Testing and Application of Biomonitoring Methods for Assessing Environmental Effects of Noxious Algal Blooms.

Gregory A. Tracey
Science Applications International Corporation
c/o U.S. Environmental Protection Agency
Environmental Research Laboratory- Narragansett
Narragansett, Rhode Island 02882

Richard L. Steele
Jennifer Gatzke
Donald K. Phelps
U.S. Environmental Protection Agency
Environmental Research Laboratory- Narragansett
Narragansett, Rhode Island 02882

Robert Nuzzi
Mac Waters
Suffolk Co. Dept. Health Services
Bureau of Marine Resources
Riverhead County Center, New York 11901

Donald M. Anderson
Woods Hole Oceanographic Institution
Woods Hole, Massachusetts 02543

INTRODUCTION

A major goal of the U.S. Environmental Protection Agency's Biomonitoring research program is to produce test methods to assess environmental effects of anthropogenic activities in marine waters (Phelps *et al.*, 1987). In support of EPA Region II involvement in the "Brown Tide Comprehensive Assessment and Management Program" (Minei, 1989), we are evaluating biomonitoring methods for application in marine waters having a history of noxious algal blooms. Activities associated with two methods are in their initial development phases. A third activity involves the use of an established biomonitoring method in the Peconic Bays system of Long Island, New York. This area has had

repeated "brown tide" bloom events since 1985, with devastating impacts on shellfish and eelgrass populations (Bricelj *et al.*, 1987; Cosper *et al.*, 1987).

Three primary objectives of this research are to determine: 1) whether existing environmental conditions are pre-disposed to development of a brown-tide bloom; 2) whether existing characteristics of suspended particulate matter, including the constituent algae, have an adverse effect on the nutrition of bivalves; and 3) whether environmental conditions at selected stations have an adverse effect on bivalve growth and physiological performance. Results pertinent to the latter two objectives are reported here in sections on mussel (Mytilus edulis) clearance (feeding) rate bioassays and mussel transplants, respectively. Progress of work relating to the first objective are reported in Steele *et al.*, 1989.

MATERIALS AND METHODS

Environmental Characteristics

Phytoplankton samples were collected at a depth of 0.3 m in 125 ml glass bottles, preserved with 4-5 drops of Lugols iodine solution (acetic acid preparation, UNESCO, 1978) and refrigerated until analyses could be performed. Cells were settled in 10 cm^3 sedimentation chambers for 18-24 hr, and counted using a Nikon model MS inverted microscope (after Utermohl, 1958). Cell counts on from 5 to 10 fields (depending on the number of cells present) were performed at a magnification of 600X. Depression slides were prepared occasionally and examined at 1500X under immersion oil to assist identifications. Features used to differentiate *Aureococcus* from other forms included; its size (2.0-3.5 μm diameter), coccoid shape (appearing somewhat irregular when preserved), and a cup-shaped chloroplast (Sieburth *et al.*, 1988). Total phytoplankton counts included "small forms" (cells < 5 μm diameter) other than *Aureococcus*. This category also may have included chroococcoid cyanobacteria since a fluorescence system was not available for further differentiation. In some cases, additional samples were counted by the immunofluorescence method of Anderson *et al.*, 1989 for verification of *Aureococcus* concentration. Chlorophyll *a* determinations were performed by methods of Strickland and Parsons (1972). Whole water samples were filtered in duplicate onto glass fiber filters and stored immediately on dry ice until analyses could be performed.

Mussel Clearance Rate Bioassays

Clearance rate bioassays were conducted in order to assess whether the constituent particulates would have adverse effects on mussel feeding. Mussels were collected from a subtidal population in lower Narragansett Bay (71° 24.0' W by 41° 29.4' N) and exposed to sub-surface water samples collected from Peconic Bay weekly to bi-weekly from June through September, 1988 (Fig. 1A). Recurrent brown tide blooms in the Long Island bays precluded the use of local mussel stocks. This was not foreseen as problematic, however, since the study emphasis was on elucidating relative differences between stations caused by localized environmental conditions. Additional water samples collected from Great South Bay (Fig. 1B) on about a monthly basis were also assayed using the mussel clearance rate test.

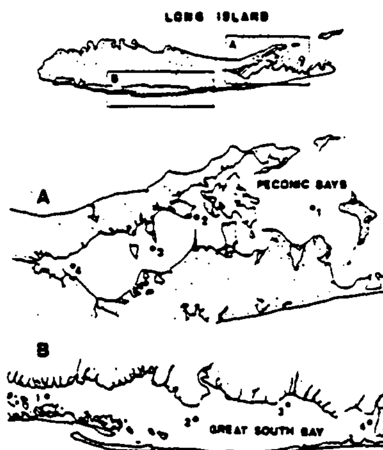

Figure 1. Sampling locations in A) Peconic Bays and B) Great South Bay, New York.

In additional experiments, mussels were fed various combinations and densities of natural particulates, the chrysophytes *Isochrysis galbana* (clone T-iso) and *Aureococcus anophagefferens*, or the diatom, *Minutocellus polymorphus*. The algae were cultured in "PES" nutrient media (McLachlan, 1973) and harvested from log-phase cultures prior to feeding experiments. *Isochrysis*, a commonly used food source for bivalves (Ewart and Epifanio, 1981), was of sufficient size (5-7 μm diameter) to allow its clearance to be measured independently of picoplankton within a mixed diet. Concentration and size-frequency

distribution of particles were measured using an electronic particle counter (Coulter Electronics, Hialeah, FL) equipped with a 50 or 100 μm aperture and calibrated with known-sized polystyrene spheres.

Bioassay mussel clearance rates were determined by placing animals individually into 1000 ml beakers (V) containing natural particulates or experimental diets at 20 °C for the determination of clearance rate. Concentrations of particles (C) greater than 4 μm were measured at 15 min intervals (T) for 1 hour. This size range was selected because effects on clearance rates due to particle size are removed, i.e. retention efficiency is 100% (Vahl, 1972; Møhlenberg and Riisgård, 1978; Silvester and Sleigh, 1984). Clearance rates (CR, ml min^{-1}) were determined after Coughlan (1969);

$$1.)\ CR = (\log C_1 - \log C_2)/(T_2 - T_1) \times V,$$

where the change in particle concentration vs. time (= d(log C)/dT) was determined by linear regression (Snedecor and Cochran, 1980). Data were inspected graphically to include only the linear portion of each curve (i.e. constant clearance rate). Clearance rates of 5 mussels were measured and averaged (± 1 S.D.) in all experiments for reporting of results. Cell concentrations in treatments containing cultured algae were maintained within 1-2 x 10^4 cells ml^{-1}, a concentration range at which clearance rates of mussels are independent and maximal yet pseudofeces production is minimal (Tenore and Dunstan, 1973; Foster-Smith, 1975).

Mussel Transplants

Mussels were transplanted in cages to sub-surface buoys at selected stations to determine whether existing environmental conditions would have adverse effects on growth (Phelps and Galloway, 1980; Phelps *et al.*, 1987). After size selection (4.9-5.1 cm) and measurement (± 0.02 cm), four replicate cages containing 10 mussels per cage were deployed in mid-July at a depth 1 meter from the bottom for 30 days. After retrieval, growth of shell (anterior-posterior length) and physiological parameters were assessed in the laboratory. Physiological measurements were made under standardized conditions of algal food (*Isochrysis galbana*, 5 x 10^4 cell ml^{-1}) and water such that any differences observed between stations could be directly attributed to environmental conditions encountered by the animals when in the field.

For the determination of clearance rate, water containing algae was passed through 500 ml exposure vessels containing mussels at 75 ml min^{-1}. Particle concentrations entering (C_1) and leaving (C_2) the exposure vessel were measured at 30 min intervals, after a 30 min acclimation period. Clearance rates, defined as the volume of water swept clear of particles per unit time assuming 100% retention, were determined by the formula of Hildreth and Crisp (1976);

$$2.) \quad CR = ((C_1 - C_2)/C_2) \times F/n$$

where F is the flow rate through the chamber, and n = number of animals in the exposure vessel (i.e. 1 in all cases).

The assimilation efficiency of food ingested by mussels was determined by the methods of Conover (1966). Feces were collected from individual exposure chambers after sieving with a 100 μm mesh to exclude pseudofeces and other non-ingested particulates. Respiration rates were determined by the methods of Widdows *et al.* (1981). Mussels were placed individually into air-tight chambers supplied with food as in clearance rates measurements. The decline in oxygen concentration over time after discontinuing water flow was monitored by O_2 electrode until a 25% reduction in O_2 saturation was observed. Scope for growth, the energy available for growth after maintenance requirements are met, was calculated from the formula of Warren and Davis (1967). The following energy conversions were applied in calculating scope for growth:

1 mg algae = 36.6×10^6 cells = 14.35 Joules (this study),
1 ml O_2 respired = 20.08 Joules (Crisp, 1971).

Food alga energy content was determined by the wet oxidation method of Maciolek (1962). Procedures used here for physiological measurements and the calculation of the scope for growth index have also been used in other studies (Widdows *et al.*, 1981; Martin *et al.*, 1984). Differences between station means were tested by analysis of variance (Snedecor and Cochran, 1980) based on 10 replicates per station.

RESULTS

Environmental Characteristics

Sub-surface water temperatures at sampling stations in the Peconic Bay system ranged from a minimum of 16 °C in early June to a maximum of about 27 °C in early August (Fig. 2A). Station 1 temperatures were 2-3 °C cooler over the course of the summer than at inshore stations, while inshore stations were not markedly different from one another. Differences in salinities among stations varied less than 2 ppt., and generally increased over time from about 28 to 31 ppt. (Fig. 2B). Water column turbidity as indicated by Secchi disk depth was not appreciably different among stations, with exception of station 1 during early summer (Fig. 2C). Chlorophyll *a* concentrations (Fig. 2D) increased steadily at all stations from early June to mid-July.

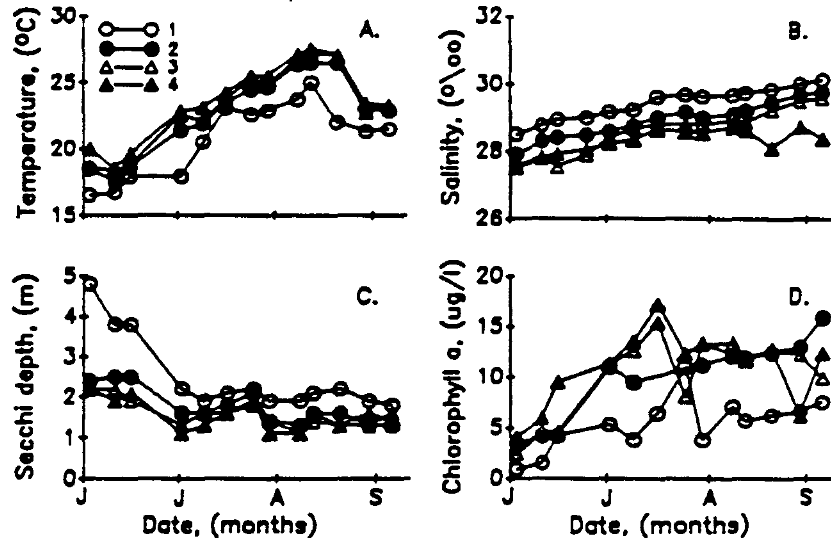

Figure 2. Temperature, salinity, water column turbidity (measured by Secchi disk depth) and chlorophyll *a* concentration at 4 stations in the Peconic Bay system, June - September, 1988.

Total phytoplankton (< 5 μm) concentration in the Peconics remained at relatively low levels through mid-June at all stations (Fig. 3A). Abundances increased markedly in late June-early July, declined in late-July, then increased steadily through the remainder of summer. In contrast, optical (hemocytometer) counts of *Aureococcus* revealed minimal densities through June and July, then increased significantly during early August (Fig. 3B). Similar

bloom concentrations were not observed at the outer-bay station in Gardiner's Bay (sta. 1). In Great South Bay near Blue Pt. (sta. 3), *Aureococcus* concentrations (determined by immunofluorescence) on 6 July and 25 July were 1.5 and 1.3 x 10^5 cell ml^{-1}, respectively. On August 1, optical examination of water samples did not indicate *Aureococcus* cells at any location.

Dynamics of phytoplankton populations within the Peconics also were investigated by following size-frequency distributions of suspended particulates over time. On 1 June, populations at all stations were dominated by cells in the 2 μm diameter range (Fig. 4). Two weeks later (13 June), the populations tended towards dominance by 4 μm diameter cells. By 6 July, this condition was evident at all stations and was accompanied by an increase in total phytoplankton numbers (Fig. 3A). A significant shift in size distributions again occurred by 21 July, when populations were dominated by 2-2.5 μm diameter cells. Shortly thereafter, a bloom of *Aureococcus* became evident in the Peconics (Fig. 3B).

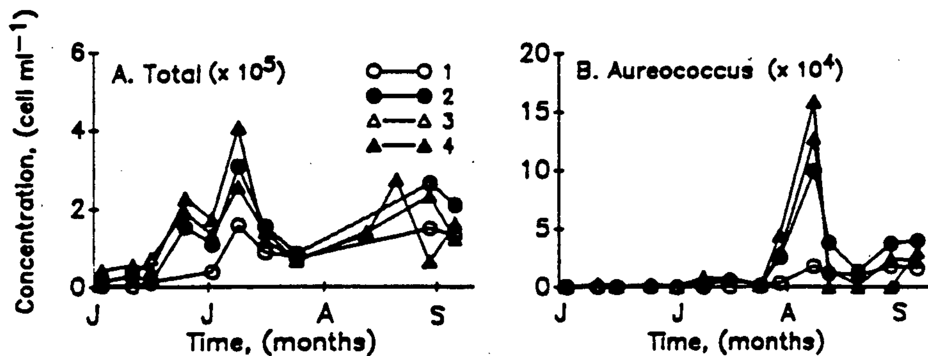

Figure 3. Concentrations of A) total phytoplankton and B) *Aureococcus anophagefferens* in Peconic Bay waters, June - September, 1988. See Figure 1 for station locations.

Mussel bioassays

Mussel clearance of ambient particulates collected from Peconic Bay stations generally declined from early June values of 40-70 ml min^{-1} to below 20 ml min^{-1} by mid-July (Fig. 5). Clearance rate reductions were most pronounced at mid-bay stations from 21 July to 17 August. Mussel clearance of Great South Bay particulates were comparable among stations, being minimal in mid-June and early August, but increased significantly in tests conducted in early September (Fig. 6).

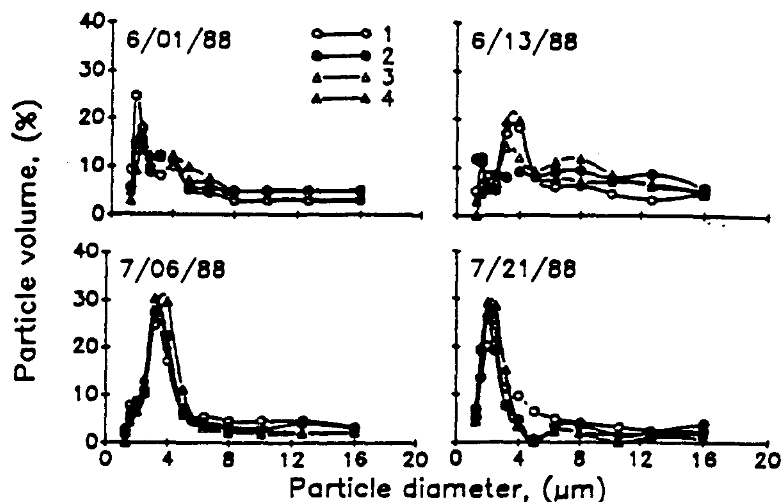

Figure 4. Size distribution of ambient particulates in Peconic Bay waters, June - September, 1988.

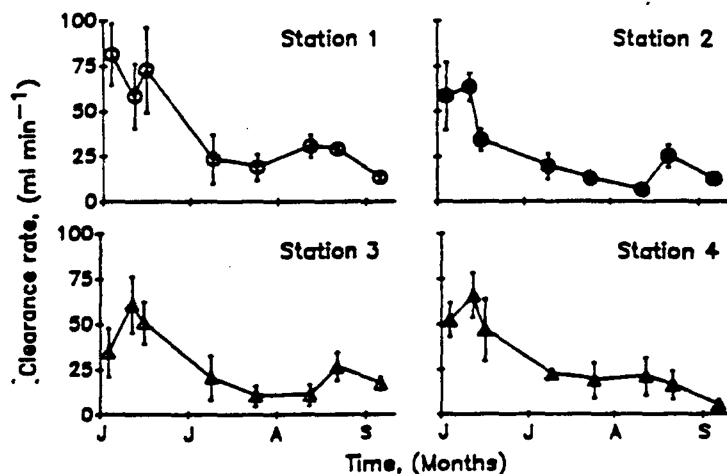

Figure 5. *Mytilus edulis*. Clearance rates of mussels fed ambient particulates collected from Peconic Bay waters, June - September, 1988.

Relationships between *Aureococcus* densities and mussel clearance rates were examined. In response to diets formulated by dilution of Great South Bay water of known *Aureococcus* concentration (determined by immunofluorescence), an inverse exponential relationship was observed over a concentration range

of about 2-15 x 10^4 cell ml^{-1} (Fig. 7). This relationship was unaffected by addition of test algae (T-iso) to the diet.

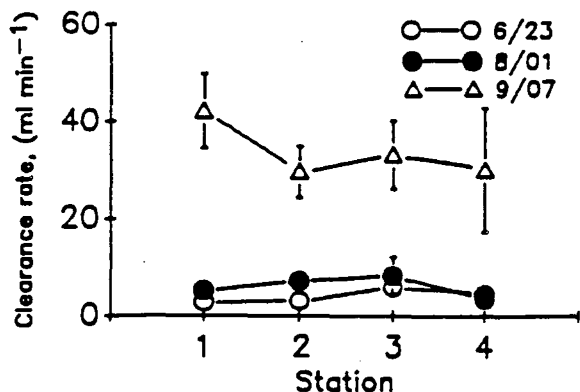

Figure 6. *Mytilus edulis*. Clearance rates of mussels fed ambient particulates collected from Great South Bay waters, June - September, 1988.

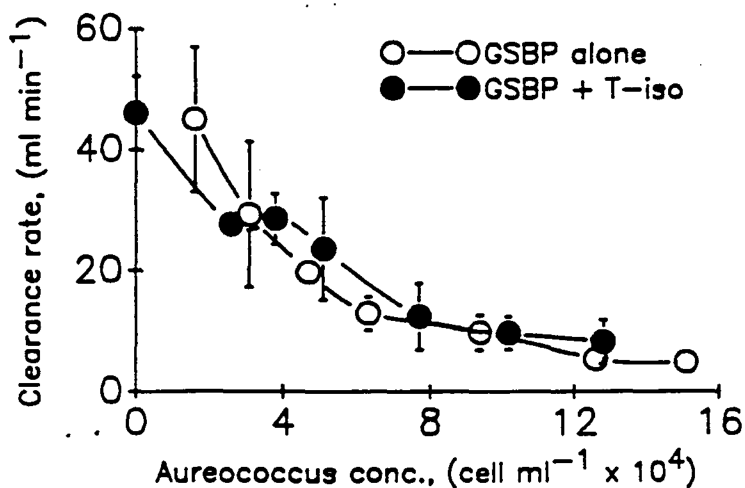

Figure 7. *Mytilus edulis*. Clearance rates of mussels fed Great South Bay particulates (GSBP) containing *Aureococcus anophagefferens* alone or in combination with the cultured food alga, *Isochrysis galbana* (T-iso).

Dose-response relationships for Peconic Bay-derived *Aureococcus* were evaluated from a scatter diagram of data pooled from mussel bioassay and cell concentration monitoring (hemocytometer counts) throughout the summer. The observed relationship also exhibited inverse exponential characteristics over

a concentration range of about 0.5-4 x 10^4 cell ml^{-1} (Fig. 8). Dose-response relationships using cultured *Aureococcus* of known concentration were examined. In two experiments using different batch algal cultures, density-dependant clearance rate reductions were not readily apparent at cell concentrations below 6-7 x 10^5 cell ml^{-1} (Fig. 9).

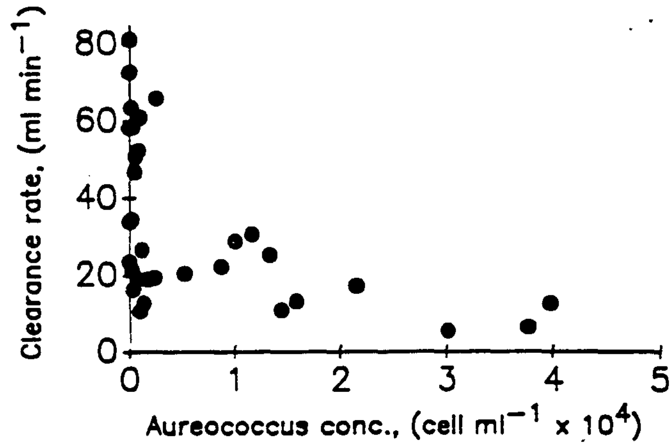

Figure 8. *Mytilus edulis*. Clearance rates of mussels vs. *Aureococcus anophagefferens* concentration for pooled data, June -September, 1988.

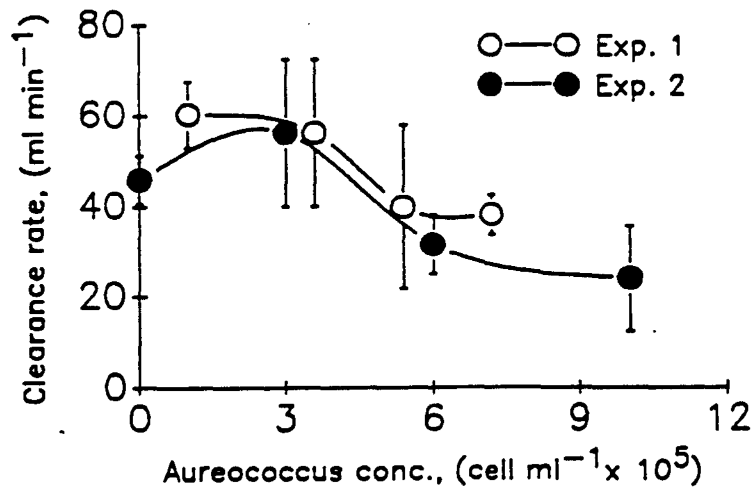

Figure 9. *Mytilus edulis*. Clearance rates of mussels fed cultured *Aureococcus anophagefferens*.

Mussel transplants

Growth of mussels transplanted in the Peconics was significantly greater at the outer-bay station than the mid-bay stations, while the inner-most bay station was intermediate in response (Table 1). In contrast, mussel survival declined from 85% at station 1 to 22.5% at station 4.

Table 1. *Mytilus edulis*. Growth and survival of mussels transplanted in the Peconic Bay System, 18 July - 19 August 1988.

Station	Growth, ($mm\ mo^{-1}$)	Survival, (%)
1	0.91 ± 0.10	85.0 ± 6.0
2	0.72 ± 0.03	53.0 ± 25.0
3	0.72 ± 0.06	40.0 ± 11.5
4	0.80 ± 0.06	22.5 ± 19.0

Table 2. *Mytilus edulis*. Physiological data (mean, (1 std. dev.)) on mussels transplanted in the Peconics, 19 July - 22 August 1988. Code: CR = Clearance rate, ($ml\ min^{-1}$); AE = Absorption Efficiency, (%); RR = Respiration rate, ($ml\ O2\ hr^{-1}$); SFG = Scope for Growth, ($Joules\ hr^{-1}$).

Station	CR	AE	RR	SFG
1	61.4 (6.7)	97.7 (1.1)	0.44 (0.08)	19.4 3.7
2	18.4 (10.0)	94.5 (3.5)	0.38 (0.13)	0.5 (7.0)
3	18.4 (10.2)	97.1 (1.3)	0.34 (0.07)	1.5 (0.7)
4	24.3 (4.9)	97.4 (0.6)	0.31 (0.04)	4.8 (0.6)

Measures of physiological performance under standardized laboratory conditions for mussels retrieved from the Peconics revealed significant reductions in test-algal clearance rates for inshore stations compared to the outer-bay station (Sta. 1; Table 2). The efficiency of dietary absorption by

mussels was high (> 95%) and did not differ among stations. Mussel respiration rates exhibited a gradual decline from offshore to inshore, with significant differences found between stations 1 and 4. Mussel scope for growth was significantly lower at the inshore stations relative to station 1 (Table 2).

DISCUSSION

Quantity and quality of available food are critical environmental parameters controlling metabolic maintenance, growth and reproduction of marine invertebrates (Gabbott, 1976; Newell and Branch, 1980). Food quality parameters include seston concentration, algal concentration, and the size, shape and ingestability of food particles (Møhlenberg and Riisgård, 1978; Kiorboe *et al.*, 1980; Bass, 1983; Bricelj *et al.*, 1984). The importance of food quality in bivalve nutrition was vividly demonstrated during the summer of 1985 in Narragansett Bay, RI, where reduced feeding, reproductive failure and massive mortalities were observed in *M. edulis* populations during an extremely dense bloom of *Aureococcus* (Tracey, 1985; Tracey, 1988). Similar effects were observed in bay scallop populations from the Peconic Bay system (Bricelj *et al.*, 1987). Observed effects were attributed to starvation induced by reductions in clearance rates at *Aureococcus* densities above 2.5-5 x 10^5 cell ml^{-1} based on experiments using natural particulates collected from the Narragansett Bay bloom. In contrast, another similar-sized picoplankter, *Synechococcus*, fed at bloom densities (> 10^6 cells ml^{-1}) did not suppress mussel clearance rates (Tracey *et al.*, 1988). Other factors, including extra-cellular exudates and species-specific sensitivity were examined but not found to be important.

Mussel bioassays

Inverse exponential relationships observed between mussel clearance rates and *Aureococcus* concentration (Figs. 7, 8, 9) corroborate earlier findings that indeed this species is noxious to mussels when present in sufficient concentrations. Although *Aureococcus* blooms did not occur in the 1988 Peconic summer to the extent of causing visual seawater discoloration (unlike the Narragansett Bay summer of 1985 or the Peconics in 1985 and 1986 (Cosper *et al.*, 1987), the data indicate significant depression in clearance rates of mussels fed particulates from Peconic Bay waters.

If the observed clearance rate reductions were dependent only on *Aureococcus* density, algal concentrations required to reduce feeding would appear to range from 1×10^4 to 6×10^5 cell ml^{-1} (Figs. 7,8,9; Tracey, 1988). Two plausible (but not exclusive) explanations for such a wide range in density-dependent effects are either that the noxious qualities of *Aureococcus* may differ significantly depending on growth conditions, or other species with noxious qualities also may occur at environmentally significant concentrations. In the Peconics, *Aureococcus* abundances greater than 1×10^4 cell ml^{-1} were not prevalent until August (Fig. 3), yet marked reductions in mussel clearance rates were observed when animals were exposed to Peconic Bay particulates as early as mid-June (Fig. 5). These responses could not be readily explained by changes in salinity, turbidity or chl *a* concentration (Fig. 2).

Effects on mussel clearance rates were concurrent with significant shifts in phytoplankton composition to a size distribution larger than *Aureococcus* (Fig. 4). It is significant to note that these dynamics were occurring even though marked changes in total phytoplankton numbers were not evident (Fig. 3). Even in Great South Bay where *Aureococcus* concentrations exceeded 10^5 cells ml^{-1}, the data suggest that this species accounted for only 20-40% of the total phytoplankton present. Other species commonly co-occurring with *Aureococcus* include the 2-4 μm diatom, *Minutocellus polymorphus* (Sieburth *et al.*, 1988). This species also may possess some properties causing clearance rate reduction in mussels as evidenced from the relationship observed in Figure 10.

Mussel transplants

Results from mussel transplant experiments indicate that environmental conditions at inshore stations are less suitable for mussel growth relative to conditions occurring at the outer-bay station. From the physiological data, it is apparent that reduced growth was caused by persistent feeding rate reduction, since clearance rate differences were observed when using *Isochrysis* as the food source (Table 2). In contrast, other effects of exposure on metabolism or food absorption were not apparent. These results are tempered by the fact that effects due to thermal stress on transplanted mussels may have been significant since temperatures (i.e. > 24 °C) were at times above the tolerance limits of *M. edulis* (Seed, 1976), and survival (Table 1) was inversely correlated with temperature (Fig. 2). However, mussel

growth tended to be lower at mid-bay stations (Table 1), a pattern not explained by trends in temperature. In addition, it was during this period of mussel transplantation that a minimum in bioassay clearance rates were observed, most notably at mid-bay stations (Fig. 5). Temperature interactions in bioassays were minimized by prior acclimation to 20 °C before exposures were initiated. Thus it is apparent that feeding reduction effects observed in bioassays are partially responsible for reduced growth of mussels in the field.

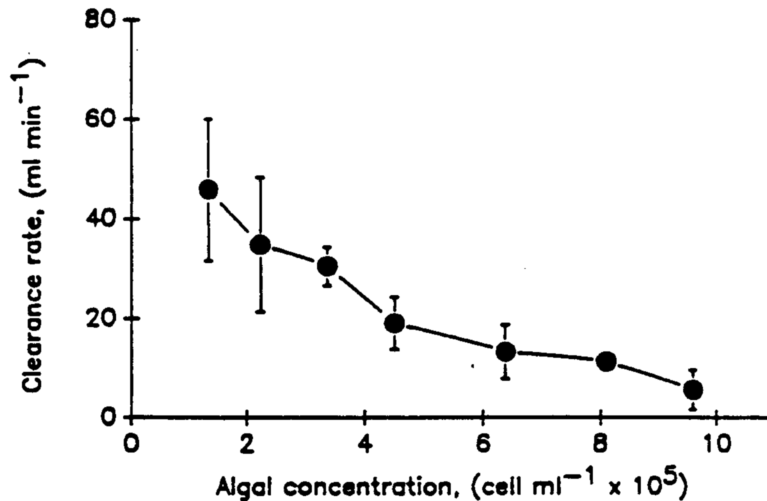

Figure 10. *Mytilus edulis*. Clearance rates of mussels fed cultures of the diatom, *Minutocellus polymorphus*.

Results of this study indicate that the type of food available for mussels in both the Peconic and Great South Bay systems changed significantly during the summer of 1988, causing both reduced feeding and slower growth of this, and presumably other, shellfish species. However, the data suggest that the toxicity of the brown tide alga, *Aureococcus*, may vary greatly depending on environmental conditions for growth, such that the observed effects could not have been predicted simply from knowing the density of this algal species. In addition, another commonly co-occurring bloom species, *Minutocellus*, may also contribute to poor nutrition of shellfish in these waters. This suggests that a complete assessment of impacts on shellfish populations due to nuisance algal blooms requires adequate monitoring of

effects caused by species within the phytoplankton as a whole. A combination of bioassay and transplant methods proposed here appear promising in their ability to contribute to such an assessment.

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