Estuaries Vol. 26, No. 2A, p. 323–338 April 2003

Seasonal Variability in Response of Estuarine Phytoplankton Communities to Stress: Linkages between Toxic Trace Elements and Nutrient Enrichment

GERHARDT F. RIEDEL[1,*], JAMES G. SANDERS[2], and DENISE L. BREITBURG[1]

[1] *The Academy of Natural Sciences, Estuarine Research Center, 10545 Mackall Road, St. Leonard, Maryland 20685*

[2] *Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, Georgia 31411*

ABSTRACT: We examined individual and interactive effects of two stressors—nutrients (nitrogen [N] and phosphorus [P]) and trace elements (a mix of arsenic [As], copper [Cu], and cadmium [Cd], and in a second experiment also zinc [Zn] and nickel [Ni])—on phytoplankton of the mesohaline Patuxent River, a tributary of Chesapeake Bay. Experiments were conducted in twenty 1-m³ mesocosms. Four mesocosm runs used two levels of nutrient loadings (0.7–1.0 × ambient N loading and enriched to 1.3–1.6 × ambient N loading) crossed with two levels of trace elements (ambient and enriched approximately 2–5 × higher than ambient concentrations) crossed with five progressive levels of ecosystem complexity. To examine seasonal patterns of responses to stressors, data from these experiments were combined with results of a similar experiment conducted during 1996 (Breitburg et al. 1999a). A second mesocosm experiment examined effects of individual and mixed trace elements, both alone and in combination with nutrients, to further examine which nutrient-trace element interactions were important. Nutrients consistently increased phytoplankton productivity and biomass. Most of the increased biomass was created by large centric diatoms, which increased the mean cell size of the phytoplankton community. Trace element additions decreased phytoplankton productivity and biomass, as well as the contribution of large centric diatoms to phytoplankton biomass. When both trace elements and nutrients were added, trace elements reduced nutrient stimulation. Although the magnitude of the response to nutrient additions tended to be somewhat greater in spring, the seasonal patterns of trace element effects, and nutrient-trace element interactions were far more striking with significant responses restricted to spring mesocosm runs. The second experiment indicated that both As and Cu were more inhibitory to phytoplankton in spring than in summer, but As was more inhibitory in the low nutrient treatments and Cu was more inhibitory in the nutrient enrichment treatments. The potential for strong seasonal patterns and high temporal variability in stressor effects and multiple stressor interactions will require close attention in the design and interpretation of management-relevant research and monitoring and may indicate the need for seasonally varying management strategies.

Introduction

Increased nutrient and contaminant loadings have been of concern in many coastal systems (e.g., Ryther and Dunstan 1971; Malone et al. 1993; Cloern 1996, 2001; Sanders and Riedel 1998; Wang et al. 2001). Evidence is strong that increased loadings of nutrients and contaminants to coastal zones are altering natural cycles of phytoplankton growth and succession in coastal systems (Cloern 1996; Sanders and Riedel 1998). Responses to increased nutrient loadings include increased productivity and phytoplankton standing stock, and the attendant problems associated with system eutrophication. Increased loadings and changes in nutrient ratios or chemical form can also change phytoplankton species composition, dominance, and succession (Schelske and Stoermer 1971; Turpin and Harrison 1979; Sanders et al. 1987; Oviatt et al. 1989; Hodgkiss and Ho 1997) because phytoplankton species vary in their requirements for the various nutrients (Tilman 1977, 1980; Kilham and Kilham 1984). High nutrient flux promotes the dominance of diatoms over flagellates (Harrison and Davis 1979; Turpin and Harrison 1979; Kilham and Kilham 1984), and contaminants such as trace elements and organic pollutants can also strongly influence phytoplankton communities. Both organic and inorganic contaminants often favor small cells and flagellates over diatoms (Menzel et al. 1970; Thomas and Seibert 1977; Sanders et al. 1981, 1994; Brand et al. 1986; Sanders and Riedel 1998).

Reductions of nutrients alone may not lead to desired water quality and health of coastal ecosystems, in part, because nutrient enrichment does not occur in isolation of other anthropogenic stressors. Instead, coastal ecosystems are continu-

* Corresponding author; tele: 410/586-9712; fax: 410/586-9705; e-mail: friedel@acnatsci.org.

ously subjected to multiple stressors resulting from both human influence and natural environmental variation. These stressors potentially interact in ways that exacerbate, mask, or alter the spatial and temporal patterns of responses of key organisms (e.g., Breitburg et al. 1999a; Cloern 2001; Breitburg and Riedel In press; papers in Breitburg et al. 1999b). As a result, research and management focusing on individual stressors may not provide the information, or produce the desired management results, when applied to systems exposed to multiple stressors. This paper focuses on one example of co-occurring and potentially interacting stressors to examine the differences between effects, including temporal patterns of effects, of individual stressors and multiple stressor interactions. Nutrient and trace element pollution are common results of urbanization and agriculture, although their sources are not identical and their loadings can vary independently in time and space. Regulatory efforts and changes in land-use patterns will continue to alter both nutrient and trace element loadings.

Stressors such as nutrients and trace elements are ordinarily considered singly when managing resources; it is quite clear from laboratory and field studies that, for a wide variety of reasons, nutrients and trace elements have interacting physiological effects. Trace elements may act as analogues of nutrient elements (e.g., AsO_4 and PO_4) producing competition for uptake or other metabolic disruptions (Riedel 1984, 1985; Sanders and Riedel 1987). Many trace elements are micronutrients required for the uptake and utilization of major nutrients (e.g., iron), and trace element limitation or interference by a second interacting trace element may lead to poor utilization of macronutrients (e.g., Rueter and Morel 1981; Sunda and Huntsman 1983; Rueter and Ades 1987). Nutrient-induced changes in phytoplankton community composition may result in a community with different trace element requirements and sensitivity than the original community. Differential sensitivity to toxic trace elements among species (e.g., Brand et al. 1986; Riedel 1988; Sanders and Riedel 1993, 1998; Moffett and Brand 1996; Moffett et al. 1997) may also change the community such that nutrient utilization is altered. Interactions between phytoplankton and trace elements and/or nutrients can be quite complex, with changes in trace element concentrations and speciation or nutrient loading leading to phytoplankton biomass and composition changes that, in turn, influence nutrient and trace element concentrations.

Interactions among phytoplankton, trace elements, and nutrients can be major factors regulating biological availability and potential toxicity for an entire ecosystem. Such alterations of the phytoplankton community occur in the context of higher trophic levels which graze upon them and recycle nutrients and trace elements. Alterations of phytoplankton assemblages as a result of changes in nutrient and trace elements may impinge on higher trophic levels. Models that predict the effects of either nutrients or toxic trace elements on ecosystems are available, but models that incorporate the interactions of nutrients and toxic trace elements are virtually unknown (but see Bartell 2003). To adequately protect and manage coastal ecosystems, we must address the interactions among important stressors in combination, not singly (e.g., Breitburg et al. 1999b; Cloern 2001; Breitburg and Riedel In press).

These experiments were carried out to address a portion of the COASTES (COmplexity And STressors in Estuarine Systems) project (Breitburg et al. 1999a). The mesocosm experiments presented in this paper were designed to address several different aspects of the interactions of nutrients, toxic trace elements, and trophic complexity in estuarine systems (see Breitburg et al. 1999a; Laursen et al. 2002; Bundy et al. 2003; Riedel and Sanders 2003). In this paper we focus on the phytoplankton biomass, production, and assemblage resulting from the additions of nutrients and trace elements. We examined the potential for temporal variability in phytoplankton responses to stressors and the potential for interactions among stressors in a series of experimental estuarine food webs of increasing trophic complexity in 1-m^3 mesocosms. We tested two categories of stressors: increased nutrient (nitrogen and phosphorus) loadings and increased trace element (a mix of copper [Cu], arsenic [As], and cadmium [Cd]) loadings across a gradient of system complexity. Each stressor category was tested alone and in combination during six mesocosm runs in 1997 and 1998. Mesocosm runs were conducted in spring and summer to examine temporal variability in their individual and interactive effects as well as the potential for interactions between nutrients and individual trace elements. Our results highlight the importance of considering multiple stressor interactions and temporal variability of stressor effects in efforts to detect and manage human influence on coastal systems.

Materials and Methods

Study Area

The Patuxent River drains the relatively heavily populated area between Washington, D.C. and Baltimore, Maryland, emptying through a partially mixed coastal plain sub-estuary into Chesapeake

Bay. Eutrophication of the estuary has been one consequence of the urbanization and agriculture. A good review of the history of land-use changes and eutrophication in the Patuxent River is provided in this volume by D'Elia et al. (2003). In summary, nutrient loadings in the Patuxent have increased several fold since pre-colonial days, peaking in approximately 1985–1986, when nutrient controls were instituted. Since that time, nutrient loadings have declined slightly, but improvements in measures of eutrophication have been minor.

The Patuxent River estuary is also subjected to a moderate enrichment of As, Cd, Cu, nickel [Ni], lead [Pb], and zinc [Zn]. A pair of studies have characterized total loadings of trace elements from the riverine portion of the Patuxent (U.S. Environmental Protection Agency [USEPA] 1996; Riedel et al. 2000). Sources of trace elements include the numerous sewage treatment plants, urban runoff (USEPA 1999), and geological formations (McCartan et al. 1998; Riedel et al. 2000). High concentrations of silver, Cd, and Cu have been observed in oysters from the Patuxent (Riedel et al. 1998).

Experimental Design

The Main Design

In order to test the individual and interactive effects of nutrients and trace elements on Patuxent River biota, four mesocosm runs were conducted over 1997–1998 (three in 1997 and one in 1998; Table 1). Mesocosm runs consisted of a 7-d acclimation period followed by a 28-d experimental period. Each run was conducted as a factorial, randomized block design in twenty 1-m^3 cylindrical fiberglass mesocosms (107 cm diam × 122 cm height). Each run of the main design was set up in a completely crossed design with two levels of nutrients × two levels of trace elements × five levels of system complexity. Nutrients and trace element concentrations were increased to target levels (see below) on days 7 and 8 by adding half of the necessary concentration each day. Beginning on day 7, both nutrients and trace elements were added continuously by peristaltic pump to maintain appropriate loadings. These runs were similar to four runs in 1996 (Breitburg et al. 1999a). We will refer to these individual runs by the year and primary month: June 1997, July 1997, September 1997, and May 1998. We designate the non-nutrient non-trace element treatment as the control (C), the nutrient addition as +N, the trace element addition as +T, and the combined addition as +N+T.

Each mesocosm contained sediments placed in circular trays 50 cm in diameter, approximately 10

TABLE 1. Starting and ending dates, means (± SD) of temperature, salinity, and pH from the mesocosm runs in 1997–1999. Temperature was monitored every 15 min, while salinity and pH were measured twice weekly.

Mesocosm Run	June 1997	July 1997	September 1997	May 1998	July 1998	May 1999
Run Type	Main	Main	Main	Main	Modified	Modified
Dates	May 13–June 16	July 1–August 4	August 19–September 22	April 14–May 18	July 7–August 10	April 20–May 24
Temperature	19.1 (1.97)	26.9 (1.3)	24.6 (1.3)	17 (1.6)	27.3 (1.1)	17.6 (2.4)
Temperature Range	14.5–25.3	22.1–32.5	17.2–29.3	13.9–22.8	24.8–30.9	12.8–24.9
Salinity	9.8 (0.4)	11.6 (0.85)	13.8 (0.7)	7.0 (0.0)	na	na
pH	8.33 (0.40)	8.07 (0.26)	8.07 (0.25)	8.51 (0.31)	na	na

TABLE 2. Dissolved organic carbon (DOC) and nutrient concentrations in filtered Patuxent River water used to infuse the mesocosms (F), non-nutrient mesocosms (C), and nutrient addition (N) mesocosms averaged over all other treatment combinations, mean (± SD). All concentrations are in μM. Nutrients were sampled weekly, while DOC was sampled twice during each mesocosm run, once shortly after the onset of nutrient and trace element additions, and three weeks after the additions.

Mesocosm Run	June 1997	July 1997	September 1997	May 1998	July 1998	May 1999
DOC–C	268 (25)	265 (11)	378 (67)	327 (37)	339 (23)	350 (40)
DOC–N	314 (62)	267 (10)	373 (40)	322 (83)	389 (60)	360 (42)
NH_4–F	1.38 (0.23)	1.41 (0.66)	0.86 (0.29)	1.13 (1.43)	0.69 (0.16)	1.50 (0.67)
NH_4–C	0.34 (0.38)	0.83 (0.96)	0.52 (0.11)	0.50 (0.27)	0.27 (0.26)	0.45 (0.22)
NH_4–N	0.34 (0.50)	0.61 (0.45)	0.52 (0.16)	0.42 (0.19)	0.27 (0.26)	0.33 (0.06)
NO_3–F	19.0 (6.2)	13.7 (3.7)	10.3 (3.0)	8.63 (4.41)	11.1 (1.7)	6.87 (3.67)
NO_3–C	11.1 (5.1)	0.34 (0.35)	0.11 (0.14)	6.87 (4.92)	0.02 (0.06)	1.26 (0.77)
NO_3–N	9.46 (11.10)	0.94 (1.62)	0.12 (0.41)	5.92 (7.69)	0.02 (0.06)	0.89 (1.86)
PO_4–F	0.21 (0.09)	0.49 (0.13)	0.68 (0.12)	0.12 (0.03)	1.31 (0.28)	0.11 (0.10)
PO_4–C	0.01 (0.04)	0.01 (0.02)	0.12 (0.12)	0.07 (0.06)	0.36 (0.26)	0.00 (0.00)
PO_4–N	0.09 (0.13)	0.02 (0.03)	0.13 (0.09)	0.10 (0.06)	0.21 (0.21)	0.01 (0.02)
SiO_4–F	24.6 (3.3)	42.2 (16.8)	32.4 (7.8)	9.9 (6.8)	81.5 (4.1)	7.3 (6.1)
SiO_4–C	14.0 (6.0)	21.9 (11.9)	36.6 (8.6)	7.1 (6.0)	73.4 (12.1)	3.2 (3.4)
SiO_4–N	11.1 (9.3)	15.6 (9.0)	32.1 (8.8)	7.2 (8.5)	35.1 (22.5)	3.7 (4.5)

cm thick (approximating a sediment surface area to water column volume of a 3 m water column, the average depth of the Patuxent River estuary). The sandy sediments were collected from shallow, sub-tidal reaches of the Patuxent, sieved to remove macrofauna, heated to 50°C to kill microinvertebrates, and homogenized by stirring before being placed in trays.

The mesocosms were maintained outdoors in raceways that contained flowing water from the Patuxent River estuary to keep the temperature of the mesocosms similar to that of the estuary. All mesocosms were filled with water from the Patuxent River and screened through 35-μm mesh nets to exclude larger organisms. The mesocosms were maintained as continuous flow-through cultures using water from the estuary, filtered to 1 μm, as the exchange water, with a turnover rate of 10% d^{-1} (20% during the acclimation phase), similar to experiments performed in previous years (e.g., Sanders and Cibik 1985; D'Elia et al. 1986; Sanders et al. 1987, 1994; Sanders and Riedel 1998; Breitburg et al. 1999a). Great effort was taken to ensure that the mesocosms were not contaminated with trace elements. All components were of acid washed, non-metallic materials, all sampling devices were acid-cleaned before use, and trace metal clean techniques were used when sampling. Some atmospheric deposition (both wet and dry) was inevitable, but was not sufficient to substantially alter the experimental concentrations.

Mesocosms were mixed by PVC 4-blade paddlewheels suspended horizontally over the mesocosms on fiberglass axles. Paddlewheels rotated at 2 rpm; every 6 h the rotation would stop for 5 min, then reverse direction. Flexible, opaque PVC liners covered the interior surfaces of each mesocosm. The liners blocked light penetration through the walls. They were removed weekly, cleaned of all epiphyte growth to minimize the influence of wall growth on water column processes using trace element clean techniques, then returned to the mesocosms.

Two nutrient loading rates were used. Ambient nutrient (C and +T) mesocosms received only the 100 l d^{-1} of filtered Patuxent River water containing ambient dissolved nitrogen and phosphorus concentrations (Table 2). Nutrient addition mesocosms (+N and +N+T) received an additional continual input of 15 μM NO_3-N and 1 μM PO_4-P. This equates to an areal loading rate of 1.8 mmol NO_3-N m^{-2} d^{-1} and 0.11 mmol PO_4-P m^{-2} d^{-1}, about 1.3–1.6 times ambient nitrogen loadings to the nearby surface layer of the Patuxent River as calculated from 1984 to 1995 averages (Breitburg et al. 1999a). Nitrogen loadings to control mesocosms were 0.7–1.0 times that calculated for the river (Hagy 1996). Because the mesocosms acted as nutrient-limited continuous flow cultures, actual dissolved nutrient concentrations in the mesocosms were substantially below the nominal additions. For a more complete discussion of the influence of the experimental treatments on nutrients in these studies, see Riedel and Sanders (2003).

The two trace element treatments included a control level (C and +N treatments), which received trace elements only from the filtered exchange water from the Patuxent River (Table 3), and a trace element addition treatment (+T and +N+T) in which a mixture of As (Na_2HAsO_4), Cu ($CuSO_4$), and Cd ($CdCl_2$) was added to produce final nominal concentrations of 10 μg l^{-1} As, 5 μg l^{-1} Cu, and 0.5 μg l^{-1} Cd. As and Cu were chosen based on their potential toxicity to phytoplankton and the relatively low enrichments over ambient Patuxent River water required to produce shifts in phytoplankton community composition in previ-

TABLE 3. Dissolved and suspended particulate trace element concentrations in the non-trace element addition (C) and trace element addition (T) mesocosms averaged over all other treatment combinations, mean (±SD). Dissolved and suspended trace elements were sampled twice during each mesocosm run, once shortly after the onset of nutrient and trace element additions, and three weeks after the additions, near the end of the run.

Mesocosm Run	June 1997	July 1997	September 1997	May 1998	July 1998	May 1999
Dissolved Trace Elements ($\mu g\ l^{-1}$)						
As–C	0.32 (0.06)	0.54 (0.07)	0.85 (0.11)	0.55 (0.05)	1.15 (0.23)	0.39 (0.07)
As–T	10.9 (2.3)	10.8 (0.9)	12.0 (1.4)	12.2 (0.9)	12.2 (1.1)	9.8 (1.4)
Cd–C	0.07 (0.02)	0.04 (0.01)	0.02 (0.00)	0.01 (0.00)	0.02 (0.00)	0.05 (0.02)
Cd–T	0.18 (0.04)	0.11 (0.07)	0.08 (0.00)	0.17 (0.02)	0.27 (0.03)	0.38 (0.02)
Cu–C	1.03 (0.27)	0.71 (0.10)	0.69 (0.18)	0.83 (0.19)	0.57 (0.15)	1.13 (0.37)
Cu–T	5.29 (0.96)	5.13 (0.44)	4.15 (0.44)	4.57 (0.65)	4.04 (0.41)	5.91 (0.80)
Particulate Trace Elements ($\mu g\ g^{-1}$)						
As–C	8.5 (3.2)	7.5 (3.4)	8.2 (4.9)	6.9 (2.2)	7.1 (2.7)	11.3 (6.3)
As–T	56.6 (75.3)	29.3 (13.7)	14.4 (4.5)	41.6 (30.5)	19.5 (10.2)	303 (452)
Cd–C	1.12 (0.06)	0.97 (0.64)	0.49 (0.20)	1.68 (0.77)	0.57 (0.36)	0.53 (0.21)
Cd–T	2.72 (3.21)	1.13 (0.92)	0.98 (0.36)	12.0 (9.1)	3.31 (1.76)	1.78 (1.01)
Cu–C	13.5 (6.9)	8.6 (3.0)	15.8 (16.5)	13.9 (3.0)	10.8 (14.9)	14.7 (6.4)
Cu–T	101 (42)	63 (25)	76 (27)	96 (14)	63 (25)	114 (32)

ous mesocosm experiments (Sanders 1979; Sanders and Cibik 1985; Sanders et al. 1991). Cd was chosen because the Patuxent has relatively high dissolved concentrations of Cd (Riedel et al. 2000), and because high concentrations of Cd are found in oysters in the upper Patuxent River estuary (Riedel et al. 1998). The concentrations of As, Cu, and Cd in this treatment were 2–5 times higher than the highest ambient concentrations measured in the river during sampling conducted as part of the COASTES program in 1995–1997 (Riedel et al. 2000) but were lower than maximum concentrations found in other studies of the Patuxent River or other Chesapeake Bay tributaries (Abbe and Sanders 1986; Sanders unpublished data; Riedel unpublished data). The levels of addition of trace elements to mesocosms were chosen to be sufficient to cause some changes in the structure and function of lower trophic levels, but below levels that would cause acute mortality of higher organisms based upon previous experiments (e.g., Sanders and Cibik 1985; Sanders and Riedel 1998; Breitburg et al. 1999a).

Five levels of system complexity were tested, building from simple to successively more complex systems. These treatments were +phytoplankton (the plankton assemblage, including phytoplankton, bacterioplankton, and microzooplankton in the Patuxent River estuary that passed through a 35-μm mesh), +zooplankton (mesozooplankton and larger microzooplankton added to the +phytoplankton assemblage), +fish (juvenile fish added to the +zooplankton assemblage), +bivalves (oysters and clams added to the +fish assemblage), and +benthos (anemones added to the +bivalve assemblage). This paper focuses on stressor effects on the phytoplankton only. Further information about the levels of complexity and their effects on phytoplankton can be found in Breitburg et al. (1999a), Bundy et al. (2003), and Wiegner et al. (2003).

The Modified Design

In the first series of mesocosm runs (the Main Design) and in a previous experiment (Breitburg et al. 1999a), there was a trend towards greater response of phytoplankton to nutrients during the spring mesocosm runs, significantly greater inhibitory response to trace elements during spring experiments, and significantly greater response of nutrient-enriched mesocosms to trace element inhibition during spring (see Results section). This suggested a link between nutrient limitation and the response of phytoplankton to both nutrient additions and trace element additions. One factor consistently different between spring and summer mesocosm runs (besides temperature) was the underlying nutrient regime. In winter and spring, the estuarine Patuxent River has low concentrations of dissolved inorganic phosphate (DIP) and high concentrations of dissolved inorganic nitrogen (DIN), yielding DIN:DIP $>$ 15:1. In summer, DIN levels fall and DIP increases, yielding DIN:DIP $<$ 15:1 (D'Elia et al. 1986, 2003; Riedel 1993). We hypothesized that relieving the phosphorus limitation in spring experiments generally produced a greater response in the phytoplankton than relieving the nitrogen limitation in summer and that phosphorus limitation was responsible for the greater inhibitory effects of trace element additions in the spring experiments compared to summer experiments. Under phosphorus limitation, As in the trace element mix would be more toxic because of the known biochemical interactions of

As and phosphorus (Blum 1966; Planas and Healey 1978; Sanders 1979; Sanders and Riedel 1987; Wangberg and Blanck 1990).

For the July 1998 and May 1999 runs the design was modified to examine the effects of the individual and combined trace elements. Four trace element treatments, control, +As, +Cu, and +mix (including As, Cu, and Cd additions at 1997–1998 levels, Ni and Zn additions at 1996 levels; see Breitburg et al. 1999a) were crossed with the same nutrient additions as in the main design, with two replicates for all non-nutrient treatments, and three replicates for nutrient addition treatments. Only the base level of complexity (+phytoplankton) was used to start these runs; copepods (+zooplankton) were added to all the mesocosms after 14 d (7 d after the nutrient and trace element treatments were started). Otherwise, sampling and analytical methods were the same as the main design.

Sampling

The mesocosms were sampled twice weekly for salinity and pH, and approximately weekly for nutrients and particulate carbon and nitrogen; temperature was measured every 15 min. Filtered (Whatman GF/F) samples for dissolved nutrient analyses (ammonia, nitrate plus nitrite, soluble reactive phosphate, silicate) were analyzed using standard analytical methods. Dissolved organic carbon and particulate carbon and nitrogen were analyzed by elemental analyzers. Dissolved and suspended trace elements were sampled twice during each mesocosm run, once shortly after the nutrient and trace element additions, and again three weeks after the additions. Sampling and analytical details can be found in Riedel et al. (2000) and in a paper on the biogeochemical aspects of these mesocosm experiments (Riedel and Sanders 2003).

A number of parameters were measured to ascertain phytoplankton growth, species composition, and productivity. Phytoplankton biomass was estimated at the same time each day by in vivo fluorescence (IVF), an indirect measure of chlorophyll *a* (chl *a*) and biomass (Goldman et al. 1973; D'Elia et al. 1986). Chl *a* was sampled approximately weekly and was highly correlated with daily IVF ($r^2 = 0.903$, $p < 0.001$). Potential phytoplankton $^{14}CO_2$ incorporation was estimated using triplicate 1-ml subsamples (Lewis and Smith 1983) from each mesocosm approximately weekly at saturating light intensities (> 250 μE m^{-2} s^{-1}; Harding et al. 1986). The small volume samples the total community at 10^6 cells l^{-1} and the volume permits direct addition of fluor to the ^{14}C-labeled community following acidification and purging for scintillation counting. Phytoplankton samples were collected weekly, preserved, and counted by inverted microscopy (Utermöhl 1958). Phytoplankton counts were converted to carbon biomass estimates using geometric estimates of cell volume and average cell volume to cell carbon ratios specific for different taxonomic groups (Verity et al. 1992; Montagnes et al. 1994). Phytoplankton cells were assigned to three size classes, 3–6, 6–20, and > 20 μm, for purposes of food chain modeling (Bartell 2003) based on their availability to higher trophic levels (Berggreen et al. 1988; Newell and Langdon 1996). The fraction of carbon in each size class was calculated from the estimated cell density and carbon for each species in the size class.

Statistical Analyses

Analysis of variance (SAS Version 8: GLM) was used to examine direct and interactive effects of stressors (C, +N, +T, +N+T) and system complexity in mesocosm runs using the main design. To test for general effects of stressors and stressor interactions across mesocosm runs the four main design mesocosm runs conducted June 1997 through May 1998 were analyzed as a randomized block design using the model:

response variable ~ f (mesocosm run, stressor, complexity, stressor × complexity)

Contrast statements were used to test the stressor comparisons of interest, i.e., C versus +N, C versus +T, and +N versus +N+T. For analyses of individual mesocosm runs the stressor × complexity interaction term was dropped, and complexity was used as a blocking factor (because of lack of replication of stressor × complexity treatments within mesocosm runs). The four April 1996 through September 1996 runs (Breitburg et al. 1999a) were included in analyses of individual mesocosm runs to increase the power to detect the consistency and seasonal patterns of responses to stressors. The effect of season on the magnitude of responses to each stressor including all eight mesocosm runs from 1996 through May 1998 was tested using the model:

response variable ~ f (season, mesocosm run (season), complexity)

The two runs of the modified design were tested separately, each using the model:

response variable ~ f (nutrients, metals, nutrients × metals)

Tukey's studentized range test was used to test for differences among stressor treatments, and Least Square Means comparisons were used to interpret interactions. ANOVAs were performed on

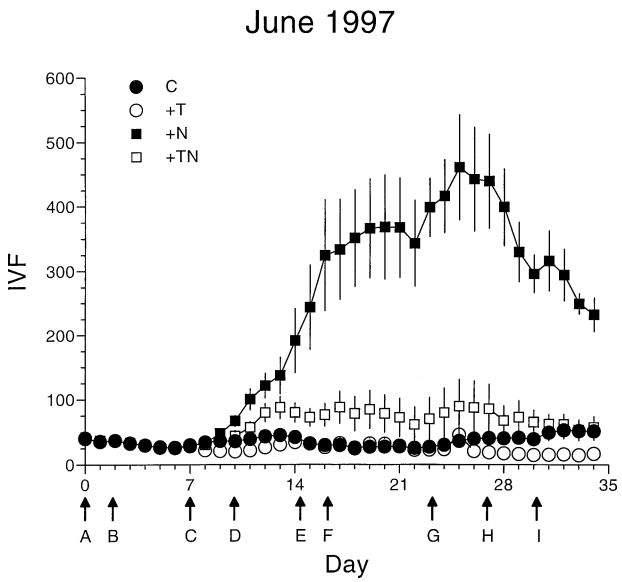

Fig. 1. In vivo fluorescence (IVF) in the June 1997 run, separated by nutrient and trace element treatment but averaged over complexity treatments (means ± SE), showing the schedule of sampling of parameters. A) Fill mesocosms, start infusions at 20%, sample phytoplankton; B) sample phytoplankton; C) switch mesocosm infusions to 10%, start nutrient and trace element additions, sample phytoplankton, nutrients, primary production; D) sample phytoplankton, primary production; E) sample trace elements; F) sample phytoplankton, nutrients, primary production; G) sample phytoplankton, nutrients, primary production, trace elements; H) sample phytoplankton; I) sample nutrients, primary production.

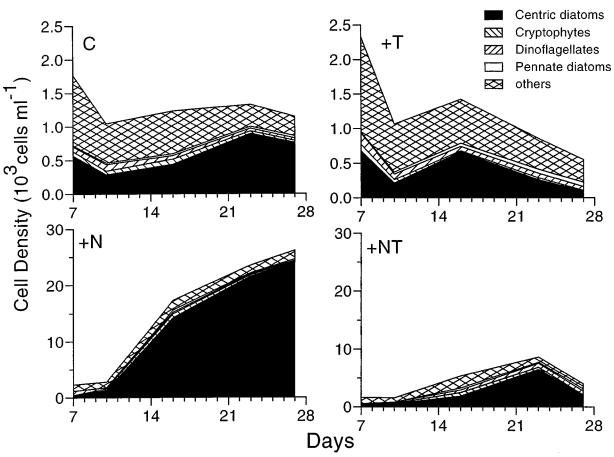

Fig. 2. Phytoplankton community data for the June 1997 mesocosm run, grouped by +N and +T treatment as in Fig. 1. Note changes in scale of the y axes.

the rank-transformed data (Potvin and Roff 1993) when variances were heteroscedastic even after log or arcsin square root transformation of proportions (Levine's test). All analyses were done on tank averages beginning with samples taken the second day of the nutrient and metal spikes. Unless otherwise specified, data are presented throughout this paper as mean ± 1 standard error.

Results

Background Data

Tables 1, 2, and 3 provide temporal, chemical, and physical data for each run. Because of space limitations, it is not possible to provide complete data for each run. However, as an example, the mean daily IVF measured for one mesocosm experiment (June 1997), grouped by +N × +T treatment, along with the schedule showing how the mesocosms were initiated and the sampling schedule for phytoplankton counts, chl *a*, ^{14}C productivity, nutrients, trace elements is shown in Fig. 1. The mean phytoplankton community found in the June 1997 mesocosm run, again grouped by +N × +T treatment, and by major algal taxa is shown in Fig. 2. The chl *a* and primary production data for all the mesocosm runs from 1996 through spring 1998 (the Main Design + the similar 1996 runs) are summarized in Table 4.

Main Design

Nutrients

Nutrient additions caused significant increases in all measures of phytoplankton. There were significant increases in phytoplankton chlorophyll (Fig. 3) and primary production (Fig. 4) in each mesocosm run individually (all $p < 0.05$) and across all four main design runs combined ($p < 0.001$). Combining both the 1996 and 1997–1998 runs, the absolute magnitude of the responses to nutrients in terms of phytoplankton chl *a* and primary production tended to be slightly greater ($p = 0.11$ and $p = 0.08$, respectively) in the spring mesocosm runs than in the summer runs (Table 4).

The addition of nutrients stimulated blooms that

TABLE 4. Seasonal means (±SE) of chlorophyll *a* and primary productivity averaged over complexity treatments for the eight mesocosm runs from 1996–1998.

Season Treatment	Chlorophyll *a* ($\mu g\ l^{-1}$)	Primary Productivity (μg carbon $l^{-1}\ h^{-1}$)
Spring		
C	8.2 (1.0)	30.2 (3.2)
+T	7.1 (0.9)	21.0 (2.2)
+N	30.4 (3.2)	134.6 (15.2)
+N+T	17.4 (2.5)	67.0 (7.2)
Summer		
C	5.0 (0.6)	34.6 (4.1)
+T	5.8 (0.8)	34.8 (4.1)
+N	18.6 (2.2)	112.9 (9.5)
+N+T	18.6 (2.0)	109.9 (10.3)

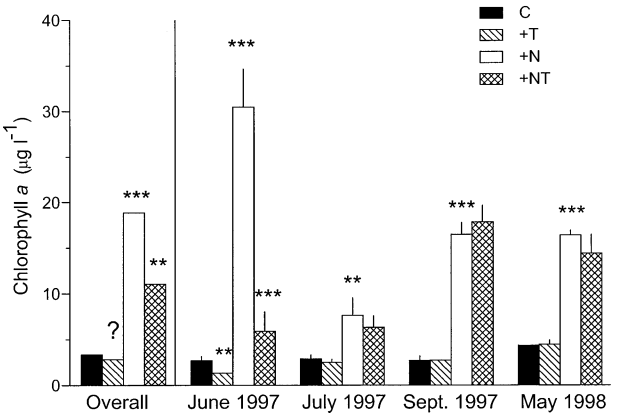

Fig. 3. Chlorophyll *a* from mesocosm runs of the main design (mean ± SE), 1997 through 1998, segregated by nutrient and trace element treatment, with averages across time and complexity treatment. The significance level of three contrasts are marked, C versus +N, C versus +T, and +N versus +NT. ? = $p \leq 0.1$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$ (ANOVA, log transform).

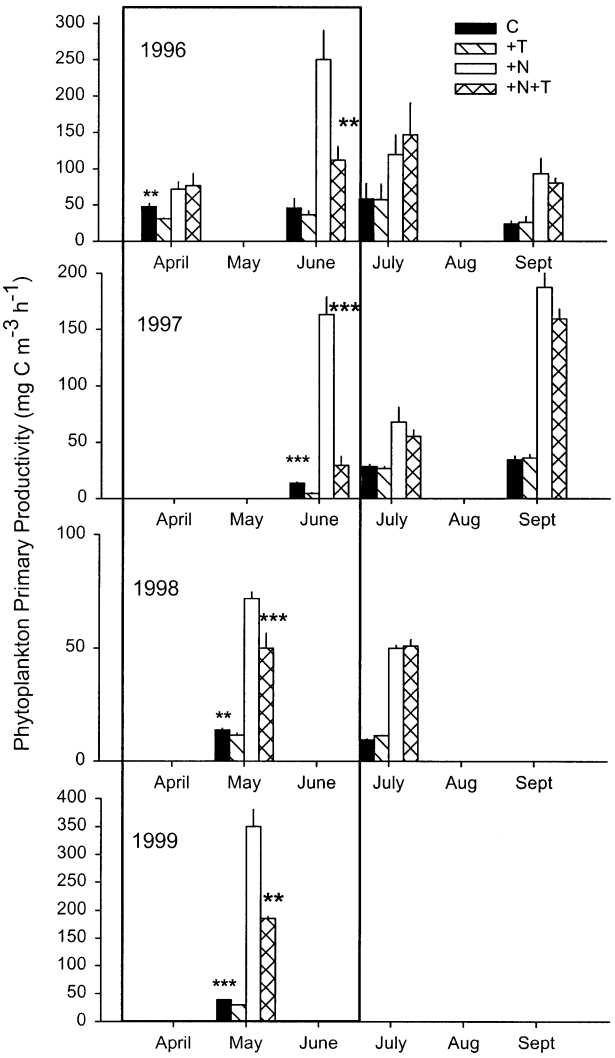

Fig. 4. ^{14}C primary production from all mesocosm runs (mean ± SE), 1996 through 1999. Averages are across time and complexity treatment. The significance level of two contrasts are marked, C versus +T, and +N versus +N+T, *** = $p \leq 0.001$. The box separates spring from summer runs. The +N treatments had significantly higher primary productivity than did the C mesocosms in all eight mesocosm runs.

were enriched in centric diatoms compared to the C treatment ($p < 0.001$; Fig. 5). In most of the mesocosm experiments, from June 1997 to May 1998, a centric diatom, *Dactyliosolen fragilissimus* (formerly *Rhizosolenia fragilissima*), was strongly stimulated by the +N treatment ($p < 0.001$ overall and $p < 0.05$ for June 1997, September 1997, and May 1998). This diatom was larger than almost all other phytoplankton species, and the carbon distribution of the phytoplankton community in the nutrient addition mesocosms often had size distributions shifted towards the largest size class (> 20 μm; Fig. 6). The shift towards large cells was significant ($p < 0.05$), or nearly so ($p < 0.06$) in the individual June 1997, July 1997, and September 1997 runs, and significant across all four mesocosm runs combined ($p < 0.01$). The mean percentage of phytoplankton carbon in cells > 20 μm increased from 76 ± 6% in controls to 86 ± 5% in the +N treatments. Most other abundant phytoplankton taxa such as dinoflagellates, cryptophytes, and unidentified flagellates also increased significantly in response to nutrients during two or more mesocosm runs (all $p < 0.05$).

Trace Elements

Unlike the consistent pattern seen in response to nutrients, trace element additions in the absence of nutrient additions (+T) were inhibitory to chl *a* biomass (Fig. 3) and primary production (Fig. 4) in only some of the mesocosm runs compared to the C treatment. Trace element addition mesocosms had significantly lower average chl *a* than did the control mesocosms in the June 1997 run, and significantly lower primary production in both June 1997 and 1998 (Fig. 4). Combining the 1996 and 1997–1998 mesocosm experiments, the mean effect of trace element additions on chl *a* and primary production in the non-nutrient mesocosms was stronger in spring experiments, but was not significant overall (Table 4).

In the June 1997 through May 1998 mesocosm runs, trace elements decreased the biomass of centric diatoms in the phytoplankton assemblage in individual spring runs ($p < 0.05$ for both June 1997 and May 1998 for *D. fragilissimus,* and June 1997 for other centric diatoms; Fig. 5) and overall ($p < 0.05$) for *D. fragilissimus.* This resulted in a

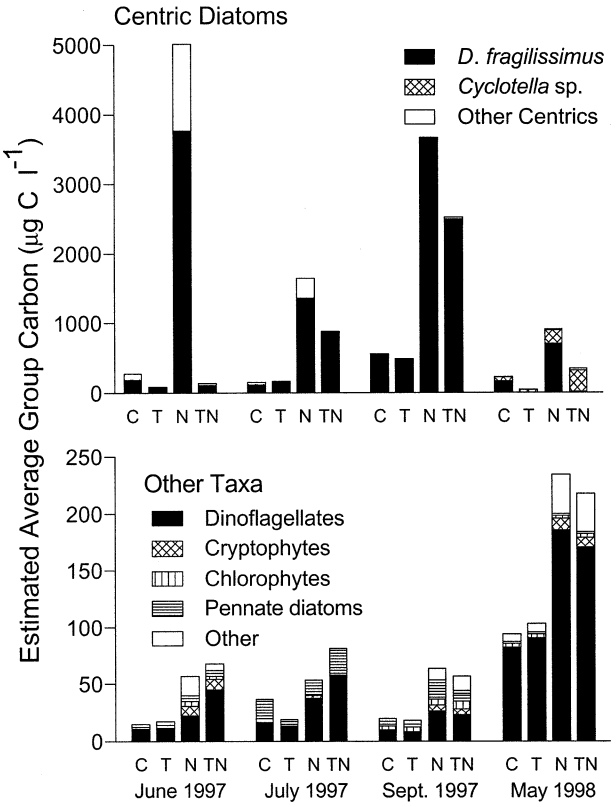

Fig. 5. Estimated mean contribution of phytoplankton taxa to phytoplankton biomass as carbon from mesocosm runs of the main design, 1997 through 1998, segregated by nutrient × trace element treatments, with averages across time and complexity treatment.

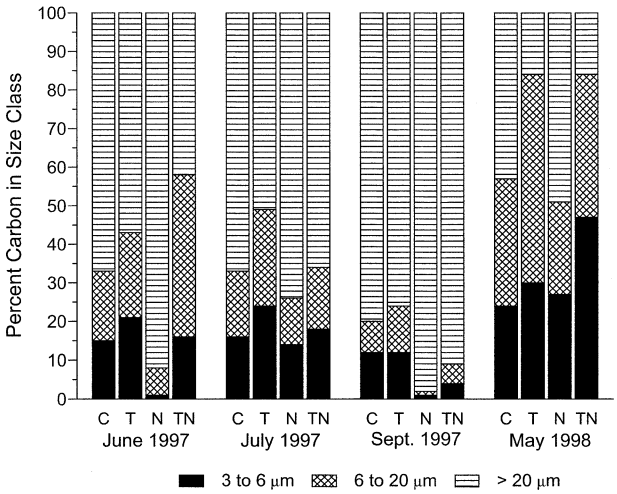

Fig. 6. Estimated mean size class frequency distributions for phytoplankton carbon biomass from mesocosms runs of the main design, 1997 through 1998, segregated by nutrient × trace element treatments, with averages across time and complexity treatment.

shift of the size spectrum of phytoplankton carbon towards smaller sizes ($p < 0.01$) to an average of 61 ± 8% of carbon in cells > 20 µm in diameter (Fig. 6). No other abundant groups of phytoplankton were significantly affected by the trace element additions.

Combined Nutrient and Trace Element Additions

Averaged across all runs, phytoplankton chl *a* and production in +N+T treatments were intermediate between the +N and +T treatments. The +N+T mesocosms had significantly lower phytoplankton chl *a* and primary production only during the spring runs (Figs. 3 and 4). Combining the 1996 and 1997–1998 mesocosm studies, the magnitude of difference between the +N+T and +N treatments was greater during spring than in other runs for both phytoplankton chl *a* and primary production (both $p < 0.01$).

During June 1997 through May 1998 large centric diatoms, the group most responsible for the increase in biomass as a result of +N treatments, was also the group responsible for the largest decrease in +N+T treatments relative to the +N treatments (Fig. 5). Both *D. fragilissimus* and other centric diatoms were significantly reduced in the +N+T treatment compared to the +N treatment ($p < 0.001$ and $p < 0.01$, respectively). As a result, +N+T treatments had smaller size frequency carbon distributions than the +N treatments (Fig. 6); less carbon (62.2 ± 8%) was in cells > 20 µm than in the +N mesocosms ($p < 0.001$). No other abundant taxa of phytoplankton showed a significant response to combined nutrient and trace element conditions, when compared to the corresponding nutrient additions alone.

The Modified Design

Nutrient Effects

Nutrient effects in the modified design studies were similar to those in previous experiments. In the May 1999 spring run the +N treatment raised average primary productivity compared to the nonnutrient control by nearly a factor of ten, while in July 1998, the summer +N treatment produced an average of roughly four times greater primary productivity (Fig. 7).

Nutrient additions produced a large increase in centric diatoms in spring. *Ceratulina pelagica* was the most abundant diatom in the C treatment, followed by *D. fragilissimus,* and the same pattern was observed in the +N treatment (Fig. 8). The spring run +N treatment also lead to a near doubling of the biomass of phytoplankton other than centric diatoms; most of this added biomass was dinoflagellates (Fig. 9), which also showed a significant response to nutrients ($p < 0.0001$). In contrast to

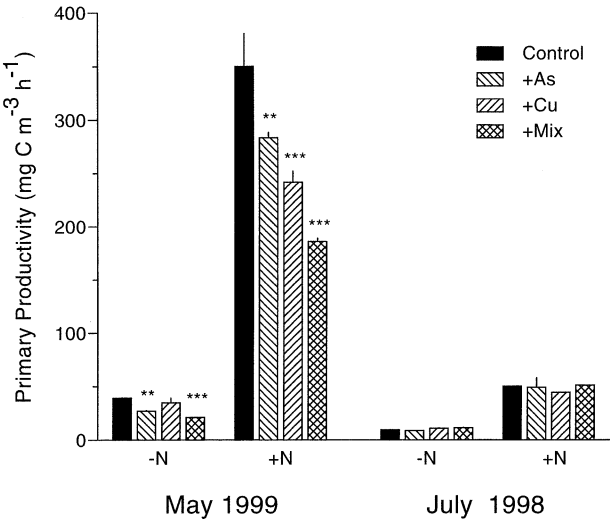

Fig. 7. ^{14}C primary productivity in the mesocosms of the modified design (mean ± SE), spring 1999 and summer 1998, segregated by nutrient × trace element treatment. The significance level of contrasts are marked for each experiment (control versus +As, control versus +Cu, and control versus +mix) within nutrient treatments, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

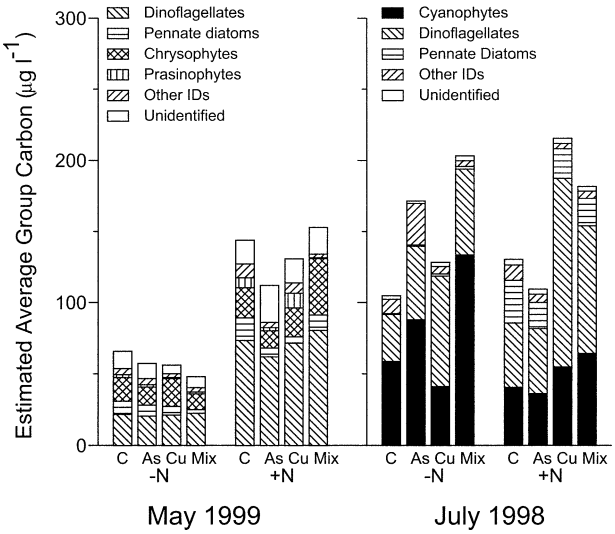

Fig. 9. Mean estimated carbon biomass of all phytoplankton taxa except centric diatoms in the mesocosms of the modified design, spring 1999 and summer 1998, segregated by nutrient × trace element treatment.

earlier spring runs, nutrient additions alone had no significant effect on the relative abundance of the three size classes; in fact, the control treatment was largely dominated (78.6 ± 2.9%) by cells > 20 µm (Fig. 10), and the nutrient addition showed a slight decrease in this fraction to 73 ± 3.6%.

In the summer run, centric diatoms, though still important members of the phytoplankton community, were not an overwhelming biomass dominant, and the stimulation by nutrient additions alone was minor (Figs. 8 and 9). Among other taxa in the summer run, cyanophytes and dinoflagellates constituted most of the biomass. There was little evidence that nutrients alone had a stimulating effect, other than increasing the biomass of pennate diatoms across all treatments receiving nutrients (Fig. 9). Nutrient additions resulted in an increase of biomass in large cells (> 20 µm) from 20.5 ± 3.4% to 42.2 ± 4.1%.

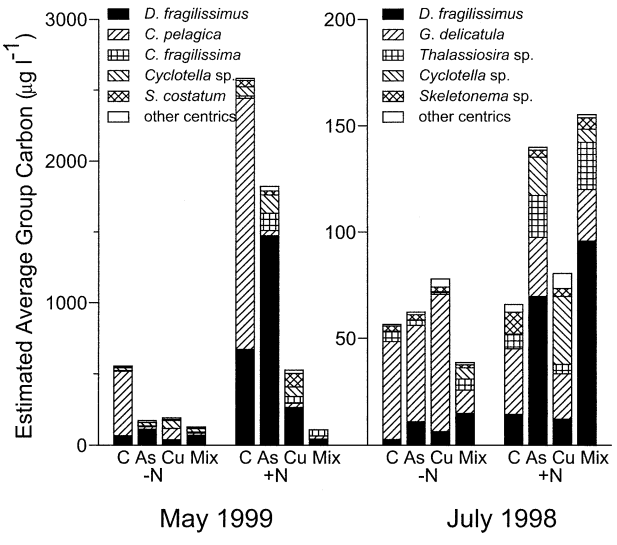

Fig. 8. Mean estimated carbon biomass of centric diatoms in the mesocosms of the modified design, spring 1999 and summer 1998, segregated by nutrient × trace element treatment.

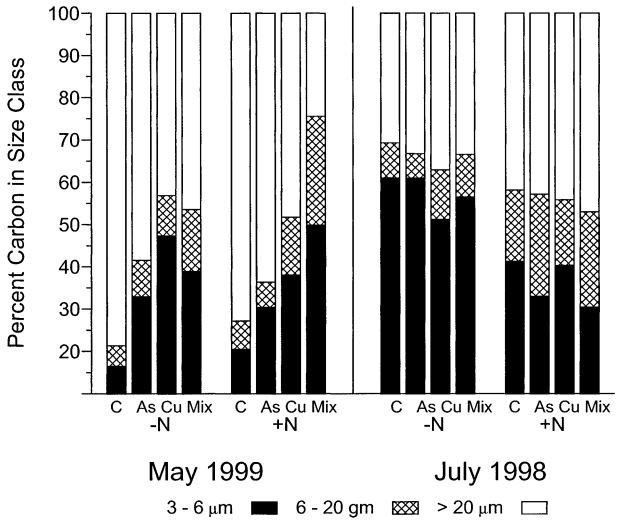

Fig. 10. Mean estimated size class frequency distributions for phytoplankton carbon of the mesocosms of the modified design, spring 1999 and summer 1998, segregated by nutrient × trace element treatment.

Trace Element Effects

Additions of As alone caused a 31% drop in productivity relative to controls (Least Square means, pairwise comparison, $p < 0.002$) in the spring run, while Cu alone had an 11% decline, which was not statistically significant (Fig. 7). The combined trace element mix (As, Cd, Cu, Ni, and Zn) caused a productivity decline of 46% (Least Square Means, pairwise comparison, $p < 0.0001$). In all cases, declines in centric diatoms in the trace element treatment mesocosms accounted for the majority of the declines in estimated cell carbon (Figs. 8 and 9), and led to significant reductions in cell size. The As addition reduced the percentage of cells > 20 μm to 58.4 ± 0.6% ($p < 0.0006$), Cu reduced it to 43.1 ± 2.6% ($p < 0.0001$), and the mix reduced it to 46.4 ± 4.7% ($p < 0.0001$).

There were no significant effects of trace elements on phytoplankton primary production, abundance of individual taxa, or phytoplankton size distribution in the absence of nutrient additions in the summer run. The As addition had a 9% decrease in productivity, while Cu had a 15% increase in productivity, and the combined trace elements had a 18% increase in productivity (Fig. 7). Trace elements treatments that included As (+As and +mix) in the absence of nutrient additions appeared to stimulate cyanophytes, while Cu additions alone tended to inhibit cyanophytes and enhance dinoflagellates.

Trace Elements + Nutrient Effects

In spring mesocosms with nutrients added, trace element additions strongly affected primary production. As additions resulted in a productivity decline of 19% ($p < 0.008$), Cu alone caused a decline of 31% ($p < 0.0001$), and the combined trace element treatment mix caused a productivity decline of 47% ($p < 0.0001$). Changes in centric diatom carbon appeared to drive the changes in productivity, with a decline of 33% due to As alone, a decline of 80% due to Cu alone, and a decline of 92% in the combined trace element treatment. Paradoxically, trace element additions containing As (+As and +mix) with nutrients appeared to stimulate *D. fragilissimus,* while the Cu treatment shifted the community structure among centric diatoms by decreasing *Guinardia delicatula* (formerly *Rhizosolenia delicatissima*), but permitting growth of a smaller diatom, *Cyclotella* sp. (Fig. 8). All of the trace element treatments were shifted to smaller phytoplankton size classes, with Cu having a greater effect (48.2 ± 2.6% carbon in cells > 20 μm, $p < 0.0001$) than As (63.7 ± 0.4% carbon in cells > 20 μm, $p < 0.03$), and the trace element mix treatments having the lowest biomass in the large size class (24.4 ± 2.6% carbon in cells > 20 μm, $p < 0.0001$).

In summer, there were no significant effects of trace elements on nutrient enriched mesocosms; productivity was reduced an average of 11% in the As treatments, reduced by 3% in the Cu treatments, and enhanced by 2% in the combined trace element treatments. The trace element treatments led to alterations of species of diatoms (Fig. 8), an outcome somewhat different from earlier runs. In treatments receiving both trace elements and nutrients, As alone had little effect, while Cu alone and the mix appeared to stimulate dinoflagellates. As in the nutrient treatment alone, the treatment containing As and nutrients appeared to favor *D. fragilissimus* (Fig. 9). There were no significant changes in the size categories with the various trace element treatments with added nutrients (Fig. 10).

Seasonal Pattern of Trace Element Effects

To further examine the seasonal influence on the response of phytoplankton to trace elements, we analyzed IVF, chl *a,* and primary production (Fig. 4) across all the mesocosms runs for which data were available (current experiments plus the four 1996 mesocosm runs described by Breitburg et al. 1999a). Controls were compared to +T treatments, and +N to +N+T or +N+mix. Six of eight comparisons from the spring time period showed statistically significant trace element effects ($p < 0.05$ or lower) for IVF, while none of twelve comparisons outside the May–June period showed significant effects of trace elements (data not shown). Chl *a* data were not available for the experiments using the modified design, but for the 1996 and 1997–1998 experiments, three of six comparisons for spring were significant compared to zero of ten for months outside this period. For primary production, seven of eight spring contrasts were significant, while only one, C versus +T for April 1996, was significant outside this period (Fig. 4).

Discussion

Diatoms inherently grow faster and can outcompete flagellates and other unicellular algae when nutrients are in good supply and other environmental conditions are favorable (e.g., Pearsall 1932; Harrison and Davis 1979; Turpin and Harrison 1979; Kilham and Kilham 1984). At the same time, evidence continues to accumulate that various kinds of contaminants exert a selective influence that favors flagellates and smaller cells at the expense of larger, centric diatoms, and recent work has emphasized the fact that toxic stressors affect the size of phytoplankton by favoring the dominance of smaller species over larger species (e.g., Menzel et al. 1970; Sanders and Riedel 1998). Such

a shift in size may indeed have significant trophic effects on coastal systems (Ryther 1969; Falkowski 1994; Sanders and Riedel 1998).

Both nutrients and trace elements caused changes in phytoplankton density, productivity and species composition, but the direction of these effects differed. Nutrients increased production and densities of all size fractions of cells, notably *D. fragilissimus* and other large centric diatoms. Trace elements often decreased production, reduced the relative contribution of *D. fragilissimus* and similar large centric diatoms and caused phytoplankton assemblages to shift to smaller size classes. Trace element effects were more pronounced in spring than in summer.

The response of *D. fragilissimus* was particularly interesting. Because of its relatively high abundance, large size, and sensitivity to both nutrient and trace element additions, this species dominated the responses both to nutrients and trace elements, when responses occurred. It was present in significant amounts in all 6 mesocosm runs during 1997–1999. It responded strongly to nutrients in all the experimental runs discussed here, except for July 1998, where another large centric diatom, *C. pelagica,* appeared to take this role. The strong response of *D. fragilissimus* usually led to almost total dominance of the biomass by this species, while non-nutrient mesocosms maintained a more even mixture of species. In the main design *D. fragilissimus* responded negatively to trace elements in the absence of added nutrients in all spring experiments, but did very well in +T treatments in summer runs, suggesting that the reason for its response to trace elements is tied to seasonal effects. In treatments with both trace elements and nutrients added, *D. fragilissimus* responded negatively in all four cases.

In the modified design, centric diatoms followed the patterns previously observed in the main design, i.e., nutrients stimulated centric diatoms, particularly in spring, and trace elements negatively affected centric diatoms, especially in spring. Although *D. fragilissimus* was present in the spring run, *C. pelagica* was more abundant, and showed a greater response to nutrients. *D. fragilissimus* became the dominant cell in the mesocosms with trace elements added. In summer, another centric diatom, *G. delicatula,* was dominant in the absence of nutrients, but *D. fragilissimus* became more important in the trace elements treatments.

D. fragilissimus and some similar large centric diatom species have been shown to be extremely sensitive to As (Sanders and Vermersch 1982; Sanders and Cibik 1985). In mesocosm experiments, As concentrations of 2.3 $\mu g\ l^{-1}$ have been shown to reduce growth rates of this species significantly, and have led to their loss from natural assemblages (Sanders and Cibik 1985; Sanders and Riedel 1998). The concentrations in the trace element treatments of these experiments (Table 3) were potentially high enough in all runs to inhibit growth; however, competitive interactions with phosphorus for uptake by phytoplankton are very important, and likely explain at least some of the seasonal differences in response.

Results of the mesocosm runs using the modified design indicate that dependence of the toxic effect of individual elements on nutrient loadings and season was more complex than we had originally hypothesized. In the spring study, As additions caused greater inhibition of productivity in the mesocosms with ambient nutrient loadings, while Cu showed greater inhibition in the nutrient enriched mesocosms, although both trace elements showed inhibitory trends in both nutrient regimes. In the +N mesocosms, although phosphorus was still limiting phytoplankton growth, phosphorus loadings were greater and As inhibition was lessened. In addition, cell leakage of carbon and complexing ligands has been shown to be less under conditions of nutrient sufficiency relative to nutrient limited phytoplankton assemblages (Hellebust 1965; Morris and Foster 1971; Sharp 1977; Wangersky et al. 1989; Zhou et al. 1989). The +N mesocosms may have contained lower concentrations of biologically-derived ligands, and had higher concentrations of free Cu^{2+} and greater Cu toxicity. Further research linking similar mesocosm experiments with chemical characterization of the speciation and complexation of Cu will be necessary to fully explore this issue.

An important point is that the responses to nutrients and trace elements in these systems does not represent only the physiological effect of the stressor on the individual species, but rather the integrated effect on the assemblage. Any particular response observed may be a direct physiological response to a stressor, an interaction of the stressors, a competitive interaction among the various species of phytoplankton as they respond to the stressors, or an indirect effect of changes in grazers and even higher trophic levels.

Implications for the Patuxent and Other Coastal Systems

Stressors can alter phytoplankton productivity, species composition, and size distribution both positively and negatively, and thereby alter carbon cycling and transfer between trophic levels. Even moderate additions of both stressors examined here led to large shifts in biomass and the size structure of the phytoplankton assemblage, most strongly reflected in the response of *D. fragilissimus*

and other centric diatoms. It is not our contention that *D. fragilissimus* is unique among large centric diatoms in its response to nutrients and toxics. Rather, it is a fairly typical member of that group which happened to be present consistently in our studies. Centric diatoms as a group are important members of phytoplankton assemblages of many marine, coastal, and estuarine systems. *D. fragilissimus* is common in the mid-Atlantic coast and Chesapeake Bay and can comprise a significant percentage of the phytoplankton carbon during the spring and early summer period (Marshall and Lacouture 1986; Marshall and Ranasinghe 1989).

The effect of adding nutrients to the phytoplankton assemblage drawn from the Patuxent River was to increase abundance of both preferred and poorly used phytoplankton size classes and taxa. Increasing the total and percent of phytoplankton carbon represented by species such as *D. fragilissimus,* which is poorly used by important suspension feeders such as the calanoid copepod *Acartia tonsa* and the eastern oyster *Crassostrea virginica* (Bundy et al. 2003), potentially increases the pool of unconsumed phytoplankton carbon that fuels oxygen depletion in subpycnocline waters during summer. In the Patuxent, an increasing fraction of phytoplankton carbon sequestered in poorly used species would indicate the potential for a non-linear relationship between nutrient loadings and negative effects of eutrophication such as hypoxia. The marked changes in the phytoplankton assemblages in these experiments occurred with average differences in nitrogen and phosphorus loadings between our control and nutrient-addition mesocosms less than the variation in nitrogen and phosphorus loadings among tributaries within the Chesapeake Bay system (Boynton et al. 1995) and less than the variation among years (1985–1999) within the Patuxent River estuary (Hagy and Boynton 2000; Sprague et al. 2000). These results do not constitute an extreme scenario, but rather situations routinely seen in this and many other areas.

Our results also indicate that the direct effects of toxic trace elements on coastal phytoplankton communities can be influenced by several factors. These include temporal and spatial patterns in loadings of the trace elements and in nutrient loadings and differences in composition of the phytoplankton community which are exposed. At times and locations where sensitive species are abundant and nutrient dynamics facilitate trace element toxicity, trace elements can strongly reduce phytoplankton abundance and production and lead to shifts in dominant species. In the Patuxent River, trace element loadings are positively correlated with river flow, and are higher in winter and spring than in summer and fall (Riedel et al. 2000), coinciding with centric diatom blooms and when centric diatoms are more responsive to trace element effects. Similar trace element loadings at other sites or at times dominated by other species or receiving different nutrient inputs will have substantially different effects.

An important implication of these results is the potential for elevated trace element loadings to mask the effects of excess nutrient inputs from anthropogenic sources—and the reverse. In general, the effect of trace elements was proportionally greater in mesocosms to which nutrients were added than in mesocosms without added nutrients, and many coastal ecosystems are exposed to both nutrient enrichment and elevated trace element concentrations. Areas with moderately elevated trace element loadings may appear to have a lessened response to excess nutrients. Where improved sewage treatment and reduced runoff from urbanized or agricultural areas reduces both trace element and nutrient loadings, the response of the phytoplankton community to nutrient reductions may be less than otherwise predicted. Management agencies charged with reducing the effects of eutrophication may believe that their efforts have not been effective. High nutrient loadings may mask the effects of trace elements on sensitive species. The magnitude of trace element effects on sensitive species or their predators may be apparent only when nutrient loadings are reduced. As we achieve success in the area of eutrophication, we may find a new, potentially very significant, issue—trace element contamination and toxicity—awaiting our understanding. Future studies and models designed to examine coastal degradation will have to address such complexity.

A number of other stressors interact with high nutrient loadings in the Patuxent, either masking or exacerbating nutrient effects. High sediment loads potentially reduce primary production and phytoplankton biomass, reducing potential responses to nutrients in much the same way that elevated trace element concentrations did in our spring mesocosm experiments. As with joint reductions of trace elements and nutrients, simultaneous reductions in both sediment and nutrient loads may reduce the apparent response to nutrient management. By contrast, disease and overfishing, which have reduced populations of suspension feeding oysters, have likely exacerbated nutrient enrichment effects on the system. Relieving these stressors and increasing stocks of suspension feeding bivalves would potentially reduce phytoplankton biomass even under current nutrient loads.

Phytoplankton occupy an important position within estuarine, coastal, and marine ecosystems.

They form the critical link between geochemical processes that occur within ecosystems and the flow of carbon through food webs. If shifts in nutrient and trace element concentrations, ratios, and speciation occur through anthropogenic influences, they can lead to shifts in phytoplankton productivity and species composition, and carbon and trophic structure of the ecosystem can be altered, perhaps yielding systems with less desirable characteristics.

Human population growth and attendant land-use changes likely increase the intensity, geographic extent, and number of anthropogenic stressors. An expanded focus on the cumulative effects of multiple stressors becomes increasingly important. Non-additive effects of multiple stressors and the variability they cause in species' responses will be important features of human-influenced systems, and will certainly be a focus of research and management in coming years and decades. Temporal and spatial variability in these effects may be a common feature of multiple stressor interactions that will overlay natural variability in coastal systems and complicate the detection and management of anthropogenic impacts.

Acknowledgments

We thank Mollie Weinstein for phytoplankton counts, Deborah Heyes for managing the mesocosm experiments, and Colleen Hatfield, Dawn Davis, Courtney Richmond, and Michael Grove for data management. We also thank other COASTES PIs (Kevin Sellner, Rick Osman, Marie Bundy, Sybil Seitzinger, and Cindy Gilmour) for their roles in the COASTES mesocosm experiments. This study was funded by the National Oceanic and Atmospheric Administration, award No. NA66RG0129, ''The Importance of Understanding Ecological Complexity to Predicting Effects of Multiple Stressors on Coastal Systems.''

Literature Cited

Abbe, G. R. and J. G. Sanders. 1986. Condenser replacement in a coastal power plant: Copper uptake and incorporation in the American oyster, *Crassostrea virginica*. *Marine Environmental Research* 19:93–113.

Bartell, S. M. 2003. A framework for estimating ecological risks posed by nutrients and trace elements in the Patuxent River. *Estuaries* 26:385–397.

Berggreen, U., B. Hansen, and T. Kiørbo. 1988. Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: Implications for determination of copepod production. *Marine Biology* 99:341–352.

Blum, J. J. 1966. Phosphate uptake by phosphate-starved *Euglena*. *Journal of General Physiology* 49:1125–1136.

Boynton, W. R., J. H. Garber, R. Summers, and W. M. Kemp. 1995. Inputs, transformations and transport of nitrogen and phosphorus in Chesapeake Bay and selected tributaries. *Estuaries* 18:285–314.

Brand, L. E., W. G. Sunda, and R. L. L. Guillard. 1986. Reduction of marine phytoplankton growth rates by copper and cadmium. *Journal of Experimental Marine Biology and Ecology* 89: 225–250.

Breitburg, D. L. and G. F. Riedel. In press. Multiple stressors in marine systems, Chapter 10. *In* L. E. Crowder (ed.), Marine Conservation Biology: The Science of Maintaining the Sea's Biodiversity (MCB). Island Press, New York.

Breitburg, D. L., J. G. Sanders, C. C. Gilmour, C. A. Hatfield, R. W. Osman, G. F. Riedel, S. P. Seitzinger, and K. P. Sellner. 1999a. Variability in responses to nutrients and trace elements, and transmission of stressor effects through an estuarine food web. *Limnology and Oceanography* 44:837–863.

Breitburg, D., S. Seitzinger, and J. Sanders (eds.). 1999b. The effects of multiple stressors on freshwater and marine ecosystems. *Limnology and Oceanography* 44.

Bundy, M. H., D. L. Breitburg, and K. G. Sellner. 2003. The responses of Patuxent River upper trophic levels to nutrient and trace element induced changes in the lower food web. *Estuaries* 26:365–384.

Cloern, J. E. 1996. Phytoplankton bloom dynamics in coastal ecosystems: A review with some general lessons from sustained investigation of San Francisco Bay, California. *Review of Geophysics* 34:127–168.

Cloern, J. E. 2001. Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series* 210: 223–253.

D'Elia, C. F., W. R. Boynton, and J. G. Sanders. 2003. A watershed perspective on nutrient enrichment, science and policy in the Patuxent River, Maryland: 1960–2000. *Estuaries* 26: 171–185.

D'Elia, C. F., J. G. Sanders, and W. R. Boynton. 1986. Nutrient enrichment studies in a coastal-plain estuary: Phytoplankton growth in large-scale continuous cultures. *Canadian Journal of Fisheries and Aquatic Sciences* 43:397–406.

Falkowski, P. G. 1994. The role of phytoplankton photosynthesis in global biogeochemical cycles. *Photosynthesis Research* 39: 235–258.

Goldman, J. C., K. R. Tenore, and H. I. Stanley. 1973. Inorganic nitrogen removal from wastewater: Effect on phytoplankton growth in coastal marine waters. *Science* 180:955–956.

Hagy, J. D. 1996. Residence times and net ecosystem processes in the Patuxent River estuary. M.S. Thesis, University of Maryland, College Park, Maryland.

Hagy, J. D. and W. R. Boynton. 2000. Controls on hypoxia in Chesapeake Bay and its major tributaries, p. 91–101. *In* M. W. Kemp, R. Bartleson, J. D. Hagy, and W. R. Boynton (eds.), Ecosystem Models of the Chesapeake Bay Relating Nutrient Loadings, Environmental Conditions, and Living Resources. Technical Report. January, 2000. Chesapeake Bay Program Office, Annapolis, Maryland.

Harding, Jr., L. W., B. W. Meeson, and T. R. Fisher, Jr. 1986. Phytoplankton production in two east coast estuaries: Photosynthesis-light functions and patterns of carbon assimilation in Chesapeake and Delaware Bays USA. *Estuarine, Coastal and Shelf Science* 23:773–806.

Harrison, P. J. and C. O. Davis. 1979. The use of outdoor phytoplankton continuous cultures to analyze factors influencing species selection. *Journal of Experimental Marine Biology and Ecology* 41:9–23.

Hellebust, J. A. 1965. Excretion of some organic compounds by marine phytoplankton. *Limnology and Oceanography* 10:192–206.

Hodgkiss, I. J. and K. C. Ho. 1997. Are changes in N : P ratios in coastal waters the key to increased red tide blooms? *Hydrobiologia* 352:141–147.

Kilham, S. S. and P. Kilham. 1984. The importance of resource supply rates in determining phytoplankton community structure, p. 7–28. *In* D. G. Meyers and J. R. Strickler (eds.), Trophic Interactions Within Aquatic Systems. AAAS Symposium 85. Westview Press, Boulder, Colorado.

Laursen, A. E., S. P. Seitzinger, R. DeKorsey, J. G. Sanders, D. L. Breitburg, and R. W. Osman. 2002. Multiple stressors in an estuarine system: Effects of nutrients, trace elements and

trophic complexity on benthic photosynthesis and respiration. *Estuaries* 25:57–69.

LEWIS, M. R. AND J. C. SMITH. 1983. A small volume, short incubation time method for measurement of photosynthesis as a function of incident irradiance. *Marine Ecology Progress Series* 13:99–102.

MALONE, T. C., W. BOYNTON, T. HORTON, AND C. STEVENSON. 1993. Nutrient loadings to surface waters: Chesapeake Bay case study, p. 8–38. *In* M. F. Uman (ed.), Keeping Pace with Science and Engineering. U.S. National Academy of Engineering, Washington, D.C.

MARSHALL, H. G. AND R. LACOUTURE. 1986. Seasonal patterns of growth and composition of phytoplankton in the lower Chesapeake Bay and vicinity. *Estuarine, Coastal and Shelf Science* 23: 115–130.

MARSHALL, H. G. AND J. A. RANASINGHE. 1989. Phytoplankton distribution along the eastern coast of the U.S.A.–VII. Mean cell concentrations and standing crop. *Continental Shelf Research* 9:153–164.

MCCARTAN, L., J. D. PEPER, L. J. BACHMAN, AND J. W. HORTON, JR. 1998. Application of geological map information to water quality issues in the southern part of the Chesapeake Bay watershed, Maryland and Virginia, eastern United States. *Journal of Geochemical Exploration* 64:355–376.

MENZEL, D. W., J. ANDERSON, AND A. RANDKE. 1970. Marine phytoplankton vary in their response to chlorine hydrocarbons. *Science* 167:1724–1726.

MOFFETT, J. W. AND L. E. BRAND. 1996. Production of strong extracellular Cu chelators by marine cyanobacteria in response to Cu stress. *Limnology and Oceanography* 41:388–395.

MOFFETT, J. W., L. E. BRAND, P. L. CROOT, AND K. A. BARBEAU. 1997. Cu speciation and cyanobacterial distribution in harbors subject to anthropogenic Cu inputs. *Limnology and Oceanography* 42:789–799.

MONTAGNES, D. J. S., J. A. BERGES, P. J. HARRISON, AND F. J. R. TAYLOR. 1994. Estimating carbon, nitrogen, protein and chlorophyll *a* from volume in marine phytoplankton. *Limnology and Oceanography* 39:1044–1060.

MORRIS, A. W. AND P. FOSTER. 1971. The seasonal variation of dissolved organic carbon in the inshore waters of the Menai Strait in relation to primary production. *Limnology and Oceanography* 16:987–989.

NEWELL, R. I. E. AND C. J. LANGDON. 1996. Mechanisms and physiology of larval and adult feeding, p. 185–230. *In* V. S. Kennedy, R. I. E. Newell, and A. F. Eble (eds.), The Eastern Oyster, *Crassostrea virginica*. Maryland Sea Grant, College Park, Maryland.

OVIATT, C. A., P. LANE, F. FRENCH, III, AND P. DONAGHAY. 1989. Phytoplankton species and abundance in response to eutrophication in coastal marine mesocosms. *Journal of Plankton Research* 11:1223–1244.

PEARSALL, W. H. 1932. Phytoplankton in the English Lakes. 2. The composition of the phytoplankton in relation to dissolved substances. *Journal of Ecology* 20:241–262.

PLANAS, D. AND F. P. HEALEY. 1978. Effects of arsenate on growth and phosphorus metabolism of phytoplankton. *Journal of Phycology* 14:337–341.

POTVIN, C. AND D. A. ROFF. 1993. Distribution-free and robust statistical methods: Viable alternatives to parametric statistics? *Ecology* 74:1617–1628.

RIEDEL, G. F. 1984. The influence of salinity and sulfate on the toxicity of chromium(VI) to the estuarine diatom *Thalassiosira pseudonana*. *Journal of Phycology* 20:496–500.

RIEDEL, G. F. 1985. The relationship between chromium(VI) uptake, sulfate uptake and chromium(VI) toxicity to the estuarine diatom *Thalassiosira pseudonana*. *Aquatic Toxicology* 7:191–204.

RIEDEL, G. F. 1988. Interspecific and geographic variation of the chromium sensitivity of algae, p. 537–548. *In* G. W. Suter and M. Lewis (eds.), Proceedings, 11th Symposium on Aquatic Toxicology and Hazard Assessment. American Society of Testing and Materials, Philadelphia, Pennsylvania.

RIEDEL, G. F. 1993. The annual cycle of arsenic in a temperate estuary. *Estuaries* 16:533–540.

RIEDEL, G. F., G. R. ABBE, AND J. G. SANDERS. 1998. Temporal and spatial variations of trace metal concentrations in oysters from the Patuxent River, Maryland. *Estuaries* 21:423–434.

RIEDEL, G. F. AND J. G. SANDERS. 2003. The interrelationships among trace element cycling, nutrient loading, and system complexity in estuaries: A mesocosm study. *Estuaries* 26:339–351.

RIEDEL, G. F., S. A. WILLIAMS, G. S. RIEDEL, C. C. GILMOUR, AND J. G. SANDERS. 2000. Temporal and spatial patterns of trace elements in the Patuxent River: A whole watershed approach. *Estuaries* 23:521–535.

RUETER, J. G. AND D. R. ADES. 1987. The role of iron nutrition in photosynthesis and nitrogen assimilation in *Scenedesmus quadricauda* (Chlorophyceae). *Journal of Phycology* 23:452–457.

RUETER, J. G. AND F. M. M. MOREL. 1981. The interaction between zinc deficiency and copper toxicity as it affects silicic acid uptake mechanisms in the marine diatom *Thalassiosira pseudonana*. *Limnology and Oceanography* 26:67–73.

RYTHER, J. H. 1969. Photosynthesis and fish production in the sea. *Science* 166:72–76.

RYTHER, J. H. AND W. M. DUNSTAN. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science* 171:1008–1013.

SANDERS, J. G. 1979. Effects of arsenic speciation and phosphate concentration on arsenic inhibition of *Skeletonema costatum* (Bacillariophyceae). *Journal of Phycology* 15:424–442.

SANDERS, J. G. AND S. J. CIBIK. 1985. Adaptive behavior of euryhaline phytoplankton communities to arsenic stress. *Marine Ecology Progress Series* 22:199–205.

SANDERS, J. G., S. J. CIBIK, C. F. D'ELIA, AND W. R. BOYNTON. 1987. Nutrient enrichment studies in a coastal plain-estuary: Changes in phytoplankton species composition. *Canadian Journal of Fisheries and Aquatic Science* 44:83–90.

SANDERS, J. G. AND G. F. RIEDEL. 1987. Control of trace element toxicity by phytoplankton, p. 131–149. *In* J. A. Sanders, L. Kosak-Channing, and E. E. Conn (eds.), Recent Advances in Phytochemistry, Volume 21. Plenum Publishing, New York.

SANDERS, J. G. AND G. F. RIEDEL. 1993. Trace element transformation during the development of an estuarine algal bloom. *Estuaries* 16:531–532.

SANDERS, J. G. AND G. F. RIEDEL. 1998. Metal accumulation and impacts in phytoplankton, p. 59–76. *In* W. J. Langston and M. J. Bebianno (eds.), Metal Metabolism in Aquatic Environments. Chapman and Hall Inc., London, U.K.

SANDERS, J. G., G. F. RIEDEL, AND D. P. FERRIER. 1991. Changes in community structure of Chesapeake Bay phytoplankton when exposed to low levels of trace metals: Management implications, p. 451–460. *In* J. Mihursky and A. Chaney (eds.), New Perspectives in the Chesapeake System: A Research and Management Partnership. Proceedings of a Conference 4–6 December 1990, Baltimore, MD. Chesapeake Research Consortium Publication No. 137. Chesapeake Research Consortium, Gloucester Point, Virginia.

SANDERS, J. G., G. F. RIEDEL, AND R. W. OSMAN. 1994. Arsenic cycling and impact in estuarine and coastal marine ecosystems, p. 289–308. *In* J. O. Nriagu (ed.), Arsenic in the Environment, Part I: Cycling and Characterization. J. Wiley and Sons, New York.

SANDERS, J. G., J. H. RYTHER, AND J. H. BATCHELDER. 1981. Effects of copper, chlorine, and thermal addition on the species composition of marine phytoplankton. *Journal of Experimental Marine Biology and Ecology* 49:81–102.

SANDERS, J. G. AND P. S. VERMERSCH. 1982. Response of marine

phytoplankton to low levels of arsenate. *Journal of Plankton Research* 4:881–893.

SCHELSKE, C. L. AND E. F. STOERMER. 1971. Eutrophication, silica depletion, and predicted changes in algal quality in Lake Michigan. *Science* 173:423–424.

SHARP, J. J. 1977. Excretion of organic matter by marine phytoplankton: Do healthy cells do it? *Limnology and Oceanography* 22:381–399.

SPRAGUE, L. A., M. J. LANDLAND, S. E. YOCHUM, R. E. EDWARDS, J. D. BLOMQUIST, S. W. PHILLIPS, G. W. SHENK, AND S. D. PRESTON. 2000. Factors affecting nutrient trends in major rivers of the Chesapeake Bay watershed. Water-Resources Investigations Report 00-4218. U.S. Geological Survey, Richmond, Virginia.

SUNDA, W. G. AND S. A. HUNTSMAN. 1983. Effect of competitive interactions between manganese and copper on cellular manganese and growth in estuarine and oceanic species of diatom *Thalassiosira*. *Limnology and Oceanography* 28:924–934.

THOMAS, W. W. AND D. L. R. SEIBERT. 1977. Effects of copper on the dominance and diversity of algae: Controlled ecosystem pollution experiment. *Bulletin of Marine Science* 27:23–33.

TILMAN, D. 1977. Resource competition between plankton algae: An experimental and theoretical approach. *Ecology* 58: 338–348.

TILMAN, D. 1980. Resources: A graphical-mechanistic approach to competition and predation. *American Naturalist* 116:362–393.

TURPIN, D. H. AND P. J. HARRISON. 1979. Limiting nutrient patchiness and its role in phytoplankton ecology. *Journal of Experimental Marine Biology and Ecology* 39:151–166.

U.S. ENVIRONMENTAL PROTECTION AGENCY (USEPA). 1996. Chesapeake Bay fall line toxics monitoring program 1994 Final Report. CBP/TRS/144/96. U.S. Environmental Protection Agency, Chesapeake Bay Program, Annapolis, Maryland.

U.S. ENVIRONMENTAL PROTECTION AGENCY (USEPA). 1999. Chesapeake Bay toxics loading and release inventory. EPA 903-R-99-006, CBP/TRS 222-100. U.S. Environmental Protection Agency, Chesapeake Bay Program, Annapolis, Maryland.

UTERMÖHL, H. 1958. Zur vervollkommung der quantitativen phytoplankton-methodik. *Mitteilung Internationale Vereinigung fuer Theoretische unde Amgewandt Limnologie* 9:1–38.

VERITY, P. G., C. Y. ROBERTSON, C. R. TRONZO, M. G. ANDREWS, J. R. NELSON, AND M. E. SIERACKI. 1992. Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. *Limnology and Oceanography* 37: 1434–1446.

WANG, W.-X., R. C. H. DEI, AND Y. XU. 2001. Cadmium uptake and trophic transfer in coastal plankton under contrasting nitrogen regimes. *Marine Ecology Progress Series* 211:293–298.

WANGBERG, S. AND H. BLANCK. 1990. Arsenate sensitivity in marine periphyton communities established under various nutrient regimes. *Journal of Experimental Marine Biology and Ecology* 139:119–134.

WANGERSKY, P. J., S. B. MORAN, R. J. PETT, D. E. SLAUENWHITE, AND X. ZHOU. 1989. Biological control on trace metal residence times: An experimental approach. *Marine Chemistry* 28: 215–226.

WIEGNER, T. N., S. P. SEITZINGER, D. L. BREITBURG, AND J. G. SANDERS. 2003. The effects of multiple stressors on the balance between autotrophic and heterotrophic processes in an estuarine system. *Estuaries* 26:352–364.

ZHOU, X., D. E. SLAUENWHITE, R. J. PETT, AND P. J. WANGERSKY. 1989. Production of copper complexing organic ligands during a diatom bloom: Tower tank and batch culture experiments. *Marine Chemistry* 27:19–30.

Received for consideration, October 23, 2001
Revised, August 14, 2002
Accepted for publication, August 29, 2002