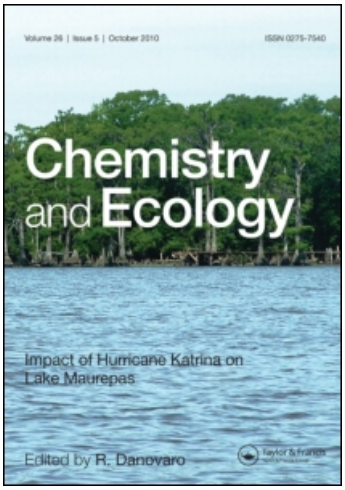

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THE INFLUENCE OF VARYING ALGAL BIOMASS ON CONTAMINANT EXPOSURE IN BENTHIC-PLANKTONIC MESOCOSMS: COPPER (II)

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A series of mesocosms was exposed to a suite of light treatments and nutrient enrichment in order to generate algal communities of varying biomass. The influence of this biomass on the speciation of copper (II) was studied. Distribution coefficients (K_d, $L\,kg^{-1}$) were relatively high (log K_d = 5 to 7), indicative of robust trace metal sequestration, and were likely controlled by the particulate organic carbon content (foc). Differences in K_d over time and among treatments were significant, as was the relationship between K_d and foc. Fluorescence quenching was used to determine binding capacities (L_t, M) and their associated binding constants (K_{cond}, M^{-1}) in order to model the solid phase copper speciation. The K_{cond} ranged between 2.1 and $5.2 \times 10^{12}\,M^{-1}$, indicating a very strong copper-ligand complex, and was higher in mesocosms that received more light. The light L_t, increased over time, dramatically after the nutrient enrichment, but did not vary systematically among light treatments. L_t ranged from 7.2×10^{-7} to 4.9×10^{-5} M. The large magnitudes of K_d, K_{cond} and L_t ensured that greater than 97% of total copper in the mesocosms was complexed by organic matter. The total copper concentration ($[Cu]_T$, M) needed to reach a target dissolved copper concentration of $10^{-12.5}$ M (pCu = 12.5) was determined for each mesocosm over time. $[Cu]_T$ was between 8.02×10^{-5} and 3.41×10^{-2} M, and increased over time. The $[Cu]_T$ normalized to the target pCu (Effective Dose Ratio, EDR) increased directly with increases in algal biomass, indicating a direct link between system productivity and copper exposure. Approximately 45% of the variance in EDR was explained by variance in total biomass, while the residual variance in EDR was due likely to differences in the strengths of particle associations and magnitude of binding capacities.

Keywords: Biomass; mesocosms; speciation; binding capacity; binding constant

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INTRODUCTION

Productive natural ecosystems feature biological feedbacks that can sequester contaminants and reduce their bioavailability to organisms (Gunnarson and Rosenberg, 1997; Jeremiason, 1997; Larrson *et al.*, 1992; Millard *et al.*, 1993). Higher productivity results in higher biomass, which subsequently reduces contaminant exposure through increased adsorption to the surfaces of particles, such as phytoplankton cells. Organic and inorganic ligand production, through processes such as excretion and cellular lysis, is also enhanced in more productive systems, and these ligands can complex with contaminants, rendering them biologically unavailable (Ko, 1994; Sijm *et al.*, 1995). McCarthy and Bartell (1988) found that increases in dissolved organic matter corresponded with lower toxicity to phytoplankton, zooplankton, benthic invertebrates and omnivorous fish.

Historically, contaminant bioavailability and toxicity has been evaluated using either a single plankton species bioassay at a bench-top scale (Ko, 1994; Sijm *et al.*, 1995; Stange and Swackhammer, 1994) or at the unconfined field population scale (Biggs *et al.*, 1980; Ko and Baker, 1995). Bench-top scale bioassays cannot take into account the degree of complexity or variability of food resources and nutrients that are found in natural ecosystems, which ultimately control the feedbacks that reduce contaminant bioavailability. Very large-scale experimental systems simply have too many variables. Small to mid-size enclosures, referred to as microcosms or mesocosms (Swartzman *et al.*, 1990), achieved a sufficient degree of complexity while maintaining a manageable size ($0.1-100\,m^3$), and were able to control parameters such as light, temperature, and number of trophic levels.

Mesocosms have been used as surrogates of existing ecosystems to evaluate the toxicity and exposure of a suite of contaminants, including pesticides and copper (Dahl *et al.*, 1996; Giddings *et al.*, 1996; Friesen *et al.*, 1995; Morris *et al.*, 1993; Howick *et al.*, 1991; Shaw & Manning, 1996). Swartzman *et al.* (1990) examined the feedback effects of algal biomass on the response of phytoplankton and zooplankton to copper toxicity using microcosms and simulation modelling. Their results suggested that the toxic effects of copper may be strongly influenced by the density and species composition of the plankton

and related differences in water chemistry. Greater algal biomass led to lower bioavailability of toxic copper. The importance of algal biomass was attributed to (1) changing copper availability due to adsorption and/or direct absorption, (2) the production of ligands by the phytoplankton that complex the copper in less toxic forms, and (3) changing pH which affects the free and toxic copper ion and copper ionization.

However, the extrapolation of contaminant fate and exposure from mesocosms to natural ecosystems must take into account the influence of system size. In experimental systems, the production of algal biomass, a major controlling factor in contaminant exposure, has been found to be scale-dependent (Perez *et al.*, 1991 and 1997; Petersen *et al.*, 1997; Howick *et al.*, 1991; Morris *et al.*, 1993; Johnson *et al.*, 1994). Perez *et al.* (1991) investigated the effect of Kepone on algal communities in three different sized mesocosms and found that planktonic biomass and the timing of their blooms were directly related to the size, in particular the wall surface area, of the experimental system. In turn, Kepone exposure was less than the applied dose and varied with system size due to the effects (adsorption and ligand complexation) of algal biomass on contaminant exposure.

Algal communities that colonize the container walls, called periphyton, are also influenced by system scale (Chen *et al.*, 1997). Periphyton biomass is directly related to the surface area of the container walls. Periphyton can be significant components of mesocosms and other experimental ecosystems, dominating the total autotrophic biomass (Rees, 1979), affecting ambient light fields, trophic interactions (Eppley *et al.*, 1978), and nutrient uptake and regeneration processes (Eppley *et al.*, 1978). Periphyton can affect the partitioning and reduce the exposure of various contaminants in the same manner as planktonic communities, by providing surfaces for adsorption and/or producing complexing ligands (Perez *et al.*, 1991).

The objective of this study was to investigate the influence of varying amounts of algal biomass on the speciation and exposure of a model trace metal, copper (II). The biomass of both planktonic and periphytic algae was varied not by size (Ashley and Baker, 1998), but by light exposure. A series of identically sized mesocosms was exposed to a suite of light treatments, ranging from zero to one hundred

percent light exposure. Additional temporal variability in algal biomass was introduced by subjecting the algal communities to a period of nutrient limitation followed by a nutrient pulse, and by physically removing the periphytic growth from the mesocosm walls. Copper was spiked into subsamples of the various mesocosms and distribution coefficients (K_d) were determined to ascertain the distribution of copper between suspended particulate and soluble phases. The inorganic and organic speciation of the soluble phase was further modelled using thermodynamic constants and measured inorganic species, experimentally-determined total ligand concentrations (binding capacities, L_t), and conditional stability constants (binding constants, K_{cond}). Since direct contaminant addition to the mesocosms was not possible, the sequestration of copper by the periphyton was modelled using empirical data gathered from the mesocosm experiment. The ability of algal biomass to reduce and control contaminant bioavailability was demonstrated by modelling copper concentrations necessary to generate a toxic response to phytoplankton.

Copper was chosen because unlike most contaminants, it has the ability to both limit and drive phytoplankton growth. Copper is a necessary electron donor or acceptor in a number of enzyme systems, such as cytochrome c oxidase (Sunda, 1991). Toxicity occurs when copper renders proteins and nucleic acids nonfunctional by outcompeting essential metals such as manganese for active enzyme sites (Sunda, 1987; Sunda *et al.*, 1981). The principal toxic species of copper is the free, "soluble" copper cation, Cu^{2+} (Sunda and Guillard, 1976; Brand *et al.*, 1986; Sunda *et al.*, 1978; Brand *et al.*, 1983; van den Berg *et al.*, 1987), along with some copper-hydroxyl species (Cowan *et al.*, 1984). Other soluble, but biologically unavailable forms of copper include complexes with inorganic ligands (*e.g.*, carbonate) and complexes with various naturally present (*e.g.*, humic substances) and/or anthropogenically-introduced (*e.g.*, EDTA) organic ligands. Particulate forms range from copper that has been adsorbed to large particles (*e.g.*, resuspended sediment or phytoplankton cells) to colloidal complexes. Anywhere from 32 to 82% of copper found in estuarine and marine systems may be particle-bound (Paulson *et al.*, 1994). Further, often greater than 99% of the remaining soluble copper has been found to be complexed by organic ligands (Tab. I; Donat *et al.*, 1994; van den Berg *et al.*, 1987; Coale and Bruland, 1988).

TABLE I Binding capacities (L_t), conditional stability constants (K_{cond}), the percentage of organically complexed soluble copper, and free copper concentrations (pCu) in estuarine and coastal waters. pCu is equivalent to the $-\log\{Cu^{2+}\}$ where {} denotes activity. Table modified from Donat *et al.* (1994)

Location	$L_t(nM)$	$Log\ K_{cond}$ (M^{-1})	*% Organic-Cu*	*pCu*	*Reference*
San Francisco Bay	13	13.5	80–92	~9	Donat *et al.* (1994)
North Sea	1.2–21.0	12.0–15.6	99.97	13.5	Donat & van den Berg (1992)
Sargasso Sea	1.5–73	10.5–15.9	99.8–99.99	13.5–15.4	Donat & van den Berg (1992)
Elizabeth River	NA	NA	98.3–99.96	10.1–12.6	Sunda *et al.* (1990)
Severn Estuary (UK)	13.3–196	11.4–12.8	99.38–99.96	11.1–12.8	Apte *et al.* (1990)
Tamar Estuary (UK)	140–500	8.1–9.4	NA	16.2–18.2	van den Berg *et al.* (1990)
Biscayne Bay	5.1	12.08	99.6	12.0–13.4	Moffet & Zika (1987)
Scheldt Estuary	28–206	13–14.8	> 99.6	12.7–14.4	van den Berg *et al.* (1987)
Narrangansett Bay	NA	NA	98–99.97	12.0–12.3	Sunda & Hanson (1987)
Cape San Blas, FL	NA	NA	98.7	11.5	Sunda & Ferguson (1983)
Mississippi River	NA	NA	99.1	11.3	Sunda & Ferguson (1983)
North Sea	NA	NA	80	10.3	Duinker & Kramer (1977)

MATERIALS AND METHODS

Mesocosm Facilities

The University of Maryland's Center for Environmental and Estuarine Studies established the Multiscale Experimental Ecosystem Research Center (MEERC) as one of the United States Environmental Protection Agency's Exploratory Research Centers. The objective of MEERC is to understand the relationships between spatial, temporal and complexity scales and ecosystem response in order to predict how ecosystems respond to perturbations. In particular, MEERC hopes to understand the influence of scale on the fate and transport of contaminants, and to predict the effect of contaminants at a given scale based on ecosystem responses at other scales. The larger environment

to which the results from these studies will be extrapolated to will be the Chesapeake Bay and its watershed.

Experimental Design

One of the MEERC experimental ecosystems consisted of interior cylindrical mesocosms of varying size and shape. Differences in both planktonic and periphytic algal growth were generated by exposing five identically sized, replicate mesocosms ($n = 2$) to a series of five light regimes, ranging from 100% of the total light (full) to 0% (no) light (Tab. II). Screens were used to alter light exposure. This experiment occurred over a period of 51 days in the summer of 1994 (July 6 to August 25). Several other studies using the same mesocosms occurred concurrently; primary productivity, respiration, and zooplankton abundance were also examined.

All mesocosms were constructed of a fiberglass-reinforced glazing material, housed in a temperature controlled facility (Horn Point Environmental Laboratory, University of Maryland System, Cambridge, Maryland) and received light from banks of fluorescent and incandescent bulbs on a 12 : 12 L : D cycle. Large, slow-moving PVC impellers created turbulence and mixing that mimicked turbulence associated with semi-diurnal tidal cycles in the Chesapeake Bay (Sanford, unpublished data). Initial conditions included a water column composed of unfiltered water from the Choptank River, a tributary of the Chesapeake Bay, that was collected in late spring (salinity varied seasonally from 8 to 12 PSU). The initial conditions also included sediments (10 cm depth) which were composed of a mixture of commercial sand and local estuarine muds (Petersen *et al.*, 1997). Once filled, the benthic, pelagic and periphytic communities with the mesocosms were allowed

TABLE II Experimental light treatments for the replicate mesocosms ($n = 2$)

Treatment	*Light intensity* ($\mu Em^{-2}sec^{-1}$)	*Exposure level*
1	320	100%
2	160	50%
3	80	25%
4	32	10%
5	0	0%

to develop. A detailed description of the design, construction, and experimental operation of the mesocosms used in this study can be found in Petersen *et al.* (1997), Chen *et al.* (1997) and Turner (1996).

During the experiment, the unfiltered seed water was replaced at a rate of 10% per day with filtered water (0.5 μm pore size) from the Choptank River. The filtered water excluded organisms larger than copepod nauplii (*e.g.*, zooplankton, fish larvae). Both the seed and the replacement waters contained ambient levels of nutrients and salinity. Since ambient nutrient concentrations were low during this experiment, a pulse of nutrients was added on day 33. Three pulses of nutrients were added at 12 hour intervals to bring the concentration of ammonium up to 50 μM, with dissolved silica and phosphate pulsed simultaneously to achieve levels of 50 μM and 3.1 μM, respectively (Chen *et al.*, 1997). All other environmental conditions (*e.g.*, temperature, mixing) were held constant.

Using an automated measuring platform, dissolved oxygen (polarographic electrodes), temperature, conductivity, pH, and conductivity were measured on a regular basis (Madden, 1995). Salinity was calculated from conductivity measurements. The automated measuring platform was also used to measure water column chlorophyll *a* concentrations, which were used in conjunction with acetone-extracted chlorophyll measurements to convert all water column chlorophyll *a* readings to concentration units ($\mu g\, l^{-1}$). Water quality was monitored biweekly by measuring nutrients (ammonium, nitrate, nitrite, phosphorus and silica) using standard automated wet chemical methods. Bacteria, phytoplankton and zooplankton were collected biweekly using a pump, and the abundance and size distribution of zooplankton determined (Chen *et al.*, 1997; Petersen *et al.*, 1997). Bacteria, phytoplankton and periphyton were not characterized by species in this experiment; future studies will address these issues.

A technique developed by Chen *et al.* (1997) was used to measure biomass of periphyton in the mesocosms. Briefly, fibreglass strips made of the same material as the mesocosm walls were attached to the mesocosm walls at the beginning of the experiment, and were retrieved periodically. Material on the strips was scraped, extracted, and the chlorophyll *a* in the extracts was measured by fluorescence. The chlorophyll *a* concentration of the scraped material was used as an index of periphytic algal biomass.

Sampling

Water samples were collected from the mesocosm facility and brought to the Chesapeake Biological Laboratory (University of Maryland Systems, Solomons, Maryland) within two hours of collection for processing and experimentation. Time restrictions and because of limits on the number and amount of samples allowed per investigator, one sample of mesocosm water from each replicate mesocosm was collected approximately once a week for six weeks. Water column samples were collected using a PVC pipe siphon apparatus that removed the water from the approximate centre of the mesocosm water column and were stored in four litre nitric acid-cleaned polyethylene bottles. Subsamples were taken in order to determine distribution coefficients, binding constants and binding capacities, and for ancillary measurements. In order to minimize contamination artifacts during sample collection, filtration, and analysis, trace metal clean procedures as described by Nriagu *et al.* (1993) were used throughout.

Ancillary Measurements

Subsamples of filtered and unfiltered water from each replicate mesocosm were analyzed for dissolved organic carbon (DOC), total suspended solids (TSS), particulate organic carbon (POC), and major ion analysis (chloride and sulphate). TSS was determined by passing a volume of sample through a tared 0.4 μm Nucleopore filtre, dried at 50°C, and reweighed. DOC was measured with a TOC analyzer (OI Corp., Model 700) using an automated persulphate digestion. POC was measured by high temperature combustion using an element analyzer (Control Equipment Corp., Model 240XA). Chloride and sulphate were measured using an ion chromatograph (Dionex DX 500) equipped with an electrical conductivity detector. The experimental methods for these analyses are described in detail in Turner (1996).

Measurement of Copper Distribution Coefficients to Suspended Solids

A distribution coefficient, K_d, in units of $L\,kg^{-1}$ was calculated from soluble and particulate phase copper concentrations by the following

equation:

$$K_d = \frac{[\mathrm{Cu(II)}]_{\mathrm{par}}/\mathrm{TSS}}{[\mathrm{Cu(II)}]_{\mathrm{sol}}} \quad (1)$$

where $[\mathrm{Cu(II)}]_{\mathrm{par}}$ and $[\mathrm{Cu(II)}]_{\mathrm{sol}}$ are the concentrations of copper ($\mathrm{ng\,ml^{-1}}$) in the particulate and soluble phases respectively, and TSS is the total suspended solid concentration ($\mathrm{kg\,l^{-1}}$). A 100 ml of each unfiltered water column sample was placed in a Teflon beaker and spiked with a known amount (500 to 5000 ng of Cu(II)) of 100 ppm copper (II) standard. The copper standard was made by dissolving cupric sulphate, 5-hydrate ($CuSO_4 \cdot 5H_2O$) in deionized water. These samples were mixed for at least 1 hour with an overhead plastic stirrer. Others have demonstrated that 1 hour is sufficient time to achieve equilibrium for the adsorption of copper to the surface of particulate matter (Gonzalez-Davila *et al.*, 1995; Harris and Ramelow, 1990). Each sample was then vacuum filtered through a glass fibre filter (0.7 μm nominal pore size). The glass fibre filter (GFF) was removed from the filter head with Teflon-coated forceps, and immersed in a measured volume of 3.0 M hydrochloric acid for four days to liberate the copper that was bound to the particles and to the filter matrix. This extract was called the "particulate" phase. The filtrate, known as the "soluble" phase, and the particulate phase extract were stored separately in polyethylene bottles. The total copper contained in these extracts was measured by graphite furnace atomic absorption spectroscopy (GFAAS) using a Perkin-Elmer Zeeman 5000. The ambient levels of copper in the mesocosms were also measured by GFAAS. The GFAAS instrumental analysis, quantification of K_d, statistical analysis and analytical quality assurance are described in detail in Turner [25].

Measurements of the Concentrations and Binding Strengths of Copper-binding Ligands

Approximately 70% of each mesocosm water sample was vacuum filtered through a GFF (0.7 μm), placed in acid-cleaned, one litre Wheaton jars, and stored in the dark at 4°C until analysis by fluorescence spectrophotometry. The theory behind fluorescence quenching method has been previously described (Newell, 1983). Briefly,

fluorescence quenching estimates dissolved ligand concentrations and their associated binding constants. If a 1 : 1 complex of copper to ligand is assumed, measured fluorescence is related to copper concentration, ligand concentration and binding constant by the following equation:

$$\mathrm{Cu}_t = \frac{(I_{\max} - I)}{(I - I_{\min})} * \left(\frac{1}{K_{\mathrm{cond}}}\right) + \frac{(I_{\max} - I)}{(I_{\max} - I_{\min})} * (L_t) \tag{2}$$

where I is the fluorescence of the ligands at each point in the titration, $I_{\max}$ is the fluorescence of the free, unbound ligands (*i.e.*, no added copper) and $I_{\min}$ is the fluorescence of the bound, saturated ligands (Newell, 1983). Cu_t is the concentration of copper at each point in the titration.

Equation 2 is derived from the mole fraction of bound ligand to total ligand, v

$$v = \frac{[\mathrm{CuL}]}{L_t} = \frac{I_{\mathrm{mac}} - 1}{I_{\max} - I_{\min}} \tag{3}$$

[CuL] is the equilibrium concentration of the copper-ligand complex. The conditional stability constant for this equilibrium is

$$K_{\mathrm{cond}} = \frac{[\mathrm{CuL}]}{[\mathrm{Cu}^{2+}][\mathrm{L}^{2-}]}. \tag{4}$$

Using the mole fraction v and the two identities

$$\mathrm{CuL} \Leftrightarrow \mathrm{Cu}^{2+} + \mathrm{CuL} \tag{5}$$

and

$$L_t \Leftrightarrow \mathrm{L}^{2-} + \mathrm{CuL}, \tag{6}$$

the conditional stability constant may be rewritten as

$$K_{\mathrm{cond}} = \frac{vL_t}{(\mathrm{Cu}_t - vL_t)(1 - v)(L_t)}, \tag{7}$$

After substituting Eq. (4) into Eq. (7) and rearranging, Eq. (2) is obtained (Newell, 1983).

A 250 ml subsample of each mesocosm water sample was vacuum filtered through a GFF (0.7 μm nominal pore size), placed in a Teflon beaker, and mixed with a plastic overhead stirrer. Each sample was first calibrated to a 1 μM quinine sulphate solution (a fluorescent standard), to determine the relative strength of I_{max}. A suite of copper (II) standards (10, 100, 1000 μg/L) were titrated into the sample until the total added copper concentration was approximately 6350 $\mu g/l^{-1}$ (100 μM). After each copper addition, the fluorescence intensities of 2 ml sample aliquots were measured at each titration step using a fluorescence spectrophotometer. In some cases, the fluorescence was fully quenched at copper concentrations lower than 100 μM, and the titration was stopped. pH was measured simultaneously and maintained at 8.0 by adding 0.1 M sodium hydroxide to the solution. The instrumental analysis, statistical analysis used to calculate L_t and K_{cond}, and analytical quality assurance is described in Turner (1996).

Chemical Distribution and Speciation Modelling

The objective of this study was to examine the influence of varying algal biomass on the exposure and bioavailability of copper. Since direct contaminant addition to the mesocosms was not possible, the distribution of copper between the soluble phase and the periphyton was modelled to be the same as the distribution of copper between the soluble phase and the suspended particles. The modelling effort was designed to begin with an arbitrarily chosen target free copper concentration sufficient to cause toxicity, and ultimately determine the total copper concentration needed to provide this target free copper concentration, in each mesocosm on each day. Experimentally determined K_d values, binding capacities, binding constants, thermodynamic stability constants from the literature (Tab. III), and ancillary measurements (activity coefficient-corrected major ion concentrations, temperature, TSS and pH) were used in the modelling effort to determine the distribution of copper between the three phases (wall, suspended and soluble) and the speciation within the soluble phase.

TABLE III Thermodynamic stability constants used in inorganic copper speciation calculations

Species	*Log K* (M^{-1})	*Reference*
$Cu(OH)_3^-$	15.10	Paulson & Kester (1980)
$Cu(OH)_4^{2-}$	16.40	Paulson & Kester (1980)
$Cu(OH)^+$	6.3	Martell & Smith (1976)
$Cu(OH)_2$ aq	11.80	Martell & Smith (1976)
$CuHCO_3^+$	1.84	Byrne & Miller (1985)
$Cu(CO_3)_2^{2-}$	10.51	Byrne & Miller (1985)
$CuCO_3$	6.77	Byrne & Miller (1985)
$CuCl_3^-$	2.29	Martell & Smith (1976)
$CuCl_2$ aq	0.16	Martell & Smith (1976)
$CuCl_4^{2-}$	4.59	Martell & Smith (1976)
$CuCl^+$	0.43	Martell & Smith (1976)
$CuSO_4$	2.36	Martell & Smith (1976)

RESULTS AND DISCUSSION

Characterization of Algal Biomass and Mesocosm Water Column

The biomass of planktonic and periphytic algae varied both according to light treatment and over time. Planktonic algal biomass, in terms of chlorophyll *a* concentration (Fig. 1) , measured on day 2 varied from 20 to 40 $\mu g/l^{-1}$, but after twelve days chlorophyll *a* levels were generally higher in treatments with more light. The levels of chlorophyll *a* in the 100% and 50% light tanks increased dramatically after the nutrient enrichment (day 33), while the 10% light and 0% light tanks showed no response.

The variability seen in the biomass of periphyton (Fig. 1) contrasted with that of planktonic algae. Periphyton biomass was highest in the 10% light tanks. It may have been lower in the higher-light tanks because of a shading effect; the higher-light tanks have a greater suspended algal biomass, which shades and inhibits periphyton growth. Periphyton biomass $\pm$ SD also increased tremendously after the nutrient enrichment in all but the 0% light tanks, going from less than $0.001 \pm 0.001\,mg\,m^{-3}$ to between 79.4 ± 40.5 (10% light) and $45.1 \pm 43.8\,mg\,m^{-3}$ (25% light). After the nutrient pulse, the biomass of periphyton in the 10% and 25% light tanks was a factor of three to ten higher than the biomass of planktonic algae in these same tanks. The inter-treatment and temporal variance of total suspended solids

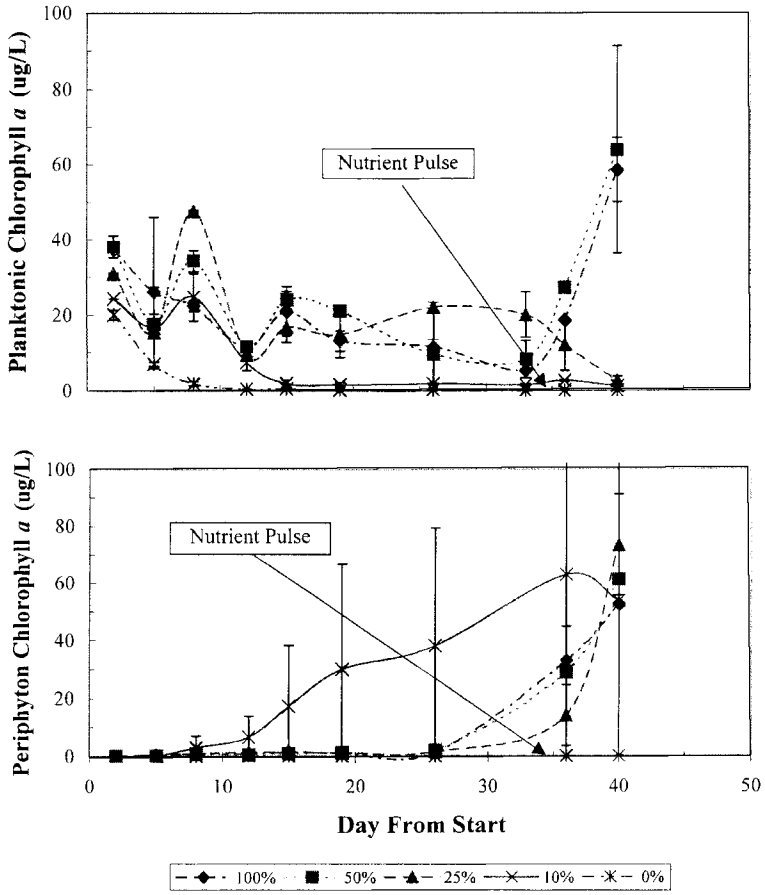

FIGURE 1 Planktonic (top) and periphytic algal biomass (bottom), measured in terms of chlorophyll *a* concentration. Measurements were taken on replicate mesocosms for each light treatment ($n = 2$). Error bars are plus/minus the standard deviation of the mean measurement. The nutrient pulse occurred on Day 33.

and their percent organic carbon (POC normalized to TSS) mimicked that of planktonic algae (Fig. 2). After two weeks these parameters increased with increasing light treatments, increased over time, and increased after the nutrient pulse. Linear correlation analysis ($\alpha = 0.05$) between TSS and chlorophyll *a* was significant ($R^2 = 0.63$), suggesting that the majority of suspended particles were phytoplankton. DOC began the experiment between 3 and 4 mg Cl^{-1} (figure not shown). Although DOC decreased to about 2.5 mg Cl^{-1} for the 10%

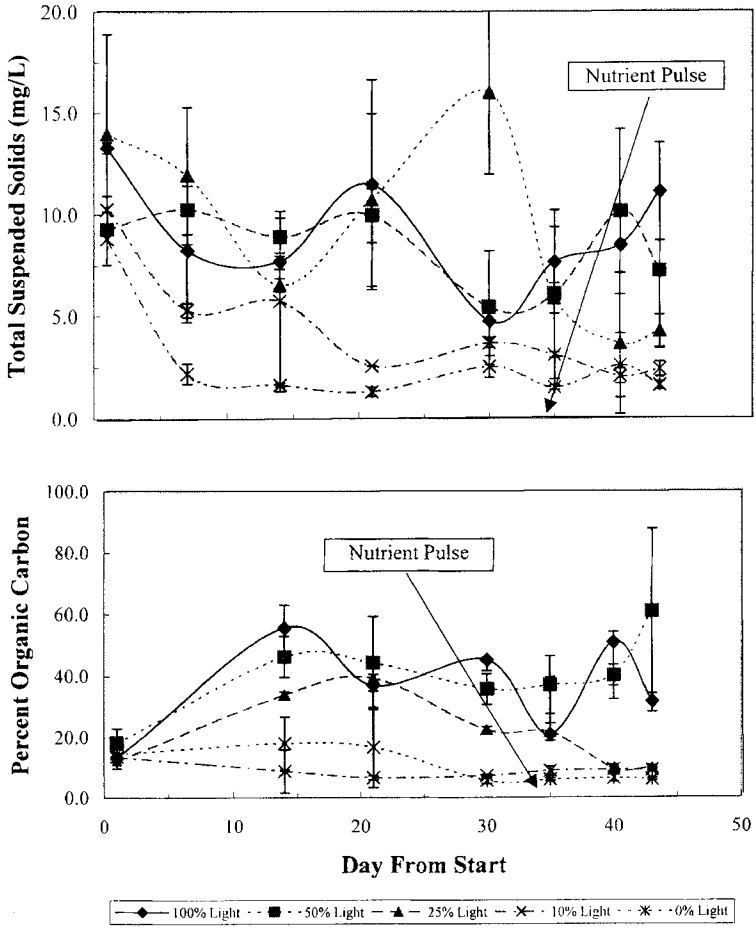

FIGURE 2 The concentration of totals suspended solids (top) and their percent organic carbon (bottom). Percent organic carbon = POC (mg C)/TSS(mg). Measurements were taken on replicate mesocosms for each light treatment ($n = 2$). Error bars are plus/minus the standard deviation of the mean measurement. The nutrient pulse occurred on Day 33.

and 0% light tanks for the duration of the experiment, it was variable for the other tanks and showed no response to the nutrient pulse. TSS correlated significantly with DOC ($R^2 = 0.63$) which suggests that algal exudates were significant contributors to the DOC pool. Salinity was relatively invariant, ranging from 11 to 13 ppt (figure not shown).

Distribution Coefficients

The distribution of copper between solution and particles was examined, both over time in individual mesocosms and among mesocosms at each time point. Distribution coefficients (Eq. (1)) were calculated as the ratio of the mass of particulate-bound copper per unit mass ($\mu g\,kg^{-1}$) over the dissolved solute mass per unit solvent volume ($\mu g\,l^{-1}$). After the first week of the experiment, K_d values were higher in higher light treatments, and varied over time (Fig. 3).

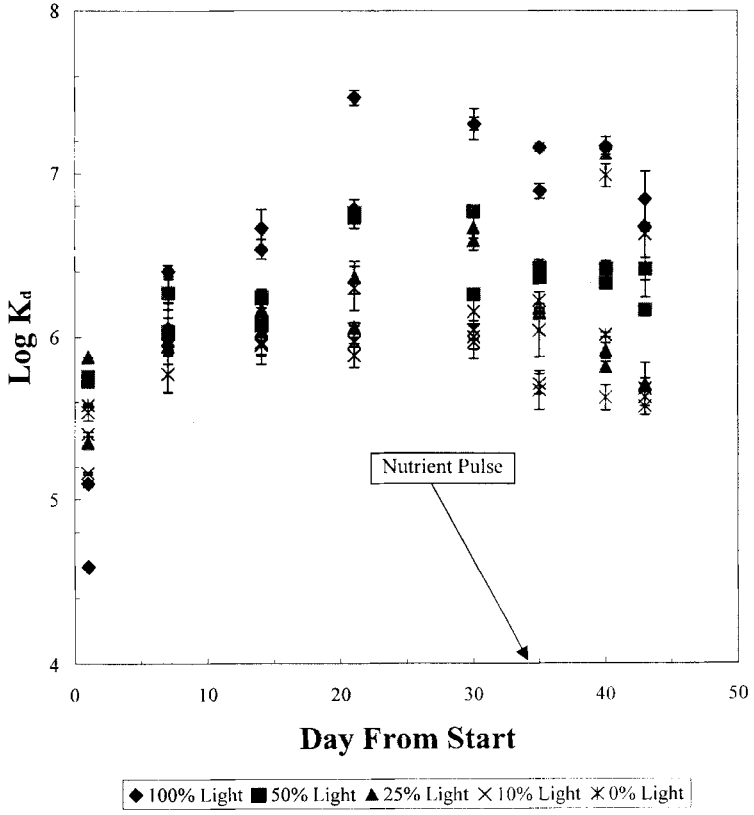

FIGURE 3 Distribution coefficients ($K_d = [Cu(II)]_{par}/[Cu(II)]^*_{sol}$ TSS, $L\,kg^{-}$). Duplicate measurements were taken on replicate mesocosms ($n = 2$) within each light treatment. Error bars are plus/minus the standard deviation of the mean of the duplicate measurements. Repeated measures analysis of variance ($n = 2$, $a = 5$, $alpha = 0.05$) revealed that distribution coefficients were significantly different among light treatments ($p = 0.0011$) and within each treatment over time ($p = 0.0001$).

Initially, $\log K_d$ ranged from ~ 5 to 6, and by day 21, K_d had increased by close to an order of magnitude in the 100%, 50% and 25% light tanks. After remaining relatively constant over days 21 to 40, K_d values varied after the nutrient pulse. Repeated measures analysis of variance ($n = 2$, $a = 5$, $\alpha = 0.05$; SAS, 1989) revealed that the differences observed in distribution coefficients both among light treatments ($p = 0.0011$) and for each treatment over time ($p = 0.0001$) were significant. Moreover, the probability that trends over time differed between light treatments was also significant ($p = 0.0008$) and these differences occurred from day 35 to 40 ($p = 0.0013$). Linear correlation analysis proved that the relationships between the distribution coefficients and percent organic carbon was statistically significant at the $\alpha = 0.05$ level, $R = 0.54$, while linear regression analysis yielded an R^2 of 0.2959.

The relatively high magnitude of K_d was indicative of very robust trace metal sequestration by the particles, and these copper distributions were controlled likely by percent organic carbon (Fig. 2). As benthic and planktonic communities evolved in the mesocosms, a profound shift between two particle types occurred. Low carbon, abiotic particles with low K_d's, primarily resuspended sediment, were replaced with carbon-rich, biotic particles characterized by high values of K_d. These biotic particles included phytoplankton cells, zooplankton and zooplankton faecal pallets, and resuspended organic detritus. This shift was reflected in the inter-treatment and temporal trends of percent organic carbon, which is a surrogate measurement of algal biomass relative to resuspended detritus. Statistically significant inter-tank differences of K_d at each time point coincided with inter-tank differences of percent organic carbon. Indeed, K_d was correlated most strongly with percent organic carbon, and the highest percentage of K_d's variance was explained by changes in percent organic carbon (30%). While Kuwabara *et al.* (1989) observed a similar temporal trend between distribution coefficients and percent organic carbon in San Francisco Bay, their K_d values were lower by two or three orders of magnitude than those from this study. Since the K_d values were normalized to TSS, a larger K_d was indicative of a greater affinity of the particles for copper, not simply more particles. This difference between the experimental K_d values and the estuarine K_d values was likely due to a large difference in the particulate percent organic carbon (mean fraction organic carbon of 0.23 for the mesocosm particles

versus a fraction organic carbon of 0.10 for San Francisco Bay particles).

Copper Complexation by Soluble Phase Organic Ligands

Fluorescence quenching was used to estimate binding capacities (L_t) and their associated binding constants (K_{cond}). One conditional stability constant estimate for each mesocosm over the entire time course of the experiment was generated and binding capacities were estimated for each mesocosm at each time point. The strength and extent of organic ligand complexation in this study was found to be robust. With one exception ($4.7 \times 10^{11}\,M^{-1}$), K_{cond} estimates ranged from 2.1 to $5.2 \times 10^{12}\,M^{-1}$, indicating a very strong copper-ligand complex, and standard errors varied from 7.9 to 13.2%. A t-test based on standard errors ($\alpha = 0.05$) was conducted in order to compare the K_{cond} generated for each mesocosm, and the 100%, 50% and 25% tanks were significantly different from the 10% and 0% light tanks. Conditional stability constants from this study compared favourably to those determined in previous studies (Tab. I) that used both similar methodology (Newell, 1983; Ryan and Weber, 1982) and alternative (primarily electrochemical) methodology (Donat *et al.*, 1994; Sunda and Hanson, 1987; Apte *et al.*, 1990; Moffett and Zika, 1987).

Binding capacities varied significantly among light treatments, but not in a systematic fashion. Generally L_t increased over time by an order of magnitude, from $\sim 1 \times 10^{-6}\,M$ on day 14 to $\sim 5 \times 10^{-5}\,M$ on the last ten days. Many of the mesocosms experienced statistically significant increases in L_t centering on the nutrient pulse. A t-test based on standard errors ($\alpha = 0.05$) examined L_t in each mesocosm over time, between individual mesocosms, and among light treatments. Consistently significant differences were found in individual tanks over time, with the 100% and 50% tanks having statistically significant differences in binding capacity from early in the experiment to late in the experiment (*e.g.*, from day 14 to 40 and day 14 to 43). Few significant and consistent differences were found among light treatments and from mesocosm to mesocosm, with the exception of replicates within a light treatment, which were not significantly different from one another. These binding capacities were an order of magnitude higher than some reported estimates from coastal and oceanic milieus (Tab. I).

Speciation Modelling

Due to the large magnitude of distribution coefficients, the percent of copper calculated in the soluble phase was less than 1% for the high light treatment tanks, and this percentage increased with decreasing light treatments to between 11 and 58% for the 0% light tanks. The extent of organic complexation was very high, greater than 97%, and was in agreement with other studies (Tab. I).

The total copper concentration ($[Cu]_T$, M) needed to reach a pCu of 12.5 ($10^{-12.5}$ M) was determined for each mesocosm over time. A target pCu of 1.5 was found to be toxic to marine ciliates (Stoecker *et al.*, 1986). At this pCu, $[Cu]_T$ ranged between 8.02×10^{-5} to 3.41×10^{-2} M, and for all treatments except for 0% light, increased over time. These values for $[Cu]_T$ were up to three orders of magnitude higher than both typical ambient estuarine copper concentrations (5×10^{-8} M) (Donat *et al.*, 1994), and the United States Environmental Protection Agency's ambient water quality criteria for copper of 4.88×10^{-5} M (U.S. EPA, 1995). Moreover, $[Cu]_T$ was relatively lower in the 0% light tanks, and prior to the nutrient enrichment (day 33), $[Cu]_T$ increased with increasing light treatments.

However, after the nutrient enrichment (day 40), $[Cu]_T$ was highest in the 10% light mesocosms. On the final day of the experiment (day 43), $[Cu]_T$ was highest in the 100% light tanks, but $[Cu]_T$ was still relatively large in the 10% light mesocosms. These inter-treatment and temporal trends mimicked those of algal biomass (Fig. 1). Planktonic biomass increased with increasing light levels and increased over time. In addition, the greatest biomass of periphyton was found in the 10% light tanks after the nutrient enrichment. On days 40 and 43, these tanks had among the highest $[Cu]_T$. These results implied that because of the presence of algal biomass, especially periphyton, the dose of contaminant required to achieve toxicity would be quite large and unrealistic. The issue then becomes whether or not to account for these wall effects, and if so, how.

The $[Cu]_T$ was normalized to the chosen target dose of $10^{-12.5}$ M, and was called the Effective Dose Ratio (EDR). A $[Cu]_T$ can be calculated for any target pCu from this EDR by multiplying the EDR by the chosen target dose. EDR was plotted *versus* total biomass (Fig. 4), and increased as total biomass increased. A three order magnitude

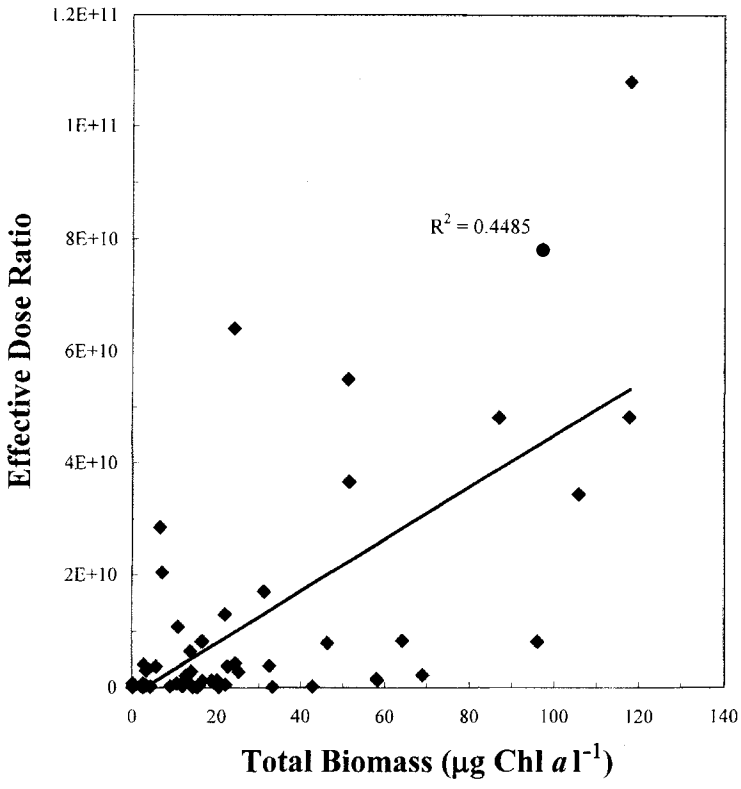

FIGURE 4 Effective Dose Ratio (EDR) *versus* total algal biomass. EDR is the ratio of the total copper concentration ($[Cu]_T$) required to produce a target free copper concentration (pCu of 12.5) (Stoecker *et al.*, 1986), to the target free copper concentration. The EDR allows the determination of $[Cu]_T$ at any target pCu. Total algal biomass is the sum of the biomass of periphyton and suspended phytoplankton.

increase in biomass (0.2 to 117.9 µg Chl $a\,l^{-1}$) corresponded with a three order magnitude increase in EDR (3.4×10^7 to 1.0×10^{11} M). Linear regression analysis indicated that 45% of the variance in EDR was explained by variance in total biomass. The residual variance in EDR can be explained primarily by differences in the strengths of particle associations and the magnitude of binding capacities. The highest EDR values were found in the 100% light and 10% light mesocosms on days 40 and 43. These mesocosms were characterized by not only the highest biomasses of suspended plankton and periphyton, but also by the highest $\log K_d$ (6.6 to 7.5) and the highest L_t (3.2

to 4.6×10^{-5} M). This suggested that the variability of contaminant bioavailability was controlled by algal biomass directly, through adsorption to particle surfaces, and indirectly, through the production of soluble complexing ligands.

Both the biomass and carbon content of suspended and wall-bound algae was, in turn, controlled by light levels, the addition of nutrients, and perhaps grazing by zooplankton. Chen *et al.* (1997) found significant linear correlations between mean light attenuation coefficients and periphyton biomass. Moreover Chen *et al.* (1997) suggest that the periphyton in these experimental systems are nutrient limited because: (1) a positive relationship was observed between periphyton biomass and nutrient concentrations; (2) exponential growth of periphyton occurred after the nutrient pulse (Fig. 1); and (3) C/N atomic ratios imply nitrogen limitation. Petersen *et al.* (1997) observed different patterns of primary productivity in different sized mesocosms, which were a function of light limitation. Furthermore, the accumulation of planktonic and periphyton biomass may have been controlled by losses to zooplankton grazing (Chen *et al.*, 1997). The dominant zooplankton was the copepod *Acartia tonsa*, with no other herbivores of significant abundance. These copepods feed on both planktonic and wall-bound algae, and were higher in abundance than typical values for the Chesapeake Bay, suggesting considerable potential for grazing. Copper complexation to the surfaces and the faecal pellets of the zooplankton was not addressed in this study.

CONCLUSIONS

Mesocosms will continue to be used as surrogates of natural systems because of their ability to achieve a significant degree of complexity while maintaining control of size and parameters. However, the extrapolation of results from experimental system to natural system is not free of artifacts. The size and shape of the mesocosm can directly and indirectly influence the biological dynamics and feedbacks of the mesocosm, which will control the exposure of biota to contaminants. $[Cu]_T$ and EDR were higher in the mesocosms containing higher levels of biomass. These copper doses would be catastrophic to systems of low algal abundance. Often the original contaminant dose will be

significantly higher, even by orders of magnitude, than the actual exposure level. The change in particle carbon content and the large magnitude of K_d implied that adsorption and particle dilution would play a more significant role than soluble organic complexation in governing contaminant exposure.

Another artifact that contributes to this contaminant sequestration is the presence of periphyton, the abundance of which is regulated primarily by light intensity (a function of mesocosm size and shape) and nutrient levels. Periphyton can dominate relatively small experimental systems, which are characterized by relatively high wall surface area: volume ratios, such as mesocosms. This periphyton can sequester significant amounts of contaminants, and removal of the periphyton may potentially remove the contaminant as well. Periphyton was the dominant source of carbon in this study, and contributed to the relatively large magnitudes of $[Cu]_T$. This artefact may be accounted for by quantifying periphyton biomass regularly and using a three-phase equilibrium approach when predicting actual exposure concentrations (Ashley and Baker, 1998). Further research that includes species-specific contaminant sequestration and the influence of algal byproducts and faecal pellets on contaminant sequestration in mesocosms is necessary to further our ability to effectively use mesocosms as surrogates of natural systems.

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