Proceedings of the Brown Tide Summit

October 20-21, 1995

New York Sea Grant

Cornell University • State University of New York • NOAA

Proceedings of the Brown Tide Summit

October 20-21, 1995

A. McElroy, Editor
Publication No. NYSGI-W-95-001
New York Sea Grant Institute

Financial support provided by the National Oceanic and Atmospheric Administration (NOAA) through the Coastal Ocean Program and the National Sea Grant College Program.

The research recommendations portion of the report was reviewed by the Brown Tide Summit Planning Committee. The technical support of Ms. Bonnie Biel who coordinated arrangements for the Summit and produced the report is also greatfully acknowledged.

Table of Contents

INTRODUCTION

William Wise
Marine Sciences Research Center, State University of New York Stony Brook

In the summer of 1995, brown tide returned to Long Island with an intensity dwarfing that of recent years and approximating the conditions of the mid-1980's, when the damaging marine algal bloom first appeared. The lack of widespread, persistent brown tides in the early 1990's, and the nascent restoration of scallop populations that had nearly been eliminated from Long Island waters by earlier brown tides, had given hope to many that brown tide was fading from view as an environmental and economic problem for Long Islanders. Perhaps brown tide had been a temporary disturbance to the ecosystem of the Island's eastern embayments, a disturbance with severe but not permanent environmental and economic impacts. Brown tide's recurrence in 1995 dimmed these hopes and galvanized a broad spectrum of elected officials, resource managers, scientists, fishermen, and environmental groups to search for the cause(s) of brown tide and for steps to prevent its occurrence or to ameliorate or mitigate its effects.

To provide direction in this search, a Brown Tide Summit was held on 20-21 October 1995 at the Holiday Inn at Ronkonkoma, New York. This is the report of that meeting. The Summit had two related objectives: to summarize extant knowledge regarding brown tide, and to identify research necessary to answer the principal questions regarding the causes of brown tide and its environmental effects. The session describing current knowledge of brown tide was a public session featuring prepared presentations by invited speakers from throughout North America. Summaries of speaker remarks are found in Section I of this report. Subsequently, small working groups of invited scientists developed a research plan detailing specific research activities to improve understanding of brown tide so that its future recurrence might be prevented or diminished. This research plan comprises Section II of this report. A list of registered Summit participants is presented in the Appendix to this report.

A History of Brown Tide on Long Island

Brown tide is a marine microalgal bloom. Microalgae, or phytoplankton, are microscopic, single-cell plants that are found in all natural freshwater and marine ecosystems. Microalgae often serve as primary producers in these systems, using solar radiant energy to build up organic compounds directly from carbon dioxide and inorganic nutrients dissolved in the water. The energy thus captured is passed along to other components of aquatic food chains through the consumption of microalgae by primary consumers, animals such as zooplankton, bivalve mollusks, and larval fish. Phytoplankton are the base of the food chain in most aquatic ecosystems.

Phytoplankton communities in temperate coastal waters display a seasonal cycle of abundance and species composition. Often, accelerated growth of one or a few species is superimposed on this overall community cycle due to a particular concurrence of environmental conditions that strongly favor growth of these species. This is termed a "bloom." most blooms are of relatively limited spatial and temporal extent. Brown tide is a bloom carried to the

extreme, often encompassing all or a major portion of the embayments of eastern Long Island for extended periods of time. It is characterized by the dominance of one species of phytoplankton, *Aureococcus anophagefferens*, often to the near-exclusion of other species. The numerical abundances achieved by the brown tide organism at the peak of a bloom can vastly exceed abundance levels of the mixed phytoplankton assemblage typically found in these waters, although total plankton biomass is not unusually high during brown tide blooms. Because it is a mono-specific (single species) bloom capable of affecting large areas over protracted periods of time, during which it can reach extreme abundance levels, brown tide can and has significantly disturbed the ecological functioning of the waters where it has occurred.

Brown tide was first reported on Long Island in June, 1985 when fishermen, clammers, and boaters reported discoloration of the water in Great South Bay. By July of that year, similar reports were being received from parts of the Peconic Bays system. By mid-summer 1985, it was clear that the bloom resulted in recruitment failure of scallops in the Peconic Bays system. Adult scallops typically spawn in the summer of their first year and do not survive to a second annual breeding cycle. Near-total loss of a crop of larvae imperiled the continued existence of populations of this commercially valuable shellfish in East End waters. Scientists and resource managers began discussions aimed at transplanting juvenile scallops purchased elsewhere to Peconic Bay waters in an attempt to provide a spawning stock that would replenish the system with larval scallops. By late September of 1985, brown tide had largely disappeared from Long Island waters and plans for transplanting activities were put on hold, pending recurrence of the bloom.

In late spring of 1986, brown tide reappeared in the eastern half of Great South Bay, Moriches Bay, Shinnecock Bay, and throughout the Peconic Bays system. The 1986 brown tide bloom again produced near-total recruitment failure of bay scallops in the Peconics and planning resumed in earnest under the auspices of the Long Island Green Seal Program to transplant hatchery-produced, juvenile scallops to selected sites in the Peconic Bays. These transplants were carried out in October, 1986; they were continued in 1987 and have been repeated periodically in subsequent years.

Regular monitoring of the Peconic Bays system for *A. anophagefferens* began in March, 1986 by the Office of Ecology of the Suffolk County Department of Health Services. Subsequently, this monitoring program was extended to Great South Bay, Moriches Bay, and Shinnecock Bay, albeit more sporadically than for the Peconic Bays system.

Reappearance of brown tide in 1986 prompted environmental resource managers and marine scientists to begin discussion of research needs relative to brown tide. Using internal funding and additional resources provided by the Suffolk County Department of Health Services (SCDHS), the New York Sea Grant Institute, and the New York State Department of Environmental Conservation, scientists at the Marine Sciences Research Center (MSRC) of the State University of New York, Stony Brook, began investigations into different aspects of brown tide. An Emergency Conference on Brown Tide and Other Unusual Algal Blooms was held in October of that year, organized by the New York State Interagency Committee on Aquatic

Resources Development, MSRC's Living Marine Resources Institute, and the Port Authority of New York and New Jersey. Like the Summit, this meeting described the then very limited state of knowledge about brown tide and outlined additional studies necessary to more fully understand and manage this phenomenon (Anonymous). By October, 1986, brown tide abundances in waters sampled by the SCDHS were nearly undetectable.

In 1987, brown tide did not reappear in most areas until mid-summer and never reached the very high levels of 1985 and 1986. However, significant numbers of *A. anophagefferens* cells remained in affected waters throughout the winter of 1987/88, the first time this had been observed. Moreover, the bloom of 1987 (and 1988) in the Peconic Bays system included species other than *A. anophagefferens*, which had been virtually the only species identified in blooms in these waters during 1985 and 1986.

At this time, the brown tide Task Force was created to advise Suffolk County and other agencies in ongoing research, monitoring, and management activities related to brown tide. The Task Force met periodically until it was supplanted in May, 1988 by the Brown Tide Comprehensive Assessment and Management Program (BTCAMP) initiated by SCDHS in response to the brown tide problem.

In 1988, relatively low levels of brown tide were present in the Peconic Bays system. Cell counts in Great South Bay in 1988 were higher than in 1986, although well below the 1+ million cells•ml^{-1} that had been characteristic of the peak bloom periods in both the Peconic Bays System and Great South Bay. At this point, Suffolk County began to develop a proposal for a program to assess and make recommendations on overall water quality and environmental issues relative to the Peconic Bays system. This became the BTCAMP program. Brown tide would be a significant issue addressed by the BTCAMP program.

By 1988, the amount of research activity directed at brown tide had grown considerably and included investigations at a number of universities and marine laboratories. Brown tide was increasingly looked at by some as just one manifestation of a more widespread proliferation of damaging phytoplankton blooms reported from various parts of the world. In October, 1988, MSRC hosted a major scientific symposium on novel phytoplankton blooms, with a major emphasis on brown tide. The papers presented at the symposium were published in book form as a proceedings (Cosper *et al.* 1989)

Since 1988, brown tide blooms on Long Island have occurred sporadically and variably in different areas of the Peconic Bays system and in several South Shore bays (Shinnecock, Quantuck, Moriches, and Great South Bays). The seeming capriciousness of the bloom is evidenced by its spatial variability among and within these systems. In 1991, significant brown tide blooms occurred in the Peconic Bays system, including West Neck Bay on Shelter Island, as well as in portions of the South Shore system (Quantuck Bay in particular). In 1992, blooms occurred in the South Shore Bays and in West Neck Bay, but not in other parts of the Peconic Bays system. A major bloom was seen in Great South Bay during the summer of 1994, but not elsewhere. In 1995, a bloom occurred in both the South Shore and Peconic Bays systems.

Blooms typically begin in early spring and peak in June/July, after which cell numbers decline, sometimes rapidly, at other times slowly, and often exhibit secondary peaks.

The level of research targeted specifically on brown tide declined after 1988 because of the lull in bloom activity and its increasingly sporadic nature and spatial variability. The limited research which was undertaken was sponsored primarily by Suffolk County and the New York Sea Grant Institute. Brown tide did focus attention on the ecological and economic importance of the Peconic Estuary, and on its fragile nature. This increased awareness played a role in the establishment in 1992 of the Peconic Estuary Program (PEP) as part of the USEPA National Estuary Program (NEP). Drawing on the recommendations of the final report of the BTCAMP Program which served as the nominating document for acceptance into the NEP, PEP has developed an Action Plan highlighting 4 areas: nitrogen pollution; coliform contamination; toxic chemicals; and living resources (SCDHS, 1992).

While brown tide has occurred most prominently in the embayments of eastern Long Island, it has also occurred in other areas of the mid-Atlantic and southern New England (Figure 1). Brown tide was present in Narragansett Bay in 1985 in bloom concentrations and has been reported twice since that time in bloom concentrations in mesocosm chambers operated by the University of Rhode Island. Brown tide occurred in New Jersey waters in 1985, although its presence was not confirmed by immunofluorescence detection techniques. It reappeared in New Jersey at bloom levels in 1995, its identity this time confirmed by immunofluorescence. *A. anophagefferens* cells have been found in coastal embayments from the Gulf of Maine to New Jersey, although usually not at high abundances. Thus, brown tide is a present or potential threat to the entire Northeast region. A closely-related species is responsible for a persistent and damaging bloom in Texas.

REFERENCES

Anonymous. undated. Proceedings of the Emergency Conference on "Brown Tide" and Other Unusual Algal Blooms, 23 and 24 October 1986. Port Authority of NY and NJ, 36 p. + appendix.

V.M. Bricelj and D.J. Lonsdale. *Aureococcus anophagefferens*: causes and ecological consequences of mid-Atlantic "brown tides" (submitted to Limnol. Oceanogr.).

Cosper, E.M., V.M. Bricelj, and E.J. Carpenter (eds.). 1989. Novel Phytoplankton Blooms. Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms. Coastal and Estuarine Studies, Volume 35. Springer-Verlag, 799 p.

SCDHS. 1992. Brown Tide Comprehensive Assessment and Management Program, 3 Vols. + Summary. Suffolk County, Hauppauge, NY.

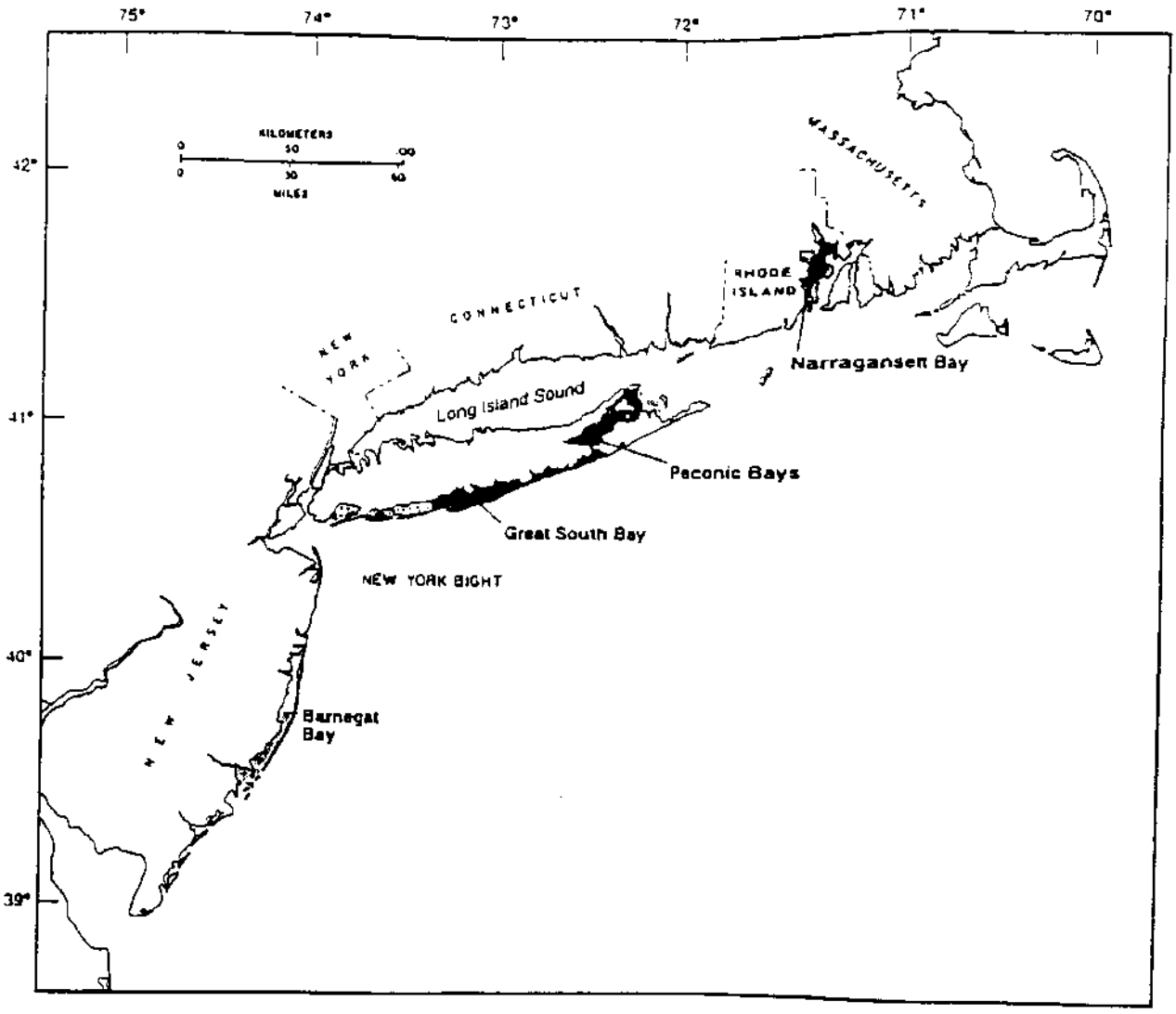


Figure 1. Distribution of *Aureococcus anophagefferens* blooms [here defined by the occurrence of densitites $> 5 \times 10^5$ *A. anophagefferens* cells•ml^{-1} (solid) or $> 2.5 \times 10^5$ cells•ml^{-1} (hatched)] along the mid-Atlantic coast of the United States (modified from Bricelj and Lonsdale submitted. The western distributional limit of brown tide in Great South Bay reflects the sampling boundaries of the Suffolk County monitoring program.

Brown Tide Summit
Friday and Saturday October 20-21, 1995
Holiday Inn, Ronkonkoma, NY
Agenda

DAY 1: Friday, October 20

INTRODUCTION

8:30-8:45 Purpose of the Summit
Dr. Anne McElroy (New York Sea Grant)
Dr. J. Kirk Cochran (Marine Sciences Research Center, SUNY-Stony Brook)
Mr. Felix Locicero (Chair of the Management Committee of the Peconic NEP)

8:45-9:30 Welcomes
Dr. Rollin Richmond (Provost, SUNY-Stony Brook)
Dr. Leon Cammen (National Sea Grant College Program, NOAA)
Dr. Donald Scavia (Director, Coastal Ocean Program, NOAA)
Hon. Robert Gaffney (Suffolk County Executive)
Hon. Michael Forbes (U.S. Representative)
Hon. Fred Thiele (N.Y. State Assemblyman)
Mr. Huson Sherman (Supervisor, Town of Shelter Island)

9:30-9:50 Regional overview of brown tide occurrence history
Dr. Robert Nuzzi (Bureau of Marine Resources, Suffolk County Dept. of Health Services)

9:50-10:10 Stakeholders' questions and perspectives
Mr. Roger Tollefsen (Citizens Advisory Committee, Peconic National Estuary Program)

ONSET AND PERSISTENCE OF BROWN TIDE

10:10-11:00 Growth physiology
Dr. Elizabeth Cosper (Coastal and Environmental Studies, Inc.)

11:00-11:25 Role of iron
Dr. Gregory Boyer (Chemistry Dept., SUNY-College of Environmental Science and Forestry)

11:25-11:50 Role of physical environment
Dr. Robert Wilson (Marine Sciences Research Center, SUNY-Stony Brook)

11:50-12:15 Mesocosm studies
Dr. Scott Nixon (Sea Grant College Program, Univ. Rhode Island)

IDENTIFICATION OF BROWN TIDE

1:30-1:55 pm — Systems for identifying brown tide
Dr. Donald Anderson (Dept. of Biology, Woods Hole Oceanographic Institution)

CONTROL AND SUBSIDENCE OF BROWN TIDE

1:55-2:20 — Trophic interactions
Dr. Darcy Lonsdale (Marine Sciences Research Center, SUNY-Stony Brook)

2:20-2:45 — Viruses
Dr. Curtis Suttle (Marine Science Institute, Univ. of Texas)

IMPACTS OF BROWN TIDE

2:45-3:10 — Overview of impacts on the environment and shellfish
Dr. V. Monica Bricelj (Marine Sciences Research Center, SUNY-Stony Brook)

LESSONS FROM ELSEWHERE

3:10-3:35 — Brown tide-like bloom in northern Gulf of Mexico
Dr. Dean Stockwell (Marine Science Institute, Univ. of Texas)

REACTION PANEL

3:35-4:15 — Scientists' reactions to what they've heard
Dr. Donald Anderson (Dept. of Biology, Woods Hole Oceanographic Institution)
Dr. Theodore Smayda (Grad. School of Oceanography, Univ. of Rhode Island)

ADJOURN DAY 1 GENERAL SESSION

4:15-4:20 — Summary and adjournment of the Day 1 General Session
Dr. J. Kirk Cochran (Marine Sciences Research Center, SUNY-Stony Brook)

WORK GROUP SESSION

4:50-5:00 — Instructions to workgroups
Dr. Anne McElroy (New York Sea Grant)
Dr. J. Kirk Cochran (Marine Sciences research Center, SUNY-Stony Brook)

5:00-6:30 — Work groups begin separate discussions
Physical factors -- *Chair, Dr. Thomas Osborn (Earth and Planetary Sciences, Johns Hopkins University)*
Chemical factors -- *Chair, Dr. Paul Falkowski (Brookhaven National Laboratory)*
Biological factors -- *Chair, Dr. David Caron (Dept. of Biology, Woods Hole Oceanographic Institution)*
Ecological impacts -- *Chair, Dr. V. Monica Bricelj (Marine Sciences Research Center, SUNY-Stony Brook)*

DAY 2: Saturday, October 21

WORK GROUP SESSION

8:30-11:00 Work groups continue separate discussions

Physical factors
Chemical factors
Biological factors
Ecological impacts

11:00-12:30 Work groups reassemble for joint discussion

Chairs lead review and discussion of individual groups' outputs
Moderator, Dr. J. Kirk Cochran (Marine Sciences Research Center, SUNY-Stony Brook)

Physical factors -- *Osborne*
Chemical factors -- *Falkowski*
Biological factors -- *Caron*
Ecological impacts -- *Bricelj*

Chairs lead consolidation and refinement of outputs into a research agenda

GENERAL SESSION

1:30-1:40 Welcome back

Dr. Anne McElroy (New York Sea Grant)

1:40-3:00 Presentation of research agenda to the public, agencies, elected officials, and Peconic National Estuary Program Policy Committee

Dr. David Caron (Dept. of Biology, Woods Hole Oceanographic Institution)
Dr. Paul Falkowski (Brookhaven National Laboratory)
Dr. Thomas Osborn (Earth and Planetary Sciences, Johns Hopkins University)
Dr. V. Monica Bricelj (Marine Sciences Research Center, SUNY-Stony Brook)

3:00-3:30 Reaction and plans

Mr. Felix Locicero (Chair of the Management Committee of the Peconic NEP)
Dr. J. Kirk Cochran (Marine Sciences Research Center, SUNY-Stony Brook)
Dr. Anne McElroy (New York Sea Grant)

3:30 Adjourn Summit

Section I

Summaries of Oral Presentations

THE BROWN TIDE - AN OVERVIEW

Robert Nuzzi
Suffolk County Department of Health Services

"Brown water in Great South Bay from Robt. Moses Causeway to West Channel. Greatest Concentration off Bayshore Marina." Complaint recorded by the Suffolk County Department of Health Services, 10 June, 1985.

Thus began the recorded history of brown tide, which now boasts a greater than 10-year life span and which, to this point, has defied efforts to determine the reason for its appearance.

Sampling efforts were mobilized during the summer of 1985, lasting until the bloom dissipated during the fall. With the reappearance of the brown tide in 1986, sampling efforts were reinitiated and have continued unabated to the present. At the same time, research programs to study the brown tide organism and its effects on the ecosystem were begun.

Although, because of the immediate and drastic effect on the bay scallop population monitoring activities have been most consistent in the Peconic Estuary, monitoring of the South Shore Estuary (Great South Bay, Moriches Bay, Shinnecock Bay) has also been undertaken.

The bloom occurred primarily in the east end and south shore bays of Suffolk County (Figure 1). Its appearance, however, in Narragansett Bay in 1985, its documented presence at what might be considered sub-bloom conditions (141,000 cells•ml^{-1}) in Barnegat Bay in 1988 (Anderson *et al. 1993)*, and as a full-fledged bloom (about one million cells•ml^{-1}) in 1995 (SCDHS), attest to its regional, rather than localized nature, and argue against a single point source trigger.

The presence, although not in bloom concentrations, of the causative organism *Aureococcus anophagefferens* (Sieburth, *et al.* 1988) has been noted in many areas along the northeast coast of the U.S., from the Gulf of Maine (Portsmouth, N.H.) to Great Bay (New Jersey). As of 1990, it had been looked for, but not found in samples collected from Delaware Bay and Chesapeake Bay (Anderson, *et al.* 1993). Our ability to discern *A. anophagefferens* amidst the noise of other picoplankton is due to the development of an immunofluorescent procedure by Anderson *et al.* (1989). Prior to 1988, population estimates, as determined by phase microscopy, are undoubtedly less accurate.

Figure 2 shows the temporal variation of *A. anophagefferens* in four embayments at which the population reached high values: Quantuck Bay (between Moriches and Shinnecock Bays), Great Cove (Bayshore Cove) in Great South Bay, West Neck Bay on Shelter Island, and Flanders Bay. As can be seen from the graph, the occurrence of blooms was not consistent within embayments. For instance, in the summer of 1990 a bloom occurred in West Neck Bay but not in Great South Bay or Flanders Bay. Quantuck Bay exhibited high cell numbers on and

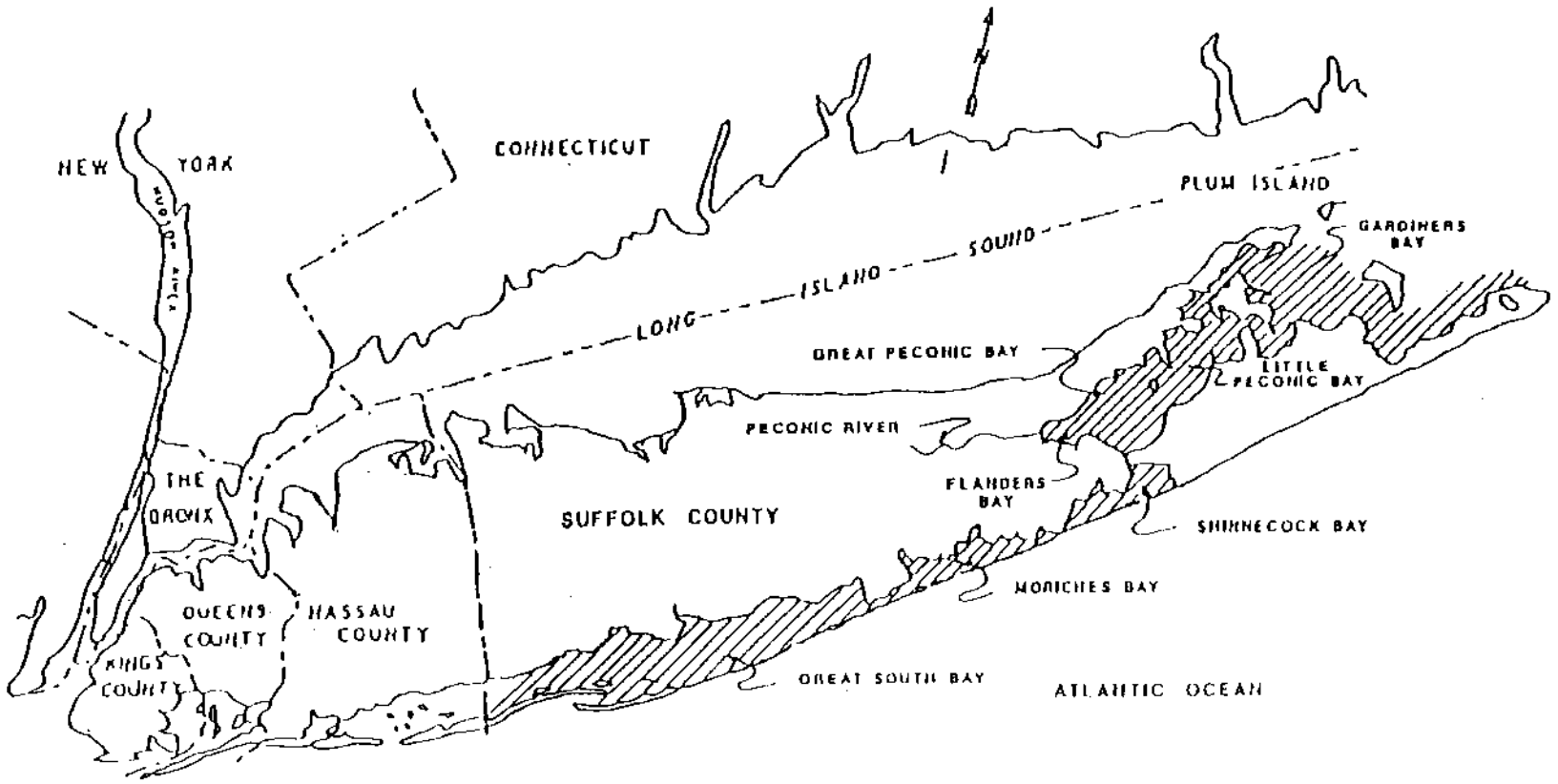


FIGURE 1 THE DISTRIBUTION OF BROWN TIDE (Aureococcus Anophagefferens) IN EASTERN LONG ISLAND

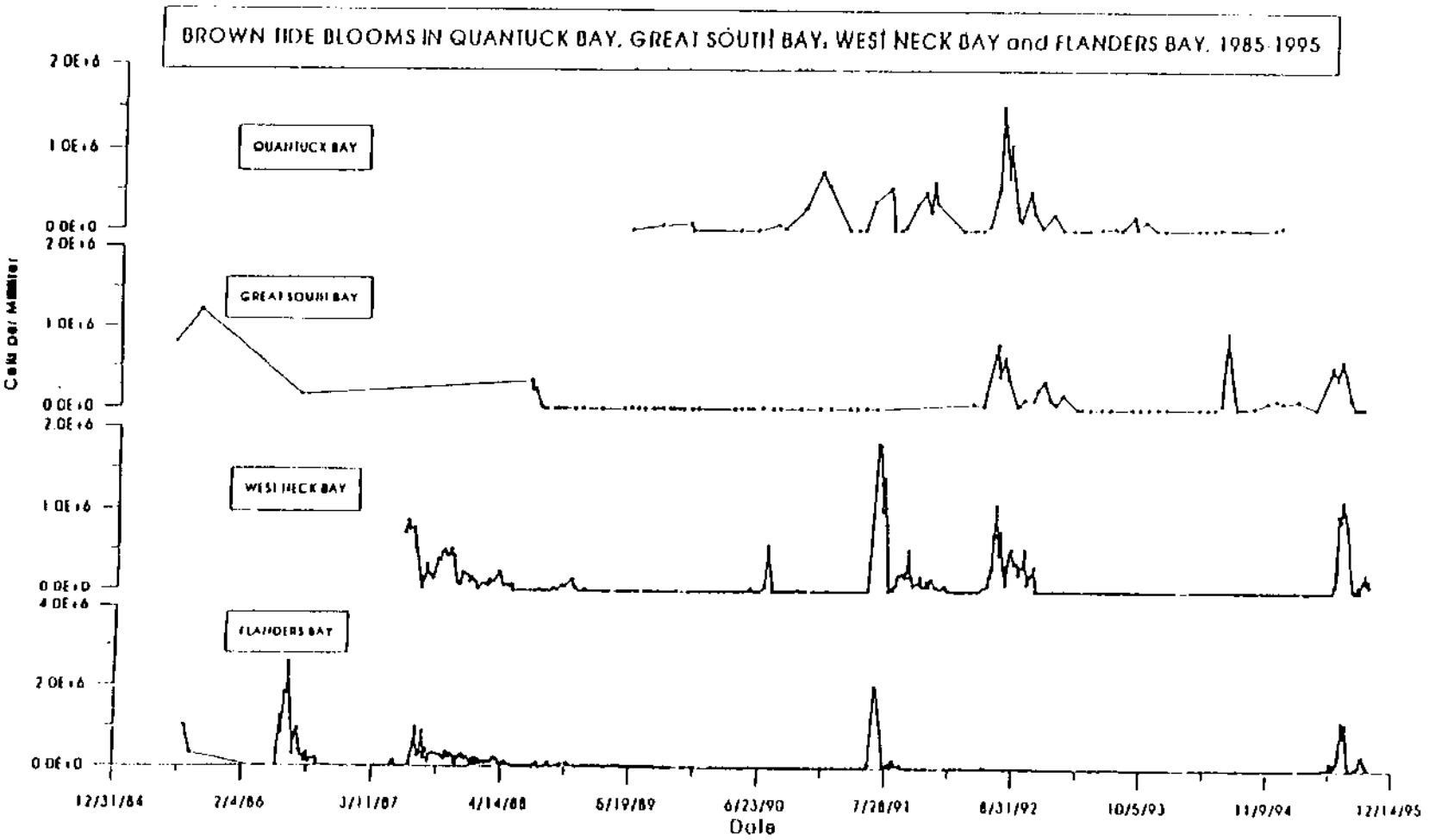


Figure 2 The temporal variation of Aureococcus anophagefferens in four Suffolk County embayments, 1985-1995

off from the summer of 1990 through the summer of 1992, during which year West Neck and Great South Bays, but not Flanders Bay, also exhibited blooms. A bloom occurred only in Great South Bay during 1994 but the summer of 1995 saw major blooms in all four bays (the 1995 data for Quantuck Bay are not plotted). The ability of *A. anophagefferens* to maintain a sizable population even during periods of low water temperature is exhibited in a number of cases.

As *A. anophagefferens* was unknown prior to its appearance in 1985, the question arises concerning its origin. Was it a low frequency member of the plankton community prior to 1985 blooming only as a result of some fundamental change in environmental conditions, or was it, perhaps, recently introduced with the bilge water of some vessel, or by some other means, ready to take advantage of an open niche? The question remains to be answered.

The regional nature of the problem suggested early on that meteorological conditions might be important in bloom onset and persistence and led to the hypothesis that increased salinities caused by drought conditions, followed by the introduction of nutrients with pulses of rainfall, might be involved in bloom formation.

Our current inability to uncover a direct relationship between meteorological conditions and the brown tide may simply be due to the paucity of reliable meteorological stations needed to provide the data. This is particularly true of rainfall, which can be quite different throughout the watershed, especially during the summer months. The use of rainfall data from distant areas often may serve only to create confusion.

It has been indicated (Najarian Associates 1992) that "recurrent brown-tide summer blooms in the Peconic Bays have been preceded by 'northeasters'".

Reduced flushing due to a reduction of wind induced subtidal sea level oscillations was hypothesized by Vieira and Chant (1993) as a mechanism for increasing the retention of land derived nutrients stimulatory to *A. anophagefferens*. Nixon *et al.* (1994) hypothesized that the blooms in Great South Bay are associated with decreased inorganic nutrient input from the coastal ocean resulting from the reductions in oscillations. In either case, whether we are dealing with an increase of *A. anophagefferens*-required nutrients, or a decrease in competitor-required nutrients, or quite possibly a combination of the two, there is, as Nixon *et al.* state, "increasing evidence from field surveys and controlled mesocosm experiments that the growth of *A. anophagefferens* is favoured by oligotrophic conditions".

Results of field (SCDHS, 1985-1995; Smayda and Villareal, 1989) and laboratory studies (Cosper *et al.* 1987), and controlled mesocosm experiments (Keller and Rice 1989; Nixon *et al.* 1994) indicated that the bloom was not related to concentrations of dissolved inorganic macronutrients (nitrogen and phosphorus) leading to the speculation that micronutrients (metals, trace organics) might be critical to bloom initiation.

Our field data, collected since 1985, suggests physico-chemical limits for the Peconic system outside of which *A. anophagefferens* is less likely to bloom. As can be seen in Figure 3.

it appears that salinities in excess of 26‰ and temperatures between 20-25 °C are factors associated with the occurrence of major bloom events. While temperature and salinity appear to be statistically linked to bloom onset (Beltrami 1995), they are not sufficient for bloom formation by themselves, *i.e.*, they apparently provide a physical window within which a bloom may occur.

These temperature and salinity limits are similar to the conditions found by Cosper to be optimal for the growth of laboratory cultures of *A. anophagefferens* (Cosper *et al.* 1989).

Growth at lower salinities was enhanced by the presence of organic phosphate (glycerophosphate), possibly due to its chelating ability.

The ability of *A. anophagefferens* to utilize organic nutrients (urea, glutamic acid, glucose), as determined by Dzurica *et al.* (1989), would almost certainly convey upon it a competitive advantage.

It should, however, be noted that interpretation of metabolism based on these, and subsequent studies to date, must be tempered by the fact that the laboratory cultures utilized were bacterized. To the best of my knowledge, an axenic culture is not yet available, although a number of people are currently attempting to develop one.

The trace metals, iron and selenium, have been found to be stimulatory to the growth of both laboratory grown *A. anophagefferens* and natural populations (Cosper *et al.* 1993), especially if citric acid is provided as a chelator. Based on these results Cosper speculates on the possible relationship between citric acid-containing detergents, coupled with iron enrichment from the pumping of deep iron-rich aquifers, and bloom formation.

Bloom dynamics are influenced by predation as well as by the availability of required nutrients and the proper environmental conditions. Recent studies by Lonsdale (1995) indicate that microzooplankton, primarily protists, are the major phytoplankton grazers in the Peconic estuary. Further, grazing experiments undertaken during a brown tide bloom in Great South Bay "strongly suggest that microzooplankton consume alternate phytoplankton and avoid *A. anophagefferens* cells."

Laboratory studies by Bricelj and Kuenstner (1989) Tracey (1988), Tracey *et al.* (1989), Ward and Targett (1989), Gainey and Shumway (1991) and others, as well as field observations have demonstrated a deleterious effect of brown tide on bivalve benthic grazers, which are thus not likely to exert significant grazing pressure during *A. anophagefferens* blooms. The lack of feeding is illustrated by Figure 4 which compares similarly aged hard clams, *Mercenaria mercenaria*, taken from Great South Bay during a major brown tide bloom to those collected from non-bloom areas.

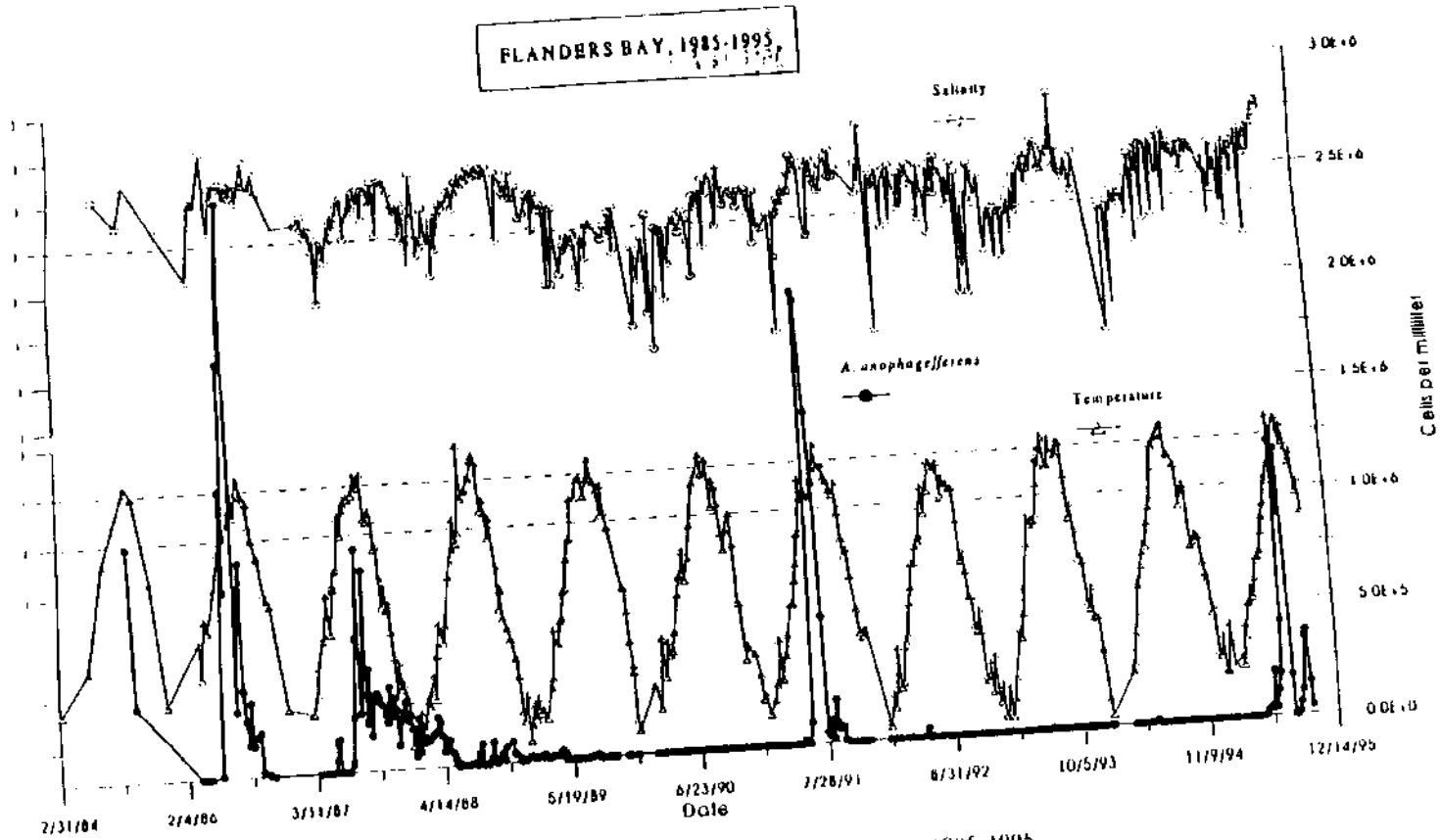


Figure 3 Brown tide blooms in Flanders Bay as related to salinity and temperature, 1985-1995

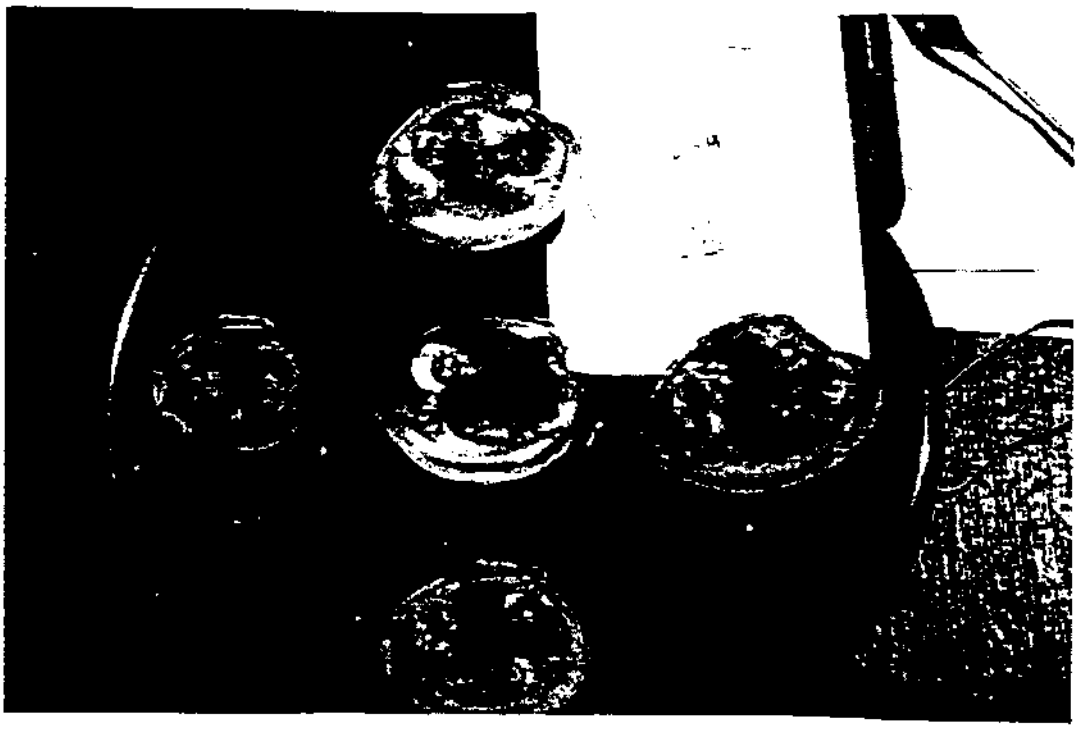


Figure 4. Hard clam (*Mercenaria mercenaria*) collected from Great South Bay (center) during a brown tide bloom, compared to clams collected from waters not affected by brown tide (clockwise from top: Northport, Connecticut, Oyster Bay, New Jersey). The meat of the Great South Bay clam is about equal in size to that of the New Jersey clam which appears to be about one-half the shell size, and smaller than the meats in the clams of similar shell size.

The potential for a viral effect on bloom dynamics was suggested by Sieburth *et al.* (1988) and lysis of *A. anophagefferens* cells by a bloom associated virus was demonstrated by Milligan and Cosper (1994).

Allelochemical interactions between *A. anophagefferens* and other algal populations have been postulated but not demonstrated (Cosper *et al.* 1989) but the apparent aversion of microzooplankton to *A. anophagefferens* may reflect primary allelochemistry in that it provides a survival advantage likely resulting from the production of a metabolite. Allelochemistry in aquatic systems has been discussed by Keating (1987).

It has been shown that the concentration of dimethyl sulfide (DMS) in Peconic Bay waters is related to the presence of brown tide (SCDHS). The concentration of acrylic acid, which was noted by Sieburth (1960) to have allelopathic effects, would be expected to be similarly related as it is the other half of the molecule of dimethylsulfoniopropionate from which DMS is derived.

Numerous scenarios have been created in an attempt to explain the sudden appearance of the brown tide including such things as:

- The increased usage of anthropogenic products containing substances conducive to *A. anophagefferens* growth, along with the introduction of those substances to the ecosystem.

- The introduction of increased iron by the pumping of submerged aquifers

both of which have already been mentioned.

Other scenarios include, but are certainly not limited to:

- The possibility of acid rain modifying the mobility and/or bioavailability of sedimentary metals, coupled with a competitively advantageous ability of *A. anophagefferens* to utilize or, perhaps, to detoxify those metals.

- The decreased introduction of an essential nutrient caused by a decrease in groundwater seepage into the bay during dry years, or a reduction of anthropogenic nutrient loading, coupled with a similarly competitive advantage.

- The disruption of the microbial loop, particularly predation, perhaps by previously used farm chemicals being introduced with the slowly moving groundwater.

- Sundry other possibilities, some of which may be more realistic than others but, considering the history of this bloom, none of which should be rejected out of hand.

It is the consideration of various scenarios with which we will be concerned for the next day-and-a-half in an effort to develop a realistic research agenda.

REFERENCES

Anderson, D.M., Keafer, B.A., Kulis, D.M., Waters, R.M. and Nuzzi, R. (1993). An immunofluorescent survey of the brown tide chrysophyte *Aureococcus anophagefferens* along the northeast coast of the United States. J. Plankton Rsch., **15**, 563-580.

Anderson, D.M., Kulis, D.M. and Cosper. E.M. (1989). Immunofluorescent detection of the brown tide organism *Aureococcus anophagefferens*. In Cosper, E.M., Bricelj, V.M. and Carpenter. E.J. (eds.), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 213-228.

Beltrami, E. (1994). Inferring brown tide dynamics in Peconic Bay from models and data. Report to Suffolk County Department of Health Services.

Bricelj, V.M. and Kuenster, S.H. (1989). Effects of the "brown tide" on the feeding physiology and growth of bay scallops and mussels. In Cosper, E.M., Bricelj, V.M. and Carpenter, E.J. (eds.), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 491-509

Cosper, E.M., Dennison, W.C., Carpenter, E.J., Bricelj, V.M., Mitchell, J.G., Kuenstner, S.H., Colflesh, D.C. and Dewey, M. (1987). Recurrent and persistent "brown tide" blooms perturb coastal marine ecosystem. Estuaries **10**, 284-290.

Cosper, E.M., Dennison, W., Milligan, A., Carpenter, E.J., Lee, C., Holzapfel, J. and Milanese, L. (1989). An examination of the environmental factors important to initiating and sustaining "brown tide" blooms. In Cosper, E.M., Bricelj, V.M. and Carpenter, E.J. (eds.), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp.317-340.

Cosper, E.M., Garry, R.T., Milligan, A.J. and Doall, M.H. (1993). Iron, selenium and citric acid are critical to the growth of the "brown tide" microalga, *Aureococcus anophagefferens*. In Smayda, T.J. and Shimizu,Y. (eds.), *Toxic Phytoplankton Blooms in the Sea*. Elsevier Press, Amsterdam, pp. 667-673.

Dzurica, S., Lee, C., Cosper, E.M. and Carpenter, E.J. (1989). Role of environmental variables, specifically organic compounds and micronutrients, in the growth of the Chrysophyte *Aureococcus anophagefferens*. In Cosper, E.M., Bricelj, V.M. and Carpenter, E.J. (eds.), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 229-252.

Keating, K.I. (1987). Exploring allelochemistry in aquatic systems. In Waller, G.R. (Ed.) *Allelochemicals: Role in Agriculture and Forestry*. ACS Symposium Series 330, ACS, Wash., D.C., pp. 136-146.

Keller, A. And Rice, R.L. (1989). Effects of nutrient enrichment on natural populations of the brown tide phytoplankton *Aureococcus anophagefferens* (Chrysophyceae). J. Phycology **25**, 636-646.

Lonsdale, D.J. (1995). A field study of microzooplankton biomass and grazing rate. Final Report to the Suffolk County Department of Health Services, 17 p + app.

Milligan, K.L.D. and Cosper, E.M. (1994). Isolation of virus capable of lysing the brown tide microalga, *Aureococcus anophagefferens*. Science **266**, 805-807.

Najarian Associates (1992). Hydraulic and water quality screening analysis of Sag Harbor: a small tidal embayment of the Peconic Bays, New York. Report prepared for the Sag Harbor waterfront Committee, Sag Harbor, NY. 45pp + app.

Nixon, S.W., Granger, S.L., Taylor, D.I., Johnson, P.W. and Buckley, B.A. (1994). Subtidal volume fluxes, nutrient inputs and the brown tide--an alternate hypothesis. Estuarine, Coastal and Shelf Sci. **37**, 303-312.

SCDHS. Unpublished data

Sieburth, J. McN., Johnson, P.W. and Hargraves, P.E. (1988). Ultrastructure and ecology of *Aureococcus anophagefferens* gen. et sp. nov. (Chrysophyceae): the dominant picoplankter during a bloom in Narragansett Bay, Rhode Island, Summer 1985. J. Phycol., **24**, 416-425.

Sieburth, J. McN. (1960). Acrylic acid, an "antibiotic" principle in *Phaeocystis* blooms in Antarctic waters. Science **132**, 676-677.

Smayda, T.J. and Villareal, T.A. (1989). An extraordinary, noxious brown-tide in Narragansett Bay. I. The organism and its dynamics. In Okaichi, T., Anderson, D.M. and Nemoto, T. (Eds.), *Red Tides--Biology, Environmental Sciences, and Toxicology*. Elsevier, New York, pp. 129-132.

Tracey, G.A. (1988). Feeding reduction, reproductive failure, and mortality in *Mytilus edulis* during the 1985 "brown tide" in Narragansett Bay, Rhode Island. Mar. Ecol. Prog. Ser. **50**, 73-78.

Tracey, G.A., Steele, R.L., Gatzke, J., Phelps, D.K., Nuzzi, R., Waters, R.M., and Anderson, D.M. (1989). Testing and application of biomonitoring methods for assessing environmental effects of noxious algal blooms. In Cosper, E.M., Bricelj, V.M. and Carpenter, E.J. (eds.), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 557-574.

Ward, J.E. and Targett, N.M. (1989). Are metabolites from the brown tide alga, *Aureococcus anophagefferens*, deleterious to muscle feeding behavior? In Cosper, E.M., Bricelj, V.M. and Carpenter, E.J. (eds.), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 543-556.

Vieira, M.E.C. and Chant, R. (1993). On the contribution of subtidal volume fluxes to algal blooms in Long Island estuaries. Estuarine, Coastal and Shelf Sci. **36**, 15-29.

STAKEHOLDERS' QUESTIONS AND PERSPECTIVES

Roger C. Tollefsen
Citizens Advisory Committee, Peconic National Estuary Program

When I was recently asked by the Citizen's Advisory Committee of the Peconic Natural Estuary Program to speak at this Summit, I expressed concern about my position. I certainly could not represent the feelings of everyone on the Committee; however, I felt I could restrict my comments to available data, published reports and observations of citizens. This perspective may by helpful to those in attendance at this Summit.

It has been said that observation is the window to science. Indeed, it is through questioning of observed facts that research may be planned. I would like to start by observing the year 1975, a point in time that may be shown as significant.

The ratio of nitrogen to phosphorous (N/P) is one of the first basic concepts to which a beginning marine biologist may be exposed. A ratio of 15:1 is considered as healthy allowing for a rich bio-diversification and ecosystem balance. The recorded level of the 1975 ratio of N/P in the Peconics according to data supplied by the Suffolk County Board of Health was 15:1. It was also in that year that a regional approach to water quality issues entitled the "208 Water Quality Study" clearly identified a source of concern for the Peconics. They concluded that the sewage treatment plant in the Town of Riverhead was located at an extremely sensitive point in the estuary. They stated that the level of nitrogen being discharged at this location was critically high; if levels were allowed to increase by as little as ten percent, they reasoned, explosive algae blooms may occur. The situation was considered to be so serious that the panel of distinguished researchers and scientists recommended that the outfall of this plant be relocated either to the Long Island Sound or extended into the deeper waters of the Peconic Bay. Despite the strong warnings, the recommendations were not followed.

According to the data, the N/P ratio was dramatically changing. Starting in 1975 this ratio increased from 15:1 to 30:1 to 45:1 to over 60:1 during the next ten years. In 1985 the Peconics experienced a major algae bloom. Whether this bloom was dominated by *A. anophagefferens* (the culprit of the current brown tide) or was a multi-species bloom remains as unclear as the water during the bloom. You will note that technical information published concerning the brown tide in the Peconics only begins in 1986. If the data is correct, wasn't the first algae bloom of 1985 in the Peconics the one that was predicted because of too much nitrogen for the estuary. Don't the facts of N/P ratio support this conclusion?

After the 1985 bloom, data showed the N/P ratio plummeted to 1:1. This low ratio of nitrogen to phosphorous is known to set up a condition where a single algae will dominate at the expense of the natural populations, a population that normally include hundreds if not thousands of different types of algae.

Observations of the conditions at that time in 1985 must include information concerning the operation of the sewage plant in the Town of Riverhead. Despite the prophetic warnings of the "208 Water Study" that nitrogen discharges must not be increased, the municipal plant was discharging nitrogen in excess of 200% greater than that documented in 1975. In addition, a scavenger plant was added at the same location. Although that plant was designed to denitrify the effluent, it incredibly operated much differently. Tertiary treatment to denitrify involves two extra steps. Part "A" oxidizes the nitrogen to a nitrate form and part "B" adds a source of carbon to the nitrified effluent in an anaerobic (without oxygen) environment to strip the nitrate of oxygen; the resultant nitrogen gas harmlessly discharges to the air. For over two and one-half years, the scavenger plant operated by nitrifying all its effluent nitrogen while de-nitrifying none of it. This preposterous condition persisted, in part, because there are no New York State laws that limit nitrogen discharges to a marine environment. I offer these two observations not to form conclusions; however, this information may spark some insight from the scientists into the possible consequences of these acts.

The first definitively reported bloom of *A. anophagefferens* was in late 1985. Water quality data for several years after that event was limited and questionable due to inconsistencies in analytical methods used.

Since the late 70's and mid-eighties, despite the over-sights at the Riverhead sewage complex, there has been a substantial effort to reduce the amount of nitrogen entering our bays. In the 1970's, five duck farms discharged their nitrogen laden waste directly to the bays. Although every bayman can provide first hand experience that the best clamming was in the areas of highest concentration of duck farm discharge, that discharge was heavily burdened by bacteria. The elimination of these sources of effluent surely reduced the bacteria sources but also substantially reduced the amount of nitrogen carried with it.

From the time the Riverhead sewage complex was clearly identified as a major polluter (a fact stated by the commissioner of Suffolk County Board of Health) impressive progress towards nitrogen reduction has been achieved. According to the firm of Malcolm Pirnie, the current sewage consultant, the plant had been operating out-of-limits over 87% of the time from 1985 until December 1988. But after 1989, because of changes to the process, the plants had reduced their nitrogen output by 40% and were then in compliance over 95% of the time.

Along with the municipal successes of nitrogen reduction, there was a growing citizen awareness of the problem. Many town initiated projects combined with individual efforts have further reduced nitrogen inputs.

Despite the proven effort to reduce the amount of nitrogen into our bays, the threat of a re-occurrence of the brown tide remains. After an absence of a year or two, the brown tide has, once again, almost destroyed the obvious scallop crop and surely contributed to the demise of a significant number of finfish and shellfish that depend upon our bays as a nursery and home. The Peconics have been described by the NYS Department of Environmental Conservation as the

cleanest body of salt water in the state. It seems to many people that the more we do to "improve" water quality according to what we have been told is better, the more we suffer from the brown tide.

It should be of some consequence to note that immediately before and after the occurrence of a brown tide, baymen have recorded water depth visibly in excess of 16 feet. Contrast this to other estuaries such as the Chesapeake, where Senator Bernie Fowler has for the last ten years ventured out into the bay with white sneakers. Being able to see to a depth of 20 inches is considered an improvement. What factors could account for the wide swing in water clarity of the Peconics when we have not been able to identify concurrent swings in any known variable? Doesn't clear water visibility indicate a lack of any algae? Could it be that the food for the algae that is normally present in our bays is too low, rather than too high.

Of all the perplexing problems associated with the brown tide, perhaps none is more intriguing than the appearance of the brown tide at multiple locations. Indeed, multiple occurrences over wide areas seem to rule out single causes at any one location. No one has been able to link the occurrences. Perhaps we have been looking in the wrong direction.

In 1985, the team of Keller and Rice of the University of Rhode Island were conducting a mesocosm experiment with the waters of Narragansett Bay in Rhode Island. Their purpose was to evaluate the effect of adding nitrogen to the waters of that bay. Coincident to their experiment, Narragansett experienced a bloom of what was later determined to be the brown tide. Throughout their year-long experiment Keller and Rice concluded, "the persistence of the brown tide species in control mesocosms and Narragansett Bay appears related to its ability to grow at very low concentrations of dissolved inorganic nitrogen, levels previously shown to limit diatom growth." They recorded that the levels of dissolved inorganic nitrogen were low at the time of the brown tide bloom and "the brown tide species appeared to persist because its nutrient requirements and/or uptake capabilities gave it a competitive advantage." If the conclusions are correct, have we, in the process of attempting to reduce nitrogen in our bays, gone too far?

It makes sense, and has been observed, that a range exists for the level of nitrogen relative to phosphorous in which we would describe the bay as healthy and bio-diversity is high. How wide that range is may not have been clearly defined; however, we know that one of the consequences of having too much nitrogen in our bays is that major algae blooms will occur. In the extreme, these blooms will result in imbalances and eventually deplete oxygen. We can reason that if all the nitrogen were possibly removed, algae life would cease to exist in any form. We also know that there is another limit, below the range of "normal" balance, but above absolute zero, that will favor certain algal forms and allow them to flourish to the detriment of others. If we have entered that range of unispecies dominance, it may be a thin line that is subject to any number of marginal changes such as the weather, temperature, or salinity. Is that unispecies the brown tide? Should we be attempting to understand this organism that dominates in a range that we may have created, or may we conclude that we should adjust the range back to the levels that supported our bays prior to our haste to control it.

In addition to the work in Narragansett Bay, there have been other conclusions that low, not high, levels of nitrogen are driving the brown tide. Nixon *et al.* concluded in a paper dated May 10, 1993 that, "while a reduction in nutrient input may seem inconsistent with the initiation of a nuisance algal bloom, there is increasing evidence from field surveys and controlled mesocosm experiments that the growth of *Aureococcus* is favoured by oligotrophic conditions." Smayda and Villareal (1989) state that "high nutrient loadings appeared to suppress *Aureococcus* abundance."

What about the Great South Bay? Is it possible that we have reduced the nitrogen into that bay in a way that plays into the hand of the brown tide? While it may seem that such a reduction is not possible, we have made significant changes that could account for the observations. Only ten years ago, the Great South Bay was the source of one of the highest densities of hard shell clams in the world. It supported thousands of baymen who supplied these clams to a hungry market. Today, that resource is less than ten percent of what it was. Some are quick to blame overharvesting; brown tides have also taken their toll. However, have we also reduced the food source for the algae that, in turn, serves as food for the clam? Have we starved the hard clam populations?

With the increasing development of the lands surrounding the Great South Bay, perhaps inevitable problems occurred with increasing residential sewage demands. In some cases, the overflows of inadequate sewage systems resulted in bacteria entering the bays and required the closure of areas bountifully filled with clams. The southwest sewer system was planned to unite all the individual sewage facilities into a single processing plant. While this effort clearly reduced the potential of bacteria leaching into our bays, it also removed the influx of nitrogen that previously flowed through groundwater into the bay. This sewage system currently processes 22,000,000 gallons per day. Although nitrogen loading of this system was not immediately available, the amount is estimated to be in excess of 3,500 lbs of nitrogen per day. When the plant was completed in 1981, this flow of an important nutrient that appeared to be then in balance with a healthy ecosystem was eliminated by discharging the processed sewage south of the bay and directly into the Atlantic Ocean.

The massive loss of the nitrogen previously supplied through groundwater into a balanced ecosystem must be considered significant. The results of this act would be discovered years later as the levels of nitrogen in the groundwater were diluted and reduced. Have we, in the Great South Bay, succeeded too well in our quest to reduce nitrogen that we have created an ideal environment for the brown tide to out-compete other resident algae forms?

There are many opportunities for theories; however, can we ignore such massive changes? Are we at the point that we need to characterize nitrogen as a villain, against which every effort should continue to rid it from our bays, or should we look at nitrogen is an important, manageable, marine resource? We need to answer this basic question before we can ever hope to understand what causes the brown tide.

Research is an important tool in eliminating the brown tide: but how much do we need to act? It is clear to some researchers that low levels of dissolved inorganic nitrogen favor the brown tide and others have discovered that adding nitrogen will stop a bloom of it. Are these conclusions wrong? While no action can ever be taken without risk, we now know the risk of not acting. Is it possible for research to recommend a calculated risk based upon our best science at the time or will we be forever looking for absolute truths while squandering our best chances?

RECURRING BROWN TIDE BLOOMS OF *AUREOCOCCUS ANOPHAGEFFERENS*: A SEARCH FOR THE CAUSES

Elizabeth M.Cosper, Christopher J.Gobler and Sharon S.Benmayor
Marine Sciences Research Center, State University of New York Stony Brook

SUMMARY

In the mid-1980's new and unusual blooms of a small, eucaryotic chrysophyte, *Aureococcus anophagefferens*, appeared in several embayments along the northeast coast of the USA and were particularly harmful to bivalve communities. The monospecific blooms were termed the brown tide due to the resulting water color. Historically, a diverse group of small microalgal species dominate the phytoplankton biomass and productivity in Long Island bays during the summer. The continued dominance through several months at high cell densities ($> 10^9$ cells$\cdot l^{-1}$) of *A. anophagefferens* was the distinctive feature of consequence during these blooms. Extensive monitoring programs have demonstrated the recurrence of these brown tides to greater or lesser extents during the summer months almost every year since at certain sites, particularly in Long Island embayments in New York State.

Environmental variables which may contribute to the occurrence of the brown tide include elevated salinities due to drought conditions, pulses of rainfall delivering organic and/or micronutrients to bay waters, reduced grazing and restricted flushing of bays. The brown tide species appears to be closely related to an open ocean chrysophyte, *Pelagococcus subviridis*. It is possible that the brown tide was seeded into northeast coastal bays in 1985, when environmental conditions were particularly favorable for its growth. The ability of this species to maintain minimal populations during the winter months seems to allow for its recurrence during subsequent summers. Culture studies have shown that for a maximal growth rate this species has specific requirements for trace elements, notably iron and selenium, chelators and organic nutrients; some of which are different from common estuarine and coastal phytoplankton species. The competitive advantage of *A. anophagefferens* over other potentially co-occurring species may relate to its heterotrophic and photoadaptive capabilities.

Field studies have confirmed the importance of iron in the occurrence of *A. anophagefferens* blooms. Total and labile iron in Long Island embayments flucuate above and below concentrations required by cultures for a maximal growth rate. Additionally, iron-rich freshwater input has been observed to stimulate blooms. Uptake rates of rapidly growing blooms (based on this species' cellular demand) are capable of depleting the dissolved iron in bays within hours. The continued importance of salintiy to bloom occurrence in the 1990's and these field observations indicate that the supply of iron from freshwater input and the salinity distibution within bays are interacting temporally and spatially to create ideal bloom conditons.

Original electron micrographs from bays affected during 1985 showed viral-like particles inside brown tide cells and in 1992, several viral isolates were obtained from Long Island bays. Field and laboratory studies of brown tide growth dynamics indicate that sudden crashes in *A. anophagefferens* populations might be the result of viral infections. The effects of viral activity in the occurrence and potential dissipation of blooms will be important in the assessment of bloom dynamics.

BLOOM OCCURRENCE AND DISTRIBUTION

Since 1985, several coastal embayments along the northeast coast of the USA have experienced novel microalgal blooms for which there is no previous record. These monospecific blooms were popularly called the brown tide due to the resulting water color. In the early summer of 1985, the first appearance of the brown tide occurred over a wide geographic range along the coast in non-contiguous bodies of water: Narragansett Bay, Rhode Island, Long Island embayments, New York and Barnegat Bay, New Jersey (Fig. 1) [1,2,3,4,5,6]. The extent of the blooms was restricted to these coastal bay systems; blooms did not appear to follow a pattern of spreading from one bay system to the next. This suggests that the environmental factors contributing to these brown tide blooms were not just localized to specific conditions in a bay system but probably were more regional, e.g. involving meteorological induced changes. The blooms on Long Island markedly reduced the extent of eelgrass (*Zostera marina*) beds because of increased light attenuation and decimated populations of commercially valuable bay scallops (*Argopecten irradians*) since the scallops were unable to graze adequately and starved to death [1,7,8]. Similarly, in Narragansett Bay the mussels were unable to feed and populations were severely reduced [9].

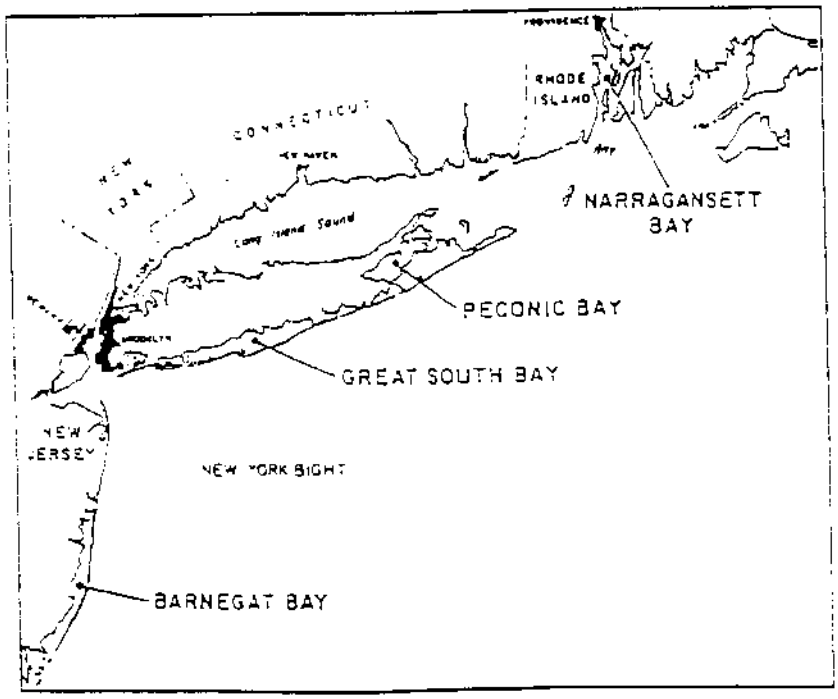


Figure 1. Regional chart of northeast coastal areas including bays affected by the "brown tide."

In 1986, the blooms recurred throughout the summer months in the same Long Island embayments as 1985. During the summers of 1987 and 1988, the brown tide blooms returned in diminishing levels to Long Island and Barnegat Bay, N.J (Fig. 2) [10,2,3]. Since 1985, extensive monitoring programs have demonstrated the brown tide recurred in Long Island bays and Barnegat Bay almost every year[10,2,3], but has not returned to Narranganseti Bay [4,6]. This paper will document and evaluate the findings to date of many scientific efforts concerning the causes and factors contributory to these unusual blooms.

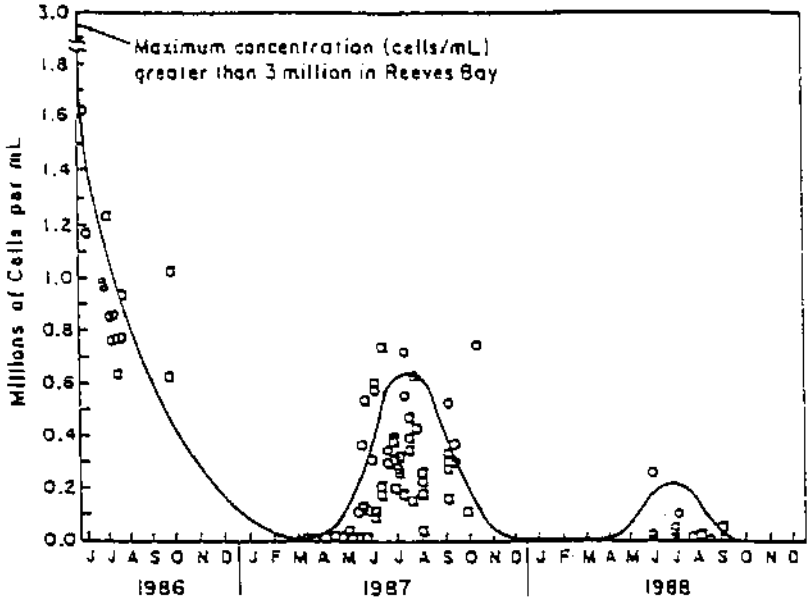


Figure 2. Brown tide cell concentrations for sites sampled in the Peconic and Great South Bays during 1986, 1987, and 1988.

BLOOM DYNAMICS ON LONG ISLAND

The brown tide species was dominant in terms of cell number and contributed greater than 80% of total cellular phytoplankton volume throughout most of the bloom period during the summer months [1,10]. During the blooms phytoplankton biomass (as indicated by chlorophyll a levels) was not particularly elevated for Long Island bays in comparison to other years since concentrations were less than 30 $\mu g \cdot l^{-1}$ (Fig. 3 A), and the majority of chlorophyll was concentrated in the smaller (< 5 μm) fraction (Fig. 3 B) [11,12,1,10]. Primary productivity levels were high but also were not different from pre-bloom years. The less than 10 μm fraction of the phytoplankton contributed greater than 90% of the total photosynthetic activity throughout the bloom period and estimates of picoplankton carbon turnover were rapid, on the order of hours [10]. Levels of inorganic nutrients (nitrate, nitrite, phosphate and ammonium) were also not different from pre-bloom years [13,11,12]. Additionally, variations in inorganic macronutrients were not correlated with variations in the productivity of the brown tide (Fig. 4 A and B) and there is no evidence to support increased macronutrient loading as a cause of the blooms [10,14]. These findings are consistent with similar studies in Narragansett Bay, Rhode Island [6].

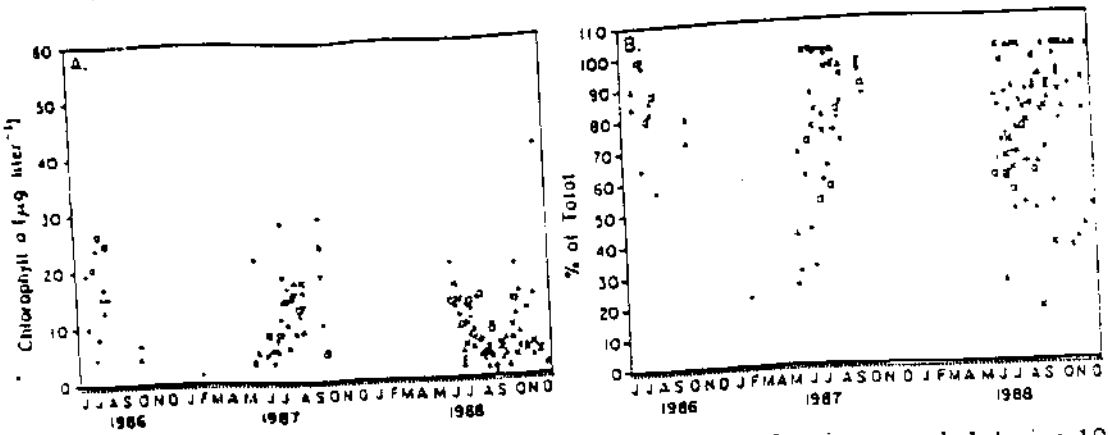


Figure 3A. Chlorophyll a concentration of the < 5µm fraction for sites sampled during 1986, 1987, and 1988. B. Chlorophyll a concentration of the < 5µm fraction as a percentage of total chlorophyll for sites sampled during 1986, 1987, and 1988.

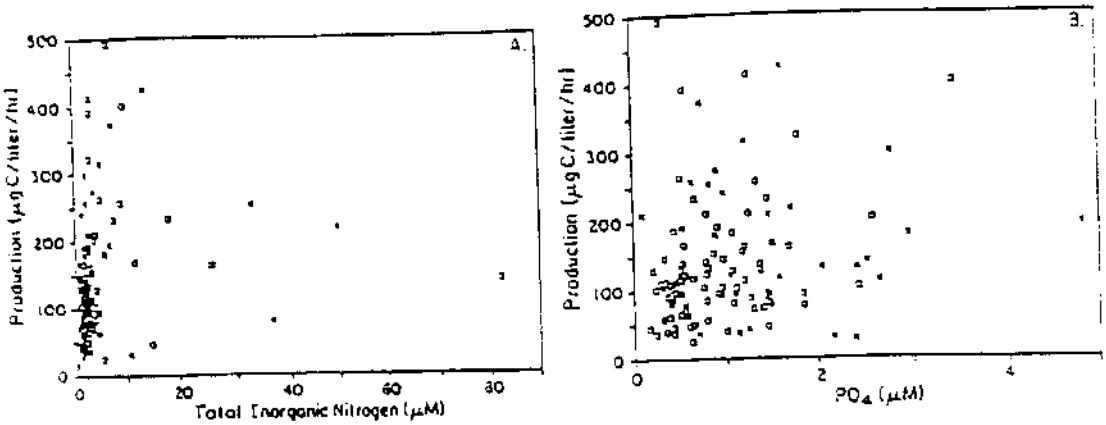


Figure 4A. Inorganic nutrients as µM of (nitrate + nitrite, ammonium) total dissolved nitrogen and B. orthophosphate (PO_4) versus primary productivity for sites sampled in East End and Southern Bays from 1986 through 1988.

The continued dominance over extensive periods of time (~ 6 months) of a single, particularly small algal species was the distinctive feature during these blooms [13,11,12,10,14]. When rapid growth rates were inferred from the rapid turnover of photic zone phytoplankton carbon or calculated using the frequency of dividing cell method, changes in phytoplankton biomass did not reflect such potential increases in populations [10]. Neither sinking of this small microalga nor flushing of the bays could account for such constant population densities, since daily sinking rates of such a small microalga would be small [15], and flushing of the bays is on the order of weeks [13,12]. Grazing by protozoans on the brown tide was evaluated in culture experiments and in field studies during the summer of 1988 [16] and was found to be a potential controlling factor on population densities.

Small microalgae known as "small forms" generally dominate the phytoplankton biomass and productivity in Long Island embayments such as Great South Bay [12] and

Peconic Bay [11] during the summer. Usually the small forms are composed of many species of diatoms, chlorophytes, cryptomonads and small flagellates which vary in percent contribution to the total phytoplankton throughout the summer months. Similar to the brown tide, dense blooms of two minute chlorophyte species occurred during the mid 1950's and affected oyster populations in Great South Bay [17,18].

BLOOM SPECIES

The brown tide microalga had not been previously identified and was named, *Aureococcus anophagefferens* [4]. *A. anophagefferens* was isolated into culture during the summer of 1986 [19] and again during the winter of 1987. The use of immunofluorescent detection techniques for *A. anophagefferens* [20] have already indicated the presence of *A. anophagefferens* in northeast coastal waters unaffected by the brown tide blooms, implying that the blooms were caused by a unique set of environmental events in particular bay systems [14]. Further immunochemical analyses [21], electron microscope analyses [4,5] and pigment analyses [22], comparing *A. anophagefferens* to other ultraplankton have demonstrated a close affinity between *A. anophagefferens* and a ubiquitous, open ocean microalga, *Pelagococcus subviridis*, isolated and identified from many areas in the Pacific Ocean [23,24] as well as from Norwegian waters [25]. *A. anophagefferens* is also closely related to another brown tide causing alga isolated from the Laguna Madre, Texas (TBA-2) where it has been causing similar devastating blooms since 1991 [26].

ENVIRONMENTAL CONDITIONS CONTRIBUTORY TO THE BLOOMS

Physical and chemical factors

The first year of the bloom occured when the annual rainfall level was the third lowest in the past 37 years and coincided with the start of a drought period that continued for several years thereafter (Fig. 5 A) [1]. The temporal pattern of rainfall appears to be important to the formation of the blooms. Low levels of rainfall during the winter and spring months of 1985 and 1986 were followed by abnormally large pulses of rain and potential freshwater inputs into the bays via runoff and/or ground water flow during the early summer. (Fig. 5 B). This pattern was not as definitive in 1987. Blooms did not recur until later in the summer and the cell densities were lower than the previous two summers (10^8 cells$\cdot l^{-1}$). The drought led to elevated salinities in the bays; since 1986 the salinities have been close to 30‰ (Fig. 6 A), whereas, they were previously around 25‰ [1,12]. During the summer of 1987, when bay salinities were initially lower, the *A. anophagefferens* blooms first occured in areas where salinities remained within the halotolerance of the species. As salinities increased during the summer and into the fall months, the brown tide regained dominance. Elevated salinities have been observed over the years to be a significant contributing factor to the initial blooming of the brown tide species, *A. anophagefferens*.

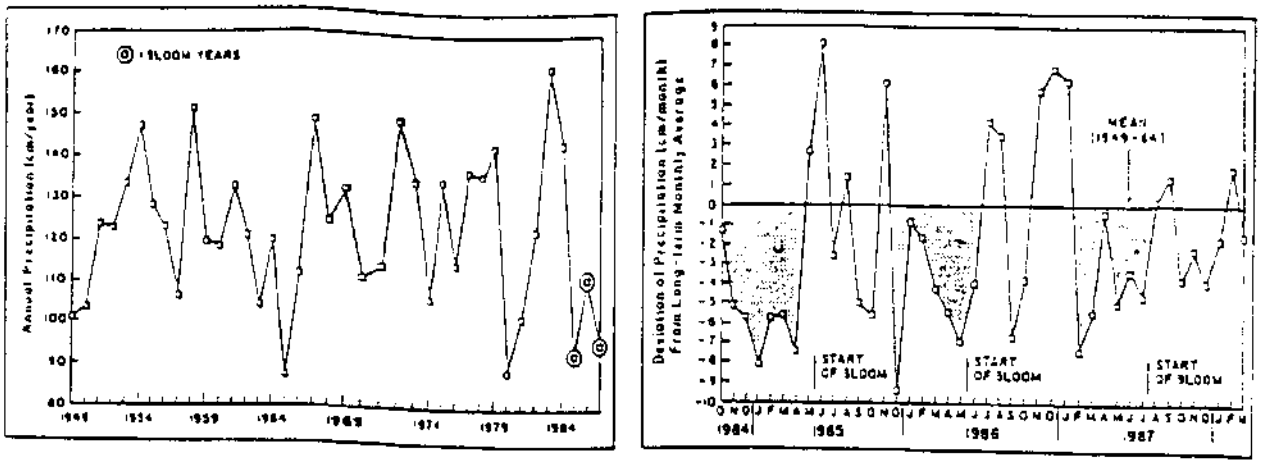


Figure 5A. Total monthly precipitation for Brookhaven National Laboratory, Upton, Long Island, NY, from 1984 to 1987. B. Monthly precipitation from Oct. 1984 to March 1988 as deviations from the mean for each month from 1949 to 1984.

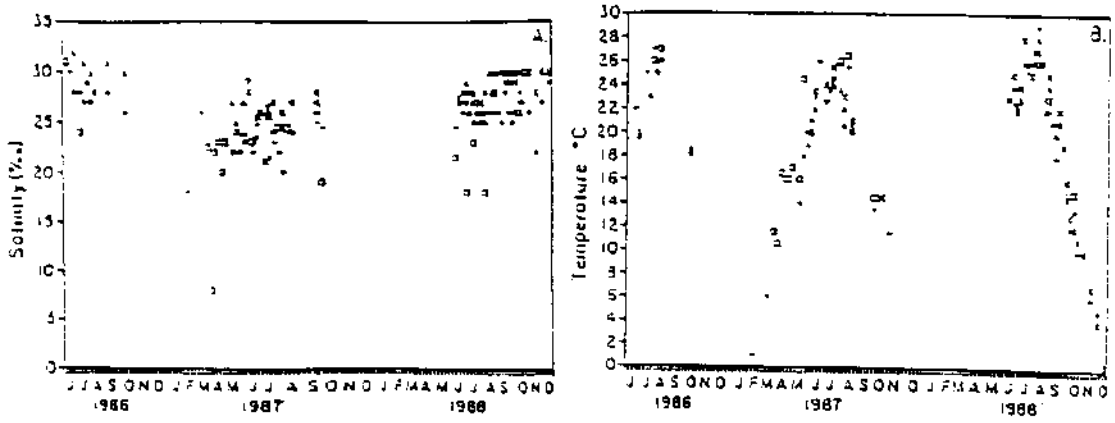


Figure 6A. Salinity and B. Temperature at stations sampled in Great South Bay and the Peconic Bays during 1986, 1987, and 1988.

Laboratory studies with *A. anophagefferens* cultures adapted to 30‰ indicate a severe reduction in growth rates below 28‰ as compared to optimal growth rates at 30‰ in standard Enriched Instant Ocean (EIO) f/2 media with inorganic phosphate (Pi) at f/20 (3.33 μM) [27] (Fig. 7). Growth in EIO f/2 with Pi at f/2 (33.3 μM) was never obtained at any salinity. When glycerophosphate is substituted for inorganic phosphate at f/2 and f/20 concentrations (f/2 GP, f/20 GP), growth of *A. anophagefferens* was enhanced substantially at lower salinities (Fig. 7) when compared to growth in inorganic media [14]. Experiments to evaluate the ability of *A. anophagefferens* to grow at higher salinities representative of neritic, 32‰, and oceanic, 35‰ waters, (Fig. 7) showed that this species could grow well at these higher salinities particularly in media with inorganic phosphate. Salinity is important to the growth of *A. anophagefferens* in Long Island Bays, but it must be evaluated in light of the presence of organic nutrients.

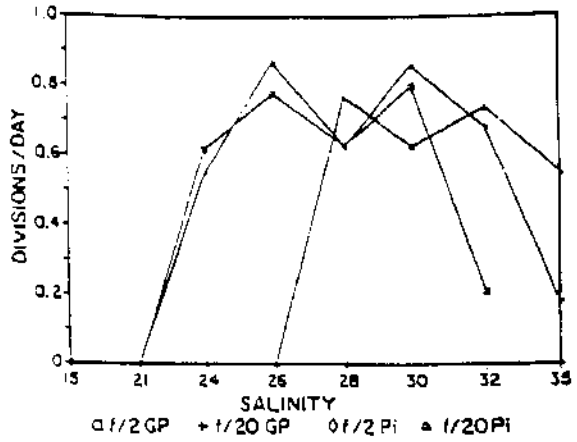


Figure 7. Growth rate of *A. anophagefferens* versus salinity in EIO media with either glycerophosphate (GP) or inorganic phosphate (Pi).

Analysis of field samples obtained from helicopter flights throughout the Peconic Bays and Great South Bay on August 12, 1986 showed the reverse of what would be predicted from laboratory analyses and rainfall data; the greatest cellular concentrations were at 26‰ and the lowest at salinities above 30‰ [14]. The high inverse correlation ($r = -0.94$) between salinity and brown tide cell concentration found during August, 1986 was not found during June and July, 1987 ($r = -0.64$ and $r = -0.46$, respectively) when salinities were reduced relative to the previous summer, although the inverse trend still prevailed [14]. During the fall, on the October 1 trip, when the brown tide was reestablished and dominant, even if at reduced concentrations, the high inverse correlation ($r = -0.96$) was again found [14].

Correlation analyses were performed between salinity and inorganic nutrient levels along transects sampled [14]. Significant nutrient distributions were positively correlated with salinity, indicating higher levels in further offshore areas or in areas where coastal waters had mixed into the bays. The distribution of brown tide cells relative to salinity cannot simply be explained by variations in inorganic macronutrient concentrations. In addition, no correlation between primary productivity of brown tide bloom waters and inorganic macronutrient levels can be demonstrated (Fig. 5 A and B) [10].

Temperature, in contrast, varied in a similar pattern in 1986, 1987 and 1988 (Fig. 6 B) and does not appear to be different from previous summers [11,12] nor to be contributory to the brown tide. The highest growth rates of summer and winter isolates were obtained at 20 and 25°C confirming that *A. anophagefferens* is a warm water species consistent with its formation of summer blooms [14]. Growth, however, over a wide temperature range can be obtained, if given enough time to adapt, so that even at 5°C doubling times of 10 days are realized. Minimal growth rates at low temperatures would be adequate to maintain populations during the winter months in the poorly flushed bays of Long Island [14,2] and could be consequential in the ability of *A. anophagefferens* to bloom.

Comparative Growth Experiments

Since the addition of organic phosphorus compounds such as glycerophosphate and fructose-1,6-diphosphate [28] to artificial seawater media affected the growth of *A. anophagefferens* relative to growth on inorganic phosphorus (Fig. 7) [14], consideration was given to the idea that the alga may be benefiting from these compounds acting as chelating agents [29,30]. If so, substitute chelators might have the same effect as the addition of organic phosphate. The addition of nitrilotriacetic acid (NTA) and citric acid (CA), chelators commonly used in culture media [27], to media resulted in growth on inorganic phosphate (Pi f/2) which was higher than that on ethylenedinitrilotetracetic acid (EDTA) (Fig. 8)[30]. Thus, NTA and CA may be better chelators than EDTA for growth factors important to *A. anophagefferens*.

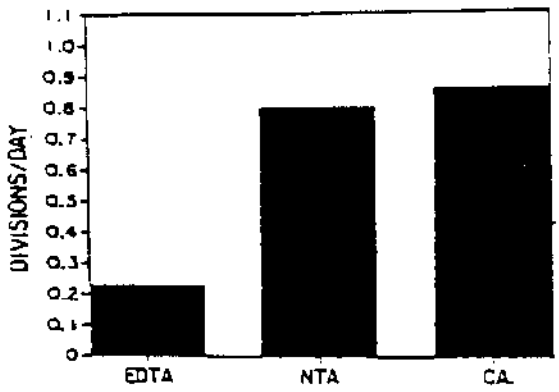


Figure 8. Growth of *Aureococcus anophagefferens* as a function of the chelator, EDTA, CA and NTA.

Further laboratory experiments were conducted to evaluate the role of chelators and a variety of essential trace elements to promote the growth of *Aureococcus anophagefferens* [31]. The use of the ultra-pure and chemically defined media Aquil to grow cultures revealed that iron (Fe) and selenium (Se) additions were critical for the growth of *A. anophagefferens*. Growth rates were higher with the addition of the chelator CA than with EDTA or NTA. Cultures grown with 9×10^{-6} M Fe and 10^{-9} M Se yielded division rates and biomass yields significantly higher than cultures grown at normal levels of media Fe (4.5×10^{-7} M Fe) and lower levels of Se ($< 10^{-10}$ M). Calculation of equilibrium complexations with the different chelators indicated that CA complexed only 25% as much Fe as EDTA and only 33% as much as NTA. Citric Acid has partially replaced phosphates in some commercial detergents used in New York and a better understanding of its role in helping to promote brown tide blooms is needed.

During the summer of 1990, small, sporadic blooms of *A. anophagefferens* were observed. Experiments were performed on natural seawater collections from bloom and non-bloom areas in order to evaluate the control of the Fe and Se above in bloom dynamics [31]. Sub-samples of water were filtered through 5µm Nitex screens to remove grazers and

were incubated at ambient temperatures for 24 hours under 42% and 4% of natural sunlight. Treatments entailed no additions, single additions of 10^{-8} M Se and 9 x 10^{-6} M Fe and Se and Fe combined. *In situ* growth rates indicated additions of Fe significantly enhanced growth rates at a bloom site under both light levels, suggesting that Fe was limiting the growth of this bloom. At a non-bloom site, combined additions of Fe and Se in low light conditions (4% level) enhanced growth.

Based on these findings, a comprehensive laboratory and field study was undertaken to better understand the role of Fe in the occurrence of *A. anophagefferens* blooms [32]. In laboratory studies, cultures were grown under a range of Fe concentrations (10 μM - 5 nM), and cellular and fluorescence characteristics were analyzed to determine levels of Fe necessary for maximal growth of this species. Cultures grown at less than 5μM total Fe (100 nM labile) experienced significantly reduced growth rates and intracellular Fe content, which impaired the photosynthetic apparatus as indicated by reduced chlorophyll a per cell, reduced maximum quantum efficiency of photosystem II (Fv/Fm), and increased *in vivo* fluorescence per unit chlorophyll a. While the cellular Fe:C of *A. anophagefferens* is larger than most marine phytoplankters, it is similair to that of the red tide forming dinoflagellate, *Gymnodinium sanguineum*. This is of particular interest since Fe has been previously implicated in the initiation and limitation of blooms of this genus [33,34].

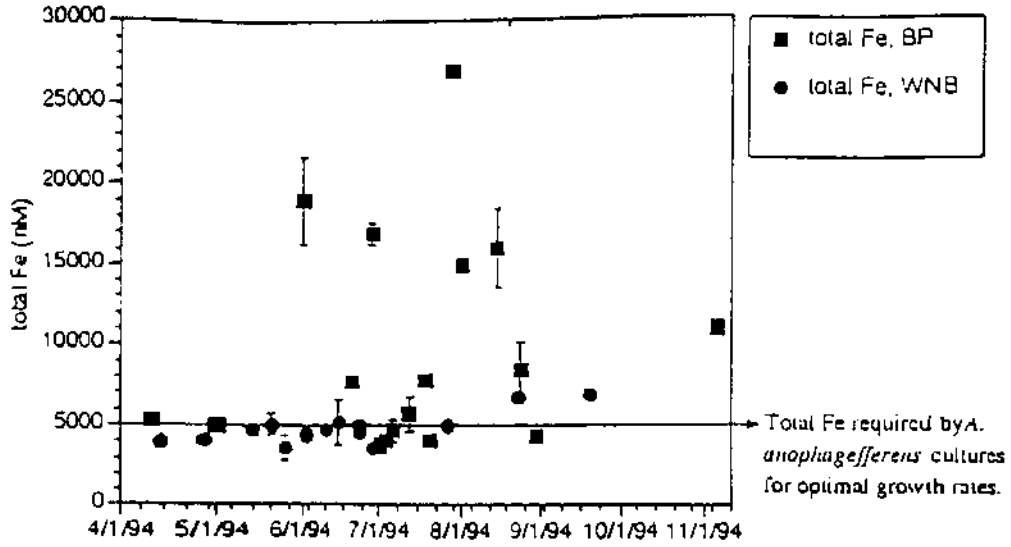
Field analysis of total and labile Fe in Long Island embayments indicated a flucuation above and below the levels required by cultures for a maximal growth rate (Fig.9 A and B) and sediment resuspension was identified as a process capable of significantly enhancing these forms of Fe within bays. Since only dissolved forms of Fe are taken up by phytoplankton, and dissolved Fe can be rapidly consumed by these blooms ([32]; see below), these resuspension events may be important for indirectly supplying Fe to *A. anophagefferens* blooms.

Observations at West Neck Bay (WNB) within the Peconic Bay system over a four year period provided further evidence of the importance of Fe and salinity in bloom occurrence (Fig. 10). In June of 1992, the largest single day rainfall event of the four years period occurred causing bay salinities to drop and dissolved Fe levels to rise. Soon after this freshwater input of Fe, a brown tide bloom occurred, during which dissolved Fe levels were depleted to a three year low. Once the bloom subsided, Fe levels were replenished to normal levels. An application of *A. anophagefferens* measured cellular Fe content (6.73 x 10^{-16} mol•cell^{-1}) [32] to the observed change in cell density during this bloom indicates that the cellular demand for Fe (390 nM) was nearly equivalent with the observed decrease in the dissolved pool (340 nM). If a similiar pattern of uptake occurred during larger blooms of 1986 (3.0 x 10^{9} cells•l^{-1}), a bloom growing at a doubling per day would deplete dissolved Fe levels in five hours. Since only a portion of the dissolved Fe pool is directly available for uptake by phytoplankton, dissolution, cycling, and/or input rates of Fe could limit growth rates of blooms.

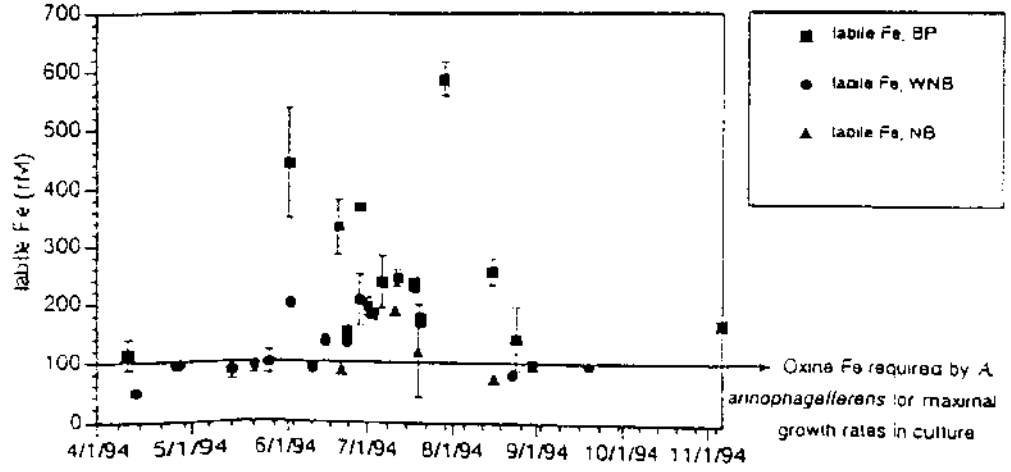
During the two years subsequent to the 1992 bloom event at WNB (Fig. 10), bay salinities were much lower earlier in the year, there were no large precipitation events or sudden increases in dissolved Fe, and blooms did not occur (note change in y-axis). Although

Figure 9. Variations of: A. total Fe and B. labile Fe within Long Island embayments during 1994.

A.



B.



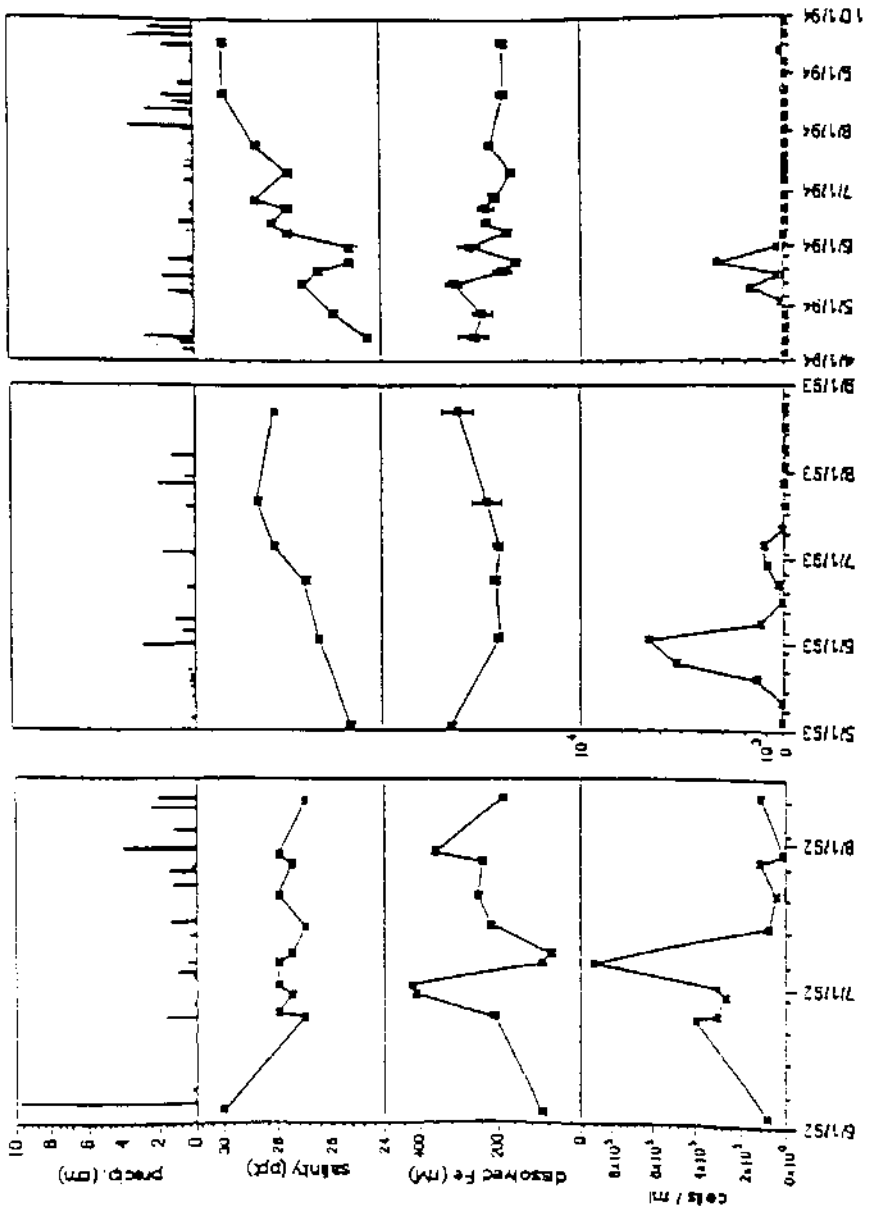


Figure 10. Precipitation, salinity, dissolved Fe, and *A. anophagefferens* densities at West Neck Bay (WNB), 1992-1994.

it was not included in this figure, bay salinities in 1995 at West Neck Bay were near 30‰ in early spring, similiarly to 1992, and once again massive blooms returned to this bay. The importance of higher bay salinities was also demonstrated during a 1994 brown tide bloom in Great South Bay, during which *A. anophagefferens* reached bloom densities only in bay waters of 26‰ and higher, an observation in concordance with earlier reports of the haline tolerance of this species [14].

Levels of dissolved Fe in Long Island embayments are inversely correlated with salinity (Fig. 11). Hence, the supply of Fe from freshwater inputs and the salinity distribution within bays interact spatially and temporally to create the ideal scenario for bloom occurrence: dry winters and springs which result in high bay salinities, followed by intense pulses of rain that deliver Fe-rich freshwater to bays. This may explain the excellent negative correlations between salinity and brown tide cell counts found previously during 1986 and 1987 blooms.

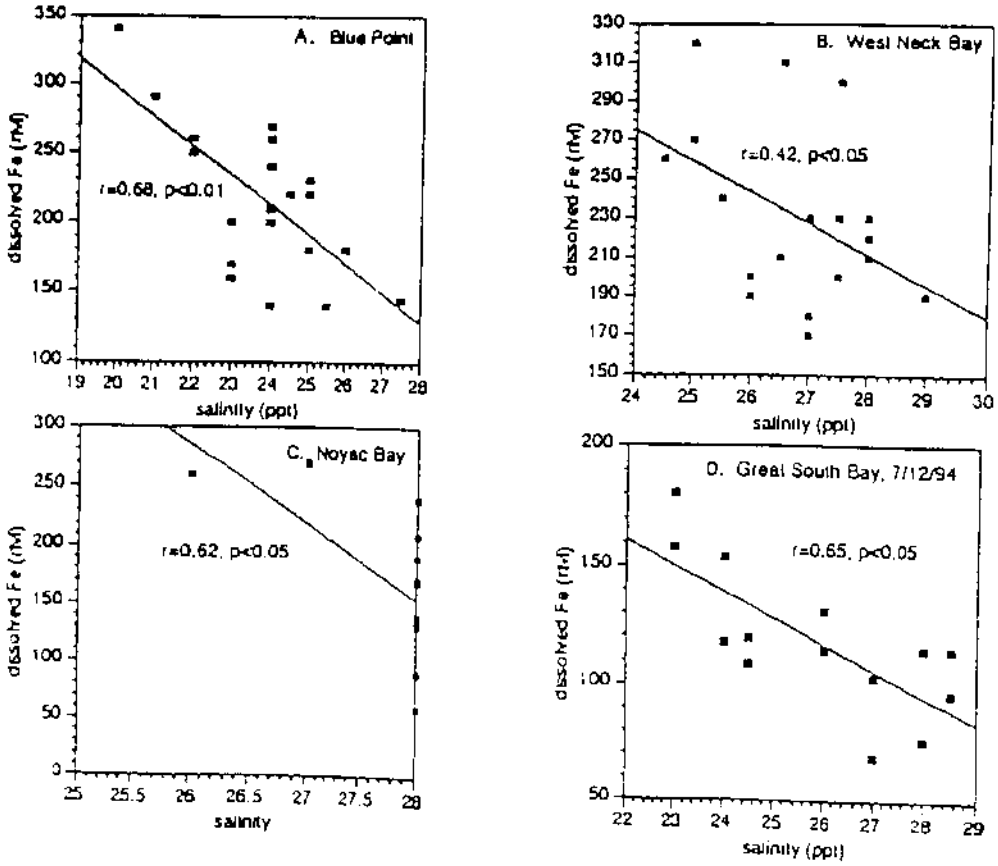


Figure 11 Correlations between dissolved Fe and salinity at Blue Point station of Great South Bay, West Neck Bay, Noyac Bay and across Great South Bay on 7/12/94.

Iron may be an important determinate in the geographic distributution of *A. anophagefferens* blooms. While this species has been detected throughout the NE United States [35], blooms of this species have been limited to the bays of Long Island during the past decade. *A. anophagefferens* blooms create one of the highest documented biological demands for Fe and Long Island bays contain some of the highest Fe levels for the salinity range they encompass. Hence, it seems this species has found an ecological niche in these embayments, in which high Fe levels are an essential component.

Heterotrophic Uptake Experiments

A. anophagefferens can grow on urea as the sole nitrogen source at rates comparable to growth on nitrate; growth on ammonium was never obtained (Fig. 12). Urea can contribute substantially to the total pool of available nitrogen in Long Island bays [12]. Growth on glutamic acid as the sole nitrogen source has also been obtained [28], and *A. anophagefferens* has shown significantly higher uptake rate constants per unit cell volume for glutamic acid than other potentially co-occuring species tested (Fig. 13 A) [28]. Similarly, glucose uptake rate constants on a per unit cell volume basis showed that *A. anophagefferens* would have a significant uptake advantage over *Nannochloris* sp. and *Minutocellus polymorphus* (Fig. 13 B) [28]. The ability of *A. anophagefferens* to effectively compete for and utilize organics for growth appears to be consequential in its ability to maintain blooms in Long Island coastal bays.

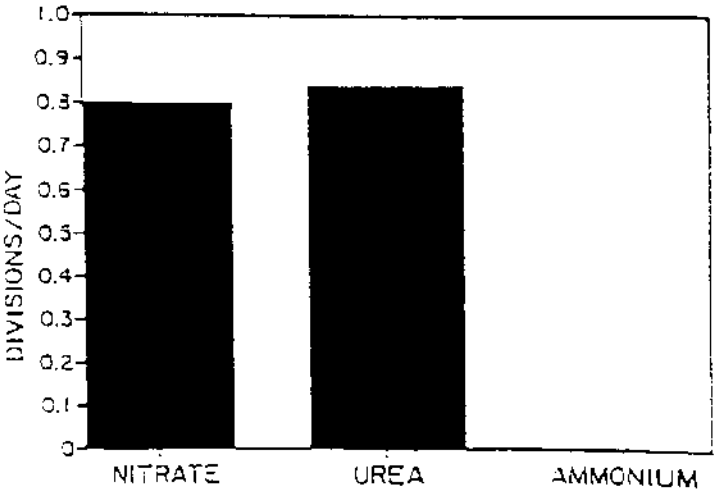


Figure 12. Growth of *Aureococcus Anophagefferens* on different nitrogen sources.

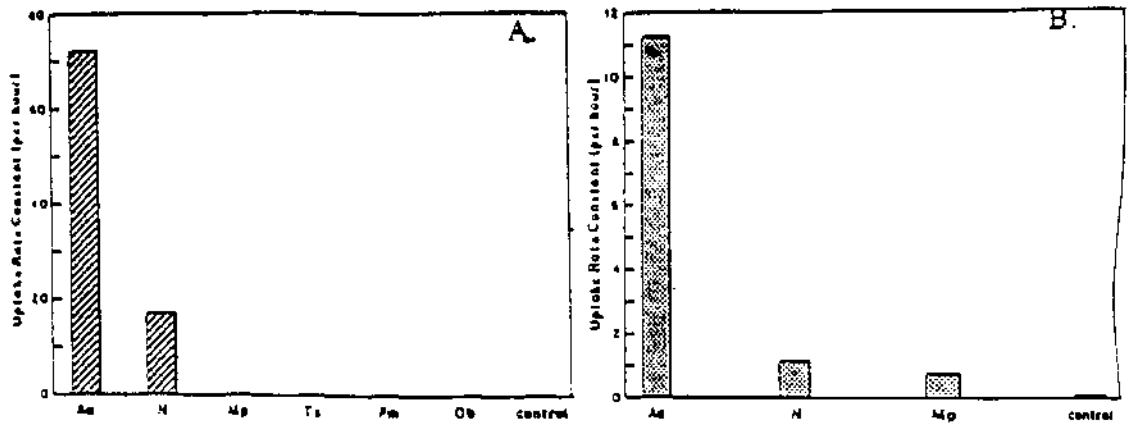


Figure 13A. Glutamic acid uptake rate constants per unit cell volume for algae grown in the presence of f/2 nitrate (N03) or of 10 μM glutamic acid (glu) as the sole nitrogen source. Algal species: *Aureococcus Anophagefferens* (Aa), *Nannochloris* sp. (N), *Minutocellus polymorphus* (Mp), *Thalassiosira pseudonana* (Ts), *Prorocentrum minimum* (Pm) and *Ditylum brightwellii* (Db). B. Glucose uptake rate constants per unit cell volume *for Aureococcus Anophagefferens* (Aa), *Nannochloris* sp. (N) and *Minutocellus polymorphus* (Mp).

Biological Interactions

It does not appear that *A. anophagefferens* excretes any alleleopathic compounds that inhibit the growth of other phytoplankton commonly found in Long Island embayments. Filtrates of media used to grow the brown tide alga were obtained from all stages of the growth cycle of the brown tide (early, mid, and late exponential phase), were added at concentrations from 0.1% to 100% to fresh enriched media. For all four species, tested, *Thalassiosira pseudonana* (3H), *Prorocentrum minimum*, *Ditylum brightwellii*, *Nannochloris* sp. (WNB 7/22), the growth was either enhanced or there was little effect [14]. At only 10% of its own filtrate taken from a senescent culture, *A. anophagefferens* was growth inhibited. We suspected and now know that this is due to viral activity.

During the summer of 1992 viruses infective of this particular phytoplankton species were isolated from bloom waters of the Peconic and Great South Bay systems [36]. Laboratory experiments with these viral isolates indicated the capability of these viruses to infect cells of this microalga within minutes of inoculation, and of lysing dense algal cultures within days. Preliminary experiments have indicated high Fe levels (50 μM) are capable of delaying viral infection and lysis of *A. anophagefferens*. The adsorption of viruses onto ferric hydroxide precipitates or other such colloidal particles [37] would likely decrease the algal/viral encounter rate, and thus delay onset of viral lysis. This result suggests that resuspension events within Long Island embayments could play an important role in bloom occurrence by not only supplying Fe to *A. anophagefferens* [32], but also by adsorbing viruses out of the water column. This may temporarily release the *A. anophagefferens* population from viral control and allow for a bloom to occur.

CONCLUSIONS

Brown tide blooms may have resulted from several factors 1) higher than average salinities in bays during the spring and early summers, 2) freshwater runoff or groundwater inputs of organic compounds and inorganic micronutrients, particularly Fe, which may be essential to the rapid growth of the *A. anophagefferens*, 3) restricted flushing by coastal waters of the Long Island bays resuling in long residence times for water on the order of weeks [13,38]), and allowing for the retention and maintenance of large populations of brown tide cells within these embayments (Fig. 14).

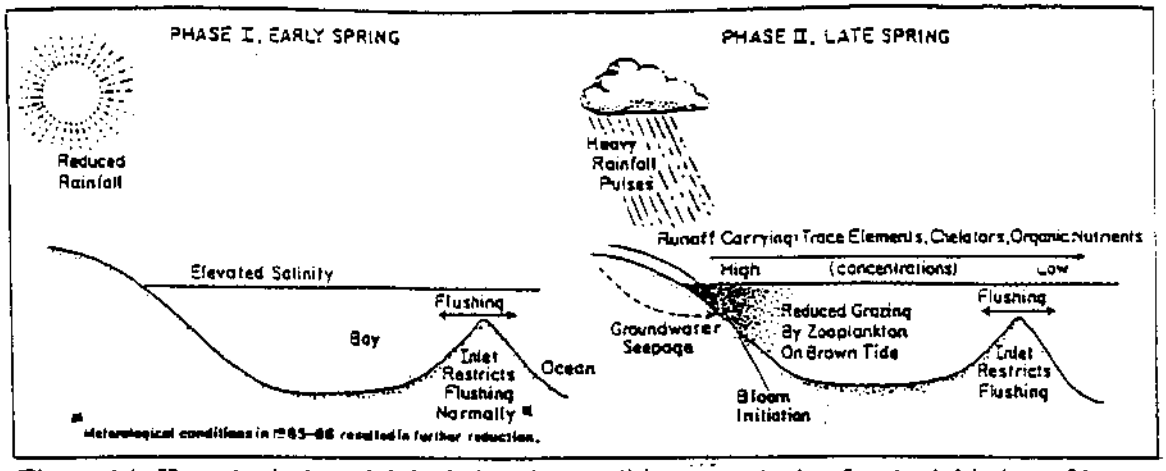


Figure 14. Hypothetical model depicting the conditions conducive for the initiation of brown tide blooms.

Further reduction in flushing of the bays by coastal waters because of changes in sea level [39] could have also been contributory. Grazing pressure during the early stages of bloom initiation was possibly also reduced [40,16]. Inactivation of viral control due to certain factors still under investigation are possibly involved and the subsidence of blooms must in part relate to viral infectivity.

The brown tide bloom scenario has some similarity to the "green tide" blooms of the 1950's in Great South Bay [17,18]. During the early fifties a lowering of salinity selected for two estuarine species, *Nannochloris* sp. and *Stichococcus* sp., with a salinity of 17‰ optimal for growth. The recurrence of the green tides for several summers afterwards appeared to depend on the restricted circulation of the inshore bays and the overwintering of large enough seed populations to initiate the next summer's growth. Effluents from duck farms, which flowed into Great South Bay through creeks, were found to be supplying nitrogeneous nutrients and promoting the growth of these two species of microalgae and these effluents were subsequently restricted [17,18].

The ability of *A. anophagefferens* to outcompete all other phytoplankton species and maintain dominance throughout the summer possibly relates to its specific micronutrient needs, heterotrophic capabilities and photoadaptive characteristics. The photosynthetic abilities of *A. anophagefferens* relative to other species under severe light limitation [1] might be particularly important since recent evidence indicates that light absorption characteristics and pigments of this species are more characteristic of a deep-dwelling oceanic species than a coastal form [41,22].

Other unusual phytoplankton blooms both past and present have resulted from subtle but long term anthropogenic eutrophication combined with altered environmental conditions [14]; examples of such are: the sewering of Providence, R.I. and the red tide of 1898 [42], duck farm effluents and the green tides of the 1950's [17,18], nutrient loading and acid rain leachates in Scandinavian waters associated with red tides and unusual *Chrysochromulina* blooms of 1988 [43,44], and unusual Gulf Stream meanders seeding *Ptychodiscus brevis* into enriched coastal waters off North Carolina [45].

Since *A. anophagefferens* is a species not previously known to cause blooms, environmental conditions contributory to the blooming could in part relate to new anthropogenic influences in these bays such as different chelators in detergents [28] or new lawn treatments [46]. Drought conditions, elevated salinities, pulses of rain delivering specific nutrients to the bay waters, along with restricted flushing of bay waters [39] set the scenario for the formation of a phytoplankton bloom. The selection of the particular brown tide species would then have related both to specific chemical conditions and any selective grazing pressures [16,40] extant. The role of viral infective activity in controlling the subsidence of these blooms [47] needs to be further evaluated as well as the possibility that viral inhibition is actually involved in allowing for the accumulation of large cell populations and blooms.

ACKNOWLEDGMENTS

Research supported by Suffolk County, New York; The Living Marine Resources Institute of the State Of New York; United States Environmental Protection Agency and New York Sea Grant Institute. We thank Saou-Lien Wong, Allen Milligan, Kristen Drewes (Milligan), Valerie Philbrick, Jim Carretta and Michael Doall for excellent technical assistance. This research represents in part the Master Theses of Susan Dzurica, Mathew Cottrell, Allen Milligan, Chris Gobler and an undergraduate thesis from Evergreen College to Kristen Drewes (Milligan). Support for C. Lee and for HPLC analyses was also provided by the Office of Naval Research. Special thanks go to the New York Air National Guard, 106 ARRG, Westhampton Beach, Long Island for making the aerial surveys possible.

REFERENCES

1. E.M. Cosper, W.C. Dennison, E.J. Carpenter, V.M. Bricelj, J.G. Mitchell, S.H. Kuenstner, D.C. Colflesh, and M. Dewey, Estuaries 10, 284-290 (1987).
2. R. Nuzzi, and R.M. Waters, in: Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms, E.M. Cosper, E.J. Carpenter and V.M. Bricelj, eds. (Springer-Verlag, Berlin 1989).
3. P. Olsen, ibid.
4. J.McN. Sieburth, P.W. Johnson and P.E. Hargraves, J. Phycol. 24, 416-425 (1988).
5. J.McN. Sieburth, and P.W. Johnson, in: Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms, E.M. Cosper, E.J. Carpenter and V.M. Bricelj, eds. (Springer-Verlag, Berlin 1989).
6. T.J. Smayda, and T.A. Villareal, ibid.
7. M. Bricelj, and S. Kuenstner, ibid.
8. W.C. Dennison, G.J. Marshall and C. Wigand, ibid.

9. G.A. Tracey, R.L. Steele, J. Gatzke, D.K. Phelps, R. Nuzzi, M. Waters and D.M. Anderson, ibid.
10. E.M. Cosper, E.J. Carpenter and M. Cottrell, ibid.
11. S.F. Bruno, R.D. Staker, G.M. Sharma, and J.T. Turner, Estuaries 6, 200-211 (1983).
12. J.S. Lively, Z. Kaufman, and E.J. Carpenter, Est. Coast. Shelf Sci. 16, 51-68 (1983).
13. C.D. Hardy, Marine Sciences Research Center Special Report, 3, State University of New York, Stony Brook, N. Y. (1976).
14. E.M. Cosper, W. Dennison, A. Milligan, E.J. Carpenter, C. Lee, J. Holzapfel and L.Milanese, in: Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms, E.M. Cosper, E.J. Carpenter and V.M. Bricelj, eds. (Springer-Verlag, Berlin 1989).
15. T.J. Smayda, Marine Geol. 11, 105-122 (1971).
16. D.A. Caron, E.L. Lim, H. Kunze, E.M. Cosper and D.M. Anderson, in: Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms, E.M. Cosper, E.J. Carpenter and V.M. Bricelj, eds. (Springer-Verlag, Berlin 1989).
17. J.H. Ryther, Biol. Bull. 106, 198-209 (1954).
18. J.H. Ryther, in: Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms, E.M. Cosper, E.J. Carpenter and V.M. Bricelj, eds. (Springer-Verlag, Berlin 1989).
19. E.M. Cosper, Appl. Phycol. Forum 4, 3-5 (1987).
20. D.M. Anderson, D.M. Kulis and E.M. Cosper, in: Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms, E.M. Cosper, E.J. Carpenter and V.M. Bricelj, eds. (Springer-Verlag, Berlin 1989).
21. L. Campbell, L.P. Shapiro, E.M. Haugen and L. Morris, ibid. 22.R.R. Bidigare, ibid.
23. J. Lewin, R.E. Norris, S.W. Jeffrey and B.E. Pearson, J. Phycol. 13, 259- 266 (1977).
24. M. Vesk, and S.W. Jeffrey, J. Phycol. 23, 322-336 (1987).
25. J. Throndsen, and S. Kristiansen, Abstrs, second International Phycol. Congr., Copenhagen p. 160 (1985).
26. H.R. DeYoe and C.A. Suttle, J. Phycol. 30, 800-806 (1994).
27. J. McLachlan, in: Handbook of Phycological Methods, Culture Methods and Growth Measurements, J.R. Stein, ed. (Cambridge University press, Cambridge 1973) pp. 25-51.
28. S. Dzurica, C. Lee, E.M. Cosper and E.J. Carpenter, in: Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms, E.M. Cosper, E.J. Carpenter and V.M. Bricelj, eds. (Springer-Verlag, Berlin 1989).
29. L.G. Sillen, and A.E. Martell, Stability Constants of Metal- Ion Complexes (Burlington House. London 1964).
30. F.M.M. Morel, Principles of Aquatic Chemistry (John Wiley & Sons. New York 1983).
31. E.M. Cosper, R.T. Garry, A.J. Milligan and M.H. Doall, in: Toxic Phytoplankton Blooms in the Sea, T.J. Smayda and Y. Shimizu, eds. (Elsevier Science Publishers. New York, 1993).
32. C.J. Gobler, The Role of Iron in the Occurrence of *Aureococcus Anophagefferens* Blooms, Masters Thesis, MSRC, SUNY at Stony Brook, (1995).
33. R.M. Ingle and D.F. Martin, Environ. Lett. 1, 69-74 (1971).
34. H.E. Glover, Limnol. Oceanogr. 23, 534-537 (1978).
35. D.M. Anderson, B.A. Keafer, D.M. Kulis, R.M. Waters and R. Nuzzi, J. Plankt. Res. 15, 563-580 (1993).

36. K.L.D. Milligan and E.M. Cosper, Science 266, 805-807 (1994).
37. R. B. Kapusciniski and R. Mitchell, Water Res. 14,363-371 (1980).
38. D.W. Pritchard, and E. Gomez-Reyes, Marine Sciences Research Center, SUNY, Stony Brook, N.Y., Special Report No. 70 (1986).
39. M. Vieira, in: Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms, E.M. Cosper, E.J. Carpenter and V.M. Bricelj, eds. (Springer-Verlag, Berlin 1989).
40. L.E. Duguay, D.M. Monteleone and C. Quaglietta, ibid.
41. C.S. Yentsch, D.A. Phinney and L. Shapiro, ibid.
42. S.W. Nixon, ibid.
43. E. Graneli, P. Olsson, P. Carlsson, B. Sundstrom and O. Lindahl, ibid.
44. E. Dahl, O. Lindahl, E. Paasche and J. Throndsen, ibid.
45. P.A. Tester, P.K. Fowler and J.T. Turner, ibid.
46. J.McN. Sieburth, ibid.
47. L.B. Slobodkin, ibid.

THE ROLE OF IRON IN BROWN TIDES: AN OVERVIEW

Gregory L. Boyer
State University of New York College of Environmental Science and Forestry

The occurrence of unusual plankton blooms (brown tides) by the chrysophyte *Aureococcus anophagefferens* has caused havoc with the shellfisheries and eelgrass beds of the Peconic and Great South Bay estuary system. Considerable effort has gone into the study of those factors responsible for the outbreak of these *A. anophagefferens* blooms. Environmental variables which may trigger or contribute to these brown tides include elevated salinities arising from drought conditions during the preceding winter, pulses of rainfall delivering organic or micronutrients to the embayments, reduced grazing, and restricted flushing of the bays. Examination of macronutrients (nitrate-nitrite and phosphate) did not show a marked difference between pre and post bloom conditions, suggesting that these inorganic macronutrients do not explain the increased incidence of brown tides in these embayments [1]. Mesocosm studies with natural population of *Aureococcus anophagefferens* from Narragansett Bay suggest that the 1985 bloom there was not simply a response to eutrophication. When dissolved macronutrients (ammonia, phosphate, silica) were added to the mesocosm containing moderate seed populations, a full scale bloom failed to appear [2].

These results have prompted investigators to look at other factors that may trigger brown tide formation. Cosper and coworkers have investigated the role of chelators and various essential trace elements in promoting the growth of *Aureococcus anophagefferens*. While several metals including vanadate and arsenate were investigated, primary efforts focused on iron and selenium [3, 4]. Iron forms a key cofactor in the enzymes needed for nitrogen assimilation (nitrate reductase, nitrogenase, GOGAT) and therefore strongly affects a cell's ability to grow at limiting nitrogen levels. Its importance in limiting primary productivity is well established in the high nitrate low chlorophyll regions of the open ocean where a combination of physiological measurements and the large scale nutrient additions have shown that the nanophytoplankton productivity in these regions is limited by iron. The effects of iron in coastal environments is less definitive. Iron and selenium are reported to enhance natural blooms of the red tide dinoflagellate *Gymnodinium nagasakiense*, the diatom *Thalassiosira pseudonana*, the raphidophytes *Chattenella antiqua* and *Heterosigma* sp. (formerly *H. akashiwo)*. Unfortunately these bioassay observations have rarely been followed up by more detailed physiological measurements that would confirm iron deficiency. Initial estimates of the minimal iron required for *Aureococcus anophagefferens* are around 9 µM, a level that is very high in comparison to most marine species [5] and well above the levels (< 1 µM) expected to be found in most inshore marine ecosystems. Addition of iron to natural waters containing a bloom of *Aureococcus anophagefferens* markedly increased their growth rate, suggesting these organisms *in situ* may be iron-limited.

Iron addition experiments such as those described above are fraught with difficulties. Iron is a common contaminant in many laboratory chemicals, requiring extensive cleaning techniques for "glassware" and laboratory equipment. Contamination from the steel hulls of research vessels will bias field bioassay results. Iron is also a very labile compound. It is

rapidly oxidized to Fe(III) in oxic solution and precipitates as its insoluble ferric hydroxide. To keep iron in a biologically available form, chelators such as EDTA are often added to media formulations. These chelators may cause their own problems, either tying up other essential trace metals or degrading to toxic products. The common chelator EDTA is especially bad in this regard, breaking down upon exposure to light to form formaldehyde. *Aureococcus anophagefferens* did not grow well with EDTA as the chelator [4]. Without further information, the reason for that lack of growth is unknown. Natural chelators may serve similar functions, either by helping to solubilize a needed trace metal or removing a potentially toxic one. Addition of such chelators through natural or anthropogenic inputs to the system can dramatically perturb the normal trace metal speciation.

Different organisms may also use different biochemical approaches to meet their cellular needs for iron. Reductive approaches, such as that used by strategy I higher plants and most chlorophytes, rely of the reduction of ferric iron to the more soluble ferrous form. This involves induction of a membrane bound ferric reductase capable of reducing a number of iron chelates. The ferrous iron is then taken up via an iron transport protein for use in the cell. Most bacteria, cyanobacteria and strategy II higher plants use a different approach. Under low iron conditions, they excrete a small molecular weight chelator, termed siderophore, which has an extremely high affinity for iron. The iron siderophore complex is then absorbed by the cell through a specific ferric siderophore receptor. Some cells do not use either of these approaches. They stop growing, waiting for the levels of iron in the environment to increase to a point which will support cell growth.

Outside environmental variables may also dramatically affect an organisms ability to sequester trace metals. Since siderophores are excreted to the surrounding environment, a stable water with little mixing may be necessary for an organism to use a siderophore-mediated uptake system. River run-off may provide the necessary trace metals, but humic materials may also complex trace metals in a form unavailable to phytoplankton. The presence of chelators in the water column may also stimulate phytoplankton growth by sequestering trace metals (namely copper) that may be toxic to a given organisms. Biological interactions between organisms may also influence trace metal uptake. Siderophore formation may sequester iron in a form that is biologically unavailable to competing species. Alternatively, an organism could produce the uptake receptors to use siderophores made by completing species. This would allow it to save the energy and materials normally expended for its own siderophore biosynthesis. In some cases, siderophore formation may be directly detrimental. Coliform bacterinphages use the siderophore receptor as a means to enter the cell. This is of interest to brown tides since recent work has suggested that *Aureococcus anophagefferens* blooms may be controlled by a phage-like virus and this phage is sensitive to iron levels. It was hypothesized that colloidal iron may bind the virus and prevent cell lysis. An alternative and equally likely explanation is that colloidal iron represses the inducible siderophore receptor that the virus needs to enter the cell. Thus iron input into the water column may not only promote the growth of the brown tide species, but may also prevent the cell lysis by the phage-like viruses.

All these factors combined indicate the need for further study on the physiological mechanisms of iron and trace metal uptake in *Aureococcus anophagefferens*. These studies need to be integrated into work on the availability of iron and natural chelators in the Peconic Estuary system. There is no guarantee that understanding the iron uptake mechanisms used by *Aureococcus anophagefferens* will provide a means to control brown tides. However it is the first logical step in determining if this organism is limited by iron *in situ*. Identification of an uptake receptor, siderophore, or iron-repressed chelate reductase would provide a needed metabolic marker for iron limitation. It may be possible to detect the presence or absence of this marker in a field population. These studies will also increase our understanding of the role of trace metals and chelators in mediating the interaction between *A. anophagefferens* and other organisms, be that a potential bloom inhibiting virus or possible diatom blooms that produce growth-stimulating natural chelators.

REFERENCES

Cosper, E. M., E. J. Carpenter, and M. Cottrell (1989) Primary productivity and growth dynamics of the "Brown Tide" in Long Island embayments. In: "Novel Phytoplankton Blooms" E. M. Cosper, V. M. Bricelj, and E. J. Carpenter, Eds., Springer Verlag, New York, NY., pp. 139-158.

Keller, A. A., and R. L. Rice (1989) Effects of nutrient enrichment on natural populations of the brown tide phytoplankton *Aureococcus anophagefferens* (Chrysophyceae). J. Phycol. 25:636-646.

Dzurica, S., C. Kee, E. M. Cosper, and E. J. Carpenter (1989) Role of environmental variables, specifically organic compounds and micronutrients, in the growth of the crysophyte *Aureococcus anophagefferens*. In: "Novel Phytoplankton Blooms" E. M. Cosper, V. M. Bricelj, and E. J. Carpenter, Eds., Springer Verlag, New York, NY., pp. 229-252.

Cosper, E. M., R. T. Garry, A. J. Milligan, and M. H. Doall (1993) Iron, selenium and citric acid are critical to the growth of the brown tide microalga, *Aureococcus anaphafferens*. In: "Toxic phytoplankton blooms in the Sea" T. J. Smayda, and Y. Shimizu, Eds., Elsevier, Amsterdam, Netherlands., pp. 667-674.

Brand, L. E., W. G. Sunda, and R. R. L. Guillard (1983) Limitation of phytoplankton reproductive rates by zinc, manganese, and iron. Limnol. Oceanogr. 28:1182-1198.

ASPECTS OF TIDAL AND SUBTIDAL FLUSHING WITHIN THE PECONIC BAYS ESTUARY

Robert Wilson
Marine Sciences Research Center, State University of NewYork Stony Brook

HYDROGRAPHY

The Peconic Bays Estuary represents a system of very shallow interconnected bays situated between the north and south forks of Eastern Long Island. It consists of Flanders Bay. Great Peconic Bay, Little Peconic Bay and Shelter Island Sound. Gardiner's Bay to the east of Gardiner's Island could also considered as part of the system. A useful description of the hydrography of the system is provided by Hardy (1976). Areal and volumetric statistics for the entire bay system are tabulated below (from Hardy):

Surface area	218 km^2
Average depth	4.7 m
Maximum depth	20 m
Width range	0.5 to 9.8 km
Volume, mean low water (mlw)	$11.6x10^8$ m^3
Tidal prism	$17.0x10^7$ m^3
Mean tidal range	0.76 m
Annual mean surface fresh water inflow	3 to 5 $m^3 \cdot s^{-1}$

These statistics are exclusive of Gardiner's Bay which has a surface are of approximately 90.5 km^2 and a mean depth of approximately 11.0 m.

Groundwater inflow is potentially important to the budgets of water and salt as well as dissolved materials within the Peconics. Measurements which can describe the temporal and spatial patterns of groundwater inflow are being considered. It is possible to say something about the magnitude of groundwater inflow to the Peconics using the groundwater flux measurements in the adjacent Great South Bay reported by Bokuniewicz (1980) and Bokuniewicz and Zeitlin (1980). They determined that the groundwater flux to Great South Bay was $8.6x10^3$ liters per day per meter of shore line. If we use the surface area A of the Peconics to infer the perimeter of an equivalent circular basin we obtain a perimeter $P=2/(\pi A)$ of 52 km. So without considering the fractal complexities of the Bay perimeter we estimate the groundwater flux to the Peconics system to be $4.5x10^8$ $l \cdot day^{-1}$ or 18.7 $m^3 \cdot s^{-1}$. Klonowski (1979) reported an annual groundwater inflow to Flanders Bay of $7.06x10^4$ m^3. Flanders Bay has a surface area of 10.1 km^2 and an equivalent perimeter of 11 km. This gives a mean annual flux per meter of shore line appreciably lower than Bokuniewicz reported for Great South Bay. Observations are required to reconcile these types of discrepancies.

Hydrographic observations reported by Hardy and more recently by Vieira (1990a, 1990b) emphasize that thermal, haline and density stratification is generally weak but detectable throughout the bays. Haline stratification is high near the mouth of the Peconic River. Conductivity, temperature, depth (CTD) measurements in the Peconics in 1984 (Vieira, 1990) show that surface to bottom density differences range from 0.5 to a maximum of approximately 1.0 sigma-t units. Data from surface salinity surveys also in 1984 show that longitudinal salinity differences from the interior of Flanders Bay to Shelter Island Sound reach a maximum of approximately 3 practical salinity units. There is some indication that maximum vertical and longitudinal density (salinity) differences occur in early spring.

We can use the information to estimate the two controlling parameters associated with the Hansen and Rattray (1966) estuarine classification. This is useful because it not only serves to establish the classification of the estuary, but also to determine approximately what fraction of longitudinal salt transport is due to dispersion, and what fraction may be due to density-driven circulation. These parameters depend on the total fresh water inflow (including groundwater), the density stratification, the maximum tidal current strength and the depth. They are a bulk densimetric Froude number defined by F_m and an estuarine Richardson number R_i (see for example Fisher *et al.* (1979)). Estimates for these parameters yield $F_m=10^{-4}$ and $R_i=10^{-2}$. These values actually place the estuary in the parameter space of a TYPE 2 estuary for which there can be significant salt flux by density driven circulation. For these parameter values the fraction of the longitudinal salt flux due to the density-driven circulation is approximately 0.3.

TIDAL FLUSHING AND RECIRCULATION

The volume of water exchanged every semidiurnal tidal cycle is approximately 15% of the mlw volume of the basin. This is equivalent to a maximum flushing rate for the entire basin of 0.28 day^{-1}. This would be a relevant flushing rate only if the water entering the bays on flood were entirely new ocean water. With recirculation, however, only a fraction of this exchange may be "new" water and only this fraction would contribute to flushing of the estuary. Using the development presented by Fisher *et al.* (1979), the volume of water entering the estuary through the constrictions at the mouth on flood is decomposed into a fraction which left the estuary on the previous ebb and a fraction of new ocean water. They defined the fractional exchange ratio as the ratio of the volume of new ocean water entering on flood to the total flood volume. Using observational data it is possible to make direct estimates for the exchange ratio. Preliminary estimates obtained using data from selected moored instruments from the 1984 National Ocean Service survey indicate that recirculation is appreciable and that a significant fraction of the volume which flowed out on a previous ebb returns on flood. Numerical results by Signell and Butman (1992) emphasize the importance of accounting for recirculation in assessing embayment flushing.

Density induced circulation can lead to reduced recirculation and thereby to enhance flushing efficiency. Garcon *et al.* (1986) determined, for example, that the presence of density induced circulation contributed to enhanced tidal flushing of an embayment. They concluded also that wind mixing and low fresh water inflow would lead to a reduction of the efficiency of

exchange through their effects on the density induced circulation. Interannual variations in freshwater inflow and wind mixing affect the density-driven circulation and thereby the efficiency of tidal flushing.

SUBTIDAL FLUSHING AND CLIMATIC VARIABILITY

Vieira (1989) and Vieira and Chant (1993) have shown that the 1986 brown tide events in the Peconics were associated with periods of reduced wind-induced subtidal sea level variations. This has led Vieira and Chant and other investigators to suggest that meteorological forcing has a direct influence on the onset of algal blooms through its effects on low frequency flushing and rainfall.

Preliminary analyses by DiLorenzo and Vieira (1992) suggest relationships between cell counts for the period 1985 through 1992 and both the northeast component of wind and precipitation. The results are compelling and they provide further evidence for the importance of meteorological forcing and climatic variability. The mechanisms by which this meteorological forcing affect cell counts need to be established, whether they be through flushing alone, wind induced bottom resuspension or through groundwater inflow.

SHINNECOCK CANAL

One unique feature of the Peconic Bays is the Shinnecock Canal which connects Great Peconic Bay with Shinnecock Bay. This canal was opened in 1893. It served to enhance the flushing of Shinnecock Bay, to aid in the maintenance of Shinnecock Inlet, and as a pathway for light boat traffic between the two bays. In its present operation it allows a rectified flow directed from Great Peconic Bay to Shinnecock Bay.

The prism represented by the flow through the canal is approximately 1.5×10^7 m^3. This represents approximately 9% of the prism of the entire bay, but it represents 25% of the prism of Great Peconic Bay whose prism is 6.04×10^7 m^3. It represents an even larger fraction of the prism of Shinnecock Bay.

At present the canal transports water from Great Peconic Bay containing Brown tide algal cells into Shinnecock Bay and subsequently out through Shinnecock Inlet. Once outside the inlet, this water is transported down the coast by the along shore currents and could inoculate other bays.

This raises the interesting possibility of a management strategy for enhanced flushing and for the control of water column salinity and temperature of the Peconic Bays involving a reversed flow operation of the canal. Although the prism associated with the discharge through the canal represents only 25% of the prism of Great Peconic Bay, the proximity of the canal to the Shinnecock Inlet and the ocean would make this an efficient exchange pathway because there would be reduced recirculation.

REFERENCES

Bokuniewicz, H.J. 1980. Characteristics of groundwater seepage into Great South Bay, New York. Estuarine and Coastal Marine Science, 10:437-444.

Bokuniewicz, H.J. and M. Zeitlin. 1980. Characteristics of groundwater seepage into Great South Bay. Special Report No. 35, The Marine Sciences Research Center, SUNY, Stony Brook, NY, Reference 80-4.

Fischer, H.B., J. Imberger, E.J. List, R.C.Y. Koh and N.H. Brooks. 1979. Mixing In Inland And Coastal Waters. Academic Press, New York.

Dilorenzo, J.L. and M.E.C. Vieira.1992. Personal Communication.

Garcon, V C., K.D. Stolzenback and D.M. Anderson. 1986. Tidal flushing of an estuarine embayment subject to recurrent dinoflagellate blooms. Estuaries, 9:179-187.

Hansen, D.V. and M. Rattray. 1966. New dimensions in estuarine classification. Limnology and Oceanography, 11:319-325.

Hardy, C.D. 1976. A preliminary description of the Peconic Bay Estuary. Special Report No. 3, The Marine Sciences Research Center, SUNY, Stony Brook, NY, Reference 76-4.

Klonowski, J.E. 1979. The water budget of Flanders Bay, New York. Masters Thesis, Earth and Planetary Sciences, City College of the City University of New York.

Signell, R.P. and B. Butman 1992. Modeling tidal exchange and dispersion in Boston Harbor, Journal of Geophysical Research, 97:15591-15606.

Vieira, M.E.C. 1989. The case for meteorological driven fluctuations in residence times of Long Island waters subject to algal blooms. In: Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms, E.M. Cosper, E.J. Carpenter, and V.M. Bricelj (eds.), Lecture Notes on Coastal and Estuarine Studies, Springer-Verlag, Berlin, pp. 295-305.

Vieira, M.E.C. 1990a. Observations of currents, temperature salinity and sea level in the Peconic Bays, 1984. Special Data Report No. 4, The Marine Sciences Research Center, SUNY, Stony Brook, NY. Reference 90-9.

Vieira, M.E.C. 1990b. Synoptic surveys of salinity and temperature in the Peconic Bays, 1984. Special Data Report No. 5, The Marine Sciences Research Center, SUNY, Stony Brook, NY. Reference 90-10.

Vieira, M.E.C., and R. Chant. 1993. On The Contribution of Subtidal Volume Fluxes to Algal Blooms in Long Island Estuaries. Estuarine, Coastal and Shelf Science 36: 15-29.

MODERN APPROACHES TO THE IDENTIFICATION, ENUMERATION, AND SEPARATION OF *AUREOCOCCUS ANOPHAGEFFERENS* IN NATURAL SAMPLES

Donald M. Anderson
Woods Hole Oceanographic Institution

INTRODUCTION

Certainly one of the most significant challenges in field investigations of the brown tide alga, *Aureococcus anophagefferens* relates to its small size and non-descript morphology (Sieburth *et al.* 1988). Measuring only 2 μm in diameter, this organism lacks morphological features which can be used to distinguish it from other similar-sized algae, bacteria, and detritus with either phase contrast or epifluorescence microscopy. Identification with the standard microscopes is uncertain unless the species is at such high abundance that it dominates a particular sample.

In recognition of this problem, an immunofluorescent technique was developed to label or "tag" the cell surface of *A. anophagefferens* so that it could be visualized using an epifluorescence microscope (Anderson *et al.* 1989). This antibody-based procedure has since been used in a number of field studies investigating grazing impacts on the brown tide organism (e.g., Tracy *et al.* 1989; Caron *et al.* 1989), as well as in a large-scale biogeographic survey of the distribution of this organism within this region (Anderson *et al.* 1993). Thus far, however, the antibody has only been used in identification and enumeration of the brown tide alga, and then only with manual microscope techniques. In this paper, the methods for identification of this algae using the transmission electron microscope and the antibody are briefly reviewed. Examples are then drawn from immunological investigations of other algal species to demonstrate new approaches that could greatly facilitate autecological studies of brown tides.

TRANSMISSION ELECTRON MICROSCOPE (TEM)

Until the advent of the antibody technique developed by Anderson *et al.* (1989), the only means of positive identification of *A. anophagefferens* was through the use of the TEM. This is because the cell has a rather non-descript morphology, and does not fluoresce in any unique manner. For the TEM procedure, cells are concentrated, fixed, dehydrated, embedded in resin, and cut into thin sections that are then viewed under high magnification according to the methods of Johnson and Sieburth (1982). With this instrument, Sieburth *et al.* (1988) demonstrated that the nucleus of this spherical picoplankter is ovoid in shape, and that one, cup-shaped chloroplast was present with an embedded pyrenoid. There is no cell wall, but the cell is often surrounded by a diffuse layer of extracellular organic material. These and other features are sufficient for the positive identification of *A. anophagefferens* among co-occurring picoplankton, but the method is tedious and is of little use in enumeration of the species.

IMMUNOFLUORESCENT IDENTIFICATION

Following procedures developed earlier for other picoplankton (e.g., Campbell and Carpenter 1987), a polyclonal antibody was raised against cell-surface proteins of *A. anophagefferens* by injecting preserved, cultured cells of this species into rabbits (Anderson *et al.* 1989). An indirect immunofluorescent protocol has been used with this antibody to label brown tide cells in culture and in field samples. Briefly, the protocol starts with a concentration step in which cells are collected on a filter, followed by a blocking step, in which a protein solution (normal goat serum) is applied to the sample to eliminate non-specific binding. The primary antibody that is specific for *A. anophagefferens* is then added to a sample. Those antibodies bind to the cell-surface proteins of the brown tide cell, but visualization of this complex is only possible after the addition of a secondary antibody, typically conjugated to a fluorescent compound such as FITC (fluorescein isothiocyanate). Cells that have been treated in this manner are easily visualized on an epifluorescent microscope, since they have a green "halo" around them (Fig. 1). The entire procedure takes several hours, of which less than 1/3 is actual "hands-on" time.

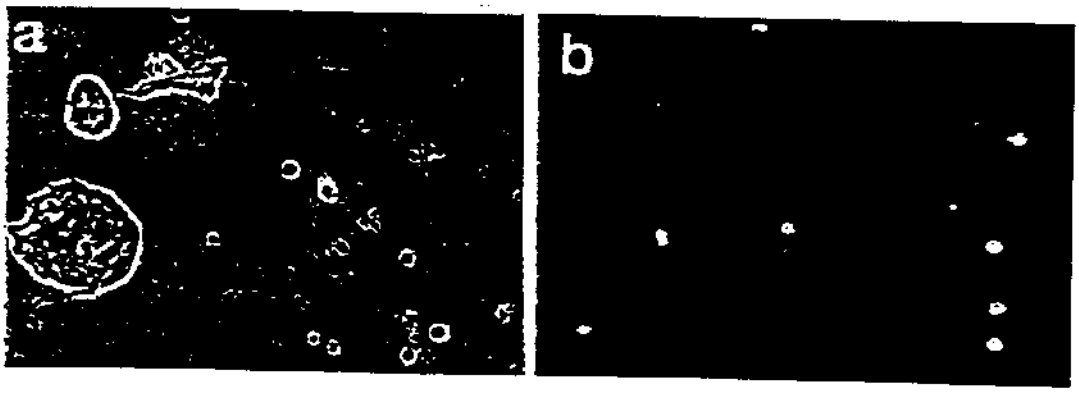


Figure 1. Phase contrast and epifluorescent micrographs of *A. anophagefferens* from a Long Island field sample. a) Phase contrast image of field sample showing detritus and cells of many types and sizes. Cells of the brown tide organism are not easily identified. b) Epifluorescence image of the same field, clearly showing the *A. anophagefferens* cells with a fluorescent halo.

The polyclonal antibody has proven to be highly specific for *A. anophagefferens*. Anderson *et al.* (1989) tested 46 algal species selected on the basis of their phylogenetic or morphological similarity to the brown tide alga. No cross-reactions were observed at antiserum dilutions of 1:3200, which was selected as the working concentration for field samples.

When the immunofluorescent technique is applied to field samples, it is easy to enumerate *A. anophagefferens* cells using a standard epifluorescence microscope. As few as 10-20 cells•ml can be detected with this procedure. This low detection limit allowed Anderson *et al.* (1993) to conduct a biogeographic survey for *A. anophagefferens* cells over a large area between Massachusetts and Delaware Bay.

NEW APPLICATIONS OF ANTIBODY TECHNOLOGY

Thus far, the antibody to *A. anophagefferens* has been used entirely for manual microscope counts. There are, however, several new technologies that could be applied to this organism which would greatly improve enumeration speed and accuracy, and which would also permit a variety of physiological measurements to be made on natural brown tide populations. Application of these methods to the brown tide alga is seen as a high priority activity that will greatly facilitate autecological studies searching for mechanisms underlying the massive blooms.

Flow-cytometry

One obvious application of the fluorescent antibody technique involves the use of a flow-cytometer to enumerate, and possibly to sort *A. anophagefferens* cells. A flow-cytometer is a sophisticated instrument used heavily in hospitals and other medical facilities to characgterize cell types. The instrument uses a laser to probe the optical characteristics of individual cells that are passed single-file in a sample stream at rates of thousands of cells per second. Fluorescence (at several different wavelengths) as well as light scattering properties, (which are sometimes related to cell-size) are recorded for each cell.

The flow-cytometer has been used in studies of phytoplankton which have unique size or autofluorescence characteristics (i.e. the natural pigments emit fluorescence at wavelengths which allow them to be distinguished from co-occurring organisms). For example, the cyanobacterium *Synnechoccus* and the prochlorophyte *Prochlorococcus phave* been investigated throughout the worlds oceans (i.e., Chisholm *et al.* 1988; Olsen *et al.* 1988). Only recently has flow-cytometry been applied to phytoplankton cells which have been labeled with antibodies, however. Vrieling *et al.* (1993, 1994) have used an antibody specific for the toxic dinoflagellate *Gyrodinium aureolum* in flow cytometric analyses of natural samples. Likewise, an antibody to the toxic dinoflagellate *Alexandrium tamarense* is being used in efforts to enumerate and separate that species from co-occurring phytoplankton and detritus in samples from the Gulf of Maine (Anderson, unpublished data).

No significant effort has yet been made to use the flow-cytometer and antibody labeling for brown tide studies, although there's every reason to believe that with some development effort, this could prove to be a useful tool. There are, however, some problems that should be anticipated based on studies of other organisms. The first relates to the natural autofluorescence of planktonic organisms. Even though the human eye distinguishes between antibody-labelled cells and other organisms in a sample, the output from a flow-cytometer when a natural sample is analyzed is often a continuum of fluorescence intensities spanning several orders of magnitude. This sometimes makes it difficult to identify a unique population with optical characteristics that do not overlap with other co-occurring organisms. The first step to circumvent this problem involves making the antibody label brighter, which can be accomplished with several different techniques (Anderson 1995). Even then, it is unlikely that the organism will be separable from others solely on the base of the fluorescence of the antibody, and thus other optical characteristics are needed. In the case of *A. anophagefferens*, its chlorophyll fluorescence and its size can be used as two additional parameters on which to define a population. It remains to be

seen whether these characters are sufficient to define a population on the screen of the flow-cytometer that can be enumerated with confidence that other organisms are not included and that the bulk of the brown tide population is represented.

Once it is possible to identify and enumerate brown tide cells among other organisms in a sample using a flow cytometer, the sorting capability of the instrument can be exploited. Despite the tremendous speed with which cells are passed through the laser beam and analyzed, it is possible for the computer to sort or collect cells with particular characteristics (e.g., those with green. antibody fluorescence, red chlorophyll fluorescence, and a certain size as suggested by light scatter). A pure sample of the species of interest can thus be obtained, and used for subsequent analysis. There are constraints to the number of organisms that can be collected in this manner due to the time involved in sorting, but the method would allow physiological or biochemical measurements to be made at the species level without interference from detritus or other organisms. These and other flow cytometric applications of the immunofluorescent assay are clearly an area where developmen effort is needed with respect to the brown tide.

ELISA

Flow-cytometers are sophisticated and expensive instruments, and many investigators and agencies will not have access to them. An alternative technique that can also be used to enumerate cells that are labelled with an antibody probe is called the "enzyme linked immunosorbent assay" or ELISA. With this method, labelled cells are not visualized through the attachment of primary or secondary antibodies, as in the immunofluorescence method described above, but rather by the fluorescence or color produced in solution by an enzyme which has been linked to one of those antibodies. The enzyme portion of the cell/antibody/enzyme complex is able to act on its appropriate substrate, producing color or fluorescence which can be quantified. An ELISA approach to enumeration of phytoplankton species would involve the filtration of samples into individual wells of a special tissue culture plate system fitted with membranes at the bottom of each well, followed by the same blocking and antibody labeling steps described above. An enzyme such as alkline phosphatase is then linked to the cell/antibody complex, typically using biotin-streptavidin linkages, and a substrate is then added. After a suitable incubation time, a vacuum is applied and the liquid surrounding the cells trapped on the membrane is drawn into tissue culture wells lying directly below the membranes, capturing the colored or fluorescent product which is then easily quantified using an automated plate reader.

ELISA procedures have long been used in many areas of medicine and biology, but are only now being applied to the detection of phytoplankton species. In on-going studies of the toxic dinoflagellate *A. tamarense*, we have found that this method holds great promise for the rapid and accurate identification of cells in a sample. Once again, developmental work is required, however, as problems with cross reactions are accentuated by the signal amplification action of the enzymes used for detection. For example, if a sample contains 100 cells of the target species for which the antibody is specific, as well as 10,000 cells of another species for which the antibody has a weak but positive cross reaction, the color or fluorescence produced by enzymes attached to the target cells may well be swamped by that produced by the weak but more numerous cross reactions. Based on our experience, this problem can be circumvented in

part by use of the primary antibody at an appropriate dilution, and by careful attention to the blocking steps in the procedure. Thus far, a detection limit of approximately 100 - 200 *Alexandrium* cells seems possible with this method.

As is the case with flow-cytometry, ELISA techniques have not yet been applied to *A. anophagefferens*, but there is every reason to expect that this method can greatly simplify and accelerate enumeration of the species

Magnetic beads

Those studying field populations of *A. anophagefferens* are presently unable to obtain species-specific measurements of important physiological parameters that would yield information about the nutritional status of the cells, their growth-rate, or other physiological parameters. Standard techniques for productivity, nutrient uptake, chlorophyll, and so forth all provide estimates at the community level, and are not readily adapted to species-specific measurements. This problem is especially severe with a species such as *A. anophaefferens* given that its small size precludes microscopic isolation (e.g., Rivkin and Seliger 1981; Rivkin 1985).

We have recently developed a technique which will allow the separation of a phytoplankton species for which an antibody is available from complex planktonic assemblages. This method (Aguilleras *et al.* submitted ms.) involves the use of tiny magnetic beads which can be linked to cells that are labelled with an antibody. The cell/bead complex is then removed from solution using a magnet. Immunomagnetic separation has been a reliable medical tool for the purification and characterization of a wide range of cell types such as tumor and lymphoid cells. This method is also used routinely for the isolation, identification and analysis of DNA or RNA sequences. The viability of certain cell types does not seem to be affected by the attachment process and in some cases, bead detachment is possible without harming the cells. When applied to phytoplankton cells, the process is fundamentally similar to the standard immunological procedures described above. Experiments with *A. tamarense* have yielded samples of better than 90 - 95% purity, both with mixtures of cultured cells, and with natural plankton communities.

Due to loss of some cells during the antibody labelling procedure, this method is not presently suitable for enumeration of a target species, but instead is intended for separation of a species from a sample for subsequent physiological or biochemical measurements. Some measurements would be straight-forward, requiring little additional developmental effort once the actual removal of target cells from solution is accomplished. For example, if this method were applied to the brown tide organism (which seems perfectly feasible since beads are used to collect bacteria), samples of brown tide water could be incubated for a normal ^{14}C protocol. The *A. anophagefferens* cells could then be removed from solution using magnetic bead, and their activity determined directly using a scintillation counter. This would provide estimates of carbon uptake at the species level, something which is presently only possible during the stages of a bloom when *A. anophagefferens* is completely dominant. Other measurements would be more problematic if it proved necessary to remove the beads from the cells to eliminate contamination or interference. For example, given the recent work of Gobler and Cosper (1995) linking the

brown tide distribution to levels of iron, it would be of great interest to determine the iron quota of *A. anophagefferens* cells in various waters and at different times during a bloom. Magnetic bead separation offers some promise in this respect, but the obvious problem with iron contamination from the beads must be avoided. In like manner, measurements of nutrient quotas such as C:N:P ratios should be possible, but not before studies have been undertaken to determine the amount of contamination from the beads, or methods are developed for detachment and removal of the beads without damage to the cells.

SUMMARY

The development of an antibody probe for *A. anophagefferens* provided a useful tool which has done much to improve the accuracy and efficiency of cell counts of this organism in natural samples. In this brief overview, new methods have been introduced which rely on this same antibody, and which could be used with great utility in studies of the brown tide. Each method has its own promises and limitations, and in all cases, development work is needed to bring the concepts proposed here to full application. In most cases, the methods are being developed for other organisms, including some that are harmful or toxic such as the dinoflagellate *A. tamarense*, so extension of those procedures to *A. anophagefferens* should be relatively straight-forward. With a suitable investment of time and funds, not only can the identification and enumeration of this species become faster and more accurate, but physiological measurements which at present are impossible at the species level will become feasible.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the NOAA National Sea Grant College Program Office, Department of Commerce grants No. NA46RG0470 (WHOI Sea Grant Projects R/B-121 and R/B-130), and by the National Marine Fisheries Service through Saltonstall- Kennedy grant (No. NA47FD00447). Contribution No.9082 from the Woods Hole Oceanographic Institution.

REFERENCES

Aguilera Bazan, A., S. Gonzales Gil, B.A. Keafer, and D. M. Anderson. Submitted ms. Immunomagnetic separation of toxic dinoflagellate cells from natural plankton samples

Anderson, D.M. 1995. Identification of harmful algal species using molecular probes: An emerging technology. *In*: Lassus, P., Arzul, G., Erard, E., Gentien, P. & Marcaillou, C. [Eds.] *Harmful Marine Algal Blooms*. Lavoisier Sci. Publ., Paris, France. pp.3-13.

Anderson, D.M., Kulis, D.M. & Cosper, E.M. 1990. Immunofluorescent detection of the brown tide organism *Aureococcus anophagefferens*. *In*: Cosper, E.M., Bricelj, V.M. & Carpenter, E.J. [Eds.] *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer Verlag, Berlin, pp. 213-28.

Anderson, D.M., Keafer, B.A., Kulis, D.M., Waters, R.M. & Nuzzi, R. 1993. An immunofluorescent survey of the brown tide chrysophyte *Aureococcus anophagefferens* along the northeast coast of the United States. *J.Plankton Res.* 15:563-80.

Campbell, L. & Carpenter, E.J. 1987. Characterization of phycoerythrin-containing *Synechococcus* spp. populations by immunofluorescence. *J.Plankton Res.* 9:1167-81.

Chisholm, S.W., Olson,R.J., Zettler,E.R; Goericke,R.,Waterbury, J.B., Welschmeyer,N.A. 1988. A novel free-living prochlorophyte abundant in the oceanic euphotic zone. *Nature* 334:340-343

Gobler, C. J. and E.M. Cosper. 1995. Stimulation of "brown tide" blooms by iron. Abstract, 7th International Conf. on Toxic Phytoplankton. Sendai, Japan.

Johnson, P.W., and J.McN. Sieburth. 1982. In situ morphology and occurrence of eucaryotic phototrophs of bacterial size in the picoplankton of estuarine and oceanic waters. J. Phycol. 18:318-327.

Olson, R.J., Chisholm, S.W., Zettler, E.R. & Armbrust, E.V. 1988. Analysis of *Synechococcus* pigment types in the sea using single and double beam flow cytometry. *Deep Sea Res.*, 35:425-40.

Rivkin, R. B. 1985. Carbon-14 labelling patterns of individual marine phytoplankton from natural populations. Marine Biology 89: 135-142.

Rivkin, R. B. and H. H. Seliger. 1981. Liquid scintillation counting for ^{14}C uptake of single algal cells isolated from natural samples. Limnol. Oceanogr. 26: 780-784.

Vrieling, E.G., Draaijer, A., van Zeijl, W.J.M., Peperzak, L., Gieskes, W.W.C. & Veenhuis, M. 1993. The effect of labelling intensity, estimated by real time confocal laser scanning microscopy, on flow cytometric appearance and identification of immunochemically labelled marine dinoflagellate species. *J.Phycol.* 29:180-8.

Vrieling, E.G., Gieskes, W.W.C., van Zeijl, W.J.M. & Veenhuis, M. 1994. Detection of*Gyrodinium aureolum* in Dutch coastal waters by particle analysis based on immuno-flow cytometry. *Neth.J.Aquat.Ecol.* 28:249-54.

PLANKTONIC FOOD WEBS AND BROWN TIDE IMPACTS

Darcy J. Lonsdale
Marine Sciences Research Center, State University of New York Stony Brook

SUMMARY

Investigations regarding the dynamic relationship between blooms of *Aureococcus anophagefferens* and planktonic grazers is essential for a proper perspective on bloom-promoting factors (i.e., grazing inhibition vs. physical/chemical factors); and to measure what, if any, negative impacts brown tides have on pelagic communities, including fish populations, in impacted bays. Herein, I briefly describe findings on what is known about the structure of planktonic food webs under non-bloom and bloom conditions, especially for Long Island bays. These studies strongly suggest that protozooplankton play a key role in energy transfer through planktonic food webs. Protozoa are major grazers of phytoplankton and, in turn, are prey for other zooplankton taxa (e.g. copepods). This central role of protozoa is most pronounced during the warmer months when brown tide occurs. The impact of *A. anophagefferens* on microbial food webs is likely concentration dependent. Grazing inhibition of protozooplankton by *A. anophagefferens* may contribute to bloom initiation and maintenance.

NON-BLOOM CONDITIONS

In order to understand impacts of brown tide on pelagic food webs, it is first necessary to have an adequate understanding of food web structure and rates of energy transfer among trophic levels under non-bloom conditions. Several lines of evidence from studies of zooplankton grazing and production indicate that protozooplankton play a central role in the planktonic food webs of Long Island bays during the warmer months. Using ^{14}C labeled algae as tracers of zooplankton grazing, experiments conducted in Great South Bay, on the southern margin of Long Island, show that larger zooplankton (i.e. late-stage copepodites and adult copepods) consume on average $< 5\%$ of the total depth-integrated primary production throughout late spring and summer (Lonsdale *et al.* in press). Smaller micrometazoa, mostly copepod nauplii, graze an additional 12-52% of total primary production throughout much of the year. Thus, in Great South Bay, community grazing rates attributable to the metazoan zooplankton are usually less than 50% of primary production. In the Peconics Bays system at the east end of Long Island, total grazing pressure by metazoan zooplankton is usually $< 15\%$ (Kim 1993). Because biomass remains relatively constant throughout much of the spring and summer despite higher rates of primary productivity in these bays (Lonsdale *et al.* in press), grazing control by other taxa is strongly implicated.

A recent study during the 1994 non-bloom year in the Great Peconic Bay and in West Neck Bay (Shelter Island, NY) confirmed the central role of protozooplankton as grazers of phytoplankton. Using the dilution technique (Landry and Hassett 1982), we found that microzooplankton, mostly protozooplankton by number, had a significant impact on total

phytoplankton stocks from mid-June through September (Lonsdale *et al.* in review). Microzooplankton grazed between 79% and 114% of the net growth of phytoplankton per day in Great Peconic Bay (Fig. 1 Mehran *et al.* in preparation). In West Neck Bay, microzooplankton were also important consumers of phytoplankton, although at times during the summer of 1994 their impact on phytoplankton biomass was significantly less compared to Great Peconic Bay (e.g. 0% and 30% of the net growth of phytoplankton was grazed per day on two dates. Thus, as found in other aquatic environments, we have established that the primary consumers of phytoplankton in Long Island bays are the protozooplankton.

Under non-bloom conditions, population growth rates of ciliates, an important component of the protozooplankton, are not food limited. Multiple regression analysis of the net growth rate of ciliates as the dependent variable revealed that water temperature was the most significant factor explaining variation in growth rate, and that measures of primary productivity, including total and < 10-μm depth-integrated primary productivity, were not significant (Lonsdale *et al.* in press). Although ciliate populations exhibit positive growth rates, especially in summer, their standing stocks, in general, do not show any strong temporal pattern (Lonsdale *et al.* in review). Ciliate populations are controlled by intense predation from copepods and smaller micrometazoa (Lonsdale *et al.* in press). Ciliates are crucial to copepod nutrition, especially during the summer months, and support production of these abundant zooplankton. We have found that in Long Island bays, the rate of copepod reproduction is directly related to the net growth rate of ciliates (Lonsdale *et al.* in press). Water temperature and measures of primary production (total and > 10-μm depth-integrated primary productivity) did not correlate to egg production rate of copepods.

Thus, the emerging picture of food web dynamics in the plankton of Long Island bays during the summer is one in which microbial processes play a key role, the constituents of which are the major consumers of phytoplankton biomass and fuel production of higher taxa, including copepods and other planktonic inhabitants such as fish larvae that prey upon protozoa.

BLOOM CONDITIONS

Studies of the impacts of *Aureococcus anophagefferens* blooms on planktonic organisms present a "mixed" picture. In laboratory studies on the effects of cultured brown tide cells on protozoa, Caron *et al.* (1989) found that two of five species of cultured protozoa grew in the presence of *A. anophagefferens* (at 1.0×10^6 cells•ml^{-1}), with or without an alternate bacterial food source, and consumed *A. anophagefferens* cells. They also noted covariation of bacterioplankton with *A. anophagefferens* density. Addition of cultured *A. anophagefferens* cells to a natural seawater sample taken from Vineyard Sound, MA resulted in an increase in protozoan density and a decrease in the concentration of *A. anophagefferens*. In a field study in Long Island bays, the authors found that densities of bacteria, ciliates and heterotrophic nanoplankton were not correlated with the density of *A. anophagefferens*, and not negatively impacted by bloom concentrations (at least from $\sim 2\text{-}7 \times 10^5$ cells•ml^{-1}). At these concentrations, *A. anophagefferens* had no obvious effect on the composition of the heterotrophic microplankton.

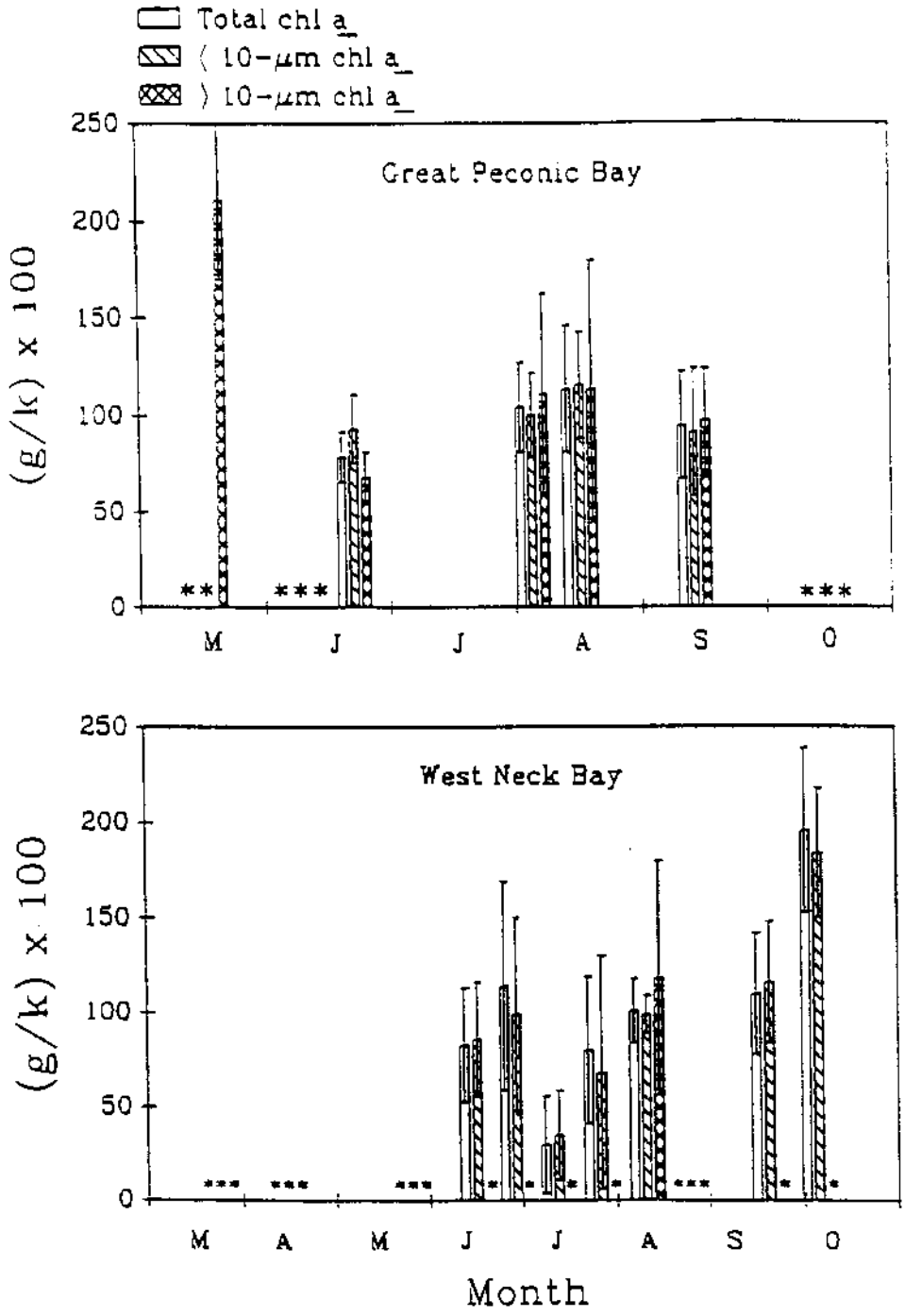


Fig. 1. Mean percent of phytoplankton production grazed (d^{-1}) by microzooplankton = (g/k) x 100 in Great Peconic Bay and West Neck Bay, Long Island in 1994 under non-bloom conditions (g = community grazing rate, k = net production rate of phytoplankton determined from dilution experiments). Error bars represent 95% confidence intervals of the slope. Non-significant grazing is noted by *.

Using fluorescently-labeled algae (not *A. anophagefferens*), Caron *et al.* also found no clear correlation between protozoan grazing rates and the density of *A. anophagefferens* cells in Long Island bays. The greatest grazing impact was found at intermediate cell concentrations. These latter findings are consistent with a recent study of microzooplankton grazing under similar concentrations of brown tide (~ 3-5 x 10^5 cells•ml^{-1}) in Great South Bay using the dilution technique. Protozooplankton were found to consume phytoplankton in the presence of brown tide (Lonsdale *et al.* in review). However, the grazers were selective, and avoided ingestion of brown tide cells. This finding, therefore, also suggests that it is unlikely that grazing by zooplankton plays a major role in the demise of brown tides, and that grazing inhibition by *A. anophagefferens* may actually contribute to the initiation and maintenance of blooms. Further work is necessary to support or refute these hypotheses.

At higher *A. anophagefferens* concentrations (> 1.0 x 10^6 cells•ml^{-1}), however, detrimental impacts on protozoa have been found. During a brown tide in West Neck Bay in 1991, *A. anophagefferens* reached a density of about 1.5 x 10^6 cells•ml^{-1} and was associated with negative growth rates of both aloricate ciliates and tintinnids (Lonsdale *et al.* in press). As brown tide declined to 5 x 10^5 cells•ml^{-1}, ciliate growth rates recovered and were equivalent to those under non-bloom conditions during the summer. Thus, it appears that the impacts of brown tide on protozoa are concentration dependent, and reflect the relative availability of alternate food sources.

During the early outbreaks of brown tide in Long Island bays, studies were underway to enumerate larger zooplankton and fish stocks. Duguay *et al.* (1989) found that in 1985 and 1986, brown tides were not associated with reduced copepod abundances in Great South Bay. Castrow and Cowen (1989) reported that larval fish growth also appeared to be relatively unaffected during the blooms of 1986 and 1987 in Great South Bay. In 1986, when extensive monitoring of *A. anophagefferens* cell concentrations was conducted by the Suffolk County Department of Health Services, the average cell concentration was 1.4 x 10^5 cells•ml^{-1}, and reached a peak of only 6-7 cells•ml^{-1} (Nuzzi and Waters 1989). Moreover, other phytoplankton such as *Nannochloris* sp. were common. Under these lower bloom concentrations, it is likely that the microbial food web remained intact, and allowed for normal zooplankton productivity. It is unlikely that the presence of brown tide at these lower concentrations interrupts copepod feeding and production if alternate food (i.e., protozoa and/or suitable phytoplankton) are present. In a laboratory study, no detrimental effects of cultured *A. anophagefferens* cells at a concentration of 5 x 10^5 cells•ml^{-1} on copepodite and naupliar survival was observed if alternate food was available (Lonsdale *et al.* in press). But, a monospecific diet of *A. anophagefferens* was insufficient for juvenile survival and growth as survival was similar to that in only filtered seawater. Durbin and Durbin (1989) also provide evidence that lower concentrations (7.6 x 10^5 cells•ml^{-1}) of *A. anophagefferens* are not especially detrimental to zooplankton production. They reported that copepod weight, "condition factor", and egg production rate during a brown tide in Narragansett Bay (1985) were low, but not unlike those sometimes found in non-bloom years. Thus, as suggested is the case for protozoa, it appears that the effects of brown tide on metazoan zooplankton are concentration dependent, and depend on the availability of alternate food. A

prolonged period of high concentration (> 1.0 x 10^6 cells•ml^{-1}) of *A. anophagefferens*, however, is likely to have widespread, detrimental effects on production rates of higher trophic levels in impacted bays via its impacts on protozooplankton. Such trophic level impacts have been found during the prolonged Texas brown tide (Buskey and Stockwell 1993).

REFERENCES

Buskey, E.J. and D.A. Stockwell (1993) Effects of a persistent 'brown tide" on zooplankton populations in the Laguna Madre of South Texas, p. 659-665, In: Samyda, T.J. and Y. Shimizu (eds.) Toxic Phytoplankton Blooms in the Sea. Elsevier Science Publishers, B.V.

Caron, D.A., E. Lim, H. Kunze, E.M. Cosper, and D.M. Anderson (1989) Trophic interactions between nano- and microzooplankton and the "brown tide", p. 265-294, In: Cosper, E.M., V.M. Bricelj, and E.J. Carpenter (eds.) Novel Phytoplankton Blooms: Causes and Impacts of recurrent brown tides and other unusual blooms. Vol. 35. Springer-Verlag, Berlin

Castro, L.R. and R.K. Cowen (1989) Growth rates of bay anchovy (Anchoa mitchilli) in Great South Bay under recurrent brown tide conditions, sumers 1987 and 1988, p. 663-674, In: Cosper, E.M., V.M. Bricelj, and E.J. Carpenter (eds.) Novel Phytoplankton Blooms: Causes and Impacts of recurrent brown tides and other unusual blooms. Vol. 35. Springer-Verlag, Berlin

Duguay, L.E., D.M. Monteleone, and C. Quaglietta (1989) Abundance and distribution of zooplankton and ichtyoplankton in Great South Bay, New York, p. 599-623, In: Cosper, E.M., V.M. Bricelj, and E.J. Carpenter (eds.) Novel Phytoplankton Blooms: Causes and Impacts of recurrent brown tides and other unusual blooms. Vol. 35. Springer-Verlag, Berlin

Durbin, A.G. and E.G. Durbin (1989) Effect of the "brown tide" on feeding, size and egg laying rate of adult female Acartia tonsa, p. 625-645, In: Cosper, E.M., V.M. Bricelj, and E.J. Carpenter (eds.) Novel Phytoplankton Blooms: Causes and Impacts of recurrent brown tides and other unusual blooms. Vol. 35. Springer-Verlag, Berlin

Kim, W.-S. (1993) Zooplankton community effects on the phytoplankton community in Long Island bays. Ph.D. Dissertation, State University of New York at Stony Brook, 242 p.

Landry, M.R. and R.P. Hassett (1982) Estimating the grazing impact of marine microzooplankton. Mar. Biol. 67:283-288

Lonsdale, D.J., M.A. Frey, R. Mehran, G.T. Taylor, and R.M. Waters (submitted) Domination of herbivorous grazing by protozooplankton in Long Island bays. Aquat. Micro. Ecol.

Lonsdale, D.J., E.M. Cosper, W.-S. Kim, M. Doall, A. Divadeenam, and S.H. Jonasdottir (in press) Food web interactions in the plankton of Long Island bays, with preliminary observations on brown tide effects. Mar. Ecol. Prog. Ser.

Nuzzi, R. and R.M. Waters (1989) The spatial and temporal distribution of "brown tide" in eastern Long Island Sound, p. 117-138, In: Cosper, E.M., V.M. Bricelj, and E.J. Carpenter (eds.) Novel Phytoplankton Blooms: Causes and Impacts of recurrent brown tides and other unusual blooms. Vol. 35. Springer-Verlag, Berlin .pa @43

VIRUSES AS BIOLOGICAL CONTROL AGENTS FOR BLOOMS OF MARINE PHYTOPLANKTON

Curtis Suttle
Marine Science Institute, The University of Texas at Austin

INTRODUCTION

Virus-like particles (VLPs) are very abundant in coastal seawater with typical concentrations of 10 to 50 million per ml (Proctor and Fuhrman 1990, Bergh *et al.* 1989), and are now recognized as being significant players in marine ecosystems (Fuhrman and Suttle 1993, Bratbak *et al.* 1994). The VLPs in these communities range widely in morphology and size, suggesting that they are pathogens for a variety of organisms. Although most of these viruses probably infect bacteria, viruses which infect phytoplankton can also be abundant. For example, viruses infecting individual phytoplankton species are ubiquitous in coastal waters and can occur at concentrations $> 10^5 \cdot ml^{-1}$ (Cottrell and Suttle 1991, 1995a, Suttle and Chan 1993, 1994). These virus assemblages are also extremely dynamic, and current estimates suggest that they are replaced every two to four days (Suttle and Chen 1992, Suttle 1994). As viruses are obligate pathogens which only replicate by infecting organisms, and as millions of viruses per ml are being produced daily, this indicates that a lot of infection is occurring in nature. Moreover, as VLPs were observed within cells of *Aureococcus anophagefferens* during a bloom (Sieburth *et al.* 1988), and a virus has recently been isolated which infects this alga (Milligan and Cosper 1994), it implies that viruses are responsible for some fraction of the mortality of these cells in nature. This suggests that it might be possible to use viruses as biological control agents to regulate blooms of this alga.

VLPs have long been known to occur in numerous taxa of microalgae (Van Etten 1991); yet, it was not until 1979 that researchers first reported the isolation of a virus which infects a marine phytoplankter, *Micromonas pusilla* (Mayer and Taylor 1979). Although there was evidence that these viruses were abundant, and the implications for control of populations were obvious, further data on viral infection of marine phytoplankton were not forthcoming until a decade later. At this time it was reported that viruses which infect a number of important taxa of phytoplankton could be readily isolated from seawater (Suttle *et al.* 1990).

Our knowledge of viruses that infect marine phytoplankton is limited and based on the few isolates which have been established in culture. These include the viruses which infect the cyanobacterium, *Synechococcus spp.* (Suttle and Chan 1993, Waterbury and Valois 1993),and the photosynthetic flagellates *Micromonas pusilla* (Mayer and Taylor 1979, Cottrell and Suttle 1991) and *Chrysochromulina brevifilum* (Suttle and Chan 1995). Viruses which infect marine *Synechococcus spp.* are tailed, contain double-stranded (ds) DNA, and appear to be closely related to those which infect freshwater cyanobacteria (Safferman *et al.* 1983, Suttle and Chan 1993, Waterbury and Valois 1993). In contrast, the virus isolates which infect eukaryotic phytoplankton are large polyhedrons that contain ds DNA (Cottrell and Suttle 1991, Suttle and Chan 1995). Sequence analysis has recently shown that these viruses belong within the family

Phycodnaviridae and are relatively closely related to the Herpes viruses (Chen and Suttle 1996). Previously the Phycodnaviridae was only known to include a group of viruses that infect Chlorella-like algae which occur as symbionts in Hydra and Paramecium (Van Etten and Ghabrial 1991).

Although viruses that infect phytoplankton can be abundant, this does not mean that they are responsible for significant mortality. It is possible that viruses persist in seawater for long periods of time, so that little infection would be required to maintain the high concentrations of infectious viruses that are observed. This seems unlikely given the large seasonal changes in the concentration of viruses which infect *Synechococcus spp.* (Waterbury and Valois 1993, Suttle and Chan 1994), *Micromonas pusilla* (Cottrell and Suttle 1995a), and *Chrysochromulina brevifilum* (Suttle and Chan 1995). These data suggest that there is considerable production and destruction of viruses, which implies that significant viral induced mortality of phytoplankton may also be occurring.

A challenge is to obtain quantitative estimates of the effect that viruses have on the mortality of phytoplankton populations in nature. One approach has been to infer the amount of virus production that must occur in order to balance the measured removal rates of infectious viruses. If one knows the number of viruses that are produced per lytic event then one can infer the number of cells that must be lysed in order to sustain the required virus production. This approach was used to investigate the effect that viruses have on the mortality of the photosynthetic picoplankter, *Micromonas pusilla* (Cottrell and Suttle 1995a). It was estimated that the turnover time of viruses infecting *M. pusilla* was approximately 1.3 days, which would require that 2 to 10% of the algal cells must be lysed on a daily basis to support the required rates of virus production. These are quite similar to other estimates that have been obtained for coastal phytoplankton populations (Waterbury and Valois 1993, Suttle 1994, Suttle and Chan 1994).

Although viruses may account for significant mortality of phytoplankton it does not necessarily follow that this is accompanied by decreases in the abundance of the species that are infected. In fact, the highest viral abundances frequently occur when the concentration of potential host cells is also highest (Waterbury and Valois 1993, Suttle and Chan 1994, Cottrell and Suttle 1995a). This is because viruses and the cells that they infect represent highly co-evolved systems, and over evolutionary time selection has resulted in systems in which stable coexistence occurs. This is not necessarily the case with all viruses, and there are a number of examples in which the appearance of viruses has been associated with the demise of phytoplankton blooms.

One example of apparent regulation of a phytoplankton population by viruses was observed during mesocosm studies in Norway. The collapse of a bloom of the coccolithophorid *Emiliania huxleyi* occurred synonymously with the appearance of large icosahedral VLPs in the surrounding water and within the algal cells (Bratbak *et al.* 1993). Similarly, Nagasaki *et al.* (1994a, b) reported that the collapse of a red-tide bloom of the raphidophyte *Heterosigma akashiwo* was associated with the appearance of VLPs within the

cells. There have been other reports of the sudden demise and autolysis of phytoplankton blooms which are consistent with viral infection. Studies have also suggested that the presence of viruses may affect community structure by preventing the establishment of a species. In one study, attempts to establish freshwater cyanobacterial blooms in mesocosms met with mixed results because of the presence of viruses which infected the cells (Dejardins and Olson 1983). Similar results have been obtained using marine bacteria. When a marine bacterial isolate was added to natural seawater, viruses which infected these cells rapidly increased and lysed the bacterial population (Hennes *et al.* 1995).

Viruses have been used as biological control agents in other systems. Is it feasible that they could be used to control nuisance and toxic algal blooms? There are a number of features which make viruses very attractive as biological control agents. First, they recognize receptors on cell surfaces, which tends to make them very host specific. This means that one could potentially target a very specific group of organisms, while leaving closely related organisms unaffected. In fact, a potential problem is that viruses are often so host specific that they are unable to infect different strains of the same species. This is probably the reason that many viruses are able to coexist with the species that they infect. Another attractive feature is the way in which viruses replicate. A single infected cell can produce hundreds of viral particles when lysis occurs. This mean that viruses can propagate through a population extremely rapidly. During a bloom situation the rate of propagation would potentially be accelerated because the rate of infection depends upon the frequency with which the viruses encounter host cells. As the encounter rates are much greater at high host densities (Wiggins and Alexander 1985), when host abundance gets high, viruses should propagate rapidly through the system. This would serve as a natural regulatory system to prevent bloom formation.

There are many problems that potentially stand in the way of using viruses as biological control agents for algal blooms. A bloom occurs when phytoplankton production exceeds removal. This can stem from an increase in production or because of a decrease in removal rates. Viruses are one of the agents that can be responsible for some of the removal; however, the occurrence of a bloom suggests that indigenous viruses were unable to keep the algal population in check.

The control of a bloom by the introduction of a virus requires the isolation and amplification of a pathogen that is capable of causing lysis of the bloom organism. If such a pathogen is present in nature then the persistence of the bloom is puzzling. There are a number of explanations of how this can occur. Some viruses are temperature sensitive, while others have specific ion requirements for infection. It is possible that a virus has different requirements than the host organism, and that the bloom is occurring in an environment which is unsuitable for viral infection or replication. This may occur in some halotolerant bacteria, for example, where high salinities have been suggested to provide a refuge from infection by lytic viruses (Daniels and Wais 1990). If the bloom is the result of a species introduction, it is possible that the bloom organism has become geographically separated from viruses that would normally keep it in check. Examples of this are known for terrestrial systems. For example,

the introduction of a fungal pathogen from Europe decimated chestnut trees in North America, but in Europe a virus controls the fungus, resulting in less damage to the trees (Nuss 1992).

Perhaps the greatest obstacle to controlling phytoplankton blooms with viruses is the diversity among phytoplankton and viral populations. There is enormous selective pressure exerted on a phytoplankton population by lytic viruses, as infection will result in cell death. Conversely, because viruses are dependent upon finding suitable hosts in order to reproduce, there is tremendous pressure for viruses to overcome resistance mechanisms that the host cells may develop. Yet, it is also imperative that viruses not completely eliminate their hosts. It is likely these selective pressures are responsible for the high genetic diversity that can be found among viruses, even among those which infect the same host strain (Cottrell and Suttle 1991, 1995b). Even though the cells in a phytoplankton bloom may appear identical, and the viruses infecting the bloom organism appear morphologically indistinguishable, there is likely to be a great deal of variation in the populations. An individual virus will likely be able to infect only a small proportion of the bloom organisms. Evidence of this can be seen in cyanobacterial and cyanophage populations in the sea. High concentrations of infectious cyanophages ($>10^3 \cdot ml^{-1}$) that cause the lysis of several marine strains of *Synechococcus*, occur in the presence of high concentrations of *Synechococcus spp.* (Suttle and Chan 1993, 1994, Waterbury and Valois 1993). The contact rates between the phytoplankton and viruses under these conditions are so high that only a small percentage of the collisions can result in infection. In other words, most of the viruses that are present are unable to infect most of the phytoplankton cells. The complex pattern of host range that occurs in these viruses provides some insight into what probably also happens in nature (Suttle and Chan 1993, Waterbury and Valois 1993).

Ultimately, viruses are one of the natural agents that prevent the initiation and establishment of blooms. The occurrence of a bloom implies that the usual controls on biomass and diversity, including pathogens and grazers, have failed. Consequently, viral control of an established bloom would likely require the introduction of a virus or viruses that have a broad host range, and which were isolated from a location or at a time when the bloom was not present. Although such an approach may be feasible, the probability of success given our current level of knowledge would not be high. Before viruses can be used as biological control agents for algal blooms, it is necessary to focus more effort into understanding the relationships between viruses and phytoplankton under non-bloom conditions.

REFERENCES

Bergh, O., K.Y. Borsheim, G. Bratbak, and M. Heldal. 1989. High abundance of viruses found in aquatic environments. Nature (London) 340:476-468.

Bratbak, G., J. K. Egge, and M. Heldal. 1993. Viral mortality of the marine alga *Emiliania huxleyi* (Haptophyceae) and termination of algal blooms. Mar. Ecol. Prog. Ser. 93:39-48.

Bratbak, G., T. F. Thingstad, and M. Heldal. 1994. Viruses and the microbial loop. Microb. Ecol. 28:209-221.

Chen, F., and C. A. Suttle. 1996. Evolutionary relationships among large double-stranded DNA viruses that infect microalgae and other organisms as inferred from DNA polymerase genes. Virology (in press).

Cottrell, M.T. and C. A. Suttle. 1991. Wide spread occurrence and clonal variation in viruses which cause lysis of a cosmopolitan, eukaryotic marine phytoplankter, *Micromonas pusilla*. Mar. Ecol. Prog. Ser. 78:1-9.

Cottrell, M. T. and C. A. Suttle. 1995a. Dynamics of a lytic virus infecting the photosynthetic marine picoflagellate *Micromonas pusilla*. Limnol. Oceanogr. 40:730-739.

Cottrell, M. T., and C. A. Suttle. 1995b. Genetic diversity of algal viruses which lyse the photosynthetic picoflagellate *Micromonas pusilla* (Prasinophyceae). Appl. Environ. Microbiol. 61:3088-3091.

Daniels, L.L. and A.C. Wais. 1990. Ecophysiology of bacteriophage S5100 infecting *Halobacterium cutirubrum*. Appl. Environ. Microbiol. 56:3605-3608.

Dejardins, P.R. and G.B. Olson. 1983. Viral control of nuisance cyanobacteria (blue-green algae) II. Cyanophage strains, stability studies of phages and hosts, and effects of environmental factors on phage host interactions. Contribution No. 185. California Water Resources Center, University of California, Davis. 35 pp.

Fuhrman, J. A. and C. A. Suttle. 1993. Viruses in marine planktonic systems. Oceanography 6:50-62.

Hennes, K. P., C. A. Suttle and A. M. Chan. 1995. Fluorescently labeled virus probes show that natural virus populations can control the structure of marine microbial communities. Appl. Environ. Microbiol. 61:3623-3627.

Mayer, J. A. and F. J. R. Taylor, 1979. A virus which lyses the marine nanoflagellate *Micromonas pusilla*. Nature 281:299-301.

Nagasaki, K., M. Ando, I. Imai, S. Itakura and Y. Ishida. 1994a. Virus-like particles in *Heterosigma akashiwo* (Raphidophyceae): a possible red tide disintegration mechanism. Mar. Biol. 119:307-312.

Nagasaki, K., M. Ando, S. Itakura, I. Imai and Y. Ishida. 1994b. Viral mortality in the final stages of *Heterosigma akashiwo* (Raphidophyceae) red tide. J. Plankton Res. 16:1595-1599.

Nuss, D.L. 1992. Biological control of chestnut blight: an example of a virus-mediated attenuation of a fungal pathogenesis. Microbiol. Rev. 56:561-576.

Proctor, L. M. and J. A. Fuhrman. 1990. Viral mortality of marine bacteria and cyanobacteria. Nature (London) 343:60-62.

Safferman, R. S., Cannon, R. E., Desjardins, P. R. Gromov, B. V., Haselkorn, R., Sherman, L. A. and Shilo, M. 1983. Classification and nomenclature of viruses of cyanobacteria. Intervirology 19:61-66.

Sieburth, J. M., Johnson, P. W. and Hargraves, P. E. 1988. Ultrastructure and ecology of *Aureococcus anophagefferens* gen et sp. nov. (Chrysophyceae): the dominant picoplankter during a bloom in Narragansett Bay, Rhode Island, Summer 1985. J. Phycol. 24:416-425.

Suttle, C. A. 1994. The significance of viruses to mortality in aquatic microbial communities. Microb Ecol 28:237-243.

Suttle, C. A. and Chan, A. M. 1993. Marine cyanophages infecting oceanic and coastal strains of *Synechococcus* : abundance, morphology, cross-infectivity and growth characteristic. Mar. Ecol. Prog. Ser. 92:99-109.

Suttle, C. A. and Chan, A. M. 1994. Dynamics and distribution of cyanophages and their effects on marine *Synechococcus* spp. Appl. environ. Microbial. 60: 3167-3174

Suttle, C. A. and Chan, A. M. 1995. Viruses infecting the marine prymnesiophyte *Chrysochromulina* spp.: isolation, preliminary characterization and natural abundance. Mar. Ecol. Prog. Ser. 118:275-282.

Suttle, C. A., Chan, A. M. and Cottrell, M. T. 1990. Infection of phytoplankton by viruses and reduction of primary productivity. Nature (Lond) 347:467-469.

Van Etten, J. L. and S. A. Ghabrial. 1991. Phycodnaviridae. Arch. Virol. 1991(Suppl. 2):137-139.

Van Etten, J. L., Lane, L. C. and Meints, R. H. 1991. Viruses and virus-like particles of eukaryotic algae. Microbiol Rev. 55:586-620.

Waterbury, J. B. and F. W. Valois. 1993. Resistance to co-occurring phages enables marine Synechococcus communities to coexist with cyanophages abundant in seawater. Appl. Environ. Microb. 59:3393-3399.

Wiggins, B.A. and M. Alexander. 1985. Minimum bacterial density for bacteriophage replication: implications for significance of bacteriophages in natural ecosystems. Appl. Environ. Microb. 49:19-23.

ECOLOGICAL IMPACTS OF BROWN TIDE

V. Monica Bricelj
Marine Sciences Research Center, State University of New York Stony Brook

The most noteworthy documented impacts of brown tide blooms of *Aureococcus anophagefferens*, since their first occurrence in the mid-1980's, have been on shellfish (commercially exploited species of bivalve molluscs) and eelgrass (*Zostera marina*). Suspension-feeding bivalves pump and concentrate phytoplankton, their primary food source, from large volumes of water and have relatively low motility. Therefore, they often experience early, direct food web effects during harmful algal blooms, and are commonly used as indicators of water quality. Thus, severe reductions in grazing (feeding rates) of mussels during the first brown tide in Narragansett Bay, RI in 1985, provided early warning of a harmful algal outbreak. Effects of brown tide on other filter-feeders and secondary consumers, e.g. larval fish and crabs, have been poorly documented. The effects of brown tide on the planktonic and benthic biota of shallow estuaries, where brown tide has occurred, are the subject of this presentation, and of a recent comprehensive review (Bricelj and Lonsdale, submitted). An attempt is made to focus attention on informational gaps, and suggest future avenues of research.

Brown tide causes severe water column light attenuation, i.e. about 50% reduction in Secchi depth during peak bloom conditions. Eelgrass provides an important nursery habitat for commercially important fish and shellfish, especially bay scallops, within shallow east coast estuaries. The depth distribution, biomass and growth of eelgrass in relatively eutrophic (nutrient-rich) coastal bays is largely controlled by light availability. During the first outbreak in 1985, brown tide caused significant reduction in the depth penetration and leaf biomass of eelgrass in Great South Bay and Peconic Bays, Long Island, NY. This effect was superimposed on long-term declines of eelgrass within Peconic Bays over the past three decades attributed to other unknown factors (wasting disease, habitat degradation, etc.). Little information is available, however, on the relative effects of brown tide and other environmental factors on long-term losses of eelgrass habitat.

Algal blooms that occur at high biomass levels can in some instances settle to the bottom, increase the biological oxygen demand in sediments and consequently lead to hypoxia/anoxia in the overlying water column and mortalities of benthic macrofauna. Brown tide is typically not associated with a marked increase in biomass (chlorophyll) levels, and shallow eastern and southern Long Island bays are generally characterized by a relatively well mixed, vertically unstratified water column. No anomalies in dissolved oxygen concentrations have been associated with brown tide in Narragansett Bay or Peconic Bays. Therefore, negative effects on bivalves during brown tide have not been attributed to oxygen limitation.

Brown tide typically occurs in the summer (late May through September, with peak abundance in June or July) and therefore coincides with the critical period of spawning, larval development and juvenile growth of commercially important bivalves in mid-Atlantic estuaries.

Brown tide caused recruitment failure of local bivalve populations, e.g. blue mussels, *Mytilus edulis*, in Narragansett Bay, and bay scallops, *Argopecten irradians*, in Long Island estuaries. However, the causal mechanisms involved, i.e. brown tide-induced failure of adults to develop gonads and spawn, reduced survival of planktonic larvae, and/or effects on survival and growth of benthic, post-settlement stages, are not well understood.

Both lethal and sublethal effects of brown tide on bivalves have been documented. Mass mortalities (30 to 100%) of adult mussels occurred during the 1985 Narragansett Bay brown tide, when *A. anophagefferens* densities reached 1.5×10^6 cells•ml^{-1}. Concentrations of 0.2 to 0.8×10^6 cells•ml^{-1} caused significant mortalities of bay scallop larvae in laboratory experiments using a cultured isolate of *A. anophagefferens*. Unusually high mortalities (up to 82%) of adult bay scallops, were determined immediately following the recent, 1995 brown tide in Peconic Bays at sites where brown tide reached $0.8\text{-}1.1 \times 10^6$ cells•ml^{-1}. However, long-term documentation of scallop mortalities that can be unequivocally assigned to the effects of brown tide and related to known field concentrations of *A. anophagefferens*, is lacking.

Brown tides have resulted in substantial economic losses in the Long Island region. For the New York State bay scallop fishery, largely centered in Peconic Bays, economic losses were estimated at $2 million per year. Considerable funds were subsequently invested in annual scallop reseeding programs designed to rehabilitate decimated stocks. These efforts, and the survival of relict scallop populations in local waters, have contributed to partial, slow recovery of stocks in Peconic Bays in recent years. Stock enhancement practices show considerable promise for this species because it can grow rapidly and reach market size within less than one year. Landings of oysters, *Crassostrea virginica*, in Peconic-Gardiners Bays, dropped from a 1974-1984 average of about 127,000 bushels to 836 bushels by 1986, following two consecutive years of brown tide, although landings had already dropped to 42,000 bushels in 1984, for reasons unrelated to brown tide. The recurrence of brown tide in two consecutive years (1985-1986) is believed to have resulted in closure of a private, commercial aquaculture operation, which contributed significantly to oyster landings via bottom plantings in Peconic Bays.

Sublethal effects of brown tide have been documented in several bivalve species. Bay scallops experienced dramatic reduction in adductor muscle weight and in gonadal index during the 1985 brown tide in Peconic bays relative to pre-bloom (1984) conditions. Reduction or complete cessation of growth has been noted for hard clams, *Mercenaria mercenaria*, by several Long Island hatcheries and more recently, during the 1995 outbreak, by New Jersey growers. Most notably, brown tide causes marked reduction in feeding (clearance) rates of adult hard clams and mussels, and typically results in poor meat quality of bivalves, i.e. thin, emaciated tissues. Sublethal effects for juveniles and adults of some species have been documented at brown tide levels exceeding a threshold of about 2×10^5 cells•ml^{-1}. Dose (time and concentration-dependent) responses are likely to be species-specific, however, and influenced by the presence of other co-occurring algae, and need to be established for different life history stages (larvae, postset, juveniles and adults).

Considerable advances have been made towards understanding the underlying mechanism of action responsible for brown tide impacts. Elucidation of the causes of grazing inhibition could in the future provide a means to counteract or mitigate these negative effects. *A. anophagefferens* cells adversely affect bivalve feeding only upon direct contact, rather than via a dissolved metabolite released into the water column. Although specific toxins produced by *A. anophagefferens* have not yet been identified, the polysaccharide-like layer surrounding its cell surface has been shown to contain a bioactive compound that interferes with the ciliary beat of isolated gills, the organ involved in food capture in post-metamorphic bivalves. Brown tide cells were also shown to interfere with larval ingestion of algae commonly used as food for bivalves. *A. anophagefferens* has been found to contain adequate levels of polyunsaturated fatty acids known to be essential for bivalve nutrition, and ingested cells are readily digested and absorbed by bivalve larvae and adults. However, due to their small size (2 μm), *A. anophagefferens* cells are inefficiently captured by the bivalve gill of juveniles and adults, but not by bivalve larvae. In summary, current evidence strongly suggests that toxicity of brown tide cells, rather than small size, poor nutritional value or high cell density, is responsible for the observed detrimental effects on bivalve feeding, growth and ultimately survival.

Preliminary studies suggest that different bivalve species may vary in their susceptibility to brown tide. This possibility has important management implications, since it would allow selection of more tolerant species for culture in regions that are recurrently affected by brown tide. Brown tide blooms vary in their duration and intensity between years and locations. Therefore, site selection and temporary relocation of stocks during peak abundance of brown tide could serve to mitigate losses of cultured stocks. Optimization of stock enhancement strategies (e.g. scallop reseeding or broodstock management) can also accelerate the recovery of natural populations affected by episodic brown tides.

REFERENCE

V.M. Bricelj and D.J. Lonsdale. *Aureococcus anophagefferens* : causes and ecological consequences of mid-Atlantic "brown tides" (submitted to Limnol. Oceanogr.).

TEXAS COASTAL LAGOONS AND A PERSISTENT BROWN TIDE

Dean A. Stockwell, Terry E. Whitledge, Edward J. Buskey, Hudson DeYoe, Kenneth C. Dunton, G. Joan Holt, Scott A. Holt, Paul A. Montagna, Curtis A. Suttle
Marine Science Institute, The University of Texas at Austin

The Laguna Madre of South Texas is part of an extensive barrier beach island and lagoon complex, extending from Corpus Christi south to the Rio Grande River. Overlying extensive seagrass beds, waters of this shallow, hypersaline bay system tend to be low in nutrients and relatively clear. In January, 1990, an occurrence of brown water (i.e., the Texas brown tide) spread quite rapidly throughout Baffin Bay and adjoining Upper Laguna Madre. This dense, persistent algal bloom has reduced the penetration of sunlight, threatening to shade out seagrass beds and disrupting sport fishing activities. Economic losses to local communities are estimated to be several million dollars each year, primarily a result of lost revenues in tourism and recreational fisheries.

Table 1 characterizes cellular properties of the Texas brown tide organism while drawing comparisons with *Aureococcus anophagefferens*. The Texas brown tide organism has been identified as a 4-5 µm Chrysophyte. Pigment data, ultrastructural studies and 18s rDNA gene sequencing have placed this previously undescribed species in a newly described class of algae, the Pelageophycae. The onset of the bloom began after a series of complex environmental interactions. An unusually hard freeze which co-occurred with extremely low tides during December of 1989, led to extensive fish and invertebrate kills. Prior to the bloom, conditions of abnormally low rainfall had led to hypersalinity within the lagoons. Concentrations of dissolved inorganic nitrogen appeared elevated prior to the bloom. These conditions have been outlined in detail during a 1991 Brown Tide Symposium and Workshop (Whitledge and Pulich 1991). Table 2 summarizes comparative bloom characteristics of the east coast and Texas "brown tides". A striking difference between the two bloom events is the tremendous increase in phytoplankton biomass within the Laguna Madre, suggesting a reallocation of carbon within the system.

Subsequent and ongoing studies of the Texas brown tide organism indicate the following:

- Seagrasses have shown reduced biomass and rates of growth (Dunton 1994; Dunton in press).
- Complete loss of seagrass beds in some areas where depth is greater than 1.3 meters (Onuf, personal communication).
- Benthic biomass, abundance, and diversity greatly reduced (Work of Montagna in Whitledge and Buskey 1994).
- A reduction of zooplankton populations and zooplankton grazing has occurred (Buskey and Stockwell, 1993; Whitledge and Buskey 1994; Buskey and Hyatt, 1995).
- The density of larval fish appear reduced in areas impacted by brown tide (Work of S. Holt in Whitledge and Buskey 1994).

- The growth and survival of larval fish are reduced by brown tide (Work of J. Holt in Whitledge and Buskey 1994).
- No effects on feeding rates of a common bivalve, *Mulinia lateralis* are apparent (Montagna *et al.* 1993).
- The brown tide organism does not utilize nitrate (DeYoe and Suttle, 1994).
- Concentrations of dissolved organic nitrogen have been reduced to relatively low concentrations (Whitledge 1993; Zheng 1994; Shormann 1992).

Table 1. A comparison of the Texas and East Coast brown tide organisms. Data for *A. anophagefferens* provided from Cosper *et al.* 1989.

Cell Properties	East Coast	Texas
Cell size (μm)	2-3	4-5
Pigments	Type III Chrysophyte	Type III Chrysophyte
External Polysaccharide layer	+	+
Cell wall	none	none
Chloroplast	1	1
Pyrenoid	immersed	single-stalked
Flagella	-	?
Flagellar basal bodies	-	2
Viral inclusions	+	+
DMS/cell	0.13 pg	0.76 pg
Chlorophyll/cell	-	0.033-0.161 pg
Polyclonal antibody for *Aureococcus*	+	-

Table 2. Comparative bloom characteristics of the Texas and the East Coast brown tide organisms. Data for *A. anophagefferens* provided from Cosper *et al.* 1989.

Bloom Characteristics:	East Coast	Texas
Cell density (cells x $10^6 \cdot ml^{-1}$)	0.66	1.9
Chlorophyll a ($\mu g \cdot l^{-1}$)	18	44
C^{14} uptake rates $mgC \cdot m^{-2} \cdot h^{-1}$	200 - 400	(20-140)
Growth rates		230 - 840
doublings -1 day	6.0 - 8.3	0.67 - 0.90
Carbon:Chlorophyll	?	318 : 1
Maximum Duration	6 months	65 months
Reoccurrence	3 - 5 years	?
Deleterious effects	Yes*	Loss of seagrass

*Mussels, clams, scallops, eelgrass, zooplankton

The Texas brown tide is still present in the Laguna Madre, having persisted for over 65 months. This is the longest monospecific phytoplankton bloom ever documented. To date, no massive fish kills or permanent resource losses have been documented for this bloom. Of great local concern, however, is the resultant impact of the brown tide on the structure of seagrass communities and ultimately the long term effects on local fisheries. Residence time within this system is long and flushing events rare. Is this bloom an initial symptom portending a future collapse of the Laguna Madre ecosystem and a possible permanent loss of resource habitat? Much work is still needed regarding the biology and impact of this Texas brown tide.

Table 3. Continued research needs

Causative Organism
Life history
Autecological studies, including organism's physiology
Biochemical parameters
Bloom Interactions
Inhibition of grazing
Allelopathy
Benthic inputs
Importance of residence times, circulation, flushing
Ecological Effects
Decline of seagrass
Reductions of benthic communities/diversity shifts
Larval fish survival
Analyses on populations of adult fish
Management
Identification and reduction of anthropogenic inputs
Evaluation of importance of circulation, residence times, and flushing events.
Nutrient regulation/modification
Enhancement of natural grazing or mortality

Acknowledgements

These studies were supported by the Texas Higher Education Board, Advanced Technology Program under Grants 4541, 3658-254 and 3658-426. Principal investigators in these studies included; A. Amos, R. Benner, K. Dunton, J. Holt, S. Holt, R. Scalan, P. Montagna, P. Parker, D. Stockwell, C. Suttle, and T. Whitledge.

REFERENCES

Buskey, E.J. & Hyatt, C.J. 1995. Effects of the Texas 'brown tide' alga on planktonic grazers. Mar. Ecol. Prog. Ser. 126: 285-292.

Buskey, E.J. & Stockwell, D.A. 1993. Effects of a persistent brown tide on zooplankton populations in the Laguna Madre of south Texas. In: Smayda,T.J. & Shimizu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier Science Publishers, Amsterdam, pp. 711-16.

DeYoe, H.R. & Suttle, C.A. 1994. The inability of the Texas brown tide alga to use nitrate and the role of nitrogen in the initiation of a persistent bloom of this organism. J. Phycol. 30: 800-806.

Dunton, K. 1994. Seasonal growth and biomass of the subtropical seagrass *Halodule wrightii* in relation to continuous measurements of underwater irradiance. Mar. Biol. 120:479-489.

Dunton, K. (In press). Photosynthetic production and biomass of the subtropical seagrass *Halodule wrightii* along an estuarine gradient. Estuaries.

Montagna, P.A., Stockwell, D.A., & Kalke, R.D. 1993. Dwarf surfclam *Mulinia lateralis* (Say, 1882) populations and feeding during the Texas brown tide event. J. Shellfish Res. 12:433-42.

Shormann, D.E. 1992. Short term variability, the effects of freshwater inflow, and factors influencing the distribution of brown tide in South Texas bays. M.A. thesis, The University of Texas at Austin, 111 pp.

Whitledge, T.E. 1993. The nutrient and hydrographic conditions prevailing in Laguna Madre, Texas before and during a brown tide bloom. In: Smayda,T.J. & Shimizu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier Science Publishers, Amsterdam, pp. 711-16.

Whitledge, T.E., & Buskey, E.J. 1994. Texas Brown Tide. In: Proceedings of Workshop on the Ecology and Oceanography of Harmful Algal Blooms at the Snow Mountain Ranch Conference Center, Colorado, August 31, 1994.

Whitledge, T.E., Stockwell, D.A., Buskey, E.J., DeYoe, H., Dunton, K.C., Holt, G.J., Holt, S.A., Montagna, P.A., & Suttle, C.A. 1995. Persistent brown tide bloom in Laguna Madre, Texas. Gulf of Mexico: Large Marine Ecosystem Symposium, St. Petersburg, FL, August, 1995.

Whitledge, T.E. and Pulich, W. M., Jr. 1991. Report: Brown Tide Symposium and Workshop. 15-16 July 1991. The University of Texas at Austin, Marine Science Institute, Technical Report No. TR/91-002, 44pp.

Zheng, Z. 1994. A multivariate study of the ecosystem of Baffin Bay and Laguna Madre, Texas. M.A. thesis, The University of Texas at Austin, 126 pp.

Section II

Research Recommendations

RESEARCH RECOMMENDATIONS

William Wise[1], J. Kirk Cochran[1], Anne McElroy[2], and Cornelia Schlenk[2]
Marine Sciences Research Center, State University of New York Stony Brook[1] and New York Sea Grant Institute[2]

SUMMARY

Brown tide has many dimensions and it affects a number of components of the coastal marine ecosystem. An effective brown tide research program must, perforce, be a multi-disciplinary one involving the work and collaboration of biologists, chemists, and physical oceanographers. While prior brown tide research included biological, chemical, and physical investigations, individual projects proceeded in isolated fashion and only modest funding was available. The distinguishing characteristic of the research proposed here is its comprehensive and coordinated approach developed from a consensus among scientists and environmental managers on priority research needs. Another key element is the need to conduct coordinated experiments in both the laboratory (where experimental conditions can be controlled precisely) and the field (under the complex array of environmental conditions that actually exist in our bays). Only through the use of laboratory and field-based manipulations can the factors that cause and potentially control brown tide blooms be discovered.

When implemented, the proposed research program will produce a large amount of data and information. This information will be useful to scientists studying the brown tide, to environmental managers and government officials charged with developing management measures to deal with brown tide, various industry groups whose businesses are affected by brown tide, and the general public concerned with brown tide as an assault on the overall quality of life on Long Island. To maximize the value of information already obtained and the proposed research plan, it is imperative that a mechanism be established to make historic data and information on brown tide available in an understandable format to all interested parties.

Brown tide is not only a regional phenomenon in the Northeast; it is frequently cited as an example of a broader phenomenon-a global increase in the frequency and severity of harmful algal blooms. The research suggested below will contribute not only to greater understanding of brown tide; it will aid wider efforts presently underway to understand and control the growing problem of disruptive and damaging blooms of marine algae.

The Summit identified the following major questions that serve to organize the proposed research agenda.

1. What are the population dynamics of brown tide blooms?

The interplay of biological, chemical, and physical factors that produce a brown tide bloom can be modeled using analytical computer models of increasing sophistication. Such models are increasingly used nationwide by environmental managers to evaluate the likely

consequences of alternative management strategies for the problem at hand. A modeling approach building on historic data and existing monitoring programs is suggested. This effort should begin with the simplest possible formulation, including an evaluation of existing models, and proceed to increasing levels of sophistication as high quality data becomes available. The goal would be development of a model capable of accurately simulating spatially and temporally the biological-chemical-physical interactions producing brown tide blooms. The management utility of this model(s) would be enhanced by interfacing it with watershed/land use development and water quality models.

2. What initiates brown tide blooms?

This is a key question and a very important element of the research plan. Research would encompass chemical, physical, and biological factors. The recommended approach is a coordinated series of field and laboratory studies, including the use of mesocosm-type facilities. These mesocosms could range from land-based systems, to experimental enclosures in the field, to manipulative experiments with whole embayments. Once a suspected causative agent(s) is identified, its sources and sinks in the system must be identified. For prospective chemical agents, this implies the development of budgets.

3. What causes brown tide to grow?

This is a critical research area that includes three, inter-related areas of investigation: defining the nutritional requirements of *A. anophagefferens*; competitive interactions between *A. anophagefferens* and other phytoplankters; and determination of *in situ* growth rates. The principal approach to the nutritional requirement issue is serial laboratory addition or manipulative experiments. Assessing competitive interactions and *in situ* growth rates will require coordinated laboratory and field studies.

4. What controls the rate of removal of brown tide?

Brown tide can be removed (reduced in abundance) through grazing pressure, the activity of viruses or other pathogens, physiological controls, or physical advection out of the affected area. To adequately assess the nature and relative importance of these separate factors, integration of laboratory and field studies is highly recommended.

5. How does brown tide impact the ecosystem, in particular bivalve shellfish?

Integrated laboratory and field studies are also necessary to answer questions regarding the ecological impacts of brown tide. The mechanisms of more direct impacts on primary consumers (bivalves) can be elucidated within a shorter time frame. The complexity of estuarine food webs suggest that indirect impacts to secondary consumers such as finfish or SAV-associated biota will be harder to determine, requiring a long term research effort. High priority is assigned to determining brown tide toxicity to shellfish among different brown tide isolates and as a function of environmental conditions and shellfish life history stage. Other high priority

research areas are identification of brown tide-resistant or -tolerant shellfish species and optimization of shellfish stock enhancement practices.

6. Management of brown tide

The overall view of participants in the work groups was that current understanding of the causes of the brown tide is not sufficient to enable recommendations to be made at this time on how to prevent or control it. However, there is a body of information developing on how shellfish management programs can accommodate, to some degree brown tide outbreaks, especially when they are relatively restricted in space and time, allowing wild fisheries to be sustained and culture operations to continue. As noted above, further refinement of shellfish management practices in the face of brown tide are encouraged.

Funding Considerations

The convenors of the Brown Tide Summit stress that the proposed research program should take full advantage of the extant knowledge regarding brown tide. Additionally, future brown tide research should complement to the fullest extent possible on-going research, monitoring, and management programs related to brown tide. Dedicated brown tide research funding nationwide from all sources over the past decade probably amounts to less than $1.5 million. This level and intensity of funding has been inadequate. The convenors of the Summit suggest that funding of approximately $1.5-2.0 million annually over a 3 to 5-year period will be necessary to adequately support the research proposed here and to provide answers to the critical questions about what causes brown tide and how to prevent or control it.

As information from existing and proposed research activities becomes available, field-based demonstration projects should be conducted to test out control or mitigation procedures identified. Funds should be sought from appropriate programs to support these activities. Use of these funds would be conditioned on a bloom actually occurring and a determination that the proposed demonstration project rests on sound scientific principles and current understanding of brown tide and its functioning in the estuarine environment.

RECOMMENDATIONS OF THE WORK GROUPS

Introduction

The research recommendations described below were developed through the efforts of separate summit work groups dealing with the following topics: biological factors; chemical factors; physical factors; and ecological effects. The charge to the work groups is presented in Table 1. On the second day of the summit, the chair of each work group presented a summary of that group's recommendations for priority research on brown tide. What follows is a consolidated summary of the work group reports. The work groups did not present their findings and recommendations in precisely the same format. In preparing this document, the editors attempted to standardize the format in which the work group reports are presented, but did not add or delete anything substantive from the materials provided by the work group chairpersons, who have each reviewed their section of the report. Chairpersons of the work groups were Dr. David Caron of the Woods Hole Oceanographic Institution (biological factors), Dr. Paul Falkowski of Brookhaven National Laboratory (chemical factors), Dr. Thomas Osborn of Johns Hopkins University (physical factors), and Dr. V. Monica Bricelj of the Marine Sciences Research Center, SUNY Stony Brook (ecosystem factors).

Table 1.

CHARGE TO WORK GROUPS

Workgroups on chemical (nutrients, metals), physical (light, temperature, salinity, and water movements) and biological (grazing, competition, ecology/physiology, viruses) factors affecting brown tide:

Given the present state of knowledge concerning brown tide, how would you go about determining the chemical, physical, and/or biological factors responsible for producing and sustaining a bloom of the brown tide organism *Aureococcus anophagefferens*?

Some of the research topics may include:

- Isolation and axenic culture of the organism
- Elucidation of growth requirements: chemical
- Elucidation of growth requirements: physical
- Study of predator-prey relationships and other biological forms of control
- Allelochemical relationships
- Physical oceanography as related to bloom formation and persistence
- Meteorology/climatology as related to bloom formation and persistence

Each of the three workgroups is being asked to develop a research program designed to determine the factors that contribute to a bloom, and to estimate the costs associated with that program. What currently available information would allow managers to avoid recurrence of blooms or to minimize their impacts? Identify courses of action that could minimize bloom events.

Workgroup on ecological effects:

Assuming that the brown tide will recur, what is known, and/or what is needed to be determined, that would allow mitigation of its effects on the ecosystem, especially the shellfish populations. What management actions should be taken before, during and/or after a brown tide event?

Biological Factors

As with all members of the biological communities found in coastal waters, the brown tide organism exists in a complex web of interactions involving its physio-chemical environment and the other organisms that exist there. It is likely that the cause(s) of the brown tide's growth, sustenance, and eventual decline encompass its interactions with both the physio-chemical environment and with other organisms. Research is needed to determine and document which interactions with its abiotic and biotic environment are key to controlling the dynamics of brown tide.

I. What factors control the growth of brown tide?

Much important brown tide research has focused on what environmental conditions stimulate its growth, often to the near-exclusion of other phytoplankters. This remains a key area of inquiry, with a variety of facets as listed below.

A. Nutritional requirements of brown tide

Research needed to provide information on this topic would primarily involve experimental studies using cultures of *A. anophagefferens*. Isolation of several additional brown tide strains will be very important, as will assuring that these cultures are axenic, or free of associated bacteria. Studies conducted over both the short-term (3 years) and long-term (up to 10 years) are recommended.

1. Role of macro-, micro-, and trace organic nutrients in *A. anophagefferens'* growth. Research to date indicates that traditional inorganic macronutrients do not play a major role in triggering brown tide blooms. Research is needed to ascertain what, if any, inorganic micronutrient or trace organic nutrients (e.g., vitamins) are involved in initiating brown tide blooms.

2. Role of variation in light (including shade adaptation and photoperiod) in affecting the nutritional requirements or preferences of *A. anophagefferens*. brown tide is capable of growing at low ambient light levels. Does its ability to use various substances as nutrients vary with light intensity? Does photoperiod play a role in affecting the growth of brown tide?

3. Role of various metals and chelating compounds in altering the nutritional requirements/preferences of *A. anophagefferens*. Interactions with chemical chelators can modify the activity of trace metals in marine ecosystems; metals that were unavailable to phytoplankton as a nutrient source can be made available, those that were toxic can become less so. Some phytoplankton manufacture low molecular weight chelators, such as siderophores, that can make them more efficient in their ability to utilize trace metals, possibly giving them a competitive advantage over other phytoplankters. It has been suggested that trace metals and

chelation may be critical to the nutrition of the brown tide organism. Research is needed to determine if this is so and to identify the metals(s) and/or chelator(s) involved.

4. Role of heterotrophy as a means of supplemental nutrition for *A. anophagefferens*. Many marine microalgae have been shown to be capable of directly using pre-formed organic substances as a nutritive source. Evidence exists that the brown tide-like organism involved in the Texas bloom is capable of heterotrophy as a means of supplemental nutrition. Research is needed to determine whether *A. anophagefferens* is similarly capable.

B. Competitive interactions involving the brown tide organism

Many brown tide blooms appear uni-algal, involving *A. anophagefferens* and few, if any, other species. Does the brown tide organism gain and maintain a competitive advantage over the other microalgae that form the normal phytoplankton community? If it does, how? Research necessary to address questions on competitive interactions involving the brown tide organism should encompass both laboratory-scale examinations using cultured isolates as well as manipulative experiments using large mesocosm experimental systems. Substantial progress can be made within a short-term (3-year) time frame, although definitive answers to these questions will likely take much longer to achieve (10 years).

1. Role of allelopathy in securing for the brown tide a competitive edge over other microalgae. Allelopathy is the term given the process whereby one organism affects the growth capability of other organisms through production of a toxin or other growth-inhibiting or -enhancing substance. Research is needed to determine if allelopathy is involved in brown tide blooms.

2. Role of bacterial associates in mediating the brown tide organism's response to environmental conditions and particularly in affecting its nutrition. All cultures of *A. anophagefferens* contain accompanying bacteria. So far, attempts to make these cultures axenic (bacteria free) have failed. Are these bacteria natural associates of this organism? If they are, what role do they have in mediating the interaction of the brown tide with its environment?

II. What factors control the removal of brown tide and how do they relate to bloom dynamics?

In the dynamic interactions within marine food webs, an excessive increase in the abundance of any organism is, under normal circumstances, at least partially prevented by the presence of predators that consume the organism. The consumption of phytoplankton is generally termed "grazing" and the consuming organisms "grazers." Grazers include a wide variety of organisms, from microzooplankton to filter-feeding molluscan shellfish. A brown tide bloom requires two conditions: environmental conditions and nutrient levels capable of supporting a dramatic increase in the biomass of *A. anophagefferens* and at least a temporary failure or inability of the grazing community to consume the increased production. Several

topics are involved in developing a better understanding of the role of grazing in the onset and termination of brown tide blooms.

A. Timing of grazer presence and grazing activity

A temporary absence or reduction in the abundance of grazers, in particular microzooplankton, may contribute to the onset of a brown tide bloom. Determination of the composition of the microzooplankton assemblage immediately prior to the onset of a bloom is difficult as the occurrence of a bloom cannot presently be predicted. Research is needed to both document microzooplankton community variability in space and time and also, via semi-controlled field experiments, assess the impact on *A. anophagefferens* abundances of changes in the zooplankton grazing communities (e.g., manipulations such as removing the larger zooplankton).

1. Extensive examination of potential grazers. Limited work has been done examining the relative ability/inclination of bacterivorous and herbivorous microflagellates and protozoans to graze on the brown tide organism. Experimental work has shown that, given a choice of several picoplankton species, many grazers will avoid *A. anophagefferens*. More work of this type is required, involving uni-algal cultures of brown tide and mixed cultures of several phytoplankton species to elucidate grazing preferences of micro-grazers. A suite of studies is recommended here, encompassing culture-based lab and field manipulations. Studies using experimental mesocosm systems are also important. Significant knowledge gains are achievable in the short-term (3 years); work conducted over a longer time frame (5-10 years) will produce more fundamental understandings.

2. The palatability/susceptibility of the brown tide organism to grazers. *A. anophagefferens* may produce a natural metabolite that makes it unpalatable to most grazers. The small size of the organism alone will provide it a refuge from predation by most large metazoans and some smaller microzooplankton. Either or both of these factors would give the brown tide a competitive advantage over other microalgae. Studies are needed to examine these possibilities and to document whether they, in fact, occur and, if they do, what part they play in initiating or sustaining brown tide blooms. As with several of the above research areas, this work would benefit tremendously from additional isolates of *A. anophagefferens*. The research would be largely culture-based. Both short- (3-year) and long-term (10-year) studies are recommended.

B. Activity of viruses

Limited experimental work suggests that viral pathogens may play a major role in halting brown tide blooms. Moreover, field observations of the dissipation of rampant brown tide blooms within a week or so are consistent with the aggressive and fast-acting affect of viral agents. More work is recommended to determine the nature and extent of the role of viral pathogens in the demise of brown tide blooms. This research would require the development of additional brown tide isolates. Both laboratory and field-based studies would be desirable. Short-term (3-year) research should produce important knowledge advances.

C. Autolysis

Bloom termination in some marine microalgae has been shown to be attributable to the organism breaking down and lysing itself. It is important to document whether this process is involved in termination of brown tide blooms.

III. What aspects of benthic-pelagic coupling may be important in brown tide blooms?

Numerous pathways exist by which energy flows between the pelagic and benthic components of shallow, nearshore marine ecosystems; the two are tightly coupled. What effect does this coupling have on the propensity for a brown tide bloom, or the prolongation of a bloom?

A. Benthic filter-feeders and the removal of suspended particles

It is suggested that clearance rates of filter-feeding, benthic bivalves may impact the habitability of the water column for *A. anophagefferens*. Large-scale reduction of bivalve populations, by natural or anthropogenic causes (e.g., fishing), may favor the dominance of picoplankton of the size of the brown tide organism. It would appear that the biomass of bivalve molluscs in the Great South Bay was greater prior to the mid-1980's, when brown tide first appeared. There is little evidence that this is so in the Peconic Bays system, however. Research to assess the impact of benthic filter-feeders on water column suspended particle loads and the size structure of phytoplankton communities is recommended. This research should use experimental mesocosm chambers to compliment laboratory studies and include both short-term and long-term studies.

B. Resuspension of bottom material and "conditioning" of the water column

Mechanical clam harvesting resuspends large amounts of bottom sediments, pore waters, etc. into the water column. In Long Island's shallow embayments, this resuspended sediment load can be readily mixed throughout the water column by tidal and wind action. This resuspended material may be a source of trace metals, organics, etc. that play a stimulatory role for the brown tide and perhaps also has an inhibitory effect on other species of microalgae. Research is recommended to address this question over both a short (3-year) and long (5-10 year) time frame. This question is also best addressed experimentally using mesocosm chambers.

Physical Factors

The work group stressed the need to take a quantitative approach to understanding the population dynamics of bloom occurrence, persistence, and subsidence in the system. Modeling is the appropriate, and one could argue, the only tool to address this issue. The key elements supporting this understanding would relate the occurrence and growth of brown tide to predation by other organisms, water circulation inside affected bays, and removal of water to areas not affected by brown tide. The eventual goal of this effort would be to predict why brown tides appear in one place versus another, allow evaluation of potential response/remediation strategies, and identify mechanisms to prevent or eliminate the problem. There are a wide range of modeling approaches that can be used to address this issue. Future efforts should take full advantage of insights and approaches obtained from past and present efforts to model the key elements involved in the areas affected by brown tide.

I. What relationship exists between historical data on meteorological and oceanographic parameters and the occurrence and distribution of brown tide in the Peconic Bays System and Great South Bay?

A thorough review of all available data on the physical, chemical, and biological factors potentially related to bloom dynamics should be conducted to allow an initial comparison between trends in space and time. Data sets on the following groups of parameters should be considered:

A. wind patterns and precipitation;
B. groundwater inflow, tributary streamflow, and current velocity and direction;
C. temperature, salinity, dissolved oxygen;
D. brown tide cell counts; and other appropriate parameters suggested by the chemical and biological work groups.

II. Can a simple quantitative model be developed that explains historic and current trends in the variation of these parameters throughout the system?

The approach here would be to develop a relatively simple model within a 2- to 3-year time frame that would allow quantitative assessment of the relative importance of various parameters to brown tide distribution and occurrence. Such a model would allow an initial evaluation of various remediation strategies and provide estimates of flushing and circulation within bays affected by brown tide. It is anticipated that on-going measurement of key factors would be required to generate the data needed to develop and test the model.

III. How can we best quantitatively describe the temporal and spatial (3-dimensional) distribution of biological, chemical, and physical parameters associated with brown tide?

Development of a fully integrated 3-dimensional model relating biological, chemical, and physical factors associated with brown tide occurrence is likely to be a longer-termed effort requiring 3-10 years. Such a model would tie together all available data on parameters connected

with bloom formation, and would provide a tool for managers to evaluate various mitigation strategies and predict the location and persistence of future blooms.

It is important that whatever modeling efforts are supported by this research initiative be closely linked to other, current work on brown tide. Modeling work should be evaluated by technical experts not directly involved in the effort at regular intervals to ensure that the most effective approach is being pursued.

Chemical Factors

Chemical factors can play an important role in initiating phytoplankton blooms. Such factors help select one species or group of species over another. As a stimulator of blooms, perhaps the most important are those that serve as nutrients - either as macronutrients (N, P, Si) or as micronutrients (e.g. trace elements). Major research questions are summarized below.

I. What is the role of major nutrients (e.g. N,P), including organic nutrients, in stimulating a brown tide bloom?

Dissolved nitrogen and phosphorous have several forms, both inorganic and organic, that can be used by organisms. Sources of these nutrients include fluxes from sediments, groundwater, sewage treatment facilities, and run-off. Nutrient budgets help to identify the relative importance of the different nutrient sources and the relative proportions of N and P available to organisms. Nutrient budgets have been estimated for portions of the Peconic system. This approach should be expanded and refined as appropriate.

Because organic nutrients such as dissolved organic nitrogen may play an important role in stimulating a brown tide bloom, it is important to include these forms of N and P in any budget. The ability of the brown tide organism to take up organic nutrients and the cellular fate of this material (whether incorporated or respired) should be investigated.

In the context of the major nutrients, it is important to assess the variability in nutrient supply and partitioning among the different forms, both within a given year and between years.

II. What is the role of micronutrients in stimulating brown tide blooms?

Micronutrients such as trace elements also can play an important role in triggering phytoplankton blooms. For example, the role of iron as a limiting micronutrient for some open ocean phytoplankton has been demonstrated. Iron enters the Peconic system via bottom sediments, groundwater, terrestrial run-off, and the atmosphere. Relative to macronutrients, less is known about micronutrient sources in the Peconics, and an iron budget is not available. Moreover, the speciation or chemical forms of the iron is not known, and this can play a crucial role in determining its availability to organisms.

For iron and a host of other potential micronutrients, the cellular quotas for growth are unknown. These quotas are important to determine for *A. anophagefferens* and should be compared with the requirements for other species. Such determinations should be made both in the field and the laboratory; the latter would require an axenic culture.

III. Research objectives

These considerations of the role of the macro- and micronutrients in causing brown tide suggest the following research objectives. The approach taken should successively eliminate candidate chemical factors based on a series of culture-based laboratory and field experiments.

A. Calculate budgets for the major nutrients (N, P, Si), to the extent possible using existing data. This calculation should be done especially for the bays most strongly affected by brown tide. Inadequacies in the existing data should be remedied by additional sampling.

B. Continuously monitor various chemical and physical parameters in the field before, during, and after brown tide blooms. This can be a labor-intensive exercise and is facilitated through the use of moored instrumentation.

C. In an effort to determine the relative importance of macro- and micronutrients in stimulating the growth of *A. anophagefferens*, a suite of experiments should be conducted in the field, with mesocosms and with bottle experiments. The goal of these experiments is to determine the growth response to additions of selected nutrients and trace elements. A parallel set of measurements should be conducted in the laboratory using axenic cultures.

D. As the efforts proceed to identify chemical factors important in stimulating brown tide blooms, it is necessary to characterize important sources and sinks of such factors. Sources include, but are not limited to, the flux from bottom sediments, groundwater inflow, sewage treatment plant effluent, atmospheric deposition, and storm water run-off. The identification of causative factors and sources can provide the kind of information that suggests management strategies to control brown tide blooms.

Ecological Effects

Given the structure and functioning of inshore marine ecosystems, many ecosystem impacts caused by a perturbation of the phytoplankton community such as brown tide can be hypothesized. Only two such impacts, however, have been clearly documented to date: a reduction of eelgrass biomass and distribution attributable to brown tide-caused light attenuation and severe impacts (recruitment failure, mortalities, growth inhibition) on several commercially important filter-feeding bivalve molluscs, in particular the bay scallop. Even for these documented impacts, however, the mechanisms by which they occurred generally remain obscure and their long-term significance for the ecology of these bays remains a matter of speculation. The effects of brown tide on secondary consumers (e.g. crabs and finfish) remain to be determined.

The following are key research needs to better understand the broad ecological significance of brown tide and to evaluate resource management strategies to, if necessary, accommodate its persistence in Long Island waters. Answering most questions related to the ecological impacts of brown tide will require both field and laboratory studies.

I. How does brown tide impact commercially important bivalves and other filter-feeders?

A. Brown tide's effect on bivalve physiology

1. What is the *in vivo* mechanism responsible for grazing suppression and other adverse effects? Grazing suppression by bivalves exposed to brown tide is frequently noted; it has been suggested that this reflects chronic toxicity of the organism. The precise mode of action responsible for grazing suppression and other negative impacts should be determined.

2. What are the density- and time-dependent effects of brown tide on survival, growth, and reproduction of bivalves? It is clear that effects of *A. anophagefferens* on bivalves depend on both cell concentrations and duration of exposure to brown tide. What are the response thresholds and how do they vary by bivalve species and, within a single species, by life history stage. Do these responses vary depending on whether the animals are exposed to mono-cultures of brown tide as opposed to mixed species assemblages? Can bivalves acclimate or adapt to long-term and recurrent exposure to brown tide?

3. How does brown tide cause recruitment failure and other reproductive impacts in bivalve mollusks? Little is known about the most sensitive life history stage in terms of brown tide impacts. Is the effect felt principally by adults at the reproductive stage? Is the timing of spawning affected? Is recruitment failure caused by effects on larval survival in the water column, or during and following metamorphosis, as the larvae settle to the bottom. How does the timing of the brown tide, which varies between locations within a year and between years, interact with various life stages of key shellfish to produce catastrophic losses?

B. Development of a brown tide bioassay

What organism(s) would be useful in assessing the relative toxicity of various brown tide isolates? Development of a standard bioassay for brown tide would help immeasurably in comparing response effects derived from work in different labs undertaken with different strains or isolates of *A. anophagefferens*.

II. How can shellfish management programs be optimized in the presence of brown tide?

A. Determination of management candidate species

1. Are some bivalve mollusc species less vulnerable/sensitive to brown tide? Should brown tide remain a permanent problem, the long-term fate of certain sensitive shellfish species (e.g. bay scallop and, perhaps, oysters) may become problematic. Work should be undertaken to screen an assortment of bivalve species to identify those that, in the face of a chronic infestation of brown tide, stand the best chance of sustaining viable, reproducing populations in the affected area or that might form the basis of an intensively managed "put and take" shellfishery.

B. Refinement of management approaches

1. How can management practices be improved to reduce losses from brown tide? If brown tide continues to recur periodically in Long Island waters, fisheries for such traditionally important shellfish species as bay scallop and hard clam might remain viable. Much has been learned in Great South Bay over the past two decades on how to artificially enhance hard clam stocks. Seeding efforts have contributed to the rehabilitation of bay scallop stocks in the Peconic Bays system. Applied research should be conducted to continue to refine and improve the effectiveness of such management measures as creation of spawner sanctuaries, transplanting, seeding, habitat improvement, and related activities.

II. What is the effect of brown tide on other ecosystem elements?

A. Impacts on submerged aquatic vegetation (SAV)

What are the long-term impacts of brown tide on SAV? Past research has documented widespread, but apparently temporary declines in the abundance, distribution, and general condition of SAV's, especially eelgrass, in brown tide-affected waters. SAV communities are affected by a multitude of other factors--general eutrophication, disease, sediment resuspension, salinity and temperature, availability of suitable substrate, light, and the activity of epiphytic organisms. Research is needed that would ascertain the relative impact of brown tide-related light attenuation among the various other determinants of the health of SAV communities.

B. Impacts on secondary consumers

1. Does brown tide-related light attenuation and increased turbidity affect organisms, such as finfish, that rely on visual cues in feeding and predator avoidance? Many key resource or forage species of finfish in local waters depend on visual cues. Fishermen contend that fish avoid areas experiencing a brown tide bloom. Research is needed to ascertain whether and to what extent shading and increased turbidity associated with brown tide blooms interferes with feeding and predator avoidance in these species.

2. What are the affects of brown tide-related eelgrass losses on secondary consumers? Many species of finfish and invertebrates are found in close association with beds of eelgrass. Should brown tide blooms reduce the abundance/distribution of eelgrass in the bays, this could represent a significant loss of habitat for these species. Research is recommended to assess the consequences of such habitat reduction for these species and the effects of reduced food availability (e.g. zooplankton) on secondary consumers.

III. Are there multiple strains of brown tide of varying relative toxicity?

Development of new brown tide isolates is key to understanding the range of ecological effects that might ensue from an outbreak of brown tide. *A. anophagefferens* appears to be somewhat plastic in its toxicity, with isolates from closely adjoining water bodies having different impacts on shellfish. The extent of this plasticity and its implication must be determined if a true understanding of this organism and its likely ecological impacts to particular areas are to be assessed.

Appendix

Registered Participants

Assemblywoman Pat Acampora
New York State Assembly
County Seat Professional Complex
Riverhead, NY 11901
Tel: 516 727-1363
Fax: 516 369-3869

Ms. Jennifer Albert
Biology Dept.
Dowling College
Oakdale, NY 11769
Tel: 516 244-3011

Dr. Donald M. Anderson
Dept. of Biology MS #32
Woods Hole Oceanographic Institute
Woods Hole, MA 02543-1049
Tel: 508 289-2351
Fax: 508 457-2134
E-mail: danderson@whoi.edu

Ms. Susan Antenen
The Nature Conservancy
250 Lawrence Hill Road
Cold Spring Harbor, NY 11724
Tel: 516 367-3225

Ms. Melanie Baacke
Box 43
Southampton College, LIU
Southampton, NY 11968
Tel: 516 287-7549

Mr. Joseph Baier
22 Southview Court
Wading River, NY 11792
Tel: 516 929-8629

Mr. Rick Balla
US EPA Peconic Estuary Program, Reg. II
290 Broadway - 25th Floor
New York, NY 10007
Tel: 212 637-3803
Fax: 212 637-3772

Dr. Ed Beltrami
Applied Math and Statistics
1-110 Mathematics
SUNY at Stony Brook
Stony Brook, NY 11794
Tel: 516 632-8367
Fax: 516 632-8490
E-mail: Beltrami@ams.sunysb.edu

Ms. Sharon Benmayor
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000

Ms. Barbara Blass
Town of Riverhead
200 Howell Ave
Riverhead, NY 11901
Tel: 516 727-3200 x267

Mr. Mike Bortini
34 Chapel Lane
East Hampton, NY 11937
Tel: 516 267-3289

Dr. Malcolm Bowman
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-8669
Fax: 516 632-8820

Dr. Gregory L. Boyer
Chemistry Dept., Baker Lab
One Forestry Drive
SUNY - CESF
Syracuse, NY 13210-2778
Tel: 315 470-6825
Fax: 315 470-6856
E-mail: GLBoyer@mailbox.syr.edu

Bold = Invited Participants
Bold Italicized = Planning Committee

Dr. V. Monica Bricelj
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-8663
Fax: 516 632-8820

Mr. Len Brown
35 Overlook Drive
Aquebogue, NY 11931
Tel: 201 235-5500

Mr. Martin Byrnes
224 Sherry Street
East Islip, NY 11730
Tel: 516 277-6634

Mr. Gregg Cademartori
624 Main Street
Port Jefferson, NY 11777
Tel: 516 474-8461

Dr. Leon Cammen
NOAA Nat'l Sea Grant College Prog.
1335 East-West Hwy.
R/OR1, SSMC-1, Rm. 5200
Silver Spring, MD 20910
Tel: 301 713-2435
Fax: 301 713-0799
E-mail: LCAMMEN@rdc.NOAA.GOV

Ms. Bethanie Carney
Box 1527, 239 Montauk Hwy.
Southampton College, LIU
Southampton, NY 11968
Tel: 516 287-5416

Dr. David A. Caron
Department of Biology
MS32
Woods Hole Oceanographic Institute
Woods Hole, MA 02543
Tel: 508 289-2358
Fax: 508 457-2169
E-mail: dcaron@whoi.edu

Ms. Amy Chan
Marine Science Institute
University of Texas.
Port Aransas, TX
Tel: 512 749-6763

Mr. Louis Chiarella
NYS DEC
Building 40
SUNY at Stony Brook
Stony Brook, NY 11790-2356
Tel: 516 444-0295

Dr. Andrei Chistoserdov
Marine Sciences Research Center
153 Discovery Hall
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-9233

Ms. Karen Chytalo
NYS DEC
205 Belle Mead Road
Setauket, NY 11733
Tel: 516 444-0468

Mr. Todd Ciccone
PO Box 446
Hampton Bays, NY 11946
Tel: 516 728-8478

Mr. Rowland V. Clark
Cherry Lane Box 782
Shelter Island, NY 11964
Tel: 516 749-2064

Dr. J. Kirk Cochran
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-8700
Fax: 516 632-8820
E-mail: KCochran@ccmail.sunysb.edu

Bold = Invited Participants
Bold Italicized = Planning Committee

Ms. Amy Codella
33 Haytown Road
Lebanon, NJ 08833
Tel: 908 932-8012

Dr. Daniel Conley
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-9251
Fax: 516 632-8820

Ms. Allesandra Conversi
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-8694
Fax: 516 632-8820

Dr. Elizabeth M. Cosper
Coastal and Environ. Studies, Inc.
83 Carlough Road
Bohemia, NY 11716
Tel: 516 563-8899
Fax: 516 563-2999

Dr. Dewitt S. Davies
Suffolk County Dept. of Planning
220 Rabro Drive, PO Box 6100
Hauppauge, NY
Tel: 516 853-4865

Mr. Walter Dawydiak
Suffolk County Dept. Health
Office of Ecology
Griffing County Center
Riverhead, NY 11901
Tel: 516 852-2077
Fax: 516 852-2743

Dr. Cynthia Decker
NYS DEC
Bureau of Marine Habitat Protection
205 Belle Mead Road
East Setauket, NY 11733
Tel: 516 444-0468
Fax: 516 444-0474

Mr. Thomas Deland
PO Box 110
Aquabogue, NY 11931
Tel: 516 722-3202

Mr. Charlie deQuillfeldt
NYS DEC
Bureau of Shellfisheries
205 Belle Meade Road
E. Setauket, NY 11733
Tel: 516 444-0482

Dr. Hudson DeYoe
Corpus Christie Bay NEP
Campus Box 290, 6300 Ocean Drive
Corpus Christie, TX 78412
Tel: 512 985-6767
Fax: 512 985-6301
E-mail: deyoe@tamucc.edu

Dr. Joseph DiLorenzo
Najarian & Associates, Inc.
1 Industrial Way West
Eatontown, NJ 07724
Tel: 908 389-0220
Fax: 908 389-8546

Dr. Percy L. Donaghay
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882
Tel: 401 792-6944
Fax: 401 792-6240
E-mail: p.donaghay

Bold = Invited Participants
Bold Italicized = Planning Committee

Dr. Gregg Doucette
NMFS Southeast Fisheries Science Center
Charleston Lab.-PO Box 12607
Charleston, SC 29422-2607
Tel: 803 762-8528
Fax: 803 762-8700
E-mail:
Greg_Doucette@ccgate.ssp.nmfs.gov

Ms. Sandra A. Dumais
PO Box 3016
East Quoque, NY 11942
Tel: 516 728-4881

Ms. Susan Dzurica
Dept. of State
162 Washington Avenue
Albany, NY
518 474-6000

Dr. Paul Falkowski
Brookhaven National Lab.
Building 318
Upton, NY 11973
Tel: 516 282-2961
Fax: 516 282-3246
E-mail: falkowsk@bnlux1.bnl.gov

Ms. Stacy Ferguson
4 Coca Ct.
Southampton College, LIU
Hampton Bays, NY
Tel: 516 728-9486

Ms. Lauretta Fischer
Suffolk County Planning Dept.
220 Rabro Drive, PO Box 6100
Hauppauge, NY 11788-0099
Tel: 516 853-6044

Dr. Nicholas S. Fisher
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-8649
Fax: 516 632-8820
E-mail: nfisher@ccmail.sunysb.edu

Congressman Michael Forbes
United States Congress
1500 William Floyd Parkway
Shirley, NY 11967

Mr. Jeff Fullmer
South Shore Estuary CAC
550 Smithtown By-Pass, Suite 205
Smithtown, NY 11787
Tel: 516 360-0480

Mr. Robert Gaffney
Suffolk County Executive
H. Lee Dennison Bldg.
Veteran's Memorial Highway
Hauppauge, NY 11788

Mr. Ken Gall
New York Sea Grant
125 Nassau Hall
SUNY at Stony Brook
Stony Brook, NY 11794
Tel: 516 632-8730
Fax: 516 632-8216

Mr. Christopher Gill
Senator Michael Tully's Office
201 Expressway Plaza One
Roslyn Hts., NY 11577
Tel: 516 484-7070

Mr. Chris Gobler
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000

Bold = Invited Participants
Bold Italicized = Planning Committee

Ms. Janet Goltz
E. Patchogue 11772
Tel: 516 475-1129

Ms. Liti Haramaty
78 Ficus Rd.
Rocky Point, NY 11778

Ms. Louise Harrison
NYS DOS
Marine Sciences Research Center
Stony Brook, NY 11794-5000
Tel: 516 632-9425

Mr. Emerson Hasbrouck
Cornell Cooperative Extension
3059 Sound Avenue
Riverhead, NY 11901
Tel: 516 727-3910

Ms. Mara Hogan
Box 404 LIU
Southampton College, LIU
Southampton, NY 11968
Tel: 516 287-5079

Dr. Dave Hutchins
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-8772
Fax: 516 632-8820
E-mail:
DAHUTCHINS@ccmail.sunysb.edu

Mr. Andrew Jones
PEP CAC

Mr. Jeffrey Kassner
Division of Environmental Protection
Town of Brookhaven
3233 Route 112
Medford, NY 11763
Tel: 516 451-6455
Fax: 516 451-6419

Ms. Sharon Kast
Town of Shelter Island
Box 3019
Shelter Island Heights, NY
Tel: 516 749-0666

Dr. Kathleen Irwin Keating
33 Haytown Road
Lebanon, NJ 08833
Tel: 908 932-8012
Fax: 908 932-8644
E-mail: kkeating@RCI.RUTGERS.edu

Dr. John F. Kelly
PO Box 3035
92 Gardiner's Bay Drive
Shelter Island Heights, NY 11965

Mr. Robert Kent
New York Sea Grant
3059 Sound Avenue
Riverhead, NY 11901
Tel: 516 727-3910
Fax: 516 369-5944

Mr. Ronald L.C. Kienhuis
121 Maple Street
Great Neck, NY 11023
Tel: 516 829-0470

Mr. Jim King
220 East Mill Road
Mattituck, NY 11952
Tel: 516 298-4718

Mr. Greg King
SSER CAC

Mr. Mike King
Tel: 516 725 1096

Mr. Zbigniew Kolber
Tel: 516 282-4086

Bold = Invited Participants
Bold Italicized = Planning Committee

Ms. Maureen Krause
Natural Science Division
Southampton College, LIU
Southampton, NY 11968
Tel: 516 287-8397

Mr. David Laby
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 751-0366

Mr. Kenneth Landversicht
132 Moriches Avenue
Mastic, NY 11950
Tel: 516 281-0543

Dr. Julie LaRoche
Dept. Applied Science
Brookhaven National Lab.
Building 318, P.O. Box 5000
Upton, NY 11973-5000
Tel: 516 282-7938
Fax: 516 282-3246
E-mail: Laroche@bnlux1.bnl.gov

Mr. Richard Larsen
Town of Riverhead
200 Howell Ave.
Riverhead, NY 11901
Tel: 516 727-3200 x 267

Mr. Senjie Lin
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5001
Tel: 516 632-8697

Ms. Lisa Liquori
Peconic Estuary Program TAC Chair
300 Pantigo Place, Suite 105
East Hampton, NY 11937
Tel: 516 324-2178
Fax: 516 324-1476

Mr. Felix Locicero
US EPA - Region II
290 Broadway - 25th Floor
New York, NY 10007
Tel: 212 637-3708
Fax: 212 637-3772

Dr. Darcy J. Lonsdale
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-8712
Fax: 516 632-8820
E-mail: Darcy.Lonsdale@sunysb.edu

Dr. Stuart R. Lowrie
The Nature Conservancy
PO Box 5125
East Hampton, NY 11937

Ms. Erika Magaletti
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-8820

Dr. John B. Mahoney
NOAA/NMFS/NEFSC
James J. Howard Marine Science Lab.
74 McGruder Road
Highlands, NJ 07732
Tel: 908 872-3055
Fax: 908 872-2088

Mr. Mark Malchoff
New York Sea Grant
3059 Sound Avenue
Riverhead, NY 11901-1098
Tel: 516 727-3910
Fax: 516 369-5944
E-mail: sgriverh@cce.cornell.edu

Bold = Invited Participants
Bold Italicized = Planning Committee

Mr. Manny Marks
27 Middleline Hwy.
Southampton, NY 11968
Tel: 516 287-1193

Ms. Gayle Marriner-Smith
1035 Hobart Road
Southold, NY 11971
Tel: 516 765-1766

Mr. Bob McAlevy
PO Box 300
Hampton Bays, NY 11946
Tel: 516 728-3189

Mr. Kevin McDonald
PO Box 569
Bridgehampton, NY 11932

Dr. Anne E. McElroy
New York Sea Grant
SUNY at Stony Brook
115 Nassau Hall
Stony Brook, NY 1794-5001
Tel: 516 632-6905
Fax: 516 632-6917
E-mail: amcelroy@ccmail.sunysb.edu

Mr. Bill McGroarty
Tel: 516 444-0438

Dr. Mike McKay
DAS-OASD, Building 318
P.O. Box 5000
Upton, NY 11973-5000
Tel: 516 282-4077
Fax: 516 282-3246
E-mail: mckay@bnlux1.bnl.gov

Ms. Melanie Meade
Marine Science Center
Southampton College, LIU
Southampton, NY 11968
Tel: 516 287-8393

Ms. Reyhan Mehran
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook. NY 11794-5000

Dr. Lynn Mendelman
421 Three Mile Harbor Road
East Hampton, NY 11937-1298
Tel: 516 324-5424

Ms. Lisa Metz
Southampton College, LIU
Southampton, NY 11968

Mr. Allen Milligan
Dept. of Oceanography
6270 University Boulevard
University of Britisb Columbia
Vancouver, BC V6T 1Z4 CANADA
Tel: 604 822-3355
Fax: 604 822-6091
E-mail: allenm@unixg.ubc.ca

Mr. Vito Minei
Office of Ecology
Suffolk County Dept. of Health Services
Griffing County Center
Riverhead, NY 11901
Tel: 516 852-2077
Fax: 516 852-2743

Prof. Henry W. Moeller
Biology Dept.
Oakdale, NY 11769
Tel: 516 244-3011

Dr. James Moffett
Fye Laboratory
Woods Hole Oceanographic Inst.
Woods Hole, MA 02543
Tel: 508 289-3218
Fax: 508 457-2164
E-mail: JMoffett@WHOI.EDU

Bold = Invited Participants
Bold Italicized = Planning Committee

Mr. Joe Mulle

Ms. Helen Murray-Tobin
Brookhaven National Lab.
DAS/OASD
1 South Technology Street
Upton, NY 11973
Tel: 516 282-2963

Mr. Bud Newins
Burnett Marine Service
34 Homan Ave.
Bay Shore, NY 11706
Tel: 516 665-0293

Dr. Robert Nuzzi
Bureau of Marine Resources
Suffolk County Dept. of Health Services
Riverhead, NY 11901
Tel: 516 852-2082
Fax: 516 852-2743

Mr. Paul Olsen
NJ DEP, Division of Science Research
35 Arctic Pkwy
CN 422
Trenton, NJ 08625
Tel: 609 292-0427
Fax: 609 633-1095

Dr. Thomas Osborn
Dept. Earth & Planetary Sciences
34th & N. Charles Street
Johns Hopkins University
Baltimore, MD 21218
Tel: 410 516-7039
Fax: 410 516-7933

Mr. Mario Paula
USEPA Peconic Estuary Program, Region II
290 Broadway
New York, NY 10007-1866
Tel: 212 637-3819
Fax: 212 637-3889/3891
E-mail: Paula.Mario@EPAMail.EPA.GOV

Mr. Chris Pranis
Bluepoints Co. Inc.
PO Box 8
W. Sayville, NY 11796
Tel: 516 589-0123

Mr. George Proios
Suffolk County Exec. Office
H. Lee Dennison Building, Vets Mem. Hwy
Hauppauge, NY 11788
Tel: 516 853-4000
Fax: 516 853-4818

Dr. Rollin C. Richmond
Provost's Office
407 Administration Building
SUNY at Stony Brook
Stony Brook, NY 11794-1401

Mr. West Ruroede
PO Box 254
Greenport, NY 11944

Ms. Kelly Ryder
925 S. Fifth Street
Lindenhurst, NY 11757
516 287-8943

Ms. Lisa Santoro

Dr. Sergio Sanudo-Wilhemy
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-8615
Fax: 516 632-8820
E-mail: SSANUDO@ccmail.sunysb.edu

Mr. Donald L. Scanlon
94 Stratford Avenue
Garden City, NY 11530
Tel: 516 571-2201

Bold = Invited Participants
Bold Italicized = Planning Committee

Dr. Donald Scavia
NOAA Coastal Ocean Program
1315 East-West Highway, Room 15140
Silver Spring, MD 20910
Tel: 301 713-3338
Fax: 301 713-4044
E-mail: dscavia@HQ.NOAA.GOV

Ms. Rebecca Schaffner
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-5137

Ms. Cornelia Schlenk
New York Sea Grant
115 Nassau Hall
Stony Brook, NY 11794-5001
Tel: 516 632-6905
Fax: 516 632-6917
E-mail: CSchlenk@ccmail.sunysb.edu

Ms. Nancy J. Schlotter
91 S. Lewis Place
Rockville Centre, NY 11570
Tel: 516 678-0032

Mr. Trent R. Schneider
New York Sea Grant
115 Nassau Hall
SUNY at Stony Brook
Stony Brook, NY 11974-5001
Tel: 516 632-9123
Fax: 516 632-6917
E-mail: TSchneider@sunysb.edu

Dr. Oscar Schofield
Institute of Marine & Coastal Sciences
PO Box 231
Rutgers University
New Brunswick, NJ 08903-0231
Tel: 908 932-6555 ext. 54
Fax: 908 932-8578
E-mail: oscar@ahab.rutgers.edu

Ms. Florence Sharkey
74 Vernon Street
Patchogue, NY 11772

Mr. Huson Sherman
Supervisor, Town of Shelter Island
Shelter Island Town Hall
Shelter Island, NY 11964
Tel: 516 749-0015
Fax: 516 749-0728

Dr. Sandra E. Shumway
Natural Science Division
Southampton College, LIU
Southampton, NY 11968
Tel: 516 287-8407
Fax: 516 287-8419
E-mail: sshumway@sunburn.liunet.edu

Mr. Al Siegel
Southampton College, LIU
Southampton, NY 11968

Dr. Theodore J. Smayda
Grad. School of Oceanography
Narragansett Bay Campus
University of Rhode Island
Kingston, RI 02881
Tel: 401 792-6171
Fax: 401 792-6682

Mr. Christopher Smith
Cornell Cooperative Extension
3059 Sound Avenue
Riverhead, NY 11901-1098
Tel: 516 727-3910
Fax: 516 369-5944
E-mail: csmith@cce.cornell.edu

Ms. Marisella Sollano
Campus Box 903
Southampton College, LIU
Southampton, NY 11968
E-mail: 516 728-4514

Bold = Invited Participants
Bold Italicized = Planning Committee

Ms. Melissa Southworth
Box 26B East Hill Road
Munnsville, NY 13409
Tel: 315 495-5681

Mr. John St. John
Hydroqual, Inc.
1 Lethbridge Plaza
Mahwah, NJ 07430
Tel: 201 529-5151

Dr. Dean A. Stockwell
Marine Science Institute
PO Box 1267
University of Texas at Austin
Port Aransas, TX 78373-1267
Tel: 512 749-6705
Fax: 512 740-6777
E-mail: d.stockwell

Mr. Jeffrey L. Strong
Strong's Neck Marine Inc.
PO Box 1409
Mattituck, NY 11952
Tel: 516 298-4770

Dr. William G. Sunda
NMFS/SE Fisheries Center
Beaufort Laboratory
Beaufort, NC 28516
Tel: 919 728-8754
Fax: 919 728-8784

Dr. Curtis Suttle
Marine Science Institute
PO Box 1267
Port Aransas, TX 78373-1267
Tel: 512 749-6733
Fax: 512 749-6777
E-mail: curtis@utmsi.zo.utexas.edu

Mr. Alan Svoboda
Town of Islip Planning Dept.
655 Main Street
Islip, NY
516 224-5450

Ms. Sandra Talerico
309 North Corona Ave.
Valley Stream, NY 11580
Tel: 516 872-0936

Mr. Jay Tanski
New York Sea Grant
125 Nassau Hall
SUNY at Stony Brook
Stony Brook, NY 11794-5001
Tel: 516 632-8730
Fax: 516 632-8216

Mr. John Targrey
309 North Corona Ave.
Valley Stream, NY 11580
Tel: 516 872-0936

Dr. Gordon Taylor
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-8688

Dr. Stephen T. Tettlebach
Long Island University
Southampton, NY 11968
Tel: 516 287-8418
Fax: 516 287-8419

Assemblyman Fred Thiele
New York State Assembly
PO Box 9001
Wainscott, NY 11975
Tel: 516 537-2583
Fax: 516 537-2836

Dr. Alan Thomas
NOAA OAR # 11651, SSMC3
1315 East-West Highway
Silver Spring, MD 20910
Tel: 301 713-2458
Fax: 301 713-0163

Bold = Invited Participants
Planning Committee

Mr. Roger Tollefsen
New York Sea Food Council
252 East Montauk Highway
Hampton Bays, NY 11946
Tel: 516 728-3474
Fax: 516 728-3690

Dr. Mario Vieira
Oceanography Department
Annapolis, MD 21402-5026
Tel: 410 293-6567
Tel: 410 293-2137
Tel: vieira@nadn.navy.mil

Mr. Thomas Villano
325 Montauk Hwy
Southampton College, LIU
Southampton, NY 11968
Tel: 516 728-4514

Mr. Robert M. Waters
Suffolk County Dept. of Health Services
County Center
Riverhead, NY 11901
Tel: 516 852-2087
Fax: 516 852-2743

Mr. Thomas Wickham
Supervisor, Town of Southold
Southold, NY 11971
Tel: 516 765-1889

Dr. Gary Wikfors
Nat'l Marine Fisheries Service
212 Rogers Avenue
Milford, CT 06460
Tel: 203 783-4225
Fax: 203 783-4217

Dr. Robert E. Wilson
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-8689
Fax: 516 632-8820
E-mail: RWILSON@ccmail.sunysb.edu

Dr. Creighton Wirick
Brookhaven National Lab.
Building 318
P.O. Box 5000
Upton, NY 11973-5000
Tel: 516 282-3063
E-mail: wirick@bnl.gov

Mr. William Wise
Marine Sciences Research Center
SUNY at Stony Brook
Stony Brook, NY 11794-5000
Tel: 516 632-8656
Fax: 516 632-8820
E-mail: BWise@ccmail.sunysb.edu

Mr. Brian Wysor
20 Magee Drive
Southampton, NY 11968
Tel: 516 287-1192

Ms. Julie Zeidner
New York Sea Grant
115 Nassau Hall
SUNY at Stony Brook
Stony Brook, NY 11794-5001
Tel: 516 632-6905
Fax: 516 632-6917

Ms. Kimberly Zimmer
New York Sea Grant
125 Nassau Hall
SUNY at Stony Brook
Stony Brook, NY 11974-5002
Tel: 516 632-9216

Bold = Invited Participants
Bold Italicized = Planning Committee

New York Sea Grant

115 Nassau Hall
SUNY at Stony Brook
Stony Brook NY 11794-5001
Phone: (516) 632-6905

207 Jarvis Hall
SUNY College at Buffalo
Buffalo NY 14260-4400
Phone: (716) 645-2088

Cornell Cooperative Extension Center
21 South Grove Street
East Aurora NY 14052-2398
Phone: (716) 652-7874

Surge III
Cornell University
Ithaca NY 14853-3001
Phone: (607) 255-2832

101 Rich Hall
SUNY College at Oswego
Oswego NY 13126-3599
Phone: (315) 341-3042

Harriwell Hall
SUNY College at Brockport
Brockport NY 14420-2928
Phone: (716) 395-2638

Cornell University Laboratory
3059 Sound Avenue
Riverhead NY 11901-1098
Phone: (516) 727-3910

Cornell Cooperative Extension Center
74 John Street
Kingston NY 12401-3824
Phone: (914) 338-3494

125 Nassau Hall
SUNY at Stony Brook
Stony Brook NY 11794-5002
Phone: (516) 632-8730