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THE EFFECT OF COPPER ON THE LIFE HISTORY STAGES OF THE HARPACTICOID COPEPOD *Tigriopus californicus*

by

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B.Sc., the University of British Columbia, 1984

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
in
THE FACULTY OF GRADUATE STUDIES
(Department of Oceanography)

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA
July 1987

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Date September 25, 1987

Abstract

Significant differences were found to exist in the copper tolerance of the life-history stages of the marine copepod, Tigriopus californicus, using artificial seawater as medium. The copper response of Tigriopus to copper was found to occur predominantly over the concentration range 1.0×10^{-6}M to 1.5×10^{-5}M. The N-2 was the most sensitive and the C-6 was the most tolerant life-history stage.

Copper equilibrated with the food of Tigriopus did not significantly affect adult fecundity (between 1.0×10^{-10} and 1.0×10^{-6} M Cu) or mortality (between 1.0×10^{-10} and 1.0×10^{-5} M). Copper equilibrated with SOW did not significantly alter the rate of egg survival (between 1.0×10^{-9} and 1.0×10^{-6} M) or naupliar activity (between 1.0×10^{-8} and 1.0×10^{-6} M). Exposure to widely varying but natural ecological conditions appears to have increased the tolerance of Tigriopus to unnatural stress.

The copper-manganese interaction observed for some species of phytoplankton such as Thalassiosira pseudonana was not found to hold for Tigriopus in SOW medium. No significant reduction in copper toxicity was observed upon addition of manganese (between 1.0×10^{-6} and 1.0×10^{-4} M) to copper (1.0×10^{-6} M) solutions.

An attempt was made to quantify the proportion of copper and manganese in biologically available forms (i.e., able to react with or pass through biological membranes) using the cation resin technique of Zorkin et al., (1986). It is thought that this attempt failed due to the resin columns being super-saturated with metal ions.

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ACKNOWLEDGEMENT

I would like to thank Dr. A.G. Lewis for his patience in my undertaking of this endeavor. I would also like to thank the members of my research committee, Dr. P.J. Harrison and Dr. T.F. Pedersen, for their time and helpful criticisms during the writing of this thesis.

Introduction

The Effects of Copper on the Life History Stages of the marine copepod, Tigriopus californicus

Copper is a required trace metal which can be both detrimental and beneficial to plants (Anderson and Morel, 1978, and Stauber and Florence, 1985) and animals (Bengtsson, 1978; Arnott and Ahsanullah, 1979; Barber and Trefry, 1981; Harrison and Rice, 1981; Lewis and Cave, 1982; and Bouquegneau, Martoja, and Truchet, 1984) at elevated concentrations. It is a transition metal capable of forming strong bonds with proteins and other ligands which accounts for a major part of its biological effect (Cherian and Goyer, 1978; Talbot and Magee, 1978; Overnell and Trewhella, 1979; Fisher, 1980; Roesijadi, 1980; Engel and Brouwer, 1982, 1984; and White and Rainbow, 1982). Copper is responsible for the conformation of some proteins (metalloproteins), the production of organic molecules such as haemes and chlorophylls, and is a component of some enzymes (Cherian and Goyer, 1978; Roesijadi, 1980).

Copper has been shown to be important in the growth and development of plants (Hipkins, 1983; and Hewitt, 1983), affecting the development of some phytoplankton populations (Anderson and Morel, 1978) and reducing the rate of photosynthesis by blocking electron transport in photosystem II

(Hipkins, 1983) in kelp (Clendenning and North, 1960) and micro- and nano-flagellates (Rajendran, Sumitra-Vijayaraghaven, and Wafer, 1978). Excess copper has also been shown to be deleterious to a myriad of organisms, including yeasts and bacteria (Sunda and Gillespie, 1979), oysters (Engel and Brouwer, 1982, 1984), rainbow trout (Dixon and Sprague, 1981), molluscs (Coombs and George, 1978), barnacles (Rainbow, Scott, Wiggins and Jackson, 1980), and shrimp (White and Rainbow, 1982).

While copper has been demonstrated to be toxic to most species of phytoplankton, the addition of manganese has been demonstrated to reverse copper-induced growth reduction (Sunda and Huntsman, 1983; Sunda et al., 1981). Additions of FeCl2, MnCl2, or of the chelating agents ethylenediaminetetraacetic acid (EDTA) or nitrilotriacetic acid (NTA) stimulated growth of cultures of Chaetoceros socialis and also reduced copper toxicity (either totally or partially) (Sunda et al., 1981). Copper toxicity to the diatom Thalassiosira pseudonana was reversed by the addition of manganese (Sunda and Huntsman, 1983). This was interpreted to be a physiological interaction between copper and manganese with copper competing for manganese uptake sites which would upset cellular manganese metabolism.

Work on the diatom Thalassiosira pseudonana demonstrated that culture growth was dependent on the cellular manganese

concentration (Sunda and Huntsman, 1983) which was found to be a function of manganese ion activity and an inverse function of the cupric ion concentration. Sunda and Huntsman (1983) developed a competitive binding model in which copper blocks either cellular manganese uptake or the binding of manganese within the cell to explain the toxicity of copper.

In contrast, it has been proposed that manganese hydroxides attached to the cell wall of the diatom Nitzschia closterium, markedly reduced copper toxicity (Stauber and Florence, 1985). This mechanism involves no physiological interaction or competition for binding sites. Thus, the copper-manganese effect may be due to manganese-copper competition at manganese uptake sites in the cell (Sunda et al., 1981) or sorption of copper by manganese oxides on the cell surface (Stauber and Florence, 1985) or to some as of yet undiscovered mechanism.

The interaction of copper and manganese reported by Sunda and Huntsman (1983) was verified by Kazumi (1985) with Thalassiosira pseudonana. Using AQUIL (Morel et al., 1979), a well defined medium, and water from a British Columbian fjord, the reduction in cell division rate produced by excess copper was ameliorated by high levels of manganese.

While the copper-manganese interaction has been studied for some plants it has not been explored for any animals. This

study includes a test of the hypothesis that manganese may reduce copper toxicity to animals. Determining if there is an effect of manganese on the copper toxicity to an aquatic animal would be a natural extension of the phytoplankton work and may help to determine the mechanism of metal toxicity in marine animals.

Intertidal marine animals are subjected to a great variety of conditions because of tidally-induced changes in water level combined with varying atmospheric conditions. Organisms living in the intertidal and splashpool zones may be expected to tolerate a wider range of environmentally-induced stress than would planktonic organisms. Tigriopus californicus is an intertidal harpacticoid copepod common to splash and tide pools from California to Alaska. It has a discontinuous life history (Feldman, 1985), and is readily cultured in the laboratory. The copper tolerance of Tigriopus was examined not only because of its hardiness as an intertidal organism, but also to determine if exposure to naturally-occurring stress would increase an organism's tolerance to unnatural stress (perhaps through the production of metallothioneins which bind metals into non-toxic forms: Cherian and Goyer, 1978; Fisher, 1980; and Roesijadi, 1980) and if there was a marked difference in the copper tolerance of any of its life history stages. Roesijadi (1980) suggested that once some critical metal-complexing capacity of an organism has been reached, it may no longer be able to process or store metal into non-toxic forms; increased mortality

would be expected.

Copper and manganese experiments used "total" metal concentrations where known amounts of metal were added to known volumes of synthetic and natural seawater to produce an experimental medium. However, both copper and manganese can be involved in a complex series of reactions with seawater ions. Only some metal species are biologically "available" and able to react with or pass through membranes of living cells (Cross and Sunda, 1977). While elevated concentrations of metal in an available form can be toxic, metal in an unavailable form will not be harmful to organisms. The proportion of total metal which is in a biologically-available form can be quantified with the resin column technique of Zorkin et al., (1986) which retains weakly charged metal species.

The medium used in the majority of bioassays was a salt solution (SOW) from AQUIL. Similar bioassays were run in natural seawater to allow comparison of results in a defined medium, SOW, with those in natural seawater.

While metal speciation confers metal bioavailability, a biological effect is determined by the tolerance of the organism. Tolerance tends to vary both with organism type and with its life-history stage. The response of the life-history

stages of Tigriopus californicus to copper was used to test the hypothesis that exposure to naturally-occurring stress would increase an organism's tolerance to both natural and unnatural stress.

Materials and Methods

Organism

Laboratory cultures of the harpacticoid copepod Tigriopus californicus were maintained in 2.8 L Pyrex® Fernbach flasks in the chelexed salt solution (SOW) of an artificial seawater medium (AQUIL; Morel et al., 1979) at 35 ppt salinity and pH 8.0. Temperature was maintained at 16 ± 1 C on 16:8 light:dark cycle. Culture medium was replaced every 96-120 h.

Animals to be used in bioassays were isolated from the laboratory cultures at the egg stage. Females with mature egg clusters were collected on a glass slide, in a drop of water and egg sacs were removed with stainless steel dissecting tweezers using a Bausch and Lomb® dissecting microscope. Eggs were washed three times in SOW before their transfer to 20 ml incubation chambers containing SOW (this washing was intended to remove particulate and soluble materials which may have been associated with the adult female or the egg cluster). Between 4 and 8 clusters were kept in each chamber until the nauplii were released; approximately 24 clusters were used in each experiment.

Hatched nauplii were maintained in acid-washed (1N HCL) Pyrex® evaporating dishes containing SOW until they were at the life history stage to be tested. Specimens to be used in

bioassays were transferred through three rinses of SOW using a micro-pipette and a dissecting microscope. The animals were then isolated in SOW for 6 to 8 h prior to their use, to remove any excreted materials which could affect the experiment. Organisms were finally isolated in solutions identical to the experimental solutions immediately prior to being used in bioassays. Any solution carried into the chamber with the copepods would thus be very similar to the test solution and medium carry-over would be expected to have minimal effect on the composition of the test solutions.

Cultures and batches of Tigriopus were fed finely ground fish food (Wardley's® basic food flakes) every 24 h. There were two advantages to using a commercially-available fish food instead of phytoplankton for feeding the copepod cultures. First, Tigriopus is a detritus-feeding benthic copepod and the variable size of the available food particles was more suitable for all of the feeding stages than was cultured phytoplankton. Second, increased survival was obtained when the copepods were isolated from the food flakes by micro-pipette than when isolated from phytoplankton-containing medium by filtration.

A second source of nourishment for the copepods was bacteria which accumulated in the culture flasks (Lewis, pers comm). The importance of the bacterial food source is unknown at this time. Tigriopus may graze: (i) solely on bacteria growing in culture medium, (ii) solely on the ground food

flakes, or on a combination of (i) and (ii). No attempt was made to keep the culture axenic.

Preparation of SOW

The major seawater salts (except for $MgCl_2.6H_2O$) were dissolved in 12-L of glass-distilled water (GDW) in a 40-L Pyrex® vessel. Once all of the major salts were dissolved the previously-dried $MgCl_2$ was added and the final volume was made up to 40-L. The SOW was then bubbled with filtered (0.4 um Nuclepore® air passed through 1N H_2SO_4 until the solution pH was 8.0 $\pm$ 0.05, then passed through an ion-exchange column (Chelex-100, 100-200 mesh, Bio-Rad® Laboratories) to reduce the level of trace metal contamination. All transfers, including trace metal and chemical solutions and organism transfers, were carried out in a class 100 laminar flow hood with all possible metal parts replaced with polypropylene. Solutions were equilibrated for 2 h before they were used.

Natural Seawater

Seawater was collected from the Fisheries and Oceans' Fisheries Research Branch in West Vancouver. It was filtered through a Gelman Metricel® membrane filter (0.45 um pore size) shortly after collection and stored at 16 C in 20-L polypropylene bottles. Water salinity was 33 ppt and pH was adjusted to 8.0 $\pm$ 0.05.

Trace Metals

Copper stocks were prepared by dissolving copper metal in reagent grade 1N HNO_3. Glass-distilled water was added to adjust the final copper concentration to 1.0×10^{-3} M. Fresh solutions were prepared daily for bioassay medium replacement. Only polypropylene bottles and volumetric flasks were used in the preparation of metal stocks. All bottles were rinsed three times with GDW and then soaked in 1N HCl for seven days. Immediately prior to use, the bottles were rinsed three times with GDW. Volumetric flasks were handled in a similar manner except that they were soaked in 3N HCl for 24 h.

Incubation Chambers

Polystyrene Nunclon® incubation chambers were initially soaked in 1N HCL for 7 d and then rinsed three times in GDW. Prior to subsequent use, the chambers were soaked in 1N HCl for a minimum of 4 d and then rinsed 3 times in GDW. Experimental solutions were allowed to equilibrate for at least 2 h before use. Ten ml of the appropriate metal-containing solution was pipetted into the incubation chambers and allowed to equilibrate with the chamber surface for 2 h; these solutions were then replaced with fresh experimental solutions and the bioassay organisms added.

Changes in metal chemistry or concentration may have

occurred in the experimental solutions during a bioassay due to a number of factors, including: (i) adsorption of metal to copepod cuticle, (ii) complexation of metal ions with organic contaminants, or (iii) continuing, non-equilibrium adsorption of metal to the walls of the incubation chambers. Relative to the metal concentration used, adsorption is not considered to have caused a major change in the metal concentration or chemistry. The daily changing of experimental solutions would remove most of the organic contaminants capable of binding metals, and would reduce the effect of metal loss through adsorption.

Bioassay Procedure

Tigriopus was studied to determine: (i) the effect of copper on egg viability, (ii) the effect of copper on naupliar survival, (iii) the effect of copper on copepodite survival, (iv) the effect of copper and food on egg cluster production, (v) the effect of copper and food on adult survival, (vi) the effect of copper on naupliar activity, (vii) the effect of manganese on copper-stressed first naupliar survival, and (viii) the effect of manganese on egg viability. The experimental procedure used for each section will be discussed.

(i) The Effect of Copper on Egg Viability

Eggs were collected 48 $\pm$ 1 h after their appearance on previously-berried females, rinsed in SOW, then exposed to SOW

solutions spiked with copper (from preliminary work it was found that egg clusters less than 48 h old suffered high mortality when removed from the donor females). One cluster was used for each of three replicates for each concentration. Collecting an adequate number of clusters at the 48 h stage of development was difficult and limited the number of clusters to three for each concentration of copper. The percentage of the eggs that hatched into nauplii at 96 h was calculated for each replicate for each concentration and averaged to produce a percent survival value. Concentrations of copper used in this series of experiments ranged from 1.0×10^{-10} to 1.0×10^{-4} M.

Unless stated otherwise, the following conditions were in effect for this and every bioassay: (i) all experimental solutions were replaced every 24 h, (ii) all bioassays were run for 96 h, (iii) three replicates were run for every concentration used, (iv) all bioassays were run in a food-free environment, (v) sample size was 20 individuals per replicate, and (vi) observations were made and dead organisms were removed at 24, 48, 72, and at 96 h.

(ii) The Effect of Copper on Naupliar Survival

Naupliar stages from N-1 to N-6 were maintained in conditions identical to those of the laboratory cultures. Nauplii to be used in bioassays were isolated in SOW three times over and finally isolated in solutions identical to experimental

solutions and counted into pre-equilibrated incubation chambers containing 10 ml of experimental solution. LC50 and LC95 values were calculated and survival data were plotted against copper concentration for each naupliar stage (an LC50 is a concentration of a material which was found to result in 50% mortality of a group of organisms within a specified time e.g. 48 h, 96 h. Similarly, an LC95 is a concentration which results in 95% mortality within the specified time).

(iii) The Effect of Copper on Copepodite Survival

An identical procedure to that used in the naupliar mortality studies was employed in the copepodite mortality studies. Each copepodite stage was grown and maintained for bioassay usage. Bioassays were run as they had been for naupliar bioassays and LC50 and LC95 values were calculated for each life-history stage. The percentage survival was plotted against copper concentration for each copepodite stage.

Preparation of Copper-Treated Food for Pre-Equilibration Experiments

Wardley's® fish food flakes (0.1 g) were finely ground with a mortar and pestle and allowed to equilibrate with 250 ml of copper solution overnight in 500 ml Nalgene® polypropylene bottles. The "equilibrated" food flakes were then removed from suspension by centrifugation for 10 min in an International

Equipment Company® centrifuge (model PR-J). The supernatant was decanted and the food flakes were re-suspended as "pre-equilibrated" food flakes in fresh experimental solutions (control and copper-enriched solutions) immediately prior to the start of bioassays.

Two series of bioassays were run using this procedure. One series was concerned with egg cluster production and one was concerned with adult tolerance to copper.

(iv) The Effect of Copper and Food on Egg Cluster Production

Previously-berried adults were placed in incubation chambers containing experimental solutions with pre-equilibrated food (Tigriopus will not produce egg clusters when food is not available). Copper concentrations used in these bioassays ranged from 1.0×10^{-10} to 1.0×10^{-5} M. The number of clusters produced between replicates was averaged to produce a mean value. Four replicates of fifteen organisms were used for each concentration.

(v) The Effect of Copper and Food on Adult Survival

The method from section (iv) was used for a second series of bioassays to determine the tolerance of the adult copepod to copper-labelled food. Animals were collected from cultures with a pipette and placed in SOW for 6 to 8 h before the start of a

bioassay. This isolation was intended to remove contaminants such as phytoplankton and bacteria which may have been in the culture medium. To minimize carry-over and dilution of experimental solutions, copepods were isolated in solutions identical to the experimental solution prior to their being used in bioassays. Solution carry-over to the incubation chambers would thus be expected to have minimal effect on the composition of experimental solutions.

Bioassays were run with the pre-equilibrated food replaced daily. The percent adult mortality at 96 h was determined for each of the copper concentrations used. Percent survival data was transformed into arcsin (Zar, 1984) values to generate LC50 and LC95 values.

(vi) The Effect of Copper on Naupliar Activity

Observations made during initial experiments suggested that the activity of the early naupliar stages (especially of the N-1) decreased with increasing copper concentration (activity was defined as a voluntary motion elicited in response to a stimulus: in this case, the stimulus was a jet of water from the tip of a micro-pipette).

The first naupliar stage was selected for this experiment because it was found to be active and sensitive to stimulation, and easy to isolate. Mature egg clusters were removed from

adults and placed in SOW until release of the nauplii occurred. Nauplii were 0-2 h old when they were collected from the SOW with a micro-pipette and placed in 20 ml multi-well incubation chambers containing experimental solutions. (The dilution effect of adding 50 ul of SOW to 10 ml of solution is << 1.0%). Copper concentrations in the culture medium were varied between $1.0x10^{-10}$ and $1.0x10^{-5}$ M Cu.

Activity was quantified by counting the number of seconds a nauplius was active once stimulated; a test time of 10 s was found to be ideal (from preliminary observations, it was found that control nauplii would often stop moving 15 s after stimulation). Organisms used in bioassays were stimulated three times in one minute. If no response was noted from any of the stimulations, the organism was termed dead and removed from the medium. If a response was observed, the three time-values were recorded and averaged to produce a mean response value. Activity was followed every 24 h for the duration of the bioassay.

(vii) The Effect of Manganese on Copper-Stressed First-Naupliar Survival

The first naupliar stage was chosen for this metal-metal study because it had a low copper tolerance. N-1 nauplii were collected and isolated as previously discussed in section (ii). Manganese concentrations used in experiments varied from

$1.0x10^{-6}$ to $1.0x10^{-4}$ M; in preliminary experiments using concentrations of $1.0x10^{-9}$ to $1.0x10^{-7}$ M, it was found that the effect on naupliar mortality was statistically indistinguishable from control mortality. Copper concentration was maintained at $1.0x10^{-6}$ M.

(viii) The Effect of Manganese on Egg Viability

Mature egg clusters were removed from previously-berried females when the clusters were 48 $\pm$ 1 h old. They were rinsed with SOW and isolated in experimental solution as previously discussed. The percentage of eggs that hatched into nauplii within 96 h was averaged to produce a percent survival value. The manganese concentration was varied from $1.0x10^{-9}$ to $1.0x10^{-5}$ M.

Statistics used in Analysis of Data

Both Anova and Tukey tests were used to compare means of experimental groups. Anova is a statistical technique used to analyze multigroup experiments. The test provided one overall comparison to determine if there was a significant difference between the means of the groups due to the independent variable (whenever the term significant is used, it is to be understood that the level of significance intended is alpha = 0.05).

The Tukey test compared all pairs of means in a multigroup

experiment to determine if any one pair was significantly greater than a critical value. If the mean of any group was significantly different than the mean of any other group, it was readily identified. If differences existed, it was of interest to know which of the conditions differed from the others.

In cases where the mean of a group was significantly greater than some critical value, the independent variable was plotted against the dependent variable and the closeness of fit to a straight line was examined. One example of this was mortality bioassays where percent survival was plotted against copper concentration. These were non-linear plots and data manipulation was required since it was of interest to determine LC50 and LC95 values from these plots.

Two transformations which were found to be applicable were the Probit and the Arcsine transformations. The Probit transformation has been used to straighten cumulative curves by changing the ordinate of the cumulative normal into a probability scale graduated in standard deviation units (Finney, 1962; Sokal and Rolf, 1981). This test provided acceptable variability for the LC50 values but the data for LC95 data contained too much uncertainty to be useable.

One transformation which is especially appropriate to work with percentages is the arcsine transformation (Zar, 1984). It stretches out both tails of a distribution of percentages and

compresses the middle. This manipulation provided improved fit of the data to a straight line with acceptable variability for both LC50 and LC95 values.

Results

The experimental series has been broken up into 8 sections, each of which explore a facet of the response of *Tigriopus californicus* to copper and to manganese.

(i) The Effect of Copper on Egg Viability

In this study the term egg was used for the embryonic phase of the organism, after fertilization and before hatching. Copper concentrations ranged from $1.0x10^{-9}$ to $1.0x10^{-5}$ M. Results are presented in Table 1 (all Tables are contained in the appendix).

The percent of eggs in a cluster which were found to hatch was high (78.8 to 81.5%) over the copper range $1.0x10^{-9}$ to $1.0x10^{-6}$ M. There was a marked decrease in the number of eggs that hatched at the $1.0x10^{-5}$ M level; egg survival plunged from over 80% at $1.0x10^{-6}$ M to less than 5% at $1.0x10^{-5}$ M Cu. Because of variability in the data, specific comments about differences in the copper response over the range $1.0x10^{-9}$ to $1.0x10^{-6}$ M cannot be made. Using the Tukey HSD test (alpha = 0.05) and the One-way Anova (alpha = 0.05), the only level of copper that was found to significantly affect the mean percentage of eggs that hatched was $1.0x10^{-5}$ M. The LC50 value for the egg stage was $3.6x10^{-6}$ M Cu, and the LC95 value was $9.6x10^{-6}$ M Cu (Table 2, Figure 1 and Figure 2).

(ii) The Effect of Copper on Naupliar Survival

Ninety-six hour LC 50 and LC 95 values are listed for each stage in Table 2. The percent survival of each naupliar stage is given in Figures 3 to 8 and in Tables 3 to 8. Added copper concentrations ranged from $1.0x10^{-10}$ to $1.0x10^{-4}$ M.

Both one way Anova and the Tukey HSD tests suggested that there was a significant difference in the effect of copper on the survival of nauplii over the concentration range tested. Between $1.0x10^{-6}$ and $4.0x10^{-6}$ M Cu the survival of the N-1 (the first nauplius) dropped from 53.3 to 3.3%. The rate of survival decreased rapidly between $1.0x10^{-6}$ and $2.0x10^{-6}$ M (from 53.3 to 33.3%), then more linearly to $4.0x10^{-6}$ M Cu. The LC50 was $1.2x10^{-6}$ M Cu and the LC95 was $3.7x10^{-6}$ M Cu.

Over the same concentration range, the N-2 appeared to be more sensitive to the lower concentrations of copper and less sensitive to the higher concentrations than the N-1. Survival declined from 31.7 to 11.7% over the concentration range $1.0x10^{-6}$ M to $4.0x10^{-6}$ M Cu. The LC50 for the N-2 was $0.3x10^{-6}$ M Cu and the LC95 value was found to be $4.7x10^{-6}$ M Cu.

The N-3 had an increased tolerance to copper relative to the N-1 and N-2. The rate of survival decreased from 65.0 to 21.7% over the range $1.0x10^{-6}$ to $6.0x10^{-6}$ M Cu. The survival rate dropped off rapidly at $7.0x10^{-6}$ M (to 15.0% $\pm$ 5.0) and was

1.7% ±2.9 at 8.0×10^{-6} M. The LC50 and LC95 values were 2.5×10^{-6} M Cu and 8.0×10^{-6} M Cu.

The N-4 appeared to be more tolerant to high concentrations of copper than were the first three naupliar stages. The N-4 survival was high (61.7 to 55.0%) over the range 1.0×10^{-6} to 3.0×10^{-6} M Cu but between 4.0×10^{-6} and 8.0x10-6 M Cu the survival dropped from 45.0 to 8.3%. The LC50 value was 2.9×10^{-6} M Cu and the LC95 was 8.7×10^{-6} M Cu.

The N-5 appeared to be more copper-tolerant than the N-4 stage. There was a regular decrease in naupliar survival (from 85.0 to 13.3%) over the range 1.0×10^{-6} to 8.0×10^{-6} M Cu. The LC50 value was 4.1×10^{-6} M Cu and the LC95 value was 8.8×10^{-6} M Cu.

The N-6 was the most copper-tolerant naupliar stage, and possessed the highest naupliar LC50 and LC95 values (Figures 1 and 2). Survival remained relatively high (86.7 to 61.7%) from 1.0 to 4.0×10^{-6} M Cu but dropped off rapidly (from 53.3 to 11.7%) with increasing copper concentration (5.0×10^{-6} to 8.0×10^{-6} M). The LC50 value was 4.8×10^{-6} M Cu, and the LC95 was 9.6×10^{-6} M Cu.

There was a progressive increase in the 96 h LC95 values from the N-1 to N-6 stage (3.7, 4.7, 8.0, 8.7, 8.8, and 9.6×10^{-6} M Cu). There was a similar trend in the LC50 values, with the

exception of the the N-2 which was lower than the N-1 value (1.2, 0.3, 2.5, 2.9, 4.1, and 4.8×10^{-6} M Cu).

(iii) The Effect of Copper on Copepodite Survival

The survival of each copepodite stage (including the adult) is given in Figures 9-14 and in Tables 9 to 14. The LC50 and LC95 values are given in Table 2. Copper concentrations tested varied from 1.0×10^{-10} to 1.0x10-@ 4 M.

The C-1 (first copepodite) appeared to be more copper-tolerant than the N-6. The LC50 for the C-1 was 5.9×10^{-6} M Cu and the LC95 was 1.1×10^{-5} M Cu while the N-6 LC50 and LC95 values were slightly lower (4.8×10^{-6} M and 9.6×10^{-6} M Cu). The C-1 survival remained high (96.7 to 88.3%) at the lower copper concentrations (1.0×10^{-6} to 2.0×10^{-6} M) but decreased steadily (from 75.0 to 8.3%) at the higher copper concentrations (3.0×10^{-6} to 1.0×10^{-5} M) (Figure 9).

Survival of the C-2 remained high (95.0 to 71.6%) over the lower copper concentrations (1.0×10^{-6} to 7.0×10^{-6} M) (Figure 10). Over the higher copper concentrations (8.0×10^{-6} to 1.1×10^{-5} M) the C-2 survival dropped markedly from 60.0 to 5.0%. The LC50 was 7.8×10^{-6} M Cu and the LC95 was 1.4×10^{-5} M Cu which made the C-2 more tolerant than the C-1.

The C-3 survival rate was high (96.7 to 65.0%) over the

copper concentration range $1.0x10^{-6}$ to $7.0x10^{-6}$ M. Survival dropped off rapidly (from 33.3 to 8.3%) at the higher concentrations ($8.0x10^{-6}$ to $1.0x10^{-5}$ M). The LC50 value was $6.5x10^{-6}$ M Cu and the LC95 value was $1.1x10^{-5}$ M Cu. Both the LC50 and the LC95 values were less than the C-2 values ($7.8x10^{-6}$ M and $1.4x10^{-5}$ M Cu).

The C-4 survival rate was relatively stable (93.3 to 68.3%) from $1.0x10^{-6}$ to $4.0x10^{-6}$ M Cu. From $5.0x10^{-6}$ to $1.1x10^{-5}$ M Cu, the rate of survival dropped from 48.3 to 11.7%. The LC50 for C-4 copepodites was $6.2x10^{-6}$ M Cu and the LC95 was $1.3x10^{-5}$ M Cu. The LC50 value for this stage was slightly less than the LC50 value for the C-3 stage (at $6.5x10^{-6}$ M Cu), while the LC95 value was slightly greater than the C-3 LC95 ($1.1x10^{-5}$ M Cu).

The C-5 survival was high (96.7 to 70.0%) over the copper range $1.0x10^{-6}$ to $5.0x10^{-6}$ M. There was an abrupt drop in survival from 50.0 to 11.7% between $7.0x10^{-6}$ and $9.0x10^{-6}$ M Cu. The LC50 value for this stage was $6.2x10^{-6}$ M Cu, and the LC95 value was $1.1x10^{-5}$ M Cu. The LC50 value was close to the C-3 and C-4 LC50 values ($6.5x10^{-6}$ M and $6.2x10^{-6}$ M Cu), whereas the LC95 value was less than the four previous stages (the C-1 through C-4 at $1.1x10^{-5}$ M, $1.4x10^{-5}$, $1.1x10^{-5}$ M, and $1.3x10^{-5}$ M Cu).

The survival plot of the C-6 (adult copepods) was distinguished from all others by containing the highest LC50 and

LC95 values as well as by a very abrupt decrease in adult survival (from 33.3 to 0.0%) between 1.4 and $1.5x10^{-5}$ M Cu. Survival remained high (98.3 to 80.0%) between $8.0x10^{-6}$ and $1.0x10^{-5}$ M Cu. With increasing copper concentrations ($1.1x10^{-5}$ to $1.5x10^{-5}$ M) the percent survival dropped from 68.3 to 0.0%. The LC50 value was $1.2x10^{-5}$ M Cu and the LC95 was $1.5x10^{-5}$ M Cu, significantly higher than the C-5 values ($6.2x10^{-6}$ M and $1.1x10^{-5}$ M Cu).

(iv) The Effect of Copper and Food on Egg Cluster Production

The percent of previously-berried females which produced a second cluster when exposed to copper-labelled food solutions was determined. Results are presented in Table 15. Over 80% of the females produced a second cluster when placed in solutions containing between $1.0x10^{-10}$ and $1.0x10^{-8}$ M Cu. There was a decrease in cluster production below 80% in females exposed to copper concentrations between 4.0 and $8.0x10^{-8}$ M Cu, then an increase between $1.0x10^{-7}$ and $1.0x10^{-6}$ M Cu where 87% of the females were berried. There was an abrupt decrease in egg production between $1.0x10^{-6}$ and $1.0x10^{-5}$ M Cu (none of the copepods in the $1.0x10^{-5}$ M group produced a cluster).

(v) The Effect of Copper and Food on Adult Survival

The percent survival for adults exposed to copper-labelled food ($1.0x10^{-10}$ to $1.0x10^{-5}$ M) was determined using the

technique discussed in section (iv). The percentage survival for each of the three replicates was averaged and the mean percent survival for each concentration was compared (Table 16). One-way Anova and the Tukey HSD test failed to establish that any significant difference existed between the mean percentage survival rates for adult copepods at copper concentrations less than $1.0x10^{-5}$ M. There was no significant difference in mortality rates between copper-labelled food and copper equilibrated in SOW alone between $1.0x10^{-7}$ and $1.0x10^{-5}$ M Cu.

(vi) The Effect of Copper on Naupliar Activity Levels

A series of experiments was run to determine if the activity of the N-1 stage decreased with increasing copper concentration. The level of activity observed was assigned a value between 0 and 10. One-way Anova and the Tukey HSD test were used to determine if there was any significant difference between the activity levels of nauplii exposed to the experimental solutions.

Nauplii exposed to copper concentrations between $1.0x10^{-8}$ and $1.0x10^{-6}$ M Cu were active for an average of 7.0 s (Table 17). At $1.0x10^{-5}$ M Cu, the nauplius was active for 0.41 s (there was only one nauplius alive in the $1.0x10^{-5}$ M Cu bioassay).

The only statistically significant effect of copper on

naupliar activity levels was found at $1.0x10^{-5}$ M Cu. There was no significant difference in activity values for any of the experimental groups between $1.0x10^{-8}$ and $1.0x10^{-6}$ M Cu.

(vii) The Effect of Manganese on Copper-Stressed First Naupliar Survival

The first naupliar stage was used to establish a manganese tolerance for one life-history stage of Tigriopus and to examine the hypothesised manganese-copper interaction in a series of 72-h bioassays. The copper concentration was held constant ($1.0x10^{-6}$ M) in experimental solutions and the manganese concentration was varied between $1.0x10^{-6}$ to $1.0x10^{-4}$ M. The rate of N-1 survival lay between 35.0% ±28.2 and 50.0% ±14.1 over this concentration range (Table 18).

Concentrations of manganese less than $1.0x10^{-6}$ M had been used in preliminary studies but the percent survival was statistically indistinguishable from control survival (95% ±5.0 and 100% ±0.0). The mean percent survival values were compared using One-way Anova (alpha = 0.05) and the Tukey HSD test (alpha = 0.05). There was no apparent statistically significant effect of manganese on the copper toxicity for the N-1 naupliar stage.

(viii) The Effect of Manganese on Egg Survival

Tigriopus eggs were exposed to manganese concentrations

between $1.0x10^{-9}$ and $1.0x10^{-5}$ M Mn (Table 19). Between $1.0x10^{-9}$ to $1.0x10^{-6}$ M Cu, the rate of survival to hatching was consistently high (77.0 to 84.0%); at $1.0x10^{-5}$ M Mn the survival rate has dropped to 54.0% $\pm$41.3. Control survival was 85.0% $\pm$17.9, and 74.0% $\pm$18.83. Using the One-way Anova and the Tukey HSD tests, there was no statistically significant effect of manganese on egg survival at any of the concentrations tested.

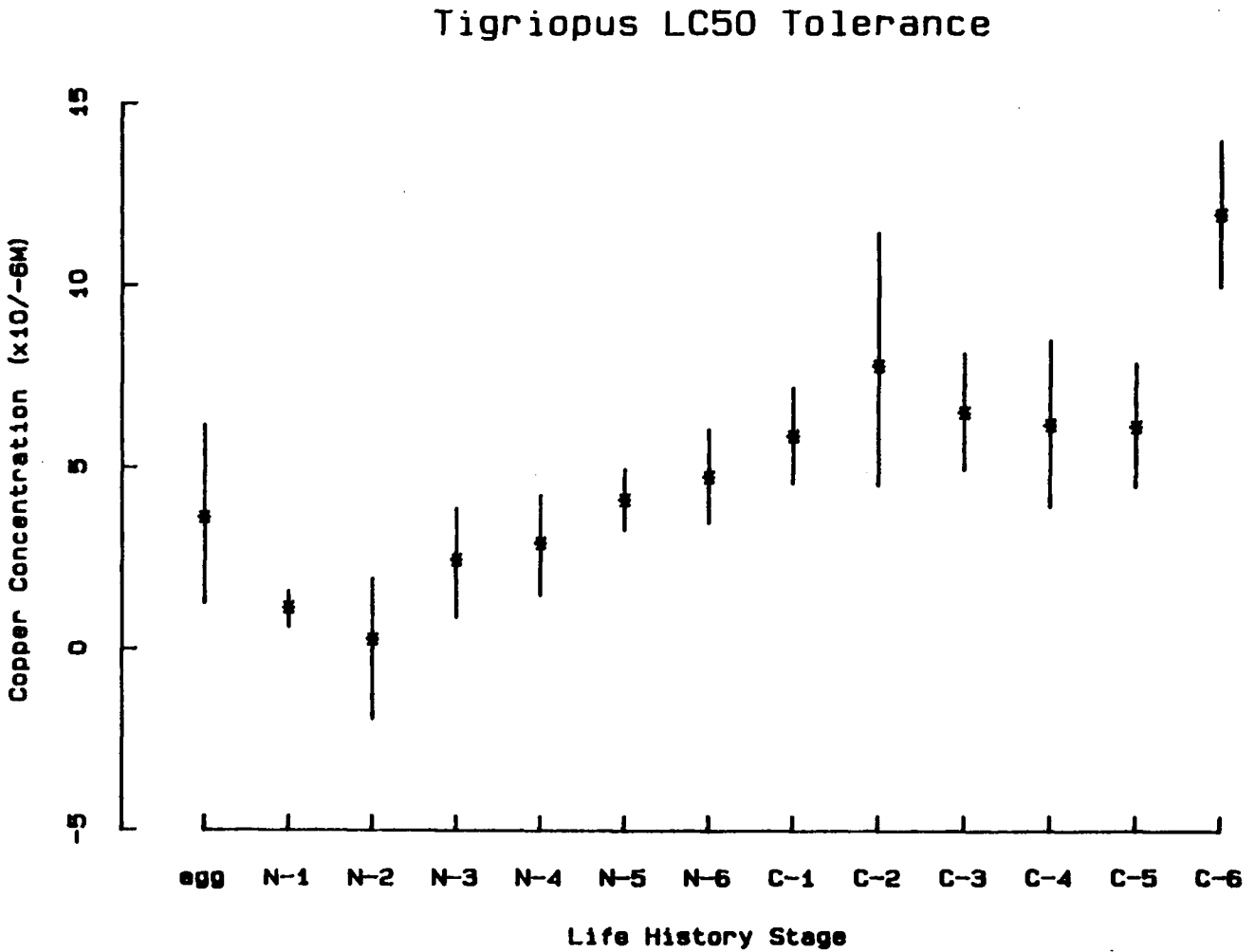

Figure 1. LC50 values for the life-history stages of Tigriopus californicus upon exposure to copper solutions (* signifies the mean value and the vertical bar represents the 95% confidence interval for this and every figure).

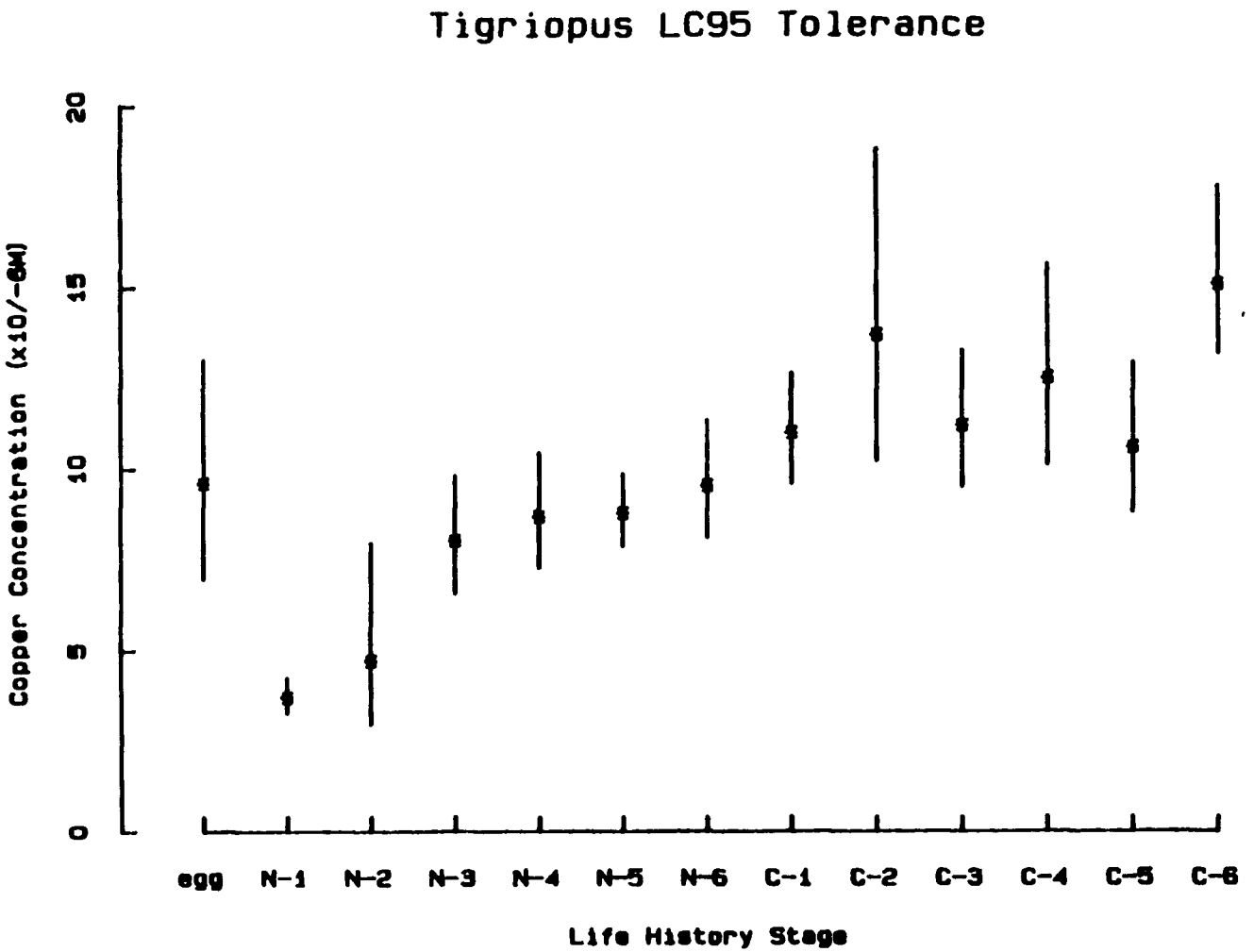

Figure 2. LC95 values for the life-history stages of Tigriopus californicus upon exposure to copper solutions.

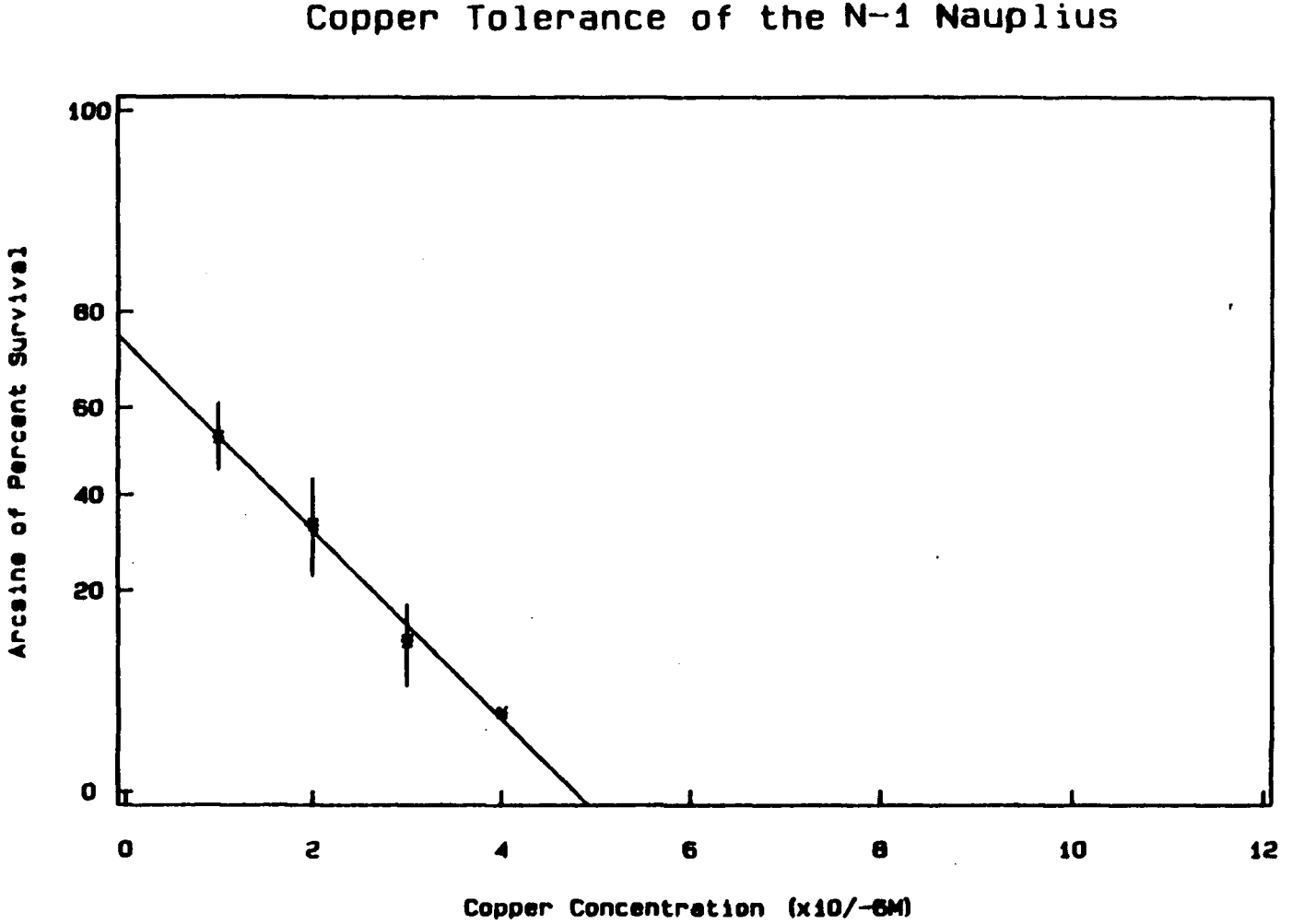

Figure 3. Arcsine of percent survival of the first naupliar stage of Tigriopus californicus upon exposure to copper solutions.

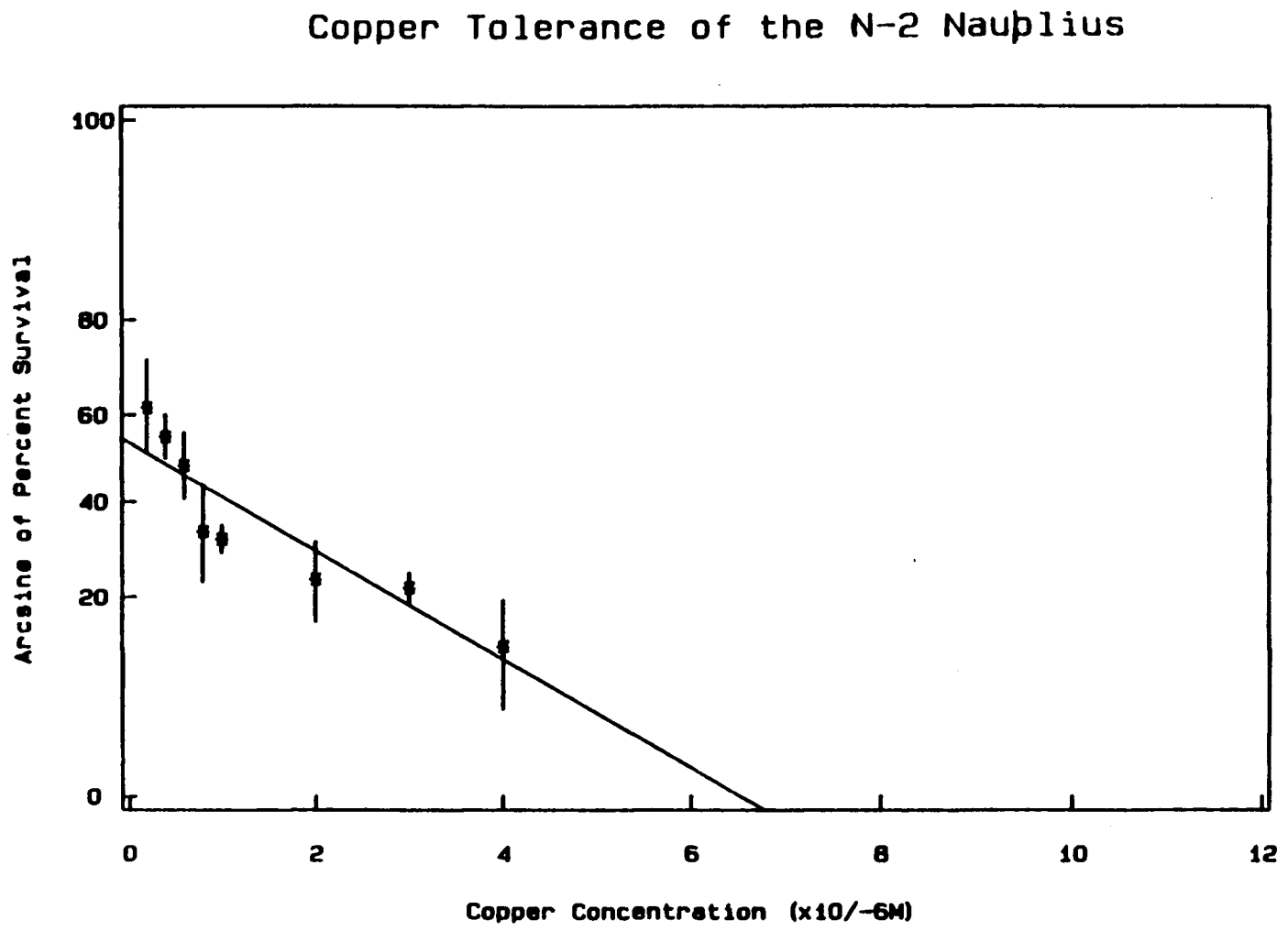

Figure 4. Arcsine of percent survival of the second naupliar stage of Tigriopus californicus upon exposure to copper solutions.

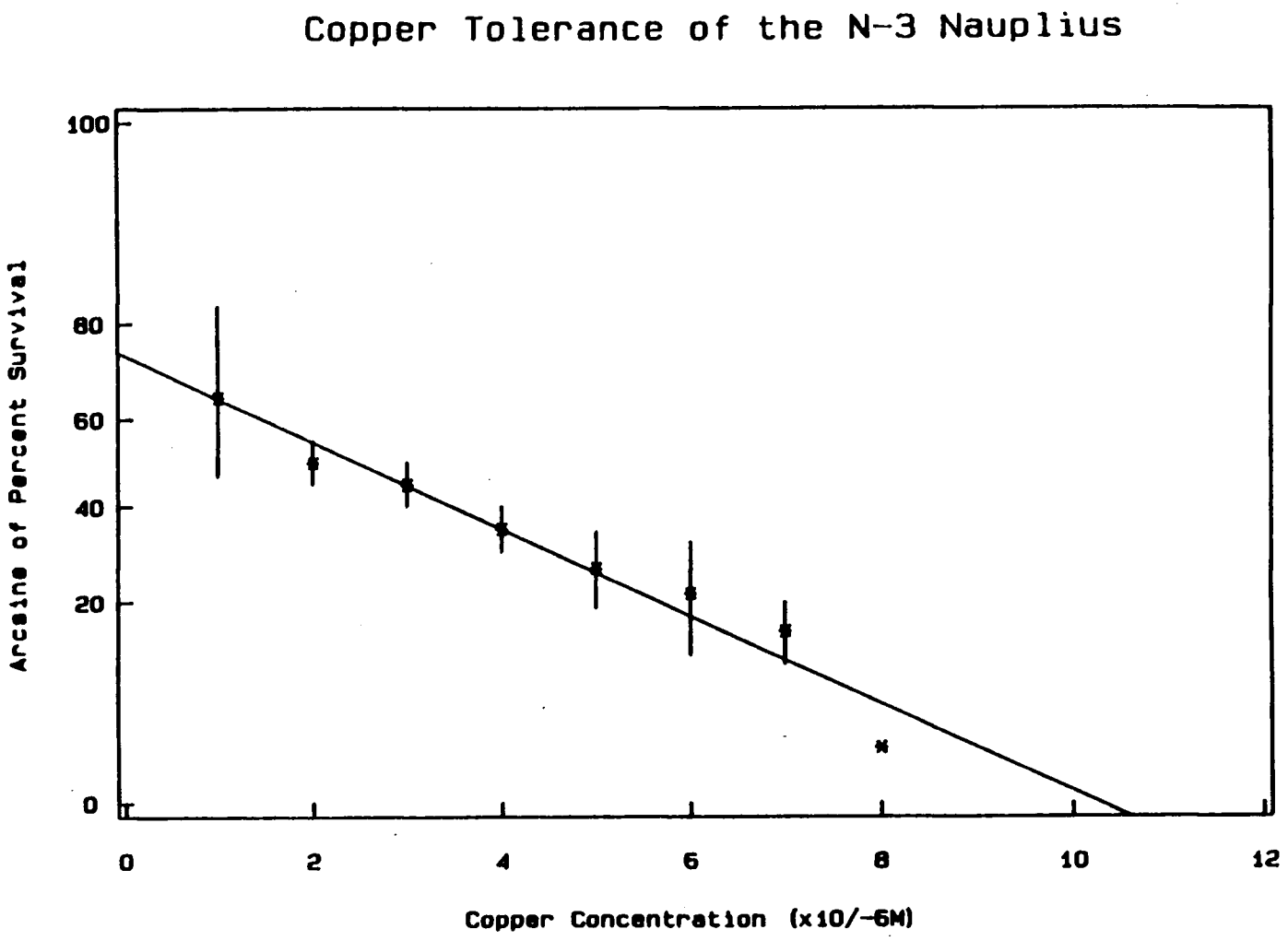

Figure 5. Arcsine of percent survival of the third naupliar stage of Tigriopus californicus upon exposure to copper solutions.

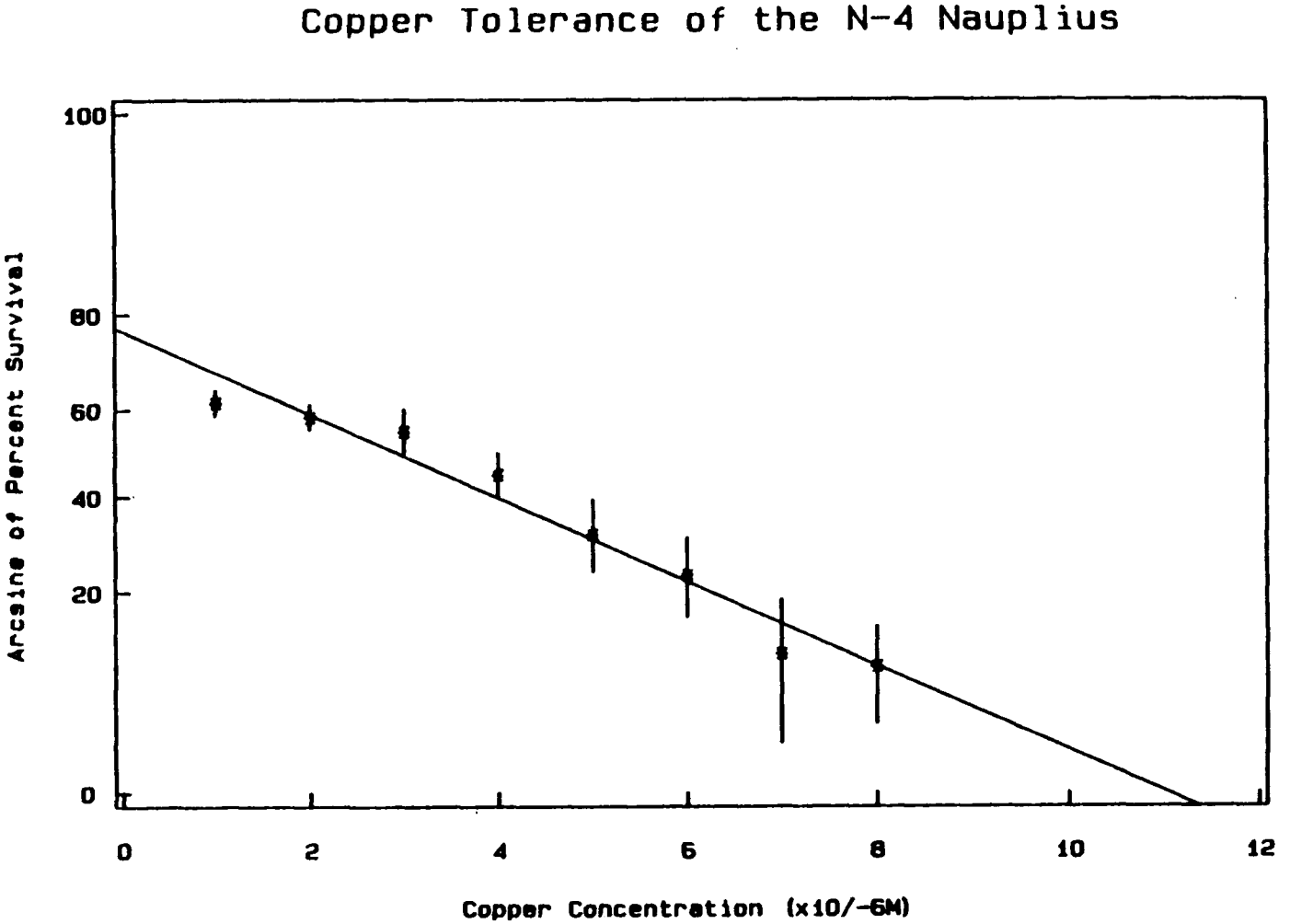

Figure 6. Arcsine of percent survival of the fourth naupliar stage of Tigriopus californicus upon exposure to copper solutions.

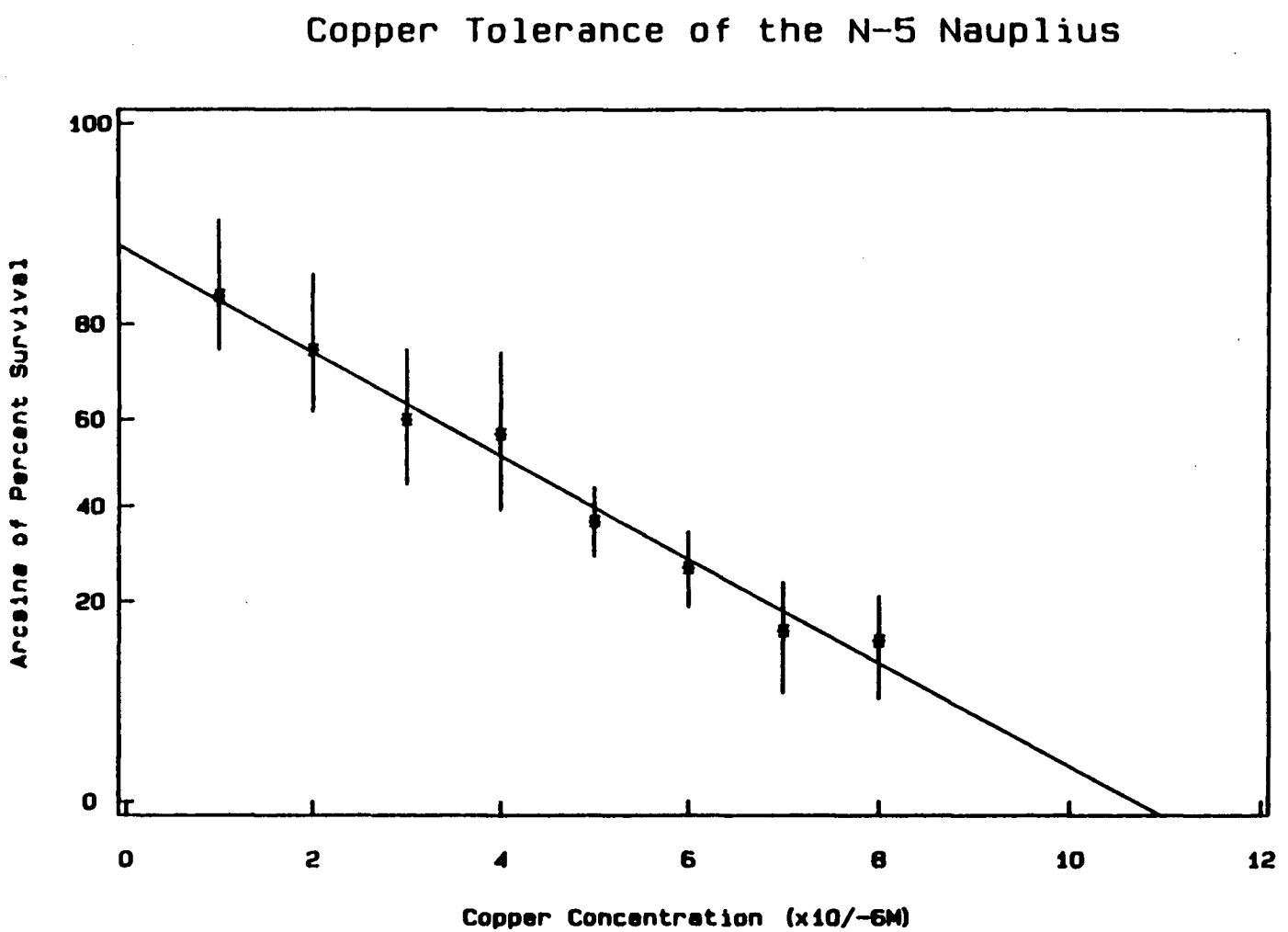

Figure 7. Arcsine of percent survival of the fifth naupliar stage of Tigriopus californicus upon exposure to copper solutions.

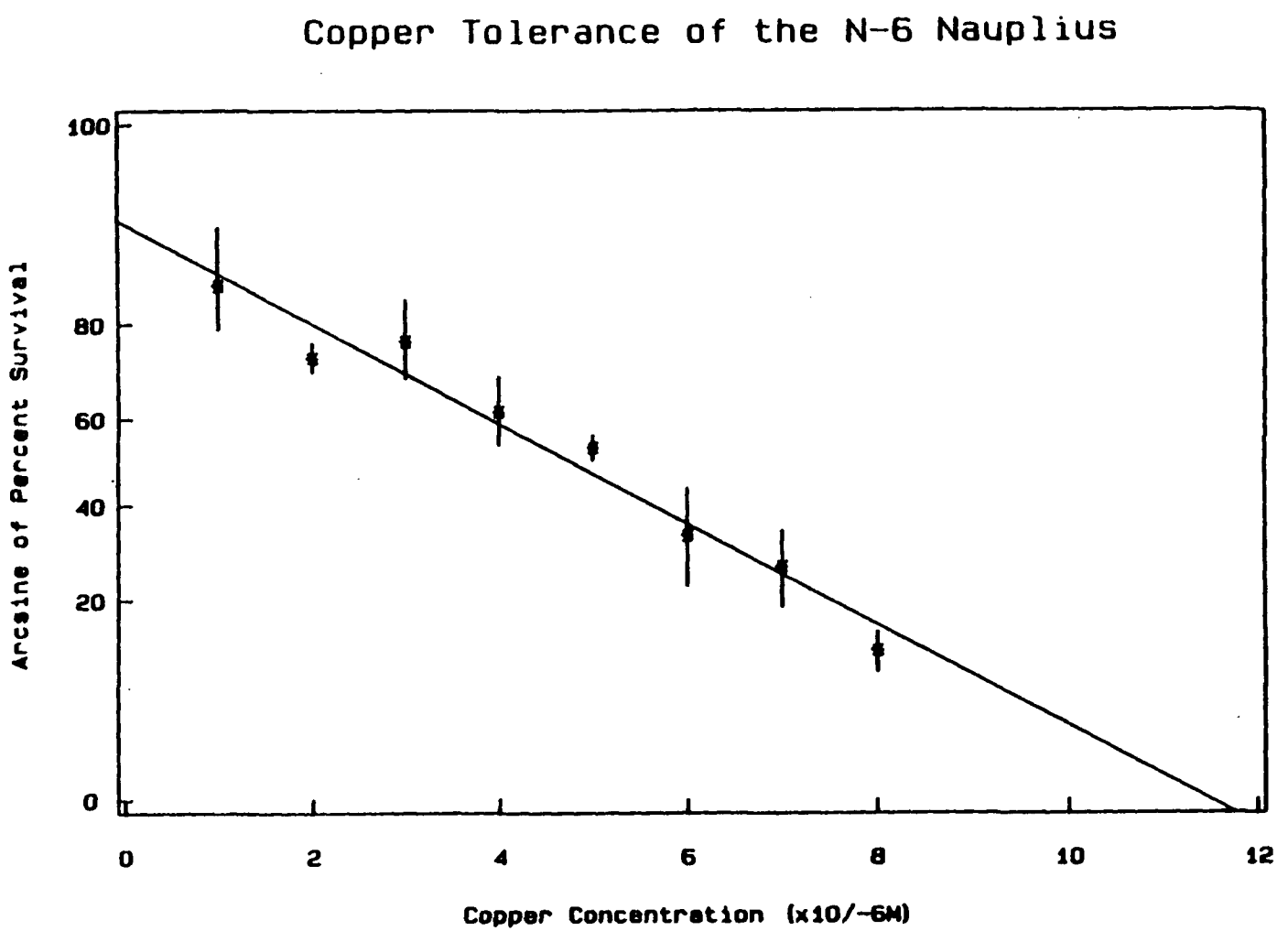

Figure 8. Arcsine of percent survival of the sixth naupliar stage of Tigriopus californicus upon exposure to copper solutions.

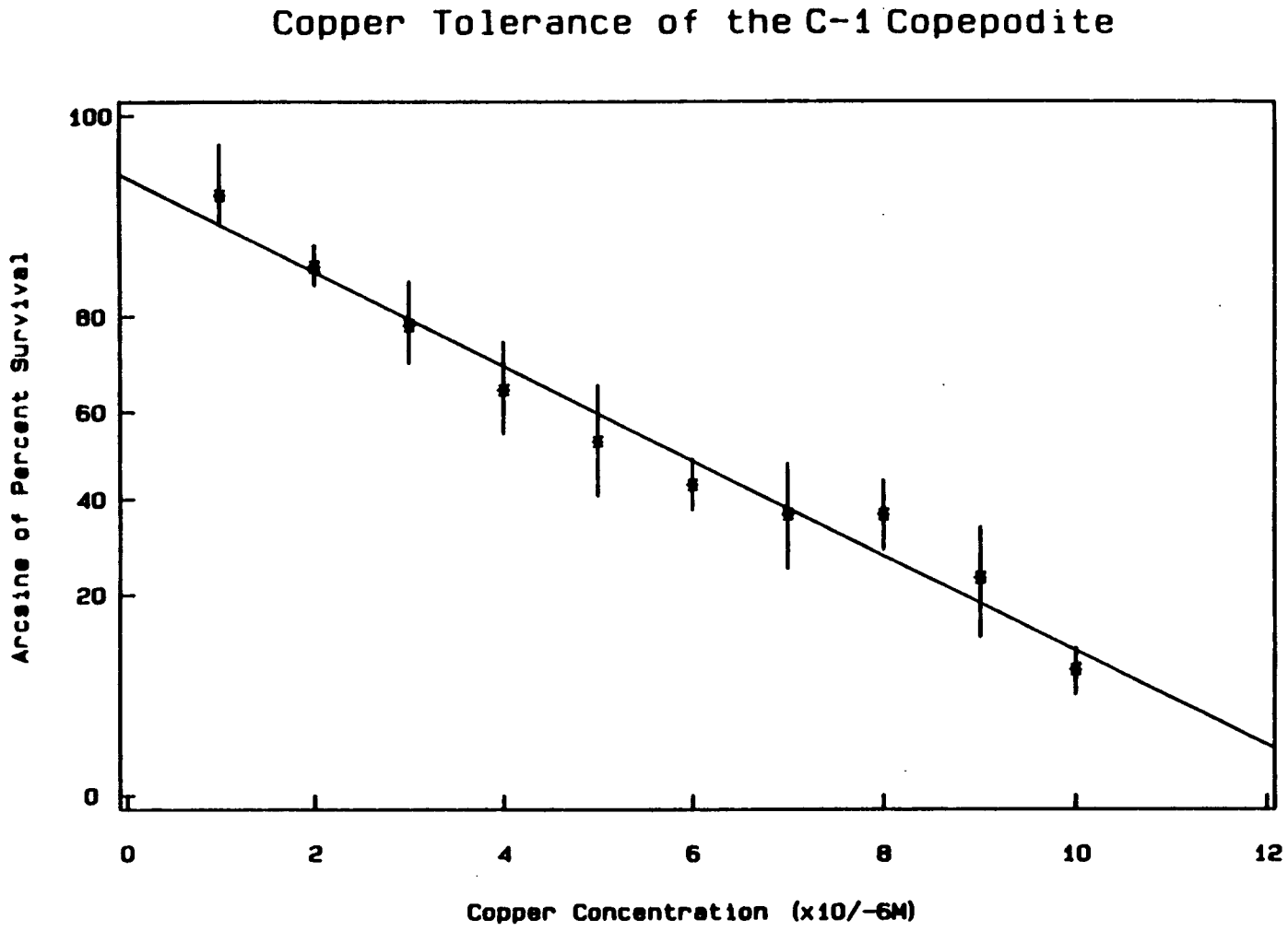

Figure 9. Arcsine of percent survival of the first copepodite stage of Tigriopus californicus upon exposure to copper solutions.

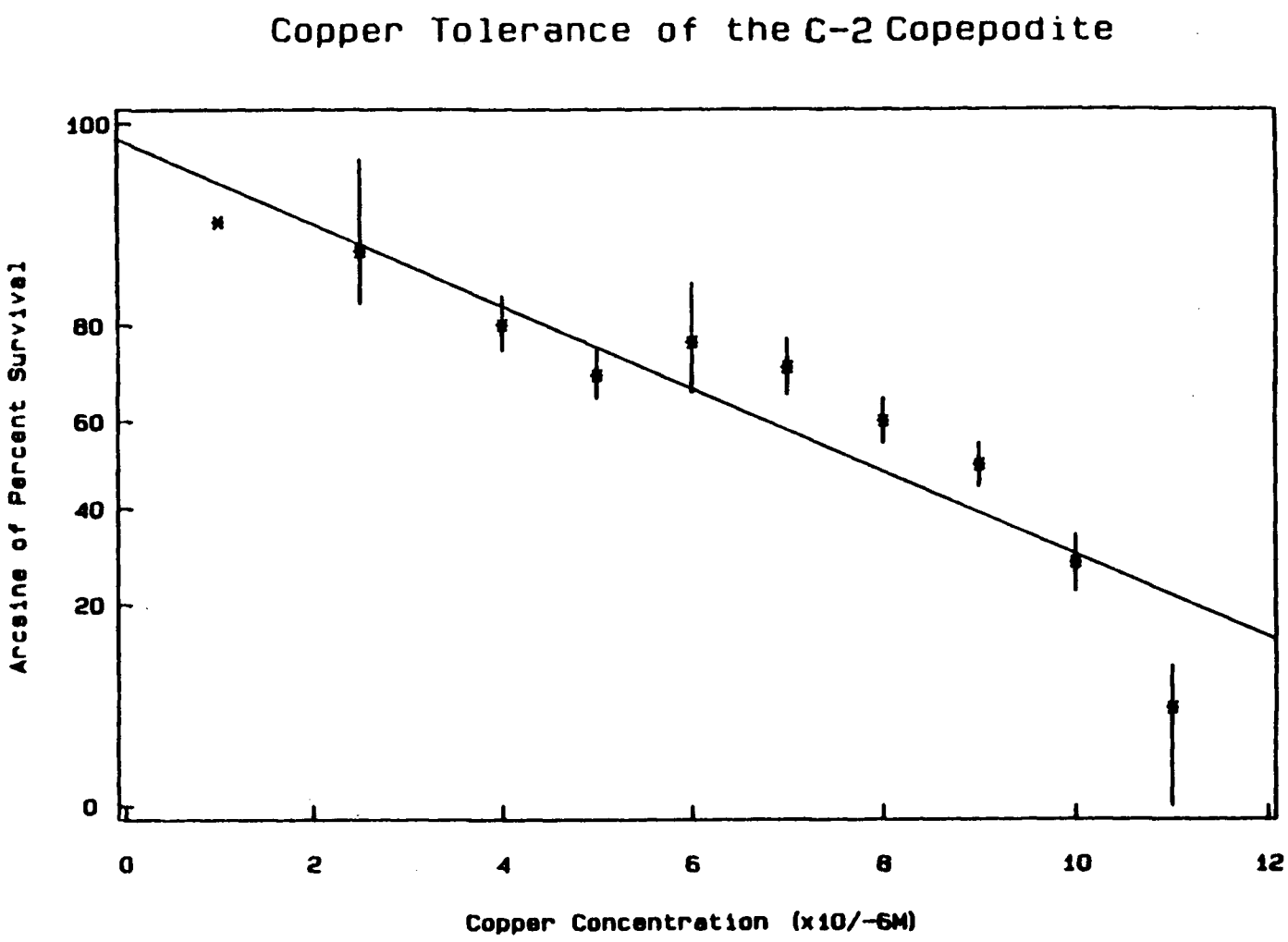

Figure 10. Arcsine of percent survival of the second copepodite stage of Tigriopus californicus upon exposure to copper solutions.

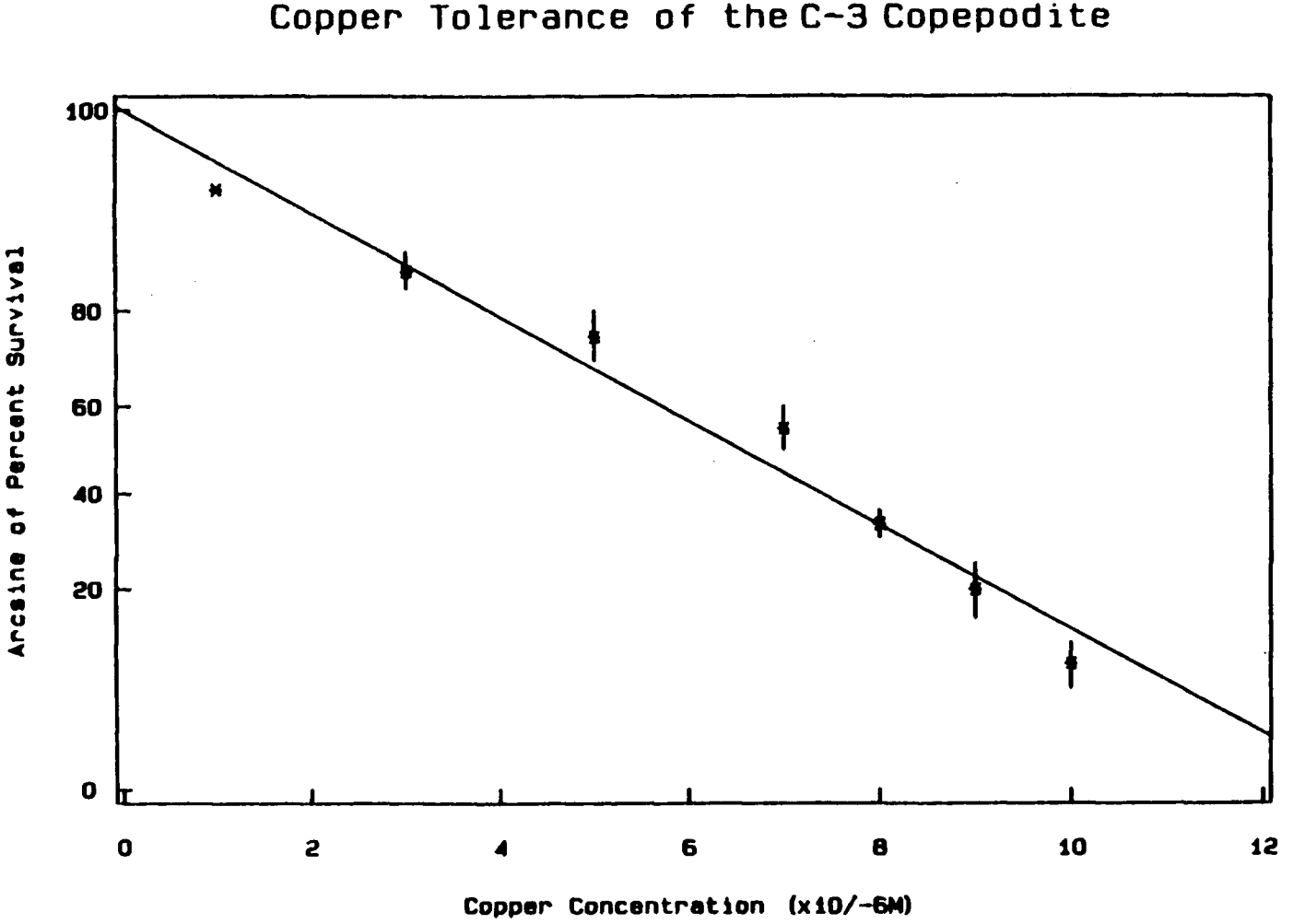

Figure 11. Arcsine of percent survival of the third copepodite stage of Tigriopus californicus upon exposure to copper solutions.

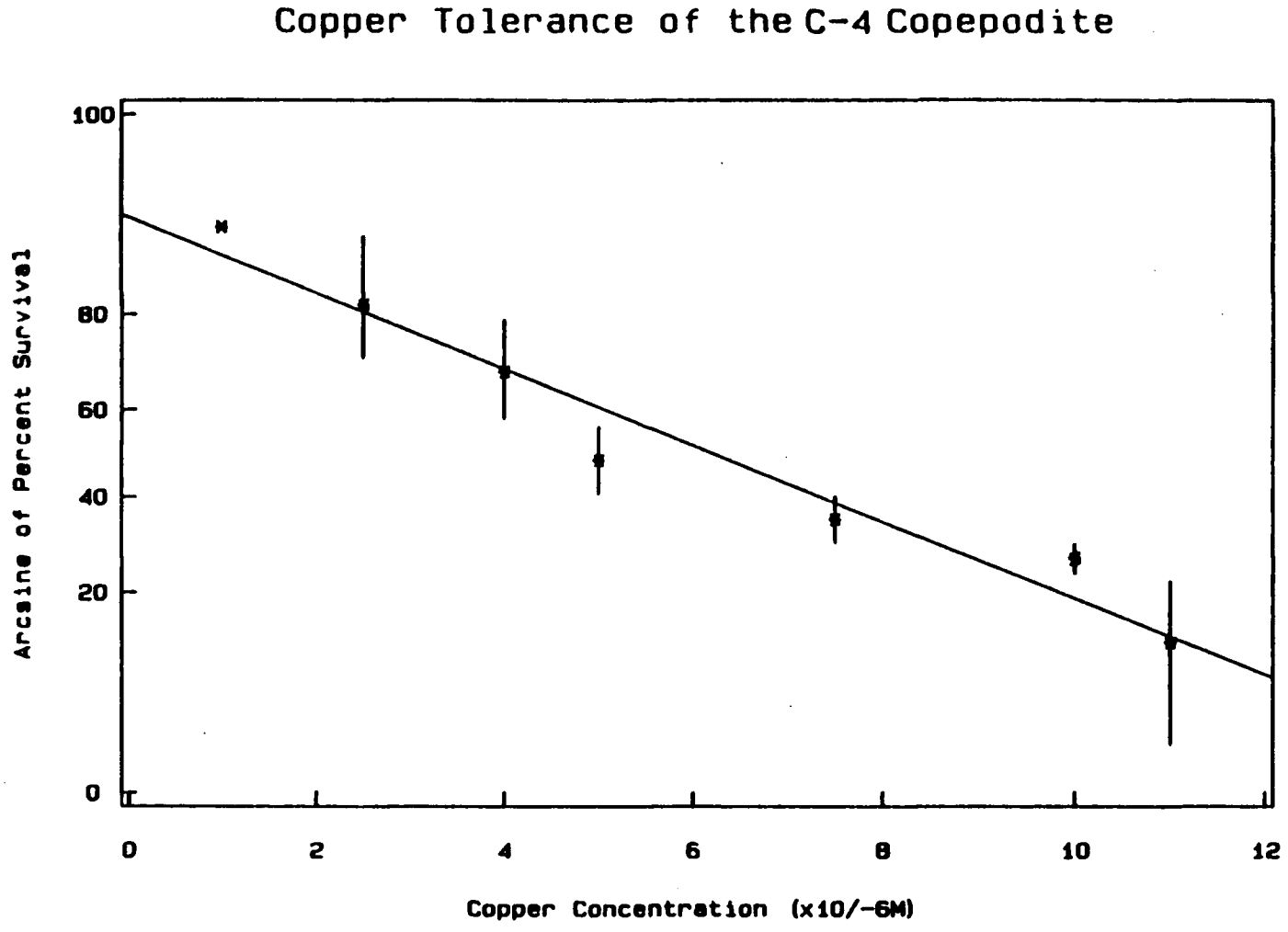

Figure 12. Arcsine of percent survival of the fourth copepodite stage of Tigriopus californicus upon exposure to copper solutions.

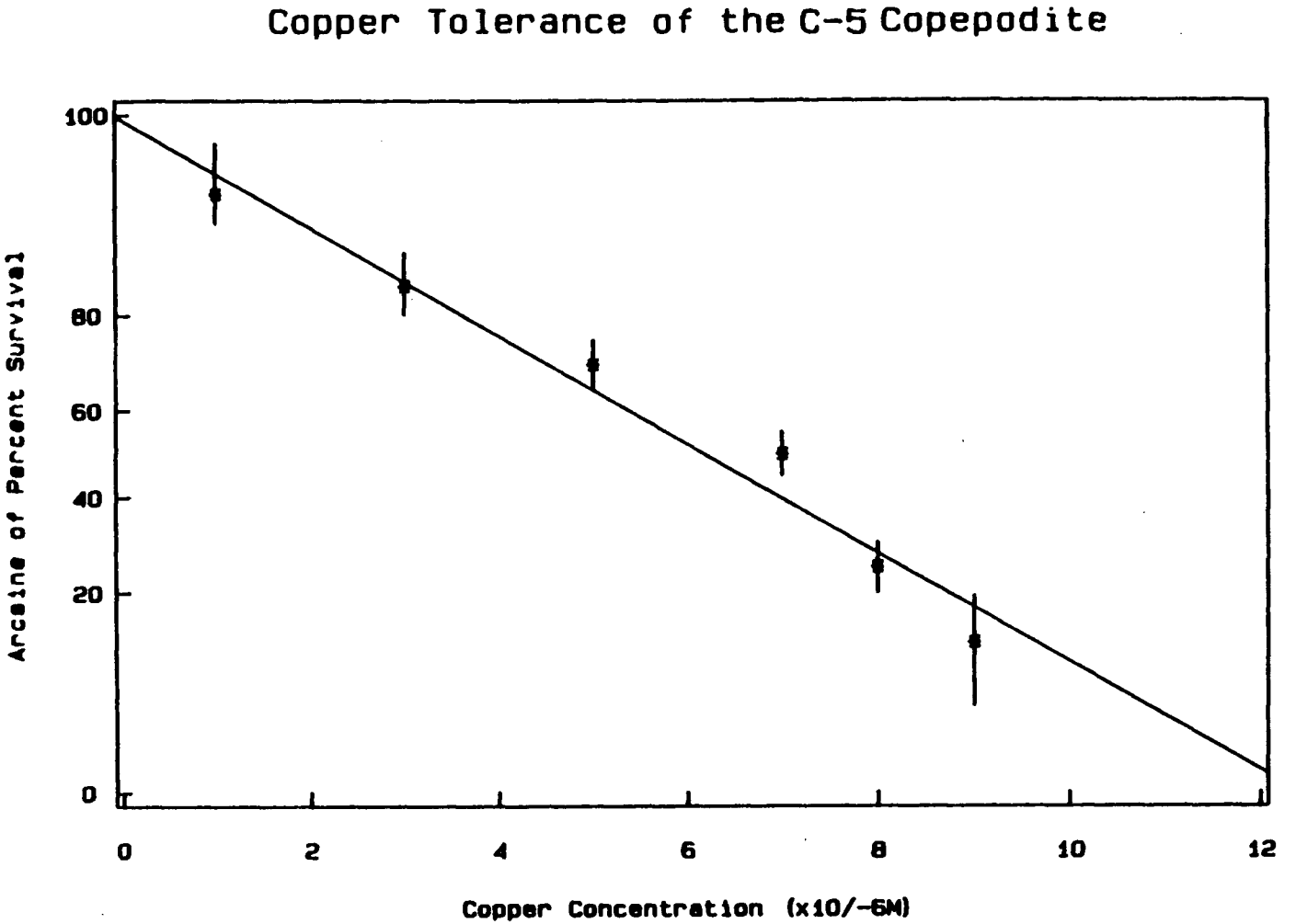

Figure 13. Arcsine of percent survival of the fifth copepodite stage of Tigriopus californicus upon exposure to copper solutions.

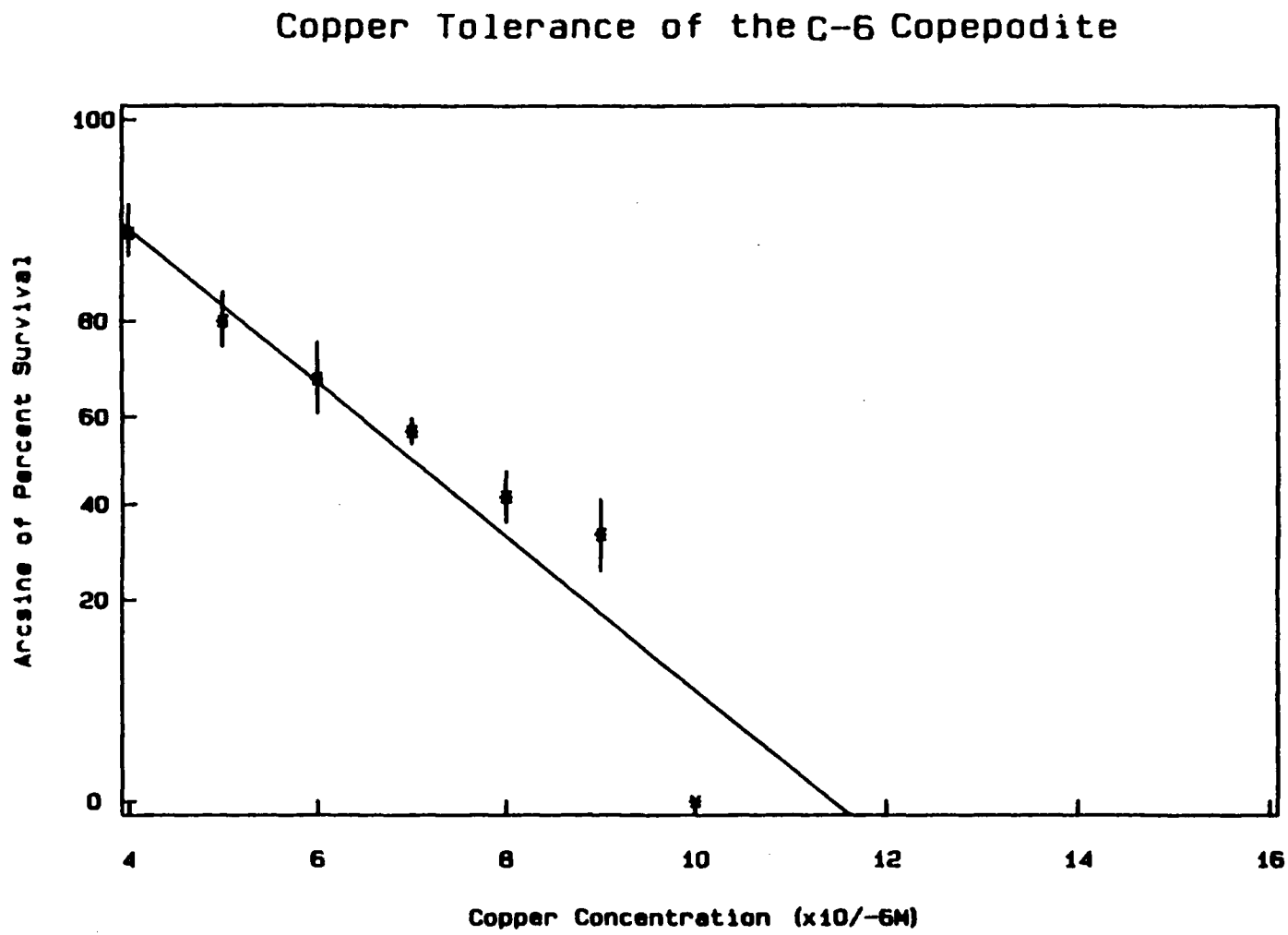

Figure 14. Arcsine of percent survival of the sixth copepodite stage of Tigriopus californicus upon exposure to copper solutions.

Discussion

As a splash pool inhabitant, Tigriopus californicus is subjected to marked changes in water temperature, salinity, and organic matter content. It survives in part because very few other organisms can exist under these conditions. Dethier (1980), suggests that Tigriopus is a a poor competitor and, for this reason, has been excluded from all except splash pool ecosystems. Although the natural habitat of Tigriopus is very harsh it is usually pristine; splash pools are infrequently subjected to contamination.

This study tested the hypothesis that exposure to widely varying but natural ecological conditions would increase tolerance to unnatural as well as natural stresses. Very high levels of copper in experimental solutions containing various life-history stages of Tigriopus were used as the unnatural stress with which to test this hypothesis.

The egg stage of Tigriopus is deposited into an egg sac attached to the mother and must be able to withstand the same environmental stress which the adults are exposed to, or they will not survive. The egg stage was found to be tolerant to total copper levels approaching 1.0×10^{-5} M (the LC50 and the LC95 values were 3.6×10^{-6} M and 9.6×10^{-6} M Cu: see Table 1). The adult stage LC50 and LC95 values were 1.2×10^{-5} M and 1.5×10^{-5} M Cu. The embryonic stage is carried by, and is

exposed to the same conditions, as the mother until the eggs are released or hatch. It is not known if the egg sac increases an egg's tolerance to the environment.

It was expected that either the N-1 or the embryonic stage would have the lowest LC50 and LC95 values since they are the least developed stages and would be expected to have the least resistance to environmental changes. The N-1 is the first post-embryonic life-history stage and would have the least energy reserves and strength of any of the naupliar stages while the egg stage would be even less developed and have smaller energy pools. The egg has, however, been exposed to the same environment that the mother copepod experienced.

Once the eggs are released as nauplii, they aggregate in the sedimentary debris and swim actively about. The copper tolerance of each naupliar stage was determined in a series of bioassays. The N-2 stage appeared to be the most sensitive to copper with each succeeding stage being more tolerant (Table 2). Each stage past N-1 was physically larger, further developed and more mobile than the previous stages. It is interesting to note that the egg stage was found to be more tolerant of copper than the first four naupliar stages based on LC95 values.

Natural perturbation of the environment (such as dessication of a tidepool or the introduction of a predator to a community) may cause mortality approaching 50 to 95% of the

population. The probability of population recovery after half (or 95%) of a population is lost due to natural causes would likely be greater than if the cause was unnatural. A 96 h bioassay is artificial in that it determines the affect of an added material on the survival of a group of organisms at 96 hours. It does not take into account the longer term impact of the chemical or the metal on the bioassay organisms after the test period. It would be expected that an LC50 or LC95 value would underestimate the true mortaltiy; an LC50 value would not mean that half of the population would survive.

The sequence of increasing metal tolerance with increasing developmental state observed for the naupliar stages does not hold for the copepodite stages. The LC95 copepodite data suggests the following trends in copper tolerance: C-6>C-4>C-2>C-3>C-1>C-5. The C-6 appeared to be the most tolerant copepodite stage and C-5 the most sensitive copepodite stage. The adult (C-6) copepod appeared able to tolerate significantly more copper than any other stage of Tigriopus. Being more developed and having survived longer than previous stages, the C-6 could be expected to have developed more tolerance to stress than the other stages.

In contrast to this predicted regime of increasing tolerance with increasing life-history stage is the C-5 copepodite which appears to be the most copper-sensitive copepodite stage, tolerating the least copper of any copepodite

stage (see Table 2). An explanation of this may be found upon examination of the LC50 and LC95 values in Table 2 and in Figure 1 and 2. There is no statistically significant difference in the tolerance of any copepodite stage from the others. One may obtain only an estimate of the tolerance of the various copepodite stages from these data.

The LC50 data suggests a different sequence than the LC95 data: C-6>C-2>C-3>C-4>C-5>C-1. Again, large standard deviation values preclude the sequencing of the copepodite stages into a copper-tolerance series of significance. The majority of LC50 values (C-1 to C-5) lie between 5.9 (C-1) and 7.8×10^{-6} M Cu (C-2). As in the LC95 series, the C-6 stage appears to be the most tolerant copepodite stage (rather than the C-5) and C-1 the least tolerant stage. The C-2 LC50 and LC95 values (7.8×10^{-6} M and 1.4×10^{-5} M Cu) are both higher than one might expect if it is assumed that each successive life-history stage would be more tolerant than the previous stage. The C-2 LC50 and LC95 values were greater than the C-3, C-4, and C-5 values (6.5×10^{-6} M and 1.1×10^{-5} M Cu, 6.2×10^{-6} M and 1.3×10^{-5} M Cu, and 6.2×10^{-6} M and 1.1×10^{-5} M Cu, respectively).

The survival plot of the adult stage was distinguished by a marked jump in both the LC50 and the LC95 values (1.2×10^{-5} M and 1.5×10^{-5} M Cu) over the C-5 values (6.2×10^{-6} M and 1.1×10^{-5} M Cu) as well as by a very abrupt decrease in adult survival (from 33.3 to 00.0%) between 1.4×10^{-5} and 1.5×10^{-5} M Cu. Survival

remained high (98.3 to 80.0%) between $8.0x10^{-6}$ and $1.0x10^{-5}$ M Cu, but between $1.1x10^{-5}$M and $1.5x10^{-5}$ M Cu, percent survival dropped from 68.3 to 00.0%. Once a crucial level of copper was reached, a further increase produced a more pronounced effect than was expected.

It appears that once the first copepodite stage has been reached factors other than the organism's developmental stage affect its ability to tolerate copper stress. These factors may be associated with or be part of the organism's biology and may include metal-complexing organics such as metallothionein, or they may be as simple as adsorption of metal onto the copepod cuticle.

Metal uptake may be active or passive in Tigriopus californicus. The fate of metal in this copepod was not determined, although it has been suggested that the copepod cuticle has been found to be a sink for metal (pers comm. A.G. Lewis). Once metal is in the gut of the copepod, it is subject to change in speciation since the low pH of the gut (relative to seawater pH) will cause metals to be freed from chelators as ionic species).

Metallothioneins (low molecular weight proteins with high thiol content and a high affinity for Hg, Cd, Zn, Ag, and Cu initially found in vertebrates: Cherian and Goyer, 1978; Roesijadi, 1980; Overnell and Trewhella, 1979; Talbot and Magee,

1978) play an important role in the metabolism and regulation of essential metals (such as Zn and Cu) which are important in metalloenzymes, membranes, and nucleic acids. Metallothionein production has been found to be induced by the presence of toxic metals (particularly Cd and Hg), suggesting that the occurrence of this protein will increase during periods of metal stress (metallothioneins contain cysteinyl residues which are efficient metal binding residues, forming three mercaptide bonds per metal atom).

Metallothioneins, or very similar proteins, have also been found in several invertebrate species (such as mussels Mytilus edulis (Talbot and Magee, 1978), crab Cancer pagurus (Overnell and Trewhella, 1979) and Scylla serrata (Fisher, 1980), oyster Crassostrea virginica (Engel and Brouwer, 1982), clam Protothaca staminea (Roesijadi, 1980), blue-green algae Anacystis nidulans and yeast Candida utilis (Fisher, 1980; Lerch, 1980)) suggesting that these groups have the ability to bind metals into non-toxic forms.

It would thus be entirely possible that the harpacticoid copepod Tigriopus californicus would be able to produce metallothioneins, or similar proteins, capable of binding toxic metals into non-available forms.

Roesijadi (1980) suggests that a threshold concentration may exist for metals below which the production of

metallothionein is non-detectable and that once this threshold is passed protein production is increased. From the data in Table 2, there does appear to be a threshold concentration for Tigriopus at approximately 6.0×10^{-6} M Cu; below this level, there is an increase in LC50 copper tolerance values with increasing developmental stage suggesting that the metallothionein may not be present in significant quantities to bind the copper. There is, however, very little change in copper tolerance with increasing developmental stage at concentrations above this "threshold" value, suggesting that the metallothionein may be functioning to bind metals.

There is also thought to be a "saturation capacity" of metallothionein, which, when exceeded, will allow metal to "spill" over as biologically available metal. Spillover may be occurring with Tigriopus at concentrations between 1.4 and 1.5×10^{-5} M Cu producing the observed increase in adult mortality (going from 41.7% $\pm$5.8 survival at 1.3×10^{-5} to 00.0% survival at 1.5×10^{-5} M Cu).

It is not known what proportion of total metal added to bioassay solutions was in a form available to Tigriopus. Experiments were run to determine the ability of materials in seawater and SOW to bind metals into unavailable forms. An unsuccessful attempt was made to determine the amount of metal in an available state with the resin column technique of Zorkin et al., (1986). It is believed that the lack of success was due

to insufficient resin being used in the columns which may have lead to the columns becoming supersaturated with copper. As such, there was no significant difference found in the amount of biologically available copper in any of the natural and synthetic seawater tests.

While it was not possible to determine the amount of metal which was biologically available, known concentrations of copper were equilibrated with ground fish food and added to cultures of Tigriopus in an attempt to determine if the ingestion of copper would affect egg production (without a food source, Tigriopus will not produce eggs).

There was no significant effect of the copper-enriched food on adult fecundity between $1.0x10^{-10}$ and $1.0x10^{-6}$ M Cu (Table 15). The percentage of berried females ranged from 65.0% $\pm$5.8 ($8.0x10^{-8}$) to 85.0% $\pm$10.0 ($1.0x10^{-10}$ M Cu). There was a significant effect of copper on egg cluster production at $1.0x10^{-5}$ M. At this concentration, there was no egg production.

Between $1.0x10^{-9}$ and $1.0x10^{-6}$ M Cu, there was no discrete concentration threshold above which Tigriopus would not produce eggs. The percentage of berried females dropped from 86.5% $\pm$16.4 at $1.0x10^{-6}$ to zero at $1.0x10^{-5}$ M Cu. It is not known whether egg case production past the first cluster was nclu affected by the copper solutions since the bioassay lasted for only 96 hours, not enough time for the release of a second or

third egg sac.

The effect of copper-labelled food on Tigriopus mortality was determined as it was thought that food containing copper in a SOW solution may be more toxic to Tigriopus than copper in SOW alone. Survival in medium containing food and metal was compared to survival in SOW solutions containing copper alone. There was no significant difference in adult survival between organisms exposed to copper solutions containing metal-equilibrated food and those exposed to copper solely in solution over the range $1.0x10^{-10}$ to $1.0x10^{-5}$ M Cu (Table 15).

This result is surprising since the pH of the copepod gut is lower than the pH of SOW or natural seawater. This would result in metal bound to the organic food material being released in the lower pH environment: this would be expected to increase adult mortality.

An apparent relationship between copper concentration and naupliar activity was studied with N-1 nauplii being exposed to copper over the range $1.0x10^{-8}$ to $1.0x10^{-5}$ M. The only level of copper which produced a significant reduction in naupliar activity was $1.0x10^{-5}$ M Cu.

While manganese has been found to reduce copper toxicity to some species of phytoplankton, (Sunda et al., 1981; Sunda and Huntsman, 1983; Stauber and Florence, 1985) it has not been

documented to produce the same result in aquatic animals. The addition of manganese to copper solutions did not significantly reduce copper toxicity to Tigriopus N-1 nauplii (Table 18). There was some indication, however, that manganese may reduce the copper toxicity to an aquatic animal such as Tigriopus californicus (at a non-significant level of confidence).

Although the mechanism by which manganese acts to ameliorate copper toxicity in phytoplankton is unknown at this time, it is thought to be either through competition at a manganese uptake site (Sunda et al., 1981) or manganese hydroxide sorption of copper onto the surface of the cell (Stauber and Florence, 1985). The latter mechanism could reduce total copper levels in both plants and animals.

The addition of manganese to bioassay solutions containing eggs of Tigriopus did not reveal the existence of a threshold of response over the manganese concentration range of 1.0×10^{-9} to 1.0×10^{-5} M (Table 19). There was no concentration of manganese which produced a statistically significant change in the percentage of eggs found to be viable.

It is interesting to compare the tolerance of Tigriopus, a splashpool inhabitant, to a planktonic calanoid copepod such as Euchaeta japonica whose metal tolerance has been studied in some detail (Lewis et al., 1973). This would allow one to determine if exposure to fluctuating environmental conditions may affect

the response of an organism's life-history stages relative to an organism that does not know a harsh environment. Tigriopus is a specialist at coping with stress in its natural environment while Euchaeta is not exposed to the same environmental changes as open marine waters do not undergo the rapid and extreme changes in conditions found in intertidal waters. Euchaeta does not have to cope with major changes in its environment since its environment is stable. One would thus not expect Eucheata to be as stress tolerant as Tigriopus.

This work with Tigriopus californicus has shown that it can tolerate more copper than Euchaeta japonica. Calibrating the tolerance of each life-history stage of Tigriopus to copper demonstrated that the most sensitive stage was the first naupliar (with a copper LC50 of 1.1×10^{-6} M Cu). Although LC values were not calculated for Euchaeta the copper tolerance of its egg stage was 1.0×10^{-8} M (Lewis et al., 1973). Physiological adaptations allow Tigriopus to survive in splashpools; the production of metallothioneins may be an organism's response to bind available metals into non-toxic forms. The physiological adaptations are thought to be evolutionary adaptations and the metallothionein production is believed to be a response to anthropogenic influences.

The tolerance to copper observed in the egg, naupliar, and copepodite life-history stages of Tigriopus californicus upon exposure to copper solutions, together with the adult tolerance

to copper-labelled food in the fecundity and mortality experiments, and the N-1 tolerance in the activity and manganese experiments, may be interpreted as support of the hypothesis that exposure to stress increases tolerance to stress.

The fact that Tigriopus survives in its niche and copes with inhospitable conditions in nature suggests that it is a tolerant organism. It may be that natural selection removes the weaker, or less tolerant, organisms and only the flexible, stress-resistant individuals survive. This ability to cope with natural stress does not suggest that it would be at all able to tolerate anthropogenic sources of stress. It has been demonstrated, however, that Tigriopus can tolerate severe metal stress in the form of copper concentrations far beyond those it would be likely to encounter in nature.

Since Tigriopus has been demonstrated to be stress-tolerant and is readily cultured in the laboratory, it was thought that it may be useful for bioassay applications. Most bioassay organisms are tolerant to stress or they could not be cultured in the laboratory. Preliminary experiments with copper, however, revealed that Tigriopus was too tolerant to be useful in most bioassay work.

Conclusion

This work attempted to verify the hypothesis that exposure to widely varying but natural ecological conditions would increase an organism's tolerance to unnatural stress. Exposure to high concentrations of copper was selected as the agent with which to induce a stress response in Tigriopus californicus.

The response of each life history stage of Tigriopus to copper was determined and LC50 and LC95 values were calculated. The egg stage is the first life-history stage and was expected to be one of the most sensitive stages; it is also the only life-history stage which is exposed to the same environmental conditions as the adult until it is released. The egg stage was approximately as copper-tolerant as the N-4.

The egg sac is actually a gel or coat of chitinous protein which binds the eggs together and this material is thought to be highly selective for metals. When egg clusters are exposed to metals, the metal solution may wash through the gel with some of the metal being adsorbed or absorbed into the coating.

The adult (LC95 = 1.5×10^{-5} M Cu) was approximately 50% more tolerant than the egg stage (LC95 = 9.6×10^{-6} M Cu). There was a general increase in the ability of Tigriopus to tolerate copper with increasing developmental stage from the first naupliar to the last copepodite.

It was thought that copper solutions allowed to equilibrate with ground fish food might be more toxic to Tigriopus than copper equilibrated with SOW alone as metal would be ingested both with its food and absorbed from solution. There was no significant effect of copper-labelled food on egg case production, except at $1.0x10^{-5}$ M, nor was there any significant difference in survival in solutions containing copper-labelled food, except at $1.0x10^{-5}$ M. There was no significant difference in adult mortality between those animals exposed to copper equilibrated with SOW and those containing copper-labelled food.

It was suggested from preliminary observations of Tigriopus nauplii that the level of activity exhibited was a function of the copper concentration to which they were exposed. However, the naupliar activity level was not found to vary significantly upon exposure to copper solutions between $1.0x10^{-8}$ and $1.0x10^{-6}$ M. It is apparent that the natural stress associated with life in a tidepool environment has increased the tolerance of Tigriopus to unnatural stress.

There was some suggestion that manganese could reduce copper toxicity to Tigriopus, but not to any significant degree. It is thought that the copper tolerance of Tigriopus may have masked some of the manganese effect.

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APPENDIX

Table 1. The effect of copper on the viability of *Tigriopus californicus* eggs.

Total Added Copper Concentration (M)	Average Survival (%)	Standard Deviation
1.0×10^{-9}	78.8	13.0
1.0×10^{-8}	86.8	8.5
1.0×10^{-7}	74.0	22.1
1.0×10^{-6}	81.5	18.7
1.0×10^{-5}	3.0	7.0
Control	85.0	17.9

Table 2. LC50 and LC95 values for each life-history stage of Tigriopus californicus (including 95% confidence limits).

Life History Stage	LC50 ($x10^{-6}$ M Cu)	LC95 ($x10^{-6}$ M Cu)
N-1	1.15 (0.62-1.60)	3.72 (3.28-4.24)
N-2	0.28 (-1.92-1.95)	4.72 (2.96-7.95)
N-3	2.46 (0.88-3.87)	8.03 (6.57-9.80)
N-4	2.92 (1.49-4.23)	8.69 (7.28-10.43)
N-5	4.12 (3.28-4.96)	8.79 (7.87-9.85)
N-6	4.75 (3.48-6.05)	9.55 (8.12-11.33)
C-1	5.88 (4.57-7.20)	11.0 (9.60-12.63)
C-2	7.82 (4.52-11.45)	13.7 (10.23-18.81)
C-3	6.54 (4.96-8.16)	11.2 (9.52-13.26)
C-4	6.19 (3.94-8.50)	12.5 (10.13-15.64)
C-5	6.15 (4.50-7.88)	10.6 (8.82-12.94)
C-6	12.00 (1.00-1.40)	15.1 (1.32-1.78)
egg	3.63 (1.28-6.17)	9.60 (6.97-13.1)

Table 3. Percent survival of Tigriopus californicus N-1 nauplii upon exposure to copper solutions. LC50 = 1.2×10^{-6} M Cu. LC95 = 3.7×10^{-6} M Cu.

Total Added Copper Concentration ($\times 10^{-6}$ M)	Percent Range Survival	Average Value (Percent)	Standard Deviation
1.0	45-60	53.3	7.6
2.0	25-45	33.3	10.4
3.0	5-15	11.7	5.8
4.0	0-10	3.3	5.8
Control	90-100	95.0	14.1

Table 4. Percent survival of Tigriopus californicus N-2 nauplii upon exposure to copper solutions. LC50 = 0.28×10^{-6} M Cu. LC95 = 4.72×10^{-6} M Cu.

Total Added Copper Concentration ($\times 10^{-6}$ M)	Percent Range Survival	Average Value (Percent)	Standard Deviation
0.2	50-70	61.7	10.4
0.4	50-60	55.0	5.0
0.6	40-55	48.3	7.6
0.8	25-45	33.3	10.4
1.0	30-35	31.7	2.9
2.0	15-30	23.3	2.6
3.0	20-25	21.7	2.9
4.0	5-20	11.7	7.6
Control	85-95	90.0	14.1

Table 5. Percent survival of Tigriopus californicus N-3 nauplii upon exposure to copper solutions. LC50 = 2.46×10^{-6} M Cu. LC95 = 8.03×10^{-6} M Cu.

Total Added Copper Concentration ($\times 10^{-6}$ M)	Percent Range Survival	Average Value (Percent)	Standard Deviation
1.0	50-85	65.0	10.0
2.0	45-55	50.0	5.0
3.0	40-50	45.0	5.0
4.0	30-40	35.0	5.0
5.0	20-35	26.7	2.9
6.0	10-30	21.7	2.9
7.0	10-20	15.0	5.0
8.0	0-5	1.7	2.9
Control	80-85	82.5	7.1

Table 6. Percent survival of Tigriopus californicus N-4 nauplii upon exposure to copper solutions. LC50 = 2.92×10^{-6} M Cu. LC95 = 8.69×10^{-6} M Cu.

Total Added Copper Concentration ($\times 10^{-6}$ M)	Percent Range Survival	Average Value (Percent)	Standard Deviation
1.0	60-65	61.7	18.3
2.0	55-60	58.3	5.0
3.0	50-60	55.0	5.0
4.0	40-50	45.0	5.0
5.0	25-40	31.7	2.9
6.0	15-30	23.3	2.9
7.0	0-15	10.0	5.0
8.0	5-15	8.3	2.9
Control	85-90	87.5	7.1

Table 7. Percent survival of Tigriopus californicus n-5 nauplii upon exposure to copper solutions. LC50 = 4.12×10^{-6} M Cu. LC95 = 8.79×10^{-6} M Cu.

Total Added Copper Concentration ($\times 10^{-6}$ M)	Percent Range Survival	Average Value (Percent)	Standard Deviation
1.0	75-95	85.0	10.0
2.0	60-85	75.0	13.2
3.0	45-75	60.0	15.0
4.0	40-75	56.7	17.7
5.0	30-45	36.7	7.6
6.0	20-35	26.7	7.6
7.0	10-25	15.0	8.7
8.0	5-15	13.3	7.6
Control	85-95	90.0	14.1

Table 8. Percent survival of Tigriopus californicus N-6 nauplii upon exposure to copper solutions. LC50 = 4.75×10^{-6} M Cu. LC95 = 9.55×10^{-6} M Cu.

Total Added Copper Concentration ($\times 10^{-6}$ M)	Percent Range Survival	Average Value (Percent)	Standard Deviation
1.0	80-95	86.7	7.6
2.0	70-75	73.3	2.9
3.0	70-85	76.7	7.6
4.0	55-70	61.7	7.6
5.0	50-55	53.3	2.9
6.0	25-45	33.3	10.3
7.0	20-35	26.7	7.6
8.0	10-15	11.7	2.9
Control	80-85	82.5	4.5

Table 9. Percent survival of Tigriopus californicus C-1 copepodites upon exposure to copper solutions. LC50 = 5.88×10^{-6} M Cu. LC95 = 1.10×10^{-5} M Cu.

Total Added Copper Concentration ($\times 10^{-6}$ M)	Percent Range Survival	Average Value (Percent)	Standard Deviation
1.0	95-100	96.7	2.9
2.0	85-90	88.3	2.9
3.0	70-80	75.0	5.0
4.0	55-75	65.0	5.0
5.0	40-65	53.3	12.6
6.0	40-50	43.3	5.8
7.0	30-50	36.7	11.6
8.0	30-45	36.7	7.6
9.0	15-25	20.0	5.0
10.0	5-10	8.3	2.9
Control	85-95	90.0	14.1

Table 10. Percent survival of Tigriopus californicus C-2 copepodites upon exposure to copper solutions. LC50 = 7.82×10^{-6} M Cu. LC95 = 1.37×10^{-5} M Cu.

Total Added Copper Concentration ($\times 10^{-6}$ M)	Percent Range Survival	Average Value (Percent)	Standard Deviation
1.0	85-100	95.0	8.7
2.5	85-100	91.7	7.6
4.0	75-85	80.0	5.0
5.0	65-75	70.0	5.0
6.0	65-85	76.7	10.4
7.0	65-75	71.7	5.7
8.0	55-65	60.0	5.0
9.0	45-55	50.0	5.0
10.0	25-35	28.3	5.8
11.0	0-10	5.0	5.0
Control	90-100	95.0	14.1

Table 11. Percent survival of Tigriopus californicus C-3 copepodites upon exposure to copper solutions. LC50 = 6.54×10^{-6} M Cu. LC95 = 1.12×10^{-5} M Cu.

Total Added Copper Concentration ($\times 10^{-6}$ M)	Percent Range Survival	Average Value (Percent)	Standard Deviation
1.0	90-100	96.7	5.8
3.0	85-90	86.7	2.9
5.0	70-80	75.0	5.0
7.0	50-60	65.0	5.0
8.0	30-35	33.3	2.9
9.0	15-25	20.0	5.0
10.0	5-10	8.3	2.9
Control	85-100	92.5	21.2

Table 12. Percent survival of Tigriopus californicus C-4 copepodites upon exposure to copper solutions. LC50 = 6.19×10^{-6} M Cu. LC95 = 1.25×10^{-5} M Cu.

Total Added Copper Concentration ($\times 10^{-6}$ M)	Percent Range Survival	Average Value (Percent)	Standard Deviation
1.0	85-100	93.3	7.6
2.5	70-90	81.6	10.4
4.0	60-80	68.3	10.4
5.0	40-55	48.3	7.6
7.5	30-40	35.0	5.0
10.0	25-30	26.7	2.9
11.0	0-20	11.7	10.4
Control	100	100.0	00.0

Table 13. Percent survival of Tigriopus californicus C-5 copepodites upon exposure to copper solutions. LC50 = 6.15×10^{-6} M Cu. LC95 = 1.06×10^{-5} M Cu.

Total Added Copper Concentration ($\times 10^{-6}$ M)	Percent Range Survival	Average Value (Percent)	Standard Deviation
1.0	95-100	96.7	2.9
3.0	80-90	85.0	5.0
5.0	65-75	70.0	5.0
7.0	45-55	50.0	5.0
8.0	20-30	25.0	5.0
9.0	5-20	11.7	7.6
Control	90-95	92.5	7.1

Table 14. Percent survival of Tigriopus californicus C-6 copepodites upon exposure to copper solutions. LC50 = 1.20×10^{-5} M Cu. LC95 = 1.51×10^{-5} M Cu.

Total Added Copper Concentration ($\times 10^{-6}$ M)	Percent Range Survival	Average Value (Percent)	Standard Deviation
8.0	95-100	98.3	2.9
9.0	90-95	93.3	2.9
10.0	75-85	80.0	5.0
11.0	60-75	68.3	7.6
12.0	55-60	56.7	2.9
13.0	35-45	41.7	5.8
14.0	25-40	33.3	7.6
15.0	00-00	00.0	0.0
Control	100.0	100.0	0.0

Table 15. The effects of copper-equilibrated food on Tigriopus californicus egg production.

Copper Concentration (M)	Percent Females Berried	Standard Deviation
$1.0x10^{-10}$	85.0	10.0
$1.0x10^{-9}$	81.5	17.1
$3.0x10^{-9}$	80.0	5.9
$6.0x10^{-9}$	82.5	15.2
$1.0x10^{-8}$	84.0	10.0
$4.0x10^{-8}$	76.5	17.1
$8.0x10^{-8}$	65.0	5.8
$1.0x10^{-7}$	80.0	14.1
$3.0x10^{-7}$	77.5	12.6
$6.0x10^{-7}$	83.5	7.0
$1.0x10^{-6}$	86.5	16.4
$1.0x10^{-5}$	00.0	00.0
Control	81.3	15.5

Table 16. The effect of copper-equilibrated food on Tigriopus californicus adult mortality.

Copper Concentration (M)	Percent Survival (%)	Standard Deviation
$1.0x10^{-10}$	85.0	10.0
$1.0x10^{-9}$	82.5	17.0
$3.0x10^{-9}$	82.5	5.0
$6.0x10^{-9}$	95.0	10.0
$1.0x10^{-8}$	95.0	5.8
$4.0x10^{-8}$	87.5	9.6
$8.0x10^{-8}$	95.0	5.8
$1.0x10^{-7}$	100.0	0.0
$3.0x10^{-7}$	100.0	0.0
$6.0x10^{-7}$	92.5	9.6
$1.0x10^{-6}$	95.0	5.8
$1.0x10^{-5}$	82.5	24.5
Control	96.3	5.2

Table 17. The effect of copper on activity levels of the N-1 stage of Tigriopus californicus .

Copper Concentration (M)	Time Active (s)	Standard Deviation
$1.0x10^{-8}$	6.87	3.7
$1.0x10^{-7}$	7.75	1.6
$1.0x10^{-6}$	6.33	3.3
$1.0x10^{-5}$	0.41	2.0
Control	9.66	4.8

Table 18. The Effects of Copper and Manganese on Survival of the N-1 of Tigriopus californicus . The copper concentration in all tests was 1.0×10^{-6} M.

Manganese Concentration (M)	Percent Naupliar Survival	Standard Deviation
1.0×10^{-6}	35.0	28.2
1.0×10^{-5}	50.0	14.1
1.0×10^{-4}	40.0	28.2
Control	97.5	7.1

Table 19. The Effect of Manganese on The Viability of Tigriopus californicus Eggs (no copper added).

Total Added Manganese Concentration (M)	Average Survival (%)	Standard Deviation
$1.0x10^{-9}$	84.0	8.4
$1.0x10^{-8}$	77.0	17.8
$1.0x10^{-7}$	77.0	15.6
$1.0x10^{-6}$	82.0	10.7
$1.0x10^{-5}$	54.0	41.3
Control	85.0	17.9