Top-down and bottom-up regulation of phytoplankton assemblages in a eutrophic estuary

by

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*Abstract.* This study was prompted by the results of a multiyear monitoring study of water quality trends and plankton composition in Raritan Bay, an estuary between the states of New York and New Jersey with a long history of cultural eutrophication and harmful algal blooms. Multivariate analysis of the monitoring dataset led to the generation of a number of hypotheses regarding phytoplankton composition, individual nuisance species, environmental factors, and zooplankton composition. The objective of this study was to supplement field observations with a series of controlled microcosm experiments designed to investigate the effects and interaction of varying mesozooplankton abundance (sieved, unsieved and enriched) and enrichment with two nutrients (nitrate and dissolved iron) on spring and summer phytoplankton assemblages. Results indicate that shifts in phytoplankton species composition following nutrient enrichment were magnified when mesozooplankton were removed from microcosms, enrichment with iron resulted in significant increases in diatoms, and enrichment with both iron and nitrate resulted in significant increases in dinoflagellates and the centric diatom *L. minimus*. The results of microcosm experiments mostly confirm the patterns that have emerged through ordination of our monitoring dataset. All together, results demonstratethat top-down and bottom-up factors interact in complex ways to alter phytoplankton community structure.

Keywords: Raritan Bay; microcosm; phytoplankton; nutrient enrichment; zooplankton grazing; eutrophication; harmful algal blooms

**INTRODUCTION**

Cultural eutrophication, the nutrient over-enrichment of surface waters by human activity, has been described as one of the most pressing problems of the 21st century (Turner and Rabalais, 2003). More than 60% of coastal waters in the United States are exhibiting the symptoms of eutrophication which include dense, and sometimes toxic, algal blooms, depletion of dissolved oxygen, and loss of marine biodiversity (National Research Council [NRC], 2000). Therefore, it is not surprising that eutrophication has represented and defined one of the central themes of coastal research since the 1970s when scientists first began expressing serious concern about the impact of nutrient enrichment on the structure and function of coastal ecosystems (Nixon, 1995). Cloern (2001) describes three phases in the development of our conceptual model of the cultural eutrophication problem in coastal ecosystems. Most of the early research conducted during Phase I of the conceptual model emphasized the relationship between nutrient loading, increasing phytoplankton biomass, and enhanced depletion of oxygen from bottom waters. Phase II, the contemporary phase of the conceptual model, recognizes that the processes that determine primary productivity and food web structure vary considerably from one system to the next. As a result, Phase II emphasizes *system-specific* responses to nutrient enrichment and includes research aimed at clarifying the way that nutrient loading, nutrient ratios, and habitat characteristics interact to affect the abundance and composition of the phytoplankton and overall food web structure. There is no question that a number of monitoring studies in different coastal ecosystems have provided important phase II insights (e.g., Smayda, 1989; MacIntyre et al., 2004; Irigoien et al., 2005; Mitra and Flynn, 2006; and Sunda et al., 2006). Cloern (2001) goes on to describe a third phase of the eutrophication model that includes consideration of the interaction between nutrient enrichment and other stressors (e.g., invasive species, climate change) and application of our scientific understanding of eutrophication to develop restoration strategies. However, certain pieces of the Phase II model still remain poorly understood despite our common understanding of the eutrophication concept and the vast knowledge we have gained over the past four decades.

One part of the phase II model that needs further clarification is the origin and development of harmful algal blooms (HABs). Coastal scientists generally agree that increased nutrient enrichment is promoting the development of many HABs (Heisler et al., 2008), but the environmental processes that select for blooms of certain species is still not well understood, especially in estuaries. Reviews of HAB research indicate that both the quantity and quality of the nutrient pool can contribute to the success of individual bloom species and that both chronic and episodic nutrient delivery can promote HABs (Anderson et al., 2002; Glibert and Burkholder, 2006; Heisler et al., 2008). Furthermore, the ultimate success of a given species, and its response to nutrient enrichment, will depend upon the rate at which zooplankton grazers are consuming that species and constraining its production. Studies have shown that selective feeding by zooplankton on certain species can alter the structure of a phytoplankton community that developed under a specific nutrient regime (e.g., Porter, 1977; Granéli et al., 1989; Turner and Tester, 1989; Gobler et al., 2002; Irigoien et al., 2005). Although this research has permitted generalizations to be drawn about the role of nutrients and grazers in generating HABs, it is important that more phase II eutrophication studies provide *system-* and *season-specific* insights about how “bottom up” (nutrient concentrations and ratios) and“top down” (zooplankton abundance and composition) factors interact to create change in phytoplankton species composition and lead to bloom formation.

Therefore, the objective of this study was to experimentally investigate the effects of artificial nutrient enrichment and zooplankton grazers on the phytoplankton species composition using microcosms in Raritan Bay, a eutrophic estuary located at the southern portion of Lower New York Bay between the states of New York and New Jersey. Microcosms are artificial, simplified ecosystems that can be used to predict the response of natural phytoplankton assemblages to specific actions under controlled conditions. Although microcosms have been criticized for yielding misleading results when they exclude important features of ecosystems (e.g., secondary consumers, seasonal effects; Carpenter 1996), they are among the few tools available for studying the systemic responses of phytoplankton to nutrient enrichment in the presence of grazers (Cloern, 2001). Furthermore, microcosm experiments are often more constructive when they are prompted and verified by results of larger, field monitoring programs, as they can be used to test hypotheses and study the effects of disturbance (Carpenter 1996). This study was prompted by the results of a multiyear monitoring study of water quality trends and plankton composition in Raritan Bay, and multivariate analysis of this three-year dataset led to the following hypotheses regarding plankton dynamics in Raritan Bay: 1) abundance of HAB species is associated with reduced grazing pressure, 2) river discharge and Si:N ratios, particularly during spring months, are positively related to diatom abundance and negatively related to abundance of flagellates, including certain HAB species (e.g., *Heterosigma akashiwo* (Hada) Hada ex Hada et Chihara, *Prorocentrum minimum* (Pavillard) Schiller, *Pfiesteria*-like species), and 3) iron is not an important predictor of phytoplankton species composition in this system (Rothenberger et al., 2013). The present study was driven by these hypotheses and designed to supplement field observations with controlled experiments. The series of experiments described here examine the effect and interaction of three zooplankton treatments (i.e., sieved, unsieved and enriched) and enrichment with two nutrients (nitrate and dissolved iron) on spring and summer phytoplankton assemblages. Because this system been the site of an ongoing monitoring program since 2010, results of experimental manipulations can be related back to patterns observed under natural conditions.

**METHODS**

*Study Site*

The Raritan Bay system (New York-New Jersey, U.S.A.; Fig. l) is both ecologically and economically significant, supporting numerous recreational and commercial fisheries and providing habitat for waterfowl, shellfish, and marine, estuarine, and anadromous fish (Kane and Kerlinger, 1994). However, the water quality and ecological integrity of this system have long been threatened by a dense human population and urban and industrial overdevelopment. By the turn of the 20th Century, shoreline development, discharges of industrial waste, and inadequate waste disposal methods led to water contamination, beach closures, reduced fish abundance and diversity, and collapse of the oyster industry (Environmental Protection Agency [EPA], 2007). By the early 1960s, the rich nutrient supplies and sluggish circulation of the Raritan Bay were contributing to extremely dense phytoplankton populations and low summer dissolved oxygen concentrations (Jeffries, 1962).

Although a number of studies in this system have demonstrated some improvements to water quality (e.g., increases in oxygen and decreases in fecal coliforms) following passage of the Clean Water Act in 1972 and improvements to sewage treatment (Brosnan and O’Shea, 1996; O’Shea and Brosnan, 2000), more recent water quality data indicate that the system is still polluted. In fact, full utilization of these waters for commercial fishing and recreational activities is restricted by poor water quality. The water and sediments are contaminated with a variety of pollutants such as heavy metals, PCBs, polynuclear aromatic hydrocarbons (PAH), and pesticides (Breteler, 1984). Nitrate and soluble reactive phosphorus (SRP) concentrations (grand mean 12 *µ*mol NO3 l-1 and 0.5 *µ*mol SRP l-1) in Raritan Bay continue to indicate eutrophic conditions and are as much as 50 and 20 times higher, respectively, than concentrations reported in the literature fifty years ago (Jeffries, 1962; Rothenberger et al., 2013). The Raritan Bay also continues to exhibit numerous symptoms of eutrophication, including high algal biomass, high turbidity, seasonal hypoxia, fish kills, and blooms of potentially harmful phytoplankton species (Olsen and Mulcahy, 1991; Reid et al., 2002; Rothenberger et al., 2013).

Chronic phytoplankton blooms of various species, including the dinoflagellates *Ceratium tripos* (Müller) Nitzsch, *Prorocentrum micans* Ehrenberg, and *Heterocapsa rotundata* (Lohmann) Hansen, and the raphidophyte *Heterosigma akashiwo* (formerly misidentified as *Olisthodiscus luteus* Carter, Smayda, 1998; Olsen and Mahoney, 2001), have occurred in the HRE and New Jersey coastal waters for over three decades (Gastrich, 2000). These blooms have been associated with moderate bather discomfort and/or illness, diminished aesthetic value of beaches, hypoxia, and fish mortality (Gastrich, 2000). A more recent study of water quality and plankton dynamics at six sampling locations in in Raritan Bay (Rothenberger et al., 2013; Fig. 1) has indicated that these species are still present and seasonally abundant and that approximately 18% of the taxa identified are capable of causing HABs (i.e., they cause harm either through toxin production, cell physical structure, or accumulated biomass, Anderson et al. 2002).

*Experimental Design*

Experimental manipulations to assess the effect of zooplankton grazers on phytoplankton biomass and composition were initiated on 9 July 2010 and 4 September 2010 using 1 l polyethylene containers incubated *in situ*. A third enclosure experiment was designed to assess the effects and interactions of *both* nutrients and zooplankton grazers on phytoplankton biomass and composition. The third enclosure experiment was initiated on 17 May 2013, also using 1 l polyethylene containers incubated *in situ*. Microcosms were incubated near site 6 of our monitoring study out of the way of boat traffic for seven days (Fig. 1). Experiments were carried out in late spring and summer because our multiyear monitoring study indicated that phytoplankton biomass as chlorophyll *a* generally peaks in late spring and that the majority of the identified bloom-forming species are most abundant during summer months (Rothenberger et al., 2013).

The first two experiments (i.e., the experiments designed to assess the effect of zooplankton grazers alone), consisted of two treatments and a control each with three replicates. For the first treatment, mesozooplankton grazers (> 160 μm) were removed (i.e., ~ 0 organisms per L-1), and, for the second treatment, grazer density was enriched to approximately three times the natural concentration (i.e., ~ 3 x 102 organisms per L-1). For control containers, ambient zooplankton density was maintained (i.e., ~ 1 x 102 organisms per L-1). Grazer densities in the three experimental treatments were chosen to reflect the typical range in spring and summer grazer density as documented by our multiyear monitoring study (i.e., after excluding outliers, total zooplankton density during spring and summer months from 2010 – 2013 ranged from 1 organism L-1 to 3 x 102 organisms per L-1). Natural plankton assemblages and water for the microcosms were collected at site 6 using a vertical polycarbonate Van Dorn water sampler (2.2 L) thoroughly rinsed with bay water prior to collection. Sample water was either poured directly into acid-cleaned buckets (i.e., for treatments with enriched zooplankton density and controls with ambient zooplankton density) or through a ~160 μm net into a third acid-cleaned bucket (i.e., for grazerless treatments). Because they cannot be removed by sieving as they are similar in size to phytoplankton, microzooplankton (heterotrophic organisms < 160 µm) were still present even in the grazerless treatments. For the enriched zooplankton treatment, additional zooplankton were collected with a Schindler-Patalas trap lowered 1.5 m below the water surface and transferred into the “enriched” bucket. Microcosm containers were acid-cleaned and rinsed with bay water before experiments to eliminate impurities. Containers were sequentially filled from the respective buckets a third at a time to improve initial homogeneity among containers (Carter et al. 2005). The order of filling the containers was random, and an air bubble was left in the containers to aid in mixing by wave action. All samples were weighted with a 15 mL centrifuge tube filled with steel shot, attached to a rope in random order, and suspended from a surface buoy that was anchored to the sea floor on a 40 kg concrete block. The surface buoy and weights allowed the bottles to remain at ~1 m depth, regardless of the tide.

The third experiment (i.e., the experiment designed to assess the effects and interactions of both nutrients and zooplankton grazers) was arranged in a 23 full-factorial design with eight manipulation types: no added nutrients/ambient zooplankton density, added nitrogen (0.5 *µ*mol l-1 as NaNO3)/ambient zooplankton, added iron (1.0 x 10-3 *µ*mol l-1 as Fe2(SO4)3)/ambient zooplankton, added iron and nitrogen (0.5 and 1.0 x 10-3 *µ*mol l-1, respectively)/ambient zooplankton, no added nutrients/mesozooplankton absent (water collected was sieved through a ~160 μg net to remove mesozooplankton grazers), added nitrogen/mesozooplankton absent, added iron/mesozooplankton absent, added nitrogen and iron/mesozooplankton absent. Each treatment consisted of three replicates. Samples were collected and microcosms were prepared using the same methods as described above for the first two experiments, and nutrients were added to containers, alone or in combination, on day 0 as a single pulse as described above.

*Sample Analyses*

 For the first two experiments, phytoplankton and zooplankton abundance were determined before and after the experiments. For the third experiment, phytoplankton and zooplankton abundance, nitrate (NO3-), and ferrous iron (Fef) were determined before and after the experiments. Because analyses of our three-year monitoring dataset indicated that dissolved N:P and Si:N ratios are important factors governing phytoplankton species composition and bloom development in Raritan Bay, particularly in spring, SRP and silicon (Si) were also determined before and after experiments so that the effect of nutrient ratios could also be analyzed.

 Phytoplankton samples were poured into amber bottles and preserved with acidic Lugol’s solution on site. Fresh, unpreserved phytoplankton samples were also brought back to the laboratory to aid microscopic identification. Zooplankton samples were preserved with 4% buffered formalin. All plankton samples were held at 4°C until analysis. Samples for nutrient analyses were poured into acid-cleaned bottles, maintained in darkness on ice for transport to the laboratory, and refrigerated or frozen as appropriate until analysis (EPA, 1993). Nutrient analyses were performed using standard methods (American Public Health Association [APHA], 2012). Concentrations of all nutrients were determined colorimetrically with a HACH (Loveland, CO) DR/2500 spectrophotometer. Accuracy checks, including the use of reagent blanks and standard solution adjusts, followed HACH procedures (Hach Manual for DR/2500 spectrophotometer). NO3- was determined within 12 h of collection using the method of cadmium reduction, and the 1, 1, 10 phenanthroline method was used to determine ferrous iron (hereinafter just called iron). Samples for analysis of iron were analyzed as soon as possible after collection (i.e., within 8 h) to prevent oxidation of ferrous to ferric iron. Samples for analysis of SRP were first filtered in the field with a Whatman Puradisc 0.45 micron syringe filter and analyzed within 48 h using the ascorbic acid-molybdate blue method, and Si was determined using the silicomolybdate method.

 Phytoplankton counts were conducted using a Palmer Maloney cell (X400) under a Leica DM 1000 phase contrast microscope. Cells were identified and counted until at least 100 specimens of the most common species were counted, and taxa abundances, including colonial and filamentous forms, were quantified by enumerating single cells. Identifications were taken to the lowest taxonomic level. Many identifications were taken to genus or species level, but for some flagellates, individual genera were difficult to consistently differentiate from one another in preserved samples and were mostly identified to the class level (i.e., Cryptophyceae, Prymnesiophyceae, Prasinophyceae, Raphidophyceae). *Pfiesteria piscicida* Steidinger and Burkholder, *Pfiesteria shumwayae* Glasgow and Burkholder, cryptoperidiniopsoids, and other physically similar taxa were classified as “*Pfiesteria*-like” under light microscopy (Seaborn et al. 2006). For enumeration of zooplankton, a subsample of ~10% of the total sample was examined (Goswami 2004). Counts were conducted using a Sedgewick Rafter counting cell (100X), also under a Leica DM 1000 phase contrast microscope. Identifications were taken to genus or species level according to Todd et al.(2006) and Johnson and Allen (2005). Juvenile or nauplii stages of zooplankton were counted separately from their adult stage because of differential feeding patterns (Johnson and Allen, 2005) and difficulty in identifying them to genus level under light microscopy.

*Statistical Analyses*

For the enclosure experiments conducted in 2010, a one-way ANOVA (*p<*0.05) was used to compare the effect of zooplankton abundance on change in phytoplankton abundance and species richness between grazerless treatments (n=3), ambient grazer treatments (n=3), and enriched grazer treatments (n=3). Change was calculated as final abundance/initial abundance or final richness/initial richness. To evaluate compositional changes in phytoplankton among the three treatments (i.e., grazerless, ambient, and enriched), changes in abundance of cyanobacteria, diatoms, dinoflagellates, other flagellates, and HAB taxa were also used as dependent variables. HAB taxa are those that have been identified as capable of causing harmful algal blooms (i.e., they cause harm either through toxin production, cell physical structure, or accumulated biomass). If a significant effect was determined using a one-way ANOVA, than Tukey’s HSD (honest significant difference) tests were used to make post hoc comparisons among the three zooplankton treatments.

For the enclosure experiment conducted in 2012, a three-way ANOVA was used with the independent factors being experimental nitrate level (ambient and +0.5 *µ*mol l-1), experimental iron level (ambient and +1.0 x 10-3 *µ*mol l-1), and grazer density (ambient and absent). The dependent variables were change in total phytoplankton abundance, species richness, diatom abundance, dinoflagellate abundance, and the abundance of HAB taxa. Cyanobacteria and other flagellates were very low in abundance in the initial samples from these microcosms and therefore were not included as dependent variables for this experiment. Data were square root transformed prior to analysis to fulfill the requirements of normality (tested by Shapiro-Wilk’s W Test) and homogeneity of variances.

In addition to using a three-way ANOVA to examine the main effects and interactions of zooplankton abundance, nitrate concentration, and iron concentration on phytoplankton abundance and functional groups, indicator species analysis (Dufrêne and Legendre 1997) was performed on the phytoplankton dataset (29 taxa; log-transformed change in abundance) by microcosm matrix (8 treatments x 3 replicates = 24 microcosms) in order to examine compositional changes at the species and genus level. Phytoplankton indicator taxa (abundant and consistently “faithful” to a particular group; significant index value p ≤ 0.05) were determined by running indicator species analysis in PC-ORD version 5.31 software separately by zooplankton abundance (groups = ambient zooplankton and absent zooplankton), nitrate concentration (groups = ambient nitrate and enriched nitrate), and iron concentration (groups = ambient iron and enriched iron).

RESULTS

*In Situ Environmental Conditions*

During the enclosure experiments conducted in July and September 2010, drought conditions were affecting the coastal counties of the Raritan River Basin (Office of the New Jersey State Climatologist [ONJSC], 2010). As a result, Raritan River discharge rates were 6.7 m3 s-1 for Julyand 5.7 m3 s-1 for September, which is below average (monthly averages measured by United States Geological Survey at Bound Brook ~ 20 km upstream from study area are 18.9 m3 s-1 for July and 21.0 m3 s-1 for September). Ambient surface temperatures during summer 2010 at site 6 averaged 22°C, dissolved oxygen concentrations averaged 9.0 mg L-1, nitrate concentrations averaged 6.5 *µ*mol NO3 l-1, SRP concentrations averaged 0.4 *µ*mol SRPl-1, and total zooplankton density averaged 1 x 102 organisms per L-1. These conditions are typical of this system during summer months (Rothenberger et al. 2013). However, salinity, which averaged 34 during summer 2010, was higher than average and most likely reflected reduced river discharge rates associated with drought conditions (Rothenberger et al. 2013).

During the enclosure experiment conducted in May 2013, precipitation totals and river discharge were near average (Office of the New Jersey State Climatologist [ONJSC], 2013). Ambient temperatures during May 2013 at site 6 were 14°C, salinity was 27, dissolved oxygen concentration was 10.0 mg L-1, ferrous iron concentration was 0.9 *µ*mol l-1, and total zooplankton density was 50 organisms per L-1. Again, these conditions are near average for this system during late spring (Rothenberger et al. 2013). However, nitrate concentration in May 2013 at site 6 was 21.5 *µ*mol NO3 l-1 and SRP concentration was 0.6 *µ*mol SRPl-1. These concentrations for nitrate and SRP are already three and two times higher respectively than the spring average (Rothenberger et al. 2013). Because ambient nitrate concentrations were already higher than average, treatments with added nitrogen received just 0.5 *µ*mol l-1 as NaNO3 to make nitrate concentration roughly comparable to maximum nitrate concentrations detected for spring in this system.

*Effect of zooplankton grazers alone*

In the microcosms, increasing zooplankton abundance caused a significant decrease in total phytoplankton abundance (Fig. 2, Tables 1 and 2). Although zooplankton abundance did not have a significant effect on phytoplankton species richness (Table 1), the number of species in the microcosms did decrease in the ambient and enriched treatments (Table 2). The change in abundance of cyanobacteria, diatoms, and HAB taxa was positive (i.e., final abundance was greater than initial abundance). However, the magnitude of the positive change for each of these algal groups decreased with increasing zooplankton abundance (Table 2). In contrast, dinoflagellates decreased in abundance in microcosms during the one week incubation period (i.e., initial abundance was greater than final abundance, Table 2). However, statistical analyses indicated that zooplankton abundance did not have a significant effect on the abundance of cyanobacteria, diatoms, dinoflagellates, or HAB taxa (Table 1). After one week incubation, treatments with enriched grazer densities did have a significant reduction in the abundance of other flagellates, which included chlorophytes, cryptomonads, euglenoids, haptophytes, and raphidophytes in this study (Tables 1 and 2). A closer examination of change in the abundance of other flagellates indicates that, while there was no significant difference in the change in cryptomonad, euglenoid, haptophyte, and raphidophyte density among treatments, chlorophyte density was significantly reduced when zooplankton were enriched (p < 0.01).

*Effects and interactions of both grazers and nutrients*

There was a significant effect of zooplankton abundance on phytoplankton abundance and composition (Table 3). There was a significantly greater increase in total phytoplankton abundance when zooplankton were absent from the microcosms (p < 0.0001) (Fig. 3A). Zooplankton abundance did not have a significant effect on phytoplankton species richness or diatom abundance, but the removal of zooplankton from the microcosms resulted in a significantly greater increase in dinoflagellates and HAB taxa (Table 3, Figs. 3D and E). Indicator species analysis suggested that the best indicators of lowered zooplankton abundance, or taxa that were consistently abundant in microcosms in which mesozooplankton grazers were removed, were large centric and chain-forming diatoms (i.e., *Coscinodiscus* spp. and *Leptocylindrus minimus* Gran), *Cylindrotheca closterium* (Ehrenberg), and several dinoflagellates, including the HAB taxa *Heterocapsa triquetra*, *Karlodinium* spp., and *Scrippsiella trochoidea* (Table 4).

There was also a significant effect of nitrate addition and iron addition on phytoplankton abundance and composition (Table 3). Interestingly, there was a significantly greater increase in total phytoplankton abundance and diatom abundance at ambient nitrate concentrations (p < 0.01) (Figs. 3A and C). However, dinoflagellates and HAB taxa increased to a significantly greater extent when nitrate was enriched (p < 0.05) (Figs. 3D and E). Various pennate diatoms and green flagellates in the genus *Pyramimonas* were indicators of ambient nitrate concentrations, whereas centric and chain-forming diatoms were indicators of enriched nitrate concentrations (Table 4). Potentially harmful dinoflagellates in the genus *Dinophysis* also emerged as indicators of enriched nitrate concentrations (Table 4). Iron addition also resulted in a significantly greater increase in total phytoplankton abundance and the abundance of dinoflagellates and HAB taxa (p < 0.0001) (Table 3, Figs. 3A, D, and E). Again, various diatoms and *Pyramimonas* spp. were indicators of ambient iron concentrations and centric diatoms and *Dinophysis* spp. were indicators of enriched iron concentrations (Table 4).

Results of the three-wayANOVA also indicate that interactions among the experimental factors produced significant changes in phytoplankton abundance and composition (Table 3). There was a significant two-way interaction of zooplankton abundance and nitrate concentration on total phytoplankton abundance and diatom abundance (p < 0.05) (Table 3). The pattern of this interaction was that total phytoplankton and diatom abundance increased to a greater extent in microcosms with ambient nitrate concentrations, and this effect was stronger when zooplankton were removed from microcosms (Figs. 3A and C). There was also a significant two-way interaction of zooplankton abundance and iron concentration on total phytoplankton abundance, dinoflagelles and HAB taxa (p < 0.05) (Table 3). When microcosms contained ambient concentrations of zooplankton, the difference in the change in phytoplankton abundance and composition between ambient iron and iron enriched microcosms was small (Figs. 3A, D, and E). However, the removal of zooplankton and addition of iron interacted to significantly alter phytoplankton assemblages. When zooplankton were removed, the addition of iron resulted in a significantly greater increase in total phytoplankton abundance (Fig. 3A). There was also a significant interaction of nitrate and iron concentration on phytoplankton composition (Table 3), but the results of this interaction differed depending on the algal group (Fig. 3). For example, the change in diatom abundance was similar in ambient and enriched nitrate treatments when iron concentrations were low (Fig. 3C). However, diatoms increased to a greater extent in microcosms in which nitrate was at ambient levels and iron was enriched (Fig. 3C). On the other hand, dinoflagellates and HAB taxa increased to a greater extent in microcosms in which both nitrate and iron were enriched (Figs. 3D and E).

Finally, there was a significant three-way interaction on all of the dependent variables measured (Table 3). For each of the dependent variables, the pattern that resulted from nutrient enrichment was magnified when zooplankton were removed from the microcosms (Fig. 3). At ambient zooplankton concentrations, nutrient enrichment had little effect on phytoplankton abundance and composition (Fig. 3). When zooplankton are removed, however, enriched concentrations of both nutrients resulted in a significant increase in dinoflagellates and HAB taxa whereas enriching iron but not nitrate concentrations resulted in a significant increase in diatoms (Fig. 3C, D, and E).

DISCUSSION

Our water quality monitoring study in Raritan Bay represents one of the few available studies, and the first for the Raritan Bay ecosystem, to include accurate and consistent data over a 4-year period (i.e., data collection began in April 2010 and is ongoing) on phytoplankton assemblage composition in relation to both nutrients and zooplankton composition. Although our multiyear dataset cannot yet be considered “long-term” (i.e., 10-40 years), it has been valuable both for documenting seasonal, spatial, and temporal change in this system and for generating and analyzing testable hypotheses. For example, the results of multivariate analyses led to the following hypotheses regarding plankton dynamics in Raritan Bay: 1) abundance of HAB species is associated with reduced grazing pressure, 2) river discharge and Si:N ratios, particularly during spring months, are positively related to diatom abundance and negatively related to abundance of flagellates, including certain HAB species (e.g., *Heterosigma akashiwo*, *Prorocentrum minimum*, *Pfiesteria*-like species), and 3) iron is not an important predictor of phytoplankton species composition in this system (Rothenberger et al. 2013). The microcosm experiments described here were driven by these hypotheses and verified by the results of the larger field program.

In sum, the results of these *in situ* microcosm experiments indicate that both top-down (lowered grazing rates) and bottom-up (enriched nutrient concentrations) factors can cause significant changes in the total abundance and species composition of phytoplankton assemblages in Raritan Bay. These general findings are not surprising, as they have been confirmed by a large number of similar studies in other systems (e.g., Vanni 1987, Yasuno et al. 1993, Gonzalez 2000, Gobler et al. 2002, Carter et al. 2005). However, closer examination of how controlled manipulations of grazer abundance and nutrient concentrations interacted to alter the relative abundance of phytoplankton groups and individual taxa revealed some interesting trends.

First, results indicate that, at ambient and reduced concentrations of mesozooplankton, there was always a net increase in total phytoplankton abundance. The net increase in most algal groups, however, decreased with increasing mesozooplankton abundance. This finding suggests that, although zooplankton exert top-down control of phytoplankton abundance, the rate of grazing in Raritan Bay generally lags behind the rate of phytoplankton growth. In addition, low levels of zooplankton grazing may even have a stimulatory effect on phytoplankton abundance through nutrient cycling and regeneration (Bergquist and Carpenter 1986, Flynn and Fielder 1989). It was only when mesozooplankton were enriched to approximately three times the natural concentration in the first two experiments that there was a significant net decrease in total phytoplankton abundance. In particular, chlamydomonad-like species were significantly reduced. No much of a change in species composition. L. minimus did well when zoops lower. Cyanobacteria when higher. Why small flagellates reduced? Microzooplankton also enriched in enriched treatments. Are copepods grazing on small algae, or when we increase copepods, do we initiate a cascade effect? But, ciliates and rotifers also 3 times greater in enriched treatments. Opposite of what would expect? Meso up, micro down, small edible up…Hypothesis accept?

In the third experiment, mesozooplankton abundance was either maintained at ambient concentrations or removed. Again, there was always a net increase in phytoplankton abundance, and the net increase in phytoplankton abundance was significantly greater when mesozooplankton were removed from microcosms. This outcome was expected; however, the interaction between lowered grazer abundance and enriched nutrients had an interesting effect on phytoplankton assemblages. First, the pattern that resulted from nutrient enrichment (described below) was magnified when zooplankton were removed from the microcosms. This finding may indicate that zooplankton grazing can mask the effects of nutrient loadings on phytoplankton community structure. When mesozooplankton were present in the microcosms, the effect of enriching nitrate and iron on phytoplankton abundance and composition was minimal. When mesozooplankton were removed, nutrient enrichment resulted in significant changes in the phytoplankton assemblage. In other words, the nutrient pool was always selecting for growth of a particular group of species, but in the presence of strong top-down control exerted by grazers, the relationship between nutrients and phytoplankton abundance and composition was not as apparent (Reynolds 1984, Sterner 1989, Cottingham and Schindler 2000, Glibert and Burkholder 2006).

The nutrient pool influenced whether diatoms or dinoflagellates became dominant, especially when grazer abundance was lowered. Ambient nitrate and iron concentrations and higher Si:N ratios were associated with increases in the abundance of pennate diatoms, particularly the chain-forming *Asterionellopsis glacialis* (Castracane) Round. Enriched iron, but not nitrate concentrations, was associated with a significant increase in centric and chain-forming diatoms, including *Coscinodiscus* spp., *Skeletonema costatum* (Greville) P.T. Cleve, and *Thalassiosira gravida* P.T. Cleve. The diatom *Leptocylindrus minimus* Gran, however, emerged as an indicator of enriched nitrate concentrations. When mesozooplankton were removed, enriched concentrations of both nitrate and iron and lowered Si:N ratios were associated with increases in dinoflagellates, including the HAB taxa *Heterocapsa triquetra* (Ehrenberg) Stein and *Dinophysis* spp. These findings are consistent with spatial and temporal patterns that have emerged through ordination of our multiyear monitoring dataset. Multivariate analyses indicated that pennate diatoms, such as *A. glacialis* and *Navicula* spp., are more abundant in Raritan Bay in spring when river discharge rates and Si:N ratios are higher (Rothenberger et al. 2013). Likewise, lowered river discharge rates and Si:N ratios and enriched nitrate and SRP concentrations are associated with increased abundance of certain dinoflagellates and the centric diatom *L. minimus* (Rothenberger et al. 2013).

The phytoplankton taxa that emerged as indicators of lower nitrate and iron concentrations are generally smaller in size with greater surface-to-volume ratios. Previous laboratory and field studies have demonstrated that small size is generally associated with higher maximum growth rates (Sarthou et al. 2005) and lower half-saturation constants for uptake of both nitrate (Eppley et al. 1969) and iron (Timmermans et al. 2004). Therefore, small cells or cells with high surface-to-volume ratios with greater efficiency in nutrient uptake may have had the competitive advantage when nitrate and iron concentrations were lower in the microcosms. Silica availability may also have been regulating the abundance of diatoms within microcosms. Although silica availability was not limiting (i.e., Si:N < 1, Dortch et al. 2001) at the start of the incubation period in any of the microcosms, mean Si:N ratios were lower in microcosms that were enriched with nitrogen (*M* = 4) compared with those that were not enriched with nitrogen (*M* = 8). By the end of the incubation period, Si:N ratios in the microcosms that were enriched with nitrogen dropped below 1 (*M* = ), while those that were not enriched with nitrogen maintained a Si:N ratio above 1 (*M* = ). This may explain why enrichment with iron but not nitrate led to a significant increase in most diatoms but enrichment with both iron and nitrate, and therefore a reduction in Si:N ratios, led to a significant increase in dinoflagellates. Similar shifts in phytoplankton species composition have been measured in both experimental and field monitoring studies. In enclosure experiments, diatom abundance and productivity is significantly higher in treatments amended with N, P, and Si than in treatments amended only with N and P (Egge and Aksnes 1992, Domingues et al. 2011). Long-term field studies in eutrophic estuaries have also documented a decline in the relative abundance of diatoms and increase in the relative abundance of flagellates as N and P (but not Si) concentrations increase (Conley et al. 1993).

*L. minimus* was one diatom species that did not seem to follow this overall pattern for diatoms in our microcosm experiments in Raritan Bay. One possible explanation is that *Leptocylindrus* has a higher half-saturation constant (Ks) for uptake of nitrate than other Raritan Bay diatoms and therefore reproduces more effectively at higher nitrate concentrations (Eppley et al. 1969). In addition, studies suggest that *Leptocylindrus* has a significantly lower level of silicification than some of the other seasonally abundant diatoms in Raritan Bay (i.e., *A. glacialis*, *Coscinodiscus* spp., *S. costatum*, and *Thalassiosira* spp.; Rousseau et al. 2002). This may explain why *L. minimus* was more abundant, and therefore an indicator species, of enriched nitrate and lowered Si:N ratios.

The appearance of *Dinophysis* spp., a group of species capable of causing diarrhetic shelfish poisoning (DSP, Yasumoto et al. 1980), as indicators of enriched nitrate and iron is also notable. Our monitoring dataset indicates that, from 2010 through 2014, *Dinophysis* spp. only appeared in samples collected from Raritan Bay in either May or June (i.e., the same time period that the third enclosure experiment was initiated). This finding is consistent with monitoring data for this species in other systems where peak cell densities were recorded in early summer when temperature increased and stratification of the water column was established (e.g., Séchet et al. 1990). In addition, *Dinophysis* spp. never exceeded a concentration greater than 3 x 102 cells ml-1 in Raritan Bay, which is consistent with other studies that have recognized this group for their relatively low growth rates (Smayda and Reynolds 2001). Although *Dinophysis* spp. abundance was also relatively low in experimental microcosms (i.e., < 3 x 102 cells ml-1), net increase of *Dinophysis* spp. was higher in treatments amended with *both* nitrate and iron regardless of whether mesozooplankton were present or not. This finding is somewhat difficult to explain given that previous studies have found no relationship between *Dinophysis* spp. growth and availability of dissolved inorganic nutrients (Delmas et al. 1992). *Dinophysis* spp. are known to be obligate mixotrophs that feed on ciliates, particularly *Mesodinium rubrum*, by myzocytosis (Jacobson and Andersen 1994, Kim et al. 2008, Reguera et al. 2012). Closer examination of the data indicates that there was not a significant difference in ciliate density between microcosms enriched with both nutrients and those that were not enriched with both nutrients. It is possible that the observed positive relationship between nutrient concentrations (i.e., both nitrate and iron) and *Dinophysis* cell density, and cell density of mixotrophic and heterotrophic dinoflagellates in general, is an indirect one. The nutrition of *natural* populations of *Dinophysis* is still not well understood (Reguera et al. 2012), and other mixotrophic and heterotrohic dinoflagellates are known to feed on a diverse array of taxa including bacteria, cryptophytes, haptophytes, chlorophytes, raphidophytes, diatoms, and other dinoflagellates (Jeong et al. 2010). Increases in both nitrate and iron in microcosms could have led to initial increases in various prey items such as bacteria, dinoflagellates, and other flagellates, which in turn increased the growth rates of ciliates and mixotrophic dinoflagellates, including *Dinophysis* spp. Since we only measured cell densities at the start and end of the microcosm experiment, it is not possible to examine finer-scale spatio-temporal predator-prey dynamics. One other possibility is simply that *Dinophysis* density was inaccurately quantified in microcosm samples. Low cell densities are a common feature of *Dinophysis*, and this feature of its biology makes it difficult to acquire accurate quantitative information (Reguera et al. 2012).

Finally, the hypothesis that iron is not an important predictor of phytoplankton species composition in this system does not seem to be supported by the results of this microcosm study. This finding is interesting considering the conflicting opinions regarding the importance of iron in regulating coastal phytoplankton dynamics. It is well known that iron additions in open-ocean environments stimulate phytoplankton productivity and bloom potential (e.g., Martin and Fitzwater 1988, Coale et al. 1996). By comparison, it has been suggested that iron is unlikely to exert any serious regulation on phytoplankton dynamics in coastal ecosystems such as Raritan Bay since the amount of iron supplied by terrestrial soils is relatively high (e.g., Martin et al. 1989, Reynolds 2006). Yet, episodic pulses of iron from land drainage or sediment resuspension have been implicated in red tide dinoflagellate blooms (Doucette and Harrison 1991). Other studies suggest that nitrate stimulation of coastal phytoplankton production is enhanced in the presence of iron and that this synergistic interaction reflects the essential role of iron in nitrate reduction (Paerl 1997). In this study, additions of iron to microcosms lead to significant increases in overall phytoplankton abundance, and, as described above, interactions between iron and nitrate enrichment determined whether diatoms or dinoflagellates increased in abundance. Our monitoring dataset indicates that late spring and early summer phytoplankton assemblages are dominated by certain diatoms (i.e., *A. glacialis*, *D. brightwellii*, *Coscinodiscus* spp., and *Leptocylindrus*) and, to a much lesser extent, dinoflagellates (i.e., *Dinophysis* spp., *Hetercapsa triquetra*, *H. rotundata*). This microcosm study suggests that episodic pulses of both nitrate and iron, either from land drainage, sediment resuspension or heavy rainfall, would shift spring phytoplankton assemblages in Raritan Bay toward greater dinoflagellate dominance.

*Conclusions*

 While it is often difficult to extrapolate experimental results to system-level scenarios due to the complexity of the forces governing phytoplankton dynamics in estuaries, our approach – combining routine field monitoring (Rothenberger et al. 2013) with controlled microcosm experiments (this study) – synthesizes evidence from a variety of sources. Our small-volume, short-term nutrient addition experiments indicate that shifts in phytoplankton species composition following nutrient enrichment were magnified when mesozooplankton were removed from microcosms, enrichment with iron only resulted in significant increases in diatoms, and enrichment with both iron and nitrate resulted in significant increases in dinoflagellates and the centric diatom *L. minimus*. The results of the microcosm experiments mostly confirm the patterns that have emerged through ordination of our multiyear monitoring dataset. Phytoplankton blooms have coincided with periods of lowered zooplankton biomass, and nitrate concentrations and Si:N ratios are important factors governing phytotplankton dynamics in Raritan Bay, especially in spring. Lower nitrate concentrations and higher Si:N ratios are correlated with increased abundance of diatoms such as *A. glacialis* and *Thalassiosira* spp., and increased nitrate and lower Si:N ratios are correlated with increased abundance of flagellates. One surprising result of this study is the significant effect of iron on coastal phytoplankton assemblages. Based on the results of our multivariate analysis of the field dataset, iron did not seem to be an important factor explaining variation in phytoplankton structure during spring. In contrast, microcosm results suggest that small pulses of iron can affect phytoplankton growth and community structure, even in nutrient-enriched estuaries, and this finding will warrants further examination. In sum, these results demonstratethat top-down and bottom-up factors interact in complex ways to alter phytoplankton community structure and both must be considered when attempting to use monitoring data to predict a HAB.

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