

Zooplankton changes associated with grazing pressure of northern quahogs (*Mercenaria mercenaria* L.) in experimental mesocosms

Darcy J. Lonsdale^{a,*}, Robert M. Cerrato^a, David A. Caron^b, Rebecca A. Schaffner^b

^a Marine Sciences Research Center, Stony Brook University, Stony Brook, NY 11794-5000, USA

^b Department of Biological Sciences, 3616 Trousdale Parkway, AHF 301, University of Southern California, Los Angeles, CA 90089-0371, USA

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Abstract

Over the last three decades, the abundance of the bivalve *Mercenaria mercenaria*, a benthic suspension feeder, has declined dramatically in the Great South Bay, Long Island, NY. This decline undoubtedly has had significant impacts on planktonic dynamics in the estuary and may be a contributing factor to the appearance of blooms of *Aureococcus anophagefferens* (brown tides) that began in 1985. We conducted three, 300-L mesocosm experiments that manipulated clam abundance in seawater containing an inoculum of *Aureococcus anophagefferens* obtained from bloom water. Within a week, differences in phytoplankton and zooplankton composition emerged between control and experimental tanks. In two of three experiments, biomasses ($\mu\text{g C L}^{-1}$) of brown tide and copepods, mostly *Acartia tonsa*, were lower in tanks with clams compared to controls while the opposite was found for ciliates. Redundancy analysis indicated that total clearance rate (L h^{-1}) by *Mercenaria mercenaria* was the single best predictor of differences in the composition of the planktonic community. The analysis also showed that the reason for increased ciliate biomass associated with clams was lower than average brown tide biomass rather than reduction in predation pressure due to lower than average copepod biomass in these same tanks. And, although food (i.e., diatoms and dinoflagellates) limitation could have contributed to low copepod abundance in tanks with clam competitors, these copepod reductions may also have resulted from direct predatory impacts of clams on early life stages (eggs and nauplii) of *Acartia tonsa*. Our findings indicate a potentially complex trophic role for *Mercenaria mercenaria* in estuarine food webs.

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1. Introduction

Benthic suspension feeders affect not only the total biomass of phytoplankton in shallow waters, but also the size-structure and taxonomic composition of the phytoplankton community. Changes in aquatic ecosystems following the introduction and establishment of exotic benthic species have been well documented and have indicated dramatic shifts in the standing stocks and food web structure of pelagic communities (Carlton et al., 1990; Caraco et al., 1997; Karatayev et al., 1997;

Bastviken et al., 1998; MacIsaac et al., 1999; Chauvand et al., 2000). Less, however, is known about changes resulting from the decline or restoration of important native species, but these impacts may also be highly significant (Newell, 1988; Dayton et al., 1995; Coen and Luckenbach, 2000; Jackson et al., 2001). For example, Noren et al. (1999) measured substantial reductions in phytoplankton biovolume (74% decrease) as water passed over a natural population of blue mussels (*Mytilus edulis* L.). Moreover, the greatest reduction in biomass occurred for cells $>12 \mu\text{m}$ in diameter.

Benthic suspension feeders may also have direct or indirect impacts on other components of the plankton, including microzooplankton and larger copepods. A decrease in tintinnid, rotifer and copepod nauplii abundances was associated with the recent invasion of the zebra mussel into the freshwater portion of the

* Corresponding author.

E-mail addresses: dlonsdale@notes.cc.sunysb.edu (D.J. Lonsdale), rcerrato@notes.cc.sunysb.edu (R.M. Cerrato), dcaron@usc.edu (D.A. Caron), rschaffn@usc.edu (R.A. Schaffner).

Hudson River, NY (Pace et al., 1998). Pearl oysters (*Pinctada margaritifera* L.) inhabiting a Polynesian lagoon obtained their greatest carbon ration from heterotrophic and mixotrophic protists whereas the dominant autotrophs, cyanobacteria, contributed little directly to their diet (Loret et al., 2000; also see LeGall et al., 1997). A decline in the abundance of the calanoid copepod *Eurytemora affinis* (Poppe) in San Francisco Bay was associated with the introduction of the Asian clam *Corbula (Potamocorbula) amurensis* (Schrenk) (Kimmerer et al., 1994; Coan, 2002). Food limitation did not appear to explain the decline because copepod egg ratios were not lower following the clam invasion. Moreover, clam feeding experiments using naupliar prey suggested that this mechanism could explain the change in the copepod population.

In mesocosm experiments, Sullivan et al. (1991) (also see Sullivan and Banzon, 1990) found an inverse relationship between copepod abundances and benthic macrofaunal numbers. Moreover, in mesocosms without a benthos, macrozooplankton such as ctenophores and fish also attained higher abundances. Two mechanisms proposed by Sullivan et al. (1991) to explain these results were the following: (1) predation by benthic organisms on planktonic larvae, and (2) competitive interactions such as between meroplankton and holoplankton.

Over the last 30 years, the decline of northern quahogs in a shallow, Long Island embayment, Great South Bay, has greatly reduced this population's filtering capacity of the Bay. Kassner (1993) estimated that the turnover time of the Bay's waters by this species increased from about 2.6 days in the 1970s to approximately 25 days by 1993. It is probable that this reduction in benthic suspension feeding altered the ecology of the plankton community in the Bay.

We have conducted mesocosm experiments that included manipulations of nutrients and *Mercenaria mercenaria* populations to investigate the physical and biological factors that may lead to the initiation of blooms of *Aureococcus anophagefferens* Hargraves et Sieburth (brown tides) that have occurred in Long Island coastal embayments since 1985 (Schaffner, 1999; Cerrato et al., 2004). Herein, we report on changes in mesozooplankton (adult copepods and copepodites) and microzooplankton (copepod nauplii and ciliates) associated with the introduction of clams to mesocosms. In addition to the grazing importance of *Mercenaria mercenaria* in the prevention of brown tides, analysis of the mesocosm data supported our hypothesis that clams had direct and indirect trophic interactions with zooplankton. For example, the presence of clams in mesocosms had a positive influence on non-loricated ciliates that was best explained by the concomitant reduction in brown tide abundance rather than lower copepod (ciliate predators) densities observed in the same tanks.

2. Materials and methods

2.1. Experimental design

Three hundred liter Nalgene® tanks, contained within a ~4.6 m diameter enclosure, were partially filled with

seawater from Coecles Harbor, Shelter Island, NY, the site of our field study. Seawater was continuously pumped into the enclosure to maintain ambient water temperature (within 1 °C) in the tanks. The mesocosms were amended with natural seawater containing *Aureococcus anophagefferens* cells collected from West Neck Bay, Shelter Island where a brown tide was occurring. The experiments were run either 8 or 10 days between late spring and early summer in 1997 and 1998. Experimental treatments ($n = 3$ for each) included manipulations of clam density and presence or absence of a pump, sediments and inorganic nutrient inputs (Table 1). Clams were added to some tanks to test the hypothesis that their suspension feeding activity could control the development of brown tide. Submersible aquarium pumps (Rio Mini 180, TAAM, Inc.) suspended at ~5–10 cm below the surface circulated water at a rate of ~450 L h⁻¹ and were utilized to minimize food depletion by the clams at the bottom of the tank. Sandy sediment was used to allow the clams to burrow, and was obtained from a Long Island beach. The sediment was rinsed with freshwater and stored dry prior to the experiment. To investigate the role of inorganic nutrients in brown tide dynamics (e.g., LaRoche et al., 1997), nutrients were added daily to some tanks, and consisted of nitrate and phosphate; the daily additions during 1997-1 were 2.5 μM NO₃⁻ and 0.16 μM PO₄⁻³; for 1998-2, initial concentrations were 2 μM NO₃⁻ and 0.2 μM PO₄⁻³, and the nutrient additions were doubled each day thereafter until the end of the experiment. This change in experimental design was for the purpose of meeting the increased nutrient requirements of the developing phytoplankton bloom. All “control” mesocosms contained only seawater from Coecles Harbor and the brown tide inoculum. The initial mean abundance of *Aureococcus anophagefferens* across all treatments in the first experiment (1997-1) was 1.4 × 10⁴ cells ml⁻¹, and was intentionally lower for the second year's experiments (6.6 × 10³ cells ml⁻¹ and 5.3 × 10³ cells ml⁻¹ for 1998-1 and 1998-2, respectively) to minimize feeding inhibition of *Mercenaria mercenaria* from contact with brown tide cells at the start of the experiment.

Initial (T_0) and final (T_f) abundances of total chlorophyll *a* and microplankton were determined using standard methods (Parsons et al., 1984; Stoecker et al., 1994). *Aureococcus anophagefferens* abundance was determined using an immunofluorescent technique (Anderson et al., 1989). To determine zooplankton abundances at the termination of an experiment, 20 L of water was removed from each tank and sieved through a 64-μm Nitex® mesh, and the contents on the mesh were preserved in ~4% formalin. Initial abundances of zooplankton for the 1998 experiments were determined by setting up two additional mesocosms concurrently with the other treatments and sampling immediately. Zooplankton were enumerated using a Wild dissecting scope.

For the community analysis (see Section 2.2), plankton population abundances at T_f were converted to μg C L⁻¹. To estimate copepod carbon, we utilized the dry weights for three life stages of *Acartia tonsa* Dana (the overwhelmingly dominant copepod species in these experiments) reported by Heinle (1966), and assumed that carbon comprised 41.6% of the dry

Table 1

Mean (± 1 s.d.; $n = 3$ for each treatment) abundances of planktonic taxa (cells mL⁻¹) and total chlorophyll *a* ($\mu\text{g L}^{-1}$) in experimental and control mesocosms on day 0 (initial) and on the last sampling day for three mesocosm experiments (1997-1, 1998-1, 1998-2)

	<i>Aureococcus</i>	Dinoflagellates	Diatoms	Total chl <i>a</i>
1997-1				
Initial	1.4 (0.1) $\times 10^4$	112.4 (20.0)	7.0 (4.5)	2.7 (0.3)
Control	1.1 (2.0) $\times 10^1$	4.9 (5.5)	3.3 (3.3)	1.7 (0.1)
Sediment + pump	2.3 (2.6) $\times 10^4$	2.8 (1.4)	4.9 (4.6)	3.5 (0.3)
Clams + sediment + pump	9.3 (5.4) $\times 10^4$	5.3 (2.8)	4.9 (2.8)	4.3 (0.5)
Inorganic nutrients	2.9 (4.1) $\times 10^2$	154.6 (134.8)	3.5 (0.7)	9.3 (3.7)
1998-1				
Initial	6.7 (0.3) $\times 10^3$	67.9 (25.7)	5.5 (2.3)	2.6 (0.7)
Control	1.3 (1.2) $\times 10^3$	6.8 (8.6)	21.5 (9.9)	1.1 (0.4)
Clams	1.9 (1.3) $\times 10^2$	1.5 (0.8)	19.4 (9.4)	2.0 (0.3)
Pump	3.9 (4.1) $\times 10^4$	4.6 (0.5)	25.6 (2.9)	1.1 (0.4)
Clams + pump	2.5 (2.2) $\times 10^1$	0.2 (0.3)	114.0 (40.7)	2.0 (0.8)
Sediment	3.2 (4.6) $\times 10^3$	159.6 (205.9)	89.0 (18.2)	4.8 (1.2)
Clams + sediment + pump	1.3 (2.3) $\times 10^1$	0.0 (0.0)	288.4 (262.6)	4.2 (4.2)
1998-2				
Initial	5.2 (1.0) $\times 10^3$	66.4 (17.1)	5.3 (2.4)	3.4 (0.3)
Control	5.9 (3.3) $\times 10^3$	2.2 (1.7)	4.0 (0.9)	1.1 (0.2)
Inorganic nutrients	7.7 (1.0) $\times 10^4$	15.9 (5.9)	165.0 (45.0)	30.0 (2.4)
Pump	6.9 (1.2) $\times 10^4$	0.5 (0.2)	18.3 (4.5)	2.4 (0.5)
Inorganic nutrients + pump	1.6 (0.8) $\times 10^5$	0.7 (0.3)	213.0 (52.0)	26.9 (7.2)
High clams + inorganic nutrients + pump	3.5 (3.4) $\times 10^1$	0.9 (1.5)	0.3 (1.0)	0.9 (0.1)
Low clams + inorganic nutrients + pump	6.3 (3.1) $\times 10^3$	1.3 (0.0)	1.4 (1.0)	1.6 (0.1)

weight (Beers, 1966). Because nauplii and copepodites were not staged in our study, we utilized the average dry weights for NIII and CIII (1.49 μg and 3.18 μg copepod⁻¹, respectively), and 7.33 μg for adults (Heinle, 1966). The carbon contents of non-loricate ciliates (4.15 ng C cell⁻¹), dinoflagellates (6.81 ng C cell⁻¹) and diatoms (2.75 ng C cell⁻¹) used in our analysis were mean values estimated for microplankton cells at three sites in the Peconic Bay estuary during May and June of 1998 (Lonsdale et al., 2006). Microplankton carbon values were based on biovolume estimates from acid Lugol's (10% final concentration) preserved cells. The biovolume conversion to cell carbon was based on the equations of Putt and Stoecker (1989), Stoecker et al. (1994), or the modified Strathman equation (Smayda, 1978) for the three microplanktonic taxa, respectively. The carbon content of *Aureococcus anophagefferens* (2.4 pg C cell⁻¹) was from Gobler and Coper (1996). In almost all cases, T_f plankton abundances were available for each tank of experiment, with two exceptions in the 1998-2 experiment. Copepod biomasses for one tank of the inorganic nutrients + pump treatment were based on the average densities of the two other tanks, and diatom biomass for each treatment was based on the average cell density of the triplicated tanks.

Total clearance rates by clams on T_f were estimated by measuring the rate of total chlorophyll *a* disappearance compared to a control (Coughlan, 1969). For the latter, we used water taken at T_f from the mesocosms with clams. The mean total clearance rates were $2.9 \pm 1.0 \text{ L h}^{-1}$ in 1997-1, $15.6 \pm 1.7 \text{ L h}^{-1}$ in 1998-1 and $16.8 \pm 2.2 \text{ L h}^{-1}$ and $7.7 \pm 1.20 \text{ L h}^{-1}$ for the high clam and low clam treatments in 1998-2, respectively. The clam density in the 1997-1

experiment (20 clams of ~ 35 mm in length) was between the low and high clam treatments in 1998-2 (15 and 30 clams, ~ 42 mm in length, respectively). However, the low clearance rate of the clams in 1997-1 was most likely due to the fact that during this experiment brown tide in clam mesocosms reached a high mean abundance (9.3×10^4 cells mL⁻¹; Table 1) demonstrated to inhibit clam feeding (Bricelj et al., 2001).

2.2. Statistical 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 (ter Braak, 1986; ter Braak and Verdonschot, 1995; ter Braak and Similauer, 2002). In this study, RDA was carried out using Canoco 4.5 (Microcomputer Power, 111 Cove Lane, Ithaca, NY 14850). Preliminary analysis of the biotic data with correspondence analysis was used to determine that gradient lengths were short, and the data were more appropriately analyzed in RDA rather than canonical correspondence analysis, a better-known direct gradient method (ter Braak and Similauer, 2002). Several techniques common in linear regression are available within Canoco's direct gradient analysis algorithm, and the following were utilized in the present study: multiple regression, stepwise regression (forward selection), and covariate analysis.

Biotic data consisted of natural log transformed biomasses ($\mu\text{g C L}^{-1}$) of six plankton groups: brown tide, diatoms,

dinoflagellates, ciliates, mesozooplankton, and copepod nauplii. Biomass, rather than abundance, was used since it was thought to better represent trophic interactions. Because biotic variables with large values can dominate RDA, we applied a log transformation so that variables with low biomass would not be obscured and would contribute to the results.

Environmental/experimental variables (i.e., explanatory variables) consisted of both nominal (categorical) variables and continuous variables. Nominal variables included experiment (i.e., 1997-1, 1998-1, 1998-2) and the presence/absence of clams, a pump, sediments, and added inorganic nutrients. Clam density and clearance rate were the only continuous variables used in the analysis.

The basic model we used to examine the relationship between community structure and explanatory variables was a fractional factorial regression model of degree 2 containing all main effects and all 2-way interactions. Variance was decomposed using a pure effects approach (Whittaker, 1984; ter Braak and Similauer, 2002) where all other main and interaction terms than the one being tested were treated as covariates. This was necessary because of the unbalanced design of the experiments and because terms involving the continuous variables covaried; both these conditions give rise to shared variance 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). The three experiments (1997-1, 1998-1, 1998-2) were treated as blocks and permutations were restricted to within blocks. A somewhat less conservative, forward selection approach was also used where main effect and 2-way interaction terms were added in a stepwise manner in the order of the magnitude of their explained variance. At each step of the analysis, previously included factors were treated as covariates, removing any shared variance between the factor being considered and those previously included.

The fractional factorial regression approach described above when applied to single and 2-factor cases with nominal variables in a balanced and complete design is equivalent to a nonparametric analysis of variance. To test this approach against a more widely used method, we analyzed subsets of our experimental results for combinations where it was possible to obtain a balanced, complete, 1- or 2-way design with nominal variables. Analysis results were compared to the results obtained by PERMANOVA, an implementation of a nonparametric multivariate analysis of variance written by Anderson (2005). Outcomes were identical in terms of significant main effects and interactions, although the F - and P -values differed. This comparison also suggested that significant differences observed in the present study were not due to changes in the correlation structure created by the permutation approach, since PERMANOVA is not sensitive to differences in correlation (Anderson, 2001). PERMANOVA was not used extensively in the present study because it is limited to completely balanced designs.

3. Results

3.1. Plankton abundance in mesocosms

At the termination of both 1998 experiments, a visual inspection of the data suggested substantial variation in copepod abundance among treatments. For mesocosm experiment 1998-1, a lower mean density of copepodites and adults compared to the appropriate control was found in two out of the three treatments containing clams (Fig. 1). Copepod naupliar abundance was lower in all tanks containing clams (Fig. 1). Decreased densities of copepods and, in particular, copepod nauplii in presence of clams in the 1998-2 experiment were noted (Fig. 2). In contrast, the 1997-1 experiment showed no

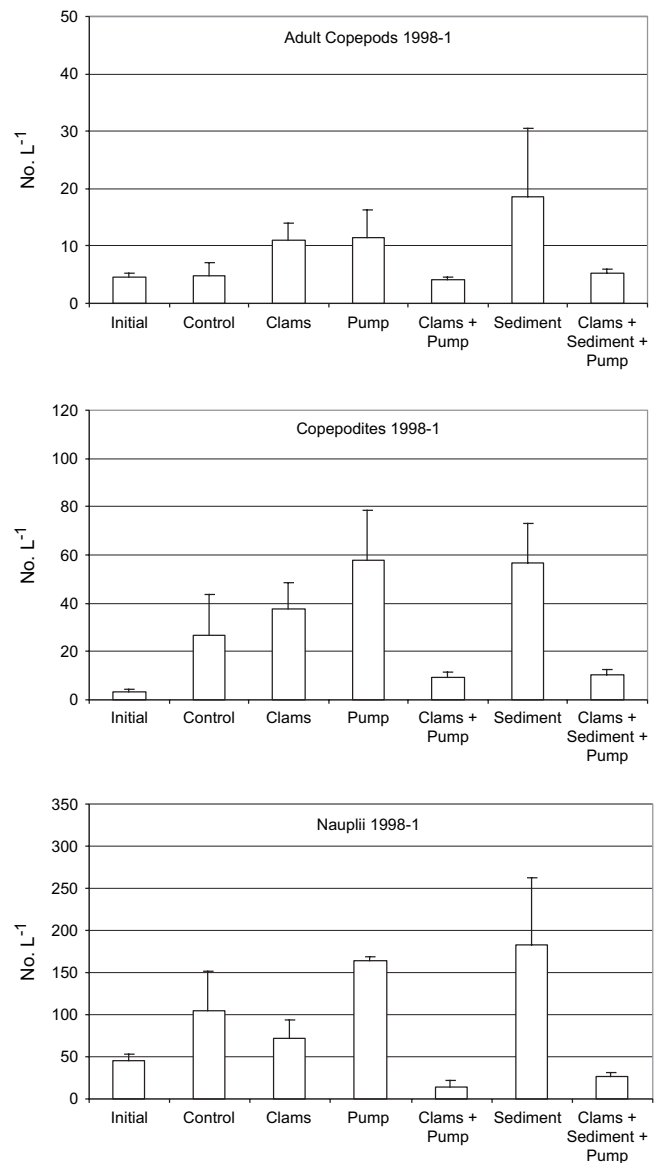


Fig. 1. Initial and final (T_f) abundances (no. L^{-1} ; mean + 1 s.d.) of copepod life stages in mesocosm experiment 1998-1. Clam treatments consisted of 30 adult *Mercenaria mercenaria* per mesocosm and the experiment was terminated on day 8.

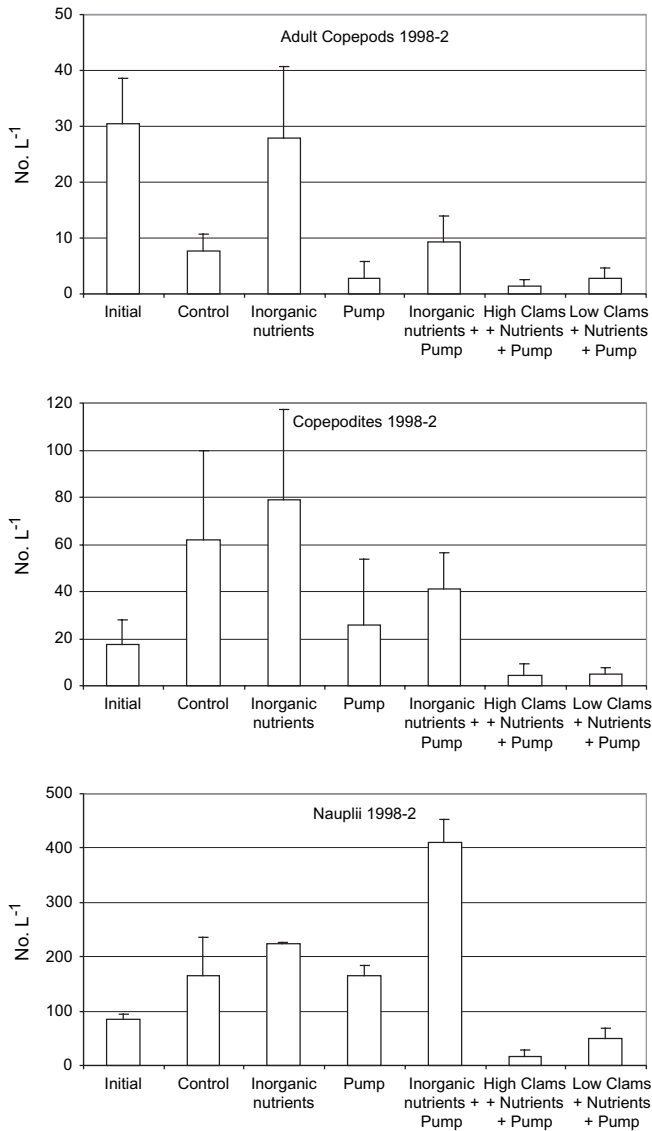


Fig. 2. Initial and final abundances (no. L⁻¹; mean + 1 s.d.) of copepod life stages in mesocosm experiment 1998-2. High clam treatments were 30 adult *Mercenaria mercenaria* per mesocosm and 15 in the low clam treatment. The experiment was terminated on day 10.

apparent relationship between the presence of clams and copepod abundance (Fig. 3). During the 1998 experiments, clams were clearing the water at a higher rate compared to the 1997-1 experiment (see Section 2).

The final abundances of ciliates (composed almost entirely of non-loricate species) were opposite to that of the copepods (Fig. 4). Ciliate abundances were higher in tanks containing clams (Fig. 4) and low *Aureococcus anophagefferens* abundances (Table 1). There appeared to be no relationship of ciliate abundance with food availability as estimated from total chlorophyll *a* (Table 1).

3.2. Statistical analysis

In the RDA analysis of all three experiments (1997-1, 1998-1, and 1998-2), about 19% of the variation in the

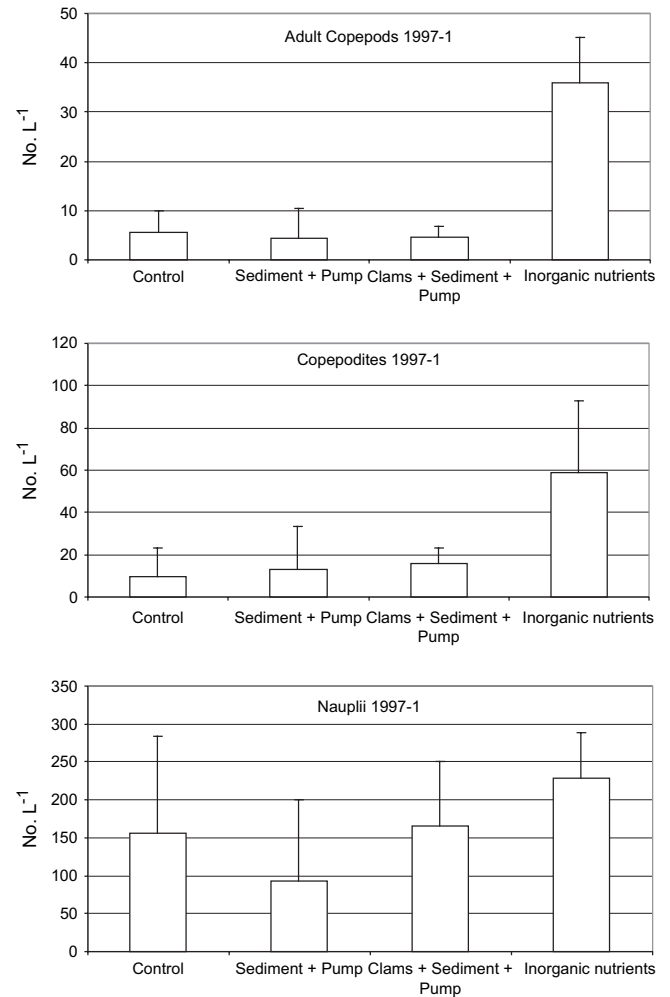


Fig. 3. Final abundances (no. L⁻¹; mean + 1 s.d.) of copepod life stages in mesocosm experiment 1997-1. Mesocosms with clams contained 20 adult *Mercenaria mercenaria* and the experiment was terminated on day 10.

plankton data was due to experimental differences (Table 2). One major difference between 1997-1 and either experiment conducted in 1998 was the initial (T_0) concentration of *Aureococcus anophagefferens*. The average T_0 cell concentration for 1997-1 was substantially higher compared to the 1998 experiments (Table 1). It is possible that this difference in *Aureococcus anophagefferens* abundances resulted in initial differences in clam clearance rate. In a laboratory study, Bricelj et al. (2001) determined that toxic isolates of brown tide inhibited feeding by *Mercenaria mercenaria* when cell abundances reached 3.5×10^4 to 5.0×10^4 ml⁻¹. In the field, the growth rate of juvenile clams was negatively affected at a density as low as 2.0×10^4 *A. anophagefferens* cells ml⁻¹ (Wazniak and Glibert, 2004; but see Greenfield and Lonsdale, 2002).

Total clam clearance rate was the best single descriptor of the impact of clams on the plankton community structure. As a single main effect, clearance rate explained 18.5% of the total variance in community structure, while presence/absence of clams and clam density explained 12.5% and 15.9%, respectively. Using the fractional factorial model with all main and 2-way interactions included, the residual variance for

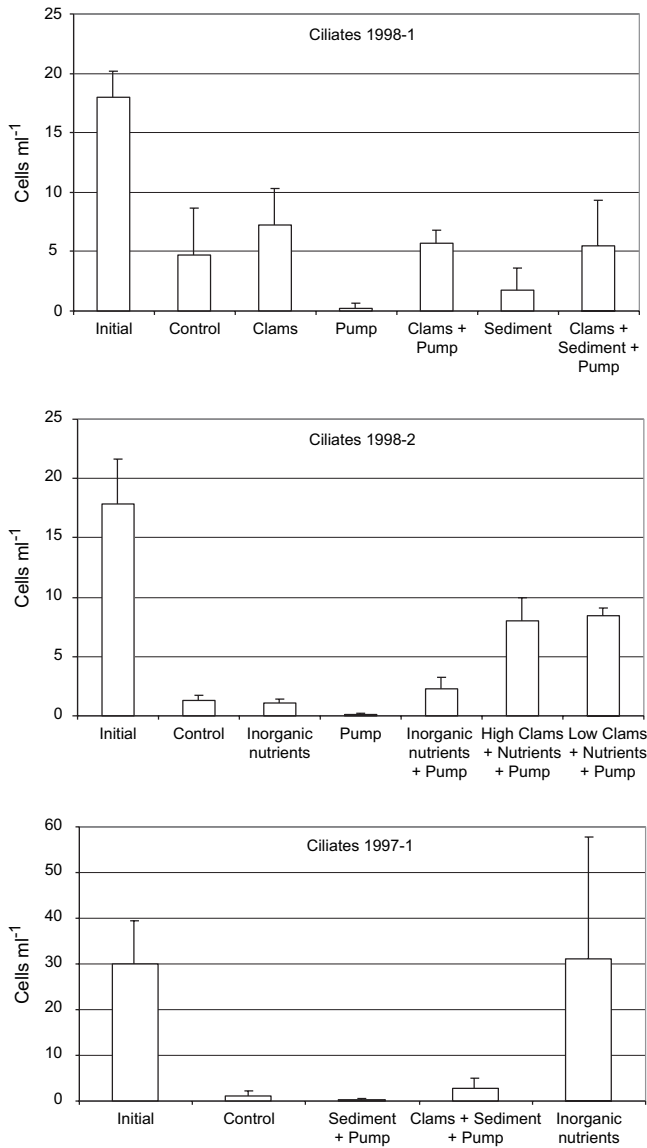


Fig. 4. Initial and final abundances (cells ml^{-1} ; mean + 1 s.d.) of ciliates, mostly non-loricated cells, in mesocosm experiments (1997-1, 1998-1, 1998-2). Note y-axis is different in panel 3.

models with clearance rate, presence/absence of clams, and clam density as explanatory variables was 23.5%, 28.3%, and 27.0% of the total variance, respectively. Because of the smaller unexplained variance, only analysis results using clearance rate will be presented in detail for the remainder of this section.

Plankton community structure was significantly affected by clam clearance rate, presence/absence of a pump, nutrient additions, sediments and most interactions between these factors (Table 2). The fractional factorial regression model containing these factors explained 57.2% of the total variance. The clearance rate \times sediment interaction was the only non-significant factor; however, pure effects variances were too small to adequately test nutrient and sediment main effects and the nutrients \times sediment interaction. In the less conservative forward selection analysis, all main effects, including nutrients

Table 2

Sum of squares decomposition of the effect of experiment, clam clearance rate, and other environmental variables on plankton community structure based on a fractional factorial model of degree 2. Total sum of squares (SS) equaled 528.29 and has been normalized to 1

	df	Normalized SS	F	P
Experiment (blocks)	2	0.187		
Shared		0.300		
Clearance rate	1	0.061	9.262	<0.001
Pumps	1	0.088	13.391	<0.001
Nutrients	1	—	—	—
Sediments	1	—	—	—
Clearance rate \times pumps	1	0.031	4.766	<0.001
Clearance rate \times nutrients	1	0.053	8.148	<0.001
Clearance rate \times sediments	1	0.006	0.940	0.409
Pumps \times nutrients	1	0.021	3.248	0.012
Pumps \times sediments	1	0.018	2.818	0.025
Nutrients \times sediments	1	—	—	—
Residual	36	0.235		

and sediments, and all interactions except for clearance rate \times sediment and nutrients \times sediments were selected (Table 3). Overall, the two analyses agreed reasonably well and suggested that all main effects and all interactions except clearance rate \times sediment and nutrients \times sediment were significant.

The RDA ordination diagram (Fig. 5) is complicated by the presence of significant interactions, but major features can be identified, including a strong negative relationship between clam clearance rate and brown tide biomass. With a pump present (Fig. 5C), clearance rate had a negative effect on brown tide, diatoms, nauplii, mesozooplankton, and dinoflagellates. Ciliates were, however, positively associated with increased clearance rate. Pumps were utilized for continuous delivery of food particles to clams, and hence, to enhance their feeding. Without a pump, clam clearance rate had a much diminished effect on plankton composition (Fig. 5A). When nutrients were added, clearance rate again had a negative effect on brown tide, diatoms, nauplii, mesozooplankton, and dinoflagellates, and a positive effect on ciliates. When no nutrients were added, clearance rate had a stronger negative impact on

Table 3

Forward selection results examining the effect of clam clearance rate, pump, nutrients, and sediments on plankton community structure based on a fractional factorial model of degree 2. Experiments (1997-1, 1998-1, 1998-2) were treated as covariables and their effect was removed prior to selection. Non-significant terms involving sediment are not shown. Total sum of squares (SS) equaled 528.29 and has been normalized to 1

	Added SS	Cumulative SS	F	P
Clearance rate	0.185	0.185	12.975	<0.001
Pump	0.116	0.301	9.748	<0.001
Clearance rate \times nutrients	0.098	0.399	9.896	<0.001
Nutrients	0.070	0.469	8.300	<0.001
Clearance rate \times pump	0.034	0.502	4.366	<0.001
Sediments	0.035	0.537	4.907	0.003
Pump \times nutrients	0.017	0.554	2.526	0.019
Pump \times sediment	0.017	0.572	2.638	0.021

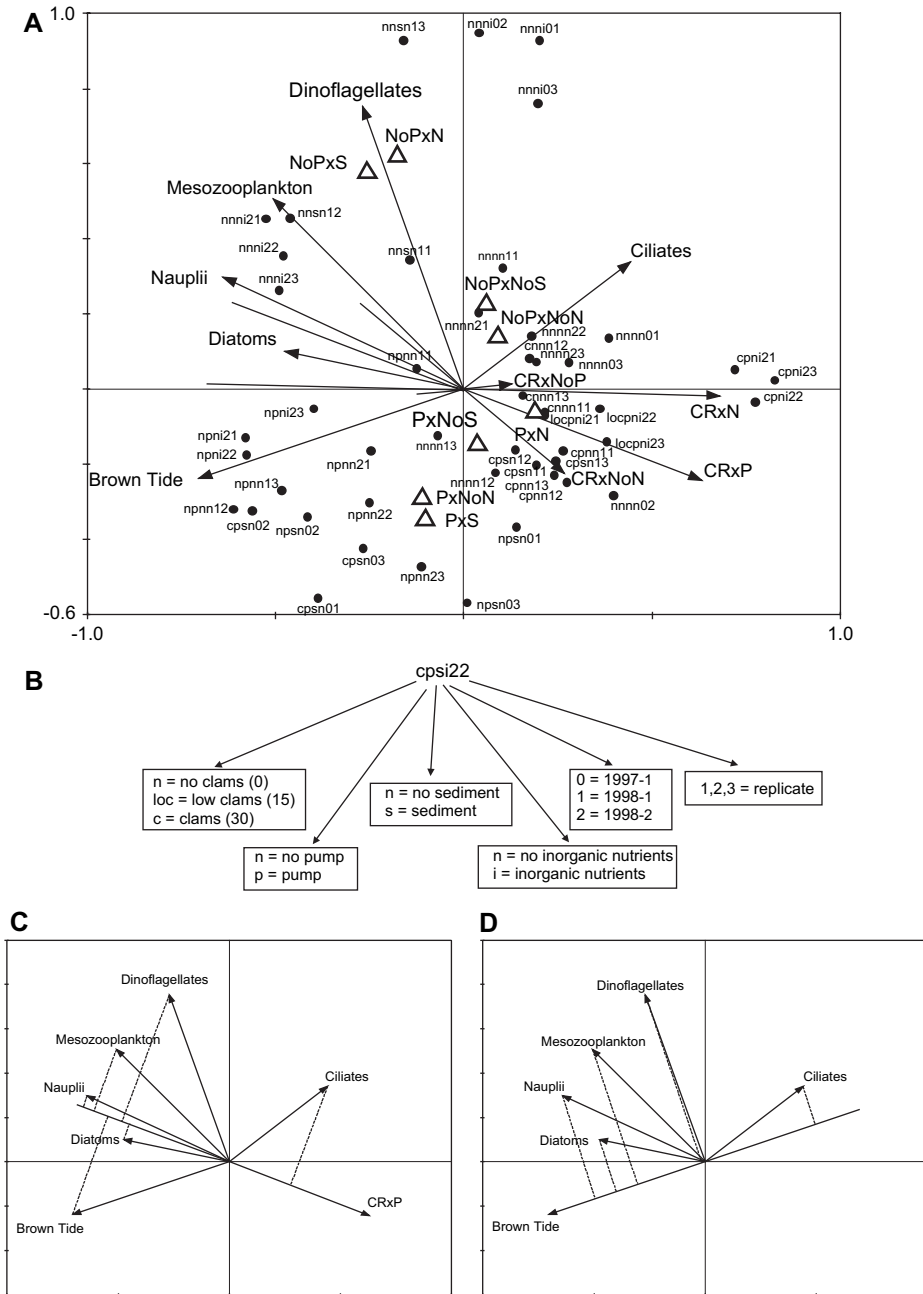


Fig. 5. Results of the RDA analysis showing the relationship between plankton community structure and explanatory variables. (A) Correlation biplot with sample scores for individual mesocosms plotted as points and planktonic taxa plotted as vectors. Continuous explanatory variables are depicted as vectors, and nominal variables are plotted as triangles. Differences between experiments (1997-1, 1998-1, and 1998-2) have been removed by treating them as covariates. For vectors, the arrowhead represents high, the origin average, and the tail (when extended through the origin) low values. Arrows always point in the direction of increase. Projections of sample points onto an individual taxa vector approximate the log transformed biomass values of each sample for that taxon. For nominal environmental variables, the plotted point is the centroid of all the sample points that have that treatment characteristic. CR = clearance rate, P = pump, and N = nutrients, S = sediments. (B) Legend for identifying treatment combinations of individual samples. (C) Effect of total clearance rate in the presence of a pump on the biomass of each plankton group. Diagram was derived from (A) but with unneeded detail removed and projections from each taxon to the CR × P vector added. Distance of a projection from the origin is proportional to the magnitude of the effect. A projection on the arrowhead side indicates a positive effect and on the tail side a negative effect. Note especially the strong negative relationship between total clam clearance rate and brown tide biomass. (D) Correlation between brown tide and each of the remaining planktonic taxa. Diagram was derived from (A) but with unneeded detail removed and projections from each taxon to the brown tide vector added. Distance of a projection from the origin is proportional to the magnitude of the correlation. A projection on the arrowhead side indicates a positive correlation and on the tail side a negative correlation.

mesozooplankton and dinoflagellates relative to the nutrient addition treatment. Brown tide was still negatively associated with high clearance rates, but the effect of clearance rate was not as strongly negative as in the nutrient addition treatment. Ciliates were still positively associated with high clearance rate, but the positive effect of clearance rate was weaker without nutrients. The negative relationship between clearance rate and diatoms or nauplii was unchanged between the presence and absence of nutrients.

Brown tide was positively associated with above average biomass of diatoms, nauplii, and mesozooplankton and negatively related to ciliates (Fig. 5D). There was little relationship between brown tide and dinoflagellate biomass. It is also notable that the association between ciliates and diatoms or nauplii was negative and almost opposite to that of brown tide. Ciliates were positively associated with dinoflagellates and unrelated to variations in mesozooplankton biomass.

Diatoms, nauplii, mesozooplankton, dinoflagellates, and ciliates were all positively associated with the absence of a pump (Fig. 5A). For diatoms, nauplii, mesozooplankton, and dinoflagellates this positive association was especially strong in the presence of nutrients or sediment. Only brown tide was positively related to the presence of a pump, although the positive effect was clearly evident only for treatments without clams.

4. Discussion

The results of this study indicate that *Mercenaria mercenaria* had significant effects on the structure of planktonic food webs that included direct and indirect interactions with other grazers, specifically copepods and ciliates. Our findings were in agreement with several studies, including Sullivan and Banzon (1990), Sullivan et al. (1991), and Kimmerer et al. (1994), in that a negative relationship between benthos and copepods was found. Food limitation of copepod populations, which could be caused by competition with *Mercenaria mercenaria* suspension feeding, may in part explain the inverse relationship between these populations in the mesocosm experiments. For example, *Acartia tonsa* females demonstrated 50% mortality when starved for more than 6 days (Dagg, 1977). The RDA analysis revealed that diatom and dinoflagellate biomasses, potential food sources for copepods were positively associated with mesozooplankton and naupliar biomasses (Fig. 5A). On the other hand, the higher than average biomass of ciliates, also a common food source for copepods such as *Acartia tonsa* adults and nauplii (Stoecker and Egloff, 1987; Stoecker and Capuzzo, 1990; Lonsdale et al., 1996) in clam tanks suggests that food limitation may not be the sole explanation for lower copepod abundance in tanks with clams.

The RDA analysis also revealed that clam filtration had a direct, negative impact on abundances of nauplii and mesozooplankton in our experiments. Above average biomasses of nauplii and mesozooplankton (adult copepods and copepodites) were associated most strongly with below average total clam clearance rate (Fig. 5C). These two zooplankton groups did not appear to be adversely affected by brown

tide; mesozooplankton and nauplii biomass showed a positive relationship with brown tide biomass (Fig. 5D). Nauplii are not likely to grow on brown tide. Copepod nauplii fed only a bloom abundance of *Aureococcus anophagefferens* exhibited a mortality rate equivalent to nauplii held in filtered seawater (Lonsdale et al., 1996). Thus the positive relationship between these two taxa cannot be explained by a trophic relationship.

An alternate explanation for the inverse relationship between copepods and clam clearance rate is that *Mercenaria mercenaria* exerted direct predatory impacts on the early life stages of copepods that were overwhelmingly dominated by *Acartia tonsa*. For example, the blue mussel, *Mytilus edulis*, has been shown in laboratory and field studies to capture and digest crustacean zooplankton (Davenport et al., 2000; Lehane and Davenport, 2002; also see Zeldis et al., 2004). Adult *Mercenaria mercenaria* have been shown to filter their larvae (pediveligers ~170–220 µm) and although the larvae were found to be diverted to the pseudofeces, they did not survive the entanglement (Kurkowski, 1981). *Acartia tonsa* are egg broadcasters and the eggs are around 70 µm in diameter. Early naupliar stages of *Acartia tonsa* are also within the pediveliger size range (NI = 94 µm to NVI = 211 µm in length at 25 °C; Heinle, 1966).

Our experiments further suggest that clams had a positive effect on ciliates. This latter outcome could reflect the release of ciliates from copepod predation pressure in the tanks with clams. In Long Island waters during the summer, predation by copepod nauplii, copepodites and adults resulted in significant mortality of ciliate populations, sometimes $>1.0 \text{ d}^{-1}$ (Lonsdale et al., 1996). However, the RDA indicated little relationship between mesozooplankton and ciliates in our experiments. Also associated with the presence of clams in mesocosms was lower than average biomass of *Aureococcus anophagefferens* (1998-1 and 1998-2 experiments). Some field and laboratory studies have shown a decline in ciliate abundance and population growth rate during brown tides (Lonsdale et al., 1996; Caron et al., 2004). The RDA indicated that the primary factor associated with the increased ciliate biomass in tanks with clams was lower than average brown tide biomass (Fig. 5D). Despite the presence of a pump, which was related to lower than average ciliate biomass without clams, above average ciliate biomass developed when clams were also present. Noted also was that in the 1997-1 experiment, brown tide developed in tanks with *Mercenaria mercenaria* and ciliate abundance was lowest in that treatment (Table 1, Fig. 4). We recognize, however, that in other experimental situations or in nature where brown tide is not an overwhelming environmental factor, we cannot predict from these mesocosm experiments what net effect the presence of clams would have on ciliates. In such a situation without brown tide, a reduction in copepods from clam predation could have a pronounced, positive influence on ciliate populations. On the other hand, predation by clams and other bivalves also has the potential to regulate ciliate populations in the absence of brown tides.

The nutritional role of heterotrophic prey in the diets of many shellfish, including *Mercenaria mercenaria*, is poorly

known (Grizzle et al., 2001). When investigated (e.g., LeGall et al., 1997; Loret et al., 2000), however, it appears that heterotrophic prey may contribute significantly to bivalve nutrition and energy budgets. Wong et al. (2003) found that zebra mussels ingested and assimilated rotifers in the laboratory, and calculated that in the Hudson River, NY, rotifers could contribute substantially to this bivalve's scope for growth. Baldwin and Newell (1991) found that heterotrophic prey, including flagellates and ciliates, were consumed by eastern oyster (*Crassostrea virginica* Gmelin) larvae, and concluded that phytoplankton availability alone may not be "a reliable measure of food supply" (p. 285). Any model that addresses the effects of *Mercenaria mercenaria* restoration on planktonic communities and "feedback" responses to the stock needs to include trophic linkages to zooplankton.

5. Conclusions

Much remains to be studied about the potential trophic interactions between *Mercenaria mercenaria* and zooplankton in shallow estuaries. Our research focus has been to better understand what potential effects the restoration of *Mercenaria mercenaria* to Long Island Bays may have on plankton dynamics. There is mounting evidence that in nature increased abundance of benthic organisms, and in particular of bivalves, can also result in dramatic changes in zooplankton composition and abundance (Kimmerer et al., 1994; Pace et al., 1998; Davenport et al., 2000; Lehane and Davenport, 2002; Zeldis et al., 2004). Zooplankton declines may be linked directly to predatory feeding behavior in benthic animals as originally proposed by Sullivan et al. (1991). We have also found, however, positive effects on some zooplankton (ciliates) that may result from the ability of clams to prevent the formation of harmful brown tides.

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