Journal Title: Marine Biology

ISSN:

Volume: 66
Issue: 1
Month/Year: 1982
Pages: 77-82

Article Author: Zamuda CD
Article Title: Bioavailability of dissolved copper to the American oyster Crassostrea virginica. I. Importance of chemical speciation

Cited In:

Marine Biology 66, 77–82 (1982)

MARINE BIOLOGY

Bioavailability of Dissolved Copper to the American Oyster *Crassostrea virginica*. I. Importance of Chemical Speciation

C. D. Zamuda [1,*] and W. G. Sunda [2,**]

[1] Department of Environmental Sciences, University of Virginia; Charlottesville, Virginia 22903, USA

[2] National Marine Fisheries Service, NOAA, Southeast Fisheries Center, Beaufort Laboratory; Beaufort, North Carolina 28516, USA

Abstract

The effect of complexation on the accumulation of dissolved copper by the American oyster *Crassostrea virginica* was determined in chemically defined exposure media. The speciation of copper was varied by varying the concentrations of total copper and model chelator, nitrilotriacetic acid (NTA). Accumulation of copper in 14-d experiments was related to the cupric ion activity and not the concentration of chelated copper. Rapid accumulation of copper occurred at cupric ion activities above 10^{-10} M and there was no measurable accumulation at 10^{-11} M.

Introduction

The availability of trace metals to aquatic organisms is influenced by the metal's chemical speciation. Although trace metals can exist in a variety of chemical forms or species, metal toxicity to many aquatic organisms is related to the metal ion activity rather than the total metal concentration (Sunda and Guillard, 1976; Anderson and Morel, 1978). Studies on the accumulation of dissolved trace metals by bivalve molluscs, however, usually have not considered the chemical speciation of trace metals in exposure media (Galtsoff, 1964; Brooks and Rumsby, 1965; Shuster and Pringle, 1969).

Certain trace metals, such as copper, are essential for normal growth and development, but elevated levels can be toxic, resulting in altered productivity and a decreased capacity to withstand environmental stress. The toxicity of copper to bivalve molluscs is well documented (Shuster and Pringle, 1969; Nelson *et al.*, 1976; Davenport, 1977).

Bivalve molluscs have been used extensively as biological indicators of heavy metal pollution (Goldberg, 1975; Phillips, 1980); but the processes and factors controlling metal availability to these organisms are poorly understood. Studies assessing the effects of chemical speciation on accumulation of dissolved metal have not established unequivocably whether organic complexation increases or decreases the bioavailability of trace metals. George and Coombs (1977) reported that cadmium complexation to EDTA, humic and alginic acids, and pectin doubled the rate of cadmium accumulation by the common mussel *Mytilus edulis* (L). In contrast, Harrison (1979) reported that glycine slightly increased and EDTA strongly decreased the accumulation of copper and zinc by oysters.

Much of the past difficulty in determining the effects of chemical speciation on biological availability has resulted from an inability to quantify the chemical forms or species of metals in experimental media. To overcome this difficulty, chemically defined model systems have been developed in which the speciation of metals can be calculated, controlled and systematically varied (Sunda and Guillard, 1976; Morel *et al.*, 1979). Synthetic chelators, such as EDTA (ethylenediaminetetraacetic acid) and NTA (nitrilotriacetic acid) whose binding properties with metals are well-known, have been used in these systems as analogues of natural organic ligands (Anderson and Morel, 1978; Sunda and Gillespie, 1979). We used a chemically defined seawater medium to investigate the effects of organic complexation on the accumulation of dissolved copper by the American oyster *Crassostrea virginica*. Exposure solutions were selected to achieve similar values of cupric ion activity within an environmentally realistic range (10^{-11} to $10^{-8.6}$ M), using different combinations of NTA and total dissolved copper.

Materials and Methods

Oysters were collected from beds located near the mouth of the Newport River estuary near Beaufort, North

* Present address: Chesapeake Biological Laboratory, University of Maryland; Solomons, Maryland 20688, USA

** Southeast Fisheries Center Contribution No. 81 – 45B. Reprint request should be addressed to this author

0025-3162/82/0066/0077/$ 01.20

Carolina, USA (Lat. 24°43'N and Long. 76°41'W). They were maintained in the laboratory in flowing seawater for 48 h to clear their guts and then exposed to dissolved copper both in metal-buffered synthetic seawater media and in metal-buffered filtered (0.45μm) natural seawater.

Oysters were exposed to a range of total copper and NTA concentrations over a 14-d period during the winter and summer (Table 1). Ten oysters were sampled on Day zero (January 23 and July 5, 1979) to obtain initial tissue copper concentrations. Periodically during the exposure period, three to five individuals were removed from each treatment tank and analyzed for metal accumulation. The four liters of exposure medium in each polyethylene tank was changed daily. Water temperature and salinity were maintained at 20°–21 °C and 35 ppt. The tanks were aerated and the pH of each exposure solution was measured periodically. The pH was 7.8 and did not vary appreciably with either time or treatment. Oyster tissues were analyzed for trace metals by digesting the entire shell contents in hot concentrated nitric acid for several days. Digests were evaporated to dryness, redissolved in 0.25 N HCl and analysed for copper and zinc by flame atomic absorption spectrophotometry (Perkin Elmer Model 403).

Cupric ion activities in exposure media containing NTA were determined from thermodynamic calculations described by Sunda (1975) and by Sunda and Gillespie (1979). The equation used in these calculations was:

$$[Cu_{TOT}] = \frac{A_{Cu}}{10^{-1.5}} + \frac{A_{Cu}[NTA_{TOT}]\,10^{12.96}}{A_{Cu}\,10^{12.96} + A_{Ca}\,10^{6.41} + A_{Mg}\,10^{5.41}},$$

where $[Cu_{TOT}]$ and $[NTA_{TOT}]$ are the total dissolved copper and NTA concentrations; A_{Cu}, A_{Ca}, and A_{Mg} are the free ion activities of copper, calcium, and magnesium; and $10^{12.96}$, $10^{6.41}$, and $10^{5.41}$ are stability constants for copper, calcium, and magnesium chelation by NTA selected from Sillen and Martell (1964). Activities of calcium and magnesium are based on formulated or measured concentrations of these metals and on total free ion activity coefficients for calcium and magnesium in seawater of 0.21 and 0.26 (Whitfield, 1975). The value $10^{-1.5}$ is the total cupric ion activity coefficient at pH 7.8 as based on the interaction of copper with inorganic ions in seawater (Sunda, 1975; Sund and Gillespie, 1979).

To compute cupric ion activities, one needs to know the concentrations of total copper in solution which may vary if there is appreciable adsorption on container surfaces or accumulation by the oysters. We used ^{64}Cu as a radiotracer during the summer experiment to determine if there was a significant loss of copper from solution. The ^{64}Cu was added at a known specific activity for all treatments on Day 3 and repeated on Day 13 for those treatments that had exhibit the greatest loss of ^{64}Cu from solution. Water samples were taken initially and after 24 h. Oyster tissue and shell and container surfaces also were analyzed for ^{64}Cu accumulation after the 24-h exposure period. Radioanalysis was by gamma-ray spectrometry (Nuclear Data Model 512) with a NaI detector.

Losses of ^{64}Cu from solution ranged from 29.2 to 87.1% for treatments on Day 3 (Table 2). For treatments of similar dissolved copper, loss of ^{64}Cu from solution decreased significantly with increasing NTA concentration due to the metal ion buffering action of NTA. Loss of ^{64}Cu was approximately equally distributed between the oyster tissue and shell surface, with negligible adsorption onto container surfaces. There was a significant reduction in the loss of radioisotope from solution during the later days of the experiment. The maximum ^{64}Cu loss for treatments examined on Day 13 was 39.9%. The removal of oysters from the experimental tanks through the 14-d exposure period apparently reduced radioisotope losses as the experiment progressed.

Due to the loss of copper from solution, the dissolved copper concentrations and, therefore, cupric ion activities did not remain strictly constant. Results from the ^{64}Cu analysis were used to estimate the mean total dissolved copper concentration and the resultant mean cupric ion activity for each treatment in the summer experiment (Table 2). Cupric ion activities for the winter experiment, for which there was no ^{64}Cu data, were based on the initial concentrations of copper. This procedure for the winter experiment should have resulted in only small error in computed pCu (-log cupric ion activity) as based on

Table 1. Composition of exposure media [a]

Trace metals	Concentration (molar)	Computed pM (-log free ion activity)
$CuSO_4 \cdot 5H_2O$		
winter	$1.0\times10^{-8} - 3.4\times10^{-6}$	11.0 – 9.3
summer	$3.2\times10^{-8} - 2.5\times10^{-6}$	10.5 – 8.5
$ZnSO_4 \cdot 7H_2O$		
winter	2.0×10^{-8}	9.5 – 8.9
summer	$1.0\times10^{-8} - 6.2\times10^{-8}$	9.0
$FeCl_3 \cdot 6H_2O$	1.0×10^{-8}	
$MnCl_2 \cdot 4H_2O$	2.3×10^{-9}	9.3
$CoCl_2 \cdot 6H_2O$	2.5×10^{-9}	10.1 – 9.3
$(NH_4)_6\,Mo_7O_{24} \cdot 4H_2O$	1.6×10^{-9}	
Chelator NTA		
winter	$1.0\times10^{-6} - 1.0\times10^{-5}$	
summer	$2.0\times10^{-7} - 1.0\times10^{-5}$	

[a] Oysters were exposed to dissolved copper in both synthetic seawater and filtered (0.45 μm pore size) natural seawater. The levels of trace metal and NTA additions and computed ionic activities are presented. Vitamin and nutrient additions corresponded to f/2 and approximately f/40, respectively (Guillard and Ryther, 1962). Vitamin and nutrients were included because this medium was also used to culture phytoplankton in subsequent studies examining the contribution fo particulate ingestion to copper accumulation by oysters. The concentration of the major seawater salts in the synthetic medium are those of S.O.W. (FWPCA, 1969). Trace metal impurities associated with the reagent grade salts were reduced to acceptable levels by passage through Chelex-100 ion exchange columns (Davey *et al*, 1973). All glassware was presoaked in 10% HNO_3 to further minimize trace metal contamination

Table 2. ^{64}Cu loss from solution and estimates of cupric ion activity for Cu-NTA exposure media in the summer experiment

Total	Treatment copper (μM)	NTA (μM)	Percent ^{64}Cu remaining after 24 h		Computed pCu			
					initial	mean [a]		mean overall [b]
			Day 3	Day 13		Day 3	Day 13	
(1)	0.864	1.0	32.6	–	8.5	8.9	–	8.7
(2)	0.254	0.2	12.9	60.1	8.5	9.1	8.7	8.9
(3)	2.54 [c]	10.0	70.8	–	9.5	9.5	–	9.5
(4)	0.254	1.0	34.0	65.2	9.5	9.7	9.6	9.6
(5)	0.254 [c]	1.0	33.8	–	9.5	9.8	–	9.7
(6)	0.254 [c]	8.1	60.2	–	10.5	10.6	–	10.6
(7)	0.254	8.1	60.0	–	10.5	10.6	–	10.6
(8)	0.032	1.0	37.1	60.2	10.5	10.6	10.6	10.6

[a] Computed on the basis of mean dissolved copper concentration. This mean was determined from ^{64}Cu data and from initial formulated copper concentrations
[b] Estimated overall mean pCu for the 14-d exposure period based on mean pCu values on Days 3 and 13
[c] Metal-buffered filtered (0.45 μm pore size) seawater used in place of the synthetic seawater

the similarity in the summer experiment between pCu values computed from initial copper concentrations and those computed from estimated mean copper concentrations (Table 2).

Results

Copper concentrations in the tissues increased with time of exposure (except at pCu 11); however, the shapes of the uptake curves differed between experiments (Fig. 1 A and B). In the summer experiment (Fig. 1 B) the rates of copper accumulation decreased with time of exposure, a typical pattern in metal accumulation experiments caused by saturation of tissue binding sites. By contrast, uptake rates in the winter experiment increased with time at cupric ion activities $> 10^{-10}$ M (Fig. 1 A). There was no oyster mortality due to copper exposure in either experiment.

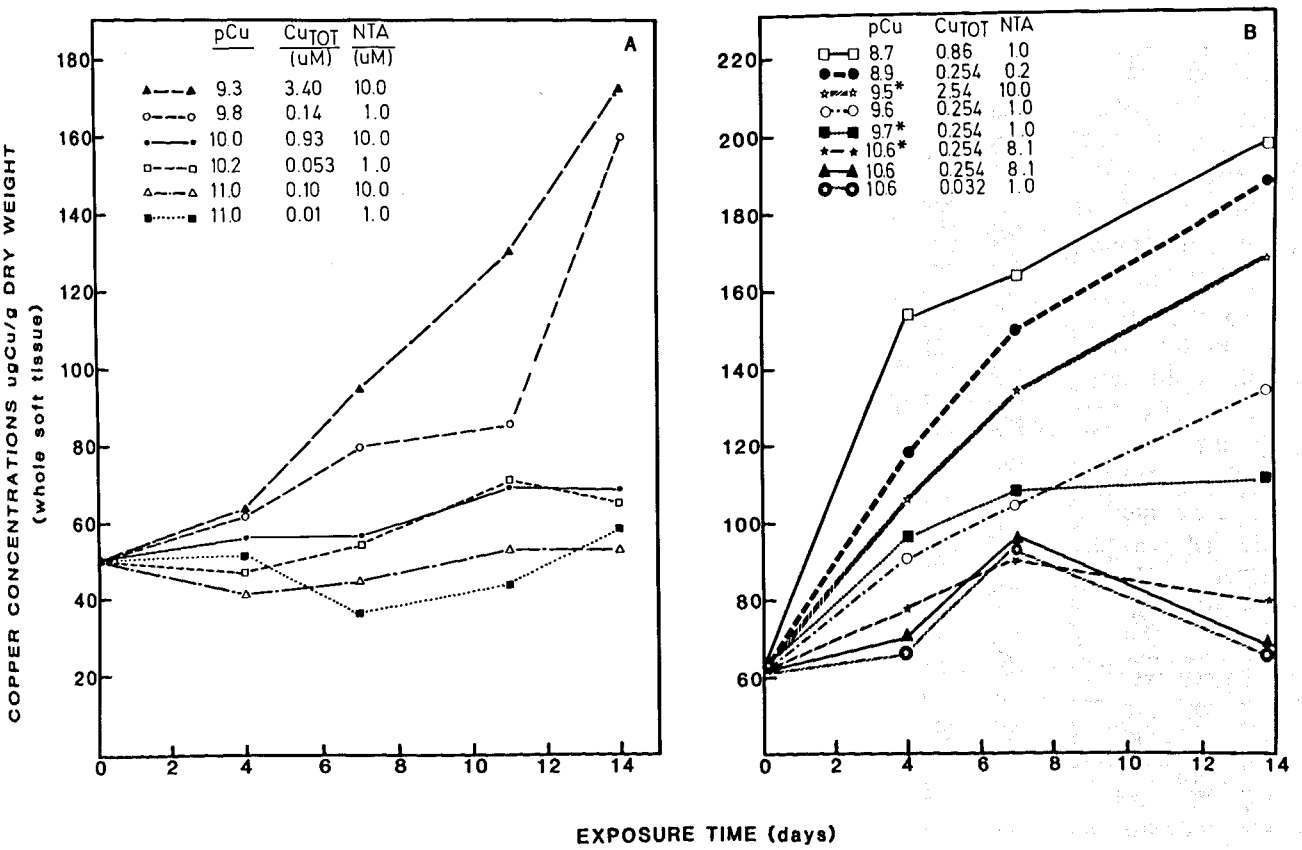

Fig. 1. *Crassostrea virginica.* Copper accumulation at different concentrations of total dissolved copper and NTA. Copper concentrations of oyster tissue are expressed on a dry weight basis and represent the means of 3–5 samples. The dry weight represented 9.5±1.7% and 13.0±1.3% (mean±1 SE) of the wet weight, for winter and summer oysters. (A) Winter experiment. (B) Summer experiment; asterisks denote the use of metalbuffered filtered natural seawater in place of the synthetic seawater medium

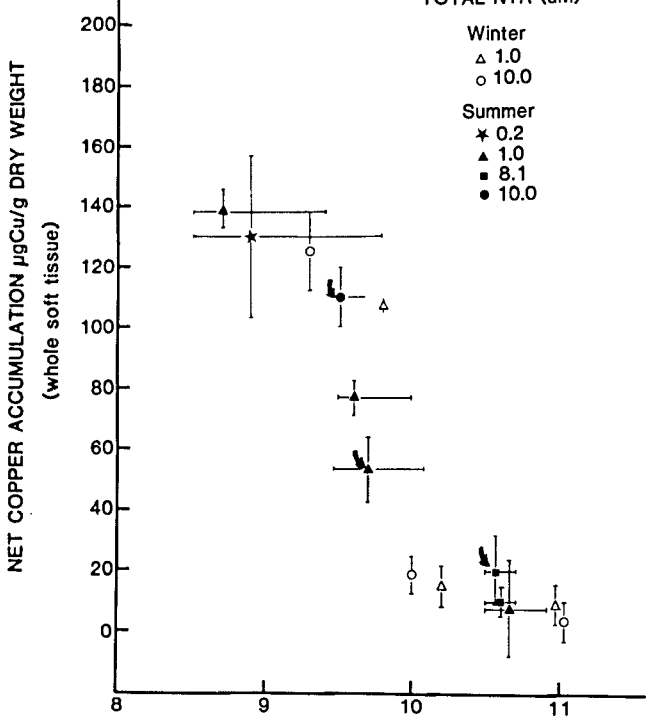

Fig. 2. *Crassostrea virginica.* Net copper accumulation after 14 d as a function of computed pCu (-log cupric ion activity). Initial copper concentrations for the winter and summer oysers were 49.2±12.6 and 58.9±11.0 µg Cu·g dry weight (mean±1 SE). Verticle error bars represent±SE. Horizontal error bars (summer experiment only) represent the range in pCu as estimated from ^{64}Cu data (Table 2). Arrows denote the use of metal-buffered filtered natural seawater in place of the synthetic seawater medium

The accumulation of copper by *Crassostrea virginica* was highly dependent on chemical speciation. Uptake during both the winter and summer experiments was related to cupric ion activity irrespective of the total concentration of copper or NTA (Fig. 1 A and B). After 14-d exposure to treatments with a total dissolved copper concentration of 0.25 µM in the summer experiment, the mean tissue accumulation of copper ranged from 10 to 130 µg Cu · g dry weight^{-1} (mean ± 1 SE), depending on the concentration of NTA (Fig. 1 B). There was no significant difference (95% confidence level) in the accumulation of copper by the oysters when exposure treatments were selected to achieve similar values of cupric ion activity with different combinations of NTA and total dissolved copper (Fig. 2).

Curves for copper accumulation with time indicate that the rates of uptake, as well as the final tissue concentrations are related to cupric ion activity (Fig. 1 A and B). The relation between net accumulated copper at 14-d and pCu was sigmoidal with a sharp inflection at a pCu of about 9.7 and no accumulation at a pCu of 11 (Fig. 2).

Discussion

Potential mechanisms that would explain the dependence of accumulation rate on cupric ion activity are: (1) copper transport directly across external cell membrane as the free cupric ion (i.e., $Cu(H_2O)_6^{2+}$), or as inorganic complex species whose concentration covaries with that of cupric ion (e.g. $CuCO_3$, $CuOH^+$, $CuCl^+$), or (2) uptake mediation by binding of copper to ligand sites at cell surfaces. Because biological membranes (i.e., lipid bilayer membranes) are relatively impermeable to charged or highly polar species (e.g. $CuCO_3$) (Finkelstein and Cass, 1968), the second mechanism is more probable. According to this second mechanism, the first event in the accumulation of copper is the binding with surface membrane sites (probably functional groups of proteins) exposed directly to the water with subsequent passage into the tissues. Such binding would concentrate copper at external membrane surfaces. If the rate of reaction of copper with surface sites is fast, relatively to the rate of passage into tissues, then copper bound to surface sites would approach equilibrium with that in the external medium. The rate of passage of copper into the tissues would be proportional to the amount bound to appropriate membrane sites, and that amount, according to thermodynamics, would be directly related to cupric ion activity in the external medium. The presence of chelators would decrease rates of accumulation by competing with membrane ligands for available copper.

Measurable accumulation of copper occurred only at cupric ion activities above 10^{-11} M (Fig. 2). Since we might expect net accumulation only at activities above those in the field, our results suggest that ambient mean cupric ion activity in the Newport estuarine seawater from which the oysters were collected was $\leqq 10^{-11}$ M. This upper limit agrees with previous estimates for this seawater obtained using bacterial toxicity assays of cupric ion activity (Sunda and Gillespie, 1979; W. G. Sunda and A. Palumbo, unpublished data).

There is considerable evidence that copper is highly complexed in marine and estuarine waters and that complexation can vary both temporally and spacially (Batley *et al.*, 1978; Harrison *et al.*, 1980; Srna *et al.*, 1980). Variations in chelation could at least partly explain observed seasonal variations in trace metal content of oysters (Frazier, 1975; Harrison, 1979). Other factors would include variations in total trace metal concentrations and in food availability and composition. Seasonal variations in oyster physiology and metabolism also appear to be important (Frazier, 1975).

A number of previous studies also have demonstrated the importance of free metal ion activity in controlling biological availability and toxicity of trace metals. A majority of these studies have dealt with copper toxicity to microorganisms (algae and bacteria) in the presence of both synthetic and natural chelators (Sunda and Guillard, 1976; Anderson and Morel, 1978; Sunda and Lewis, 1978; Jackson and Morgan, 1978; Sunda and Gillespie, 1979).

Others have shown that zinc nutritional availability to phytoplankton (Anderson *et al.,* 1978), copper accumulation by phytoplankton (Sunda and Guillard, 1976), and cadmium toxicity to a species of shrimp (Sund *et al.,* 1978) are all related to free metal ion activities. In all these studies increases in trace metal complexation decreased metal availability or toxicity by reducing free metal ion activities.

In contrast to these findings, some researchers have reported stimulation of heavy metal accumulation in the presence of both naturally occurring and synthetic chelating agents (George and Coombs, 1977; Harrison, 1979). George and Coombs observed a two-fold increase in cadmium accumulation by *Mytilus edulis* in the presence of EDTA, humic acid, sodium alginate or pectin, which they interpreted as resulting either from rapid exchange of chelated metal with membrane carrier ligands or from direct uptake and transport of metal chelates. In interpreting such studies, however, one must keep in mind that the mere addition of a chelator does not guarantee the occurrence of significant chelation with a particular metal. Computations of Mantoura *et al.* (1978) show that the combined effect of low stability constants and strong competition by chloride ions should effectively prevent complexation of cadmium by humic acid in seawater. Based on these calculations, it is unlikely that enhanced accumulation of cadmium in the presence of humates is due to increased biological availability of cadmium-humate complexes. An alternative hypothesis to explain George and Coombs' (1977) results is that addition of chelators reduced the activities of other metals such as copper or zinc and thereby reduced competition by these metals for available tissue binding sites. The result of this reduced competition could have been an increase in the tendency for tissue sites, to bind cadmium. In support of this hypothesis, we note that stability constants for copper chelation are usually orders of magnitude higher than for cadmium chelation. Leber (1974) and Brown and Parsons (1978) have also reported cadmium displacement of copper and zinc from metal binding proteins (metallothioneins) for aquatic organisms.

The strong affinity of NTA for copper reduced the possibility of metal competition effects in our experiments since NTA binds copper in seawater by at least an order of magnitude more strongly than it does most competing trace metals (e.g. 30, 1000 and 10000 fold more than for zinc, cadmium and manganese, respectively). According to our calculations, the 0.1 to 10 μM differences in NTA concentration in the experimental media would have no significant effect on the activities of manganese and cadmium but would have some effect on the activity of zinc. In the winter experiment the dissolved zinc concentration was held constant at 2×10^{-8} M and the computed zinc ion activity varied from $10^{-9.5}$ to $10^{-8.9}$ M due to changes in NTA concentration. In the summer experiment, however, total zinc concentrations were varied such that the computed zinc ion activity remained constant thereby eliminating possible effects due to variable zinc ion activity.

A major difficulty in previous experiments with trace metal accumulation by marine organisms has been the lack of control and quantitative definition of metal speciation. Natural seawater, with its variability in minor constituents, is often ill-defined with regard to trace metal chemistry. Background trace metal concentrations are usually unknown and even when the concentration of the metal is known, the activity usually is not, due to unknown levels of natural complexation. With a metal-buffered system, sufficient total metal and chelator can be added to mask background levels of the metal and natural chelators, which often allows the use of natural seawater in place of synthetic seawater. Results from our study indicate similar patterns of copper accumulation by oysters in artificial seawater and metal-buffered natural seawater. Media poorly buffered for trace metals can lead to significant loss of metal from solution due to accumulation by the organisms or adsorption onto surfaces. Such changes will result in the loss of definition of the level of trace metal exposure. In our experiments, this problem could not be completely circumvented even at NTA concentrations of 10^{-5} M (Table 2), although high chelator concentrations did sharply reduce loss of copper from solution.

In summary, this study indicates the importance of free ion activity in controlling bioavailability of dissolved copper to oysters and demonstrates the utility of metal-chelate buffers in studies of trace metal accumulation by marine animals. With the use of trace metal buffered media, difficulties with the lack of control and definition of the metal chemistry are minimized and metal accumulation and toxicity can be examined at the typically low concentrations of free metal ions representative of the natural environment.

Acknowledgements. This research was supported by the Ocean Dumping Program of NOAA's Office of Marine Pollution Assessment and by an Interagency agreement between the National Marine Fisheries Service and the Department of Energy.

Literature Cited

Anderson, D. M. and F. M. Morel: Copper sensitivity of Gonyaulax *tamarensis.* Limnol. Oceanogr. *23,* 283–295 (1978)

Anderson, M. A., F. M. Morel and R. R. L. Guillard: Growth limitation of a coastal diatom by low zinc ion activity. Nature, Lond. *276,* 70–71 (1978)

Batley, G. E. and D. Gardner: A study of copper, lead and cadmium speciation in some estuarine and coastal marine waters. Estuar. coast. mar. Sci. *7,* 59–70 (1978)

Brooks, R. R. and M. G. Rumsby: The biochemistry of trace element uptake by some New Zealand bivalves. Limnol. Oceanogr. *10,* 521–527 (1965)

Brown, D. A. and T. R. Parsons: Relationships between cytoplasmic distribution of mercury and toxic effects to zooplankton and chum salmon (*Oncorhynchus keta*) exposed to mercury in a controlled ecosystem. J. Fish. Res. Bd Can. *35,* 880–884 (1978)

Davenport, J.: A study of the effects of copper applied continuously and discontinuously to specimens of *Mytilus edulis* (L.) exposed to steady and fluctuating salinity levels. J. mar. biol. Ass. U.K. *57,* 63–74 (1977)

Davey, E. W., M. J. Morgan and S. J. Erickson: A biological measurement of copper complexation capacity of seawater. Limnol. Oceanogr. *18,* 993–997 (1973)

Federal Water Pollution Control Administration: FWPCA methods for chemical analysis of water and wastes, 240 pp. Cincinnati, Ohio: Federal Water Pollution Control Administration 1969

Finkelstein, A. and A. Cass: Permeability and electrical properties of thin lipid membranes. J. gen. Physiol. *52,* 1450–1730 (1968)

Frazier, J. M.: The dynamics of metals in the American oyster, *Crassostrea virginica.* I. Seasonal effects. Chesapeake Sci. *16,* 162–171 (1975)

Galtsoff, P. S.: The American oyster *Crassostrea virginica* Gemlin. Fishery Bull. Fish. Wildl. Serv. U.S. *64,* 1–480 (1964)

George, S. G. and T. L. Coombs: The effects of chelating agents on the uptake and accumulation of cadmium by *Mytilus edulis.* Mar. Biol. *39,* 261–268 (1977)

Goldberg, E. O.: The mussel watch – A first step in global marine monitoring Mar. Pollut. Bull. *6,* 111 (1975)

Guillard, R. R. L. and J. H. Ryther: Studies on marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula conferuacea,* (Cleve) gran. Can. J. Microbiol. *8,* 229–238 (1962)

Harrison, F. L.: Effect of the physicochemical form of trace metals on their accumulation by bivalve molluscs. *In:* Chemical modeling in aqueous systems, pp. 611–634. Ed. by A. Jenne. Am. Chem. Soc. Symp. Ser. 93 (1979)

Harrison, F. L., D. J. Bishop, R. R. Emerson and D. W. Rice, Jr.: Concentration and speciation of copper in waters collected near the San Onofre and Diablo Canyon Nuclear Power Stations, 66 pp. U.S. Nuclear Regulatory Commission, NUREG/CR-0750 (1980)

Jackson, G. A. and J. J. Morgan: Trace metal chelation interactions and phytoplankton growth in sea water media: theoretical analysis and comparison with reported observations. Limnol. Oceanogr. *23,* 268–282 (1978)

Leber, A. P.: A mechanism for cadmium and zinc-induced tolerance to cadmium toxicity: involvement of metallothionein, 72 pp. Ph. D. thesis, Purdue University, Lafayette, Ind. 1974

Mantoura, R. F. C., A. Dickson and J. P. Riley: The complexation of metals with humic materials in natural waters. Estuar. coast. mar. Sci. *6,* 387–408 (1978)

Morel, F. M. M., J. G. Rueter, D. M. Anderson and R. R. L. Guillard: Aquil, a chemically defined phytoplankton media for trace metal sudies. J. Phycol. *15,* 135–141 (1979)

Nelson, D. S., A. Calabrese, B. A. Nelson, J. R. MacInnes and D. R. Wenzloff. Biological effects of heavy metals on juvenile bay scallops, *Argopecten irradians,* in short-term exposures. Bull. environ. Contam. Toxicol. *16,* 275–282 (1976)

Phillips, D. J. H.: Quantitative aquatic biological indicators, 488 pp. London: Applied Science Publishers 1980

Shuster, C. N. and B. H. Pringle: Trace metal accumulation by the American eastern oyster, *Crassostrea virginica.* Proc. natn. Shellfish. Ass. *59,* 91–103 (1969)

Sillen, L. G. and A. E. Martell: Stability constants of metal ion complexes. Chem. Soc. spec. Publ., *17,* 754 pp. (1964)

Srna, R. R., K. S. Garrett, S. M. Miller and A. B. Thum: Copper complexation capacity of marine water samples from southern California. Environ. sci. Technol. *14,* 1482–1486 (1980)

Sunda, W. G.: The relationship between cupric ion activity and the toxicity to phytoplankton, 168 pp. Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge 1975

Sunda, W. G., D. W. Engel and R. M. Thuotte: Effect of chemical speciation on toxicity of cadmium to grass shrimp, *Palaemonetes pugio:* Importance of free cadmium ion. Environ. sci. Technol. *12,* 409–413 (1978)

Sunda, W. G. and P. A. Gillespie: The responses of a marine bacterium to cupric ion and its use to estimate cupric ion activity. J. mar. Res. *37,* 761–777 (1979)

Sunda, W. G. 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)

Sunda, W. G. and J. M. Lewis: Effect of complexation by natural organic ligands on toxicity of copper to a unicellular alga, *Monochrysis lutheri.* Limnol. Oceanogr. *23,* 870–876 (1978)

Whitfield, M.: Seawater as an electrolyte solution. *In:* Chemical oceanography, Vol. 1, 2nd ed, pp. 43–171. Ed. by J. P. Riley and G. Skirrow. New York: Academic Press 1975

Date of final manuscript acceptance: October 24, 1981.
Communicated by S. K. Pierce, College Park

Abund
(*Rhizo*
in the

A. L. Alld

[1] Oceanic
Santa Barb

[2] Center f

Abstract

Abundar
floating
tracanei
Current
during C
maximu
duction
colony^{-1}
equal vo
their lov
mats cor
duction.
most all
Rhizosol
mats, wh
by a di
species i
containe
rates of
since th
bacteriu
N-fixati
cate tha
cuss the
trogen f

Introdu

Most pl
microsc
vironm
there a
penter
chains

Volume 66 Number 3 1982

Indexed in Current Contents

Marine Biology

International Journal on Life in Oceans and Coastal Waters

Volume 66 Number 1 1982

Indexed in Current Contents

Springer International

Marine Biology ISSN 0025-3162 MBIOAJ 66 (1) 1–108 (1982) January 1982

Marine Biology

International Journal on Life in Oceans and Coastal Waters

Volume 66, 1982

Springer International