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The Effect of Salinity on the Acute Toxicity of Total Dissolved and Free Cadmium to the Copepod *Eurytemora affinis* and the Larval Fish *Cyprinodon variegatus*

Chesapeake Bay Program

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Report

The Effect of Salinity on the Acute Toxicity of Total Dissolved and Free Cadmium to the Copepod *Eurytemora affinis* and the Larval Fish *Cyprinodon variegatus*

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ABSTRACT

The objective of this study was to determine the influence of a range of salinities (5, 15 and 25 ppt) on the acute toxicity of total dissolved and free cadmium to sheepshead minnow, *Cyprinodon variegatus* larvae and the copepod, *Eurytemora affinis* nauplii. Data were analyzed to determine if the acute toxicity (96 h LC50s) was different among salinities for the test species. Total dissolved cadmium was measured in selected test conditions and the proportion of total cadmium as Cd^{+2} (free ion or toxic form) was determined at each salinity. Ninety six hour LC50 values for *C. variegatus* were 180.3, 312.4 and 495.5 µg/L total cadmium at 5, 15 and 25 ppt, respectively. A significant increase in LC50 values with salinity was likely related to a decrease in the free ion as salinity increased. Ninety-six hour LC50 values for *E. affinis* were 51.6, 213.2 and 82.9 µg/L total cadmium at 5, 15 and 25 ppt, respectively. A comparison of LC50 values for the copepod between salinities showed a significant difference between 5 and 15 ppt and between 15 and 25 ppt. There was no difference in LC50 values between 5 and 25 ppt. The physiological characteristics of *E. affinis* were likely responsible for the higher tolerance at the middle salinity. Cadmium speciation in the various test salinities was dominated by association with inorganic binding ligands; organic complexation was negligible. The speciation at all salinities was dominated by $CdCl^{+}$ and $CdCl_2^{\circ}$. The free ion accounted for 20, 8 and 4.5 % of the total cadmium at 5, 15 and 25 ppt, respectively. As current water quality criteria do not

distinguish among individual cadmium species these data have important implications for estuaries such as Chesapeake Bay because the presence of the toxic form of cadmium will increase as salinity decreases.

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INTRODUCTION

One commitment of the 1988 Chesapeake Bay Basinwide Toxics Reduction Strategy was to give contaminants on the Toxics of Concern List priority in the development of water quality criteria (U.S. EPA, 1991a). Presently the United States Environmental Protection Agency develops water quality criteria for both freshwater and marine systems. Estuarine organisms are supposed to be protected under the marine criteria. There are ,however, compelling biological and chemical factors that may prevent estuarine biota from being protected under marine criteria and these factors justify the need for specific estuarine criteria. Estuarine organisms, because of their inherent physiological differences from freshwater and marine organisms, may differ substantially in sensitivity to some toxic substances. For example, recent toxicity studies with an estuarine zooplankter and fish showed that salinity ranging from 5 to 25 ppt significantly influenced the toxicity of atrazine (Hall et al., in press). The unique water chemistry of estuarine environments may also be responsible for differences in bioavailability of some toxic substances, thus affecting their toxicities.

Four metals listed on the Toxics of Concern list for Chesapeake Bay were potential candidates for assessing the effects of salinity on their toxicity (cadmium, chromium, copper and lead). We eliminated chromium and copper because of potential problems with the speciation chemistry. Lead was eliminated due to

solubility problems at various salinities and possible differential precipitation rates at various salinities. Cadmium was, therefore, selected for this study due to less projected problems with speciation chemistry. The toxicity data base for cadmium with Chesapeake Bay species was also more extensive when compared with the other metals considered for this project (Hall et al., 1994).

The environmental impact of cadmium in aquatic systems is determined by its total concentration, by partitioning between dissolved and particulate phases, and by its "chemical speciation" (i.e. by the physicochemical forms in which the element is found). The toxicity and/or bioavailability of a trace metal to aquatic organisms has in most instances been found to correlate with the activity of the free metal ion rather than with the total metal concentration (e.g. Brand et al., 1983, 1986; Sunda et al., 1987, 1990). The concentration of the free ion may be much lower than the total metal concentration due to complexation by various inorganic and organic ligands in solution. The association of metals with natural complexing ligands may therefore serve to buffer the system with respect to the toxicity of a given metal.

The inorganic speciation of cadmium in natural waters is a function of pH, temperature, the ionic strength of the solution, and the relative concentrations of potential binding ligands. In seawater, the inorganic speciation of cadmium is predicted to be dominated by association with the chloride ion, while in freshwater total cadmium will be dominated by the free hydrated ion (Cd^{2+}) at pH 6 and partitioned between the free ion and carbonate complexes

at higher pH (Turner et al., 1981; Byrne et al., 1988). Byrne et al. (1988) estimated that 97.2% of dissolved inorganic Cd in seawater exists as chloride complexes, predominantly $CdCl^+$ and $CdCl_2^\circ$. In an estuarine environment, where freshwater and seawater mix and chemical reactions can occur, the change in cadmium speciation from the free ion and carbonate species to predominately chloride complexes occurs at low salinities (< 5 ppt). Chloride complexes will therefore dominate the cadmium speciation over most of the estuary.

In contrast to its well-characterized inorganic speciation, the speciation of dissolved cadmium with respect to natural organic ligands is poorly known. In the central North Pacific, cadmium appears to be 60-70% complexed by strong, relatively Cd-specific, organic ligands. The latter are present, however, at very low concentrations (approx. 0.1 nM) (Bruland, 1992). Similar behavior has been observed for cadmium in coastal waters of the northwest Atlantic, with complexing ligand concentrations on the order of 0.3 nM (Lewis and Luther, unpublished data).

The study described in this report was conducted to determine the influence of salinity on the toxicity of total dissolved and free cadmium to estuarine species. Specific objectives were to determine the acute toxicity (96 h LC50s) of cadmium to two Chesapeake Bay resident species, the sheepshead minnow, *Cyprinodon variegatus* larvae and copepod *Eurytemora affinis* nauplii, at salinities of 5, 15 and 25 ppt. These data were analyzed to determine if acute toxicity (96 h LC50 values) was different among

salinities for each species. Total dissolved cadmium was measured in selected test conditions. The solution speciation of cadmium with respect to free hydrated ions and inorganic complexes in the sample solution was also determined using MINEQL+, an interactive PC version of the original MINEQL equilibrium modeling program (Schecher and McAvoy, 1991). MINEQL+ utilizes equilibrium constants to solve mass balance expressions, using a modified Newton-Raphson iterative procedure.

METHODS

Test Organisms

Eurytemora affinis cultures were maintained at 8, 15, and 22 ppt salinity and 23-25 C in our laboratory. Copepods were reared in autoclaved estuarine water (14 ppt) obtained from the Choptank River at Horn Point Center for Environmental and Estuarine Studies (CEES). Salinity was adjusted with H-W Marinemix or deionized water. Copepods were fed a diet consisting of equal volumes of two phytoplankton species, *Thalassiosira fluviatilis* and Isochrysis *galbana*, each maintained in log-phase growth. The phytoplankton were also cultured in autoclaved estuarine water supplemented with F/2 media (Guillard, 1975).

Cyprinodon variegatus larvae were obtained from Aquatic Biosystems, Inc. (Fort Collins, CO). Larvae were <24-h old and shipped at three salinities (8, 15, and 24 ppt). Larvae were placed in aquaria containing salinity adjusted (5, 15, and 25 ppt) Choptank River water and fed *Artemia* nauplii for overnight acclimation.

Test Procedures

Cadmium chloride (lot number 50H-0879) used in these experiments was obtained from Sigma Chemical Company (St. Louis, MO). Autoclaved estuarine water from the Choptank River (10 ppt) was used as control water and diluent for all toxicity tests. Salinity adjustments to the desired test salinities (5, 15, and 25

ppt) were made with HW Marinemix or deionized water. Acute toxicity tests (96 h) with *Eurytemora* and sheepshead larvae were conducted as static non-renewal. All tests were conducted in a biological incubator to maintain a constant temperature of 25 C and a photoperiod of 16-h light:8-h dark. Standard water quality parameters (temperature, dissolved oxygen, pH, and salinity) were recorded initially and at the end of the exposure period for each test condition. Selected test conditions were sampled initially and at 96 h for total cadmium analysis and speciation.

The *Eurytemora* experiment at 5 ppt salinity was conducted at the following nominal cadmium concentrations: 0, 32, 56, 100, and 180 µg/L. The test at 15 ppt salinity was conducted at the following nominal concentrations: 0, 32, 56, 100, 180, 320, and 560 µg/L cadmium. Nominal concentrations of cadmium in the 25 ppt salinity test were: 0, 56, 100, 180, 320, and 560 µg/L. Each test concentration was prepared by diluting a stock solution (100 ug cadmium/L) of cadmium chloride with salinity adjusted Choptank River water. The salinity adjusted diluent for all conditions was prepared by diluting the estuarine water to 5 ppt with deionized water and adding synthetic seasalt to the desired salinity. Stock solutions were prepared 1-2 days prior to testing by dissolving 40.77 mg of anhydrous cadmium chloride in 250 mL of deionized water.

Toxicity tests were initiated with copepodids (48 to 72-h old). Copepodids were obtained by isolating adult gravid copepods in polycarbonate jars containing salinity adjusted estuarine water

for 24-h and collecting the recently hatched neonates. Neonates were held for 48-h prior to starting each test. Three replicate 150 mL glass beakers containing 100 mL of test solution were used for each condition. A test chamber was suspended within each beaker to contain the organisms. Chambers were constructed from 3.8 cm diameter rigid polycarbonate tubing cut to a length of 5.0 cm to provide a 40 mL volume when suspended in the beaker. The bottom of the chamber was covered with 53 um mesh Nitex screen. Copepodids were counted by drawing small aliquots of copepods and water into a wide-bore glass pipet and examining under a dissecting microscope (15x magnification). The initial number of copepods (10-15) and the corresponding test chamber were recorded. *Eurytemora* were fed daily with 1.0 mL of a two-species phytoplankton mixture (50/50; v/v). Algal densities within the test chamber were generally 1-2 x 10^4 *Isochrysis* cells/mL and 2-3 x 10^3 *Thalassiosira* cells/ml. Algal cell counts were conducted with a Spencer improved Neubauer corpuscle counting chamber. Survival was evaluated in each condition after a 96 h exposure. Copepods were counted by first lowering the volume of solution in the test chamber, then removing the remaining copepods and water in small aliquots with a pipet. Each aliquot within the pipet was examined with the aid of a dissecting microscope for the presence of live copepods.

Cyprinodon acute toxicity tests (96 h) were conducted following U.S. EPA protocol (U.S. EPA., 1991b). For the test conducted at 5 ppt salinity, larvae were exposed to the following

nominal cadmium concentrations: 0, 56, 100, 180, 320, 560, and 1000 µg/L. The 15 ppt salinity test was conducted with the following nominal concentrations: 0, 100, 180, 320, 560, 1000, and 1800 µg/L. The 25 ppt salinity test was conducted with the following nominal concentrations: 0, 180, 320, 560, 1000, 1800, and 3200 µg/L. Each concentration was prepared by diluting a stock solution of cadmium (1000 µg/L) with the appropriate volume of salinity adjusted diluent. Tests were initiated with 48-h old larvae following a 24-h acclimation period. The maximum salinity change was ≈ 5ppt. Three replicate 600 mL glass beakers containing 400 mL of test solution were used for each condition. Each replicate received ten larvae by transferring them from the acclimation water to a test beaker with a fire-polished, wide-bore glass pipet. Larvae were fed at 48 h with 100 uL of concentrated *Artemia* nauplii. At that time, dead larvae were counted and removed from each beaker. Larval survival was evaluated in each condition after 96 hours of exposure.

Cadmium Analysis

General Procedures

Total dissolved cadmium and cadmium speciation measurements were conducted by the College of Marine Studies, University of Delaware (Lewes, DE). Samples for total dissolved cadmium and speciation analyses were filtered (0.4 um polycarbonate membrane) and collected in precleaned polyethylene containers. Total dissolved cadmium samples were preserved with Seastar ultrapure

nitric acid (Seastar Chemicals, Inc., Seattle, WA). Samples collected in polyethylene bottles for speciation measurements were immediately frozen and shipped to the University of Delaware on dry ice by Federal Express courier service. The following test conditions were sampled initially and at 96h for total dissolved cadmium and speciation measurements: *Eurytemora* test at 5 ppt (32, 100, 180, and 560 µg/L); *Eurytemora* test at 15 ppt (32, 100, and 560 µg/L); *Eurytemora* test at 25 ppt (56, 180, and 560 µg/L); *Cyprinodon* test at 5 ppt (56, 180, and 1000 µg/L); *Cyprinodon* test at 15 ppt (100, 320, and 1800 µg/L); *Cyprinodon* test at 25 ppt (180, 560, and 3200 µg/L).

Total Dissolved Cadmium Analyses

Total dissolved cadmium concentrations were measured by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES). Concentrations were determined by comparison of sample emission values with a linear calibration curve. Working standards were prepared by serial dilution of a 1000 ppm commercial ICP cadmium standard (Inorganic Ventures, Inc., Toms River NJ). Standards were prepared in an organic-free (ultraviolet-irradiated) natural seawater matrix, diluted to the appropriate salinity (5, 15 or 25 ppt) and acidified to 0.1 N with high purity quartz-distilled nitric acid. Thus the matrix of the standards was equivalent to that of the samples, correcting for any signal suppression due to seasalts in the samples. Analytical sensitivity was 1.46±0.1 emission units/µg/L-Cd, with a detection limit of approximately 3

μg/L.

Organic Complexation

Voltammetric measurements of cadmium were conducted using an EG&G Princeton Applied Research (PAR) Model 384B-4 polarographic analyzer with a PAR Model 303A mercury drop electrode. Analyses were performed in the square wave anodic stripping voltammetry (SWASV) mode with a hanging mercury drop electrode (HMDE). Cadmium was reduced and deposited in the mercury drop for one minute at a deposition potential of -1.0 V. Instrumental parameters for reoxidation and stripping of the cadmium from the mercury drop were: scan rate = 200 mV sec^{-1}, pulse amplitude = 20 mV, pulse frequency = 100 Hz, scan range = -1.0 to -0.4 V.

The form of the metal deposited at a mercury electrode is dependent upon both the thermodynamic stability and the kinetic lability of the complexes, as well as the thickness of the diffusion layer at the electrode surface and the diffusion rate of a complex through this layer. For a metal-ligand complex, the dissociation of the complex and subsequent reduction of the metal to form the mercury amalgam can be represented as:

$$ML_p^{n+} \underset{k_f}{\overset{k_d}{<===>}} M^{n+} + p\ L$$

$$M^{n+} + n\ e^- \text{ ---> } M^{\circ}(Hg)$$

where M and L are the metal and complexing ligand respectively, and k is the rate of reaction for the dissociation (k_d) or the formation of (k_f) of the metal-ligand complex ML.

For a given deposition potential a stripping voltammetric method will detect only the free ionic form of the metal plus those complex species which dissociate to the free ion within the timespan required for diffusion of the complex into and out of the electrode diffusion layer. This is illustrated schematically in Figure 1. Such complexes can be defined as electrochemically active or "labile" in the context of detection by stripping voltammetry. All other complexes (e.g. strong organic complexes) will be electrochemically inert, or "non-labile", passing through the electrode diffusion layer without dissociation and hence yielding no measurable current.

Assuming that there are no electroactive organic cadmium complexes in solution, the measured current will be directly proportional to the concentration of Cd', where [Cd'] is the total concentration of dissolved inorganic cadmium species present (i.e. free hydrated Cd^{2+} plus inorganic complexes) (Bruland, 1992). Cd' is related to the concentration of the free ion, $[Cd^{2+}]$, by an inorganic side reaction coefficient, such that $[Cd'] = [Cd^{2+}]\alpha_{Cd'}$ (Ringbom and Still, 1972).

In the absence of electrochemically inert cadmium complexes, titration of the samples with cadmium should yield Cd' concentrations equivalent to the total dissolved cadmium concentration measured by ICP-AES. For the purposes of this study, the extent of cadmium complexation by natural organics in the source water should be negligible. Even if we assume an initial binding ligand concentration in the diluted source water of 10 nM

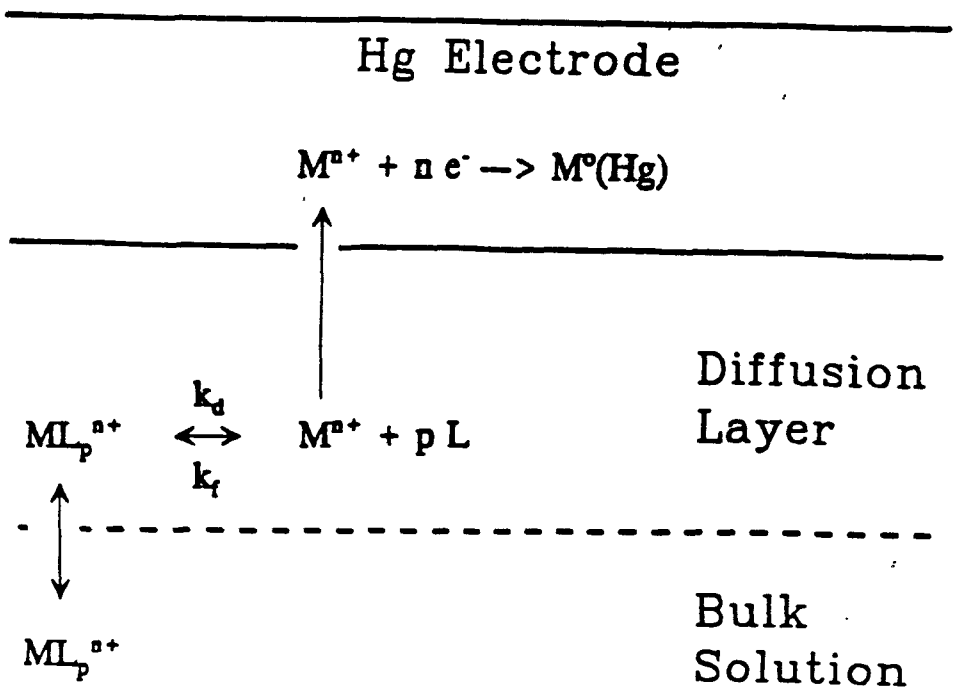


Figure 1. Dissociation and reduction of a metal complex at a mercury electrode (after Florence, 1986). (M and L are the metal and complexing ligand respectively, and k is the rate of reaction for the dissociation (k_d) or the formation (k_f) of the metal-ligand complex ML.

(an order of magnitude above reported values for seawater), this represents less than 4% of the total cadmium in the lowest concentration sample (32 ppb). The organic complexation of cadmium will be unimportant assuming that the test organisms did not produce prodigious amounts of complexing material over the course of the experiment.

Cadmium Speciation Calculations

The solution speciation of cadmium with respect to free hydrated ions and inorganic complexes in the sample solutions was estimated using MINEQL$^+$ (Schecher and McAvoy, 1991), an interactive PC version of the original MINEQL equilibrium modeling program. MINEQL (Westall et al., 1976), and its predecessor REDEQL (Morel and Morgan, 1972), utilizes equilibrium constants to solve mass balance expressions, using a modified Newton-Raphson iterative procedure.

The input for MINEQL$^+$ consists of the total solution concentrations of all components to be modeled and an extensive data base of equilibrium constants for the formation of solution species and solid phases consisting of combinations of the basic components. For the purposes of this study, the components consisted of the cadmium ion, the major and minor seawater cations and anions, and H^+ (Table 1).

The concentrations of the seawater ions were calculated based upon the sample salinity and the relative proportions of each ion in natural seawater. Based on information supplied by the

Table 1. Dissolved solution components for MINEQL equilibrium modeling program.

Cations	Anions
Na^+	Cl^-
K^+	Br^-
Ca^{2+}	F^-
Mg^{2+}	CO_3^{2-}
Sr^{2+}	SO_4^{2-}
Cd^{2+}	
H^+	

manufacturer, the major ion composition of the "MarineMix" used to amend sample salinities to 15 and 25 ppt is equivalent to that of natural seawater. Therefore, no correction was necessary for the use of commercial salts rather than natural seawater. The hydrogen ion concentration (pH) was considered as a fixed parameter, set to the pH measured in each sample. In the absence of total inorganic carbon or alkalinity measurements, the samples were assumed to be in equilibrium with the atmosphere with respect to the carbonate system ($pCO_2 = 10^{-3.5}$ atm). It was further assumed that no precipitation of the mineral phase otavite ($CdCO_3$) occurred over the course of the 96 h experiments. Total cadmium concentrations were within 8% and 16% of the nominal concentrations added for *Cyprinodon* and *Eurytemora* tests, respectively, indicating no significant removal of cadmium due to precipitation reactions.

Statistical Analysis

The 96h LC50 values with 95% confidence limits were generated from the mortality data using the Trimmed Spearman-Karber method (Hamilton, et al., 1978). The LC50 values from adjacent salinities were compared by standard error of means (USEPA, 1985a) to determine significant effect ($p<0.05$).

RESULTS

Water Quality and Cadmium Chemistry

Water quality conditions measured every other day during the six 96 h toxicity tests are presented in Table 2. All conditions appeared adequate for survival of test species. Nominal and measured concentrations of total cadmium on day 0 and 4 during the acute tests are shown in Table 3 and Figure 2. Measured concentrations were very similar to the nominal concentrations in all tests as the relative standard deviation ranged from 0.42 to 6.89 %. In most instances, the relative standard deviation was less than 3%. All three saline controls contained no detectable cadmium at a detection limit of 3 μg/L.

Four samples were selected for voltammetric analysis (Table 4). The resulting titration curve in Figure 3 was linear in each instance thus suggesting no significant organic complexation (r^2 > 0.997). The presence of strong, electrochemically inert complexes would result in a significant deviation from linearity at low cadmium concentrations (Bruland, 1992). The electroactive cadmium concentrations agreed within 2.5 to 5 % with the total cadmium values determined by ICP-AES, indicating that the speciation of cadmium in the test solutions is dominated by the free ion and inorganic complexes (Table 4). Voltammetric analysis therefore yeilded no additional information beyond the total dissolved cadmium concentrations, rendering it unnecessary to perform ASV measurements with every sample. Note that the voltammetric analyses also serves as an independent check upon the accuracy of

Table 2. Ranges in water quality conditions during the 96-h cadmium toxicity tests.

Species	Test Salinity (ppt)	Temp (C)	pH	Sal (ppt)	D.O. (mg/L)
E. affinis	5	24 - 25	7.53 - 8.76	5 - 6	7.5 - 8.9
	15	24	8.09 - 8.38	14 - 16	7.5 - 7.9
	25	24 - 25	7.89 - 8.45	24 - 27	6.4 - 7.2
C. variegatus	5	24 - 25	7.45 - 7.63	5 - 6	6.7 - 7.7
	15	24 - 25	7.73 - 8.06	14 - 16	6.0 - 7.4
	25	24 - 25	7.91 - 8.22	25 - 26	5.4 - 6.9

Table 5. Total cadmium concentrations as determined by ICP-AES. Nominal cadmium values represent the original cadmium concentrations added to the samples at the beginning of each experiment. (Analytical precision is reported as the standard deviation and the coefficient of variation (C.V.) based upon replicate analyses (n=5) of the same sampl

Organism	Time	Salinity	Nominal [Cd] (µg/L)	Measured [Cd] (µg/L)	Std. Dev.	C.V.
Cyprinodon	D0	5	56	52.98	1.12	2.12
Cyprinodon	D0	5	180	166.10	2.04	1.23
Cyprinodon	D0	5	1000	963.01	24.78	2.57
Cyprinodon	D4	5	56	55.88	0.74	1.33
Cyprinodon	D4	5	180	177.09	1.88	1.06
Cyprinodon	D4	5	1000	994.23	18.69	1.88
Cyprinodon	D0	15	100	97.53	2.66	2.73
Cyprinodon	D0	15	320	312.99	6.89	2.20
Cyprinodon	D0	14	1800	1736.46	19.47	1.12
Cyprinodon	D4	15	100	99.81	3.25	3.25
Cyprinodon	D4	15	320	318.12	6.15	1.93
Cyprinodon	D4	16	1800	1893.76	8.64	0.46
Cyprinodon	D0	25	180	175.01	1.84	1.05
Cyprinodon	D0	25	560	559.11	15.81	2.83
Cyprinodon	D0	25	3200	3274.89	125.69	3.84
Cyprinodon	D4	26	180	183.77	6.02	3.28
Cyprinodon	D4	26	560	561.38	2.36	0.42
Cyprinodon	D4	26	3200	3252.98	101.08	3.11
Eurytemora	D0	5	32	27.01	0.87	3.23
Eurytemora	D0	5	100	93.17	0.97	1.04
Eurytemora	D0	5	560	550.10	15.67	2.85
Eurytemora	D4	6	32	28.16	1.94	6.89
Eurytemora	D4	6	100	92.27	1.11	1.21
Eurytemora	D4	6	180	168.86	3.65	2.16
Eurytemora	D0	14	32	30.21	1.10	3.64
Eurytemora	D0	14	100	100.19	1.47	1.47
Eurytemora	D0	14	560	535.90	7.96	1.49
Eurytemora	D4	14	32	28.16	1.30	4.61
Eurytemora	D4	15	100	88.29	1.98	2.24
Eurytemora	D4	14	560	501.66	17.70	3.53
Eurytemora	D0	24	56	50.51	0.52	1.03
Eurytemora	D0	24	180	176.37	5.90	3.34
Eurytemora	D0	24	560	523.50	4.95	0.94
Eurytemora	D4	26	56	49.44	2.80	5.66
Eurytemora	D4	27	180	160.28	1.99	1.24
Eurytemora	D4	26	560	518.72	13.29	2.56

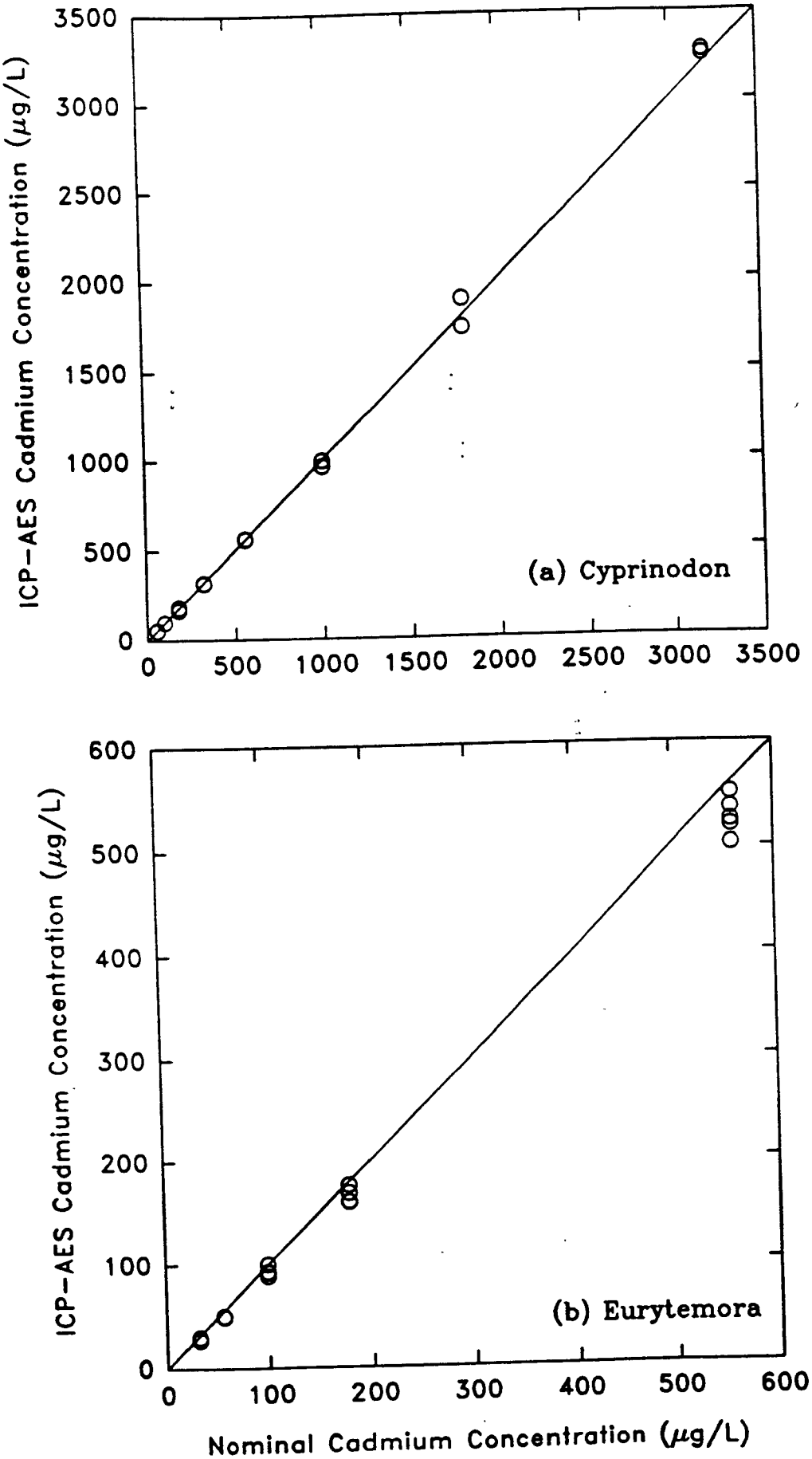


Figure 2. Total cadmium as determined by ICP-AES versus the nominal cadmium concentrations added to samples at the beginning of the experiments. The solid lines represent the expected 1:1 lines for perfect agreement between the data sets.

Table 4. Voltammetric analysis of selected samples. [Cd'] represents the sum of all electroactive cadmium species. [Cd] is the value from ICP-AES analysis.

Organism	Time	Salinity	ICP-AES [Cd] (µg/L)	[Cd'] (µg/L)
Cyprinodon	D4	15	99.81	95.10
Cyprinodon	D4	26	183.77	179.81
Eurytemora	D4	6	28.16	27.49
Eurytemora	D4	14	28.16	28.92

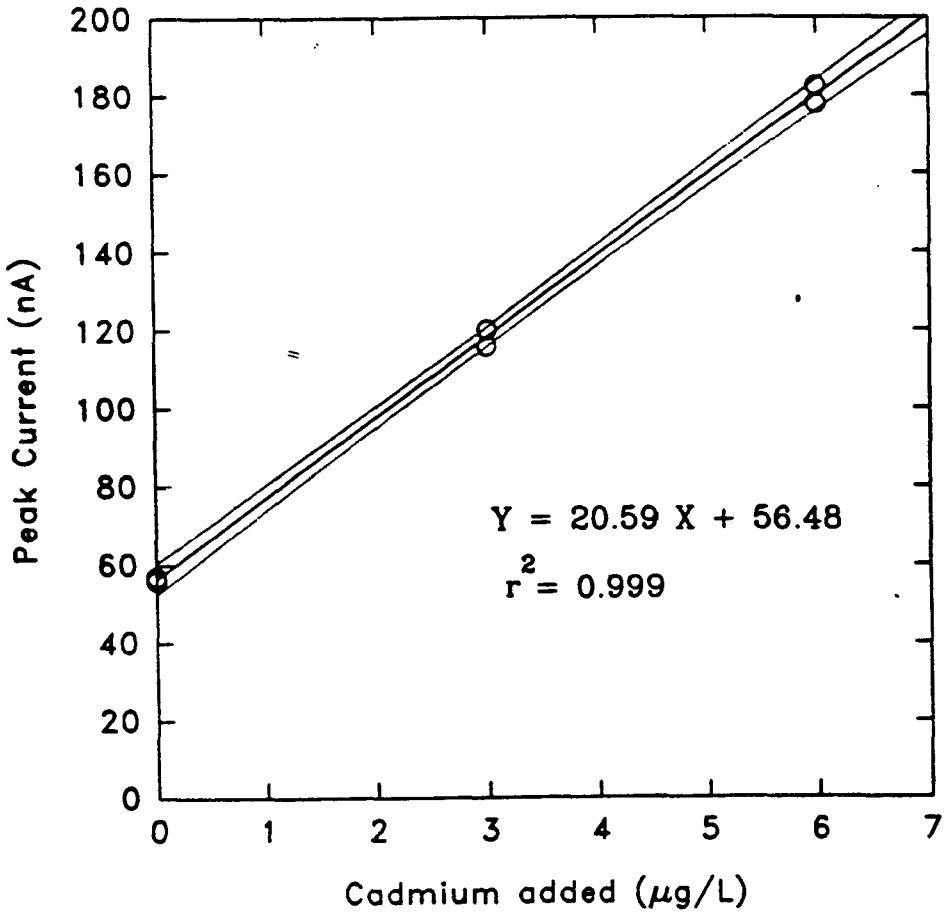


Figure 3. Titration curve for the voltammetric analysis of Eurytemora, 5 ppt salinity, 32 µg/L Cd, t=4. The solid and dotted lines represent the linear regression and 95% confidence intervals, respectively, through the data.

the ICP-AES results for total dissolved cadmium.

The inorganic cadmium speciation as predicted by MINEQL+ is present in Table 5 and 6. The predicted concentrations for each dissolved species in terms of concentration is presented in Table 5 while Table 6 lists the species as a percentage of the total cadmium in solution. Seven species were identified as significant (>1%): the free hydrated ion (Cd^{2+}); carbonate and sulfate complexes ($CdCO_3$ and $CdSO_4$) and the four chloride complexes ($CdCl^+$, $CdCl_2°$, $CdCl_3^-$, and CdOHCL°. The speciation was dominated at all salinities by $CdCl^+$ and $CdCl_2°$. The free ion accounted for 20, 8 and 4.5% of the total dissolved cadmium at 5, 15 and 25 ppt, respectively. Small variations between tests were due to small differences in sample pH and salinity.

Toxicity Data

Ninety-six hour LC50 values for *E. affinis* and *C. variegatus* are presented in Table 7 (See Appendix A for raw data). The copepod values were 51.6, 213.2 and 82.9 µg/L total cadmium at 5, 15 and 25 ppt, respectively. Acute toxicity values for *Cyprinodon* were 180.3, 312.4 and 495.5 µg/L total cadmium at 5, 15 and 25 ppt, respectively. A comparison of LC50 values for *E. affinis* between various salinities showed a significant difference between 5 and 15 ppt and between 15 and 25 ppt (Table 8). There was no significant difference in LC50 values between 5 and 25 ppt. A comparison between the various LC50 values for *Cyprinodon* showed a significant increase with salinity (Table 8). The LC50 at 5 ppt was

Table 5. Thermodynamic equilibrium model results as a function of species concentration. Equilibrium modeling was done using the MINEQL+ interactive personal computer program.

Org.	Time	Sal	pH	Total [Cd] (µg/L)	Predicted Conc. of Dissolved Complexes (ug/L) Cd(2+)	CdOHCl	CdCl +	CdCl3 -	CdCl2.	CdCO3	CdS
Cyp.	D0	5	7.63	52.98	10.33	0.57	32.26	0.35	6.87	0.66	1.
Cyp.	D0	5	7.62	166.10	32.48	1.74	101.38	1.08	21.58	1.98	5.
Cyp.	D0	5	7.56	963.01	188.83	8.85	590.10	6.31	125.89	8.74	29.
Cyp.	D4	5	7.5	55.88	10.99	0.45	34.28	0.37	7.31	0.39	1.
Cyp.	D4	5	7.46	177.09	35.07	1.30	109.37	1.17	23.27	1.02	5.
Cyp.	D4	5	7.57	994.23	194.45	9.34	608.08	6.51	129.26	9.44	30.
Cyp.	D0	15	8.03	97.53	7.81	2.26	51.14	4.11	27.31	2.63	1.
Cyp.	D0	15	8.06	312.99	24.84	7.72	162.98	13.04	87.00	9.62	5.
Cyp.	D0	14	8.06	1736.46	146.12	43.39	916.06	64.74	460.84	57.32	31.
Cyp.	D4	15	7.75	99.81	8.27	1.26	54.06	4.34	28.89	0.77	1.
Cyp.	D4	15	7.73	318.12	26.41	3.83	173.10	13.83	92.06	2.24	5.
Cyp.	D4	16	7.87	1893.76	146.12	30.46	996.99	90.03	560.88	23.27	31.
Cyp.	D0	25	8.21	175.01	7.74	5.02	74.97	15.85	62.83	5.63	2.
Cyp.	D0	25	8.21	559.11	24.62	15.96	239.41	50.36	200.07	17.98	7.
Cyp.	D0	25	8.22	3274.89	143.87	95.65	1393.76	294.49	1168.96	109.59	43.
Cyp.	D4	26	7.93	183.77	8.26	2.83	80.48	18.10	69.01	1.63	2.
Cyp.	D4	26	7.91	561.38	25.40	8.28	247.28	55.30	211.31	4.55	6.
Cyp.	D4	26	8.01	3252.98	145.00	59.80	1416.24	318.09	1213.92	41.36	38.
Eur.	D0	5	7.62	27.01	5.26	0.28	16.41	0.18	3.50	0.32	0
Eur.	D0	5	7.60	93.17	18.21	0.94	56.87	0.61	12.14	1.01	2
Eur.	D0	5	7.63	550.10	107.23	5.90	334.95	3.57	71.26	6.85	16
Eur.	D4	6	8.09	28.16	4.42	0.79	15.62	0.23	3.87	2.28	0
Eur.	D4	6	8.11	92.27	14.27	2.69	50.58	0.76	12.59	8.09	2
Eur.	D4	6	8.1	168.86	26.30	4.82	92.84	1.39	23.04	14.16	4
Eur.	D0	14	8.09	30.21	2.54	0.81	15.85	1.12	8.00	1.15	0
Eur.	D0	14	8.13	100.19	8.31	2.90	52.04	3.69	26.19	4.50	1
Eur.	D0	14	8.13	535.90	44.51	15.51	278.75	19.67	140.50	24.05	9
Eur.	D4	14	8.32	28.16	2.09	1.16	13.49	0.97	6.89	2.75	0
Eur.	D4	15	8.38	88.29	5.91	4.00	40.35	3.32	22.03	10.21	1
Eur.	D4	14	8.37	501.66	35.74	22.59	232.67	16.75	119.14	59.57	9
Eur.	D0	24	8.18	50.51	2.37	1.38	22.14	4.30	17.87	1.51	0
Eur.	D0	24	8.16	176.37	8.37	4.64	77.78	15.06	62.61	4.83	2
Eur.	D0	24	8.23	523.50	24.05	15.85	225.92	44.29	183.21	19.33	7
Eur.	D4	26	8.24	49.44	2.06	1.48	20.57	4.69	17.98	1.71	0
Eur.	D4	27	8.15	160.28	6.62	3.93	67.33	16.30	60.13	3.60	1
Eur.	D4	26	8.45	518.72	19.78	23.27	200.07	45.75	175.34	43.50	6

Table 6. Thermodynamic equilibrium model results as a percentage of the total cadmium concentration. Equilibrium modeling was done using the MINEQL+ interactive personal computer program.

Org.	Time	Sal	pH	Total [Cd] (µg/L)	Predicted Cadmium Speciation As A Percentage of Total [Cd] Cd(2+)	CdOHCl	CdCl + CdCl3 -	CdCl2	CdCO3	CdS(
Cyp.	D0	5	7.63	52.98	19.5	1.1	60.9	0	13.0	1.2	3.
Cyp.	D0	5	7.62	166.10	19.5	1.0	60.9	0	13.0	1.2	3.
Cyp.	D0	5	7.56	963.01	19.6	0	61.2	0	13.0	0	3.
Cyp.	D4	5	7.5	55.88	19.7	0	61.5	0	13.1	0	3.
Cyp.	D4	5	7.46	177.09	19.7	0	61.6	0	13.1	0	3.
Cyp.	D4	5	7.57	994.23	19.6	0	61.2	0	13.0	0	3.
Cyp.	D0	15	8.03	97.53	8.0	2.3	52.4	4.2	28.0	2.7	1.
Cyp.	D0	15	8.06	312.99	7.9	2.5	52.1	4.2	27.8	3.1	1.
Cyp.	D0	14	8.06	1736.46	8.5	2.5	52.9	3.7	26.6	3.3	1.
Cyp.	D4	15	7.75	99.81	8.3	1.3	54.2	4.3	28.9	0.0	1.
Cyp.	D4	15	7.73	318.12	8.3	1.2	54.3	4.4	28.9	0.0	1.
Cyp.	D4	16	7.87	1893.76	7.7	1.6	52.8	4.8	29.7	1.2	1.
Cyp.	D0	25	8.21	175.01	4.4	2.9	42.8	9.0	35.9	3.2	1.
Cyp.	D0	25	8.21	559.11	4.4	2.9	42.8	9.0	35.9	3.2	1.
Cyp.	D0	25	8.22	3274.89	4.4	2.9	42.6	9.0	35.8	3.3	1.
Cyp.	D4	26	7.93	183.77	4.5	1.5	43.9	9.8	37.7	0	1.
Cyp.	D4	26	7.91	561.38	4.5	1.5	44.0	9.9	37.7	0	1.
Cyp.	D4	26	8.01	3252.98	4.5	1.8	43.6	9.8	37.4	1.3	1.
Eur.	D0	5	7.62	27.01	19.5	1.0	60.9	0	13.0	1.2	3.
Eur.	D0	5	7.60	93.17	19.6	1.0	61.0	0	13.0	1.1	3.
Eur.	D0	5	7.63	550.10	19.5	1.1	60.9	0	13.0	1.2	3.
Eur.	D4	6	8.09	28.16	15.7	2.8	55.3	0	13.7	8.1	2.
Eur.	D4	6	8.11	92.27	15.5	2.9	54.8	0	13.6	8.8	2.
Eur.	D4	6	8.1	168.86	15.6	2.9	55.0	0	13.7	8.4	2.
Eur.	D0	14	8.09	30.21	8.4	2.7	52.5	3.7	26.5	3.8	1.
Eur.	D0	14	8.13	100.19	8.3	2.9	52.0	3.7	26.2	4.5	1.
Eur.	D0	14	8.13	535.90	8.3	2.9	52.0	3.7	26.2	4.5	1.
Eur.	D4	14	8.32	28.16	7.4	4.1	47.9	3.4	24.5	9.8	1.
Eur.	D4	15	8.38	88.29	6.7	4.5	45.8	3.8	25.0	11.6	1.
Eur.	D4	14	8.37	501.66	7.1	4.5	46.5	3.4	23.9	11.9	1.
Eur.	D0	24	8.18	50.51	4.7	2.7	43.8	8.5	35.4	3.0	1.
Eur.	D0	24	8.16	176.37	4.7	2.6	44.1	8.5	35.5	2.7	1.
Eur.	D0	24	8.23	523.50	4.6	3.0	43.2	8.4	35.0	3.7	1.
Eur.	D4	26	8.24	49.44	4.2	3.0	41.7	9.5	36.3	3.5	1.
Eur.	D4	27	8.15	160.28	4.1	2.4	41.9	10.2	37.4	2.2	1.
Eur.	D4	26	8.45	518.72	3.8	4.5	38.6	8.8	33.8	8.4	1.

* 0 = less than 1%

Table 7. Ninety-six h LC50 values (µg/L) (with 95% confidence limits) and mean control survival for *E. affinis* and *C. variegatus* tested at three salinities.

Species	Test Salinity (ppt)	Mean Control Survival % (S.E.)	96-H LC50 (95% C.L.)
E. affinis	5	91.7 (8.3)	51.6 (36.2-73.5)
	15	97.0 (3.0)	213.2 (182-249.7)
	25	80.0 (5.8)	82.9 (51.1-134.3)
C. variegatus	5	100	180.3 (151.5-214.5)
	15	93.3 (3.3)	312.4 (275.1-354.7)
	25	100	495.5 (420.9-583.2)

Table 8. A comparison of LC50 values between adjacent salinities using the Standard Error of Means Method.

Species	Salinity (ppt)	Z Value	H Value	Significant (p<.05)
E. affinis	5-15	4.1348	1.4736	*
	15-25	2.5729	1.6614	*
	5-25	1.6071	1.8192	
C. variegatus	5-15	1.7222	1.2166	*
	15-25	1.6129	1.2112	*
	5-25	2.7778	1.2384	*

significantly lower than 15 ppt and the value at 15 ppt was significantly lower than 25 ppt. The value at 5 ppt was also significantly lower than at 25 ppt.

DISCUSSION

Voltammetric analysis of selected samples were in excellent agreement with the ICP-AES measurements. Standard addition curves were linear, indicating no significant organic complexation. The "cadmium complexing capacity" of the experimental sample matrix was therefore negligible. Furthermore, there was no significant production of cadmium binding ligands by the test organisms over the course of the experiments. The data presented above were important for these experiments because it was demonstrated that cadmium speciation was dominated by association with inorganic binding ligands.

In freshwater at pH of 6, Turner et al. (1981) predicted that the inorganic cadmium would be approximately 96% free with carbonate complexes becoming important at higher pH. In seawater at 35 ppt and a pH of 8.2, the chloride complexes were predicted to account for approximately 97% of the inorganic species with the free ion responsible for less than 3% of the total (Turner et al., 1981; Bryne et al., 1988). Assuming that only chemical factors are important and the organic ligands are negligible, the cadmium toxicity due to Cd^{+2} would be expected to decrease significantly over the course of an entire estuary (i.e., Chesapeake Bay) where salinity may range from 1 to greater than 26 ppt. At natural total cadmium concentrations, binding of cadmium by strong complexing ligands may reduce toxicity even further.

The inorganic complexation of cadmium in the sample solutions was estimated by the use of the MINEQL+ thermodynamic equilibrium

model. Model results indicated that the cadmium speciation was largely dominated by complexation with the chloride ion, with small contributions by the free ion and carbonate and sulfate species. The free ion decreased from 20% of the total dissolved cadmium at 5 ppt to less than 5% of the of the total at 25 ppt salinity. Similar data have been reported by other investigators as more free cadmium was present at low salinities varing in logarithmic fashion from about 23% of total as free cadmium at 5 ppt to only about 4% at 32 ppt (Sunda et al., 1978; Engle and Fowler, 1979). Our data would suggest a four fold decrease in toxicity from 5 to 25 ppt assuming that the free ion is bioactive form of the element and physiological factors of the test species are negligible. The toxicity data with *Cyprinodon* generally support this prediction as there is approximately a three fold decrease in toxicity from 5 to 25 ppt. The *Eurytemora* data however do not support this prediction as the 96 h LC50 values at both 5 and 25 ppt were similar. This species was most resistant to cadmium at the middle salinity (15 ppt). The physiological characteristics of *Eurytemora* may have been responsible for higher tolerance at the middle salinity. Sprague (1985) has suggested that euryhaline species are most resistent to toxic conditions at isosmotic salinities due to minimization of osmotic stress. Other investigators have also reported there is decreased osmotic stress in various aquatic species as salinity increases toward the isosmotic point, with a decreased inward flow of water, which presumably would be accompanied by reduced intake of toxic ions (Herbert and Wakefield,

1964; Herbert and Shurben, 1965).

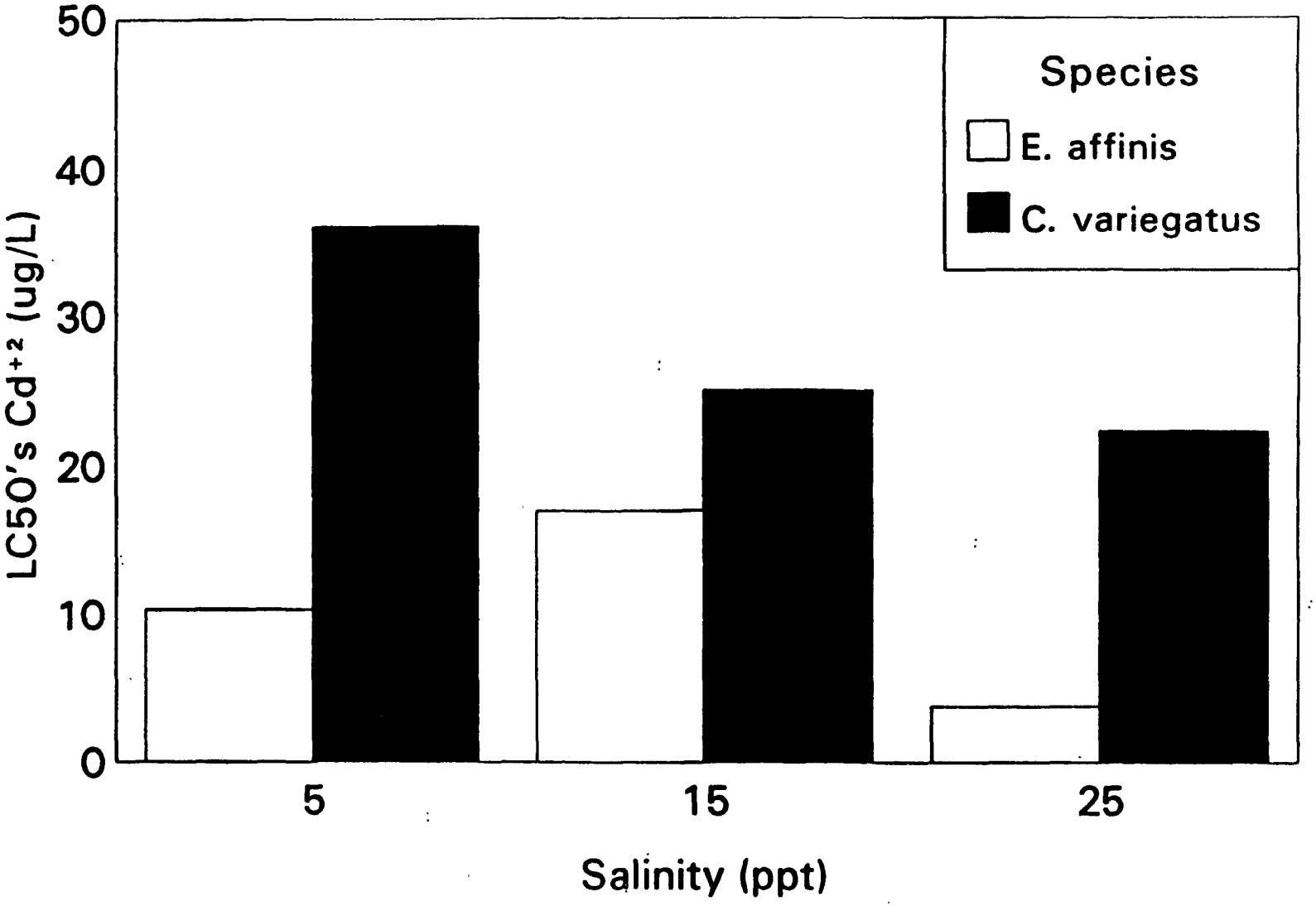
The 96 h LC50 values for *E. affinis* reported from the three cadmium toxicity tests at the various salinities ranged from 51.6 to 213.2 µg/L total cadmium. Sullivan et al. (1983) reported a similar 96 h LC50 of 147.7 µg/L total cadmium for *E. affinis* at 10 ppt. Another investigator reported a substantially higher 96 h LC50 of 1,080 µg/L total cadmium for *Eurytemora* (Gentile, 1982). Acute toxicity values ranging from 90 to 337 µg/L total cadmium at 30 ppt for a similar copepod species, *Acartia tonsa* were also similar to our *Eurytemora* data (Gentile, 1982; Sosnowski and Gentile, 1978). The lowest LC50 value (51.6 µg/L) reported for *E. affinis* in our experiments was only slightly higher than the U.S. EPA acute marine water quality criterion of 43 µg/L (U.S. EPA, 1987). The range of acute cadmium values reported for saltwater invertebrate species in the U.S. EPA cadmium water quality criteria document was 41.29 to 135,000 µg/L total cadmium (U.S. EPA, 1985b). These data suggest that *Eurytemora* is very sensitive to cadmium when compared with other estuarine aquatic biota.

The 96 h LC50 values for *Cyprinodon* larvae ranged from 180.3 to 495.5 µg/L total cadmium at the three test salinities. Cardin (1982) reported acute toxicity values of 577 to 602 µg/L for the larval stages of the Atlantic silverside and the winter flounder, respectively at 20 ppt. These values are similar to the acute LC50s we reported for *Cyprinodon*. Other investigators have reported higher acute values for other larval estuarine fish. Middaugh and Dean (1977) reported 48 h LC50s of 9,000 and 32,000 µg/L for 7 and

14 day larval mummichogs, respectively at 20 ppt. These investigators also reported 48 h LC50s of 3,800, and 2,200 µg/L for 1 and 14 day old Atlantic silverside larvae at 20 ppt.

In a recent review synthesizing the influence of salinity on the toxicity of various classes of chemicals, it was reported from 33 cadmium toxicity studies that cadmium toxicity generally increased with decreasing salinity (Hall and Anderson, 1994). The *Cyprinodon* data from the experiments follow this trend as 96 h LC50 values were significantly lower at lower salinities thus suggesting that cadmium bioavailablity (chemical factors) were the predominant mechanism for toxicity. The *Eurytemora* data do not follow this trend due to the physiological factors previously discussed. One important aspect of this study was the calculation of the free cadmium along with the total cadmium concentration in the experiments. The free cadmium ion (Cd^{+2}) is generally considered to be the toxic form of cadmium to aquatic biota in the water column and it is virtually inversely proportional to salinity (Sunda et al., 1978; Engle and Fowler, 1979). A calculation of 96 h LC50s for free cadmium for *Eurytemora* based on using 20, 8 and 4.5% of the total cadmium at 5, 15 and 25 ppt, respectively, showed that free cadmium was still less toxic at the middle salinity (Figure 4). The calculated free cadmium LC50 values for *Cyprinodon* were similar at both 15 and 25 ppt but were somewhat higher at 5 ppt (Figure 4).

Figure 4. Ninety-six hour LC50 values for *E. affinis* and *C. variegatus* at various salinities based on calculating Cd^{+2} from total cadmium values.



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APPENDIX A

Raw data from the *Eurytemora* and *Cyprinodon* 96 h toxicity tests at three salinities

96 h *Eurytemora* Tests

Salinity	Cd conc (mg/L)	# Alive/Total per Rep A	B	C	$\bar{x}$ % Survival
5 ppt	0	8/8	8/8	6/8	91.7
	.032	2/8	6/8	6/8	66.7
	.056	3/8	4/8	5/9	47.7
	.100	4/7	0/7	1/8	23.2
	.180	0/7	0/7	0/7	0
15 ppt	0	10/10	10/10	10/11	97
	.032	8/10	12/12	9/10	90
	.056	8/11	11/13	9/10	83
	.100	10/11	10/10	11/12	94
	.180	9/10	8/11	8/10	81
	.320	1/11	1/10	0/11	6
	.560	0/11	0/10	0/12	0
25 ppt	0	9/10	7/10	8/10	80
	.056	7/10	6/10	6/10	63.3
	.100	0/10	3/10	8/11	35.5
	.180	5/10	5/12	4/10	43.8
	.320	1/11	0/11	0/11	3
	.560	0/12	0/10	0/10	0

96 h *Cyprinodon* Tests

Salinity	Cd conc (mg/L)	# Dead/10 per Rep A	B	C	$\bar{x}$ % Mortality
5 ppt	0	0	0	0	0
	.056	1	0	2	10
	.10	1	1	1	10
	.18	8	4	7	63
	.32	9	8	5	73.3
	.56	10	10	9	96.7
	1.00	10	9	10	96.7
15 ppt	0	1	0	1	6.7
	.10	0	0	0	0
	.18	2	1	0	10
	.32	4	8	2	46.7
	.56	10	10	9	96.7
	1.00	10	10	10	100
	1.80	10	10	10	100
25 ppt	0	0	0	0	0
	.18	1	0	0	3.3
	.32	2	3	3	26.7
	.56	6	5	5	53.3
	1.00	9	9	9	90
	1.80	10	10	10	100
	3.20	10	10	10	100