The Interrelationships among Trace Element Cycling, Nutrient Loading, and System Complexity in Estuaries: A Mesocosm Study

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ABSTRACT: Biogeochemical interactions between a suite of trace elements and nutrients were examined in a series of experimental mesocosm experiments to understand how multiple stressors affect estuarine environments and how these effects are modified by the complexity of the system used to examine them. Experimental treatment included additions of nutrients and trace elements separately and combined, along with a gradient in experimental system complexity. Eight mesocosm experiments were carried out from 1996 through 1998. Increased nutrients generally decreased dissolved trace element concentrations, in large part through an increase in phytoplankton biomass, but also by increasing the concentration of metals in the particles. Trace element additions increased dissolved nutrients by decreasing phytoplankton biomass. The presence of sediments reduced both dissolved trace element and nutrient concentrations. Other complexity treatments had weaker effects on both dissolved nutrients and trace elements. Many of the observed effects appeared to be seasonal, occurring only in spring, or their magnitude was greater in spring. This may be linked to a change from phosphorus to nitrogen limitation that often occurs in the Patuxent River estuary in the late spring or early summer period.

Introduction

Enrichment of estuarine systems with nutrients and trace elements is a common occurrence in the United States and throughout the world (Gray and Paasche 1984; Howarth 1988; Malone et al. 1993; Cloern 1996; Sanders and Riedel 1998). Nutrient enrichment can increase algal growth (including harmful algal blooms), decrease light penetration, cause accumulation of organic carbon in the benthos, and consequent hypoxia or anoxia in bottom waters (Schelske and Stoermer 1971; Turpin and Harrison 1979; Sanders et al. 1987; Oviatt et al. 1989; Riegman 1995). Trace element enrichment suppresses primary production, alters the species composition and size of the phytoplankton community (Sanders and Cibik 1985; Brand et al. 1986; Sanders et al. 1994), and increases accumulation of trace elements in the sediment and the food chain (Goldberg et al. 1978; Han and Hung 1990).

Stressors such as nutrients and trace elements are ordinarily considered individually when managing resources even though laboratory and field studies show that nutrients and trace elements have interacting effects. Trace elements may mimic nutrients producing competition for uptake or other metabolic disruptions (e.g., Sanders and Riedel 1987), they may be micronutrients required for the uptake and utilization of macronutrients, or

trace element limitation or interference by a second interacting trace element may cause poor utilization of macronutrients (e.g., Rueter and Morel 1982; Rueter and Ades 1987; Sunda and Huntsman 1983).

Interactions between nutrients and trace elements may also occur in less direct ways in natural phytoplankton communities. Nutrient or trace element enrichment may alter the phytoplankton community, producing a community with different nutrient and trace element requirements and sensitivity than the original. The resulting changes in the concentrations or ratios of nutrients may impact the concentration, and speciation of trace elements, via their effect on the community, and trace elements may exert similar effects on nutrient concentrations and ratios. Alterations of the phytoplankton community can affect higher trophic levels which graze upon them and recycle nutrients and trace elements. Any alterations of phytoplankton communities as a result of changes in nutrient and trace elements may alter higher trophic levels, which in turn, can impact the phytoplankton community.

The potential for interactions between nutrients and a suite of trace elements was examined in a series of mesocosm experiments as part of a larger study (COASTES—COmplexity And STressors in Estuarine Systems; Breitburg et al. 1999). These mesocosms used water and biota from the mesohaline Patuxent River, and additions of nutrients

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and trace elements, separately and combined, along a gradient of increasing system complexity. The objective of this project was to increase understanding of how multiple stressors affect estuarine environments, and how the complexity of the model system used to study the multiple stressors affects the outcome.

This paper describes the biogeochemical interactions of nutrient and trace element in these mesocosm systems, and their interactions with the complexity treatments. The interactions of biota and other system complexity with nutrients and trace elements in these mesocosm have been previously discussed (Breitburg et al. 1999; Laursen et al. 2002) and are in other papers in this volume (Bundy et al. 2003; Riedel et al. 2003).

Study Area

The Patuxent River drains an area between Washington, D.C., and Baltimore, Maryland, emptying through a partially mixed coastal plain subestuary into Chesapeake Bay. Urbanization of the estuary has led to eutrophication. Pre-European settlement annual loadings were estimated to be approximately 0.36×10^6 kg N and 0.010×10^6 kg P (Boynton et al. 1995). By 1985–1986, the annual loadings of nutrients into the estuary were estimated to be 2.2×10^6 kg N and 0.15×10^6 kg P, and the estuary showed increased chlorophyll and turbidity, and decreased oxygen content (D'Elia et al. 2003). Beginning in 1987, nutrient loading controls were put in place for both nitrogen (N) and phosphorus (P) in the watershed. Following the implementation of controls, loadings to the estuary have been reduced to 1.6×10^6 kg N and 0.12×10^6 kg P, but indicators of eutrophication (e.g., chlorophyll, turbidity, and oxygen) have only improved slightly. Further information concerning historical changes in the Patuxent River can be found in D'Elia et al. (2003).

The estuary is also subjected to trace element enrichment. Total loadings of metals from the riverine portion of the Patuxent have been relatively well characterized (EPA 1996; Riedel et al. 2000). Important anthropogenic sources include sewage treatment plants, urban runoff, and atmospheric deposition (Scudlark et al. 1994; Riedel et al. 2000). Geology may also be important in trace element enrichment of the estuary (McCartan et al. 1998; Riedel et al. 2000).

Methods and Materials

Mesocosm Methods

Experiments used twenty 1-m³ cylindrical fiberglass mesocosms, filled with water from the estuarine Patuxent River, screened through a pair of 35-μm plankton nets. The mesocosms were kept in outdoor raceways in flowing water from the estuary to maintain the temperature near ambient conditions, and operated as continuous flow cultures with a flow rate of 10% d⁻¹ (100 l d⁻¹), using 1-μm filtered estuarine water. Polyvinyl chloride (PVC) paddle wheels were used to gently mix the mesocosms. Opaque PVC liners were placed inside of the mesocosms, and were cleaned weekly to remove wall growth. Mesocosms were operated for 7 days prior to the start of the nutrient and trace element treatments and the addition of biota.

Four mesocosms experiments were carried out in 1996, in April, June, July, and September. These are designated 196, 296, 396, and 496. Similarly, there were four experiments in May, July, and August of 1997 and May 1998, designated 197, 297, 397, and 198, using a somewhat different experimental design. Five levels of experimental system complexity were tested, encompassing a gradient from simple to more complex. In 1996, treatments were phytoplankton (the plankton assemblage, including phytoplankton and associated bacterioplankton and microzooplankton) that passed through the 35-μm screen, +zooplankton (mesozooplankton and larger microzooplankton added to the phytoplankton assemblage), +fish (juvenile mummichogs, *Fundulus heteroclitus,* added to the +zooplankton assemblage), +sediment (sediment added to the +fish assemblage), and +benthos (benthic invertebrates, *Crassostrea virginica, Macoma balthica,* and *Diadumene leucolena,* added to the +sediment assemblage). In 1997–1998, sediment was added to all the treatments, so that there was no separate +sediment treatment, and an additional treatment +mollusc, in which the *Diadumene leucolena* was eliminated from the +benthos treatment. Sediments were placed in circular trays 50 cm diameter, approximately 10–15 cm thick. In 1996, the sediment was a sandy-silt collected from approximately 6-m depth in St. Leonard's Creek on the Patuxent River, while in 1997 and 1998, a muddy sand collected in approximately 1 m of water from the Patuxent River was used. Sediments were heat treated (~50°C for 3 d) to kill macroinvertebrates prior to use.

Nutrients and trace elements were added continuously by peristaltic pump. Half of the concentrations required to bring nutrients and trace elements in the addition treatments up to the steady state concentration were added on both days 7 and 8. Control tanks received no added nutrients, while nutrient treatment tanks received 1.6 mmol of NO_3^- and 0.1 mmol of PO_4^{3-} daily (final nominal concentrations of 16 μM N and 1 μM P, respectively). Different trace element addition treatments were used in the two years. In 1996, a mixture of five trace elements was used, 10 μg l⁻¹ arsenic (As),

1 µg l^{-1} cadmium (Cd), 5 µg l^{-1} copper (Cu), 5 µg l^{-1} nickel (Ni), and 5 µg l^{-1} zinc (Zn; final nominal concentrations). These reflect an enrichment of 5–10 times the concentrations recorded in the estuarine Patuxent River (Riedel et al. 2000). In 1997–1998, Ni and Zn were dropped from the mixture, and the nominal concentrations of As, Cd, and Cu were adjusted to 12.5, 0.5, and 7.5 µg l^{-1}. As and Cu were adjusted to compensate for loss of dissolved trace elements observed in the 1996 experiments, while Cd was lowered to produce a more realistic enrichment.

CHEMICAL SAMPLING AND ANALYSIS

A peristaltic pump with acid-washed C-Flex pump tubing and acid-washed PFA sampling tubing was used to collect dissolved trace elements samples using an acid-washed polypropylene 0.4 µm filter (Flegal et al. 1991; Hurley et al. 1996). Filtration blanks were taken prior to use of the filters. Duplicate samples were collected during every sampling to evaluate precision. Samples for Cd, Cu, Ni, and Zn were collected in acid-washed PFA bottles and preserved with 0.2% Baker Ultrex grade HCl. Samples for As were collected in acid-washed polyethylene bottles, and quick frozen to preserve As speciation. Suspended particles were concentrated from 8 l using a 500,000 NMWC cross-flow filtration cartridge, and collected on 0.4 µm polycarbonate filters (Petruševski et al. 1995; Wen et al. 1996; Riedel et al. 2000).

Samples for dissolved Cd, Cu, Ni, and Zn were pre-concentrated for analysis using APDC/ammonium DDDC chelation-chloroform extraction (Bruland et al. 1979; Nolting and de Jong 1994). Cadmium, Cu, and Ni were analyzed by graphite furnace atomic absorption spectrometry, using a Perkin Elmer 5100 ZL, calibrated using the method of standard additions. Zinc was analyzed by flame atomic absorption spectrometry. A sample of a Standard Reference Material (SRM) from the National Research Council of Canada (NRCC), SLEW-2 was concentrated and analyzed along with each group of samples to check accuracy. Arsenic was analyzed by hydride generation, cryogenic trapping, chromatographic separations, and detection using a hydrogen-burning quartz cuvette (Andreae 1977; Braman et al. 1977; Riedel 1993) in a Perkin Elmer 2380 atomic absorption spectrometer. This method determines arsenate, arsenite, monomethylarsenic (MMA), and dimethylarsenic (DMA). Seston samples were wet digested in $HNO_3/HCL/HF/HClO_4$ in an open Teflon vial (Van Loon 1985). The resulting digestates were analyzed for As, Cu, Cd, Ni, and Zn by the same techniques cited for dissolved trace elements. Analytical methods for nutrients, dissolved organic carbon, and chlorophyll have been previously documented (Breitburg et al. 1999).

TABLE 1. Mean ratios (by mole) of dissolved inorganic nitrogen (NH_4^+ + NO_2^- + NO_3^-) to dissolved inorganic phosphate (PO_4^{3-}) during the mesocosms runs.

Mesocosm Run	Starting Month	Seasonal Classification	Mean DIN : DIP Ratio	
			Non-Nutrient	Nutrient
196	April	Spring	314	129
296	June	Spring	115	79
396	July	Summer	4.8	3.8
496	September	Summer	5.6	6.0
197	May	Spring	219	98
297	July	Summer	23	29
397	August	Summer	6.8	5.6
198	April	Spring	106	63

STATISTICAL ANALYSIS

Analysis of variance (SAS version 6.12) was used to determine the effects of trace element and nutrient additions and system complexity treatments on dissolved trace element and nutrient concentrations, and particulate trace element distribution coefficients (K_Ds). Data sets were tested for homogeneity of variance, and based on these results, many of the data sets were log or rank transformed for analysis. While many analyses showed significant ($p < 0.05$) effects of trace element additions, a number also had ($p < 0.25$) interactions too large to be neglected between trace elements and other variables. Because substantially greater biological response to the metal additions occurred during spring runs (Riedel et al. 2003), the runs were separated into spring (April–June) and summer (July–September) runs. This also reflected seasonal differences in potential nutrient limitation status of the experiments, as determined by the average ratios of dissolved inorganic N (DIN) to dissolved inorganic P (DIP) in the mesocosms (Table 1). The spring experiments showed ratios greater than 16:1, while all but one of the summer experiments yielded ratios much less than 16:1. Experiment 297 had an average DIN/DIP ratio of 26 and very low levels of both DIN and DIP, suggesting joint N and P limitation.

Because biological uptake, transformations, and changes in phytoplankton community composition require time to occur, only statistical results for trace elements from the second of two sampling dates in each mesocosm run are presented. The analysis for the distribution coefficient (K_D) of particulate trace elements initially combined the ambient trace elements and the trace elements additions, to determine whether trace element additions changed the K_D. In subsequent analysis to examine the effects of nutrients and complexity treatments on K_D, these data were also separated
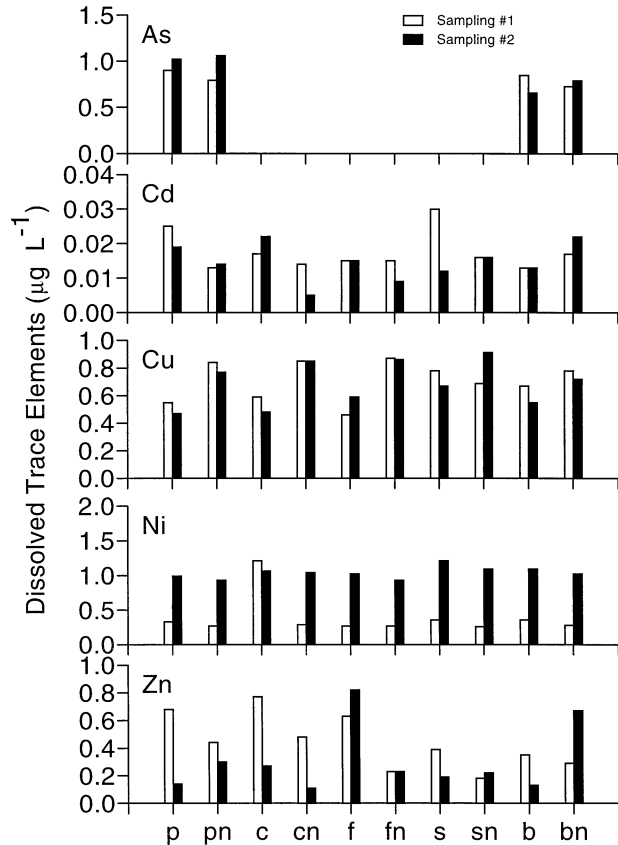
Fig. 1. Concentrations of dissolved trace elements from the treatments without added trace elements in the mesocosms from the 396 experiment at two sampling times. p = phytoplankton only, pn = phytoplankton plus nutrients, c = copepods addition, cn = copepod addition plus nutrients, f = fish addition, fn = fish addition plus nutrients, s = sediment addition, sn = sediment addition plus nutrients, b = benthic fauna addition, bn = benthic fauna addition plus nutrients. Open bars = sampling 1, filled bars = sampling 2.
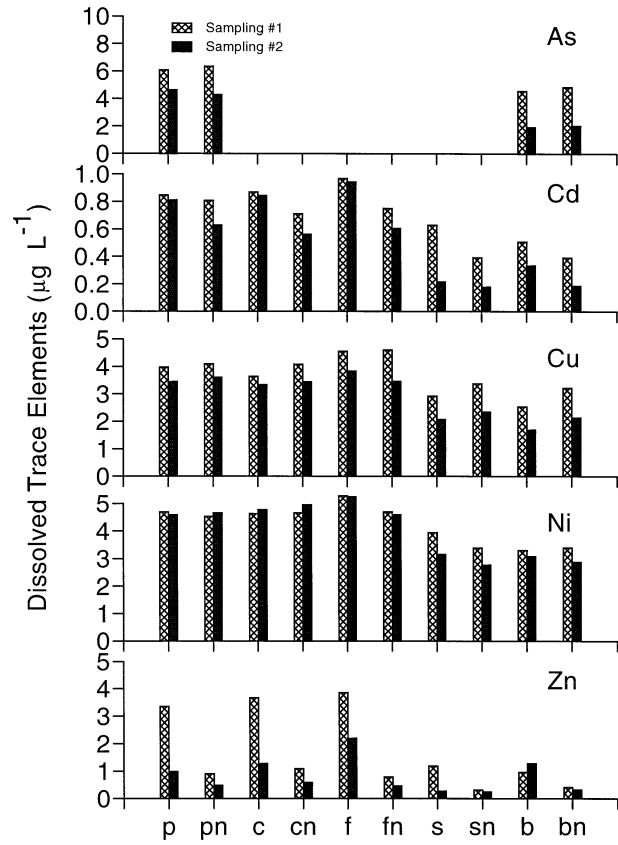

Fig. 2. Concentrations of dissolved trace elements from the treatments with added trace elements in the mesocosms from the 396 experiment at two sampling times. p = phytoplankton only, pn = phytoplankton plus nutrients, c = copepods addition, cn = copepod addition plus nutrients, f = fish addition, fn = fish addition plus nutrients, s = sediment addition, sn = sediment addition plus nutrients, b = benthic fauna addition, bn = benthic fauna addition plus nutrients. Coarse cross hatch bars = sampling 1, fine crossed hatched bars = sampling 2.

into ambient trace elements and trace element addition treatments.

Results

Effects of Nutrients and System Complexity on Trace Elements

Dissolved Cd, Ni, and Zn in tanks with nutrients added often decreased (Figs. 1 and 2, Table 2), presumably due to enhanced uptake by the additional biomass. This reduction more often occurred in the trace element addition treatments. Dissolved Cu concentrations were unaffected by nutrient addition in the trace element addition tanks, but were higher in the nutrient addition tanks than in controls receiving ambient trace elements.

In the 1996 experiments, there were no significant effects of nutrients on the concentration of

total dissolved or of any species of As (Table 2). In the 1997–1998 spring experiments there were no significant changes in total As concentration, but nutrient addition enhanced the transformation among the various forms of As. In the 1997–1998 summer experiments, nutrient additions decreased total As concentrations, and led to significant differences in the concentrations of most of the component species. Methylated As species were present at much higher concentrations during the spring experiments (Fig. 3).

The addition of sediments led to losses of trace elements from the water column; this was the most striking effect of complexity treatments noted. Because of the modifications to the experimental design in 1997–1998, this only occurred in the 1996 experiments. This effect was significant for all trace elements in the 1996 summer experiment, but in spring, a significant decrease was observed only for

TABLE 2. Statistical summary of effects of nutrients on the dissolved trace element concentrations in the mesocosm study. For main effect ns = p > 0.05, * = 0.05 > p > 0.01, ** = 0.01 > p > 0.001, *** = 0.001 > p, Pos. = an increase in response to nutrients, Neg. = a decrease in response to nutrients. MMA = monomethylarsenic, DMA = dimethylarsenic, nd = not determined.

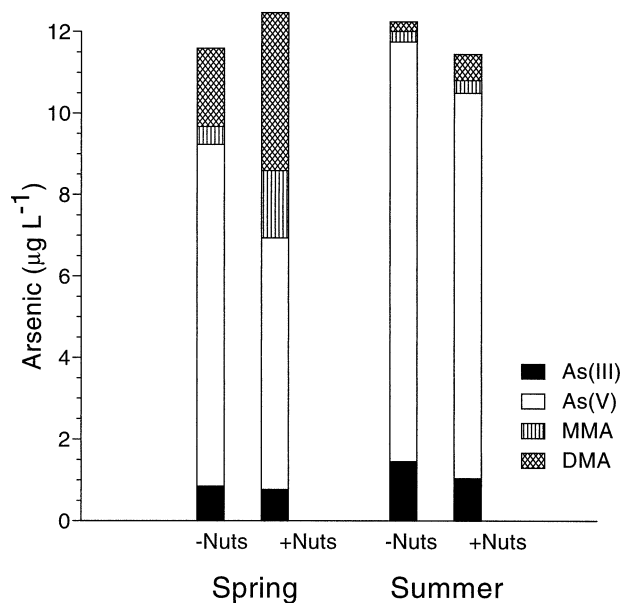| Trace Element	Trace Element Treatment	Spring 1996	Summer 1996	Spring 1997–1998	Summer 1997
As(III)	+	nd	nd	ns	ns
	−	nd	nd	Pos***	ns
As(III+V)	+	ns	ns	Neg***	Neg***
	−	ns	ns	Neg***	Neg***
MMA	+	ns	ns	Pos***	ns
	−	ns	ns	ns	Pos**
DMA	+	ns	ns	Pos***	Pos**
	−	ns	ns	Pos***	Pos**
Total As	+	ns	ns	ns	Neg**
	−	ns	ns	ns	ns
Cd	+	ns	Neg**	Neg**	Neg***
	−	ns	ns	Neg***	Neg**
Cu	+	ns	ns	ns	ns
	−	Pos**	Pos***	Pos***	Pos***
Ni	+	ns	Neg*	nd	nd
	−	ns	ns	nd	nd
Zn	+	ns	Neg***	nd	nd
	−	ns	ns	nd	nd

Fig. 3. Average concentrations of dissolved As species in the treatments with added As in the 1996 mesocosm experiments, separated by nutrients additions and season.

Cu and Ni in mesocosms with trace elements added, and for As when trace elements were not added (Table 3).

In the 1997–1998 experiments, where sediment was added to all mesocosms, few effects of the complexity treatments were observed. In the spring 1997–1998 experiments, only As(III) showed a significant effect with complexity (Table 3), with high concentrations of As(III) found in the mesocosms with benthic organisms added to the sediment (both of the benthos and mollusc treatments). In summer 1997 experiments, both total As and DMA concentration in the benthos and mollusc treat-

TABLE 3. Summary of effects of complexity on the dissolved trace element concentrations in the mesocosm studies. For main effects ns = p > 0.05, * = 0.05 > p > 0.01, ** = 0.01 > p > 0.001, *** = 0.001 > p. Complexity treatments with significant results are ranked from high to low, and Tukey groupings not significantly different (α ≤ 0.10) are underlined. P = phytoplankton, C = copepods, F = fish, S = sediment, B = benthos, M = mollusc, MMA = monomethylarsenic, DMA = dimethylarsenic, nd = not determined.

Trace Element	Trace Element Treatment	Spring 1996	Summer 1996	Spring 1997–1998	Summer 1997
As(III)	+	nd	nd	M B F C P**	ns
	−	nd	nd	ns	ns
AS(III+V)	+	ns	P B***	ns	ns
	−	ns	P B*	ns	ns
MMA	+	ns	ns	ns	ns
	−	ns	ns	B P M C F*	ns
DMA	+	ns	ns	ns	F P C B M*
	−	ns	ns	ns	ns
Total As	+	ns	P B**	ns	C F P B M*
	−	P B*	P B*	ns	ns
Cd	+	ns	F P C S B**	ns	ns
	−	ns	ns	ns	ns
Cu	+	C F P S B**	F P C S B**	ns	ns
	−	ns	ns	ns	ns
Ni	+	P C F B S*	F C P S B*	nd	nd
	−	ns	ns	nd	nd
Zn	+	ns	C P F S B*	nd	nd
	−	ns	ns	nd	nd

TABLE 4. Results of analysis of variance on the particle distribution coefficients (K_D) for 1996 and 1997–1998 mesocosm studies, showing the significance of the main effects of the trace element additions on the K_D. For significant results, the rank order of the means is shown by N = no trace elements added, T = trace elements added. ns = p > 0.05, * = 0.05 > p > 0.01, ** = 0.01 > p > 0.001, *** = 0.001 > p. MMA = monomethylarsenic, DMA = dimethylarsenic, nd = not determined.

Trace Element	Spring 1996	Summer 1996	Spring 1997–1998	Summer 1997
Inorganic As	ns	N>T***	N>T***	N>T***
MMA	ns	ns	N>T***	ns
DMA	N>T**	ns	N>T***	ns
Total As	ns	N>T*	N>T***	N>T***
Cd	ns	N>T***	N>T***	N>T**
Cu	ns	N>T***	T>N***	ns
Ni	N>T***	N>T***	nd	nd
Zn	ns	N>T**	nd	nd

ments ranked lowest in the concentrations (Table 3).

EFFECTS OF TRACE ELEMENT ADDITIONS, NUTRIENTS, AND SYSTEM COMPLEXITY ON PARTICULATE TRACE ELEMENTS

To normalize for the potential effects of the differences in dissolved trace element concentrations, particulate data are presented as the distribution coefficient (K_D):

$$K_D = \frac{P}{C}$$

where P is the concentration of the trace element in suspended particles ($\mu g\ g^{-1}$) and C is the concentration of the trace element in solution ($\mu g\ l^{-1}$; Balls 1989). The influence of trace element additions on the K_D for trace elements was relatively strong and consistent (Table 4). With only one exception, Cu in spring 1997, the K_D was less in the treatments receiving added trace elements, as illustrated in Fig. 4 for the July 1996 experiment. The effect was weakest in the spring 1996 experiments, where only Ni and DMA showed significant differences, and summer 1997, where Cd, inorganic As, and total As were significantly affected (Table 4).

In the spring 1996 experiments, the only significant nutrient effect occurred for DMA without added trace elements, which was significantly elevated in the nutrient additions compared to the ambient nutrients. In the summer 1996 experiments, no significant nutrient effects were observed for any As species, however, significantly higher K_D values were observed for both Cd and Zn. In 1997, the effect of nutrients was greater; in spring 1997, there were significant positive nutrient effects on DMA, total As, and Cd, and a negative effect on Cu. In summer 1997, the K_D values
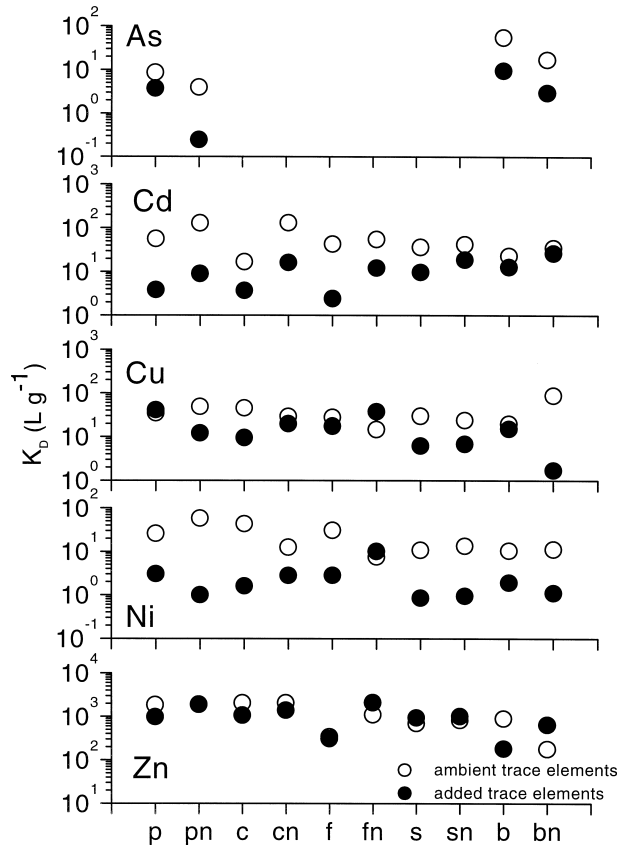

Fig. 4. K_D values for trace elements from the 396 mesocosm experiment. p = phytoplankton only, pn = phytoplankton plus nutrients, c = copepods addition, cn = copepod addition plus nutrients, f = fish addition, fn = fish addition plus nutrients, s = sediment addition, sn = sediment addition plus nutrients, b = benthic fauna addition, bn = benthic fauna addition plus nutrients. Open circles = ambient trace elements, closed circles = added trace elements.

for Cd were significantly increased by nutrients (Table 5).

The effect of experimental system complexity on K_D appeared to be largely linked to sediment or benthic activity. When a significant overall effect of system complexity on K_D was noted, the sediment and benthic (1996), or benthic and mollusc treatments (1997), were ranked as the two lowest, although they were not always a clearly defined group by themselves (Table 6).

EFFECTS OF TRACE ELEMENTS AND COMPLEXITY TREATMENTS ON NUTRIENTS

If the trace element additions had a negative effect on overall primary productivity, it would be reasonable to expect that trace element treatments would have contained higher dissolved nutrient concentrations than their non-nutrient pair. Because it was anticipated that the results might de-

TABLE 5. Statistical summary of effects of nutrients on the particle distribution coefficients (K_D) of trace elements in suspended particles for the mesocosm study, separated by metal treatment. For main effect ns = $p > 0.05$, * = $0.05 > p > 0.01$, ** = $0.01 > p > 0.001$, *** = $0.001 > p$. MMA = monomethylarsenic, DMA = dimethylarsenic, nd = not determined.

Trace Element	Trace Element Treatment	Spring 1996	Summer 1996	Spring 1997–1998	Summer 1997
As(III+V)	+	ns	ns	ns	ns
	−	ns	ns	ns	ns
MMA	+	ns	ns	ns	ns
	−	ns	ns	ns	ns
DMA	+	Pos*	ns	Pos*	ns
	−	ns	ns	ns	ns
Total As	+	ns	ns	ns	ns
	−	ns	ns	Pos*	ns
Cd	+	ns	Pos***	ns	Pos**
	−	ns	Pos**	Pos*	ns
Cu	+	ns	ns	ns	ns
	−	ns	ns	Neg***	ns
Ni	+	ns	ns	nd	nd
	−	ns	ns	nd	nd
Zn	+	ns	Pos**	nd	nd
	−	ns	ns	nd	nd

pend on the potential nutrient limitation status (P versus N limitation), spring and summer mesocosm experiments were analyzed separately. Similarly, because of the modifications in the experimental design, the 1996 and 1997–1998 mesocosm experiments were analyzed separately. The results of these ANOVAs are summarized in Table 7. There were significant effects of trace element additions, nutrient additions, and complexity treatments on nutrient concentrations.

Because of the uptake of nutrients by phytoplankton in the continuous cultures, nutrient additions would not necessarily result in accumulation of excess dissolved nutrient concentrations. NH_4^+, NO_3^-, and $Si(OH)_4$ concentrations in the nutrient treatments were often significantly lower than in the non-nutrient treatments (Table 7) while PO_4^{3-} in the nutrient treatments was significantly greater in 3 of the 4 seasonal-mesocosm combinations and significantly less in the 4th mesocosm experiment (summer 1996).

In all instances where significant effects of trace element additions on nutrient concentrations occurred, there were higher nutrient levels in the trace element addition treatments. The strength of this appeared to be affected by the design of the experiment; in the 1996 mesocosm experiments (where sediments were placed in only the sediment and benthos treatments), this result was observed in 3 of 8 opportunities. In the 1997–1998 experiments, where sediment was added to all mesocosms, this effect was observed in 6 of 8 possible combinations; only NH_4^+ in both spring and summer failed to show increased concentrations in response to trace element additions (Table 7).

Complexity treatments often significantly influenced nutrient concentrations. In the 1996 mesocosm experiments, significant complexity effects were observed in 5 of the 8 possible combinations. With only one exception, the sediment and benthos treatments ranked at the bottom of the list of treatments in rank orders, showing that the presence of sediment in the mesocosms reduced the nutrient concentrations. In the 1997–1998 meso-

TABLE 6. Summary of effects of complexity on distribution coefficient (K_D) of trace elements in suspended particles in the mesocosm studies. For main effects ns = $p > 0.05$, * = $0.05 > p > 0.01$, ** = $0.01 > p > 0.001$, *** = $0.001 > p$. Complexity treatments with significant results are ranked from high to low, and Tukey groupings not significantly different ($\alpha \leq 0.10$) are underlined. P = phytoplankton, C = copepods, F = fish, S = sediment, B = benthos, M = mollusc, MMA = monomethylarsenic, DMA = dimethylarsenic, nd = not determined.

Trace Element	Trace Element Treatment	Spring 1996	Summer 1996	Spring 1997–1998	Summer 1997
As(III+V)	+	ns	P B*	ns	ns
	−	ns	P B**	ns	ns
MMA	+	ns	P B*	ns	ns
	−	ns	ns	F P C M B*	ns
DMA	+	P B*	ns	ns	ns
	−	ns	ns	F C P M B*	ns
Total As	+	ns	P B**	ns	ns
	−	ns	P B*	F C P M B**	ns
Cd	+	ns	ns	F P C M B***	ns
	−	ns	ns	P F C B M*	ns
Cu	+	ns	ns	F C P M B*	ns
	−	ns	ns	F P C M B*	ns
Ni	+	ns	ns	nd	nd
	−	ns	P F C B S*	nd	nd
Zn	+	ns	ns	nd	nd
	−	ns	ns	nd	nd

TABLE 7. Results of Analysis of Variance on the mean nutrient concentrations for 1996 and 1997–1998 mesocosm studies, showing the significance of the main effects of the nutrient additions, trace element additions, and system complexity treatments. ns = p > 0.05, * = 0.05 > p > 0.01, ** = 0.01 > p > 0.001, *** = 0.001 > p. Direction or rank order of treatment effects from high to low are shown below significance indicator. Complexity treatments with significant results are ranked from high to low, and Tukey groupings not significantly different ($\alpha \leq 0.10$) are underlined. P = phytoplankton, C = copepods, F = fish, S = sediment, B = benthos, M = mollusc, Pos. = increased mean nutrient concentration, Neg. = decreased mean nutrient concentration.

Variables	NH_4^+	NO_3^-	PO_4^{3-}	$Si(OH)_4$
Spring 1996				
Trace elements	ns	ns	ns	Pos.*
Nutrients	Neg.*	ns	Pos.*	ns
Complexity	ns	C P F̲ B̲ S**	ns	P̲ C̲ F S B***
Summer 1996				
Trace elements	ns	ns	ns	ns
Nutrients	ns	ns	Neg.*	Neg.***
Complexity	ns	B P̲ C̲ S F*	C P̲ F̲ S B**	P̲ F̲ C S B***
Spring 1997–1998				
Trace elements	ns	Pos.*	Pos.**	Pos.***
Nutrients	ns	Neg.*	Pos.***	Neg.*
Complexity	ns	B M̲ C̲ F P*	B M̲ C̲ F P*	F P̲ C̲ B M*
Summer 1997				
Trace elements	ns	Pos.**	ns	Pos.***
Nutrients	Neg.*	ns	Pos.**	Neg.***
Complexity	C B̲ P̲ M̲ F**	B C̲ M̲ F P*	ns	ns

cosm experiments, complexity treatments usually had a significant effect on nutrient concentrations (Table 7).

Discussion

TREATMENT EFFECTS ON DISSOLVED TRACE ELEMENTS

The influence of nutrients on the concentrations of trace elements appeared to be strongly affected by seasonal factors, and by whether or not the trace elements were those initially present or were those added in the treatments. Cu increased in concentration from 28% to 42% in the mesocosms with no trace elements added. This was the only case where trace elements showed significant increases with nutrient additions. One potential source for the increased Cu in the nutrients was the nutrient stock solutions. These were tested and determined not have enough Cu to account for the increase. Another logical source for additional Cu was the sediments (e.g., Riedel et al. 1997, 1999), but the effect of nutrients was observed in the treatments without sediment added in the 1996 experiments. Mesocosms that received nutrient additions exhibited greatly increased rates of primary productivity (Breitburg et al. 1999; Riedel et al. 2003). This increase in total dissolved Cu may be due to the production of dissolved organic ligands which served to keep more of the added Cu in solution (e.g., McKnight and Morel 1979; Teresa et al. 2001). In support of this, a small but signif-

icant increase (4–6%) in mean dissolved organic carbon occurred in the nutrient treatments.

The implication of this finding in the Patuxent and similar estuaries is that the mobility of Cu can be enhanced by nutrient enrichment. With an average Cu enrichment of 34% compared to the non-nutrient mesocosms, the residence time of Cu in the mesocosm water columns was increased several days as a result of the added nutrients. An increase of the residence time of several days in the water would increase the average distance that Cu moves down the estuary from a source in the river before becoming absorbed onto particles or phytoplankton, and raise the fraction of Cu that exits the river into the Chesapeake Bay, or for the entire Chesapeake Bay, the fraction of Cu that enters the coastal zone. It is interesting to note that dissolved Cu concentrations in the Patuxent River do not exhibit the common mixing profile of declining concentrations with increasing salinity; concentrations are relatively constant throughout the saline portion of the estuary (Riedel et al. 2000). Increased Cu mobility caused by nutrient enrichment may be responsible for this behavior. Evaluating the relationship of nutrient enrichment to Cu mobility is difficult from our data set alone, given that there were only two trace element levels and two nutrient levels, simulating only a single region of the estuary. These results suggest that additional examination of this topic is warranted.

In the summer 1996 experiments, the other cat-

ionic metals added (Cd, Ni, and Zn) exhibited significantly lower concentrations with added nutrient treatments when trace elements were added. The result carried through in the 1997–1998 experiments with Cd (Ni and Zn were not added in the 1997–1998 experiments). The mean decrease was 22.5% for Cd, 8.4% for Ni, and 56.5% for Zn. The loss of cationic metals from solution was likely due to the addition of biomass resulting from the nutrient additions and metal incorporation into that biomass. For at least Cd and Zn, the addition of nutrients sometimes significantly increased the concentrations of metal in the biomass, further decreasing the dissolved fraction.

Arsenic concentrations and speciation in the Patuxent River have a well established seasonal cycle, with low concentrations and dominance by methylated species in winter and spring, and higher concentrations and much lower fractions of methylated forms in summer and fall (Riedel 1993; Riedel et al. 2000). The period in winter and spring when methyl species dominate coincides with a period when phosphate was potentially limiting based on DIN/DIP ratios, and when dense blooms of algal species that are known to methylate As are present. In these experiments, both a seasonal signal and a nutrient effect were observed. Arsenic was added to all treatments as sodium arsenate, As(V). In spring, a conversion of a substantial fraction of the added As(V) to DMA and MMA occurred during the course of the mesocosm runs, with greater concentrations of MMA and DMA in mesocosms receiving added nutrients. Arsenite [As(III)] production was not influenced strongly by either season or nutrients, although mean concentrations were somewhat higher in the summer experiments. These experiments support our previous studies that suggest that a switch between P limitation in winter and spring and N limitation in summer and fall was responsible for the production of large amounts of methylated As species during winter and spring (Riedel 1993; Riedel et al. 2000).

The occurrence of high methylation rates for As under conditions of P limitation suggests that the presence of methylarsenicals might be used as a long-term indicator for P limitation in estuarine systems. Arsenic methylation is slow relative to nutrient cycling, requiring days to convert a majority of the As from inorganic As to organic As even at times of high production and low N:P ratio (Sanders and Windom 1980; Sanders and Riedel 1993). Demethylation is also relatively slow (Sanders 1979; Scudlark and Johnson 1982). Conversion of a substantial fraction of As in an estuarine system would require conditions of P limitation for substantial periods to convert a majority of the available As to

DMA and MMA. This signal would persist for days to weeks after P limitation ends before demethylation and additional inputs of inorganic As reduce the predominance of methylated As.

TREATMENT EFFECTS ON SUSPENDED TRACE ELEMENTS

The decrease in K_D with trace element additions for Cd, Cu, Ni, and Zn observed in most cases could have several origins. It may have been caused by saturation of adsorption sites for the metals on the particles. It may also have been an artifact of dilution by an inert fraction of the suspended particles. If we assume that the particles have an inert fraction of Cu of 15 $\mu g\ g^{-1}$, and adsorb metal with a proportionality constant (a true K_D) of approximately 40 l g^{-1}, at 0.5 $\mu g\ l^{-1}$ dissolved Cu, the Cu content of the particles would be 35 $\mu g\ g^{-1}$, and have an apparent K_D of 70 l g^{-1}. If the dissolved Cu were raised to 5.0 $\mu g\ l^{-1}$, the Cu content of the particles would be 230 $\mu g\ g^{-1}$, and the apparent K_D would be 43 l g^{-1}. Another likelihood is that the character of the particles was different between added trace elements and the control treatments, such that particles from the trace element treatments had reduced affinities for the trace elements (Sanders and Riedel 1998). The trace element treatments often had different phytoplankton community composition, particularly in the nutrient addition treatments, where larger diatoms appeared to be stimulated by nutrient additions, but inhibited by trace metal additions (Riedel et al. 2003). If the larger cells had higher K_D than the smaller cells replacing them, the K_D of the particles in the trace element treatments could be reduced. Finally, the added trace elements may have stimulated the production of dissolved or colloidal ligands, which would also lower the K_D (McKnight and Morel 1979; Moran et al. 1996).

An increase in K_D for Cd with nutrient addition was observed. A similar increase in K_D for Cd in suspended material has been observed during the spring bloom in South San Francisco Bay, where dissolved metals, including Cd, were greatly reduced in concentration while the concentration of these metals in the particles rose substantially (Luoma et al. 1998). Wang and coworkers (Wang and Dei 2001; Wang et al. 2001) demonstrated that two species of common bloom-forming centric diatoms accumulated higher concentrations of Cd, and that both flagellates and diatoms accumulated Cd and Zn at much greater levels when grown under conditions of high nitrate concentrations. They saw little effect under high phosphate concentrations. In our experiments, increases in K_D of Cd with nutrient additions occurred primarily in summer, when nitrogen was the limiting nutrient, paralleling the

findings of Wang and Dei (2001) and Wang et al. (2001). This is also contrary to the particle concentration effect observed in some estuaries, where increased concentrations of particles show generally decreased K_D values (e.g., Balls 1989; Moran et al. 1996), since the nutrient additions greatly increased phytoplankton biomass.

The effect of nitrate enrichment on the accumulation of Cd may have significant consequences. The Patuxent River is relatively high in dissolved and suspended particulate Cd, probably as the result of a geologic phenomenon (Riedel et al. 2000). Concentrations of Cd in Patuxent River oysters are among some of the highest known in the United States, often well above Food and Drug Administration recommendations for Cd in shellfish (Riedel et al. 1998). If an ample supply of N causes phytoplankton to accumulate higher concentrations of Cd during the spring bloom when the Patuxent River is P limited and high in N, our results predict higher concentrations of Cd in phytoplankton, further contributing to the high levels of Cd in Patuxent River oysters.

The appropriateness of calculating a distribution coefficient, K_D, for different As species is questionable due to the speciation of As and transformations between those species. Even the use of a K_D from total As in both water and particles is difficult to interpret because of the movements of As species between the dissolved and particulate pools. Since the methylarsenicals in the water and tissues are produced by the same biochemical processes, the K_Ds for MMA and DMA are a comparison of the biomass producing the compound compared to the amount produced. In both sets of spring experiments (P-limited), but in neither set of summer experiments (N-limited), the mean K_D for DMA was significantly lower in the nutrient treatments. This suggests that the increase in the production of dissolved DMA due the nutrient addition was more than proportional to the increase in particulate DMA stimulated by the nutrient additions.

The effects of system complexity on the K_D of most of the trace element treatments are due to the sediment treatment (in 1996) or the benthic invertebrate treatments (in 1997). In the 1996 experiments, significant effects of the system complexity treatment for K_D were only observed for As, and in all cases the benthic treatment had higher K_D values than the phytoplankton treatment. In the benthic treatments the concentration of dissolved As species was reduced, and in most cases, the concentration of As in the particles increased. The decreases in the dissolved concentration was clearly the result of adsorption to the sediment. A mechanism for the increases in the concentration

of As in the suspended particles in the benthic treatments is not clear. Resuspended bottom sediments may have contributed higher concentrations of As in treatments with added sediment. This is unlikely because surface sediments in this region of the Patuxent River contain approximately 8–12 $\mu g\ g^{-1}$ As, composed largely of inorganic As, with traces of DMA (Riedel unpublished data). Suspended sediments in the mesocosms without trace element additions had similar concentrations (10.7 ± 4.4 $\mu g\ g^{-1}$), so that the contribution of resuspended sediments would be relatively low. The speciation of the As in the mesocosm suspended particles was also dominated by organic As compounds, primarily DMA, which averaged 6.9 ± 4.2 $\mu g\ g^{-1}$, further suggesting that most of the As in suspended particles was derived from dissolved As incorporated by phytoplankton.

In the 1997–1998 mesocosm runs, the benthic and mollusc treatments had the lowest K_D for As (and all other metals). All mesocosms in the 1997–1998 mesocosm runs contained sediments, but the benthic and mollusc treatment contained benthic invertebrates that removed phytoplankton. By filtering out larger particles, it appears that the filter feeders left particles with lower K_D. The exact process behind this is not clear, and further examination is warranted.

Treatment Effects on Nutrient Concentrations

Additions of nutrients (N and P) generally increased dissolved PO_4^{3-} concentrations, and the trend was more consistent in 1997 experiments (Table 7). This was likely due to greater coherence in the data due to similar sediment additions to all treatments. It was also stronger in the spring compared to summer, a period when the mesocosms were generally P limited. Nutrient additions had only minor effects on NO_3^- concentrations. Dissolved silicate was usually reduced by the N and P additions; presumably increases in diatom biomass resulted in greater depletion of the silicate. An increase in diatoms was a common result of the nutrient additions (see Riedel et al. 2003). Similar effects have been observed in several areas in the field receiving increasing N and P additions, such as the Great Lakes (Schelske and Stoermer 1971).

Metal additions generally resulted in increased dissolved concentrations of NO_3^-, PO_4^{3-}, and $Si(OH)_4$, but not NH_4^+. In several cases (primarily in spring), the metal additions caused significant decreases in phytoplankton productivity and biomass (Riedel et al. 2003). By decreasing productivity and biomass, trace element additions resulted in less nutrient removal. It is difficult to conceive of an example where this effect could be observed

in the field; without a control site for comparison it would require substantial inhibition of phytoplankton production to observe the accumulation of nutrients. This and similar experiments may provide data for trace element cycling models that can predict how changes in trace element loadings influence estuarine nutrient cycling.

There were significant effects of the system treatments on the residual nutrient concentrations. In the 1996 experiments, with one exception, the sediment and benthic treatments had the lowest concentrations of NO_3^-, PO_4^{3-}, and $Si(OH)_4$. Since considerable benthic primary productivity was associated with sediments in these tanks, it is reasonable to presume that the additional benthic production depleted the nutrients in these tanks to lower levels. Denitrification and adsorption of PO_4^{3-} could also contribute to losses of these nutrients (Stumm and Morgan 1981).

In the spring 1997 experiments, the benthic and mollusc treatments contained the highest concentrations of NO_3^- and PO_4^{3-}, but the lowest $Si(OH)_4$ concentrations. The increase in NO_3^- and PO_4^{3-} associated with the benthic community was likely a result of both a reduction in phytoplankton biomass, and hence less nutrient utilization, and increased regeneration by the organisms. The reduction of $Si(OH)_4$ suggests that the removal and sedimentation of diatoms by the benthic community significantly altered the silicate biogeochemistry by packaging of diatom frustules in fecal material. In the summer experiments, the effect of system complexity was less clear, the sediment and benthic treatments generally ranked in the middle for NO_3^- and PO_4^{3-}, and no significant effect was observed for $Si(OH)_4$.

Summary and Conclusions

Trace elements and nutrients, both common contaminants in developing watersheds, interact strongly with a variety of trophic components within estuaries. The effects of nutrient additions on phytoplankton biomass appeared to be controlled by seasonal factors, in which nutrient limitation (P versus N) was likely an important regulator. Nutrient additions, which caused substantial changes in the biomass and phytoplankton community composition, generally resulted in reductions in dissolved trace element concentrations (with the exception of Cu in the non-trace element additions), increased fractions of trace elements on particles, and in some instances (Cd and Zn) actual increases in the concentrations of metals in the particles. All of these promote shorter residence times for trace elements in the water column. Nutrient enrichment of estuaries should reduce dissolved trace elements, and cause most trace elements to be re-

moved to the sediments closer to their source. In the case of Cd and Zn, the increase in concentration in particulate material may increase the amount transferred to higher trophic levels. For Cu at low concentrations the effect appears to be reversed; nutrient enrichment may promote the solution of Cu, resulting in longer residence times and greater transport. These predictions are contingent on the hypothesis that nutrient enrichment in estuaries will lead to greater eutrophication; in some cases estuaries may be saturated with nutrients or light-limited, and added nutrients may not substantially increase production.

The opposite case exists as well. As a result of clean-up efforts, some estuaries, including the Patuxent, are on the opposite course. After many years of increasing nutrients and eutrophication, nutrient reductions are beginning to produce evidence of lowered production. In this situation the question becomes, how will future reductions in nutrient loadings and eutrophication alter trace element cycling and behavior in estuaries?

Trace element additions obviously raised the concentrations of trace elements in the suspended particles, but not usually in proportion to the change in concentration. Trace elements also tended to increase free nutrient concentrations, presumably by inhibiting the phytoplankton. Trace element additions that reach levels inhibitory to plankton tend to increase the residence time of nutrients, and promote their transport through the estuary.

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