NUREG/CR-3476
UCRL-53486

Chemicals in Effluent Waters from Nuclear Power Stations: The Distribution, Fate, and Effects of Copper

F. L. Harrison

Prepared for
U.S. Nuclear Regulatory Commission

Lawrence
Livermore
National
Laboratory

NOTICE

Availability of Reference Materials Cited in NRC Publications

Most documents cited in NRC publications will be available from one of the following sources:

1. The NRC Public Document Room, 1717 H Street, N.W.
   Washington, DC 20555
2. The NRC/GPO Sales Program, U.S. Nuclear Regulatory Commission,
   Washington, DC 20555
3. The National Technical Information Service, Springfield, VA 22161

Although the listing that follows represents the majority of documents cited in NRC publications, it is not intended to be exhaustive.

Referenced documents available for inspection and copying for a fee from the NRC Public Document Room include NRC correspondence and internal NRC memoranda; NRC Office of Inspection and Enforcement bulletins, circulars, information notices, inspection and investigation notices; Licensee Event Reports; vendor reports and correspondence; Commission papers; and applicant and licensee documents and correspondence.

The following documents in the NUREG series are available for purchase from the NRC/GPO Sales Program: formal NRC staff and contractor reports, NRC-sponsored conference proceedings, and NRC booklets and brochures. Also available are Regulatory Guides, NRC regulations in the *Code of Federal Regulations*, and *Nuclear Regulatory Commission Issuances*.

Documents available from the National Technical Information Service include NUREG series reports and technical reports prepared by other federal agencies and reports prepared by the Atomic Energy Commission, forerunner agency to the Nuclear Regulatory Commission.

Documents available from public and special technical libraries include all open literature items, such as books, journal and periodical articles, and transactions. *Federal Register* notices, federal and state legislation, and congressional reports can usually be obtained from these libraries.

Documents such as theses, dissertations, foreign reports and translations, and non-NRC conference proceedings are available for purchase from the organization sponsoring the publication cited.

Single copies of NRC draft reports are available free, to the extent of supply, upon written request to the Division of Technical Information and Document Control, U.S. Nuclear Regulatory Commission, Washington, DC 20555.

Copies of industry codes and standards used in a substantive manner in the NRC regulatory process are maintained at the NRC Library, 7920 Norfolk Avenue, Bethesda, Maryland, and are available there for reference use by the public. Codes and standards are usually copyrighted and may be purchased from the originating organization or, if they are American National Standards, from the American National Standards Institute, 1430 Broadway, New York, NY 10018.

NUREG/CR-3476
UCRL-53486
RE

Chemicals in Effluent Waters from Nuclear Power Stations: The Distribution, Fate, and Effects of Copper

Manuscript Completed: September 1983
Date Published: April 1984

Prepared by
F. L. Harrison

Lawrence Livermore National Laboratory
7000 East Avenue
Livermore, CA 94550

Prepared for
Division of Health, Siting and Waste Management
Office of Nuclear Regulatory Research
U.S. Nuclear Regulatory Commission
Washington, D.C. 20555
NRC FIN A0119

ABSTRACT

This report provides a summary of research performed to determine the physicochemical forms and fate of copper in effluents from power stations adjacent to aquatic ecosystems with water that differs in salinity, pH, and concentrations of organic and inorganic constituents. In addition, research performed to evaluate responses of selected ecologically and economically important marine and freshwater organisms to increased concentrations of soluble copper is reviewed. The same parameters were measured and the same analytical techniques were used throughout the study.

Copper concentration and speciation, in influent and effluent waters collected from eight power stations using copper alloys in their cooling systems, showed that the quantities of copper associated with particles, colloids, and organic and inorganic ligands differed with the site, season, and mode of operation of the station. Under normal operating conditions, the differences between influent and effluent waters were generally small, and most of the copper was in bound (complexed) species except when low pH water was circulated. However, copper was high in concentration and present in labile species during start-up of water circulation through some cooling systems and during changeover from open-cycle to closed-cycle operation.

The toxic response to copper differed with the species and life stage of the organism and with the chemical form of copper in the water. Our primary emphasis was on acute effects and most of the testing was performed under controlled laboratory conditions. However, sublethal effects of copper on a population of bluegills living in a power station cooling lake containing water of low pH and on a population exposed to increased soluble copper in the laboratory were also assessed.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PREFACE

This study had three purposes: (1) to study the behavior of potentially toxic substances introduced to surface waters from nuclear power stations; (2) to determine the magnitude of the impact of these substances on representative, economically important species; and (3) to develop models to predict the behavior and the impact of these substances. The thrust of this research was to investigate the impact of corrosion products from cooling systems, in particular, copper. Copper is of special interest because it is toxic to aquatic organisms.

The author thanks the staff of the participating power companies for their excellent cooperation and advice and the members of their research teams for their assistance in the execution of this research project. Special thanks goes to Dr. Phillip Reed, the NRC manager for this project, for his encouragement and support.

The author acknowledges the continuing support received from the Ecological Research Division of the Office of Health and Environmental Research of the U.S. Department of Energy for research that complemented this study and facilitated its execution.

CHEMICALS IN EFFLUENT WATERS FROM NUCLEAR POWER STATIONS: DISTRIBUTION, FATE, AND EFFECTS OF COPPER

EXECUTIVE SUMMARY

Use of surface waters in the operation of nuclear power stations may degrade the quality of the ambient water. The objectives of this research project were: (1) to obtain data on the behavior of potentially toxic substances introduced into surface waters by nuclear power stations or produced by chemical reactions of substances released from nuclear power stations in the receiving water; (2) to determine the magnitude of the effects of these substances on representative, economically important aquatic species and (3) to develop models to predict their behavior and impact. These substances include corrosion products that are leached by water circulating in cooling systems and chemicals that are added to the coolant. Of special interest are corrosion products of copper because of their adverse effects on aquatic organisms.

The kinds and quantities of copper species present in influent and effluent waters were determined in samples collected from power stations located adjacent to marine, estuarine, and freshwater ecosystems. Copper concentration and speciation differed with the site, season, and mode of operation of the station. Under normal operating conditions, the differences between influent and effluent waters were generally small and, at those sites where measurements of chemical form were taken, most of the copper was in bound (complexed) species. An exception of this was found at H.B. Robinson Nuclear Power Station where water circulated through the condensers was low in pH (<6). Here, copper concentrations were higher than those from other stations, the differences between influent and effluent waters were greater, and larger amounts of labile copper were found.

During start-up of water circulation through cooling systems and during changeover from open-cycle to closed-cycle operation, however, copper was high in concentration. The size, chemical form, and duration of the copper pulse released during start-up and changeover differed with factors such as the interval between shutdown and start-up, preconditioning of the condenser tubing, and hydrodynamic conditions.

Copper sensitivity of a group of ecologically and economically important aquatic organisms was evaluated. Our primary emphasis was on acute effects, and most of the testing was performed under controlled laboratory conditions and in flowthrough bioassay systems. The toxic response to copper differed with the species and life stage of the animal and with the chemical form of copper in the water. In general, early life history stages were more sensitive than adults. Some primary producers and early life stages of higher trophic organisms were found to be very sensitive to low levels of labile copper.

Sublethal effects of copper on bluegills were assessed in populations living in the intake and discharge zones of the H.B. Robinson Nuclear Power Station. These populations showed structural abnormalities and reduced reproductive capacity. Tissue analyses showed elevated copper concentrations in the livers. Liver metalloproteins were separated and quantified. The results indicated that the metallothionein detoxification system in the bluegills was saturated and copper was present in some metalloprotein pools in levels that may impair metabolic functions.

Experiments were conducted in the laboratory to determine the concentrations of copper in effluents and the durations of exposure that bluegills can tolerate without adverse sublethal effects. Bluegills exposed to increased concentrations of labile copper showed increases of copper in the fractions of the soluble proteins in their liver that contain metallothionein and metalloenzymes. These increases were related to exposure time and concentration.

INTRODUCTION

Pollutants released into aquatic ecosystems as byproducts of nuclear power production vary with the type of cooling system used and with the chemicals added to the water to control specific maintenance problems. Those substances from cooling systems include metals such as Cu, Ni, Al, Zn, and Ti in condenser tubing; those from additives include chlorine, chromate, ammonia, dispersants, and detergents. Of these pollutants, copper is of special interest because of its adverse effect on aquatic organisms. Copper was implicated in the abalone kill that occurred in the discharge cove of the Pacific Gas and Electric nuclear power station at Diablo Canyon before the Cu-Ni tubing in the cooling system was replaced by Ti tubing. Copper was also responsible for the reduced commercial value of oysters harvested at Chalk Point, the site of the fossil-fueled power station operated by Baltimore Gas and Electric.

An important factor affecting the distribution and final fate of copper released into aquatic environments is its physicochemical form. When the metal is discharged, it is partitioned among the soluble and particulate fractions of the water and the bedload sediments (Fig. 1). Discharged soluble copper may be sorbed onto either particulate material that is suspended in the water column or particles in the surface layer of the bedload sediments. Discharged particulate copper may remain in suspension or may be deposited in the bedload sediments. The form in the soluble fraction is related to the inorganic and organic constituents in the water; the form in the particulate fraction is related to the kinds of chemical sites present on the particles.

Physicochemical form also affects the toxicity of copper. Considerable information is available on the response of aquatic organisms to copper (Refs. 1-7). Environmental factors known to alter the physicochemical form of copper and, in turn, its toxicity include pH, the presence of inorganic carbon and phosphorus, exchange reactions between suspended sediments and water, and the presence of other metals or toxicants.

Numerous attempts have been made in the past to quantify the physicochemical forms of copper in water systems (Refs. 7-11). Field measurements have been made using a variety of techniques. The validity of some measurements is in question because of the sampling and analytical methods used. Furthermore, comparison of results from different sites is difficult because the techniques used by different investigators are rarely the same.

This study was initiated (1) to determine the physicochemical forms and effects of copper in effluents from power stations adjacent to ecosystems with water systems that differed in salinity, pH, and concentration of organic and inorganic constituents and (2) to evaluate the responses of a selected group of ecologically and economically important marine and freshwater organisms to increased concentrations of soluble copper. The same parameters were measured and the same analytical techniques were used throughout the study.

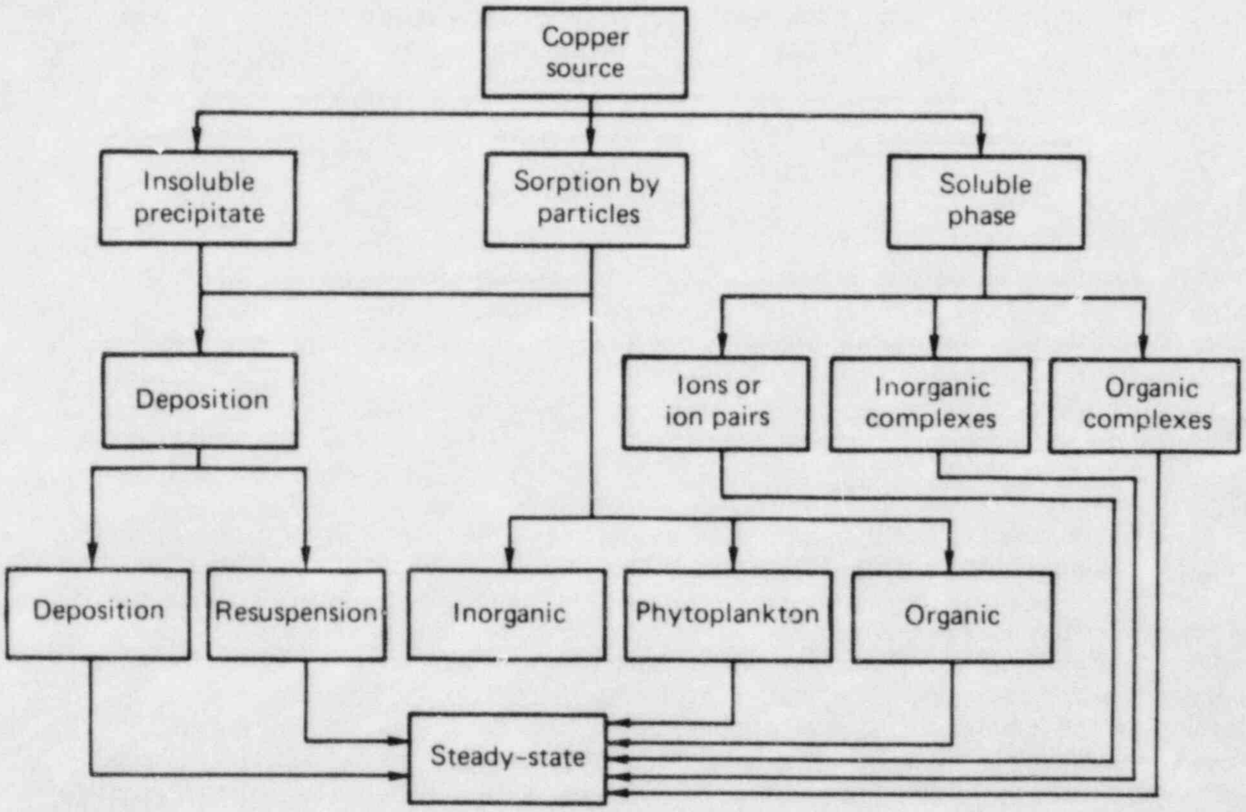

Figure 1. Hypothetical model of the partitioning of copper among compartments in aquatic ecosystems.

To accomplish our first objective, sampling programs were conducted in the intake and discharge zones of eight different nuclear power stations situated in widely different geographical locations (Table 1). At each station we made a series of collections and measured a number of parameters in the water, suspended particles, and bedload sediments using state-of-the-art methods (Table 2). Detailed information on the sites, collection methods, and analytical techniques is available in a series of reports (Refs. 12-16).

To accomplish our second objective, toxicity studies were conducted on both marine and freshwater organisms. Responses to acute levels were defined by exposing organisms to a range of concentrations from those producing immediate mortality to those producing no visible effect. We exposed early life and adult stages of organisms to copper in temperature-controlled, flowthrough bioassay systems and characterized the physicochemical form of the copper in each test system. Responses to sublethal levels were evaluated by exposing the organisms to low levels of copper and quantifying the changes in detoxification systems, in morphology, and in growth and development. Results from our investigations are available in a series of reports (Refs. 12, 17-24). Information from our investigations provides the kinds of data needed to understand the behavior of copper in ecosystems and the potential toxic effects on organisms of copper released from cooling systems and from other urban and industrial sources.

EXPERIMENTAL PROCEDURES

COPPER ANALYSES

Water samples were collected through an all-plastic system that had been acid-washed and rinsed with double-distilled water, then rinsed again with double-distilled water immediately before use. Water samples were filtered *in situ* through a 0.4-µm (pore size), Nuclepore 293-mm (diam) membrane filter and collected into specially cleaned containers. The water was analyzed for total, labile, and bound copper, the particles for dry and wet weights and for total copper content.

Total soluble copper in freshwater and estuarine samples was determined by direct injection of acidified samples into a HGA 2100 model graphite furnace attached to a model 303 Perkin Elmer Atomic Absorption Spectrophotometer (AAS); marine samples were preconcentrated by solvent extraction of organic chelates (Ref. 25) before analysis on the AAS. Labile copper was measured by ion exchange and polarographic analysis. The Riley and Taylor method (Ref. 26) was used to determine the Chelex-100-labile copper; the Florence and Batley method (Ref. 27) was used to determine the anodic stripping voltametry (ASV)-labile copper. Particulate copper was determined on the material collected on the Nuclepore filters after wet and dry weights were determined. The samples were ashed for 48 hours at 450°C, digested with acid, and then diluted to volume in preparation for analysis on the AAS.

DISTRIBUTION COEFFICIENTS

The copper distribution coefficient [illegible]$_d$) of particles was determined for each of several samples of unfiltered water by radiolabel techniques. Triplicate 1-L bottles of water were spiked with ^{64}Cu, placed in a water bath at 12 to 14°C, and then shaken continuously for 24 hours before filtration. The entire sample of water was filtered through a 0.45-µm (pore size) Millipore filter. After filtration, the bottle and filtration apparatus were rinsed with 25 mL of 0.1 N HCl to remove sorbed

Table 1. Location of power stations in the survey, the source of the cooling water used, the total number of field collections made, and the year(s) in which the study was conducted.

Power station	Nearest major city	Cooling water	Collections Number	Collections Years
Marine Ecosystems				
Diablo Canyon	San Luis Obispo, CA	Pacific Ocean	3	1977-78
San Onofre	San Clemente, CA	Pacific Ocean	4	1977-78
Estuarine Ecosystems				
Salem	Salem, NJ	Delaware River	4	1978-80
Surry	Williamsburg, VA	James River	3	1978-80
Freshwater Ecosystems				
Fort St. Vrain	Denver, CO	Well water and St. Vrain creek	3	1978
H.B. Robinson	Florence, SC	Pee Dee River	3	1978-80
Kewaunee	Manitowoc, MI	Lake Michigan	3	1978
Vermont Yankee	Brattleboro, VT	Connecticut River	5	1978-79

Table 2. Parameters measured in field studies conducted at power stations.

Compartment	Parameter	Instrument
Water[a]	Conductivity	Conductivity meter
	Temperature	Thermometer
	pH	pH meter
	Total dissolved copper	Atomic absorption spectrometer
	Labile vs bound copper	Polarograph Ion-exchange resin (Chelex 100)
	Dissolved organic carbon	Carbon analyzer
	Complexing capacity	Polarograph
Suspended particles	Total, nonfilterable residue copper	Atomic absorption spectrometry
	Total, nonfilterable residue	Filtration apparatus
	Copper distribution coefficient	Gamma-well radionuclide counter
	Particulate organic carbon	Carbon analyzer

[a] Water analyzed was filtered through a 0.4 µm Nuclepore filter.

^{64}Cu. Activities of ^{64}Cu were determined in the unfiltered water, in duplicate samples of the filtrate, in the material on the filter, and in the acid wash. Recovery of the added radionuclide was generally >95%.

Each K_d was calculated from the data and the following relationship:

$$K_d = \frac{fs}{(1 - fs)} \; \frac{V}{W} \; , \qquad (1)$$

where fs = fraction of ^{64}Cu on filter,
(1 - fs) = fraction of ^{64}Cu in filtrate,
V = weight of water (g),
W = dry weight of particles (g).

The K_d as defined is dimensionless, and greater sorption of ^{64}Cu to the particulate fraction results in a higher K_d.

MOLECULAR-WEIGHT FRACTIONATION

Two 2-L aliquots of filtered water that had been stored at 4°C since collection were spiked with ^{64}Cu, allowed to equilibrate a minimum of 12 hours, and then subjected to ultrafiltration (Fig. 2). One of the 2-L aliquots was photo-oxidized with UV light to reduce the concentration of dissolved organic matter. Two drops of 35% H_2O_2 were added to each 100 mL of water to accelerate the oxidation. The reaction rate was also increased by the increase in temperature that occurs during irradiation (60°C). Ultrafiltration was performed with standard Model 402 cells, which contained magnetic stirrers (Amicon Corporation, Lexington, Mass.). The nominal molecular weight retentions of the membranes used in this test are as follows: XM-100A, 100,000; UM-10, 10,000; and UM-2, 1,000.

Each ultrafiltration experiment was initiated by transferring 270 mL of the ^{64}Cu-spiked water to an ultrafiltration cell. The water was stirred for 3 minutes, three 10-mL aliquots were removed for counting, the remaining 240 mL were subjected to positive N_2 pressure and constant stirring until 120 mL of filtrate was collected, and then the cell was vented. The filtrate was sampled serially to monitor the changes in ^{64}Cu concentration; a 10-mL sample was taken from each of the first, second, and third 40-mL aliquots of the filtrate. The retentate was sampled only after venting. The filters were removed and placed in vials for counting. The cell assembly was rinsed well with 50 mL of 0.1 N HCl, and a 10-mL aliquot of this acid wash was counted. Recovery of the ^{64}Cu present in the initial test solution was calculated from the ^{64}Cu concentrations in the different fractions.

APPARENT COMPLEXING CAPACITY

The complexing capacity was determined using the method of Ref. 28. The 10-mL samples of filtered water were aliquoted into cups and then spiked with quantities of copper that would result in a range in concentrations of added copper. The spiked samples were equilibrated 15 to 18 hours at 10°C, and then an analysis of labile copper was performed on each sample. The area of the copper peak on each voltamogram was determined and plotted on the Y-axis versus concentration of added Cu^{2+}. The complexing capacity of the sample corresponds to the labile copper concentration intercepted on the abscissa.

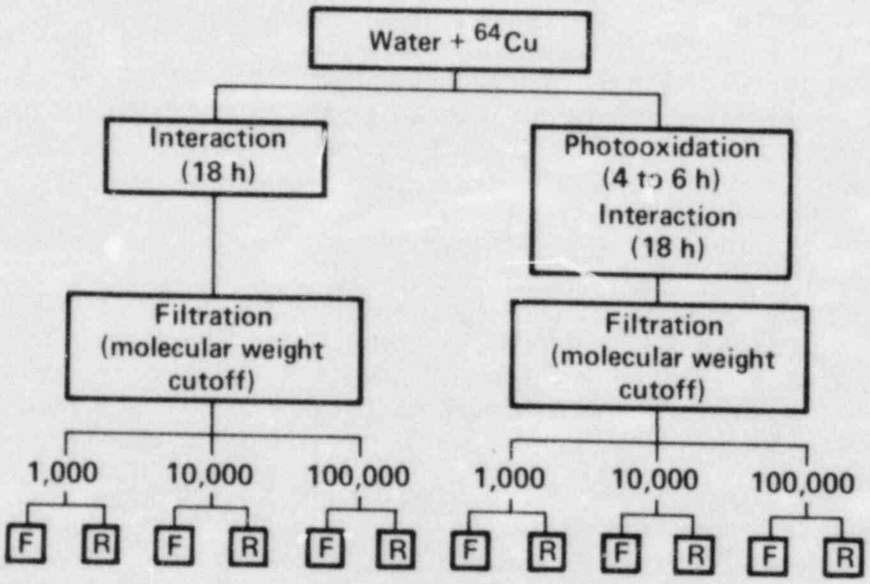

Figure 2. Ultrafiltration-procedure flow diagram. The filtrate (F) and retentate (R) were analyzed for ^{64}Cu.

ORGANIC CARBON ANALYSIS

Analyses for organic carbon were performed on the soluble and particulate fractions of water filtered through glass equipment that had been heated to 450°C to destroy organic material. The filter and filtrate were analyzed for particulate and soluble organic carbon, respectively, with an Oceanography International Carbon Analyzer by the standard persulfate oxidation method (Ref. 29).

BIOASSAYS

Each species was exposed to concentrations of copper that ranged from those producing no apparent effect to those producing an immediate effect. Most bioassays were conducted in flowthrough systems. Mortalities were generally recorded daily for adult stages, every 4 to 6 hours for early life stages. From the family of curves derived from cumulative mortality versus duration of exposure, lethal concentrations (LC_{50}/time) were determined. The concentration of Chelex-100-labile copper in the water was determined in each test system.

METALLOPROTEIN ISOLATION

Livers from bluegills were placed in three volumes per weight of nitrogen-saturated, 50 m<u>M</u> Tris-HCl (pH 7.6) containing 10% sucrose, 1% 2-mercaptoethanol, and 200 K.I.U. of Trasylol and then homogenized on ice with a polytron. Duplicate aliquots of each homogenate were reserved for metal analysis.

Homogenates were centrifuged at 115,000 gravities for 90 minutes at 0°C. The final clear supernatants (S-115s) were saturated with N_2(g), frozen immediately with liquid nitrogen, and stored at -70°C until they were chromatographed. Duplicate aliquots of each S-115 and the pellets were reserved for metal analysis.

The S-115s of the livers were processed on a Waters high performance liquid chromatograph (HPLC) fitted with a Varian TSK 3000 SW gel permeation column (22 x 300 mm). To separate copper metalloproteins, aliquots of the S-115s were injected and eluted with a 0.15 M NaCl and 50 mM Tris-HCl buffer solution (pH 7.6 at 25°C) at a flow rate of 4 mL/min. Molecular absorbance at 280 nm was monitored continuously and the 35 fractions collected were analyzed for metals. The column was standardized using proteins of known molecular weights.

COPPER PARTITIONING IN ABIOTIC COMPARTMENTS

WATER SAMPLES

Total Copper Concentration

Total copper in influent and effluent waters of power stations during normal operations differed greatly (Table 3). Some of the differences between influent and effluent waters are related to the quantities of water circulated through the condensers, the inorganic and organic constituents in the water, and the age and kind of alloy used in the condenser tubing. In this study, the number of sample collections was limited; consequently, the data are not meant to characterize each site but to indicate the variation that can be expected between different types of ecosystems and between different sampling times in a given type of ecosystem.

Total copper concentrations in effluents following start-up of water circulating through the cooling system and changeover from open-cycle to closed-cycle operation were much larger than those during normal operation (Table 3). The greatest differences between intake and discharge waters were found during start-up of water through the condenser tubing after an enforced shutdown (Refs. 13,30). At the Diablo Canyon Nuclear Power Station, the highest concentration reported at the time of start-up in 1975 was 7700 µg Cu/L; concentrations after 10 minutes and 1440 minutes (24 hours) of continuous operation fell to 900 and 67 µg Cu/L, respectively. This pulse was released during the testing of the cooling system; Diablo Canyon Station has never generated electricity. Warrick et al. (Ref. 30) also reported that, in general, the longer the period between shutdown and start-up, the greater the amount of corrosion products that were formed on the Cu-Ni alloy surface. The large volume of loose corrosion products found in the condenser tubings consisted of copper trihydroxlchloride [$CuCl_2 \cdot 3\, Cu(OH)_2$].

Increased concentrations in the discharge waters at Diablo Canyon were seen immediately after water began to circulate on October 11, 1977 (Ref. 12). Although titanium replaced the copper-nickel tubing in the main system at Diablo Canyon, part of the auxiliary pumping system still contained copper. Our first sample, taken about 5 minutes after the water began to flow, contained 28 µg Cu/L. Copper levels dropped rapidly; after 3.5 hours little difference could be seen between the intake and discharge waters.

The first water discharged after a start-up at Salem Unit II contained large quantities of particles that were high in copper; copper concentration in the particles was 170 µg/g dry weight compared to 6.6 in those in the influent water (Ref. 13). Copper in the soluble fraction of the water was only 0.6 µg/L. This was lower than the 0.9 measured before start-up in the water from the intake zone.

Pulses of copper were released during changeover at Vermont Yankee Nuclear Power Station. The mode of operation of the station is changed from open-cycle to closed-cycle before the onset of the warm summer months. Two mechanical draft-type towers are put into operation. The wood in both towers has been treated with Cupranol, a copper-containing preservative.

Young et al. (Ref. 31) reported that the total copper contributed from power station cooling systems into the Southern California Bight is 2.1 metric tons per year. Although this is a considerable amount, it is smaller than they reported from other sources. They estimated the annual input of copper in metric tons per year into the Southern California Bight from known sources to be the following: municipal waste, 507; antifouling paints, 180; storm runoff, 42; and dry fallout, 31.

Soluble and Particulate Copper

We found large differences in the quantities of copper in the soluble (<0.4-µm) and particulate (>0.4-µm) fractions of effluent from power stations (Table 3). Because soluble copper discharged into waters from a point source may not remain in solution but be sorbed onto particles, an increase of copper in the particulate fraction may not indicate a release of particulate copper but only an increase in the amount of copper sorbed to particles already present in the water.

Table 3. Ranges in concentration of soluble (<0.4 µm) and particulate (>0.4 µm) copper, maximum total copper, and maximum difference in copper (ΔCu) between influent and effluent waters. Copper concentrations are in µg/L.

Power station	Fraction		Maximum total copper	Maximum ΔCu
	Soluble	Particulate		
Marine Ecosystems				
Diablo Canyon	0.5 - 1.9	<0.1 - 1.3	3.2	--
Diablo Canyon[a]	26	1.9	28	27
San Onofre	0.2 - 1.8	<0.1 - 1.5	3.3	∿2
Estuarine Ecosystems				
Salem	2.3 - 5.0	2.9 - 5.6	11	2.1
Salem[a]	0.6	>2500	>2500	>2500
Surry	2.0 - 3.7	4.9 - 21	25	4.2
Freshwater Ecosystems				
Fort St. Vrain	3.3 - 5.2	7.8 - 26	31	20
H.B. Robinson	18 - 61	13 - 19	79	22
Kewaunee	3.1 - 4.1	0.7 - 15	18	10
Vermont Yankee	3.2 - 4.0	0.8 - 5.0	8.4	5.1
Vermont Yankee[b]	24	25	49	41

[a] Concentration following start-up of water circulating through the condenser system after a shutdown.

[b] Concentration following changeover from open-cycle to closed-cycle operation.

The properties of particles suspended in the water column depend in part on their mineral composition and on the seasonal changes in biological productivity in the waters. Binding sites on the particles are generally heterogeneous, and different types of copper associations with the particles may ensue. Copper may be sorbed at metal oxide surfaces, exchange with ions within clay minerals, bind to organic matter, or complex with a ligand sorbed at a binding site. Because of the heterogeneity of the sites and the complexity of the interactions occurring, the chemical reactions taking place are difficult to define. Consequently, we performed empirical measurements of distribution coefficients and copper concentrations to provide useful information for determining the fate and distribution of the released copper.

Particles suspended in the water column differed in copper content, weight, organic carbon content, and affinity for copper as measured by their distribution coefficients (Table 4). Increases in copper concentration in the particulate fraction of the water may result from increases in number of particles of a given concentration, or increases in the amount of copper associated with each particle.

The amount of particulate material in suspension in the water differed greatly with the ecosystem (Table 4). The amounts of nonfilteraterable residues were in water collected during a period of high runoff from land at Salem and Surry. High amounts probably

Table 4. Maximum values of parameters measured in particles collected in effluent from different power stations.

Power station	Copper, µg/g	Nonfilterable residue, mg dry wt/L	Particulate organic carbon, mg/L	Distribution coefficients
Marine Ecosystems				
Diablo Canyon	290	11	0.5	48,000
San Onofre	70	54	0.6	45,000
Estuarine Ecosystems				
Salem	160	120	2.8	17,000
Surry	470	84	2.1	16,000
Freshwater Ecosystems				
Fort St. Vrain	700	51	1.9	36,000
H.B. Robinson	2,600	12	1.1	25,000
Kewaunee	3,600	8	1.1	46,000
Vermont Yankee	1,300	12	0.5	61,000

result in increased quantities of the copper that are available to filter feeders and deposited in the vicinity of the power station. The latter result would depend on the rate of water flow in the ecosystem and the size and density of the particles in the water column. A measure of the copper affinity to particles that are natural to the waters was obtained from the distribution coefficient (K_d). Average values were high at San Onofre, Diablo Canyon, Vermont Yankee, and Kewaunee (Table 4). The lower values obtained at Fort St. Vrain, Salem, and Surry may be related to the high complexing capacities of these waters.

An important factor controlling the copper concentration in particulate material is uptake by planktonic organisms. The process is most evident during the time of plankton blooms (Ref. 32). Knauer and Martin (Ref. 33) studied the uptake of copper and other metals by plankton in Monterey Bay, Calif., and observed seasonal changes in the metal content of the plankton. They attributed these changes to accumulation of these metals during seasons of low productivity when the availability of the metal was high.

Young _et_ _al_. (Ref. 31) measured the concentrations of soluble and particulate copper in influent and effluent waters from eight power stations in the Southern California Bight that have copper-nickel alloys in their cooling systems. They reported a mean value of total copper of 1.12 µg/L for the influent waters and an increment of 0.31 µg/L for the effluent over the influent water. These mean values were derived from all observations on all eight stations. The particulate copper species were approximately 29% of the total copper in both the influent and effluent waters. The concentrations they reported for the particulate fraction may be lower than those reported by others because they leached the particles collected on the filters with acid rather than put them totally into solution.

Copper Speciation

Species of copper have been classified as labile or bound (Ref. 34) and as very labile, moderately labile, slowly labile, and nonlabile (Ref. 35). The groups are defined by the experimental conditions under which the measurement is made. Techniques used to differentiate between copper species include solvent extraction (Refs. 25, 36-38), ion exchange (Refs. 12-15, 26, 39,), electrochemistry (Refs. 13-15, 20, 27, 28, 40-44,), ultrafiltration (Refs. 12-15, 45, 46), gel filtration chromatography (Ref. 44), and ion specific electrodes (Refs. 47-49). For convenience, we will use the terms labile and bound as proposed by Florence and Batley (Ref. 39) even though they are imprecise. We refer to the following as labile species: ions, ion pairs, readily dissociable (labile) inorganic and organic complexes, and easily exchangeable copper sorbed on either colloidal inorganic or organic matter. Inorganic anions to which the copper may be complexed are hydroxides, carbonates, chlorides, sulfates, phosphates, and nitrates; organic anions are amino acids, amino sugars, alcohols, urea, etc. We shall refer to those forms that are irreversible or slowly reversible as bound species: stable copper-organic complexes, copper bound to high-molecular-weight organic material, some inorganic complexes, and copper occluded in, or sorbed tightly on, highly dispersed colloids. Included in this group of bound species is a large fraction of copper complexed to humic substances (refractory organic material that makes up a large fraction of organic material in natural waters).

Large differences were found in the quantities of labile and bound copper in effluent waters at different power stations (Table 5). Quantities were found to differ with season as well as with the type of ecosystem. Concentrations of labile and bound copper were measured in 1977 during start-up at Diablo Canyon (Ref. 12), in 1979 during changeover from open-cycle to closed-cycle operation at Vermont Yankee (Ref. 14), and in 1980 during start-up of Unit II at Salem (Ref. 13). Following start-up at Diablo Canyon and changeover at Vermont Yankee, the pulse of copper discharged was primarily in solution and in Chelex-100 (ion exchange resin) labile forms. We can expect, then, that the labile copper released would remain as such until it is diluted with sufficient volume of water to provide the ligands required to complex the copper.

The results obtained at Diablo Canyon and Vermont Yankee were in contrast to those obtained at Salem. During start-up at Salem, the pulse of copper was primarily in the form of particles, and only small amounts of labile copper were released. At Salem the condenser tubes were pretreated with a commercially available product, CI-50. Additional studies of copper speciation in effluents during start-up are required to confirm the usefulness of pretreatment with chemicals.

Discussions of chemical forms of copper in the soluble fraction of waters are included in some recent reviews of copper (Ref. 7, 10, 11, 50). It is well accepted that the chemical form of copper is important in controlling geochemical and biological processes. Consequently, attempts have been made both to calculate and to measure copper speciation.

Concentrations of copper species have been calculated from equilibrium chemical models. These models are used to predict the way in which specified amounts of metals and ligands are partitioned by competing reactions in the solution and solid phases. Nordstrom et al. (Ref. 51) compared the 12+ available computerized models for equilibrium calculations in aqueous systems. They evaluated consistency among programs by comparing the log of the molar concentrations of free ions and

Table 5. Ranges in concentration of labile and bound species of copper (μg/L) in effluents from power stations.[a]

Labile	Bound	Power station	References
Marine Ecosystems			
0.1-0.4	0.4-1.8	Diablo Canyon, CA	12
0.4-21.2[b]	0.2-6.5	"	"
0.2-0.7	0.1-1.6	San Onofre, CA	"
Estuarine Ecosystems			
0.9-4.2	<0.2-3.2	Salem, NJ	13
0.5-3.3[b]	--[c]	"	"
0.5-2.9	0.2-2.6	Surry, VA	"
Freshwater Ecosystems			
0.4-0.7	2.9-4.5	Fort St. Vrain, CO	14
13-34	4.9-26.8	H. B. Robinson, SC	15
1.7-2.3	1.4-1.7	Kewaunee, WI	14
1-2.1	1.2-2.4	Vermont Yankee, VT	"
130[d]	21[d]	"	"

[a] Chelex-100 ion exchange resin was used to differentiate between labile and bound species. At a minimum, three collections were made, and triplicate samples were analyzed.

[b] Values obtained in water collected after start-up of water circulation through the cooling system.

[c] No data available.

[d] Value obtained in water collected after changeover from open-cycle to closed-cycle operation.

complexes for two test solutions: a hypothetical seawater and a hypothetical river water. They proposed that the lack of agreement for minor species reflects primarily differences in the thermodynamic data base of each chemical model, although other factors such as activity coefficient calculations, redox assumptions, temperature corrections, alkalinity corrections, and the number of complexes used also may affect the results. Many of the models do not include interactions with organic ligands. Others suffer from the omission of polynuclear and mixed-ligand complexes (Ref. 9). When more experiments are performed and the data generated are incorporated into the models, we can expect the predictive capabilities of the models to improve.

Molecular-Weight Fractionation

A better understanding of the kinds of speciation reactions occurring can be obtained if information is available on the quantities of soluble copper in different molecular-weight fractions (MWFs). The distribution of copper among MWFs differed with the ecosystem (Table 6). The average percentage of soluble copper that was in the four different MWFs in untreated water from San Onofre was about

Table 6. Average percentages of ^{64}Cu in different molecular-weight fractions of ultrafiltered effluent waters collected at power stations.

Power station	UV treatment (h)	Molecular-weight fractions			
		<1,000	>1,000 <10,000	>10,000 <100,000	>100,000
Marine Ecosystems					
San Onofre	0	28	24	25	22
	4	48	17	12	23
Diablo Canyon	0	15	21	41	23
	4	37	14	12	37
Estuarine Ecosystems					
Surry	0	10	20	55	15
	6	20	0	13	67
Salem	0	10	38	40	12
	6	25	0	0	75
Freshwater Ecosystems					
Vermont Yankee	0	2	3	75	20
	4	3	0	2	95
Kewaunee	0	4	10	58	28
	4	14	1	22	63
Fort St. Vrain	0	5	32	55	8
	4	9	7	6	78
H.B. Robinson	0	12	22	55	11
	6	29	1	66	4

the same. Photo-oxidation of the water resulted in an increased percentage in the <1000 MWF containing the labile forms which are considered to be more toxic than bound forms. In untreated water from Diablo Canyon, the largest percentage of copper was in the >10,000 < 100,000 MWF; photo-oxidation of the water resulted in increases in both the <1000 and the >100,000 MWFs. The increased percentage of copper in the >100,000 MWF is probably the result of the formation of colloidal copper or of the adsorption of copper to other metal colloids.

The pattern of copper distribution among MWFs in treated and untreated water was similar for effluents from Salem and Surry. The percentage of copper in the <1000 MWF was smaller in these samples than in those from marine ecosystems and larger than in those from freshwater ecosystems. An important factor controlling speciation in estuarine waters may be the formation of chloro complexes; samples with increased salinity had increased percentages of copper in the <1000 MWF.

In untreated waters from Fort St. Vrain, Kewaunee, and Vermont Yankee, the lowest percentage of copper was found in the <1000 MWF and the highest in the >10,000 <100,000 MWF. The greatest changes that occurred in copper distribution upon photo-oxidation of the waters were a decrease in percentage in the >10,000 <100,000 MWF and an increase in percentage in the >100,000 MWF.

The copper distribution in the MWFs of waters from H.B. Robinson differed from those from the other freshwater sites; higher percentages were found in the <1000 and >1,000 < 10,000 MWFs. Destruction of organic compounds by UV treatment resulted in increases in the percentages in the <1,000 and >10,000 <100,000 MWFs. A possible explanation for the higher percentage in the >10,000 < 100,000 than in the >100,000 MWF is that smaller colloids are present in waters of lower pH.

Apparent Complexing Capacity (ACC)

The impact on ecosystems of the addition of copper from point sources is related to the concentration in the water of ligands that bind copper tightly. If, in the water, the concentration of ligands is considerably higher than that of copper, then ligands are available to react with added copper and this results in a change in the physicochemical form of the added copper. The formation of tightly associated copper-ligand complexes results in a form of copper that is less toxic to some biota (see following discussion on speciation effects) and that has a lower affinity for suspended matter (Ref. 16).

The measurement of ACC of waters has been reviewed recently (Ref. 52). The methods used to determine ACC include solubilization, biological response, voltametry, ion exchange, and selective ion electrodes. Investigators using solubilization include Kunkel and Manahan (Ref. 53), Campbell et al. (Ref. 54), Elder et al. (Ref. 55), Kerr and Quinn (Ref. 56), Avnimelech and Raveh (Ref. 57); those using biological response include Davey et al. (Ref. 58), Gachter et al. (Ref. 59), Gillespie and Vaccaro (Ref. 60); those using voltametry include Chau (Ref. 61), O'Shea and Mancy (Ref. 62), Smith (Ref. 46), Campbell et al. (Ref. 54), Duinker and Kramer (Ref. 63), Hanck and Dillard (Ref. 64), Shuman and Woodward (Ref. 65), Figura and McDuffie (Ref. 66), Harrison et al. (Ref. 12), Hoffman et al. (Ref. 67), Hart and Davies (Ref. 68), Lazar et al. (Ref. 69), Harrison et al. (Refs. 13,14); those using ion exchange include Van den Berg and Kramer (Ref. 70) and those using selective ion electrode include Ramamoorthy and Kushner (Ref. 71) and Giesy et al. (Ref. 72). The values obtained for complexing capacity range from 32,000 for an oil field brine (Ref. 53) to 1 for pristine waters off the coast of California (Ref. 12). In general, the complexing capacities of marine waters are lower than those for estuarine and freshwater ecosystems.

Data on ACC are available for a limited number of effluents from seven power stations (Table 7). The ACC for the receiving water of the marine ecosystems at both San Onofre and Diablo Canyon Nuclear Power Stations was low, approximately 1 μg Cu/L. Consequently, it can be expected that any labile copper added to the effluent waters at these stations will remain primarily as such and be in chemical forms that are considered to be toxic to the biota. The ACC at estuarine and freshwater ecosystems was generally higher and more variable. It is expected that higher concentrations of humic and fulvic acids would be present in estuarine and freshwater ecosystems than in marine ecosystems because of both runoff from land and the high productivity of most estuarine and freshwater ecosystems.

BEDLOAD SEDIMENTS

Distribution Coefficients

The copper affinity of intact bedload sediments from the intake and discharge zones at the stations differed greatly (Table 8). In samples from a given collection, distribution coefficients of the <150-μm fraction were higher than intact sediment.

Table 7. The apparent complexing capacity of effluent waters from power stations.

Station	Complexing capacity, μg Cu/L			
	Fall	Winter	Spring	Summer
Marine Ecosystems[a]				
Diablo Canyon, CA	1	--[b]	1	1
San Onofre, CA	1	1	1	1
Estuarine Ecosystems[c]				
Salem, NJ	5	60	20	--
Surry, VA	8	30	9	--
Freshwater Ecosystems[d]				
Fort St. Vrain, CO	--	12	100	36
Kewaunee, WI	--	3	29	36
Vermont Yankee, VT	--	8	1	1

[a] Ref. 12.
[b] No collection.
[c] Ref. 13.
[d] Ref. 14.

The distribution coefficients of suspended particles were considerably higher than those of bedload sediments (cf. Tables 4 and 8).

Copper Concentrations

Copper concentrations in intact bedload sediments differed by more than a factor of ten from station to station (Table 9). Highest copper concentrations were found at Fort St. Vrain, the lowest at San Onofre. Increased copper in sediments is important because of potential contamination of bottom-dwelling animals with copper and of potential remobilization of copper from the sediments.

Table 8. Distribution coefficients of bedload sediments collected at nuclear power stations.

Power station	Intake		Discharge	
	Intact	<150µm	Intact	<150µm
Marine Ecosystems				
San Onofre	240	1,000	190	460
Diablo Canyon	--[a]	--	200	7,500
Freshwater Ecosystems				
Vermont Yankee	1,600	2,500	2,300	3,300
Kawaunee	--	--	40	110
Fort St. Vrain	--	--	2,200	3,100
Estuarine Ecosystems				
Surry	1,900	5,400[b]	4,700	14,000[b]
Salem	7,800	13,000[b]	1,600	11,000[b]

[a] No samples collected.

[b] The <62-µm fraction was used in these analyses.

Table 9. Copper concentrations of bedload sediments (µg/g dry weight) collected at nuclear power stations.

Power station	Intake		Discharge	
	Intact	<150µm	Intact	<150µm
Marine Ecosystems				
San Onofre	4.4	6.6	4.2	6.9
Diablo Canyon	--	--	9.9	9.2
Freshwater Ecosystems				
Vermont Yankee	16	20	17	20
Kewaunee	--	--	5	8
Fort St. Vrain	--	--	130	170
Estuarine Ecosystems				
Surry	7.3	36[a]	22	47[a]
Salem	42	58[a]	28	78[a]

[a] The <62-µm fraction was used in these analyses.

IMPACT OF COPPER RELEASES

The data acquired from our field sampling at nuclear power stations can be used to assess the extent to which ecosystems may be impacted by the copper released. Many of the parameters measured had large seasonal differences. Consequently, average values do not reflect the conditions that may have adversely affected biota. In Table 10 are compiled the maximum values of each parameter measured. By comparing these values, we can better identify those stations where copper releases may be a problem.

MARINE ECOSYSTEMS

Copper concentrations in the abiotic compartments measured at San Onofre were low (Table 10). Values were also low at Diablo Canyon except during start-up of water circulating through the cooling system. At both sites differences in copper concentrations between the intake and discharge waters were small during normal operations. Most of the copper in the discharge water was in bound forms under normal operating conditions. Because the addition of copper to the water was small and sufficient organic ligands were present to complex most of the copper, we can expect little or no impact from the release of copper from San Onofre or Diablo Canyon Power Stations during normal operation and without addition of chemicals to the effluents.

Adverse effects from copper releases may occur when water circulation is started up again through the condenser systems after a shutdown and when the coolant waters are chlorinated to prevent fouling of the condenser tubing. We detected elevated concentrations of copper in the discharge waters during start-up at Diablo Canyon. The maximum concentration of 28 µg Cu/L that we obtained was low compared to the 1800 and 7700 µg Cu/L reported earlier, because the Cu-Ni alloy in the main cooling system was replaced by titanium. The only copper now present is in a small auxiliary system. Most of the increase in concentration was due to labile copper, the chemical form that is toxic to biota.

The presence of large amounts of labile copper probably resulted from the absence of ligands to complex the copper; our analyses of the waters show that the complexing capacities were low. We can expect then that any pulse of labile copper released into the waters at San Onofre and Dablo Canyon will remain labile until it is diluted with a sufficient volume of water to provide the additional ligands to complex the copper.

Chlorination results in the degradation of organic matter in the water. This practice may affect the biota by changing the chemical form of potentially toxic metals in the discharge waters. Organic ligands that bind copper may be destroyed and the concentrations of labile copper increased. The impact of the destruction of organic matter would be greatest when copper concentrations are at seasonal highs.

Bedload sediments collected in the discharge area of both the San Onofre and Diablo Canyon Power Stations were low in copper, and the copper distribution coefficients of the sediments were low. The sediment from both sites consisted primarily of sand, and little organic matter was present. These results indicate little or no build up of copper in the sediment at either site and a small potential for build up in the future.

Table 10. Maximum values of parameters measured in the discharge zone of nuclear power stations.

Parameter	Type of ecosystem										
	Marine			Freshwater					Estuarine		
	San Onofre	Diablo Canyon		Kewaunee	Fort St. Vrain	H.B. Robinson	Vermont Yankee		Surry	Salem	
	Normal[a]	Normal	Start-up[b]	Normal	Normal	Normal	Normal	Changeover[c]	Normal	Normal	Start-up
Water column											
Copper (µg/L)											
Total	3	3	28	18	31	79	10	190	25	11	>2500
ΔCu	2	--	27	10	20	22	5	180	4	2	>2500
Labile	0.7	0.4	21	2.3	1	34	2.1	70	2.9	3.8	3.3
Bound	1.6	1.8	6	1.7	4	27	2.4	110	2.6	3.2	3.2
Particles	1.5	1.3	2	15	26	19	5	81	21	5.6	>2500
Complexing capacity											
(µg/L)	1	1	--	36	100	27	8	--	30	60	60
DOC[d] (mg/L)	2	2	--	2	7	5	3	--	4	5	5
Particles											
Copper (µg/L)	70	0.3	--	3,600	700	2,600	1,300	--	470	160	--
Residue (mg/L)	54	11	--	8	51	12	12	--	84	120	--
K_d	45,000	48,000	--	46,000	36,000	25,000	61,000	--	16,000	17,000	--
POC[e] (mg/L)	0.6	0.5	--	1.1	1.9	1.1	0.5	--	2.1	2.8	--
Chlorophyll[f]	--	--	--	0.6	7	--	0.3	--	0.3	1	--
Intact Bedload Sediment											
Copper (µg/g)	8	12	--	5	130	72	17	--	22	28	--
K_d	400	600	--	40	2,200	21,000	2,300	--	4,700	1.600	--
Size class	Sand	Sand	--	Sand	Silt	Gravel-sand	Silt	--	--	--	--

[a] Observed during normal operation of the station.
[b] Observed during start-up of water circulating through the cooling system.
[c] Observed during changeover from closed-cycle to open-cycle operation.
[d] DOC, dissolved organic carbon.
[e] POC, particulate organic carbon.
[f] Height of absorbance peak at 660 nm.
[g] TOC, total organic carbon.

FRESHWATER ECOSYSTEMS

During the normal operation of power stations, the total copper concentrations in the discharge waters were considerably higher in freshwater ecosystems than in marine ecosystems. Maximum values ranged from 10 at Vermont Yankee to 79 µg Cu/L at H.B. Robinson (Table 10). Also, copper concentrations differed greatly between the intake and discharge waters. Most of the copper in the water column at both Kewaunee and Fort St. Vrain was in the particulate fraction. Except at H.B. Robinson, labile copper was only a small fraction of the total. The high concentration of labile copper at H.B. Robinson is most likely because the pH of the water is <6.

The total concentrations of copper in the effluent waters from power stations located at freshwater sites is in the range reported as toxic to aquatic organisms. However, not all of the copper may be available to the organisms. Of concern would be the effects on filter-feeding organisms because they may accumulate copper from both the soluble and particulate fractions of the water. When most of the copper is bound or associated with particles, the important entry route of copper into the biota may be from the food chain rather than from the water. Concentrations of copper in the biota living in the discharge areas of Fort St. Vrain, H.B. Robinson, and Vermont Yankee may be higher than in those at other sites. Of special interest is the impact of the copper released at Fort St. Vrain. Not only are the total copper concentrations high here, but also the discharge zone impacted may be the largest. At this site discharge waters are delivered to a 25-acre pond before they overflow into offsite waters. To assess copper availability to food chain organisms at Fort St. Vrain, concentrations of copper in organisms in different trophic levels in the holding pond must be compared to concentrations in the same organisms from a control site.

The maximum complexing capacity of the water measured at each of the four freshwater sites was much higher than any determined at marine sites. During the seasons of the year when the complexing capacity is large, addition of labile copper would probably have a minimum impact.

Of concern in freshwater systems that have large quantities of copper present is the effect of chlorination on biological availability of the copper. Not only may chlorination destroy organic ligands in solution, it may also destroy organic ligands associated with the particles in suspension. Chlorination may result in sufficient change in the physicochemical form of the copper to produce a toxic response in sensitive organisms.

High concentrations of copper were detected in suspended particles in the freshwater ecosystems sampled. The maximum concentration determined at Kewaunee, 3600 µg Cu/g, was more than ten times higher than the maximum determined at the two marine sites. This high value does not seem to be correlated with high particulate organic carbon, chlorophyll, or copper in the bedload sediments.

Bedload sediments at Fort St. Vrain contained high concentrations of copper. The total organic carbon at this site was also high, and the sediment contained a fair amount of silt. Oxidation of organic material in the sediments may cause Eh changes in the sediment that result in the release of copper into interstitial waters. This copper may enter the water column by diffusion across the sediment-water interface or by mixing with the overlying water when bedload sediments are resuspended by currents or from feeding or locomotion of animals.

ESTUARINE ECOSYSTEMS

Copper concentrations in water collected at Surry and Salem in January were higher than those obtained at other times and are given in Table 10. In January the salinity of the water was low, and the nonfilterable residue was high; these data suggest that runoff from the land was large.

Total copper concentrations in the discharge waters at both Salem and Surry were high, and when a large percentage of the copper was in the particulate fraction, little difference was detected between intake and discharge waters (Ref. 13). The copper contributions from land runoff may have obscured any additions of copper from the cooling systems.

At both Salem and Surry very little of the total copper was in labile forms and the complexing capacity of the waters was high. These results indicate that sufficient ligands are available in the water to bind considerable quantities of copper. However, estuarine ecosystems may be affected by copper during chlorination. Because total copper concentrations are in the range that results in toxic effects on some biota, destruction of soluble and particulate organic matter may result in changes in form that affect the copper availability to the biota.

Bedload sediments at Surry and Salem had similar concentrations of copper, but were not as high as those at Fort St. Vrain. Sediments were collected relatively close to the stations; areas may exist that have higher concentrations than we measured.

More data are needed before the impact of the release of copper into estuaries can be assessed. The information obtained to date indicates that potential problems may exist because of the high concentrations that were found and the changing conditions in the water column. The combination of high copper concentrations and high particle loads indicate that significant quantities of copper may be accumulated by filter feeders such as oysters.

COPPER TOXICITY TO REPRESENTATIVE AQUATIC ORGANISMS

We found the response of aquatic organisms to copper differed greatly with trophic level and with life history stage; the 48-hour LC_{50}s determined ranged from ∿10 to 9900 (Table 11). Also, the toxicity curves generated from the mortality date differed in slope and placement with species and life stage (Fig. 3). Of the organisms tested, oyster embryos were the most sensitive and crayfish the least sensitive to copper.

PRIMARY PRODUCERS

Macrocystis pyrifera

Gametophytes of the giant kelp, Macrocystis pyrifera, were found to be sensitive to copper (Ref. 17). Complete inhibition of growth occurred when gametophytes were exposed continuously to copper concentrations of 500 µg/L or more. Vegetative growth was inhibited significantly by continuous exposure to 50 µg Cu/L or more. The production of eggs by female gametophytes was inhibited by spikes of 30 µg Cu/L. The total concentrations of copper were measured in most experiments. In those where labile copper concentrations were measured, they were considerably lower than that of total copper.

Table 11. Toxicity of copper to some representative aquatic organisms.

Copper[a] (μg/L)	Endpoint	Organism	Lifestage
Marine Organisms			
>30	Growth inhibition	Giant kelp	Gametophyte
650	48-h LC_{50}	Oysters[b]	Adult
∿10	48-h LC_{50}	Oysters	Embryonic
460	48-h LC_{50}	Anchovy	Adult
400	24-h LC_{50}	Anchovy	Larval
200	96-h LC_{50}	Anchovy	Embryonic
440	24-h LC_{50}	Herring	Adult
2000	24-h LC_{50}	Herring	Larval
240	96-h LC_{50}	Herring	Embryonic
Freshwater Organisms			
>2600	96-h LC_{50}	Clam	Adult
28	24-h LC_{50}	Clam	Larval (veliger)
600	24-h LC_{50}	Clam	Larval (juvenile)
9900	48-h LC_{50}	Crayfish	Adult
1200	48-h LC_{50}	Crayfish	Larvae
3700	480-h LC_{50}	Crayfish	Embryonic
540	24-h LC_{50}	Carp	Adult
180	24-h LC_{50}	Carp	Larval
240	24-h LC_{50}	Carp	Embryonic

[a] Copper concentration to achieve endpoint.

[b] Corrected for shell closure.

Photomicrographs of the gametophytes on the grids on which they were grown recorded the physical appearance of control and treated cultures. Fig. 4 shows a control culture after 6 weeks of growth; Fig. 5 shows the effect of exposure to 50 μg Cu/L for the same period. The copper-exposed gametophytes were stubbier in appearance than the controls and lacked the distincitve sexual characteristics of control plants. Photomicrographs showing the effects of 6 weeks of exposure to 75, 100, 150, and 200 μg Cu/L are presented in Figs. 6, 7, 8, and 9, respectively. The treated cultures show a growth pattern characterized by fewer and shorter filaments that seem to branch randomly. At 200 μg Cu/L, the plants exposed for 6 weeks did not seem to have progressed past the growth level that would have been reached by some control cultures after only 1 or 2 weeks.

The physiological toxic effects of copper on *M. pyrifera* gametophytes can be divided into three general categories. First, acute copper toxicity is characterized by traumatic expulsion of the cell contents. This is probably the result of cell membrane disintegration and was observed at spikes of 500 μg Cu/L or more.

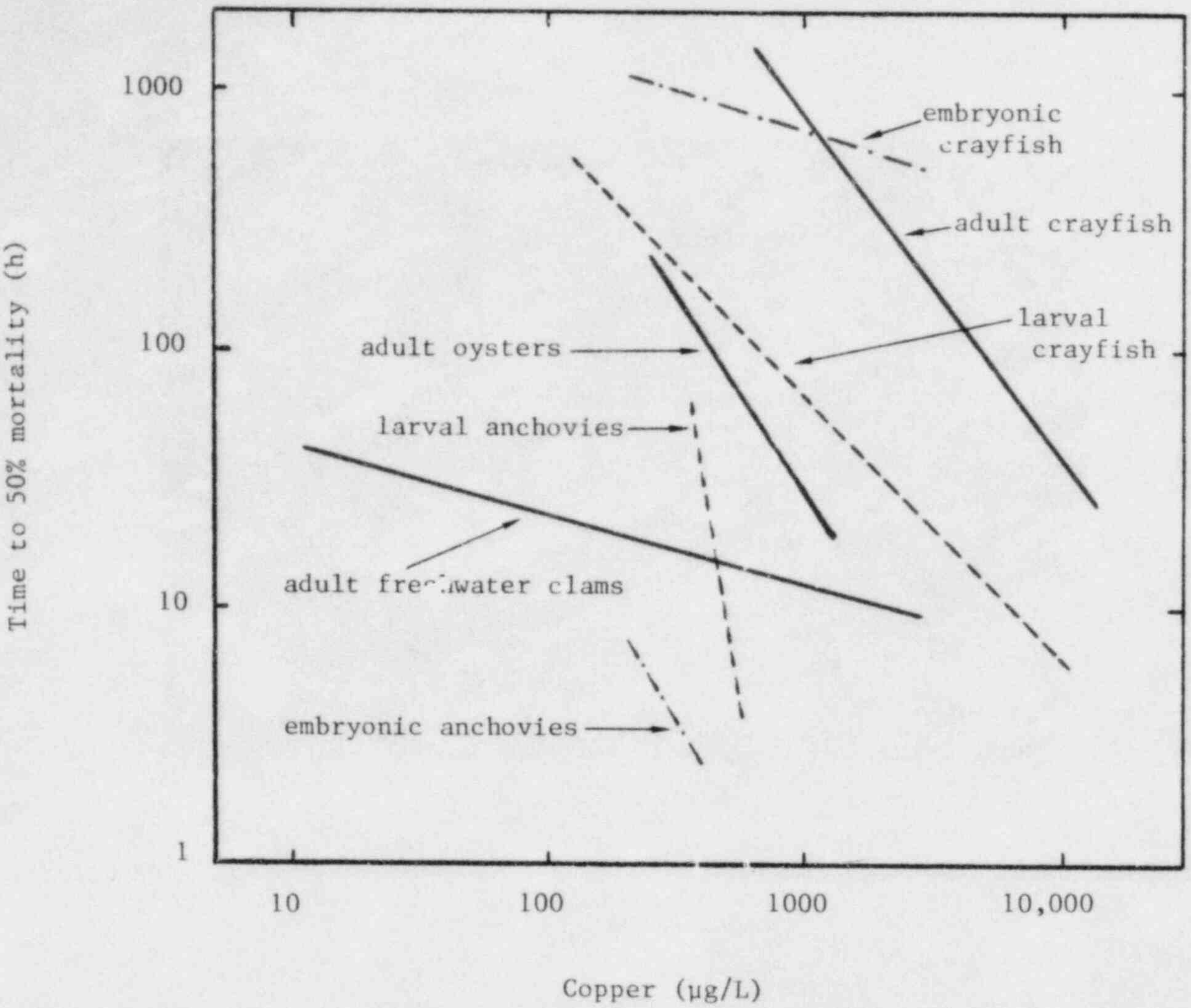

Figure 3. Toxicity curves constructed for some different species and life stages of aquatic organisms.

Second, acute copper toxicity is characterized by varying degrees of growth inhibition, resulting from the interference of copper with photosynthesis, respiration, and enzyme function. At copper concentrations nominally between 50 and 200 µg Cu/L, when the metal-induced stress is continued for 4 to 6 weeks, the gametophytes disintegrate at a rate directly related to the amount of copper in the medium. For pulsed exposures of 1-week-old gametophytes to mid-range copper concentrations, the recovery of vegetative growth to control levels takes approximately 2 to 3 weeks. Whether sexual maturation is impaired by the metal-induced stress during the vegetative phase is a question that needs to be answered.

Third, subacute toxic effects were observed with initial spikes of 30 and 50 µg Cu/L. At these levels, initial concentrations of labile copper (probably less than 10 µg Cu/L) block the production of eggs in female gametophytes. Whether this interference occurs at one or several key enzymatic reactions is unknown. With initial copper spikes of 10 and 20 µg Cu/L, little or no labile copper was available to the gametophytes because the biologically active metal forms are readily complexed by the ligands naturally present in the seawater and by those ligands exuded by the plant.

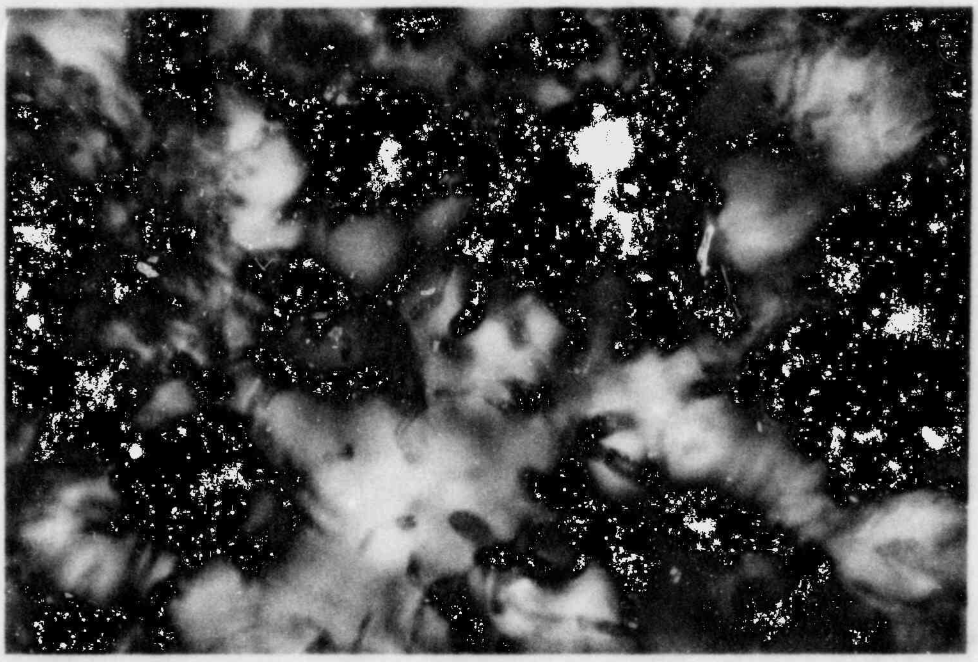

Figure 4. Six-week-old culture of Macrocystis pyrifera gametophytes: control.

Figure 5. Six-week-old culture of Macrocystis pyrifera gametophytes: continuous exposure to 50 µg Cu/L.

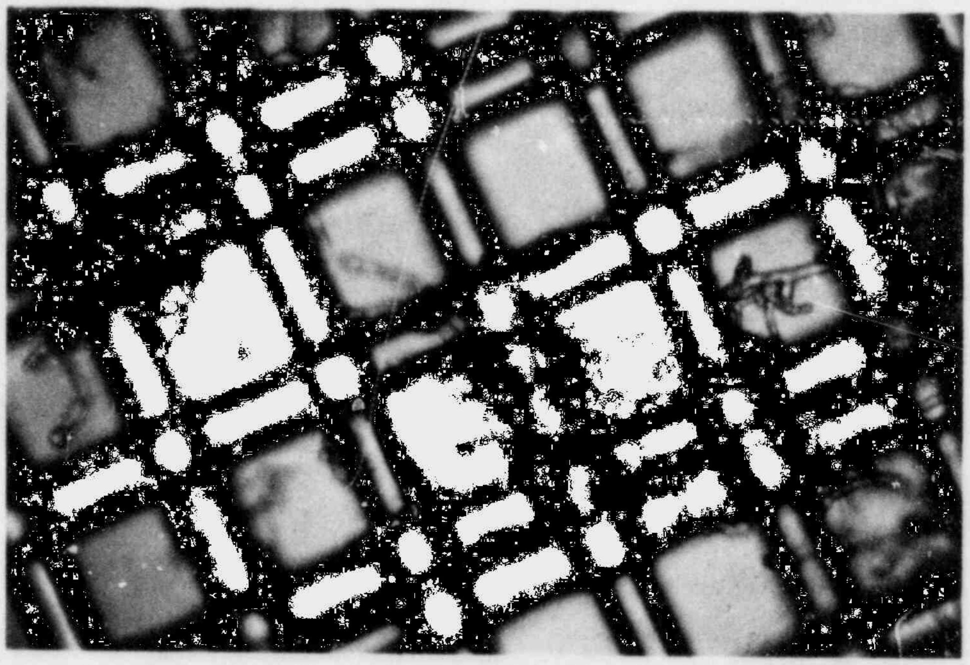

Figure 6. Six-week-old culture of Macrocystis pyrifera gametophytes: continuous exposure to 75 µg Cu/L.

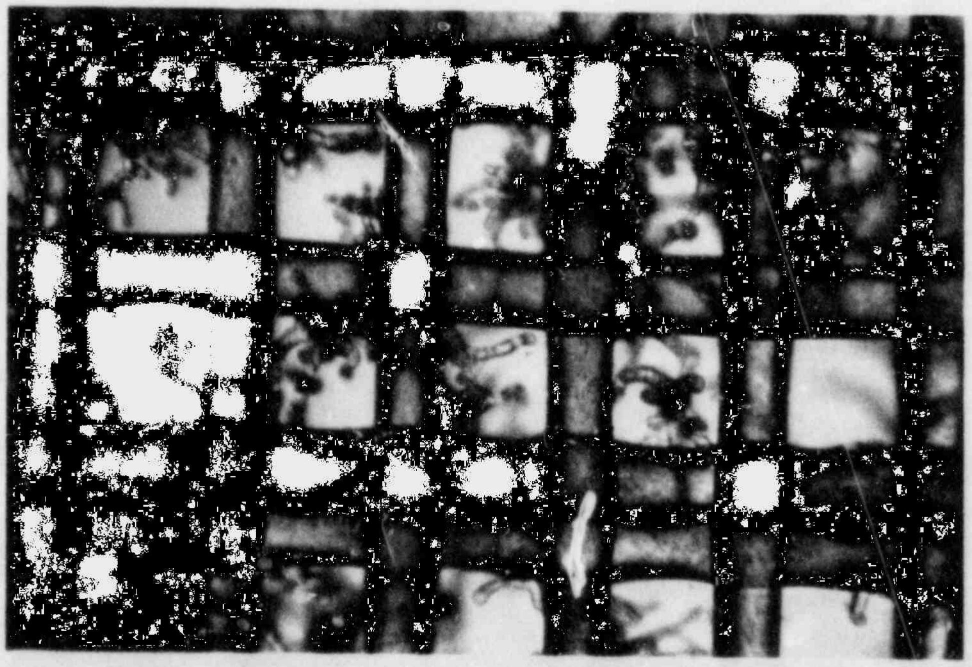

Figure 7. Six-week-old culture of Macrocystis pyrifera gametophytes: continuous exposure to 100 µg Cu/L.

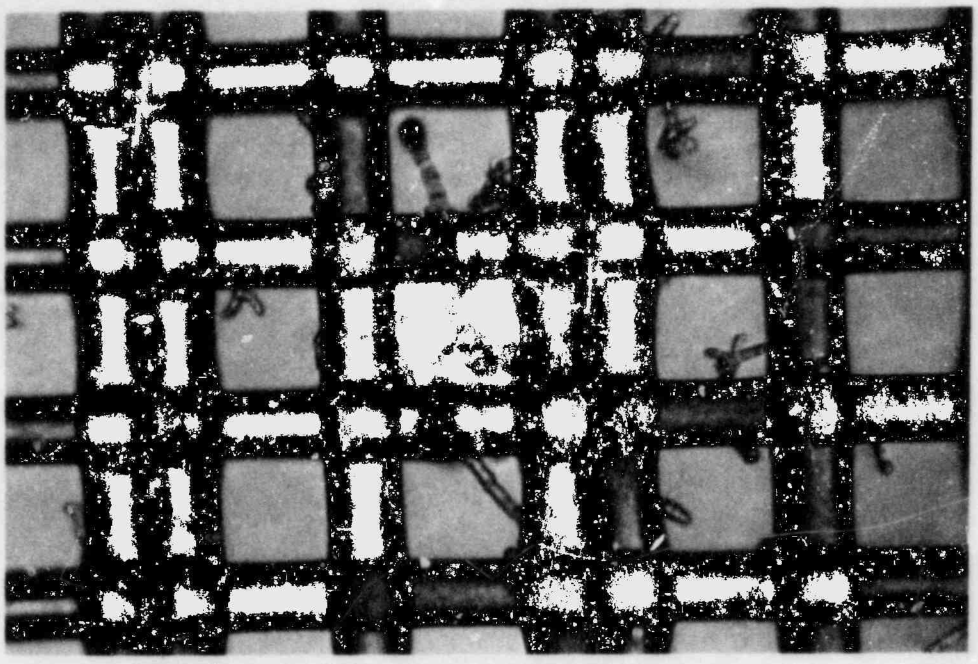

Figure 8. Six-week-old culture of Macrocystis pyrifera gametophytes: continuous exposure to 150 µg Cu/L.

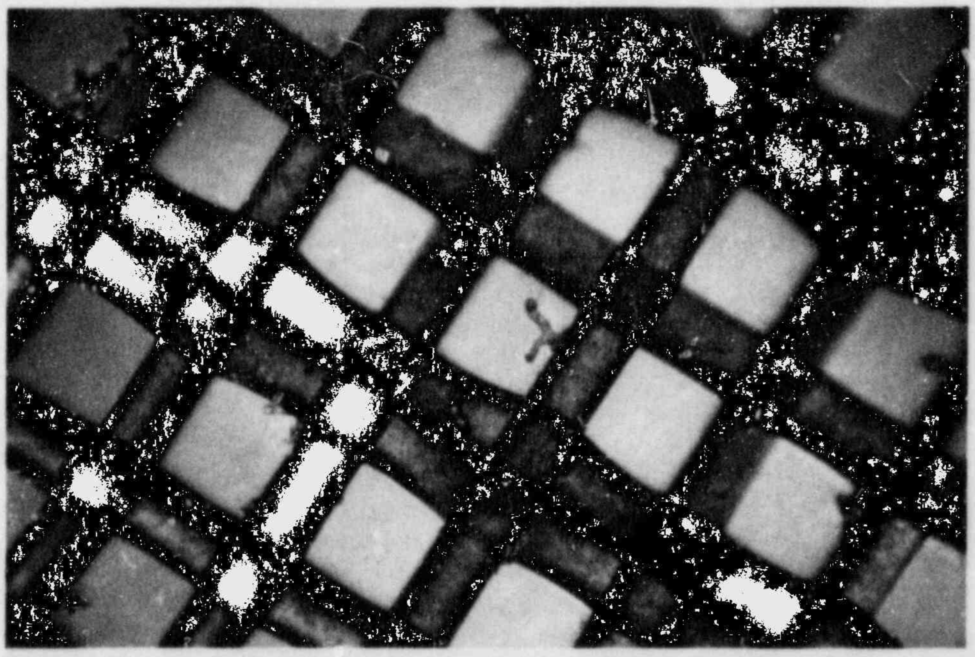

Figure 9. Six-week-old culture of Macrocystis pyrifera gametophytes: continuous exposure to 200 µg Cu/L.

Other Primary Producers

A wide range of response of primary producers to copper has been demonstrated (Refs. 7,8). Copper appears to affect basic physiological processes such as photosynthesis, growth, and nitrogen fixation and to result in distinct morphological changes. However, little is known about its primary mode of action.

EFFECTS OF COPPER SPECIATION

Reduction in copper toxicity to primary producers has been observed both in the presence of chemicals known to bind copper and of organic chelators found in natural waters (Refs. 48, 73-80). The reduction has been attributed to the decrease in concentration of the free metal ion (Refs. 48, 76-78, 80-83). However, Wagemann and Barica (Ref. 79) state that the effectiveness of copper as an algicide in six prairie, hard-water lakes (pH 8.0 to 9.4 and high concentrations of dissolved organic matter) can be understood more readily if in addition to Cu^{2+} and $CuOH^{+}$, the species $Cu(OH)_2^0$ were also considered to be toxic to algae. They call the sum of the three copper species the "total toxic copper."

Some investigators have proposed that primary producers may excrete organic substances and thus alter the toxicity of their environment (Refs. 84, 85). Gnassia-Barelli *et al.* (Ref. 84) demonstrated that *Cricosphaera elongata* liberated organic substances into culture media that complexed and detoxified copper; the molecular weight range of the organic substances was between 500 and 10,000 daltons.

BIVALVE MOLLUSCS

Both a freshwater bivalve and a marine bivalve were tested (Refs. 20, 19). The adult stages of both species were considerably less sensitive to copper than were the early life history stages (Table 11). Also, the adult oyster *Crassostrea gigas* was considerably more sensitive than the freshwater clam, *Corbicula manilensis*. Our studies indicated that the closure response of shelled bivalves is important for the prevention of adverse effects. Both adult and larval stages tolerate pulses of copper that are high in concentration by closure of the valves of the shell. Because of the high sensitivity of oyster larvae to copper, they were used to evaluate the effect of organic chelators on the response to copper (Ref. 86).

Oysters

The family of mortality curves (time *vs* concentration) from oysters was unlike that from other organisms in that the greatest mortality at 96 hours occurred at intermediate rather than high copper concentrations. However, by correcting the duration of each oyster's exposure to copper by a factor related to the amount of time the shells were open, we obtained typical mortality curves.

Significant mortalities occurred at copper concentrations of ≥ 200 µg/L and higher. Data analysis yields a 48-hour LC_{50} of 650 µg/L, a 96-hour LC_{50} of 430 µg/L, and an incipient lethal concentration (ILC) of 230 µg/L. The incipient lethal concentration is the concentration that kills 50% of the population during an exposure that is sufficiently long that acute lethal action has ceased. The percentage of copper in labile forms in the bioassay water ranged from 73 to 89.

Distribution of ^{64}Cu in oysters after 24-hour exposure differed among oysters maintained in water containing either high or low copper concentrations. Copper flux into tissues increased with the copper concentrations in the water, indicating little metabolic regulation. These data indicate that copper accumulated by oysters is proportional to the exposure concentration.

Oysters can reduce their exposure to copper by preventing the entrance of water into their mantle cavities by tightly closing the valves of their shell. This closure may protect the oyster from episodic releases of copper. Therefore, oysters may not be the best biological indicator of metal pollution, because they do not circulate water continuously, but close the valves of their shell during adverse environmental conditions.

The effect of copper on oyster embryo mortality was determined in a series of 48-hour experiments in a static bioassay system. Fertilized eggs were exposed to copper concentrations ranging from 5 to 100 µg/L in filtered, sterilized, natural seawater. All embryos that failed to reach the D-hinge larval stage by 48-hour were recorded as abnormal. The 48-hour LC_{50} was 12 µg copper/L.

The effects of copper on development were determined by observing exposed populations of embryos at 12-hour intervals from 24 to 72 hours. The greatest effect on the exposed populations occurred between 24 and 36 hours after fertilization. This is the time of shell deposition and transition from trochophore to veliger larvae; affected larvae failed to deposit a shell and died as trochophores by 72 hours.

Destruction of the naturally occurring chelators in the culture water by UV photo-oxidation resulted in doubled embryo abnormality at 10 µg Cu/L. Adding any of five organic chelators (EDTA, sodium citrate, glycine, oxalate, and humic matter) to the seawater increased embryo survival.

Analysis of the bioassay water for copper by differential pulse anodic stripping voltametry (ASV) revealed that a significant fraction of the copper was labile. Embryo abnormality was attributed to the labile copper species because abnormality decreased when copper chelators were added.

Freshwater Clams

The sensitivity to copper of larval and adult stages of the freshwater clam, Corbicula manilensis, was determined by exposing clams to concentrations ranging from 10 to 12,000 µg Cu/L. Concentrations of total and labile copper were measured in the bioassay water. In addition, copper concentrations were determined in tissues of adult clams.

Sensitivity of adult clams was assessed in four different experiments that were 4 to 10 weeks long. At concentrations of <3000 µg Cu/L in the bioassay water, the valves of the shell were open about the same amount of time. However, at concentrations of 6,000, 9,000, and 12,000 µg Cu/L, the valves remained closed.

The toxicity curve obtained from the combined mortality data indicates a 96-hour LC_{50} of >2,600 µg Cu/L for adult clams. An ILC could not be determined from the data but seems to be <10 µg Cu/L. Because the animals responded to high concentrations of copper by tightly closing valves of their shells, 48-hour and 24-hour LC_{50}s could not be determined.

Copper concentrations in adult clams held in water to which copper was added were higher than in those in the control water and were dose related. Evidence for loss of copper just before or at death was obtained.

A timetable of development was determined to interpret the effects of copper exposure of larvae. The response to copper depended on life stage and decreased markedly for successive developmental stages. Experiments conducted on the veliger and juvenile larvae yielded 24-hour LC_{50}s of 28 and 600 µg Cu/L, respectively. Trochophore larvae were sensitive to copper concentrations as low as 5 µg Cu/L. The sensitivity of each stage was related to the amount of larval shell deposition.

The fully shelled veliger and juvenile larvae immediately closed their shells at all copper concentrations and kept them closed throughout the experiment. Control larvae were always actively swimming with the velum or foot extended. (Fig. 10a). The initial high mortality of veliger larvae exposed to low levels of copper may be attributed to the presence of early veligers or late trochophores that do not have fully developed shells (Fig. 10b). These larvae cannot close their shells and were therefore constantly exposed. Dead veliger and juvenile larvae were found either intact with gaping shells or disintegrating with closed shells (Fig. 10c).

Other Bivalve Molluscs

More data are available for bivalve molluscs than for other classes of molluscs (Refs. 7, 8). Lower LC_{50} values are usually obtained using flowthrough rather than static bioassay systems. Some of the lowest values were obtained for marine clams.

CRUSTACEA

Crayfish

The copper sensitivity of adult, embryonic, and larval stages of the crayfish Procambarus clarkii was determined with flowthrough bioassay methods (Ref. 23).

Four adult experiments were performed lasting up to 1,350 hours and using copper concentrations ranging from 230 to 11,600 µg Cu/L. Tissue analysis was performed on adult crayfish exposed to copper.

The toxic response to copper was determined for two groups of crayfish embryos that differed in age at the time exposure was initated. The first group used embryos in which 50% had hatched 240 hours after the start of exposure; the second group used embryos in which 50% had hatched 600 hours after the start of exposure.

The toxic response to copper was determined for three groups of crayfish larvae. The first group of larvae was exposed to copper for 250 hours as embryos and then exposed through larval development. The second group was exposed to copper 48 hours after hatching and consisted of first instars. The third group was exposed to copper 120 hours after hatching and consisted of second instars. Embryonic and larval exposures ranged from 100 to 2750 µg Cu/L.

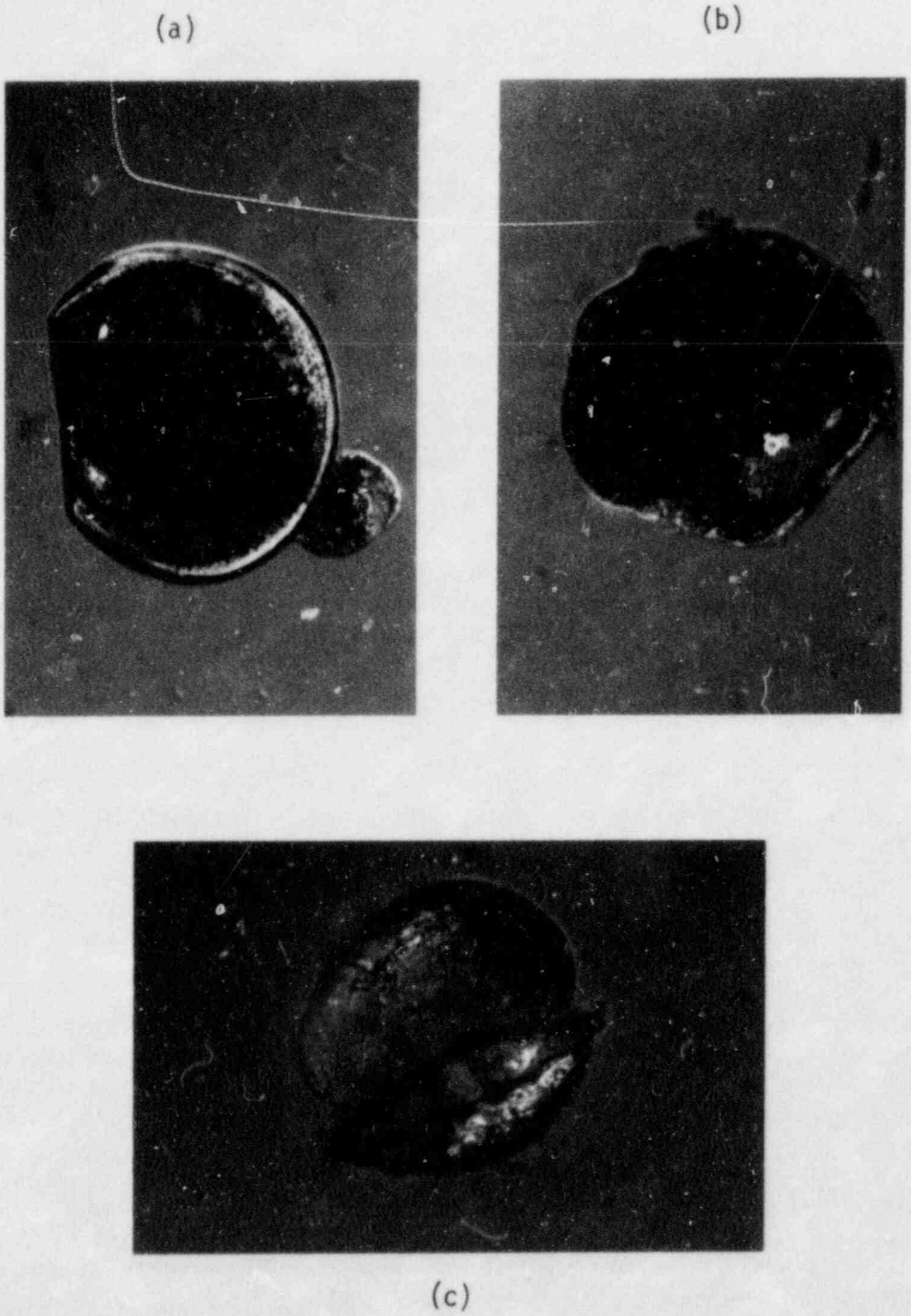

Figure 10. Photomicrographs of early life stages of clams: (a) control larva showing velum and foot extended; (b) control late trochophore or early veliger larva; (c) veliger larva at death after exposure to copper.

The 480-hour LC_{50} for adult crayfish was determined to be 1300 µg Cu/L. The remains of adult crayfish showed significant dose-related increases in tissue copper concentration over controls. Though not dose related, the gills showed significant increase in copper concentration at exposures of 480 µg Cu/L and greater.

The mortality of crayfish embryos was related to their age at the start of copper exposure. Embryos exposed early in their development had much poorer hatching success than those exposed later. The 480-hour LC_{50} for early crayfish embryos was calculated to be 5700 µg Cu/L.

Larvae exposed to copper just after hatching appeared to be more sensitive than larvae exposed through embryonic development. Crayfish larvae exposed to copper developed black discolorations of the gill filaments. No difference between first and second instar larval copper sensitivity was found. The 480-hour LC_{50} for crayfish larvae was calculated to be 120 µg Cu/L. The larva was the most sensitive life-history stage for *P. clarkii*. Also, larvae exposed to copper were smaller and appeared to be at earlier stages of development than control larvae (Fig. 11).

Other Crustacea

Sensitivity to copper has been defined for a number of both marine and freshwater crustacea (Refs. 7, 8). Sensitivity of crustacea to copper, like that of other aquatic organisms, is dependent on the chemical form of the copper and on bioassay conditions. As in other groups of organisms, crustacea early life stages are generally more sensitive than adults. Copper in low concentrations is of special importance to crustacea because the primary respiratory pigment is hemocyanin, a copper-containing pigment, and not hemoglobin.

EFFECTS OF COPPER SPECIATION ON INVERTEBRATES

Data are available on the reduction of heavy metal toxicity to invertebrates by organic chelating agents. Lewis *et al.* (Refs. 87, 88) reported an increase in the survival of the calanoid copepod *Euchaeta japonica* in water enriched with EDTA and in water to which soil extracts had been added. Andrew (Ref. 89) showed that toxicity of copper to *Daphnia* correlated well with cupric ion activity measured with an ion-specific electrode. Young *et al.* (Ref. 90) suggested a relationship between polarographically labile copper and copper toxicity; in their studies, an increase of 7 µg/L labile Cu resulted in significant mortality to developing shrimp zoeae. Borgmann (Ref. 91) evaluated the effect of complexing agents on copepod production. Harrison *et al.* (Ref. 86) found that added organic chelators reduced the toxicity of copper to *Crassostrea gigas* embryos; the ability of the chelator to reduce toxicity was related to its log K value. They also reported that destruction of the naturally occurring copper-binding organic matter in the seawater by UV photo-oxidation reduced embryo survival.

FISHES

The responses of different life stages of both a marine and freshwater fish to acute levels of copper were evaluated. The northern anchovy, *Engraulis mordax*, was selected as a representative marine fish because of its economic importance and because early life stages are present in the vicinity of power stations. The common carp, *Cyprinus carpio*, was selected because it is present in the vicinity of power station and is an important aquaculture species. The response of the freshwater bluegill, *Lepomis macrochirus*, to sublethal concentrations of copper was

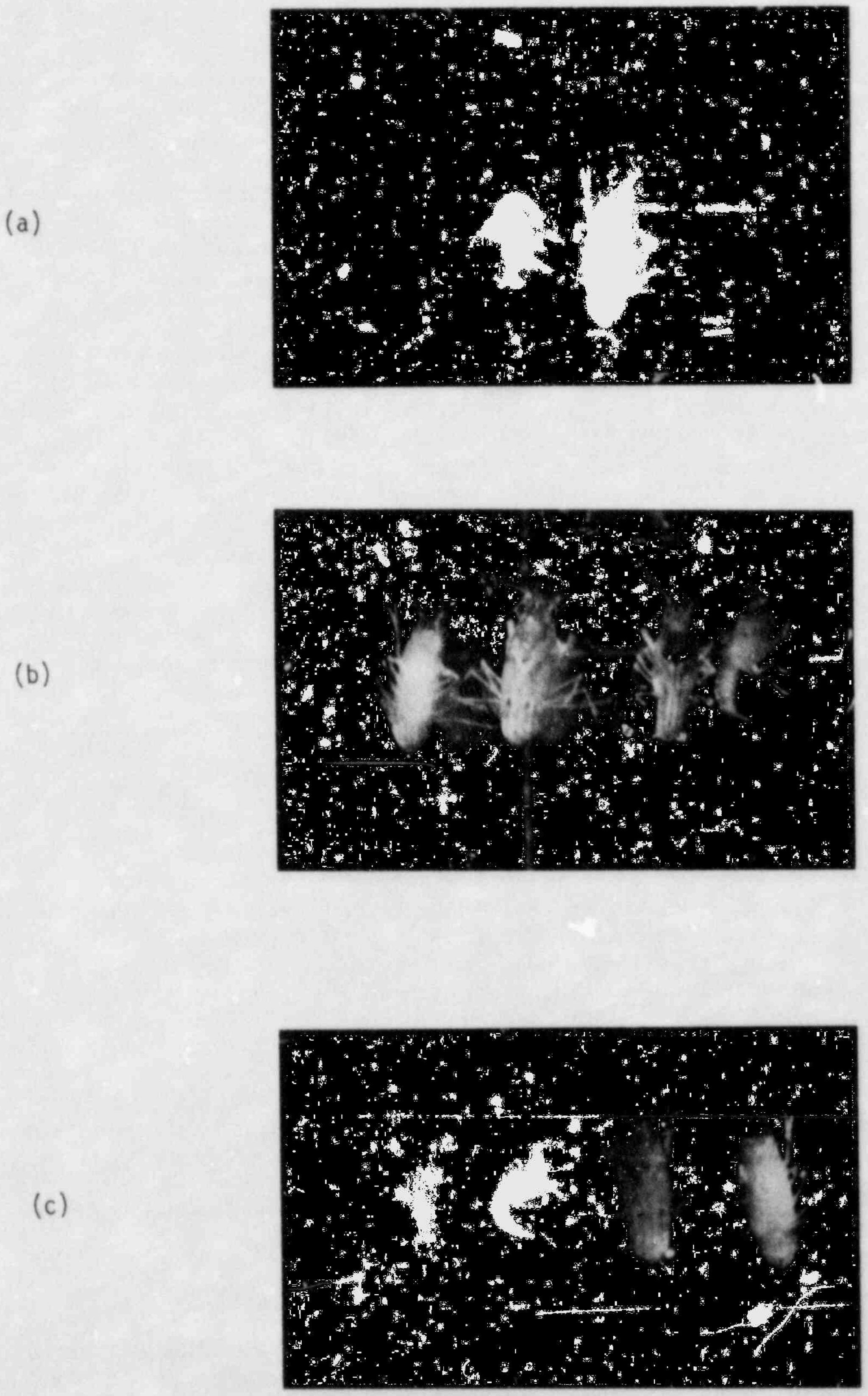

Figure 11. Photomicrographs of crayfish larvae: (a) control larva on left was newly hatched and on right was 13 days old; (b) two larvae on left were exposed to 25 µg Cu/L for 2 weeks and on right to control water; (c) two larvae on left were exposed to 50 µg Cu/L and on right to control water.

evaluated to try to establish the cause of the reduced reproductive capacity and increased structural deformities that were observed in bluegills from the impoundment adjacent to the H.B. Robinson Steam Electric Plant.

Northern Anchovy

The effects of copper on the embryonic and larval stages of the Northern anchovy were investigated (Ref. 18) because copper is known to be one of the most toxic heavy metals to a variety of marine species, and it is discharged into coastal waters by the release of municipal waste waters, power station effluents, and the use of marine antifouling paints. Anchovy early life stages were used because natural mortalities that occur during the early stages of marine fish have been suggested to be a major factor in reducing the size of a given year-class of fish. Pollutants that have an impact on the survival of fish embryos or larvae might further reduce the size of a given year-class of fish.

The anchovy life stage most sensitive to copper was the embryo. For anchovy embryos, the 12-hour LC_{50} was 200 µg Cu/L, and the estimated ILC was 190 µg Cu/L. A sensitive period of embryonic development was noted to be prior to closure of the blastopore. The 12-hour LC_{50}, 24-hour LC_{50}, and ILC_{50} for anchovy larvae were 460, 400, and 370 µg Cu/L, respectively. The percent of the total copper in the labile (noncomplexed) form for all concentrations used during testing averaged 96%. Some larvae that hatched from embryos that were exposed to 200 µg Cu/L showed structural abnormalities (Fig. 12).

During an earlier study, we examined the sensitivity of the early stages of another marine fish, the Pacific herring. The anchovy and herring have very different spawning strategies. The anchovy spawns a fragile, pelagic egg and the herring spawns a tough, demersal egg. For both species the embryo was most sensitive, though the demersal embryo was six times more sensitive than the pelagic embryo.

Common Carp

Experiments were performed to determine the acute toxicity of copper to adults, newly hatched larvae, and embryos of the common carp, Cyprinus carpio (Ref. 21). Concentrations of total and Chelex-100-labile copper were measured in the bioassay water. In addition, we determined the amount of copper in muscle and liver tissues of some experimental adult carp.

Sensitivity of adult carp was assessed in three different experiments. The toxicity curve generated from the combined mortality data indicated that the 48-hour LC_{50} was 170 µg Cu/L and the ILC was 120 µg Cu/L. Analyses of the bioassay water showed that more than 65% of the copper was in Chelex-100-labile forms. Copper concentrations in muscle and liver tissues taken from carp exposed to increased copper concentrations in the bioassay water did not significantly differ from concentrations in the same tissues in control animals.

Toxic response to copper was determined for groups of embryos that were 4 to 6, 8 to 10, and 20 to 24 hours old. The sensitivity to copper appeared to decrease with increased age. The 48-hour LC_{50} and ILC for 6 to 8-hour carp embryos was 230 µg Cu/L. The percent of larvae that hatched decreased with increasing copper concentrations in the water and increased with increasing age of the embryo at the

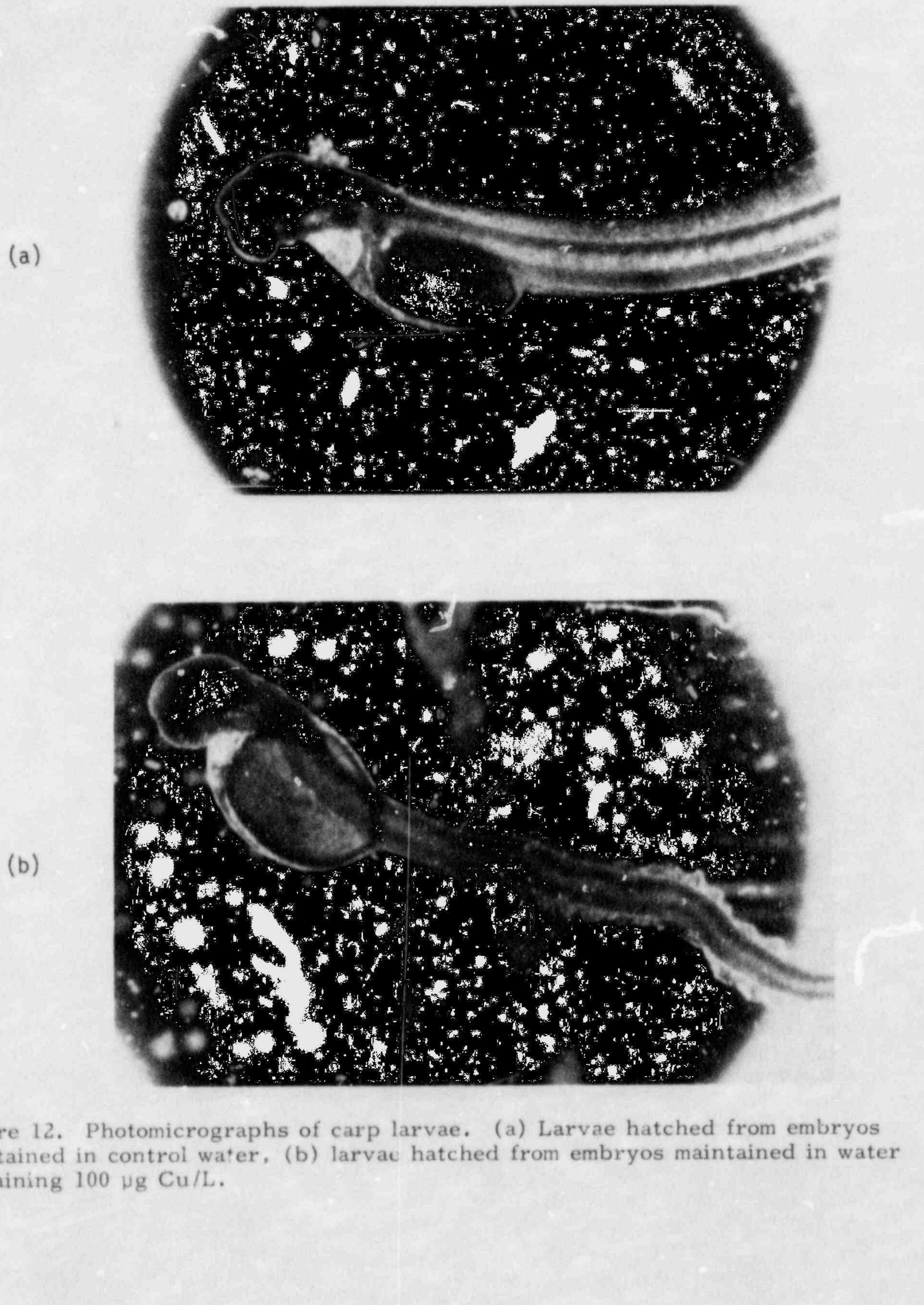

Figure 12. Photomicrographs of carp larvae. (a) Larvae hatched from embryos maintained in control water, (b) larvae hatched from embryos maintained in water containing 100 μg Cu/L.

time the experiment began. More than 65% of the copper in the bioassay water was in Chelex-100-labile forms. Also, newly hatched larvae of embryos exposed to 100 μg Cu/L had structural deformities (Fig. 13).

Effects of copper on newly hatched larvae were determined; the 48-hour LC_{50} and ICL were 120 and 110 μg Cu/L, respectively. No change in sensitivity with age was established. Copper in the bioassay water was primarily in Chelex-100-labile forms.

Bluegills

In a previous study of copper concentration and speciation in the intake and discharge waters of the H.B. Robinson Nuclear Power Station, we determined that copper was present in chemical forms and amounts that could potentially cause adverse effects on some fish populations (Fig. 14). Also, our results and those of others indicated that liver copper concentrations were high in some bluegills from the copper-impacted area of the cooling lake. We investigated the kinds and quantities of metals associated with the metalloproteins in livers of bluegills, because cadmium, copper, mercury, and zinc can be detoxified by binding to metallothioneins (MTs) (Ref. 22).

Bluegills were collected from the cooling lake near the effluent discharge from the power station, near the water intake to the cooling system, and from a control pond and were examined for total metal in muscle and liver tissues. Much lower concentrations of copper were found in muscle than in liver tissue. Also, copper levels in the environment were reflected in liver but not in muscle tissue.

Livers from bluegills from the three sampling sites were removed, homogenized, and centrifuged and the supernatant fluids processed on a high performance liquid chromatograph (HPLC) to separate proteins on the basis of their molecular weight. Eluate from the gel-permeation column was monitored continuously for UV absorbance (proteins containing aromatic amino acids absorb UV light at 280 nm) and the 35 fractions collected were analyzed for copper, cadmium, and zinc. Elution profiles of the supernatant fluids showed large differences in the kinds and quantities of metals associated with metalloproteins present in the bluegills from the three sites. Copper concentrations in the low molecular weight (LMW) protein fraction, which contains MTs considered to be sites of detoxification, were highest in bluegills from the discharge site and lowest in those from the control pond. Increased quantities of copper in the intermediate molecular weight (IMW) and high molecular weight (HMW) protein fractions, which contain metalloenzymes considered to be sites of toxic action, were found in bluegills living in the discharge site. Also, data were obtained on these same fish that indicate that copper displaced zinc from metalloenzymes in the HMW and IMW protein fractions.

Our results indicate that the labile copper released from the cooling system of the H.B. Robinson Nuclear Power Station accumulated in the livers of bluegills living in the intake and discharge sites. Furthermore, copper was present in livers of bluegills from the discharge site at concentrations that could potentially interfere with normal metabolic activity and hence impair reproduction and development.

Bluegills were also exposed to increased soluble copper under controlled laboratory conditions (Ref. 22). Laboratory populations were exposed to copper concentrations ranging from 20 to 160 μg/L at pH 5.5 and to 80 ppb at pH 7.5 (Fig. 15). Liver

Figure 13. Photomicrographs of carp larvae: (a) larvae hatched from embryos maintained in control water; (b) larvae hatched from embryos maintained in water containing 100 µg Cu/L.

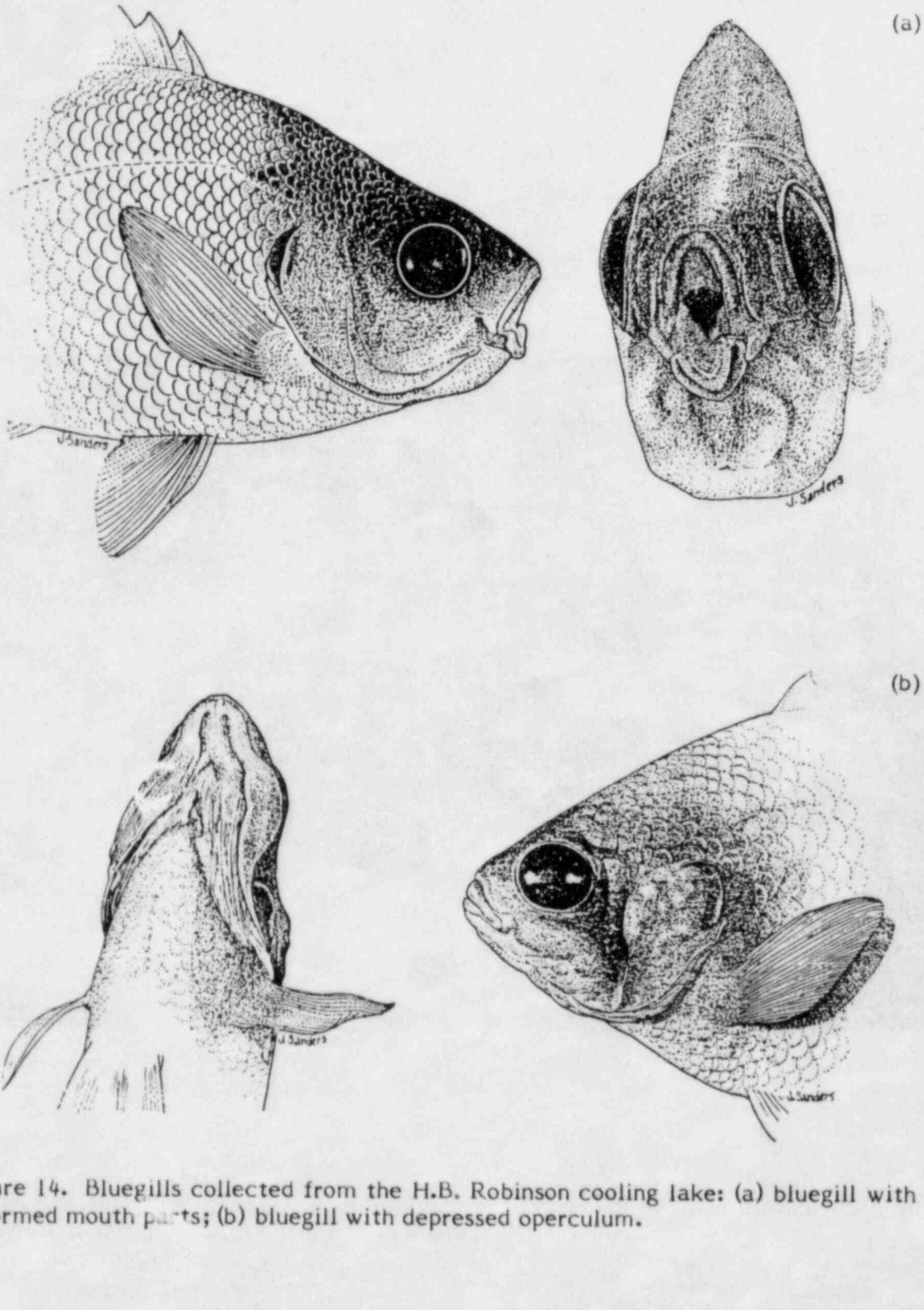

Figure 14. Bluegills collected from the H.B. Robinson cooling lake: (a) bluegill with deformed mouth parts; (b) bluegill with depressed operculum.

Figure 15. Facility where bluegills were exposed to increased soluble copper. Water was circulated continuously in the 760-L tanks. Initially, each tank contained about 70 bluegills that were 6 to 8 cm in length.

metalloproteins were separated into LMW, IMW, and HMW protein fractions using HPLC. There was an increase in the amount of copper associated with the LMW, IMW, and HMW protein fractions with increased exposure concentration and duration. Comparison of the distribution of copper among the metalloprotein fractions in fish exposed to 80 µg Cu/L at pH 5.5 and 7.5 indicated that larger amounts of copper were associated with the HMW protein fraction in fish maintained at the lower pH.

Other Fishes

Large differences have been seen in the sensitivity of fishes to copper (Refs. 7, 8). Considerable more data on copper toxicity are available for freshwater than marine fish. Of special interest are the effects of copper on early life stages. The mortalities that occur in these stages influence the strength of a given year-class and it is generally these stages that are most sensitive to copper.

EFFECTS OF COPPER SPECIATION ON FISHES

Reduced toxicity of copper to fishes has been related to the formation in the water of organic and inorganic complexes of copper. A number of investigations indicated that the presence of organic chelators in the water reduced the toxic response (Refs. 92-96). The toxic response is also reduced by the formation of copper inorganic complexes that are related to the concentration of inorganic ions in the water. It has been well documented that sensitivity to copper is inversely related to hardness and alkalinity (Refs. 97-100). According to Stiff (Ref. 98) and Chapman and McCrady (Ref. 99), the phenomenon occurs because more copper carbonate complexes form at the higher alkalinities that accompany the higher hardness values.

Increased toxicity of copper to fishes has been related to decrease in the pH of the water. Howarth and Sprague (Ref. 101) examined the interactions of pH and hardness on the toxicity of copper to rainbow trout. An increase in toxicity with a decrease in pH was apparent only in low-hardness waters. Adverse effects on bluegills living in the cooling lake at the H. B. Robinson Nuclear Power Station were attributed to increased concentrations of labile copper in this low-pH, blackwater ecosystem (Ref. 22).

It is generally accepted that Cu^{2+} is toxic to fishes. However, it is not clear that it is the only toxic form of copper. Shaw and Brown (Ref. 96) concluded that Cu^{2+} and $CuCO_3$ are the forms toxic to rainbow trout (*Salmo gairdnerii*). Pagenkopf *et al.* (Ref. 102) indicated that Cu^{2+} and $CuOH^+$ are toxic. Andrew *et al.* (Ref. 103) stated that copper toxicity is directly related to activities of Cu^{2+}, $CuOH^+$, and $Cu_2(OH)_2^{2+}$. Engel and Sunda (Ref. 104) attributed the toxicity of copper to the activity of Cu^{2+}. Chakoumakos *et al.* (Ref. 100) considered Cu^{2+}, $CuOH^+$, and $Cu(OH)_2^0$ to be important copper species that caused toxicity within the pH range they tested. However, the calculated importance of the $Cu(OH)_2^0$ depended on the stability constant used in the model to calculate species information.

There is only limited information on the toxicity of different chemical forms of copper, and it is not known whether all aquatic organisms are sensitive to the same chemical forms. For some organisms, the sites on the membranes critical in accumulating copper may bind only Cu^{2+}. However, the loss of Cu^{2+} from the water (as for example, when it is taken up by an aquatic organism) will result in the formation of more Cu^{2+} from dissociation of the other labile forms of copper with which it is in equilibrium. Under these circumstances, it is expected that the entire labile pool of copper could be available to the organisms.

Toxicity to copper is also related to the physiological state of the organism (Refs. 7, 8). Factors affecting the physiological response of the organism include salinity, temperature, dissolved O_2, pH, and light. Also important is the condition of the organism (life history stage, life cycle change, size, activity) and any previous exposure to copper or other metals that results in acclimation or adaptation.

Copper toxicity is also affected by the rate at which copper is absorbed by the organism. This could result from differences among organisms in the permeability to copper of surfaces across which transport occurs. Quantities accumulated may differ with the concentrations of copper and competing metals in the water, the salinity, and the physiological state of the organism.

The relationship between concentration in the water and quantities accumulated depends on how copper is metabolized. In some organisms, copper concentrations are not regulated and tissue concentrations are dependent on the concentration in the water and the duration of the exposure. In others, concentrations are regulated and selected tissue copper levels are relatively constant except when concentrations in the environment are so high that the influx of copper exceeds the efflux and total storage capacity of the tissues.

MODEL DEVELOPMENT

A mathematical model was developed to simulate the fate of copper in a marine environment (Ref. 105). The model describes the kinetics of copper transformation in solution from copper ions and labile complexes to tightly bound organic complexes, and the kinetics of sorption on suspended particles. It is imbedded in a two-dimensional finite-element model that can simulate advection and diffusion processes in natural waters. The model was tested by simulating realistic conditions of a slug discharge of copper ions with the cooling water from a nuclear power plant. The model performed correctly under the conditions assumed.

RECOMMENDATIONS FOR FUTURE STUDY

COPPER PARTITIONING STUDIES

1. Behavior of Copper in Cooling Lakes and Ponds. When copper is discharged continuously into either cooling ponds or lakes having slow turnover rates, a large potential exists for accumulation of copper in the ecosystem. An example of such accumulation was found at the pond at the Fort St. Vrain Nuclear Power Station. Here, blowdown from the cooling tower is discharged into a 25-acre pond; the water from the pond overflows into St. Vrain Creek, part of the natural, offsite water system. In this pond we found high concentrations of copper. Not only was the difference in total copper concentration large between the waters entering and discharging from the station property, but in one collection the concentration was greater in discharge water than in the blowdown water. In addition, the copper concentrations in bedload sediments were about ten times higher than those from other sites.

The distribution and ultimate fate of copper discharged into bodies of water with slow turnover rates must be determined in representative cooling ponds and lakes.

2. Sediment/Water Interface. In marine and freshwater ecosystems containing sediments with a high affinity for copper, the deposition and mobilization of copper into and from the bottom sediments are very significant components of the copper cycle. Bedload sediments are in a dynamic stage with the overlying water and can act as a copper sink or source depending on reactions occurring in the sediment and water.

Two important ways that copper in bedload sediments can reenter the water column are by resuspension of particles and by diffusion across the sediment-water interface. Resuspension depends on the size and composition of the particles, the rate of water flow across the surface, and disturbances from the locomotion or feeding of biota. Diffusion depends on physical, biological, and chemical processes occurring in the sediment. These processes can result in changes in redox potential and pH in the sediment and alter the physicochemical form and behavior of the copper. The rates of movement of copper across the interface under conditions that occur in the discharge zones of nuclear power stations must be evaluated.

3. Chlorination Effects. Water is commonly chlorinated to reduce fouling in the condenser tubing of power station cooling systems. This practice may affect the biota directly as free chlorine or its reaction products or indirectly by affecting the chemical form of potentially toxic metals in the discharged water. Because one of the results of chlorination is the degradation of organic matter, organic ligands that bind copper may be destroyed and the concentration of labile copper increased. Such a result would be important in estuarine and freshwater ecosystems when copper concentrations are high but the copper is in bound forms rather than labile forms. Information is needed on the chemical form of copper in water during chlorination. Chemical forms should be monitored when the complexing capacity is both high and low in the ecosystem.

4. Start-up of Coolant Water Flow. It has been argued that the high concentrations observed after start-up at Diablo Canyon were unique to that power plant. Changes in the concentration and chemical forms of copper discharged during start-up at other power stations must be documented to assess the impact of continued use of Cu-Ni alloys in cooling systems and to determine the need for countermeasures. Information on changes in copper concentration are needed from representative power stations on marine, freshwater, and estuarine ecosystems.

5. Effect of Ionic Composition on Copper Speciation. In estuarine ecosystems the percentage of soluble copper in each of the molecular-weight fractions examined differed greatly in samples collected when runoff from land was high and when it was low. At both the Salem and Surry Stations, a greater percentage of the soluble copper was present in the <1000 molecular-weight fraction at $10^{o}/oo$ salinity than at $1^{o}/oo$. The decrease in the percentage of copper that is associated with this low molecular-weight fraction with decreased salinity, although evident both in the presence and absence of naturally occurring, dissolved organic matter, was more pronounced in its absence. At higher salinities copper-organic complexes may dissociate, and small copper-inorganic complexes may form. Information is needed on the chemical speciation of copper in water of different anionic and cationic composition and in water containing different kinds and quantities of organic ligands. These data can be used in mathematical models to predict the chemical forms of copper under changing environmental conditions.

6. Impact on Kelp-Forest Communities. An important resource of open coastal ecosystems is the kelp community. Because kelp beds provide food and shelter for a large number of species, they are important in maintaining the productivity of an ecosystem. We must assess the effects of periodic chlorination, heavy metal leachates, and elevated temperature regimes on the structure of forest communities.

7. Effect of Organic Matter Content in Particles. Additional field and laboratory experiments are needed to determine the effect of the organic matter content in bedload sediments and in suspended particles on copper partitioning.

8. Effects of Titanium Tubing in Cooling Systems. Copper-nickel tubing has been replaced by titanium tubing in units at a number of nuclear power stations. We must determine the quantity and fate of titanium leached from cooling systems of nuclear power stations and assess the toxicity of titanium to biota.

9. Effects of Particle Size. Sorption of copper is related to particle size. More information is needed on the role of particle size on the K_d of bedload sediments and suspended particles. Also, further study is required of the particulate material released during start-up when copper-nickel tubing is treated with Cl-50 to reduce corrosion. Our data indicate that the treatment resulted in low labile copper concentrations, the form of copper considered to be most toxic to biota. However, before this treatment is adopted for general use, the total quantities of particulate copper released and the chemical composition, size distribution, and toxicity of the particles need to be evaluated. This information would permit an assessment of the potential distribution of the material in the environment and impact on indigenous biota.

10. Seasonal Changes in Complexation to Organic Matter. Our ultrafiltration results indicate that dissolved organic matter plays an important role in determining the chemical form of copper in water, but little information is available about the quantities of organic matter involved in copper binding and how these vary with season. This organic matter must be characterized more precisely so that the source can be identified and isolated. Organic matter could be added to effluent waters during start-up to minimize the impact of releasing labile copper.

11. Variability in Complexation to Dissolved Organic Matter. Large differences in ACC were noted in the water samples collect at the different nuclear power stations. Also, our ultrafiltration studies indicated that the distribution of soluble copper among different molecular-weight fractions differed with sample collection time. However, the number of field studies we performed was too few to characterize this variability. Because complexation affects the bioavailability and distribution of copper released in effluents, more data are neded on the seasonal changes in kinds and quantities of dissolved organic matter in the water.

12. Effect of Low pH Waters. The major physicochemical forms of copper in the H.B. Robinson water appear to be different from those at other freshwater sites. Additional data are needed to establish whether the copper partitioning and binding behavior was attributable to the lower pH of the water system and/or to differences in the kinds and quantities of organic ligands present.

The partitioning between the soluble and particulate forms of copper as well as the nature of the physicochemical forms may affect its availability to the biota.

Therefore, it is important to further study the effects of environmental factors and seasonal variations on the speciation, sedimentation, and bioavailaability to copper in the ecosystem.

TOXICITY STUDIES

1. Effect of Salinity on the Concentration and Toxicity of Copper to Oysters. As part of our study of marine ecosystems, we determined the effect of copper on oysters. Although data are available that indicate oysters accumulate increased quantities of copper at decreased salinities, the effect of changes in salinity on the toxic response is unknown.

Oysters are transferred to areas of lower salinity by some growers to "fatten them up." This practice may result in detrimental changes in oysters if the sensitivity to copper is increased at lower salinities. Alterations that might be expected are degenerative changes in condition (increased water and decreased organic matter in the meat), increased copper content, and discoloration of the shell. The effect of changing salinity on the sensitivity of both adult and embryonic oysters must be evaluated.

2. Copper Concentrations in Oysters. Copper concentrations in oysters living in the vicinity of power stations are higher during some seasons of the year than the proposed guidelines of 100 µg Cu/g wet weight. The concentrations in water that result in these high copper concentrations in oysters are not known. In laboratory experiments, oysters living in water containing 25 µg Cu/L had concentrations as high as 700 µg Cu/g wet weight as steady state conditions of uptake and loss were approached. Because copper concentrations in effluent waters are generally less than 25 µg Cu/L but more than the ambient concentration, the relationship between concentration in the water and that in oysters must be defined in water with copper concentration less than 25 µg Cu/L.

3. Availability of Particulate Copper. Filter-feeding shellfish commonly inhabit and reproduce in areas of estuaries receiving effluents from nuclear power stations. In the past, adverse effects on oysters living in the vicinity of other power stations have been reported. These include reduced commercial value because of discoloration of shells and greening of soft tissues. An evaluation is needed of the bioavailability of particulate copper released during start-up and periods of high runoff and of the potential impact of this copper on the loading of detoxification systems. The quantities of copper bound to metal-binding proteins and incorporated in granules should be monitored with changes in season and following start-up of water flowing through the condensers. These kinds of data could be used to to determine whether detoxification capabilities are being exceeded during start-up and are resulting in adverse effects on the general health of shellfish populations.

4. Sublethal Effects of Copper. The effect of copper on mortality has been evaluated in a group of aquatic organisms. These data are useful in assessing the effects of episodic releases of high levels of copper, but are of limited value in assessing the effects of continuous exposure to the considerably lower levels of copper that are present in the vicinity of many power stations.

Low levels of pollutants, singly or in combination, result in stressed populations. Stress may reduce the potential for growth of an organism, its reproductive capacity, its ability to resist further change, or its chances of survival.

Manifestations of stress may take place at different levels of organization in the organism. Small changes may occur in the morphological, physiological, cytological, and biochemical conditions of the organisms and may involve both somatic and germ cells.

Representative aquatic organisms must be evaluated with sensitive tests to identify populations in jeopardy so that appropriate measures can be adopted before irreparable changes occur in the environment.

5. Interactions of Power Station Pollutants. Biota living in the discharge area of nuclear power stations might be exposed to increased copper, detergents, dispersants, chlorine, and temperatures. Some information is available on the response of organisms to these factors separately, but little or no data are available on the interaction of these environmental stresses. Data are needed on the synergistic effects of pollutants on different life stages of important food organisms and on key food-chain organisms.

6. Effects of Copper-Laden Sediments on Demersal Embryos. Experiments should be conducted to determine the role that copper-laden interstitial waters and sediments play in the toxic exposure of demersally spawned fish embryos. Fish embryos spawned in near-bottom environments are exposed not only to copper in the water column, but also to sediments that may contain high concentrations of copper. This copper may be mobilized from the sediments to the sediment-water interface at concentrations many times above ambient water concentrations. Based on copper exposure in water only, the demersal spawning behavior, as represented by the Pacific herring embryo, seems to be more sensitive than the pelagic anchovy embryo (ILCs of 33 and 190 µg Cu/L respectively). Demersal embryos should be exposed to sediments contaminated with known levels of copper.

7. Relationship of Ovoviviparous Spawning Strategy to Copper Sensitivity. Experiments should be conducted in which the effect of sublethal adult exposure is related to the embryonic and larval development of ovoviviparous fishes. The anchovy and herring represent the pelagic and demersal spawning strategies, respectively. An additional spawning strategy that should be examined is that employed by the ovoviviparous fishes. Embryos of such fishes are retained in the lumen of the ovary until shortly before yolk sac absorption is complete. Fewer embryos and larvae are produced compared with other spawning strategies, and sublethal impacts on adults carrying embryos may have gross effects on fecundity.

8. Effects of Copper in Food. Aquatic plants have been shown to concentrate copper from the water, and because some aquatic organisms are detritus feeders, they accumulate copper body burdens through ingestion of copper-laden plant material. Further studies examining the toxicity of copper uptake through food are required before the impact of power-station-related copper releases on aquatic organism can be completely assessed.

9. Copper Accumulation by Freshwater Clams Exposed to Sublethal Concentrations of Copper. Copper concentrations in clam tissues were higher in all animals held in water to which copper was added. We do not know whether the copper concentrations in tissues increase with increasing concentrations in the water or reach a maximum value at a given copper concentration. The relationship between copper concentrations in tissues and water should be investigated both in field and laboratory studies to obtain data on concentration factors and the effect of the

chemical form of copper on its accumulation in tissues. These data are needed for models to predict tissue copper burdens and to recommend limits on copper concentrations to prevent harm to the biota.

10. Physiological Effects of Exposure to Sublethal Concentrations of Copper on Freshwater Clams. Copper concentrations as low as 10 µg/L increased the mortality of adult clams. In addition we obtained evidence of reduced feeding as a result of increased copper. More data are needed on the effects of continuous exposure to low levels of copper on other physiological processes. This information can be used to assess impairment of the ability of these animals to compete effectively with others in the environment.

11. Sensitivity of Early Life Stages Brooding in the Gill Chambers of Freshwater Clams. The brooding of prejuvenile larvae in the gill chamber may protect them from high concentrations of toxic chemicals. We should compare concentrations and chemical forms of copper in the fluid in the mantle cavity and in surrounding waters to determine whether the mantle changes the availability of copper to the organism.

12. Effects of Sublethal Exposure on Reproductive Capacity. If carp live in the discharge zones of nuclear power stations whose cooling systems contain copper alloys, the fish are exposed throughout their entire life cycle to small increases in copper concentration in the water. Information is needed about the effects of such exposures on reproductive capacity. Carp early life history stages from pristine and polluted ecosystems must be examined to determine whether there are differences in mortality rates, number of abnormal individuals, and percent of eggs that hatch.

13. Detoxification of Copper in Carp. Copper concentrations in muscle and liver tissues of carp were highly variable and did not appear to be related to exposure concentrations. The effect of increased concentrations of copper in tissues may not be related to the total copper present but to the concentration of an active form. Information is available indicating that heavy metals can be detoxified by complexing with MTs. Data are needed on the concentrations of copper in the metalloprotein pools of adult carp exposed to increased concentrations of copper and on the relationship of the organo-metal concentrations to deleterious effects and total tissue copper concentrations.

14. MTs Residence Time in Adult Bluegills. The kinetics of MT production in bluegills should be evaluated. The time required for depuration of the copper bound to MTs will determine in part the frequency of intermittent high concentrations of copper in the water that can be tolerated with a minimum of adverse effects on bluegill populations.

15. MTs in Early Life History Stages. The role of MTs in early life stages should be determined. No information is available regarding how the MT-detoxification system is activated and how the capacity of the system changes with development. These kinds of data are required to schedule pulse releases from nuclear power stations so that the impact to eggs, larvae, and fry of bluegills is minimized.

17. Criteria must be Established for the Selection of Appropriate Concentration Factors (CFs) for Models to Predict the Quantities of Copper Transferred Along Food Chains. The CFs calculated from field and laboratory data may differ greatly. More information is needed on factors affecting the metabolism of copper in aquatic organisms to select the appropriate CF value to evaluate a specific ecosystem.

REFERENCES

1. H. V. Leland, E. D. Copenhaver, and L. S. Corrill, "Heavy Metals and Other Trace Metals," *J. Water Pollut. Control Fed. 46*, 1452-1475 (1974).

2. H. V. Leland, E. D. Copenhaver, and D. J. Wilkes, "Heavy Metals and Other Trace Elements," *J. Water Pollut. Control Fed. 47*, 1635-1656 (1975).

3. H. V. Leland, D. J. Wilkes, and E. D. Copenhaver, "Heavy Metals and Related Trace Metals," *J. Water Pollut. Control Fed. 48*, 1459-1486 (1976).

4. D. Saward, A. Stirling, and G. Topping, "Experimental Studies on the Effects of Copper on a Marine Food Chain," *Mar. Biol. 29*, 351-361 (1975).

5. A. G. Lewis and P. H. Whitfield, *The Biological Importance of Copper in the Sea*, Report No. 223, International Copper Research Association (1974).

6. P. V. Hodson, U. Borgmann, and H. Shear, "Toxicity of Copper to Aquatic Biota," Part II, pp. 307-372, in *Copper in the Environment*, J. O. Nriagu, Ed., John Wiley and Sons, NY (1979).

7. F. L. Harrison, *A Review of the Impact of Copper Released into Marine and Estuarine Environments*, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-53042 and NUREG/CR-2823 (1982).

8. F. L. Harrison and D.J. Bishop, *A Review of the Impact of Copper Released into Freshwater Environments*, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-53488 and NUREG/CR-3478 (1984).

9. R. L. Schmidt, "Copper in the Marine Environment--Part I," *CRC Critical Rev. Environ. Control 8*, 101-152 (1978).

10. E. A. Boyle, "Copper in Natural Waters," Part I, pp. 77-88, *Copper in the Environment*, J. O. Nriagu, Ed., John Wiley and Sons, NY (1979).

11. J. O. Leckie and J. A. Davis, "Aqueous Environmental Chemistry of Copper," Part I, pp. 89-121, *Copper in the Environment*, J. O. Nriagu, Ed., John Wiley and Sons, NY (1979).

12. F. L. Harrison, D. J. Bishop, R. R. Emerson, and D. W. Rice, Jr., *Concentration and Speciation of Copper in Receiving Waters of the San Onofre and Diablo Canyon Nuclear Power Stations*, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-52706 and NUREG/CR-0750 (1980).

13. F. L. Harrison, D. J. Bishop, D. W. Rice, Jr., and J. P. Knezovich, *Copper in the Intake and Discharge Zones of the Surry and Salem Nuclear Power Stations*, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-53325 and NUREG/CR-2965 (1983).

14. F. L. Harrison, D. J. Bishop, D. W. Rice, Jr., R. Emerson, and B. J. Smith, *Concentration and Distribution of Copper in Effluents from Three Freshwater Nuclear Power Stations*, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-53482 and NUREG/CR-3358 (1983)

15. F. L. Harrison, D. J. Bishop, D. W. Rice, Jr., and J. P. Knezovich, Concentration and Distribution of Copper in Effluents from the H. B. Robinson Nuclear Power Station, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-53047 and NUREG/CR-3132 (1983).

16. R. R. Emerson and F. L. Harrison, Copper Distribution in Abiotic Compartments of Aquatic Ecosystems Near the Diablo Canyon and San Onofre Nuclear Power Stations, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-52555 and NUREG/CR-1090 (1981).

17. B. M. Smith and F. L. Harrison, Sensitivity of Macrocystis Gametophytes to Copper, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-52481 and NUREG/CR-0684 (1978).

18. D. W. Rice, Jr. and F. L. Harrison, Copper Sensitivity of the Northern Anchovy Engraulis mordax, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-52499 and NUREG/CR-0748 (1979).

19. F. L. Harrison and D. W. Rice, Jr., Effect on Oysters of Increased Levels of Copper in Seawater Lawrence Livermore Laboratory, Livermore, CA, UCRL-52706 and NUREG/CR-0747 (1978).

20. F. L. Harrison, J. P. Knezovich, and D. W. Rice, Jr., Effects of Copper on Adult and Early Life Stages of the Freshwater Clam, Corbicula manilensis, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-52741 and NUREG/CR-1379 (1981).

21. F. L. Harrison and D. W. Rice, Jr., The Sensitivity of Adult, Embryonic, and Larval Carp, Cyprinus carpio, to Copper, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-52726 and NUREG/CR-1089 (1981).

22. F. L. Harrison and J. R. Lam, Concentrations of Copper-Binding Proteins in Livers of Bluegills from the Cooling Lake at the H. B. Robinson Nuclear Power Station, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-53041 and NUREG/CR-2822 (1982).

23. D. W. Rice, Jr. and F. L. Harrison, The Sensitivity of Adult Embryonic, and Larval Crayfish, Procambarus clarkii, to Copper, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-53048 and NUREG/CR-3133 (1983).

24. F. L. Harrison and J. R. Lam, Concentrations of Copper-Binding Proteins in Livers of Bluegills Exposed to Increased Concentrations of Soluble Copper, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-53487 and NUREG/CR-3477 (1984).

25. J. D. Kinrade and J. C. Van Loon, "Solvent Extraction for Use with Flame Atomic Absorption Spectometry," Anal. Chem. 48, 1984 (1976)

26. J. P. Riley and D. Taylor, "Chelating Resins for the Concentration of Trace Elements from Sea Water and Their Analytical Use in Conjunction with Atomic Absorption Spectrophotometry," Anal. Chim. Acta 40, 479-487 (1968).

27. T. W. Florence and G. E. Batley, "Determination of Copper in Seawater by Anodic Stripping Voltametry," J. Electroanal. Chem. 75, 791-798 (1977).

28. Y. K. Chau and K. Lum-Shue-Chan, "Determination of Labile and Strongly Bound Metals in Lake Water," Water Res. 8, 383-388 (1974).

29. J. D. H. Strickland and T. R. Parson, A Practical Handbook of Seawater Analysis, (Fisheries Research Board of Canada, Ottawa, Canada, 1962).

30. J. W. Warrick, S. G. Sharp, and S. J. Friedrick, Chemical, Biological, and Corrosion Investigations Related to the Testing of the Diablo Canyon Unit 1 Cooling Water System, Pacific Gas and Electric Co., Dept. of Engineering Research, Report No. 7333, 129 (1975).

31. D. R. Young, Tsu-Kai Jan, and M. D. Moore, "Metals in Power Plant Cooling Water Discharges," Coastal Water Research Project Annual Report (1977).

32. M. I. Abdullah and L. G. Royle, "A Study of the Dissolved and Particulate Trace Elements in the Bristol Channel," J. Mar. Biol. Ass. U.K. 54, 581-597 (1972).

33. G. A. Knauer and J. H. Martin, "Seasonal Variations of Cadmium, Copper, Manganese, Lead and Zinc in Water and Phytoplankton in Monterey Bay, California," Limnol. Oceanog. 18, 597-604 (1973).

34. G. E. Batley and T. M. Florence, "Determination of the Chemical Forms of Dissolved Cadmium, Lead and Copper in Seawater," Mar. Chem. 4, 347-363 (1976).

35. P. Figura and B. McDuffie, "Use of Chelex Resin for Determination of Labile Trace Metal Functions in Aqueous Ligand Media and Comparison of the Method with Anodic Stripping Voltametry." Anal. Chem. 51, 120-125 (1979).

36. R. R. Brooks, B. J. Presley, and I. R. Kaplan, "APDC-MIBK Extraction System for the Determination of Trace Elements in Saline Waters by Atomic-Absorption Spectrophotometry," Talanta 14, 809-816 (1967).

37. K. Kremling and H. Petersen, "APDC-MIBK Extraction System for the Determination of Copper and Iron in 1 CM^3 of Sea Water by Flameless Atomic-Absorption Spectrometry," Anal. Chim. Acta 70, 35-39 (1974).

38. D. S. Flett, "Chemical Kinetics and Mechanisms in Solvent Extraction of Copper Chelates," Accounts of Chemical Research 10, 99-104 (1977).

39. T. M. Florence and G. E. Batley, "Trace Metals Species in Seawater. I. Removal of Trace Metals from Seawater by a Chelating Resin," Talanta 23, 197-186 (1976).

40. T. M. Florence, "Anodic Stripping Voltametry with a Glassy Carbon Electrode Mercury Plated in Situ," J. Electronanal. Chem. 35, 237-245 (1970).

41. J. Gardiner and M. J. Stiff, "The Determination of Cadmium, Lead, Copper, and Zinc in Groundwater, Estuarine Water, Sewage, and Sewage Effluent by Anodic Stripping Voltametry," Water Res. 9, 517 (1975).

42. W. Lund and D. Onshus, "The Determination of Copper, Lead and Cadmium in Seawater by Differential Pulse Anodic Stripping Voltametry," Anal. Chim. Acta 86, 109-122 (1976).

43. H. W. Nurnberg, P. Valenta, L. Mart, B. Raspor, and L. Sipos, "Application of Polarography and Voltametry to Marine and Aquatic Chemistry," Z. Anal. Chem. 282, 357-367 (1976).

44. S. F. Sugai and M. L. Healy, "Voltammetric Studies of the Organic Association of Copper and Lead in Two Canadian Inlets," Mar. Chem. 6, 291-308 (1978).

45. M. A. Rashid, "Role of Humic Acids of Marine Origin and Their Different Molecular Weight Fractions in Complexing Di- and Tri-Valent Metals," Soils Science 3, 298-305 (1971).

46. R. G. Smith, "Evaluation of Combined Applications of Ultrafiltration and Complexation Capacity Techniques to Natural Waters," Anal. Chem. 48, 74-76 (1976).

47. P. M. Williams and R. J. Baldwin, "Cupric Ion Activity in Coastal Seawater," Marine Science Commun. 2, 161-181 (1976).

48. W. Sunda and R. R. L. Guillard, "The Relationship Between Cupric Ion Activity and the Toxicity of Copper to Phytoplankton," J. Mar. Res. 34, 511-529 (1976).

49. W. G. Sunda and J. A. M. Lewis, "Effect of Complexation by Natural Organic Ligands on the Toxicity of Copper to a Unicellular Alga, Monochrysis lutheri," Limnol. Oceanog. 23, 870-876 (1978).

50. A. Demayo, M. C. Taylor, and K. W. Taylor, "Effects of Copper on Humans, Laboratory and Farm Animals, Terrestrial Plants, and Aquatic Life," CRC Critical Rev. Environ. Control 12, 183-255 (1982).

51. D. K. Nordstrom, L. N. Plummer, T. M. L. Wigley, T. J. Wolery, J. W. Ball, E. A. Jenne, R. L. Bassett, D. A. Crerar, T. M. Florence, B. Fritz, M. Hoffman, G. R. Holdren, Jr., G. M. Lafon, S. V. Mattigod, R. E. McDuff, F. Morel, M. M. Reddy, G. Sposito, and J. Thrailkill, "A Comparison of Computerized Chemical Models for Equalization Calculations in Aqueous Systems," pp. 857-892, in Chemical Modeling in Aqueous Systems, Everett A. Jenne, Ed.; American Chemical Society, Washington, D.C., (1979).

52. T. A. Neubecker, and H. E. Allen, "The Measurement of Complexation Capacity and Conditional Stability Constants for Ligands in Natural Waters," Water Res. 17, 1-14 (1983).

53. R. Kunkel and S. E. Manahan, "Atomic Absorption Analysis of Strong Heavy Metal Chelating Agents in Water and Waste Water," Anal. Chem. 45, 1465-1468 (1973).

54. P. G. C. Campbell, M. Bisson, R. Gagne, and A. Tessier, "Critical Evaluation of the Copper (II) Solubilization Method for the Determination of the Complexation Capacity of Natural Waters," Anal. Chem. 49, 2358-2363 (1977).

55. J. F. Elder, K. E. Osborn, and C. R. Goldman, "Iron Transport in a Lake Tahoe Tributary and Its Potential Influence Upon Phytoplankton Growth," Water Res. 10, 783-788 (1976).

56. R. A. Kerr, and J. G. Quinn, "Chemical Comparison of Dissolved Organic Matter Isolated from Different Oceanic Environments," Mar. Chem. 8, 217-229 (1980).

57. Y. Avnimelech, and A. Raveh, "Decomposition of Chelates Leached from Waste Disposal Sites," J. Environ. Qual. 11, 69-72 (1982).

58. E. W. Davey, M. J. Morgan, and S. J. Erickson, "A Biological Measurement of the Copper Complexation Capacity of Seawater," Limnol. Oceanog. 18, 993-997 (1973).

59. R. Gachter, J. S. Davis, and A. Mares, "Regulation of Copper Availability to Phytoplankton by Macromolecules in Lake Waters," Environ. Sci. Technol. 12, 1416-1421 (1978).

60. P. A. Gillespie and R. F. Vaccaro, "A Bacterial Bioassay for Measuring the Copper Chelation Capacity of Seawater," Limnol. Oceanog. 23, 543-548 (1978).

61. Y. K. Chau, "Complexing Capacity of Natural Water--Its Significance and Measurement," J. Chromat. Sci. 11, 579 (1973).

62. T. A. O'Shea and K. H. Mancy, "Characterization of Trace Metal Organic Interactions by Anodic Stripping Voltametry," Anal. Chem. 48, 1603-1607 (1976).

63. J. C. Duinker and C. J. M. Kramer, "An Experimental Study on the Speciation of Dissolved Zinc, Cadmium, Lead, and Copper in the River Rhine and North Sea Water by Differential Pulse Anodic Stripping Voltametry," Mar. Chem. 5, 207-228 (1977).

64. K. W. Hanck and J. W. Dillard, "Evaluation of Micromolar Compleximetric Titrations for the Determination of the Complexing Capacity of Natural Waters," Anal. Chim. Acta 89, 329-338 (1977).

65. M. S. Shuman and G. P. Woodward, "Stability Constants of Copper-Organic Chelates in Aquatic Samples," Environ. Sci. Technol. 11, 809-813 (1979).

66. P. Figura and B. McDuffie, "Determination of Labilities of Soluble Trace Metal Species in Aqueous Environmental Samples by Anodic Stripping Voltametry and Chelex Columns and Batch Methods," Anal. Chem. 52, 1433-1439 (1980).

67. M. R. Hoffmann, E. C. Yost, S. J. Eisenreich, and W. J. Maier, "Characterization of Soluble and Colloid Phase Metal Complexes in River Water by Ultrafiltration. A Mass-Balance Approach." Environ. Sci. Technol. 15, 655-661 (1981).

68. B. T. Hart and S. H. R. Davies, "Copper Complexing Capacity of Waters in the Magela Creek System, Northern Australia," Environ. Technol. Lett. 2, 205-214 (1981).

69. B. Lazar, A. Katz, S. Ben-Yaakov, "Copper Complexing Capacity of Sea Water: A Critical Appraisal of the Direct Anodic Stripping Voltametry," Mar. Chem. 10, 221-232 (1981).

70. C. M. G. Van den Berg and J. R. Kramer, "Determination of Complexing Capacities of Ligands in Natural Waters and Conditional Stability Constants of the Copper Complexes by Means of Manganese Dioxide," Anal. Chim. Acta 106, 113-120 (1979).

71. S. Ramamoorthy and D. J. Kushner, "Heavy Metal Binding Components of River Water," J. Fish Res. Bd. Can. 32, 1755-1766 (1975).

72. J. P. Giesy, Jr., L. A. Briese, and G. J. Leversee, "Metal Binding Capacity of Selected Maine Surface Waters," Environ. Geol. 2, 257-268 (1978).

73. S. J. Erickson, N. Lackie, and T. E. Maloney, "A Screening Technique For Estimating Copper Toxicity to Estuarine Phytoplankton," J. Water Pollut. Cont. Fed. 48, 270-278 (1970).

74. E. Steeman-Nielsen and S. Wium-Andersen, "The Influence of Cu on Photosynthesis and Growth in Diatoms," Physiol. Plant. 24, 480-484 (1971).

75. S. J. Erickson, "Toxicity of Copper to Thalassiosira pseudonana in Unenriched Inshore Seawater," J. Phycol. 8, 318-323 (1972).

76. O. P. Morris and G. Russell, "Effect of Chelation on Toxicity of Copper," Mar. Pollut. Bull. 4, 159-160 (1973).

77. D. M. Anderson and F. M. M. Morel, "Copper Sensitivity of Gonyaulax tamarensis," Limnol. Oceanog. 23, 283-295 (1978).

78. N. M. L. Morel, J. G. Bueter, and F. M. M. Morel, "Copper Toxicity to Skeletonema costatum," J. Phycol. 14, 43-48 (1978).

79. R. Wagemann and J. Barica, "Speciation and Rate of Loss of Copper from Lakewater with Implication to Toxicity," Water Res. 13, 515-523 (1979).

80. G. S. Canterford and D. R. Canterford, "Toxicity of Heavy Metals to the Marine Diatom Ditylum brightwellii (West) Grunow," J. Mar. Biol. Ass. U.K. 60, 227-242 (1980).

81. G. A. Jackson and J. J. Morgan, "Trace Metal-Chelator Interactions and Phytoplankton Growth in Seawater Media: Theoretical Analysis and Comparison With Reported Observations," Limnol. Oceanog. 23, 268-282 (1978).

82. N. S. Fisher and D. Frood, "Heavy Metals and Marine Diatoms: Influence of Dissolved Organic Compounds on Toxicity and Selection for Metal Tolerance Among Four Species," Mar. Biol. 59, 85-93 (1980).

83. R. Petersen, "Influence of Copper and Zinc on the Growth of a Freshwater Alga, Scenedesmus quadricauda: The Significance of Chemical Speciation," Environ. Sci. Technol. 16, 443-447 (1982).

84. M. Gnassia-Barelli, M. Romeo, F. Laumond, and D. Pesando, "Experimental Studies on the Relationship Between Natural Copper Complexes and Their Toxicity to Phytoplankton," Mar. Biol. 47, 15-19 (1978).

85. C. M. G. Van den Berg, P. T. S. Wong, and Y. K. Chan, "Measurement of Complexing Materials Excreted from Algae and their Ability to Ameliorate Copper Toxicity," J. Fish. Res. Bd. Can. 36, 901-905 (1979).

86. F. L. Harrison, J. S. Knezovich, and J. S. Tucker, The Sensitivity of Embryos of the Pacific Oyster Crassostrea gigas to Different Chemical Forms of Copper, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-52725 and NUREG/CR-1088 (1980).

87. A. G. Lewis, A. Ramnarine, and M. S. Evans, "Natural Chelators--An Indication of Activity with the Calanoid Copepod Euchaeta japonica," Mar. Biol. 11, 1-4 (1971).

88. A. G. Lewis, P. H. Whitfield, and A. Ramnarine, "Some Particulate and Soluble Agents Affecting the Relationship Between Metal Toxicity and Organism Survival in the Calanoid Copepod, Euchaeta japonica," Mar. Biol. 17, 215-221 (1972).

89. R. W. Andrew, "Toxicity Relationships to Copper Forms in Natural Waters," in Proc. Workshop on Toxicity of Biota to Metal Forms in Natural Water, Duluth, Minnesota, October 7 to 8, 1975, R. W. Andrew, P. V. Hodson, and D. E. Konasewich, Eds., Great Lakes Research Advisory Board, International Joint Commission (1976).

90. Y. S. Young, J. M. Gurtisen, C. W. Apts, and E. A. Crecelius, "The Relationship Between the Copper Complexing Capacity of Sea Water and Copper Toxicity in Shrimp Zoeae," Mar. Environ. Res. 2, 265-275 (1979).

91. U. Borgmann, "Determination of Free Metal Ion Concentrations Using Bioassays," Can. J. Fish. Aquat. Sci. 38, 999-1002 (1981).

92. N. Grande, "Effect of Copper and Zinc on Salmonid Fishes," Adv. Water Poll. Res. 1, 97-111 (1967).

93. J. B. Sprague, "Promising Anti-Pollutant: Chelating Agent NTA Protects Fish from Copper and Zinc," Nature 220, 1345-1346 (1968).

94. R. C. H. Wilson, "Prediction of Copper Toxicity in Receiving Waters," J. Fish Res. Bd. Can. 29, 1500-1502 (1972).

95. P. Zitko, W. V. Carson, and W. G. Carson, "Prediction of Incipient Lethal Levels of Copper to Juvenile Atlantic Salmon in the Presence of Humic Acid by Cupric Electrode," Bull. Environ. Contam. Toxicol. 10, 265-271 (1973).

96. T. L. Shaw and V. M. Brown, "The Toxicity of Some Forms of Copper to Rainbow Trout," Water Res. 8, 377-382 (1974).

97. R. Lloyd, and D. W. M. Herbert, "The Effect of the Environment on the Toxicity of Poisons to Fish," J. Inst. Public Health Engr. 61, 132-43 (1962).

98. M. J. Stiff, "The Chemical States of Copper in Polluted Fresh Water and a Scheme of Analysis to Differentiate Them," Water Res. 5, 585-599 (1971).

99. G. A. Chapman and J. K. McCrady, "Copper Toxicity: a Question of Form," in *Recent Advances in Fish Toxicology*, a Symposium, Ecological Research Series, EPA 600 13-77-085, 132-151 (1977).

100. C. Chakoumakos, R. C. Russo, and R. V. Thurston, "Toxicity of Copper to Cutthroat Trout (*Salmo clarki*) Under Different Conditions of Alkalinity, pH and Hardness," *Environ. Sci. Technol.* *13*, 213-219 (1979).

101. R. S. Howarth and J. B. Sprague, "Copper Lethality to Rainbow Trout in Waters of Various Hardness and pH," *Water Res.* *12*, 455-462 (1978).

102. G. K. Pagenkopf, R. Russo, and R. Thurston, "Effect of Complexation on Toxicity of Copper to Fishes," *J. Fish. Res. Bd. Can.* *31*, 462 (1974).

103. R. W. Andrew, K. E. Biesinger, and G. E. Glass, "Effects of Inorganic Complexing on the Toxicity of Copper to *Daphnia magna*," *Water Res.* *11*, 309-315 (1977).

104. D. W. Engel and W. G. Sunda, "Toxicity of Cupric Ion to Eggs of the Spot *Leiostomus xanthurus* and the Atlantic Silverside *Menidia menidia*," *Mar. Biol.* *50*, 121-126 (1979).

105. G. T. Orlob, D. Hrovat, T. Wakeman, and F. Harrison, *A Mathematical Model for Simulation of the Fate of Copper in a Marine Environment*, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-52728 and NUREG/CR-1098 (1982).

NRC FORM 335
(7-77)

U.S. NUCLEAR REGULATORY COMMISSION

BIBLIOGRAPHIC DATA SHEET

1. REPORT NUMBER (Assigned by DDC)
NUREG/CR-3476
UCRL-53486

4. TITLE AND SUBTITLE (Add Volume No., if appropriate)
Chemicals in Effluent Waters From Nuclear Power Stations:
The Distribution, Fate and Effects of Copper

2. (Leave blank)

3. RECIPIENT'S ACCESSION NO.

7. AUTHOR(S)
F.L. Harrison

5. DATE REPORT COMPLETED
MONTH September
YEAR 1983

9. PERFORMING ORGANIZATION NAME AND MAILING ADDRESS (Include Zip Code)
Lawrence Livermore National Laboratory
P.O. Box 5507, Mail Code L-453
Livermore, CA 94550

DATE REPORT ISSUED
MONTH April
YEAR 1984

6. (Leave blank)

8. (Leave blank)

12. SPONSORING ORGANIZATION NAME AND MAILING ADDRESS (Include Zip Code)
Division of Health, Siting and Waste Management
Office of Nuclear Regulatory Research
U.S. Nuclear Regulatory Commission
Washington, DC 20555

10. PROJECT/TASK/WORK UNIT NO.

11. CONTRACT NO.
A0119

13. TYPE OF REPORT
Topical

PERIOD COVERED (Inclusive dates)

15. SUPPLEMENTARY NOTES

14. (Leave blank)

16. ABSTRACT (200 words or less)

This report provides a summary of research performed to determine the physicochemical forms and fate of copper in effluents from power stations adjacent to aquatic ecosystems with water that differs in salinity, pH, and concentrations of organic and inorganic constituents. In addition, research performed to evaluate responses of selected ecologically and economically important marine and freshwater organisms to increased concentrations of soluble copper is reviewed.

Copper concentration and speciation showed that the quantities of copper associated with particles, colloids, and organic and inorganic ligands differed with the site, season, and mode of operation of the station. Under normal operating conditions, the differences between influent and effluent waters were generally small, and most of the copper was in bound (complexed) species except when low pH water was circulated. However, copper was high in concentration and present in labile species during start-up of water circulation through some cooling systems and during changeover from open-cycle to closed-cycle operation.

The toxic response to copper differed with the species and life stage of the organism and with the chemical form of copper in the water. Our primary emphasis was on acute effects. However, sublethal effects of copper on a population of bluegills living in a power station cooling lake containing water of low pH and on a population exposed to increased soluble copper in the laboratory were also assessed.

17. KEY WORDS AND DOCUMENT ANALYSIS
copper, copper corrosion in products, copper toxicity, sediments, nuclear power stations, effluents, fish, clams, crayfish, oysters

17a. DESCRIPTORS

17b. IDENTIFIERS/OPEN-ENDED TERMS

18. AVAILABILITY STATEMENT
UNLIMITED

19. SECURITY CLASS (This report)
UNCLASSIFIED

21. NO. OF PAGES

20. SECURITY CLASS (This page)
UNCLASSIFIED

22. PRICE
$

NRC FORM 335 (7-77)

UNITED STATES
NUCLEAR REGULATORY COMMISSION
WASHINGTON, D.C. 20555

OFFICIAL BUSINESS
PENALTY FOR PRIVATE USE, $300

FOURTH-CLASS MAIL
POSTAGE & FEES PAID
USNRC
WASH. D.C.
PERMIT No. G67

120555078877 1 IANIRE
US NRC
ADM-DIV OF TIDC
POLICY & PUB MGT BR-PDR NUREG
W-501
WASHINGTON DC 20555

THE DISTRIBUTION, FATE, AND EFFECTS OF COPPER