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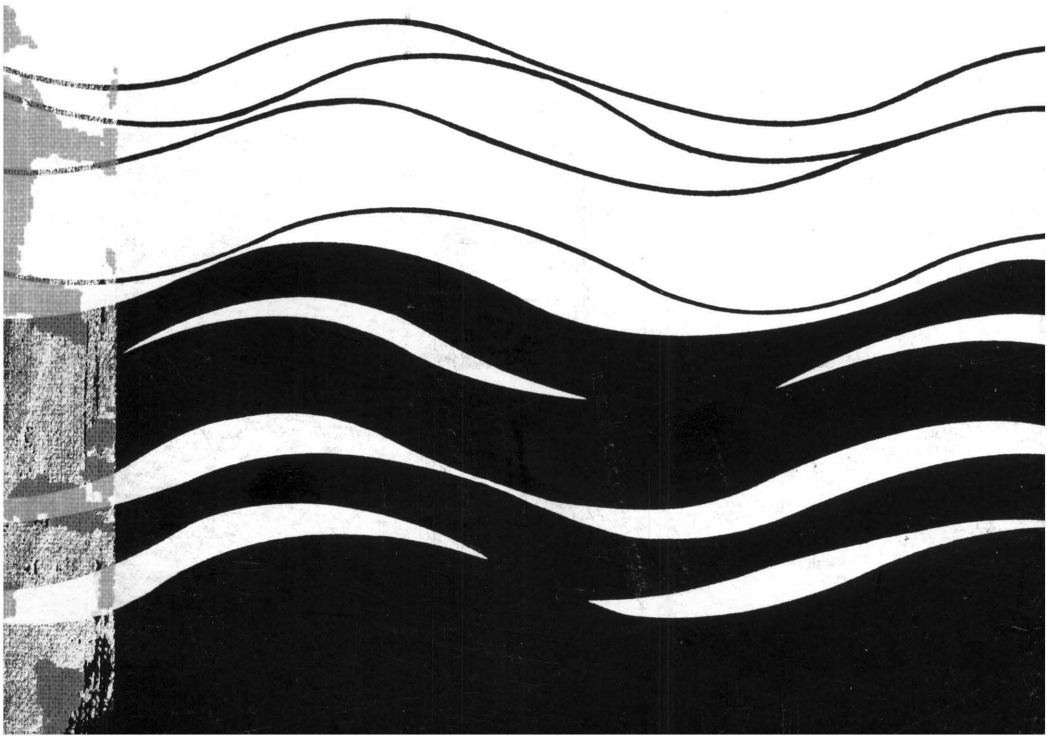

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PREFACE

Most current environmental legislation deals only with the regulation of single toxicants and thermal wastes. Little consideration has been given to the potential effects of temperature on chemical toxicity to aquatic organisms. These organisms may be important sport or commercial fishes or eventual food for fishes. The objective of this research was to provide information on the influence of temperature on toxicity (adverse effects) of chemicals on 15 species that represent various positions in the food web of an aquatic ecosystem. The scope of this research was to provide information for estimating the extent of additional research needed to define the problem and for developing hypotheses on the nature of the problem.

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Dr. John Cairns, Jr. was responsible for the overall design of the project. Responsibilities for components were as follows:

Algae—Dr. Bruce C. Parker and Kenneth G. Seaburg*
Invertebrates—Dr. Arthur L. Buikema, Jr., Dr. Cairns, Robert Honig*, Matthew McGinniss*, and Jeanne Miles Potter*
Fish—Alan G. Heath and Micki J. Smith*

*Graduate research assistant.

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ABSTRACT

This research evaluates the effect of temperature on the sensitivity of food web organisms to selected chemicals. Test organisms included planktonic algae *(Cyclotella meneghiniana, Scenedesmus quadricauda, Chlamydomonas* sp. [species], and *Lyngbya* sp.), zooplankton *(Daphnia pulex, Daphnia magna,* and *Chilomonas paramecium),* microbenthos *(Aeolosoma headleyi, Philodina acuticornis,* and *Lepidodermella* sp.), macrobenthos *(Nitrocris* sp.), and fishes *(Salmo gairdneri, Notemigonius crysoleucus, Lepomis macrochirus, Carassius auratus,* and *Ictalurus punctatus).*

The chemicals used in this research were copper, zinc, chromate, chlorine, cyanide, and phenol. The sensitivity of the test organisms to these chemicals was studied over a temperature range of 5° to 35° C. The specific temperatures studied varied with each species.

The results of this research indicate that there is considerable variation in the sensitivity of each species, and this variation depends on the chemical and the test temperature. Generally, the algal responses to the temperature and chemicals varied depending on the thermal sensitivity of the algal species in question. The invertebrates usually exhibited the classic response of increased sensitivity to chemicals at higher temperatures. Two notable exceptions to this observation were the "reverse" response of temperature and cyanide to the oligochaete *Aeolosoma* and the temperature independent response of the invertebrates to phenol. For fish, survival was enhanced (a) at low concentrations of chemicals and high temperatures and (b) at high chemical concentrations and low temperatures. Some fish species had chemical threshold concentrations which were not influenced by temperature.

Key words: Toxicity, Bioassay, Thermal Pollution, Interaction of Toxicants, Aquatic Organisms

INTRODUCTION

I. Environmental Legislation

Most of the current legislation as well as regulations and environmental laws already on the books attempt to deal on an item-by-item basis with environmental problems, including the introduction of heated wastewater from steam electric power plants and waste discharges containing various chemicals. As a consequence, control efforts are focused largely on regulating either discharge concentrations of chemicals or thermal input in the receiving system. Rarely is consideration given to the possible effects between and among these two variables which might alter the severity of their deleterious effects on aquatic organisms. The National Academy of Sciences' *Water Quality Criteria of 1972* [1973] recommends that some attention be paid to in-systems standards such as diversity indices. These would be helpful in assessing the cumulative impact on aquatic communities of the various point-source waste discharges together with non-point source discharges. Implementation of such recommendations would require a substantial revision in the administrative approach toward environmental quality control, however, and is therefore unlikely to occur in the very near future. If the current trend in environmental legislation continues, general statements about protecting the indigenous biota or maintaining fisheries, shell fisheries, and so on will be of limited value. Instead, the great need is for documentation of chemical toxicity changes that result from heated waste discharges. This data would provide the basis for modification of laws and regulations to insure that such discharges are held to environmentally acceptable levels. Modifying clauses of this sort are conspicuously absent in both the marine and freshwater sections of *Water Quality Criteria of 1972,* yet there are a substantial number of places where they would be quite appropriate, if proper scientific justification were available. Referenced works such as *Water Quality Criteria of 1972* should be updated every three to five years at the very least, and possibly every year. Thus, it would be helpful if appropriate information of this type were available for one of the forthcoming revisions.

Knowledge of the general classes of chemical compounds most likely to have their toxicity mediated by changes in temperatures would also be helpful. This would have a major effect on the protocols required by such books as *Principles for Evaluating Chemicals in the Environment* [1975] which attempts to define the bioassays and other tests necessary

before a new chemical is discharged or introduced into the environment.

Some of the current legislation refers to "zero discharge of pollutants." An attempt now is being made by the National Commission on Water Quality to define the word "pollutant." If this is done on a chemical-by-chemical or a pollutant-by-pollutant basis, it is unlikely to reflect the fact that discharge which might pollute one system might not pollute another because the quality of the environment and the nature of the ecosystem into which the material is introduced will determine whether or not the material pollutes. For legislation of this type, knowledge of the effect of temperature changes upon the toxicity of specific chemicals to aquatic organisms is mandatory. Such information also is essential for developing reliable prediction models of the assimilative capacity of various freshwater ecosystems. Such models must be produced in an overall management plan for environmental quality control if they are to be most useful in achieving beneficial, non-degrading use of ecosystems.

The most compelling support for this study is, however, the energy crisis and the nature of our response to it. The national goal for energy self-sufficiency or reduced dependency on other nations is likely to be based initially on a switch from oil to coal as the predominant fossil fuel. Perhaps the most conspicuous short-term impact of this shift will be seen in increased numbers of coal-fueled electric generating plants. A second component of the response is likely to be increased development of the atomic energy segment of the electric power generation industry. At least for the near future (i.e., slightly past the year 2000), this will result in the proliferation of power plants essentially similar in operational characteristics to those already in existence. The lead time for development of steam power plants is in excess of 10 years, and the process of acquiring permits for such plants requires several years. Thus, substantial numbers of plants of an entirely different nature than those now existing are not likely to come on line before the year 2000 at the earliest. As a result, even if the most conservative predictions of electrical energy and total energy consumption are realized—namely the reduction to a two percent total energy consumptive increase per year in the United States—a substantial number of steam electric power plants will be required within the next 25 years. Furthermore, it will probably be impossible to locate all of these plants in areas remote from other waste dischargers. Even if this were possible, steam electric power plant use on a regular basis would increase the use of various slime control measures, such as chlorination, which are needed to maintain the efficiency of the

cooling system. It is inevitable that coolant and other discharge waters will contain significant contributions of heavy metals from the pipes of cooling systems and other parts of the power generation operation. Therefore, even for steam electric power plants situated at considerable distances from other waste dischargers, the effects of temperature changes on the toxicity of chlorine, chloroamines, and heavy metals to aquatic life should be thoroughly investigated. Because a number of these steam electric power plants probably will be near chemical industries (e.g., the power plants on the Kanawha River and others of the Ohio Basin), the effects of thermal changes on the toxicity of a number of other potential chemicals should also be investigated.

II. Ecological Significance

Recently, much attention has been focused on the need for bioassays. With increased emphasis on improving the nation's waters, the laboratory bioassay has proved to be a valuable tool in evaluating problems of chemical toxicity. Bioassays are laboratory tests in which the toxic concentration of a particular substance may be determined under controlled environmental conditions. The objective of a bioassay is to determine the biological effect of acute or chronic stress upon an organism. This research was concerned primarily with determining the effects of temperature on the toxicity of chemical compounds that are frequently found in industrial wastes.

Much data has been collected on the use of fish bioassays to determine heavy metal and temperature toxicity [Doudoroff and Katz, 1953; Skidmore, 1964; Jarvaid and Anderson, 1967; Coutant, 1971]. Current bioassay procedures for fish have been reviewed by Sprague [1970, 1971].

Within the last few decades the use of algae and invertebrates in controlled standarized bioassays for toxicity testing has been minimal [American Public Health Association (APHA), 1974]. Algae and invertebrates are important members of the aquatic food web [Buikema et al., 1974a and 1974b; Cairns et al., 1975].

The algae are recognized as the most important primary producers in both aquatic and marine ecosystems. In the past few decades, researchers have begun to focus attention on the problem of chemical and heated discharges being introduced into water ecosystems. While many investigations have been carried out on these two problems separately, few

have focused attention on the effect of temperature on chemical toxicity within these aquatic and marine ecosystems, and very little of this involved algae [Cairns et al., 1975].

Invertebrates are important intermediate components of the food web. They are readily classified by the food they eat (e.g., herbivores) and their position in energy flow (e.g., secondary consumers). Most invertebrate research has been conducted on the cladoceran *Daphnia magna.* Invertebrates used in recent bioassays have included other cladocerans, oligochaetes, planarians, protozoans, rotifers, snails, and commercially important molluscs and crustaceans [Environmental Protection Agency (EPA), 1971]. Because a variety of methods were used in invertebrate bioassay, speculative data comparison among species is all that is possible [Buikema et al., 1974a and 1974b]. Similarly, little research has been conducted on the effects of temperature on the sensitivity of invertebrates to chemicals.

For fish, temperature is an environmental variable of primary importance. Virtually every physiological process in fish is affected by temperature [Fry, 1971], and many fish species are subjected to seasonal temperature changes of 25°C or more.

Cairns et al. [1975] reviewed the literature on the effect of temperature on chemical toxicity to fish and, based on rather scattered and limited information, concluded that temperature is a larger factor in short-term acute toxicity than it is in long-term exposure.

Higher temperatures are usually assumed to make a pollutant more toxic [Sprague, 1970]. Cairns et al. [1975] state that an increase in temperature may potentiate the effect of toxicants that act on cellular enzymes. The toxicity (LC50) of zinc to Atlantic salmon *(Salmo salar),* when exposed to acclimation temperatures from 3°C to 19°C, increased with temperature [Hodson and Sprague, 1975]. O'Hara [1973] showed that there is more accumulation of cadium by the fiddler crab at higher temperatures. Using the rainbow trout *(Salmo gairdneri),* MacLeod and Pessah [1973] concluded that for acclimation temperatures of 5-10-20°C the response to zinc was linearly related to temperature. The toxicity (TL_m 96 hr) of chromate and arsenate to the rotifer, *Philodina roseola,* increased with acclimation temperatures over a range of 5°C to 35°C [Schaefer and Pipes, 1973]. For the fiddler crab, *Uca pugilator,* the effect of mercury was temperature dependent [Vernberg et al., 1973; Vernberg

and O'Hara, 1972]. At higher temperatures mercury depressed metabolic rates, possibly accounting for increased mercury toxicity. Jones [1973] conducted bioassays using two marine and two estuarine isopods. These tests showed that as temperature increased, mercury toxicity also increased. The resistance of rainbow trout to phenol increases with increases in temperature from 6°C to 18°C for 48-hour LC50's [Brown et al., 1967].

However, no assumptions should be made about temperature effects on toxicity [EPA, 1975]. No difference in TL_m's (median tolerance limit) for copper, zinc, nickel, cadmium, and chromate between 15°C and 28°C were found for six species of freshwater fish [Rehwoldt et al., 1972]. Using the snail, *Physa heterostropha,* and the bluegill, *Lepomis macrochirus,* Cairns and Scheier [1957, 1958] found that 18°C and 30°C had no effect on the toxicity of zinc. When the bluegill was tested at the same temperatures as above, but with potassium dichromate, again no significant difference was found [Cairns and Scheier, 1959].

Taking into account the contradictory information on the effect of temperature on chemical toxicity, Sprague [1970] recommended that tests should be continued whenever possible until the toxicity curve shows a lethal threshold concentration. For a general review of literature on temperature toxicity interactions, the reader is referred to Cairns et al. [1975].

PROJECT OBJECTIVES

This study was designed:

1. To provide information to estimate the additional work necessary to define adequately the problem of temperature effects on chemical toxicity;
2. To provide information for developing tentative hypotheses on the nature of the problem, and
3. To identify interim measures that could be taken to cope with the problem.

Specific research objectives were:

1. To compare the sensitivity of representative aquatic organisms to selected chemicals, and
2. To evaluate the effect of temperature on the sensitivity of these organisms to the selected chemicals.

MATERIALS AND METHODS

I. Chemicals and Temperature

The chemicals used in this research were copper ($CuSO_4 \cdot SH_2O$), zinc ($ZnSO_4 \cdot 7H_2O$), chromate ($K_2Cr_2O_7$), cyanide (KCN), phenol and chlorine (as NaOCl or $Ca(OCl)_2 \cdot 3H_2O$). Except for chlorine (NaOCl), American Chemical Society reagent grade chemicals were used. Chemical concentrations were made by dilution and usually were verified by analytical procedures. Chlorine concentrations were determined on a Wallace and Tierman amperometric titrator and were made on the day of use. Phenol was made on the day of use and the concentrations were measured with the Lowry et al. [1951] modification. Metal concentrations were determined by atomic absorption or colorimetrically [APHA, 1974] from stock solutions made with glass distilled water.

The sensitivity of the test organisms to these chemicals was studied over a temperature range of 5° to 35°C. The specific temperatures studied varied with each species because of their different thermal tolerances.

II. Algae

Four algae *(Table 1)* representing diverse taxonomic groups were obtained from reliable sources and cultured using standard phycological aseptic techniques. Tests to insure axenic cultures of the first three algal species listed in *Table 1* were continuously applied during the periodic culture transfers. *Lyngbya* sp. was maintained as a uni-algal culture. The concentrations of the six chemicals studied *(Table 2)* were chosen with regard to published literature [Becker and Thatcher, 1973].

The basic assay procedure essentially followed that of EPA's Algal Assay Procedure [Bartsch, 1971] with several modifications. Since minimal nutrient media have not been determined for all of these assay algae, 0.85x the concentration of Bold's modified Bristol's medium [Nichols, 1973] was chosen which could provide each assay alga with adequate amounts of nutrients. This medium was enriched with micronutrients, sodium silicate, and vitamin B_{12} and adjusted to pH 6.8 with KOH (potassium hydroxide). Copper was omitted from the micronutrient solution since Eyster [1973] reported that relatively large amounts of this

metal are present in other compounds as a contaminant. The calcium hardness of this medium was determined to be 68 ± 2 mg/l.

Two culturing apparati were employed during the study. *Chlamydomonas* sp. and *Lyngbya* sp. were assayed in an inexpensive water bath system utilizing aquaria and 10-watt aquarium heaters while *Scenedesmus quadricauda* and *Cyclotella meneghiniana* were grown in a Sherrer Model 4005-0 incubator. Both systems were reliable in that temperatures of 5°, 15°, 25°, and 35°C ± 1°C could be maintained. The water bath system provided approximately 200 foot-candles (ft-c) of continuous cool-white fluorescent light while the incubator provided approximately 400 ft-c of continuous cool-white fluorescent light. The cultures were not bubbled or shaken.

Since the number of replicates (six) of each toxicant concentration necessitated an easy growth monitoring procedure, optical density was chosen as the easiest, most rapid method of quantitating growth. Therefore, initial standardization assays were used to correlate optical density with direct cell counts and dry weights. The sample cultures were grown in optically tested 13 mm *Pyrex* test tubes fitted with *Teflon* caps. These tubes could be placed directly into a Bausch and Lomb Spectronic-20 spectrophotometer without exposure to contamination after gently mixing with a touch plate vortex mixer. A wave length of 525 nannometers (nm) was chosen to monitor growth, since results obtained at this wavelength should be little affected by anticipated pigment changes induced by the toxicants. This monitoring procedure allowed frequent measurements to be carried out. *Scenedesmus quadricauda* and *Cyclotella meneghiniana* were monitored daily for five days, while *Chlamydomonas* sp. was monitored at zero, one, two, four, six, eight, and ten days.

Direct cell counts were carried out by fixing samples with Lugol's solution and counting with the use of a haemacytometer. Dry weights were determined by weighing prewashed, predried Whatman GFC filters on a Mettler Type H6 analytical balance, filtering samples under a slight vacuum, redrying the filters and algal residue (at 105°C for 20 hours), and finally reweighing.

Rapidly growing, temperature-adapted batch cultures were centrifuged gently to provide the algal inocula which were delivered in a volume of 0.4 ml. The final volume of each sample was 4 ml derived by the addition of the 0.6 ml chemical inoculum and the 0.4 ml algal inoculum to

3 ml of sterile medium (autoclaved at 15 psi for 20 minutes). The chemical assay for each alga employed 45 randomly arranged sample tubes at each test temperature. Fifteen of these were optical density blanks containing the various toxicant concentrations, and 30 were actual samples.

The *Lyngbya* sp. assay involved only a direct dry weight assay due to the filamentous nature of this alga, and therefore, only a mean specific growth rate ($\bar{\mu}$) or yield was determined at the end of an 18-day incubation period. This assay utilized 16 mm disposable culture tubes fitted with *Teflon* caps and containing a final culture volume of 10 ml.

Specific growth rates (μ) were determined by substituting growth measurements (optical density or dry weight) in the formulae [Bartsch, 1971; Guillard, 1973] :

$$\mu = \frac{\ln(x_1/x_0)}{t_1 - t_0}$$ where

x_0 = growth measurement at time t_0

x_1 = growth measurement at time t_1

Mean specific growth rates ($\bar{\mu}$) were obtained by substituting in the formula:

$$\bar{\mu} = \frac{\ln(x_f/x_0)}{t_f - t_0}$$ where

x_f = growth measurement at end of assay period (t_f)

x_0 = growth measurement at initial time of assay (t_0).

The mean maximum growth rate, $\bar{\mu}$max, was obtained by averaging the maximum growth rates observed for each sample. $\bar{\mu}$ and $\bar{\mu}$max for each toxicant concentration were tested for significance by comparison to the values of the controls.

III. Invertebrates

A. Unicellular Organisms

Pure cultures of the cryptomonad flagellate, *Chilomonas paramecium,* were developed from a mixed protozoan culture obtained from Carolina Biological Supply Co., Burlington, North Carolina. This mixed culture

was inoculated into an organic medium developed for *Chilomonas paramecium* [Provasoli, 1958] and allowed to grow for a week. The medium consisted of: tryptone (DIFCO) (0.1 w/v percent); yeast extract (DIFCO) (0.2 w/v percent); and sodium acetate (0.1 w/v percent). Glass distilled water was added to make the required volume and pH was adjusted to 6.3 ± 0.1. The media was routinely vacuum filtered with Whatman No. 2 filter paper on a large Büchner Funnel. The media was then autoclaved at 15 psi for 15 minutes.

Pure cultures were obtained by making several sterile transfers into flasks containing this organic medium and 36 mg/l each of streptomycin fulfate and penicillin-g (Sigma Chemical Co., St. Louis, Missouri). When *Chilomonas paramecium* was the only species of protozoan evident and bacterial contamination was not apparent, the use of antibiotics was discontinued since low levels of bacterial contamination would not affect electronic cell counts (see below).

Stock cultures were maintained in 100 mm x 16 mm culture tubes with polypropylene caps containing 5 ml of media. The cultures were maintained under 100-200 ft-c light intensity, a 12L:12D photoperiod, and approximately 10°, 20°, and 30°C, with subculturing at one to three-day intervals for the 30°C cultures, at three to five-day intervals for the 20°C cultures, and five to ten-day intervals for the 10°C cultures.

Toxicity tests with *Chilomonas* involved determination of log-phase growth rate constants over a range of chemical concentrations at 10°, 20°, and 30°C. At each temperature, two replicate culture tubes were used for each chemical concentration, and four replicates were used for controls (distilled water was added instead of chemical solution). Preliminary tests with a wide range of chemical concentrations at room temperature were conducted to obtain an approximation of concentrations suitable for the actual tests.

For toxicity tests with chromium, copper, and zinc, 1 ml each of metal solution or distilled water was added to culture tubes containing 5 ml of media. The culture tubes were then autoclaved, and after cooling to the appropriate temperature, inoculated with 0.2 ml of a well-mixed log-phase *Chilomonas* culture. Autoclaving after dosing was not possible with chlorine and phenol due to their volatile nature. Therefore, using the sterile technique, 1 ml of these solutions was added to culture tubes which were then inoculated with 0.2 ml of *Chilomonas* culture.

Concentrations of *Chilomonas paramecium* were monitored with an electronic particle counter (Electrozone/Celloscope Model 112 LTH, Particle Data Inc., Elmhurst, Illinois) using a 95μ orifice. *Chilomonas paramecium* was observed to have a size range of approximately 20-30 microns. The settings of the particle counter were adjusted to obtain empirical counts which had a good correlation with direct visual counts. Visual counts were obtained both with a Sedgwick-Rafter counting cell and a Whipple ocular micrometer disc calibrated with a stage micrometer [Everhart, 1972]. Low levels of bacterial contamination did not interfere with the electronic counts since the lower size limit of particles recorded by the 95μ orifice is approximately 2 microns. This obviated the need for antibiotics in the *Chilomonas* cultures. Cell concentrations immediately following inoculation (time = t_0) were determined with the particle counter. An aliquot was taken from each culture tube (after mixing with a vortex mixer) and diluted in an isotonic electrolytic solution (Isoton, Scientific Products, McGaw Park, Illinois) for counting. Three replicate counts were made for each tube and averaged with counts from the other tube(s) of the same toxicant, concentration, and temperature. Final cell counts (time = t_1) were made at 19-25 hours for the 30°C assays, 44-48 hours for the 20°C assays, and 98-163 hours for the 10°C assays.

The hourly growth rate constant (μ) was calculated using the formula of Bartsch [1971] and Guillard[1973]. Multiplication of this growth rate constant by 24 gives a constant (μ24) with dimensions of day^{-1}. All negative growth rates were converted to zero. Growth rates were expressed as a percentage of the growth rate in the control tubes.

B. Multicellular Organisms

The toxicity procedures used are based on recommendations in *Standard Methods* [APHA, 1974] and modified as appropriate by Cairns and Messenger [1974], Buikema et al. [1974a, 1974b], and Newman [1975] for static tests. All studies were conducted in environmental growth chambers maintained at the appropriate temperature (±1°C); photoperiod was approximately 12L:12D, and light intensity was approximately 60 ft-c. All animals were preacclimated to test conditions for two to four days. The tests were conducted without aeration or renewal of toxicant. The temperatures studied were 5°, 10°, 15°, 20°, and 25°C. Invertebrates used included the annelid, *Aeolosoma headleyi;* the cladocerans, *Daphnia magna* and *Daphnia pulex;* the gastrotrich, *Lepidodermella* sp.;

the rotifer, *Philodina acuticornis;* and the snail, *Nitrocris* sp. *Aeolosoma, Lepidodermella,* and *Philodina acuticornis* were obtained from Carolina Biological Supply Co., Burlington, North Carolina. *Nitocris* sp. was collected from the McCoy area of the New River, Virginia. The cladocerans, *Daphnia pulex* and *Daphnia magna,* were from stock cultures at Virginia Polytechnic Institute and State University.

The number of live organisms was recorded at 24 and 48 hours. The criteria for determining if the organisms were dead varied. For the cladocerans, gastrotrichs, rotifers, and annelids, the lack of visible external or internal motion was used. For the snail, failure to respond to a gentle probing on the foot with a blunt dissecting needle was used. For both the cladocerans and snail, observations were made with the unaided eye. The other animals were observed using a Wild M-5 stereoscope.

Animals dead prior to the end of the test were not removed. With the rotifers and gastrotrichs, formalin was added to the sample at the end of each experiment, and the total number of organisms was counted. Control mortality of greater than 10 percent resulted in exclusion of data as suggested by *Standard Methods* [APHA, 1974] and EPA [1975]. LC50 values were obtained by probit analyses [Finney, 1971] using the Statistical Analysis System [Barr et al., 1976].

Except for tests with rotifers and cyanide, all experiments were conducted with charcoal-dechlorinated Blacksburg tapwater. The chemical characteristics of the water were: pH, 7.5 ± 0.05; total hardness, 45 ± 5 mg/l; and total alkalinity, 42 ± 5 mg/l as $CaCO_3$. Rotifers were cultured and tested in a synthetic medium *(Table 3)* made with glass distilled water which was bubbled with CO_2 until all the calcium and magnesium precipitate was dissolved. The water then was bubbled with air for 24 hours. The sensitivity of all organisms to cyanide also was tested in this synthetic medium. At the initiation and termination of each bioassay, the pH was measured with an Orion pH meter, water hardness with a Hach Chemical Kit, and alkalinity by sulfuric acid titration [APHA, 1974]. With the exception of cyanide, there was no significant effect of toxicants on water chemistry. Cyanide increased the pH of the test solution to 8.2 ± 0.3.

C. Specific Test and Culture Conditions

Culturing methods for the oligochaete *Aeolosoma headleyi* were based

on work by Newman [1975]. *Aeolosoma* were cultured in the synthetic medium which contained food *(Table 3).* Nineteen parts of the synthetic medium and one part trout chow suspension were mixed to prepare the culture medium. The trout chow suspension was made by blending 1.5 to 2 g trout chow *(Trout Chow #3,* Ralston-Purina Co., St. Louis, Missouri) in 200 ml of distilled water. This solution was then passed through a fine mesh bolting cloth, and the medium was autoclaved and stored in the refrigerator. Before it was added to the cultures, the medium was aerated at room temperature for two to three hours. The cultures of *Aeolosoma* were maintained in 13-cm glass culture bowls containing 150 ml of the medium. Twice a week, one-half of the stock cultures were replaced with fresh medium. The cultures also contained protozoans and algae. *Aeolosoma* cultures were maintained at room temperature, 21° ± 2°C. Light intensity was 40-60 ft-c. Whenever possible, experiments were started the day the animals were fed, and animals were preacclimated to the test temperatures for four days. All tests were performed in Syracuse watch glasses. Ten *Aeolosoma* and 1 ml of culture medium were transferred to the watch glass using a large bore pipette. Two ml of diluent were added, and 1 ml of a specific 4x chemical concentration was then added. The total volume in the test containers was 4 ml. The three replicates and controls were covered with *Saran Wrap* to retard evaporation.

Both cladocerans were reared in separate 10-gallon aquaria containing dechlorinated Blacksburg tapwater. Gentle aeration using "oil free" air was supplied to maintain an O_2 concentration of 6 mg/l [Lee, 1976]. Water temperature was approximately 21° ± 2°C and light intensity was between 50 and 60 ft-c. *Daphnia* were fed 5 to 10 ml of a fine suspension of trout chow every 48 hours. The trout chow was made by blending 1.5 to 2.0 g trout chow in 200 ml of distilled water for one minute. This solution was then diluted to 500 ml with distilled water and allowed to settle for 10 minutes. The supernatant was collected and stored in the refrigerator. The *Daphnia* were collected for tests with a large coarse net (1.0 mm mesh) from the aquarium and then transferred to a 600 ml *Pyrex* beaker. A large bore pipette (1.5 mm) was used to transfer 10 animals to each of the test containers containing 200 ml of the diluent. To minimize the effects of handling, 100 ml of a 3x solution of chemical was added to obtain the desired range of concentrations. The total volume in the 600 ml beakers was 300 ml. Three replicates of each concentration and controls were run. *Saran Wrap* was used to cover each beaker to retard evaporation.

The snail, *Nitocris* sp., was maintained in 10 and 29-gallon aquaria containing carbon-dechlorinated Blacksburg tapwater. Constant aeration with "oil free" air was supplied to maintain oxygen levels above 6 mg/l; the water temperature was maintained at 21° ± 2°C, and the light intensity at 60 ft-c. The snails were fed boiled lettuce every other day and were kept under laboratory conditions at least one week before testing. *Nitocris* were transferred by hand to each beaker containing the appropriate concentration of chemical (total volume 500 ml). Three replicates and controls were run, and the beakers were covered with *Saran Wrap* to retard evaporation.

The rotifer, *Philodina acuticornis,* was cultured in a soft synthetic dilution water *(Table 3),* based on the methods of Buikema et al. [1974a, 1974b]. The rotifers were cultured in the same manner as the oligochaetes (see above). Cultures of the rotifer were kept in 250 ml Erlenmeyer flasks filled with 150 ml of culture medium. Every two days ap-approximately 50 to 75 ml of the medium were replaced with fresh medium; dog multivitamins (Vionatte, Squibb) also were added in minute quantities (0.01 g). Cultures were maintained at 21° ± 2°C at a light intensity of 40 to 60 ft-c. Rotifer tests were conducted in small glass culture dishes. Animals were preacclimated to the test temperature for two to four days, and the animals were fed 24 hours before testing. Four ml of *Philodina* were pipetted from the cultures (population size averaged 90 animals per 4 ml) into test containers. Because the animals were too small to be handled separately, 1 ml of a 5x concentration of chemical was added to obtain the desired concentration range. The total volume of the test container was 5 ml. Three replicates of each concentration and controls were placed in a flat *Tupperware* tray and covered with *Saran Wrap.*

The same culture methods used for the rotifers and the oligochaetes were used to culture the gastrotrich, *Lepidodermella.* Three ml of animals were pipetted from the cultures to the small glass culture dishes used as test containers. Population size averaged 63 animals per 3 ml. Animals were preacclimated to test conditions for 48 hours. One ml of a 4x concentration of a specific chemical was added to the test container to obtain the desired concentration. Three replicates of each concentration and controls were covered with *Saran Wrap* to retard evaporation.

IV. Fish

ORSANCO 24 bioassay procedures were used for prophylactic treatment of the fish [Smith et al., 1974]. The bioassay procedures of Sprague [1970] were used to determine the sensitivity of freshwater fish to phenol, cyanide, copper, zinc, and chromium. The fish used were rainbow trout, *Salmo gairdneri;* golden shiner, *Notemigonius crysoleucus;* bluegill, *Lepomis macrochirus;* goldfish, *Carassius auratus,* and channel catfish, *Ictalurus punctatus.* Young fish less than 10 cm total length were used *(Table 4).* The fish were held and tested in soft (≈40 ppm hardness) carbon-dechlorinated Blacksburg tapwater. Trout were preacclimated for at least three weeks to 5°, 12°, and 18°C. The other fish were preacclimated to 5°, 15°, and 30°C for at least three weeks.

The toxicity tests were conducted in 40 l nalgene containers which were submerged in a water bath for temperature control (±1°C). Four groups of 10 fish each were exposed to chemical concentrations bracketing a preliminary estimate of the 24-hour LC50, and a fifth group served as a control. The percent survival at 1, 2, 4, 8, 12, and 24 hours in each concentration was recorded. All dead and surviving fish were measured for length and weight *(Table 4).* The LC50, 24-hour LC-1-99 and LT-50 for each temperature, toxicant, and species was then calculated by the probit analysis described for the invertebrates.

A constant record of the temperature of the test chambers was kept with a thermograph. At the beginning and end of each run, dissolved oxygen, pH, and test chemicals were measured *(Table 4).* Where the concentration of the chemicals declined over the 24-hour period *(Table 5),* the mean concentration was used as the effective concentration. Dissolved oxygen was measured by a YSI (Yellow Springs Instrument) oxygen probe and pH was measured with a Sargent Model LS pH meter.

RESULTS AND DISCUSSION

I. Algae

The standardization assays confirmed that optical density measurements correlated well (e.g., *Table 6)* with direct cell counts and dry weights which indicated that Baer's law was obeyed. *Table 7* displays the overall mean specific growth rates obtained for each algal species at each temperature. As expected, growth at 5°C was very slow and inconsistent. However, toxicity tests were carried out for *Cyclotella meneghiniana* which displayed the greatest growth at this temperature. Thirty-five de-degrees C was beyond the maximum temperature tolerance limits for *Chlamydomonas* sp. and *Cyclotella meneghiniana;* therefore, no tests were conducted for these two species at that temperature. In general, the growth rates agree with the data of Trainor and Shubert [1973] of unshaken algal cultures grown in a similar medium.

Generally, higher copper concentrations inhibited growth at higher temperatures for *Cyclotella meneghiniana (Figure 1), Scenedesmus quadricauda (Figure 2),* and *Chlamydomonas* sp., while the data on *Lyngbya* sp. were inconclusive *(Table 8).* There was no temperature effect on copper sensitivity of *Cyclotella* between 5° and 15°C, and a plateau existed between 1 and 4 mg copper per liter. Growth inhibition of *Scenedesmus quadricauda* by copper was more obvious between 15° and 25°C than between 25° and 35°C.

Observing the daily specific growth rates (μ) of these organisms shows that *Cyclotella meneghiniana* was inhibited severely within the first day at all temperatures, while *Scenedesmus quadricauda* was inhibited only after one day's time at all temperatures. *Chlamydomonas* sp. was inhibited during the first day at 15°C, but inhibition occurred after one day at 25°C. Microscopical examination of copper-treated cells after the full incubation period revealed extremely chlorotic cells. When subcultured in a fresh copper-free medium, these cells produced healthy populations, indicating that the effect of copper was algistatic. Of note is the observed stimulation of *Scenedesmus quadricauda* and *Chlamydomonas* sp. at lower concentrations of copper.

The toxicity of zinc varied considerably *(Table 9); Chlamydomonas* sp. *(Figure 3)* was slightly more inhibited than *Cyclotella meneghiniana (Figure 4),* while *Scenedesmus quadricauda (Figure 5)* and *Lyngbya* appear-

ed to be quite tolerant of this toxicant. The higher concentrations of zinc produced precipitates which hampered the optical density measurements. However, the careful use of optical density blanks containing the same concentrations of zinc permitted accurate readings, assuming that any precipitates formed were not affected by the presence of algae in the sample cultures. Microscopical examination of zinc-treated cultures revealed apparently healthy cells which would suggest a different kind of inhibition than that of copper. The erratic variations in daily specific growth rates suggest that zinc toxicity may be a slower, more gradual process than copper toxicity. The effect of temperature on zinc toxicity would be difficult to ascertain with the data obtained.

According to Becker and Thatcher [1973], at a pH of 6.8, hydrochromate ($HCrO_4^-$) is the most common ion of the chromates and is more toxic than either $CrO_4^=$ or $CrO_7^=$, possibly because monovalent ions tend to be more readily absorbed than divalent ions. In this research, however, we refer to all the ions as chromate. *Cyclotella meneghiniana (Figure 6)* was inhibited severely by as little as 0.5 mg/l chromate at all three temperatures with inhibition greatest at 25°C *(Table 10). Scenedesmus quadricauda (Figure 7)* was inhibited by 0.5 mg/l at 15°C but not at 25° or 35°C. Examination of the *Chlamydomonas* sp. daily specific growth rates indicated that after an initial decrease in population within the first four to six days, the cultures began to recover, possibly indicating that the chromate ions were somehow being deactivated or that a percentage of the population was less susceptible to chromate toxicity. Subculturing of chromate-treated cultures of *Cyclotella meneghiniana* into a chromate-free medium produced no new growth.

Preliminary testing showed that all traces of residual chlorine and chloramines disappeared from sterile assay medium within 24 hours at 25°C. The data display this short-termed presence of residual chlorine since first day specific growth rates of the algae, in general, decreased proportionally to the concentration of residual chlorine inoculated *(Table 11).* This initial decrease was eventually followed by a recovery of specific growth rates equal to or greater than those of the controls. However, *Scenedesmus quadricauda,* grown at 35°C, displayed no inhibition in growth, while *Cyclotella meneghiniana,* grown at 5°C, displayed an erratic recovery from 1.52 mg/l residual chlorine. An inspection of the specific growth rates after two days shows that *Cyclotella meneghiniana, Scenedesmus quadricauda,* and *Chlamydomonas* sp. were more inhibited at 15°C than at 25°C. The assay procedure for *Lyngbya* sp. did not allow

daily monitoring; therefore, any initial retardation of growth could not be ascertained. Cultures grown at 15° and 35°C and treated with 0.52 mg/l residual chlorine produced yields with significantly lower dry weights when compared to controls.

No trends were observed during the phenol assays which would indicate that this chemical was toxic to the assay algae *(Table 12).* In fact, *Scenedesmus quadricauda* and *Chlamydomonas* sp. sometimes displayed significantly stimulated growth rates. The only alga utilized in the study of cyanide, *Chlamydomonas* sp., did not display any significant inhibition of growth by any of the concentrations utilized at either 15° or 25°C *(Table 13).*

Very little literature exists concerning the interactions of chemical toxicants and temperature on the growth of algae. However, much research has been carried out on chemical toxicity alone since certain algae have been implicated as being nuisances in freshwater supplies. Comparing concentrations of toxicants found to be inhibitory to various algae by different laboratories is virtually useless because different culturing and assaying methods obviously produce large variations in results.

Algae cultured in dilute media (and often natural waters) generally appear to be inhibited by lower concentrations of toxicants than algae grown in more concentrated media [Hervey, 1949]. Although Patrick [1971] found little difference in the tolerance of two freshwater diatoms grown in culture media representing two distinctly different habitats (soft water and hard water), increasing water hardness reduces the availability of toxic metal ions due to the formation of precipitates which are relatively non-toxic [Cairns et al., 1972b].

Several authors [Maloney and Palmer, 1956; Hervey, 1949; Fisher and Wurster, 1973] have produced data which indicate that certain groups of algae seem to be more tolerant of chemicals than others. The Chlorophyta (in particular the Chlorococcales) appear to be the most tolerant while the diatoms are the least tolerant. The tolerance of other groups of algae falls somewhere between these two groups. Jensen et al. [1976] have shown that concentrations of copper as small as 10 μg/l, when added to Norwegian fjord seawater, inhibited the growth of three marine diatoms, while Eyster [1973] has shown that *Chlorella sorokiniana* can grow optimally in Knop's medium containing up to 10^{-3} m of copper.

Each species of algae is known to exist only within a particular range of temperatures and some species are eurythermal while others are stenothermal. Studies on the effects of temperature on the toxicity of polychlorinated biphenyls (PCB's) to marine phytoplankton suggest that the phytoplankton tolerance to this toxicant decreases as the temperature tolerance limits for each species is approached [Fisher and Wurster, 1973]. Reynolds et al. [1974] have shown that phenol toxicity to *Selenastrum capricornutum* increases with increasing temperature according to the Arrhenius function. Patrick [1971], working with various toxicants and two freshwater diatoms in two distinctly different media, has shown that different toxicants produce unpredictable inhibition of growth rates at different temperatures.

No literature reporting the effects of temperature on the tolerance of algae to copper exists, even though this chemical is often used as an algicide. The data in this report indicate that copper toxicity generally increases between 5° and 15°C and between 15° and 25°C for all algae tested *(Figures 1* and *2, Table 8).* The data do not show any increasing toxicity between 25° and 35°C for *Scenedesmus quadricauda,* while copper toxicity appears to decrease between 25° and 35°C for *Lyngbya* sp. At 35°C, the upper temperature tolerance limit for *Scenedesmus quadricauda* is being approached [Komarek and Ruzicka, 1969], and at 5°C the lower temperature tolerance limit for *Cyclotella meneghiniana* should be approached. These observations, therefore, are contrary to Fisher and Wurster's [1973] observations that phytoplankton are more vulnerable to chemical toxicity when existing in suboptimal environments. It could be possible that an organism might be "preconditioned" by one stress that will assist in tolerating additional stress.

The fact that sublethal concentrations of copper actually stimulated the growth rates of *Scenedesmus quadricauda* and *Chlamydomonas* sp. agrees with Eyster's [1973] data on the nutrient requirements of *Chlorella sorokiniana.* This alga grew most optimally at copper concentrations (10^{-3} m) just below the concentrations (1.8×10^{-3} m) that proved inhibitory. The concentrations that produced stimulatory growth rates (up to 4 mg/l) in our study are much greater than are found in most natural bodies of water [Kopp, 1969].

Patrick [1971] found that zinc toxicity to *Nitzschia linearis* in synthetic soft water increased as temperature increased from 22° to 30°C, while zinc toxicity to *Navicula seminulum* in synthetic hard water produced

irregular results. Jensen et al. [1974] showed that three marine diatoms varied extensively in their growth response to zinc enrichment of fjord water.

Because zinc toxicity increased for *Cyclotella meneghiniana* and decreased for *Scenedesmus quadricauda* and *Chlamydomonas* sp. as temperature increased, no firm conclusions can be made as to the effect of temperature on zinc toxicity. However, it should be noted that each alga responded with either increasing or decreasing tolerance to zinc as temperature increased. Results with *Lyngbya* sp. were inconsistent, but a general trend toward slightly more inhibition at 15° and 25°C than at 35°C was ascertained.

As with copper, zinc stimulated *Chlamydomonas* sp. and *Scenedesmus quadricauda* at lower concentrations although no stimulation was observed for *Scenedesmus quadricauda* at 35°C. Eyster[1973] reported that *Chlorella sorokiniana* grew very well with as much as 1.5×10^{-3} m zinc added to Knop's medium. The stimulation of *Chlamydomonas* sp. and *Scenedesmus quadricauda* by both copper and zinc at near-lethal concentrations is difficult to explain but should be noted by water management personnel.

Patrick [1971] noted a slight decrease in chromate toxicity to *Nitzschia linearis* with increasing temperature in a synthetic soft-water medium, but an increase in toxicity to *Navicula seminulum* with increasing temperature in a synthetic hard water medium. Our data showed that chromate toxicity increased at higher temperatures for three species while *Scenedesmus quadricauda* displayed opposite results. Obviously, the effects of temperature on chromate toxicity varies from species to species. Hervey [1949] observed that diatoms were as sensitive to chromate toxicity as yeasts and bacteria, while the green algae *Chlorella variegatus, Chlorococcum humicola,* and *Scenedesmus obliquus* were much more tolerant. In the present study, *Scenedesmus quadricauda* was found to be much more tolerant than *Cyclotella meneghiniana.*

Unlike zinc and copper, chromate produced no stimulated growth response in *Chlamydomonas* sp. and *Scenedesmus quadricauda,* but Hervey [1949] did note that 0.032 mg/l chromium stimulated the growth of the euglenoid *Lepocinclis steinii.* Because chromium has never been found to be a necessary trace element for the growth of algae, its presence in trace amounts may be extremely detrimental to algae in general and to diatoms in particular.

Although chlorine is often used as a biocide, little data measuring its effects on algae have been published. Hirayana and Hirano [1970] observed that five to ten minutes of exposure to 1.15 mg/l chlorine produced a time lag in the growth of a marine species of *Chlamydomonas.* This species recovered from a five-minute exposure to 20 mg/l Cl_2 after nine days, while cultures of a marine diatom, *Skeletonema costatum,* did not grow after a five-minute exposure to 2 mg/l Cl_2. This observation, coupled with the data presented in this paper, indicates that chlorine has a time-related inhibitory effect on algae. Because the inherent chlorine demand of natural waters [Becker and Thatcher, 1973] and synthetic media deactivates the introduced residual chlorine, sublethal doses will cause an initial decline in population followed by a recovery where the growth rates are comparable to the control's growth rates. Obviously the length of time that residual chlorine remains present in solution would depend on the properties of the medium, the dose, and, in all probability, the temperature. Note that *Scenedesmus quadricauda* was not inhibited at 35°C and that *Cyclotella meneghiniana* at 5°C did not recover as well as cultures grown at 15° and 25°C. Possibly, the major effect of temperature on chlorine toxicity to algae could simply be the effect on the rate of change of toxic residual chlorine into non-toxic compounds.

Reynolds et al. [1974] reported that 20 mg/l phenol inhibited the growth of *Selenastrum capricornutum* in a continuous-flow assay, while Ruthven and Cairns [1973] observed that *Euglena gracilis* tolerated 750 mg/l phenol in a bottle-type assay. *Euglena gracilis* required greater than 100 mg of phenol/l before lethal effects were observed. Obviously, the differences in assay procedures could produce these discrepancies. The length of time that phenol is present in a bottle-type assay could be very short, while in a continuous-flow assay, fresh solutions of phenol are constantly being resupplied to the medium. The results presented in this paper do not reflect any inhibition at all by phenol with stimulation occurring in several assays with *Scenedesmus quadricauda* and *Chlamydomonas* sp. If phenol is indeed being decomposed, then these two algae could possibly have utilized the carbon fragments as energy sources.

The paucity of published literature on the toxicity of cyanide to algae, coupled with the inconclusive data gathered in this study, do not allow any conclusions to be drawn on the effect of temperature on cyanide toxicity. Patrick [1971] did observe that cyanide toxicity to *Nitzschia linearis* in a soft water medium did increase slightly with increasing temperature, while results on *Navicula seminulum* in a hard water medium

were inconclusive. Toxicity did appear greater in soft water than in hard water.

The influence of temperature on the effects of the six chemicals to algae appears to be unique with each chemical. It does become apparent that no "universal law" exists which might easily describe these effects. Species of algae from widely diverse taxonomic groups possess many different bio-chemical and physiological characteristics and, therefore, should reflect these differences by reacting differently to toxicants. Our data support the observations of others that certain groups of algae (the diatoms) may be much more intolerant of chemical toxicity than other groups (the Chlorococcales). Because certain groups of algae (the Cyanophyta and, to some extent, the green algae) seem to tolerate warmer waters than other groups (diatoms and important flagellates), heated wastewater discharges into natural bodies of water containing chemicals can only assist the decline of the more intolerant diatom species which, in respect to total biomass, are the more important group of algae in most larger bodies of water. Therefore, a major alteration in community structure would be likely to occur.

II. Invertebrates

A. Unicellular Organisms

The ranges of growth rates in the control tubes at the three temperatures are summarized in *Table 14.* With the concentrations of chemicals used in this study, the effects on the growth rate of *Chilomonas (Table 15)* can be described as basically "all or nothing"; growth rates were either fairly close to that of the controls or else very little growth occurred. There were a few intermediate values recorded for chromate, chlorine, copper, and zinc, but no true graded effects were observed. Thus, the concentration at which the chemical begins to exert an effect cannot be accurately determined. However, with the available data, one may make some observations by noting the highest concentration at which the growth rate remains high and the lowest concentration at which growth rate has been depressed. This suggests the range in which each chemical exhibits a graded effect, but it does not allow for an accurate estimation of median inhibitory levels [Sudo and Aiba, 1973].

Three separate tests were made with copper at 30°C and the results were quite variable. The pH for one of the 30°C tests (#3) was 6.3 at 0.284

mg/l copper and dropped to 5.4 at 48.5 mg/l copper. The data indicates no change in toxic effects of copper at different temperatures. However, the effects of copper may be influenced by the media. Nielsen and Kamp-Nielsen [1970] noted in studies with a unicellular green alga, *Chlorella pyrenoidosa,* that for every milligram of "protein digest" (protein hydrolysate) in the media approximately 0.01 mg of copper may be bound. They also observed that this binding of copper was most pronounced during the first 24 hours, but after 72 hours harmful effects of the copper might become more evident. The *Chilomonas* medium contains approximately 3 mg/l of protein hydrolysate (1 mg/ml tryptone, 2 mg/ml yeast extract). A test culture tube of 6 ml (5 ml media, 1 ml of water or toxicant solution) would contain about 15 mg of protein hydrolysate. This would be capable of binding approximately 0.15 mg of copper, resulting in a reduction of copper available of up to 25 mg/l. Furthermore, the effects of copper may not have been evident for the 30°C tests which covered a time span of approximately 24 hours compared to 44-48 hours and 98-163 hours for the 20° and 30°C tests respectively (this effect of different exposure times is equally applicable to the assays of the other toxicants in this study). The complication of binding by the medium also may apply to the tests with chromate and zinc.

The results of the chromate assays indicate that *Chilomonas paramecium* were less able to tolerate chromate at 20° and 30°C than 10°C *(Table 15).* The results of the tests with zinc indicate a decrease in zinc toxicity to *Chilomonas* with increased temperature. It should be noted that the pH of the medium (measured after the test) decreased from 6.8 for the control to 5.8 at a concentration of 110.7 mg/l zinc. Additionally, at high concentrations of zinc, sterilization of the dosed medium by autoclaving frequently resulted in the formation of a precipitate, possibly making unavailable some of the zinc originally in solution.

Chlorine appeared to exhibit a slight decrease in toxicity with increased temperature. This effect may be due to the volatile nature of chlorine which would result in greater loss from solution at higher temperatures. No effect of temperature on phenol toxicity was observed. Volatilization of phenol may have some influence on toxicity at the different temperatures.

Protozoa have been widely used as medical and pharmacological tools, for example, in assays for antibiotics. However, little work has been done concerning effects of pollution on protozoa. General reviews of the

pollution ecology of protozoa have been written by Cairns [1974] and Cairns et al. [1972b]. Since then, Carter and Cameron [1973] estimated lethal threshold concentrations at 23-25°C for several metal compounds using the ciliate protozoa *Tetrahymena pyriformis.* Their results were 4.5 mg/l $HgCl_2$ in hard and soft water; 6.67 mg/l $ZnSO_4$ in distilled water; 42 mg/l $Pb(NO_3)_2$ in soft water; >250 mg/l $Pb(NO_3)_2$ in hard water; 3.33 mg/l $CoSO_4$ in distilled water; and 1.67 mg/l $CdSO_4$ in distilled water.

Ruthven and Cairns [1973] used several protozoan species in toxicity tests with a number of compounds. The ranges (in mg/l) of lethal concentrations that killed all organisms within 10 minutes were: 160-5,000 for chromium (for 7 species); 1,000-3,200 for phenol (4 species); 0.056-500 for copper (13 species), and 1.8-1,000 for zinc (8 species). For *Chilomonas parameciurn,* the lethal concentrations (mg/l) over a temperature range of 19-25°C were: 1,000 for chromate; 1,500 for phenol; 0.056 for copper, and 10-18 for zinc. The ranges (mg/l) of tolerated concentrations (the highest concentration at which some of the organisms were living after three hours were: 18-100 for chromate (7 species); 560-1,000 for phenol (7 species); 0.024-5.6 for copper (12 species), and 0.056-5,000 for zinc (11 species). Between 19° and 25°C, *Chilomonas paramecium* tolerated the following concentrations (mg/l): 18 for chromate; 560 for phenol; 3.2-5.6 for zinc, and 0.024 for copper.

Sudo and Aiba [1973] found median inhibitory levels (the concentration that reduced the growth rate to 50 percent of that of a control) for three ciliates: 0.25 mg/l copper and 0.53 mg/l chromium for *Vorticella microstoma;* 0.32 mg/l copper and 12.9 mg/l chromium for *Colpidium campylum,* and 0.27 mg/l copper and 20.2 mg/l chromium for *Opercularia* sp. The growth rate of a marine ciliate, *Cristigera,* was reduced 8.3 percent by 0.125 ppm $ZnSO_4$ [Gray and Ventilla, 1973]. Cairns and Dickson [1970] noted that the number of species in protozoan communities held in plastic troughs recovered 120 hours after a 24-hour exposure to 24 ppm zinc. However, a 24-hour exposure to copper resulted in very poor recovery, indicating that residual copper effects on protozoan communities may be considerable. Water temperature in this study ranged from 15° to 25°C. In another study, free chlorine in concentrations above 1.15 ppm administered three times over two hours and 0.66 ppm administered every 20 minutes over two hours produced significant decreases in the number of protozoan species [Cairns and Plafkin, 1975].

In general, this method seems fairly easily adaptable for use in the rapid assessment of water quality. *Chilomonas paramecium,* or other protozoans such as *Tetrahymena pyriformis* [Carter and Cameron, 1973], can be easily cultured on simple and inexpensive media. However, metals in solution may be bound by organic compounds in the media. In a study of the effects of mercury and other heavy metals upon both freshwater and marine algae, tests were carried out in diluted media to avoid alteration of toxicant effects resulting from interactions with more concentrated media [Hannan and Patouillet, 1972]. Gray and Ventilla [1973] studied the effects of mercury, lead, and zinc on a ciliate marine protozoan. The assay was conducted in a dilute peptone (0.03 w/v percent) in seawater medium with bacteria being the main food source for the protozoans. Thus, further tests of this nature with pure cultures of protozoans would best be carried out in a dilute assay medium capable of supporting log-phase growth for several days. In addition, since autoclaving of dosed medium may affect the availability of metals by causing precipitation or enhancing binding with components of the medium, dosing could be carried out after the medium is sterilized.

B. Multicellular Organisms

The sensitivity of the invertebrates to chemicals varied among the chemicals and over time *(Table 16).* Except for the annelid, *Aeolosoma,* the most to least toxic metals were copper, zinc, and chromate. These data compare favorably with previous data collected for the rotifer, *Philodina* [Buikema et al., 1974a, 1974b] and the flatworm, *Polycelis* [Jones, 1940]. According to their solution pressures, the order of toxicity should be copper, chromate, and zinc [Jones, 1940]. The reversed order of chromate and zinc may be due to the fact that Jones [1940] introduced chromium as a chromic ion while we used the dichromate ion. Similar data for fish were summarized by Doudoroff and Katz [1953], Jones [1964], and Schweiger [1957].

Copper was toxic to all the animals tested. The cladocerans, *Daphnia pulex* and *Daphnia magna,* were the most sensitive and the snail, *Nitocris* sp., was least sensitive. With the exception of *Philodina acuticornis,* chromate was very toxic to all the animals. Order of sensitivity from most to least was *Aeolosoma, Daphnia pulex, Daphnia magna, Nitocris,* and *Philodina acuticornis.* Zinc also was toxic; the rotifer, *Philodina acuticornis,* was the most sensitive and *Aeolosoma* was the least sensitive.

Of the nonmetals, phenol was the least toxic to all animals studied *(Table 15).* Except for the snail, *Nitocris,* chlorine was more toxic than cyanide. Chlorine was quite toxic to all the animals (exposed) with the rotifer, *Philodina acuticornis,* most sensitive and the snail, *Nitocris* sp., the least sensitive. The limited data obtained on cyanide covers a wide range of sensitivity. Cyanide was very toxic to *Daphnia pulex* followed by *Nitocris* sp. and the rotifer, *Philodina acuticornis.* Cairns and Scheier [1963] reported that volatilization of cyanide varied from 16 to 68 percent, and Broderius [1973] reported on the effect of pH on cyanide toxicity. These phenomena may account for the range in sensitivity that was found. Phenol toxicity decreased as temperature increased, and all animals were extremely tolerant to phenol.

The cladocerans, *Daphnia pulex* and *Daphnia magna,* were the most sensitive to chlorine, chromium, copper, cyanide, and phenol. *Philodina acuticornis* was the most sensitive to chlorine and zinc. The snail, *Nitocris* sp., and oligochaete, *Aeolosoma,* were the least sensitive animals (exposed).

The cladoceran, *Daphnia pulex,* is a cosmopolitan species distributed over the entire North American continent and found in a variety of habitats. *Daphnia magna* is found mainly in hard water areas and is thus more restricted in habitat [Lee, 1976; Buikema et al., 1976]. Anderson [1944] reported the threshold concentration of 94 mg/l of phenol for *Daphnia magna.* Dowden and Bennet [1962] reported a TL_m of 21 mg/l of phenol for *Daphnia.* Malacea [1966] showed that hexavalent chromium was more toxic than trivalent chromium. For *Daphnia hyalina,* Baudouin and Scoppa [1974] determined a 48-hour LC50 value of 0.22 mg/l for chromium and a 48-hour LC50 value of 0.04 mg/l for zinc and 1.0 mg/l for cyanide. Biesinger and Christensen [1972] reported a value of 2.0 mg/l trivalent chromium for a three-week LC50 using *Daphnia magna.* Winner [1976] noted that sensitivity to acute chromium stress was quite variable among *Daphnia.* Using the same species, Freeman and Fowler [1953] calculated a 100-hour LC50 for chromium of 0.42 mg/l. Winner [1976] exposed six species of *Daphnia* to copper and found that larger species were less sensitive than smaller species. A MATC (maximum allowable toxicant concentration) of 40 ppb copper was estimated for four species.

Aeolosoma, a small, freshwater oligochaete, is worldwide in distribution. It has been collected in standing water as well as in large rivers [Maciorow-

ski et al., 1975]. Knibs [1910] studied the effect of chemicals, including heavy metals, on the behavior and reproduction of *Aeolosoma.* Temperature and chemical interactions were studied by Kamemoto and Goodnight [1956] and Parker and Kamemoto [1957] with *Aeolosoma hemprichi.* Encystment of some species of *Aeolosoma* at low temperatures was noted by Beddard [1889, 1895] and Herlant and Meewis [1950]. Newman [1975], who conducted acute chronic bioassays using *Aeolosoma headleyi,* found that toxicity of sulfates from highest to lowest was copper, cadmium, zinc, cobalt, and nickel.

A cosmopolitan genus, *Philodina,* is found in standing waters from littoral zones of lakes and small ponds to Antarctic springs [Buikema et al., 1974a]. Most work on the sublethal effects of chemicals on *Philodina* has been chronic studies [Finesinger, 1926; Gilbert, 1968; Meadow and Barrows, 1971] or field-oriented studies [Edmondson, 1944; Needham, 1966; Anderson, 1970]. However, recently rotifers have been used in controlled acute bioassays using industrial effluents [Sullivan and Buikema, 1972; Buikema et al., 1974a, 1974b]. The following 48-hour LC50's were reported respectively for copper, chromium, zinc, and phenol: 0.8, 50.0, 3.0, and 205.0 mg/l [Buikema et al., 1974a, 1974b]. Our results with chromate-temperature interactions are similar to those obtained by Schaefer and Pipes [1973].

Lepidodermella is a small, freshwater gastrotrich. It is a merofaunal animal uncommon in running water [Pennak, 1953], but found in shallow litteral zones where it feeds upon algae, bacteria, and decaying organic matter [Dujardin, 1841]. Few toxicity studies have been conducted on gastrotrichs, although Hummon [1974] reported on the effects of DDT on longevity and reproduction in *Lepidodermella squammata.*

Nitocris sp. is a member of the family Pleuoceridae, the river snails. To date, little work on pollution ecology has been done with *Nitocris* except for the work of Lee [1976] and Buikema et al. [1976]; however, there have been studies on effects of temperature on other aquatic snails [EPA, 1973; Cairns et al., 1975 and 1976].

As temperature increased, the sensitivity of *Nitocris, Philodina, Daphnia pulex, Daphnia magna,* and *Aeolosoma* to the metals and chlorine also increased. The toxicity of cyanide to all organisms, except for *Aeolosoma,* increased as temperature increased. *Aeolosoma* was less sensitive

to cyanide at higher temperatures. Generally, there was no effect of temperature on the sensitivity of the test organisms to phenol.

The increased sensitivity of the test organisms to chemicals at higher temperatures was probably the result of increased metabolic activity coupled with temperature potentiation of toxicant action on enzymes [Cairns et al., 1975]. For the cladocerans, an increase in temperature speeds the molting process. At the time of molting, the organism is most susceptible to toxicants entering the body [Lee, 1976]. Consequently, an increase in temperature increases the sensitivity of cladocerans to chemicals. The temperature-independent activity of phenol probably reflects a combination of short-term sensitivity of organisms to acute exposure and the effect of high temperature on the evaporation and decomposition of phenol. The observation that cyanide is less toxic to the oligochaete *Aeolosoma* at higher temperatures is an enigma. Perhaps the worm is capable of shifting its metabolic pathway from the election transport system which is the site of inhibition [Lehninger, 1970]. It is also possible that the cyanide evaporated from solution [Cairns and Scheier, 1963].

Temperature did not appear to affect the sensitivity of the protozoan *Chilomonas paramecium* to phenol and chromium. The protozoan appeared to be more tolerant of chlorine and zinc at higher temperatures. It was less sensitive to copper at 20°C than at 10° and 30°C. In terms of the order of toxicity, the most to least toxic metals were copper, zinc, and chromate. Generally, chlorine was more toxic than cyanide, and phenol was the least toxic compound studied. The cladocerans usually were the most sensitive organisms studied, and the snail *Nitocris* and the oligochaete *Aeolosoma* were the least sensitive organisms studied. Because a variety of planktonic and benthic organisms were studied, it would be inappropriate to speculate on the potential effects of temperature on chemical toxicity on community structure until more evidence is available. It would be surprising if alterations in structure did not occur.

III. Fish

The 24-hour LC50 values for each chemical and fish species are summarized in *Table 17.* An increase in temperature caused an increase in sensitivity to cyanide in goldfish, golden shiners, and bluegills. The sensitivity of goldfish to cyanide increased with each increase in test temperature. However, with golden shiners and bluegills, there was only a slight

difference in cyanide sensitivity between 5° and 15°C and no difference between 15° and 30°C. With channel catfish and rainbow trout, temperature had little influence on cyanide sensitivity.

Lower LC50 values were obtained for zinc for the four species (exposed) when they were tested at 15°C compared to 5°C. However, this thermal effect on the shiners and bluegills was slight. At 30°C goldfish and bluegills showed an even greater sensitivity to zinc. But at 15° and 30°C, golden shiners and rainbow trout showed no significant change in sensitivity to zinc.

At 15°C the LC50 for chromate tended to be lower than at 5°C with goldfish and golden shiners. A further decrease in LC50 occurred with goldfish tested at 30°C but did not occur with golden shiners. Bluegills tested at 5° and 30°C do not differ significantly in chromate sensitivity. However, catfish and trout tested at 30°C and 18°C, respectively, appear to be less sensitive than those tested at 5°C.

No significant differences in copper LC50's were noticeable with goldfish or bluegills tested at 5° and 15°C. A decrease in copper LC50 occurred with goldfish tested at 30°C, but an increase occurred with the bluegills at 30°C. With channel catfish and rainbow trout, an increase in temperature lowered the copper LC50 values. For golden shiners, temperature did not affect copper sensitivity.

The lethal response to phenol followed a very erratic pattern in some cases *(Figure 8).* This made it difficult to determine an LC50. At 30°C no deaths could be directly attributed to phenol because of the rapid growth of bacteria introduced with the fish which caused severe depletion of the dissolved oxygen. Goldfish and golden shiners tested at 15°C tended to have lower LC50's for phenol compared to those tested at 5°C. However, rainbow trout showed the reverse situation in this temperature range. There was no difference in phenol toxicity between 12° and 18°C trout. The erratic response of the 15°C bluegills to phenol makes it impossible to evaluate the influence of temperature on phenol toxicity to this species.

The range of concentrations *(Figures 9* to *12)* estimated to give from 1 to 99 percent kill in 24 hours was obtained from computer drawn probability curves. These may be used to indicate the relative precision with which the LC50 can be estimated, although this is not the same as a con-

fidence interval. In most cases temperature affected this range, and thus, the precision in calculating the LC50. For example, goldfish exposed to chromate at 5°C show a large span of concentrations which cause from 1 to 99 percent kill in 24 hours *(Figure 9)* as well as a large standard deviation in the calculated LC50 *(Table 17).* This is believed to indicate that at 5°C, low chromate concentrations may kill a few goldfish, but much higher concentrations are required to kill them all in 24 hours. Since a relatively large change in concentration is required for each percent change in kill, there is a decrease in the precision for calculating the LC50. At 15° and 30°C, the span from 1-99 percent kill is much narrower *(Figure 9)* and the standard deviations are much smaller *(Table 17).* Out of all species and toxicants tested (except phenol), 13 had the widest span at 5°C, 3 at 30° or 18°C, and 3 at 15° or 12°C.

The LT-50 (time at which 50 percent died in a given concentration) was calculated for cyanide, chromate, and copper *(Figures 13* to *15).* LT-50's were not determined for phenol or zinc because of the erratic responses to phenol and the lack of much death before 12 hours in zinc solutions. The smaller the slope, the greater the ability to survive longer over a relatively wide range of concentrations. Also of interest are crossover points. For example, in copper below 4 mg/l, bluegills survive longer at 30°C *(Figure 15),* but above 4 mg/l, they survive longer at 15°C (which has the smaller slope). With cyanide *(Figure 13),* all species except trout were more capable of surviving at 5°C. A crossover is suggested for catfish such that below 0.36 mg of cyanide they survive longer at 15°C.

The warmwater species survived longest at low concentrations of chromate *(Figure 14)* when tested at 15° or 30°C. At higher concentrations, these temperatures permitted less survival time than at 5°C. The trout, a coldwater species, exhibited the longest survival time at low concentrations in 5°C water, but 12°C permitted the longer survival at higher concentrations.

With copper *(Figure 15),* all species except the very sensitive trout and golden shiners show a crossover between 3.0 and 4.0 mg/l. Goldfish survived longer at 3.5 mg/l at 15°C than at 5°C. Bluegills survived better at 15°C than at 30°C. Initially, catfish showed the reverse, surviving better at 15°C (assuming the line can be extended to higher concentrations) than at 5°C. If the slope of the 5°C LT-50 is extended to lower concentrations, another crossover would occur around 3.0 mg/l. Below this concentration catfish may survive longer at 30°C. Thus, a response similar

to that for chromate occurred in which a higher test temperature resulted in longer survival at low chemical concentrations, and a low test temperature resulted in longer survival at higher chemical concentrations. From the data available, temperature appeared to have no real influence on the survival time of the trout or golden shiners exposed to copper.

These results show that temperature affects acute chemical toxicity to fish but not in a simple manner. A summary of the results is given in *Table 18.* Temperature crossovers seen in the LT-50 graphs *(Figures 13* to *15)* indicate that the concentration of the toxicant may determine which temperature results in longer survival. Where temperature crossovers occur *(Table 19),* warmwater species tended to survive longer at a higher acclimation temperature when the toxicant concentration was low (below the crossover) than when the concentration was high (above the crossover). Part of *Table 19* does involve an interpolation of data. It is possible that the threshold concentration would be reached first; in these cases the lower temperature would always result in longer survival.

By relating to the temperature for longest survival only at the concentration at which the highest LC50 occurred, correlation is obtained between the temperature which gives the most tolerance and longest survival *(Table 18).* Thus, where the LC50's are above the crossover, the low acclimation temperature had the highest LC50 *(Table 20)* and longest survival. From this it could be concluded that a decrease in temperature will decrease toxicity. When the LC50's were below the crossover, a higher acclimation temperature had the highest LC50 *(Table 20)* and longest survival. From this it could be concluded that an increase in temperature will decrease toxicity. The LC50's for rainbow trout in cyanide were in the range of the crossover *(Table 20),* and no effect of temperature on toxicity was found. By noting only the conclusions, it would appear that the response of each species differs when, in actuality, the response was the same. At concentrations below the crossover, an increase in temperature reduces toxicity, and at concentrations above the crossover, a decrease in temperature reduces toxicity. Rainbow trout showed the reverse with chromate and cyanide.

When a range of concentrations (LC 1-99) was examined, the lower temperature (5°C) always required the highest concentration to cause a 99 percent kill except where no crossover occurred. Crossovers do not always occur; but in the three (out of 15) times they did not occur, the

lower temperature either definitely resulted in longer survival or was not different from the higher acclimation temperatures.

Studies of temperature's effect on zinc toxicity have mainly been performed on rainbow trout, Atlantic salmon, and bluegills. In this report, rainbow trout acclimated at 12° and 18°C had very similar LC50 values, but at 5°C the LC50 had doubled *(Table 17).* Lloyd [1960] determined by five-day tests that the zinc threshold concentration for rainbow trout (acclimated over a range of temperatures from 13.5° to 21.5°C) was influenced very little by temperature. Lloyd also found that the survival time was more than doubled at an acclimation temperature of 12°C as compared to 22°C. Observation of the LT-50 data shows that the threshold concentrations are very much alike, but once threshold is reached an increase in temperature caused a greater reduction in survival time. Lloyd's and our reports cover zinc concentrations from 1 to 30 mg/l. A temperature crossover was not observed, nor seems likely, because the lower temperature definitely resulted in longer survival. This contrasts with the chromate results for rainbow trout where survival was longer at the higher temperature after crossover.

In our study, bluegills indicated a decreased toxicity (higher LC50 and LC 1-99) of zinc as temperature decreased *(Table 17, Figure 11).* Burton et al. [1972] also showed that bluegills exposed to lethal (32 mg/l) and sublethal (10 mg/l) zinc concentrations survived longer at a lower acclimation temperature. This survival time was reduced in relation to the speed at which the temperature was increased. Cairns and Scheier [1957] found little difference in zinc toxicity (96-hour TL_m) to bluegills acclimated at 18° and 30°C. A later report [Cairns et al., 1972a] stated that this lack of difference does not rule out the possibility that a combination of acclimation temperature, exposure time, and concentration exists which would decrease zinc toxicity to bluegills. This resulted from a comparison of 24, 48, and 96-hour TL_m's of bluegills acclimated to 7-9°C and 21-24°C and exposed to a high concentration of zinc (32 mg/l). The cold-acclimated fish died slower which resulted in differences in the three TL_m's. Thus, temperature affected the time until death [Cairns et al., 1972a]. We have shown that concentration affects which acclimation temperature (high or low) will increase the time until death. At a low concentration, death occurs sooner at lower temperatures, while at high concentration, death occurs sooner at higher temperatures. The importance of this can be seen in the interpretation of the data of Hodson and Sprague [1975].

In a study of acute zinc toxicity to Atlantic salmon, Hodson and Sprague [1975] state that "lower acclimation temperatures were associated with longer survival but less tolerance, i.e., lower LC50's." LC50's were determined over a two-week period, but since all mortality occurred in less than one week, they were estimates of the threshold LC50's. Observation of the LT-50 slopes which occurred at higher concentrations (from approximately 5-30 mg/l) shows that temperature crossovers occur at the concentration for the threshold of the 19°C acclimated salmon (approximately 5 mg/l). Below this concentration the 19°C acclimated salmon are more tolerant and also survive longer than at 5°C. At the concentrations above the 5 mg/l threshold, the low temperature results in longer survival but also a lower one-week LC50; thus, crossovers occur. When an exposure time of less than one week is examined (for example, 24 or 48 hours, which is at or below the time for the LT-50's when using the higher concentrations), the lower temperature would have been concluded to result in greater tolerance (highest LC50). Therefore, the results of Hodson and Sprague [1975] do support the concept that temperature affects the time until death, and concentration affects which temperature is best for survival. Because of this, both exposure time and concentration must be carefully examined in a temperature-toxicity study.

The effects of temperature crossovers in LT-50's were best demonstrated in this report by the copper and chromium data. Little information existed until now regarding temperature influence on their toxicity. Rehwoldt et al. [1972] compared the ranges of 96-hour TL_m's obtained from pooling the data of several species of fish acclimated at 15°C and 28°C. From this, it was concluded that temperature does not influence the toxicity of copper, zinc, nickel, cadmium, or chromium. Cairns and Scheier [1959] found the 96-hour TL_m for chromium (as dichromate) to be the same for bluegills acclimated at 18° and 30°C. However, it was also noted that at higher concentrations, the higher temperature lowered the resistance time. As discussed earlier with zinc, Cairns et al. [1972a] have since demonstrated that exposure time influences temperature-toxicity results, and it appears that at 96 hours an effect may go unnoticed. The LT-50's and 24-hour LC50's of copper and chromium for all species studied in this report (except the shiners which were very sensitive to copper) did show an effect of temperature on their toxicity.

Cairns and Scheier [1963] found that bluegills exposed to KCN (potassium cyanide) at 30°C had slightly lower 48, 72, and 96-hour TL_m's

than those at 18°C. Temperature showed no influence on the cyanide threshold to bluegills, nor did it affect the 24-hour LC50 in our study.

With rainbow trout, temperature did not influence the 24-hour LC50 which appeared to be near the threshold concentrations, since at 5°C a line parallel to the time axis was obtained in the LT-50 graph *(Figure 13).* Burdick et al. [1958] reported the threshold value for trout at 60°F (15.6°C) to be 0.09. In this report the 24-hour LC50 for rainbow trout at 5°C was 0.09. Thus, all trout appear to have the same threshold to cyanide which is not influenced by temperature. Alexander et al. [1935] reported that rainbow trout and salmon smolts exposed to an extremely high cyanide concentration of 3 ppm died twice as fast at 19°C as at 6°C. Using a lower lethal concentration (0.5 ppm), Wuhrman [1952] found that raising the temperature from 5° to 20°C reduced the survival of trout, tench, and minnows by 60, 65, and 75 percent respectively. This indicated little variation between species as to temperature effect on acute cyanide toxicity, although tench can survive longer than trout [Cairns et al., 1975]. In this report the lack of a temperature effect on acute toxicity has been emphasized, but it is also notable that the sensitivity of the species to the toxicants does differ *(Table 17, Figures 13* to *15).*

The results of Alexander et al. [1935] and Wuhrman [1952] do not agree with the prediction, from the cyanide LT-50 crossover with rainbow trout *(Figure 13),* that a higher temperature would result in longer survival at these higher concentrations. However, agreement with this projection has been mentioned in a preliminary study by Brown [1968] who found HCN (hydrocyanic acid) to be more toxic to trout fry at a low temperature (3°C) than at a higher one (13°C). Further study is required to resolve these contradictions.

Although erratic and uninterpretable results were obtained for phenol in this study, other reports indicate the temperature response of fish to phenol is similar to that demonstrated by the other toxicants studied. Gersdorff [1943] showed that at high concentrations of phenol, goldfish survival time decreased as temperature increased. Brown et al. [1967] showed the same response to high concentrations by rainbow trout acclimated from 6.3° to 18.1°C. Again, this is the opposite of the trend shown by trout in response to chromium and cyanide by this report.

Brown et al. [1967] also tested lower concentrations and found at 18.1°C

the 48-hour LC50 (9.8 mg/l) was almost twice that at 6.3°C (5.4 mg/l). The same response was found in the 24-hour LC50's of our study (5°C – 5.6 mg/l; 15° and 30°C – 11.0 and 11.3 mg/l). Brown et al. [1967] reported that all deaths occurred within 25 hours.

These values represent the phenol threshold for rainbow trout which is delayed at the higher temperature. Observation of their LT-50 graph shows that once the threshold is reached at the higher temperature, a larger decline is obtained in survival time than occurred at the lower temperatures. Thus, crossover occurred at about 10 mg/l phenol with rainbow trout. Low phenol concentrations (below the crossover) resulted in higher LC50's and delayed thresholds (longer survival) as the temperature increased. High phenol concentrations (above the crossover) resulted in longer survival and higher LC50's (when shorter exposure time was used) as temperature decreased.

The temperature effect on time until death and the concentration effects on which temperature is best for survival suggest a relationship between the toxification and detoxification processes of fish. Increasing temperature may increase toxicity by increasing the diffusion of the toxicant across membranes by directly or indirectly affecting metabolism, which must function at a higher rate. Increasing ventilation which increased the rate of water and toxicant exposure across the gills [Cairns et al., 1975] may also increase toxicity. However, detoxification mechanisms and excretory processes will also increase and may influence temperature effects on toxicity. The results of this study suggest that at low toxicant concentrations an increase in diffusion, metabolism, and ventilation may not be as significant as the increased detoxification and excretion. Thus, at low concentrations a higher temperature would result in longer survival. As the concentration of the toxicant increases, diffusion and ventilation of the toxicant becomes more significant and detoxification becomes overwhelmed. The evidence that at higher concentrations a lower temperature caused longer survival indicates that decreasing diffusion and ventilation ultimately become more important for survival once detoxification and excretion are overwhelmed.

For fish, temperature influenced toxicity by affecting the time to death. The concentration or exposure time used to determine toxicity influenced which temperature (high or low) resulted in longer survival. This occurred because of temperature crossovers which possibly were caused by a delay in reaching threshold at higher acclimation temperatures. Interestingly,

the only cold water species tested, rainbow trout, exhibited a reverse trend with chromate and copper. Some species and toxicants had threshold concentrations which were not influenced by temperature. However, they still showed longer survival at lower temperatures as the concentration increased. By carefully examining exposure time and concentration, a remarkably similar response can be seen in all temperature-toxicity studies reviewed. Although this response is similar, the species do differ in their sensitivity to each toxicant.

SUMMARY AND CONCLUSIONS

The tentative hypothesis discussed in earlier papers—the influence of temperature upon chemical toxicity is both chemical and species specific—was fully confirmed by this research. The prospects of developing in the near future a practical, predictive model which would enable one to predict the responses of one trophic level (e.g., algae) from the responses of another (e.g., fish) are poor. Similarly, the precise effects of temperature upon the response of a specific group of aquatic organisms (e.g., fish) to a particular category of chemicals (e.g., heavy metals) is not as uniform as a regulatory official might desire.

A very important conclusion from this study is that for fish, chemical concentration affects which acclimation temperature (high or low) will increase the time until death. At a low concentration, death occurred sooner at lower temperatures and at high concentration death occurred sooner at higher temperatures. In short, temperature affects the time until death and concentration affects which temperature is best for survival. The "temperature crossover" effect deserves special attention.

In evaluating response according to taxonomic grouping, this study confirms the earlier observation of Patrick [1971] that different toxicants (particularly chromate) produce unpredictable inhibition of growth rates at different temperatures. The paucity of algal responses to the commonly used biocide chlorine is worth noting as is the lack of information on the effects of temperature on the algal tolerance of copper.

The invertebrates had different responses but not grossly so. Most of the results indicated that increased temperature did, in fact, reduce the tolerance to the various test chemicals. Occasional departures from this pattern indicate that toxicity-temperature generalizations should be viewed with caution.

The fish data also show that temperature affects the relative precision with which an LC-50 can be estimated. There were several additional points of interest in the fish results. First, the smaller the slope of the LT-50 curves *(see Figures 13* to *15)* the greater the ability to survive longer over a relatively wide range of concentrations. Second, the "crossover" phenomenon is of considerable interest. For example, in copper below 4 mg/l, bluegill sunfish survive longer at 30°C *(Figure 15),* but above 4 mg/l they survive longer at 15°C. Where temperature crossovers occur,

warmwater species tend to survive longer at a higher acclimation temperature when the toxicant concentration was low (below the crossover) than when the concentration was high (above the crossover). The results of this study suggest that at higher acclimation temperatures an increase in diffusion, metabolism, and ventilation may not be as significant as the increased detoxification and excretion. Thus, at low concentrations a higher temperature would result in longer survival. As the concentration of the toxicant increases, diffusion and ventilation of the toxicant becomes more significant and detoxification is overwhelmed. The evidence that at higher concentrations a lower temperature resulted in longer survival suggests that decreasing diffusion and ventilation ultimately became more important for survival once detoxification and excretion are overwhelmed.

These results suggest that temperature-toxicity interactions are far more complex than earlier literature has indicated. They indicate clearly that waste discharges containing chemicals and heated wastewater discharges cannot be regulated independently unless they are widely spaced. This is just one more indication that attempts to control pollution by regulating components (i.e., zinc, heat, etc.) individually on a site-by-site basis without examining the collective impact on the biota of the receiving system are doomed to failure. This is particularly true when the stresses approach critical thresholds and a change in one environmental characteristic might well create lethal or deleterious conditions.

On a site specific basis, when environmental concentrations or temperatures approach critical limits, bioassays with important indigeneous aquatic organisms should be mandatory. These studies should be designed to determine:

1. The existing amount of chemical-temperature interaction affecting toxicity;
2. The probability of these interactions producing lethal or deleterious conditions, and
3. The group of organisms most likely to be affected, and, as a consequence, most useful in a biological monitoring program.

If potentially dangerous conditions are likely to exist, more extensive studies should be carried out to define them more precisely. Appropriate monitoring systems to signal their approach should be put in place, and management plans to prevent adverse effects should be developed and implemented.

LITERATURE CITED

Alexander, W.B.; Southgate, B.A., and Bassindale, R., 1935. "Survey of the River Tees, Part II: The Estuary-Chemical and Biological." Water Pollution Research, London No. 5 (cited by Jones, J.R.E., 1964. *Fish and River Pollution.* Butterworth and Co., London).

American Public Health Association, 1974. *Standard Methods for the Examination of Water and Wastewater.* 13th edition.

Anderson, B.G., 1944. "The Toxicity Thresholds of Various Substances Found in Industrial Wastes as Determined by the Use of *Daphnia magna." Sewage Works Journal.* 16:1156-65.

Anderson, R.S., 1970. "Effects of Rotenone on Zooplankton Communities and a Study of Their Recovery Patterns in Mountain Lakes in Alberta." *Journal of the Fisheries Research Board of Canada.* 27:1335-56.

Barr, A.J.; Goodnight, J.H.; Sall, J.P., and Helwig, J.T., 1976. *A User's Guide to SAS 76.* Statistical Analyses System Institute Inc. Raleigh, North Carolina.

Bartsch, A.F., 1971. "Algal Assay Procedure Bottle Test." Environmental Protection Agency. Corvallis, Oregon.

Baudouin, M.F. and Scoppa, P., 1974. "Acute Toxicity of Various Metals to Freshwater Zooplankton." *Bulletin of Environmental Contamination and Toxicology.* 12(6):745-64.

Becker, C.D. and Thatcher, T.O., 1973. *Toxicity of Power Plant Chemicals to Aquatic Life.* United States Atomic Energy Commission. Publication Wash-1249.

Beddard, F.E., 1889. "Notes on Certain Species of *Aeolosoma." Annals and Magazine of Natural History.* (1971) 6:262-5.

———, 1895. *A Monograph of the Order of Oligochaeta.* Clarenaron Press, Oxford.

Biesinger, K.E. and Christensen, G.M., 1972. "The Effects of Various Metals on Survival, Growth, Reproduction, and Metabolism of *Daphnia magna.*" *Journal of the Fisheries Research Board of Canada.* 29:1691-1700.

Broderius, S.J., 1973. "Determination of Molecular Hydrocyanic Acid in Water and Studies of the Chemistry and Toxicity to Fish of Metal-cyanide Complexes." Ph.D. Dissertation. Oregon State University.

Brown, V.M., 1968. "The Calculation of the Acute toxicity of Mixtures of Poisons to Rainbow Trout." *Water Research.* 2:723-33.

Brown, V.M.; Jorda, D.H.M., and Tiller, B.A., 1967. "The Effect of Temperature on the Acute Toxicity of Phenol to Rainbow Trout in Hard Water." *Water Research.* 1:587-94.

Buikema, A.L., Jr.; Cairns, J., Jr., and Sullivan, G., 1974a. "Development and Assessment of Acute Bioassay Technique for the Littoral Rotifer, *Philodina acuticornis.*" *Office of Water Resources Research Bulletin* No. 71.

———, 1974b. "Evaluations of *Philodina acuticornis* (Rotifera) as a Bioassay Organism for Heavy Metals." *Water Resources Bulletin.* 10:(4) 648-61.

Buikema, A.L., Jr.; Lee, D.R., and Cairns, J., Jr., 1976. "A Screening Bioassay Using *Daphnia pulex* for Refinery Wastes Discharged into Freshwater." *Journal of Testing and Evaluation.* 4:119-125.

Burdick, G.E.; Dean, H.J., and Harris, E.J., 1958. "Toxicity of Cyanide to Brown Trout and Smallmouth Bass." *New York Fish and Game Journal.* 5:133.

Burton, D.T.; Morgan, E.L., and Cairns, J., Jr., 1972. "Mortality Curves of Bluegills *(Lepomis macrochirus* Raf.) Simultaneously Exposed to Temperature and Zinc Stress." *Transactions America Fisheries Society.* 101: 435-41.

Cairns, J., Jr., 1974. "Protozoans (Protozoa)." In *Pollution Ecology of Freshwater Invertebrates.* Fuller and Hart, editors. Academic Press, Inc., pp. 1-28.

Cairns, J., Jr., et al., 1972a. "The Effects of pH, Solubility, and Temperature upon the Acute Toxicity of Zinc to the Bluegill Sunfish *(Lepomis macrochirus* Raf.)." *Transactions of the Kansas Academy of Science.* 74: 81-92.

Cairns, J., Jr. and Dickson, K.L., 1970. "Reduction and Restoration of the Number of Freshwater Protozoan Species Following Acute Exposure to Copper and Zinc." *Transactions of the Kansas Academy of Science.* 73:1-10.

Cairns, J., Jr.; Heath, A.G., and Parker, B.C., 1975. "The Effects of Temperature upon the Toxicity of Chemicals to Aquatic Organisms." *Hydrobiologia.* 47(1):135-71.

Cairns, J., Jr.; Lanza, G.R., and Parker, B.C., 1972b. "Pollution Related Structural and Functional Changes in Aquatic Communities with Emphasis on Freshwater Algae and Protozoa." *Proceedings of the Academy of Natural Sciences of Philadelphia.* 124(5):79-127.

Cairns, J., Jr. and Messenger, D., 1974. "An Interim Report on the Effects of Prior Exposure to Sublethal Concentrations of Toxicants upon the Tolerance of Snails to Thermal Shock." *Archiv Fuer Hydrobiologie.* 74(4):441-7.

Cairns, J., Jr.; Messenger, D., and Calhoun, W.F., 1976. "Invertebrate Response to Thermal Shock Following Exposure to Acutely Sublethal Concentrations of Chemicals." *Archiv Fuer Hydrobiologie.* 77:164-75.

Cairns, J., Jr. and Plafkin, J.L., 1975. "Response of Protozoan Communities Exposed to Chlorine Stress." *Archiv Fuer Protistenkunde.* 117:47-53.

Cairns, J., Jr. and Scheier, A., 1957. "The Effects of Temperature and Hardness of Water upon the Toxicity of Zinc to the Common Bluegill *(Lepomis macrochirus* Raf.)." *Notulae Naturae (Philadelphia).* 299:12.

———, 1958. "The Effects of Temperature and Hardness of Water upon the Toxicity of Zinc to the Pond Snail, *Physa heterostropha* (Say)." *Notulae Naturae (Philadelphia).* 308:11.

———, 1959. "The Effects of Temperature and Hardness upon the Toxicity of Potassium Dichromate to the Common Bluegill Sunfish." *Transactions of the Northeast Wildlife Conference.* 10:86-97.

———, 1963. "Environmental Effects upon Cyanide Toxicity to Fish." *Notulae Naturae (Philadelphia).* 361:11.

Carter, J.W. and Cameron, I.L., 1973. "Toxicity Bioassay of Heavy Metals in Water Using *Tetrahymena pyriformis." Water Research.* 7:951-61.

Coutant, C.C., 1971. "Effects on Organisms of Entrainment in Cooling Water: Steps Toward Predictability." *Nuclear Safety.* 12:600-7.

Doudoroff, P. and Katz, M., 1953. "Critical Review of Literature on the Toxicity of Industrial Wastes and Their Components to Fish." *Sewage and Industrial Wastes.* 25:802-39.

Dowden, B.F. and Bennett, H.J., 1965. "Toxicity of Selected Chemicals to Certain Animals." *Journal Water Pollution Control Federation.* 37(9): 1308-16.

Dujardin, F., 1841. "Historire Naturelle les Zoophytes Infusoires." *Liboririe Encyclopedique de Rortet.* Paris. pp. 568-70.

Edmondson, W.T., 1944. "Ecological Studies of Sessile Rotifers. I: Factors Affecting Distribution." *Ecological Monographs.* 14:31-66.

Environmental Protection Agency, 1971. "The Effects of Chemicals on Aquatic Life." Water Pollution Control Series, *Water Quality Criteria Data Book, Volume 3.*

———, 1973. "Effects of Temperature on Growth and Reproduction of Snails." Office of Research and Monitoring. EPA-R3-73-021.

———, 1975. "Methods for Acute Toxicity Test with Fish Macroinvertebrates and Amphibians." Ecological Research Series. EPA-660/3-75-009.

Everhart, C.P., Jr., 1972. "Methods with *Tetrahymena."* In *Methods in Cell Physiology, Volume 5.* D.M. Prescott, editor. Academic Press, Inc. pp. 220-288.

Eyster, C., 1973. "Optimal Nutrient Concentration Requirements for *Chlorella sorokiniana.*" Environmental Sciences Division, United States Air Force.

Finesinger, J.E., 1926. "Effect of Certain Chemical and Physical Agents on Fecundity and Length of Life and Their Inheritance in a Rotifer, *Lecane (Distyla) inermis* (Bryce)." *Journal of Experimental Zoology.* 44: 63-96.

Finney, D.F., 1971. *Statistical Methods in Biological Assay.* 2nd edition. Hafner.

Fisher, N.S. and Wurster, C.F., 1973. "Individual and Combined Effects of Temperature and Polychlorinated Biphenyls on the Growth of Three Species of Phytoplankton." *Environmental Pollution.* 5:205-12.

Freeman, L. and Fowler, I., 1953. "Toxicity of Combinations of Certain Inorganic Compounds to *Daphnia magna." Sewage and Industrial Wastes.* 25:1191-5.

Fry, F.E.J., 1971. "The Effect of Environmental Factors on the Physiology of Fish." In *Fish Physiology, Volume 6.* W.S. Hoar and D.J. Randall, editors. Academic Press, Inc.

Gersdorff, W.A., 1943. "Effect of Change of Temperature on Relative Toxicity of Rotenone and Phenol." *Journal of Agricultural Research.* 67:65-80.

Gilbert, J.J., 1968. "Dietary Control of Sexuality in the Rotifer, *Asplanchna brightwelli,* Gorse." *Physiological Zoology.* 41:14-45.

Gray, J.S. and Ventilla, 1973. "Growth Rates of a Sediment-Living Marine Protozoan as a Toxicity Indicator for Heavy Metals." *Ambio.* 2: 118-21.

Guillard, R.R.L., 1973. "Division Rates." In *Handbook of Phycological Methods—Culture Methods and Growth Measurements.* J.R. Stein, editor. pp. 280-311.

Hannan, P.J. and Patouillet, C., 1972. "Effect of Mercury on Algal Growth Rates." *Biotechnology and Bioengineering.* 14:93-100.

Herlant-Meewis, H., 1950. "Cyst Formation in *Aeolosoma hemprichi* (Ehr.)." *Biological Bulletin.* 99:173.

Hervey, J.R., 1949. "Effect of Chromium on the Growth of Unicellular Chlorophyceae and Diatoms." *Botanical Gazette.* 111(1):1-11.

Hirayama, K. and Hirano, R., 1970. "Influences of High Temperature and Residual Chlorine on Marine Phytoplankton." *Marine Biology.* 7(3): 205-13.

Hodson, P.V. and Sprague, J.B., 1975. "Temperature-Induced Changes in Acute Toxicity of Zinc to Atlantic Salmon *(Salmo Salar)." Journal of the Fisheries Research Board of Canada.* 32(1):1-10.

Hummon, W.D., 1974. "Effect of DDT on Longevity and Reproductive Rate in *Lepodermella squaminata (Gastortricha chaetonotide)." American Midland Naturalist.* 92:327-39.

Jarvaid, M.Y. and Anderson, J.M., 1967. "Thermal Acclimation Temperature Selection in Atlantic Salmon, *Salmo salar,* and Rainbow Trout, *Salmo gairdneri." Journal of the Fisheries Research Board of Canada.* 24: 1507-13.

Jensen, A.; Rystad, B., and Melson, S., 1974. "Heavy Metal Tolerance of Marine Phytoplankton. I: The Tolerance of Three Algal Species to Zinc in Coastal Seawater." *Journal of Experimental Marine Biology and Ecology.* 22:249-56.

———, 1976. "Heavy Metal Tolerance of Marine Phytoplankton. II: Copper Tolerance of Three Species in Dialysis and Batch Cultures." *Journal of Experimental Marine Biology and Ecology.* 22:249-56.

Jones, J.R.E., 1940. "A Further Study of the Relation Between Toxicity and Solution Pressure, with *Polycelis nigra* as Test Animal." *Journal of Experimental Biology.* 17:408-15.

———, 1964. *Fish and River Pollution.* Butterworth and Co., London.

Jones, M.B., 1973. "Influence of Salinity and Temperature on the Toxicity of Mercury to Marine and Brackish Water Isopods." *Estuarine and Coastal Marine Science.* 1:425-31.

Kamento, F.I. and Goodnight, G.J., 1956. "The Effects of Various Concentrations of Ions on Asexual Reproduction of the Oligochaete, *Aeolosome hemprichi.*" *Transactions of the American Microscopial Society.* 75:219.

Knibs, H.G., 1910. "The Reactions of *Aeolosoma* to Chemical Stimuli." *Journal of Experimental Zoology.* 8:43-74.

Komarek, J. and Ruzicka, J., 1969. "Effect of Temperature on the Growth and Variability of *Scenedesmus quadricauda* (Turp.) Breb." In *Studies in Phycolocy.* B. Fott, editor. E. Schweizerbart'sche Verlags buchhandlung, Stuttgart, Germany. pp. 262-92.

Knopp, J.F., 1969. "The Occurrence of Trace Elements in Water." In *Proceedings of the Third Annual Conference on Trace Substances in Environmental Health.* University of Missouri. pp. 59-73.

Lee, D.R., 1976. "Development of an Invertebrate Bioassay to Screen Petroleum Refinery Effluents Discharged into Freshwater." Ph.D. dissertation. Virginia Polytechnic Institute and State University.

Lehninger, A.L., 1970. *Biochemistry.* Worth Publishers, Inc.

Lloyd, R., 1960. "The Toxicity of $ZnSO_4$ to Rainbow Trout." *Annals of Applied Biology.* 48:84-94.

Lowry, O.H.; Rosebrough, J.; Farr, A.L., and Randall, R.J., 1951. "Protein Measurement with the Folin Phenol Reagent." *Journal of Biological Chemistry.* 193:265-75.

Maciorowski, A.F.; Benfield, E.F., and Hendricks, A.C., 1975. "Freshwater Oligochaetes of the Kanawha River." Abstract. *Association of Southeastern Biologists Bulletin.* 22:65-70.

MacLeod, J.C. and Pessah, E., 1973. "Temperature Effects on Mercury Accumulation, Toxicity, and Metabolic Rate in the Rainbow Trout *(Salmo gairdneri).*" *Journal of the Fisheries Research Board of Canada.* 30:485-92.

Malacea, I., 1966. "Contributions to Knowledge on the Toxic Effect of Cyanides, Ammonia, Mercury, and Arsenic on Some Species of Fish and on *Daphnia.*" English summary. *Studii de Protectia si Epurarea Apelor.* 7:751-92.

Maloney, T.E. and Palmer, C.M., 1956. "Toxicity of Six Chemical Compounds to Thirty Cultures of Algae." *Water and Sewer Works.* 103(11): 509-13.

Meadow, N.D. and Barrows, C.H., Jr., 1971. "Studies on Aging in a Bdelloid Rotifer. II: The Effects of Environmental Conditions and Maternal Age on Longevity and Fecundity." *Journal of Gerontology.* 26:302-9.

National Acedemy of Sciences, 1973. *Water Quality Criteria of 1972.* Ecological Research Series, EPA-R3-73-033.

———,1975. *Principles for Evaluating Chemicals in the Environment.*

Needham, R.G., 1966. "Effects of Toxaphene on Plankton and Aquatic Invertebrates in North Dakota Lakes." In *Investigations in Fish Controls, Number 4.* U.S. Fish and Wildlife Resource Publication.

Newman, J.P., Jr., 1975. "The Effects of Heavy Metals on Asexual Reproduction of the Annelid *(Aeolosoma headleyi),* Beddard (1888)." Master's thesis. Virginia Polytechnic Institute and State University.

Nichols, H.W., 1973. "Growth Media—Freshwater." In *Phycological Methods.* J.R. Stein, editor. Cambridge University Press. pp. 7-24.

Nielsen, E.S. and Kamp-Nielsen, L., 1970. "Influence of Deleterious Concentrations of Copper on the Growth of *Chlorella pyrenoidosa." Physiological Plant Pathology.* 23:828-40.

O'Hara, J., 1973. "Cadium Uptake by Fiddler Crabs Exposed to Temperature and Salinity Stress." *Journal of the Fisheries Research Board of Canada.* 30:846-8.

Parker, R.A. and Kamemoto, F.I., 1957. "The Effects of Various Concentrations of Ions on the Asexual Reproduction of the Oligochaete *Aeolosome hemprichi,* Part II." *Transactions of the American Microscopial Society.* 78:207-11.

Patrick, R., 1971. Report in *Water Quality Criteria,* 1972. National Academy of Sciences-National Academy of Engineering.

Pennak, R.W., 1953. *Freshwater Invertebrates of the United States.* Ronald Press Company.

Provasoli, L., 1958. "A Catalogue of Laboratory Strains of Free-Living and Parasitic Protozoa." *Journal of Protozoology.* 5:1-38.

Rehwoldt, R.; Menapace, W.; Nerrie, B., and Allessardrello, D., 1972. "The Effects of Increased Temperature upon the Acute Toxicity of Some Heavy Metal Ions." *Bulletin of Environmental Contamination and Toxicology.* 8:91-6.

Reynolds, J.H.; Middlebrooks, S.E., and Porcella, D.B., 1974. "Temperature-Toxicity Model for Oil Refinery Waste." *Journal of the Environment, Engineering Division,* American Society of Chemical Engineers. 100(EE3):557-76.

Ruthven, J.A. and Cairns, J., Jr., 1973. "Response of Freshwater Protozoan Communities to Metals." *Journal of Protozoology.* 70:127-35.

Schaefer, E.D. and Pipes, W.O., 1973. "Temperature and the Toxicity of Chromate and Arsenate to the Rotifer, *Philodina roseola." Water Research.* 7:1781-90.

Schweiger, G., 1957. "Die Toxicologische Einwirkung von Schwermettallsalzen auf Fische und Fischnahrtiere." *Archiv Fuer Fischereiwissenshaft.* 8:57-78.

Skidmore, J.F., 1964. "Toxicity of Zinc Compounds in Aquatic Animals, with Special Reference to Fish." *Quarterly Review of Biology.* 227-48.

Smith, L.L. et al., 1974. *ORSANCO 24-Hour Bioassay.* Ohio River Valley Water Sanitation Commission.

Sprague, J.B., 1970. "Measurement of Pollutant Toxicity to Fish, II: Utilizing and Applying Bioassay Results." *Water Research.* 4:3-32.

———, 1971. "Measurement of Pollutant Toxicity to Fish, III: Sublethal Effects and 'Safe' Concentrations." *Water Research.* 5:245-66.

Sudo, R. and Aiba, S., 1973. "Effect of Copper and Hexavalent Chromium on the Specific Growth Rate of Ciliata Isolated from Activated Sludge." *Water Research.* 7:1301-7.

Sullivan, G.W. and Buikema, A.L., 1972. "The Effect of Hardness on Toxicity of Zinc Sulfate to the Rotifer, *Philodina* sp." Abstract. *Association of Southeastern Biologists Bulletin.* 19:104.

Trainor, F.R. and Shubert, L.E., 1973. "Growth of *Dictyosphaerium, Selenastrum,* and *Scenedesmus* (Chlorophyceae) in a Dilute Algal Medium." *Phycologia.* 12:35-9.

Vernberg, W.B.; DeCoursey, P.J., and Padgett, W.J., 1973. "Synergistic Effects of Environmental Variables on Larvae to *Uca pugilator." Marine Biology.* 22:307-12.

Vernberg, W.B. and O'Hara, J., 1972. "Temperature-Salinity Stress and Mercury Uptake in the Fiddler Crab, *Uca pugilator." Journal of the Fisheries Research Board of Canada.* 29:1491-4.

Winner, R.W., 1976. "Toxicity of Copper to Daphnids in Reconstituted and Natural Waters." Ecological Research Series. EPA-600/3-76-051.

Wuhrman, K., 1952. "Sur Quelques Principles de la Toxicologie du Poisson." *Bull. de C.B.E.D.E.* 15:49-60. (Cited by Jones, J.R.E., 1964. *Fish and River Pollution.* Butterworth and Co., London).

TABLES

TABLE 1
Algae Utilized in Assays

Algae	Taxonomic Group	Source	Strain#
Cyclotella meneghiniana	Bacillariophyceae	F. Trainor	
Scenedesmus quadricauda	Chlorococcales	Indiana Culture Collection	76
Chlamydomonas sp.	Volvocales	Indiana Culture Collection	608
Lyngbya sp.	Cyanophyta	Indiana Culture Collection	B487

TABLE 2
Chemical Tested in Algal Assays

Toxicant	Concentrations (mg/l)	*Lyngbya* assay
Copper (Cu^{++})	1, 2, 4, 8	0.1, 1.0, 10
Zinc (Zn^{++})	5, 10, 15, 20	0.1, 1.0, 10
Chromate	0.5, 1, 2, 4	0.1, 1.0, 10
Chlorine (residual)	0.15, 0.38, 0.76, 1.52	0.1, 0.26, 0.52
Cyanide*	0.01, 0.03, 0.06, 0.10	Not run
Phenol	1, 5, 10, 20	Not run

*Only utilized with *Chlamydomonas* sp.

TABLE 3

Chemical Composition of Synthetic Water (Modified from Cairns and Scheier, 1957)

Compound	Concentration (g/l)
KCl	1.46×10^{-2}
NH_4NO_3	3.60×10^{-2}
$NaHCO_3$	2.30×10^{-2}
$H_2S{\cdot}O_3$	1.00×10^{-2}
Fe citrate	2.00×10^{-2}
$CaCO_3$	2.00×10^{-2}
$CaCO_4$	3.44×10^{-2}
$MgSO_4{\cdot}7H_2O$	2.46×10^{-2}

TABLE 4

Fish Sizes and Chemical Characteristics of Water in Test Tanks

	Fish weight (g)			Fish length (cm)				
	$\bar{X}$	SD	N	$\bar{X}$	SD	N	Wt (g)	Length (cm)
Goldfish	1.93	1.05	800	4.59	0.73	800	0.6-5.8	3.1-6.6
Golden shiner	2.56	1.02	700	6.42	0.78	700	0.6-4.9	3.2-7.6
Bluegill	0.64	0.67	650	3.43	0.72	650	0.1-3.0	2.2-5.8
Channel catfish	8.81	3.86	350	9.72	1.42	350	2.0-20.3	6.3-13.4
Rainbow trout	4.42	1.95	750	7.25	3.19	750	1.1-11.8	4.0-10.6

Hardness (mg/l as $CaCO_3$) 36

Oxygen (mg/l)	$\bar{X}$	SD	N	Range	$\bar{X}$ decrease/24 hr (SD)
5 C	11.0	0.75	125	9.0-12.5	0.53 (0.35)
15 C	8.5	0.56	100	6.2-9.9	0.65 (0.49)
30 C	6.0	0.91	100	4.2-7.9	0.41 (0.30)

pH (by toxicant)	$\bar{X}$	SD	N	Range
control	7.4	0.4	60	6.5-8.1
CN^-	7.6	0.3	46	6.7-8.1
Zn^{++}	7.1	0.4	40	5.8-7.8
Cr^{+6}	5.8	0.8	50	4.6-7.5
Cu^{++}	7.2	0.4	60	6.3-7.8
Phenol	7.2	0.4	30	6.6-7.6

TABLE 5
Percent Decline in Toxicant Concentration During a 24-Hour Period

	Temperature (°C)					
	5		15		30	
	$\bar{X}$	SD (N/T)*	$\bar{X}$	SD (N/T)	$\bar{X}$	SD (N/T)
% Δ $[CN^-]$/24 hrs.†	32.9	30.0 (3/18)	27.4	11.6 (9/16)	32.0	18.6 (12/12)
% Δ $[Zn^{++}]$/24 hrs.	9.6	17.0 (10/16)	2.7	2.6(4/12)	2.0	0.8 (3/12)
% Δ $[Cr^{+6}]$/24 hrs.	8.8	8.1 (8/20)	19.1	9.1(8/10)	7.1	6.2 (3/20)
% Δ $[Cu^{++}]$/24 hrs.	11.0	8.0 (13/26)	18.5	15.7(16/16)	17.0	15.0 (20/20)
% Δ [Phenol]/24 hrs.	13.0	4.0 (3/18)	13.1	5.2 (5/12)	–	–

*Mean and standard deviation with sample size in which a decline was observed (N)/total number of concentrations tested (T).

†Analytic method for cyanide at the low concentrations effective upon trout was near its limit of sensitivity thus giving poor precision.

TABLE 6

Standardization of *Chlamydomonas* sp.
(Optical Density, Direct Cell Counts, and Dry Weights)

35°C	Day 0	1	2	4	6	8	10
Optical Density	0.026	0.018	0.016	0.012	0	0	0
Direct Cell Counts	0.18	0.07	0.03	0.01	0	0	0
Dry Weight	0.10	-*	-	-	-	-	0

r = 0.99 for O.D. to Direct Cell Counts.

25°C							
Optical Density	0.042	0.046	0.048	0.053	0.064	0.066	0.069
Direct Cell Counts	0.58	0.67	0.74	0.91	0.98	1.05	1.12
Dry Weight	0.16	-	-	-	-	-	0.28

r = 0.98 for O.D. to Direct Cell Counts.

15°C							
Optical Density	0.038	0.043	0.046	0.050	0.056	0.062	0.065
Direct Cell Counts	0.58	0.64	0.64	0.70	0.80	1.04	1.09
Dry Weight	0.135	-	-	-	-	-	0.25

r = 0.97 for O.D. to Direct Cell Counts.

5°C							
Optical Density	0.038	0.042	0.044	0.040	0.045	0.042	0.042
Direct Cell Counts	0.68	0.78	0.72	0.77	0.82	0.76	0.77
Dry Weight	0.135	-	-	-	-	-	0.175

r = 0.59 for O.D. to Direct Cell Counts.

Optical Density = absorbance units at 525nm
Direct Cell Counts = $X10^6$ cells/ml
Dry Weights = 10^{-1} mg/4 ml sample
*- No Sample Taken

TABLE 7
Growth of Assay Algae at Each Test Temperature

	35°C	25°C	15°C	5°C
Cyclotella meneghiniana[a]	death	0.190	0.076	0.068
Scenedesmus quadricauda[a]	0.264[c]	0.224	0.143	negligible
Chlamydomonas sp.[b]	death	0.064	0.065	negligible
Lyngbya sp.[b]	0.291	0.278	0.207	negligible

[a]Incubated at ≈400 ft-c light intensity.
[b]Incubated at ≈200 ft-c light intensity.
[c]Values represent mean specific growth rates.

TABLE 8
Effect of Temperature on Copper Toxicity to Selected Algae

Concentration (mg/l)	Temperature (°C) 5	15	25	35
		Chlamydomonas sp.		
0.0		0.060* (0.224)	0.045 (0.184)	
1.0		0.071 (0.160)	0.073 (0.220)	
2.0		0.079 (0.210)	0.065 (0.157)	
4.0		0.074 (0.195)	0.062 (0.162)	
8.0		-0.014 (0.141)	-0.080 (0.140)	
		Scenedesmus quadricauda		
0.0		0.136 (0.281)	0.208 (0.273)	0.272 (0.439)
1.0		0.124 (0.248)	0.227 (0.320)	0.288 (0.514)
2.0		0.145 (0.320)	0.231 (0.367)	0.288 (0.610)
4.0		0.136 (0.274)	0.237 (0.384)	0.256 (0.556)
8.0		0.073 (0.297)	0.091 (0.261)	0.122 (0.464)
		Cyclotella meneghiniana		
0.0	0.072 (0.140)	0.084 (0.154)	0.206 (0.354)	
1.0	0.077 (0.197)	0.075 (0.182)	0.137 (0.274)	
2.0	0.059 (0.143)	0.076 (0.194)	0.144 (0.256)	
4.0	0.070 (0.138)	0.070 (0.133)	0.139 (0.279)	
8.0	-0.023 (0.131)	-0.050 (0.137)	-0.059 (0.146)	
		Lyngbya sp.		
0.0		0.207	0.278	0.291
0.1		0.187	0.286	0.311
1.0		0.187	0.223	0.272
10.0		0.159	0.172	0.208

*Data are presented as mean growth rates, $\overline{\mu}$, and maximum growth rates, $\overline{\mu}$max (in parentheses).

TABLE 9
Effect of Temperature on Zinc Toxicity to Selected Algae

Concentration mg/l	Temperature (°C) 5	15	25	35
		Chlamydomonas sp.		
0.0		0.068*(0.141)	0.059 (0.259)	
5.0		0.081 (0.154)	0.084 (0.305)	
10.0		0.055 (0.108)	0.070 (0.328)	
15.0		0.022 (0.096)	0.024 (0.155)	
20.0		0.001 (0.106)	0.002 (0.148)	
		Scenedesmus quadricauda		
0.0		0.122 (0.298)	0.214 (0.288)	0.270 (0.548)
5.0		0.159 (0.340)	0.235 (0.405)	0.260 (0.521)
10.0		0.137 (0.289)	0.214 (0.444)	0.227 (0.516)
15.0		0.130 (0.360)	0.188 (0.337)	0.223 (0.711)
20.0		0.079 (0.266)	0.168 (0.402)	0.207 (0.475)
		Cyclotella meneghiniana		
0.0	0.070 (0.178)	0.067 (0.154)	0.182 (0.346)	
5.0	0.062 (0.173)	0.070 (0.162)	0.138 (0.242)	
10.0	0.061 (0.144)	0.063 (0.198)	0.142 (0.254)	
15.0	0.074 (0.218)	0.054 (0.128)	0.111 (0.248)	
20.0	0.036 (0.199)	0.013 (0.142)	0.070 (0.262)	
		Lyngbya sp.		
0.0		0.207	0.278	0.291
0.1		0.190	0.274	0.291
1.0		0.165	0.234	0.279
10.0		0.180	0.247	0.278

*Data are presented as mean growth rates, $\overline{\mu}$, and maximum growth rates, $\overline{\mu}$max (in parentheses).

TABLE 10
Effect of Temperature on Chromate Toxicity to Selected Algae

Concentration mg/l	Temperature (°C) 5	15	25	35
		Chlamydomonas sp.		
0.0		0.067*(0.212)	0.071 (0.269)	
0.5		0.052 (0.145)	0.043 (0.138)	
1.0		0.044 (0.152)	0.004 (0.204)	
2.0		0.025 (0.120)	-0.058 (0.144)	
4.0		-0.009 (0.073)	-0.038 (0.013)	
		Scenedesmus quadricauda		
0.0		0.165 (0.300)	0.240 (0.441)	0.249 (0.504)
0.5		0.130 (0.285)	0.227 (0.360)	0.252 (0.500)
1.0		0.118 (0.262)	0.220 (0.400)	0.206 (0.478)
2.0		0.088 (0.297)	0.229 (0.382)	0.210 (0.413)
4.0		0.066 (0.222)	0.151 (0.297)	0.132 (0.386)
		Cyclotella meneghiniana		
0.0	0.064 (0.119)	0.076 (0.167)	0.198 (0.388)	
0.5	0.028 (0.167)	-0.001 (0.123)	-0.274 (0.085)	
1.0	-0.003 (0.131)	-0.098 (0.109)	-0.472 (0.134)	
2.0	0.006 (0.162)	0.135 (0.128)	-0.331 (0.098)	
4.0	-0.047 (0.106)	-0.116 (0.066)	-0.480 (0.052)	
		Lyngbya sp.		
0.0		0.207	0.278	0.291
0.1		0.193	0.230	0.303
1.0		0.162	0.232	0.285
10.0		0.118	-0.028	0.152

*Data are presented as mean growth rate, $\overline{\mu}$, and maximum growth rate, $\overline{\mu}$max (in parentheses).

TABLE 11
Effect of Temperature on Chlorine Toxicity to Selected Algae

Concentration mg/l	Temperature (°C) 5	15	25	35
		Chlamydomonas sp.		
0.0		0.071*(0.120)	0.113 (0.192)	
0.15		0.058 (0.139)	0.116 (0.211)	
0.38		0.066 (0.177)	0.111 (0.241)	
0.76		0.055 (0.160)	0.049 (0.374)	
1.52		0.027 (0.159)	-0.038 (0.146)	
		Scenedesmus quadricauda		
0.0		0.134 (0.296)	0.238 (0.395)	0.263 (0.514)
0.15		0.113 (0.258)	0.229 (0.378)	0.275 (0.526)
0.38		0.128 (0.286)	0.212 (0.386)	0.265 (0.480)
0.76		0.114 (0.276)	0.191 (0.407)	0.286 (0.516)
1.52		0.074 (0.348)		0.258 (0.491)
		Cyclotella meneghiniana		
0.0	0.070 (0.183)	0.077 (0.148)	0.168 (0.322)	
0.15	0.067 (0.172)	0.072 (0.130)	0.176 (0.308)	
0.38	0.051 (0.151)	0.071 (0.133)	0.162 (0.478)	
0.76	0.054 (0.161)	0.069 (0.171)	0.144 (0.477)	
1.52	-0.007 (0.179)	0.058 (0.141)	0.109 (0.552)	
		Lyngbya sp.		
0.0		0.207	0.278	0.291
0.1		0.190	0.234	0.269
1.0		0.187	0.255	0.278
10.0		0.130	0.248	0.238

*Data are presented as mean growth rate, $\overline{\mu}$, and maximum growth rate, $\overline{\mu}$max (in parentheses).

TABLE 12
Effect of Temperature on Phenol Toxicity to Selected Algae

Concentration mg/l	Temperature (°C) 5	15	25	35
		Chlamydomonas sp.		
0.0		0.136*(0.196)	0.171 (0.381)	
1.0		0.137 (0.275)	0.159 (0.352)	
5.0		0.156 (0.324)	0.166 (0.331)	
10.0		0.168 (0.297)	0.163 (0.335)	
20.0		0.163 (0.278)	0.173 (0.335)	
		Scenedesmus quadricauda		
0.0		0.159 (0.347)	0.222 (0.358)	0.266 (0.489)
1.0		0.139 (0.292)	0.238 (0.408)	0.289 (0.442)
5.0		0.178 (0.338)	0.240 (0.467)	0.314 (0.610)
10.0		0.156 (0.282)	0.289 (0.540)	0.343 (0.658)
20.0		0.191 (0.323)	0.312 (0.631)	0.305 (0.628)
		Cyclotella meneghiniana		
0.0	0.062 (0.176)	0.060 (0.241)	0.194 (0.379)	
1.0	0.062 (0.154)	0.070 (0.180)	0.206 (0.329)	
5.0	0.064 (0.150)	0.071 (0.253)	0.168 (0.297)	
10.0	0.064 (0.174)	0.078 (0.260)	0.183 (0.348)	
20.0	0.065 (0.158)	0.067 (0.250)	0.210 (0.458)	

*Data are presented as mean growth rate, $\overline{\mu}$, and maximum growth rate, $\overline{\mu}$max (in parentheses).

TABLE 13

Effect of Temperature on Cyanide Toxicity to *Chlamydomonas* sp.

Concentration mg/l	Temperature (°C) 15	25
0.0	0.058* (0.170)	0.068 (0.212)
0.01	0.052 (0.177)	0.058 (0.107)
0.03	0.052 (0.171)	0.067 (0.129)
0.06	0.056 (0.151)	0.066 (0.118)
0.10	0.052 (0.185)	0.066 (0.125)

*Data are expressed as mean growth rate, $\overline{\mu}$, and maximum growth rate, $\overline{\mu}$max (in parentheses).

TABLE 14

Range of Growth Rates Observed for *Chilomonas paramecium*, in Controls, at Three Acclimation Temperatures

Temperature (°C)	Range of growth rate (day^{-1})
30	1.99 -2.99
20	1.05 -1.45
10	0.185 -0.354

Note: 30°C growth rates are based on a time interval of 19-25 hours.
20°C growth rates are based on a time interval of 44-48 hours.
10°C growth rates are based on a time interval of 98-163 hours.

TABLE 15

Growth Response of *Chilomonas paramecium* to Selected Toxicants Tested at Three Temperatures

Toxicant concentration (mg/l)	Temperature (°C) 10	20	30
	Copper		
0.15	*		–
0.30			–
0.45		0	0
1.0	+	0	–
2.5		–	0
5.0		0	––
8.6		0	–
10.0	+		––
25.0	––	0	0
50.0	––		––
100.0	––		––
	Chromium		
1.0	–		0
2.0	––	0	
4.2	––	––	––
5.5	––	––	––
10.0	––	––	––
18.0	––	––	
50.0	––		
	Zinc		
0.18		0	0
1.3		––	+
4.4			0
5.0	––		
10.0	––	0	
25.0	––	––	+
60.0	––	––	––
100.0	––	––	––
	Chlorine		
0.015	0	0	0
0.15	0	+	+
0.7	––	––	––
1.4	––	––	––
2.8	––	––	––
	Phenol		
25.0	–	0	0
200.0	––	––	––
500.0	––	––	––
1000.0	––	––	––

*No data were available if blank.
0 No change, ± 10% of the control.
– 10 to 50 % decrease from the control.
–– >50% decrease from the control.
+ 10 to 50% increase from the control.

TABLE 16
Toxicity of Selected Compounds to Representative Aquatic Invertebrates

Chemical	24-hr LC50's Temperature (°C) 5	10	15	20	25
			Nitrocris		
Chlorine	15.6	14.0	11.9	9.6	8.3
Chromium	9.6	9.0	7.4	5.6	5.0
Copper	3.3	3.2	1.8	0.56	0.48
Zinc	5.71	5.6	3.8	2.1	1.8
Phenol	401.0	361.0	370.0	384.0	400.0
Cyanide	14.0	13.0	11.0	10.0	7.6
			Philodina		
Chlorine	0.13	0.1	0.09	0.07	0.065
Chromium	56.0	51.0	42.0	38.0	36.2
Copper	1.5	1.43	1.3	1.21	1.15
Zinc	1.57	1.4	0.83	0.61	0.54
Phenol	362.0	347.0	331.0	325.0	371.0
Cyanide				0.50 to 250.0	
			Daphnia pulex		
Chlorine	0.14	0.13	0.1	0.095	0.05
Chromium	7.0	5.6	1.0	0.8	0.56
Copper	0.08	0.07	0.03	0.02	0.01
Zinc	2.1	1.8	1.1	0.56	0.46
Phenol	109.0	102.5	97.0	101.0	111.0
Cyanide	0.42	0.33	0.32	0.15	0.003
			Daphnia magna		
Chlorine	0.16	0.15	0.145	0.14	0.076
Chromium	7.9	6.06	4.8	1.0	0.76
Copper	0.1	0.09	0.05	0.01	0.01
Zinc	2.8	2.3	1.8	1.0	0.79
Phenol	115.0	102.0	100.0	94.0	102.0
			Aeolosoma		
Chlorine	3.2	2.75	2.2	1.96	1.7
Chromium	1.4	10.8	10.0	7.8	5.6
Copper	2.8	2.6	2.4	1.8	1.6
Zinc	19.6	18.3	16.0	15.6	14.2
Phenol	421.0	390.0	411.0	401.0	400.0
Cyanide	11.0	100.0	120.0	160.0	160.0

TABLE 16 (Continued)

Chemical	48-hr LC50's Temperature (°C) 5	10	15	20	25
			Nitrocris		
Chlorine	12.8	10.0	7.7	6.0	5.3
Chromium	9.1	7.8	3.7	1.2	0.8
Copper	3.0	2.4	1.0	0.3	0.21
Zinc	4.8	4.6	2.8	1.9	1.65
Phenol	389.0	351.0	353.0	360.0	391.0
Cyanide	13.6	12.8	10.0	8.0	7.0
			Philodina		
Chlorine	0.1	0.078	0.065	0.05	0.047
Chromium	54.0	50.6	39.2	31.0	29.0
Copper	1.3	1.2	1.13	1.0	0.95
Zinc	1.55	1.3	0.78	0.60	0.50
Phenol	300.0	282.0	245.0	205.0	292.0
Cyanide				20.0 to 145.0	
			Daphnia pulex		
Chlorine	0.11	0.091	0.075	0.04	0.03
Chromium	4.8	3.2	0.9	0.76	0.4
Copper	0.07	0.06	0.02	0.01	0.056
Zinc	1.6	1.2	0.94	0.5	0.28
Phenol	93.0	87.8	85.0	81.0	79.0
Cyanide	0.33	0.33	0.18	0.11	0.001
			Daphnia magna		
Chlorine	0.15	0.13	0.12	0.116	0.085
Chromium	7.6	5.6	4.3	0.9	0.56
Copper	0.09	0.07	0.04	0.01	0.007
Zinc	2.3	1.7	1.1	0.28	0.56
Phenol	100.0	92.0	91.0	88.0	91.2
			Aeolosoma		
Chlorine	2.6	2.3	2.0	1.84	1.68
Chromium	12.1	10.0	8.6	7.0	4.8
Copper	2.6	2.3	2.0	1.65	1.0
Zinc	18.1	17.6	15.6	15.0	13.5
Phenol	360.0	351.0	381.0	356.0	341.0
Cyanide	10.0	9.0	120.0	160.0	160.0

TABLE 17

Median Lethal Concentration (ppm±SD) of Selected Chemicals after 24 Hours

Species Toxicant Temp.* (°C)	*Carrassius auratis* (Goldfish)	*Notemigonius crysoleucus* (Golden Shiner)	*Lepomis macrochirus* (Bluegill)	*Ictalurus punctatus* (Channel Catfish)	*Salmo gairdneri* (Rainbow Trout)
Cyanide (as CN)					
5	3.25±1.95	0.54±0.19	0.24	0.20±0.2	0.090
15	0.44	0.31±0.06	0.16	0.31±0.1	0.098
30	0.28	0.30±0.08	0.19±0.04	0.23	0.092
Zinc (as Zn^{+2})					
5	103±36	11.4±5.43	23.0±5.9	–	2.8
15	40±13	7.76±2.76	19.1±4.4	–	1.56±0.35
30	24±18	8.33±2.06	8.85±2.4	–	2.1
Chromate (as Cr^{+6})					
5	354±296	151±35	228±78	50	58.9±33
15	213±39	109±35	280±151	58±37	141.0±114
30	109±50	104±30	214±38	72	95.5±20
Copper (as Cu^{+2})					
5	2.7±0.85	0.33±0.15	2.59±0.80	3.7	0.95±6.41
15	2.90	0.23±0.05	2.50	2.6±0.4	0.43±0.22
30	1.51±0.51	0.27±0.23	3.82±0.06	3.1±0.3	0.15±0.02
Phenol					
5	200	129±79	60	–	5.6
15	60-120	35±48	19-160	–	11.0
30	–	–	–	–	11.3

**Salmo gairdneri* acclimated at 5°, 12°, 18°C.

TABLE 18

Summary of Results Showing Temperature of Highest LC50, Widest Range of LC 1-99, and Longest Survival at Highest LC50

	Cyanide	Chromate	Copper	Zinc
Goldfish	5 , 5 , 5*	5, 5 , 5	15 , 15†, 15‡	5, 5, –
Golden Shiner	5 , 5 , 5	5, 5§, 5	ND‖, 30 , ND‖	5, 5, –
Bluegill	5 , 5§, 5	15, 15 , 15	30 , 5 , 30‡	5, 5, –
Channel Catfish	15 , 5 , 15‡	30, 5 , 30‡	5 , 5 , 5	–
Rainbow Trout	ND‖, 5 , 12	12, 12 , 12	5 , 5 , ND‖	5, 18, –

*Response at 5°C was not graphed since no death occurred until 24 hours even with the relatively high concentrations used.

†Fifteen slightly higher than 5°.

‡As determined below a crossover in the LT-50's and in the range of lower concentrations at which a higher temperature resulted in longer survival.

§Five slightly higher than 15° or 30° (addition of zero to 1% would make this wider).

‖No difference between the three temperatures.

TABLE 19

Instances Where a Temperature Crossover Occurred in LT-50's

	Cyanide		Chromate		Copper	
	(low)*	(high)†	(low)*	(high)†	(low)*	(high)†
Rainbow Trout	5	12	5	12	–	–
Channel Catfish	15	5	30	5	30	5/15§
Goldfish	–	–	15	5‡	15	5
Golden Shiner	15	5‡	30	5‡	–	–
Bluegill	30	5‡	–	–	30	15

*Temperature of longest survival below the crossover at low concentration.

†Temperature of longest survival above the crossover at high concentrations.

‡Extrapolation of data.

§With extrapolation, longest survival is at 30°C from 0-3 mg/l, 5°C from 3-4 mg/l, and 15°C for greater than 4 mg/l.

TABLE 20

Comparison of Concentration and Temperature of Highest LC50 Value to Concentration at Which a Temperature Crossover Occurred in LT-50

	Cyanide			Chromate			Copper		
	Highest LC50 Conc.	(Temp.)*	Crossover Conc.	Highest LC50 Conc.	(Temp.)*	Crossover Conc.	Highest LC50 Conc.	(Temp.)*	Crossover Conc.
Rainbow Trout	0.09-0.098	(ND)†	0.095	141	(12)	80	–	–	–
Channel Catfish	0.31	(15)	0.36	72	(30)	80	3.7	(5)	3.0
Goldfish	–	–	–	354	(5)	180	2.9	(15)	3.5
Golden Shiner	0.54	(5)	0.33	151	(5)	116	–	–	–
Bluegill	0.24	(5)	0.15	–	–	–	3.6	(30)	4.0

*Temperature given in °C.

†No data.

FIGURES

Figure 1

Influence of Various Temperatures and Toxicant Concentrations on Mean Specific Growth Rates of *Cyclotella meneghiniana* and Copper at Five Days

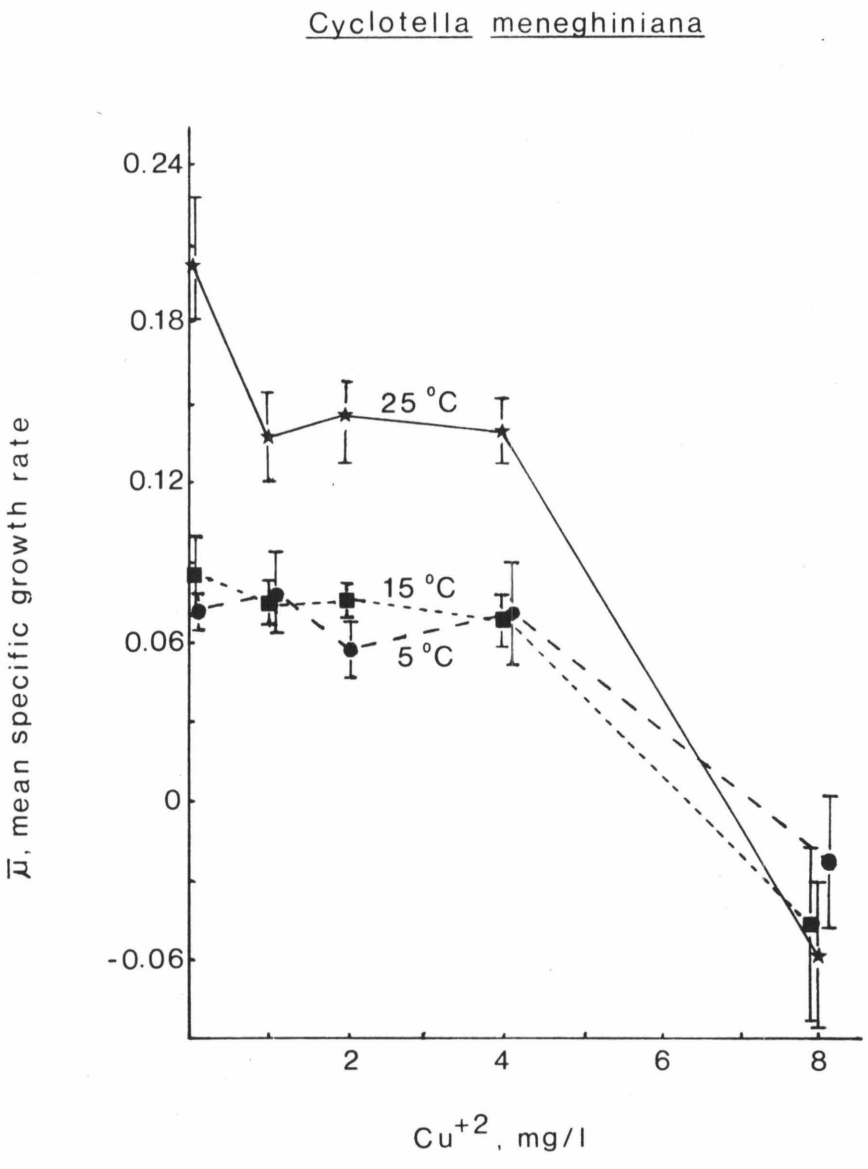


Figure 2

Influence of Various Temperatures and Toxicant Concentrations on Mean Specific Growth Rates of *Scenedesmus quadricauda* and Copper at Five Days

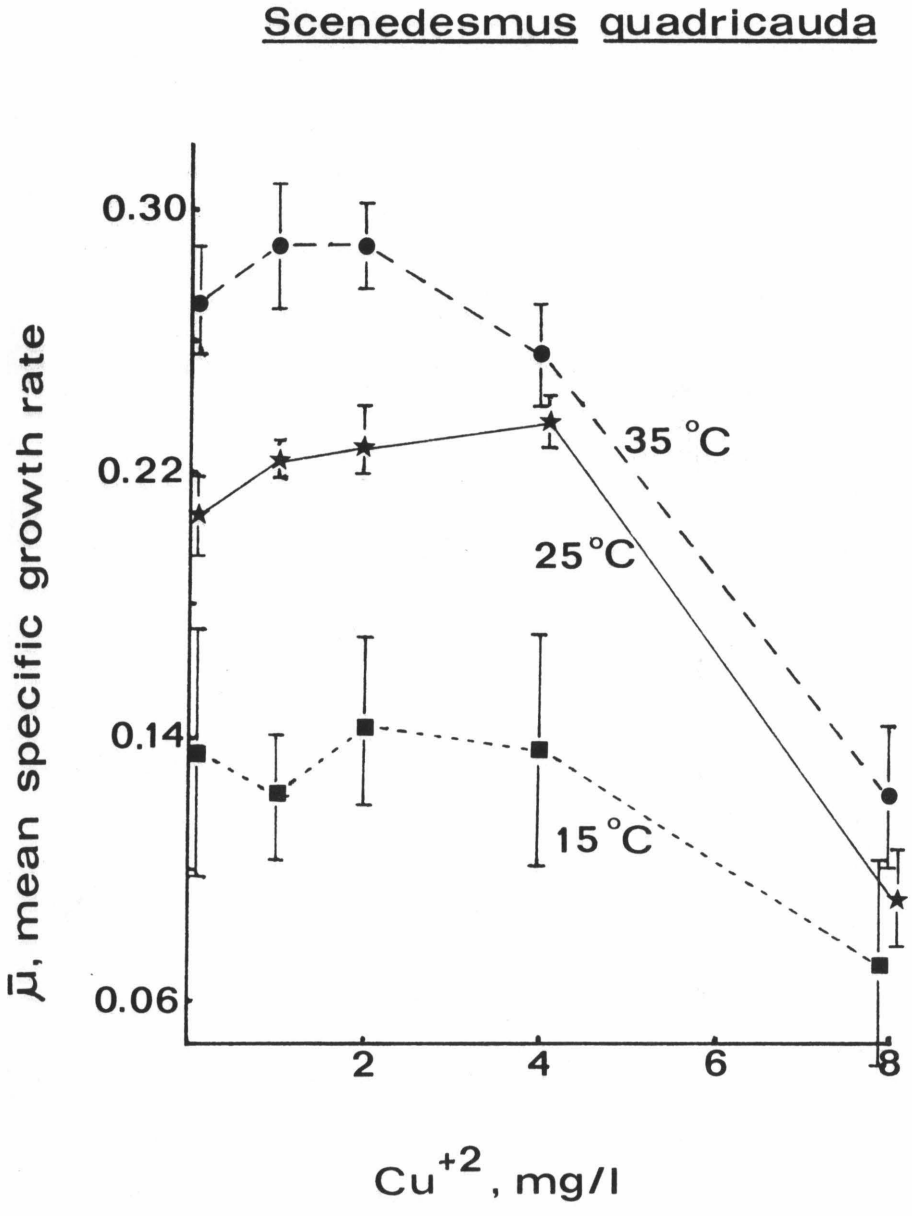


Figure 3

Influence of Various Temperatures and Toxicant Concentrations on *Chlamydomonas* sp. and Zinc at 10 Days

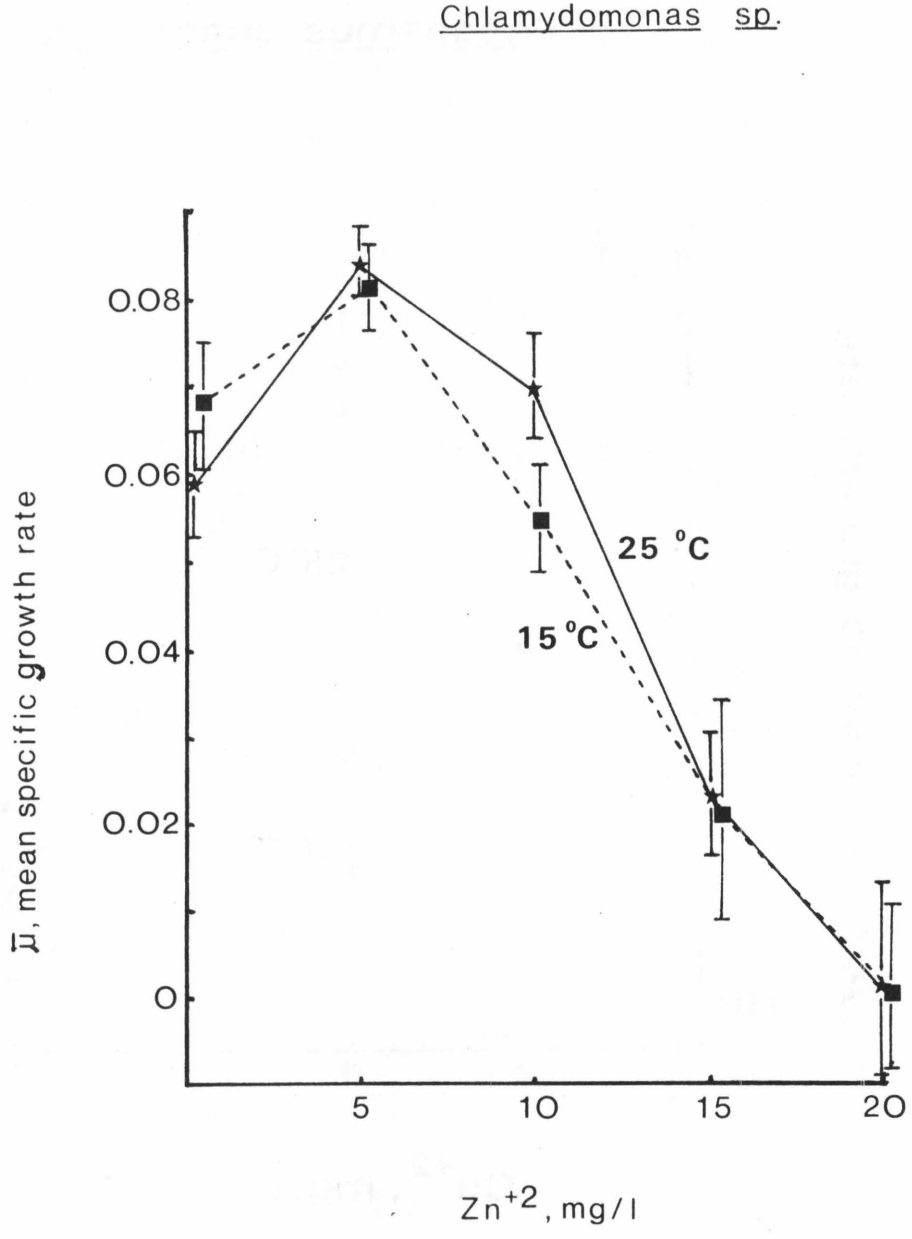


Figure 4

Influence of Various Temperatures and Toxicant Concentrations on Mean Specific Growth Rates of *Cyclotella meneghiniana* and Zinc at Five Days

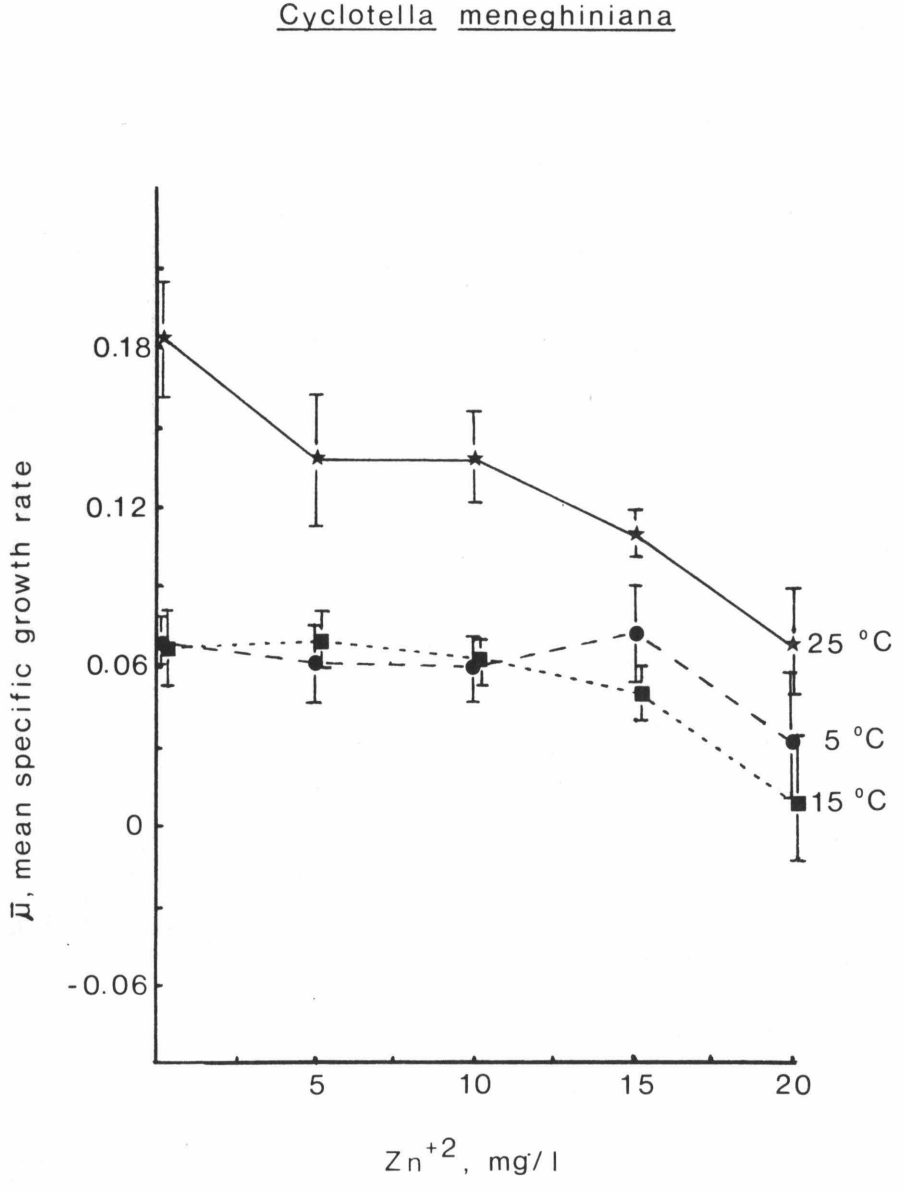


Figure 5
Influence of Various Temperatures and Toxicant Concentrations on *Scenedesmus quadricauda* and Zinc at Five Days

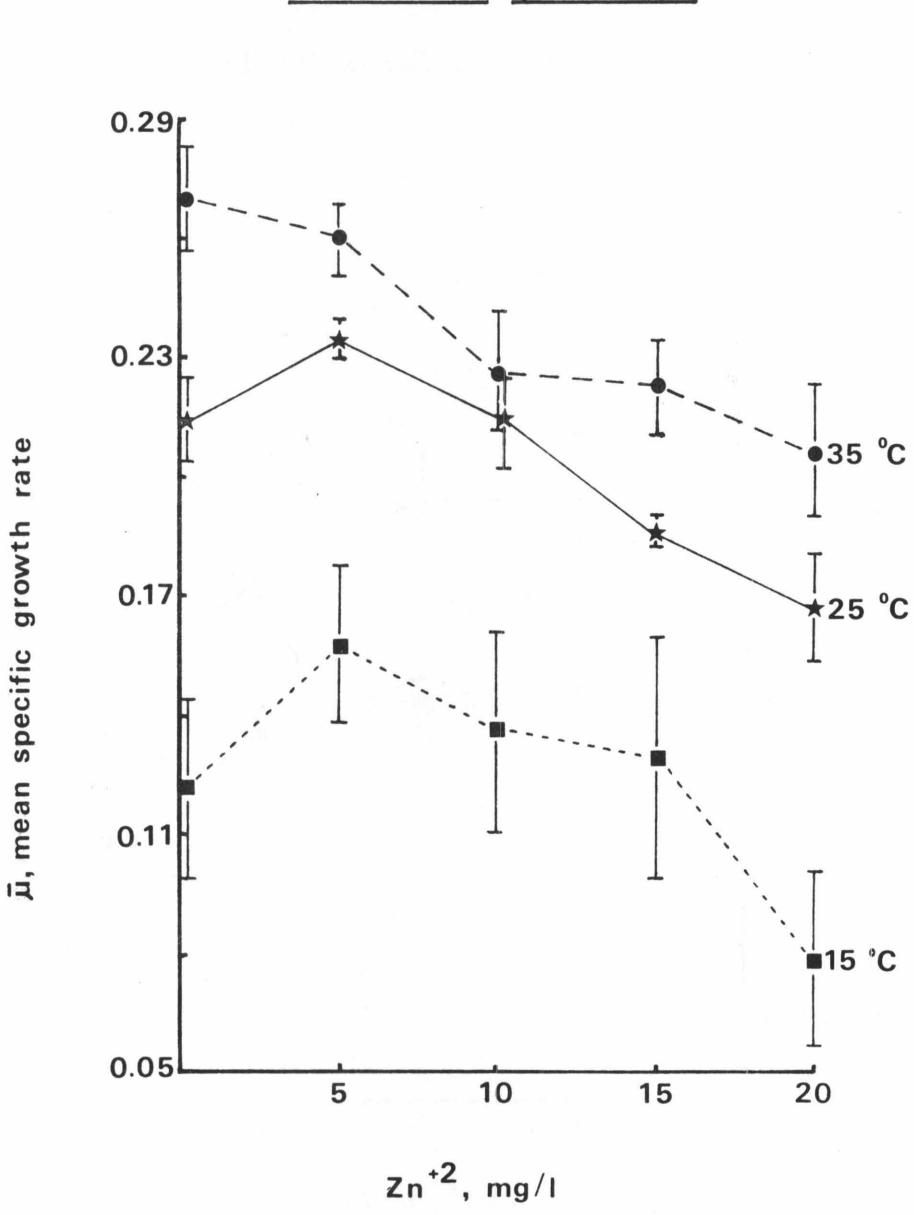


Figure 6

Influence of Various Temperatures and Toxicant Concentrations on Mean Specific Growth Rates of *Cyclotella meneghiniana* and Chromium at Five Days

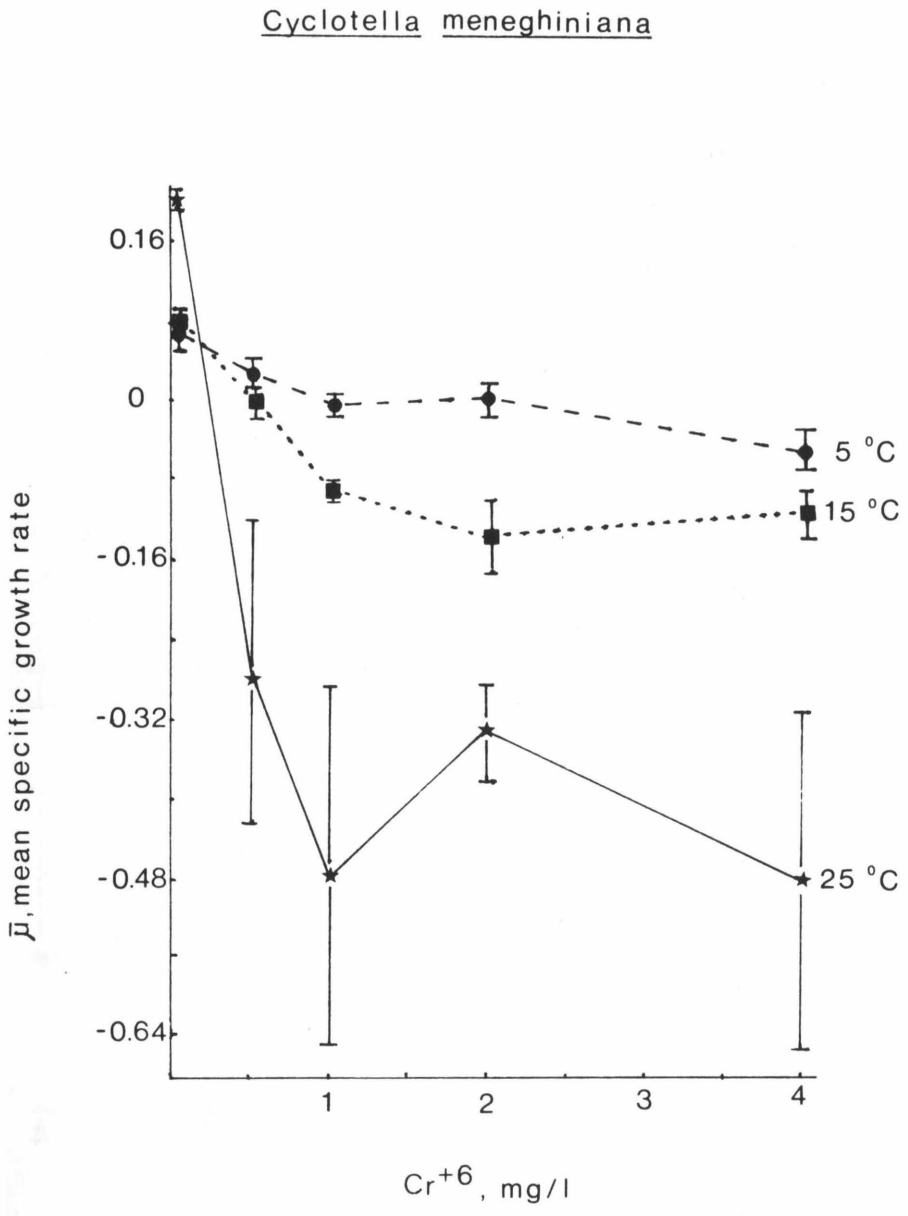


Figure 7
Influence of Various Temperatures and Toxicant Concentrations on Mean Specific Growth Rates of *Scenedesmus quadricauda* and Chromium at Five Days

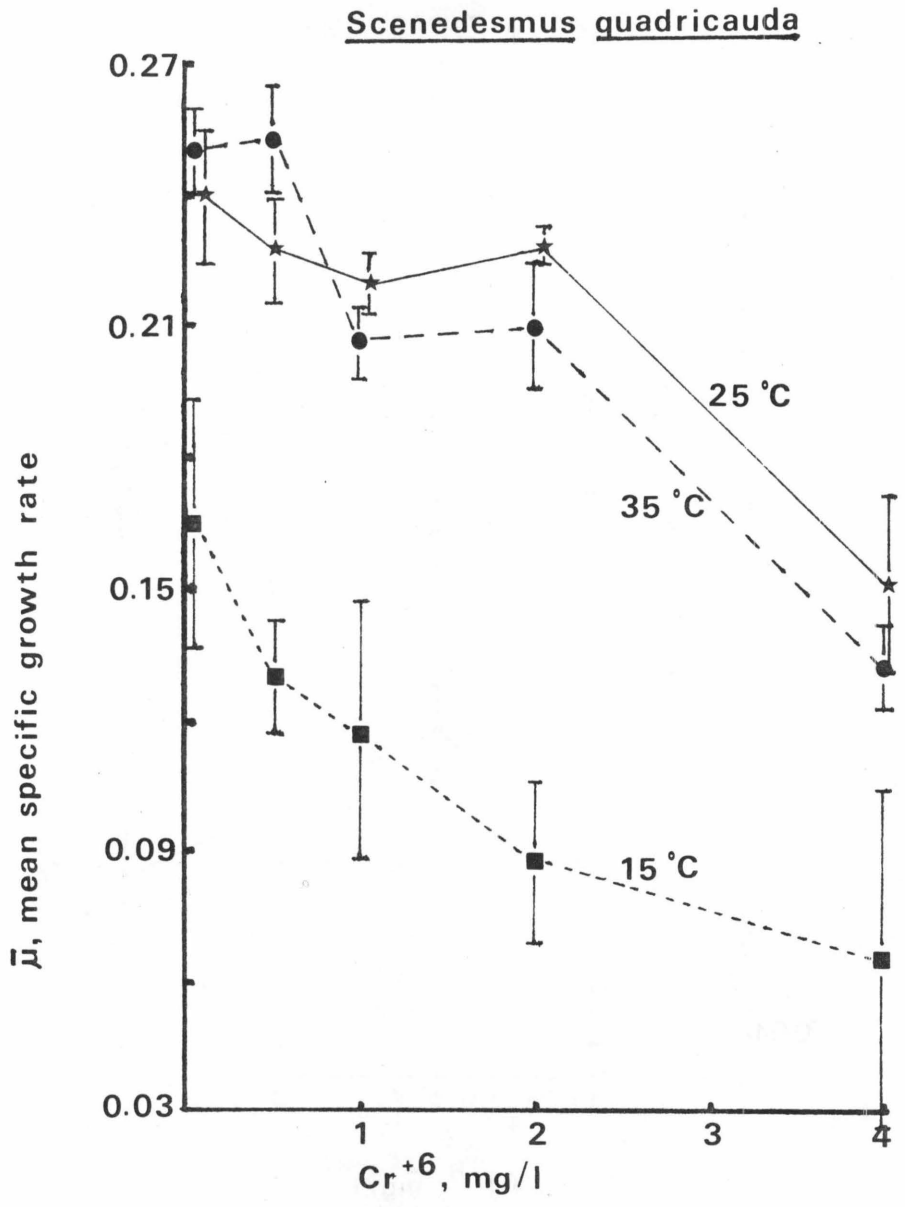


Figure 8
Percent Death of Goldfish and Bluegill at 24 Hours
in Phenol Concentrations

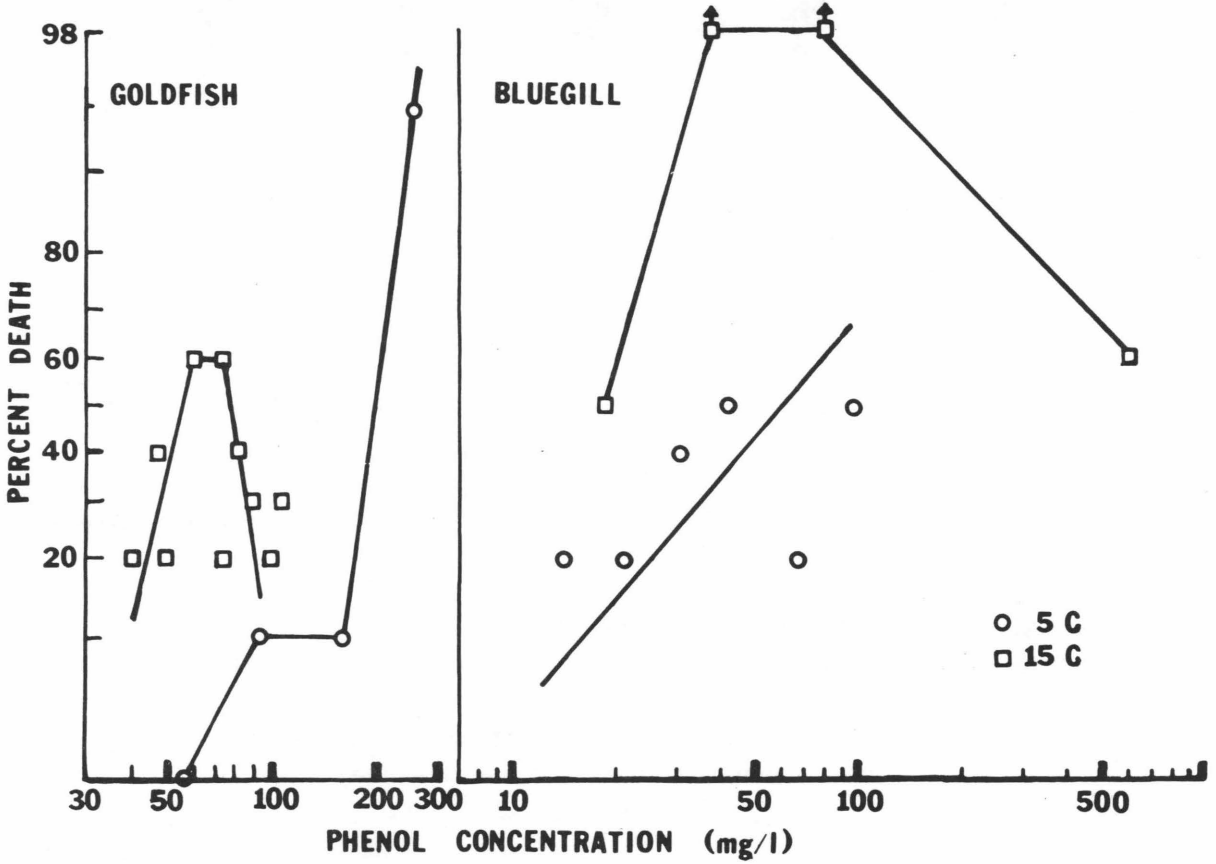


Figure 9
Effect of Temperature on Range of Chromate Concentrations
Estimated to Give from 1 to 99 Percent Kill at 24 Hours

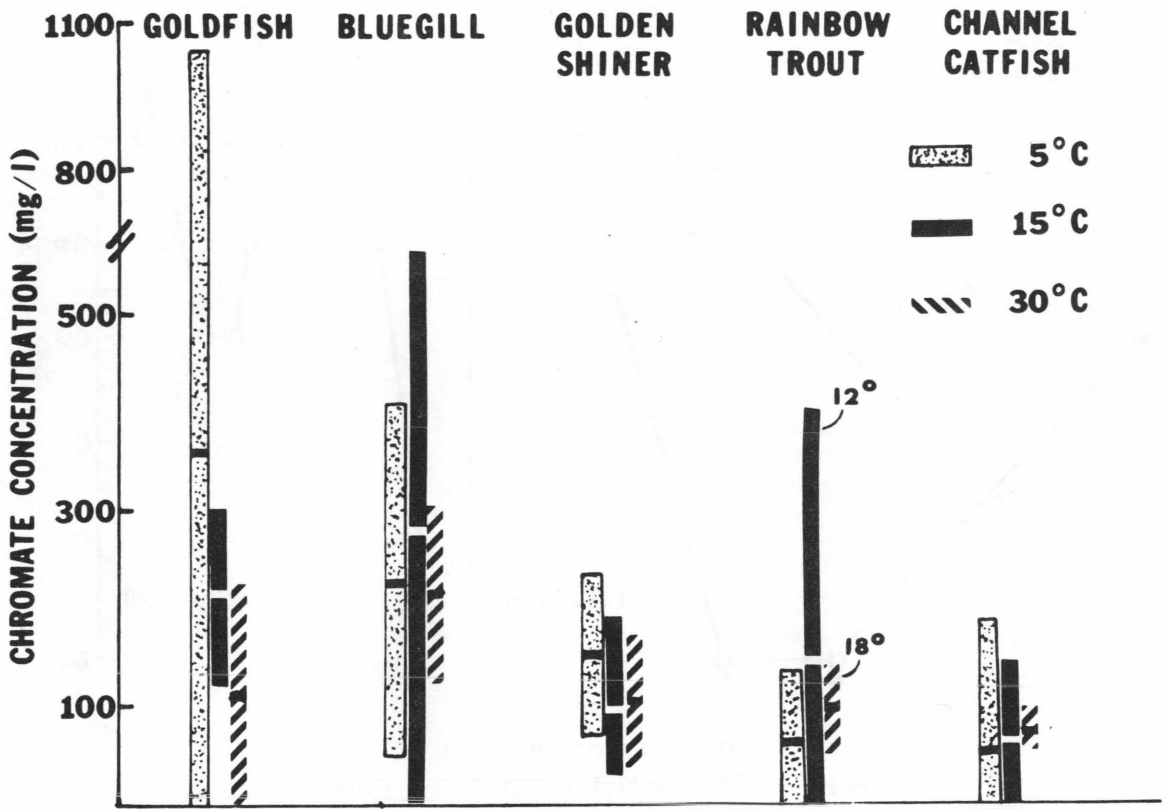


Figure 10
Effect of Temperature on Range of Cyanide Concentrations
Estimated to Give from 1 to 99 Percent Kill at 24 Hours

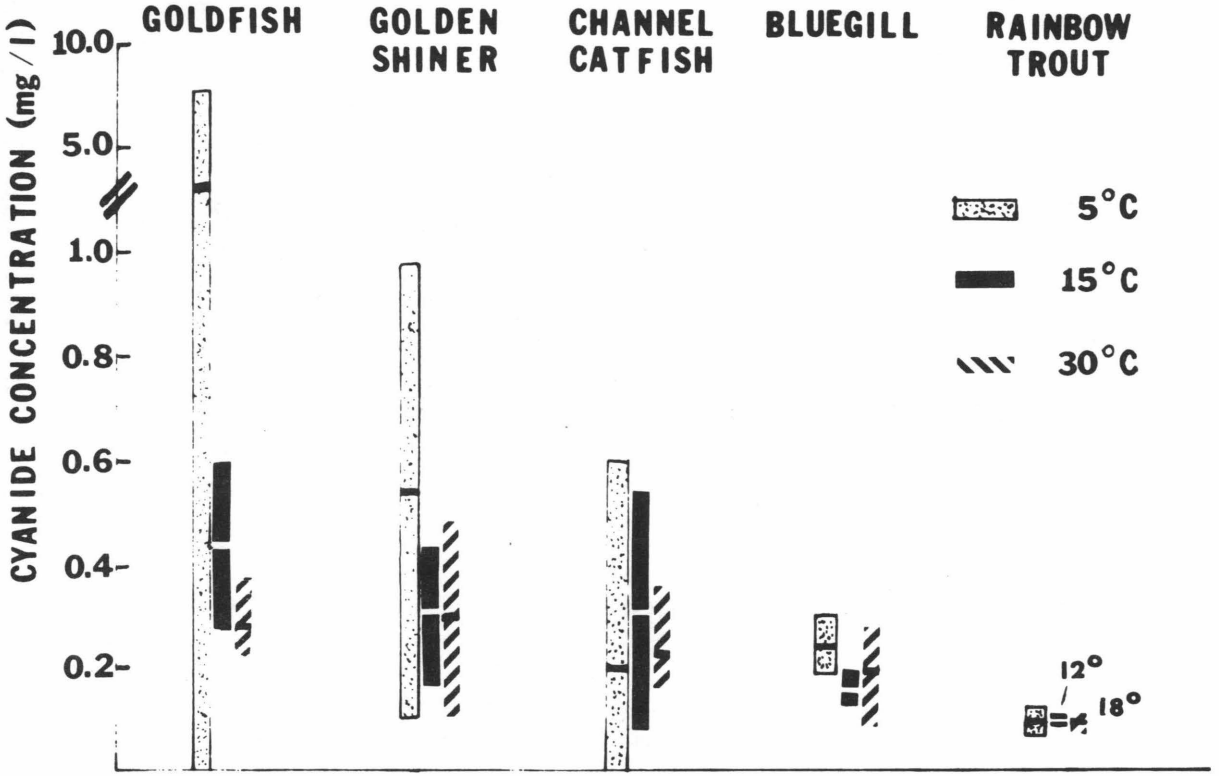


Figure 11
Effect of Temperature on Range of Copper Concentrations
Estimated to Give from 1 to 99 Percent Kill at 24 Hours

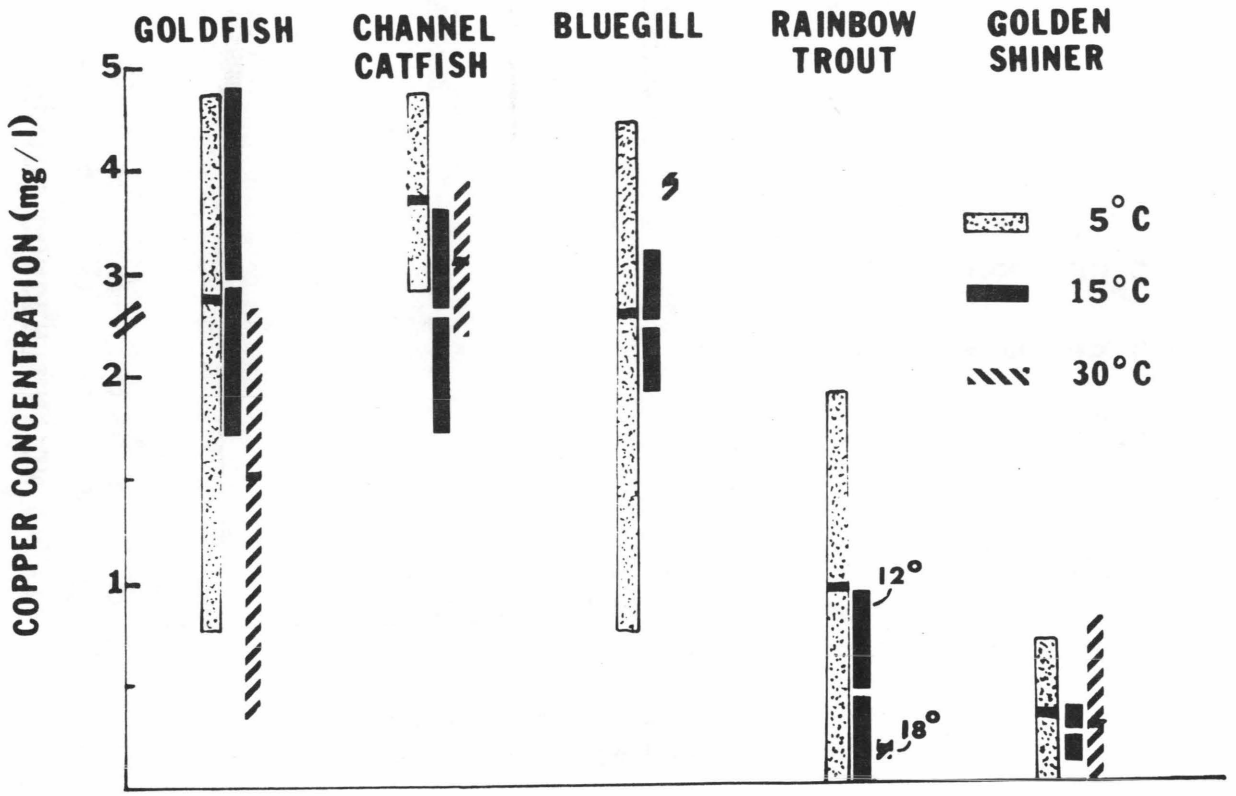


Figure 12

Effect of Temperature on Range of Zinc Concentrations Estimated to Give from 1 to 99 Percent Kill at 24 Hours

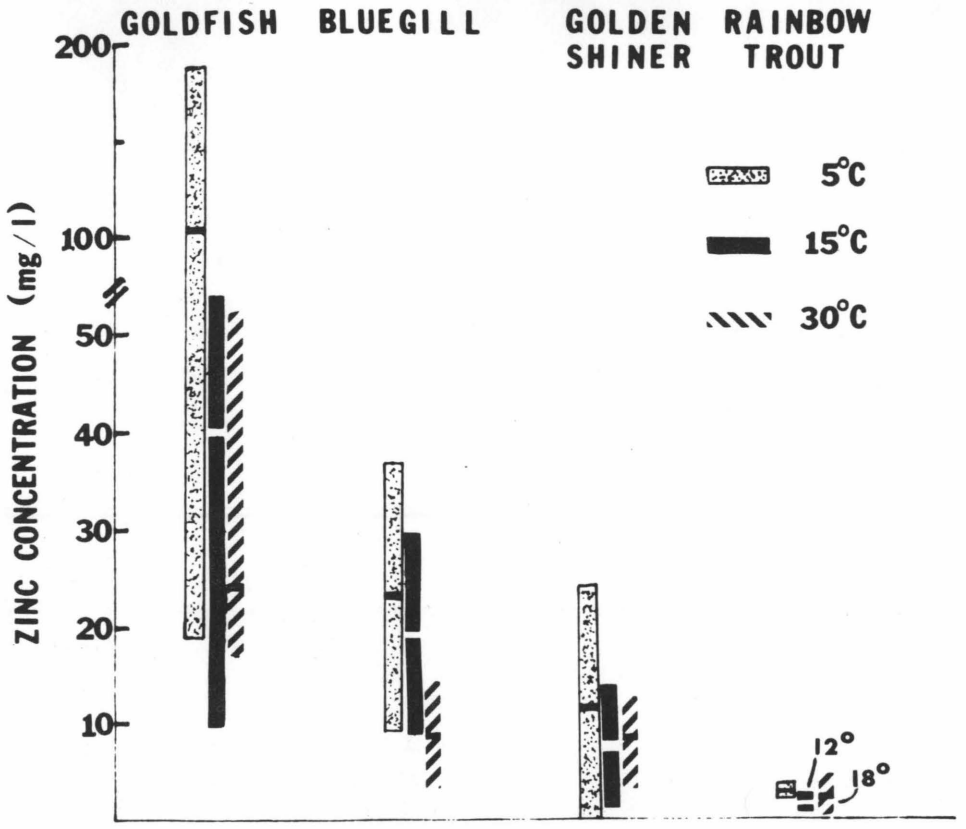


Figure 13
Effect of Temperature on Median Lethal Time at Various Concentrations of Cyanide

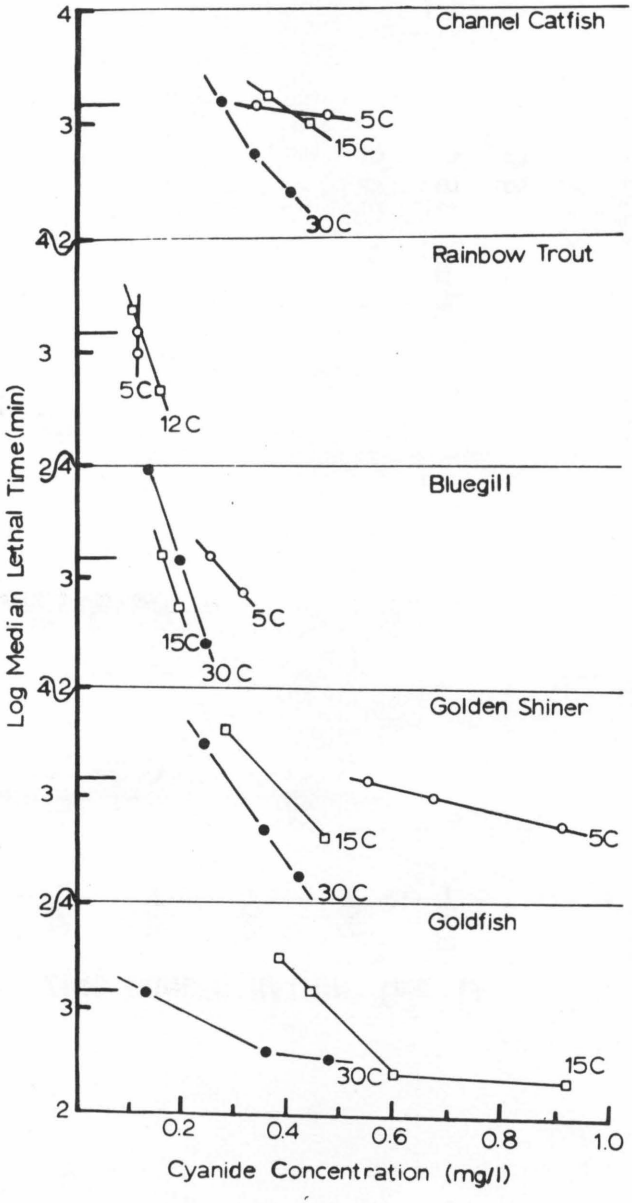


Figure 14
Effect of Temperature on Median Lethal Time at Various Concentrations of Chromate

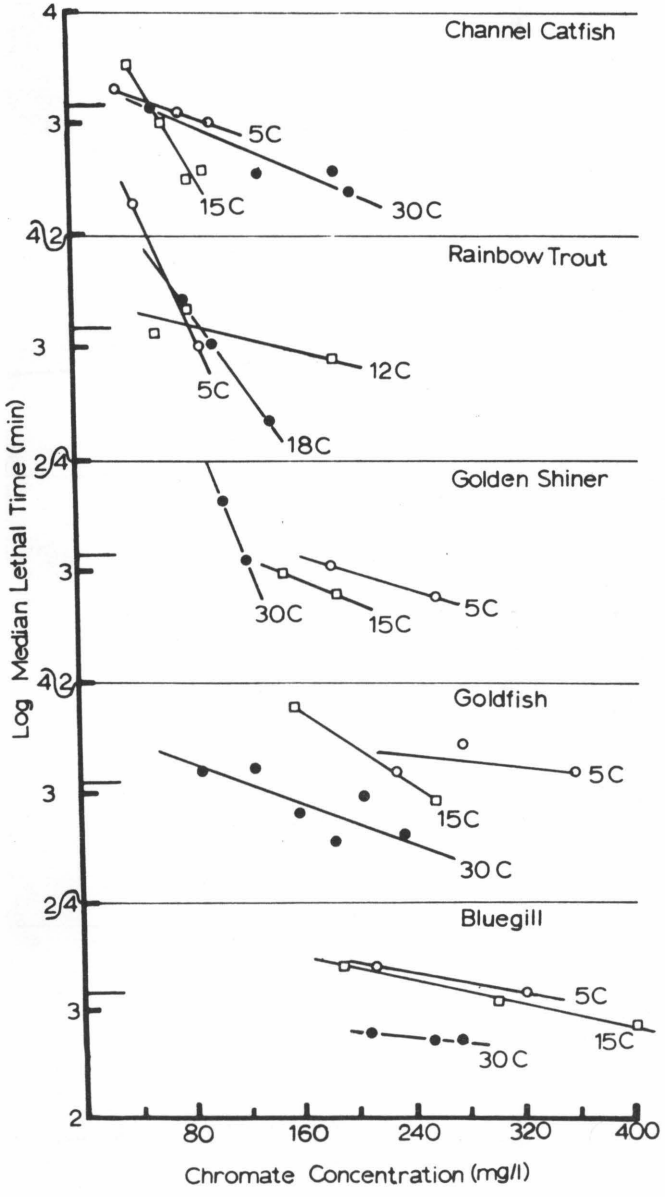
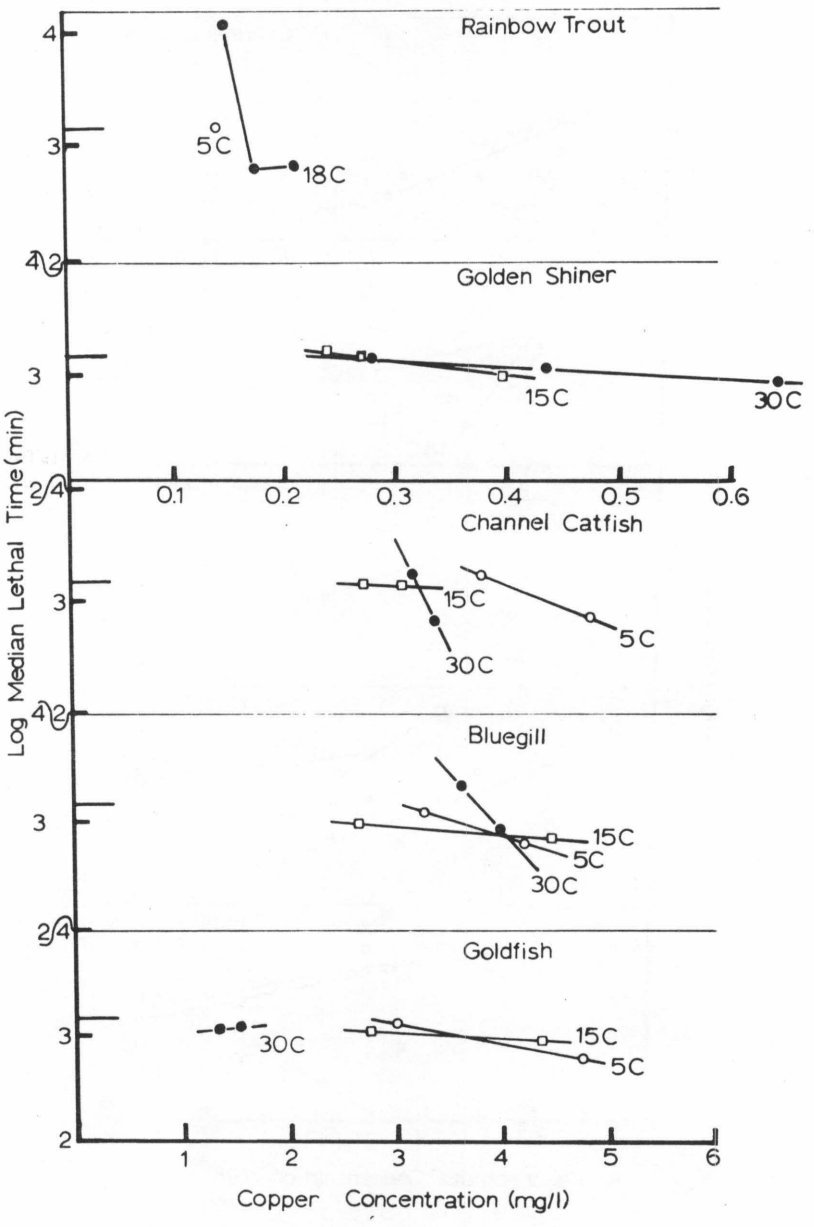


Figure 15
Effect of Temperature on Median Lethal Time at Various Concentrations of Copper



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