BROWN TIDE 1999 and 2000

Data supplied by Suffolk County Department of Health Services (SCDHS) showed that, except for West Neck Bay, the Peconic Bays of Long Island remained free of brown tide for the fifth consecutive year in 1999. South shore bays, however, did experience brown tides. Samples taken in Shinnecock Bay showed brown tide counts as high as 200,000 cells/ml in June and July 1999. During this same period, brown tide counts in Moriches Bay ranged between 100,000 - 300,000 cells/ml. Starting in late September and continuing through late November 1999, a second smaller bloom event ranged between 90,000 - 125,000 cell/ml.

A different pattern of longer and multiple blooms emerged in Great South Bay (GSB) in 1999. Samples taken at various locations showed a bloom starting in late October with counts exceeding 600,000 cells/ml and persisting through December with counts reaching over 730,000 cells/ml. High counts (300,000 cells/ml) continued through January 2000, when monitoring was suspended due to a freeze. Monitoring was reinitiated in mid-February 2000, after the thaw, at which time cell counts were lower (a maximum of 50,000 cells/ml) but still relatively high for this time of year. Cell numbers declined throughout February to a low of about 5,000 cells/ml, and began to increase from March to June 2000 reaching in excess of 700,000 cells/ml (see figure 1). Dr. Robert Nuzzi suggests that the 1999 bloom may have been triggered by the release of dissolved organic nitrogen during the late summer-fall decomposition of a major summer Cladophora bloom in GSB.

Continued on page 3

Figure 1: Brown tide at station 150, Hog Neck Bay, in Great South Bay, Long Island New York 1999-2000. (Graph supplied by Dr. Robert Nuzzi, Suffolk County Department of Health Services.)
Tribute to Dr. Maureen Keller

Dr. Maureen D. Keller, a marine scientist at Bigelow Laboratory for Ocean Sciences, passed away on November 17, 1999. Dr. Keller was an internationally renowned expert in the ecology and physiology of marine phytoplankton. She was especially known for her expertise in growing phytoplankton and her work on the production of sulfur compounds by algae. Her recent work on the ecology of the toxic red tide organism, *Alexandrium*, in the Gulf of Maine, and the brown tide in the Long Island bays has contributed greatly to the understanding of how these harmful blooms form. Maureen’s perceptive observations have furthered understanding of these unusual phenomena. She was the originator of the “picoplankton niche hypothesis” as a possible explanation for brown tide blooms. Maureen’s scientific insight, ability to distill technical information and easy sense of humor made her one of several scientists the BTRI relied upon for presentations to both the scientific community and the public. In addition to her research, Dr. Keller was a leading advocate for teaching science and exposing students of all ages to the excitement of marine research. She sponsored scores of undergraduate student interns who worked in her lab over the years. She initiated and ran the annual Bigelow BLOOM program that brings 16 high school students, one from each county of Maine, to Bigelow for an intensive one-week course in marine science. She organized the Maine/New Hampshire regional competition of the first National Ocean Science Bowl. Held at the University of New England in 1998, this is now an annual academic competition for high school teams in the field of oceanography. Maureen Keller was a gifted scientist, a treasured colleague, and a pleasure to know. She is very much missed.

A memorial service was held for Dr. Keller on Monday, November 22, 1999, and a celebration of Dr. Keller’s professional life and scientific career was held at Bigelow Laboratory in West Boothbay Harbor on December 3, 1999.

The Keller family and Bigelow laboratory have established the Keller Memorial Education fund to support young scientists. Contributions may be sent to the Bigelow Laboratory for Ocean Sciences, 180 McKown Pt. Rd., W. Boothbay Harbor, ME, 04575.
Several other states have reported brown tides. *Aureococcus* has been reported in Little Egg Harbor and Barnegat Bay, New Jersey. Suffolk County Department of Health Services (SCDHS) analyses of Maryland’s Coastal Bays Program water samples taken from 20 different field stations revealed brown tide counts between 100,000 - >470,000 cell/ml (Spence Cove) between late May through June 1999. More information about brown tide in 2000 will appear in BTRI Report #6.

**Brown tide working session**

BTRI teams and other brown tide researchers came together for a working session hosted by NYSG at Suffolk County Community College in March 2000. This focused group of investigators presented their latest results to the BTRI Steering Committee and project sponsors, discussed new ideas and coordinated the 2000 field season. Information presented at the working session is incorporated in the following project briefs. The BTRI researchers agreed that two multi-authored and coordinated papers will be developed to synthesize project and working session results. These papers will be submitted to peer review journals for publication (see bibliography for currently published papers resulting from BTRI projects page 11). One paper will focus on *Aureococcus* physiology and the second paper will focus on field data synthesis.

This report builds on BTRI Reports Numbers 1-4, concludes some of the BTRI 1996-1999 projects and introduces the three BTRI 1999-2001 projects. This report follows the same format as the previous issues for easy project tracking. Boldfaced terms are defined under Key Terms adding to those defined in the earlier reports.

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**Andersen: Multiple Culture Isolates (Xenic and Axenic), Biodiversity and Ultrastructure of *Aureococcus anophagefferens***

This team’s original objective was to establish and maintain culture strains of *A. anophagefferens*. Additionally, they examined *A. anophagefferens* genetic diversity and structure.

Andersen’s team successfully established 17 culture strains of *Aureococcus* that have been deposited in the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP). These cultures were isolated from samples collected in Great South Bay and several bays in the Peconics Estuary, both on Long Island, and from Barnegat Bay New Jersey, during a thirteen year span between 1985 - 1998. To date, bacteria-free or axenic cultures have not been established, however, efforts beyond the scope of this project will continue at the CCMP to achieve this goal.

Different strains showed slightly different growth characteristics while maintained in culture. *A. anophagefferens* demonstrated better growth in cultures with higher bacterial numbers, when small amounts of organic nutrients were added, and under high light conditions. *A. anophagefferens* growth was adversely affected by high bacterial growth under high organic nutrient conditions. Therefore, these investigators hypothesized that some of the observed growth differences may be due to the bacteria present in the cultures. This team also noted that even low amounts of ammonia killed *A. anophagefferens*, a rather unexpected finding since most photosynthetic organisms are able to utilize...
ammonia. Work is currently underway at the CCMP to determine the lethal dose of ammonia for *A. anophagefferens*.

Detailed analysis of three DNA regions (18S rRNA, *rbcL* & RUBISCO from 17 different strains; see BTRI Reports Numbers #2 - #4 for DNA analysis details), indicated no genetic variability at the DNA level. Therefore, investigators concluded that there is a single species of brown tide, *Aureococcus anophagefferens* in New York and Barnegat Bay New Jersey. When ribosomal DNA sequences (ITS) were analyzed, this organism demonstrated some degree of genetic diversity at the individual organism level rather than at the population level. Multiple, but different, copies of these regions were found but not all strains had the same different sequence. DNA sequencing of the different strains of *A. anophagefferens* will continue beyond the scope of this project. This team compared two gene sequences (*rbcL* & RUBISCO) between *Aureococcus* and the Texas brown tide organism *Aureoumbra lagunensis* and found these two species to be significantly distinct.

The DNA sequence data also allowed an analysis of *Aureococcus anophagefferens* phylogeny. It was previously thought that *Aureococcus anophagefferens* was in the class Chrysophyceae, however, this analysis confirms that *Aureococcus anophagefferens* belongs to the class Pelagophyceae. A phylogenic comparison was also made between *Aureococcus anophagefferens* and the Texas brown tide organism *Aureoumbra lagunensis*. It was determined that *Aureococcus anophagefferens* belongs to the order Pelagomonadales, primarily a group of open ocean organisms, and *Aureoumbra lagunensis* belongs in the order Sarcinochrysidales, a group of predominantly coastal organisms.

These results suggest that *Aureococcus anophagefferens* either invaded the bays from the open ocean or that its ancestors were open ocean organisms.

While examining an *A. anophagefferens* strain from Long Island, a distinct cell wall was observed. *A. anophagefferens* cell wall appears to have a reticulated structure. However, the reticulated nature may be an artifact caused by sample preparation for electron microscopy. If the cell wall is not reticulated, it may act to protect brown tide cells in the sediments during non-bloom periods, however, a multi-stage life history has not been reported for *A. anophagefferens*.

### Research Project Brief: Ecology

**Glibert & Kana:** Mechanisms for Nutrient and Energy Acquisition in Low Light: Successful Strategies of *Aureococcus anophagefferens*.

In order to investigate if *A. anophagefferens* possesses certain physiological characteristics allowing it to outcompete other phytoplankton, this team examined *A. anophagefferens*’s ability to assimilate nitrogen and to photosynthesize in the turbid Long Island bays relative to co-occurring phytoplankton. Confirming results of several other BTRI projects, this team reported difficulty growing and maintaining *A. anophagefferens* cultures in the laboratory. Surmounting this problem, these investigators developed new technology employing a computer controlled culture system, called a...
turbidostat, to maintain *A. anophagefferens* at an exponential growth phase.

Turbidostat cultures used to examine the effect of organic carbon on *A. anophagefferens* confirmed that *Aureococcus* is capable of utilizing organic carbon influencing principally the nighttime metabolism.

Evaluating *A. anophagefferens*’s nitrogen nutritional regime unusual results were discovered. The activity of urease, the enzyme usually used to metabolize the organic nitrogen compound urea, is also high in cultures grown under inorganic nitrogen (nitrate) conditions. The activity of this enzyme was a function of *A. anophagefferens* growth rate regardless of the nitrogen source. This suggests that the enzymatic activity of urease can supply both carbon and nitrogen from urea. The team also found that *A. anophagefferens* does not grow when given ammonia as a nitrogen source (see also Andersen page 3).

Under low light conditions, such as in the southern bays of Long Island, when photosynthesis can no longer supply an adequate amount of carbon, *A. anophagefferens* can utilize organic carbon, such as urea, to supplement growth. Additionally, unlike other algae, *Aureococcus* apparently does not regulate photosynthesis over a wide light range. Therefore, its photosynthetic apparatus can be damaged under high light conditions suggesting that low light or turbid waters are needed for *Aureococcus* to establish itself.

*Aureococcus* thus displays characteristics of a mixotroph. It can obtain its needed carbon autotrophically through photosynthesis or, under light limiting conditions, heterotrophically by metabolizing organic carbon sources directly. Thus, *A. anophagefferens* possesses physiological mechanisms giving it a competitive edge in turbid bays such as those on Long Island. Building on methodology and findings from this project, Kana and his team will continue their investigations in BTRI 1999-2001 (see page 9).

Dr. Glibert reviewed historic plankton pigment samples from several Maryland bays and discovered that brown tide has been in these Maryland Bays since 1993 (detection of presence or absence only). This new finding may have implications for the scallop restoration program currently underway in this area and suggests that *Aureococcus* may be present in yet unidentified bays.

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**Research Project Briefs: Bloom Triggers**

**Sañudo-Wilhelmy, Hutchins & Donat:**

Biogeochemical and Anthropogenic Factors that Control Brown Tide Blooms: The Effects of Metals and Organic Nutrients in Long Island’s Embayments.

Collecting field measurements of organic, inorganic nutrients and bioactive trace metals, this team set out to investigate the relative importance of natural versus anthropogenic processes in bloom dynamics in Long Island’s embayments. They analyzed over 25 parameters in more than 200 samples of surface water, groundwa-ter and interstitial water. They also carried out more than two hundred field experiments using water from several Long Island embayments including collecting the first dissolved trace metal data for the Peconics system. Field manipulation experiments as well as field sample collections were performed under bloom, pre- and post-bloom conditions.

Continued on page 6
These investigators found that brown tide blooms appear to be caused by a succession of events and that the nutrient requirements of the brown tide change according to what is abundant in the environment. For example, brown tide blooms appear to be preceded by a non-Aureococcus spring phytoplankton bloom caused by high flow of inorganic nutrients such as nitrate in groundwater. The timing of this spring bloom appears to be related to the timing of the maximum spring groundwater seepage rate. During the non-brown tide spring phytoplankton bloom, Aureococcus abundance is insignificant. After a few weeks, the spring groundwater input is reduced, light penetration within the water column decreases as the spring bloom density increases, and then the non-brown tide phytoplankton bloom subsides. Because Aureococcus is adapted to grow under low light conditions, brown tide start to proliferate while other algal species are shaded out as water column light penetration decreases. Bacterial decomposition of the initial non-Aureococcus spring bloom also releases organic nutrients from decaying material into the water column. The reduced groundwater seepage combined with an organically enriched environment provides the ideal niche for the brown tide bloom, as it utilizes organic nutrients. Other species which rely on inorganic nutrients and higher light levels for photosynthesis cannot compete in this environment. See Figure 2 on page 6 for a conceptual diagram.

**Boyer & La Roche**: *Ferredoxin and Flavodoxin as a Metabolic Marker for Iron Stress in Aureococcus anophagefferens*

In order to investigate the role of the trace metal iron in brown tide blooms, this team examined six different potential biomarkers for iron limitation, five more than originally proposed. Using carefully defined artificial seawater and trace metal clean conditions, this team determined a minimum iron quota of 10 nM to support A.
A. anophagefferens growth in culture. A. anophagefferens is efficient in taking up its needed trace amounts of iron from the environment. Therefore, it is unlikely that iron levels will limit growth in bloom populations or play a role in bloom initiation.

The primary focus of this project has been evaluating the use of the flavodoxin to ferredoxin ratio as an indicator of iron-stress. The basis for this iron-stress marker is that under iron-limited conditions, the iron-containing protein ferredoxin is replaced with the non-iron containing protein flavodoxin. After this project was initiated, the unexpectedly low iron requirement of 10 nM was determined making iron-limiting experimentation difficult. Since protein isolation proved problematic, this team used antibodies derived from spinach for ferredoxin and another marine algae, Phaeodactylum, for flavodoxin antibodies for protein purification. The results showed that A. anophagefferens replaces ferredoxin with flavodoxin under iron limiting conditions in laboratory tests validating the principal of this assay, however, this assay could not be developed into a suitable test to measure iron limitation in the field.

Aureococcus anophagefferens showed a shift to a lower photosynthetic capacity under iron-stressed or limiting laboratory conditions (see BTRI Report #4). Utilizing a ratio (variable to maximal chlorophyll fluorescence) that indicates the change in photosynthetic efficiency, results from six different field populations of A. anophagefferens showed that these organisms were not iron-limited in the field. To date, this ratio is the best indicator to assess potential iron limitation for A. anophagefferens. Since A. anophagefferens cells undergo nutrient stress, chlorophyll measurements do not make a good biomarker for iron limitation.

Since freshwater and marine bacteria (up to 60%) produce siderophores to aid in iron uptake, presence of siderophores was examined as another way to measure iron stress. Results from several experiments showed no evidence of siderophore production by A. anophagefferens. There is evidence, however, that suggests that A. anophagefferens might be able to reduce iron from bacterial siderophores. Ferric chelate reductase (FCR) is an enzyme capable of reducing iron, releasing it from potential metal chelators, potentially playing an important role in the iron dynamics of A. anophagefferens. Results suggest that field populations contain about 10,000 times the minimum FCR activity needed to support maximum A. anophagefferens growth. Little is known about the iron pool available to A. anophagefferens, and most organic iron complexes can be reduced by FCR. This research indicates the FCR activity could be developed into a metabolic marker for iron limitation.

This team also developed techniques for large-scale culture of A. anophagefferens in defined seawater and provided brown tide to other researchers.
**Smayda:** Analysis of Physical, Chemical and Biological Conditions Associated with the Narragansett Bay Brown Tide.

The primary objective of this research was to quantify the role of physical, chemical and biological factors in the initiation, maintenance and demise of *A. anophagefferens* in the 1985 Narragansett Bay bloom event using an extensive two-year (1985-87) data set. A thorough statistical analysis considering these and several other parameters was completed and compared to a 32-year data time series. Similar to the Long Island bloom events, the Narragansett Bay bloom was supported in part by dissolved organic nitrogen (DON) from various sources (river inflow, groundwater and sewage treatment discharge). However, the analysis suggest that DON (or the ratio of dissolved organic to inorganic nitrogen) was not the controlling mechanism. Neither reduced bay flushing nor nutrient levels could account for the 1985 Narragansett Bay bloom. When compared to the 32-year time series, 1985 along with 1965-66, stand out as unique years in that they were drought years with high irradiance and low river flow. Dr. Smayda speculates that reduced rain and flushing may have altered the water quality of Narragansett Bay setting the stage for a bloom, however they did not directly cause the bloom.

This team concluded that the *A. anophagefferens* bloom dynamics in Narragansett Bay during 1985 was promoted by a progressive and cascading failure of grazing processes at several trophic levels. Pelagic grazing pressure was reduced by failed populations of the dominant copepod and high numbers of ctenophores (comb jellyfish), bay anchovy and other larvae in the water column. Combined with the loss of benthic filter feeding due to the bay-wide mortality of the blue mussel, these conditions severely limited grazing pressure on *A. anophagefferens*, and regulated the 1985 bloom event. These multiple failures did not occur in subsequent years.

The simultaneous brown tide bloom of 1985 in Narragansett Bay, RI, and the Peconic Bay of Long Island, and possibly in Barnegat Bay, New Jersey but not in Buzzards Bay or Delaware Bay, suggests some regional scale regulation. Similar climatological events between Narragansett Bay and the Long Island embayments, such as the North Atlantic Oscillation Index (a groundwater index) as suggested by La Roche et al., wind direction and strength, rain fall, cloudiness, and temperature suggests a regional synchronicity for the 1985 brown tide blooms. Conversely, brown tide did not bloom in Buzzards Bay and Delaware Bay despite regional contiguity. This supports the notion that local scale habitat regulation can be decisive in regulating subsequent *Aureococcus* bloom dynamics regionally following the climatological triggering of its initial bloom event in 1985.
**Sieracki:** The Effects of Microbial Food Web Dynamics on the Initiation of Brown Tide Blooms.

Expanding on work from the Keller and Sieracki BTRI 1996-1999 project, this investigator plans to examine the growth and grazing of *Aureococcus* within the context of the microbial plankton community. This area has been identified as an important research topic by several investigators. The hypothesis is that a picoalgae niche is typically occupied by an algae called *Synechococcus* and that *Synechococcus* must be selectively removed or reduced to open the niche to *A. anophagefferens*. This team will also focus on the picoplankton community including phototrophic and heterotrophic components such as bacteria and protozoan grazers.

**Kana, MacIntyre, Cornwell & Lomas:** Benthic-Pelagic Coupling and Long Island Brown Tide.

To gain insight into the regional differences in the occurrence of brown tide across the Long Island bays, the group will examine several hypotheses regarding the control of brown tide by nutrients and the coupling between the water column and the bottom (benthic-pelagic coupling). The central focus of this project is on the role of sediment and benthos as mediators of nutrient exchange with the water column. A coupled benthic-pelagic model is used as a framework for studying the role of sediments in brown tide dynamics. Field sampling will include south shore bays, West Neck Bay and Great Peconic Bay. Physiological experiments utilizing technology developed in Glibert’s BTRI 1996-1999 project, the turbidostat, will allow for accurate bioenergetic measurements of *A. anophagefferens* growth and photosynthesis under diverse organic nutrient conditions.

**Lonsdale, Caron & Cerrato:** Causes and Prevention of Long Island Brown Tide.

This project continues efforts utilizing mesocosms to study and understand the factors leading to brown tide outbreaks and possible bloom prevention or mitigation. This team will examine several topics including changes in the plankton community structure that take place as *A. anophagefferens* increases in relative and absolute abundance within a natural plankton assemblage. They will also examine the effects that perturbation to the pelagic food web have on success or failure of brown tide. Investigations will continue exploring how suspension-feeding bivalves affect planktonic food web structure, and how their activities affect the absolute and relative abundance of *A. anophagefferens*. This investigation will consider the effects of the chemical form of growth-limiting nutrients and the rate of nutrient loading as factors affecting brown tide initiation and bloom magnitude.
KEY TERMS

A compiled list of Key Terms can be found on the New York Sea Grant web page at www.seagrant.sunysb.edu

autotrophic
Organisms such as plants and some bacteria which produce their own food from inorganic substances such as carbon dioxide and inorganic nitrogen (i.e., photosynthesis).

benthic
Pertaining to the floor or deepest part of the sea or ocean; also includes the bottom-dwelling forms of marine life that live there.

bioenergetic
The biology of energy transformations and energy exchanges within and between living things and their environments.

biomarkers
A change in cell content that can be used as an indicator of the cell or its physiological state.

chrysophyceae
Unicellular golden-brown algae that inhabit fresh and salt water environments.

copepod
A small crustacean with both freshwater and marine forms. Important as a food source for higher trophic organisms such as fish.

electron microscopy
The technique used to produce an enlarged image of a tiny object that utilizes an electron microscope, an instrument that uses a beam of electrons focused by an electron lens. This type of microscopy is necessary when items or features are too small to be imaged by light. In this case, the image is created by the bending/reflection of an electron beam rather than a light beam.

eukaryotic
A cell with a distinct membrane-bound nucleus.

exponential growth phase
Or logarithmic growth phase is the period of growth during which the population grows at an exponential rate.

groundwater
All subsurface water, especially that part in the zone of saturation

heterotrophic
Organisms that obtains nourishment from the ingestion and breakdown of organic matter such as plants and animals.

interstitial water
Subsurface water contained in pore spaces between the grains of rock and sediments.

mesocosm
Experimental apparatus or enclosure in which environmental factors can be manipulated.

microplankton
Small, single-celled planktonic organisms in a size range 20 - 200 mm.

mixotrophic
Obtaining nutrition by combining autotrophic and heterotrophic mechanisms.
**BIBLIOGRAPHY**

Published papers from BTRI projects, to date:


This glossary was compiled with input from an assortment of sources:

*Webster’s New Collegiate Dictionary* (1981), Henry B. Woolf, Editor-in-Chief
Various BTRI investigators and Steering Committee Members

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**nanoplankton**
Small, single-celled planktonic organisms in a size range 2.0 - 20 mm. Can be animals (nanozooplankton) or plants (nanophytoplankton).

**niche**
A unique ecological role of an organism in a community.

**pelagic**
Open water that is above the bottom and below the surface.

**pelagophyceae**
A class of alga that includes *Aureococcus*, *Aureoumbra* and related species.

**phylogeny**
The history of the development of a species of related organisms.

**picoalgae**
Very small, single-celled planktonic algae in a size range 0.2 - 2.0 mm.

**reduce**
The process by which electrons are added to a substance to reduce it (e.g., the conversion of a metallic oxide or sulfide to the free metal).

**reticulated**
Having or resembling a network of fiber or lines.
The Brown Tide Research Initiative (BTRI) is funded by the National Oceanic and Atmospheric Administration's Coastal Ocean Program and administered by New York Sea Grant. The first (1996-1999) three-year $1.5 million BTRI program was developed to increase knowledge concerning brown tide by identifying the factors and understanding the processes that stimulate and sustain brown tide blooms. Continued funding for BTRI (1999-2001), as a $1.5 million three-year effort, comes once again from NOAA's COP. The COP, National Sea Grant Office, National Science Foundation, Environmental Protection agency, office of Naval Research, and National Aeronautics and Space Administration are jointly sponsoring research on Harmful Algal Blooms (HAB) ecology and oceanography in the interagency research program, Ecology and Oceanography of Harmful Algal Blooms (ECOHAB).

BTRI projects are composed of eight and three research projects respectively that were selected from national calls for proposals. To involve concerned parties and aid in decision-making, New York Sea Grant formed the BTRI Steering Committee of invited state, local and government agency representatives, and citizen's groups. The research projects chosen for BTRI funding were selected following peer review and evaluation by a technical review panel with local input from the BTRI Steering Committee and ECOHAB.

This Report Series will aid in the dissemination of general brown tide information. The results and conclusions of the projects will help determine the directions of potential management and future research.

If you have questions about brown tide, would like a copy of Report #1, 2, 3, 4 or 5, or would like to be added to our mailing list, please contact Patrick Dooley at New York Sea Grant (pdooley@notes.cc.sunysb.edu or 631-632-9123). You may also read these reports by visiting our website: <<http://www.seagrant.sunysb.edu>>. This publication may be made available in an alternative format and is printed on recycled paper.