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Ecological Effects of Benthic Suspension Feeding on Plankton Community Structure in Coastal Systems

A Dissertation Presented

by

Jerónimo Pan

to

The Graduate School

In Partial Fulfillment of the

Requirements

For the Degree of

Doctor of Philosophy

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Abstract of the Dissertation

Ecological Effects of Benthic Suspension Feeding on Plankton Community Structure in Coastal Systems

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This dissertation explored biological benthic-pelagic coupling in model shallow systems (coastal lagoons) currently at alternative ecological states, most likely the product of intense anthropogenic exploitation of estuarine resources (extractive shellfisheries). Currently, dominant grazers of algae include planktonic microorganisms (nanoflagellates, heterotrophic dinoflagellates, ciliates) instead of significant filtration by macrobenthos. The research focused on the role of benthic suspension-feeding animals in modulating composition, trophic structure, and ecological processes in the planktonic food web at current and hypothetically-increased population densities that would result from restoration.

Firstly, laboratory experiments explored the potential of marine mussels (*Geukensia demissa*, *Mytilus edulis*) and clams (*Mercenaria mercenaria*) in grazing and assimilating toxic benthic (*Amphidinium carterae*) and planktonic (*Prorocentrum minimum*) dinoflagellates, and another harmful algal species (*Aureococcus anophagefferens*). Most bivalves tested had the physiological capacity to clear harmful, bloom-producing microalgae.

A second stage specifically looked into potential top-down controls exerted by ribbed mussels *G. demissa* on shallow coastal systems. Microcosm field experiments (0.06 m^3) were run with ambient seawater from Long Island bays differing in planktonic biomass and compositional structure. Overall, the results indicated that, when subjected to a mixture of sizes and types of food items, including heterotrophs and toxic algae, the ribbed mussel behaved mostly as a non-selective feeder. When present at high abundances, ribbed mussels might therefore have the potential to improve general water quality.

Finally, mesoscale (0.4 m^3) field incubations incorporated commercial and non-commercial bivalves (*M. mercenaria*, *G. demissa*) and a recently introduced invasive colonial ascidian (*Didemnum vexillum*) at varying densities, to assess the potential ecological effects of increased benthic suspension-feeding on the current (alternative-state) structure of a coastal lagoon. The response of several planktonic components (picocyanobacteria, picoeukaryotes, auto- and heterotrophic nano- and microplankton, and micrometazoans) was analyzed. In general, the macrobenthic assemblages had interactive effects on the structure (biomass, composition) and functioning (growth rates of primary producers, and growth and grazing rates of nano- and microheterotrophs) of the plankton community.

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Acknowledgments

A long, long time ago… I can still remember
How that music used to make me smile
And I knew if I had my chance
That I could make those people dance
And maybe they'd be happy for a while...
(D. McLean, American Pie)

It's so strange, when you're down
And lying on the floor
How you rise, shake your head
Get up and ask for more.
(B. Andersson & B. Ulvæus, When all is said and done)

If I can make it there
I'm gonna make it anywhere
It's up to you...
(F. Ebb, New York, New York)

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GENERAL INTRODUCTION

This dissertation focused on the ecological effects of benthic suspension feeding on plankton community structure in coastal systems. The coastal systems in question were lagoonal estuaries (Great South Bay, Quantuck Bay), and other shallow embayments (West Neck Bay, Flax Pond) in NY that have experienced declining shellfish harvests and large reductions in other bivalve abundances.

Probably the most studied of the systems mentioned above, the Great South Bay has been in an ecological state that does not support large, functionally significant benthic suspension feeders for the past >30 years. The Great South Bay supported a significant American oyster (*Crassostrea virginica*) commercial fishery from 1880s-1940s that collapsed after peak landings of 4,630 metric tons in 1938 (McHugh and Williams, 1976). The causes for the decline were linked to significant changes in circulation patterns and the dominance and persistence of small (2-4 μm) chlorophytes (e.g. *Nannochloris atomus* and *Stichococcus* spp.), attributed to the input of organic nitrogenous wastes (ammonium, urea) from a land-based duck industry (Ryther, 1954). After the collapse of the oyster fishery, exploitation turned to hard clams (*Mercenaria mercenaria*), which soon constituted the principal commercial fishery for NY. In the early 1970s, NY provided more than 60% of the American market demand for hard clams (coming mostly from extractive practices on natural populations in the Great South Bay). *M. mercenaria* commercial landings reached their annual peak in 1976 at over 3,932 metric tons (McHugh, 1977). Recreational and subsistence catches were also substantial (e.g. the estimated recreational catches in 1974 for NY and NJ together was about 1,247 metric tons, or ~34% of commercial landings; McHugh, 1979). Soon after, hard clam landings in NY started dropping dramatically and never recovered to previous levels.

Among the biological causes, bottom-up effects such as changes in plankton composition and dynamics (Weiss et al., 2007), together with the invasion and recurrence of brown tides caused by the pelagophyte *Aureococcus anophagefferens* since the mid-1980s (reviewed by Bricelj and Lonsdale, 1997; Gobler et al., 2005), have been cited.

The ribbed mussel, *Geukensia demissa*, constitutes another example of a suspension-feeding shellfish that has declined. Ribbed mussels are a keystone species in the *Spartina alterniflora* marshes fringing coastal embayments (Bertness, 1984), and the

current trend for these fringing marshes indicates substantial area loss (Valiela et al., 2004). Thus, it is reasonable to expect a comparable loss of ribbed mussels.

At high densities suspension-feeding bivalves provide a number of positive ecosystem services that include reduced turbidity and an increase light penetration, facilitating the establishment of seagrass beds and recruitment grounds for fin- and shellfish; prevention of harmful algal blooms; removal of nutrients by burial of nitrogen and phosphorus in the form of biodeposits and the ultimate removal of nitrogen from the system via denitrification (reviewed by Dame, 1996; Prins et al., 1998; Newell et al., 2002; Newell, 2004). It is therefore understandable that restoration efforts (e.g. a hard clam re-stocking initiative currently under course by The Nature Conservancy) aim to achieve a state that provides ecosystem services similar to those in the past.

In the current state there appears to be no tendency for benthic suspension-feeding metazoans to recover to their former abundances or influences to the systems in question. The systems' current degraded and undesirable state appears to be stable and resilient on its own (*sensu* Boesch and Goldman, 2009). The restoration of a functionally significant benthic suspension-feeding population would probably require a large-scale perturbation.

Many estuarine systems that have gone through substantial reductions in benthic biomass (e.g. Chesapeake Bay; Newell, 1988; Jackson et al., 2001; Boesch and Goldman, 2009) have experienced an ecological shift towards a new stable state dominated by water column microbial processes and an increased planktonic secondary production. For example, the dominant grazers in the Great South Bay are now protists, mainly heterotrophic nanoflagellates and ciliates (Boissoneault-Cellineri et al., 2001; Deonarine et al., 2006). These planktonic grazers exhibit considerable feeding selectivity compared to benthic suspension feeders, and are capable of inducing compositional changes in the plankton community (Stoecker et al., 1986; Griffin and Rippingale, 2001; Gobler et al., 2004b). Primary productivity levels for Great South Bay are high as in most other coastal lagoons (Kjerfve, 1994), with ultraphytoplankton (<5 μm) dominating biomass over microplankton (>20 μm; Lonsdale et al., 1996b; Sieracki et al., 2004; Lonsdale et al., 2006). Under the present conditions there is abundant phytoplankton biomass, but it does not seem to be readily available to large metazoan consumers, partly because of size-

related issues (i.e. cells might fall below the optimum particle range to be efficiently processed by suspension feeders; Bricelj and Malouf, 1984).

The main objectives of this dissertation were:

1) to examine how a non-selective bivalve suspension feeder (the ribbed mussel, *Geukensia demissa*) processes different components of the current plankton community of coastal bays in NY differing in structure and community composition.

2) to test whether *Geukensia demissa* filtration physiology is affected by the brown tide organism, *Aureococcus anophagefferens*, from laboratory-cultured and wild-type sources.

3) to study the potential of the mussels *Geukensia demissa* and *Mytilus edulis*, and the clam, *Mercenaria mercenaria*, to clear bloom-forming and potentially toxic dinoflagellates (*Prorocentrum minimum*, *Amphidinium carterae*) that are recurrently found in NY embayments.

4) to study how the current planktonic community of Great South Bay responds to increased densities of benthic suspension feeders, and comparatively study how benthic suspension feeders may alter planktonic food web structure in single-species and multi-species assemblages, testing for additive or interactive effects in the latter.

5) to study how changes in planktonic composition exerted by increased densities of benthic suspension feeders are reflected in ecological rates within the pelagic food web of the Great South Bay (determining phytoplankton growth rates, and microzooplankton community grazing and growth).

The first objective was explored through short-term microcosm grazing experiments with *G. demissa* monitoring changes in biomass and composition of different components of natural planktonic communities (Chapter 1). For the second objective, feeding experiments were conducted with ribbed mussels exposed to mixtures of a microalga known to support good bivalve growth (*Isochrysis galbana*, clone CCMP 1323), and *Aureococcus anophagefferens* (CCMP 1708) proportional gradient with a constant C content. Additionally, ribbed mussels were exposed to dilutions of a brown tide to test feeding responses with a wild-type *A. anophagefferens* (Chapter 2). Laboratory feeding experiments were also conducted for the third objective (Chapter 3),

with mussels and the hard clam exposed to different proportions of mixtures of *I. galbana* (CCMP 1323) and either one of the toxic dinoflagellates *Prorocentrum minimum* (CCMP 696) and *Amphidinium carterae* (CCMP 1314).

The question of what ecological interactions would arise in the pelagic food web and benthic-pelagic coupling in Great South Bay should substantial filtration by benthic organisms be reintroduced, motivated the third and fourth objectives. Mesoscale enclosure experiments with increased benthic densities of single or multiple species studied changes in biomass and structure resulting from benthic suspension feeding (Chapter 4). These long term (72- or 120-h) incubations were coupled with short (24-h) incubations in order to characterize and compare how ecological rates and processes of the planktonic community are altered as a result of the activity of suspension feeders (Chapter 5).

It is expected that the scientific knowledge produced in this project would contribute to ongoing modelling efforts of the current ecological relationships in Great South Bay. This in turn could be translated into ecosystem-based management strategies, and ecological restoration policies.

CHAPTER 1

Effects of ribbed mussel (*Geukensia demissa*) grazing on the planktonic community of coastal embayments with different trophic structure

ABSTRACT

The grazing potential of the ribbed mussel (*Geukensia demissa*) on different components of the natural planktonic community was evaluated on field microcosm experiments (0.06 m^3). Shallow coastal embayments around Long Island, NY differing in planktonic biomass and compositional structure were contrasted. No significant differences were found in clearance rates for chlorophyll fractions and microplankton taxa. However, significant differences in clearance were found for pico- and nanoplankton. Overall, the results indicate that, when subjected to a mixture of sizes and types of food items, the ribbed mussel behaves mostly as a non-selective feeder. Moreover, ribbed mussels were capable of assimilating organic matter from environments with different planktonic structure at >50% efficiencies.

INTRODUCTION

Coastal bays, lagoonal estuaries and salt-marshes on Long Island (New York) and in other coastal areas have experienced a decline in suspension-feeding bivalves and consequently there has been a shift from benthic grazing (by heavily-exploited, formerly-dense stocks of shellfish) to pelagic grazing (Jackson et al., 2001; Lonsdale et al., 1996b; Lonsdale et al., 2006). The ribbed mussel, *Geukensia demissa*, is a suspension feeder found in *Spartina alterniflora* marshes that fringe coastal bays, partially embedded in the sediment or in superficial aggregations of individuals attached by byssal threads (Franz, 1997). Ribbed mussels are known for their positive effects on the marsh cordgrass, deriving water-column nitrogen through excretion and biodeposition (Bertness, 1984). As in many other areas, the current trend of tidal salt marshes on Long Island indicates substantial area loss, and therefore it is reasonable to expect a comparable loss of ribbed mussels. Average salt marsh loss around Long Island from 1974 to the early 2000s amounted to 50% on the north shore, 35% in the Peconic Bays, and 25% on the south shore (Mushacke and Picard, 2004). Fragmentation, perimeter erosion, extension and widening of tidal creeks, subsidence and filling or wetland 'reclamation' are cited as the most common causes of loss of coastal wetlands (Valiela et al., 2004).

Many aspects of the feeding ecology of *Geukensia demissa* have been described from laboratory- and field-based experiments. Ribbed mussels typically live in detritus-dominated salt marshes, which experience significant daily (tidal) and seasonal variations in concentration and composition of seston particles (Huang et al. 2003b). This latter fact has forced mussels to be true omnivores, meeting their nutritional requirements by utilizing a wide variety of living and dead material (Kreeger and Newell, 1996). In other words, ribbed mussels can feed on broad range of particle sizes, and on non-conventional planktonic sources for bivalves. The literature provides numerous examples that ribbed mussels can ingest and assimilate carbon from bacteria and detrital aggregates (Wright et al., 1982; Langdon and Newell, 1990; Huang et al., 2003a); heterotrophic nanoflagellates (Kreeger and Newell, 1996); and refractory cellulosic material derived from vascular plants (Kreeger et al., 1988; Kreeger and Newell, 2001; Huang et al., 2003a).

By ingesting and assimilating carbon from numerous sources, ribbed mussels are part of complex trophic pathways within the salt marsh. For example, Bushaw-Newton et al. (2008) incubated heterotrophic bacteria native to a salt marsh, with ^{14}C-labelled DOM derived from *Spartina* and the invasive *Phragmites australis*. The bacterial intermediate was then fed to ribbed mussels, which were capable of assimilating bacterial carbon derived from cordgrass and reed, with 74% and 90% efficiencies, respectively. The grazing pressure exerted by ribbed mussels is also thought to cause shifts in the size distribution of the water-column microbiota (Kemp et al., 1990) to larger cells through the removal of small cells in proportion to the fraction of the total biomass.

The present study represents a field test of how *Geukensia demissa* processes different components of the natural planktonic community in water bodies with different planktonic composition and structure. Experiments were carried out to test the hypothesis that *Geukensia* is a non-selective benthic suspension feeder with the potential to alter planktonic community structure (in relation to size and taxonomic composition).

MATERIALS AND METHODS

Study sites

Experiments were conducted *in situ* at three shallow bays around Long Island (New York, USA). Locations (Figure 1.1) were selected based on planktonic structure, with the purpose of conducting a field test on grazing on ambient seawater containing different proportions of total, <20 μm and <5 μm phytoplankton fractions. Also, differences in floristic composition found in previous studies were considered for the selection of sites (Lonsdale et al., 2006; 2009).

Flax Pond (FP; 40°57' N, 73°08' W) is a 57 ha tidal marsh with influence from the adjacent Long Island Sound. It has 26 ha of *Spartina alterniflora* marsh and 27 ha of tidal channels and bare sediments (Woodwell et al., 1979). A single inlet connects the marsh to the Long Island Sound and through it, ~80% of the water at high tide is removed twice daily in the tidal flushing. There is no freshwater input beyond the precipitation that falls directly on the marsh and its small drainage basin (Woodwell et al., 1977).

West Neck Bay (WNB; 41°03' N, 72°21' W) is a shallow enclosed embayment, fringed with *Spartina alterniflora* marsh and connected to the eastern half of the Peconic Bays estuary (Dulaiova et al., 2006). There is continual exchange of water with the Peconic Estuary throughout the tidal cycle, through a long restricted channel (Gobler and Sañudo-Wilhelmy, 2001); but the limited exchange through the narrow channel yields a residence time of ~12 d (DiLorenzo and Ram, 1991).

Quantuck Bay (QB; 40°48' N, 72°36' W) is one of several interconnected coastal lagoonal estuaries along the south shore of Long Island, with an area of ~5 km^2. QB has limited flushing due to the absence of an inlet to the Atlantic Ocean, and is flushed by tidal activity through the canals connecting it to Moriches Bay to the west, and Shinnecock Bays to the east (Lomas et al., 2004). As in other estuaries along the south shore of Long Island, wind-mixing and shallow depth (1-2m in average) results in a homogenous water column (Wilson et al., 1991).

Experimental design

Experiments were conducted in 58-l (50 cm inner diameter), opaque, polypropylene cylindrical tanks filled with ambient seawater by immersion. Prior to each experiment, all tanks were scrubbed and rinsed with fresh water. The use of pumps for filling the tanks and/or sampling was avoided, since pumps disrupt some fragile

microzooplankton such as naked ciliates and dinoflagellates (James, 1991; Suzuki et al., 2002). Each tank was covered with a 0.9 cm-thick polystyrene fitted cover to limit light penetration and help keep temperature close to ambient. Empirical measurements of light attenuation made with a standard PAR quantum sensor (Skye Instruments Ltd., model SKP 200), showed that the polystyrene lids cut PAR by ~98%. Additionally, experimental tanks had a plastic grate (1 cm-tall, with 1×1 cm openings) on the bottom, on which the experimental shellfish were placed, to prevent resuspension of biodeposits when the water in the tank was mixed. Upon filling, the tanks remained *in situ*, semi-submerged in the water (~60% of tank height), and resting on the bottom of the bay, to prevent rocking which might create an experimental artifact.

Individual ribbed mussels were collected during a flood tide from wild populations at the experimental sites, carefully brushed to remove epibionts, and their byssal threads were trimmed. Mean shell heights were (mm ± SE) 71.4 ± 1.1, 70.5 ± 1.0, 71.3 ± 0.9 for FP, WNB and QB respectively. At the beginning of their normal immersion time, mussels were placed into tanks with ambient seawater and allowed to acclimate for ~30 min. Then mussels were gently transferred to new tanks, and nutrients were added to all tanks at the start of the clearance rate experiment at 2 μM nitrogen as NH_4Cl and 0.12 μM phosphorus as NaH_2PO_4 (after Granéli et al., 1993; calculations based on excretion budgets for *Geukensia* by Jordan and Valiela, 1982). The addition of nutrients compensated for the lack of mussel excretion in control tanks. Clearance rate experiments lasted 2-3 h and the water was gently agitated manually every 45 min. The normal feeding activity of mussels (e.g. opened valves, extended mantle edges; Riisgård, 1988) was checked periodically throughout the experiment. There were 3 replicate treatment tanks with 6-8 mussels each and 3 control tanks with no mussels; average mean dry tissue weight (g ± SE) per tank was 6.58 ± 0.29, 7.1 ± 0.11, 13.25 ± 0.15 for FP, WNB and QB respectively. The density of mussels was adjusted to the volume of the container and the duration of each experiment, so that chlorophyll concentration would decline sufficiently to accurately estimate clearance rates, but not allow mussels to graze below ~70% of the initial concentration. Three experiments were run between June and September 2006, and one experiment in May 2007. Environmental parameters (temperature, salinity, dissolved oxygen) were recorded at the beginning, mid-point and

end of each experiment with a handheld YSI model 85 (YSI Inc., Yellow Springs, OH). Experimental dates and environmental data are reported in Table 1.1.

Sampling, processing and calculated parameters

Bivalve clearance rates were estimated for the total, <20 µm and <5 µm components of the plankton from the decline in chlorophyll *a* between the beginning and end of the experiment. Total and size-fractionated chlorophyll *a* was calculated from duplicate 30 ml water samples for whole water, <20 µm water partitioned by gravity filtration on a Nitex mesh, and <5 µm water partitioned using a polycarbonate membrane filter. Water samples were concentrated onto Whatman GF/F filters and chlorophyll *a* was extracted in acetone for 24 h, and measured fluorometrically (Turner Designs, model 10-AU), after Arar and Collins (1997).

Initial and end-time water samples (250 ml) were collected for characterization of microplankton (20-200 µm), preserved in 10% acidic Lugol's iodine in amber jars, and stored in the dark (Stoecker et al., 1994). Microplankton samples were processed using standard settling techniques (Utermöhl, 1958; Edler and Elbrächter, 2010) and counted using an inverted light microscope (Olympus, model CK2). In most cases individual cells were counted; for colonial or filamentous algae, cells were counted when the colony was >20 µm. Taxa were identified to the lowest possible taxonomic level (Maeda and Carey, 1985; Maeda, 1986; Tomas, 1997; Taylor et al., 2003) and classified into the following taxonomic groups: euglenoids, dinoflagellates (hetero- and autotrophic), centric and pennate diatoms, loricate and aloricate (oligotrich) ciliates. Other groups found included non-euglenoid flagellates (e.g. Prymnesiophyceae, Rhaphidophyceae, Cryptophyceae, Haptophyceae, Pyramimonadales and Ebriids), radiolarea and rhizopoda; some tychopelagic or benthic species usually found within the water column of well-mixed environments were included in the counts. Standard measurements of cell linear dimensions were performed for biovolume estimations (Hillebrand et al., 1999; Sun and Liu, 2003). Standard conversion factors published in Putt and Stoecker (1989) and Strathmann (1967) were applied to estimate biomass.

Seawater samples (5 ml) for picoplankton community analysis were collected at the beginning and end of the experiment, preserved in 1% glutaraldehyde (from a 10% stock solution prepared with filtered natural seawater) and stored at 4°C until processed (Boissonneault-Cellineri et al., 2001). The densities of heterotrophic bacteria, picocyanobacteria (*Synechococcus*), photosynthetic pico-eukaryotes and small cryptophytes (2-4 µm) were estimated from these samples by flow cytometry (Becton-Dickinson, model FacsCalibur; Olson et al., 1993). Sample aliquots (0.5-2 ml) were run twice, before and after staining with SYBR green I dye (Sigma-Aldrich). Absolute counts were obtained using a known concentration of fluorescent beads (Spherotech Inc., rainbow fluorescent particles, 1.93 µm diameter). Data was analyzed with the WinMDI 2.9 software package.

At the end of each experiment, feces were collected in order to estimate bivalve absorption efficiency with the ash-free dry weight:dry weight ratio method (Conover, 1966). In the lab, feces were collected onto precombusted, preweighed Whatman GF/F filters and rinsed with distilled water; also, known volumes (~500 ml) of ambient water were filtered onto precombusted, preweighed Whatman GF/F filters to obtain estimates of organic content within the food. Filters were dried at 60ºC to constant weight (>24 h), and then ashed at 450ºC in a muffle furnace (Barnstead Thermolyne, model 1400).

Mussels were collected, dissected and dried at 60ºC for >48 h to estimate soft tissue dry weight. Clearance rates are expressed in units of dry tissue weight.

Parameters and data analysis

Clearance rates based on chlorophyll *a* concentrations and from the biomass obtained for the micro- and picoplanktonic components of the community were calculated using the equation in Coughlan (1969) as:

$$CR = \frac{V}{n}\left[\frac{(\ln C_0 - \ln C_t)}{t} - a\right] ,$$

where V is the water volume, n dry tissue weight of *Geukensia*, C_0 is the chlorophyll content or biomass at t=0, C_t is the chlorophyll content or biomass at t final, and a is the background cell growth rate (positive or negative) from control tanks without bivalves.

Differences in planktonic structure, in terms of chlorophyll *a* size fractions across and within experimental sites were analyzed with a repeated measures ANOVA (Zar, 1999), with chlorophyll fraction and site as factors, and treating the two experimental dates at WNB as different sites. Differences in chlorophyll-based CRs were analyzed with a repeated measure ANOVA with size-fraction and site as factors. *Geukensia*'s selectivity for microplanktonic food particles based on their size was analyzed by means of a 2-way repeated measures ANOVA performed on log-transformed biovolumes (μm^3), with treatment (control, *Geukensia*) and sampling time (t_0 and t_{final}) as factors. Absorption efficiencies were analyzed by means of a 1-way ANOVA, with site as nominal factor. These analyses were done with the Statistica software package (version 9).

Micro- and picoplankton biomass were treated as multivariate data. For both sets of data, a redundancy analysis (RDA) was performed to test for site effects on initial community composition, with location as nominal (qualitative) environmental variable. RDA is a direct gradient ordination technique that combines ordination of multivariate data (e.g. plankton biomass by taxa, clearance rates by taxa) with regression on the environmental data (Jongman et al., 1995). Significance of environmental variables in explaining community variation was evaluated by means of permutation tests (Manly, 1991). Biomass data were ln (x + 1) transformed. Another RDA was performed to test for differences in taxa-specific clearance rates with site as factor. Negative CRs were considered = 0 (Lonsdale et al., 2009). The statistics reported correspond to the test of significance of all canonical axes, and include λ (trace, or the sum of all eigenvalues) which represents the fraction of the variance explained by environmental variables, and the *F*-ratio and p-value. The analyses were performed with Canoco 4.5 and plots were generated with CanoDraw software packages. All values in the figures and text are reported as means ± SE.

RESULTS

The three embayments considered in this study showed significant differences in the structure of phytoplankton (Figure 1.2) and in pico- and microplanktonic biomass and composition (Figures 1.3, 1.4). Chlorophyll *a* contents in ambient whole seawater ranged

from 2.26 ± 0.06 μg l^{-1} (FP) to 10.17 ± 0.15 μg l^{-1} (QB), with intermediate values of 2.54 ± 0.12 and 3.22 ± 0.13 μg l^{-1} for WNB 2 and WNB 1, respectively. In all bays, small (< 5μm) phytoplankton dominated the autotrophic biomass. Thus, the experiments were run under conditions ranging from moderate concentrations to very high dominance by small forms of phytoplankton. The autotrophic biomass structure of the bays (related to chlorophyll *a* concentration) was significantly different in terms of size fractions and between sites (repeated measures ANOVA, size: $F(2,48)= 52.9$, $p<<0.001$; site: $F(3,24)= 1213.51$, $p<<0.001$; Figure 1.2).

Most of the variation in picoplankton was concentrated along one axis of the RDA triplot (Figure 1.3). Differences in picoplanktonic community structure were statistically significant among bays ($\lambda= 0.97$, $F= 117.74$, $p<0.01$). *Synechococcus* densities were highest at QB (5.8×10^5 cells ml^{-1}) and lowest at WNB1 ($\sim 1.5 \times 10^4$ cells ml^{-1}). Bacterioplankton and >2 μm cryptophyte densities were higher at WNB2 and QB compared to WNB1. WNB2 presented a group of picoeukaryotes (1.0×10^5 cells ml^{-1}) that was not detected for any of the other bays/dates (not included in RDA, nor triplot).

Microplankton biomass and composition also varied significantly with site ($\lambda= 0.82$, $F= 15.49$, $p<0.01$; Figure 1.4). Total (auto- and heterotrophic) microplankton biomass ranged from ~10 μg C l^{-1} in FP, to 574 μg C l^{-1} in WNB2. Except for FP, heterotrophic forms dominated the microplanktonic biomass in all bays. Oligotrich ciliates (e.g. *Strombidium* spp., *Strobilidium* spp., *Mesodinium* sp.) were dominant for both dates in WNB (63-471 μg C l^{-1}) and in QB (55 μg C l^{-1}). Unarmoured dinoflagellates (e.g. *Akashiwo sanguinea*, *Gyrodinium dominans*, *Karlodinium veneficum*, *Polykrikos kofoidii*, *Amphidinium* sp.) and armoured dinoflagellates (e.g. *Prorocentrum* spp., *Dinophysis acuminata*, *Scrippsiella* sp.), were especially abundant in WNB on both dates (41-28 μg C l^{-1}). Chain-forming centric diatoms (mostly *Leptocylindrus minimus*) and phytoflagellates (e.g. *Chroomonas* spp.) were dominant in FP (making up 7 μg C l^{-1}) and, abundant in WNB2 (making up 59 μg C l^{-1}).

Chlorophyll-based clearance rates for whole seawater ranged between 1.01 and 1.69 l h^{-1} g DW^{-1} at the experiment sites (Figure 1.5). Despite the dominance of <5 μm forms in all locations and the 4.5-fold difference in total chlorophyll *a* concentration

between the bays, there were no significant differences in clearance rate by size fraction or by site (repeated measures ANOVA, size: $F(2,20)= 0.12$, $p>0.05$, site: $F(3,10)= 0.69$, $p>0.05$). Thus, the results suggest that ribbed mussels were consistently removing phytoplankton independently of size, and size-proportions in the field; and that the elevated phytoplankton biomass in some locations (e.g. >10 µg chl *a* l^{-1}) was not affecting filtration activity of ribbed mussels.

Clearance rates of picoplankton were significantly different among bays ($\lambda= 0.65$, $F= 6.36$, $p<0.05$; Figure 1.6). Ribbed mussels at WNB1 were most activly filtering bacterioplankton (1.40 ± 0.09 l h^{-1} g DW^{-1}), *Synechococcus* (2.18 ± 0.28 l h^{-1} g DW^{-1}) and >2 µm cryptophytes (5.34 ± 0.53 l h^{-1} g DW^{-1}), a result in accordance with the highest clearance rate for the <5 µm chlorophyll fraction (1.36 ± 0.65 l h^{-1} g DW^{-1}; Figure 1.5) found for this bay. Clearance rates for heterotrophic bacteria ranged in all bays ranged from 0.78 ± 0.31 (WNB2) to 1.40 ± 0.09 l h^{-1} g DW^{-1} (WNB1); this range of clearance rates is comparable to the rate reported by Wright et al. (1982; 0.46 ± 0.03 l h^{-1} g DW^{-1}). Clearance rates for *Synechococcus* ranged from 0.46 ± 0.34 (QB) to 2.18 ± 0.28 l h^{-1} g DW^{-1} (WNB1); although they measured grazing with a different methodology, Kemp et al. (1990) also report considerable grazing by ribbed mussels on *Synechococcus*. Not surprisingly, >2 µm cryptophytes had the highest clearance rates of all microorganisms measured by flow cytometry, with a range of 1.62 ± 0.48 (QB) to 5.34 ± 0.53 l h^{-1} g DW^{-1} (WNB1). Picoeukaryotes in WNB2 were grazed at a rate of 1.08 ± 0.48 l h^{-1} g DW^{-1} (not included in RDA, nor triplot).

Biomass-based clearance rates for the different components discussed above are summarized on Table 1.2.

With the possible exception of centric diatoms, there was no clear evidence that clearance rates differed for individual components of the microplankton (Figure 1.7). Clearance rates estimated for microplankton groups were marginally non-significantly different among sites ($\lambda= 0.36$, $F= 1.68$, $p= 0.07$). Clearance rates of centric diatoms were the highest of any group (ranging from 3.1 to 7.5 l h^{-1} g DW^{-1}); however, centric diatoms were not abundant in QB, so comparing results between sites for this group was not

possible. Similarly, clearance rates of euglenoids ranged between 2.4 and 4.3 l h^{-1} g DW^{-1}, but this group was not abundant in FP, preventing comparisons between sites.

Results from a 2-way ANOVA performed on microplankton biovolumes (log-transformed) are reported on Table 1.3. In general, the outcomes of the ANOVA test were non-significant, indicating that at least for organic particles in the microplankton size range (>20 µm), ribbed mussels were not selecting on the basis of size (cell volume). The few statistically significant differences are not consistent across groups or locations, and may not be of any biological relevance. For example, the significant interaction for treatment and time for QB dinoflagellates followed by a HSD multiple comparison test, which indicated that cell volumes in tanks with *Geukensia* differed between initial (mean 942 ± 80 μm^3) and final (1324 ± 128 μm^3) sampling times. This suggested an apparent selection for smaller-sized dinoflagellate cells; however, the difference in cell volume represents just about ~25%. Statistics for euglenoids and loricate ciliates are not reported because variances were inhomogeneous; however, the outcomes of the ANOVA test were non-significant as well.

Absorption efficiencies were high (>50%) for all locations. WNB2 presented the highest efficiencies (mean ± SE) of 77 ± 2%, followed by FP (73 ± 4%), QB (66 ± 2%) and WNB1 (54 ± 1%). A one-way ANOVA showed significant differences among sites [F (3,8) =8.18, $p < 0.01$].

DISCUSSION

Differences in planktonic structure and dominance of ultraphytoplankton

The differences in planktonic structure among the three bays considered in this study can be mainly related to three factors: physical forcing, bottom-up (i.e. eutrophication), and top-down effects (i.e. the effects of grazers).

Physical forcing most likely plays a part in determining the planktonic structure of the systems considered in this study. These being relatively small embayments with limited circulation, the most evident physical forcing is residence time of the water. As previously described, FP exchanges ~80% of the water twice daily due to tidal flushing.

On the other hand, WNB has high residence time (~12 d; DiLorenzo and Ram, 1991) and a rough estimate of flushing rate for QB (tidal range:mean depth) indicates that only ~20% of the water is exchanged in each tidal cycle. Limited flushing may contribute to the development of 'unique' plankton communities, compared to those of adjacent bays (e.g. significantly higher numbers of *Aureococcus anophagefferens* cells, during brown tide events in QB; Nuzzi and Waters, 2004), and likely plays a role in retaining phytoplankton within the system, and allowing biomass levels to increase (~10 µg chl *a* l^{-1}).

The size distribution of phytoplankton characterized by a dominance of small forms (i.e. <5 µm, ultraphytoplankton) is not a novel feature in systems analogous to the ones considered in this study. For example, during 1952-53, the chlorophytes *Nannochloris atomus* and *Stichococcus* sp. (2-4 µm) dominated phytoplankton biomass reaching cell densities $>10^7$ cells ml^{-1} in Great South Bay (GSB) and Moriches Bay (Ryther, 1954). These chlorophyte blooms drastically reduced pelagic diversity and interfered with the seasonal succession of phytoplankton, remaining at high densities throughout most of the year. Their occurrence and persistence was related to human eutrophication (increased organic N, and low N:P ratios; Ryther, 1954). A survey of primary productivity in GSB during the late 1970s found a great variation in the densities of diatoms and dinoflagellates, while cryptomonad and chlorophyte species were abundant throughout the year contributing to approximately half of the total phytoplankton biomass (Lively et al., 1983). Even more recent studies (Lonsdale et al., 1996b; 2006; Sieracki et al., 2004) report that autotrophic biomass and production in GSB are often dominated by small phytoplankton (>80% of the chlorophyll *a* corresponds to organisms <5 µm).

As for the causes of this skewed distribution, the evidence is not conclusive over an eutrophic state of the systems. For instance, an increase in organic nutrients has been pointed to as an important factor in the initiation of picoplankton-dominated brown tides in QB (Nuzzi and Waters, 2004), but Cloern and Dufford's (2005) review of phytoplankton dynamics in estuarine systems emphasizes that cell size of phytoplankton communities is determined both by nutrient supply and selective grazing. Small size (high surface:volume ratio) provides a competitive advantage in nutrient-impoverished

systems (e.g. the open ocean, oligotrophic lakes), but this advantage disappears in systems where high nutrient concentrations promote selective growth of large cells. Cloern and Dufford (2005) cite the nutrient-rich San Francisco Bay estuary as an example of the latter, where phytoplankton biomass is dominated by large taxa with cells >30 μm contributing 40%, and cells <8 μm contributing only 4% of community biomass.

The causes for the dominance of ultraphytoplankton seem to lie in the relationship between bottom-up and top-down effects (and the strength of their interaction, or lack of it). At peak historical densities during the mid-1970s hard clams (*Mercenaria mercenaria*) cleared about 30-40% daily of the entire volume of GSB (Cerrato et al., 2004), and it has been well documented that, at high population densities, suspension-feeding bivalves can have a strong influence on the pelagic food webs of numerous coastal bays (Dame, 1996; Newell, 2004; Lonsdale et al., 2009). A consequence of the decline in bivalves has likely been the release of nano- and microzooplankton grazers from a direct source of competition and predation. This probably caused an ecological shift towards an alternate state that supports increased planktonic secondary production, such as in Chesapeake Bay after the demise of native oysters (Newell, 1988; Jackson et al., 2001; Boesch and Goldman, 2009).

Several studies (Boissonneault-Cellineri et al., 2001; Deonarine et al., 2006) indicate that, in the absence of historically high abundances of benthic suspension feeders, the primary consumers of small cells (<5 μm) are now protists such as nanoflagellates and ciliates (mostly <40 μm). Planktonic grazers usually are much more selective than bivalve suspension feeders, presenting increased rates of consumption of certain species of microalgae and rejection of less nutritious or toxic species (Stoecker et al., 1986; Griffin and Rippingale, 2001; Gobler et al., 2004). Consequently, it has been hypothesized that this lower predation rate provides the latter phytoplankton with a net growth advantage relative to other phytoplankton, contributing to the flourishing of noxious and nutritionally-poor species. On the other hand, providing further credence to the strength of top-down benthic influences in regulating ultraphytoplankton densities, Cerrato et al. (2004) demonstrated that at peak historical densities *M. mercenaria* hard clams could have provided an effective top-down control mechanism to prevent the initiation of brown tide blooms and/or modulate numeric outbreaks.

Non-selective grazing by ribbed mussels

Clearance rate as defined by Bayne et al. (1976) is the product of retention efficiency and pumping rate, therefore differences in clearance rate among chlorophyll size-fractions or different taxonomic groups reflect differences in retention efficiencies. The only significant differences in clearance found in this study were among pico- and nanoplanktonic groups (Figure 1.6), with clearance rates as high as 5.3 l h^{-1} g DW^{-1} for small (>2 µm) cryptophytes. Few other studies have reported suspension-feeding bivalves grazing on cryptophytes. Pastoureaud et al. (2003) described *Crassostrea gigas* grazing on a bloom of the cryptophyte *Plagioselmis prolonga*, but they provide no conclusive evidence for selective grazing.

Retention efficiencies in most suspension-feeding bivalves decrease steeply below a particle size of ~5 µm (Møhlenberg and Riisgård, 1978; Riisgård, 1988), but that does not seem to be the case for ribbed mussels, which have >75% efficiencies for 2-µm particles (Riisgård, 1988). The mechanisms of particle retention have to do with the degree of development and the distance between laterofrontal cirri in the ctenidia (Jørgensen, 1990). Wright et al. (1982) described a closer spacing of laterofrontal cirri in *Geukensia demissa* when compared to other marine mussels (every 1.57 µm), and concluded that it confers an anatomical advantage to ribbed mussels when feeding on small-size particles. This could well explain the high clearance rates found for nanoplankton, and the ability of *G. demissa* to graze phytoplankton equally well across size ranges (Figure 1.5).

The finding of no apparent size-selectivity for cells in the microplankton size-range (>20 µm) is interesting. On a first approach, it would seem reasonable to assume that an organism like *Geukensia demissa*, which has the capability to feed on a vast array of particle sizes, would tend to maximize its carbon ingestion by selecting bigger prey items. Food quality has generally been assumed to correlate directly with carbon content, and thus also with cell size (Dame, 1996), but the latter is not necessarily the case for all microplanktonic prey items. For instance, up to 85% of the cell volume in marine diatoms is occupied by the central vacuole, and diatoms generally have lower cellular carbon concentration than dinoflagellates of an equivalent volume (Strathmann, 1967;

Hitchcock, 1982; Menden-Deuer and Lessard, 2000). There are, however, other chemical cues that may influence selection of prey items, and have little to do with size-selectivity. For example, Beninger and Descottignies (2005) demonstrated that centric diatoms possess an organic coating (the perifrustular envelope) that renders the cells more palatable for scallops, independently of the carbon content of the cell. In this study, clearance rates were higher for centric and pennate diatoms compared to other microphytoplankton (although marginally non-significant), even though they represented a minor proportion of the microplanktonic biomass in all bays (with the exception of FP; 43%).

Absorption efficiency results related more to differences in planktonic composition between sites, and not so much to the total amount of food in the system. FP and both dates in WNB presented similar phytoplankton biomass (in terms of chlorophyll *a*), while the biomass at QB was significantly higher. Moreover, WNB2 presented a high proportion (83%) of <5 μm phytoplankton and high densities of bacteria and pico-eukaryotes in the plankton; also, ciliates made up 82% of the microplankton biomass in this bay. On the other hand in WNB1, which presented the lowest absorption efficiency of all three bays, the proportion of <5 μm phytoplankton was only ~50% of the total, and while ciliates accounted for 55% of the microplanktonic biomass, carbon-dense armoured dinoflagellates (e.g. *Dinophysis*, *Scrippsiella*, *Prorocentrum*) accounted for 36% of the biomass. The latter may not be as easily assimilable a food source as the centric diatoms (Hitchcock, 1982) that were abundant in FP, where absorption efficiencies were the second highest.

Interestingly, ribbed mussels presented comparable clearance rates for autotrophs and heterotrophs, and high absorption efficiencies for all bays, regardless of the proportion of heterotrophs in them. The literature dealing with bivalves as herbivores is far more extensive than that regarding them as consumers of secondary productivity. However, in recent years a growing body of literature has demonstrated that suspension-feeding bivalves are capable of feeding on a wide range of prey including protozoan microzooplankton (Le Gall et al., 1997; Dupuy et al., 1999; Nielsen and Maar, 2007; Trottet et al., 2008) and marine and freshwater crustacean micro- and mesozooplankton (Wong et al. 2003a; Zeldis et al. 2004; Prins and Escaravage, 2005; Lehane and

Davenport, 2006). Other than the caloric superiority of secondary production (Kozlovsky, 1968), there are some other advantages to consuming heterotrophic components of the microplanktonic community. For instance, ciliates can play a significant role in microbial food webs by trophic upgrading, or improving the food quality available to higher trophic levels by adding sterols or sterol-like compounds (Martin-Creuzburg et al., 2005) not ordinarily sythesized by invertebrates (Müller-Navarra, 2008). On the other hand, it has been demonstrated that a number of ciliates feed on toxic red-tide dinoflagellates (Jeong et al., 1999; Jeong et al., 2002; Rossetta and McManus, 2003) and therefore make certain carbon sources, that by prey avoidance would not be incorporated into higher levels, available to other predators.

Concluding remarks

In general, these field-based experiments showed that, when subject to a vast array of food items (in terms of size and food quality), the ribbed mussel *Geukensia demissa* behaves as a non-selective feeder for the most part. Moreover, it is capable of assimilating organic matter from environments with different planktonic structure at >50% efficiencies.

The ultimate purpose underlying research of this nature is to enhance the ecological understanding of the ways to improve the quality of water, supporting healthy trophic interactions in estuaries. There is debate about the efficacy of shellfish restoration for improving water quality in estuaries (Newell, 1988; Gerritsen et al., 1994; Pomeroy et al., 2006). In that sense, results from this study indicate that with its non-selective suspension feeding and capability to consume ultraphytoplankton, ribbed mussels represent a species for tidal wetland re-stocking that could exert potentially important effects on the planktonic community structure.

ACKNOWLEDGMENTS

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A. Fournier and M.A. Marcoval-Pan helped in the field with experiments. JP was partially supported by a Fulbright fellowship during this research.

Table 1.1: Ambient environmental parameters (temperature, salinity, dissolved oxygen; mean ± SD) for all experimental dates and locations. FP: Flax Pond, QB: Quantuck Bay, WNB: West Neck Bay.

	date	temp (ºC)		S (PSU)		DO (mg l^{-1})	
FP	**5/8/2007**	17.5	± 1.1	23.5	± 0.3	9.7	± 0.5
QB	**6/28/2006**	23.9	± 0.4	22.9	± 0.1	6.1	± 0.7
WNB1	**6/26/2006**	25.4	± 0.9	22.4	± 0.6	5.7	± 0.8
WNB2	**9/22/2006**	21.2	± 0.8	22.9	± 0.5	7.2	± 0.7

Table 1.2: Biomass-based clearance rates (l h^{-1} gDW^{-1}) of *Geukensia demissa*, for clorophyll, pico- and nanoplankton, and microplankton; mean (SE). WNB1: West Neck Bay (6/26/06), WNB2: West Neck Bay (9/22/06), Flax: Flax Pond, Qua: Quantuck Bay..

Site	whole chl *a*	<20 µm chl *a*	<5 µm chl *a*
WNB1	1.69 (0.72)	0.57 (0.59)	1.36 (0.65)
WNB2	0.51 (0.79)	1.17 (0.8)	1.12 (0.48)
Qua	1.28 (0.16)	1.26 (0.13)	1.27 (0.11)
Flax	0.80 (0.61)	0.50 (0.43)	0.41 (0.87)

Site	hetero. bacteria	*Synechococcus*	pico-eukaryotes	>2 µm cryptophytes
WNB1	1.40 (0.09)	2.18 (0.28)		5.34 (0.53)
WNB2	-0.12 (0.62)	1.73 (0.38)	0.76 (0.47)	0.67 (1.46)
Qua	0.95 (0.14)	0.30 (0.25)		1.04 (0.65)

Site	phytoflagellates	euglenoids	dinoflagellates	centric diatoms	pennate diatoms	loricate ciliates	aloricate ciliates
WNB1		2.44 (1.05)	1.70 (1.03)	7.53 (1.96)	3.98 (0.61)	2.68 (0.84)	0.96 (0.57)
WNB2	-0.67 (0.45)	4.33 (2.05)	3.47 (4.32)	3.07 (0.42)	2.89 (1.63)	-0.13 (4.28)	3.11 (5.32)
Qua		2.87 (1.29)	1.19 (1.24)		1.73 (0.34)	1.05 (0.99)	1.23 (0.94)
Flax	2.45 (0.26)		0.14 (1.33)	3.81 (0.23)	2.61 (1.67)		0.54 (0.49)

Table 1.3: p-values for 2-way ANOVA performed on log-transformed biovolumes (µm3) for different groups of microplankton. Each location was run independently, with treatment (control, *Geukensia*) and sampling time (t_0 and t_{final}) as factors. Significant differences indicate selectivity. FP: Flax Pond, QB: Quantuck Bay, WNB1: West Neck Bay (6/26/06).

p-value		dinoflag	centric	pennate	aloricate
FP	treat	0.08	0.27	0.46	0.63
	time	0.87	0.73	0.44	0.91
	treat*time	0.62	0.96	0.98	0.96
QB	treat	0.61		0.23	**0.002**
	time	0.14		0.96	0.94
	treat*time	**0.006**		0.15	**0.0006**
WNB1	treat	0.42	0.19	0.07	0.51
	time	0.94	0.84	0.35	0.82
	treat*time	0.45	0.07	**0.005**	0.73

Figure 1.1: Map of Long Island (New York) showing the location of the three shallow embayments where experiments were performed. Flax Pond (FP; 40°57' N, 73°08' W) is a pocket marsh with influence from the Long Island Sound; Quantuck Bay (QB; 40°48' N, 72°36' W) is a coastal lagoonal estuary; and West Neck Bay (WNB; 41°03' N, 72°21' W) is a marsh-fringed bay within the Peconic Bays estuary.

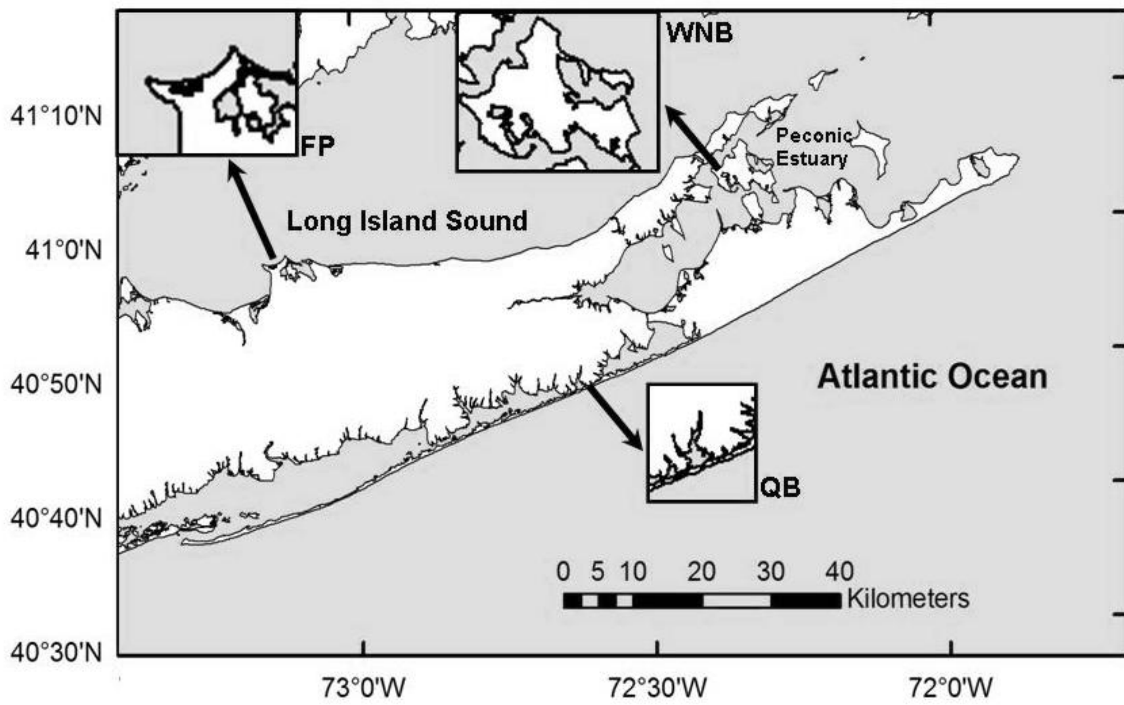
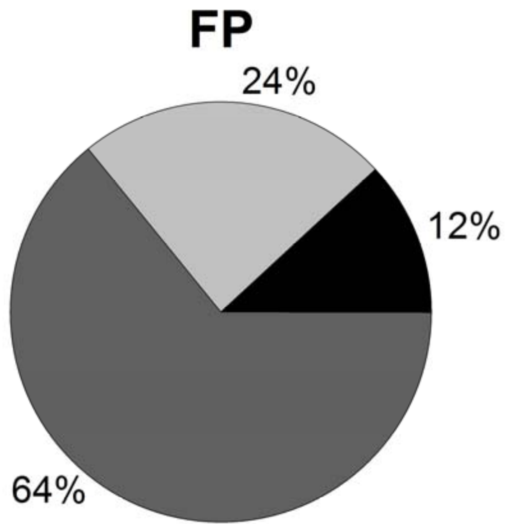
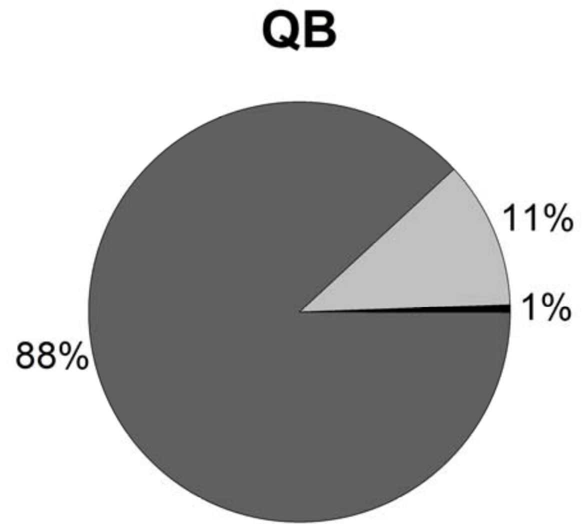
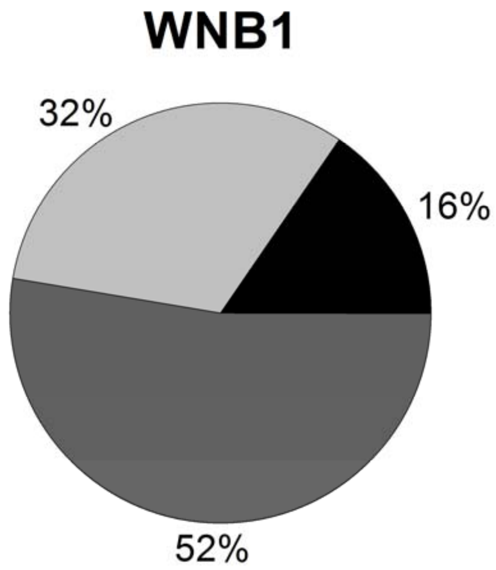
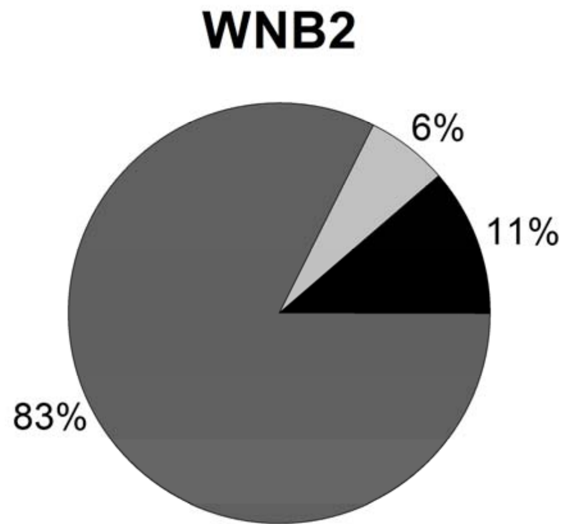

WNB
41°10'N
FP
Peconic
Estuary
Long Island Sound
41°0'N
40°50'N
Atlantic Ocean
40°40'N
QB
0 5 10 20 30 40
Kilometers
40°30'N
73°0'W
72°30'W
72°0'W

Figure 1.2: Size-fractionated proportions of the phytoplankton community (based on whole chlorophyll *a* concentration for the different fractions). Whole chlorophyll *a* concentration (µg l^{-1}) by location was 2.26 (FP), 10.17 (QB), 3.22 (WNB1) and 2.54 (WNB2). Repeated measures ANOVA showed significant differences between sites [$F_{(3,24)} = 1213.51$, $p << 0.001$] and in size fractions within sites [$F(2,48) = 52.9$, $p << 0.001$]. FP: Flax Pond, QB: Quantuck Bay, WNB1: West Neck Bay (6/26/06), WNB2: West Neck Bay (9/22/06).


FP
24%
12%
64%


QB
11%
1%
88%


WNB1
32%
16%
52%


WNB2
6%
11%
83%

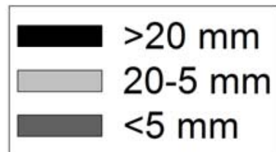
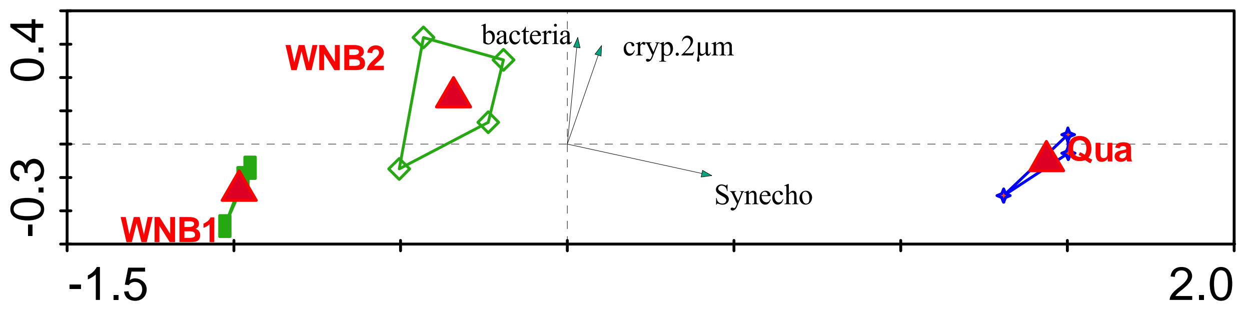

>20 mm
20-5 mm
<5 mm

Figure 1.3: Triplot diagram for redundancy analysis, showing abundances (cells ml^{-1}) for bacterioplankton, *Synechococcus* and nanoplanktonic (>2 μm) cryptophytes, in Qua: Quantuck Bay, WNB1: West Neck Bay (6/26/06), and WNB2: West Neck Bay (9/22/06). A test of significance of all canonical axes showed significant differences in community structure among bays ($\lambda = 0.97$, $F = 117.74$, $p<0.01$).



0.4
-0.3
-1.5
2.0
WNB2
WNB1
bacteria
cryp.2µm
Synecho
Qua

Figure 1.4: Triplot diagram for redundancy analysis, showing abundances (mg C l^{-1}) for autotrophic (euglenoids, phytoflagellates, pennate and centric diatoms) and heterotrophic (dinoflagellates, aloricate oligotrich ciliates, and loricate ciliates) microplankton in Flax: Flax Pond, Qua: Quantuck Bay, and WNB1: West Neck Bay (6/26/06). A test of significance of all canonical axes showed significant differences in community structure among bays (λ= 0.82, F= 15.49, $p<0.01$).

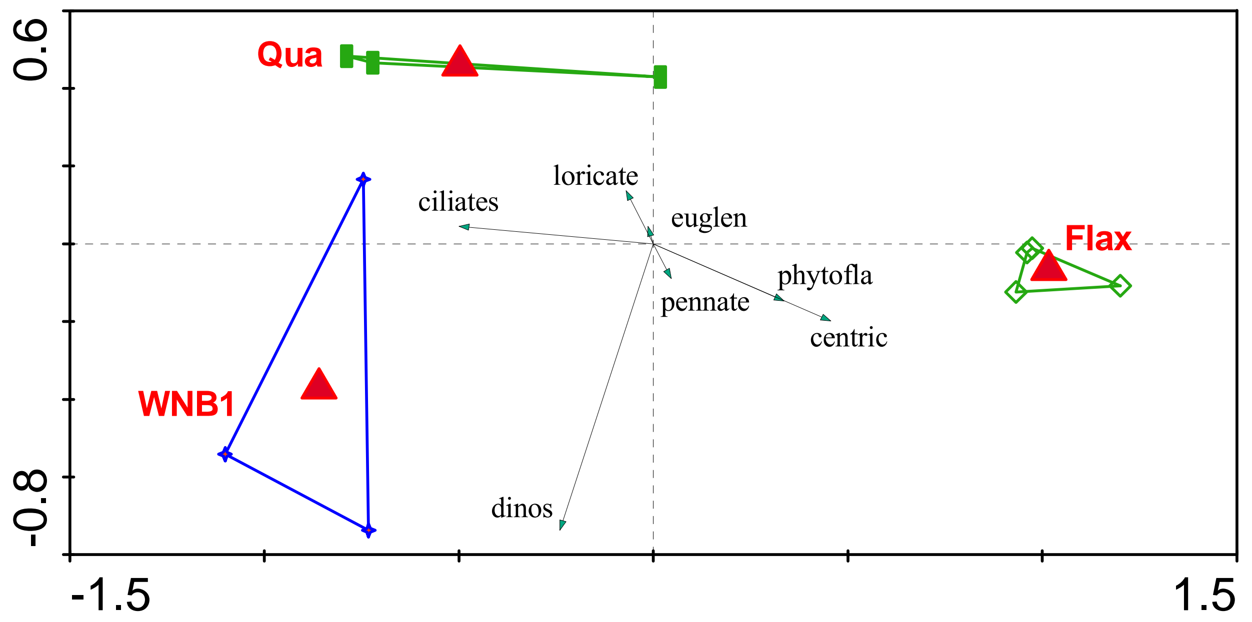

0.6
-0.8
-1.5
1.5
Qua
WNB1
Flax
loricate
ciliates
euglen
phytofla
pennate
centric
dinos

Figure 1.5: Clearance rates (mean ± SE) of *Geukensia demissa*, based on chlorophyll *a* concentration for different size fractions. A repeated measures ANOVA showed no significant differences for size [$F(2,20) = 0.12$, $p > 0.05$] or among sites [$F(3,10) = 0.69$, $p > 0.05$]. FP: Flax Pond, QB: Quantuck Bay, WNB1: West Neck Bay (6/26/06), WNB2: West Neck Bay (9/22/06).

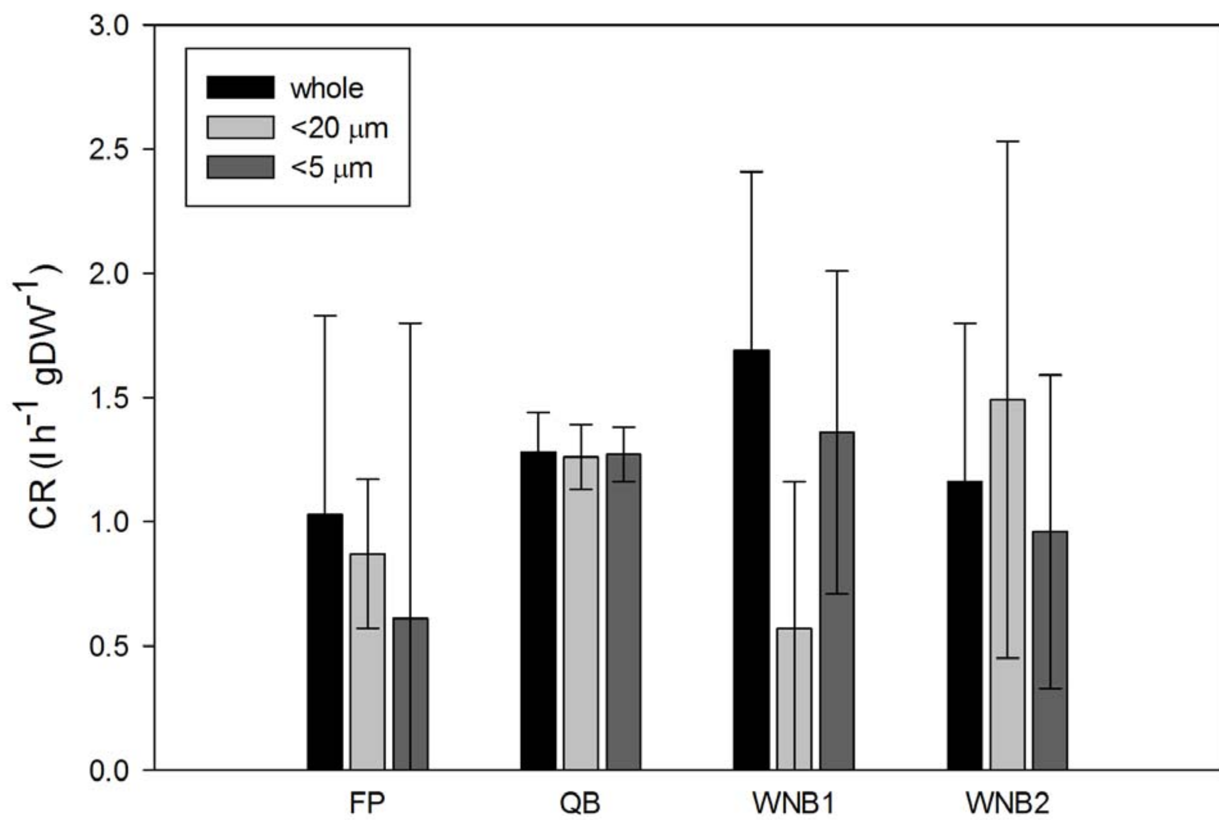

whole
<20 μm
<5 μm
CR (l h-1 gDW-1)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
FP
QB
WNB1
WNB2

Figure 1.6: Triplot diagram for redundancy analysis of *Geukensia demissa* clearance rates based on cell densities of bacterioplankton, *Synechococcus* and nanoplanktonic (>2 μm) cryptophytes in Qua: Quantuck Bay, WNB1: West Neck Bay (6/26/06), and WNB2: West Neck Bay (9/22/06). A test of significance of all canonical axes showed significant differences in clearance rate among bays ($\lambda= 0.59$, $F= 5.09$, $p<0.05$).

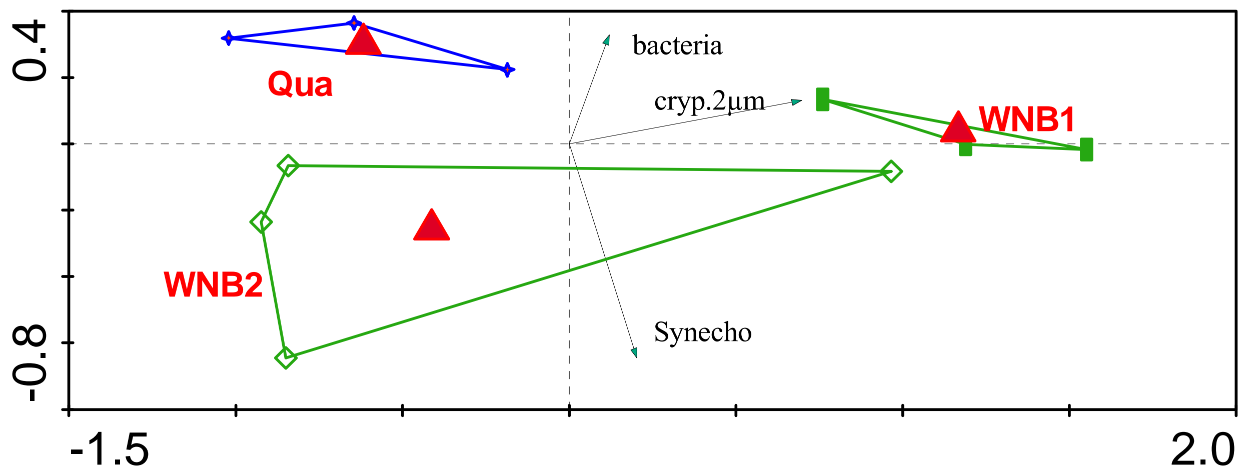
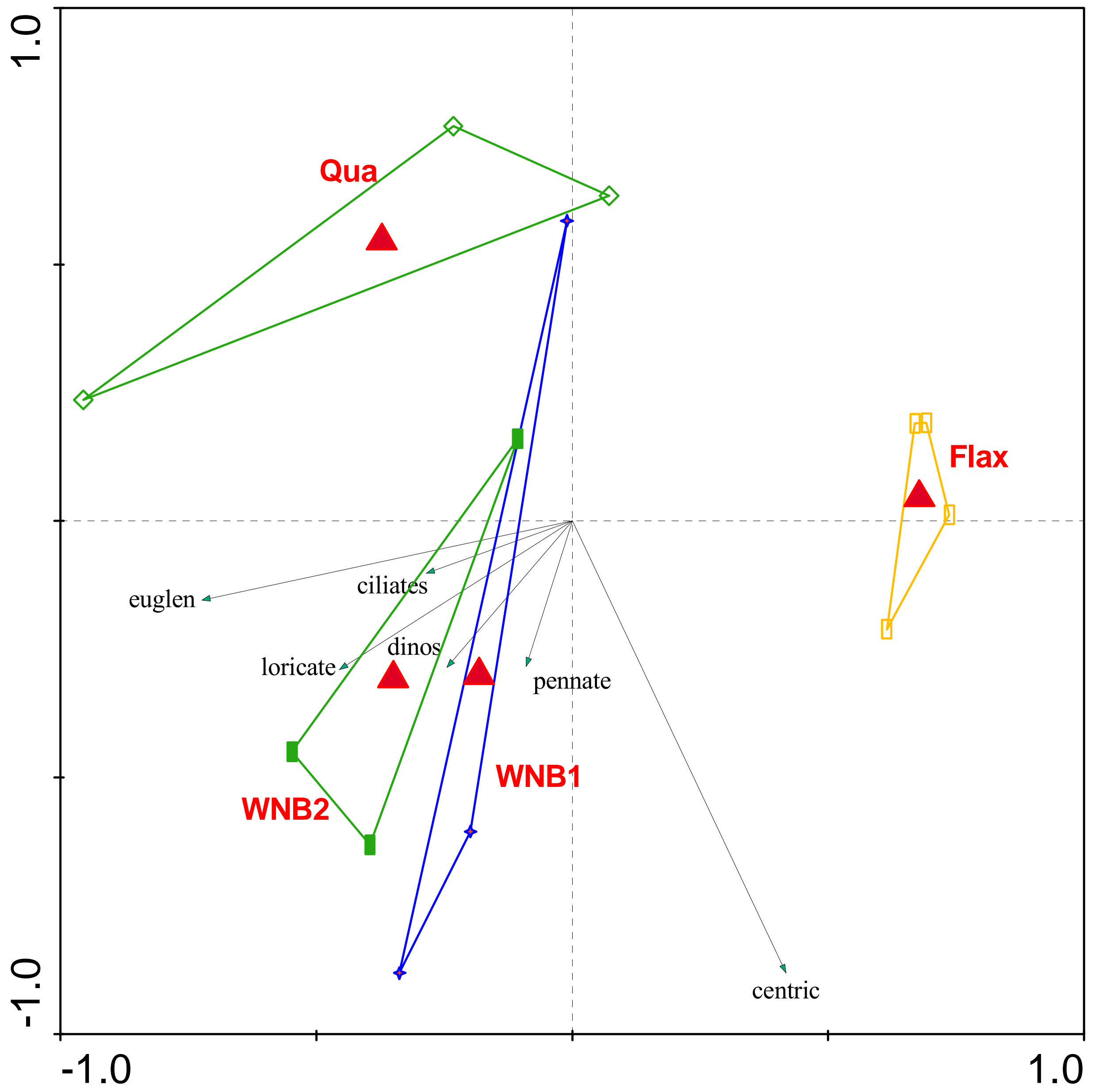

0.4
-0.8
-1.5
2.0
bacteria
cryp.2µm
Synecho
Qua
WNB1
WNB2

Figure 1.7: Triplot diagram for redundancy analysis of *Geukensia demissa* clearance rates based on biomass estimates of microplankton. A test of significance of all canonical axes showed marginally non-significant differences in clearance rate among bays (λ= 0.36, F= 1.68, p= 0.07). Flax: Flax Pond, Qua: Quantuck Bay, WNB1: West Neck Bay (6/26/06), WNB2: West Neck Bay (9/22/06).


1.0
-1.0
-1.0
1.0
Qua
Flax
WNB1
WNB2
euglen
ciliates
loricate
dinos
pennate
centric

CHAPTER 2

Ribbed mussel (*Geukensia demissa*) grazing on cultured and wild-type strains of the brown tide organism (*Aureococcus anophagefferens*)

ABSTRACT

The grazing potential of the ribbed mussel (*Geukensia demissa*) on the brown tide organism (*Aureococcus anophagefferens*) was tested in laboratory short term (1 h) grazing experiment, and contrasted with grazing experiments using blue mussels (*Mytilus edulis*). One set of experiments used proportional (vol:vol) mixtures of cultures of *A. anophagefferens* (CCMP1708) and a microalga known to support good bivalve growth (*Isochrysis galbana* CCMP1323) at a constant particulate organic C content. Another set of experiment considered proportional (vol:vol) mixtures of a natural brown tide and ambient seawater from a non-brown tide location. Clearance rates were estimated from total chlorophyll content and absolute cell counts by flow cytometry. Ribbed mussels were able to effectively graze cultured *A. anophagefferens* from monospecific suspensions ~5×10^5 cells ml^{-1}, and at natural bloom threshold levels below 1.4×10^5 cells ml^{-1}. Clearance rate of blue mussels was significantly lowered by the presence of cultured and wild-type brown tide. *G. demissa* assimilated C from *A. anophagefferens* more efficiently than *M. edulis*. Thus, ribbed mussels may play an important role in regulating the initiation and build-up of brown tide events.

INTRODUCTION

Aureococcus anophagefferens is a coccoid pelagophyte of ~2-3 µm ESD that has been detected in several noncontiguous bays of the midwestern Atlantic coast of the US, either at bloom (>1.0×10^6 cells ml^{-1}) or background non-bloom concentrations since 1985 (Bricelj and Lonsdale, 1997). More recently it has also occured in Saldanha Bay, South Africa (Gobler et al., 2005). *A. anophagefferens* is capable of using a wide variety of nitrogen and carbon compounds (Mulholland et al., 2002), but its advantage over other phytoplankters lies in its ability to use organic nitrogen (Nuzzi and Waters, 2004). Blooms are not necessarily related to the absolute amount of nitrogen in the system (i.e. they are not a consequence of eutrophication), but to the type of prevalent nitrogen (Sunda et al., 2006). The most plausible scenario for the establishment of blooms corresponds to low levels of dissolved inorganic nitrogen combined with high (particulate

and dissolved) organic nitrogenous compounds (Gobler et al., 2004a; 2005; Kana et al., 2004; Lomas et al., 2004; Mulholland et al., 2002). *A. anophagefferens* adaptation to low light regimes also encourages rapid cellular growth and provides it with a competitive advantage over other phytoplankton (Gobler et al., 2005).

Extensive blooms of *A. anophagefferens*, called 'brown tides', have had significant effects both on aquatic ecosystems and on individual species. The ecological impacts have been extensively reviewed in Bricelj and Lonsdale (1997) and Gobler et al. (2005). The harmful effects of *Aureococcus* are mostly related to the sheer biomass (although it can present toxic effects to juvenile shellfish at 35×10^3 cells ml^{-1}; Bricelj et al., 2001) and persistence of monospecific blooms that can last for several months, resulting in numerous direct and indirect effects on aquatic organisms and their habitats. Of particular importance are the effects on bivalve molluscs and the shellfisheries sector. For example, the 1985-86 brown tides were responsible for the demise of the bay scallop (*Argopecten irradians*) fishery on Long Island, New York, by inducing starvation and subsequent recruitment failure (Tettelbach and Wenczel, 1993). Many studies have focused on the effects of *Aureococcus* on juvenile and adult shellfish. Growth rates of juvenile hard clams (*Mercenaria mercenaria*) were significantly lower during blooms (Greenfield and Lonsdale, 2002; Wazniak and Glibert, 2004), and short- and long-term exposure to certain strains of *A. anophagefferens* had toxic effects on juvenile hard clams and blue mussels (Bricelj et al., 2001; Bricelj et al., 2004). However, suspension-feeding function is not equally impaired by *A. anophagefferens* in all molluscs. Harke et al. (in press) found that the gastropod *Crepidula fornicata* was able sustain high clearance rates during dense brown tides, while *Mercenaria mercenaria* fed at significantly lower rates.

In general, toxins can be classified by the level of activity; at the cellular level microalgal toxins are usually distinguished either as cytotoxins or biotoxins. Biotoxins are able to affect whole organisms in bioassays, whereas cytotoxins do not (Carmichael, 1992). Some bioactive compounds or metabolic byproducts produced by microalgae can affect cell membranes or particular organ systems and, although not necessarily lethal, can affect normal physiological function, perhaps ultimately causing sublethal effects (Landsberg, 2002). The latter seems to be the case for *Aureococcus anophagefferens*, for which a few laboratory studies have focused on the toxicity of intact and lysed cells, but

to date no specific toxin has been chemically characterized. However, the work of Gainey and Shumway (1991) with dissected ctenidia of several bivalves found that for most species (e.g. *Mytilus edulis*, *M. mercenaria*), cells of *A. anophagefferens* caused a significant decrease in the activity of the lateral cilia that enable normal feeding. However, for a few species (including *Geukensia demissa*), this impairment of ciliary action did not occur. This physiological impairment was related to a response of lateral cilia to a dopamine-like compound in the extracellular polysaccharide layer of the alga that requires direct cell contact and is not elicited by dissolved metabolites in cell-free filtrates of intact or lysed cells. According to that, it would be appropiate to classify *Aureococcus* with other species documented to harm aquatic organisms through nontoxic mechanisms. For simplicity the term 'toxic' is used broadly throughout this text, mostly with reference to adverse effects of *A. anophagefferens* on a particular metabolic function (usually suspension feeding).

Studies addressing the nutritional value of *Aureococcus* (usually related to lipid composition) date back to Bricelj et al. (1989). This study reported a high fatty acid content, and through a chromatographic characterization, concluded that essential ω-3 polyunsaturated fatty acids (PUFAs) were present in *A. anophagefferens* at levels comparable to those of microalgae of high nutritional value. The nutritional quality of *A. anophagefferens* has been recently revisited in studies with shellfish larvae, in relation to the poor recruitment of bivalves in bays experiencing chronic brown tides. The results are contradictory to those of Bricelj et al. (1989); for example, it was demonstrated that a toxic strain of *A. anophagefferens* (CCMP 1708) impaired larval development in *M. mercenaria* by significantly affecting growth (Padilla *et al.*, 2006; Bricelj and MacQuarrie, 2007), while the effects on larval survivorship did not show a clear trend. Following up on that research line, Przeslawski et al. (2008) demonstrated that larvae fed only an *A. anophagefferens* diet had significantly less lipid stores and smaller size than those in mixed- and control (*Isochrysis galbana*) diets.

Ribbed mussels are known for their capacity to feed on various planktonic food sources ranging in size, nutritional quality and assimilatory efficiency (Wright et al., 1982; Kreeger and Newell, 1996; Huang et al., 2003b). Ribbed mussels reach their highest population densities along their distribution range in Long Island bays, New York

(~5,750 m^{-2} at the marsh edge; Franz, 1993), where brown tides are recurrent (Bricelj and Lonsdale, 1997). Jamaica Bay presents even higher densities than other estuaries, averaging 10,000 m^{-2} (Franz, 2001). It is noteworthy that until 2008, no brown tide episodes had taken place in the section of Great South Bay that lies west of the Robert Moses Causeway and into South Oyster Bay, where there are a number of sedimentary islands and tidal wetlands with dense populations of *Geukensia demissa*, suggesting that this species might have played a role controlling densities of *A. anophagefferens*.

Other than *in vitro* studies (Gainey and Shumway, 1991), no previous research has focused on the effects of toxic microplankton on *Geukensia demissa*'s feeding. This study is the first to test the hypothesis that *Geukensia demissa* can graze on (remove from suspension and process into biodeposits) and metabolize carbon coming from the brown tide organism more efficiently than another intertidal mussel, *Mytilus edulis*. Laboratory-based, short-term grazing experiments were performed with cultures of a toxic strain and water from a natural bloom ('wild-type') of *A. anophagefferens*. If shown, this study would provide further support for shellfish stock enhancement (currently under development) and salt marsh habitat restoration strategies for estuaries experiencing recurring brown tides.

MATERIALS AND METHODS

Two types of grazing experiments were performed: 1) experiments with mixtures of monospecific algal cultures; and 2) experiments mixing an *Aureococcus* bloom and a non-bloom natural plankton community.

1. Grazing experiments with monospecific algal cultures

Feeding experiments were conducted with ribbed mussels exposed to different proportions of *Isochrysis galbana* (CCMP 1323) and *Aureococcus anophagefferens* (CCMP 1708). The choice of the prymnesiophyte *I. galbana* was based on a number of reasons. Firstly, it is known to support good bivalve growth due to its high content of long chain PUFAs (Jeffrey et al., 1994; Barsanti and Gualtieri, 2006). Secondly, *I. galbana* is of comparable shape (nearly spherical; Riisgård, 1988) to *A. anophagefferens*

and, although the *Isochrysis* cell is motile (van den Hoek et al., 1996), its swimming is minimal, much like the case of the coccoid cell of *A. anophagefferens*.

Both microalgal species were grown in a modified-batch culture (Hoff and Snell, 2001) in 20-l carboys of autoclaved, 0.2-μm filtered seawater from Long Island Sound (S =27) at 20ºC, with gentle air bubbling. *I. galbana* was grown in f/2-Si medium (Guillard and Ryther, 1962), while *A. anophagefferens* was cultured in a modified f/2 medium using citric acid as a chelator, iron as $FeCl_3$, selenium, and β-glycerophosphate as the phosphorus source (Bricelj et al., 2001). The light source was 40W cool-white fluorescent bulbs; the light regime was set at a 16:8 h light:dark cycle, with an average irradiance of 38 × 10 μmol quanta m^{-2} s^{-1} (Quantum sensor model SKP 200, Skye Instruments Ltd.). All cultures were periodically checked under the microscope for eukaryote contamination, and monitored for exponential growth using either cell counts with a hemocytometer or *in vivo* chlorophyll spectrophotometry. For the experimental assays, *A. anophagefferens* was harvested at late-exponential or stationary phase (for being significantly more toxic than early-exponential phase; Bricelj et al., 2001), and *Isochrysis* was harvested at the exponential phase.

Ribbed mussels (mean shell height ± SE =64.6 ± 2.7 mm; n =35) were collected at low tide from wild populations at Flax Pond, NY, a location that has never experienced brown tides. Mussels were cleaned of epibionts, their byssal threads trimmed, and allowed to purge their guts in 0.2-μm filtered seawater for >24 h prior to the start of an experiment. To test the toxicity of the cultured brown tide strain, a standard mussel feeding inhibition bioassay (Bricelj et al., 2001) was conducted with *Mytilus edulis* (56.9 ± 1.9 mm; n =9) collected at the same location as *G. demissa*.

Indirect determinations of clearance rate were conducted in static chambers (cylindric, 19.8 cm base diameter, semi-clear polypropylene). Prior to the experiment mussels were placed into beakers containing 3 L of the treatment mixture and left to acclimate for 1 h, then they were gently transferred to fresh experimental mixtures. There was a single mussel per beaker. The start of a clearance rate experiment was determined by visually checking for signs of normal feeding function (e.g., opened valves, extended mantle edges; Riisgård, 1988). Each experimental run lasted for 1 h, so that, on average, mussels were not allowed to graze the microalgal biomass below 20% of the original

concentration, and accumulation of metabolic wastes in the suspension was prevented (Bayne et al., 1976). Experiments were carried out at room temperature (~20ºC) in a darkened lab (~0.25% incubator irradiance) to prevent growth of photosynthetic microalgae during the course of the experiments. The design followed a randomized complete blocks design (Sokal and Rohlf, 1995), doing at least three independent replicate runs of all treatments with the same batch of algae.

The proportions of *Isochrysis* : *Aureococcus* used in the experiments were 100:0, 80:20, 50:50, 20:80, 0:100. These proportions were calculated on a C-content basis, adjusting cell densities to obtain a similar total organic C content of (mean ± SE) 566.0 ± 10.6 µg C l^{-1} across treatments, matching a detrimental brown tide concentration of ~500,000 cells ml^{-1} as a reasonable experimental level (Bricelj et al., 2001). To estimate organic content for each treatment, conversion values of 8.2 pg C $cell^{-1}$ for *I. galbana* (Strathmann, 1967) and 1.08 pg C $cell^{-1}$ for *A. anophagefferens* strain CCMP 1708 (Bricelj et al., 2001) were assumed. Additionally, combusted clays from the Hudson River, NY (1 mg l^{-1}) were added to the experimental mixture in order to provide an inorganic non-combustable fraction to the suspension (Bricelj and Malouf, 1984).

Clearance rates were determined from the rate of total chlorophyll *a* and cell removal considering the concentration and cell density at initial and final times, and the background growth rate (positive or negative) from control beakers without mussels (Coughlan, 1969). Total chlorophyll *a* was calculated from duplicate 30 ml water samples concentrated onto Whatman GF/F filters, extracted in acetone for 24 h, and measured fluorometrically (Turner Designs, model 10-AU) after Arar and Collins (1997). *Isochrysis* and *Aureococcus* cell densities were estimated from 5-ml samples preserved in 1% glutaraldehyde (from a 10% stock solution prepared with filtered natural seawater); cell counts were performed by flow cytometry (Becton-Dickinson, FACSCalibur; Olson et al., 1993), concentrating cells by a factor of 6 by gentle centrifugation (Sorvall RT6000B; 1,500 rev min^{-1}, 6 min), and using a known dilution of 2-µm fluorescent latex beads (Sigma-Aldrich Co). Cell detection was based on size and fluorescence properties, using FL-3H (670 nm) and FL-4H (661 nm) channels. Data were interpreted using the CellQuest software package for flow cytometry analysis.

Retention efficiencies (*RE*) of *A. anophagefferens* were calculated relative to *I. galbana* following Bricelj et al. (2001). For mixed treatments, RE was determined as

$$RE = 100 \times \frac{CR_{A.anophagefferens}}{CR_{I.galbana}}$$

and for single species treatments (100% *Aureococcus*)

$$RE = 100 \times \frac{CR_{A.anophagefferens100\%}}{mean\ CR_{I.galbana}}.$$

Absorption efficiency was estimated after Conover's (1966) ash-free dry weight:dry weight ratio method. For that, at the end of the exposure to the treatment media mussels were transferred to 0.2-μm filtered seawater and allowed to depurate for >12 h, after which feces were collected. At the completion of the experiment, mussels were dissected and soft tissue dry weight was determined by drying at 60ºC for >48 h in order to express clearance rates in units of tissue dry weight.

2. Grazing experiments diluting a natural bloom of* Aureococcus anophagefferens *with non-bloom natural plankton

A brown tide occurred during late June and July 2007 in Quantuck Bay (QB). Cell densities reached 6 × 10^5 ml^{-1} (R. Nuzzi, Suffolk County Department of Health Services, pers. comm.). Batches of sub-surface ambient water were collected from QB at high tide on 7/11 and 7/14/2007 in 20-l acid-washed cubitainers, and subsequently kept in a cooler and in the dark, to prevent light stress and warming during transit to the lab. Additionally, sub-surface ambient water was collected at high/incoming tide from Stony Brook Harbor (SBH), a location adjacent to Long Island Sound where no blooms of *A. anophagefferens* have ever been recorded, on the same dates within ~2.5 h of collection at Quantuck Bay. Upon arrival at the laboratory, both batches were kept in an incubator at 20°C until the setup of experiments.

On average, chlorophyll *a* concentrations at QB (mean ± SE =18.7 ± 0.8 μg l^{-1}) were about 5× greater than at SBH (3.73 ± 0.4 μg l^{-1}). Converting chlorophyll concentrations to phytoplankton carbon was done with the empirical model of Cloern et al. (1995), using parameters (irradiance and μ' values) typical of a Long Island brown tide

published by Milligan and Cosper (1997) and those published by Chang and Carpenter (1991) typical for a pelagic community of Long Island Sound, for QB and SBH respectively. The obtained C:chl ratios were 42.0 ±1.3 (± SE) and 58.0 ±1.7 for QB and SBH respectively, which are close to the ratio of ~60 reported by Boissonneault-Cellineri et al. (2001) for an analogous estuarine system. Additionally, duplicate microplankton samples (50 ml, preserved in 10% acidic Lugol's iodine solution) were taken in order to characterize the plankton community of both locations. These samples were quantified following standard settling techniques and counting 1-ml aliquots in a Sedgewick-Rafter chamber (LeGresley and McDermott, 2010) with a Zeiss compound microscope. Biovolume was estimated from linear cell dimensions (Sun and Liu 2003), and biomass conversion factors published by Menden-Deuer and Lessard (2000), Putt and Stoecker (1989), Smayda (1978), and Strathmann (1967) were applied.

QB water was diluted by mixing with SBH water, obtaining vol:vol proportions of 80:20, 50:50 and 20:80%; additionally, full strength QB (100% volume) and SBH (100% volume) treatments were utilized. In terms of phytoplankton carbon (μgC l^{-1}), the treatments ranged from a concentration of 216.2 (100% SBH) to 784.3 (100% QB). These fall within the range of 'moderate' particulate organic carbon reported by Malouf and Bricelj (1989) for a number of bivalves subject to experimental culture (300-700 μgC l^{-1}).

Clearance rate experiments were conducted under controlled conditions in the laboratory following a randomized complete block design (Sokal and Rohlf, 1995). Cylindrical (19.8 cm base diameter) polypropylene beakers were filled with 3 L of seawater mixtures, with two replicated runs for each of the two collection dates. Experimental ribbed mussels (mean shell height ± SE =66.4 ± 1.2 mm; n =20) and blue mussels (56.2 ± 0.9 mm; n =20) were collected at low tide from wild populations at Flax Pond, NY, a tidal marsh adjacent to Long Island Sound. All mussels were cleaned of epibionts, and allowed to purge their guts in 0.2-μm filtered seawater for >24 hours prior to the experiment. A single mussel (*Geukensia* or *Mytilus*) was gently transferred to each experimental beaker, after being acclimated for 1 h to the corresponding experimental mixture. The start of the experiment was determined by visually checking for signs of

normal feeding function (e.g., opened valves, extended mantle edges; Riisgård, 1988) and experiments lasted for ~1 h.

Clearance rates were determined from the rate of total chlorophyll *a* and *A. anophagefferens* cell removal considering the chlorophyll concentration and cell density at initial and final times, and the background growth rate (positive or negative) from control beakers without mussels (Coughlan, 1969). Chlorophyll content determination was done as described in the previous section. *Aureococcus* cell densities were determined from 5-ml samples preserved in 1% glutaraldehyde (from a 10% stock solution prepared with filtered natural seawater) using the monoclonal antibody technique (ELISA) developed by Caron et al. (2003); this technique provides more accurate and rapid detection of *A. anophagefferens* cells in mixed algal samples over both the immunofluorescent staining with a polyclonal antibody (PAb) method and traditional microscopy techniques (Caron et al., 2003). Absorbance was measured by a microplate reader (Molecular Devices, model Spectramax Plus 384) at 450 nm. Absorption efficiencies were estimated with the method published by Conover (1966), as described in the previous section.

Data analysis

For experiments with microalgae cultures, clearance rates on total chlorophyll *a* across treatments were analized by regression; if results were non-significant, a single-factor ANOVA was run to confirm that the outcome was not dependent on the choice of a linear model. Clearance rates based on cell counts of individual microalgae species were analyzed by 2-factor ANCOVAs with blocks as random factors and the proportion of a species as the covariate, testing first for homogeneity of slopes. If not significantly different, slopes were pooled, and adjusted means of the resulting regressions were tested. Retention efficiencies were compared to published values by means of a two-tailed t-test.

For the experiments mixing natural water (*Aureococcus* bloom and non-bloom), the microplankton community (cell concentration) was contrasted via a 2-way ANOVA, with location and taxa as factors. Clearance rates were analyzed by ANCOVAs with the absolute carbon content of the treatment as the covariate and species and blocks (random)

as factors, testing first for homogeneity of slopes. This was followed by an ANOVA, with species, water collection date and dilution proportion (nominal) as factors. If significant, both a *post-hoc* multiple comparison test (Tukey HSD) and an *a priori* planned comparison of species at a dilution proportion were run (Sokal and Rohlf, 1995). Absorption efficiencies were analyzed firstly by ANCOVAs, and then by a 3-way ANOVA with species, dilution proportion and blocks as factors. All statistical analyses were done with the Statistica software package (version 9).

RESULTS

1. Experiments with monospecific cultures

A noxious effect of *A. anophagefferens* (CCMP 1708) was confirmed by the blue mussel bioassay. Clearance rate based on total chlorophyll *a* significantly declined with increasing percent *A. anophagefferens* (regression $t = 2.63$, $df = 7$, $p < 0.05$, Figure 2.1a). On average, clearance rate for *A. anophagefferens* was 39% of the clearance rate measured for *I. galbana* in the single species treatments, for *Mytilus edulis*. The same was confirmed from cell counts (Figure 2.1b), in which clearance rates of *A. anophagefferens* were only 31% of those for *I. galbana*, based on adjusted means obtained by ANCOVA. This difference was statistically significant (ANCOVA $F(1,5) = 59.8$, $p < 0.001$). Interestingly, *M. edulis* maintained elevated clearance rates for *I. galbana* (3.9 l h^{-1} gDW^{-1}) in the mixed suspension treatment, indicating that feeding function was normal for the non-toxic algae in the mixture, and some sort of particle sorting was involved.

Clearance rate of ribbed mussels on total chlorophyll *a* did not vary with the proportion of *A. anophagefferens* present in the mixture (regression, $t = 0.15$, $df = 33$, $p > 0.05$, Figure 2.2a). Clearance rates ranged from 7.1 to 8.5 l h^{-1} gDW^{-1}, very high values for any suspension feeding bivalve. Analyzing the data by ANOVA also yielded a nonsignificant result ($F(4,30) = 0.16$, $p > 0.05$), indicating that the outcome was not dependent on the choice of a linear model. Count-based clearance rates of *A. anophagefferens* were about the same of those for *I. galbana*, based on adjusted means

obtained by ANCOVA (Figure 2.2b). Differences in clearance rate were not significant (ANCOVA $F(1,45) = 0.18$, $p > 0.05$), and all values were above 6 l h^{-1} gDW^{-1}, again high rates for any bivalve. Thus, unlike blue mussels, no reduction in clearance rate was observed for ribbed mussels feeding on mixed algal cultures containing the brown tide organism.

Clearance rate is the product of pumping rate and filtration efficiency (also called retention efficiency; Bayne et al., 1976). With mixtures of algae, the ratio of clearance rates between species provides an estimate of relative retention efficiency, since the pumping rate of the mussel is the same for all algae in the suspension. The CCMP 1323 strain of *I. galbana* used in the experiments measured [mean length (SD) × width (SD) µm] 5.6 (1.4) × 4.3 (0.3), and the CCMP 1708 strain of *A. anophagefferens* measured [mean ESD (SD) µm] 2.05 (0.08), the latter value comparable to the size reported by Bricelj et al. (2001) for this strain. With those linear dimensions, both shellfish should retain *I. galbana* with 100% efficiency and retention efficiencies for *A. anophagefferens* were 43% (13) and 96% (7) for *M. edulis* and *G. demissa*, respectively. According to Møhlenberg and Riisgård (1978) and Riisgård (1988), both mussels should retain spherical particles of equivalent size to *A. anophagefferens* with efficiencies in the range of 75-90%. On average, *M. edulis* retention was significantly lower than expected (mean =43%; t-test $p < 0.05$), and this result contrasts sharply with the retention efficiency of ~74% relative to *I. galbana* for a nontoxic strain of brown tide (CCMP 1784) obtained by Bricelj et al. (2001). Thus, the much larger reductions observed could not have been due to small particle size alone, and noxious effects are clearly indicated for blue mussels. On the other hand, *G. demissa* retention was above the expected value (mean =96%; t-test, $p > 0.05$), indicating tolerance to the toxic strain of *A. anophagefferens*.

Average absorption efficiencies for *Geukensia* across treatments were 61.0 ± 2.4%, indicating that ribbed mussels were not only clearing out particles from unialgal and mixed suspensions, but also efficiently digesting the material.

2. Experiments diluting a natural bloom of **Aureococcus anophagefferens**

The analysis of microplankton communities from QB and SBH showed that water batches were significantly different with respect to location (ANOVA $F(1,30) = 20.86$,

p<0.001) and taxonomic composition (ANOVA $F(4,30) = 18.48$, $p<0.001$). In the microplanktonic size range (>20 µm), water from QB was dominated by large heterotrophic dinoflagellates such as *Gyrodinium* cf. *spirale* and *Gyrodinium dominans* (mean =582 cells ml^{-1}), followed by aloricate oligotrichous ciliates (mean =104 cells ml^{-1}) of the genus *Didinium* and Scuticociliates. On the other hand, water from SBH was mostly comprised of small autotrophic forms (centric diatoms reaching mean densities ~3,900 cells ml^{-1} and dominated by the genus *Cerataulina*, and small phytoflagellates with average densities ~400 cells ml^{-1}; Figure 2.3).

Biomass estimations of microplankton groups showed that microheterotrophs (i.e. ciliates and dinoflagellates >20 µm) comprised 97% of the total microplanktonic biomass in QB, while the proportion was 7% of the total in SBH, where most dinoflagellates were autotrophic. Autotrophic forms dominated the microplanktonic biomass in SBH; phytoflagellates (cryptophytes and prymnesiophytes) and dinoflagellates (*Prorocentrum* spp.) made up 20% of the total biomass, while diatoms dominated the system, with a 74% abundance.

Clearance rates based on total chlorophyll *a* (Figure 2.4a) were contrasted by homogeneity of slopes in ANCOVA, and ANOVA tests, both yielding significant differences ($p<0.001$) between mussel species, carbon concentration of the mixture (homogeneity of slopes), or the dilution of QB bloom water (ANOVA, nominal factor), and their interaction. A random factor was added to the ANOVA to account for the effect of water batches (collection dates); it was non-significant ($p>0.05$). An *a priori* planned-comparison test found significant differences among the clearance rates for *G. demissa* and *M. edulis* for the whole SBH water treatment and the 20% bloom dilution ($p<0.001$) treatments (C content 216 and 256 µgC l^{-1}, respectively), while clearances in the other treatments (503-784 µgC l^{-1}) were non-significant ($p>0.05$), albeit marginally for the 80% dilution ($p = 0.06$). *M. edulis* clearance rates were low (0.7~0.0 l h^{-1} gDW^{-1}) across all dilutions. Consistent with results from lab experiments using brown tide cultures, the average clearance rate of ribbed mussels on diluted QB water was 63% greater than that of blue mussels. Moreover, *G. demissa* clearance rates were ~45% lower but comparable to the rates found in the lab experiments for the SBH and the higher dilution of QB water

treatments, dropping (~62%) when the dilution was 50% or higher. This suggests that either the higher absolute concentration of carbon in the mixture or the higher proportion of the *A. anophagefferens* bloom had a detrimental effect on *G. demissa* clearance.

The pattern for clearance rates based on *A. anophagefferens* cell counts (Figure 2.4b) was similar to that for total chlorophyll *a* (Figure 2.4a). The homogeneity of slopes test and an ANOVA yielded significant differences ($p<0.05$) between mussel species, carbon content in the mixture, and their interaction. As with results based on total chlorophyll, an *a priori* planned-comparison test showed clearance rates were significantly different ($p<0.001$) for the treatment corresponding to a 20% QB dilution (1.4×10^5 cells ml^{-1}) and the rest of bloom dilutions (*A. anophagefferens* densities ranging from $3.2–5.9 \times 10^5$ cells ml^{-1}) were non-significant. Although there might be some confounding effects of the mussels grazing at different absolute C levels and seawater sources, the latter evidence strongly suggests a threshold effect for *G. demissa* grazing on cell concentrations $>1.4 \times 10^5$ cells ml^{-1} of the *A. anophagefferens* wild-type. A threshold effect of *Aureococcus* on normal bivalve feeding is congruent with Bricelj et al.'s (2001) findings for *Mercenaria mercenaria* juveniles. However they cite a threshold value of 35×10^3 cells ml^{-1}, which is roughly an order of magnitude lower than the cell densities tested in this experiment.

Absorption efficiencies (Figure 2.5) showed the same pattern for both mussels, with higher values (>63%) in the 100% SBH and 100% QB treatments, meaning that both species were able to assimilate carbon from both water sources. Differences across dilution treatments were significant (ANOVA $F(3,13) = 17.2$; $p<0.001$). It is noteworthy that for the full strength brown tide treatment, which showed the lowest clearance rates, absorption of carbon showed the highest values both for *Geukensia* and *Mytilus* (69% and 65%, respectively).

DISCUSSION

The toxic effect of the CCMP 1708 strain of *Aureococcus anophagefferens* demonstrated for *Mytilus edulis* in this study is congruent with findings by Bricelj et al. (2001) for the same strain. However, it is noteworthy that in this study, toxic effects were demonstrated for blue mussels at *A. anophagefferens* concentrations of 5×10^5 cells ml^{-1}

(for the 100% *Aureococcus* treatment), roughly half of those tested by Bricelj et al. (2001; 1×10^6 cells ml^{-1}), pointing out the virulence of the strain. At the moment these experiments were run, the strain had been kept in non-axenic culture for ~11 years, and unlike other isolates (e.g. CCMP 1784) it had maintained its toxicity throughout this period. With the evidence gathered in this study, on plausible explanation for the low clearance rates is that ciliary function was not completely altered by the presence of *A. anophagefferens* (i.e. the retention efficiency for *I. galbana* remained high), but that cell size in relation to the dopamine-like organic coating of the cell (Gainey and Shumway, 1991) induces some sort of rejection for *A. anophagefferens* cells at the ctenidium.

The high clearance rates found in *G. demissa* demonstrate that the strain did not impair filtration in ribbed mussels, a finding which is in agreement with that of Gainey and Shumway (1991). Moreover, no inhibition of uptake or suppressed feeding on a non-toxic prey (i.e. *I. galbana*) was observed for mixed suspensions, contrary to what Bricelj et al. (2001) found for *M. mercenaria* juveniles. The significantly higher retention efficiency for CCMP 1708 cells (2.05 μm) confirms that *G. demissa* is capable of effectively processing small-sized particles at the ctenidium (Wright, 1982).

On the other hand, high absorption efficiencies indicate that ribbed mussels were able to metabolize carbon from *A. anophagefferens* cells relatively well. Ribbed mussels do have the ability to digest non-conventional and refractory sources of carbon, and assimilate it into tissue with varying efficiencies (Kreeger et al., 1988; Langdon and Newell, 1990). Kreeger et al. (1990) hypothesized that increased gut residence time for refractory material ingested by ribbed mussels results in higher absorption efficiencies. In these experiments, ribbed mussels started producing feces shortly after the experiments were over, indicating relatively short gut residence times and a high digestibility for *A. anophagefferens*. The latter may be related to the simplicity of the *A. anophagefferens* cell structure. Some studies have related the digestibility of microalgae by bivalves to cell wall structure and complexity (Brillant and MacDonald, 2003); in that sense *A. anophagefferens* has a simple coccoid cell that lacks thick cell walls present in other microplankters. However, a sufficient carbon intake does not ensure growth and survival if the diet is deficient in essential micronutrients. Bricelj et al. (1989) found no chemical composition defficiencies in *A. anophagefferens*, and the evidence of dense populations

of *G. demissa* in areas that are recurrently subject to brown tides provides some indication that *A. anophagefferens* might partially match the nutrition requirements of certain organisms. Further studies incorporating long-term exposure to diets based on *A. anophagefferens* (e.g. Bricelj et al., 2004) would ultimately demonstrate if *A. anophagefferens* can be a nutritious food for ribbed mussels and sustain growth.

Microplankton community analysis during the 2007 brown tide bloom at QB showed that microheterotrophs (i.e. ciliates and dinoflagellates >20 µm) comprised 97% of the total microplanktonic biomass. Similarly, Gobler et al. (2004b) found a marked increase in microzooplankon grazing rates during July and August following a peak of brown tide density in QB. Numerous reports indicate that microzooplankton grazing rates on *A. anophagefferens* are usually lower than grazing rates on the total community (e.g., Gobler et al., 2002; Deonarine et al., 2006), but Gobler et al. (2004b) argue that during late stages of brown tides the establishment of a protozoan community might be able to grow and graze robustly in the presence of *A. anophagefferens*. The high microheterotrophic biomass found during the QB brown tide might provide support to the latter hypothesis. Although this study focused only on macrobenthos grazing directly upon the brown tide organism, the exploration of alternative trophic pathways in bloom-dominated systems and the transfer of organic carbon from *A. anophagefferens* to higher trophic levels through benthic predation on microheterotrophs might prove an interesting research line to explore.

The dilution of a well-developed brown tide bloom with seawater from a different source recreated, to a certain extent, the earlier stages of the development of a brown tide. In this study, both mussel species had lower clearance rates when exposed to 50% or higher proportions and full strength brown tide water (QB). This observation suggests that ribbed mussels can be overwhelmed when suddenly exposed to a developed toxic bloom, and indicated a threshold effect for *G. demissa* grazing on wild-type *A. anophagefferens* above cell concentrations 1.4×10^5 cells ml^{-1}. This threshold concentration is an order of magnitude higher than found for juvenile hard clams (Bricelj et al., 2001), but still three-times lower than the cell concentrations of 5×10^5 cells ml^{-1} tested in the experiments using laboratory cultures. It is puzzling that *G. demissa* did not

present higher clearance rates at higher bloom proportions but that does not negate the results from experiments using cultures, which demonstrated that ribbed mussels were less sensitive than blue mussels to toxic effects of *A. anophagefferens*. Bivalves are more effective at preventing the build-up of toxic species than controlling a fully developed bloom (Cerrato et al., 2004). In this regard, ribbed mussels would continue to remove toxic species longer and would be more effective at removing toxic algae than blue mussels. It is well known that environmental conditions may have an effect on the toxicity of microalgae (Landsberg, 2002), and thus the wild *A. anophagefferens* strain from the bloom may have been more toxic than the cultures. Mussels might have been responding negatively other components in QB water (e.g. the co-occurring nanoplanktonic centric diatom *Minutocellus* sp., or pennates such as *Nitzschia longissima*). Intraspecific variability in harmful algae has been long recognized (Burkholder and Glibert, 2009), and is usually evidenced in chemical composition and toxicity differences among strains, among other traits. For instance, quantitative aspects of toxin production may arise from environmental variability and epi-genetic factors, leading to a high variability in the quantity of toxin produced within a given species (Burkholder and Glibert, 2009). Contrasting the toxicity of a laboratory-cultured and a wild-type strain of *A. anophagefferens*, differences in the response of a molluscan suspension feeder were found suggesting differences in the virulence of both strains. This is not surprising, given that cultured strains substantially alter their physiological traits over time, and that the laboratory clone used in this study, had been in culture >10 years at the time of experiments.

The fact that absorption efficiencies for both mussels were highest for the full strength brown tide treatment might relate to the biomass dominance of *A. anophagefferens* in this treatment, and the simple structure of the cell (discussed above). Another explanation might be cell aggregation enhanced by extracellular polysaccharides, increasing the effective diameter of the cells. Moreover, Bricelj et al. (1989) reported a high content of easily digestible molecules like fatty acids in this picoplankter.

Concluding remarks

The main findings of this study are that *Geukensia demissa*'s suspension feeding physiology, as well as its ability to incorporate carbon, remain at high rates when subjected to mixed and monospecific suspensions ~5×10^5 cells ml^{-1} of *Aureococcus anophagefferens*, and at natural bloom threshold levels below 1.4×10^5 cells ml^{-1}. This result provides experimental support for shellfish stock-enhancement and salt-marsh habitat restoration strategies in estuaries experiencing recurring brown tides.

The question remains as to what extent can *G. demissa* exert control over brown tides. Several interplaying factors should be considered when attempting a response to this question. On the one hand, *G. demissa* presents naturally dense populations in areas affected by brown tides. On the other hand, despite being a non-commercial bivalve with non-fluctuating stock levels, its distribution limited to fringing marshes in the intertidal zone might limit its controls over the development of brown tides in shallow embayments.

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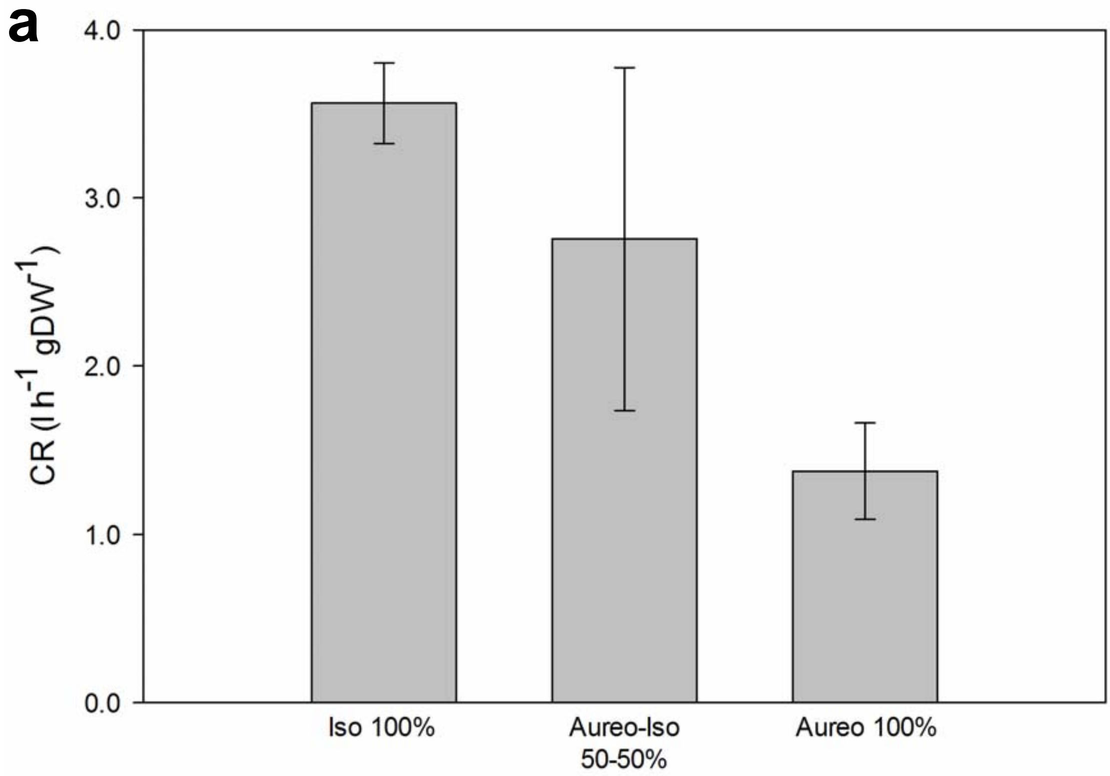
Figure 2.1: *Mytilus edulis* clearance (mean ± SE) on mixtures of *Aureococcus anophagefferens* (CCMP 1708) and *Isochrysis galbana* (CCMP 1323). Mixtures were done on C-content basis; absolute C content across treatments =566.0 µg C l^{-1}. **a)** CRs based on total chlorophyll *a* (regression t =2.63, df =7, $p<0.05$). **b)** CRs based on cell counts performed by flow cytometry (ANCOVA $F(1,5) = 59.8$, $p<0.001$).


a
4.0
3.0
2.0
1.0
0.0
CR (l h^{-1} gDW^{-1})
Iso 100%
Aureo-Iso
50-50%
Aureo 100%

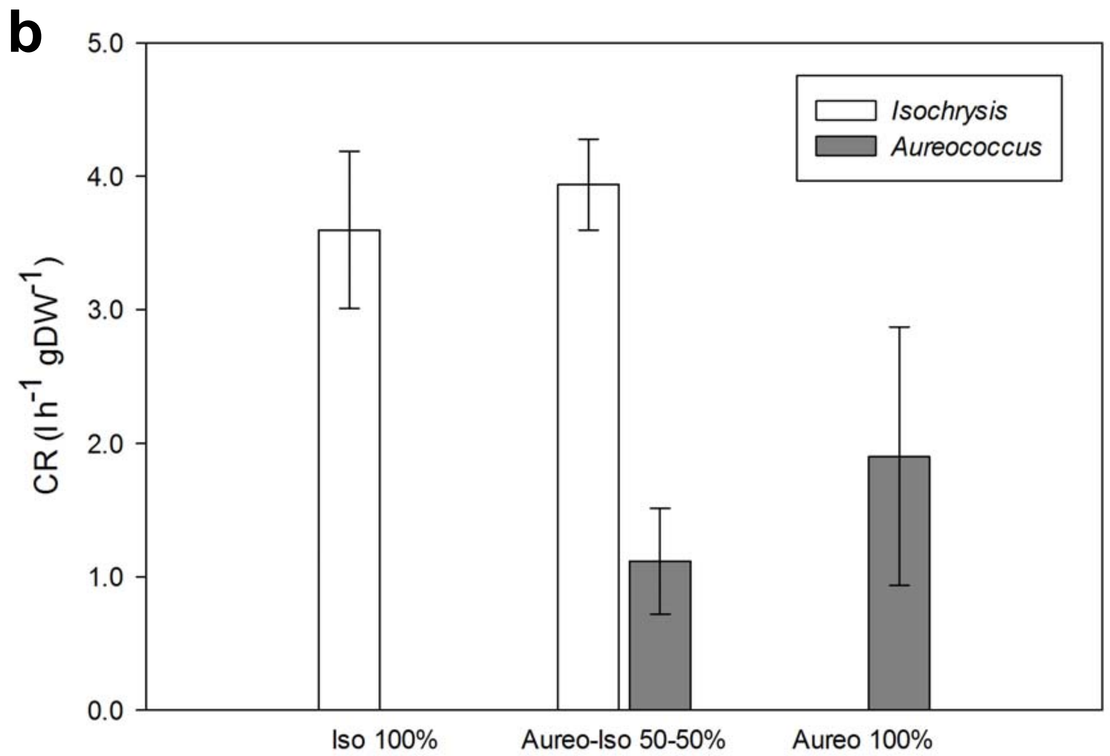
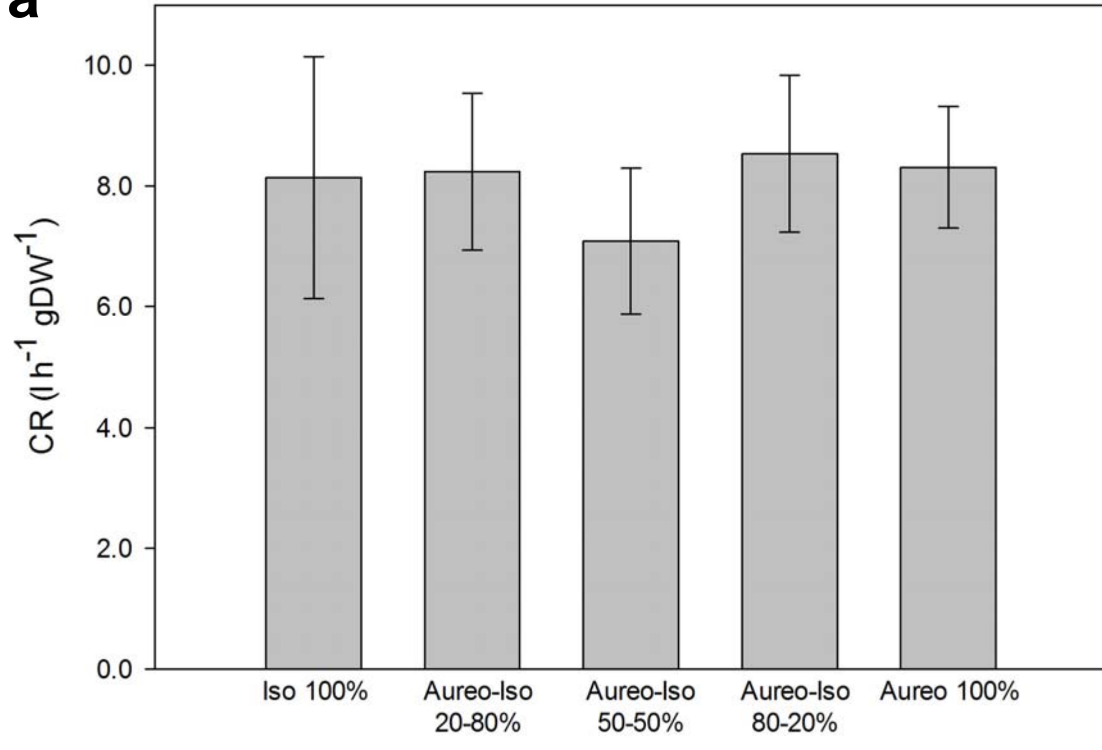

b
5.0
4.0
3.0
2.0
1.0
0.0
CR (l h^{-1} gDW^{-1})
Isochrysis
Aureococcus
Iso 100%
Aureo-Iso 50-50%
Aureo 100%

Figure 2.2: *Geukensia demissa* clearance (mean ± SE) on mixtures of *Aureococcus anophagefferens* (CCMP 1708) and *Isochrysis galbana* (CCMP 1323). Mixtures were done on C-content basis; absolute C content across treatments =566.0 µg C l^{-1}. **a)** CRs based on total chlorophyll *a* (regression t =-0.15, df =33, p>0.05; ANOVA $F(4,30)$= 0.16, p>0.05). **b)** CRs based on cell counts performed by flow cytometry (ANCOVA $F(1,45)$ =0.18, p>0.05).


a
CR (l h^{-1} gDW^{-1})
10.0
8.0
6.0
4.0
2.0
0.0
Iso 100%
Aureo-Iso 20-80%
Aureo-Iso 50-50%
Aureo-Iso 80-20%
Aureo 100%

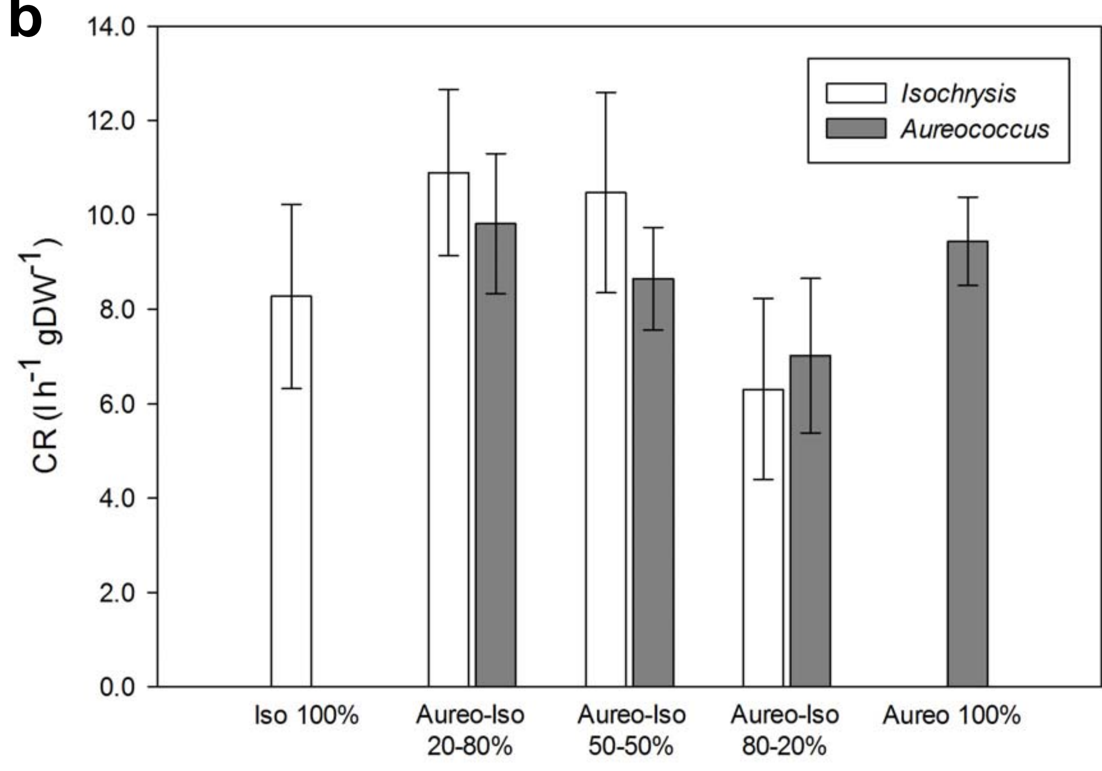

b
CR (l h^{-1} gDW^{-1})
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Isochrysis
Aureococcus
Iso 100%
Aureo-Iso 20-80%
Aureo-Iso 50-50%
Aureo-Iso 80-20%
Aureo 100%

Figure 2.3: Microplankton community composition (mean ± SE) for Quantuck Bay (QB) and Stony Brook Harbor (SBH). Water batches collected on 7/11 and 7/14/2007. ANOVA locations $F(1,30) = 20.86$, $p < 0.001$; ANOVA taxonomic groups $F(4,30) = 18.48$, $p < 0.001$.

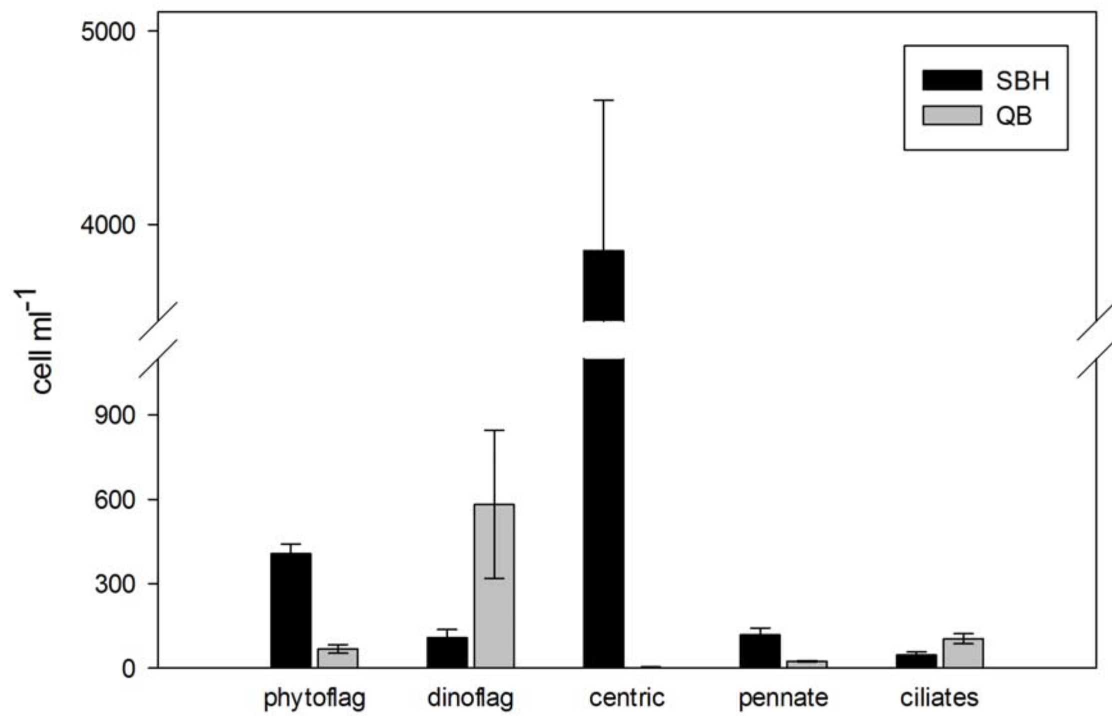
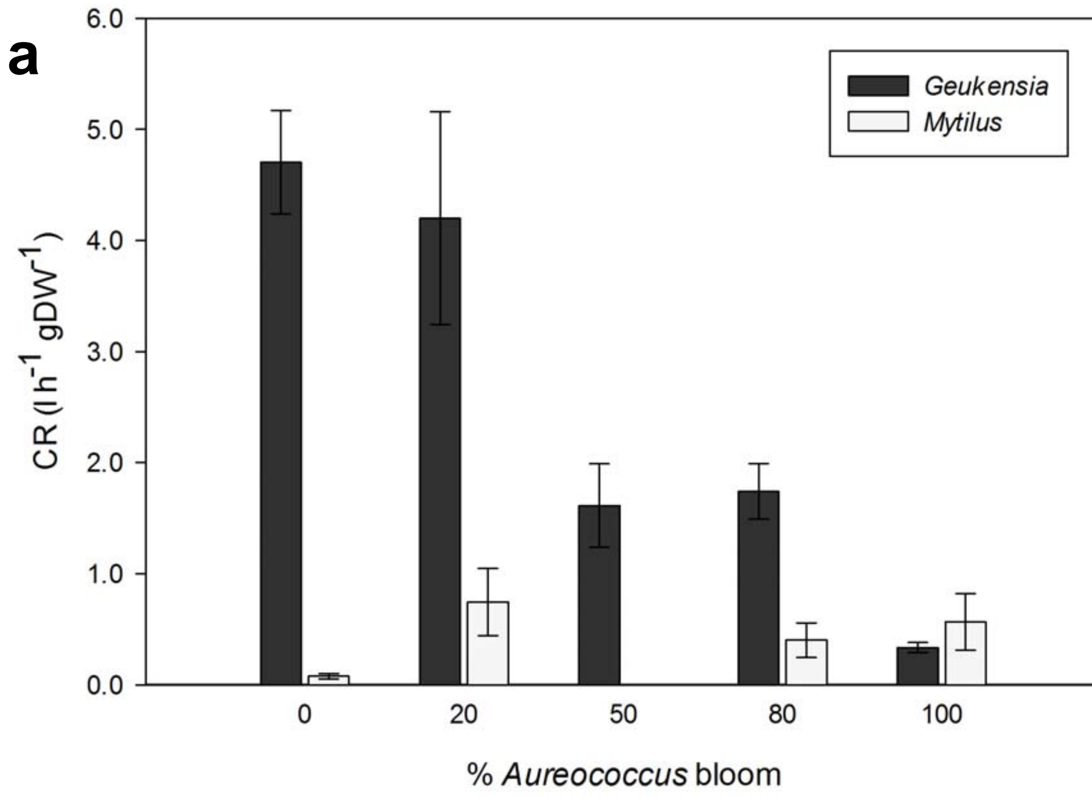

5000
4000
900
600
300
0
cell ml^{-1}
SBH
QB
phytoflag
dinoflag
centric
pennate
ciliates

Figure 2.4: *Geukensia demissa* and *Mytilus edulis* clearance (mean ± SE) on dilutions of an *Aureococcus anophagefferens* bloom from Quantuck Bay with Stony Brook Harbor natural plankton. Absolute C content across treatments range of 216.2 - 784.3 μg C l^{-1}. **a)** CRs based on total chlorophyll *a* (species homogeneity of slopes test in ANCOVA, and ANOVA $p<0.001$). **b)** CRs based on cell counts of *A. anophagefferens* (all factors homogeneity of slopes test, and ANOVA $p<0.05$).


a
Geukensia
Mytilus
CR (l h-1 gDW-1)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
20
50
80
100
% Aureococcus bloom

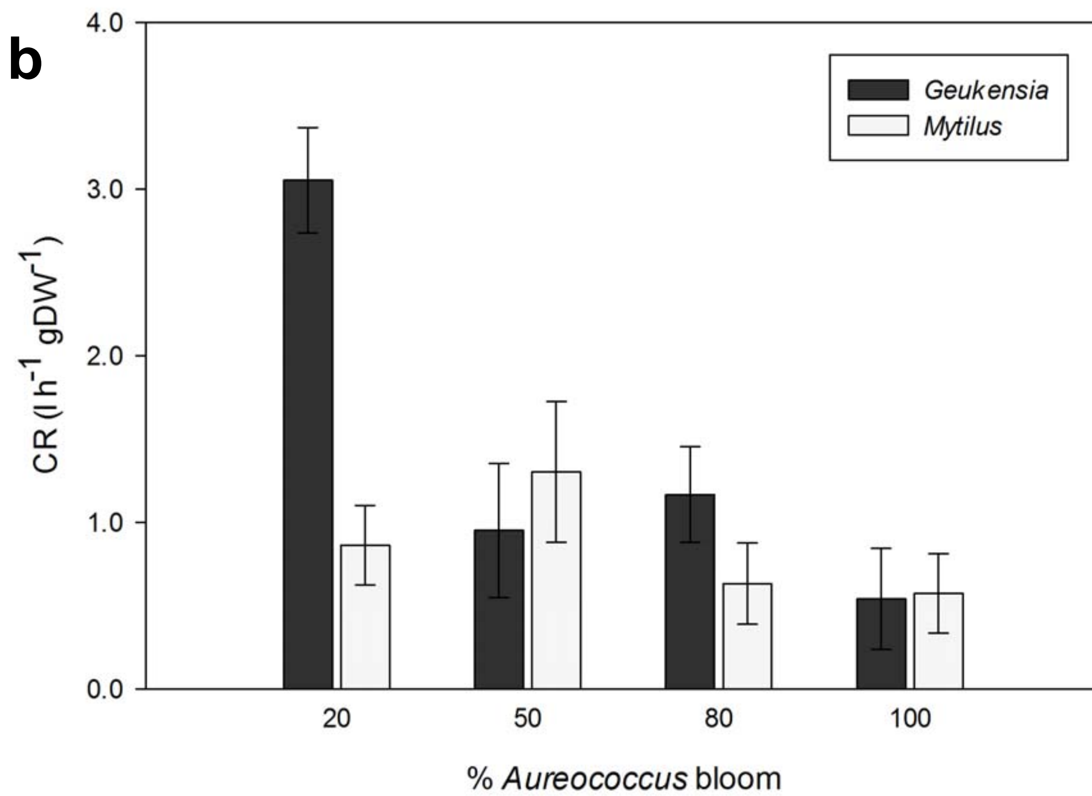
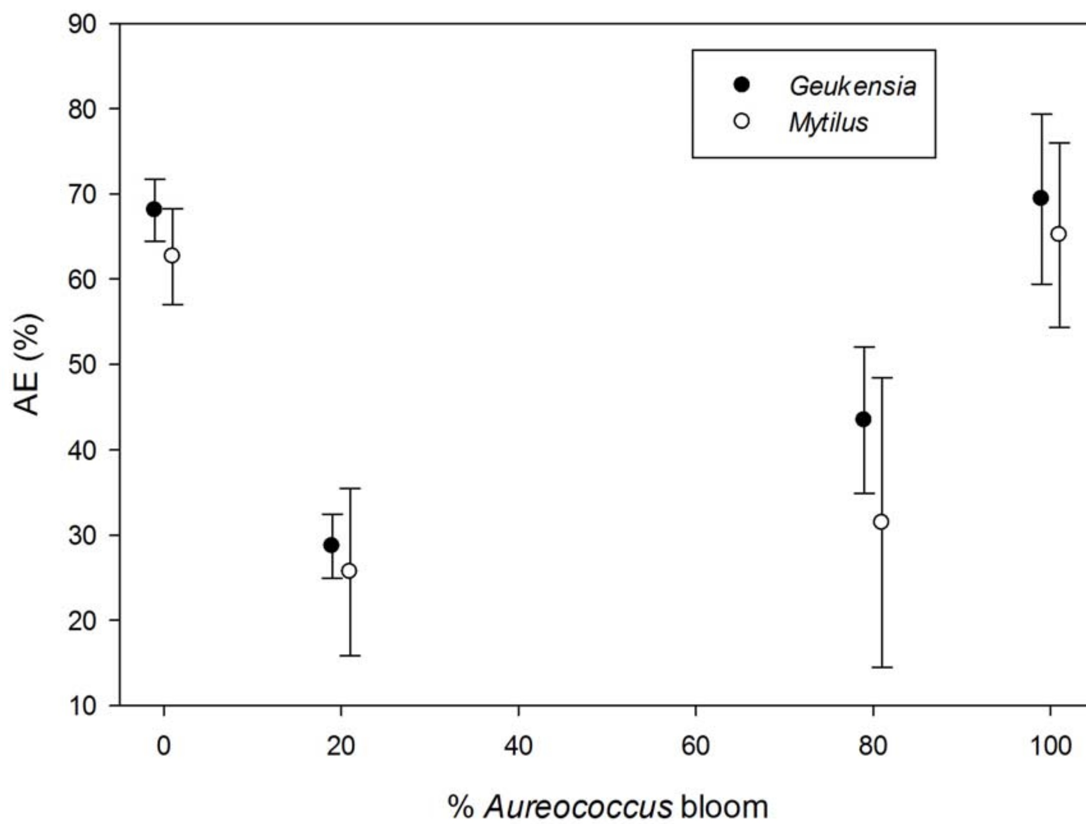

b
Geukensia
Mytilus
CR (l h-1 gDW-1)
4.0
3.0
2.0
1.0
0.0
20
50
80
100
% Aureococcus bloom

Figure 2.5: Absorption efficiencies (mean ± SE) of *Geukensia demissa* and *Mytilus edulis* feeding on an *Aureococcus anophagefferens* bloom (from Quantuck Bay) diluted (vol:vol) with Stony Brook Harbor natural plankton. ANOVA treatments $F(3,13) = 17.2$; $p < 0.001$.



Geukensia
Mytilus
AE (%)
% Aureococcus bloom
90
80
70
60
50
40
30
20
10
0
20
40
60
80
100

CHAPTER 3

Grazing of marine mussels (*Geukensia demissa* Dillwyn, *Mytilus edulis* L.) and clams (*Mercenaria mercenaria* L.) exposed to a proportional gradient of toxic planktonic and benthic dinoflagellates

ABSTRACT

Bloom-producing toxic dinoflagellates are an increasing nuisance in coastal and estuarine systems. The dinoflagellates *Amphidinium carterae* and *Prorocentrum minimum* are capable of developing blooms in the order of 10^4 - 10^5 cells ml^{-1}, and adverse effects have been described for shellfish and zooplankton. Potential top-down controls on dinoflagellates exerted by grazing activity of marine mussels (*Geukensia demissa*, *Mytilus edulis*) and clams (*Mercenaria mercenaria*) were studied on laboratory-based experiments. Proportional gradients (maintaining a constant level of total particulate organic C) of a microalga known to support good bivalve growth (*Isochrysis galbana*, CCMP1323) and either toxic dinoflagellate (*A. carterae* CCMP1314; *P. minimum* CCMP696) were fed to bivalves in short term (1 h) static chamber experiments. Bivalve clearance rates were estimated from absolute chlorophyll content depletion and automated counts of cell concentrations (Coulter Counter or flow cytometry). The significantly lower retention efficiencies for the two dinoflagellates compared to expected published values may be indicative of toxic effects on shellfish. Nonetheless, all three shellfish showed no significant differences across proportional treatments of either *A. carterae* or *P. minimum*. When contrasted to the two mussels, *M. mercenaria* exhibited significantly lower clearance rates in the presence of a toxic species.

INTRODUCTION

Harmful algal blooms (HABs) occur when cell densities of (generally) a single microalgal species are significantly elevated in relation to background concentrations in a short period of time, leading to a disruption of an aquatic ecosystem (Sunda et al., 2006). The disruptive mechanisms are varied, some have to do with the promotion of anoxic events, or the modification of the light environment with adverse consequences to submerged aquatic vegetation, but most usually relate to chemical compounds produced by HAB species. Most of the microalgae involved in the formation of HABs produce potent biotoxins that have adverse effects on the physiology of other aquatic biota, and that, through trophic biomagnification and transfer, can impact all levels of marine food

webs, including humans (Landsberg, 2002; Sunda et al., 2006). HABs occur in marine, estuarine, and freshwater systems and their frequency has increased globally due to anthropogenic influences (Smayda, 1990; Hallegraeff, 1993; Anderson 1997; Sunda et al., 2006).

Of the ~5000 described species of marine microalgae only dozens species of phytoplankton have been reported to be harmful; 90% of these being flagellates (most notably dinoflagellates; Smayda, 1997). Several authors (e.g. Sournia, 1995; Smayda, 1997) have linked specific features of dinoflagellate biology (e.g. mixotrophy, vertical migration, and typical swimming and aggregation patterns) to their supremacy among HAB species. Dinoflagellates are a significant component of protistan nano- and microplankton, with recent studies demonstrating complex trophic modes (e.g. facultative heterotrophy, mixotrophy) among representatives of this group (Jeong et al., 2005; Seong et al., 2006).

Toxic species of dinoflagellates are an emerging topic of research and concern in coastal ecosystems. The unarmoured dinoflagellate *Amphidinium carterae* has been reported to produce ichthyotoxic hemolysins (Yasumoto, 1990). A closely related species, *A. klebsii*, is known to produce an antifungal agent with hemolytic activity (Satake et al., 1991). Some allelopathic properties have also been attributed to bioactive compounds synthesized by *Amphidinium* (amphidinols) inhibiting growth of the diatom *Nitzschia* sp. (Paul et al., 1996).

Amphidinium carterae is an epibenthic dinoflagellate, often abundant in association with macroalgae (Baig et al., 2006), but can also be resuspended by currents into the pelagic environment (i.e. tychopelagic) where it can form blooms reaching cell densities of 1.2×10^4 cells ml^{-1} (Baig et al., 2006). The majority of benthic dinoflagellates occuring in the nepheloid layer produce toxins (Holmes, 1996), and despite recent research interest in other benthic dinoflagellates (e.g. *Gambierdiscus* and *Ostreopsis*), few studies have examined the ecological impacts of *Amphidinium*. Working with natural zooplankton communities from the lower Hudson River estuary, Lonsdale et al. (1996a) found that neither the larger mesozooplankton (adult copepods and copepodites), nor micrometazoa (nauplii) grazed on radiolabeled *Amphidinium* sp. Similarly, in studies with cultured strains, Jeong *et al*. (2001b) noted that the copepod

Acartia avoids *Amphidinium carterae* as a prey item, but still, there is some carbon transfer to the copepod from *Amphidinium* via the heterotroph *Oxyrrhis marina*.

Prorocentrum minimum is an armoured dinoflagellate with a wide geographic range throughout coastal areas, and most strains are considered to be nontoxic, at least to humans (Landsberg, 2002). In addition to demonstrated allelopathic effects of high-density *P. minimum* filtrates on the growth of diatoms (Tameishi et al., 2009), the toxicity of certain *P. minimum* strains has been confirmed in laboratory exposures to mice (Denardou et al., 1995; Grzebyk et al., 1997; Denardou-Queneherve et al., 1999). However, according to Wikfors (2005) it appears that *P. minimum* is toxic only sporadically (i.e. presents transient toxin expression), with specific environmental conditions (e.g. carbon deprivation that likely occurs during an intense bloom) inducing toxin production. Some studies have even used *P. minimum* as an ideal (control) food source for copepods *versus* aldehyde-producing diatoms (Ianora et al., 2004). At present, the chemical compound(s) responsible for *P. minimum* effects on rodents and molluscs remain(s) unknown, and it is not clear if it is the same compound(s) that elicits toxic responses in these two targets (Wikfors, 2005).

Regardless of the chemical identity of the bioactive compound produced by *P. minimum*, there is substantial evidence of its adverse effects (e.g. pathological effects, inhibition of feeding, and mortality) on bivalves (Luckenbach et al., 1993; Wikfors and Smolowitz, 1993; 1995; Glibert et al., 2007; Galimany et al., 2008). Glibert et al. (2007) found severe reduction in motility of 2-week old larvae of the Asian oyster *Crassostrea ariakensis* exposed to *P. minimum*, but embryos and larvae of the eastern oyster *Crassostrea virginica* were not adversely affected (Glibert et al., 2007; Stoecker et al., 2008). Luckenbach et al. (1993) fed mixed diets of *P. minimum* at bloom densities to juvenile eastern oysters, all of which died within 14 days, or within a longer period (22 days) when the bloom density was diluted to 33%. Besides causing mortality, *P. minimum* proved to be an unsatisfactory food source at high concentrations, reducing clearance rates (Luckenbach et al., 1993). Wikfors and Smolowitz (1993) exposed hard clams (*Mercenaria mercenaria*) and bay scallops (*Argopecten irradians*) to diets based on *P. minimum* and *P. micans* in combination with the standard bivalve food *Isochrysis*. Clams survived on *P. minimum* diets, but did not grow, and a diet including *P. minimum*

caused 100% mortality of bay scallops in one week. Bay scallops ingested *P. minimum*, but histopathological observations showed poor development of digestive diverticula, attenuation of the epithelium with abnormal vacuolation, and necrosis. The authors suggested that *P. minimum* may produce an enterotoxin that systemically affects absorptive cells and the vascular system (Wikfors and Smolowitz, 1993). Additional studies showed newly settled spat of eastern oysters exposed to *P. minimum* had an abnormal accumulation of lipid in the stomach epithelium (Wikfors and Smolowitz, 1995); accumulation bodies within absorptive cells of the digestive diverticulum contained dinoflagellate autolysosomal bodies, indicating some sort of nutritional interference. Similarly, Galimany et al. (2008) found that *Mytilus edulis* subjected to monospecific diets of *P. minimum* consumed cells but the ingestion elicited an immune response in the intestine, which implied hemocyte encapsulation of the dinoflagellate.

One of the characteristic features of *P. minimum* is the production of high-density blooms, mostly in spring-summer. Literature reports of cell densities reached during blooms present considerable variation among locations. For example an April bloom in Sinaloa Bay, Mexico, presented a mean density ~7.2×10^3 cells ml^{-1}, reaching up to 2.7×10^4 cells ml^{-1} (Martínez-López et al., 2008). Yamasaki et al. (2010) mention densities up to 4.3×10^4 cells ml^{-1} occuring from May to June in Hakozaki, Japan. 'Mahogany tides' of *P. minimum* occur annually in the upper and middle regions of Chesapeake Bay, with cell concentrations sometimes reaching 1×10^5 cells ml^{-1} (Johnson et al., 2003). The highest densities reported in the literature are on the order of 3.5×10^5 cells ml^{-1}, for August in the Gulf of Gdańsk (Baltic Sea; Witek and Pliński, 2000). Blooms have also been recorded in several NY coastal bays that historically contained dense bivalve populations. For instance, a bloom of *P. minimum* of up to 8.5×10^3 cells ml^{-1} occurred in the Forge River, a tributary to Moriches Bay in late summer of 2008 (RM Waters, Suffolk County Department of Health Services, pers. comm.); and a bloom of 4.5×10^3 cells ml^{-1} happened in mid-December 2009, in Shinnecock Bay (J Pan, unpub.)

Suspension-feeding bivalves exert a dominant organizing role in shallow aquatic ecosystems by diverting production from the water column to the benthos (French McCay et al., 2003) and at high densities provide a number of positive benefits (reviewed by Newell, 2004). Studies on the mechanisms controlling phytoplankton biomass in

lagoonal systems point to a prominent role of bivalve grazing compared to mesozooplankton grazing (Nakata et al., 2000). For decades, scientists have advocated for biological control as an alternative to chemical or physical methods in the mitigation of HABs (Anderson, 1997). The objectives of this research were to study the potential of bivalve suspension feeders to graze on benthic and pelagic dinoflagellates capable of creating dense blooms and potentially toxin-producing. Three species of bivalves were considered, in consideration of their co-occurrence in coastal environments where *Amphidinium carterae* and *Prorocentrum minimum* have been recorded. These shellfish were the Northern quahog, *Mercenaria mercenaria*, a subtidal clam that burrows in shallow sandy or muddy bottoms (Wells, 1957), and two intertidal marine mussels: the ribbed mussel, *Geukensia demissa*, a keystone species in *Spartina alterniflora* marshes, partially embedded in the sediment, or in superficial aggregations attached by byssal threads (Bertness, 1984; Franz, 1997), and *Mytilus edulis*, a semi-sessile species which prefers hard substrates (Bayne et al., 1976). All three shellfish considered in this study posses well developed latero-frontal cirri (Jørgensen, 1990) which enable them to retain particles >4 µm with 100% efficiency (Møhlenberg and Riisgård, 1978; Riisgård, 1988).

This research was motivated because, firstly, to date very few studies have examined the effects of toxic, bloom-forming dinoflagellates on bivalve clearance rate, one of their most commonly measured physiological parameters (one exception being Luckenbach et al., 1993). Secondly, an extensive review of the literature revealed that no studies have yet focused on benthic organisms grazing on *Amphidinium carterae*, in spite of it being a common benthic dinoflagellate. Lastly most studies evaluating the effects of toxic microalgae on shellfish have considered commercially-exploited species, with a few exceptions (Gainey and Shumway, 1991; Lesser and Shumway, 1993) looking at ecosystem-relevant but unexploited bivalves such as the ribbed mussel, *Geukensia demissa*.

MATERIALS AND METHODS

Microalgal cultures

The three algal species involved in this study were the prymnesiophyte *Isochrysis galbana* (CCMP 1323, isolated from the Irish Sea), and the dinoflagellates *Prorocentrum minimum* (CCMP 696, isolated from the Great South Bay, NY) and *Amphidinium carterae* (CCMP 1314, isolated from Cape Cod, MA). Microalgae were grown in a modified-batch culture system (Hoff and Snell, 2001) and scaled up to 20 l, using L1 medium for dinoflagellates (Guillard and Hargraves, 1993), and f/2-Si medium for *Isochrysis galbana* (Guillard and Ryther, 1962). All cultures were prepared with 0.2-µm filtered seawater from Long Island Sound (S =27), and mild air bubbling. Incubators were set at 20ºC and a 16:8 h light:dark cycle with an average irradiance of ~38 × 10 µmol quanta m^{-2} s^{-1} (Quantum sensor, model SKP 200, Skye Instruments Ltd.). The growth of all microalgal species was monitored by *in vivo* chlorophyll *a* fluorescence (Turner Designs 10-AU fluorometer) and/or cell counts (performed microscopically with a hemocytometer). For experiments, *Isochrysis galbana* was harvested at exponential growth phase, and the dinoflagellates were harvested in late-stationary growth phase, since at this stage dinoflagellates exhibit their maximum toxin production and toxicity potential (Sakamoto et al. 1992, Grzebyk et al.,1997, Emura et al. 2004). For each experiment, linear standard measurements (n =75 for each species) were obtained with a Zeiss compound microscope. On average, length (SD) × width (SD) in µm were: 5.6 (1.4) × 4.3 (0.3) for *Isochrysis galbana*; 17.2 (1.6) × 14.1 (1.7) for *Prorocentrum minimum*; and 13.9 (1.4) × 10.2 (1.1) for *Amphidinium carterae*.

Experimental shellfish

Blue mussels (*Mytilus edulis*) and ribbed mussels (*Geukensia demissa*) were collected at low tide from wild populations in Flax Pond, NY, a tidal marsh adjacent to Long Island Sound; hard clams (*Mercenaria mercenaria*) came from natural and re-stocked populations in Great South Bay, NY, a coastal lagoon. Mean shell lengths were [mm (SE)] 39.3 ± 1.3 (n =49), 77.2 ± 1.5 (n =70), and 67.8 ± 0.5 (n =40); for *M. edulis*, *G. demissa* and *M. mercenaria*, respectively. All experimental bivalves were cleaned of epibionts, and allowed to purge their guts in 0.2-µm filtered seawater for >24 h prior to the experiments.

Grazing experiments

Bivalves were exposed to suspensions of *Isochrysis galbana* which supports good bivalve growth (Jeffrey et al. 1994; Barsanti and Gualtieri 2006), and either one of the toxic dinoflagellates *Prorocentrum minimum* and *Amphidinium carterae*. In order to eliminate confounding effects of varying food concentration (particulate organic C) across treatments, a proportional gradient of any two species was calculated on a C basis. The proportions of any two algal species considered were 100:0, 80:20, 50:50, 20:80, 0:100. Literature C conversion values of 8.2 pg C cell^{-1} for *I. galbana* (Strathmann, 1967), 150.0 pg C cell^{-1} for *P. minimum* (Kim and Jeong, 2004), and 95.4 pg C cell^{-1} for *A. carterae* (Menden-Deuer and Lessard, 2000) were used for calculations. Experiments with *Amphidinium* were performed at a particulate organic carbon concentration of (mean ± SE) 1,000.0 ± 0.3 µg C l^{-1}, and experimental runs with *P. minimum* were performed at a concentration of 1,049.6 ± 16.8 µg C l^{-1}. These values are in the lower range of the concentrations that Foster-Smith (1975) found to give optimum clearance rate for marine mussels (*Mytilus edulis*) and clams (*Cerastoderma edule*). Also, both values are close to the concentration of particulate organic carbon corresponding to monospecific blooms reported in the literature for *Amphidinium carterae* (~1.2×10^4 cells ml^{-1} =1,145 µgC l^{-1}) and *Prorocentrum minimum* (~8.5×10^3 cells ml^{-1} =1,275 µgC l^{-1}). Combusted clays from the Hudson River, NY (1000 µg l^{-1}) were added to the experimental mixture containing about the same concentration of particulate organic carbon in order to provide an inorganic fraction to the suspension (Bricelj and Malouf, 1984).

Grazing experiments were conducted in static chambers (cylindric, 19.8 cm base diameter, semi-clear polypropylene beakers) with a single bivalve per beaker. Prior to the experiment the animals were conditioned in the experimental algal mixtures for 1 h, and then they were gently transferred to fresh treatment mixtures (volume 2 l). The start of an experiment was determined by visually checking for signs of normal feeding function (*e.g.*, gaping of valves, extendend mantle edges and/or siphons; Riisgård, 1988). Each experimental run lasted for 1 h, so that, on average, bivalves were not allowed to graze the microalgal biomass below 33% of the original concentration, and to prevent accumulation of metabolic wastes in the suspension (Bayne et al., 1976). Experiments were carried out at room temperature (~20ºC) in a darkened lab (~0.25% growth

irradiance), in order to prevent growth of photosynthetic microalgae during the course of the experiments. Experiments followed a randomized complete block design (Sokal and Rohlf, 1995), with each block consisting of a control and each treatment combination, and using the same batch of microalgae.

At the beginning (t_0) and end of each block run (t_f), aliquots of the particle suspension were taken for total chlorophyll *a* determination and cell counts. Total chlorophyll *a* was determined by concentrating duplicate 30 ml samples onto Whatman GF/F filters, extracting for at least 24 hours in 100% acetone at -20ºC, and measured fluorometrically (Turner Designs, model 10-AU; Arar and Collins, 1997). Cell densities of the experiments using mixtures of *Amphidinium carterae* and *Isochrysis galbana* were estimated from 5 ml samples preserved in 1% glutaraldehyde (from a 10% stock solution prepared with filtered natural seawater) by flow cytometry (Becton-Dickinson, FACSCalibur). A known concentration of yellow-green fluorescent 2 µm polystyrene beads (Sigma-Aldrich Co.) was added as a standard for the enumeration of cells. Data were interpreted using the CellQuest software package for flow cytometry analysis. Cell densities of the experiments using mixtures of *Prorocentrum minimum* and *Isochrysis galbana* were estimated from aliquot samples (5 ml) preserved in 10% Lugol's acidic iodine solution and run in a Coulter Multisizer 3 (Beckman-Coulter) fitted with a 100 µm aperture and a counting duration of 26 s.

Clearance rates (CR) were determined from the rate of cell removal as

$$CR = \frac{V}{n}\left[\frac{(\ln C_0 - \ln C_t)}{t} - a\right] \ ,$$

where V is the water volume, n dry tissue weight of the bivalve, C_0 is the chlorophyll content or cell density at t=0, C_f is the chlorophyll or cell density at t final, and a is the background growth rate (positive or negative) from control beakers without bivalves (Coughlan, 1969). Retention efficiencies (RE) of toxic dinoflagellates were calculated relative to *Isochrysis galbana* following Bricelj et al. (2001). For mixed suspensions, RE was determined as

$$RE = 100 \times \frac{CR_{dinoflagellate}}{CR_{I.galbana}}$$

and for single species treatments (100% dinoflagellates)

$$RE = 100 \times \frac{mean\ CR_{dinoflagellate}}{mean\ CR_{I.galbana}}$$

Bivalves were then dissected and soft tissue dry weight was determined by drying at 60ºC for >48 h, in order to express clearance rate in units of dry tissue weight.

Data analysis

Clearance rates based on total chlorophyll *a* across treatments were analyzed by regression; if results were non-significant, a single-factor ANOVA was run to confirm that the outcome was not dependent on the choice of a linear model. In the analysis of clearance rates based on cell counts of individual microalgae, a test for homogeneity of slopes was run first, and if non-significant, the data was then analyzed by 2-factor ANCOVAs with blocks as random factors and with the proportion of a species as the covariate. Slopes were pooled and their adjusted means tested. This was followed by a 3-way ANOVA with blocks as random factors, and species and dilution proportion as a nominal factors; the single algal groups (0:100 and 100:0 treatments) had to be combined for this test. If significant, both a *post-hoc* multiple comparison test (Tukey HSD), and an *a priori* planned comparison of species at a proportion were run (Sokal and Rohlf, 1995). Clearance rates were contrasted between the three bivalves by means of a 2-factor ANOVA (chlorophyll-based CRs) with microalgal proportion, shellfish species and their interaction as factors; and by pooling the slopes and doing a test for adjusted means (ANCOVA) for the cell count-based CRs. Retention efficiencies were compared to expected published values by means of a two-tailed t-test. All statistical analyses were done with the Statistica software package (version 9).

RESULTS

The general trend for clearance rates based on total chlorophyll for all three shellfish showed no significant differences across treatments considering different proportions of *A. carterae* or *P. minimum* (Table 3.1; Figures 3.1a-3.6a). The few exceptions to this trend were for *Geukensia demissa* grazing on *Amphidinium* (Figure

3.2a), in which the regression test was significant (regression $t = 3.26$, $df = 17$, $p < 0.01$), indicating that clearance was reduced with increasing proportions of the toxic dinoflagellate in the diet; however a single-factor ANOVA was non-significant ($F(4,14) = 2.4$, $p > 0.05$). The other such case was for *Mytilus edulis* grazing on a *P. minimum* gradient (Figure 3.4a), in which the ANOVA test was marginally significant ($F(4,14) = 3.16$, $p = 0.048$), but the regression was non-significant ($t = -1.49$, $df = 17$, $p > 0.05$). Although non-significant, several experiments showed higher clearance rates for those treatments combining *I. galbana* and a dinoflagellate (particularly *P. minimum*; Figures 3.5a, 3.6a), indicating some preference for mixed diets.

In general, clearance rates estimated from cell counts corroborated the trends from the data set based on chlorophyll, showing no significant differences in clearance for all three bivalves between *Isochrysis galbana* and either dinoflagellate, or across the proportional gradient (Table 3.2; Figures 3.1b-3.6b). This finding suggests that neither dinoflagellate had an effect impairing grazing from either bivalve tested. However, *M. edulis* grazing on *A. carterae* showed a significant difference for the proportion (nominal) factor of the ANOVA test ($F(2,5) = 8.64$, $p = 0.02$; Figure 3.1b). The other exception was for *Mytilus* grazing on *P. minimum*, in which the proportion factor of the 3-factor ANOVA yielded a marginally significant difference ($F(3,17) = 3.31$, $p = 0.045$; Figure 3.4b). An *a posteriori* Tukey HSD multiple comparisons test showed that the 100% treatments were significantly different from the *Prorocentrum*:*Isochrysis* 80:20% treatment.

When compared (2-factor ANOVA), chlorophyll-based clearance rates were significantly different between bivalve species for the *Amphidinium* ($F(2,59) = 26.92$; $p < 0.001$) and *P. minimum* ($F(2,59) = 14.1$; $p < 0.001$) experiments. This was congruent with clearance rates based on cell counts, which yielded a significantly lower ($p < 0.001$) adjusted mean by ANCOVA for *Mercenaria* feeding on both *Amphidinium* (0.69 ± 0.15) and *P. minimum* (1.15 ± 0.14). Adjusted means for *Geukensia* and *Mytilus* were not significantly different (Tukey HSD test $p = 0.9$), and their respective values were 3.01 ± 0.35 and 2.81 ± 0.62 for *Amphidinium*; and 3.06 ± 0.43 and 3.18 ± 0.39 for *P. minimum* experiments.

The three microalgal species used in these experiments were >5 µm, and according to Møhlenberg and Riisgård (1978) and Riisgård (1988), all organic particles within this size-range should have been retained with 100% efficiency by all three species of bivalves. Mean retention efficiency (± SD) results are presented in Table 3.3, with the p-values of a two-tailed t-test. Interestingly, all retention efficiencies were significantly lower than expected for particles of their size (except RE of *Mytilus* with *A. carterae*), which suggests that a factor other than cell size was influencing particle capture. In some cases (e.g. *Geukensia* and *Mercenaria* feeding on *Amphidinium*) the remarkably low retention efficiencies corresponded to those expected for particles <2 µm. When compared by means of a 2-factor ANOVA (with bivalve and dinoflagellate species as factors), no significant differences in retention efficiency were found between bivalves ($p>0.05$), or dinoflagellate species ($p>0.05$), but the interaction of the factors was significant ($F(2,29) = 5.35$; $p = 0.01$).

DISCUSSION

The short-term experiments in this study focused on bivalve clearance rate and particle capture using mixed suspensions of toxic dinoflagellates and a recognized nutritionally rich microalga. The validity of such experimental design lies in the fact that monodiet experiments are not representative of realistic natural conditions (even for bloom-forming species). The incorporation of two species in a suspension, along a proportional gradient, is more representative of natural conditions (with the 100% treatment being equivalent to a truly monospecific bloom). The finding of no significant differences in clearance rates across proportional treatments indicates that all bivalves tested were able to cope with increasing proportions of dinoflagellates in their diets. If these clearance rates are sustained over prolonged periods of time, the bivalve species tested may exert a top-down control on HAB-causing dinoflagellates.

Considering that the chemical identity of *P. minimum*'s toxin(s) is still an unresolved matter (Wikfors, 2005), and that there are no published toxicity bioassays considering *A. carterae* and bivalves, studies like this are important. However, the results should be interpreted with caution. Firstly, the data presented here are not conclusive on

toxic effects elicited by the dinoflagellates tested. Experiments with a similar approach, using a mixed diet of a different *P. minimum* clone and the diatom *Thalassiosira weissflogii* along a proportional gradient (nutritional 'reference line'), concluded that *P. minimum* was nutritionally insufficient for the copepod *Acartia tonsa* (Dam and Colin, 2005). Except for *Geukensia* feeding on *Amphidinium* (Figure 3.2a), the calculated regressions of clearance *versus* dinoflagellate concentration in the mix were non-significant, so the 'reference line' cited by Dam and Colin (2005) does not apply to these data.

When contrasted to the two mussels, *M. mercenaria* exhibited significantly lower clearance rates (Figures 3.3 and 3.6). This result is most likely related to the concentration of particulate organic carbon used in the experimental mixtures. Tenore and Dunstan (1973) determined that the feeding rate of hard clams increases with food concentration up to ~450-650 µgC l^{-1} and declines thereafter. Likewise, Malouf and Bricelj (1989) refer to an optimal concentration of particulate organic carbon for a number of bivalves in the range of 300-700 µgC l^{-1}. Tenore and Dunstan (1973) reported consistently higher feeding rates for *Mytilus edulis* than for hard clams across a range of concentrations, consistent with the results in this study. Estuarine bivalves cope with the naturally fluctuating levels of food in their environments by two methods: keeping a constant filtration rate over a range of cell concentrations and increasing the proportion of material rejected as pseudofeces (e.g. the strategy of marine mussels), or reducing the amount of material filtered by reducing filtration rates and lower pseudofecal production (e.g. clams; Foster-Smith, 1975). Tenore and Dunstan (1973) reported comparatively lower biodeposition rates in hard clams than in blue mussels, also suggesting that they may not be as well adapted for feeding at high food concentrations as marine mussels. For consistency among experimental shellfish species and for comparative purposes, all the experiments in this study were run at ~1,000 µgC l^{-1}, which is above the optimal level of particulate organic carbon for many bivalves (~700 µgC l^{-1}; Malouf and Bricelj, 1989), and likely created an experimental artifact for *M. mercenaria*. However, the clearance rates expressed per g DW obtained in this study fall within the range reported by Malouf

and Bricelj (1989, Table 2.6) for hard clams feeding on a suspension of natural particles at the same and lower temperatures as in this study.

Despite the significantly lower retention efficiencies in some cases clearance rates for *Isochrysis* in mixed-algae suspensions were lower than those for either dinoflagellate (Figures 3.1b, 3.4b, 3.6b). This indicates that in the presence of a toxic species there might be inhibitory uptake of a non-toxic prey. This pattern has also been reported by Bricelj et al. (2001) and termed suppressed feeding for *M. mercenaria* feeding on mixed suspensions of a toxic strain of *Aureococcus anophagefferens* and *Isochrysis galbana*.

The significantly lower retention efficiencies for the two dinoflagellates compared to literature values (Møhlenberg and Riisgård, 1978; Riisgård, 1988), found with most bivalves in this study, could be related to three factors: the swimming capacity; the non-spherical cell shape; and/or the presence of bioactive metabolites (toxins) in the dinoflagellates studied. Cells of *P. minimum* are active swimmers (Parke and Ballantine, 1957), with a mean swimming speed of 107.7 $\mu m\ s^{-1}$ (Miyasaka et al., 1998). A mean swimming speed of 75.1 $\mu m\ s^{-1}$ has been reported by Gittleson et al. (1974) for *A. carterae* cells. The speed of movement in flagellated protozoa can be fast in relation to their cell size, particularly in the smaller species (<20 µm; Laybourn-Parry, 1984), which seems to be the case for the dinoflagellates considered in this study. In terms of body lengths, *P. minimum* can cover in 6.26 s^{-1} its length while *A. carterae* covers it in 5.4 s^{-1}. Flow velocities of the through current at the interfilament canals are on the order of 100 $\mu m\ s^{-1}$ (e.g. 300 $\mu m\ s^{-1}$ for *Crassostrea virginica*; Bernard, 1974), and it seems reasonable to assume that the swimming ability of the two dinoflagellates in this study might have contributed to some degree to their escape into the pallial cavity, thus lowering apparent retention in comparison to a poor swimmer like *Isochrysis galbana* (W. Day, University of Rhode Island, pers. comm.).

On the other hand, both dinoflagellates considered have cell shapes that deviate from the typical double-cone characteristic of most dinoflagellates, with *P. minimum* being laterally flattened (Faust and Gulledge, 2002), and *A. carterae* being flattened dorso-ventrally (Taylor et al., 2003). In their extensive review of particle capture and processing in marine bivalves, Ward and Shumway (2004) report that differences in cell

shape can significantly influence particle capture, with particles deviating from spherical shape (with the same volume) being more efficiently retained. If applicable to dinoflagellates, the latter would be contradictory with the findings reported in this study, but particle capture by suspension-feeding bivalves continues to be an active field of research, and many statements should be regarded as preliminary at best.

Lastly, it is possible that dinoflagellate cells are being retained differently and resuspended to a greater extent than *Isochrysis* cells by virtue of their toxicity. Bricelj et al. (1998) found that cells of a toxic strain of *Alexandrium* were captured and transported in a loose slurry on the dorsal food groove by the oyster *Ostrea edulis*. This tract has higher flow velocities than the ventral food groove, in which particles are transported in a well compacted mucus string. At the dorsal gill-labial palp junction a large proportion of the dinoflagellate cells were dispersed as a cloud into the surrounding pallial cavity, remaining free in suspension. The authors suggested that this pattern might have been related to toxin production in that *Alexandrium* strain, and raised the question that being resuspended after transport through the gills would result in lower measurements of clearance rates, when these are determined with an indirect method, as was done in this study.

Finally, conclusions drawn from short-term experiments like these may fall short in elucidating longer-term effects of exposure to noxious phytoplankton. Recently, studies have focused on the immunological response of bivalves triggered by long-term exposures to toxic dinoflagellate prey. For example, Hégaret and Wikfors (2005a) found that a long-term exposure of Eastern oysters and bay scallops to a *P. minimum* diet had a significant effect upon their immune profiles (e.g. increases in granulocytes, and death and changes in hemocyte function), these effects being dependent upon duration of exposure. Similarly, Hégaret and Wikfors (2005b) found an immunological response (i.e. increase in hemocyte numbers, and an increase in phagocytosis associated with mortality of hemocytes) in oysters exposed to blooms of *P. minimum*. Therefore, it would be interesting to contrast the findings in this study to longer-term exposure experiments.

ACKNOWLEDGMENTS

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Table 3.1: Statistical tests results for total chlorophyll-based clearance rates. See Figures for references.

		regression p-value		single-factor ANOVA p-value		Figure
A. carterae	*M. edulis*	0.82		0.32		**3.1a**
A. carterae	*G. demissa*	**0.004**	t= 3.26 df= 17	0.09		**3.2a**
A. carterae	*M. mercenaria*	0.19		0.37		**3.3a**
P. minimum	*M. edulis*	0.15		**0.04**	F(4,14)= 3.16	**3.4a**
P. minimum	*G. demissa*	0.84		0.75		**3.5a**
P. minimum	*M. mercenaria*	0.41		0.34		**3.6a**

factor: proportion

Table 3.2: Statistical tests results for cell counts-based clearance rates. See Figures for references.

		homogen. slopes p-value	2-factor ANCOVA p-value	3-factor ANOVA p-value	Figure
A. carterae	*M. edulis*	0.46	a 0.28 b 0.59	a 0.12 b **0.02** $F(2,5) = 8.64$ c 0.62	**3.1b**
A. carterae	*G. demissa*	0.28	a 0.71 b 0.71	a 0.58 b 0.47 c 0.54	**3.2b**
A. carterae	*M. mercenaria*	0.21	a 0.85 b 0.36	a 0.54 b 0.60 c 0.80	**3.3b**
P. minimum	*M. edulis*	0.27	a 0.19 b 0.25	a 0.29 b **0.04** $F(3,17) = 3.31$ c 0.71	**3.4b**
P. minimum	*G. demissa*	0.40	a 0.46 b 0.53	a 0.10 b 0.09 c 0.20	**3.5b**
P. minimum	*M. mercenaria*	0.87	a 0.62 b 0.85	a 0.72 b 0.72 c 0.91	**3.6b**

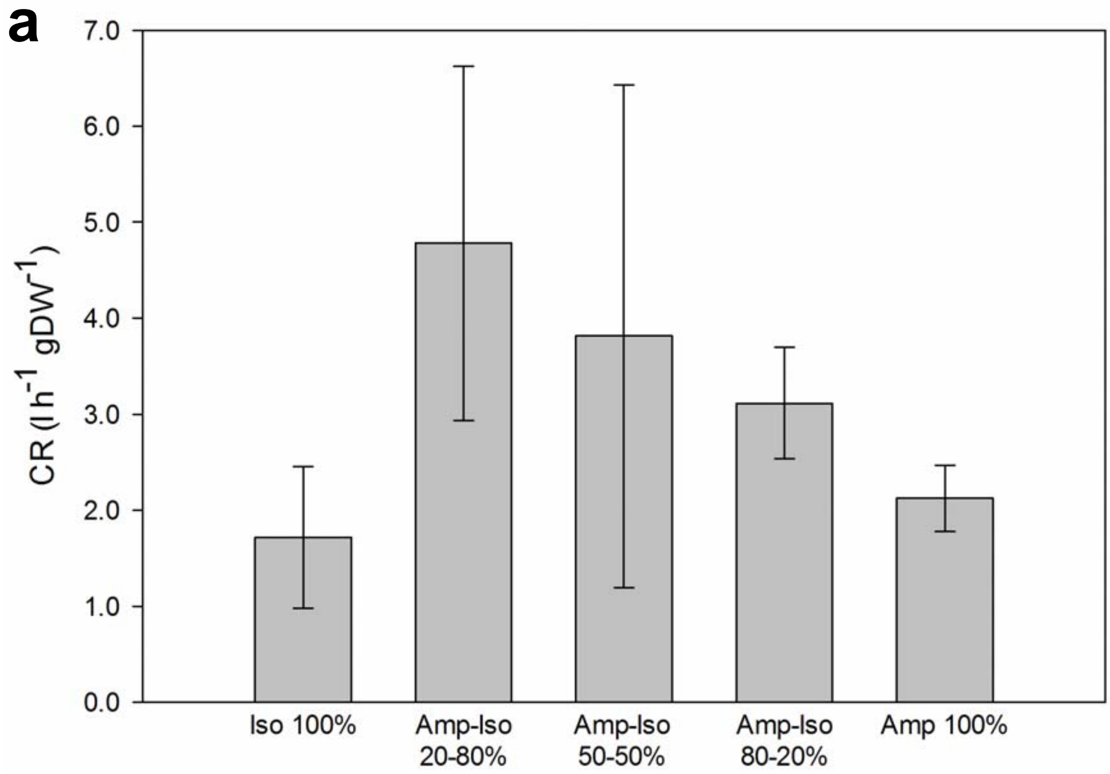
2-factor ANCOVA *factors:*
a: treatment (species)
b: proportion

3-factor ANOVA *factors:*
a: treatment (species)
b: proportion (nominal)
c: interaction

Table 3.3: Retention efficiency (mean ± SD) for the three bivalves species, grazing on *Amphidinium carterae* and *Prorocentrum minimum*. Data were compared to empirically-derived (expected) values by a two-tailed t-test.

	Amphidinium carterae		p-value	*Prorocentrum minimum*		p-value
Mytilus edulis	88.6	± 12.1	0.070	71.2	± 20.0	**0.032**
Geukensia demissa	56.3	± 30.0	**0.016**	65.8	± 26.1	**0.013**
Mercenaria mercenaria	43.3	± 19.5	**0.010**	79.8	± 13.0	**0.006**

Figure 3.1: *Mytilus edulis* clearance (mean ± SE) on proportional mixtures of *Amphidinium carterae* (CCMP 1314) and *Isochrysis galbana* (CCMP 1323). Mixtures were done on C-content basis; absolute C content across treatments =1000.0 μg C l^{-1}. **a)** CRs based on total chlorophyll *a*. **b)** CRs based on cell counts performed by flow cytometry.


a
CR (l h-1 gDW-1)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Iso 100%
Amp-Iso
20-80%
Amp-Iso
50-50%
Amp-Iso
80-20%
Amp 100%

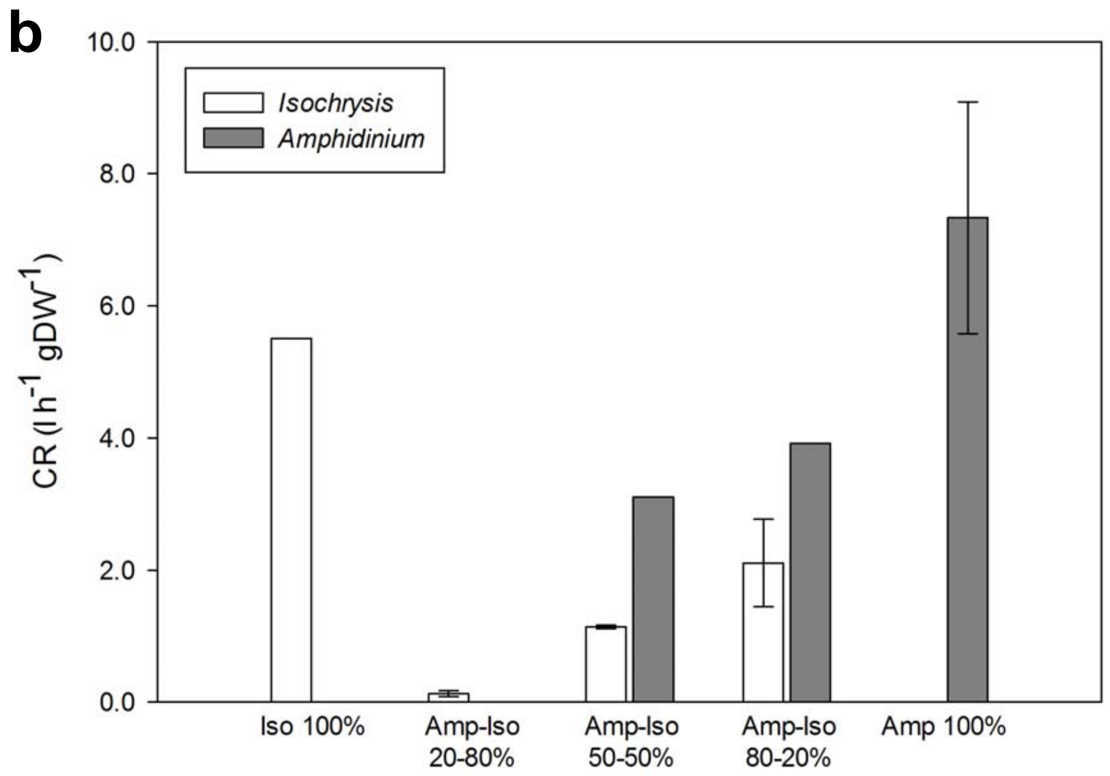
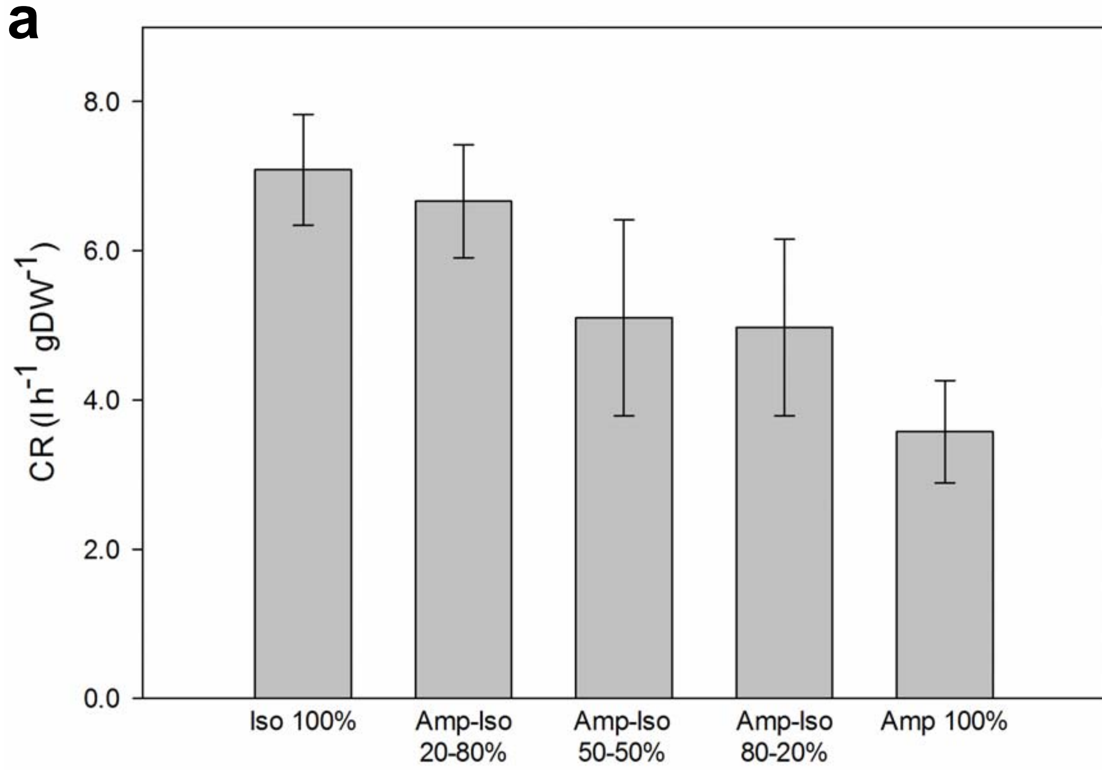

b
Isochrysis
Amphidinium
CR (l h-1 gDW-1)
10.0
8.0
6.0
4.0
2.0
0.0
Iso 100%
Amp-Iso
20-80%
Amp-Iso
50-50%
Amp-Iso
80-20%
Amp 100%

Figure 3.2: *Geukensia demissa* clearance (mean ± SE) on proportional mixtures of *Amphidinium carterae* (CCMP 1314) and *Isochrysis galbana* (CCMP 1323). Mixtures were done on C-content basis; absolute C content across treatments =1000.0 µg C l^{-1}. **a)** CRs based on total chlorophyll *a*. **b)** CRs based on cell counts performed by flow cytometry.


a
CR (l h^{-1} gDW^{-1})
8.0
6.0
4.0
2.0
0.0
Iso 100%
Amp-Iso 20-80%
Amp-Iso 50-50%
Amp-Iso 80-20%
Amp 100%

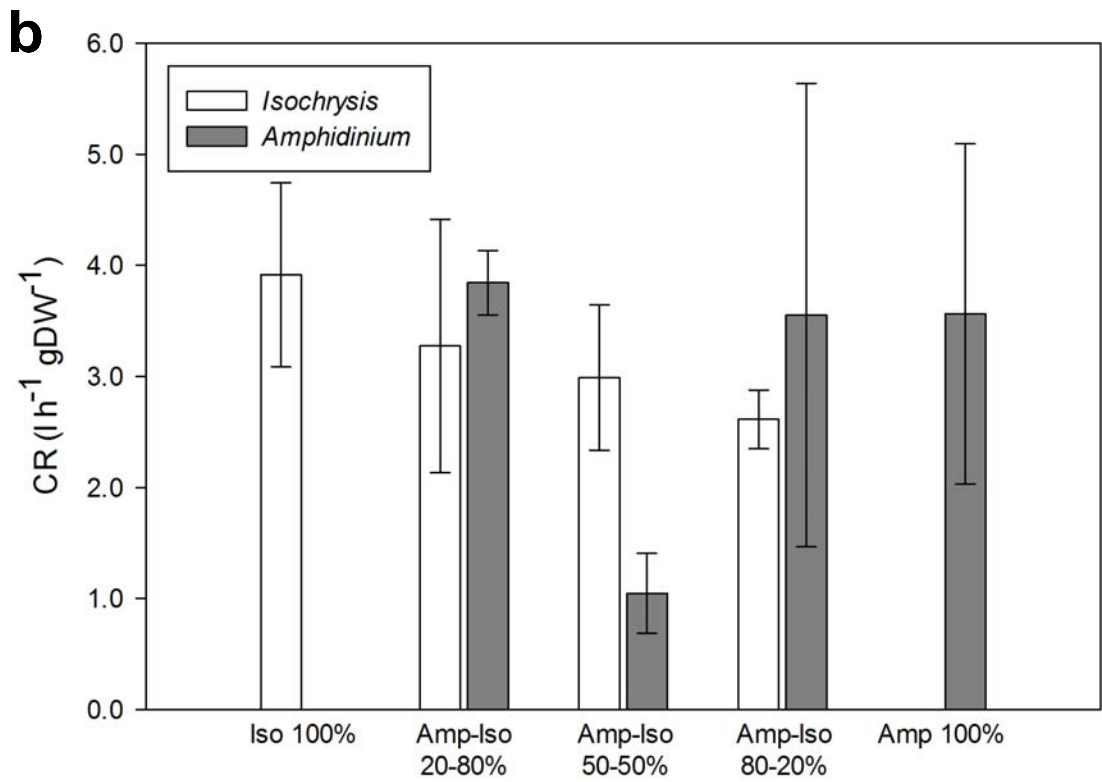
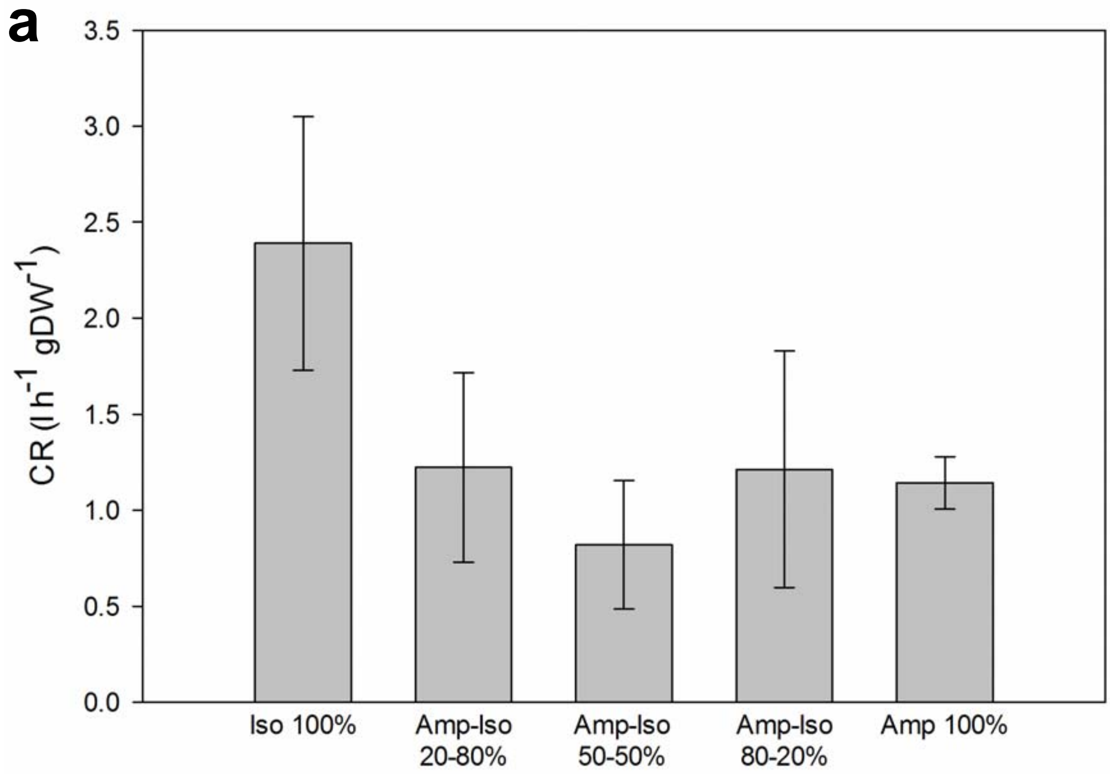

b
CR (l h^{-1} gDW^{-1})
Isochrysis
Amphidinium
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Iso 100%
Amp-Iso 20-80%
Amp-Iso 50-50%
Amp-Iso 80-20%
Amp 100%

Figure 3.3: *Mercenaria mercenaria* clearance (mean ± SE) on proportional mixtures of *Amphidinium carterae* (CCMP 1314) and *Isochrysis galbana* (CCMP 1323). Mixtures were done on C-content basis; absolute C content across treatments =1000.0 μg C l^{-1}. **a)** CRs based on total chlorophyll *a*. **b)** CRs based on cell counts performed by flow cytometry.


a
CR (l h^{-1} gDW^{-1})
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Iso 100%
Amp-Iso 20-80%
Amp-Iso 50-50%
Amp-Iso 80-20%
Amp 100%

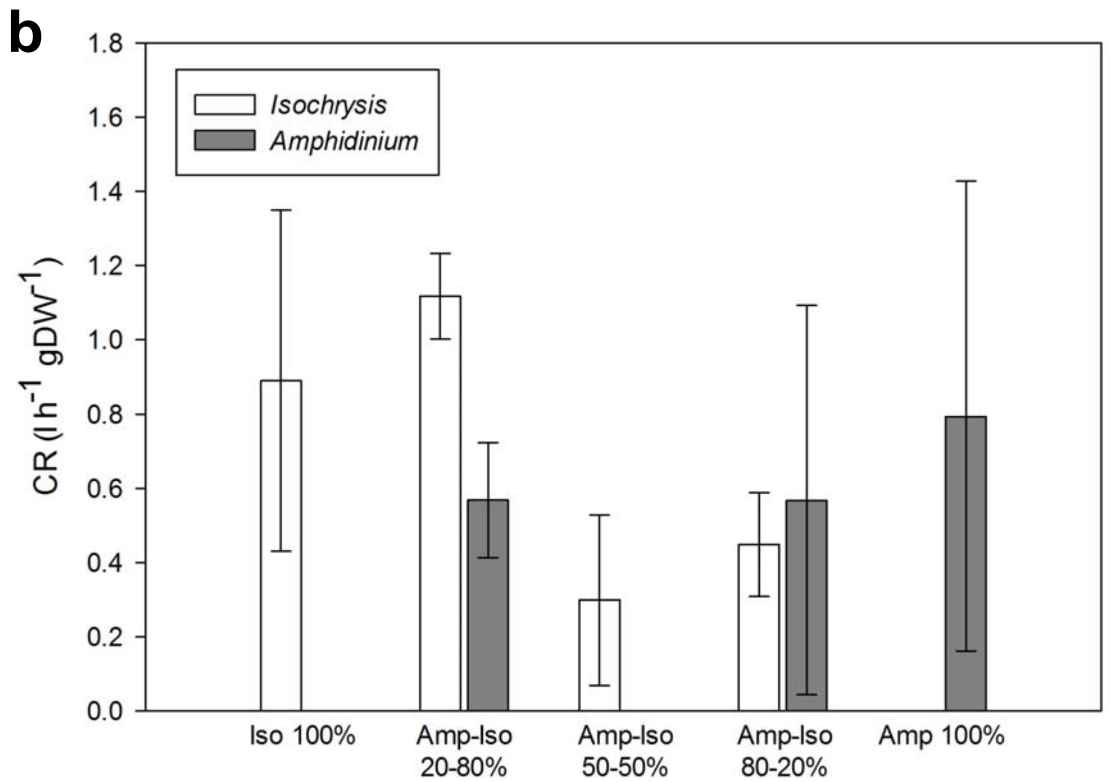
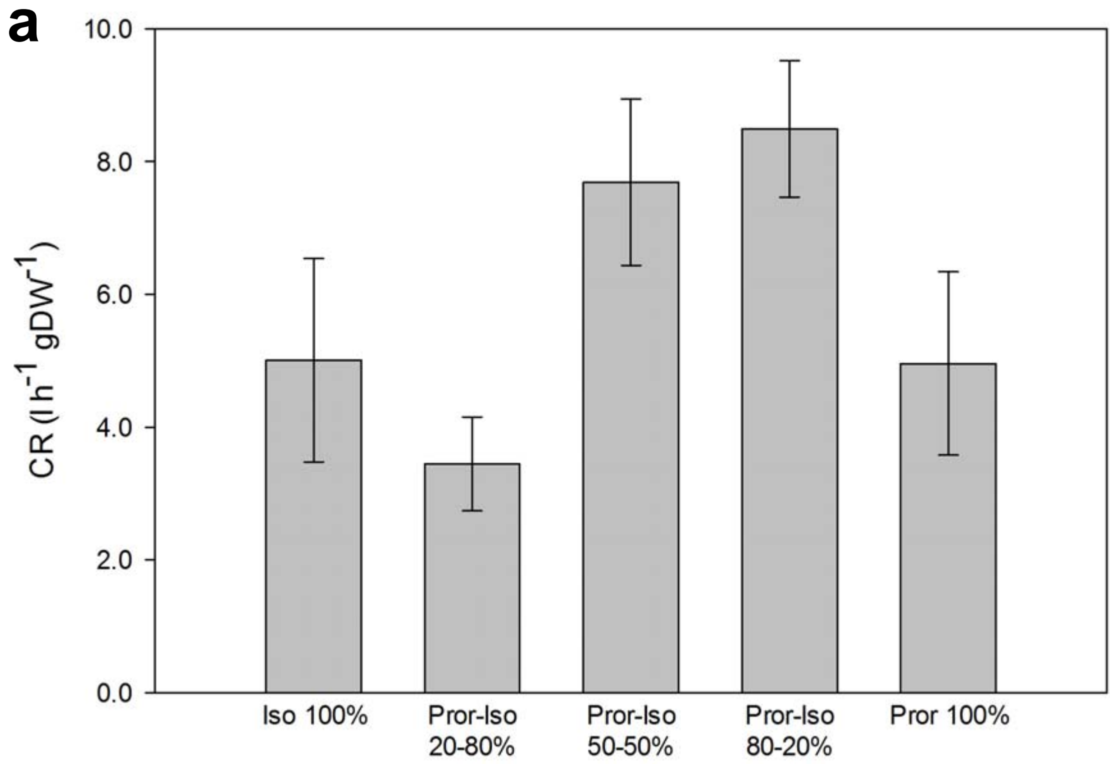

b
CR (l h^{-1} gDW^{-1})
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Isochrysis
Amphidinium
Iso 100%
Amp-Iso 20-80%
Amp-Iso 50-50%
Amp-Iso 80-20%
Amp 100%

Figure 3.4: *Mytilus edulis* clearance (mean ± SE) on proportional mixtures of *Prorocentrum minimum* (CCMP 696) and *Isochrysis galbana* (CCMP 1323). Mixtures were done on C-content basis; absolute C content across treatments =1050.0 µg C l^{-1}. **a)** CRs based on total chlorophyll *a*. **b)** CRs based on cell counts performed with a Coulter counter.


a
10.0
8.0
6.0
4.0
2.0
0.0
CR (l h-1 gDW-1)
Iso 100%
Pror-Iso
20-80%
Pror-Iso
50-50%
Pror-Iso
80-20%
Pror 100%

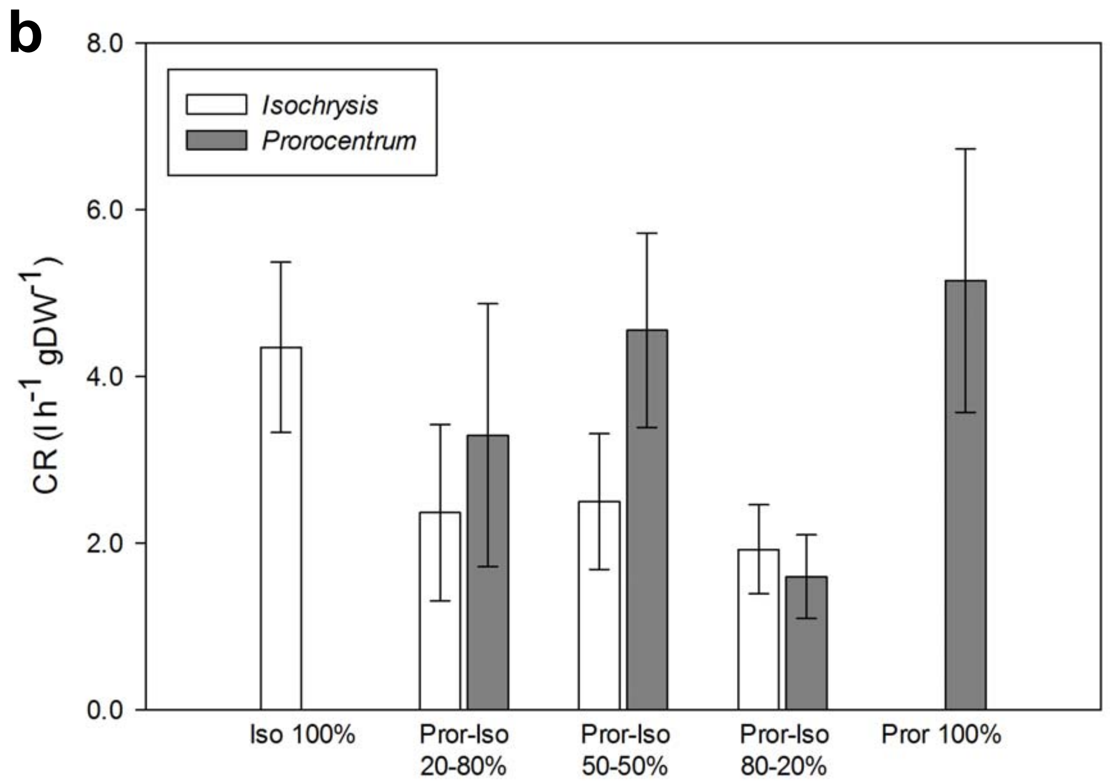
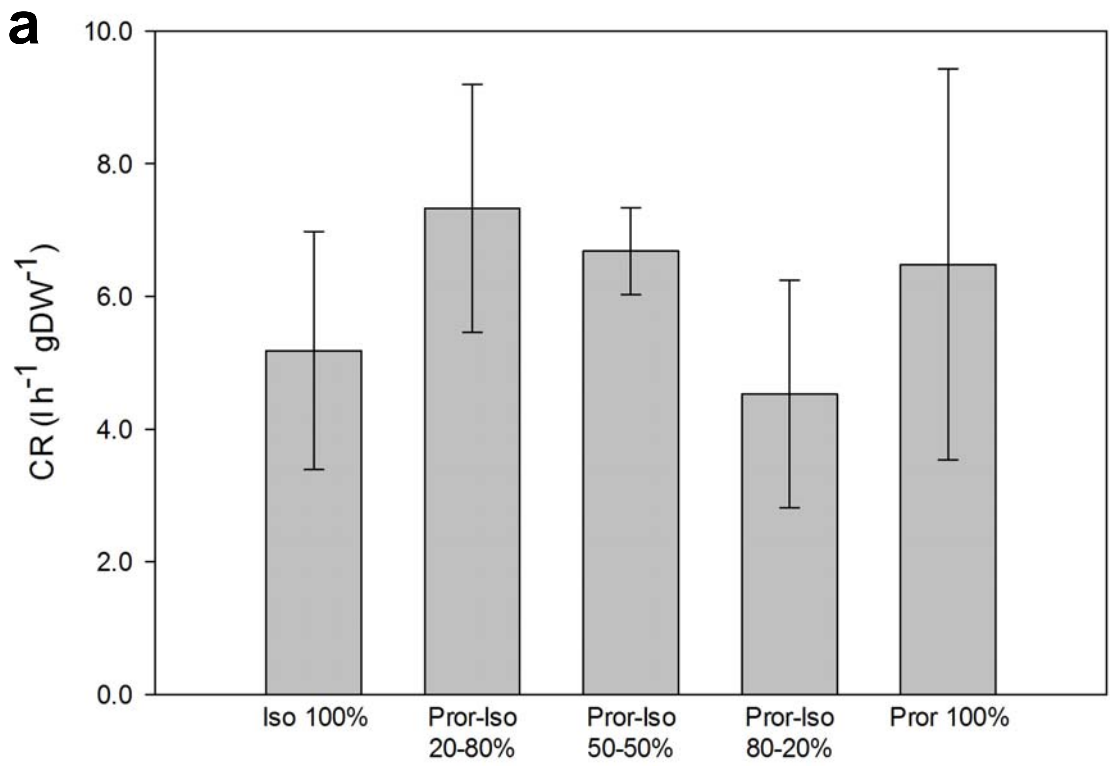

b
8.0
6.0
4.0
2.0
0.0
CR (l h-1 gDW-1)
Isochrysis
Prorocentrum
Iso 100%
Pror-Iso
20-80%
Pror-Iso
50-50%
Pror-Iso
80-20%
Pror 100%

Figure 3.5: *Geukensia demissa* clearance (mean ± SE) on proportional mixtures of *Prorocentrum minimum* (CCMP 696) and *Isochrysis galbana* (CCMP 1323). Mixtures were done on C-content basis; absolute C content across treatments =1050.0 µg C l^{-1}. **a)** CRs based on total chlorophyll *a*. **b)** CRs based on cell counts performed with a Coulter counter.


a
10.0
8.0
6.0
4.0
2.0
0.0
CR (l h-1 gDW-1)
Iso 100%
Pror-Iso
20-80%
Pror-Iso
50-50%
Pror-Iso
80-20%
Pror 100%

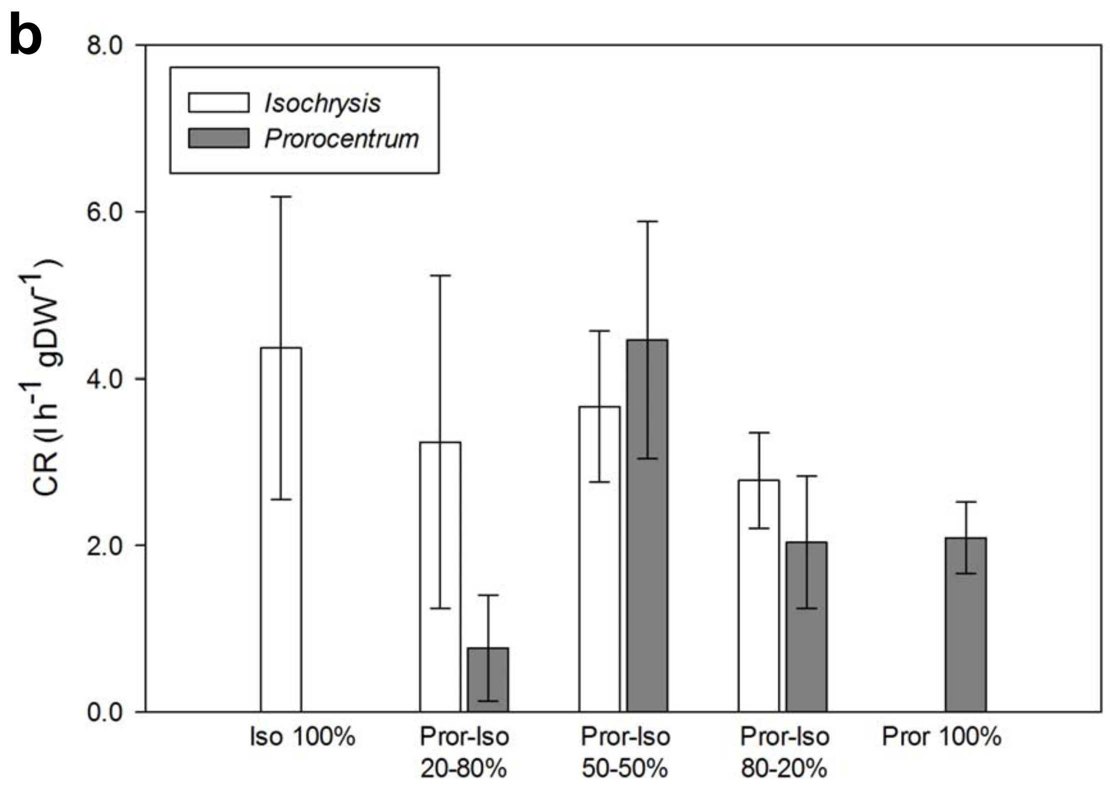
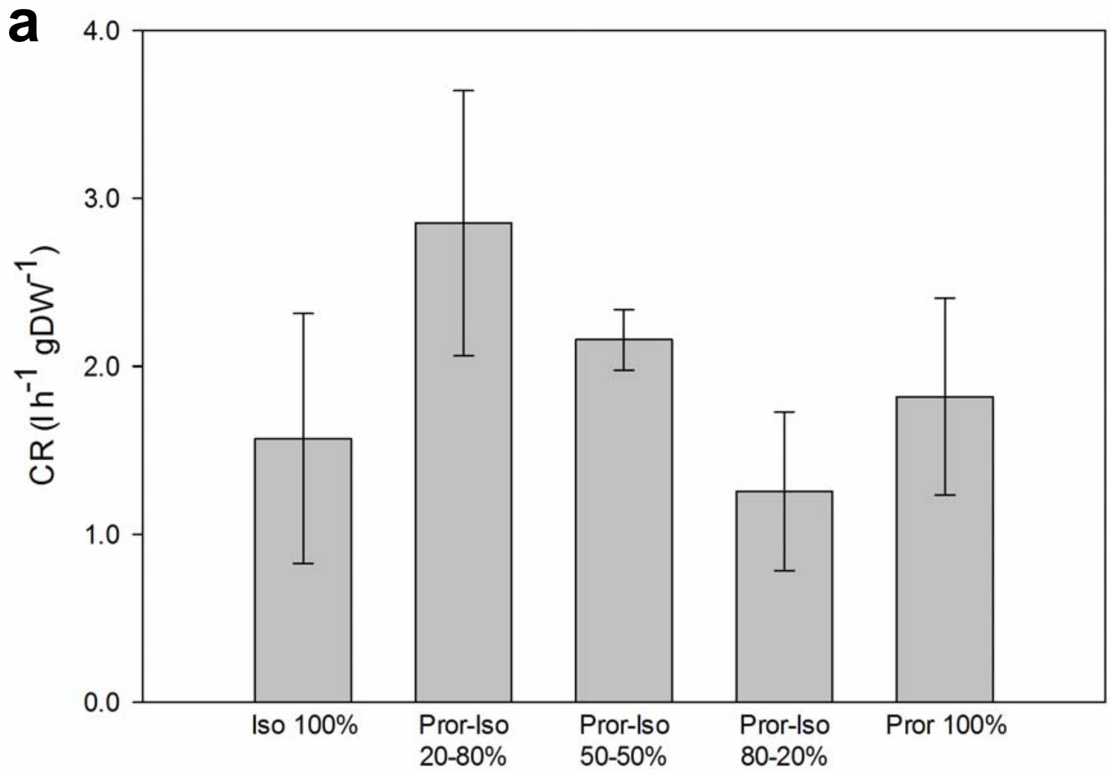
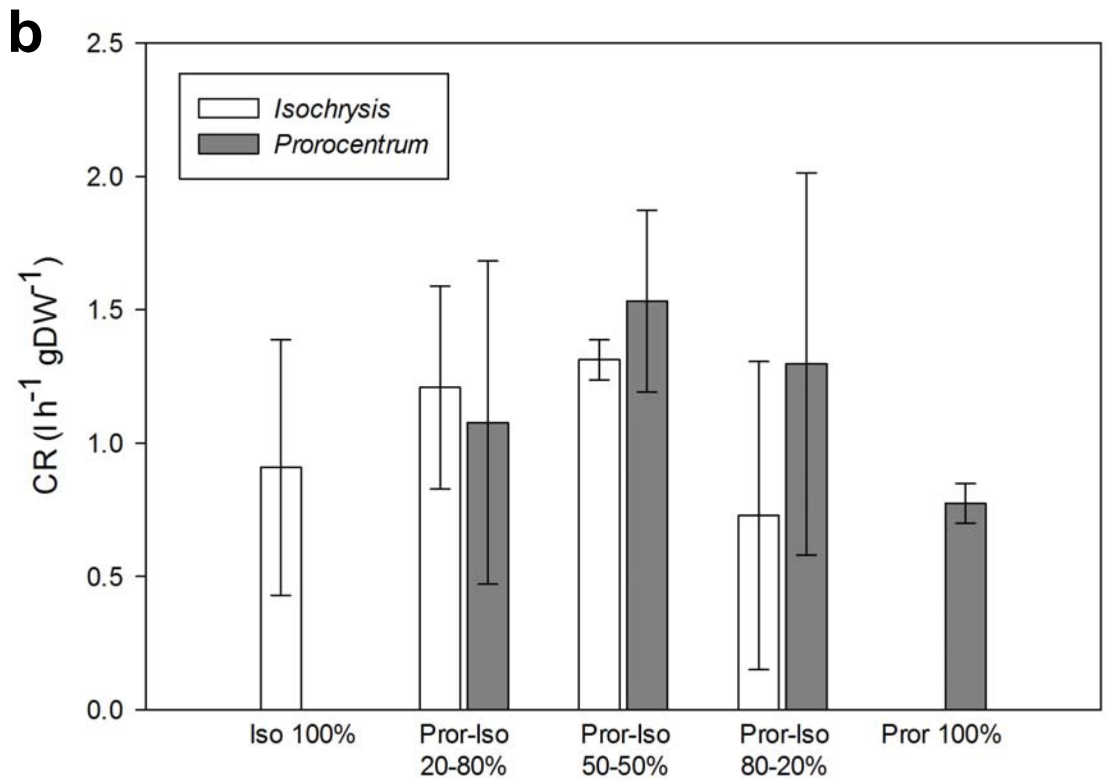

b
8.0
6.0
4.0
2.0
0.0
Isochrysis
Prorocentrum
CR (l h-1 gDW-1)
Iso 100%
Pror-Iso
20-80%
Pror-Iso
50-50%
Pror-Iso
80-20%
Pror 100%

Figure 3.6: *Mercenaria mercenaria* clearance (mean ± SE) on proportional mixtures of *Prorocentrum minimum* (CCMP 696) and *Isochrysis galbana* (CCMP 1323). Mixtures were done on C-content basis; absolute C content across treatments =1050.0 µg C l^{-1}. **a)** CRs based on total chlorophyll *a*. **b)** CRs based on cell counts performed with a Coulter counter.


a
CR (l h^{-1} gDW^{-1})
4.0
3.0
2.0
1.0
0.0
Iso 100%
Pror-Iso 20-80%
Pror-Iso 50-50%
Pror-Iso 80-20%
Pror 100%


b
CR (l h^{-1} gDW^{-1})
2.5
2.0
1.5
1.0
0.5
0.0
Isochrysis
Prorocentrum
Iso 100%
Pror-Iso 20-80%
Pror-Iso 50-50%
Pror-Iso 80-20%
Pror 100%

CHAPTER 4

Top-down (grazing) and bottom-up (nutrient regeneration) effects of suspension-feeding macrobenthos on the plankton community of a coastal lagoonal estuary: a mesocosm study in Great South Bay, New York, USA

ABSTRACT

Mesoscale (0.4 m^3) field incubations incorporated commercial (the hard clam *Mercenaria mercenaria*) and non-commercial (ribbed mussel *Geukensia demissa*) bivalves, and a recently introduced colonial ascidian (*Didemnum vexillum*) at varying densities and in single- and multispecies assemblages, to assess the potential ecological effects of increased benthic suspension-feeding on the current planktonic structure of the Great South Bay, NY. The effects on biomass of several planktonic components (picocyanobacteria, picoeukaryotes, auto- and heterotrophic nano- and microplankton and micrometazoans) and concentration of other environmental parameters of relevance (total and <5-µm chlorophyll, dissolved nutrients) was quantified and analyzed with multivariate techniques (redundancy analysis, RDA). Both bivalve species invariably exerted top-down control on phytoplankton biomass, and drove changes in composition (e.g. *G. demissa* had positive effects on the biomass of centric diatoms). Top-down control by the ascidian on most planktonic components was almost entirely absent. *D. vexillum* mostly exhibited bottom-up effects, induced by regeneration of ammonium (which was an order of magnitude higher than in the bivalves); biomass dominance of diatoms resulted from shifting Si:N ratios. Interactive effects arose in multispecies assemblages.

INTRODUCTION

The Great South Bay, together with several smaller coastal bays spreading along the south shore of Long Island, forms a system of interconnected coastal lagoonal estuaries. Coastal lagoons comprise 13% of coastal areas worldwide (Kjerfve, 1994) and represent a conspicuous physiographic feature of continental land margins (Boynton et al., 1996). Because of their large surface area and small volume, shallow coastal lagoons are more sensitive than other estuarine and shelf systems to physical forcing from winds, groundwater and surface freshwater flow. Wind-stress mixing coupled with shallowness is probably the most important physical factor determining the functioning of coastal lagoons (Wilson et al., 1991; Kjerfve, 1994) and promoting a tight trophic coupling

between plankton and benthos. Vertical turbulent diffusion and horizontal transport by tidal currents make phytoplankton much more readily available to planktonic predators throughout the water column and to the benthos. Accordingly, the macrozoobenthic community in lagoons is dominated by suspension-feeding organisms (Bazaïri et al., 2003).

The Great South Bay historically sustained a commercial hard clam (*Mercenaria mercenaria*) fishery, coming mostly from natural populations, and constituting a significant source of revenue for NY. *M. mercenaria* commercial landings reached their annual peak in 1976 at 3,932 metric tons (McHugh, 1977). Soon after, hard clam landings in NY started dropping dramatically and have never recovered. The causes of the decline have been attributed to physical forcings such as habitat loss and biological bottom-up effects mostly related to brown tides of the pelagophyte *Aureococcus anophagefferens* since the mid-1980s. At peak historical densities, hard clams in Great South Bay could have provided an effective top-down control mechanism to prevent the initiation of brown tide blooms and/or modulate outbreaks (Bricelj et al., 2001; Cerrato et al., 2004). Cerrato et al. (2004) indicate that hard clams cleared about 40% per day of the entire volume of Great South Bay prior to their decline; at current densities, it is estimated that they clear <1% per day. Under current conditions, primary productivity levels for Great South Bay remain high with a dominance of ultraphytoplankton biomass over microphytoplankton (>80% of chlorophyll *a* corresponds to organisms <5-µm; Lonsdale et al., 1996b; Sieracki et al., 2004; Lonsdale et al., 2006). Under the present conditions there is abundant phytoplanktonic biomass, but it does not seem to be readily available to large metazoan consumers such as *Mercenaria*, partly because of size-related issues (i.e. cells might fall below the particle size range that is efficiently processed; Bricelj and Malouf, 1984; Newell, 2004).

Changes in biological interactions associated with changes in the dominance of a particular species may profoundly alter the structure and function of marine ecosystems, often with cascading effects on other components (Jackson et al., 2001; van Nes et al., 2007; Pillay et al., 2008). It has been hypothesized that for the past three decades, Great South Bay has been in an ecological state that does not support large, functionally significant benthic suspension feeders. With the bay's current ecological state there

appears to be no tendency for hard clams (or any equivalent large benthic suspension feeder) to recover to their former abundances or influences on the system.

The restoration of a functionally significant, benthic suspension-feeding component to Great South Bay would probably require a large-scale perturbation to the ecosystem such as a substantial restocking of bivalves and increase in habitat and recruitment (see Boesch and Goldman, 2009). Even though several authors have been advocating for restoration strategies that target specific ecosystem functions (French McCay et al., 2003), the ecosystem services provided by shellfish (e.g. filtering capacity and increase in water clarity, benthic-pelagic coupling, nutrient sequestration) have been largely ignored or underestimated in most restoration or stock enhancement efforts (Coen and Luckenbach, 2000).

The species used in this study included two native bivalves known to have significant effects in water-colum turnover rates and benthic-pelagic coupling. For example, it has been mentioned that at peak historic densities *M. mercenaria* could cleared up to 40% per day of the entire volume of Great South Bay; and Jordan and Valiela (1982) estimated that *G. demissa* cleared a volume of water greater than the volume exchanged on each tidal cycle in a MA marsh. A non-bivalve benthic suspension feeder, the invasive colonial ascidian *Didemnum vexillum*, was also utilized. Several closely-related ascidians within the genus *Didemnum* are emerging worldwide as successful invaders undergoing massive population explosions, and establishing as dominant members in subtidal communities with firm substrates (Bullard et al., 2007; Lambert, 2007). Populations of *Didemnum vexillum* were first documented in NY waters in 2004, growing on bridge pilings and docks in Shinnecock Bay (Bullard et al., 2007). The spread into the adjacent Great South Bay seems to be very likely to happen in the near future. Ascidians exhibit a feeding physiology entirely different from that of bivalve molluscs, based on mucus nets and tangential flows. The filtration apparatus consists of the pharynx, which ventrally harbors an endostyle that secretes a complex mucoprotein (Armsworthy et al., 2001; Petersen, 2007; Riisgård and Larsen, 2010). The resulting mucus net consists of transverse and longitudinal filaments of 10-25 nm in diameter, with openings of 0.2-0.5 µm wide and 0.5-2.2 µm long (reviewed by Petersen, 2007; Riisgård and Larsen, 2010). This allows most species to trap particles in the picoplanktonic size

range. In general, ascidians are considered to be non-selective suspension feeders (Armsworthy et al., 2001).

This study focused on the potential effects that may arise as a result of substantial suspension-feeding activity by benthic organisms on pelagic community structure. Experiments were conducted in mesoscale (~0.4 m^3) water enclosures, with experimental density manipulations of native benthic suspension-feeding bivalves and a recently introduced colonial ascidian. The overall objective was to characterize and compare changes in planktonic community composition and structure induced by benthic suspension feeders. Given that functional richness could increase ecosystem properties through positive interactions such as complementarity and facilitation (Hooper et al., 2005), multi-species assemblages were considered on some experiments.

MATERIALS AND METHODS

The basic protocol consisted of 72- or 120-h grazing experiments with benthic suspension-feeding organisms and ambient seawater from Great South Bay. By conducting incubations over such periods, the effects of periphyton growth were minimized (Heath et al., 1995). In order to incorporate seasonal changes in plankton structure, the experiments were conducted from October 2007 to July 2009. Experimental dates, duration and benthic species are listed in Tables 4.1 and 4.2, and Figure 4.1.

Location and experimental organisms

Experiments were performed at the West Sayville Boat Basin located on the north shore of Great South Bay (40°43' N, 73°05' W).

Ribbed mussels (*Geukensia demissa*) were collected at the start of each experiment from wild populations at Quantuck Bay (40°48' N, 72°36' W) for all experimental dates except for 4/29, in which mussels were collected at Flax Pond (40°57' N, 73°08' W) and suspended in lantern nets for >24 h prior to the start of the experiment in order to acclimate them to Great South Bay plankton. All mussels were carefully brushed to remove epibionts, and their byssal threads trimmed. Northern quahogs (*Mercenaria mercenaria*) came from wild and re-stocked populations from an adjacent

location in Great South Bay; all clams were brushed carefully to remove epibionts. Mean shell lengths and biomasses for the experimental shellfish are provided in Tables 4.1 and 4.2, and Figure 4.1.

Colonies of *Didemnum vexillum* were collected subtidally (1-2.5 m depth at low tide) from the pilings of Ponquogue Bridge (Shinnecock Bay, NY; 40°50' N, 72°29' W) and transferred in temperature-controlled aquaria to the experimental location at West Sayville within 1 h. Most accompanying macroinvertebrates and macroalgae were carefully removed before introduction of the colonies into the experimental tanks. Portions of the colonies were placed in stacks of tissue paper to absorb excess water content, and then weighed in order to estimate the total wet weight biomass that was added to the experimental tanks. Experiments with *Didemnum vexillum* also incorporated a bivalve species, in a 'response surface' design (Inouye, 2001; see below) in which the densities of either organism (singly- or in combination) were varied to create a 'surface' of many density-treatments. A graphical representation of this is presented in Figure 4.1. All handling and manipulation of *Didemnum vexillum* colonies was kept to a minimun.

Estimates of soft tissue dry weight were obtained for all three experimental species, by dissecting and drying at 60ºC for >48 h.

Experimental design I: Bivalve grazing in single-species mesocosms

The bivalve grazing experiments were conducted in enclosed mesocosm systems filled with ambient seawater from Great South Bay. Experimental mesocosms consisted of cylindrical tanks (internal diameter =0.76 m, height =1.22 m) made of light-transmitting fiberglass reinforced polymer (Sun-Lite ®; Solar Components Corp., Manchester, NH) that allowed the water in the tanks to receive a natural light regime. Prior to each experiment, all tanks were scrubbed, rinsed with fresh water, and then filled with seawater from Great South Bay. The use of pumps for filling the tanks and/or sampling was avoided, since peristaltic pumps disrupt some fragile microzooplankton such as naked ciliates and dinoflagellates (James, 1991; Suzuki et al., 2002). On dates coinciding with the bloom of the ctenophore *Mnemiopsis leidyi* in Great South Bay (McNamara et al., 2010), a 1000-μm plankton net was used when filling each tank in order to exclude this pelagic grazer. For each experiment, the twelve experimental tanks

were deployed *in situ* by hanging them in two rows from a wooden floating platform (6.8 × 2.45 × 0.3 m), with Styrofoam floats, harbored at a boat slip directly adjacent to the inlet of the marina.

The experimental shellfish were placed in aquaculture lantern nets (base area =0.1225 m^2) suspended in line, at the center of the tanks. For each experimental run, three treatments (n =4 tanks each) were established: a control (empty bivalve shells) and two experimental bivalve treatments with different stocking densities. The different treatments yielded different estimates of water turnover per day (due to bivalve feeding; see below). The ribbed mussel (*Geukensia demissa*) is an intertidal organism that shows changes in metabolic rate while submerged or exposed to air (Hilbish, 1987; Wilbur and Hilbish, 1989); hence lantern nets with *G. demissa* were pulled out of their respective tanks daily for ~4 h, to simulate a tidal cycle.

Each experimental tank was enriched daily with 4.8 μM nitrogen as NH_4Cl, a concentration similar to the levels sometimes found in Long Island estuaries (Gobler et al., 2004a). Amending the water with excess nitrogen served the purpose of removing differences in plankton production between control and treatment tanks that may arise due to bivalve excretion. Moreover Gobler et al. (2004a) showed nitrogen limitation in this system. Environmental parameters (temperature, salinity and dissolved oxygen) were recorded throughout the experiments with a handheld YSI model 85 (YSI Inc., Yellow Springs, OH). Additionally, temperature and salinity values were obtained from a Seacat sensor (Sea Bird Electronics, SBE 16) located 5 m away from the experimental tanks (C.N. Flagg, SoMAS, Stony Brook University).

Experimental design II: Response-surface mesocosms

Most ecological experiments using two competing species use experimental designs based on either substitution (i.e. the total density of individuals in each treatment is held constant, while the proportions are varied), or additive (the density of one species is held constant, and the density of the competitor is varied). These two designs have been criticized for their limitations when interpreting results (Inouye, 2001; and references therein). Response surface experimental designs vary the densities of two

competing species independently. Competition models often describe nonlinear surfaces, and experimental designs with more than two densities are adequate to capture these nonlinearities (Inouye, 2001). Even response surface experiments with a relatively low number of replicates or combinations of densities, or those that include high densities above carrying capacities, provide more accurate parameter estimates than traditional designs (Inouye, 2001).

A series of 6 mesocosm experiments involving the bivalves *Geukensia demissa* and *Mercenaria mercenaria*, and the invasive colonial ascidian *Didemnum vexillum*, in a response surface experimental design, were conducted in the same fashion described in the previous section. A detailed description of the densities used for each combination of two benthic suspension feeders, shell lengths and biomass are given in Table 4.2 and Figure 4.1. Ranges of percent daily volume turnover are given in Table 4.3 (for estimation details, see next section).

Estimation of clearance rates

In order to estimate clearance rates at initial experimental conditions (e.g. seston concentration, temperature), parallel short term clearance-rate experiments were carried out 24 h after the setup of the mesocosm tanks. Individual bivalves or variable numbers (according to the volume of water in the container), and portions of *Didemnum* colonies (known wet weights), were placed into polypropylene cylindrical containers filled with ambient seawater (volume 2-58 l) and allowed to acclimate for ~15 min. There were 3-4 replicate treatment tanks with animals and 3-4 control tanks with no animals. Inorganic nitrogen was added as 5 μM NH_4Cl to compensate for invertebrate excretion in control tanks. Clearance rate experiments lasted 1-3.5 h and some means of gentle mixing was provided in those experiments with volumes >28 l.

The suspension of natural particles in seawater was sampled at initial and final times for total chlorophyll *a* and seston content (see below for methodological details). A handheld portable fluorometer (Turner Designs, model *Aqua*fluor™) was also used to measure *in vivo* chlorophyll *a* every 15-30 min. Clearance rates were determined from the rate of chlorophyll *a* (absolute or *in vivo* values) or seston removal, taking into account

the background growth rate (positive or negative) from control containers without animals (Coughlan, 1969).

Some experiments had two species in the same container (either two bivalve species, or a bivalve species and *Didemnum*, in addition to containers with either species alone), yielding a single clearance rate. For these experiments a 2-way ANOVA test was applied to evaluate interaction effects on clearance rate, arising from the combined presence of two suspension feeders in the same container. Results and further experimental details of the experiments estimating clearance rates are reported on Table 4.4.

Clearance rates were expressed in units of volume per h per dry tissue weight. It was assumed that the clearance rates obtained in the parallel experiments were a proxy for the clearance rates in experimental tanks. Average individual clearance rates, benthic suspension feeder biomass, immersion time (in the case of *Geukensia*), and mesocosm volumes were combined to estimate a percent daily turnover for each tank. These values are reported in Tables 4.1 (as means) and 4.3 (as ranges).

Sampling and processing

At the beginning of each experiment, water was pooled from 3-4 tanks and then subsamples were taken (n =3-4) to characterize initial conditions. At 72-h and 120-h, samples were taken from each individual tank, in order to characterize the plankton community and changes in biomass and structure resulting from benthic feeding.

Chlorophyll content. Chlorophyll *a* content was estimated from duplicate 30 ml samples of whole and <5-µm fractionated seawater. Size fractionation was done by filtering through a polycarbonate membrane filter. Plankton samples were then concentrated onto Whatman GF/F filters and chlorophyll *a* was extracted in 90-100% acetone for at least 24 h at -20ºC, and measured fluorometrically (Turner Designs, model 10-AU) after Arar and Collins (1997). Additionally, *in vivo* chlorophyll *a* in each tank was measured daily (triplicate readings) with a portable fluorometer; the instrument provided semi-quantitative values that served in the monitoring of the experiments (data not shown).

Picoplankton. Water samples (4.5 ml) were preserved in 1% formaldehyde (from a 10% stock solution prepared with filtered natural seawater), flash-frozen in liquid N_2, and stored at -80°C. The densities of heterotrophic bacteria, picocyanobacteria (*Synechococcus*), and photosynthetic picoeukaryotes (<2 µm) were estimated from these samples by flow cytometry (Olson et al., 1993). A population of >2 µm nanoeukaryotes was consistently detected by this method and included in the analysis. Sample aliquots (0.5-2 ml) were run twice in a Becton-Dickson FACSCalibur flow cytometer, before and after staining with SYBR Green I (Lonza Inc., Rockland, ME). Absolute counts were obtained using a known concentration of fluorescent beads (Spherotech Inc.; rainbow fluorescent particles, 1.93 µm diameter). Data were analyzed with the WinMDI 2.9 software package. Conversion factors published in Lee and Fuhrman (1987) and Verity et al. (1992) were applied to estimate biomass

Nanoplankton. Seawater samples (50 ml) were preserved in 1% formaldehyde for autotrophic and heterotrophic nanoplankton (2-20 µm) enumeration using epifluorescent microscopy (Porter and Feig, 1980). These samples were kept refrigerated until transfered (within 24 h) into polycarbonate filters (0.8-µm black, 25 mm diameter), and mounted into glass slides using Vectashield mounting medium with DAPI (1.5 µg ml^{-1}; Vector Laboratories Inc., Burlingame, CA). Volumes filtered ranged from 5 to 20 ml, according to the seasonal concentration of organisms. Nanoplankton slides were stored at -20°C (Sherr and Sherr, 1993) until enumeration with a Zeiss Axioskop fluorescence microscope equipped with a HBO 50/AC mercury lamp and three wavelength filter sets (UV, blue and green excitation). Nanoplanktonic organisms were enumerated at 1000× magnification, using a Zeiss 100× Plan-Neofluar oil objective. Organisms were classified into the following taxonomic and functional groups: cryptophytes, heterotrophic and autotrophic dinoflagellates, centric and pennate diatoms, heterotrophic nanoflagellates and oligotrichous ciliates. Standard measurements of cell linear dimensions were performed for biovolume estimations (Sun and Liu, 2003). Conversion factors published in Strathmann (1967), Børsheim and Bratbak (1987), Putt and Stoecker (1989), and Menden-Deuer and Lessard (2000) were applied to estimate biomass.

Microplankton. Samples of whole seawater (100-200 ml) were preserved in acidic Lugol's iodine fixative (5-10%) in amber jars and stored in the dark (Stoecker et al., 1994) for enumeration of planktonic microorganisms (20-200 μm). Microplankton samples were processed by settling for ~24 h (50 ml) in a graduated cylinder, followed by removal of 40 to 45 ml of the overlying water. A minimum 200 microorganisms were then counted in a Zeiss compound microscope, by 1 ml aliquots in a Sedgewick-Rafter counting chamber (LeGresley and McDermott, 2010). Standard measurements of cell linear dimensions were performed for biovolume estimations (Sun and Liu, 2003). Conversion factors published in Strathmann (1967), Smayda (1978), Putt and Stoecker (1989), and Menden-Deuer and Lessard (2000) were applied to estimate biomass. In most cases individual cells were counted; for chain-forming or filamentous algae, cells were counted only if the colony was >20 μm. Taxa were identified to the lowest possible taxonomic level (Maeda and Carey, 1985; Maeda, 1986; Tomas, 1997; Taylor et al., 2003) and classified into the following taxonomic groups: euglenoids, heterotrophic and autotrophic dinoflagellates, centric and pennate diatoms, loricate and aloricate (oligotrich) ciliates. Other groups found included other phytoflagellates (e.g. Prymnesiophyceae, Rhaphidophyceae, Cryptophyceae, Haptophyceae, Pyramimonadales and Ebriids) and Rhizopoda. Some tychopelagic or benthic species usually found within the water column of well-mixed environments were included in the counts.

Zooplankton. Larger zooplankton were collected by pouring buckets of water (18-20 l) through a 40-μm Nitex sieve. Initial samples were taken from the ambient water (n =2-4). At the end of each experiment, the same seawater volume was removed from each experimental and control tank (n =1). Animals caught on the sieves were rinsed with 0.45-μm filtered seawater into glass jars, and preserved in 5% buffered formalin with Rose Bengal. Eggs and nauplii of the calanoid copepod *Acartia tonsa* Dana, and other zooplanktonic micrometazoa were enumerated in subsamples taken with a Stempel pipette under a dissecting microscope (Wild Heerbrugg, model M3); a minimum of 200 organisms were counted for each sample (Omori and Ikeda, 1984).

Dissolved nutrients. Seawater samples filtered through GF/F filters (0.7 μm) were taken for dissolved nutrient analysis. Water samples (n =3) were pooled from 4 tanks at the beginning of the experiment. Samples were then taken from each tank (n =1)

at 24, 72 and 120-h. Nutrients were analyzed colorimetrically using a spectrophotometric microplate reader (Molecular Devices, SpectraMax, model 384 Plus). Nitrate was analyzed by reducing the nitrate to nitrite using spongy cadmium (Jones, 1984); nitrite, ammonium, phosphate, and silicate were analyzed following modified techniques from Parsons et al. (1984); urea was analyzed with a protocol modified from Price and Harrison (1987). Molar ratios of N:P and Si:N were calculated considering inorganic and organic forms of nitrogen. Molar nutrient ratios were analyzed by means of a 2-way ANOVA, with time and treatment as factors.

Data analysis

Multivariate data analysis was carried out using redundancy analysis (RDA). RDA is a form of direct gradient analysis that combines ordination of multivariate biotic data with regression of the ordination scores against environmental/experimental variables in order to examine the relationship between community structure and the set of explanatory variables (terBraak, 1986; ter Braak and Verdonschot, 1995; ter Braak and Similauer, 2002). RDA was carried out using Canoco 4.5 (Microcomputer Power, Ithaca, NY).

Biotic data consisted of natural log transformed biomasses. Biomass, rather than abundance, was preferred since it was thought to better represent trophic interactions. Because biotic variables with large values can dominate RDA, a log transformation was applied so that variables with low biomass would not be obscured and would contribute to the results. RDAs were also run on nutrient and chlorophyll data; these data were centered and standardized to remove the effect of different measurement units.

Environmental/experimental variables (i.e. explanatory variables) were suspension feeder biomasses in each tank and the time of measurement. In the single species experiments, the option of treating suspension feeder treatments as nominal variables, with replicated treatments of each (e.g. 0, 18, and 36 *Geukensia* with n =4 for each treatment), was possible. This could not be done for the two-species experiments, since no treatment was replicated. To be consistent across experiments, all explanatory variables were, therefore, considered continuous variables. Initial measurements at the beginning of an experiment (t =0), while collected, were not used in the statistical

analysis. They were included in the RDA as passive or supplementary samples at the end of the analysis and plotted in the ordination diagram as indicators of the initial conditions in the tanks.

The basic model used to examine the relationship between community structure and explanatory variables was a regression model containing all main effects and all interactions. Variance was decomposed using a pure effects approach (Whittaker, 1984; ter Braak and Similauer, 2002) where any main effect not being tested was treated as a covariate. Interaction terms were not included as covariates in testing for main effects as suggested by Anderson (2001).

Because of the unbalanced design of the experiments and because terms involving the continuous variables covaried, it was expected that shared variance would occur between terms. The pure effects approach removes this shared variance prior to testing the significance of the factor. Significance of each factor was tested by Monte Carlo permutation test, using a model-based method that randomly permuted the residuals of the regression of the biotic data on the covariates (ter Braak and Similauer, 2002).

Time was considered a repeated measures factor, since multiple measurements collected from a tank could not be considered independent. To carry out the permutation tests, tanks were considered whole plots and time was designated as a split-plot factor (ter Braak and Similauer, 2002). To test for the effect of suspension feeders, tanks (whole-plots) were permuted as units across treatments, and time (split-plot) was not permuted. To test for time as a main effect, the tanks (whole-plots) were not permuted and time (split-plot) was permuted as a series where permutations were restricted to cyclic shifts (e.g . [t0, t1, t2], [t2, t0, t1], [t1, t2, t0]). This preserved the autocorrelation structure in the time series (ter Braak and Similauer, 2002). For interaction effects, both tanks (whole-plots) and time (split-plots) were permuted. In these cases, the time permutation was again restricted to cyclic shifts.

When tanks were sampled only twice during an experiment (e.g. at t= 72 and 120 h), a simpler procedure was used to examine interactions between suspension feeder treatments and time. For biotic variables, instantaneous growth rates were computed as:

$$GR = \frac{\ln(B_{t2}) - \ln(B_{t1})}{t2 - t1}$$

Ordination diagrams involving growth rates were more easily interpreted than diagrams with interaction terms, so this technique was used when significant time interactions were found.

RESULTS

Single species mesocosms

Daily turnover times in experimental tanks ranged from 32-58% of the volume for the experiment with $t_{initial}$ 10/31/07 (Table 4.1). Hard clams had a negative effect on total and <5-µm chlorophyll, and on the biomass of nano- and picoplanktonic groups. *Mercenaria* also had negative effects on rotifers and micrometazoan larvae. On the other hand, hard clams had a marked positive effect on eggs, nauplii and copepodites of *Acartia tonsa*, and also on other invertebrate eggs (Figure 4.2; trace-λ= 0.163; *F*-ratio= 1.751; p-value= 0.035).

On the experiment with $t_{initial}$ 4/29/08, daily turnover times in experimental tanks ranged from 64-118% of the volume (Table 4.1). Ribbed mussels had a negative effect on total and <5-µm chlorophyll. *Geukensia* had a positive effect on most nanoplanktonic groups, but the biomass of nanoplanktonic diatoms was negatively affected. On the other hand, ribbed mussels had a negative effect on the biomass of most microplanktonic groups (centric diatoms, euglenophytes, dinoflagellates), and positive effects on the phytoflagellates and aloricate ciliates. Similarly to the 10/31/07 experiment with *Mercenaria*, ribbed mussels had negative effects on rotifers, and a marked positive effect on micrometazoan larvae, and nauplii and copepodites of *A. tonsa* (Figure 4.3; trace-λ= 0.154; *F*-ratio= 1.637; p-value= 0.049). Dissolved nutrients were not measured for these two experiments.

Daily turnover times ranged from 43-91% of the volume for the experiment with $t_{initial}$ 6/10/08 (Table 4.1). Hard clams had a negative effect on total and <5-µm chlorophyll. Hard clams had positive effects on nanoplanktonic groups (cryptophytes, pennate diatoms and autotrophic dinoflagellates), while most microplanktonic groups (except dinoflagellates) were negatively affected, as were nanoplanktonic ciliates. *Mercenaria* also had negative effects on rotifers and copepodites, but on the other hand,

positive effects were shown for *Acartia* copepodites and nauplii, and other micrometazoan eggs and larvae. Hard clams had positive effects on nitrogenous compounds (ammonium and nitrate), and negative effects on silicate and phosphate (Figure 4.4; trace-λ= 0.176; *F*-ratio= 1.708; p-value= 0.035).

The experiment starting on 7/21/08 introduced longer incubation times (120 h) than the previous experiments. Daily turnover times ranged from 48-103% of the volume (Table 4.1). Ribbed mussels decreased total and <5-µm chlorophyll. *Geukensia* decreased most nano- and microplanktonic groups, but had a positive influence on nano- and microplanktonic centric diatoms (e.g. *Coscinodiscus* sp., *Thalassiosira* sp.); pennate diatoms, however, were negatively influenced (Figure 4.5; trace-λ= 0.323; *F*-ratio= 8.891; p-value= 0.001).

Daily turnover times for the experiment with $t_{initial}$ 8/03/08 ranged from 126-239% of the volume (Table 4.1). Ribbed mussels decreased total and <5-µm chlorophyll (Figure 4.6a; trace-λ= 0.521; *F*-ratio= 7.265; p-value= 0.001). The biomass of *Synechococcus* and picoeukaryotes also decreased in the presence of ribbed mussels with respect to control tanks (Table 4.6). Moreover, *Geukensia* had a negative effect on most nano- and microplanktonic groups, but presented a positive effect on nano- and microplanktonic centric diatoms (e.g. *Coscinodiscus* sp., *Thalassiosira* sp.), and a minor positive effect on euglenophytes (Figure 4.6a). Conversely, time as an environmental factor had a positive effect on the biomass of most nano- and microplanktonic components except small pennates, autotrophic dinoflagellates and aloricate ciliates. Ribbed mussels had a positive effect on nauplii of *Acartia* and eggs of non-crustacean invertebrates, and a negative effect on other invertebrate larvae (data not shown). The significant interaction of *Geukensia**time depicted in Figure 4.6a, was further analyzed in the triplot presented in Figure 4.6b, which is based on growth rate estimates. *Geukensia* positively affected the growth rates of centric diatoms, ciliates and phytoflagellates and small dinoflagellates. Conversely, the growth rates of pennate diatoms, microplanktonic dinoflagellates and cryptophytes were negatively affected by ribbed mussels. The growth rates of total chlorophyll and of the <5-µm/total chl ratio were negatively affected by ribbed mussels (Figure 4.6b).

Response-surface mesocosms

The experiment starting on 9/18/08 showed comparable daily turnover times for those tanks with either bivalve or with both species, ranging from 25-81% of the volume (Table 4.3). All dissolved nutrients increased with time, as did the <5-µm chlorophyll fraction, nanoplanktonic centric diatoms and microplanktonic phytoflagellates (Figure 4.7). Even though *Geukensia* alone had a marked effect, there also was a significant interactive effect of the two bivalves in the same direction (Figure 4.7; trace-λ= 0.452; *F*-ratio= 5.442; p-value= 0.001). In general, nanoplanktonic groups and all nutrients were positively affected by the bivalves, while the biomass of all microplanktonic groups except aloricate ciliates decreased. Numerically dominant microplankton representatives included *Prorocentrum micans* and *P. minimum* among dinoflagellates, *Leptocylindrus minimus* and *Chaetoceros* sp. among centric diatoms, and *Nitzschia longissima* among pennates. The biomass of the phagotrophic ebriid flagellate *Hermesium adriaticum* (labelled 'othM' in the triplot) increased in time. Ebriids were rarely found in the microplankton of Great South Bay, but Rhodes and Gibson (1981) cite a peak in abundance of this species in the lower Chesapeake Bay, also for the month of September; both bivalves had negative effects on this organism.

On the experiment with $t_{initial}$ 10/01/08, estimated daily turnover times were higher in tanks with *Geukensia* alone (range 34-105% volume) than in tanks with *Mercenaria* alone (range 19-59% volume) or with both bivalve species (range 24-49% volume; Table 4.3). Even so, hard clams alone had a stronger effect than the interaction *Mercenaria***Geukensia* (Figure 4.8a; trace-λ= 0.512; *F*-ratio= 5.115; p-value= 0.001). *Mercenaria* alone only had a positive effect on nanoplanktonic ciliates and pennate diatoms, and increased the concentration of ammonium and silicate (to a minor degree); all other plankton components as well as dissolved nutrients decreased in the presence of hard clams (Figure 4.8a). Similarly, the interactive effects of *Mercenaria***Geukensia* promoted an increase in the biomass of nanoplanktonic ciliates and pennates, with negative effects on the rest of the plankton components and all dissolved nutrients. Picoplanktonic biomass was incorporated in the RDA analysis for this experiment, showing a negative effect of bivalves on the biomass of heterotrophic bacteria, *Synechococcus*, and pico- and nanoeukaryotes. Likewise, bacteria, *Synechococcus* and

picoeukaryotes decreased their abundance in time. With regards to growth rates, *Mercenaria* had diverging effects when considered alone *versus* its interactive effects with ribbed mussels. For instance, the rates of increase of nitrate, nitrite, urea and phosphate were positively affected by *Mercenaria* alone, while the increases of ammonium and silicate were negatively affected (Figure 4.8c); the opposite pattern was observed for *Mercenaria***Geukensia* (except for no effect on urea; Figure 4.8b). Similar relationships were observed for the growth rates of several groups of pico-, nano- and microplankton; for example the growth rates of nanoeukaryotes, nanoplanktonic pennate diatoms and the <5-µm/total chl ratio were negatively affected by *Mercenaria***Geukensia* (Figure 4.8b), while the inverse effect was observed in *Mercenaria*-only treatments (Figure 4.8c).

On the experiment with $t_{initial}$ 6/15/09, despite marked differences in percent daily water turnover (in tanks with ribbed mussels daily turnover ranged 33-95%, *versus* 4-8% in tanks with *Didemnum* alone, Table 4.3), *Geukensia* had a weaker and overall opposite effect than *Didemnum* (Figure 4.9a; trace-λ= 0.634; *F*-ratio= 6.237; p-value= 0.001). *Didemnum* alone and the combination of *Didemnum* and *Geukensia*, had a strong positive effect on total and <5-µm chlorophyll. *Didemnum* and *Geukensia***Didemnum* had positive effects on bacteria and nanoeukaryotes, but decreased the biomass of *Synechococcus* and maybe picoeukaryotes. *Didemnum* and *Didemnum***Geukensia* had positive effects on most nano- and picoplanktonic groups, but also showed no major effects on nanoplanktonic centric diatoms and microplanktonic pennates. Also, *Didemnum* and *Didemnum***Geukensia* increased all dissolved nutrients, except silicate; conversely, *Geukensia* was associated with an increase in silicate. Time as an environmental factor had positive effects on most autotrophic microplankton (diatoms, phytoflagellates, dinoflagellates) and bacteria, but the biomass of nano- and picoplanktonic components decreased with time. Silicate was the dissolved nutrient that changed most with time (Figure 4.9a), its rate of change being negatively affected by *Didemnum* and *Didemnum***Geukensia* (Figure 4.9b). At the end of the experiment (t-120 h), Si:N molar ratios were significantly different among treatments (ANOVA, p<0.001), with lower values at t-120h when compared to the control (mean= 1.5). This finding suggests a significant effect due to N excretion, and an increased uptake of dissolved

silicate in tanks with *Didemnum* (Figure 4.10). In general, *Didemnum* had a positive effect on the growth rate of centric diatoms (e.g. microplanktonic *Leptocylindrus* sp., *Leptocylindrus minimus*, *Chaetoceros* sp., *Corethron* sp.), and negative effects on the growth rate of pennates (e.g. *Nitzschia longissima*, ~20-μm *Navicula* spp.; Figure 4.9b).

On the experiment with $t_{initial}$ 6/22/09, estimated daily turnover times were much higher in tanks with hard clams (range 19-56% volume) than in tanks with *Didemnum* alone (range 2-3% volume; Table 4.3). In general, *Mercenaria* and *Didemnum* had opposing effects on plankton components, with a minor significant interaction of *Mercenaria**Didemnum* (Figure 4.11a; trace-λ= 0.609; *F*-ratio= 4.414; p-value= 0.001). *Mercenaria* had positive effects on the biomass of bacterioplankton, pico- and nanoeukaryotes; all other components studied being negatively affected. Conversely, *Didemnum* and *Mercenaria**Didemnum* decreased the biomass of nanoplanktonic cryptophytes, and microplanktonic phyto- and dinoflagellates, having a positive effect on all other components. All dissolved nutrients, except silicate, increased with *Didemnum*. Regarding growth rates (Figure 4.11b), *Didemnum* had marked positive effects on bacterioplankton, picoeukaryotes and microplanktonic pennate diatoms (e.g. *Nitzschia* spp.). The biomass of microplanktonic dinoflagellates (e.g. *Prorocentrum minimum*), euglenophytes and phytoflagellates, and nanoplanktonic cryptophytes was negatively affected by *Didemnum* and *Mercenaria**Didemnum*. Growth of centric diatoms (nano- and microplanktonic) was positively affected by *Didemnum*, but not by *Mercenaria**Didemnum*. *Didemnum* and *Mercenaria**Didemnum* had positive effects on the growth rate of most nutrients, except phosphate and silicate (Figure 4.11b). Si:N molar ratios were significantly different between treatments (2-way ANOVA, p<0.001), with lower values at t-120 h for all treatment tanks compared to the control (mean =3.0; except for the *Mercenaria* treatment with lowest density). This again supports a significant positive effect increasing dissolved nitrogen likely due to N excretion (Figure 4.12).

The experiment with $t_{initial}$ 6/29/09 had marked differences in percent daily water turnover between tanks with ribbed mussels (range 51-142%) and tanks with *Didemnum* alone (range 4-8%, Table 4.3). *Didemnum* alone and *Geukensia**Didemnum* increased total and <5-μm chlorophyll; time had the same effect on these components (Figure

4.13a; trace-λ= 0.448; *F*-ratio= 5.839; p-value= 0.001). The biomass of 2-5 μm eukaryotes increased in tanks with *Didemnum*, while *Synechococcus* decreased in most treatment tanks compared to the control. *Didemnum* had positive effects on microplanktonic centric (e.g. *Leptocylindrus minimus*, *Skeletonema costatum*) and pennate diatoms (e.g. *Nitzschia* spp.), and also nanoplanktonic pennates. The biomass of micro- and nanoplanktonic ciliates and dinoflagellates was also positively affected by *Didemnum*. Most nutrients increased with *Didemnum* biomass, except nitrate and silicate (Figure 4.13a). Tanks with *Geukensia***Didemnum* had positive effects on the growth rates of most nanoplanktonic components, namely autotrophic dinoflagellates, centric and pennate diatoms and ciliates; however they had negative effects on the growth rates of cryptophytes (Figure 4.13b). The growth rate of total chlorophyll and the ratio <5-μm/total chl was negatively affected by *Geukensia***Didemnum*; a negative effect was also observed for most nutrients (except nitrate). Molar ratios of N:P for all treatment tanks were higher than the control tank at t-120 h (data not shown). A 2-way ANOVA indicated that differences in N:P were significant between incubation times ($p<0.001$), treatments ($p<0.001$) and their interaction ($p<0.001$). Si:N molar ratios were significantly different between treatments (ANOVA $p<0.001$), with significantly higher values at t-120h for the control (mean =3.7; Tukey test $p<0.001$). This further supports the afore-mentioned significant effect due to excretion, and also an increased uptake of dissolved silicate in treatment tanks (Figure 4.14).

On the experiment started on 7/06/09, tanks with *Mercenaria* had the lowest range of estimated daily turnover (ranging between 6-19% volume), but still considerably higher than those tanks with *Didemnum* alone (range 1-2% daily turnover, Table 4.3). Similarly to the experiment started on 6/22/09, *Mercenaria* and *Didemnum* had opposing effects on plankton components, althought the interaction was stronger than in the previous date (Figure 4.15a; trace-λ= 0.541; *F*-ratio= 5.607; p-value= 0.001). *Mercenaria* had positive effects on the biomass of nanoplanktonic diatoms and dinoflagellates, showing negative effects on most other planktonic components. Conversely, *Didemnum* had positive effects on the biomass of all planktonic components, except nanoplanktonic dinoflagellates. Moreover, the biomass of picocyanobacteria and bacteria increased in tanks with *Didemnum* compared to the control (Table 4.6). The interaction

*Mercenaria***Didemnum* had positive effects on the biomass of centric and pennate diatoms (e.g. ~20 µm *Nitzschia* sp., *Nitzschia longissima*) and nanoplanktonic dinoflagellates; other components (microplanktonic phyto- and dinoflagellates, and aloricate ciliates, and nanoplanktonic cryptophytes) were negatively influenced. *Didemnum* increased all nutrients but silicate, while *Mercenaria* decreased all nutrients but urea. Nitrate and nitrite were not quantified for this experiment. *Didemnum* alone had negative effects on the growth rates of centric diatoms (Figure 4.15c), while *Mercenaria* and *Mercenaria***Didemnum* increased growth rates of centric diatoms (Figures 4.15b,d). Silicate growth rates were decreased by both suspension feeders and their interaction; the interaction of *Mercenaria***Didemnum* also decreased growth rates of urea, ammonium and phosphate. Si:N molar ratios were significantly different between treatments (ANOVA $p<0.001$), with significantly higher values at t-120h for the control (mean 14.0; Tukey test $p<0.001$). This confirms the pattern of a decrease in Si:N ratios in tanks with suspension feeders outlined before, that indicates a significant effect due to excretion (Figure 4.16).

Table 4.5 lists averaged biomass (µg chl*a* l^{-1}; µg C l^{-1}) and abundance (zooplankton org l^{-1}) for all plankton groups considered, and concentrations (µM) of dissolved nutrients at the start of each mesocosm incubation (i.e. a proxy for initial ambient concentrations). Table 4.6 lists initial and end-time (after 72- or 120-h incubation) biomass for pico- (i.e. heterotrophic bacteria, *Synechococcus*, picoeukaryotes) and nanoplankton components (i.e. 2-5 µm eukaryotes) analyzed by flow cytometry. Table 4.7 summarizes the individual and interactive effects of suspension-feeding benthos on the biomass (and concentrations) of different plankton components, presented in Figures 4.2-4.15a. Table 4.8 summarizes the effects on the growth rates of the different plankton groups, presented in Figures 4.6b-4.15d.

DISCUSSION

Top-down effects on plankton community

Both bivalve species invariably exerted top-down control on phytoplankton biomass (Tables 4.7, 4.8). The same applies to most planktonic components across a size range ~1 to >40 µm, including zooplanktonic prey such as rotifers, in accordance to recent findings that pointed out the trophic link between this 'non-traditional' prey and benthic suspension feeders (Wong et al., 2003a,b). The effective control even on the smallest prey would be expected, as both bivalves have well developed laterofrontal cirri in the ctenidium (Jørgensen, 1990) which enable them to effectively trap small (<5 µm) particles in suspension.

Mercenaria exerted apparent better control than *Geukensia* on picoplanktonic biomass (Figure 4.8a). This result is interesting, since heterotrophic bacteria and nanoplankton have been suggested to be an occasional food item for ribbed mussels from which they can derive a substantial proportion of their nutrition (Wright et al., 1982; Langdon and Newell, 1990). On the other hand, Bass et al. (1990) reported effective filtration in juvenile hard clams fed *Synechococcus* and the picoplanktonic chlorophyte *Nannochloris*, but with low absorption efficiencies.

An interesting result from single-species mesocosms with *Geukensia* was the positive effect that ribbed mussels had on the biomass of centric diatoms (nano- and microplanktonic), with the opposite negative effect on pennate biomass. In one experiment in particular ($t_{initial}$ 7/21/08), the initial biomass of pennate diatoms was 14× higher than that of centric diatoms, resulting in ribbed mussels promoting a community shift among nano- and microplanktonic diatoms from dominance by pennate to centric types. The mechanism behind these changes in biomass likely involves increased diatom population growth rates modulated by top-down effects. It is known that grazing can influence the net population growth rate of phytoplankton (Smayda, 1997), and this experiment showed that *Geukensia* had positive effects on the growth rates of centric diatoms, and negative effects on the growth rates of pennates (Figure 4.6b).

Diatoms are widely recognized as a major dietary component of bivalves, and the findings from this study suggest that an elevated bivalve biomass and increased suspension-feeding function may potentially drive significant changes in the abundances of these. Porter (1977) and Power et al. (1998) mention examples of phytoplankton species that grow faster when subject to high grazing pressure. In support of the latter,

instead of seeing a switch to inedible species after the invasion and increased predation rates of zebra mussels (*Dreissena polymorpha*) in the Hudson River, Caraco et al. (1997) found that species shifts led to a preponderance of faster growing species. Among other traits, diatoms are known to have intrinsic higher growth rates than other phytoplankton (Smayda, 1997).

Ultimately, these changes in plankton standing stock and community composition may have effects on bivalve growth. For example, Pratt and Campbell (1956) were among the first to point out that phytoplankton composition has an effect on bivalve growth, with <15 µm centric diatoms (e.g. *Skeletonema costatum*, *Chaetoceros* sp.) being more beneficial than microplanktonic diatoms or flagellates for the hard clam. Several authors revisited the issue of hard clam growth in relation to plankton community composition. Greenfield et al. (2005) concluded that growth in juvenile hard clams was greatest at field locations where centric diatoms (e.g. *Thalassiosira* sp., *Skeletonema* sp., *Chaetoceros* sp.) were dominant, in contrast to field locations where nanoflagellates, pennate diatoms (e.g. *Nitzschia closterium*) and heterotrophic dinoflagellates where abundant. Weiss et al. (2007) provided further evidence that centric diatoms (e.g. *Skeletonema costatum*, *Chaetoceros* sp.) are positively correlated with *Mercenaria* growth.

A surprising finding was that the bivalve species considered in this study effectively controlled densities of some >40 µm zooplankton (i.e. rotifers; Figures 4.2-4.4), while they had a positive effect on the densities of eggs and larval stages of copepods, suggesting a weak or lack of top-down control for the latter. Marine copepods are known to exhibit preferential or selective feeding (Griffin and Rippingale, 2001; Sommer and Sommer, 2006), and high phytoplankton biomass inhibits copepod grazing (Griffin and Rippingale, 2001; and references therein). Intense benthic grazing by bivalves had a negative effect on total and <5-µm chlorophyll, diminishing phytoplankton biomass. Some trophic cascade may have been involved, in which the decrease in phytoplankton biomass may have promoted copepod grazing and population growth, explaining the positive effects of bivalves on the density of copepod eggs and larval stages. Moreover, the control bivalves exerted on rotifers may have promoted copepod population growth by releasing them from a direct source of competition.

Top-down control by *Didemnum* on most planktonic components was remarkably weak, to the point of being almost entirely absent. There was, however, one exception in which the biomass of *Synechococcus* and picoeukaryotes was lower in incubations with *Didemnum* (Figure 4.9a). Considering the characteristics of the filtration apparatus of ascidians (Petersen, 2007) and existing evidence of effective top-down control on heterotrophic bacteria (see below), a much more efficient top-down control from *Didemnum*, especially on small-forms of plankton, would have been expected. For example, Bak et al. (1998) demonstrated that the colonial ascidian *Trididemnum solidum* effectively filtered bacterioplankton from experimental cylinders, being capable of grazing bacteria at densities up to 2×10^6 cells ml^{-1} (Bak et al., 1996). The increased abundance of bacteria and other picoplankton in tanks with *Didemnum*, may be related to increased ammonium regeneration. Osinga et al. (1999) found positive feedbacks between ammonium production and heterotrophic bacteria in dying or decaying sponges.

Bottom-up effects on plankton community

Riemann et al. (1988) conducted a series of seasonal incubations of estuarine plankton manipulating the presence and densities of benthic suspension feeders, planktivorous fish, inorganic nutrients and natural, untreated sediments. They found that comparatively to enclosures without sediments, sediments acted primarily as a nutrient source, thereby increasing phytoplankton biomass. The mesocosms in this study did not incorporate sediments, and therefore a number of processes that relate to sediment biogeochemistry and enhanced benthic biodeposition (reviewed in Newell et al., 2002; Newell, 2004) could not be considered. Therefore, beyond some straightforward interpretations, such as the accumulation of nitrogenous compounds (predominantly ammonium) in experimental tanks due to excretion by macroinvertebrates, any further interpretation of the effects on dissolved nutrients is likely to be not entirely representative of natural conditions.

Goodbody (1957) provided estimates of ammonium excretion in solitary ascidians, ranging from (mean ± SE) 35.2 ± 2.3 µg NH_4-N h^{-1} gDW^{-1} in *Molgula manhattensis*, to 68.7 ± 3.5 in *Ciona intestinalis*. A rough estimation of ammonium

regeneration by *Didemnum vexillum* from concentrations measured in experimental tanks (not an *ad hoc* laboratory-based experimental determination of excretion rate), estimated ammonium production to be 168.1 ± 25.4 μg NH_4-N h^{-1} gDW^{-1}, at temperatures ranging from 20-24ºC, a value considerably higher than the rates reported by Goodbody (1957). There is also the possibility that after being detached and suspended from a non-hard substrate, parts of the ascidian colonies might have died or been subject to increased bacterial degradation. Osinga et al. (1999) reported increased ammonium production and heterotrophic bacteria densities in dying or decaying sponges.

Ammonium excretion estimates for *Mercenaria mercenaria* indicate a rate of 14.7 ± 1.1 μg NH_4-N h^{-1} gDW^{-1} (Srna and Baggaley, 1976) and 31.6 ± 3.1 for *Geukensia demissa* (Jordan and Valiela, 1982). Ammonium production by *Didemnum* was therefore an order of magnitude higher than that for either bivalve. This elevated ascidian nitrogen regeneration had remarkable positive effects on certain components of the plankton community, most notably nano- and microplanktonic diatoms. Given that no silicate was added into the enclosures, the mechanism was likely triggered by a progressive accumulation of ammonium, followed by rapid uptake of dissolved silicate. This pattern is evidenced by a progressive decline in Si:N ratios, more marked for those tanks that included ascidians (Figures 4.10, 4.12, 4.14, 4.16).

The dominance of diatoms resulting from shifting Si:N ratios is difficult to interpret in the light of some literature. For example in nutrient manipulation experiments Sommer (1994) found that diatoms became dominant at Si:N ratios >25 (much higher than the Si:N ratios found in this study, and the Si:N ratios corresponding to the phytoplankton spring bloom), while flagellates were superior competitors at lower ratios. Schöllhorn and Granéli (1996) established a similar pattern working with natural plankton communities, with a Si:N ratio of 1 favoring diatoms, and flagellates dominant at a Si:N ratio of 0.25. In contrast, this study found peaks of diatom dominance (>6,000 μg C l^{-1}) at Si:N ratios ~0.25. However, it is well established that nutrient-induced peaks of diatom dominance are transitional features (Egge and Aksnes, 1992), and it is possible that the sampling periodicity in this study might have missed mid-point events that could better explain this pattern. In any case, it is not surprising that diatoms were the taxonomic group that responded faster to these bottom-up influences, since diatom

growth rates are generally much higher than those for dinoflagellates, and other flagellates of equivalent biomass (Smayda, 1997).

The interpretation of diatom dominance in tanks with ascidians in terms of N:P ratios also presents contradictions to other authors' findings. For example, manipulating nutrient concentrations in mesocosms, Egge and Heimdal (1994) found that diatom numbers decreased when the N:P ratio increased (>80), while the present study found peaks in diatom biomass (~11,500 μg C l^{-1}) at N:P ratios >100 at t-120 h in tanks with *Mercenaria* and *Didemnum*. Compositionally this peak in diatom biomass corresponded mostly to pennates (small *Nitzschia* sp. and *Nitzschia longissima*), a result that is contradictory to the findings of Pilkaitytė and Razinkovas (2007), who found that centric diatoms were favoured over pennates in nitrogen enrichment experiments.

It is apparent that the overwhelming effect of ascidian production of ammonium (either the product of excretion or bacterial degradation of the tissue; see Osinga et al., 1999) ultimately resulted in high phytoplankton biomass (peaks >8,000 μg C l^{-1}), and supression of top-down controls exerted by bivalves in multi-species enclosures. The remarkably high concentration of particulate organic carbon, roughly an order of magnitude higher than moderate algal concentrations (Foster-Smith, 1975; Malouf and Bricelj, 1989), likely impaired the normal suspension-feeding function of bivalves.

Finally, although consistent with the very low filtration rates (~ 35 ml h^{-1} gDW^{-1}) estimated from chlorophyll reduction in experiments with natural plankton, the absence of top-down effects from *Didemnum vexillum* is puzzling, and raises the question of which carbon sources does this ascidian rely on. Although some species of the genus (i.e. *D. molle*) host symbiotic *Prochloron* spp., Koike et al. (1993) estimated that the symbiont autotrophic production is not enough to meet respiration losses, and thus *Didemnum* must rely on additional carbon sources, putatively supplied through suspension feeding. There are virtually no literature references indicating which specific planktonic food items (taxonomic group, or size-class) are captured and processed by colonial ascidians. Most studies of such type are performed with solitary animals, and even if natural plankton assemblages are considered, the parameters usually measured are generic (e.g. total particulate matter, particulate organic matter, chlorophyll concentration; Hartl and Ott, 1999). In that sense, even if it failed to provide valid conclusions, this study represents

one of the first to look into top-down effects of a colonial ascidian on specific plankton components within a natural assemblage.

Interactive effects of suspension-feeding species

Asmus and Asmus (2005) point out that diversity within suspension feeder guilds is important, because of the way it can affect the amount and sizes of particles removed from the spectrum of available food items. In other words, this follows the 'insurance hypothesis', as stated by Naeem (2002), according to which niche complementarity yields a more efficient resource use than an equivalent set of monocultures. Most of the response surface experiments in this study (4 out of 6) showed statistically significant interactive effects on biomass of two suspension-feeding species. Even though interactive effects of the two bivalves were weaker than the effects of a single bivalve (Figures 4.7 and 4.8a), the fact that these effects are exerted in the same direction indicates a likelihood in resource utilization, and both bivalve species pertaining to the same trophic guild.

On the other hand, when the colonial ascidian *Didemnum* established interactive effects with a bivalve species, these were exerted in a diverging direction compared to the effects of either species alone (Figure 4.10a). This indicates that complex interactive effects arising from the combination of suspension-feeding species belonging to different guilds were at play in these experiments.

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Table 4.1: Single-species mesocosm experiments in Great South Bay (40º43'N, 73º05'W). Suspension-feeding bivalve species were *Mercenaria mercenaria* and *Geukensia demissa*, from local sources. Estimated % daily turnover in each tank, from empirically-derived clearance rates at t_0 seston concentration and temperature. Ammonium concentrations (µM) added daily into tanks. All values reported as mean ± SD.

initial date	incubation (h)	temp (ºC)	species	n orgs	shell length (mm)	total biomass (g DW)	daily turnover (% vol)	n replicate tanks	daily ammendments NH_4 (µM)
				0				3	3.72
10/31/07	72	14.1 ± 0.5	*Mercenaria*	8	65 ± 3.7	18.1 ± 0.7	32 ± 2	4	4.09
				16	64.3 ± 4.1	35.4 ± 0.3	58 ± 5	4	4.01
				0				3	4.89
4/29/08	72	13.9 ± 0.7	*Geukensia*	9	75.5 ± 8.8	12.4 ± 0.8	64 ± 5	4	4.93
				18	73.5 ± 8.9	23.2 ± 1.5	118 ± 9	4	4.89
				0				3	5.03
6/10/08	72	25.2 ± 1.0	*Mercenaria*	10	67.4 ± 3.6	16.5 ± 0.8	43 ± 2	3	5.07
				20	67.8 ± 3.5	34.2 ± 0.8	91 ± 4	4	4.96
				0				4	5.04
7/21/08	120	26.4 ± 0.8	*Geukensia*	18	69.2 ± 8.9	18.8 ± 0.3	48 ± 2	3	5.18
				36	70.2 ± 9.6	38.7 ± 1.5	103 ± 6	3	5.41
				0				4	4.97
8/3/08	120	26.5 ± 0.5	*Geukensia*	18	73.1 ± 6.5	20.8 ± 0.5	126 ± 4	4	4.95
				36	71 ± 7.6	39.3 ± 1.4	239 ± 9	4	4.91

Table 4.2: Response-surface mesocosm experiments in Great South Bay (40º43'N, 73º05'W), incorporating two suspension feeders (combinations of *Geukensia demissa*, *Mercenaria mercenaria* and/or *Didemnum vexillum* from local sources). All values reported as mean ± SD.

initial date	incubation (h)	temp (ºC)	*Geukensia* shell height (mm)	*Mercenaria* shell length (mm)	*Didemnum*	daily ammendments NH_4 (µM)
9/18/08	120	20.6 ± 0.7	67.2 ± 6.1	66.9 ± 3.0		4.94
10/1/08	120	18.4 ± 1.2	65.4 ± 8.2	66.3 ± 3.7		4.91
6/15/09	120	19.9 ± 0.5	46.9 ± 8.4		present	4.67
6/22/09	120	20.6 ± 0.6		65.8 ± 3.9	present	4.48
6/29/09	120	24.5 ± 0.8	46.9 ± 8.4		present	4.56
7/6/09	120	23.8 ± 1		64.9 ± 3.8	present	4.68

Table 4.3: Response-surface mesocosm experiments in Great South Bay (40º43'N, 73º05'W). Ranges of percent daily volume turnover in treatment tanks, estimated from empirically-derived clearance rates at t_0 seston concentration and temperature.

treatment	9/18/08	10/1/08
Geukensia-only	27 - 81%	34 - 105%
Mercenaria-only	25 - 75%	19 - 59%
Geuk+Merc	39 - 81%	24 - 49%

treatment	6/15/09	6/29/09
Geukensia-only	33 - 95%	51 - 142%
Didemnum-only	4 - 8%	4 - 8%
Geuk+Didem	34 - 64%	52 - 93%

treatment	6/22/09	7/6/09
Mercenaria-only	19 - 56%	6 - 19%
Didemnum-only	2 - 3%	1 - 2%
Merc+Didem	20 - 39%	8 - 14%

Table 4.4: Empirically derived clearance rates ($l\ h^{-1}\ gDW^{-1}$) for *M. mercenaria*, *G. demissa* and *D. vexillum*, feeding on Great South Bay natural plankton, in single- or 2-species experimental setups. All values reported are mean ± SE. * denotes significant differences in 1 or 2-way ANOVA. See text for further details.

suspension feeder species	date	temp. (ºC)	n (orgs)/wet weight (g)	avrg. dry weight (g)	ambient biomass (µg chl *a* l^{-1})	beaker/ tank vol (l)	clearance rate based on:			
							total chl *a*	<5 µm chl *a*	seston	*in vivo* chl
Mercenaria	11/1/07	14.8	4	9.5	4.3	30	0.7 ± 0.25		0.27 ± 0.18	
Mercenaria	6/11/08	24.8	6	10.4	33.1	58	0.33 ± 0.14		0.82 ± 0.13	0.44 ± 0.14 *
Mercenaria	9/17/08	23.7	10	16.5	15.5	35	0.08 ± 0.01			0.25 ± 0.03 *
Mercenaria	10/3/08	18.5	10	16.6	16.6	28	0.03 ± 0.01			0.20 ± 0.11
Mercenaria	6/23/09	20.8	1	1.9	7.0	3	0.02 ± 0.01			0.17 ± 0.05 *
Mercenaria	7/7/09	24.5	1	2.1	6.3	2	0.06 ± 0.004			0.02 ± 0.02
Geukensia	4/30/08	14.6	9	11.5	23.4	58	0.34 ± 0.06	0.84 ± 0.11	1.07 ± 0.11	
Geukensia	5/19/08	15.5	9	14.2	30.6	58	0.41 ± 0.14		0.45 ± 0.3	1.40 ± 0.31 *
Geukensia	7/22/08	27.0	9	10.9	13.6	58	0.22 ± 0.14		0.36 ± 0.09	0.50 ± 0.09 *
Geukensia	8/4/08	27.4	10	9.5	13.7	45	0.83 ± 0.35		0.38 ± 0.28	1.23 ± 0.25 *
Geukensia	9/17/08	23.7	10	9.9	15.5	35	0.32 ± 0.04 *			0.71 ± 0.03 *
Geukensia	10/3/08	18.5	10	5.5	16.6	28	0.07 ± 0.01			0.97 ± 0.34 *
Geukensia	6/16/09	20.3	2	0.8	10.2	3	1.01 ± 0.16 *			
Geukensia	6/30/09	24.7	2	1.1	4.7	2	1.39 ± 0.42 *			1.60 ± 0.37 *
Didemnum	6/16/09	20.3	47.1	4.0	10.2	3	0.05 ± 0.01			
Didemnum	6/23/09	20.8	99.0	6.2	7.0	3	0.02 ± 0.003 *			0.04 ± 0.01
Didemnum	6/30/09	24.7	96.0	6.1	4.7	2	0.05 ± 0.01			0.03 ± 0.01
Didemnum	7/7/09	24.5	78.9	4.8	6.3	2	0.03 ± 0.01			0.01 ± 0.01
Geuk+Merc	9/17/08	23.7	10 + 10	26.8	15.5	35	0.13 ± 0.02			0.31 ± 0.07 *
Geuk+Merc	10/3/08	18.5	10 + 10	22.7	16.6	28	0.01 ± 0.01			0.19 ± 0.03 *
Geuk+Didem	6/16/09	20.3	2 + 53.4	5.1	10.2	3	0.08 ± 0.03			
Geuk+Didem	6/30/09	24.7	2 + 100.3	7.4	4.7	2	0.13 ± 0.02			0.16 ± 0.06
Merc+Didem	6/23/09	20.8	1 + 108.7	8.6	7.0	3	0.02 ± 0.002			0.03 ± 0.02
Merc+Didem	7/7/09	24.5	1 + 85.1	7.1	6.3	2	0.02 ± 0.01			0.02 ± 0.01

Table 4.5: Concentration at t_0 of total and fractionated (<5 µm) chlorophyll a (determination by fluorometry); dissolved nutrients; picoplankton and 2-5 µm nanoeukaryotes (determination by flow cytometry); nanoplankton (determination by epifluorescence microscopy); microplankton (determination by settling and microscopy); and >40 µm zooplankton. All values reported as mean (SE).

units	taxa	10/31/07		4/29/08		6/10/08		7/21/08		8/3/08		9/18/08		10/1/08		6/15/09		6/22/09		6/29/09		7/6/09	
µg l^{-1}	total chl	2.9	(0.2)	20.7	(0.4)	37.8	(0.1)	29.1	(1.9)	14.8	(1.2)	19.3	(0.3)	13.2	(0.7)	11.5	(0.9)	8.7	(0.4)	7.9	(0.1)	8.7	(0.3)
	<5 µm chl	2.1	(0.1)	13.5	(1)	26.7	(2.3)	14.0	(0.8)	13.3	(1.2)	2.3	(0)	0.6	(0)	3.2	(0.1)	1.3	(0)	1.3	(0)	2.2	(0)
µM	silicate					53.9		87.6	(2.7)	11.1	(1.5)	45.5	(0)	40.5	(1.7)	14.4	(1.1)	23.5	(0.2)	26.6	(1.9)	15.0	(0)
	ammonium					0.7		0.3	(0.1)	1.5	(0.1)	1.6	(0.3)	1.1	(0)	3.3	(0)	6.5	(0.3)	5.9	(2.1)	9.6	(1.7)
	nitrate					7.9		0.2	(0.1)	1.3	(0.2)	1.3	(0.2)	6.0	(0.1)	3.0	(0.8)	3.0	(0)	1.7	(0.5)		
	nitrite									0.5	(0)	2.5	(0)	2.2	(0.1)								
	urea									0.4	(0.1)	0.8	(0)	0.7	(0)	0.6	(0)	0.4	(0)	0.9	(0)	1.2	(0.2)
	phosphate					0.2		0.2	(0)	0.1	(0)	0.5	(0)	0.2	(0)	0.1	(0)	0.2	(0)	0.5	(0)	0.2	(0)
µgC l^{-1}	hetero bacteria	133.9		37.6		89.2		69.7		82.3		62.5		32.1	(1.8)	28.6	(1.4)	45.2	(0)	26.1		35.3	
	Synechococcus	23.4		7.3		11.9		44.8		98.9		330.7		231.7	(6.1)	69.2	(19.6)	72.8	(6.2)	342.8		285.2	
	picoeukaryotes	518.4		223.9		160.0		1267.8		1834.1		1199.3		606.4	(22.9)	39.5	(10.2)	55.1	(0.7)	338.8		233.8	
	nanoeukaryotes	775.1		6742.2		11056.7		6458.5		7683.9		6472.7		3906.8	(94.4)	1234.7	(351.4)	1932.1	(6.1)	4345.5		3951.2	
µgC l^{-1}	cryptophytes	548.5	(48.7)	1027.2	(164.7)	226.1	(42.3)	1130.3	(142.6)	764.7	(88.3)	1579.3	(344.7)	678.5	(130)	1984.2	(1155)	1886.6	(766.9)	1171.0	(255.5)	857.6	(249.8)
	autotroph dino	45.8	(45.7)	327.0	(131)	1736.5	(152.6)	1207.2	(244)	1305.7	(330.6)	516.8	(179.5)	718.8	(411.1)					639.4	(190.5)	545.9	(321.9)
	centric	18.5	(6.1)	200.5	(52.5)	191.6	(19.4)	112.0	(18.5)	196.0	(49.2)	191.3	(14.1)	271.0	(106.9)	1272.2	(305.6)	5194.4	(1313.4)	24.8	(8.8)	851.9	(98.2)
	pennate	2.1	(1.3)	88.2	(26.3)	27.9	(5)	57.9	(17.1)	29.5	(7.7)	14.6	(3.6)	77.2	(56.2)			30.3	(30.3)	13.0	(6.4)	5.5	(2.7)
	ciliates	104.0	(22.1)	121.0	(47.3)	24.7	(10.9)			53.7	(24.1)			51.7	(25.9)					40.5	(25.8)	25.8	(12.9)
µgC l^{-1}	phytoflagellates	287.8	(25.5)	149.5	(10.4)	61.1	(15.9)	105.8	(20)	160.9	(22.5)	163.6	(14.4)	143.4	(20.4)	253.4	(40.6)	272.4	(2.5)	110.2	(14.4)	47.0	(0.7)
	euglenoids	1.4	(0.5)	0.7	(0.4)	0.8	(0.5)	13.2	(4.7)	1.1	(0.4)	0.6	(0.2)	3.0	(1)	0.5	(0.3)	0.3	(0.1)	0.1	(0.1)	0.3	(0.2)
	dinoflagellates	60.0	(17.1)	539.2	(39)	1463.1	(176)	953.5	(218.9)	349.3	(14.4)	294.9	(36)	371.2	(23)	698.2	(149.6)	264.8	(51.8)	141.9	(22.6)	162.0	(12.4)
	centric	1.8	(0.4)	147.0	(5.3)	28.4	(3.4)	12.6	(12.6)	1.9	(0.8)	8.9	(0.3)	0.4	(0.3)	8.8	(0.7)	21.9	(3.8)	12.5	(2.7)	15.3	(5.3)
	pennate	11.0	(3.3)	102.4	(8.1)	249.5	(11.9)	1747.3	(103.5)	105.0	(7.2)	89.2	(7.2)	55.6	(8.1)	54.0	(4.9)	41.6	(3.2)	46.6	(2.4)	147.8	(2.7)
	loricate ciliates	3.3	(1.9)					9.9	(5.7)			3.7	(2.8)	0.8	(0.7)					1.0	(0.5)		
	aloricate ciliates	70.4	(17.5)	48.5	(17.4)	519.5	(103.8)	242.4	(103.8)	95.2	(30.4)	113.7	(23.1)	371.8	(109.3)	82.0	(28.4)	65.6	(25)	149.5	(9.6)	43.7	(19)
org l^{-1}	rotifers	8.0	(1.9)	13.4	(3)	11.3	(1.9)	13.3	(6.5)	1.0	(0.8)	0.1	(0.1)	4.1	(2.1)	0.3	(0.2)	0.6	(0.3)				
	Acartia eggs	10.9	(2.3)			14.4	(3.7)	0.9	(0.5)									0.1	(0)				
	Acartia nauplii	21.4	(1.4)	15.9	(0.5)	33.7	(7.3)	649.1	(405)	8.5	(2.2)	25.4	(4.4)	14.6	(3.4)	14.8	(0)	136.7	(119.2)	85.1	(19.5)	72.1	(28.1)
	Acartia copepod.	2.8	(0.4)	0.6	(0.4)	2.5	(0.8)	37.3	(17.2)	1.0	(0.6)	2.5	(0.7)	2.3	(1.3)	1.7	(0.8)	23.8	(17.5)	16.7	(5.7)	50.3	(28)
	other eggs	2.3	(0.4)	79.9	(1.8)	57.5	(6.7)	35.7	(8.9)	126.0	(43.4)	95.8	(4.1)	0.3	(0)	19.4	(3.4)	5.9	(1)	375.9	(348.6)	22.8	(8.5)
	invert. larvae	2.0	(0.4)	59.1	(3.1)	43.9	(4.6)	24.2	(6.4)	15.3	(3.7)	4.6	(0.6)	13.9	(1.4)	26.9	(3.7)			153.9	(62.9)	118.1	(60.4)
	veligers																	31.0	(9.6)				

Table 4.6: Picoplankton (heterotrophic bacteria, *Synechococcus*, picoeukaryotes) and 2-5 µm nanoeukaryote concentration (µg C l^{-1}) at initial and end times (either 72- or 120-h incubations) for control and treatment mesocosm tanks. Values reported are from single sample (n =1) determinations by flow cytometry.

			benthic susp feeder [g DW]	
	10/31/08	**t=72 h**	***Mercenaria***	
$\mu g\ C\ l^{-1}$	**initial**	**control**	**18.1 ± 0.7**	**35.4 ± 0.4**
hetero bacteria	134	110	121	113
Synechococcus	23	24	23	18
picoeuk	518	695	840	620
nanoeuk	775	1320	1213	1073
	4/29/08	**t=72 h**	***Geukensia***	
$\mu g\ C\ l^{-1}$	**initial**	**control**	**12.4 ± 0.8**	**23.2 ± 1.5**
hetero bacteria	38	64	70	67
Synechococcus	7	4	4	4
picoeuk	224	137	219	233
nanoeuk	6742	6858	9275	9745
	6/10/08	**t=72 h**	***Mercenaria***	
$\mu g\ C\ l^{-1}$	**initial**	**control**	**16.6 ± 0.9**	**34.3 ± 0.8**
hetero bacteria	89	173	161	161
Synechococcus	12	8	9	7
picoeuk	160	738	484	259
nanoeuk	11057	6603	7034	5894
	7/21/08	**t=120 h**	***Geukensia***	
$\mu g\ C\ l^{-1}$	**initial**	**control**	**18.8 ± 0.3**	**38.7 ± 1.5**
hetero bacteria	70	80	79	106
Synechococcus	45	4	2	2
picoeuk	1268	263	109	222
nanoeuk	6459	4000	2781	3154
	8/3/08	**t=120 h**	***Geukensia***	
$\mu g\ C\ l^{-1}$	**initial**	**control**	**20.8 ± 0.5**	**39.3 ± 1.4**
hetero bacteria	82	160	115	95
Synechococcus	99	25	9	3
picoeuk	1834	1643	266	274
nanoeuk	7684	4715	4154	3838

	9/18/08	**t=120 h**	***Geukensia***	***Mercenaria***	***Geu+Mer***
$\mu g\ C\ l^{-1}$	**initial**	**control**	**15.8**	**33.3**	**47.6**
hetero bacteria	63	207	219	208	301
Synechococcus	331	264	144	114	244
picoeuk	1,199	850	594	400	503
nanoeuk	6,473	3,172	2,891	2,720	4,106
	6/29/09	**t=120 h**	***Geukensia***	***Didemnum***	***Did+Geu***
$\mu g\ C\ l^{-1}$	**initial**	**control**	**11.6**	**19.4**	**29.0**
hetero bacteria	26	41	39	67	67
Synechococcus	343	30	1	2	6
picoeuk	339	347	230	197	391
nanoeuk	4,346	3,047	3,299	7,113	6,952
	7/6/09	**t=120 h**	***Mercenaria***	***Didemnum***	***Did+Mer***
$\mu g\ C\ l^{-1}$	**initial**	**control**	**17.7**	**11.2**	**29.4**
hetero bacteria	35	82	83	158	108
Synechococcus	285	75	37	283	54
picoeuk	234	641	468	782	769
nanoeuk	3,951	5,919	7,580	9,736	8,054

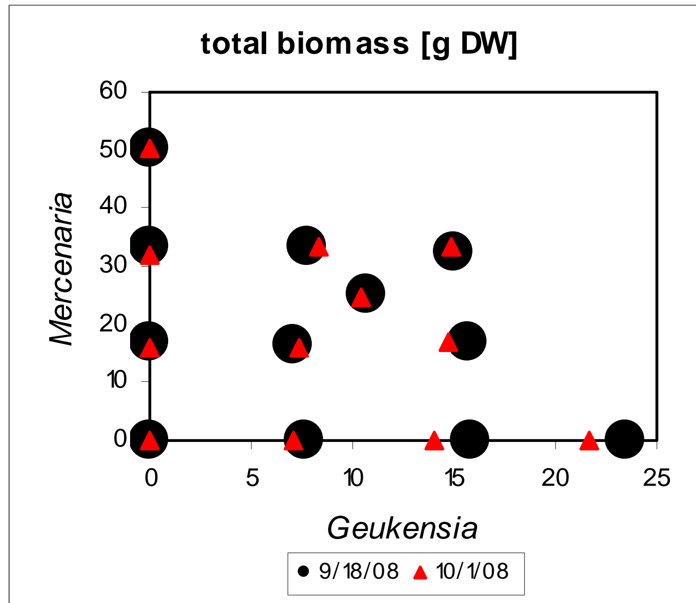
Table 4.7: Summary of positive (+), negative (**-**) and no effects (**0**) of suspension feeders (*Geu*: *G. demissa*, *Mer*: *M. mercenaria*, *Did*: *D. vexillum*) on standing stocks. The table integrates results from RDA triplots presented in Figures 4.2-4.15a. Suspension feeder references in bold type indicate stronger explanatory variables. Species references: chl tot: total chlorophyll *a*, chl <5µm: <5 µm chlorophyll *a*, nuts: nitrite, nitrate, urea, phosphate; ammon: ammonium, silicate: silicate, bact: heterotrophic bacteria, *Syn*: *Synechococcus*, picoeuk: picoeukaryotes, nanoeuk: nanoeukaryotes, cryptoN: nanoplanktonic cryptophytes, cenN: nanoplanktonic centric diatoms, cenM: microplanktonic centric diatoms, penN: nanoplanktonic pennate diatoms, penM: microplanktonic pennate diatoms, autdinN: autotrophic nanoplanktonic dinoflagellates, dinoM: microplanktonic dinoflagellates, phyflgM: microplanktonic phytoflagellates, euglenM: microplanktonic euglenoids, ciliateN: nanoplanktonic ciliates, alorcilM: aloricate microplanktonic ciliates, lorcilM: loricate microplanktonic ciliates, Acnaup: *Acartia tonsa* nauplii, Accopep: *Acartia tonsa* copepodites, Acegg: *Acartia tonsa* eggs, rotifer: >40 µm rotifers, othegg: non-crustacean invertebrate eggs, larvae: non-crustacean invertebrate larvae.

		T initial	chl tot	chl <5µm	nuts	ammon	silicate	bact	*Syn*	picoeuk	nanoeuk	cryptoN	cenN	cenM	penN	penM	autdinN	dinoM	phyflgM	euglenM	ciliateN	alorcilM	lorcilM	Acnaup	Accopep	Acegg	rotifer	othegg	larvae
Mer		10/31/07	-	-								-	-	-	-	-	-	-	-	-	-	-		+	+	+	-	+	-
Geu		4/29/08	-	-								+	-	-	-	0	+	-	+	-	+	+		+	+		-	-	+
Mer		6/10/08	-	-	+	+	-					+	-	-	+	-	+	+	-		-			+	-	+	-	+	+
Geu		7/21/08	-	-								-	+	+	-	-	+	-	0	-									
Geu		8/3/08	-	-								-	+	+	-	-	-	-	0	+	-	-							
Geu	*Geu*Mer*	9/18/08	-	-	+	+	+					+	+	-	+	-	-	-	-	0		+							
Mer		10/1/08	-	-	-	+	+	-	-	-	-	-	+	-	+	-	-	-	-	-	0	-							
*Geu*Mer*		10/1/08	-	-	-	0	-	-	-	-	-	-	+	-	+	-	-	0	-	-	+	-							
Did	***Did*Geu***	6/15/09	+	+	+	+	-	+	-	-			0	+		-		+	+			+							
Geu		6/15/09	-	-	-	-	+	-	+	+			0	-		+		-	-			-							
Did	*Did*Mer*	6/22/09	+	+	+	+	-	+	+	+	+	-	+	+		+		+	0	+		+	+						
Mer		6/22/09	-	-	-	-	0	+	-	+	0	-	-	-		-		-	-	-		-	-						
Did		6/29/09	+	+	+	+	-					+	0	+	+	+	-	+	+		+	+	+						
Did		7/6/09	+	+	+	+	-					+	+	+	+	+	0	+	+			+							
Mer		7/6/09	-	-	-	-	-					-	+	+	+	-	+	-	-			-							

Table 4.8: Summary of positive (+), negative (**-**) and no effects (**0**) of suspension feeders (*Geu*: *G. demissa*, *Mer*: *M. mercenaria*, *Did*: *D. vexillum*) on growth/increment rates of species. The table integrates results from RDA triplots presented in Figures 4.6b-4.15d. Species references: chl tot: total chlorophyll *a*, <5chl/tot: ratio of total/<5 μm chlorophyll *a*, nuts: nitrite, nitrate, urea, phosphate; ammon: ammonium, silicate: silicate, bact: heterotrophic bacteria, *Syn*: *Synechococcus*, picoeuk: picoeukaryotes, nanoeuk: nanoeukaryotes, cryptoN: nanoplanktonic cryptophytes, cenN: nanoplanktonic centric diatoms, cenM: microplanktonic centric diatoms, penN: nanoplanktonic pennate diatoms, penM: microplanktonic pennate diatoms, autdinN: autotrophic nanoplanktonic dinoflagellates, dinoM: microplanktonic dinoflagellates, phyflgM: microplanktonic phytoflagellates, euglenM: microplanktonic euglenoids, ciliateN: nanoplanktonic ciliates, alorcilM: aloricate microplanktonic ciliates, lorcilM: loricate microplanktonic ciliates.

	T initial	chl tot	<5chl/tot	nuts	ammon	silicate	bact	*Syn*	picoeuk	nanoeuk	cryptoN	cenN	cenM	penN	penM	autdinN	dinoM	phyflgM	euglenM	ciliateN	alorcilM	lorcilM
Geu	8/3/08	-	-								-	+	+	-	-	+	-	+	-	+	+	
Mer	10/1/08	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	-	-	+	-	
*Geu*Mer*	10/1/08	+	-	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	
Did	6/15/09	+	+	+	+	-	+	-	0	+	+	+	+	-	0		+	+			+	
*Did*Geu*	6/15/09	+	+	+	+	-	+	+	-	+	+	-	+	-	-		+	+			+	
Did	6/22/09	+	-	+	+	-	+	+	+	+	-	+	+		+		-	-	-		+	+
*Did*Mer*	6/22/09	+	-	+	-	+	+	+	+	-	-	0	-		+		-	-	-		+	+
*Did*Geu*	6/29/09	-	-	+	-	-					-	+	+	+	+	+	+	+		+	0	-
Did	7/6/09	+	+	+	+	-					-	-	-	+	+	+	+	+			+	
Mer	7/6/09	+	+	+	+	-					+	+	+	+	+	+	+	+			+	
*Did*Mer*	7/6/09	0	+	-	+	-					-	+	+	+	-	0	-	-			+	

Figure 4.1: Total biomass (g DW), of two suspension feeders (combinations of *Geukensia demissa*, *Mercenaria mercenaria* and/or *Didemnum vexillum*) incorporated into response-surface mesocosm experiments in Great South Bay (40º43'N, 73º05'W). 12 treatments considered, for each of both replicate dates (●,▲).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Mercenaria
Geukensia
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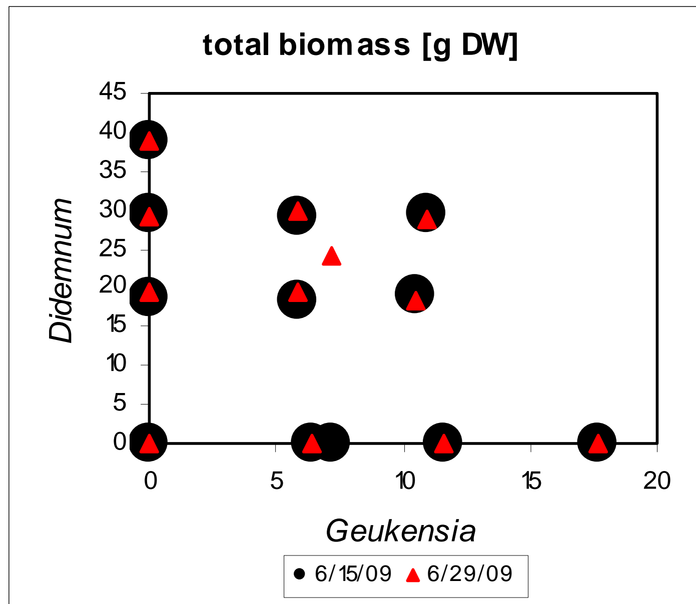
30
20
10
0
0
5
10
15
20
25
9/18/08
10/1/08


total biomass [g DW]
Didemnum
Geukensia
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40
35
30
25
20
15
10
5
0
0
5
10
15
20
6/15/09
6/29/09

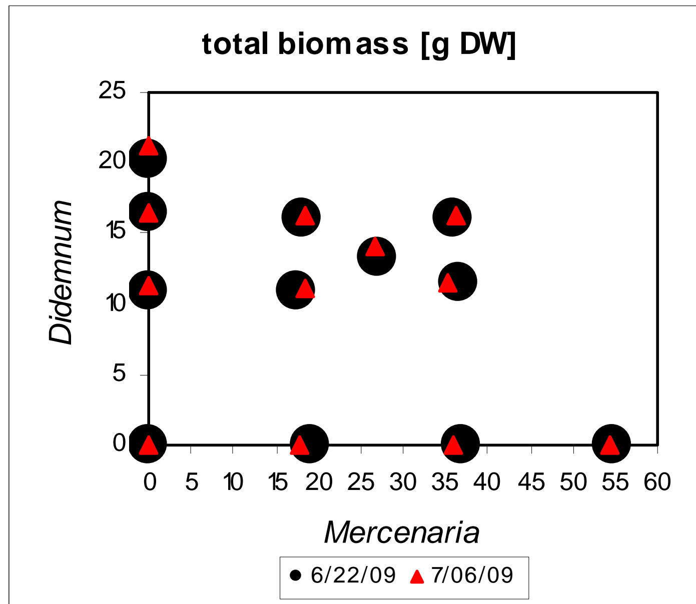
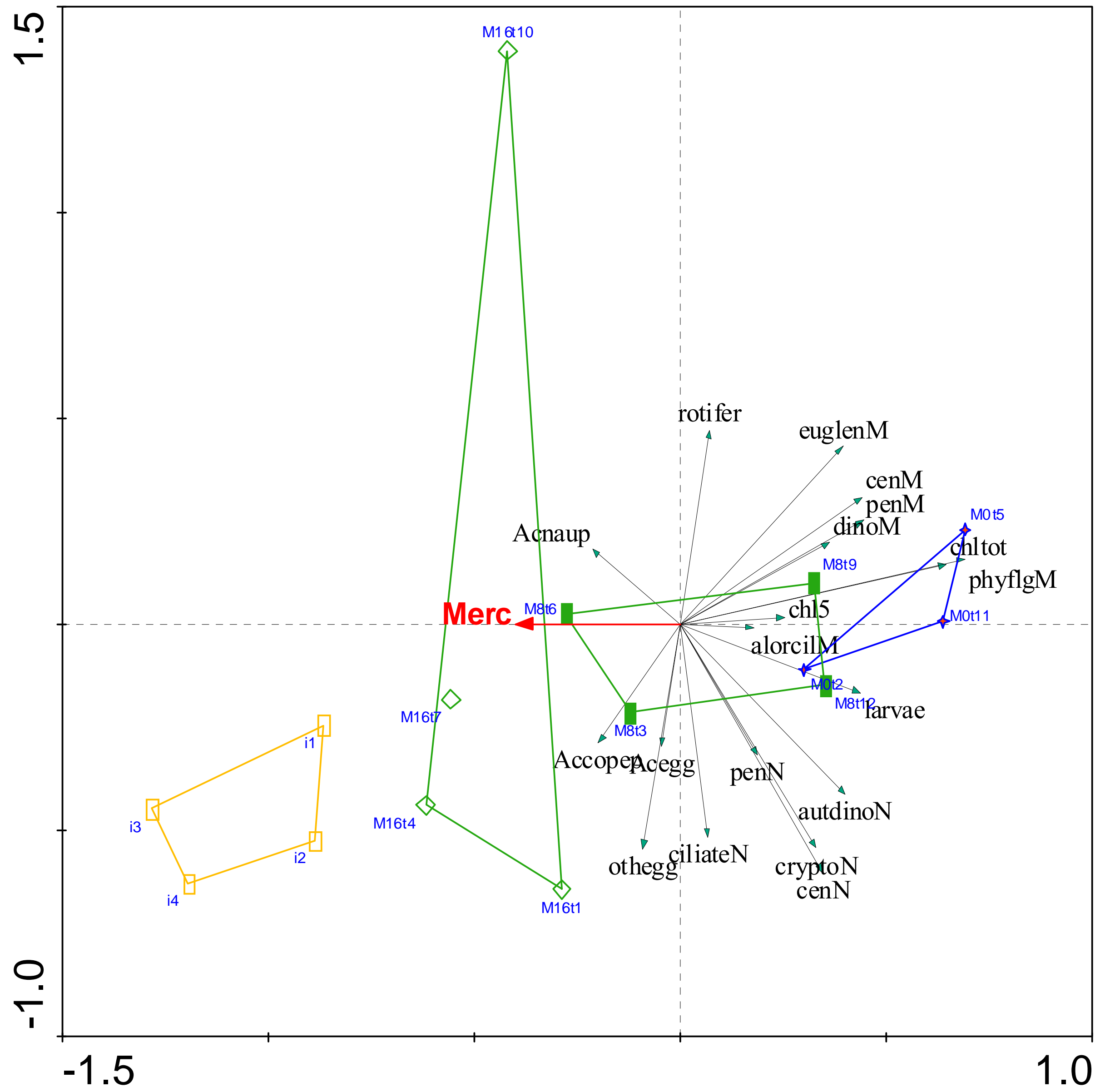

total biomass [g DW]
Didemnum
Mercenaria
25
20
15
10
5
0
0
5
10
15
20
25
30
35
40
45
50
55
60
6/22/09
7/06/09

Figure 4.2: *M. mercenaria* mesocosm experiment in Great South Bay (40º43'N, 73º05'W, initial date 10/31/07), RDA triplot for species biomass and/or concentration. Statistical significance: λ= 0.163, F-value= 1.751, p-value= 0.035. Species references in Table 4.7 legend.



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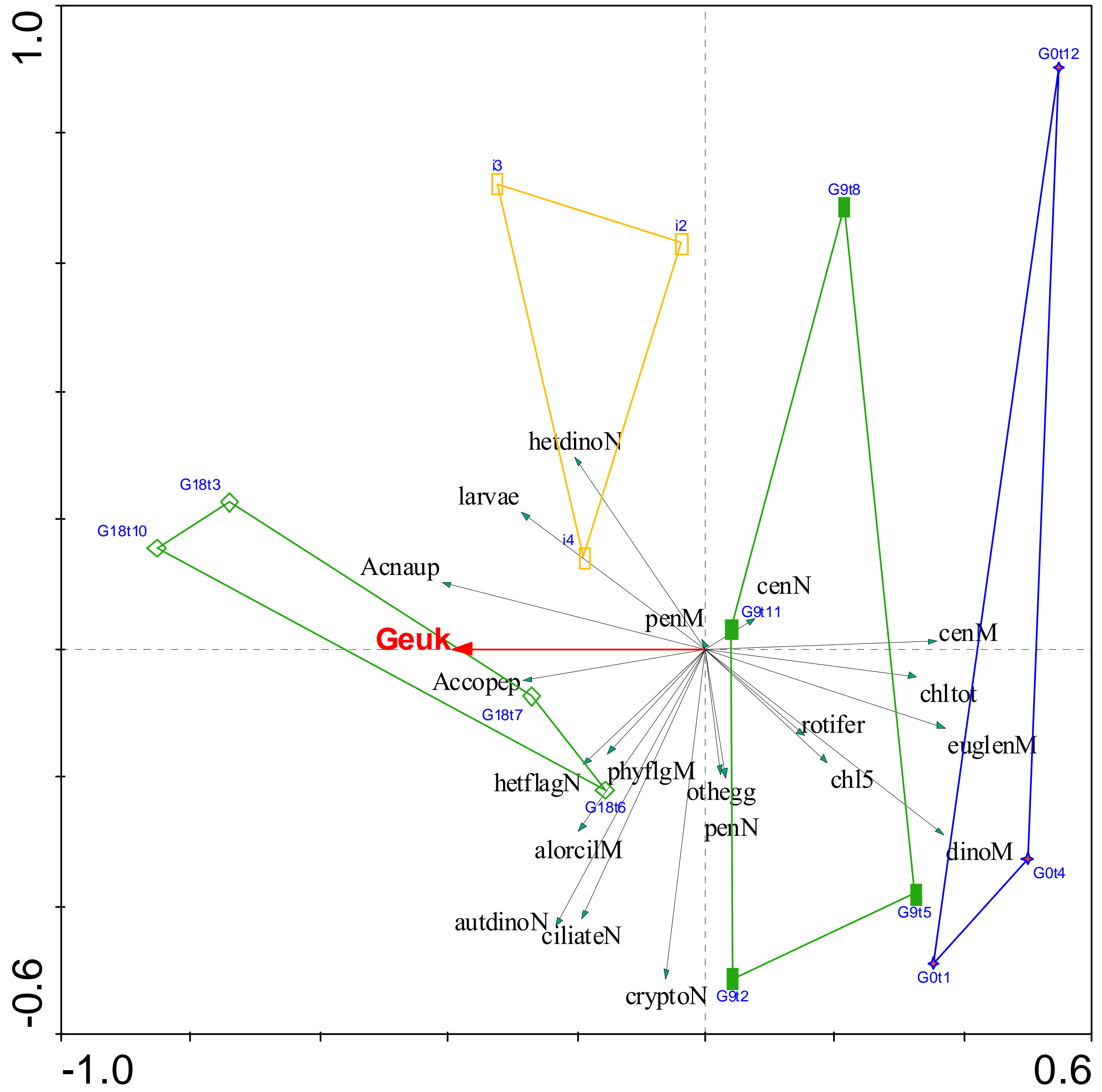
-1.5
1.0
M16t10
rotifer
euglenM
cenM
penM
dinoM
M0t5
chltot
phyflgM
Acnaup
M8t9
M8t6
Merc
chl5
M0t11
alorcilM
M0t2
M8t12
larvae
M16t7
M8t3
i1
Accopep
Acegg
penN
i3
M16t4
autdinoN
i2
othegg
ciliateN
cryptoN
cenN
i4
M16t1

Figure 4.3: *G. demissa* mesocosm experiment in Great South Bay (40º43'N, 73º05'W, initial date 4/29/08), RDA triplot for species biomass and/or concentration. Statistical significance: λ= 0.154, *F*-value= 1.637, p-value= 0.049. Species references in Table 4.7 legend.



1.0
-0.6
-1.0
0.6
G0t12
i3
G9t8
i2
hetdinoN
G18t3
larvae
G18t10
i4
Acnaup
cenN
penM
G9t11
Geuk
cenM
Accopep
chltot
G18t7
rotifer
euglenM
phyflgM
hetflagN
chl5
othegg
G18t6
penN
dinoM
alorcilM
G0t4
G9t5
autdinoN
ciliateN
G0t1
cryptoN
G9t2

Figure 4.4: *M. mercenaria* mesocosm experiment in Great South Bay (40º43'N, 73º05'W, initial date 6/10/08), RDA triplot for species biomass and/or concentration. Statistical significance: λ= 0.176, F-value= 1.708, p-value= 0.035. Species references in Table 4.7 legend.

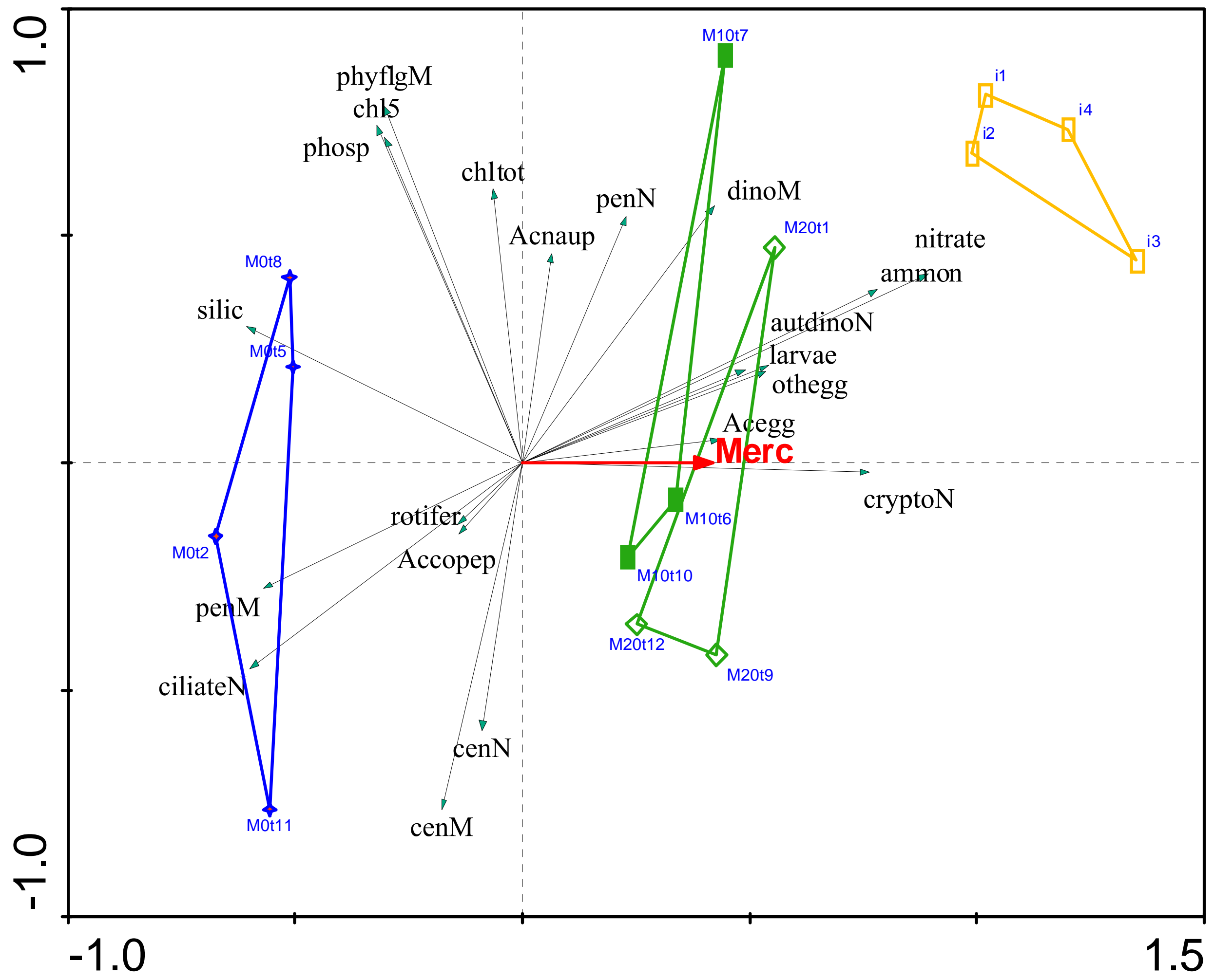

1.0
-1.0
-1.0
1.5
phyflgM
chl5
phosp
chltot
penN
dinoM
Acnaup
M10t7
i1
i4
i2
i3
M20t1
nitrate
ammon
M0t8
silic
M0t5
autdinoN
larvae
othegg
Acegg
Merc
cryptoN
rotifer
M10t6
M0t2
Accopep
M10t10
penM
M20t12
M20t9
ciliateN
cenN
M0t11
cenM

Figure 4.5: *G. demissa* mesocosm experiment in Great South Bay (40º43'N, 73º05'W, initial date 7/21/08), RDA triplot for species biomass and/or concentration. Statistical significance: λ= 0.323, *F*-value= 8.891, p-value= 0.001. Species references in Table 4.7 legend.

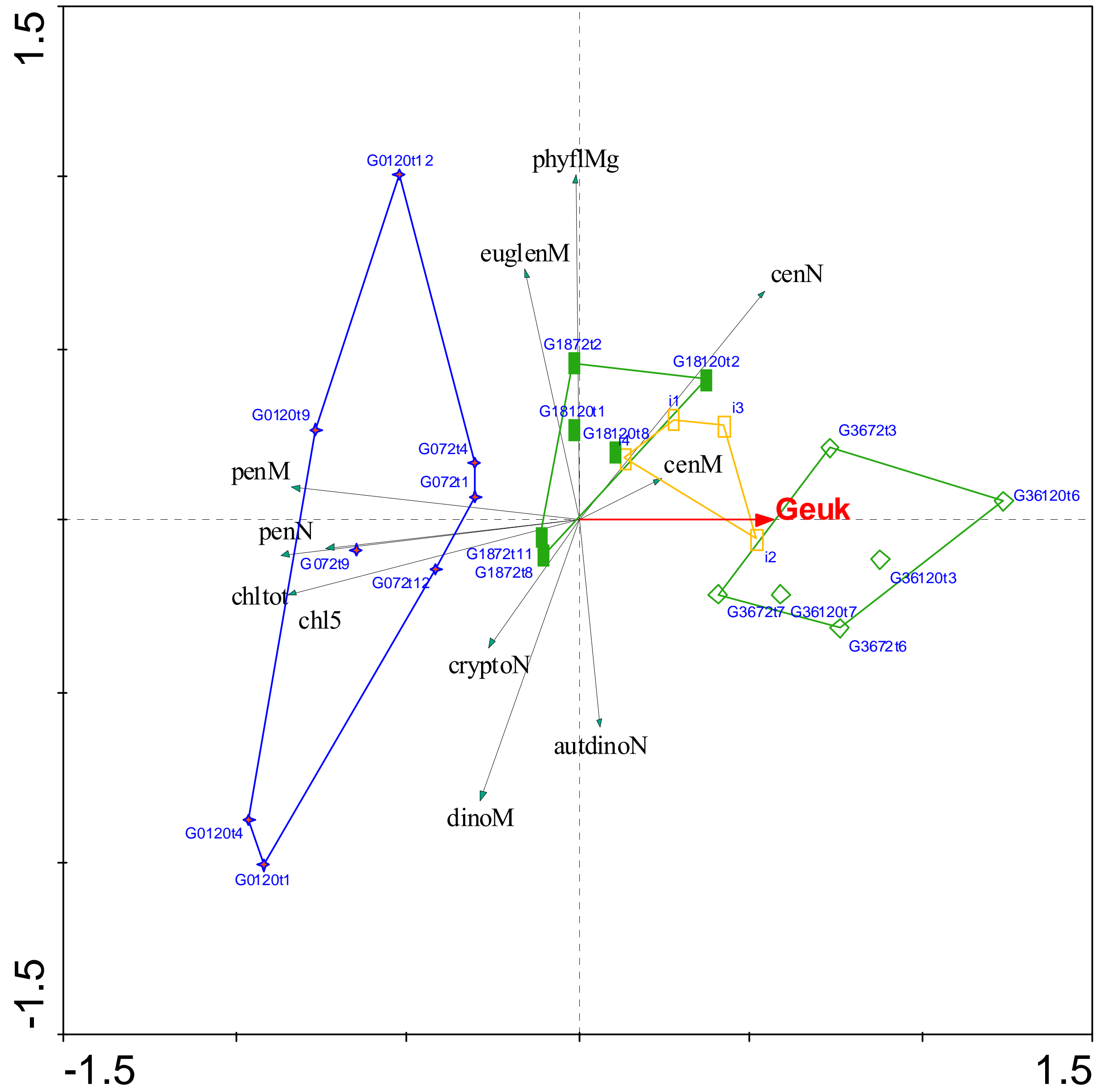

1.5
-1.5
-1.5
1.5
G0120t1 2
phyflMg
euglenM
cenN
G1872t2
G18120t2
G0120t9
i1
i3
G18120t1
G3672t3
G18120t8
i4
G072t4
penM
cenM
G072t1
G36120t6
Geuk
penN
i2
G1872t11
G072t9
G1872t8
G072t12
G36120t3
chltot
G3672t7
G36120t7
chl5
G3672t6
cryptoN
autdinoN
dinoM
G0120t4
G0120t1

Figure 4.6: *G. demissa* mesocosm experiment in Great South Bay (40º43'N, 73º05'W, initial date 8/03/08) **(a)** RDA triplot for species biomass and/or concentration. Statistical significance: λ= 0.521, *F*-value= 7.265, p-value= 0.001 **(b)** RDA triplot for species growth rates. Species references in Table 4.7 legend.

a

1.0

-1.0

-1.5

1.5

Time

Geu*Time

Geuk

cenM
phyflgM
cenN
ciliateN
dinoM
penM
chltot
chl5
euglenM
cryptoN
penN
autdinoN
alorcilM

G36120t1
G36120t7
G36120t1
G36120t4
G18120t1
G18120t5
G18120t8
G18120t2
G0120t12
G0120t6
G0120t9
G0120t3
i1
i2
i3
i4
G3672t10
G3672t7
G3672t4
G3672t1
G1872t11
G1872t2
G1872t8
G1872t5
G072t6
G072t9
G072t12
G072t3

b

1.0

-1.0

-0.8

1.0

Geuk

G18t2
G18t8
G18t11
G18t5
dinoM
penN
penM
chltot
alorcilM
cenM
autdinoN
euglenM
G0t6
G0t3
G0t9
G0t12
G36t4
G36t7
G36t1
G36t10
cenN
ciliateN
phyflgM
cryptoN
chl<5

Figure 4.7: *G. demissa* + *M. mercenaria* response-surface mesocosm experiment in Great South Bay (40º43'N, 73º05'W, initial date 9/18/08), RDA triplot for species biomass and/or concentration. Statistical significance: λ= 0.452, *F*-value= 5.442, p-value= 0.001. Species references in Table 4.7 legend.

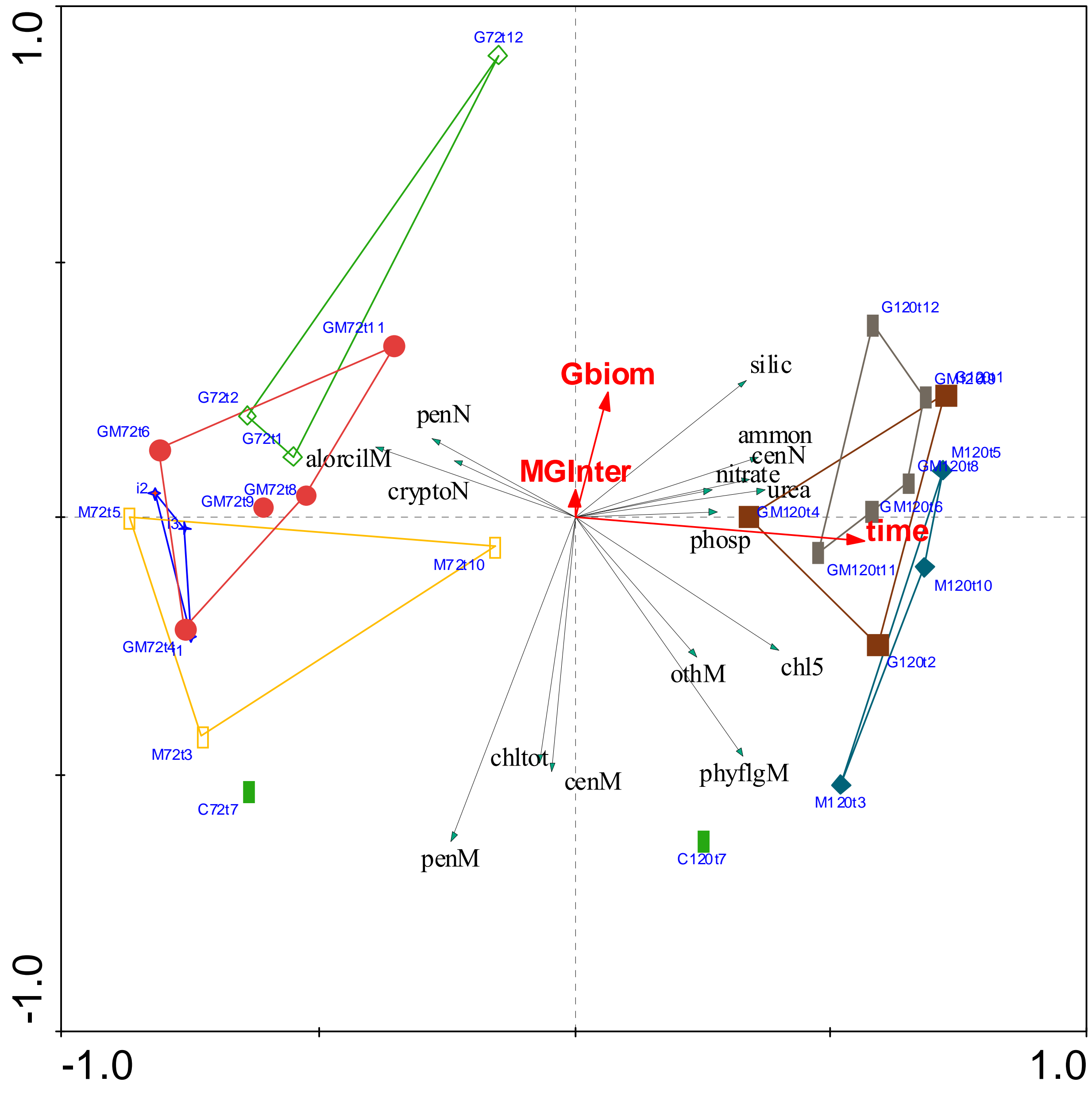
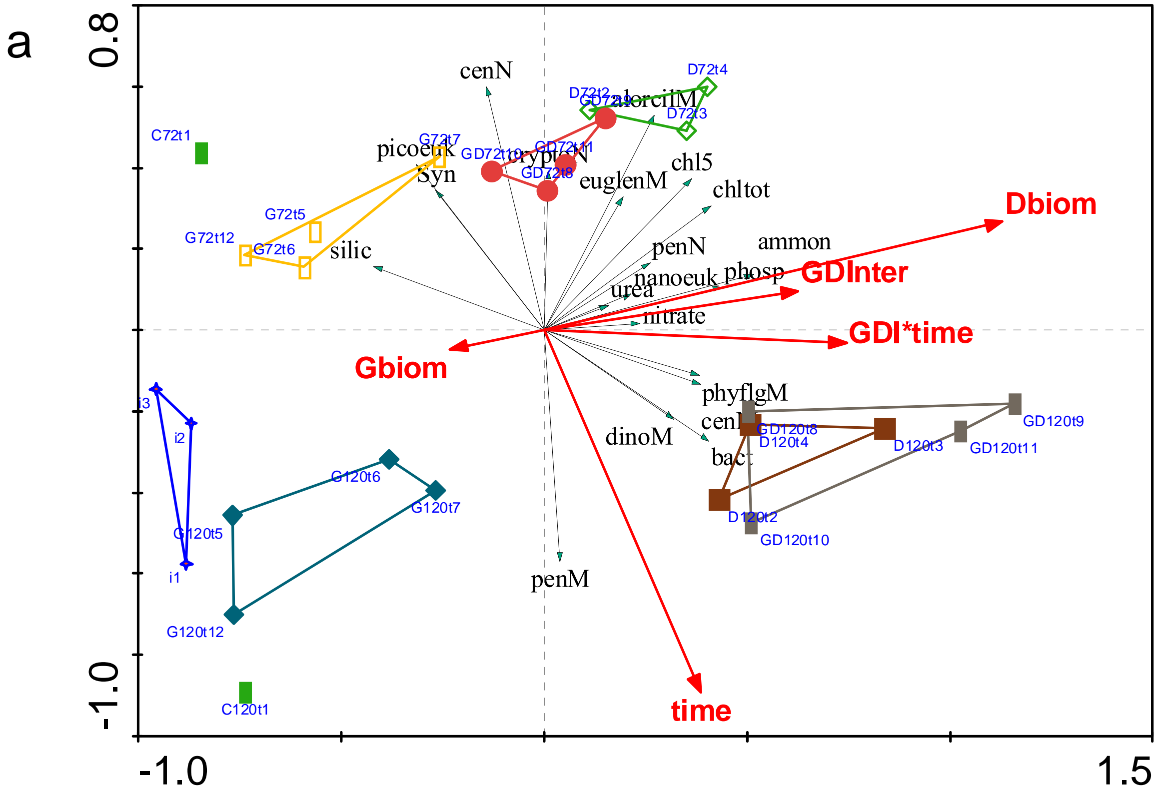

G72t12
GM72t11
G72t2
G72t1
GM72t6
GM72t8
GM72t9
i2
M72t5
M72t10
GM72t4
M72t3
C72t7
Gbiom
MGInter
time
penN
alorcilM
cryptoN
silic
ammon
cenN
nitrate
urea
phosp
chl5
othM
phyflgM
chltot
cenM
penM
G120t12
M120t5
GM120t8
GM120t6
GM120t4
GM120t11
M120t10
G120t2
M120t3
C120t7
1.0
-1.0
-1.0
1.0

Figure 4.8: *G. demissa* + *M. mercenaria* response-surface mesocosm experiment in Great South Bay (40º43'N, 73º05'W, initial date 10/01/08) **(a)** RDA triplot for species biomass and/or concentration. Statistical significance: λ= 0.512, *F*-value= 5.115, p-value= 0.001 **(b)** RDA triplot for species growth rates in relation to *Geukensia*Mercenaria* **(c)** RDA triplot for species growth rates in relation to *Mercenaria* biomass. Species references in Table 4.7 legend.

a
1.5
-1.5
-1.5
2.0
Mbiom
MGInter
MGI*time
time
penN
ciliateN
dinoM
bact
alorcilM
autdinoN
picoeuk
nanoeuk
Syn
chltot
chl5
penM
phyflgM
euglenM
phosp
urea
silic
ammon
M72t5
GM72t6
G72t1
GM72t4
GM72t8
GM72t9
M72t10
GM72t11
M72t3
G72t2
G72t12
i2
i1
i3
C72t7
C120t7
M120t5
M120t10
GM120t8
GM120t4
GM120t6
GM120t11
GM120t9
G120t1
G120t12
M120t3
G120t2
b
0.8
-0.6
-0.8
0.8
GMt11
Mt10
dinoM
cenN
nanoeuk
Gt12
GMt9
Syn
ciliateN
autdinoN
bact
Ct7
chltot
penN
alorcilM
cenM
Mt3
MGInter
chl<5
nitrite
Gt2
GMt8
nitrate
cryptoN
silic
ammon
Mt5
picoeuk
urea
phyflgM
penM
euglenM
Gt1
GMt4
GMt6
phosp
c
1.5
-1.0
-1.0
1.5
bact
dinoM
cenM
urea
penN
chltot
silic
Syn
ammon
cenN
ciliateN
alorcilM
Mbiom
nanoeuk
chl<5
nitrite
nitrate
penM
phyflgM
cryptoN
phosp
picoeuk
euglenM
autdinoN

Figure 4.9: *D. vexillum* + *G. demissa* response-surface mesocosm experiment in Great South Bay (40º43'N, 73º05'W, initial date 6/15/09) **(a)** RDA triplot for species biomass and/or concentration. Statistical significance: λ= 0.634, *F*-value= 6.237, p-value= 0.001 **(b)** RDA triplot for species growth rates in relation to *Geukensia*Didemnum* and *Didemnum* biomass. Species references in Table 4.7 legend.


a
0.8
-1.0
-1.0
1.5
cenN
alorcilM
picoeuk
Syn
cryptoN
euglenM
chl5
chltot
Dbiom
silic
penN
ammon
nanoeuk
phosp
GDInter
urea
nitrate
GDI*time
Gbiom
phyflgM
cenM
dinoM
bact
penM
time
C72t1
G72t7
GD72t10
GD72t11
GD72t8
D72t2
D72t4
D72t3
G72t5
G72t12
G72t6
i2
i1
G120t6
G120t7
G120t5
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C120t1
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D120t3
GD120t9
GD120t11
D120t2
GD120t10

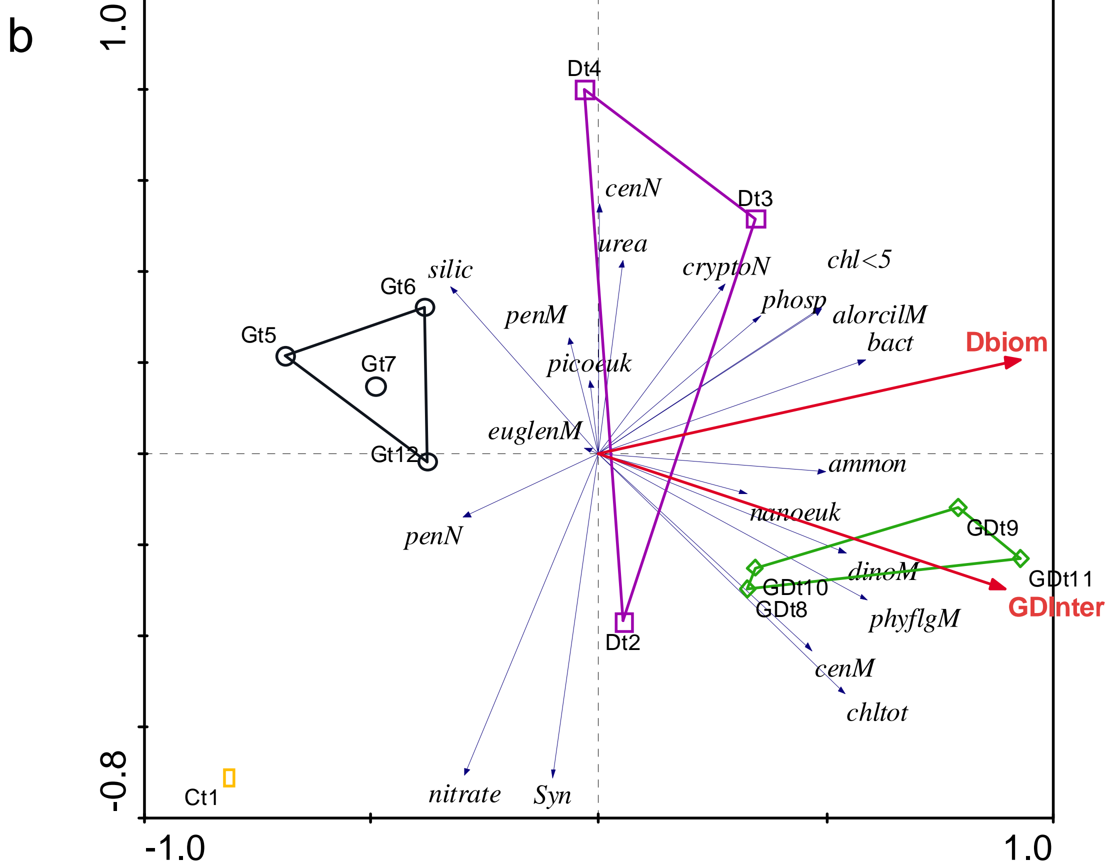
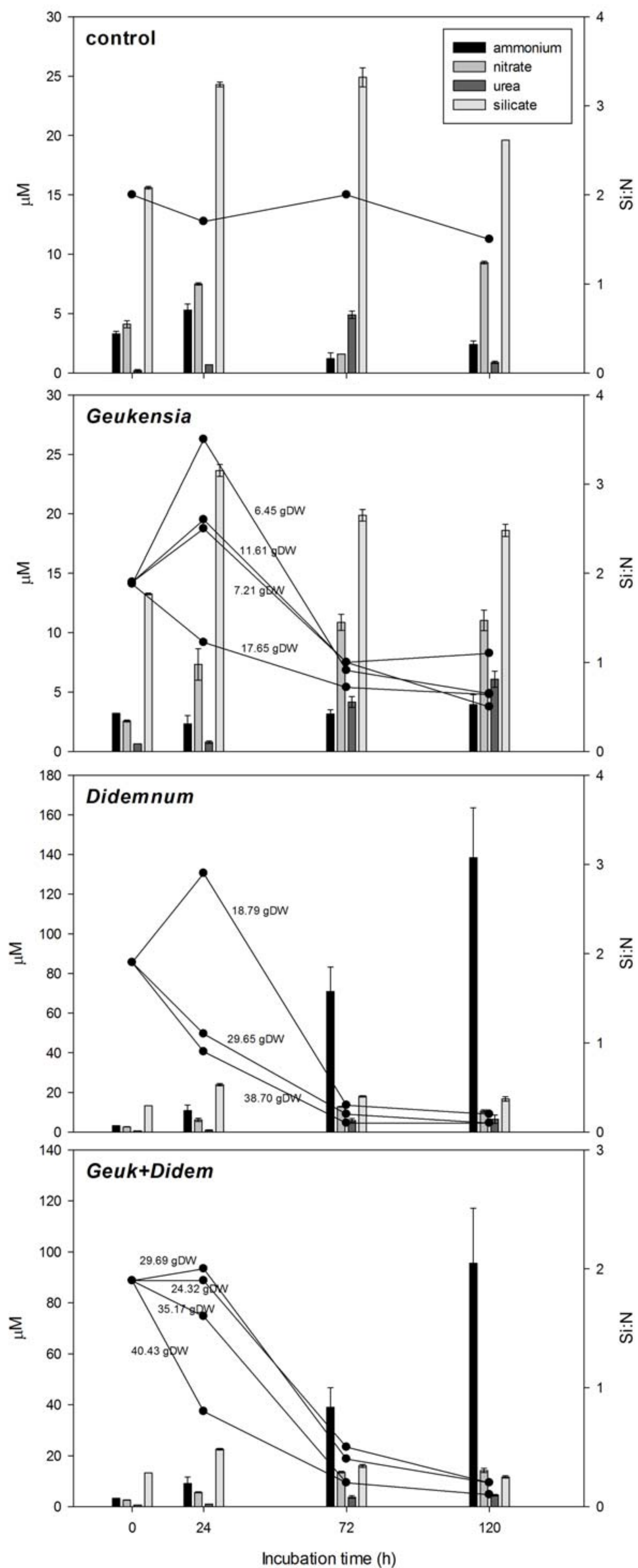

b
1.0
-0.8
-1.0
1.0
Dt4
Dt3
cenN
urea
cryptoN
chl<5
silic
Gt6
Gt5
Gt7
penM
phosp
alorcilM
bact
Dbiom
picoeuk
euglenM
Gt12
ammon
penN
nanoeuk
GDt9
dinoM
GDt10
GDt8
GDt11
GDInter
phyflgM
Dt2
cenM
chltot
Ct1
nitrate
Syn

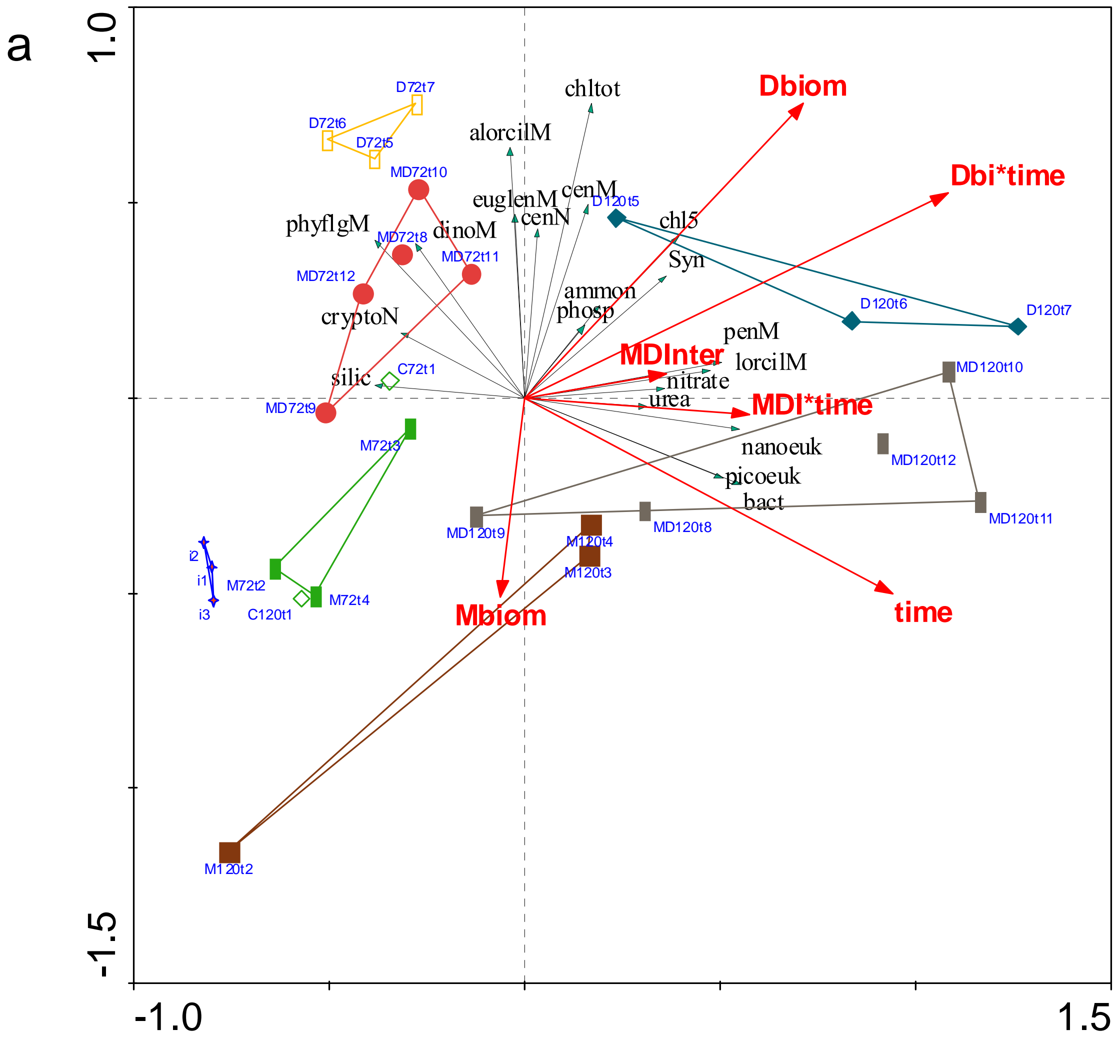
Figure 4.10: Nutrient concentrations (μM, mean ± SE) *versus* mesocosm incubation time, for response surface experiment in Great South Bay (40º43'N, 73º05'W, initial date 6/15/09). Values presented are for single control tank, and averaged for tanks with same species or combination of species of suspension feeder. Second-y axis, Si:N molar ratio *versus* mesocosm incubation time (2-way ANOVA, $p<0.001$); total biomass of suspension feeder expressed in gDW.



control
ammonium
nitrate
urea
silicate
Geukensia
6.45 gDW
11.61 gDW
7.21 gDW
17.65 gDW
Didemnum
18.79 gDW
29.65 gDW
38.70 gDW
Geuk+Didem
29.69 gDW
24.32 gDW
35.17 gDW
40.43 gDW
µM
Si:N
Incubation time (h)

Figure 4.11: *D. vexillum* + *M. mercenaria* response-surface mesocosm experiment in Great South Bay (40º43'N, 73º05'W, initial date 6/22/09) **(a)** RDA triplot for species biomass and/or concentration. Statistical significance: λ= 0.609, *F*-value= 4.414, p-value =0.001 **(b)** RDA triplot for species growth rates in relation to *Mercenaria**Didemnum* and *Didemnum* biomass. Species references in Table 4.7 legend.

a



b

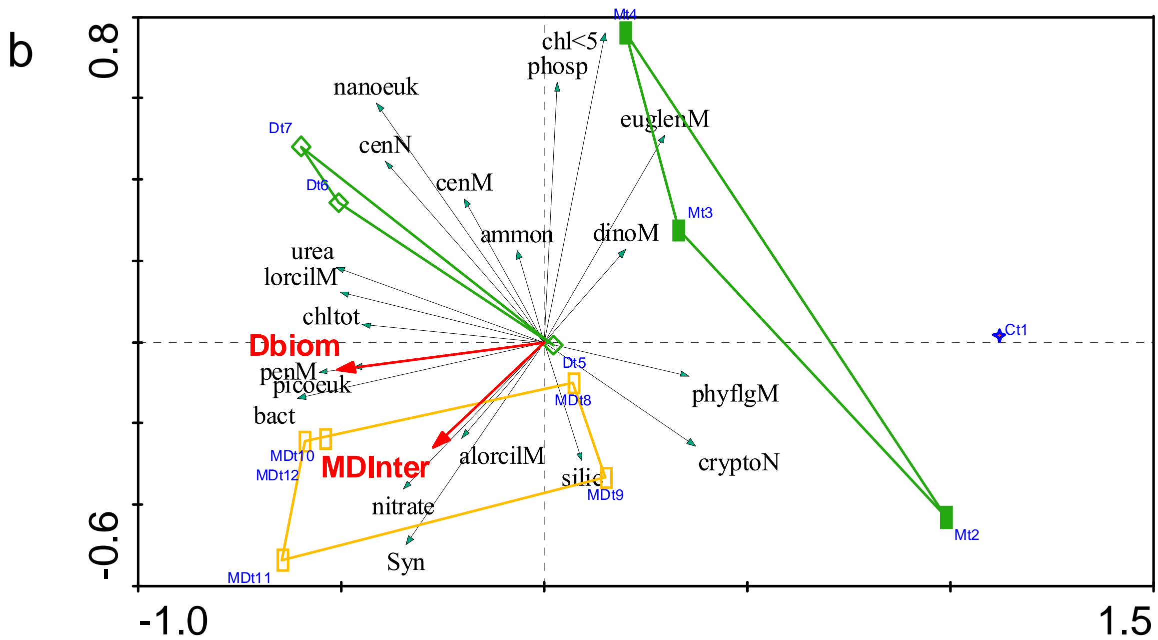


Figure 4.12: Nutrient concentrations (µM, mean ± SE) *versus* mesocosm incubation time, for response surface experiment in Great South Bay (40º43'N, 73º05'W, initial date 6/22/09). Values presented are for single control tank, and averaged for tanks with same species or combination of species of suspension feeder. Second-y axis, Si:N molar ratio *versus* mesocosm incubation time (2-way ANOVA, $p<0.001$); total biomass of suspension feeder expressed in gDW.

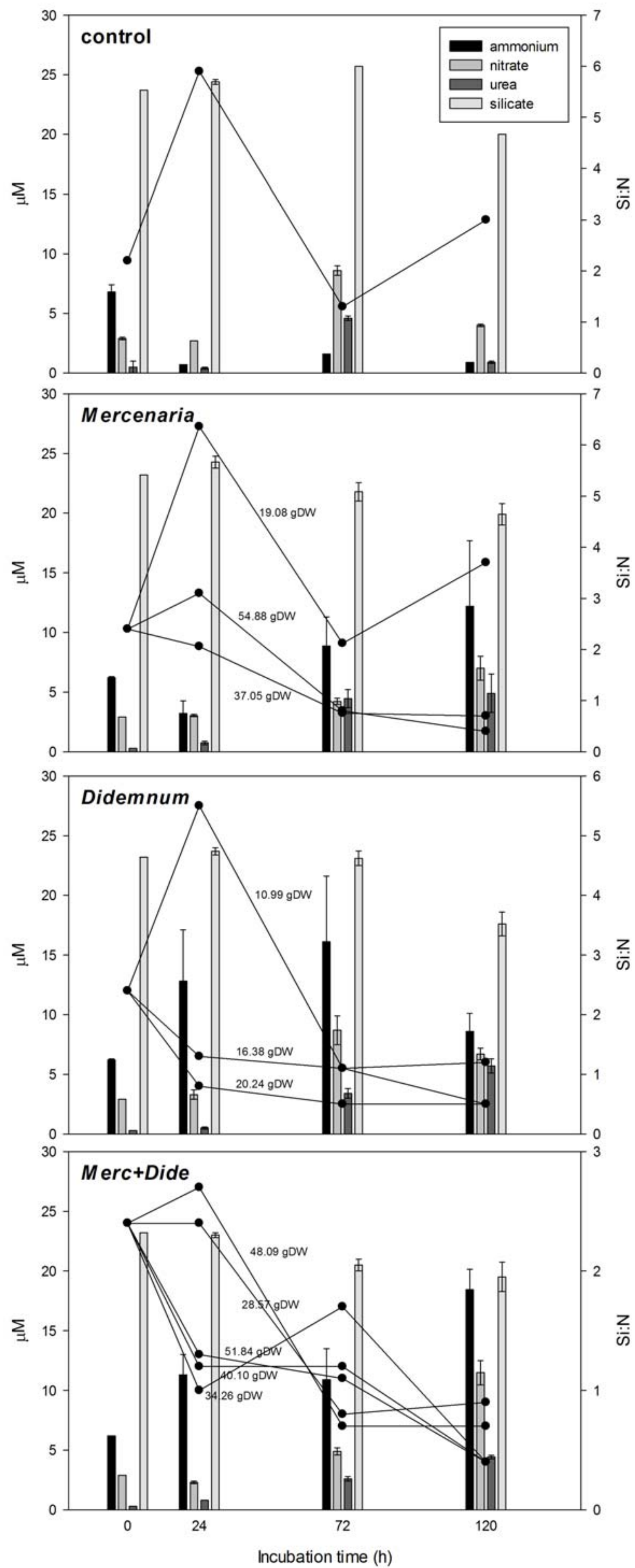
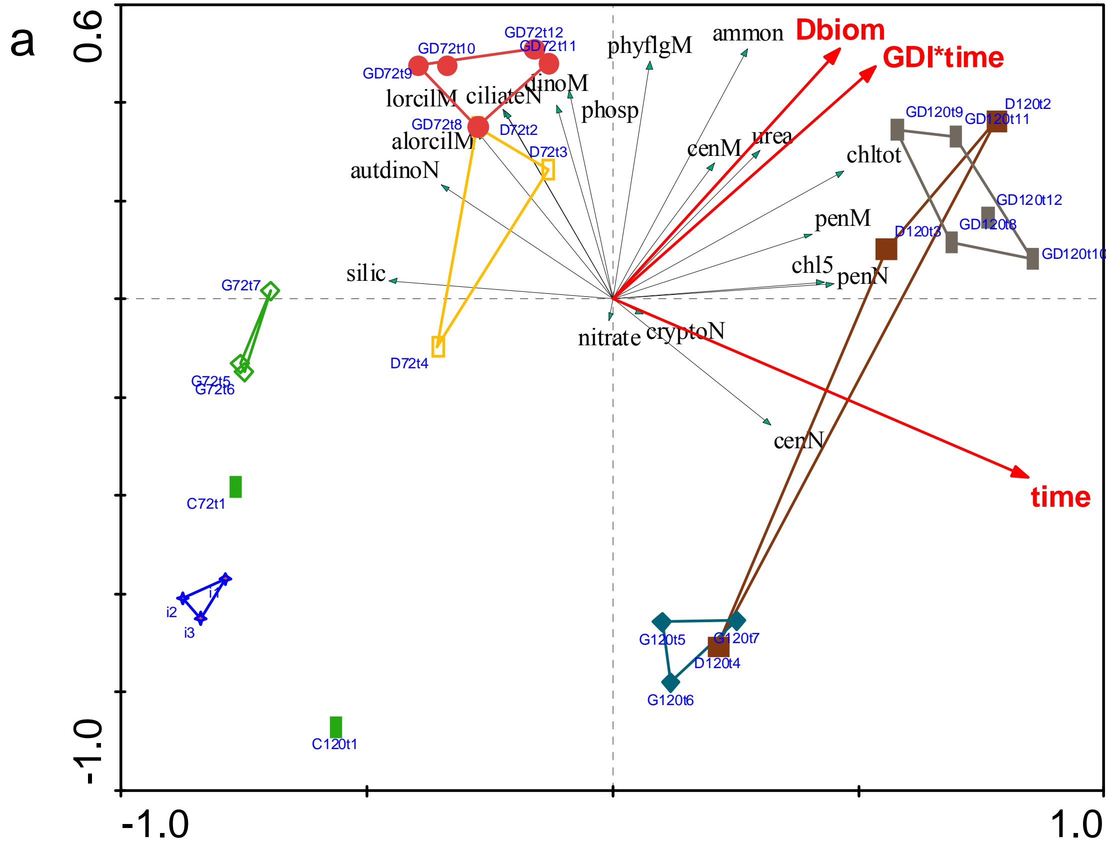

control
ammonium
nitrate
urea
silicate
Mercenaria
19.08 gDW
54.88 gDW
37.05 gDW
Didemnum
10.99 gDW
16.38 gDW
20.24 gDW
Merc+Dide
48.09 gDW
28.57 gDW
51.84 gDW
40.10 gDW
34.26 gDW
μM
Si:N
Incubation time (h)

Figure 4.13: *D. vexillum* + *G. demissa* response-surface mesocosm experiment in Great South Bay (40º43'N, 73º05'W, initial date 6/29/09) **(a)** RDA triplot for species biomass and/or concentration. Statistical significance: λ= 0.448, *F*-value= 5.839, p-value =0.001 **(b)** RDA triplot for species growth rates in relation to *Geukensia*Didemnum*. Species references in Table 4.7 legend.


a
0.6
-1.0
-1.0
1.0
Dbiom
GDI*time
time
phyflgM
ammon
lorcilM
ciliateN
dinoM
phosp
alorcilM
autdinoN
cenM
urea
chltot
penM
chl5
penN
silic
nitrate
cryptoN
cenN

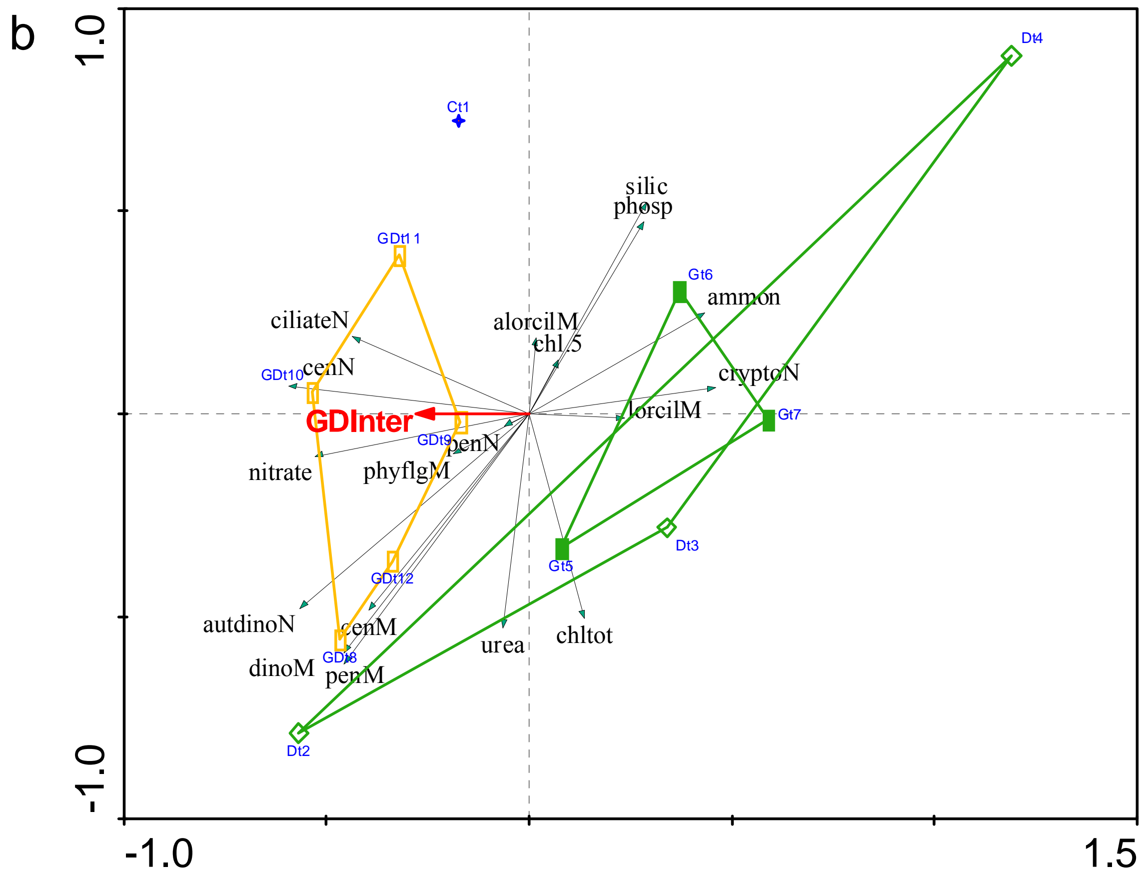

b
1.0
-1.0
-1.0
1.5
GDInter
silic
phosp
ciliateN
alorcilM
chl.5
ammon
cenN
cryptoN
lorcilM
penN
nitrate
phyflgM
autdinoN
cenM
dinoM
penM
urea
chltot

Figure 4.14: Nutrient concentrations (μM, mean ± SE) *versus* mesocosm incubation time, for response surface experiment in Great South Bay (40º43'N, 73º05'W, initial date 6/29/09). Values presented are for single control tank, and averaged for tanks with same species or combination of species of suspension feeder. Second-y axis, Si:N molar ratio *versus* mesocosm incubation time (2-way ANOVA, $p<0.001$); total biomass of suspension feeder expressed in gDW.

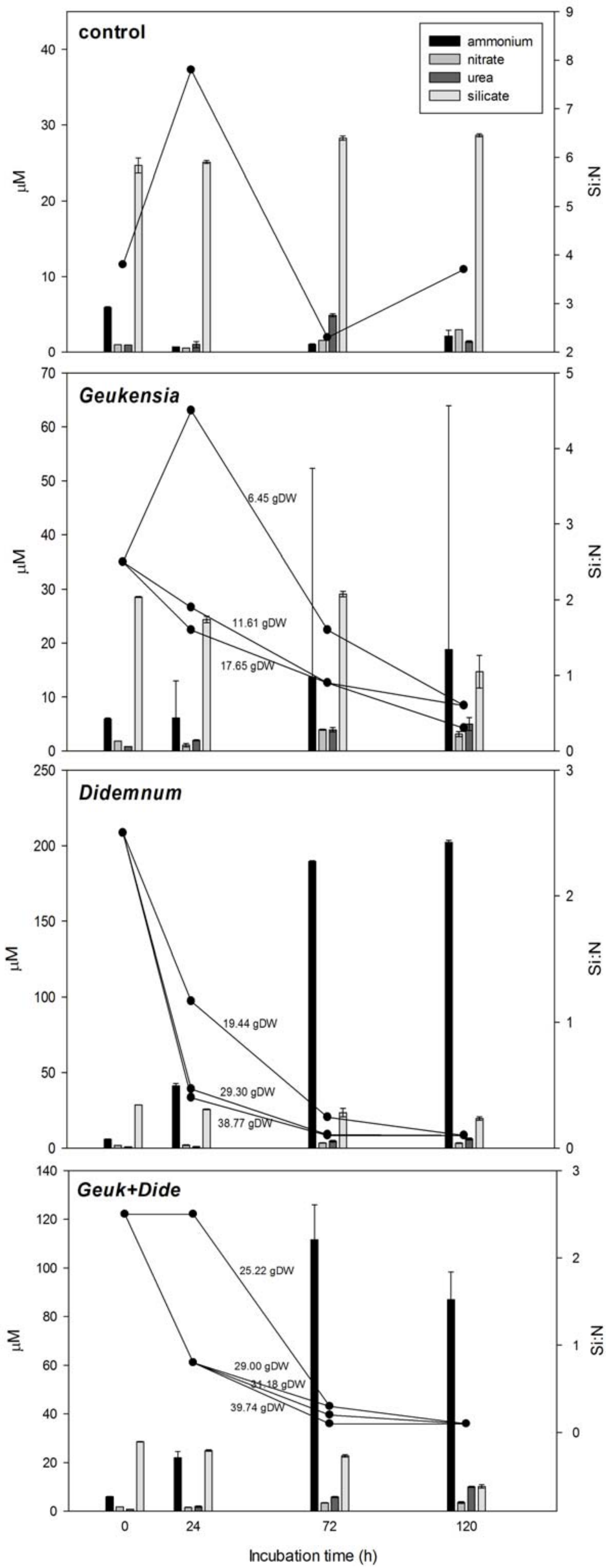
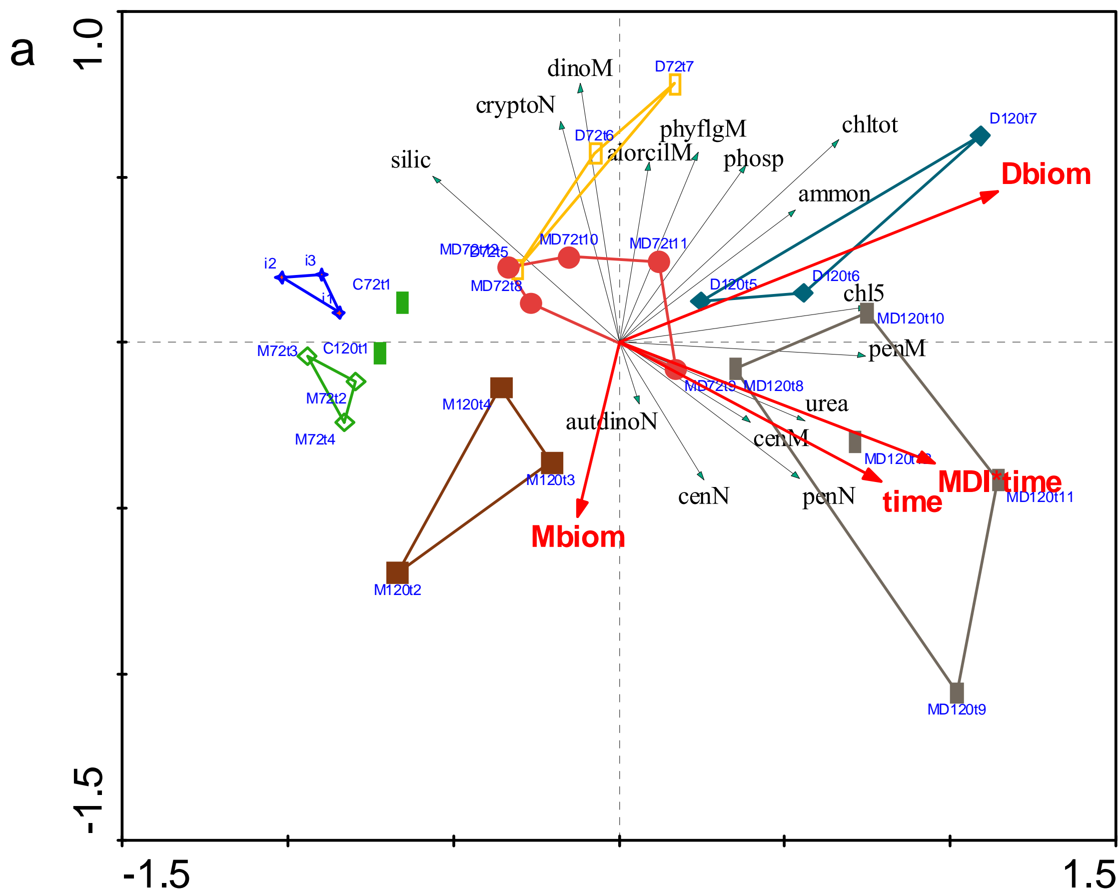
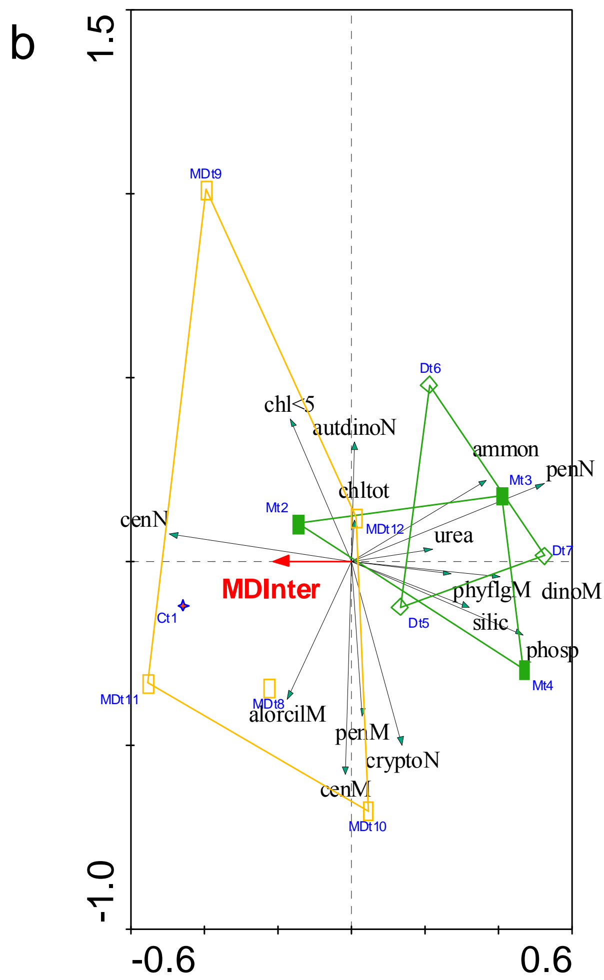
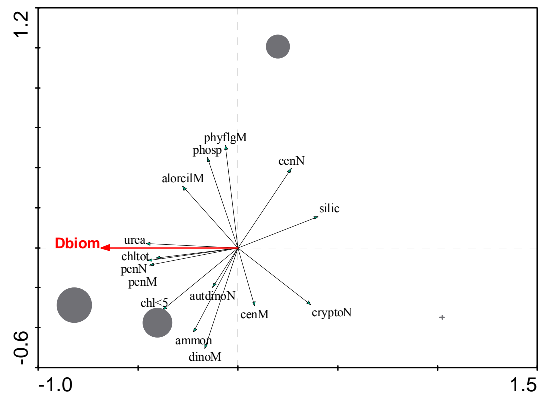

control
ammonium
nitrate
urea
silicate
μM
Si:N
Geukensia
6.45 gDW
11.61 gDW
17.65 gDW
Didemnum
19.44 gDW
29.30 gDW
38.77 gDW
Geuk+Dide
25.22 gDW
29.00 gDW
31.18 gDW
39.74 gDW
Incubation time (h)

Figure 4.15: *D. vexillum* + *M. mercenaria* response-surface mesocosm experiment in Great South Bay (40º43'N, 73º05'W, initial date 7/06/09) **(a)** RDA triplot for species biomass and/or concentration. Statistical significance: λ= 0.541, *F*-value= 5.607, p-value= 0.001 **(b)** RDA triplot for species growth rates in relation to *Mercenaria*Didemnum* **(c)** RDA triplot for species growth rates in relation to *Didemnum* biomass **(d)** RDA triplot for species growth rates in relation to *Mercenaria* biomass. Species references in Table 4.7 legend.







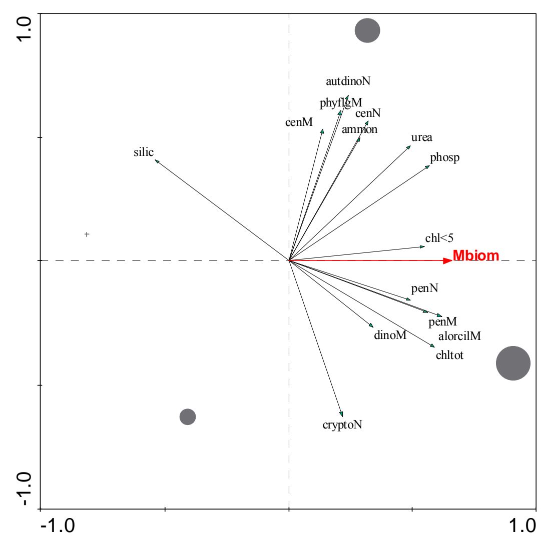
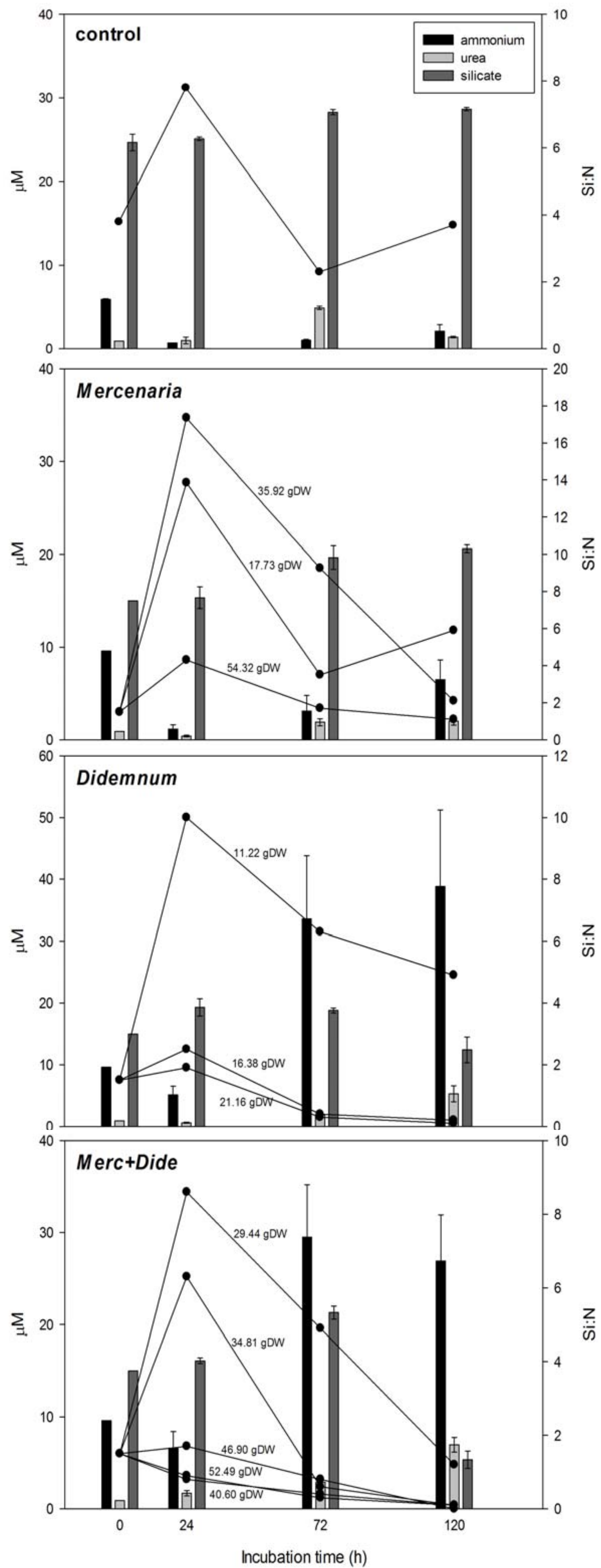


Figure 4.16: Nutrient concentrations (μM, mean ± SE) *versus* mesocosm incubation time, for response surface experiment in Great South Bay (40º43'N, 73º05'W, initial date 7/6/09). Values presented are for single control tank, and averaged for tanks with same species or combination of species of suspension feeder. Second-y axis, Si:N molar ratio *versus* mesocosm incubation time (2-way ANOVA, $p<0.001$); total biomass of suspension feeder expressed in gDW.


control
ammonium
urea
silicate
µM
Si:N
Mercenaria
35.92 gDW
17.73 gDW
54.32 gDW
Didemnum
11.22 gDW
16.38 gDW
21.16 gDW
Merc+Dide
29.44 gDW
34.81 gDW
46.90 gDW
52.49 gDW
40.60 gDW
0
24
72
120
Incubation time (h)

CHAPTER 5

Residual ecological effects of suspension-feeding benthic macroinvertebrates on planktonic growth and grazing rates in a coastal lagoonal estuary

ABSTRACT

The residual effects of high densities of benthic macroinvertebrates on the growth rates of phytoplankton and microheterotrophs, and the grazing rates of the latter, were explored with dilution and grow-out experiments. Experiments started at the end of enclosure incubations of natural plankton from the Great South Bay, NY with increased benthic suspension feeder densities (bivalves and a colonial ascidian). Benthic suspension feeders had a significant effect on phytoplankton growth rates. There were two major (diverging) trends observed: in some outcomes, phytoplankton growth rates decreased under the influence of suspension feeders (putatively the effect of intense benthic grazing), while in others benthic suspension feeders promoted an increased growth rate of the phytoplankton community (putatively compensatory growth enhance by benthos-mediated nutrient regeneration). Bivalve treatments lowered the grazing rates of microheterotrophs in relation to the control. There were significant effects of suspension feeders on population growth rates of heterotrophs (e.g. positive effects of benthic suspension feeders and the growth rates of microplanktonic heterotrophic dinoflagellates).

INTRODUCTION

The study of ecological benthic-pelagic coupling has traditionally focused on trophic interactions. Benthic suspension-feeding organisms, such as bivalve molluscs, are known for the positive effects that they provide to coastal ecosystems (reviewed by Dame 1996; Prins et al., 1998; Newell et al., 2002; Newell, 2004; Lonsdale et al., 2009). A number of studies comparing coastal systems that have experienced dramatic changes in abundances of suspension-feeding benthos (either through introductions or eliminations) have linked them to shifts in pelagic structure and function of the whole system (Newell, 1988; Caraco et al., 1997; Jackson et al., 2001; Cerrato et al., 2004; Pomeroy et al., 2006; Mann et al., 2009).

Most studies of trophic interactions induced by increased benthic-pelagic coupling have examined changes in pelagic structure (e.g. standing stock, community

composition), while few have looked into changes in planktonic ecological rates (e.g. community growth, grazing). The latter is well known to happen in pelagic systems. For example, preliminary evidence suggests that reductions in phytoplankton standing stocks are associated with high mortality rates of estuarine ciliates (Dolan and McKeon, 2005). Similarly, planktonic microheterotrophs grow at faster rates with higher food concentrations (Landry et al., 1995; Calbet and Landry, 2004).

Several examples of the direct and cascading effects of changes in phytoplankton biomass can be drawn from extensive limnological research in which the trophic structure of temperate lakes is determined by relatively linear top-down or bottom-up interactions (Dettmers and Wahl, 1999; and references therein). A simple food chain model consisting of piscivorous fish controlling the abundance of planktivorous fish, and these controlling zooplankton which in turn grazes on phytoplankton, has been studied under natural conditions or experimentally manipulating the densities of either component(s). Bertolo et al. (1999) worked with experimental mesocosms in which the abundance of planktivorous fish, zooplankton and phytoplankton were manipulated to assess the cascading effects of fish on phytoplankton standing crop. They found that fish enhanced phytoplankton biomass, and the mechanism involved release of zooplankton grazing pressure. Dettmers and Wahl (1999) experimentally manipulated the density of a pelagic predator with an intermediate trophic position (the onmivorous larvae of gizzard shad, *Dorosoma cepedianum*) in lake enclosures, to evaluate differential predation impacts on zooplankton. The omnivorous predator targeted and exerted strong predatory impacts on crustacean zooplankton, in turn facilitating an increase in rotifer populations. Due to the latter, there were no overall differences in total zooplankton biomass, but there was a shift toward a smaller-sized zooplankton assemblage of a lesser food quality. Dorazio et al. (1987) studied the effects of stocking a lake with piscivorous salmonids. One outcome of their experiment yielded higher densities of cladocerans and lower chlorophyll for summer months; linking this to phytoplankton growth rates and zooplankton grazing rates, they concluded that herbivorous zooplankton controlled algal biomass during the summer.

With a few exceptions (e.g. Heath et al., 1995; Caraco et al., 1997), to date there are virtually no studies that looked into indirect impacts of direct benthic grazing on

production rates in the coupled planktonic system. Therefore, the goal of the present study was to explore the hypothesis that benthic suspension feeding not only has the potential to influence plankton community biomass and compositional structure, but also primary and secondary productivity. This study specifically looked into the residual effects of high densities of benthic macroinvertebrates on the growth rates of phytoplankton and microheterotrophs, and the grazing rates of the latter. The effects of seasonality (variations in chlorophyll standing stocks, community composition, temperature), and different top-down (increased water turnover and grazing), and bottom-up effects (excretion) from benthic macroinvertebrates were investigated in a series of coupled-experimental enclosure incubations (Chapter 4).

MATERIALS AND METHODS

Mesocosm experiments (***modulating structure and function of plankton communities***)

From October 2007 to July 2009 a series of field mesocosm experiments (400 l, 72- to 120-h duration) were carried out, experimentally manipulating densities of benthic grazers (the hard clam *Mercenaria mercenaria*, the ribbed mussel *Geukensia demissa*, and the invasive colonial ascidian *Didemnum vexillum*) in single- or two-species assemblages, in order to study their effects on natural plankton assemblages from Great South Bay, NY (Chapter 4).

At the end of each of these mesocosm experiments (i.e. after the natural plankton community had been influenced by the activity of benthic suspension-feeding metazoans), seawater from experimental tanks was used in phytoplankton growth rate and microzooplankton community grazing (dilution) experiments, and microheterotroph population growth rate (‘grow-out’) experiments. Comparisons between control and macrobenthos-influenced treatments provide insight into how plankton trophic dynamics are modified in the presence of macrofauna.

Phytoplankton growth and microheterotroph grazing

To determine residual or lasting effects of benthic macroinvertebrates on primary productivity, phytoplankton growth rates and microheterotroph grazing rates were

estimated using the refined dilution technique (Landry et al., 1995). This method relies on dilution of herbivorous heterotrophic plankton with filtered seawater to create a gradient of phytoplankton mortality. Phytoplankton gross growth and mortality rates are determined from changes in net phytoplankton growth rate along the dilution gradient.

Benthos-influenced seawater from replicate (n= 3-4) mesocosm tanks (details listed on Table 5.2 and Chapter 4) was pooled into carboys (~20 l each, in triplicate). From this batch of pooled water, a diluent filtrate was prepared by direct gravity flow of seawater through sequential 3.0, 0.45 and 0.2 μm in-line cartridge filters (Pall Corp.). All experimental containers, silicone tubing, and cartridge filters were soaked in 10% HCl to remove dissolved organics and rinsed with distilled water prior to use. All work was performed with minimal bubbling.

The dilution series consisted of 1200 ml PAR-transparent polycarbonate bottles with 25, 50, 75 and 100% unfiltered seawater, each in triplicate. All dilution bottles were enriched with 10 μM ammonium, and 1 μM phosphate for nutrient replete growth of autotrophs, and compensation of microzooplankton excretion in the different dilutions. An additional set of whole seawater bottles with no nutrient additions were incubated in triplicate along with the nutrient-amended series, to assess the impact of nutrient addition. A control nutrient-enriched bottle of 0.2-μm filtered seawater was also incubated; chlorophyll concentrations in the diluent control bottles were near the limit of analytical detection (both with acetone extraction and *in vivo* readings performed with a handheld portable fluorometer; Turner Designs, model *Aqua*fluor™), and never showed measurable increments during any of the experiments. Experimental bottles were incubated *in situ* for ~24 h, fastened to a plexiglass board floating in mid-water, at a depth of ~30 cm below the water surface (roughly a quarter of the mean depth of Great South Bay; Wilson et al., 1991). Water temperature data (Table 5.2) were obtained from a Seacat sensor (Sea Bird Electronics, SBE 16) 5 m from the experimental incubations (C.N. Flagg, SoMAS, Stony Brook University).

Samples of microplankton (n =1; 100-200 ml, acidic Lugol's iodine 5-10% preservation), nanoplankton (n =3; 50 ml, 1% formaldehyde fixation) and picoplankton (n =3; 4.5 ml, 1% formaldehyde fixation) were taken during the setup of the experiment in order to characterize the microbial grazer community at the beginning of the

experiment. At the end of the incubation period (~24 h), replicate subsamples for total chlorophyll *a* determination were taken from all experimental bottles. Net growth rates of the phytoplankton in each bottle were calculated from changes in chlorophyll *a* concentration over the length of the experiment as $\mu = (\ln C_t - \ln C_0)/t$, where t is incubation time, C_t is final and C_0 is initial bulk chlorophyll *a* concentration. Population growth rates of the phytoplankton community (k, d^{-1}) were determined as the estimated intercepts of linear regressions of the net growth rate in nutrient amended bottles (μ_n) *versus* dilution, and adjusted for nutrient addition (μ_0); this is equivalent to the theoretical growth rate in the absence of predators (Landry et al., 1995). Microheterotroph grazing coefficients (g, d^{-1}) were calculated as the negative slopes of the regressions.

With the growth and grazing coefficients, two parameters, the chlorophyll *a* biomass removed daily (P_i, % d^{-1})

$$P_i = \frac{(C_0 - C_0 e^{g})}{C_0} \times 100$$

and the chlorophyll *a* production grazed per day (P_p, % d^{-1})

$$P_p = \frac{(C_0 e^{k} - C_0) - (C_0 e^{(k-g)} - C_0)}{(C_0 e^{k} - C_0)} \times 100$$

were calculated, after Verity et al. (2002).

Planktonic heterotroph production experiments

Additional ‘grow-out’ incubations were conducted in order to determine residual or lasting effects of benthic macroinvertebrates on planktonic nano- (<20 μm) and microheterotrophs (20-64 μm). It has been proposed (Lonsdale et al., 2009) elevated bivalve predation rates could have a substantial influence on the net population growth rate of some microplanktonic heterotrophs.

Seawater from control or benthos-influenced mesocosms, was pre-filtered using gravity filtration through 64-μm and 20-μm Nitex mesh, to exclude larger planktonic predators while minimizing damage to protists. Each batch of filtered water was then transferred to 1200 ml polycarbonate bottles (3 replicates from each batch), and incubated *in situ* alongside the dilution experiment bottles. Initial and final-time samples (n =3; 1 from each bottle) were taken for the enumeration of microzooplankton (100-200

ml, acidic Lugol's iodine 5-10% preservation) and nanoplankton (50 ml, 1% formaldehyde fixation) from the 64-µm and 20 µm-filtered bottles, respectively. Taxa-specific net growth rates (r, d^{-1}) were estimated for micro- (>20-64 µm) and nanoplanktonic (<20 µm) heterotrophs as $r = (\ln C_t - \ln C_0)/t$, where C_t and C_0 are final and initial biomass concentration. For data consolidation, the biomass of nano- and microheterotrophs was grouped into the functional/taxonomic groups of: flagellates (mostly cryptophytes, euglenophytes and ebriids), dinoflagellates, and ciliates (loricate and aloricate oligotrichs) for the calculations of r.

Sample processing

***Chlorophyll* a**. Bulk chlorophyll *a* was measured from duplicate 30 ml water samples, concentrated onto Whatman GF/F filters, extracted in 90-100% acetone for at least 24 h at -20ºC in the dark, and measured fluorometrically (Turner Designs, model 10-AU), after Arar and Collins (1997).

Dissolved nutrients. Seawater samples (n =1) filtered through GF/F filters (0.7 µm) were taken during experimental setup from batches of water coming from pooling 3-4 tanks or from individual tanks, in order to measure dissolved nutrients. Nutrients were analyzed colorimetrically after Jones (1984), Parsons et al. (1984) and Price and Harrison (1987). Molar concentrations of total N were calculated considering inorganic and organic forms of nitrogen.

Microplankton. Microplankton samples were processed by settling 50 ml for ~24 h in a graduated cylinder, followed by removal of 40 to 45 ml of the overlying water. A minimum of 100 microorganisms (20-64 µm) were then counted in a Zeiss compound microscope by resettling 1 ml aliquots in a Sedgewick-Rafter counting chamber (LeGresley and McDermott, 2010). Standard measurements of cell linear dimensions were performed for biovolume estimations (Sun and Liu, 2003). Conversion factors published in Menden-Deuer and Lessard (2000), Putt and Stoecker (1989), and Verity and Langdon (1984) were applied to estimate biomass. Taxa were identified to the lowest possible taxonomic level (Maeda and Carey, 1985; Maeda, 1986; Tomas, 1997; Taylor et al., 2003). Only mixo- or heterotrophic representatives of cryptophytes, ebriids, euglenoids, dinoflagellates, and loricate and aloricate (oligotrich) ciliates were considered

in the counts. The high concentration of Lugol's preservative used to minimize losses of ciliates and the storage of samples for extended periods before examination precluded the distinction of phototrophs from heterotrophs (Dennett et al., 2001), thus mixo- and heterotrophic organisms were identified as such, on the basis of literature reports of heterotrophic nutrition (Lonsdale et al., 2006; Table 5.1).

Nanoplankton. Seawater samples (50 ml) preserved in 1% formaldehyde (from a 10% stock solution prepared with filtered natural seawater) were kept refrigerated until collected (within 24 h) onto polycarbonate filters (0.8 µm black, 25 mm diameter), and mounted into glass slides using Vectashield mounting medium with DAPI (1.5 µg ml^{-1}; Vector Laboratories Inc., Burlingame, CA). Volumes filtered ranged from 5 to 20 ml, according to the seasonal concentration of organisms. Nanoplankton slides were stored at -20°C until enumeration (Sherr and Sherr, 1993). Cell counts were performed with a Zeiss Axioskop fluorescence microscope equipped with a HBO 50/AC mercury lamp and three wavelength filter sets (UV, blue and green excitation). A minimum of 50 organisms comprising heterotrophic dinoflagellates, heterotrophic nanoflagellates and oligotrichous ciliates were enumerated at 1000× magnification, using a Zeiss 100× Plan-Neofluar oil objective. Standard measurements of cell linear dimensions were performed for biovolume estimations (Sun and Liu, 2003). Conversion factors published in Børsheim and Bratbak (1987), Menden-Deuer and Lessard (2000), and Putt and Stoecker (1989) were applied to estimate biomass.

Heterotrophic bacteria. Water samples (4.5 ml) preserved in 1% formaldehyde (from a 10% stock solution prepared with filtered natural seawater) were flash-frozen upon return to the lab, and stored at -80°C. Counts of heterotrophic bacteria were obtained by flow cytometry (Becton-Dickinson, model FacsCalibur; Olson et al., 1993). Sample aliquots (0.5-2 ml) were run twice, before and after staining with SYBR green I dye (Sigma-Aldrich). Absolute counts were obtained using a known concentration of fluorescent beads (Spherotech Inc., rainbow fluorescent particles, 1.93 µm diameter). Data was analyzed with the WinMDI 2.9 software package. A conversion factor of 20 fg C $cell^{-1}$ was used for biomass estimations (Lee and Fuhrman, 1987).

Data analysis

For each experiment, the slopes (*g*) of the regressions fitting the observed net growth *versus* dilution factor were analyzed with a test for homogeneity of slopes. If non-significantly different then they were pooled and tested for differences in the adjusted means using an analysis of co-variance (ANCOVA). If the ANCOVA was significant, *post-hoc* pairwise multiple comparisons were performed with a Tukey HSD test (Zar, 1999). If the slopes were significantly different, then a Tukey HSD multiple comparisons test of the slopes was run, followed by a Tukey multiple comparisons test of intercepts (Table 5.3).

A Kruskall-Wallis ANOVA by ranks was performed on the μ_0/μ_n ratio to test for differences in nutrient limitation among treatments, and followed by a multiple comparisons test. Differences in averaged molar concentrations of total N, P and Si for the different treatments were analyzed by means of a one-way ANOVA, followed by a *post hoc* HSD test with unequal n (Figure 5.1).

The estimated parameters *k*, *g*, (*k*-*g*), P_i and P_p were plotted *versus* initial chlorophyll (C_0) and/or incubation temperature. These relationships were analyzed by ANCOVA as described above. Verity et al. (2002) suggested that the relationship between phytoplankton growth and temperature fits an exponential model. Before running a homogeneity of slopes test, linear and exponential models were fitted to the datasets of *k* and *g versus* temperature. Non-linear and linear models virtually had the same fit (r^2) for all cases, thus the linear regression model was followed.

The relationship between phytoplankton growth rate (*k*) and microheterotroph grazing rate (*g*) is presented as a regressions for the different treatments (Figure 5.8). Due to the fact that there is no causal relationship among each of these parameters (i.e. it cannot be assumed that either variable is dependent upon the other), a geometric mean regression was run to estimate the slope, intercept and approximate standard error of the relationship between *k* and *g* (Ricker, 1972). Then, a Tukey HSD multiple comparisons test was used to compare differences between pairs of these parameters (Sokal and Rohlf, 1995).

Redundancy analysis (RDA) was performed to examine relationships between taxa-specific net growth rates (*r*) of nano- and microheterotrophs and suspension feeder

density (terBraak, 1986). RDA was carried out using Canoco 4.5 (Microcomputer Power, Ithaca, NY).

RESULTS

Phytoplankton growth rates, and microheterotroph grazing rates are presented in Table 5.2. Although there is considerable variation (probably related to factors such as phytoplankton community composition), phytoplankton growth and microheterotroph grazing rates were comparable between experiments that started after 72- (first three dates) and 120-h incubations of seawater with benthic suspension feeders.

Of the eleven dilution experiments performed, six showed significant differences in *g* among treatments (Table 5.2). All but one (i.e. 9/23/08) experiment showed significant differences in *k* among treatments. There is some indication that benthic suspension feeders affected phytoplankton community growth rates almost invariably, while microheterotroph grazing was not always affected.

Post-hoc multiple comparisons showed lower microheterotroph grazing rates in treatments with elevated bivalve filtration in three (i.e. 6/13/08, 9/23/08 and 10/6/08) out of the six significantly different experiments. On the experiment performed on 6/27/09, the combination of 2 suspension feeders (*Mercenaria* and *Didemnum*) also yielded significantly lower *g*. On the other hand, in 2 experiments (7/26/08 and 7/4/09), suspension feeder treatments produced a higher *g* than the control.

Post-hoc multiple comparisons indicate that on four out of the eleven dates (i.e. 11/3/07, 7/26/08, 6/20/09 and 7/4/09) phytoplankton community growth rates (*k*) were significantly lower in experiments using water from control tanks, compared to water influenced by macrobenthos. For two dates (5/2/08 and 8/8/08), phytoplankton growth rates were significantly higher in experiments using control water compared to *k* calculated for experiments using water influenced by the bivalve *Geukensia*. The combination of two bivalve species (*Geukensia* and *Mercenaria*; dates 9/23/08 and 10/6/08), and of the bivalve *Mercenaria* and the ascidian *Didemnum* (dates 6/27/09 and 7/11/09), did not promote significant changes in phytoplankton growth rates in relation to

control water. However, the combination of ribbed mussels and the ascidian (dates 6/20 and 7/4/09) yielded significantly higher phytoplankton growth rates than control water.

Grazing impacts due to microheterotrophs, quantified as chlorophyll biomass removed daily (P_i) and chlorophyll production grazed d^{-1} (P_p) were substantial, averaging 131% d^{-1}, and 116% d^{-1} respectively, for all dilution experiments (Table 5.2). However, grazing losses were not correlated to the biomass of nano- and microheterotrophs and heterotrophic bacteria (data not shown). Biomass estimates of standing stocks of pico- nano- and microheterotrophs (µg C l^{-1}) at initial times are presented in Table 5.4. Excluding a late summer peak (1147 ± 234 µg C l^{-1}, for 8/8/08), the abundance of heterotrophic bacteria and protozoan nano- and microheterotrophs ranged between 137-634 µg C l^{-1}. In general, the lowest abundances of heterotrophs (< ~200 µg C l^{-1}) were present for mid-summer dates (6/13/08, 7/26/08, 7/4/09 and 7/11/09). The most abundant and recurrent microheterotrophs found in grow-out experiments were the flagellate *Cryptomonas*, the dinoflagellates *Prorocentrum micans*, *Heterocapsa* sp., *Gyrodinium* cf. *spirale*, *Gyrodinium dominans*, *Karlodinium veneficum*; and the ciliates *Tintinnopsis* sp., *Favella* sp., *Strombidium* sp., *Strobilidium* sp., *Balanion* sp., and *Laboea* sp.

Averaged molar concentrations of total N, P and Si for t_0 for different treatments are shown in Figure 5.1. Significant differences among treatments were found for total N (1-way ANOVA $F(5,98) = 11.54$, $p << 0.001$), total P (1-way ANOVA $F(5,98) = 4.08$, $p = 0.002$), and total Si (1-way ANOVA $F(5,98) = 5.42$, $p < 0.001$). *Post-hoc* multiple comparisons showed significant differences ($p<0.05$) in total N for the *Didemnum* and *Didemnum*+bivalve treatments, with respect to controls and individual bivalve treatments. Multiple comparisons showed significant differences ($p<0.05$) in total P for the 2-bivalve treatment, with respect to control, *Geukensia* and the *Didemnum*+bivalve treatments. There were significant differences ($p<0.01$) in total Si, for the 2-bivalve treatment with respect to those treatments including *Didemnum*.

Despite daily nitrogen amendments in experimental mesocosms, the μ_0/μ_n ratio evidenced nutrient limitation in all but the *Didemnum* treatments. Differences in μ_0/μ_n were significant among *Didemnum* and control, *Geukensia* and *Mercenaria* treatments (Kruskall-Wallis ANOVA, $p<0.05$).

Phytoplankton growth rate (*k*) and the initial chlorophyll standing stock (C_0) mostly had a positive relationship (Figure 5.2), with significant differences among treatments (homogeneity of slopes test $F(4,110) = 2.80$, $p = 0.03$). *Post hoc* multiple comparisons of slopes evidenced a significantly higher ($p<0.05$) *k* for the *Didemnum* treatment, and a significant negative relationship for the *Geukensia* treatment. There was a positive relationship of phytoplankton growth rate with temperature (Figure 5.3; regression $p<<0.001$), but non-significant treatment effects with temperature (homogeneity of slopes test $F(4,110) = 2.27$, $p = 0.07$). Differences in the regressions of each treatment were shown with an ANCOVA test of intercepts ($F(4,110) = 5.28$, $p < 0.001$); *Didemnum* had significantly higher *k* compared to the other treatments (Tukey HSD test, $p<0.05$).

There was a significant positive relationship between microheterotroph grazing (*g*) and initial phytoplankton standing stock (C_0), (regression $p<<0.001$, Figure 5.4). An ANCOVA test of intercepts ($F(4,110) = 2.63$, $p = 0.04$) evidenced significant differences among treatments, but pairwise differences could not be identified with the *post hoc* multiple comparison test (Tukey HSD multiple comparisons of adjusted means). The positive relationship between grazing and temperature, showed significant differences among treatments (homogeneity of slopes test $F(4,110) = 3.03$, $p = 0.02$; Figure 5.5), but again, the *post hoc* test could not distinguish between treatments.

There were significant differences among treatments (homogeneity of slopes test $F(4,110) = 3.58$, $p = 0.01$), in the relationship between *k*-*g* (i.e. the difference between phytoplankton growth and grazing rates) and initial algal biomass (C_0; Figure 5.6). The *Didemnum*+bivalve and *Geukensia* treatments were significantly different from each other (Tukey HSD test of slopes, $p<0.05$), which is probably explained by the relationship of *k* vs. C_0 described above (Figure 5.2). Pooled slopes of *k*-*g* did not evidence any significant relationship with temperature (ANCOVA test of intercepts $F(1,105) = 1.09$, $p = 0.29$; Figure 5.7), but there were temperature-independent treatment effects (ANCOVA test of intercepts $F(4,105) = 9.76$, $p<<0.001$). *Didemnum* and *Didemnum*+bivalve treatments had a higher *k*-*g* than the control; and *Geukensia* had lower *k*-*g* than *Didemnum*, but *Mercenaria* did not (Tukey HSD test of intercepts, $p<0.05$).

Phytoplankton growth rate (*k*) was positively related to microheterotroph grazing rate (*g*). As mentioned before, both parameters were measured with error, so geometric mean regressions for the different treatments were run (Figure 5.8). A Tukey HSD multiple comparisons test evidenced significant differences ($p<0.05$) in the slopes of the *Geukensia* and *Didemnum* treatments with respect to the control.

There was a significantly positive relationship between chl *a* biomass removed per day (P_i) and temperature (regression $p<<0.001$; Figure 5.9), but no treatment effects were detected (ANCOVA test of intercepts $F(4,110) = 1.93$, $p = 0.11$). Conversely, there was no apparent relationship between primary production grazed per day (P_p) and temperature [homogeneity of slopes test $F(4,110) = 1.39$, $p = 0.24$], and no treatment effects either [ANCOVA test of intercepts $F(4,110) = 0.97$, $p = 0.43$] (Figure 5.10).

The relationships between taxa-specific net growth rates (*r*) of nano- and microheterotrophs and suspension feeder density were analyzed by redundancy analysis (RDA). RDA graphical outcomes (triplots), statistical significance (*F*-ratio and p-value) and fraction of variance explained by independent variables (λ) are presented in Figures 5.11-5.15; results are summarized in Table 5.5.

Five 'grow-out' experiments out of the eleven performed, showed significant relationships between net growth rates of planktonic heterotrophs and suspension feeder density. The ribbed mussel *Geukensia*, alone and in combination treatments with *Didemnum* had more significant influences on growth rates than any other species of suspension feeder, while *Mercenaria* had significant effects only on one date (6/27/09). Regardless of the species, the effect of suspension feeders on population growth rates of heterotrophs, was size- and taxon-specific. For example, growth rates of nanoplanktonic (<20 μm) flagellates were negatively affected by suspension feeder density, but no consistent pattern was found for flagellates in the microplanktonic (20-<64 μm) fraction. However, it needs to be argued that growth rates of microplanktonic euglenoids were also negatively affected. In general, positive effects prevailed in the relationship of benthic suspension feeders and the growth rates of microplanktonic dinoflagellates and aloricate ciliates. Conversely, no consistent pattern was evident for nanoplanktonic representatives of these, nor loricate ciliates (Table 5.5).

DISCUSSION

The response of different systems to increased grazing pressure from bivalves depends strongly on the degree to which phytoplankton compensate for increased grazing losses (Caraco et al., 1997). The present study found benthic suspension feeders to affect phytoplankton community growth rates almost invariably, having 'residual effects' that lasted after their remotion from the experimental system.

There were two major trends observed with respect to phytoplankton growth rates. On the one hand, phytoplankton growth rates decreased under the influence of suspension feeders. More specifically, on two experimental dates phytoplankton growth rates were significantly lower in *Geukensia* treatments than in the control. One possible explanation of this, might be directly related to increased grazing pressure on the phytoplankton community. For example, the outcome of a model by Caraco et al. (1997) reports a 5-fold decrease in phytoplankton productivity in certain areas of the Hudson River which experienced substantial increases of the exotic benthic bivalve *Dreissena polymorpha*. They attributed this to the elevated grazing rates, shifts in phytoplankton community composition, and the inability of phytoplankton community growth rates to cope with the intensive grazing pressure exerted by the invasive bivalve.

On the other cases reported in the present study, benthic suspension feeders promoted an increased growth rate of the phytoplankton community. In more than a third of the experiments (all of them involving *Geukensia*, alone or with *Didemnum*) phytoplankton community growth rates were significantly higher in treatments than in controls. A large body of literature suggests that primary producers can compensate for increases in grazing loss by increasing growth rate (e.g. Bergquist and Carpenter, 1986). Nutrient remineralization has been pointed as one of the most prominent mechanisms behind this. Phytoplankton can compensate for direct grazing losses by increasing growth rates, due to increased nutrient supply by grazers (Doering et al., 1986; Sterner, 1986). These enhanced growth rates may be large enough that, despite greatly increased grazing losses, little or no decline in phytoplankton biomass occurs and production actually increases (Bergquist and Carpenter, 1986; Doering et al., 1986; Sterner, 1986).

Heath et al. (1995) found increased phytoplankton growth rates in enclosure experiments with elevated densities of a benthic suspension feeder (the zebra mussel, *Dreissena polymorpha*), which they attributed to elevated rates of ammonium regeneration induced by the bivalve. Suspension-feeding bivalves are known to increase nutrient availability in the water column by increased rates of nutrient remineralization in the organic matter-enriched sediment adjacent to bivalve populations (Prins and Smaal, 1994), and by releasing nutrients stored in particulate form through direct excretion. The relative importance of both mechanisms is a matter of debate (Prins et al., 1998). The mesocosm incubations preceding dilution experiments did not include sediments as a habitat component, and therefore it would be reasonable to assume that the significantly higher phytoplankton growth rates found in treatments with benthic organisms are related to increased nutrient levels generated by excretion and/or regeneration. There are numerous publications that support the later. For instance, Murphy and Kremer (1985) estimated that a dense population of *Mercenaria mercenaria* contributed >50% of benthos-produced ammonium in a California lagoon, supplying the phytoplankton community with ammonium in excess. Gardner et al. (1993) contrasted net fluxes of ammonium and nitrate from sediments, and ammonium by benthic macroinvertebrates (amphipods, decapods and polychaetes), concluding that benthic macroinvertebrate excretion contributes a substantial proportion of nitrogen regeneration in an estuarine system. Similarly, Magni et al. (2000) studied nutrient regeneration in a tidal flat where the bivalves *Ruditapes philippinarum* (Veneridae) and *Musculista senhousia* (Mytilidae) made up >86% of the biomass and concluded that nutrient regeneration through diffusive flux was more than an order of magnitude lower than due to the excretory activity of bivalves, highlighting the major role of the benthos in the regeneration of nutrients made immediately available to primary producers.

However, the previous observations should be addressed with caution. It needs to be mentioned here, that despite daily nitrogen additions in mesocosm tanks, and 5-day incubations with benthos, the μ_0/μ_n ratio reported on this study evidenced nutrient limitation in the phytoplankton community (except for *Didemnum* treatments, Figure 5.1). Nutrient limitation has previously been reported for Great South Bay (Gobler et al., 2002; Gobler et al., 2004a). It should be mentioned here that there were significant

differences in dissolved nutrient concentrations among *Didemnum* treatments with respect to bivalve treatments and the control. As discussed on Chapter 4, ammonium regeneration rates in tanks with *Didemnum* were an order of magnitude higher than those reported for *Geukensia* and *Mercenaria*. The mechanism behind this might have involved direct excretion, or be the product of an experimental artifact in which the ascidian decayed and produced ammonium in excess through bacterial degradation (Osinga et al., 1999).

Microheterotroph grazing rates were not consistently affected by suspension feeders (i.e. six out of eleven experiments had significant outcomes). Microheterotroph grazing rates can be hypothesized to be indirectly influenced by the differential changes in phytoplankton growth rates discussed above. This study provided evidence that in half of the cases (6/13/08, 9/23/08, 10/6/08), bivalves lowered the grazing rates of microheterotrophs in relation to the control. Notably, these lower than control grazing rates never occurred in treatments with *Didemnum*.

Several studies have suggested that zooplankton grazers are highly selective on the basis of nutritional quality, over a range of prey items (Stoecker et al., 1986; Griffin and Rippingale, 2001; Schatz and McCauley, 2007). If selective grazing by microheterotrophs is reduced by the activity of benthic bivalves, this might in turn have significant effects on plankton community dynamics (see Griffin and Rippingale, 2001).

The apparent positive relationship between phytoplankton growth and microheterotroph grazing with temperature reported on this study is consistent with findings by Verity et al. (2002), using similar a methodology. Boissonneault-Cellineri et al. (2001) also report a tight coupling between phytoplankton growth and heterotroph grazing for other Long Island systems.

Although the evidence presented is not entirely conclusive, significant effects of suspension feeders on population growth rates of heterotrophs are reported in this study. Among the suspension feeders considered, *Geukensia* had a preponderant effect on the growth rates of heterotrophs (i.e. four out of five significant outcomes) which is not surprising, given that the ribbed mussel is known to feed on a vast array of planktonic

prey, including heterotrophic flagellates (Kreeger and Newell, 1996; also discussed in Chapter 1).

The effects of benthic suspension feeders were differential among the functional groups of nano- and microheterotrophs considered. Positive effects prevailed in the relationship of benthic suspension feeders and the growth rates of microplanktonic dinoflagellates. An explanation for that might lie in the release of benthic predation pressure over dinoflagellates. There is some evidence that dinoflagellates are of a lesser nutritional quality than other planktonic prey (Hitchcock, 1982); therefore, in the presence of other prey items of varying food quality, suspension feeders might have released dinoflagellates from grazing pressure, what is ultimately reflected in higher net growth rates than controls.

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Table 5.1: List of microplanktonic (>20-64 μm) heterotrophic taxa, with published references to heterotrophic or mixotrophic nutrition (prey and mechanisms), considered in estimations of microheterotroph growth rates (r, d^{-1}).

taxon	heterotrophic nutrition (prey species)	references
PHYTOFLAGELLATES		
Chroomonas	glycerol (photo- and chemoheterotroph)	Antia et al. (1973)
Cryptomonas	bacteria, HNFs [freshwater]	Tranvik et al. (1989); Urabe et al. (2000)
Chattonella ovata	bacteria (FLBs)	Seong et al. (2006)
Hermesium adriaticum		Hoppenrath and Leander (2006)
DINOFLAGELLATES		
Gyrodinium cf. *spirale* (naked)	diatoms, dinoflagellates	Hansen (1991); Hansen (1992) Tiselius and Kuylenstierna (1996), Kim and Jeong (2004)
Gyrodinium dominans (naked)	*Isochrysis, Chatonella*, *Rhodomonas*, dinoflagellates, diatoms, cryptophytes	Hansen (1991); Nakamura et al. (1992); Pedersen and Hansen (2003), Kim and Jeong (2004)
Karlodinium veneficum (naked)	cryptophytes	Adolf et al. (2007)
Gyrodinium galatheanum (naked)	prymnesio-, crypto- and chlorophytes	Li et al. (2000)
Amphidinium sp. (naked)	bacteria (FLBs), ingestion mechanism: peduncle	Strom and Morello (1998)
Cochlodinium polykrikoides (naked)	ciliates (?)	Seong et al. (2006)
Akashiwo sanguinea (naked)	dinoflagellates, diatoms	
Polykrikos sp. (naked)		Jeong et al. (2001); Johnson et al. (2003)
Prorocentrum minimum (thecate)	bacteria (FLBs), *Cryptomonas*	Seong et al. (2006); Li et al. (1996)
Prorocentrum micans (thecate)	unidentified encysted cell	Jacobson and Anderson (1996)
Heterocapsa rotundata (thecate)	bacteria (FLBs)	Seong et al. (2006)
Scrippsiella sp. (thecate)	food vacuoles w/ extrusome-like bodies (??)	Jacobson and Anderson (1996)
Gonyaulax sp. (thecate)	ciliates (?)	Jacobson and Anderson (1996)
Protoperidinium sp. (thecate)	ciliates, diatoms, dinoflagellates; ingestion mechanism: pallium	Hansen (1991); Jeong and Latz (1994); Buskey (1997)
Diplopsalis sp. (thecate)	>20 um dinoflagellates; ingestion mechanism: pallium	Hansen (1991)
Dinophysis sp. (thecate)	ciliates (*Tiarina*); ingestion mechanism: peduncle	Hansen (1991)
Alexandrium sp. (thecate)	dinoflagellates	Jacobson and Anderson (1996)
LORICATE CILIATES		
Codonella		
Favella		
Helicostomella		
Tintinnopsis		
NAKED CILIATES		
Strombidium		
Strobilidium		
Mesodinium rubrum	mixotrophic	Crawford (1989); Stoecker et al. (1994)
Leegardiella		
Lohmaniella		
Balanion	*Rhodomonas*	Jakobsen and Hansen (1997)
Didinium		
Laboea		
Halteria		
Tontonia		
Scuticociliates		
Euplotes		

Table 5.2: 24-h dilution experiments from Great South Bay, following mesocosm incubations of seawater with benthic suspension feeders. C_0 is the initial standing stock [mean (SE) μg chl *a* l^{-1}] at the start of dilution experiments. Microheterotroph grazing coefficients [*g* (SE) d^{-1}] were calculated from linear regressions of the nutrient-enriched dilution series (along with p-values and r^2 for each regression). Phytoplankton population growth rate [*k* (SE) d^{-1}] were calculated as the intercepts of the regressions. μ_0/μ_n is the ratio of phytoplankton growth rate in unenriched (μ_0) and enriched (with nitrate and phosphate) bottles (μ_n). P_i (% d^{-1}) is the chl *a* biomass removed daily. P_p (% d^{-1}) is the chl *a* production grazed daily. Calculations for P_i and P_p explained in main text.

date	mesocosm incubation (h)	benthic suspension feeder	density (n tank^{-1})	temp. °C (mean/ range)	C_0 (μg l^{-1})		p regression	r^2	g (d^{-1})		k (d^{-1})		μ_0/μ_n	k-g	P_i (% d^{-1})	P_p (% d^{-1})
11/3/07	72		control	11.77	5.50	(0.11)	*	0.47	0.37	(0.13)	0.31	(0.09)	-7.22	-0.06	44.15	115.14
		Mercenaria	8	12.46 - 10.93	4.08	(0.05)	**	0.52	0.41	(0.12)	0.38	(0.08)	-0.50	-0.02	50.07	104.57
		Mercenaria	16		3.32	(0.09)	*	0.00	0.02	(0.11)	0.33	(0.07)	0.75	0.31	2.04	7.08
5/2/08	72		control	14.02	31.31	(0.39)	***	0.93	0.82	(0.07)	0.60	(0.05)	1.29	-0.23	127.22	124.75
		Geukensia	9	14.54 - 13.55	27.42	(1.12)	***	0.80	0.62	(0.1)	0.52	(0.07)	-0.79	-0.09	85.44	112.86
		Geukensia	18		28.03	(0.48)	***	0.77	0.58	(0.11)	0.39	(0.08)	4.60	-0.19	78.84	137.20
6/13/08	72		control	24.81	46.82	(3.97)	***	0.96	1.57	(0.1)	1.08	(0.07)	6.11	-0.49	381.95	119.84
		Mercenaria	10	25.92 - 24	48.53	(0.13)	***	0.84	1.12	(0.16)	0.66	(0.11)	-6.07	-0.46	205.81	139.38
		Mercenaria	20		28.03	(0.48)	***	0.96	1.09	(0.07)	1.55	(0.05)	0.83	0.45	198.87	84.54
7/26/08	120		control	26.13	36.79	(0.68)	***	0.92	0.94	(0.09)	0.36	(0.06)	4.11	-0.58	155.32	200.38
		Geukensia	18	27.23 - 25.09	23.10	(0.36)	***	0.96	1.46	(0.09)	1.20	(0.06)	-0.60	-0.26	331.90	109.91
		Geukensia	36		10.81	(0.45)	***	0.90	1.29	(0.13)	1.76	(0.09)	0.55	0.46	264.53	87.72
8/8/08	120		control	26.13	18.52	(2.2)	***	0.92	0.92	(0.09)	1.04	(0.06)	0.13	0.12	151.70	93.20
		Geukensia	18	26.79 - 25.36	15.07	(0.42)	***	0.73	0.78	(0.15)	0.34	(0.1)	-1.07	-0.44	118.58	185.96
		Geukensia	36		9.88	(1.43)	***	0.92	0.92	(0.09)	0.92	(0.06)	0.07	0.00	150.74	99.95
9/23/08	120		control	20.02	17.01	(1.54)	***	0.85	1.88	(0.24)	1.51	(0.17)	0.12	-0.36	552.54	108.62
		Geukensia	24	21.01 - 19.29	14.51	(0.11)	***	0.91	0.49	(0.05)	0.52	(0.03)	0.22	0.02	63.45	96.33
		Mercenaria	20		15.17	(1.75)	***	0.85	0.57	(0.08)	0.68	(0.05)	0.65	0.11	76.41	87.66
		Geu+Merc	24+20		15.39	(0.31)	***	0.68	0.37	(0.08)	0.11	(0.05)	-1.53	-0.25	44.12	282.64
10/6/08	120		control	16.55	37.10	(1.21)	***	0.73	0.95	(0.18)	0.73	(0.12)	0.14	-0.22	157.59	118.02
		Geukensia	12	17.38 - 15.78	20.92	(1.48)	**	0.62	0.53	(0.13)	0.24	(0.09)	2.71	-0.30	70.44	196.56
		Mercenaria	20		20.77	(0.84)	*	0.38	0.36	(0.14)	0.48	(0.1)	-7.42	0.13	42.86	78.46
		Geu+Merc	12+20		19.86	(0.49)	ns	0.04	0.08	(0.12)	0.03	(0.08)	2.28	-0.05	7.87	276.43
6/20/09	120		control	20.26	17.73	(0.54)	***	0.69	0.40	(0.08)	0.67	(0.06)	1.41	0.27	48.83	67.28
		Geukensia	24	20.76 - 19.81	10.56	(0.62)	***	0.82	0.33	(0.05)	0.86	(0.03)	1.02	0.53	38.62	48.26
		Didemnum	694 g		24.81	(1.02)	**	0.53	0.47	(0.14)	1.11	(0.1)	1.67	0.64	60.72	56.27
		Geu+Didem	24+700g		39.74	(1.09)	*	0.49	0.31	(0.1)	0.89	(0.07)	0.88	0.59	35.98	44.74
6/27/09	120		control	21.59	9.66	(0.09)	***	0.76	0.69	(0.12)	0.81	(0.08)	0.38	0.12	99.44	89.89
		Mercenaria	20		7.79	(0.27)	***	0.82	0.93	(0.14)	1.25	(0.1)	0.64	0.32	153.70	85.00
		Didemnum	280 g	22.28 - 21.05	20.69	(0.31)	***	0.87	0.93	(0.12)	1.89	(0.08)	1.24	0.96	153.81	71.41
		Merc+Didem	20+270g		16.75	(0.49)	***	0.73	0.52	(0.1)	0.83	(0.07)	0.61	0.31	68.70	72.07
7/4/09	120		control	25.12	8.14	(0.91)	**	0.60	0.29	(0.07)	-0.21	(0.05)	-1.98	-0.50	33.44	-106.01
		Geukensia	24		11.26	(0.65)	***	0.79	0.74	(0.12)	1.11	(0.08)	0.72	0.37	109.94	78.08
		Didemnum	366 g	26.2 - 24.25	53.20	(2.14)	***	0.79	1.41	(0.23)	1.99	(0.16)	1.32	0.57	311.44	87.74
		Geu+Didem	24+339g		55.84	(3.39)	***	0.96	0.78	(0.05)	1.32	(0.04)	0.95	0.54	118.10	74.00
7/11/09	120		control	23.11	15.38	(1.45)	***	0.78	0.64	(0.11)	0.12	(0.07)	-2.04	-0.52	89.09	426.07
		Mercenaria	10		13.35	(2.72)	***	0.80	0.82	(0.13)	0.35	(0.09)	-2.57	-0.47	126.66	189.80
		Didemnum	150 g		39.59	(2.84)	***	0.85	0.99	(0.13)	0.52	(0.09)	1.83	-0.47	168.05	154.78
		Mer+Didem	10+150g	24.1 - 22.43	30.33	(3)	***	0.87	0.84	(0.1)	0.75	(0.07)	-0.14	-0.09	131.11	107.55

* $p<0.05$
** $p<0.01$
*** $p<0.001$

Table 5.3: Statistical test results, comparing phytoplankton growth (*k*, intercepts) and microheterotroph grazing (*g*, slopes) from regressions presented in Table 5.2.

date		homogeneity of slopes *F*	df	p	*post-hoc* multiple comparison slopes Tukey HSD test p<0.05	ANCOVA test of intercepts (pooled slopes) *F*	df	p	*post-hoc* multiple comparison intercepts Tukey HSD test p<0.05	
11/3/07	cont	3.24	2,29	0.05		40.3	2,31	**<0.001**	***a***	cont
	M8								***b***	M8
	M16								***c***	M16
5/2/08	cont	1.88	2,28	0.17		13.83	2,30	**<0.001**	***a***	cont
	G9								***b***	G9
	G18								***a***	G18
6/13/08	cont	5.64	2,30	**0.01**	***a***				***a***	cont
	M10				***b***				***b***	M10
	M20				***b***				***a***	M20
7/26/08	cont	6.44	2,30	**0.005**	***a***				***a***	cont
	G18				***b***				***b***	G18
	G36				***ab***				***c***	G36
8/8/08	cont	0.52	2,30	0.6		52.46	2,32	**<0.001**	***a***	cont
	G18								***b***	G18
	G36								***c***	G36
9/23/08					***a***				***a***	cont
	Geu*x	33.88	1,40	**<0.001**	***b***				***a***	G24
	Mer*x	27.67	1,40	**<0.001**	***b***				***a***	M20
	Geu*Mer*x	18.81	1,40	**<0.001**	***b***				***a***	2sp
10/6/08					***a***				***a***	cont
	Geu*x	5.64	1,40	**0.02**	***ab***				***b***	G24
	Mer*x	12.84	1,40	**<0.001**	***b***				***ab***	M20
	Geu*Mer*x	0.2	1,40	0.65	***b***				***a***	2sp
6/20/09									***a***	cont
	Geu*x	1.44	1,40	0.24		84.08	1,43	**<0.001**	***b***	G24
	Did*x	0.08	1,40	0.77		50.11	1,43	**<0.001**	***c***	D
	Geu*Did*x	0.23	1,40	0.63		3.59	1,43	0.06	***d***	2sp
6/27/09					***a***				***a***	cont
	Mer*x	0.47	1,39	0.5	***b***				***b***	M20
	Did*x	0.47	1,39	0.5	***c***				***b***	D
	Mer*Did*x	7.06	1,39	**0.01**	***d***				***a***	2sp
7/4/09					***a***				***a***	cont
	Geu*x	0.43	1,40	0.51	***a***				***b***	G24
	Did*x	17.76	1,40	**<0.001**	***b***				***c***	D
	Geu*Did*x	15.51	1,40	**<0.001**	***a***				***b***	2sp
7/11/09									***a***	cont
	Mer*x	0.02	1,40	0.89		24.85	1,43	**<0.001**	***b***	D
	Did*x	2.44	1,40	0.13		12.12	1,43	**0.001**	***a***	M10
	Mer*Did*x	1.95	1,40	0.17		26.23	1,43	**<0.001**	***a***	2sp

Table 5.4: Biomass estimates (μg C l^{-1}) of pico- (heterotrophic bacteria), nano- and microplanktonic (flagellates, dinoflagellates, loricate and aloricate ciliates) heterotrophs, present at t_0 of dilution experiments. Biomass conversion factors referenced in text.

date	mesocosm treatment		heterot. bacteria	biomass (µg C l^{-1}) nano- and microplanktonic flagellates	ciliates	TOTAL
11/3/07		control	109.5	154.9	125.1	389.4
	Mercenaria	8	120.7	65.8	76.8	263.2
	Mercenaria	16	112.9	90.4	187.1	390.4
5/2/08		control	63.7	760.8	121.0	945.5
	Geukensia	9	69.8	394.2	67.4	531.4
	Geukensia	18	67.4	266.3	90.2	423.9
6/13/08		control	172.5	49.3	36.6	258.4
	Mercenaria	10	161.1	32.9	0.0	194.0
	Mercenaria	20	160.7	16.4	17.3	194.5
7/26/08		control	80.4	231.6	17.3	329.3
	Geukensia	18	79.1	34.9	0.0	114.0
	Geukensia	36	105.8	68.7	3.0	177.5
8/8/08		control	159.7	1401.5	47.0	1608.3
	Geukensia	18	114.8	742.9	125.7	983.4
	Geukensia	36	94.7	678.1	78.0	850.8
9/23/08		control	207.0	288.1	182.8	677.9
	Geukensia	24	219.4	202.5	120.4	542.3
	Mercenaria	20	207.6	210.2	89.4	507.3
	Geu+Merc	24+20	300.8	148.0	16.4	465.1
10/6/08		control	23.0	224.4	92.9	340.3
	Geukensia	12	23.3	345.5	98.4	467.3
	Geu+Merc	12+20	23.3	217.6	131.2	372.1
	Mercenaria	20	20.5	85.7	78.0	184.2
6/20/09		control	29.3	918.9	0.0	948.2
	Geukensia	24	25.6	70.1	84.4	180.1
	Didemnum	694 g	57.0	241.4	182.8	481.2
	Geu+Didem	24+700g	49.1	342.6	330.4	722.2
6/27/09		control	23.9	389.3	98.4	511.7
	Didemnum	280 g	51.1	109.0	110.1	270.2
	Merc+Didem	20+270g	74.1	93.4	215.6	383.1
	Mercenaria	20	33.3	249.2	133.6	416.1
7/4/09		control	41.2	23.4	11.6	76.2
	Didemnum	366 g	66.6	62.3	146.1	275.0
	Geu+Didem	24+339g	67.4	23.4	41.0	131.7
	Geukensia	24	39.1	23.4	31.8	94.3
7/11/09		control	81.8	23.4	36.4	141.6
	Mer+Didem	10+150g	108.0	7.8	16.4	132.2
	Didemnum	150 g	158.5	15.6	2.3	176.4
	Mercenaria	10	82.9	15.6	0.0	98.5

Table 5.5: Summary of positive (+), negative (**-**) and no effects (**0**) of suspension feeder density treatments (single-species, or interactive effects *), on growth rates (r d^{-1}) of nanoplanktonic (N, <20 µm) and microplanktonic (M, 20-<64 µm) heterotrophic protists. The table integrates results from RDA triplots presented in Figures 5.11-5.15. Species references: hetflagN: heterotrophic nanoflagellates; hetdinoN: heterotrophic dinoflagellates; ciliateN: nanoplanktonic ciliates; euglenM: heterotrophic microplanktonic euglenoids; phytflgM: heterotrophic microplanktonic flagellates; dinoM: heterotrophic microplanktonic dinoflagellates; alorcilM: microplanktonic aloricate ciliates; lorcilM: microplanktonic loricate ciliates.

		N flagellates	N dinoflag.	N ciliates	M euglenoids	M flagellates	M dinoflag.	M aloric cil.	M loric cil.
Geukensia	5/2/08	-	+	-	+		+	-	
Geukensia	8/8/08	-	+	**0**	-	+	+	+	+
*Geuk*Didem*	6/20/09	-	-	-	-	+	+	+	-
Mercenaria	6/27/09	-	-	+	-	-	+	-	-
*Geuk*Didem*	7/4/09					-	+	+	+

Figure 5.1: Molar concentrations (μM) of total N, total P and total Si at t_0 of dilution experiment incubations. Bars are averages ± SE for all experimental dates of a certain suspension feeder treatment. μ_0/μ_n is the ratio of phytoplankton growth rate in unenriched (μ_0) and enriched (with nitrate and phosphate) bottles (μ_n) and provides an indication of nutrient limitation (<1). Datapoints are averages ± SE for all experimental dates of a certain suspension feeder treatment. For data consolidation and analysis, *Didemnum+Geukensia* and *Didemnum+Mercenaria* are considered in the same category (*Did*+bivalve sp.), while *Geukensia+Mercenaria* treatments are labeled 2 biv. spp.

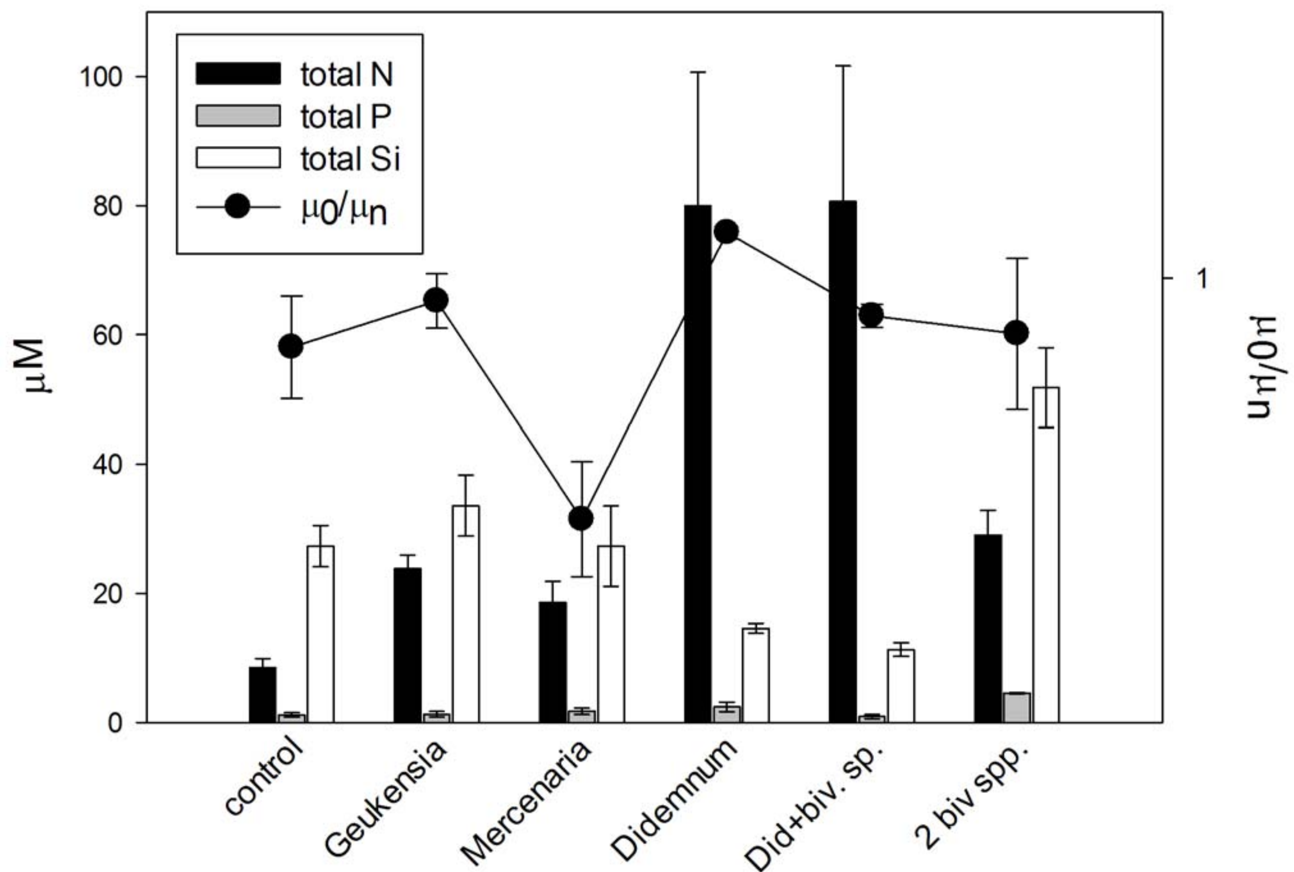
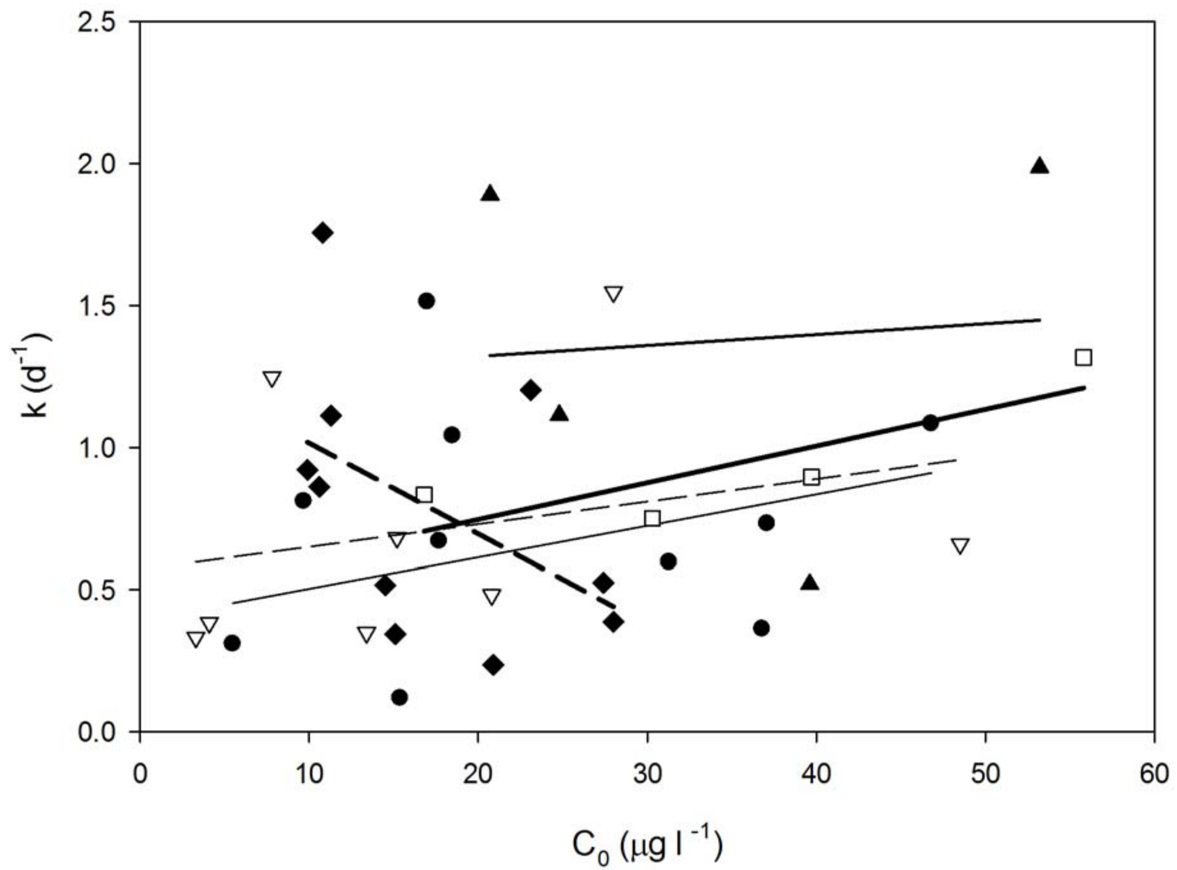

total N
total P
total Si
μ0/μn
μM
μ0/μn
100
80
60
40
20
0
1
control
Geukensia
Mercenaria
Didemnum
Did+biv. sp.
2 biv spp.

Figure 5.2: Relationship between phytoplankton growth (*k*) and the initial chlorophyll standing stock (C_0) in bottle incubations. Homogeneity of slopes test $F(4,110) = 2.80$, **p =0.03**. Multiple comparisons (Tukey HSD test) discussed in main text.


2.5
2.0
1.5
1.0
0.5
0.0
k (d^{-1})
0
10
20
30
40
50
60
C_0 (μg l^{-1})

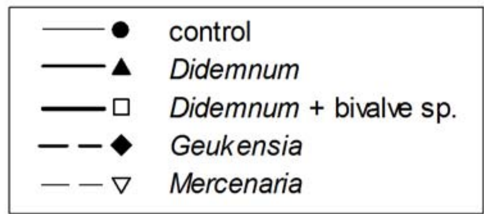
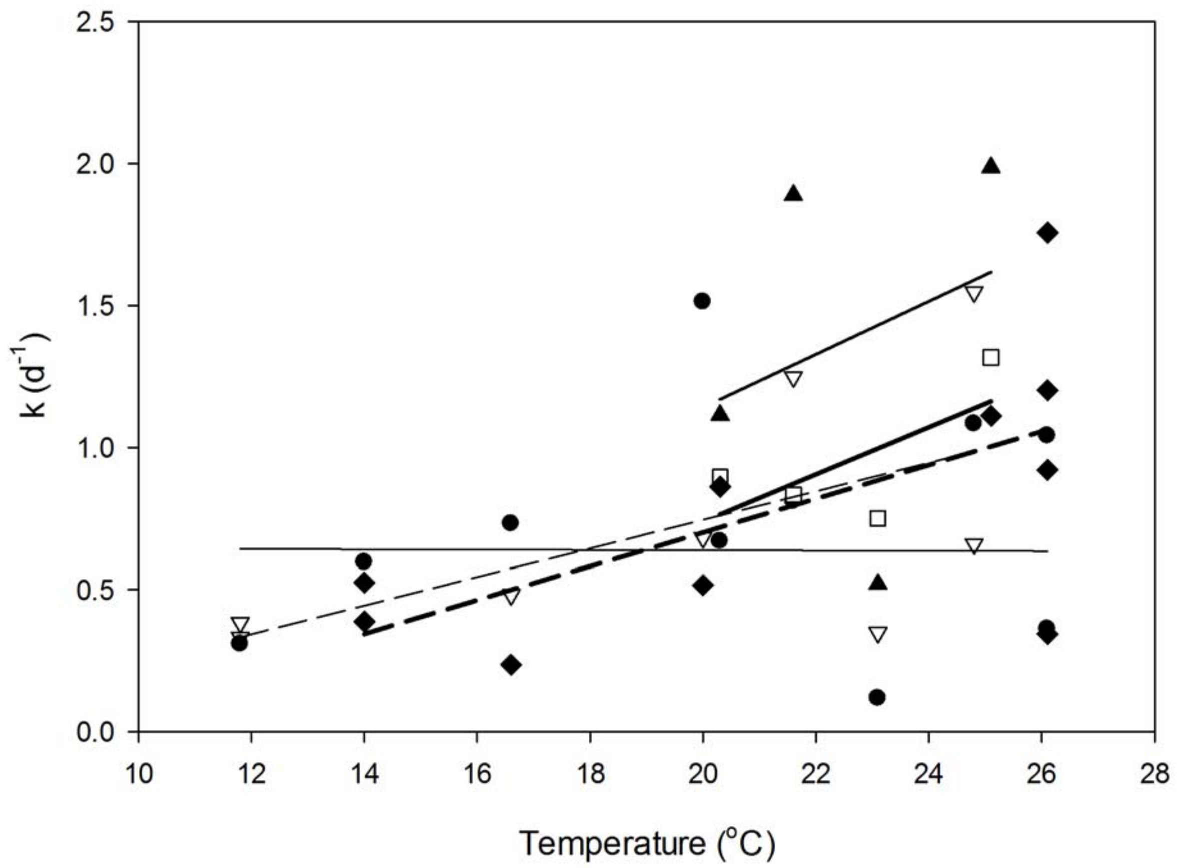

control
Didemnum
Didemnum + bivalve sp.
Geukensia
Mercenaria

Figure 5.3: Relationship between phytoplankton growth (*k*) and temperature (ºC) in bottle incubations. ANCOVA $F(4,110) = 5.28$, **p =0.001**. Multiple comparisons (Tukey HSD test) discussed in main text.


2.5
2.0
1.5
1.0
0.5
0.0
k (d-1)
10
12
14
16
18
20
22
24
26
28
Temperature (oC)

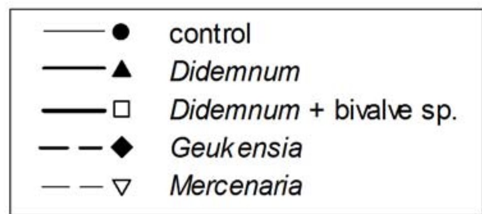
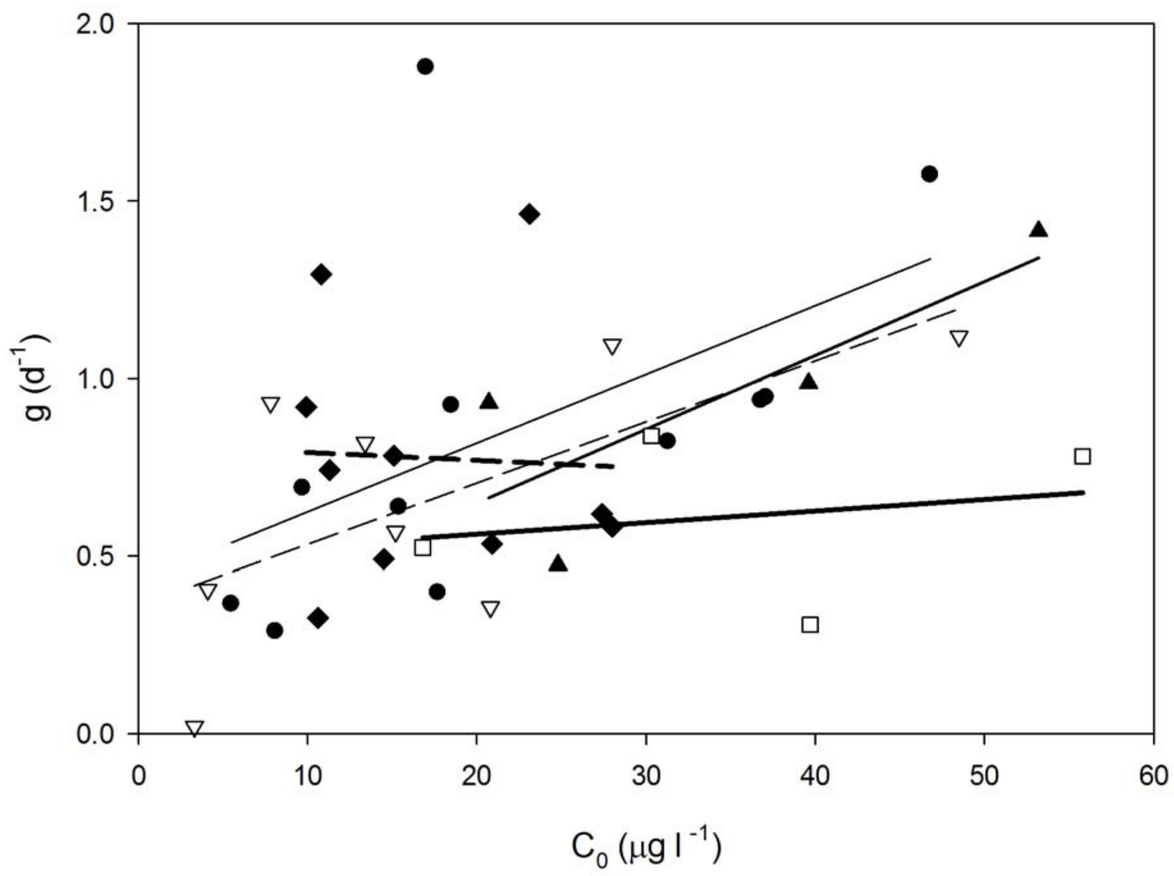

control
Didemnum
Didemnum + bivalve sp.
Geukensia
Mercenaria

Figure 5.4: Relationship between microheterotroph grazing coefficient (*g*) and the initial chlorophyll standing stock (C_0) in bottle incubations. ANCOVA $F(4,110) = 2.63$, **p =0.04**. Multiple comparisons (Tukey HSD test) discussed in main text.


g (d^{-1})
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
C_0 (μg l^{-1})

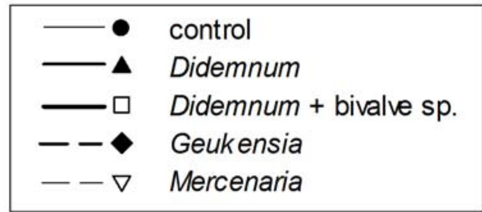
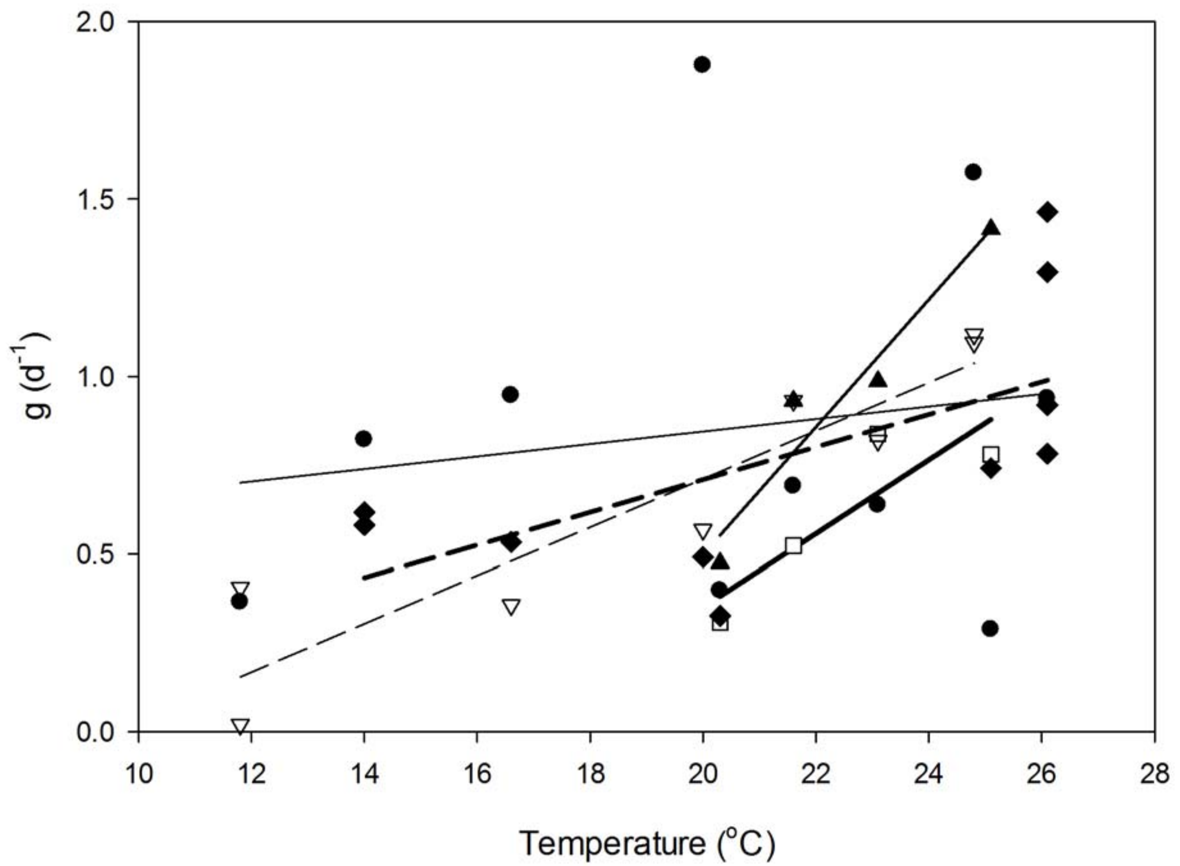
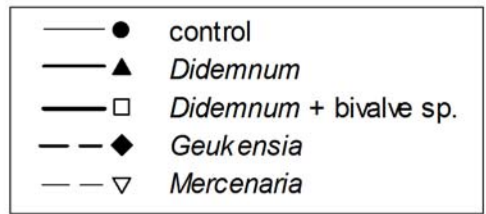

control
Didemnum
Didemnum + bivalve sp.
Geukensia
Mercenaria

Figure 5.5: Relationship between microheterotroph grazing coefficient (*g*) and temperature (ºC) in bottle incubations. Homogeneity of slopes test $F(4,110) = 3.03$, **p =0.02**. Multiple comparisons (Tukey HSD test) discussed in main text.

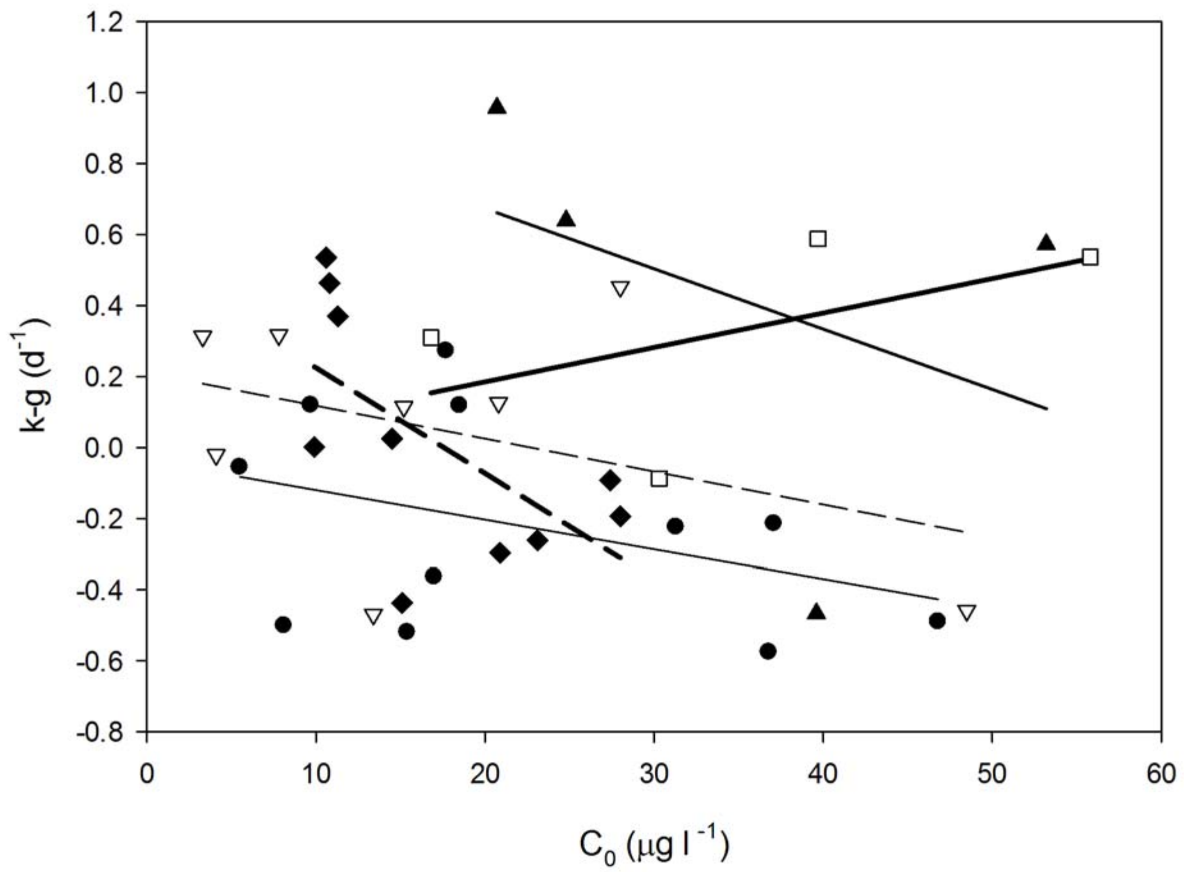


g (d^{-1})
Temperature (^{o}C)
2.0
1.5
1.0
0.5
0.0
10
12
14
16
18
20
22
24
26
28

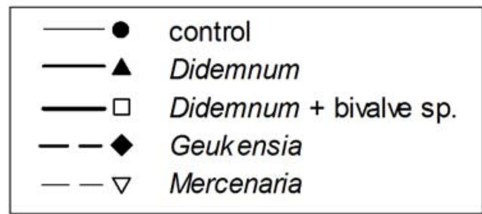


control
Didemnum
Didemnum + bivalve sp.
Geukensia
Mercenaria

Figure 5.6: Relationship between the net difference in growth and grazing coefficients (*k*-*g*) and the initial chlorophyll standing stock (C_0) in bottle incubations. Homogeneity of slopes test $F(4,110) = 3.58$, **p =0.01**. Multiple comparisons (Tukey HSD test) discussed in main text.

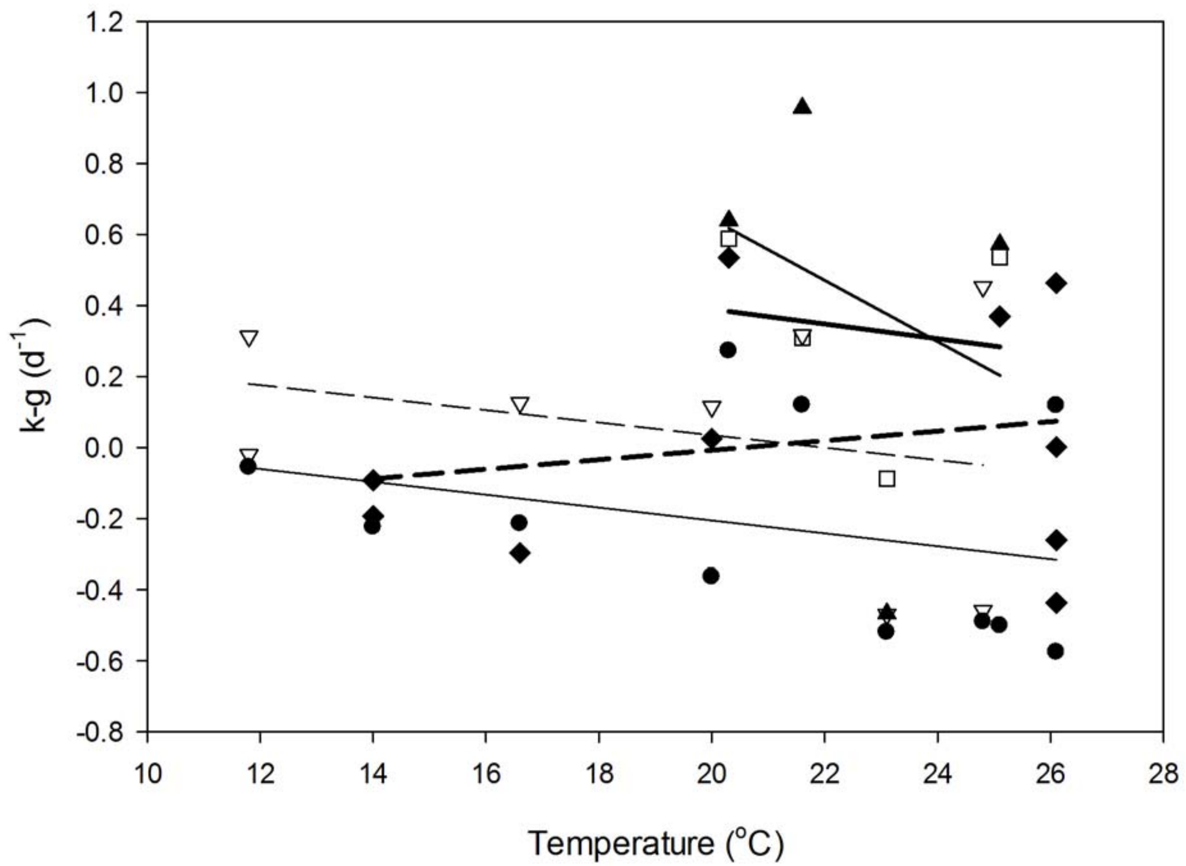


1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
k-g (d-1)
0
10
20
30
40
50
60
C0 (µg l -1)



control
Didemnum
Didemnum + bivalve sp.
Geukensia
Mercenaria

Figure 5.7: Relationship between the net difference in growth and grazing coefficients (*k*-*g*) and temperature (ºC) in bottle incubations. ANCOVA $F(4,110) = 5.28$, $\mathbf{p = 0.001}$. Multiple comparisons (Tukey HSD test) discussed in main text.


1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
k-g (d-1)
10
12
14
16
18
20
22
24
26
28
Temperature (°C)

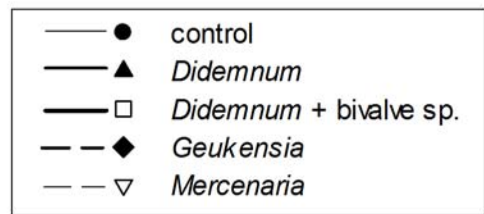
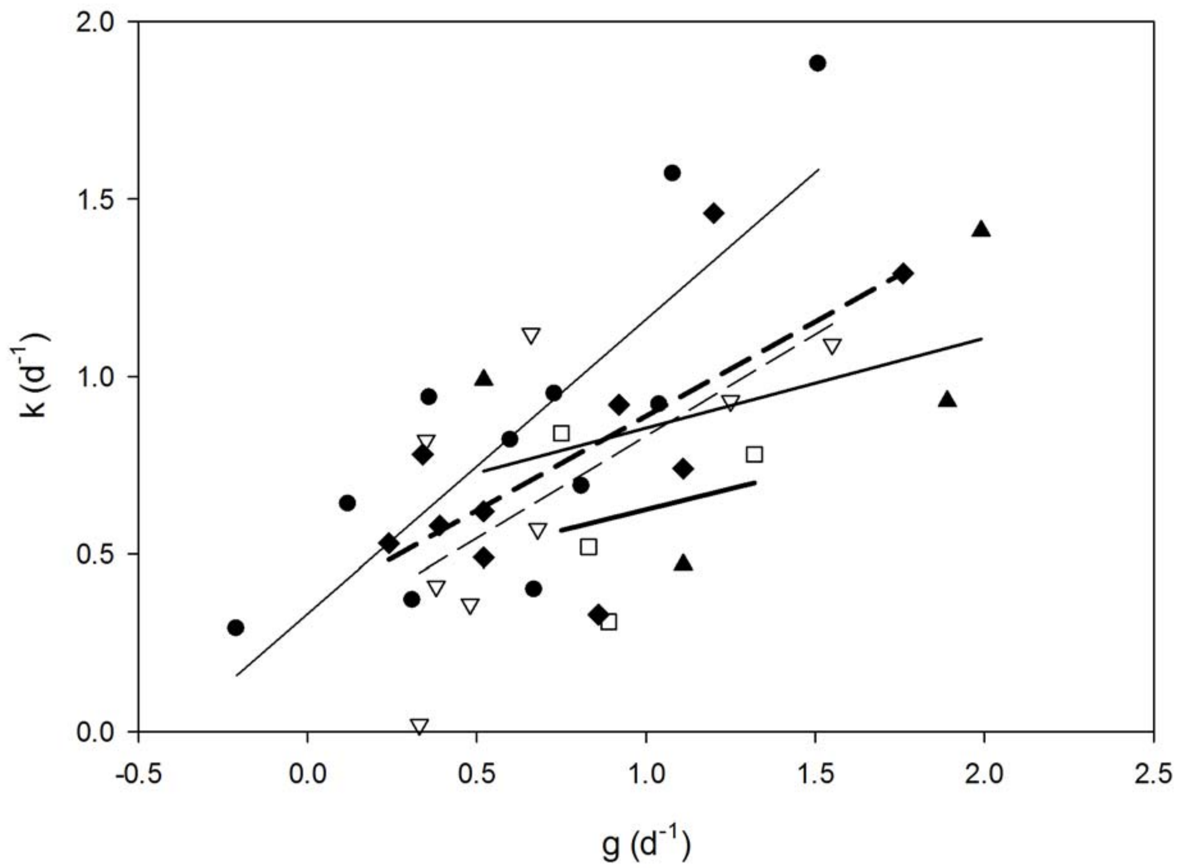
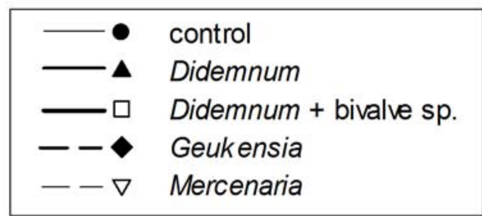

control
Didemnum
Didemnum + bivalve sp.
Geukensia
Mercenaria

Figure 5.8: Geometric mean regressions between phytoplankton growth (*k*) and microheterotroph grazing (*g*) rates in bottle incubations. Multiple comparisons (Tukey HSD test) discussed in main text.

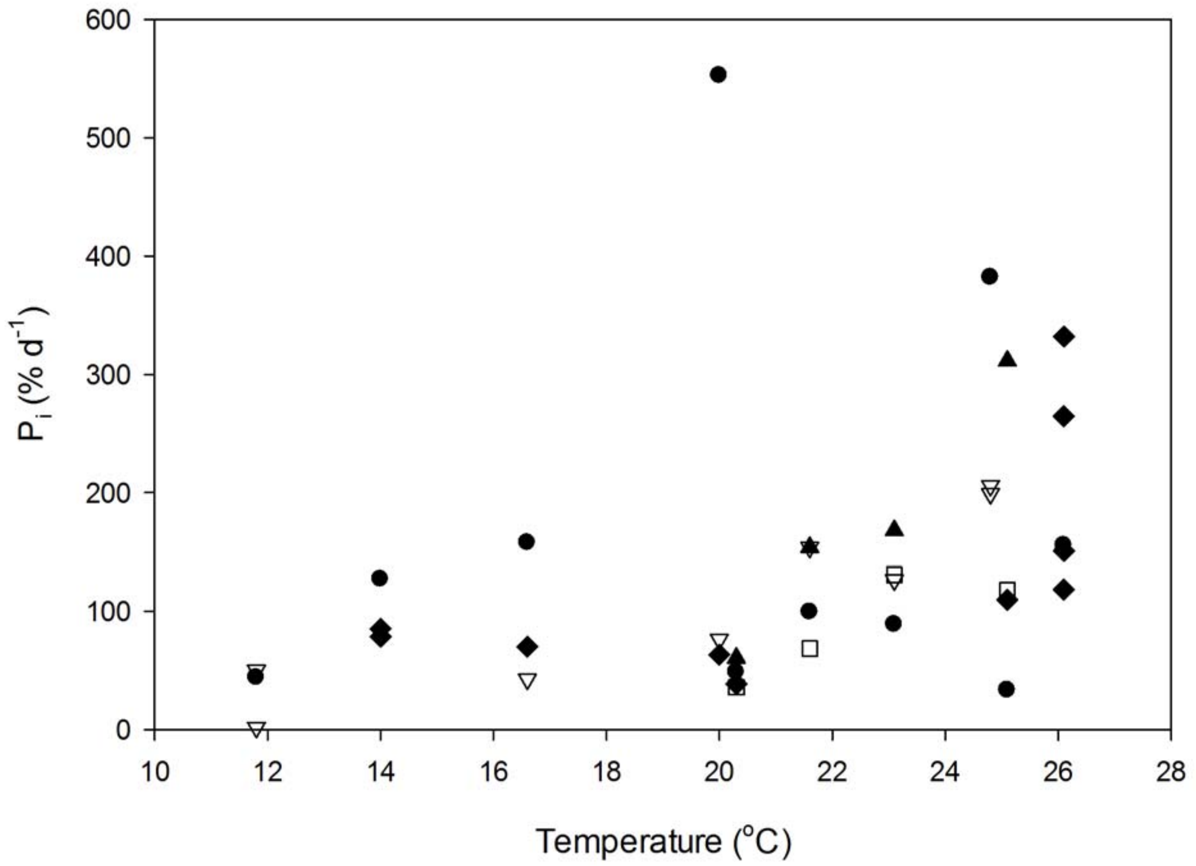


2.0
1.5
1.0
0.5
0.0
k (d^{-1})
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
g (d^{-1})



control
Didemnum
Didemnum + bivalve sp.
Geukensia
Mercenaria

Figure 5.9: Relationship between chlorophyll *a* biomass removed per day by microheterotrophs (P_i, %) and temperature (ºC). Regression **$p << 0.001$**.


600
500
400
300
200
100
0
P_i (% d^{-1})
10
12
14
16
18
20
22
24
26
28
Temperature (°C)

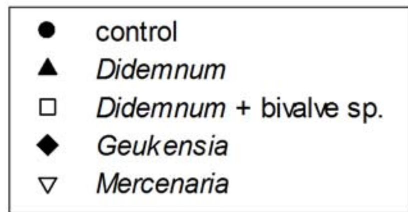
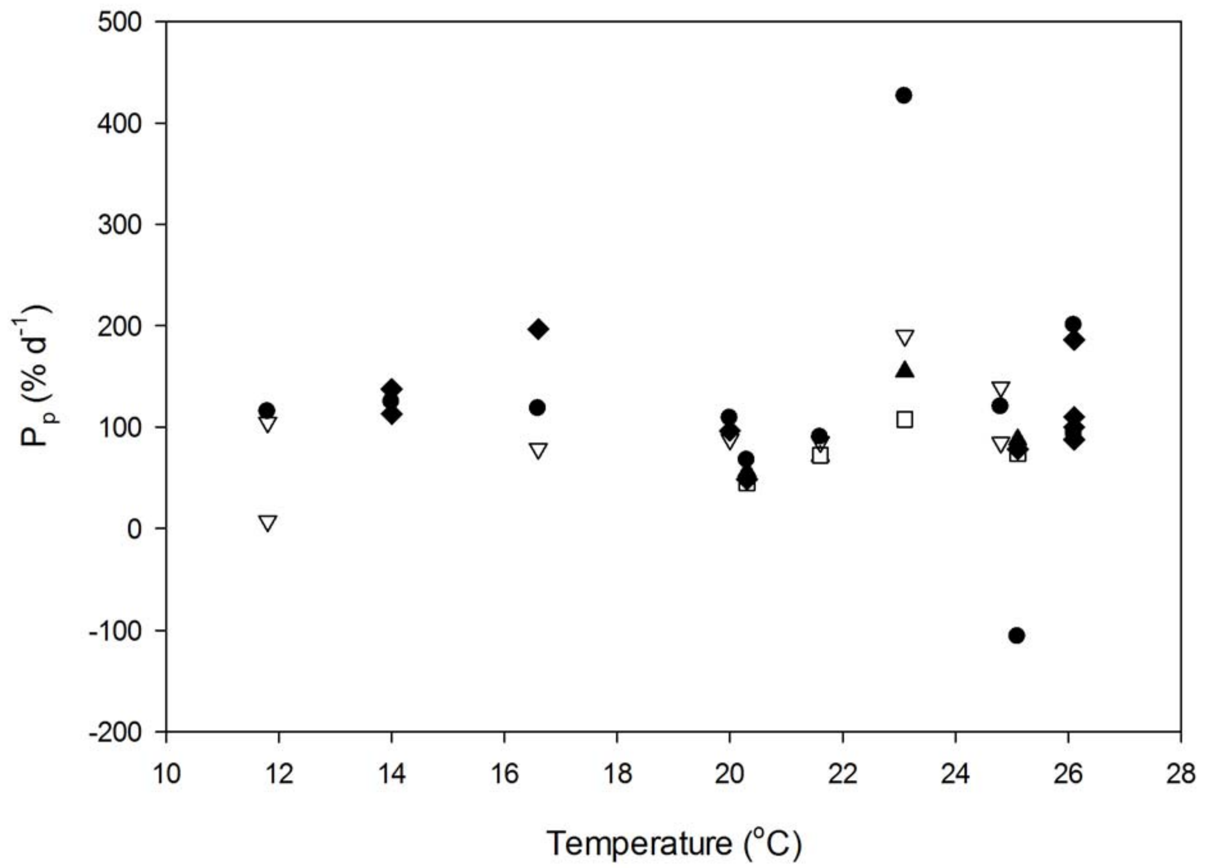
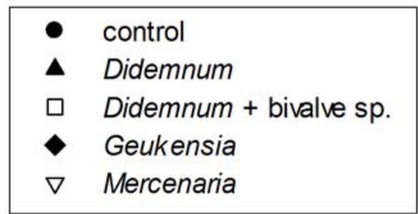

control
Didemnum
Didemnum + bivalve sp.
Geukensia
Mercenaria

Figure 5.10: Relationship between primary production grazed daily by microheterotrophs (P_p, %) and temperature (ºC). Regression $p = 0.79$.



500
400
300
200
100
0
-100
-200
10
12
14
16
18
20
22
24
26
28
P_p (% d^{-1})
Temperature (°C)



control
Didemnum
Didemnum + bivalve sp.
Geukensia
Mercenaria

Figure 5.11: RDA triplot for benthic suspension feeder (*Geukensia*) density effects on net growth rates (r, d^{-1}) of nano- and microplanktonic heterotrophic protists. Experimental date 5/2/08. Statistical significance: λ= 0.27, F-value =2.65, **p-value =0.01**. Species references in Table 5.5 legend.

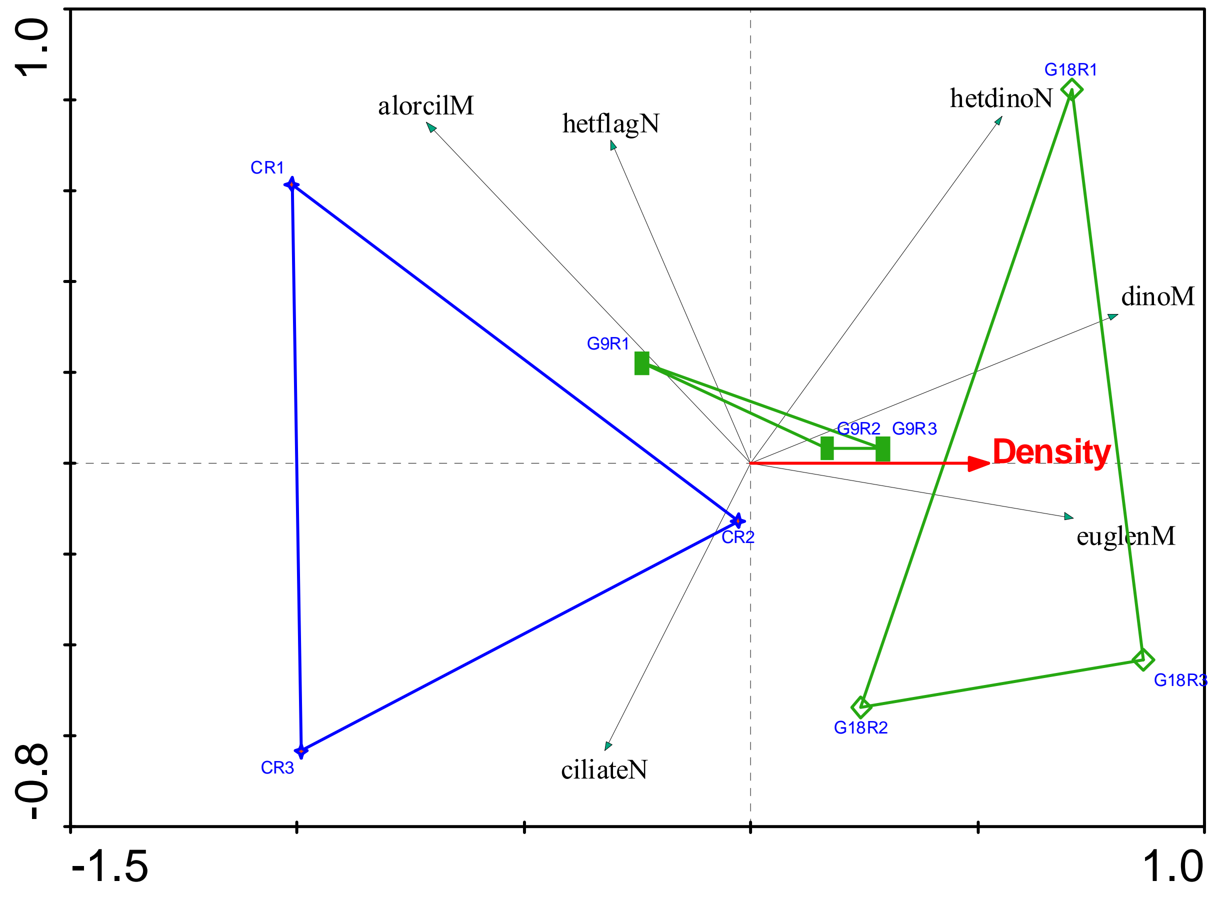

1.0
-0.8
-1.5
1.0
alorcilM
hetflagN
hetdinoN
dinoM
euglenM
ciliateN
Density
CR1
CR2
CR3
G9R1
G9R2
G9R3
G18R1
G18R2
G18R3

Figure 5.12: RDA triplot for benthic suspension feeder (*Geukensia*) density effects on net growth rates (r, d^{-1}) of nano- and microplanktonic heterotrophic protists. Experimental date 8/8/08. Statistical significance: λ= 0.22, F-value =2.02, **p-value =0.03**. Species references in Table 5.5 legend.

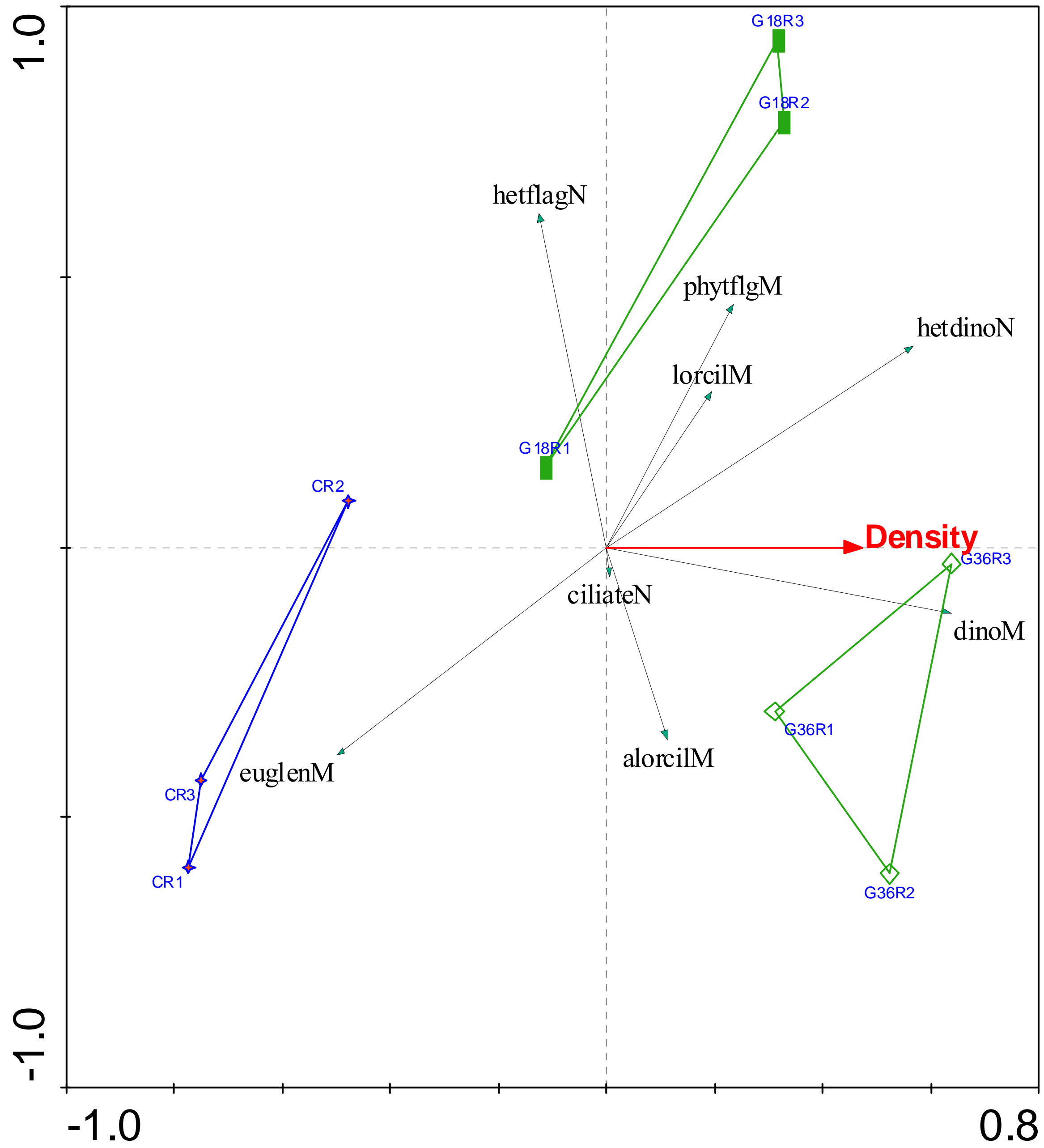

1.0
-1.0
-1.0
0.8
G18R3
G18R2
G18R1
hetflagN
phytflgM
hetdinoN
lorcilM
CR2
Density
G36R3
ciliateN
dinoM
G36R1
euglenM
alorcilM
CR3
CR1
G36R2

Figure 5.13: RDA triplot for benthic suspension feeder (*Geukensia**Didemnum*) density effects on net growth rates (r, d^{-1}) of nano- and microplanktonic heterotrophic protists. Experimental date 6/20/09. Statistical significance: λ= 0.21, *F*-value =2.48, **p-value =0.02**. Species references in Table 5.5 legend.

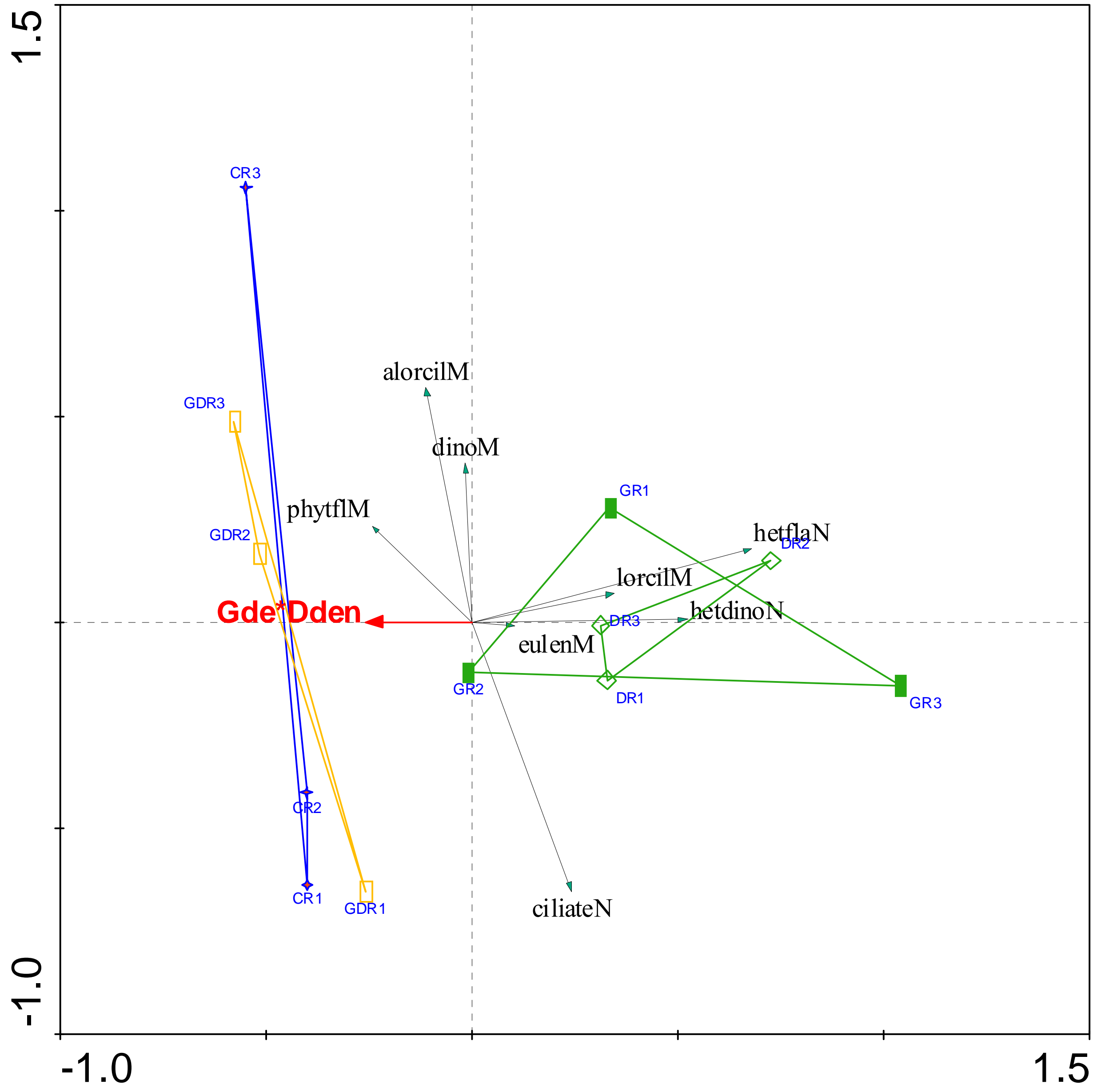
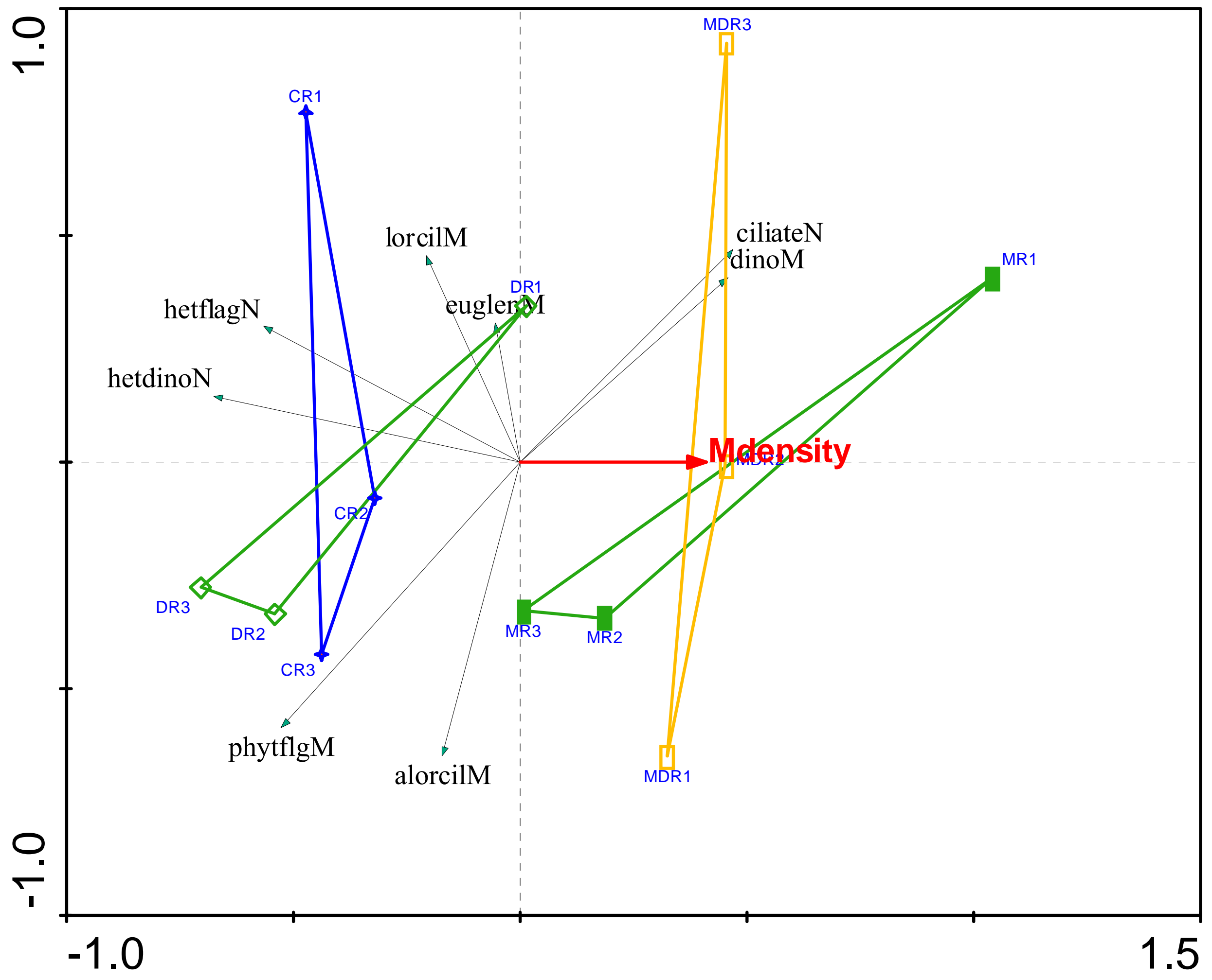
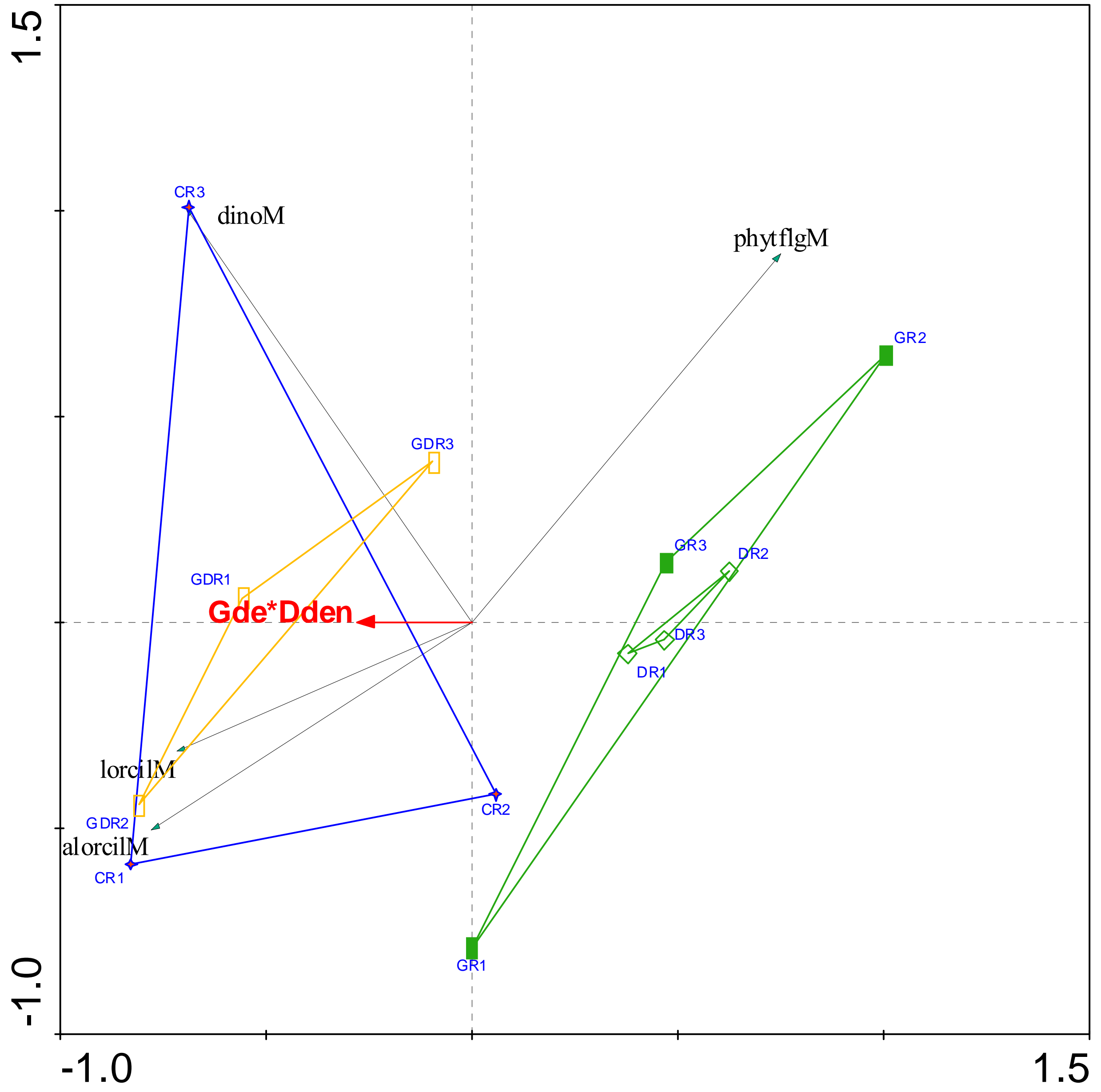

1.5
-1.0
-1.0
1.5
CR3
GDR3
GDR2
CR2
CR1
GDR1
alorcilM
dinoM
phytflM
Gde*Dden
GR1
hetflaN
DR2
lorcilM
DR3
hetdinoN
eulenM
GR2
DR1
GR3
ciliateN

Figure 5.14: RDA triplot for benthic suspension feeder (*Mercenaria*) density effects on net growth rates (r, d^{-1}) of nano- and microplanktonic heterotrophic protists. Experimental date 6/27/09. Statistical significance: λ= 0.17, F-value =2.15, **p-value =0.04**. Species references in Table 5.5 legend.



1.0
-1.0
-1.0
1.5
MDR3
CR1
ciliateN
dinoM
lorcilM
MR1
DR1
hetflagN
euglenM
hetdinoN
Mdensity
MDR2
CR2
DR3
DR2
MR3
MR2
CR3
phytflgM
alorcilM
MDR1

Figure 5.15: RDA triplot for benthic suspension feeder (*Geukensia**Didemnum*) density effects on net growth rates (r, d^{-1}) of nano- and microplanktonic heterotrophic protists. Experimental date 7/4/09. Statistical significance: $\lambda = 0.24$, F-value =3.89, **p-value =0.01**. Species references in Table 5.5 legend.


1.5
-1.0
-1.0
1.5
CR3
dinoM
phytflgM
GR2
GDR3
GR3
DR2
GDR1
Gde*Dden
DR3
DR1
lorcilM
CR2
GDR2
alorcilM
CR1
GR1

SYNTHESIS

The study of ecological benthic-pelagic coupling has traditionally focused on reproductive (reviewed by Strathmann, 1990) or trophic interactions. Through the latter, benthic suspension-feeding organisms are known to exert a dominant organizing role in shallow aquatic ecosystems by diverting production from the water column to the benthos (French McCay et al., 2003). Benthic suspension-feeding organisms such as bivalve molluscs are known for their positive effects to coastal ecosystems, including reduction of turbidity, prevention of harmful algal blooms (HABs), removal of nutrients by burial of nitrogen and phosphorus in the form of biodeposits, and the ultimate removal of nitrogen *via* denitrification (reviewed by Dame, 1996; Prins et al., 1998; Newell et al., 2002; Newell, 2004; Lonsdale et al., 2009).

Besides providing valuable ecosystem services, commercially exploited suspension-feeding molluscs are an important source of revenue for local and regional economies. Firstly, marine molluscs constitute the majority of the world's shellfish exports by tonnes (55%), largely surpassing crustacean shellfish. Moreover, considering together capture and aquaculture sources, suspension-feeding bivalves constitute the majority of the world's production of marine molluscs (66%), surpassing pelagic molluscs such as squid and cuttlefish, and generating an estimated annual gross revenue of 13,632 million dollars [from an analysis of FAO (2009) statistics].

A key physiological feature of bivalves is their high feeding rates, possibly an adaptation to living in estuarine systems that historically supported low concentrations of phytoplankton in relation to high loads of detrital and mineral particles (Newell, 2004). This organismic-level feature is to be regarded from a new perspective when scaled-up to ecosystem-level processes (Dame, 1996). A number of studies comparing coastal systems that have experienced a dramatic decline in the densities of suspension-feeding benthos have linked these reductions to shifts in pelagic structure and function (Newell, 1988; Cerrato et al., 2004; Pomeroy et al., 2006; Mann et al., 2009).

This dissertation explored biological benthic-pelagic coupling modulated by suspension-feeding macroinvertebrates (mostly bivalves, and also a colonial invasive ascidian) at the organismic and ecosystem levels, implementing laboratory and field-based experimental approaches, respectively.

Ribbed mussels (*Geukensia demissa*) typically live in detritus-dominated salt marshes, which experience significant daily (tidal) and seasonal variations in concentration and composition of seston particles (Huang et al., 2003a). This has forced ribbed mussels to be true omnivores, meeting their nutritional requirements by utilizing a wide variety of living and dead material (Kreeger and Newell, 1996). The study outlined in Chapter 1 represented a field test of how *G. demissa* processes different components of the natural planktonic community in water bodies with different planktonic composition and structure. The bays considered in this study presented a 4.5-fold difference in total chlorophyll *a* concentration. The autotrophic biomass in all bays was dominated (>52%) by ultraplankton. Except in one bay, heterotrophic forms dominated the microplanktonic biomass, and differences in picoplanktonic community structure were statistically significant among bays.

Despite these differences in planktonic structure, there were no significant differences in the chlorophyll clearance rate of *Geukensia*, suggesting that ribbed mussels were consistently removing phytoplankton independently of size, and size-proportions, in the field. The only significant differences in clearance found in this study were among pico- and nanoplanktonic groups, but both mean rates were nonetheless high and comparable to rates for microplanktonic prey. The high clearance rates found for nanoplankton, and the ability of *Geukensia* to graze phytoplankton equally well across size ranges can be attributed to a closer spacing of laterofrontal cirri in *Geukensia* compared to other marine mussels. Clearance rates were high for centric and pennate diatoms (although marginally non-significant), even though they represented a minor proportion of the microplanktonic biomass. Ribbed mussels were not selecting on the basis of cell size (biovolume). Interestingly, ribbed mussels presented comparable clearance rates for autotrophs and heterotrophs, and high absorption efficiencies (>50%) for all locations.

Zooplankton grazers (across a range of scales comprising microzooplankton to mesozooplankton) are much more selective than benthic metazoans. For example, it has long been recognized that selective feeding by microzooplankton may have important consequences both for phytoplankton dynamics and the dynamics of microzooplankton,

by mostly increasing growth rates of the latter (Stoecker et al., 1986). Through their selectivity for certain food items, zooplankton grazing can directly influence phytoplankton succession, with edible species being replaced by inedible species when zooplankton grazing rates are high (Griffin and Rippingale, 2001; and references therein). Thus, the type of dominant microalgae in a community is as significant in structuring the composition and biomass of zooplanktonic grazers (i.e. bottom-up effects), as zooplankton are in structuring phytoplankton communities (i.e. top-down effects). The experiments outlined in this chapter demonstrated that *Geukensia* behaved as a non-selective suspension feeder, and as such may have the potential to drive changes in planktonic structure that diverge from those induced by pelagic grazers.

Dramatic changes in bivalve abundance (either through introductions or eliminations) can have consequences to other components of the ecosystem (Caraco et al., 1997, and references therein), as is the case on Long Island estuaries and tidal marshes that have experienced substantial declines in populations of suspension-feeding bivalves. Following up on this, Quantuck Bay was chosen to estimate how much of the filtration capacity by *Geukensia demissa* has been compromised due to marsh loss and habitat modification. The perimeter of the estuarine/marine areas of the bay (according to the National Wetlands Inventory, U.S. Fish and Wildlife Service) was surveyed using satellite images, and classified into different types according to the current state of the fringing marsh. The four types considered were:

- bulkheaded: where an upright wall-like partition (wooden or concrete) delimits developed areas and the water (23% of bay perimeter)

- artificial beaches and boat ramps: where sand or pavement has been added to the bay to create consolidated substrates (7% of bay perimeter)

- residential waterfront: where the high *Spartina alterniflora* marsh has been transformed into developed areas (lawns, etc.), and the edge marsh has been impacted by piers and docks (43% of bay perimeter)

- 'pristine' fringing saltmarsh and marsh islands within the bay (currently, 27% of bay perimeter), being the only areas where healthy *G. demissa* populations are found. It

was assumed that historically the whole perimeter of the bay was occupied by a fringing saltmarsh with populations of *G. demissa*.

The zonation of a NY fringing saltmarsh has a ~1.5 m wide seaward edge where *G. demissa* individuals are found at ~800 m^{-2} in the tall *S. alterniflora* zone, while upshore the edge *G. demissa* is present at much lower densities (Franz, 1997; J. Pan, pers. obs.). With an area of ~5,000,000 m^2 (Lomas et al., 2004) and a tidal range of 0.17 m, Quantuck bay exchanges 850,000 m^3 on each tidal cycle. Assuming an average clearance rate of 2.1 l h^{-1} org^{-1} (experimentally determined *in situ* for early summer 2006, see Chapter 1), and considering an average immersion time of 6 h for each tidal cycle, it can be assumed that the current population of *G. demissa* in Quantuck Bay filters 10% of the volume exchanged in each cycle, whereas historically it filtered 39% of the exchanged volume. In other words, the Bay has lost ~74% of its historic filtration capacity attributed to *G. demissa*. It is worth mentioning that this filtration budget only accounts for reductions in the population filtration capacity of an intertidal mussel (which is actively filtering about half of the time of subtidal bivalves), and that furhter losses of suspension-feeding capacity should be computed if the population declines of other suspension-feeding bivalves in the system are to be considered.

Extensive blooms of the pelagophyte *Aureococcus anophagefferens* in mid-Atlantic estuaries (called ‘brown tides’) have resulted in significant effects both on individual species and aquatic ecosystems related to cell toxicity, the sheer biomass and persistence of monospecific blooms that can last for several months. Short- and long-term exposure experiments to certain strains of *A. anophagefferens* have evidenced toxic effects on juvenile hard clams and blue mussels (Bricelj et al., 2001; Bricelj et al., 2004). Although not necessarily lethal, *A. anophagefferens* produces a bioactive compound that can affect normal physiological function. Although not chemically characterized, a dopamine-like compound in the extracellular polysaccharide layer of the microalga elicits a response of lateral cilia in the ctenidium upon direct cell contact, impairing suspension-feeding function. This response, however, is species-specific. Chapter 2 examined ribbed mussels grazing on cultured and wild-type strains of the brown tide organism.

The experiments with mixtures of monospecific algal cultures showed that clearance rate did not vary with the proportion of *A. anophagefferens* present in the mixture, and were considerably high values for any suspension-feeding bivalve. Moreover, no inhibition of uptake or suppressed feeding on the non-toxic prey (*Isochrysis galbana*) was observed for mixed suspensions, contrary to what Bricelj et al. (2001) found for *Mercenaria mercenaria* juveniles. Retention efficiencies for *Aureococcus* were high (96 %), and *Geukensia* efficiently digested brown tide cells. The experiments mixing an *Aureococcus* bloom and a non-bloom natural plankton community showed a threshold effect for *Geukensia* grazing on *Aureococcus* cell concentrations >1.4 $\times 10^5$ cells ml^{-1}. Such an effect was found by Bricelj et al. (2001) for *M. mercenaria* juveniles, however the threshold for hard clams was roughly an order of magnitude lower than that for ribbed mussels.

In conclusion, *Geukensia demissa*'s suspension-feeding physiology, as well as its ability to incorporate carbon, remain at high rates when subjected to mixed and monospecific suspensions ~5 $\times 10^5$ cells ml^{-1}, and at natural bloom threshold levels below 1.4 $\times 10^5$ cells ml^{-1} of wild-type brown tide. At first hand, this provides experimental support for the ribbed mussel as a potentially viable candidate for stock-enhancement and salt-marsh habitat restoration strategies in estuaries experiencing recurring brown tides.

Most of the microalgae involved in the formation of HABs produce potent biotoxins that have adverse effects on the physiology of other aquatic biota, and that, through trophic biomagnification and transfer, can impact all levels of marine food webs, including humans (Landsberg, 2002; Sunda et al., 2006). 90% of HAB-causing microalgae are dinoflagellates (Smayda, 1997), and this predominance has been linked to specific features of their biology such as mixotrophy, vertical migration, and typical swimming and aggregation patterns (Sournia, 1995; Smayda, 1997). Chapter 3 studied grazing in marine mussels and clams exposed to a proportional gradient of toxic benthic (*Amphidinium carterae*) and pelagic (*Prorocentrum minimum*) dinoflagellates known to co-occur with these bivalve suspension feeders and create dense blooms.

Clearance rates for *Mytilus edulis*, *Geukensia* and *Mercenaria* showed no significant differences across treatments with increasing proportions of noxious dinoflagellates in the diets. Conversely, Luckenbach et al. (1993) fed mixed diets of *P. minimum* at bloom densities to juvenile *Crassostrea virginica* and observed a reduction in filtration rates. Although non-significant, several experiments showed higher clearance rates for those treatments combining *I. galbana* and a dinoflagellate indicating some preference for mixed diets. However, retention efficiencies for dinoflagellate cells were significantly lower than expected for particles of their size, suggesting that a factor other than cell size was influencing particle capture.

If clearance rates are sustained over prolonged periods of time the bivalves tested here may exert a top-down control on HAB-causing dinoflagellates. Conclusions drawn from short-term experiments like the ones in this study may fall short in elucidating further effects of exposure to noxious phytoplankton but are nonetheless important in the characterization of trophic relationships between benthic and planktonic species.

The three first chapters of this dissertation dealt mostly with the topic of particle selectivity in suspension-feeding bivalves, a topic that has been reviewed in the literature (e.g. Shumway et al., 1985; Ward and Shumway, 2004). In summary, particle selection is an important species-specific process in suspension-feeding bivalves. The differential sensitivity of blue and ribbed mussels to *A. anophagefferens* found in Chapter 2, supports this. Particle selectivity is based upon physical (e.g. cell size, shape); behavioral (e.g. swimming ability and motility of phytoplankton; Bricelj et al., 1998); and chemical or qualitative characteristics of the particles in suspension (e.g. cell stickiness, Waite et al., 1995; epicellular chemical cues, Gainey and Shumway, 1991; Beninger and Decottignies, 2005; cell physiological state, Brillant and MacDonald, 2003). The significantly lower retention efficiencies found in most bivalves for the dinoflagellates tested in Chapter 3, suggests that any of the above-mentioned factors (other than cell size) was influencing particle capture.

Chapter 4 focused on top-down and bottom-up effects of suspension-feeding macrobenthos on the plankton community of Great South Bay, a coastal lagoon. At peak historical densities of *M. mercenaria*, the former dominant benthic macroinvertebrate in

the system, 40% of the entire bay volume was cleared daily by suspension feeding while at the current densities benthic suspension feeders filter <1% per day (Cerrato et al., 2004). Experimental evidence suggested that hard clams could have provided an effective top-down control mechanism to prevent the initiation of brown tide blooms and/or modulate outbreaks (Bricelj et al., 2001; Cerrato et al., 2004). Under current conditions primary productivity is high, but there is a dominance of nano- and picoplankton biomass over microplankton (>80% of chlorophyll *a* corresponds to ultraplankton; Lonsdale et al., 1996b; Sieracki et al., 2004; Lonsdale et al., 2006; Chapter 1 of this dissertation), and likely makes the phytoplankton biomass not readily available to large metazoan consumers. It is apparent that the current ecological state of Great South Bay does not support abundant and functionally-significant benthic suspension feeders. A series of experiments were conducted in mesoscale water enclosures (~0.4 m^3), in which the densities of native benthic suspension-feeding bivalves and a recently introduced colonial ascidian were manipulated, with the objective of characterizing and comparing changes in planktonic composition and structure induced by benthic suspension feeders and testing for interactive effects (positive or negative) in multi-species assemblages.

Bivalves (*G. demissa* and *M. mercenaria*) exerted top-down control on the biomass of phytoplankton and micrometazoans. There were differential responses to top-down effects of suspension feeders among taxonomic groups. For instance, in one experiment, ribbed mussels had positive effects on the biomass of centric diatoms and negative effect on pennates. The mechanism behind these changes in biomass likely involved diatom population growth rates and their modulation by top-down effects. It is known that grazing can influence population growth rate of phytoplankton (Bergquist and Carpenter, 1986; Smayda, 1997; Sunda et al., 2006). Nutrient remineralization has been pointed as one of the most prominent mechanisms behind this; phytoplankton can compensate for direct grazing losses by increasing growth rates, due to increased nutrient supply (Doering et al., 1986; Sterner, 1986). These enhanced growth rates may be large enough that, despite greatly increased grazing losses, little or no decline in phytoplankton biomass occurs and production actually increases (Bergquist and Carpenter, 1986; Doering et al., 1986; Sterner, 1986).

Moreover, bivalve species effectively controlled densities of certain >40 μm microzooplankton (rotifers), while having positive effects on the densities of eggs and larval stages of copepods, suggesting a weak or lack of top-down control for the latter. A trophic cascade may have been involved in which the decrease in phytoplankton biomass may have promoted copepod grazing and population growth. Moreover, the control bivalves exerted on rotifers may have further promoted copepod population growth by releasing them of a direct source of competition. Ultimately, the observed changes in plankton biomass and community composition may affect bivalve growth; future research should look into these feedback mechanisms.

Top-down control by *Didemnum vexillum*, a colonial invasive ascidian, was almost entirely absent. On the other hand, bottom-up effects were induced by ammonium regeneration by *Didemnum*, which was an order of magnitude higher than excretion rates for bivalves, having remarkable positive effects on the plankton community, most notably diatoms. The mechanism involved a progressive decline in Si:N ratios which was more marked for those tanks that included ascidians; not surprisingly diatoms were the taxonomic group that responded faster to bottom-up influences, since diatom growth rates are much higher than flagellates of equivalent biomass (Smayda, 1997). Furthermore, Gobler et al. (2004a) reported nitrogen limitation for certain groups of phytoplankton in this system.

An increase in diversity is often reflected in increases in ecosystem function (Hooper et al., 2005), and functional richness could increase ecosystem properties through positive interactions such as complementarity and facilitation. Most experiments with multiple benthic species showed statistically significant interactive effects between the two suspension feeders. When two bivalves were involved, these effects were in the same direction as that of either bivalve considered singly, albeit with different intensity, suggesting utilization of the same prey resources. On the other hand, when *Didemnum* interacted with a bivalve species, the interaction diverged from the effects of either species alone, suggesting complex interactive effects arising from the combination of suspension-feeding species that belong to different guilds. Meso-scale experiments like the one in this study provide useful information for modelling the significance of suspension feeders at the ecosystem scale (Asmus and Asmus, 2005).

Chapter 5 followed up on the mesocosm incubations of the previous section, and studied the residual effects of benthic suspension feeding on the growth rates of planktonic auto- and heterotrophs, and microheterotroph grazing. The goal was to explore the hypothesis that benthic suspension feeding not only has the potential to influence plankton community composition and structure, but can also have lasting effects on functional rates.

Most dilution experiments showed significant differences in phytoplankton community growth rate between treatments, while only half showed significant differences in microheterotroph grazing among treatments, suggesting that suspension feeding had lasting (residual) effects that were more pronounced on phytoplankton growth than on grazing. In general, phytoplankton growth rates were significantly lower in experiments using unaltered water from control tanks, in contrast to higher growth rates using macrobenthos-influenced water. One explanation of this can be from bottom-up influences, e.g. suspension-feeding bivalves are known to increase nutrient availability in the water column by increased rates of nutrient remineralization (Prins and Smaal, 1994), and nitrogen regeneration rates in *D. vexillum* tanks were an order of magnitude higher than bivalves. In support of the latter, the combination of ribbed mussels and the ascidian yielded significantly higher phytoplankton growth rates, in comparison to control water, evidencing lasting bottom-up effects induced by *Didemnum*. Another possible explanation invoking top-down influences relates increased phytoplankton growth rates induced by increased benthic grazing (e.g. centric diatoms, see Chapter 4), which in this case would have had lasting or residual effects.

The relationship between growth and grazing was positive for autotrophs and negative for heterotrophs. In support of the first relationship, Boissonneault-Cellineri et al. (2001) found a tight coupling between phytoplankton growth and heterotroph grazing. On the other hand, the negative relationship between microheterotroph growth and grazing can probably be related to the nutritional quality of the plankton community. Finally, these results should be interpreted cautiously, and with the thought that most planktonic microorganisms have complex trophic roles involving shifts between mixed nutritional modes (Sherr and Sherr, 2002; Cloern and Dufford, 2005).

Integrating final comments

This dissertation characterized trophic interactions between benthic suspension feeding and planktonic auto- and heterotrophic microorganisms from estuarine environments. It is apparent that the bivalve species considered were capable of exerting top-down controls on planktonic biomass, as evidenced in field and laboratory experiments with noxious phytoplankton. Even though they behaved mostly as non-selective grazers, the extent of these controls on planktonic biomass were different among the taxonomic groups considered. The mechanisms underlying such controls involve differential modulations of growth rates of the different planktonic groups considered. The invasive colonial ascidian incorporated in mesoscale experiments had positive bottom-up influences modulated through high excretion rates. As with top-down influences, planktonic species responded differentially to these bottom-up effects. Complex interactive effects arise from multi-species assemblages of benthic suspension feeders, and these are not always exerted in the same direction as those of either species singularly.

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