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Variability in responses to nutrients and trace elements, and transmission of stressor effects through an estuarine food web

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Abstract

Aquatic systems are increasingly exposed to multiple stressors from anthropogenic sources. These stressors can vary in the consistency and magnitude of responses they elicit in biota and in how the presence of additional stressors modifies their effects. Understanding how the biological environment and temporal dynamics influence responses to stressors, and how stressors interact, is important to predicting their effects in the natural environment. We examined temporal variability in responses of an experimental estuarine food web to elevated trace elements and nutrients, as well as non-additive effects of the combination of these two stressors. Experiments were conducted four times during spring through autumn 1996 in 20 l-m^3 mesocosms. We measured a range of system-, population-, and individual-level parameters to quantify responses of phytoplankton, bacterioplankton, heterotrophic nanoflagellates, copepods, fish, and benthic invertebrates to trace element and nutrient additions.

The response to trace element additions was more variable both temporally and among phytoplankton and higher trophic level taxa than was the response to nutrient additions. Most taxa increased, either significantly or showed a trend toward increasing, in response to nutrient additions in all four mesocosm runs. In contrast, the direction as well as the magnitude of responses to trace element additions varied considerably among taxa and experimental runs. Two distinct types of nutrient×trace element interactions were important. First, temporal dynamics of nutrient ratios appeared to affect the temporal pattern of toxicity of trace elements to phytoplankton. Second, in the June mesocosm run when trace element additions reduced production, abundance, or growth of many organisms, these reductions were often proportionately greater in nutrient addition tanks than where no nutrients were added. Our results suggest that considerable temporal and taxonomic variation in responses to trace element loadings are likely to be seen in field settings even under constant loadings to the system and that trace elements may mask the magnitude of the response to high nutrient loadings in eutrophic systems. More generally, the presence of multiple stressors may either increase or dampen the temporal and spatial variability seen in aquatic systems, depending on the interactions among stressors and the influence of background environmental conditions and sensitive species on the expression of stressor effects.

Stressors vary considerably in the specificity of their effects; some stressors may affect nearly all organisms within a system, while others elicit direct responses from few species. The extent of this specificity can be influenced both by their mode of action and by the level of exposure to which species are subjected. Even when considering direct effects on a single trophic level, susceptibility can vary among species (e.g., Magnuson et al. 1989; Sanders and Riedel 1998; Diaz and Rosenberg 1995; Williamson et al. 1999) and may be influenced by the presence or intensity of other stressors in the environment (e.g., Folt et al. 1999; Lenihan et al. 1999). A potential consequence of this variation is that functioning of the susceptible trophic level may depend on species composition at any particular point in space or time as well as on the intensity and duration of the system's exposure to the spectrum of stressors in the environment.

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Transmission of stressor effects to higher trophic levels should depend on the relationship between stressor effects and species composition of trophic levels susceptible to direct effects, the presence of complementary responses within those trophic levels (Frost et al. 1995), and on feeding specificity and feeding relationships among organisms throughout the rest of the food web (Sanders et al. 1994; Breitburg et al. 1997). Where only certain taxa or trophic levels are susceptible to direct effects of stressors, the importance of particular species as prey and the potential for nutritionally similar species to exhibit complementary responses (e.g., increase while other species decrease) may determine whether stressor effects are dampened or magnified throughout the food web (Frost et al. 1995; Riegman 1995).

Increased nutrient and contaminant loadings, which can lead to enrichment and(or) degradation of the system, have been of concern in many estuaries and coastal systems (e.g., Gray and Paasche 1984; Hecky and Kilham 1988; Howarth 1988; Malone et al. 1993; Cloern 1996; Sanders and Riedel 1998). Increases in both nutrients and trace metals in coastal waters reflect the general increase in anthropogenic activities in the coastal zone. For example, 75% of the U.S. population will live within 75 km of the coastline by 2010 (Williams et al. 1991). The response to increased nutrient loadings can be increased productivity and phytoplankton standing stock (and the attendant problems associated with system eutrophication), but in addition, increased loadings and changes in nutrient ratios or chemical form can also change phytoplankton species composition, dominance, and succession (Turpin and Harrison 1979; Schelske and Stoermer 1971; Goldman and Stanley 1974; Sanders et al. 1987; Maestrini and Bonin 1981; Oviatt et al. 1989; Riegman 1995). Because phytoplankton species exhibit differing requirements for the various nutrients, community structure changes as the relative nutrient concentrations and fluxes change (Tilman 1977, 1980; Kilham and Kilham 1984). For example, high nutrient loading rates promote the growth and dominance of diatoms over flagellates (Turpin and Harrison 1979; Kilham and Kilham 1984). In a similar fashion, anthropogenically added contaminants such as organic pollutants and trace elements can strongly influence composition, dominance, and dynamics of phytoplankton assemblages (Menzel et al. 1970; Sanders and Cibik 1985; Brand et al. 1986; Sanders et al. 1994; Sanders and Riedel 1998). Both organic and inorganic contaminants often favor small cells and flagellates over diatoms (Menzel et al. 1970; Sanders and Riedel 1998). On a global scale, evidence is strong that the inputs of nutrients and contaminants to coastal zones are altering natural cycles of phytoplankton growth and succession in coastal systems (Cloern 1996).

We examined the potential for temporal variability in primary producer responses to stressors, transmission of stressor effects to higher trophic levels, and the potential for nonadditive interactions among stressors in a series of experimental estuarine food webs of increasing trophic complexity in 20 l-m^3 mesocosms. Our source of water and organisms, and therefore the model for our simplified food web, was the mesohaline portion of the Patuxent River estuary, a Maryland tributary of Chesapeake Bay. We tested two categories of stressors—increased nutrient (N and P) loadings and increased trace element (a mix of Cu, As, Cd, Ni, Zn) loadings. Each stressor category was tested alone and in combination during four 1996 mesocosm runs to examine temporal variability in their individual and interactive effects. This experiment was designed to address a portion of the overall objective of the COASTES (COmplexity And STressors in Estuarine Systems) project, which is to understand how multiple stressors affect estuarine environments and how those effects are modified by system complexity.

In this manuscript we focus on nutrient and trace element effects on general patterns and variability in phytoplankton production and abundance, and the transmission of these effects through mesocosm food webs. We predicted that direct effects of the two stressors should be restricted to lower trophic levels at the loading rates used (*see methods*). Components of the food web measured include bacterial production, heterotrophic nanoflagellate density, copepod abundance and stage distribution, growth rate of an omnivorous estuarine fish (the mummichog *Fundulus heteroclitus*), and growth rates of three benthic invertebrate species (the eastern oyster *Crassostrea virginica*, the clam *Macoma balthica*, and the anemone *Diadumene leucolena*). Direct effects of grazers on their prey are noted where this information is important to describe or explain stressor effects, but detailed effects of trophic complexity will be described elsewhere. Similarly, detailed analyses of phytoplankton dynamics, nutrient and trace element biogeochemistry, and temporal dynamics of higher trophic level responses will be emphasized elsewhere. Our purpose here is to provide a broad overview of our results across all taxa.

In our experimental systems, the response to trace element additions was more variable both temporally and among taxa than was the response to nutrient additions. Variability in phytoplankton responses to trace element additions seemed to depend on both the abundance of particularly vulnerable species and on temporal variability in whether nitrogen or phosphorus was the limiting nutrient. Transmission of stressor effects through the food web reflected both the integrated responses of the phytoplankton assemblage as a whole and specific feeding relationships within the food web. During the mesocosm run where direct trace element effects were most prominent, trace element and nutrient effects were not additive; rather, through much of the food web, trace elements disproportionately reduced the positive response to nutrients. Our results indicate that where multiple stressors occur in complex food webs, variability in biological processes and stressor interactions may lead to considerable temporal and taxonomic variation in responses even under constant loadings to a system.

Methods

Experimental design—The effects of nutrients, trace elements, and trophic complexity were tested with natural plankton assemblages from the Patuxent River estuary and higher trophic level organisms collected from the Patuxent but which are common throughout the Chesapeake Bay system and other mid-Atlantic estuaries. The experiment was conducted as a factorial, randomized block design in 20 l-

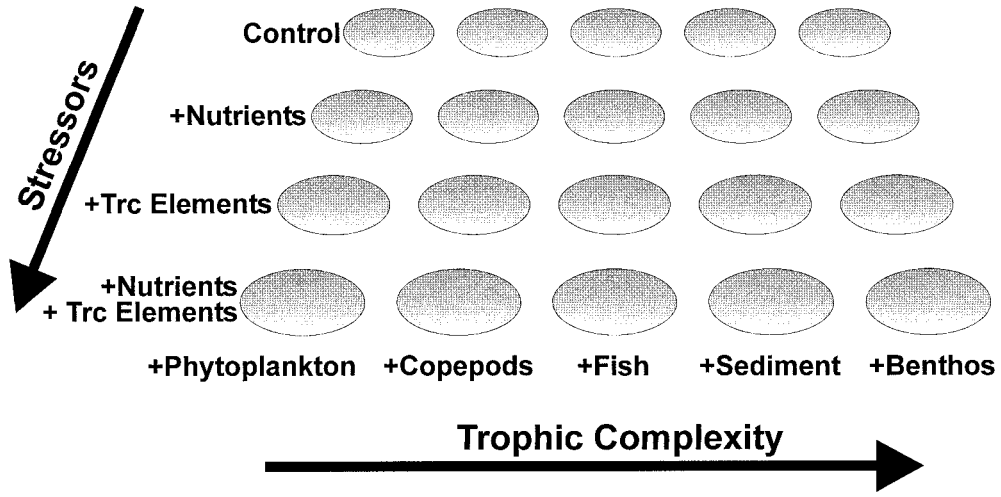

Fig. 1. Experimental design used for mesocosm experiments. Twenty mesocosm tanks were used for each mesocosm run with a completely crossed design of stressor treatment with complexity. Treatments were randomly assigned to tanks. (Trc—trace.)

m^3 cylindrical fiberglass mesocosms (107-cm diam × 122-cm total ht). During each of the four times mesocosms were run (mesocosm runs = blocks), the tank array was set up in a completely crossed design with two levels of nutrients × two levels of trace elements × five levels of system complexity (Fig. 1). This experimental design allowed us to statistically analyze the entire 1996 data set with mesocosm run as the blocking factor and to analyze each individual mesocosm run for treatment effects, albeit with lower statistical power (*see below*).

The mesocosms were outdoors in raceways that contained flowing estuary water to keep the temperature of the mesocosms similar to that of the estuary. All mesocosms were filled with water from the Patuxent River estuary, screened through 35-μm mesh plankton nets to exclude larger organisms. The mesocosms were maintained essentially as continuous flowthrough cultures using mesohaline estuary water, nominally filtered to 1 μm, as the exchange water, with a turnover rate of 10% per day. Each mesocosm run consisted of a 7-d acclimation period, followed by a 28-d experimental period (d 7–34). Nutrients and trace element concentrations were increased to target levels in +nutrient, +trace element, and +nutrient+trace element tanks on days 7 and 8 by adding half of the necessary concentration on each day. After day 8 both nutrients and trace elements were added continuously by peristaltic pump to maintain appropriate loadings. On days 9 and 10, higher trophic level organisms were added to appropriate mesocosms. The four mesocosm runs were performed consecutively in 1996 during spring and summer. Dates, temperatures, salinities, pH values, and other ancillary data are found in Table 1.

Each mesocosm was mixed by a PVC 4-blade paddlewheel suspended horizontally over the tanks on a fiberglass axle. Paddlewheels rotated at 4 rpm; every 6 h the motor would stop for 5 min, then reverse direction. Flexible, opaque PVC liners were attached to the interior surfaces of each mesocosm. These liners blocked light penetration and were removed approximately weekly and cleaned of all epiphyte growth to minimize the influence of wall growth on water column processes. Paddlewheel stirrers and liners were modeled after the University of Rhode Island seagrass mesocosms (S. Granger and S. Nixon pers. comm.); the rest of the experimental system is similar to that described by Sanders et al. (1987).

Two nutrient loading rates were used. Control tanks received only the 100 liter d^{-1} of 1-μm filtered Patuxent River estuary exchange water containing *dissolved* N and P concentrations found in the river; concentrations of N and P in exchange water varied with time and among runs (Table 1). Because exchange water was nominally filtered to 1 μm prior to use in order to remove most phytoplankton and other organisms, *total* N and P in the exchange water was lower than that in the river. Nutrient addition tanks received an additional 1.8 mmol NO_3-N m^{-2} d^{-1} and 0.11 mmol PO_4-P m^{-2} d^{-1}. Total DIN loadings to nutrient addition tanks were about 1.3–1.6× ambient loadings to the nearby surface layer of the Patuxent River as calculated from 1984 to 1995 averages. This level of nutrient loading was chosen after preliminary experiments in 1995 to depict a realistic scenario of increased nutrient inputs because of continued urbanization of the Patuxent watershed that presumably would yield increased phytoplankton production. Loadings to control tanks were 0.7–1.0× that calculated for the river (Hagy 1996).

The two trace element treatments included a control level, which received trace elements only from the exchange water and reflected background concentrations in the Patuxent River estuary and a trace element addition treatment in which a mixture of arsenic (As), copper (Cu), cadmium (Cd), zinc (Zn), and nickel (Ni) was added (*see Table 1*). Concentrations of dissolved As, Cu, Cd, and Ni in trace element addition mesocosms were 2–5× higher than the highest ambient concentrations measured in the river during routine monitoring conducted as part of the COASTES program in 1995–1996 (Riedel et al. submitted) but were lower than maximum concentrations found in other studies of the Pa-

Table 1. Environmental and test parameters for each run. Temperature and salinity are given as mean (range); dissolved nutrient and trace element concentrations are given as mean ± SE. Treatment column data refer to nutrient addition mesocosms for rows with N and P data and to trace element addition mesocosms for rows with trace element data. Nutrient loading rates are in mmol m^{-3} d^{-1}, N and P concentrations are in μmol $liter^{-1}$, trace element concentrations are in μg $liter^{-1}$, and As : P ratios are concentrations on a mol : mol basis with P averaged across both nutrient treatments.

Parameter	April run		June run		July run		September run	
Dates	1 Apr–6 May		20 May–24 Jun		9 Jul–12 Aug		26 Aug–30 Sep	
Temp (°C)	12.9 (7–21)		22.7 (17–28)		26.1 (23–29)		24.2 (20–29)	
Salinity	9.4 (8–11)		7.5 (6–8)		8.5 (7.5–9.5)		10.2 (9–11)	
pH	7.6–9.5		7.9–9.2		7.6–8.8		7.9–8.7	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Mean DIN concn	20.3 ± 1.3	19.5 ± 1.9	6.48 ± 0.45	5.36 ± 0.87	0.30 ± 0.02	0.29 ± 0.02	0.94 ± 0.04	0.70 ± 0.03
Mean N loading	3.66	5.26	1.97	3.57	1.70	3.30	1.67	3.27
Patuxent estuary DIN loading*	3.6		2.7		2.1		2.5	
Mean P concn	0.03 ± 0.008	0.20 ± 0.05	0.01 ± 0.001	0.03 ± 0.01	0.16 ± 0.01	0.19 ± 0.01	0.24 ± 0.04	0.22 ± 0.03
Mean P loading	0.04	0.14	0.04	0.14	0.09	0.20	0.13	0.23
N : P mesocosm concn	677	98	648	179	2	2	4	3
N : P mesocosm loading	92	38	49	26	19	17	13	14
Mean As concn	0.38 ± 0.01	8.24 ± 0.33	0.58 ± 0.05	8.89 ± 0.94	0.85 ± 0.05	4.34 ± 0.58	1.10 ± 0.09	7.67 ± 0.65
Mean Cu concn	0.95 ± 0.06	4.70 ± 0.90	0.96 ± 0.06	4.14 ± 0.14	0.70 ± 0.03	3.31 ± 0.18	0.66 ± 0.06	3.40 ± 0.11
Mean Cd concn	0.043 ± 0.001	0.80 ± 0.03	0.046 ± 0.009	0.62 ± 0.04	0.016 ± 0.001	0.61 ± 0.06	0.024 ± 0.002	0.69 ± 0.03
Mean Ni concn	1.21 ± 0.11	5.44 ± 0.15	1.15 ± 0.09	5.14 ± 0.15	0.71 ± 0.09	4.16 ± 0.18	0.96 ± 0.04	4.76 ± 0.10
Mean Zn concn	3.30 ± 0.33	8.05 ± 0.57	0.76 ± 0.13	4.34 ± 1.54	0.37 ± 0.05	1.23 ± 0.25	0.72 ± 0.08	2.61 ± 0.25
As : P mesocosm concn	0.05	0.91	0.32	6.9	0.06	0.33	0.06	0.52

* 1984–1995 average loading for the surface layer of the Patuxent near Battle Creek (which is the closest monitoring station to the source of our mesocosm water) calculated from Hagy 1996.

tuxent River (Abbe and Sanders 1986) or other Chesapeake Bay tributaries and their watersheds (Nanticoke: J. G. Sanders unpubl.; Rhode River: G. F. Riedel unpubl.). Zinc concentrations in mesocosms were lower than the maximum found in oligohaline portions of the Patuxent during the 1995–1996 monitoring.

Rates of addition of trace elements to mesocosms were chosen, based on previous experiments and other existing information, 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 (Sanders and Riedel 1998). An examination of the wide variety of information available concerning the direct effects of these trace elements on marine organisms (e.g., Forstner and Wittmann 1983; U.S. EPA 1984*a*,*b*,*c*, 1986, 1987) suggested that, with rare exceptions, the concentrations of the elements added to the mesocosms were below the concentrations known to have toxic effects on organisms other than phytoplankton. In general, the most sensitive groups are the primary producers, protozoa, and younger life stages of higher organisms. However, the degree of sensitivity varies enormously both among apparently similar species of the same group and among different groups. Toxicity estimates for Cu and As for marine phytoplankton are closer to the values added to the mesocosms than for the other elements. Given the high concentrations required to directly affect higher trophic level organisms relative to those we used, our presumption that effects of trace elements on growth, production, and densities of organisms in mesocosm tanks would result from effects of trace elements on primary producers seems reasonable. With the exception of Cu, none of the additions exceed Maryland water-quality criteria for estuarine systems (MDE 1993).

Five levels of system complexity were tested, building from simple to successively more complex systems. These complexity treatments were +phytoplankton (the plankton assemblage, including phytoplankton and associated bacterioplankton and microzooplankton in raw Patuxent River estuary water that passed through a 35-μm mesh net), +zooplankton (mesozooplankton and larger microzooplankton added to the +phytoplankton assemblage), +fish (juvenile fish added to the +zooplankton assemblage), +sediment (sediment added to the +fish assemblage) and +benthos (benthic invertebrates added to the +sediment assemblage) (Fig. 1).

Mesozooplankton additions were dominated by calanoid copepods; *Eurytemora affinis* was most abundant in the April run and *Acartia tonsa* dominated the June, July, and September runs. Both of these copepod species feed on both phytoplankton and microzooplankton (Heinle 1966; Heinle and Flemer 1975). Fish added to the +fish, +sediment, and +benthos treatments were juvenile mummichogs (*F. heteroclitus*). Mummichogs are among the most abundant shallow-water fish in Atlantic coast estuaries and are important as both predators and prey in estuarine and salt-marsh food webs (Kneib 1986). They feed on benthic invertebrates, benthic algae, and zooplankton. The four species of benthic invertebrates added to the benthos treatments were the eastern oyster *C. virginica,* the clam *M. balthica,* the sea anemone *D. leucolena,* and in the first mesocosm run only, the polychaete worm *Heteromastus filiformis.* All four species are very common in the Patuxent River and adjacent Chesapeake Bay but each represents a different habitat type and(or) trophic group. The oyster is a key epifaunal species throughout Chesapeake Bay, occurring on and forming hard substrate. It is a suspension-feeder feeding principally on phytoplankton but capable of ingesting other food such as detritus and attached bacteria (Langdon and Newell 1990; Crosby et al. 1990; Baldwin and Newell 1991; Kennedy et al. 1996). *Macoma* is an infaunal species found in both muddy and sandy environments. It is both a suspension-feeder and surface deposit-feeder with its feeding mode dependent on such factors as sediment type (Olafsson 1986) and population density (Olafsson 1989; Lin and Hines 1994). *Diadumene* is an epifaunal species found on hard substrates, including oyster shell. It is a predator, feeding principally on zooplankton but is capable of ingesting larger prey such as small fish and amphipods. Finally the infaunal polychaete, *Heteromastus,* is a subsurface deposit-feeder which feeds at depth within the sediment and moves material to the sediment surface. Because *Heteromastus* had no measurable effect on sediment nutrient flux in preliminary experiments, and because of extremely low local field densities during 1996, we discontinued its use after the first 1996 mesocosm run and do not report data for it in this paper. Collection, handling, and sampling of each group of organisms is described separately below.

Sampling and collection of organisms—The mesocosms were sampled twice weekly for salinity and pH, approximately weekly for nutrients and particulate C and N, and twice per run for trace element concentrations; temperature was measured continuously (Table 1). Filtered samples (Whatman glass-fiber filters GF/F) for dissolved inorganic nutrient analyses (ammonia, nitrate plus nitrite, soluble reactive phosphate, dissolved silicate) were analyzed on a Lachat QuikChem Analyzer (Lachat QuikChem Methods 31-107-06-1-A, 31-107-04-1-A, 31-115-01-3-A, and 31-114-27-1-A, respectively). Filtered samples for dissolved organic C were analyzed with a Shimadzu 5000 TOC analyzer using high temperature oxidation. Particulates retained by the filters were analyzed for particulate C and N (Exeter Analytical, Inc., CE-440 Elemental Analyzer). Cd, Cu, Zn, and Ni were concentrated using APDC/DDDC chloroform extraction (Bruland et al. 1979; Nolting and de Jong 1994) and analyzed by graphite furnace atomic absorption spectrophotometry (AAS) (Cd, Cu, Ni) or flame AAS (Zn). Arsenic was analyzed by hydride generation AAS (Riedel 1993).

Over the course of each run, we measured a number of parameters to ascertain phytoplankton growth, species composition, and productivity. Phytoplankton samples were collected twice weekly, preserved, and counted by inverted microscopy (Utermöhl 1958). Phytoplankton biomass was estimated at the same time each day by in vivo fluorescence, a measure of chlorophyll *a* and, indirectly, biomass (Goldman et al. 1973; D'Elia et al. 1986); during the course of all runs, in vivo fluorescence and measured chlorophyll *a* were significantly correlated ($r^2 = 0.91$, $P = <0.0001$). Chlorophyll *a* was sampled approximately weekly (Parsons et al. 1984). Phytoplankton production, measured as ^{14}C incorpo-

Table 2. Starting density of copepods and starting sizes of fish and bivalves in each mesocosm run. Data are $X \pm$ SE. Means and standard errors calculated from tank means for fish and benthic invertebrates. Sample size: $n = 16$ mesocosm tanks for each mesocosm run for copepods, 12 mesocosm tanks per mesocosm run for fish, and 4 mesocosm tanks per mesocosm run for benthic invertebrates.

	Apr	Jun	Jul	Sep
Copepods eggs (No. liter^{-1})*		21.3 ± 13.3	12.6 ± 9.7	
Copepod nauplii (No. liter^{-1})*		23.1 ± 4.4	169.4 ± 180.8	
Copepodites (No. liter^{-1})*	2.7 ± 0.4	1.0 ± 0.1	3.2 ± 0.3	4.9 ± 0.3
Adult copepods (No. liter^{-1})*	1.4 ± 0.2	4.3 ± 0.3	2.7 ± 0.5	3.6 ± 0.1
Fish wt (g)	0.19 ± 0.001	0.07 ± 0.000	0.15 ± 0.01	0.22 ± 0.001
Macoma area (cm^2)	0.41 ± 0.07	0.50 ± 0.002	0.74 ± 0.01	0.62 ± 0.01
Oyster area (cm^2)	1.83 ± 0.01	1.01 ± 0.01	1.26 ± 0.04	1.74 ± 0.02
Anemone area (cm^2)	0.39 ± 0.01	0.59 ± 0.08	0.37 ± 0.07	0.48 ± 0.09

* Only includes tanks to which zooplankton were added.

ration of samples incubated at saturating light intensities (Strickland and Parsons 1972), was estimated using 100-ml subsamples from each mesocosm approximately weekly. Daily rates (g C m^{-3} d^{-1}) were estimated from the hourly rate, day length, and incident radiation and vertical distributions of light within the mesocosms and assumed a linear increase in fixation rate from low to saturating light levels.

We estimated whole system photosynthesis and respiration, as well as net system metabolism (balance between organic matter production and consumption), approximately weekly in all mesocosms. Photosynthesis and respiration were estimated from consecutive dawn-dusk-dawn oxygen measurements in each mesocosm (YSI model 57) (Odum and Hoskin 1958; Oviatt et al. 1986, 1993). Changes in oxygen concentration due to air–water oxygen diffusion were calculated based on rates of air–water diffusion of SF_6, a non-reactive gas, which was added to the mesocosms in each run (Wanninkhof et al. 1987).

We measured bacterial production using the [^{3}H]leucine uptake method (Kirchman et al. 1985), including an EtOH rinse of the filters. Samples were incubated for 30 min at ambient temperature in the dark, using 50 nM added leucine. Duplicate incubations were done for each sample. Bacterial production was measured in each tank once during the acclimation period of each mesocosm run, and then weekly.

We used epifluorescent microscopy (Sherr and Sherr 1993; Sherr et al. 1993) to enumerate heterotrophic nanoflagellates (HNAN), which are defined here as cells 2–20 μm in diameter with discrete nuclei that were not identifiable as non-flagellated phytoplankton or dinoflagellates and that lacked visible chloroplasts. Samples from each of the 20 mesocosm tanks, plus raw and filtered Patuxent River water, were taken once during the acclimation period of each mesocosm run, and then weekly.

Samples were preserved with gluteraldehyde to a final concentration of 2%, and were stained and filtered within 24 h of fixing. A 5–10-ml fixed sample was filtered onto a 0.8-μm black Nuclepore polycarbonate filter using a vacuum of $<$12.7 cm of Hg; a 0.45-μm Millipore type HA filter was used as a backing filter. Filters were then stained with 1 ml of 0.2-μm filtered citrate phosphate buffer (pH 4.0) followed by 100 μl of 0.2-μm filtered DAPI (10 μg ml^{-1}) stain for 8 min. Dry filters were mounted and frozen until enumeration. For each sample, a total of 100 nanoflagellates, and no fewer than 30 each HNAN and PNAN (phototrophic nanoflagellates) were counted in different areas of the filter. Counting error, estimated by counting the same slide five separate times, was between 14 and 17%.

We collected zooplankton from the Patuxent and Potomac Rivers with repeated short net tows (1–2 min) with 202- and 35-μm mesh nets (for adult plus copepodites and nauplii plus eggs, respectively). Particulates in cod-ends were gently decanted through 1-cm mesh screens to remove medusae and ctenophores into 20-liter carboys partially filled with ambient water. On return to the laboratory, carboy contents were gently added to filtered ambient water in aquaria with very weak aeration. After addition of *Thalassiosira pseudonana* as a food source, aquaria were allowed to sit overnight; dead and injured animals were siphoned off the tank bottoms the following morning. The water from each aquarium was partitioned into equal aliquots using a Wildco Folsom plankton splitter and added to the 16 mesocosms receiving zooplankton (Table 2).

The morning after the addition and at 3, 7, 14, and 21 d thereafter, we sampled zooplankton from each mesocosm. Mesozooplankton (primarily late stage copepodite and adult copepods) were collected by pumping 50 liters from each mesocosm at 730 liters min^{-1} into a submerged 202-μm mesh sieve and preserved in 5% buffered formaldehyde. Hard-bodied microzooplankton, including rotifers and nauplii, were collected by pouring 8 liters of mesocosm water through a submerged 20-μm net, rinsed into a jar containing Lugol's iodine (final concn, 10% vol/vol), and preserved by adding 5% buffered formaldehyde. In this paper we discuss data on adult copepods and copepodites for all four mesocosm runs; data on eggs and nauplii are available only for the June and July mesocosm runs. Copepod life stages were analyzed separately because of differences among life stages in their use of different sizes and taxa of prey (Berggreen et al. 1988). Stressor-related shifts in phytoplankton size and taxonomic distribution could thus affect factors such as stage duration and egg production as well as total density.

We collected juvenile mummichogs (*F. heteroclitus*) with 3–5-mm mesh beach seines and pole nets from salt marshes and shallow embayments of the Patuxent River. Fish were held in 75-liter aquaria at ambient temperatures and fed brine

shrimp (*Artemia* sp.) nauplii and Tetra Min flake food for 1–6 d before measuring them for experiments. For the June and July runs, we spawned field-collected adults in the laboratory and reared larvae and juveniles on brine shrimp nauplii, flake food, and benthic invertebrates. Benthic algae were also available to fish on the bottoms and walls of the aquaria. Twenty-four hours before placing fish in mesocosms, we measured standard length (SL) and total length (TL) to the nearest 0.5 mm and wet weight to the nearest 0.01 g. Of the 120–130 fish measured for each mesocosm run, we selected the 96 that were most similar in weight to each other and to those used in other runs (Table 2). Fish were divided into eight groups based on weight, and one fish from each group was randomly assigned to each of the 12 mesocosms receiving fish, yielding an experimental density of eight fish per tank. This density was sufficient to allow fish growth rate in each tank to be based on several fish but avoid complete elimination of fish prey. Fish were released into appropriate mesocosms after the initial zooplankton samples were taken in mesocosm tanks.

At the end of each experimental run, we removed all fish from mesocosms, immediately weighed and measured them, and preserved the fish in 50% reagent alcohol. Contents of the stomach and first half of the intestine were examined for four fish from each mesocosm tank. Zooplankton and benthic invertebrates were identified to broad taxonomic categories and counted. The quantities of sediment and benthic algae in fish guts, as well as gut fullness, were ranked on a qualitative scale.

Sediments were included in some tanks containing fish and in all tanks that contained benthic invertebrates in order to increase the complexity of these treatments and to provide suitable habitat for clams (*see below*). The +sediment treatment also served as a control for the sediment effects within the +benthos treatment. The sediments used were sandy muds collected from a depth of 3–5 m near the mouth of St. Leonard's Creek, a tributary of the Patuxent River. Sediments were collected using a nonmetallic box corer which sampled a 15- × 15-cm area to a depth of 10–15 cm. Individual cores were transferred intact to plastic containers and transported to the laboratory. In the laboratory, cores were immersed in filtered seawater and heated to at least 50°C for 5–7 d to kill macrofauna. After heat treatment, the cores were transferred intact to cylindrical PVC trays that were 50 cm in diameter and 15 cm deep. Approximately nine cores were added to each tray and the trays held in laboratory tanks with flowthrough filtered seawater until they were placed in the mesocosms. The size of sediment trays in mesocosms yielded a sediment area : water column volume similar to that in a 4-m depth water column in the estuary. The sediment volume used was intended to be sufficiently large that sediment would influence the systems but not release nutrients in amounts that would overwhelm the effects of the nutrient addition treatments. In addition to providing habitat for benthic invertebrates and algae, sediments released nutrients into tanks. Thus, the +sediment tanks and +benthos tanks in each treatment had higher baseline nutrient loadings than did other treatments, but within each of the +sediment and +benthos complexity treatments, the difference between +nutrient and no-nutrient addition tanks was similar to that in other complexity treatments.

Oysters were purchased from a local hatchery and averaged 12–17 mm long at the start of each experiment. Five individual oysters were glued to each PVC panel and 10 panels were randomly assigned to each mesocosm tank. Prior to deployment each panel was videotaped to record the size (Table 2) and location of each oyster. The oyster panels were suspended vertically in the mesocosms.

Approximately 100 *Macoma* were added to the sediment tray in each of the four benthos treatments. These organisms were collected from St. Leonard's Creek and the Patuxent River 1–2 weeks before the beginning of each experiment. After collection, the clams were sorted into 2–3 size groups, and equal numbers from each group were assigned to each treatment. Before deployment, the clams were videotaped to record their sizes (Table 2).

Diadumene was collected from oyster shells dredged from nearby oyster beds in the Patuxent River. Individual anemones were gently detached from the shell and then placed in a small container with 100-cm^2 PVC panels to which they reattached. The number of anemones on individual panels ranged from 1 to 20. Four panels were suspended in each +benthos mesocosm with panels chosen so that the total number of anemones was similar in each treatment; a total of 14–20 individual anemones was placed in each tank. Before deployment the panels were removed from water and each anemone was gently prodded so that it contracted to its minimum size. The panels were then videotaped to record the size (Table 2) and location of each individual.

All benthic organisms were collected at the end of each experiment, counted, and re-measured by videotaping them and then analyzing their images using a computer image analysis system. Length (maximum diameter), width (minimum diameter), and cross-sectional area were measured for each individual. For oysters and anemones it was possible to identify each individual and compare its initial and final size to get a direct measure of growth. This was not possible for *Macoma* and only initial and final population means and size distributions could be compared. In general, we used area as the primary statistic of growth since it integrated growth in all directions.

Statistical analyses—Analysis of variance (SAS version 6.12: GLM) was used to examine direct and interactive effects of nutrient additions (N), trace element additions (T), and system complexity (C). For analyses including all four 1996 mesocosm runs (mesorun), we used the model: response variable = mesorun + N + T + C + (N×T) + (N×C) + (T×C) + (N×T×C). For analyses of individual mesocosm runs we dropped the 3-way interaction term (because of lack of replication for this term within mesocosm runs) and mesorun variable. Full models were simplified by successively dropping interaction terms with the highest $P \geq 0.25$ until the overall model r^2 and F statistics for the main effects were no longer improved by the simplification. Main effects were always retained in the model. All statistical results for ANOVAs are for type 3 sums of squares except as described below. Levine's test, based on full ANOVA models for tests including all four mesocosm runs, and

Table 3. Regression coefficients (r^2) for comparisons of measures of abundance and production in mesocosm experiments. Dash: regression not considered biologically meaningful; ns: $P \geq 0.05$; low: $r^2 < 0.10$, but $P < 0.05$. In addition to regressions indicated in the table, bacterial production was compared to dissolved nutrient concentrations (N and P) and to dissolved organic carbon. A minus sign indicates a negative relationship between variables. Phytoplankton cell size: small—3–6-μm phytoplankton; med—6–20-μm phytoplankton; large–>20-μm phytoplankton. Integrative measures of phytoplankton biomass and production: ivf—*in vivo* fluorescence; Chl *a*—chlorophyll *a*; PP—phytoplankton production; PC—particulate C; PN—particulate N. Whole system and higher trophic level parameters: wsP—whole system photosynthesis; BP—bacterial production; HNAN—heterotrophic nanoflagellates; cop—copepods; chir—chironomids; temp—temperature. Other abbreviations: cop45—stage 4–5 copepodites; cop13—stage 1–3 copepodites; ws-resp—whole system respiration.

	Cell size			Integrative measures					Whole system parameters					
	Small	Medium	Large	ivf	Chl *a*	PP	PC	PN	wsP	BP	HNAN	cop	chir	temp
BP ($n = 80$)	ns	ns	0.83	0.44	0.43	0.61	0.38	0.25	0.50	—	0.13	—	—	0.29
HNAN ($n = 80$)	0.10	0.13	ns	0.26	0.17	0.14	0.38	0.35	low	0.13	—	ns	—	ns
Adult cop ($n = 16$)	−0.33	−0.26	0.47	ns	ns	ns	—	—	—	—	ns	—	—	0.67
Cop45 ($n = 16$)	ns	ns	0.60	ns	ns	ns	—	—	—	—	ns	—	—	0.44
Cop13 ($n = 16$)	ns	0.37	ns	0.37	0.37	ns	—	—	—	—	ns	—	—	ns
Cop nauplii ($n = 8$)	ns	ns	ns	0.76	0.60	ns	—	—	—	—	ns	—	—	ns
Cop eggs ($n = 8$)	ns	ns	0.90	ns	ns	ns	—	—	0.91	—	ns	—	—	ns
Fish ($n = 48, 12$)*	—	—	—	—	—	—	—	—	—	—	—	0.67	0.84	0.42
Macoma ($n = 16$)	ns	0.25	ns	0.58	0.56	0.36	0.44	0.72	ns	—	0.52	—	—	ns
Oyster ($n = 16$)	−0.41	−0.58	0.49	ns	ns	ns	ns	ns	ns	—	ns	—	—	0.86
Anemone ($n = 16$)	—	—	—	—	—	—	—	—	—	—	—	ns	—	ns
ws-resp ($n = 80$)	−0.27	−0.41	−0.57	−0.72	−0.61	−0.57	−0.52	−0.53	−0.66	−0.68	−0.20	ns	−0.38	ns

* For regressions of fish growth, $n = 48$ for comparisons with plankton, $n = 12$ for comparisons with benthos.

main effects only for individual runs, was used to test for heteroscedasticity. Data were log-transformed to equalize variances and to improve deviations from normality where needed. In data sets where log-transformed data still violated assumptions of homogeneity of variance or substantially deviated from normality, we rank-transformed data and performed ANOVAs on the ranks (Potvin and Roff 1993). Where complexity effects were strong, analyses of rank-transformed data sometimes allowed a clearer determination of the presence of stressor effects. Instances where statistics were performed on rank-transformed data are reported in the text.

We used regression analyses followed by inclusion of significant regression variables in ANOVA models in order to identify potentially important food-web linkages and then tested for nutrient and trace element effects on higher trophic levels after removing variation due to predators or prey from the model. Linear regression analyses of log-transformed data were used to examine the relationships between individual response variables (e.g., production, growth, or densities of individual taxa) and the production, growth, and(or) abundance of their predators and prey as well as mean mesocosm run temperature. The matrix of regression tests performed and r^2 values for significant (i.e., $P \leq 0.05$) regressions are given in Table 3. All regressions discussed in the results were significant at $P \leq 0.05$ and had r^2 values ≥ 0.10. To examine relationships between copepod densities and factors other than fish predation that might have influenced copepod abundance, we performed regressions on data from +copepod tanks separately ($n = 16$ for adults and copepodites; $n = 8$ for eggs and nauplii). This was necessary because of the extremely strong effect of fish predation on copepod densities. All other regressions included all tanks in which a particular type of organism was present. Prey types were analyzed separately because of strong correlations among prey or between several prey types and another variable (e.g., in vivo fluorescence or temperature). Prey measures yielding the highest r^2 values were included as continuous variables in ANOVA models; type 1 sums of squares were inspected to determine whether inclusion of regression variables altered the significance of the stressor classification variables.

Data are presented as mean ± 1 SE. Unless otherwise noted, all growth analyses for fish and benthic invertebrates were done on tank averages in order to avoid problems of non-independence of samples. For plankton, treatment effects were tested on mean densities or production data from all sample dates excluding pretreatment period ''initials.'' No significant differences among stressor treatments were found for initial sizes or densities of any organisms used in these studies except that sea anemones were slightly larger in nutrient addition tanks than in tanks with no added nutrients.

Results

Nutrients—The mesocosms appeared to be P limited in spring and early summer (April and June runs) and N limited in mid–late summer (July and September runs), based on the N : P ratio of external inputs and the N : P ratios of dissolved nutrients within the mesocosms relative to the theoretical ratio of 16 : 1 required for phytoplankton growth (Table 1). For example, during the April and June runs, the inorganic N : P ratios of inputs to all mesocosms ranged from 25 to 102 and the N : P of inorganic nutrient concentrations within mesocosms ranged from 98 to 677, both strongly indicating P limitation. During the July and September runs, the N : P input ratio was close to 16 : 1, although the N : P of inorganic nutrients in the mesocosm water was very low (range 2–4)

Table 4. Average changes in algal abundance and production in response to (A) nutrient and (B) trace element additions. Only changes ≥10% and ≥0.1 × 10^6 cells liter^{-1} are shown. Data are calculated from averages of all tanks with vs. without nutrients added (n = 10,10) and with vs. without trace elements added (n = 10,10) without regard to trophic complexity or the presence of the other stressor (experimental design explained in methods). Taxa in bold italics showed a significant ($P < 0.05$) change in density in analyses of all mesocosm runs combined; parentheses indicate $0.10 > P > 0.05$. Changes in abundance shown in bold italics indicate significant ($P < 0.05$) nutrient or trace element effects for that mesocosm run; parentheses indicate $0.1 > P > 0.05$. Abbreviations and units: nd—no data; avg chn—average changes in cell density (×10^6 cells liter^{-1}) for algal taxa; machine units for *in vivo* fluorescence; μg liter^{-1} for chlorophyll *a*; g C m^{-3} d^{-1} for phytoplankton production (as carbon fixation) and whole system photosynthesis.

Taxa	April % chn, avg chn	June % chn, avg chn	July % chn, avg chn	September % chn, avg chn
A. Change in algal abundance and production with addition of nutrients				
Centric diatoms	(+131, 7.0)	***+185, 4.5***	***+134, 9.2***	***+971, 5.1***
Pennate diatoms	***+84, 0.3***	***+69, 0.1***	***+106, 0.1***	***+415, 0.4***
Dinoflagellates		+171, 0.7		
Chlorophytes	***+58, 2.9***	***+1,466, 5.7***		***+229, 0.1***
(Chrysophytes)				
Cryptophytes	+84, 1.6	***+61, 0.3***		+170, 0.1
(Cyanophytes)				***+42, 0.2***
Prasinophytes	***+104, 0.9***			
Others		***+52, 1.2***	***+67, 0.7***	***+92, 0.9***
Total cell density	***+84, 12.9***	***+205, 12.5***	***+120, 10.1***	***+314, 6.9***
Integrative measures				
In vivo fluoresc.	***+69, 75***	***+200, 130***	***+141, 131***	***+213, 81***
Chlorophyll a	***+69, 10.5***	***+255, 30.3***	***+167, 17.4***	***+388, 22.5***
Phytoplankton productivity	***+88, 0.38***	***+321, 1.55***	***+129, 0.86***	***+246, 0.62***
Whole system photo.	nd	***+262, 0.61***	***+100, 0.67***	***+79, 0.37***
B. Change in algal abundance and production with addition of trace elements				
Centric diatoms		***−65, −4.6***	+62, 5.5	
Pennate diatoms	***−10, −0.1***	+90, 0.1	+72, 0.1	+69, 0.2
Dinoflagellates				
Chlorophytes	+22, 1.3	***+1,705, 5.8***		
Chrysophytes				
Cryptophytes	***+154, 2.4***	***−34, −0.2***		
Cyanophytes			***+1,602, 0.1***	−18, −0.1
Prasinophytes	***−14, −0.2***			
Others	+17, 0.3	−10, −0.3	+57, 0.6	
(Total cell density)	+21, 4.2	***+7, 0.8***	+61, 6.3	+2, 0.1
Integrative measures				
In vivo fluoresc.	+1, 2	***−20, −29***	***+49, 62***	−6, −5
Chlorophyll *a*	−4, −1.0	***−30, −9.4***	***−20, 3.5***	−8, −1.4
(Phytoplankton productivity)	−10, −0.06	***−46, −0.74***	+15, 0.16	−9, −0.05
Whole system photo.	nd	***−24, −0.14***	***−13, −0.13***	+7, 0.04

implying strong N limitation. Nutrient loadings achieved in the nutrient addition mesocosm tanks ranged from 1.4 to 1.8× of control tanks for N and 1.8 to 3.5× of control tanks for P. Variation among runs in ratios of treatment : control nutrient loading reflected the seasonal variation in ambient nutrient concentrations in Patuxent River water.

Phytoplankton—The most invariant response of phytoplankton to both a 2-fold increase in nutrient (N and P) loadings and to a 2–5-fold increase in trace element (Cu, As, Cd, Ni, Zn) loadings was an increase in total cell density (Table 4). However, the response to trace elements was far more variable both temporally and among taxa than was the response to nutrient additions.

Total cell densities significantly increased in response to nutrient additions in all four mesocosm runs, although the magnitude of this response to nutrient additions varied from 84% in April to >300% in September (Table 4). In addition, all abundant taxa whose cell densities changed measurably (we used a change of ≥10% and ≥0.1 × 10^6 cells liter^{-1} as the criterion) increased in response to nutrients, and most of these changes were statistically significant (i.e., $P < 0.05$ for the main effect of nutrients). Nutrient additions also resulted in consistent increases in integrative measures of biomass and production of primary producers [in vivo fluorescence (Fig. 2), chlorophyll *a*, ^{14}C phytoplankton production (referred to as phytoplankton production in this paper), and whole system photosynthesis]. In general, the magnitude of the increase in total cell densities was quite similar to the magnitude of the increases seen in the integrative measures (e.g., 69–88% in April, 200–nearly 400% in June–September). The response to nutrient additions was generally weak-

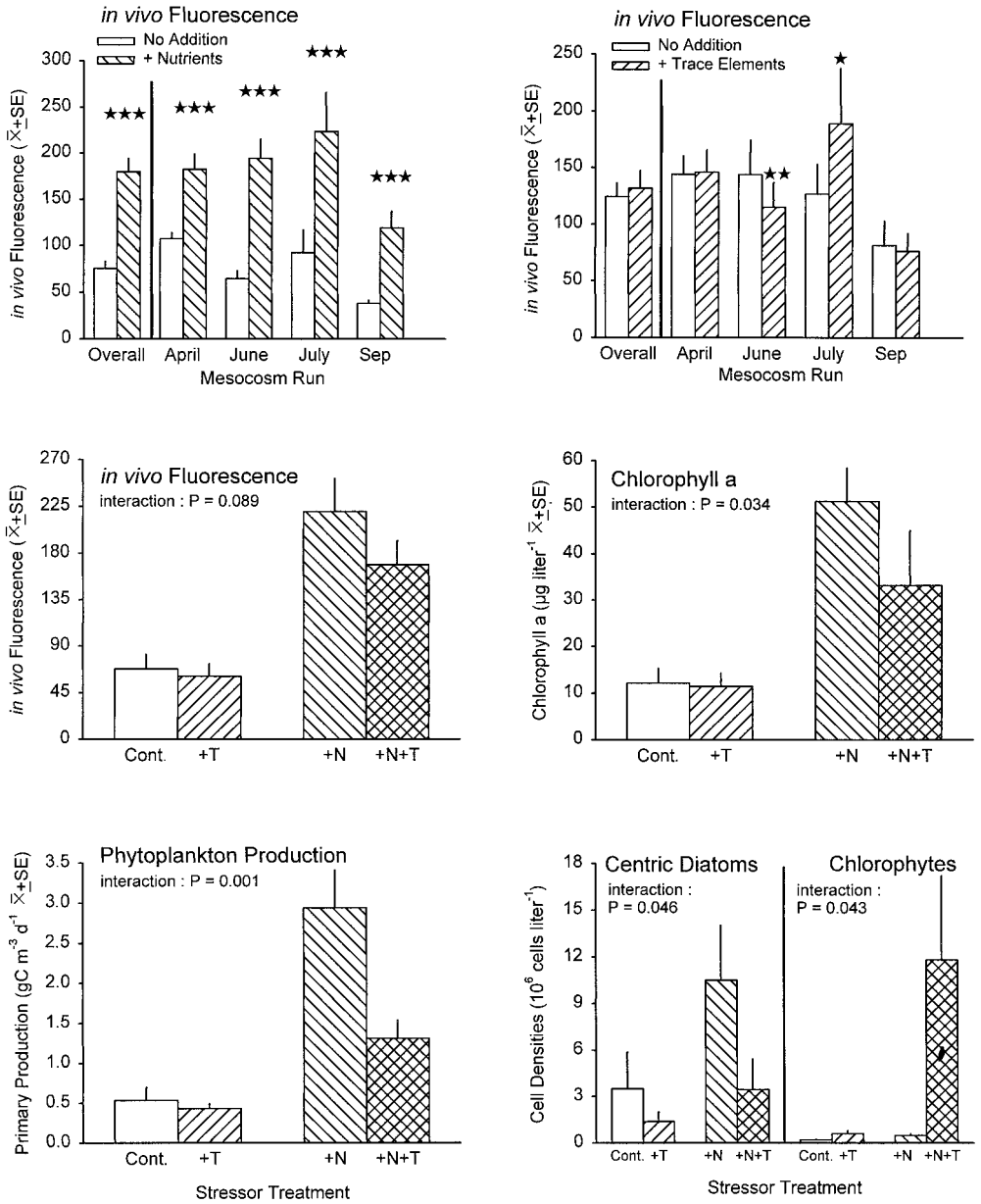

Fig. 2. Phytoplankton responses to nutrient and trace element additions. The upper panels illustrate effects of nutrients (left) and trace element additions (right) on in vivo fluorescence. (*—$P \leq 0.05$; **—$P \leq 0.01$; ***—$P \leq 0.001$; no symbol above bar indicates $P > 0.10$.) The lower panels illustrate responses to stressor treatments during the June 1996 mesocosm run. Cont.—control tanks with no stressors added; +T—trace element addition tanks; +N—nutrient addition tanks; +N+T—tanks to which both nutrients and trace elements are added. All complexity levels are included in analyses.

er during the April mesocosm run than during the June, July, or September runs. Whole system photosynthesis averaged 0.73 g C m^{-3} d^{-1} across all tanks and treatments during the June–September runs (measurements were not available for the April run), and averaged about twice as high in tanks with added nutrients as in tanks without nutrients added (1.00 ± 0.08 vs. 0.45 ± 0.06 g C m^{-3} d^{-1}).

The response of phytoplankton to trace elements was far more variable both temporally and among taxa compared to the response to nutrient additions. Trace element additions tended to increase total phytoplankton cell densities in all runs, although this increase was significant only in the July mesocosm run (Table 4). In contrast, trace element additions resulted in a mix of positive and negative responses among mesocosm runs for most individual algal taxa, as well as a mix of positive and negative responses by the various algal taxa within mesocosm runs (Table 4). Complementary responses occurred in the April, June, and September runs (i.e., some taxa decreased while others increased). Integrative measures of phytoplankton response tended to be reduced

by trace element additions except in the July run and were not in general agreement with the increases in total cell density. For example, during the June mesocosm run in vivo fluorescence, chlorophyll *a,* phytoplankton production, and whole system photosynthesis were all significantly reduced by 20–49% in trace element addition tanks, but total cell density increased by 7% and chlorophytes increased by more than three orders of magnitude in those same tanks. The increased density of small-celled chlorophytes was balanced, however, by a complementary decrease in densities of larger centric diatoms (Table 4, Fig. 2).

There was a strong nutrient×trace element interaction in phytoplankton responses during the June mesocosm run (i.e., effects of trace elements and nutrients were not additive). For many of the integrative measures, trace element additions in the June run had little or no effect in the absence of added nutrients but greatly decreased the response of phytoplankton to nutrient additions (Fig. 2). Much of the decrease due to trace elements, increase due to nutrients, and trend toward a nutrient×trace element interaction during the June run reflected changes in the density of *Rhizosolenia fragilissima,* a large (30 μm), bloom-forming diatom common in the mesohaline Patuxent River and Chesapeake Bay, and which is particularly sensitive to arsenic (Sanders and Cibik 1985). Main effects of nutrients and trace elements on *R. fragilissima* densities were both significant (both $P < 0.02$). In addition, densities of *R. fragilissima* differed between trace element addition and non-addition tanks by a factor of 2 without added nutrients, but by a factor of nearly 4 with nutrient additions; *R. fragilissima* densities averaged $1.4 \pm 0.7 \times 10^6$ cells liter^{-1} in control tanks, $0.7 \pm 0.3 \times 10^6$ cells liter^{-1} in tanks with trace elements but no nutrients added, $9.7 \pm 3.4 \times 10^6$ cells liter^{-1} with added nutrients but no trace elements, and $2.6 \pm 1.5 \times 10^6$ cells liter^{-1} in tanks with both stressors added (interaction $P = 0.06$). *R. fragilissima* was also the numerically dominant centric diatom during the July mesocosm run but showed no consistent response to trace elements during that run ($P = 0.30$). In contrast to the June run, in vivo fluorescence, chlorophyll *a,* and cyanophyte densities increased in July trace element addition tanks.

Pennate diatoms and prasinophytes decreased in response to trace element additions and also showed a significant nutrient×trace element interaction during the April mesocosm run. For pennate diatoms, the nutrient×trace element interaction followed the same pattern as that for centric diatoms in the June run (i.e., trace elements reduced pennate densities in the nutrient addition treatments). In contrast, prasinophytes were most strongly affected by trace element additions in tanks without added nutrients. In both cases, the magnitude of the negative trace element effect was small, averaging $0.1–0.2 \times 10^6$ cells liter^{-1}, and countered by a significant increase in cryptophytes in trace element addition tanks. As a consequence, neither the direct effect of trace elements nor the trace element×nutrient interaction were reflected in integrative measures of phytoplankton biomass and production. *R. fragilissima* was absent or rare in mesocosm tanks during the April mesocosm run.

Bacterioplankton—Bacterial production averaged 0.16 ± 0.013 g C m^{-3} d^{-1} across all tanks and treatments during 1996, and ranged from 0.02 to 0.75 g C m^{-3} d^{-1}. During the same period (April through October 1996), bacterioplankton production at the nearby Patuxent River station XDE5339 (Maryland Water Quality Monitoring Program) averaged 0.14 ± 0.06 g C m^{-3} d^{-1} ($n = 11$) (Gilmour unpubl. data).

Nutrient additions significantly increased bacterial production in analyses of all four mesocosm runs combined and in each mesocosm run when mesocosm runs were analyzed individually (Fig. 3). On average, bacterial production was twice as high in tanks with added nutrients compared to tanks without nutrients added (0.11 ± 0.011 vs. 0.22 ± 0.019 g C m^{-3} d^{-1}); tanks with and without added nutrients bracketed production measured in the Patuxent River. The bacterial response to nutrient additions was weakest during the April mesocosm run when bacterial production increased by only 30% compared to a 85–165% increase in the other three mesocosm runs. The percent increase in bacterial production in response to nutrient additions ranged from about one-third to two-thirds that of the increase in phytoplankton production.

There were no significant overall effects of trace elements on bacterial production. However, bacterial production was significantly lower (by ~30%) in trace element addition tanks in the June run (Fig. 3). There was also a significant non-additive nutrient×trace element interaction during the June run; as with several phytoplankton measures, trace element additions reduced bacterial production only in tanks with added nutrients (Fig. 3).

Regressions and further analyses indicated that stressors affected bacterial production directly as well as through trophic interactions. Bacterial production was positively related to all integrative measures of total phytoplankton biomass (chlorophyll *a,* particulate N and C, in vivo fluorescence), the density of large phytoplankton cells, phytoplankton production (Fig. 3), and whole system photosynthesis (Table 3), but not to dissolved nutrient concentrations. The correlation between bacterioplankton production and dissolved organic carbon was very weak ($r^2 = 0.08$, $P = 0.01$). These relationships support the paradigm of rapid release and regeneration of nutrients through the microbial loop. However, nutrient additions to tanks also affected bacterial production directly. Inclusion of phytoplankton production in ANOVAs indicated a significant direct effect of nutrient additions on bacterial production, in addition to the effect of nutrients on bacterial production through phytoplankton production, in two of four experiments (April: $P < 0.04$; and June: $P < 0.02$).

Bacterial production also varied significantly among mesocosm runs ($P = 0.0001$) and with the trophic complexity of the tanks ($P = 0.001$). Complexity differences were generally driven by higher bacterial production in tanks containing sediment. This was true even before nutrients and trace elements were added, presumably due to increased nutrient loadings to these tanks via sediment efflux. Rates of bacterial production generally followed temperature (Table 3); average bacterial production was lowest during the April mesocosm run (0.088 ± 0.004 g C m^{-3} d^{-1}) and highest

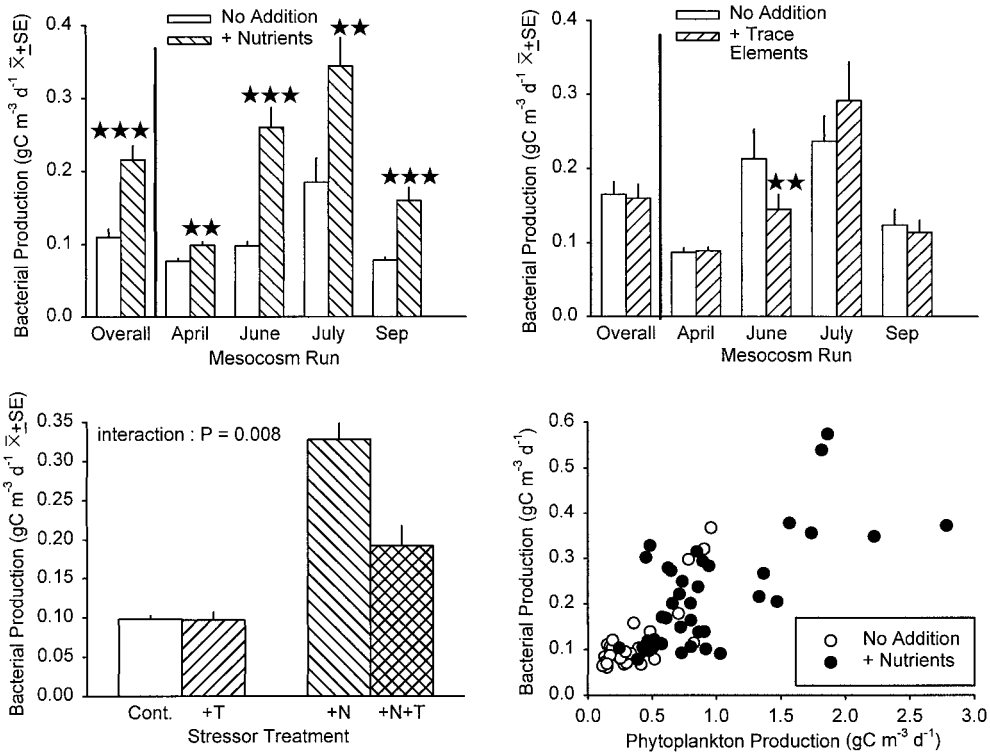

Fig. 3. Bacterioplankton responses to nutrient and trace element additions, and relationship between bacterial production and phytoplankton production. Lower left panel illustrates the effect of stressor treatments on bacterial production during the June 1996 mesocosm run. Symbols as in Fig. 2.

during the July run (0.26 ± 0.03 g C m^{-3} d^{-1}) when average water temperatures were 12.9°C and 26.1°C, respectively.

Heterotrophic nanoflagellates—Heterotrophic nanoflagellate (HNAN) densities in the mesocosms averaged 2,926 ± 138 cells ml^{-1} across all runs and treatments and ranged from about 500 to 15,000 cells ml^{-1}. The range of HNAN densities in mesocosm tanks was similar to that measured in the Chesapeake outflow plume (McManus and Fuhrman 1990) and in the nearby Rhode River estuary (Dolan and Gallegos 1991). Densities were similar (~3,200 cells ml^{-1}) in all runs except September, when density averaged about 1,900 cells ml^{-1}.

HNAN densities were significantly higher in nutrient addition tanks than in tanks without added nutrients across all four mesocosm runs (Fig. 4), in July and September runs, and on individual dates toward the end of the April and June runs. Mean densities increased by between 20 and 50% with nutrient additions, which was considerably lower than the phytoplankton production response to nutrients. The HNAN response was especially muted relative to the phytoplankton response in the June and July runs.

Trace element additions significantly affected average HNAN densities in the April and June mesocosm runs (Fig. 4) and on individual dates within the July and September runs. In the June, July, and September runs, densities were 10–35% lower in tanks with trace element additions. In contrast, HNAN counts averaged about 25% higher in tanks with added trace elements than in those without trace element additions in the April run; an April increase was also seen in densities of copepods, which can be significant predators of the larger celled flagellates (Fig. 5). A significant interaction between the effects of nutrients and trace elements was seen in the June mesocosm run (Fig. 4). Similar to the pattern seen for other taxa, but perhaps more striking, addition of trace elements had no affect alone but totally eliminated the HNAN response to nutrient enrichment in the June run.

Additional analyses indicated the potential for both trophic and direct effects of stressors. HNAN densities generally increased with increasing phytoplankton biomass and production, bacterial production, and particulate N and C (Table 3, Fig. 4). HNAN densities were also significantly lower in +copepod tanks, which had the highest densities of predatory copepods. Inclusion in ANOVA models of integrative measures of phytoplankton and particulates that yielded significant regressions indicated that nutrient effects are likely indirect (i.e., through trophic interactions as would be expected), but that trace elements may directly affect HNAN densities. Nutrients had no significant effect on HNAN densities when particulates or phytoplankton parameters were included (all $P \geq 0.40$) but effects of trace elements or trace element×nutrient interactions remained significant in the April and June mesocosm runs.

Copepods—Greater than 99% of the planktonic copepods added to mesocosm tanks were *E. affinis* in the April me-

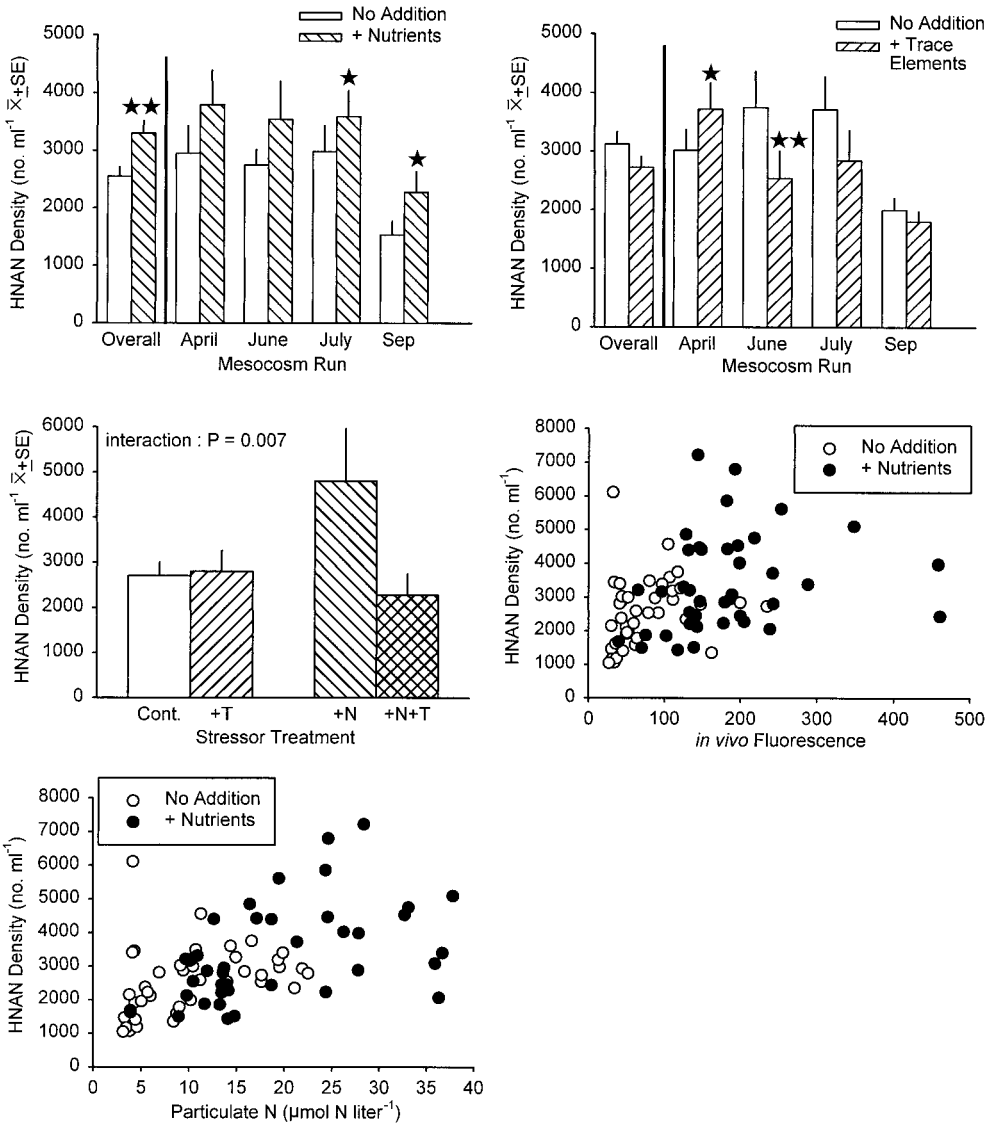

Fig. 4. Heterotrophic nanoflagellate density responses to nutrient and trace element additions, and the relationship between HNAN and in vivo fluorescence and particulate N. Center left panel illustrates the effect of stressor treatments on HNAN densities during the June 1996 mesocosm run. Symbols as in Fig. 2. Trace element effects were also significant during peak densities in the July and September runs.

socosm run, and *A. tonsa* in the other three runs, reflecting seasonal variation in zooplankton assemblages in mesohaline portions of Chesapeake Bay and its tributaries. Initial densities of older copepod stages (adults + copepodites) did not vary among stressor or organism treatments, but did vary among experimental runs ($P = 0.0001$, Table 2). Averaged across all 1996 runs, initial densities were 5.9 ± 0.3 copepods liter^{-1} in mesocosm tanks to which copepods were added and 0.4 ± 0.2 individuals liter^{-1} in +phytoplankton tanks.

Both adult and copepodite densities were significantly higher in nutrient addition tanks than in tanks without added nutrients in analyses including all four mesocosm runs and all 16 mesocosm tanks in each run to which copepods had been added. Densities of adults and copepodites averaged about twice as high in nutrient addition tanks as in tanks without added nutrients. However, the magnitude and statistical significance of nutrient effects varied among mesocosm runs and life-history stages (Figs. 5 and 6). Adult, copepodite, and egg densities were significantly higher in nutrient addition tanks than in non-nutrient tanks during both summer mesocosm runs (Fig 5; $P = 0.004$ and 0.008 for egg densities), while the effect of nutrients on nauplii densities was significant only in the July run ($P = 0.003$; data not shown). Densities of all stages (including eggs and nauplii) tended to increase by 50–>100% with nutrient additions during June and July (Figs. 5 and 6). ANOVAs of individual copepod stages were run on data that were rank-transformed within mesocosm run and complexity treatment.

The direction as well as the magnitude of copepod responses to trace element additions varied among mesocosm

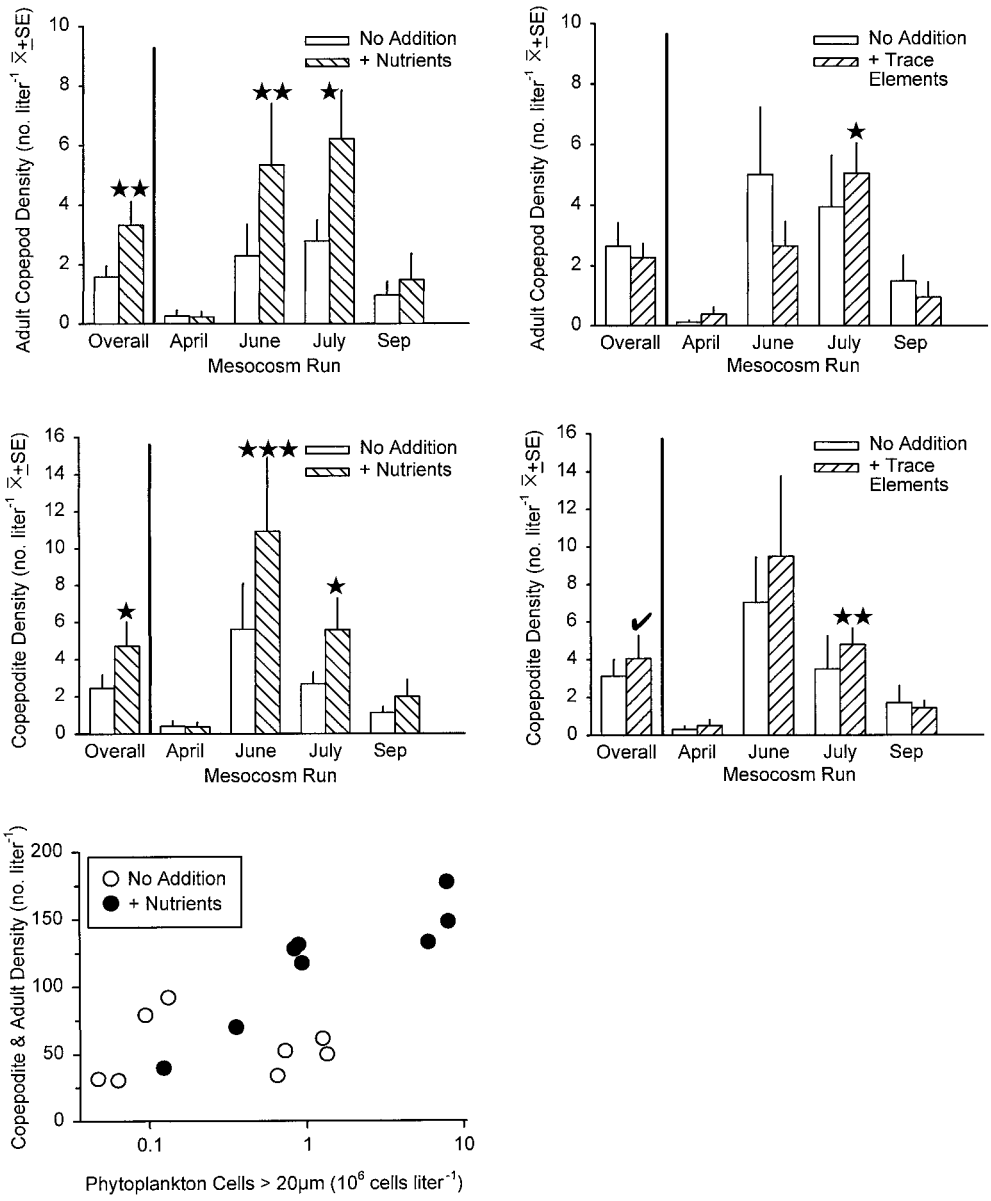

Fig. 5. Adult copepod and copepodite responses to nutrient and trace element additions. Also shown are the relationships between copepod densities and large phytoplankton cells in the +copepod tanks. Symbols as in Fig. 2. Checkmark: $0.10 < P < 0.05$.

runs and copepod stages (Figs. 5 and 6) and only the responses in the July mesocosm run were statistically significant. During that time, adult, copepodite, and egg densities were all significantly greater in trace element addition tanks than in tanks without added trace elements. In contrast, adult, nauplii, and egg densities tended to be lower (but not significantly so) in trace element tanks during the June run (Figs. 5 and 6) when phytoplankton generally declined or showed significant nutrient×trace element effects (Table 4). Both adult copepods and copepod egg densities also tended to follow the same pattern as many other components of the food web during the June run, with control, trace metal addition, and nutrient+trace metal additions being similar and lower than mean densities in the nutrient-only addition tanks (Fig. 6). The nutrient×trace element interaction was significant for adults only on the date with peak copepod densities. Copepodites showed the opposite pattern with highest densities in the +nutrient+trace metal tanks. However the trace element×nutrient interaction was not significant for any copepod stage and primarily reflected the pattern in +copepod tanks, the complexity treatment where all copepod stages were most abundant. Neither adults nor copepodites were significantly affected by trace element additions when all four mesocosm runs were combined, but copepodite densities tended to be higher in trace element addition tanks than in tanks without added trace elements ($P = 0.10$).

Copepod densities in mesocosm tanks were reduced by fish predation ($P < 0.05$) and were positively related to the

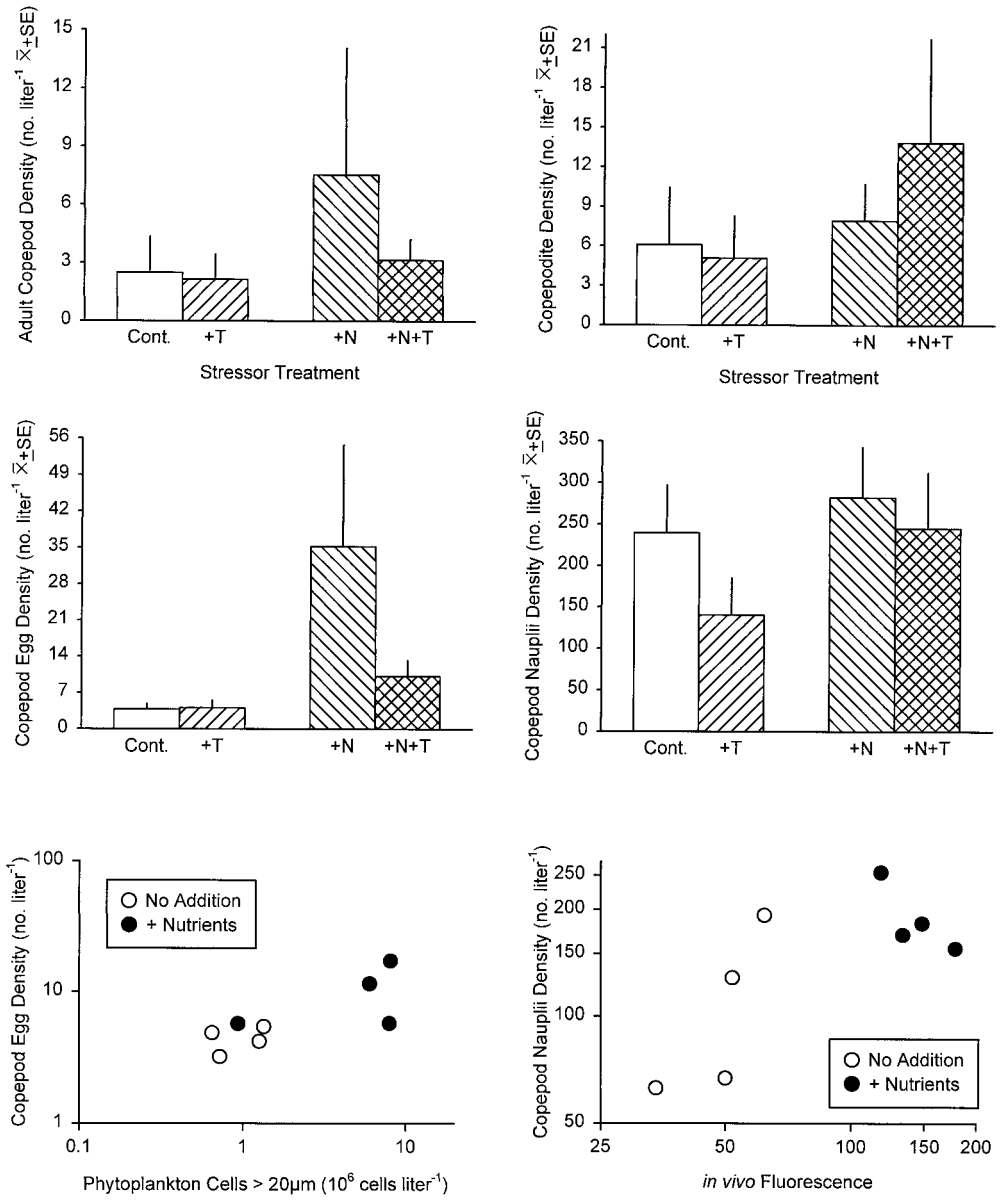

Fig. 6. Effects of nutrient and trace element treatments on all copepod stages during the June mesocosm run (upper four panels; all $P > 0.10$ when averaged across all dates and complexity treatments), and relationship between egg and nauplii stages of copepods and the density of large phytoplankton cells in +copepod tanks during the June and July mesocosm runs (lower two panels).

abundance of prey, such that prey abundance rather than direct stressor effects explained significant variation in copepod densities when included in ANOVA models. Averaged across all mesocosm runs, densities were 6.1 ± 1.1 adult copepods liter^{-1} and 9.2 ± 2.2 copepodites liter^{-1} in tanks without fish or benthic invertebrates compared with 1.4 ± 0.2 adults liter^{-1} and 2.0 ± 0.3 copepodites liter^{-1} in tanks with fish (i.e., +fish, +sediment, and +benthos tanks). Correlations between copepod abundances and phytoplankton varied among stages (Table 3, Figs. 5 and 6). Adult copepod densities were positively correlated with the densities of large phytoplankton cells and temperature and negatively correlated with medium and small phytoplankton densities. Stage 4–5 copepodites were also positively correlated with densities of large phytoplankton cells and temperature. In contrast to older copepods, stage 1–3 copepodite and nauplii densities were positively correlated with measures of total phytoplankton biomass (chlorophyll *a* and mean treatment in vivo fluorescence) but not with large cell densities or temperature. Stage 1–3 copepodites were also positively related to densities of medium-sized phytoplankton cells. Egg densities were strongly correlated with large phytoplankton cell densities and whole system photosynthesis. Adult copepod and egg densities were positively correlated with *R. fragilissima* densities across organism treatment in the June mesocosm run, but this correlation was not significant in the

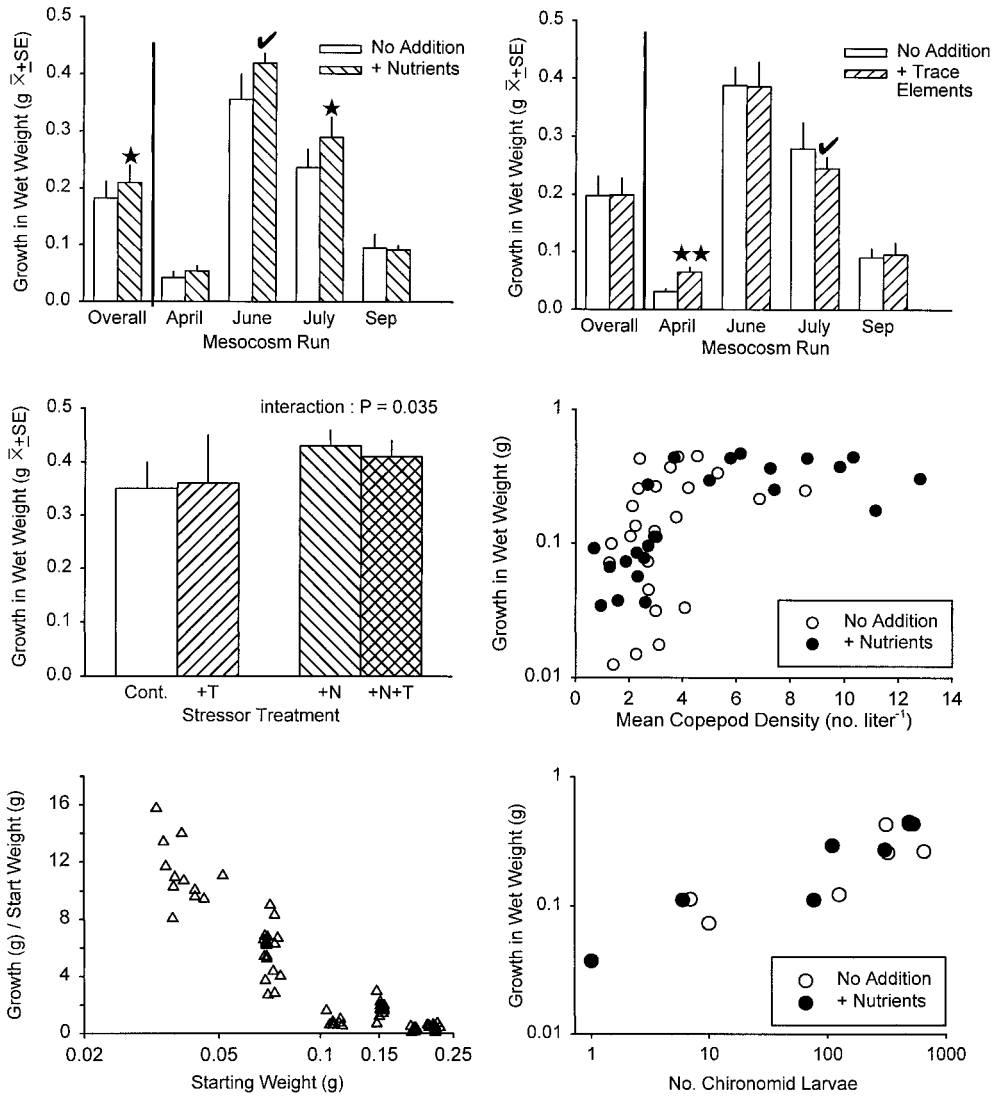

Fig. 7. Growth of fish in response to nutrient and trace element additions (upper two panels), stressor effects during the June mesocosm run (center left panel), and relationship between copepod density, starting weight, and total number of chironomid larvae in sediment trays and fish growth. Copepod comparison includes all tanks in which fish were present; chironomid comparison includes tanks in which chironomids were counted (+benthos tanks). Relationship between starting weight and weight-specific growth includes data from preliminary 1995 experiments. Symbols as in Fig. 2. Checkmarks: $0.10 < P < 0.05$.

other runs. Nutrient and trace element effects were not significant in ANCOVA models that included significantly correlated phytoplankton size-classes or the single most strongly correlated system-level phytoplankton variable for each copepod stage (all $P \geq 0.10$), indicating that stressor effects are mediated through changes in prey abundance.

Fish growth—Initial sizes of mummichogs varied among experimental runs ($P = 0.001$, Table 2), reflecting seasonal variation in field populations and spawning dates of laboratory reared fish, but did not vary among stressor or complexity treatments (all $P > 0.10$). Averaged across all runs and treatments, initial sizes of fish were 20.3 ± 0.4 mm in length and 0.16 ± 0.01 g in wet weight (averages are grand means of the 48 mesocosm tank averages). Fish grew an average of 6.3 ± 0.7 mm (21%) in SL and 0.20 ± 0.2 g (125%) in wet weight while in mesocosm tanks, and weight-specific growth rate was strongly related to initial size (Fig. 7). Results of treatment effects below are given for growth in wet weight only; similar results were found for analyses using growth in standard length. All ANOVAs of fish growth during individual mesocosm runs were based on data ranked within mesocosm run and complexity treatment because variances of log-transformed data for the September mesocosm run were significantly heteroscedastic (Levene's test $P < 0.05$).

Fish growth was significantly affected by nutrient additions (Fig. 7), the presence of sediments in the tanks ($P =$

0.0001), the interaction between system complexity and nutrient additions ($P = 0.025$), and by mesocosm run ($P = 0.0001$) in combined analyses of all mesocosm runs. Fish growth averaged 60% higher in the presence of sediments than in tanks without sediment, presumably reflecting the presence of benthic prey in sediment trays and the overall higher production associated with nutrient release from the sediments, but the difference between nutrient and nonnutrient addition tanks was reduced by both sediment and benthic invertebrate presence. Averaged across all tanks containing fish, fish grew 18% more in weight in mesocosms with added nutrients than in mesocosm tanks with no added nutrients (Fig. 7), but in the +fish mesocosms (i.e., in the absence of sediment, bivalves, and anemones) fish grew 70% more in weight in mesocosms with added nutrients than where no nutrients were added. Nutrient additions significantly increased fish growth during the July mesocosm run and tended to increase fish growth in nutrient addition tanks in the June run as well (but June $P = 0.094$) (Fig. 7). The average magnitude of the nutrient effect on growth was highest in the two runs (June and July) that had the highest overall growth rates of fish and in which calanoid copepods were most abundant and most strongly affected by nutrients.

As with several other taxa analyzed, there was considerable temporal variation among mesocosm runs in direction as well as magnitude of trace element effects. Fish growth was significantly higher with trace element additions in the April mesocosm run, but tended to be higher in the absence of trace elements in the July mesocosm run (but July $P = 0.067$). Reflecting this variation in responses among runs, trace element additions had no effect on fish growth when all 1996 mesocosm runs were included in analyses ($P = 0.673$; Fig. 7); fish grew an average of 0.20 ± 0.03 g in weight in tanks with or without trace element additions. There was an interaction between nutrients and trace elements on fish growth during the June mesocosm run (interaction $P = 0.035$, but overall model $P = 0.077$) (Fig. 7). However, differences among fish growth rates in the various June run stressor treatments was extremely small and the pattern was more evident when growth rates were ranked within complexity treatment than when mean growth rates were examined.

Comparisons of fish growth and prey abundance indicated that trophic interactions likely explained fish responses to stressors. Benthic algae (especially pennate diatoms), chironomid larvae, and harpacticoid copepods were the most abundant items in mummichog guts at the end of each experiment. Benthic algae other than diatoms were likely present but were not identifiable in guts. We assume that calanoid copepods were also an important component of fish diets early in the experiment because calanoids were rare in mesocosms containing fish by the middle of each mesocosm run (*see copepod results, above*). However only an average of <1 calanoid copepod per fish gut was found in samples taken at the end of each mesocosm experiment. The abundance of the benthic algae, chironomids, and harpacticoids in fish guts varied significantly among mesocosm runs (all $P < 0.01$) but not with nutrient or trace element treatment (all $P > 0.1$).

Fish growth was positively related to chironomid density, copepod density, and temperature, and inversely related to log-transformed starting wet weight ($r^2 = 0.55$, $P = 0.0001$, $n = 79$, including 1995 preliminary experiments) (Fig. 7; Table 3). No independent measure of benthic harpacticoid copepod density was available to use in regression analyses. Neither nutrient nor trace element treatments significantly affected fish growth when any of the three prey types (copepods, chironomids, and benthic production) were included in ANOVA models (P always >0.30).

Benthic invertebrates—Mortality of oysters and *Macoma* was consistently low in all experiments and all treatments. Oyster mortality ranged between 0 and 14% with no significant differences seen among treatments. *Macoma* mortality, which also included any losses in re-sampling and re-sieving the sediments, ranged between 5 and 16% with no differences among treatments. Because some anemones moved off the experimental panels, mortality of *Diadumene* could not be estimated.

Initial sizes of oysters varied among mesocosm runs ($P = 0.001$) (Table 2), reflecting the growth of those individuals from the initial hatchery stock maintained in a laboratory raceway with flowthrough Patuxent River water. Initial sizes of clams also varied among mesocosm runs ($P = 0.0001$) (Table 4), reflecting the growth of clams in the natural populations from which they were collected. Finally, initial sizes of anemones varied both among runs ($P = 0.001$) (Table 4) and among treatments ($P = 0.042$). In general, anemones collected at the end of summer were smaller than those at the beginning. Differences among treatments reflected an overall slightly larger initial size in the nutrient addition treatment than in the control treatment. Mean oyster growth was 1.24 ± 0.18 cm^2 or $99.1 \pm 14.5\%$, mean *Macoma* growth was 0.35 ± 0.03 cm^2 or $65.6 \pm 7.1\%$, and mean anemone growth was 0.06 ± 0.01 cm^2 or $27.0 \pm 4.8\%$. All ANOVAs of benthic invertebrates were done on rank-transformed data.

We found no significant differences or consistent patterns in growth of oysters that could be attributed to nutrient or trace element treatments (Fig. 8). Oysters grew more in nutrient addition tanks than in tanks without added nutrients from the same mesocosm run and trace element treatment in only 50% of the cases. Similarly oyster growth was greater in five tanks with added trace elements and less in three tanks when mesocosm tanks were paired by mesocosm run and nutrient treatment. Oyster growth was positively related to large phytoplankton cell densities in nutrient addition tanks and negatively related to small- and medium-sized phytoplankton cell densities (Fig. 8, Table 3). Oyster growth was negatively related to chlorophyte densities ($r^2 = 0.59$). However, temperature was the most important variable explaining variation in oyster growth (Table 3, Fig. 8). The pattern of positive, negative, and significant relationships between oyster growth, potential prey abundances, and temperature was quite similar to that found for adult copepods.

In contrast to oysters, growth of *Macoma* was significantly higher in nutrient addition tanks than in tanks to which no nutrients were added (Fig. 9). *Macoma* growth also averaged lower with trace element additions than without added trace elements in all four runs (Fig. 9). In addition, *Macoma*

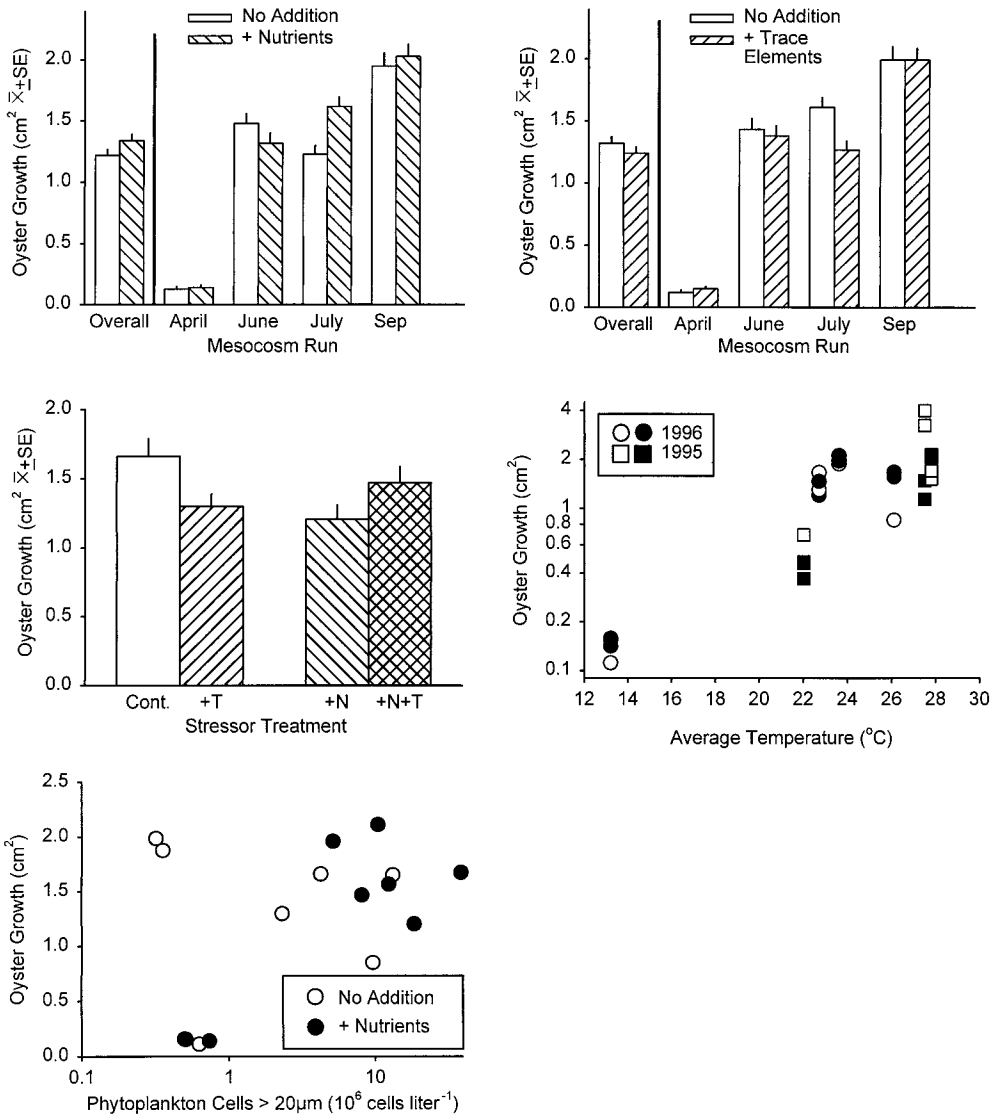

Fig. 8. Growth of oysters in response to nutrient and trace element additions (upper two panels), stressor effects during the June mesocosm run (center left panel), and relationship between temperature, density of large phytoplankton cells, and oyster growth. Temperature plot includes data from preliminary 1995 experiments. Symbols as in Fig. 2.

showed a significant nutrient×trace element interaction across all four mesocosm runs (Fig. 9). Trace element additions reduced the response to nutrient additions in all four runs, similar to the pattern seen for a number of species in June.

Growth of anemones was not consistently affected by nutrient additions (Fig. 10). However, anemones were the only taxa examined whose growth was significantly greater in trace element addition tanks, with a consistent pattern in all mesocosm runs (Fig. 10). Anemone growth averaged across all runs was nearly 3× greater in tanks with added trace elements than in tanks with no trace elements added. In addition, like *Macoma*, there was a significant nutrient×trace element interaction effect on anemone growth across all four mesocosm runs. For anemones, however, the nutrient×trace element interaction reflected a pattern that was opposite to that of *Macoma* (i.e., anemone growth was lowest in tanks with added nutrients but not added trace elements).

The experimental design did not permit analyses of separate mesocosm runs for benthic invertebrates. However, growth of *Macoma* followed the same general pattern during the June run as did phytoplankton, bacterial production, HNAN density, and several of the copepod life stages (Fig. 9). In contrast, neither oysters nor anemones conformed to this pattern (Figs. 8 and 10).

Variation in prey abundance did not appear to explain the response of *Macoma* or anemones to trace elements. Unlike the oyster, growth of *Macoma* seemed to be positively influenced by a wide range of integrative measures of phytoplankton biomass and production, as well as particulate N and C, was less strongly affected by particular size classes of phytoplankton, and was not significantly related to tem-

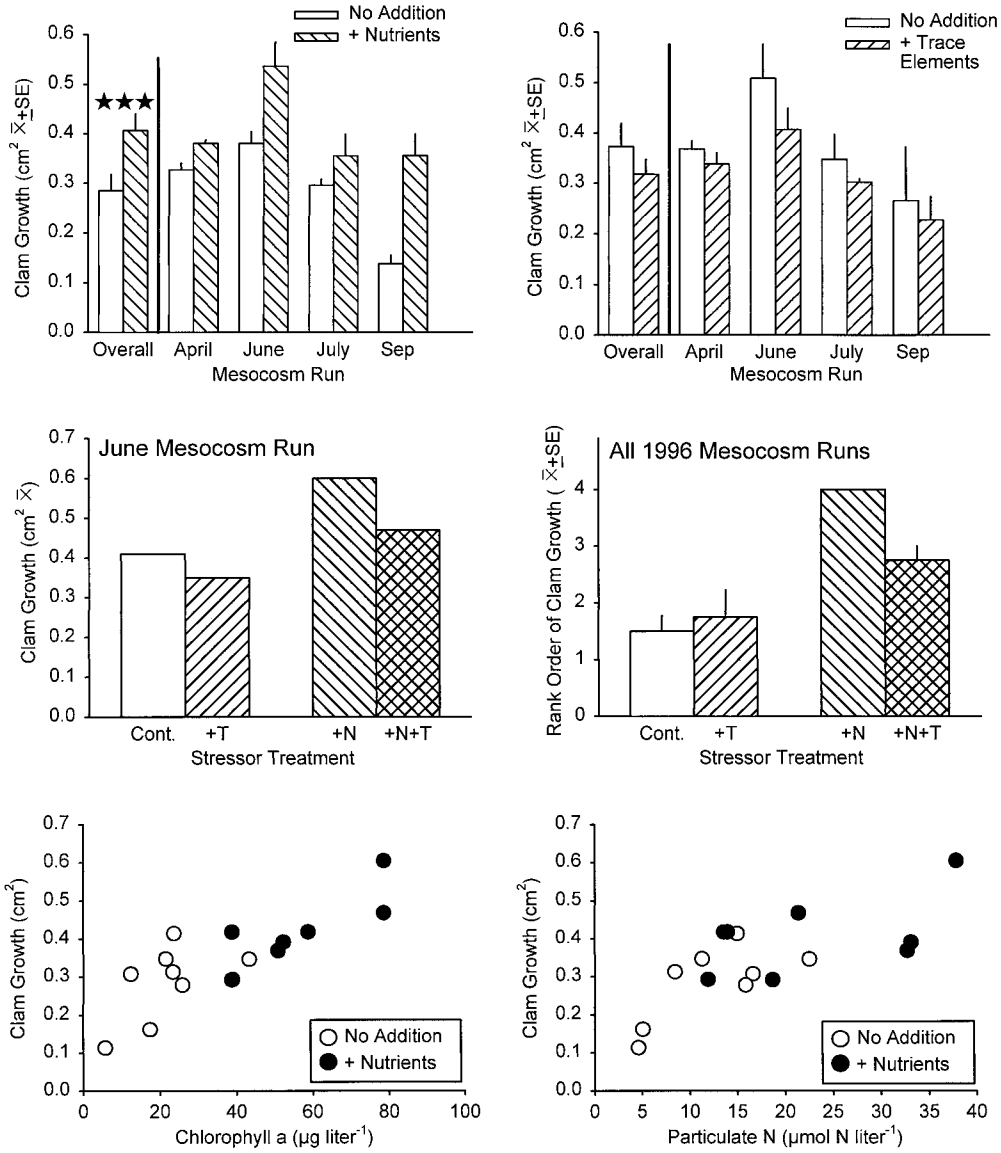

Fig. 9. Growth of *Macoma* in response to nutrient and trace element additions (upper two panels), stressor effects during the June mesocosm run and all mesocosm runs combined (center panels), and relationship between chlorophyll *a*, particulate N, and clam growth. Symbols as in Fig. 2.

perature (Fig. 9, Table 3). Inclusion of particulate N (the regression parameter with the greatest r^2) or measures of phytoplankton growth or production had little effect on ANOVA results for *Macoma* growth. Mean anemone growth was not significantly related to copepod abundance or temperature when only 1996 data were included in analyses (Table 3), but when data from 1995 preliminary experiments were included, copepod abundance explained 23% of the variability in anemone growth (Fig. 10). The regression coefficient for this relationship was negative, suggesting that anemones significantly affected copepod density rather than the reverse. Effects of trace elements and the nutrient×trace element interaction were still significant when copepod densities were included in ANOVAs.

Whole system respiration—Total respiration of all organisms in the system averaged 0.64 g C m^{-3} d^{-1} across all tanks and treatments during June–September runs in 1996 (measurements were not available for the April run) and ranged from <0.01 to 1.61 g C m^{-3} d^{-1}. On average, whole system respiration was about twice as high in tanks with added nutrients compared to tanks without (0.82 ± 0.07 vs. 0.45 ± 0.05 g C m^{-3} d^{-1}) (Fig. 11). Nutrient additions also significantly increased whole system respiration in each mesocosm run when they were analyzed individually. Trace metals significantly decreased whole system respiration only in the July experiment (13% decrease) (Fig. 11). No statistically significant nutrient×trace metal interactions were seen for whole system respiration.

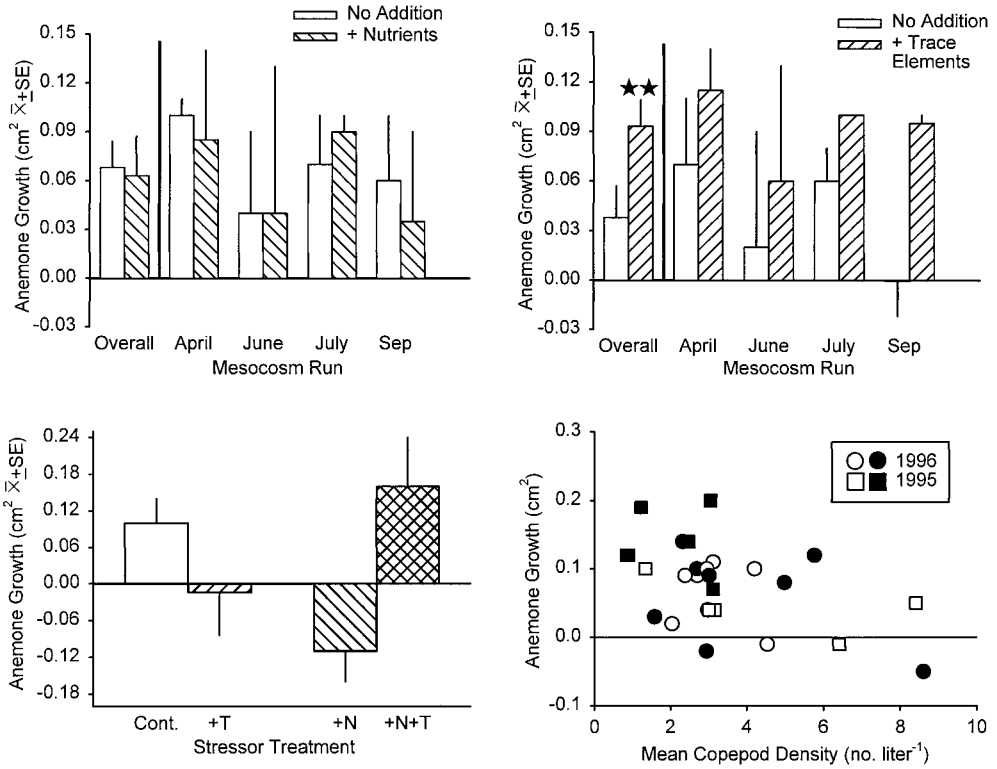

Fig. 10. Growth of sea anemones in response to nutrient and trace element additions (upper two panels), stressor effects during the June mesocosm run (center left panel), and relationship between copepod density and anemone growth. Symbols as in Fig. 2.

Discussion

Results of our mesocosm experiment indicate that temporal dynamics and interactions among stressors will be critical to predicting responses to multiple stressors in aquatic systems. Where the temporal pattern of stressor effects varies among stressors, detecting single stressor effects and stressor interactions may be strongly dependent on the timing of experiments or sampling. Two types of interactions among stressors we tested appeared important: the way in which the temporal dynamics of one stressor (nutrients) affected the temporal pattern of toxicity of the second stressor (trace elements), and the non-additive response to the two stressors when trace element effects were prominent. The non-additive effect of nutrients and trace elements seems to occur because of a combination of direct nutrient and trace element effects and complementary responses of phytoplankton taxa.

Below we discuss responses by phytoplankton and consumers to individual stressors, as well as temporal and taxonomic variation in non-additive effects of nutrients and trace elements. We also examine the importance of both direct and indirect effects of stressors to the expression of stressor effects throughout the experimental food web. These indirect effects of stressors include complementary responses within the phytoplankton assemblage and trophic effects

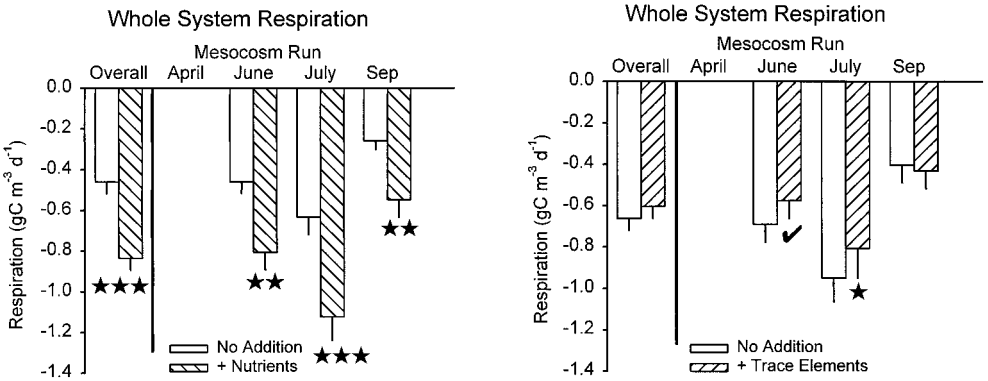

Fig. 11. Whole system respiration response to nutrient and trace element additions. Symbols as in Fig. 2.

(i.e., changes in the abundance and suitability of prey or substrate available to consumers) that result from changes in the overall abundance and production of phytoplankton, variation among taxa in their direct response to stressors, and complementary responses among phytoplankton taxa. We suggest that the effects of trace elements on consumers are less predictable from integrative measures of phytoplankton biomass and production than are effects of nutrients because of the relatively greater importance of complementary responses and variation among taxa within the algal assemblage in response to the trace element exposures used in our experiment.

Phytoplankton responses—The response of phytoplankton to nutrients and trace elements varied both temporally and among taxa. For nutrient additions, this variation was in the magnitude of the response, not the direction. There was a consistent increase in all integrative measures of phytoplankton biomass or production in response to nutrient additions (Table 4). These results are consistent with previous studies in estuaries and coastal marine ecosystems, which include studies ranging from small-scale bioassay experiments (Ryther and Dunstan 1971) to large-scale continuous culture studies (D'Elia et al. 1986), experimental mesocosms (Nixon et al. 1984), and regression analysis of data from a wide range of coastal ecosystems (Boynton et al. 1982; Nixon 1992). The magnitude of the response to nutrient additions in our mesocosm experiments (avg ~3-fold increase in biomass or production with a 1.5–2-fold increase in N loadings and a 2–5-fold increase in P loadings) is similar, although slightly higher, than the magnitude of change observed in primary production in the mainstem of Chesapeake Bay due to interannual differences in total N loading (Boynton et al. 1995). The temporal variation in response to nutrient additions was dominated by a dampened response in the April run (in absolute changes in algal biomass and production), likely due to lower temperatures and light levels.

Increased cell densities of abundant phytoplankton taxa were also measured in nutrient addition treatments in all instances where changes in cell density were meaningful (Table 2). In all runs, large increases were seen in the numerically important, relatively large centric diatoms. Such a result has been seen in the past and is presumably due to the inherent ability of centric diatoms to outcompete other taxa for scarce resources (e.g., Pearsall 1932; Parsons et al. 1978; Sanders et al. 1987; Oviatt et al. 1989). However, some smaller sized taxa, such as chlorophytes, also consistently increased in runs in which they were abundant (i.e., avg density $>0.1 \times 10^6$ cells liter^{-1}), and we saw no examples of phytoplankton taxa decreasing in response to increases in diatom densities (*see also* Oviatt et al. 1989). In general, phytoplankton taxa other than diatoms were more variable in their numerical response to nutrient additions—increasing dramatically in some runs but not in others.

In contrast to responses to nutrients, phytoplankton responses to trace elements varied in direction as well as magnitude, both temporally and among taxa. This result seems to reflect variation in the sensitivity of algal species, the abundance of particularly sensitive species, and perhaps nutrient limitation in tanks. Negative responses to trace element additions were balanced by complementary increases in other, presumably more resistant, taxa. For example, *R. fragilissima*, a large bloom-forming diatom that is particularly sensitive to arsenic (Sanders and Cibik 1985), was the numerically dominant diatom in the June run. Decreases in *R. fragilissima* were offset by increases in small-celled chlorophytes in the June run. Such patterns of replacement have been described before for trace element contaminants (e.g., Cloern 1996; Sanders and Riedel 1998) and may be important in stabilizing ecosystem processes where stressors strongly affect the dynamics of some taxa (Frost et al. 1995).

The response of phytoplankton to multiple stressors was non-additive when trace element effects were prominent (June). This non-additive response was evident in measurements at both the taxa and ecosystem (integrative phytoplankton measures) level (Fig. 2). The nutrient×trace metal interaction on phytoplankton production and biomass was largely caused by the responses of *R. fragilissima* and chlorophytes, although these two taxa showed differing responses to the multiple stressors. Trace metals dampened the response of *R. fragilissima* to increased nutrients, while the chlorophytes increased considerably more than predicted in the nutrient plus trace metal addition tanks. The overall response of phytoplankton biomass and production to the multiple stressors reflected the diatom response because of their larger cell size, although the change in cell density of chlorophytes was similar to that of the diatoms.

We believe that the negative response of *R. fragilissima* to the trace element mixture in the June mesocosm run, but not in the other runs, was primarily a response to arsenic toxicity and concomittant phosphorus limitation. Arsenate, a chemical analogue of phosphate, is taken up primarily by the phosphate transport system in phytoplankton; thus, arsenic toxicity is heavily regulated by P loading and concentration (Blum 1966; Planas and Healey 1978; Sanders and Cibik 1985; Wangberg and Blanck 1990). In the June run, the As : P concentration ratio in the trace element addition tanks averaged 6.9 (mol : mol), far higher than in the other runs (0.3–0.9 averaged across non-nutrient and nutrient addition tanks). In addition, P concentrations were very low in June compared to the other runs, increasing arsenic toxicity. P concentrations in the June nutrient addition tanks were about an order of magnitude lower than that found in the other three mesocosm runs, and P concentrations in both the June and April non-nutrient addition tanks were about an order of magnitude lower than in the July or September runs. Low ambient P concentrations coupled with high N : P ratios in spring and low ambient N concentrations coupled with low N : P ratios in summer and fall are typical in the Patuxent River estuary, Chesapeake Bay, and many other temperate coastal systems, resulting in seasonal variation in the nutrient most limiting to phytoplankton production (e.g., D'Elia et al. 1986; Sanders et al. 1987; Fisher et al. 1988, 1992; Malone et al. 1996). This temporal pattern of nutrients is reflected in the loadings from filtered Patuxent estuary water into our mesocosms that had no additional source of nutrients (Table 1). In addition, it affected the N : P input ratio in the tanks with added nutrients. Thus there was sufficient N in the June run, resulting in utilization of essentially all P inputs.

Only the April non-nutrient addition tanks had As : P ratios approaching even half of those found in all trace element addition tanks in June (i.e., 2.3 in non-nutrient addition tanks and 0.54 in +nutrient tanks with added trace elements). However, not only was the April As : P ratio lower than that in June, but the April run was dominated by *Chlorella* and small centric diatoms that have shown no particular sensitivity to As (Sanders et al. unpubl. data). Nevertheless, there were small but significant decreases in prasinophytes and pennate diatoms during the April run. *R. fragilissima* was absent or extremely rare in mesocosm tanks during the April run.

It is also particularly striking that during the July run trace element additions caused strong increases in cell densities of centric diatoms and other abundant phytoplankton taxa, phytoplankton biomass (as chlorophyll *a*), and phytoplankton production as measured by carbon fixation. Average concentrations of the two elements most likely to cause inhibition, As and Cu, were lower during the July mesocosm run than during other runs. These decreased concentrations were likely caused by biological and biogeochemical processes within the mesocosm tanks. In addition, the shift from P limitation to N limitation would reduce the potential toxicity of As. However, neither the lower dissolved trace element concentrations measured in mesocosm tanks nor the potential reduction in As toxicity explain the marked increased in phytoplankton abundance and production that was seen.

Bacterioplankton and higher trophic levels: Direct effects and transmission of stressor effects through trophic interactions—Effects of nutrient and trace element additions varied through the mesocosm food webs such that direct effects on primary producers were sometimes, but not always, reflected in bacterioplankton and higher trophic levels. As with phytoplankton, responses of bacterioplankton and other consumers to nutrients were less variable than were responses to trace element additions. Transmission of stressor effects through the food web may have depended on both the magnitude of the overall response and variation among algal taxa in direct and complementary responses to stressor additions. In addition, there were several instances in which bacterioplankton or higher trophic levels seemed to be affected by stressors directly as well as indirectly through trophic interactions.

Most consumers generally increased with nutrient additions, but the temporal pattern and magnitude of the response varied among consumers. Bacterioplankton, HNAN and *Macoma* increased or showed a trend toward increasing production, density, or growth in response to nutrient additions in all four mesocosm runs. In addition, heterotrophic respiration consistently increased with added nutrients. Copepod densities and fish growth also generally increased, but responses to nutrient additions were muted or absent in the April run (which had the weakest phytoplankton response to nutrients) and the September run (when phytoplankton biomass and production were lowest). Seasonal patterns of growth and reproduction, as well as effects of starting size on growth rates of fish and *Macoma*, likely combined with food-web influences to create the seasonal patterns of responses by consumers that were significantly affected by nutrient additions.

Unlike other consumers, the increase in bacterioplankton production in nutrient addition tanks likely reflected direct stimulation by nutrients as well as indirect stimulation through the food web. The strong positive relationship between phytoplankton production and bacterial production indicates that substrate supply was important to control bacterial growth. In addition, the importance of direct effects of nutrients on bacterial production in our study is supported by the statistical significance of nutrient effects in ANOVA models that included phytoplankton production as a covariate.

The magnitude of nutrient effects on consumers was less than that seen for phytoplankton and did not always reflect the position of taxa within the food web. Species at comparable trophic levels (e.g., oysters and *Macoma*, fish and anemones) responded quite differently to nutrient additions. An approximately 1.5–2-fold increase in N loadings and a 2–5-fold increase in P resulted in a tripling of phytoplankton production and chlorophyll *a*. In contrast, nutrient additions led to a doubling of bacterial production, a doubling of copepod densities, 30% increases in HNAN density and *Macoma* growth, and a 17% increase in fish growth. Smaller, non-significant responses were seen in growth of oysters (+12%) and anemones (−12%) with nutrient additions.

The response of bacterial production to nutrient additions ranged between one-third and two-thirds of the response of phytoplankton production in individual mesocosm runs in our experiments. This response of bacterial production was somewhat less than that found in other studies in estuarine enclosures in which the relative increase in bacterial production in response to nutrient additions exceeded the increase in phytoplankton production (Riemann et al. 1990; Baretta-Bekker et al. 1994). Lower losses of energy and C to respiratory processes under nutrient-enriched conditions can enhance the flux of C through the microbial loop, including increased percentages of C flow through bacterial production, nanoflagellates, and microzooplankton (Baretta-Bekker et al. 1994). The 30% average increase in HNAN densities with nutrient additions in our experiment was also quite attenuated relative to the increased production of their bacterioplankton and phytoplankton prey. In addition, the response of HNAN to nutrient enrichment was considerably lower than that of copepods, which may consume significant quantities of HNAN (Gasparini and Castel 1997) but also feed on prey at the same trophic level as HNAN.

Copepod responses to nutrient additions in our mesocosms were generally greater than that found in other studies, while fish and *Macoma* responses were similar to previous results. For example, Fulton (1984) observed a 30–50% increase in copepod densities with an addition of 16 mmol N m^{-3} $week^{-1}$ (equivalent to 2.3 mmol N m^{-3} d^{-1} compared to our additions of 1.6 mmol N m^{-3} d^{-1}), and Sullivan and Banzon (1990) found only a 9% increase in females in an 8× nutrient loading treatment in the MERL mesocosm. In seagrass mesocosms, growth in standard length of juvenile mummichogs was 14% higher (compared to 13% higher growth in SL in our study), and growth of juvenile winter flounder, three- and nine-spine sticklebacks, and Atlantic silversides

was 5, 9, and 50% higher, respectively, with an average 5-fold increase in N and 1.5-fold increase in P in seagrass mesocosms (E. Buckley and S. Nixon unpubl. data). Growth of Atlantic menhaden (*Brevoortia tyrannus*) from the larval through juvenile stage was increased by 50% with an 8× increase in nitrogen loading in the MERL mesocosms (Keller et al. 1990). The relationship between phytoplankton abundance and production and *Macoma* growth is close to that found in earlier studies (e.g., Lin and Hines 1994).

The effects of the relatively modest nutrient additions we used were likely transmitted throughout most of the food web because nutrient enrichment in our mesocosms increased the entire primary producer base. Although some phytoplankton species or higher taxa increased disproportionately in response to nutrient additions, there were no major reductions in the abundance of cells in any taxa or size category ≥ 3 μm (the smallest cell size included in our counts). Changes in the composition of these algal assemblages resulting from nutrient additions therefore may not have been sufficient to create bottlenecks in populations of herbivorous zooplankton or reduce growth of suspension-feeding bivalves, such as *Macoma,* that efficiently utilize a wide spectrum of phytoplankton taxa. *Macoma* is not selective with regard to particle size (Self and Jumars 1988) and ingests particles from 1-μm bacteria to 300-μm sand grains (Gilbert 1977; Harvey and Luoma 1984).

There were exceptions to the general pattern of increases throughout the mesocosm food web with nutrient additions, however; neither anemones nor oysters experienced increased growth in response to nutrient additions. Growth of anemones in our experiments was highly variable and sometimes negative, perhaps reflecting low densities of their copepod prey in tanks containing fish. Growth of oysters was always positive, but did not follow the temporal or treatment-related patterns of integrative measures of their phytoplankton prey. Unlike *Macoma,* growth of the oysters showed no significant relationship to phytoplankton production or biomass, or to particulate N or C. Studies have shown that although oysters can ingest a variety of particle types and sizes, the nutritional value can vary greatly among prey types (e.g., Crosby et al. 1990; Langdon and Newell 1990, 1996; Baldwin and Newell 1991). Growth rates of oysters therefore vary greatly with species and biochemical composition of the available phytoplankton, bacteria, and other particulates. While nutrient additions in our mesocosms increased the density of some phytoplankton taxa that are likely to be good food sources, nutrients also increased densities of other taxa, such as chlorophytes, that are particularly poor food sources for oysters (Langdon and Newell 1996). We do not know whether *R. fragilissima,* which is a chain-forming as well as a large-celled diatom and was highly responsive to nutrient as well as trace element additions, is a good or poor food source for oysters. It is possible that the species of phytoplankton accounting for differences in biomass and production among treatments were not the species contributing the most to oyster growth. However, it is not clear whether oysters are truly more sensitive to phytoplankton taxonomic composition than are copepods and *Macoma,* or whether more detailed nutritional information is simply available for this commercially important species. Given that oysters did increase in size by more than 100% in all but the April run, there clearly was sufficient food for at least moderate growth rates in all treatments. It is therefore possible factors other than those related to treatment effects—rather than shifts in taxonomic composition of phytoplankton—may have masked potential responses to increased phytoplankton by oysters. For example, oyster growth may have been affected by the parasite *Perkinsus marinus,* which is endemic within the region but which we did not measure.

The presence and direction as well as the magnitude of responses of consumers to trace element additions was much more variable among taxa and experimental runs than were responses to nutrient additions. In addition, the correspondence between responses to trace element additions by consumers and responses of integrative measures of phytoplankton biomass and production was poor. Bacterial production and adult copepod densities tended to follow the same temporal pattern of responses to trace elements as did integrative measures of phytoplankton (i.e., little change during the April and September runs, a decrease in June, and an increase in July). However, the interaction between nutrient and trace element effects seen for phytoplankton during the June mesocosm run was strongly reflected in growth or abundance of several taxa (bacterioplankton and HNAN), muted for some taxa (fish and *Macoma*), and not apparent for others (oysters, anemones, and total copepods). Furthermore, HNAN densities and growth of fish, oysters, and *Macoma* tended to decrease in the July run, although phytoplankton biomass and production increased. In addition, HNAN densities, total adult copepod plus copepodite densities (analyses not shown; $P < 0.05$), and fish growth all significantly increased during the April run, although phytoplankton biomass and production were unaffected by trace elements during that time.

Both HNAN and *Macoma* showed some evidence of trace element toxicity. HNAN densities were significantly lower in trace element tanks at least on dates of peak HNAN densities in the June, July, and September mesocosm runs. If future research confirms the sensitivity of HNAN to trace elements, these organisms could provide an important mechanism for the transmission of toxic effects to less sensitive organisms in microbial food webs because of their importance as both predators and prey. Average growth of *Macoma* was consistently lower in trace element tanks in all runs, and the nutrient×trace element interaction was significant in analyses of all 1996 mesocosm runs combined. We therefore cannot rule out the possibility that trace element toxicity led to decreased growth of *Macoma.* Copper concentrations of 16–21 μg liter^{-1} (3–6 times the concentrations used in our experiments) have been shown to cause 50% mortality of *Macoma inquinata* in 30 d (U.S. EPA 1984*c*).

Specific trophic interactions and variation among algal taxa in their response to trace elements likely influenced whether consumer and integrative phytoplankton responses to trace elements were similar or disparate. For example, the nutrient×trace element interaction during the June mesocosm run was apparent for bacterial production, which integrates phytoplankton responses through the DOC pool, and perhaps for *Macoma,* which feeds on a broad range of phytoplankton. However, responses differed among copepod

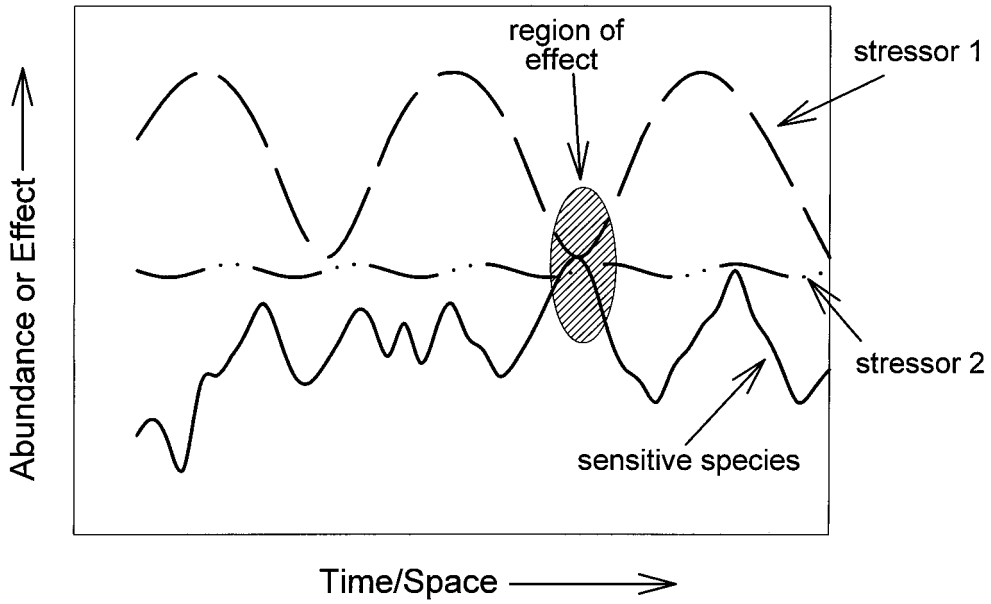

Fig. 12. The convergence of temporal and spatial patterns of multiple stressors and sensitive species can determine the region in time and space where effects of multiple stressors differ from those predicted by individual stressors effects. Temporal and spatial patterns of loadings, abundances, or effects may vary for each stressor and sensitive species independently. However, the expression of non-additive interactions among factors may occur only where and when all factors coincide and are in states conducive to producing non-additive interactions. In our study, the convergence of nutrient additions, low P, elevated trace elements, and high abundance of a sensitive phytoplankton species were all required to produce dramatic changes in production and abundance in +N+T mesocosms.

stages. Adult copepods, which can efficiently prey on phytoplankton as large as individual *R. fragilissima* cells (Berggreen et al. 1988), and copepod eggs followed a pattern during the June mesocosm run similar to that of *R. fragilissima* and integrative measures of phytoplankton abundance and production. Trace element additions decreased the magnitude of the response of adult copepods to nutrient enrichment, especially during periods of peak densities. Nevertheless, it is unlikely that *R. fragilissima* was an important food source for adult *A. tonsa* in the June mesocosm run. Egg production tended to follow similar patterns as *R. fragilissima*, but diatom diets in general have also been related to reduced copepod reproduction (Ban et al. 1997), and preliminary experiments yielded depressed egg production by *A. tonsa* fed *R. fragilissima* (Bundy and Oppert unpubl.). Copepodites did not follow the same pattern as adults and eggs. Copepodites can graze on small phytoplankton cells the size of the chlorophytes (Berggreen et al. 1988), which became dominant in the +nutrient+trace element tanks, and there was an increase in copepodite stages 1–3 and 4–5 in +nutrient+trace element tanks throughout most of the run. However, adult abundances remained low in +nutrient+trace element tanks and did not reflect the expected transition from copepodite to adult stages. This may indicate that the food source was not adequate to either facilitate molting into the adult stage or persist once the molt had occurred. Thus, copepodite stage duration may have increased and molting into the adult form may have decreased in +nutrient+trace element tanks as a result of shifts in the size and taxonomic composition of the phytoplankton assemblage.

Juvenile mummichogs eat both adult and copepodite stages of copepods, and the complementary responses of these two copepod life stages in nutrient addition tanks with and without added trace elements may have contributed to the extremely muted nutrient×trace element effect on mummichog growth. We do not have sufficient data to determine the response to trace element additions of other items found in fish guts. Chironomids were thoroughly sampled only in the +benthos tanks, and benthos as small as harpacticoid copepods were not counted in 1996 experiments. Although benthic algal production was measured once during the mesocosm run in tanks containing sediment (Seitzinger unpubl. data), previous tests with *Spartina, Spartina* detritus, and green algae (but not diatoms) suggest that plant matter does not support growth of *F. heteroclitus* (White et al. 1986).

Conclusions

A consideration of temporal variation in the quantity and availability of individual stressors, as well as natural variation in both species abundances and responses to stressors, will be critical to our understanding and management of multiple stressor effects in anthropogenically influenced systems. Temporal and spatial patterns of the quantity and level of activity of each individual stressor can be unique because of temporal and spatial patterns of relevant anthropogenic

activities, the abundance or responsiveness of sensitive species, and physical factors such as temperature or rainfall. Nevertheless, it is the convergence of these patterns of variation that will determine where and when the cumulative effects of multiple stressors are likely to be most important and strongly diverge from a simple additive model of individual effects (Fig. 12). In addition, naturally occurring temporal and spatial variation in physical factors and disturbances may strongly influence the spatial and temporal pattern of system susceptibility to anthropogenic stressors.

Our results suggest that the direct effects of trace elements on estuarine phytoplankton will exhibit considerable temporal and spatial variability related to variation in species composition and nutrient limitation, in addition to any variation caused by temporal and spatial patterns in loadings of the trace elements themselves. At times and locations where sensitive species are abundant and nutrient dynamics facilitate trace element toxicity, trace elements may strongly reduce phytoplankton abundance and production and lead to shifts in dominant species. Similar loadings at sites or times dominated by other species or receiving somewhat different nutrient inputs may have no detectable effect on parameters such as those we measured.

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. In general, the effect of trace elements was proportionately greater in nutrient addition tanks than in tanks without added nutrients. It is logical to assume that many coastal ecosystems exposed to anthropogenic stress will be 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. Similarly, high nutrient loadings may mask the effects of trace elements on sensitive species. The magnitude of the trace element effects on sensitive species or their predators may be apparent only when nutrient loadings are reduced.

Our results also indicate that trace element as well as nutrient effects will vary temporally and among species for bacterioplankton and higher trophic levels. The effects of these stressors on taxa other than phytoplankton are mostly indirect and result from trophic interactions. Because of this, an understanding of the extent of variation (or similarity) in phytoplankton species' responses may be as important to predicting the transmission of stressor effects through the food web as are measures of the magnitude of the effects of that stressor on species directly affected. Experiments designed to directly test the influences of species-specific changes in the phytoplankton assemblage on consumer populations are needed to explain specific instances in which consumer responses and integrative responses of the phytoplankton assemblage diverge.

Food-web relationships are likely to add an additional source of variation to the expression of trace element effects and nutrient×trace element interactions in aquatic systems. In our experiments, nutrient additions increased densities of most phytoplankton taxa and significantly increased production, growth, and(or) densities of most other organisms throughout the food web. In contrast, effects of trace elements on phytoplankton (including those likely due to interactions among phytoplankton taxa) varied among species in direction as well as magnitude and were often not reflected in higher trophic levels. Even where responses of other trophic levels closely matched those of integrative measures of the phytoplankton assemblage response to stressors (e.g., the response of bacterioplankton to nutrients and trace elements), variation among primary producers in their response to stressors may have been important because species-specific characteristics such as cell size and turnover rate lead to variation among phytoplankton species in their relative contribution to these integrative measures. In general, the temporal and among-species variation in indirect responses to moderately elevated trace element additions may be difficult to detect under field conditions, especially where nutrients are also elevated, even when strong direct effects of trace elements on sensitive phytoplankton species occur.

As human population growth and activities increase the intensity, geographic extent, and number of anthropogenically generated 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 likely be important features of human-influenced systems.

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