, the amount of lipid deposition in individuals may clarify the pattern of weight gain during growth and may have great significance for the population's reproductive success.

The specific objectives of this study on an aquatic insect were: 1) to determine the effect of chronic toxicant stress on growth and reproduction, 2) to determine if chronic exposure affects lipid deposition in the last larval stage, and 3) to determine if toxicant body burdens of immature and adults can be correlated with effects on reproduction and growth.

## MATERIALS AND METHODS

### Choice of Experimental Insect

The midge, Chironomus riparius, was chosen to test the objectives of this research. C. riparius can be found in depositional areas of rivers and in lake sediments (Learner and Edwards 1966, Edgar and Meadows 1969). Chironomidae represent a significant portion of many aquatic insect communities both in biomass and species number. Larval densities are commonly 50,000 per m<sup>2</sup> and may make up 50% of the total number of species present in a community (Coffman 1978). Members of the genus Chironomus are often very important as fish food and can be important in the diet of surface feeding ducks (Townsend et al. 1981).

Chironomus riparius has a short life cycle, approximately 21 days at 23.5°C, can swarm and mate in a confined space, and is easily maintained in the laboratory with minimal care. The insect was already in continuous culture in our laboratory, and previous work had established certain life history and acute toxicity information. These factors, along with its ecological importance, made C. riparius a suitable choice for this study.

### Growth and Reproduction Experiments

Growth experiments with Chironomus riparius were conducted in 21 l glass aquaria. A ground paper towel substrate was provided for burrowing and as a food source. Brown paper towels were shredded and then ground in a Waring blender for approximately 30 seconds. The towel substrate was then placed in 600 ml beakers and boiled for 20 minutes to volatilize any organic contaminants. The resulting slurry was placed in a 5 l aquaria along with 250 ml of a Trout Chow/yeast suspension. The suspension was made by adding 6.3 g Trout Chow (Purina Trout Chow No. 3, Ralston-Purina Co., St. Louis, Missouri) and 2.7 g active dry yeast (Fleischmann's, Nabisco Brands, Inc., East Hanover, New Jersey) to 500 ml of water and blending for 5 minutes in a Waring blender. The suspension was allowed to settle for 1 hr, after which 300 ml of the supernatant were poured off and saved under refrigeration at 9°C. The remainder was discarded. The paper towel slurry was then aerated for 5-7 days to facilitate microbial colonization. About 1 l of the slurry was distributed to each of 21 l experimental tanks, and an additional 200 ml of Trout Chow/yeast suspension were added. Aquaria were aerated an additional 2-10 days before the experiments were begun. This variable time interval was due to the staggered set up of aquaria during a single experiment.

Aquaria were held under constant water temperature (23.5 °C) and a 14L:10D photoperiod inside a Sherer CEL 4-4 Environment Chamber. Cool-white fluorescent lights provided approximately 1600 lux of illumination at the water surface. All aquaria contained 7 l of either diluent water (carbon dechlorinated tap water) or a chromium (VI) solution. Four liters were removed from each aquarium every other day throughout the experiments and replaced with an appropriate fresh solution of chromium or tap water. A chromium stock solution was made weekly from potassium chromate and stored at 9°C for use in preparing experimental solutions. An additional 50 ml Trout Chow/yeast suspension was added to each aquarium after each renewal.

Water samples were taken both before and after renewal, filtered through a 0.45 µm membrane, and preserved with metal-free HNO<sub>3</sub> acid. Water samples were analyzed for chromium using a Perkin-Elmer (model 460) Atomic Absorption Spectrophotometer. Diluent water was measured weekly for hardness, alkalinity, pH, and conductivity. Oxygen concentration and pH were measured periodically in the aquaria.

Egg masses were removed from stock culture tanks. The number of eggs in each mass was counted using a dissecting microscope. Egg masses were transferred to petri plates containing diluent water until introduction into experimen-

tal tanks. The number of masses placed in the 2 mg/l chromium aquaria was double that in controls to compensate for presumed mortality (extrapolated from 48 hr LC-50 values). All eggs used in experiments were <48 hr old. Because of limitations in egg production in stock cultures, the set up of all aquaria was staggered over two weeks.

Hatching of egg masses was complete 2-3 days after their introduction into aquaria. Remaining eggs were either counted under magnification or estimated as a percent of the total number of eggs in the original mass. Day 1 of the larval growth period was designated as the day on which approximately 50% of the eggs had hatched. Subsequently, subsamples of the larval population were removed along with the associated substrate at 7, 12, 17, and 22 days. Sample size ranged from twenty to thirty-five larvae. Larvae were picked from the substrate (with the aid of a dissecting microscope on the first two sampling dates) and sacrificed by freezing for a minimum of 2 hours at  $-1^{\circ}\text{C}$ . Freezing was used because it should result in less weight loss by dissolution of lipids than the more common method of sacrifice by immersion in  $100^{\circ}\text{C}$  water. Each larva was rinsed, measured for length with an ocular micrometer to the nearest 0.08 mm (1 unit of micrometer scale = 0.08 mm), and placed on a pre-weighed aluminum square. Length was measured from the

anterior end of the head capsule to the posterior end of the abdomen, excluding the anal gills. Larvae were then dried for 24 hrs. at 60°C, placed in a dessicator to cool to room temperature, and weighed to the nearest 1 ug on a Cahn Electrobalance. Growth curves were generated from these data.

Growth rates (G) were calculated for the exponential phase of each growth curve. Rates were calculated as the natural logarithm of the mean length (or dry weight) at Day 7 minus the mean length (or dry weight) at Day 1 divided by the time interval in days. Mean length and dry weight at Day 1 were established by measuring twenty larvae randomly chosen from three stock culture egg masses. Mean length and dry weight of these animals was 0.84 mm and 0.001 mg, respectively.

To study the reproductive success of Chironomus under chronic chromium exposure, adults were allowed to emerge from the same populations used for growth experiments. Adults were sexed, counted, and moved to separate 5 l aquaria covered with netting and containing a 10 cm culture dish with water for oviposition. When daily emergence declined to 1 or 2 adults, the substrate was searched for remaining larvae and pupae. These animals were counted and the number recorded for later calculation of percent survival for the population. In one set of experiments, adults were allowed

to mate and oviposit in the original test aquarium to ascertain whether the transfer process (which included aspiration into a 5 mm bore glass tube) damaged adults and inhibited mating. Dead adults were collected from mating cages, dried for 24 hrs. at 60°C, cooled to room temperature in a dessicator and weighed to the nearest 1 ug on a Cahn Electrobalance.

Egg masses laid in the culture dish were removed daily and counted. Masses were then placed in small, individual glass petri plates containing dechlorinated tap water. Numbers of eggs that hatched and/or the number of remaining unhatched eggs were counted. Thus, any decreased egg viability was the result of first generation exposure rather than the direct effect of chromium on the eggs.

#### Metal Accumulation

Fourth instar larvae, taken from control and treatment in Experiment II, soon after the first emergence of adults, were measured for chromium accumulation. Animals were picked from the substrate and frozen; they were then thawed, rinsed in dilute metal-free HNO<sub>3</sub> to remove adsorbed chromium, and placed on individual acid washed glass coverslips. After drying at 60°C for 24 hrs. and cooling to room temperature in a dessicator, larvae were removed from the cover-

slips and weighed to the nearest 1 ug on a Cahn Electrobalance. Single larvae were wet-digested using metal-free HNO<sub>3</sub> (EPA 1979). Samples were made up to 5 ml with glass distilled water and analyzed on a Perkin-Elmer Atomic Absorption Spectrophotometer with a graphite furnace. Three replicate injections were made and averaged to the nearest 1 ug/l.

Male and female adults collected after mating and oviposition were dried, weighed, and wet-digested using the same method as for the larvae. Samples were made up to a 5 ml volume and analyzed using the graphite furnace.

#### Lipid Content

Lipid content of pupae was measured as a loss in dry body weight after a chloroform-methanol extraction. Egg masses were introduced into 5 l glass aquaria containing either 0.5 mg/l chromium or dechlorinated tap water. A paper towel substrate was provided and 50% of the water was renewed every other day to maintain concentrations of chromium (VI). A Trout Chow/yeast suspension was added to the aquaria at the rate of 20 ml every 2 days.

Fourth instar larvae were isolated in separate aquaria after 16-20 days and observed every 12 hours for initiation of the pupal stage. Pupae were removed when found and their

sex determined by microscopic observation of genitalia. Only females were used in lipid analysis because of their larger size and the greater importance of lipids for egg production and reproductive success.

Prior to removal of pupae for analysis, 2 mm bore glass tubes, 15 mm in length, were stuffed with glass wool plugs to one-half their length. Tubes were then soaked for 24 hrs in a 2:1 chloroform-methanol solution with occasional gentle shaking to loosen small broken pieces of glass wool. Tubes were removed with stainless steel forceps and placed in acetone rinsed vials. The tubes were then dried at 60°C for 24 hrs. The vials were sealed with plastic caps, cooled to room temperature, and, finally, removed from the vials to be weighed to the nearest 1 ug on a Cahn Electrobalance. Tubes were handled with acetone cleaned stainless steel forceps at all times.

Female pupae were stripped of their partially shed larval exuvia, rinsed in distilled water and frozen for 10 minutes. Pupae were then placed in individual, pre-weighed, plugged tubes and dried at 60°C for 24 hrs. The tubes were cooled to room temperature in a dessicator and weighed again with the dry animal inside.

Extraction of total tissue lipids was done with a drip system diagrammed in Figure 1, modified from Buikema (1970). The reservoir contained 150 ml of a 2:1 chloroform-methanol

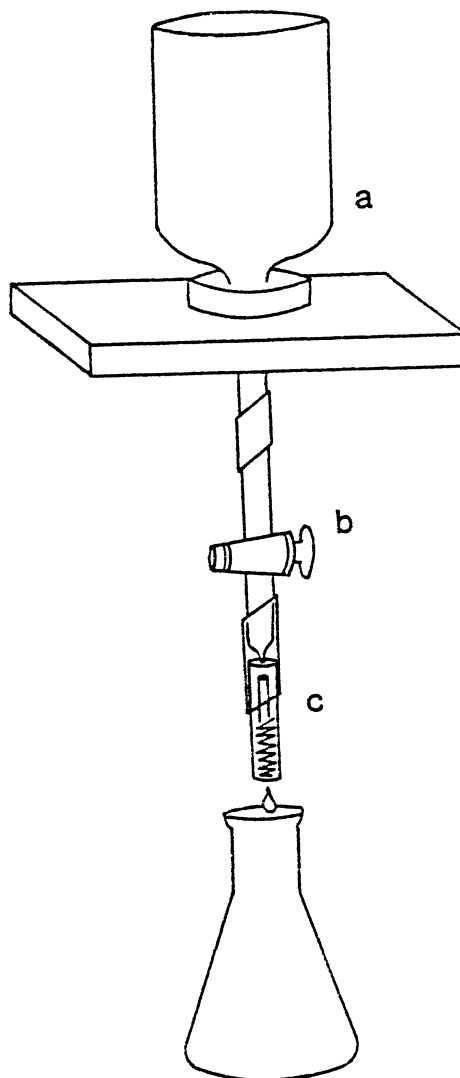


Figure 1. Lipid extraction system for individual Chironomus pupae. Components are a) a reservoir for chloroform-methanol solution, b) a teflon or glass stopcock for adjusting drip rate and c) a small glass tube containing pupa placed inside a larger, glass wool stoppered tube.

solution (Folch et al 1957) which was dripped slowly through the glass tube containing the pupae. Tubes were dried at 60°C, cooled to room temperature in a dessicator, and reweighed on the Electrobalance. The loss in weight between pre- and post-extraction weighings was considered to be the dry weight of lipids. The lipid content of pupae was then calculated as a percentage of lean weight (final weight after extraction minus the weight of the glass tube alone). Control tubes, not containing pupae, were run to check for problems in weighing, handling, and extraction procedures.

#### Direct Effect of Chromium on Egg Hatching

Masses were removed from stock culture tanks and split into either two or three approximately equal parts using a dissecting scalpel. Numbers of eggs in each section were counted and the sections were randomly placed into a 0, 2, or 5 mg/l chromium solution. Sections were isolated within individual egg cups constructed from a single 55 mm glass petri plate with a Nitex screen collar affixed with silicone sealer. When all hatching was complete (i.e., no more developing eggs were present), unhatched eggs were counted and a "percent hatching" value was calculated. Paired comparison of egg sections from a single egg mass were necessary because of the large natural variation in hatching rates among masses.

## RESULTS

### Chromium Concentrations

Chromium concentrations within experimental aquaria declined over the 48 hours between partial renewals of solutions. Average concentrations of dissolved chromium were calculated from samples taken before and after each renewal. Growth Experiments I and II with nominal concentrations of 2 mg/l chromium had averaged measured concentrations of 2.06 and 1.83 mg/l of dissolved chromium, respectively. Measured concentrations in Experiment III averaged 0.335 mg/l dissolved chromium; the nominal concentration was 0.50 mg/l (Table 1). Dechlorinated tap water used for both diluent water and the control was checked periodically for dissolved chromium and contained from 24 to less than 5 ug/l. Hardness, alkalinity, conductivity, and pH of tap water remained fairly stable throughout all three experiments (Table 2). Oxygen content in aquaria averaged 6.7 ppm with no single measurement below 6.1 ppm; the pH ranged from 6.9 to 8.0.

Table 1. Mean dissolved chromium concentrations with standard deviations, as measured by atomic absorption spectrophotometry.

Experiment	Nominal Conc'n (mg/l)	Replicate	Mean Conc'n (mg/l)	Standard Deviation	Number of Samples
I	2	1	2.06	0.22	30
	5	1	5.72	0.25	12
II	2	1	1.82	0.36	26
	2	2	1.84	0.22	24
III	0.5	1	0.32	0.24	24
	0.5	2	0.35	0.19	28

Table 2. Mean water chemistry parameters for dechlorinated tapwater used as the diluent for all experiments.

Parameter	Mean	Standard Deviation
Hardness (as mg/l CaCO <sub>3</sub> )	63	8
Alkalinity (in mg/l)	34	8
pH (units)	7.6	(7.30, 7.67) <sup>1</sup>
Conductivity (in umhos)	0.140	0.009

1. 95% confidence interval

### Growth and Reproduction Experiments

Three growth experiments were conducted, two of which yielded information about reproductive performance of adults. These experiments will be referred to as growth Experiments I, II, and III. Experiment I involved two lethal concentrations of hexavalent chromium (2 and 5 mg/l), while Experiment II was conducted with only one lethal dosage (2 mg/l). In Experiment III chromium concentrations were sub-lethal (0.5 mg/l). Figures 2-7 show graphically the growth of larvae in Experiments I, II and III in terms of mean dry weight and mean length for each sampling date.

Simple comparison of means and 95% confidence intervals around points was used to interpret growth curves. In Experiment I treatment larvae (2 mg/l) had an initial growth rate greater than controls; treatment larvae were significantly longer at Day 12 than larvae from the second control replicate (Figure 2). The first replicate control population was sampled at slightly different intervals, but appears to be smaller in length than treatment larvae through Day 15. After Day 15 larvae removed from either treatment or control were not significantly different in length. A single replicate population was exposed to 5 mg/l chromium and sampled at Day 7. Only six larvae were found; they were significantly shorter than control larvae.

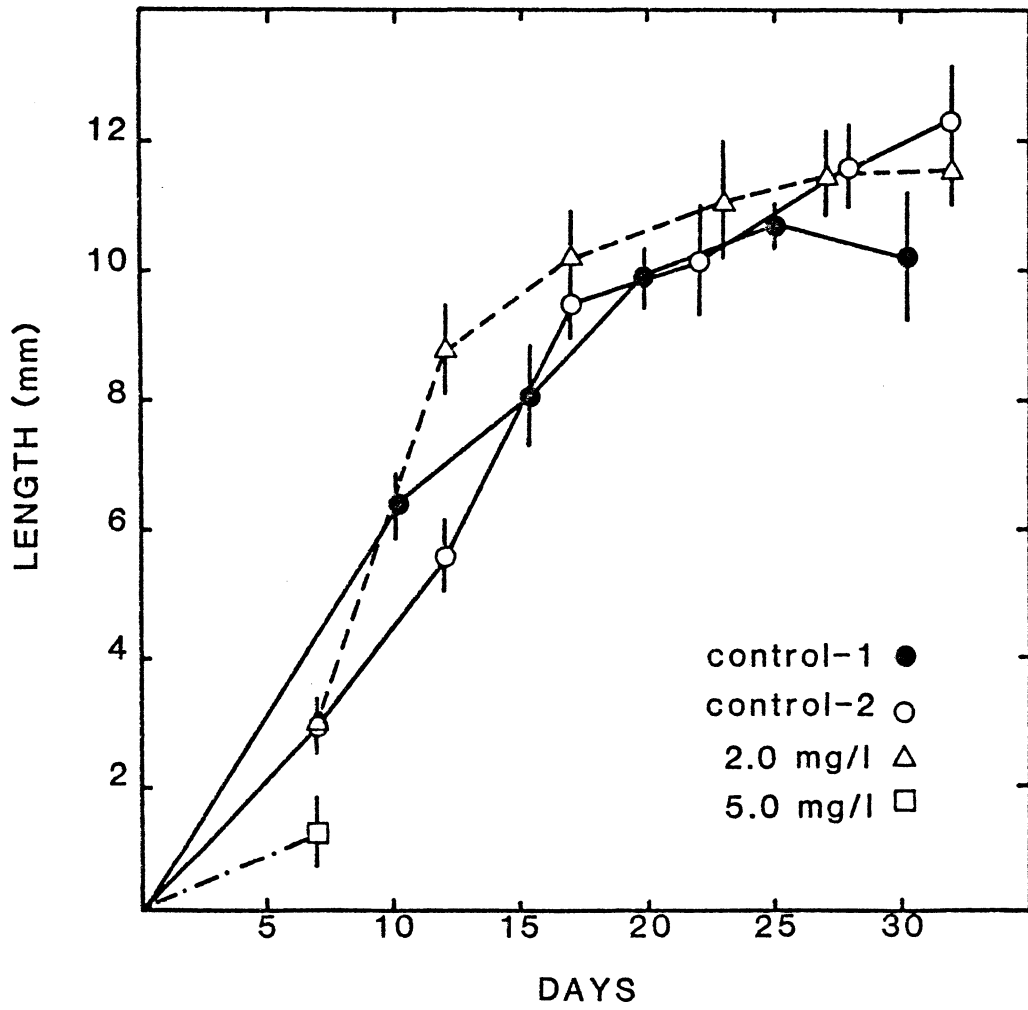


Figure 2. Growth curves for Experiment I with mean length plotted against days. Vertical bars represent 95% confidence intervals for each mean.

Comparison of weight increases in Experiment I was made difficult by the lack of confidence intervals (Figure 3). Two sampling dates are also displaced, limiting the number of points that could be compared statistically. The trend of greater dry weights for the treatment population, however, is still apparent at Day 12. Final weights were similar for both control and treatment populations.

Experiment II growth curves were quite different than those in Experiment I although a similar chromium concentration was used. In Experiment II control populations grew faster and attained greater length than treatment populations (Figure 4). By Day 7, control larvae were significantly longer than larvae exposed to chromium (2 mg/l) and remained longer in size for the following three sampling dates. Mean lengths for control and treatment populations were not equivalent on the last sampling date.

Weight comparisons were more difficult to make in Experiment II due to the small size of animals; it was necessary to pool individuals for weighing and no standard deviation could be determined. Although 95% confidence intervals are lacking for many points (Figure 5), the trend is obviously the same as that seen by examining increases in length. Control populations gained weight faster than those treated to chromium, and final weights were greater in controls than in treatment populations.

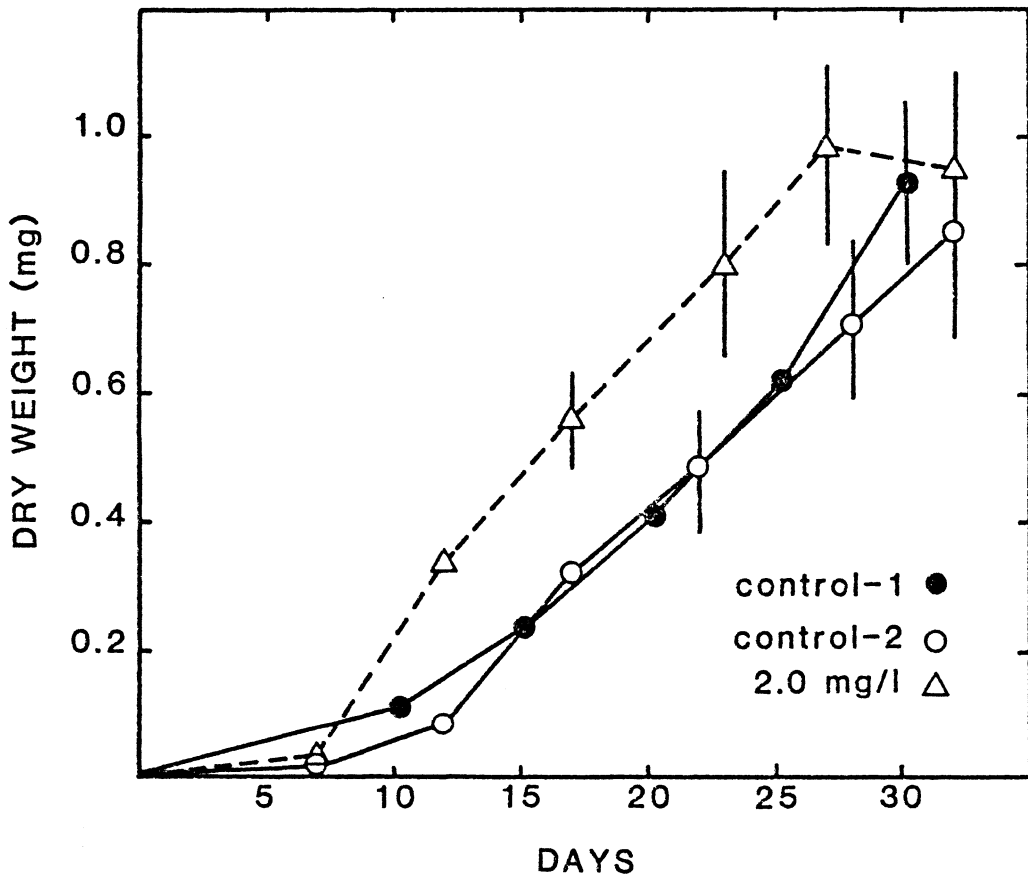


Figure 3. Growth curves for Experiment I with mean dry weight plotted against days. Vertical bars represent 95% confidence intervals for each mean.

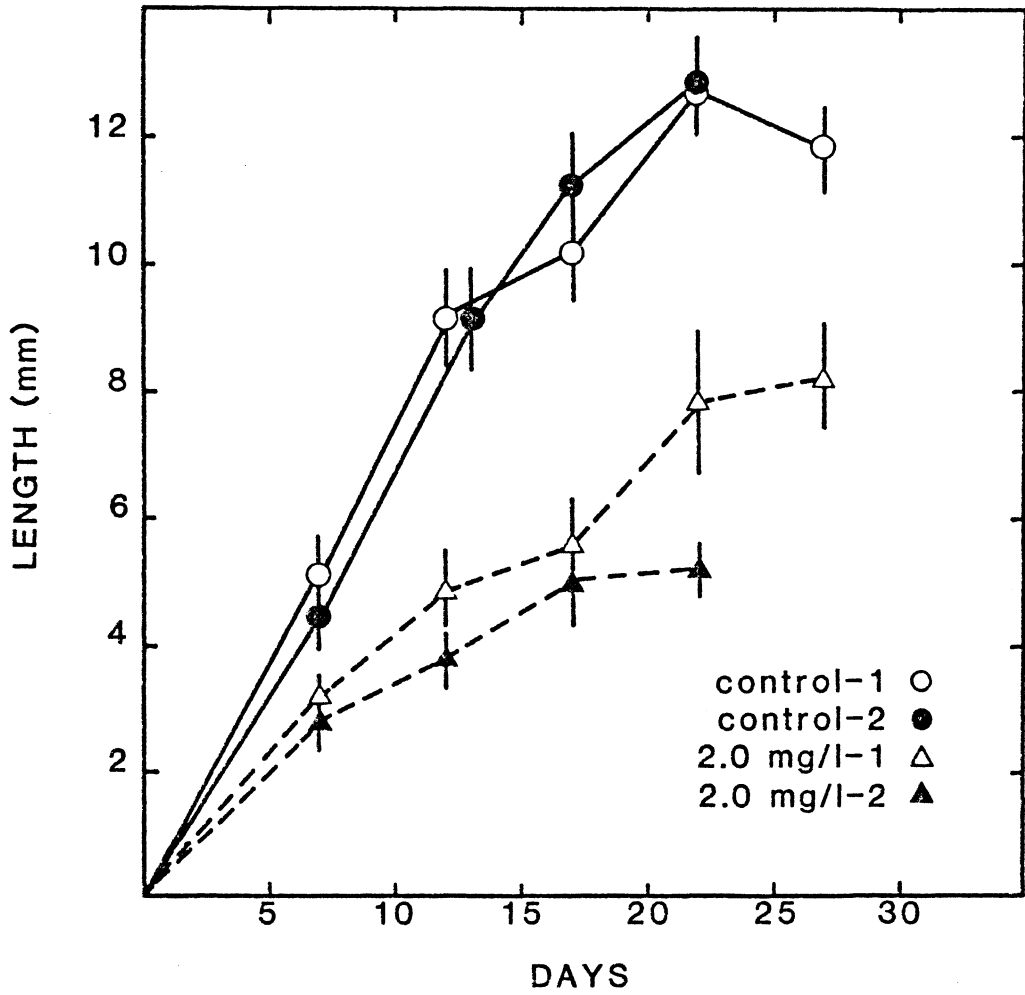


Figure 4. Growth curves for Experiment II with mean length plotted against days. Vertical bars represent 95% confidence intervals for each mean.

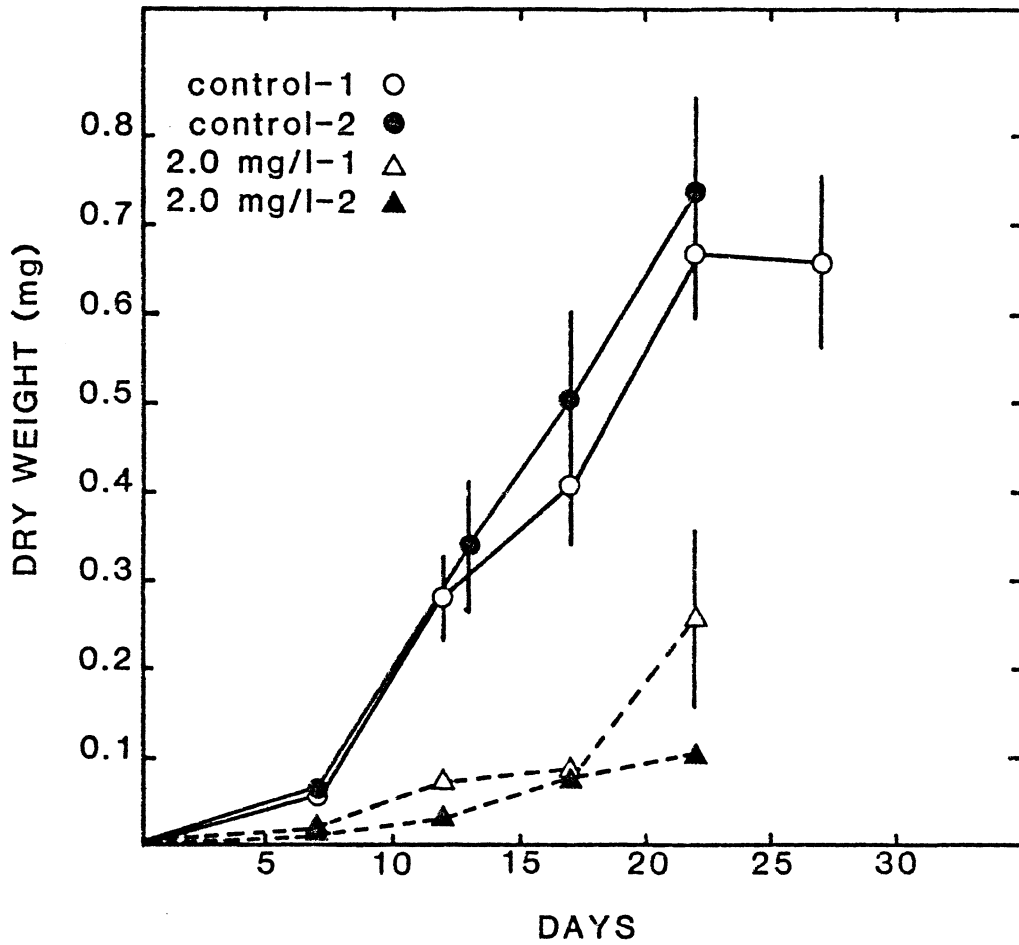


Figure 5. Growth curves for Experiment II with mean dry weight plotted against days. Vertical bars represent 95% confidence intervals for each mean.

In Experiment III the first control replicate (0.5 mg/l) was excluded from this particular analysis because of significantly retarded growth. Increases in length of treatment larvae were equivalent within replicates, but were significantly larger than the controls at Day 7 (Figure 6). After Day 12 control larvae could not be distinguished by length from treatment larvae.

Growth curves for Experiment III based on dry weight (Figure 7), did not show good agreement between replicate treatment populations, though, both contained larvae significantly heavier than the second control replicate. By Day 12 replicate treatment populations had attained equivalent mean dry weights, while remaining significantly heavier than controls.

Growth rates (G), in terms of increase in length, ranged from 0.13 to 0.28 ln mm/day in control populations, while treatment populations (Experiments I and III combined) ranged from 0.22 to 0.33 ln mm/day (Table 3). Using weight as the growth parameter, G ranged from 0.35 to 0.68 ln mg dry wt/day for control populations. Values for exposed populations ranged from 0.51 to 0.82 ln mg dry wt/day. Growth rates were not significantly different between treatment and control due to high variability.

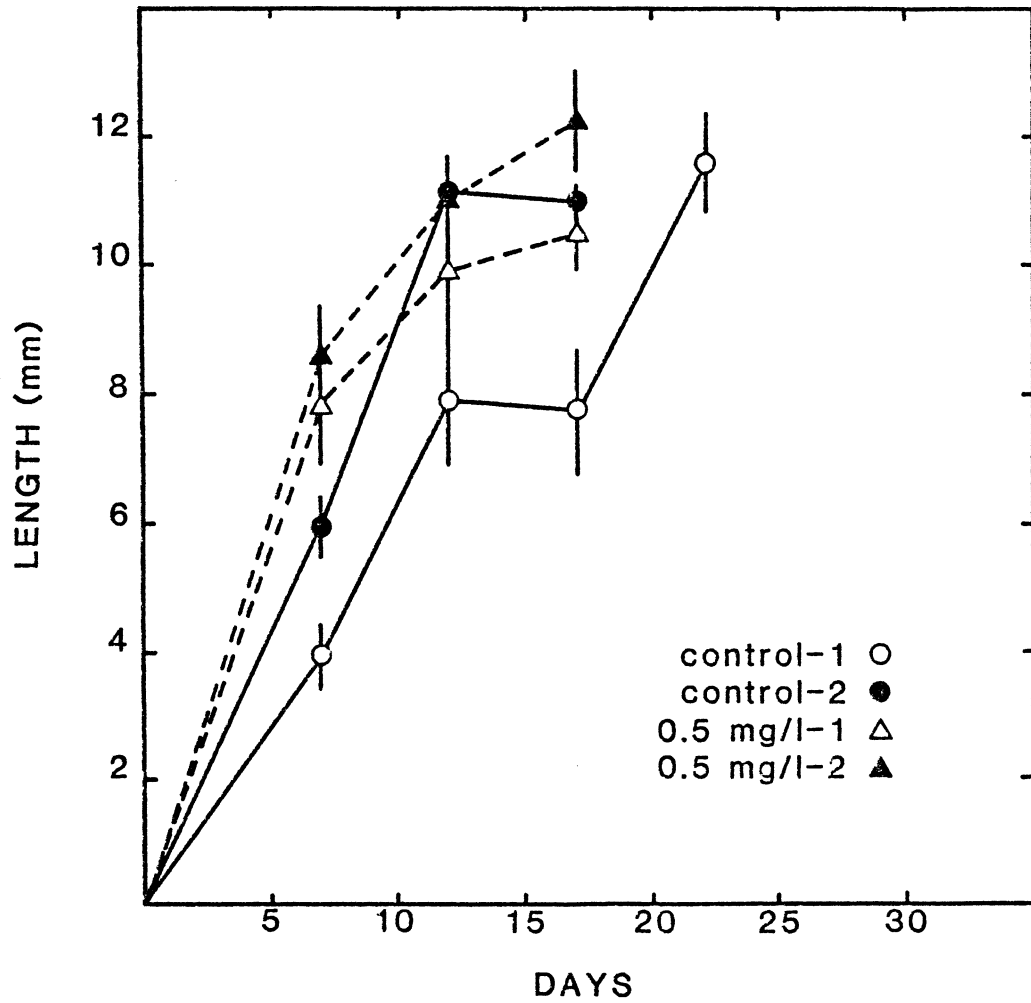


Figure 6. Growth curves for Experiment III with mean length plotted against days. Vertical bars represent .95% confidence intervals for each mean.

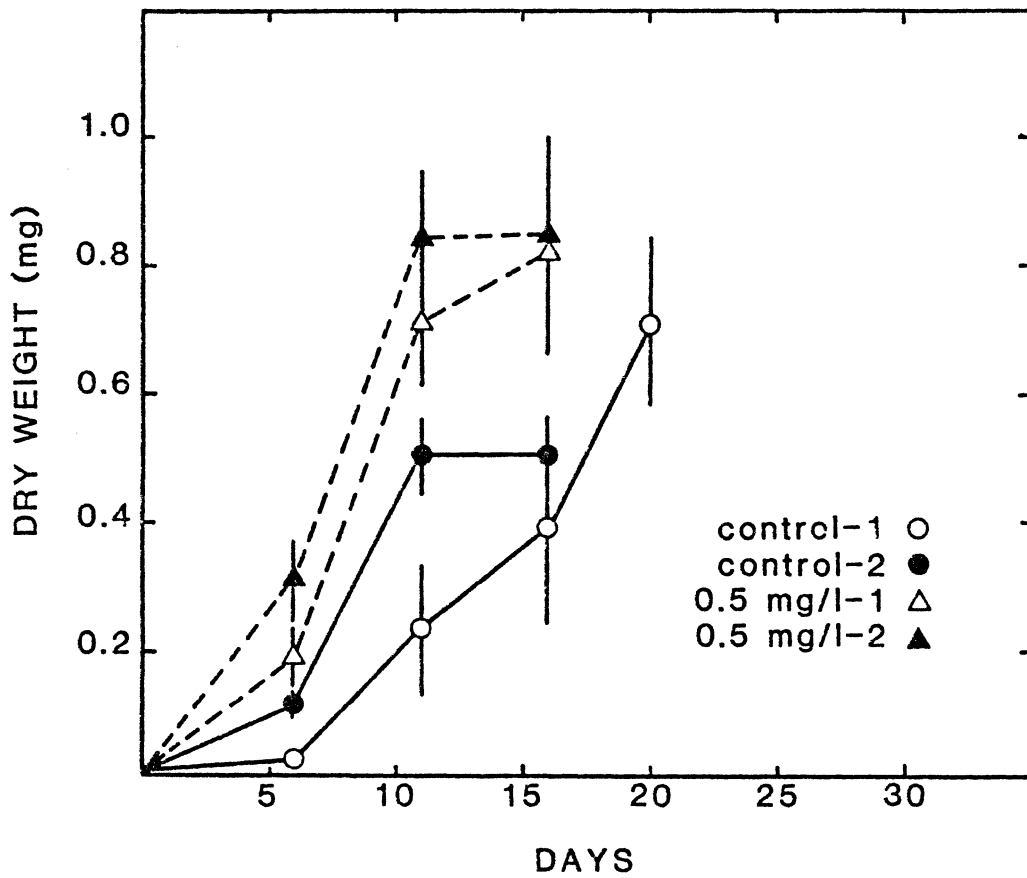


Figure 7. Growth curves for Experiment III with mean dry weight plotted against days. Vertical bars represent 95% confidence intervals for each mean.

Table 3. Growth rates (G) for larvae during the exponential phase of growth.

Experiment	Concentration (mg/l)	Rep.	Growth Period	G (ln mm/day)	G (ln mg/day)
I	Control	1	Day1-10	0.20	0.48
	Control	2	Day7-12	0.13	0.35
	2	1	"	0.22	0.51
II	Control	1	Day1-7	0.25	0.58
	Control	2	"	0.24	0.58
	2	1	"	0.20	0.44
	2	2	"	0.17	0.40
III	Control <sup>1</sup>	1	"	0.22	0.47
	Control	2	"	0.28	0.68
	0.5	1	"	0.32	0.75
	0.5	2	"	0.33	0.82

1. Anomalous control with poor survival.

The number of days to first adult emergence from Experiments I and III was inversely related to chromium concentration (Table 4, Figure 8). Number of days to first emergence was plotted as a percentage of the corresponding control because replicability was not good between experiments. Growth Experiment II was not included in these calculations.

Mean dry weight of female adults collected after death in Experiment III was 0.424 mg. Male adults averaged 0.278 mg dry weight. Statistical analysis by 2-way ANOVA (SAS:GLM) revealed no significant difference in mean weight between members of the same sex emerging from control and treatment aquaria ( $p=0.46$ ). Adults did differ significantly ( $p=.0001$ ) on the basis of sex; females were approximately 50% heavier than males. As in Experiment III, adults from Experiment I showed no significant effect of chromium exposure on dry weight. Females averaged 0.522 mg and males 0.259 mg dry weight; females averaged slightly heavier than those in Experiment III.

Reproduction was monitored in Experiments I and III. An average of 0.33 egg masses (std. dev.=0.09) per female was produced, which is slightly below the average 0.41 egg

Table 4. The number of days to first adult emergence from aquaria as a percentage of the number of days to first emergence from the corresponding control.

Experiment	Concentration (mg/l)	Replicate	Days to First Emergence	Percentage of Control
I	Control	1	20	1.00
	Control	2	20	1.00
	2	1	15	0.75
III	Control	1	16	1.00
	0.5	1	13	0.81
	Control	2	14	1.00
	0.5	2	12	0.86

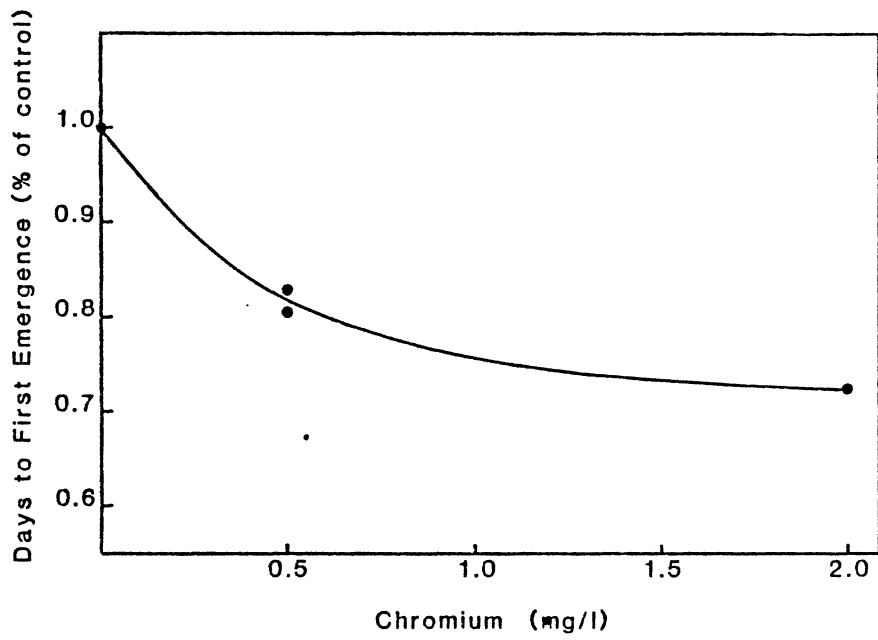


Figure 8. The number of days to first emergence in control and exposed populations, as a percentage of the corresponding control, plotted against nominal chromium concentration.

masses per female observed by Lee et al. (1980) in laboratory studies. The mean size of egg masses was 270 eggs (std. dev.=69) in Experiment I and 220 eggs per mass (std. dev.=109) in Experiment III. No significant differences in the size of egg masses were detected among groups of reproducing adults in a single experiment. Variability of egg mass size was relatively high within groups.

Under all experimental conditions (both control and treatment) a large percentage (mean=51%, std. dev.=21%) of egg masses showed no hatching. In Experiment III the percentage of fertile egg masses was not increased in replicate populations in which adults were not transferred to swarming cages. Infertile egg masses were not included in the calculation of hatching success or reproductive success. Thus, mating success does not bias the data, and conclusions are conservative. The hatching success of individual egg masses was compared between control and treatment populations. No significant differences were found except in Experiment I where hatching success was significantly lower for the population exposed to 2 mg/l chromium when compared to controls (Table 5).

Reproductive success, as measured by the number of larvae produced per adult female, was inversely related to chromium concentration (Table 6, Figure 9). Only in

Table 5. Mean hatching rate of fertile egg masses.

Experiment	Concentration (mg/l)	Replicate	Mean Hatching Rate
I	Control	1	.793
	Control	2	.597
	2	1	.020 <sup>1</sup>
II	Control	1	.517
	Control	2	.424
III	Control	1	.486
	Control	2	.545
	0.5	1	.380
	0.5	2	.345

1. Significantly different hatching rate ( $p=.0001$ ).

Table 6. Reproductive success of adults as measured by the number of second generation larvae hatching per female adult of the first, or exposed generation.

Experiment	Concentration (mg/l)	Replicate	No. Larvae	No. Females	Reproductive Success
I	Control	1	3858	129	29.9
	Control	2	1791	114	15.7
	2	1	35	187	0.2
III	Control	1	955	33	28.9
	0.5	1	1885	155	12.2

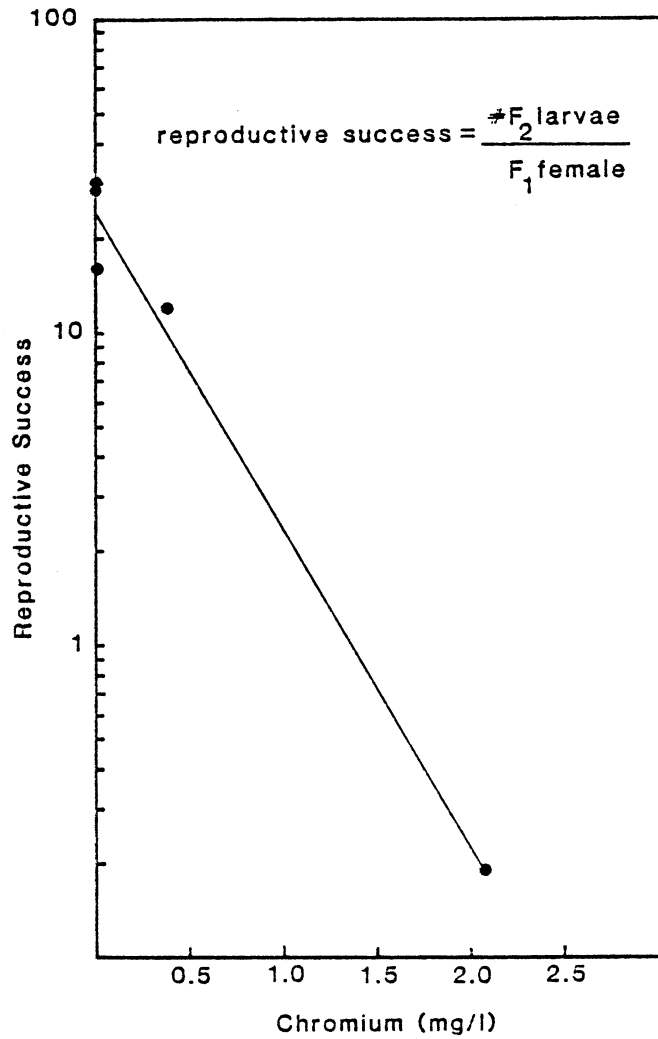


Figure 9. Reproductive success of exposed and control populations plotted against mean measured chromium concentration.

Experiments I and III were sufficient data collected for calculation of this index.

Net reproductive rate ( $R_0$ ) was calculated for control and exposed populations in Experiment I and III. Net reproductive rate equals the number of female offspring produced by an average adult female during her lifetime (Wilson and Bossert 1971); it is a measure that integrates both generation time and reproductive success. Reproductive success was calculated as before, but only production of female larvae was considered (it was assumed that half of larvae produced were female based on the approximately 1:1 ratio of male to female adults). Average generation time was calculated as the number of days to emergence of half of the adult females, plus one day for mating and ovipositing.  $R_0$  for the control population was 14.47, while  $R_0$  equaled 6.08 for the population exposed to 0.5 mg/l chromium. In Experiment I at 2 mg/l  $R_0$  equaled 0.18, while control values were 14.95 and 7.35 (Table 7).

Intrinsic growth rates (Table 7) were calculated for each population using the equation  $b_0 - d_0 = r$  (Wilson and Bossert 1971). The intrinsic growth rate of a population simply adds mortality to the information considered in  $R_0$ . The birth rate ( $b_0$ ) was calculated by dividing  $R_0$  by the average generation time. The death rate ( $d_0$ ) was

Table 7. Net reproductive rates ( $R_0$ ) and birth (b) and death (d) rates used to calculate intrinsic growth rates (r) for larval populations.

Experiment	Replicate	$R_0$	Average Generation Time	$b_0$	$d_0$	r
I	Control/1	14.95	33	.453	.009	.444
	Control/2	7.35	35	.210	.009	.201
	2 mg/l/1	0.18	23	.008	.027	-.019
III	Control/1	14.47	21	.689	.014	.675
	0.5 mg/l/1	6.08	16	.380	.019	.361

calculated by the dividing the percentage mortality (derived from Table 8) of the population by the average generation time. At lethal levels (2 mg/l) the r value is negative.

Survival was calculated by subtracting from the initial population size the number of larvae and adults removed from the aquarium and the number of remaining larvae and pupae. Survival of larval populations exposed to 2 mg/l chromium (VI) was approximately 60% of the survival rate for three replicate controls. Survival at 0.5 mg/l was equal to control survival rates, which averaged 62% of the original number of larvae hatching into aquaria (Table 8).

Table 8. Percent survival of hatched larvae over the growth period from Day 2 to adult emergence.

Experiment	Chromium (VI) Concentration (mg/l)			
	Control	0.5	2.0	5.0
I	.57	-	.32	0
	.68	-	-	-
II	.74	-	.45	-
	.49	-	.33	-
III	.20 <sup>1</sup>	.63	-	-
	- <sup>2</sup>	-	-	-
Mean	.62	.63 <sup>3</sup>	.37	0
% of Control	1.0	1.0	.60	0

1. Abnormally low survival rate, not included in mean.
2. Survival not measured in second replicates of control and 0.5 mg/l.
3. Not significantly different from control survival values.

### Accumulation

Fourth instar control and treatment (2 mg/l) larvae sampled during Experiment II showed significant differences in chromium accumulation (Student's t-test,  $p=.0005$ ) (Table 9). In Experiment I treated male adults had significantly higher ( $p=.04$ ) chromium body burdens than males from controls. Female adults from Experiment I did not contain significantly different amounts of chromium in their dry tissues ( $p=.97$ ). The concentration of chromium per unit of dry weight increased from larva to adult for both control and treatment groups, probably due to a reduction in biomass between these stages without a concomitant loss of chromium.

### Lipid Content

Analysis of lipid data revealed a significant difference (Student's t-test,  $p=.0001$ ) in lipid content of control and treatment larvae (Table 10). Control pupae contained an average of 0.116 mg lipid/mg lean dry weight, while treatment pupae averaged 0.222 mg lipid/mg lean dry weight. Extraction efficiency was 95 to 98% with the technique used.

Table 9. Mean chromium accumulation in the bodies of fourth instar larvae and adults with standard deviation in parentheses. Sample size was ten for all analyses except adult females, with a sample size of twenty.

	Control	Chromium (2 mg/l)
Larvae	0.335 <sup>1</sup> (0.310)	1.081 <sup>2</sup> (0.467)
Adult Females	1.127 (0.559)	1.133 (0.523)
Adult Males	1.250 (0.309)	1.644 <sup>3</sup> (0.472)

1. ug Cr/mg dry weight.

2. Significantly different from control (p=.0005).

3. Significantly different from control (p=.0404).

Table 10. Lipid content of female pupae as a percentage of lean dry weight.

	Control	Chromium (0.5 mg/l)
Mean	0.116 <sup>1</sup>	0.222 <sup>2</sup>
Standard Deviation	0.086	0.088
Range	(0.019, 0.352)	(0.137, 0.437)
Sample Size	24	19

1. mg lipid/mg lean dry weight.

2. Significantly different from control ( $p=.0001$ ).

### Direct Effect of Chromium on Egg Hatching

Paired comparisons of egg sections from a single mass were tested using a paired t-test after angular transformation of the data (Sokal and Rohlf 1969). No significant differences in percent hatching were found between sections exposed to 2 mg/l or 5 mg/l and control water. A paired comparison of hatching in 2 mg/l and 5 mg/l did reveal significant differences ( $p=0.01$ ); hatching rate was higher in 5 mg/l. Mean hatching rates were found to increase in the order: 2 mg/l, control, 5 mg/l. The significant difference between hatching rates in 2 and 5 mg/l may be an artifact of sample size; only 15 paired comparisons were made.

### Head Capsule Abnormalities

In growth Experiment II abnormalities in head capsule shape were noted in both chromium-exposed populations. Approximately 4.5 and 9.6% of sampled larvae showed some degree of deformity in replicates 1 and 2, respectively. Deformity is due to incomplete shedding of the head capsule during molt to the next instar, with subsequent hardening of the new head capsule underneath (Figure 10). The old head capsule may or may not be shed after hardening of the new exoskeleton. The extent to which this deformity interferes with normal feeding is unknown but many dead larvae

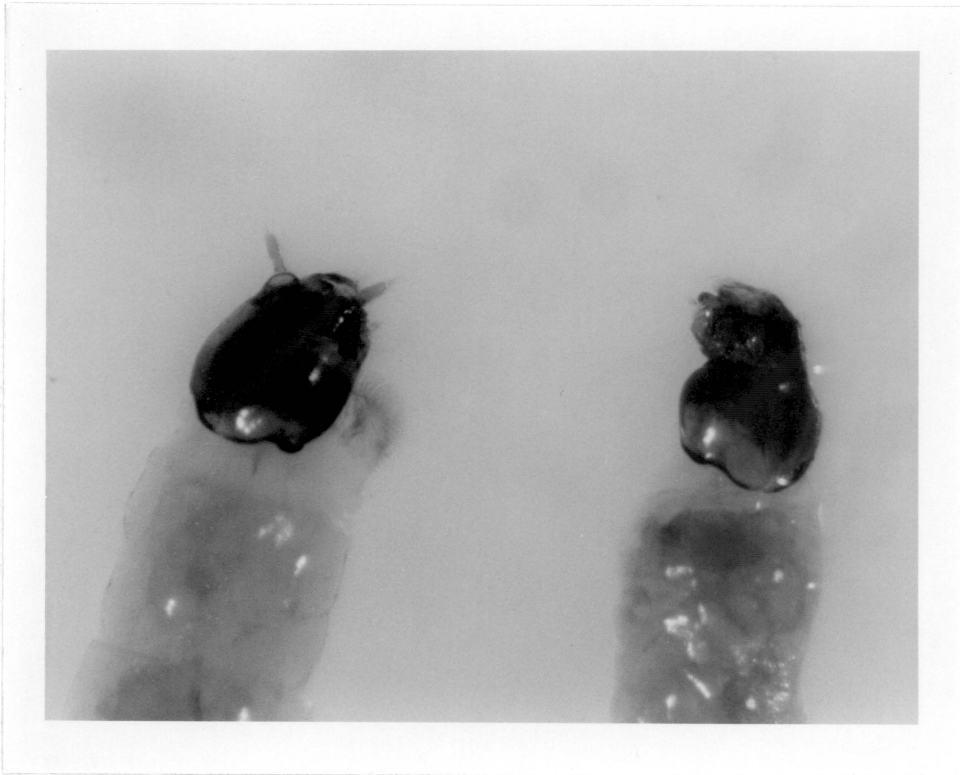


Figure 10. Head capsules of normal (left) and deformed (right) fourth instar larvae.

whose head capsules were severely deformed or still partially enclosed by the old head capsule were recovered from both chromium-treated aquaria.

## DISCUSSION

Stimulation of growth rate was observed in Chironomus riparius larvae at both lethal (2.0 mg/l) and sublethal (0.5 mg/l) concentrations of hexavalent chromium. Growth curves had a logistic shape characteristic of observations made over the entire lifetime of an organism or a single cohort (Warren 1971, Brody 1945). The steepest portion of the curve, exponential growth, becomes asymptotic as individuals reach mature size. It is during exponential growth that chromium stimulation is most apparent both graphically and by comparison of "G" values (Figures 2,3,6,7, Table 3). In Experiments I and III adults emerged from treatment aquaria from 2 to 5 days earlier than their respective controls. The consistency of this relationship is further evidence of the stimulatory effect of chromium, even at lethal levels.

The delay in stimulation seen in Experiment I (Days 7-12) as compared to the period of stimulated growth in Experiment III (Days 1-7) is probably due to the presence of moribund larvae at the higher, lethal concentration. During the period of Days 1-7, the most sensitive portion of the population was dying or had died; mortality of later instars

was probably much less significant because of their increased resistance to chromium toxicity (Niederlehner, unpublished data).

In both Experiments I and III, mean lengths of larvae following the exponential phase of growth (i.e., from Day 7 to Day 22 in III and from Day 12 to Day 27 in I) did not differ significantly within a sampling date (see 95% confidence interval for each point on Figures 2 and 6). Despite the higher initial growth rates in exposed populations, control larvae had a mean length similar to treatment larvae at the end of the experiments.

It is apparent from the growth curves that while larvae in Experiment III reached a similar final length, control animals did not attain the same final weight before pupation and emergence. After Day 7 the shapes of the curves are the same. Thus, differences in final larval weights are due to the significantly different means on Day 7, indicating the importance of early weight gains to molting and maturation. However, adult weights did not differ significantly between control and treatment populations in Experiment III. Differences in the weight of pupae were probably equalized during the intense metabolic activity of metamorphosis (Chapman 1971).

In Experiment I final weights were equivalent for control and treatment populations. Growth from Day 7 to 12 was faster for exposed larvae. From Day 12 to Day 32 the rate of weight increase of exposed larvae was similar to that of controls. Only on Day 32 did mean weights of sampled larvae overlap. Because control larvae began emerging 5 days later than exposed larvae, the weight at pupation was probably similar for both groups. These data provide evidence for a minimum weight requirement for the onset of metamorphosis.

As is true for many holometabolous insects, Chironomus riparius shows a dramatic increase in weight in its final instar without as rapid an increase in length. Much of its increase in "plumpness" in the fourth instar is due to maturation processes that will allow it to metamorphose, including the deposition of reserve materials as fat and glycogen. Because treatment larvae reached the fourth instar sooner than controls did (in some cases less than 7 days) they began to gain weight in a manner disproportionate to their increase in length. Control animals molted to the fourth instar somewhat later. At fourth instar control larvae were the same approximate length as exposed larvae, yet were lighter in weight. Physiological signals for initiation of metamorphosis are not well understood (Chapman 1971), but some minimum weight is required (Hilsenhoff 1966, Vannote

and Sweeney 1980). Factors acting on control larvae in Experiment III to induce pupation before gaining a similar amount of weight as exposed larvae are unknown.

In several studies involving mammals, fish, and various aquatic invertebrates, chromium (VI) has not proven to be a growth stimulant (Byerrum 1960, Trabalka and Gehrs 1977, Verriopoulos and Moraitou-Apostolopoulou 1981, Moraitou-Apostolopoulou and Verriopoulos 1982). Byerrum (1960) did report that rats receiving chronic, sublethal doses seemed to have a greater survival rate than controls. Female fathead minnows (Pimephales promelas) showed an initial depression in growth rate at sublethal chromium concentrations, yet at the end of chronic exposure they were similar in weight to controls (Pickering 1980). In the cases of increased rat longevity and compensatory increase in growth rate of fathead minnows it may be that low doses of chromium, an essential trace metal, provided "sufficient challenge" (Smyth 1967) to physiological systems. Adjustment to the challenge might allow the animals to surpass control groups in such parameters of health as growth rate or longevity. The mechanism for stimulation (or "challenge") of Chironomus riparius may have been through enzyme activation of catabolic pathways or increased effectiveness of energy storage.

Mortality at 2 mg/l chromium averaged 63% (or 40% of control mortality). The 48-hr LC-50 for first instar Chironomus riparius has been established as 31.5 mg/l chromium (VI) (Niederlehner, unpublished data) for the diluent water used in this study. Thus, the concentration of hexavalent chromium that causes approximately 40% mortality over a life time is only 6% of the 48-hr LC-50. The disparity between LC-50 values for 48 hour exposures and chronic exposure has been noted in several studies involving a variety of animals (e.g., Ruesink and Smith 1975, Spehar et al. 1978).

At 0.5 mg/l mortality was not appreciably different than control mortality. Average mortality in control populations agreed well with the mortality observed by Lee, Fullard, and Huntington (1980) in the same species. Lee et al. (1980) found survival to the adult stage was 23 to 55% of the number of original eggs. Average mortality from eggs to adults for control populations was 43% in this study. Biever (1968) found that the rearing temperatures for Chironomus that produced the fastest growth rate did not coincide with the optimal temperature for high survival. Nebeker (1971) reported similar findings with Pteronarcys dorsata. Thus, the experimental temperature regime for

Chironomus riparius may be partially or wholly responsible for its poor survival rate.

Hatching success of egg masses from stock culture tanks was not reduced by concentrations lethal to larvae (2 and 5 mg/l). Other investigators have reported similar insensitivity of this life stage to toxicants in aquatic insects (e.g., Townsend et al. 1981, Gauss et al. 1983). Gauss et al. reported no reduction in hatching of Chironomus tentans egg masses until a copper concentration of 5 mg/l was reached. Water hardening, a process of water uptake by the gelatinous sheath protecting the eggs, may reduce sensitivity. Water hardening is not complete in Chironomus riparius before 24 hours, making the youngest eggs, theoretically, the most sensitive. No reduction in hatching was seen in egg masses less than 24 hours old when exposed to chromium. Results of this study indicate that production in Chironomus riparius is not limited by direct exposure of eggs to chromium, even at concentrations lethal to larvae.

The similarity between adult female weights from control and chromium-treated populations within a single experiment may explain their equivalent fecundity (number of eggs per mass). In most arthropods fecundity is generally correlated with the mass of the female (Tolba and Holdich 1981, Livdahl 1982, Steinwascher 1982). Female dry weights aver-

aged 0.098 mg heavier in Experiment I than in Experiment III, possibly explaining their higher fecundity.

In all experiments hatching success of individual masses was quite variable; an average of 51% did not hatch. The lack of potential mates was not an obstacle to fertilization; the male to female ratio was approximately 1:1 and emergence of males and females was relatively synchronous. Caspary and Downe (1971) found that swarming of 10 Chironomus riparius adults could be prevented by reduction of vertical space to 4 cm or reduction of horizontal space to 4 cm. Swarming cages used in this study were considerably larger, i.e., a vertical dimension of 14 cm and a horizontal dimension of 38 cm. It was assumed that this was sufficient space for normal swarming activity. The percentage of non-hatching, presumably unfertilized eggs, was not successfully explained, and may be due to some inadequacy in swarming cages. The possibility of subtle damage to adults during their transfer from aquaria to cages was discounted because the percentage of non-hatching egg masses was not reduced when adults were not moved.

Reproductive success of control and treatment were first compared in terms of hatching rates for individual egg masses. A trend of decreasing hatching success with chromium exposure was observed within Experiments I and III, but

the reduction was significant only at concentrations of 2 mg/l. Taking a more "population-oriented" approach, reproductive success was measured as the number of second generation larvae hatching per first generation female adult. There are a limited number of data points in Figure 9, but it appears that an inverse relationship exists between chromium (VI) concentration and reproductive success for Chironomus.

Trabalka and Gehrs (1977) reported lowered reproduction in Daphnia magna exposed to chromium (VI), apparently due to decreased adult longevity. Hatakeyama and Yasuno (1981) found that copper reduced the number of eggs produced by a parthenogenic midge larva. This reduction was correlated with decreasing adult size. Hexavalent chromium did not inhibit egg production in Tisbe holthuriae (Copepoda)

(Verriopoulos and Moraitou-Apostolopoulou 1981). However, egg sac abortion increased significantly at 1 mg/l. The effect of chromium concentration on egg sac development in the copepod seems to parallel the lack of embryo development in egg masses of C. riparius in this study. Verriopoulos and Moraitou-Apostolopoulou (1981) also demonstrated lowered survival of second generation nauplii. It is possible that reduced survival or growth rates in second generation

Chironomus riparius larvae could seriously effect long-term population survival.

Net reproductive rate ( $R_0$ ) was calculated for all experimental populations because it incorporates both the growth rate and reproductive success into one measure. Net reproductive rate for the treatment population in Experiment III was much lower than that of the control population. However, its significance could not be established statistically for lack of sufficient data points. In Experiment I net reproductive rate was much lower for the treatment population than for controls; it is apparent that the increased growth rate of exposed animals at 2 mg/l was not sufficient to compensate for lowered reproductive success of adults.

It has not been determined whether  $R_0$  is a true measure of a population's success under natural conditions. There are no data for Chironomus to confirm a similar rate for succeeding generations under chronic exposure. The shorter generation time for treatment larvae might cause the population to become asynchronous with normal seasonal changes, especially where this normally multivoltine (Learner and Edwards 1966) species is restricted to fewer generations per year by food or temperature. Finally, the interaction of chromium with other natural or anthropogenic stresses may produce significantly different results than

those seen in this laboratory study. Experiment II, which will be discussed later, provides some evidence for this last point.

The intrinsic growth rate of a population incorporates both mortality and net reproductive rate. Its calculation at lethal concentrations of chromium (2 mg/l) produced a negative value, indicating a collapsing population. Because mortality is assumed to be equal in controls and at 0.5 mg/l chromium, a constant mortality rate of 38% was applied. Thus, at sublethal levels the calculation of  $r$  adds little information after  $R_0$  has been established.

The lipid content of female pupae was studied in an effort to elucidate the allocation of energy to growth and reserves. The fat body of most insects increases significantly in size with increasing age to metamorphosis. It may be completely dissolved in the pupal stage, especially in some higher Diptera (Wigglesworth 1972). The fat body also provides reserve material for egg production in females. In examining the increase in weight and length of Chironomus riparius during exposure to chromium it is apparent that weight increases in the fourth instar are not sacrificed to higher metabolic costs during this phase of growth. Lipid analysis confirmed that energy reserves were accumulated to a greater extent in treatment animals than in control ani-

mals. Thus, lack of sufficient energy reserves cannot explain lowered reproductive success of exposed animals. Similar increases in lipid content were reported by Thornton and Wilhm (1974) when Chironomus attenuatus was exposed to 600 mg/l NaCl over the period of larval development from egg to adult.

Many studies contribute information which may partially or completely explain both faster growth rates and larger fat accumulations in treatment larvae. Chromium (III) has been identified as a stimulant in the conversion of acetate to CO<sub>2</sub>, cholesterol, and fatty acids in rat livers in vitro (Curran 1954), it reportedly stimulates oxygen consumption in a succinic dehydrogenase-cytochrome system (Horecker et al. 1939), and has been shown to stimulate phosphoglucomutase (Strickland 1949). None of these effects are exclusive to chromium, but may result from activation by various metals (NAS 1974). Chromium, as an integral part of the digestive enzyme trypsin (NAS 1974) may increase assimilation of proteinaceous food. Finally, it has been established that chromium is a cofactor in the reaction of insulin with cell membrane (Mertz and Roginski 1963), increasing the entry rate of a sugar into fat tissue. Although this function of chromium is not immediately relevant to insect physiological processes, chromium may have an analogous role in the

removal of glucose from the haemolymph into the fat body for conversion to trehalose. All the above are important reactions in the biochemical processing and storage of high energy compounds, though, and may be responsible for significantly altering patterns of growth if stimulated. Many of these pathways exist in the fat body of insects (Wigglesworth 1972), providing for the immediate deposition of storage products.

Chromium accumulation in larvae and adults was measured and verified the actual assimilation of metal. Treatment larvae and male adults bioconcentrated chromium to a greater extent than controls, while analysis did not reveal any significantly greater accumulation in adult females from a treatment population. Treatment larvae contained quantities of chromium one order of magnitude lower than those found by Cherry et al. (1979) in chironomids sampled from an ash drainage system with sediment chromium concentrations of 38.4 ppm. Cover and Whilm (1982) analyzed Chironomus riparius larvae from a reservoir for zinc and iron (both essential trace metals like chromium) and reported metal levels within the same range (0.5-1.5 mg/g) as found in this study. Anderson et al. (1978) reported no significant difference in copper and zinc concentrations between chironomid larvae collected at two sites with different amounts of sed-

iment metal contamination. The authors concluded that at sublethal concentrations the animals were able to physiologically control body burdens of essential trace elements. Metal accumulation in this study was the result of exposure to lethal levels (2 mg/l) which may overwhelm the normal mechanisms for regulating chromium concentration in the body.

Absorption of chromium through the gastrointestinal tract of mammals is minimal for both trivalent and hexavalent forms (Luckey et al. 1975). Byerrum (1960) found that about 6% of ingested hexavalent chromium was absorbed in rats. Most studies reviewed by the National Academy of Sciences (1974) reported absorption of much less than 50% of ingested chromium. Dual-tracer studies of assimilation rates (e.g., Calow and Fletcher 1972) depend to a great extent on the fact that the isotope  $\text{Cr}^{51}$  is absorbed in only small amounts. However, hexavalent chromium is easily absorbed across biological membranes when introduced intravenously (NAS 1974). Hexavalent chromium may enter the body through surface absorption and reduction to a trivalent state by binding with proteins (NAS 1974). It is thought that all chromium in biological materials is in the reduced, trivalent form (Mertz 1969). Transfer within the body is by binding to blood proteins such as transferrin, the iron-

carrying protein or, in man, the albumin fraction. It seems likely that most chromium entering the body of Chironomus larvae is through absorption across the body surface rather than through the assimilation of ingested chromium. Most chromium bound to the surface of food particles is likely to be in the trivalent form (Schroeder and Lee 1974) and not easily absorbed across the gut wall.

After chromium enters the body, it is accumulated in various parts of mammalian experimental subjects, showing high affinity for the spleen, kidney, testis, and epididymis in the rat (Hopkins 1965). The location and extent of accumulation differed with age of the animals, mature rats showing greater retention in testis and epididymis. Byerrum (1960) reported accumulation of hexavalent chromium to be most persistent in spleen, liver, and kidney of rats, and in the liver and kidney of dogs. Van der Putte et al. (1981) found that chromium accumulated in the kidney, liver, and the nuclear fraction of gills of rainbow trout (Salmo gairdneri). Removal of chromium from the body is primarily through urine (Hopkins 1965, Byerrum 1960) with small amounts being found in feces, apparently after reabsorption from the body (Hopkins 1965).

Hexavalent chromium does not bind strongly to proteins until it is reduced to the trivalent form. In that state it

has been shown to stabilize and cross-link protein conformation, the basis for its use in the tanning process (NAS 1974). As reported in their review of chromium, the National Academy of Sciences (1974) states that methionine, cystine, lactic acid, hemoglobin, and globulins have all been shown to bind with trivalent chromium.

Chromium has also been found in association with nucleic acids. Hexavalent chromium reacts strongly with nucleic acids in vitro, although the reaction probably involves an initial reduction to the trivalent form (NAS 1974). Highly purified fractions of beef liver contain from 50-140 ug. of chromium per gram of tissue (Wacker and Vallee 1959). The reason for chromium's association with RNA is unknown, but it is thought to stabilize secondary structure (Luckey et al. 1975).

The lack of significantly higher amounts of chromium in adult females from chromium-treated tanks may be the result of either protein-metal or nucleic acid-metal interactions during egg formation. During yolk synthesis, special proteins in the haemolymph of the female are absorbed by the oocyte (Chapman 1971). This process provides a potential means for scavenging chromium from the body and depositing it in the eggs. The hardened egg shell or chorion is made up of layers of protein that are "tanned" usually by quinone

(Wigglesworth 1972). Chromium might conceivably replace quinone in its function as cross-linkage for protein and be withdrawn from other tissues in the process. The massive production of RNA in the nurse cells of Dipteran ovaries may serve a scavenging role, also. As RNA is produced in the nurse cells it flows into the cytoplasm of the oocyte (Wigglesworth 1972), carrying with it the stabilizing chromium ions. Because adults were not collected until after mating and ovipositing, any chromium deposited in eggs was lost. Any one or more of these mechanisms may explain the similarity in chromium body burdens for control and treatment adult females.

It is quite likely that chromium complexes with DNA and RNA during their intensive production throughout spermatogenesis in Chironomus. Hopkins (1965) found that  $Cr^{51}$  injected into mature male rats was first concentrated in the testis and was later in higher concentrations in the epididymis. The author inferred that chromium was being incorporated into sperm that was then stored in the epididymis. An excess of chromium might cause infertility in the sperm of male chironomids or in the eggs of female chironomids or cause detrimental effects in both.

The results of Experiment II did not parallel Experiment I. In an effort to increase adult numbers, twice as

many eggs were introduced into each aquarium as had been used in the previous experiment. While initial numbers in treatment populations were almost twice that of control populations, early toxic effects of chromium probably equalized population size between the control and treatment in the first week of growth. Final population sizes were similar for the two treatment populations and the first control replicate. The second control replicate had a much lower density and was not a good control for the effects of crowding. Growth of control populations was not dissimilar to the growth of controls in Experiments I and III. Growth rates (G) of controls were comparable to those found in the other experiments. Simple inspection and comparison of 95% confidence intervals in Figures 4 and 5 revealed significantly slower growth for treatment populations. Abnormalities in the formation of the head capsules of larvae were found only in the exposed populations. It appears that the additional stress of crowding produced a more severe response to chromium in Chironomus larvae.

Several investigators have reported deformities in larval chironomids sampled from lakes and channels contaminated with pesticides, heavy metals, and domestic wastes (Hare and Carter 1976, Saether and McClean 1972, Koehn and Frank 1980, Warwick 1980). All deformities referred to in these studies

were of mouthparts only, most commonly the mentum. Deformation of the teeth on the mandibles and the premandibles was also found. The deformities found in Chironomus riparius in this study were of a different nature, but both seem to be highly correlated with pollution stress. Although crowding, or increased density, of larval chironomids has been shown to slow the rate of growth and development (Kajak 1963), no such effect was apparent in control populations in Experiment II. It can be concluded that both the decreased growth rate and occurrence of head capsule deformities are the result of chromium exposure acting synergistically with increased population density.

## SUMMARY

The growth of Chironomus riparius was stimulated at sublethal concentrations of hexavalent chromium. This stimulation of growth was still apparent at lethal chromium concentrations in surviving larvae. The final mean lengths of animals from all control and treatment populations were equivalent, but final dry weights were greater for animals exposed to sublethal concentrations of chromium. The early emergence of treatment populations provided further evidence of faster growth and earlier maturation in Chironomus larvae. Direct toxicity of chromium to eggs was not seen and is unlikely to reduce production in Chironomus. Enzyme activation of energy processing pathways by this essential trace metal or production of increased amounts of the chromium-requiring, digestive enzyme trypsin may be the mechanism for stimulation of growth.

Production, as stored energy, was not reduced by chromium exposure. Lipid content of female pupae was significantly higher in treatment populations than in controls, suggesting stimulation of fat depositing biochemical pathways in the larvae. Increased amounts of lipid in

Chironomus at the time of pupation may account for the dissimilarity in dry weights of larvae removed on the last sampling dates of Experiment III.

Reproduction appeared to be impaired in adults emerging from treatment aquaria. The production of egg masses was not reduced, but the number of second generation larvae successfully hatched per adult female decreased with increasing hexavalent chromium concentration. Further investigation would be necessary to establish these observations statistically. A decrease in reproductive success may be due to metal accumulation in Chironomus tissues, specifically the eggs or sperm of adults.

Net reproductive rate, integrating both growth rate and reproductive success, was greater for control populations than for those exposed to sublethal levels of chromium within a single experiment. The intrinsic growth rate ( $r$ ) was positive under all conditions except when larvae were exposed to a lethal concentration of chromium (VI).

Under conditions of increased larval density chromium caused decreased growth rates and impaired the ability of larvae to shed the old head capsule during molting. In a natural environment the presence of such additional stress as crowding, food limitation, or other toxicants might prove synergistic with chromium, as in Experiment II. In such a

case, the measure of overall population performance ( $R_0$ ) established under controlled laboratory conditions could not be realistically extrapolated to predict a population's success in the field.

With the increasing emphasis placed on the use of growth and reproduction as parameters for measuring chronic toxicity (Lehmkuhl 1979), caution must be taken in extrapolating the information to predict survival of a population. It may be necessary to determine whether these parameters change with multigeneration exposure and how they may be affected by conditions of natural and man-made stress and variability in the environment.

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EFFECTS OF HEXAVALENT CHROMIUM ON THE GROWTH AND  
REPRODUCTION OF CHIRONOMUS RIPARIUS (DIPTERA)

by

Carolyn L. Hax

(ABSTRACT)

Effects of hexavalent chromium on the life cycle of Chironomus riparius were examined in the laboratory, using sublethal (0.5 mg/l) and 40% lethal (2.0 mg/l) concentrations. Chironomus egg masses were introduced into both control and chromium-treated 21 l aquaria. Larvae were subsampled at 5-day intervals, measured for length, and weighed to generate growth curves. Emerging adults were allowed to mate and oviposit. Twenty female pupae were collected from control and treatment (0.5 mg/l) populations within 12 hours of pupation. Their bodies were analyzed for lipid content by calculating loss in dry weight after a chloroform-methanol extraction. Chromium body burdens were measured in both larvae and adults from treatment (2.0 mg/l) and control populations. Effects of chromium on hatching rates of egg masses were studied.

Growth was stimulated at both lethal and sublethal concentrations of chromium. Lipid content of female pupae was significantly higher in treatment populations than in controls. Both stimulated growth and increased lipid

storage may be the results of metal activation of enzymes involved in energy processing and storage in the insect. Reproductive success, as measured by numbers of offspring per adult female, declined with increasing chromium concentrations. Chromium concentrations in adult males and larvae were significantly greater for treatment populations. Metal body burdens may have become concentrated in eggs or sperm of Chironomus and caused the observed decrease in reproductive success. No direct toxicity of chromium on egg masses was observed at concentrations up to 5 mg/l.