Sea Grant New York

Brown Tide Research Initiative

Report #3 March 1999

1998 BROWN TIDE

During 1998, the Peconic Bays of Long Island, New York, remained relatively free from brown tide for the third consecutive year and neither Rhode Island nor New Jersey reported any bloom activity. Brown tide bloomed, however, in isolated embayments of Long Island's east end and south shore. A short, but relatively intense bloom occurred in West Neck Bay on Shelter Island that started in June and ended by late July. This bloom peaked at approximately 600,000 cells/per milliliter (see page 6). A slightly longer but less intense bloom appeared in Great South Bay. This bloom started in April and ended in July peaking at only about 260,000 cells/per milliliter. Since 1985 when brown tide first appeared, 1989 and 1996 have been the only years in which the south shore bays and the Peconic Bays have been free from significant bloom events. (See *BTRI Report Number 1* for a timeline of bloom activity.)

Photo by Barbara Branca

West Neck Bay, June 1998.

Peconic scallop populations have not recovered to pre-1985 abundances. Since then, only three years —l992, 1994 and 1995— have had notable levels of harvest. The quantities yielded during these years were only about ten percent of the yearly harvest prior to the brown tide. Even though Peconic Bay has been relatively free of the brown tide for the past three years, the bay scallop population has not recovered despite what has appeared to be a strong natural set. After a negligible harvest in 1996, 1997 showed a significant increase in the scallop population, though well below 1992 and 1994-5 levels. The harvest for 1998, however, has declined even further to only 20 percent of l997 levels.

Building on *BTRI Reports Numbers 1 & 2*, this report presents progress made after the second of three BTRI field seasons and announces several related new initiatives sponsored by New York Sea Grant. *Report Number 3* follows the same format as the first two BTRI reports allowing for easy project tracking. Boldfaced terms are defined under *Key Terms* adding to those in BTRI *Reports Numbers 1 & 2*.

Brown Tide Research Initiative
Report #3

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Sea Grant
New York

New York Sea Grant is part of a national network of universities meeting the challenging environmental and economic needs of the coastal ocean and Great Lakes regions. Unique among the 29 Sea Grant programs nationwide because it has both marine and Great Lakes shorelines, New York Sea Grant engages in research, education, and technology transfer to promote the understanding, sustainable development, utilization, and conservation of our diverse coastal resources. NYSG facilitates the transfer of research-based information to a great variety of coastal user groups which include businesses, federal, state and local government decision-makers and managers, the media, and the interested public.

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NEW BTRI FUNDING

Funds for a "Phase 2" of the Brown Tide Research Initiative will soon be available for a suite of new research projects. Mainly through the foresight and continued efforts of NY Congressman Michael Forbes, substantial federal funding for brown tide work has again been secured through NOAA's Coastal Ocean Program to carry BTRI through the year 2002. The success of this program to date demonstrates the value of this larger-scale and coordinated approach. Each year, the Symposium, Reports and investigator interactions are becoming more and more exciting as we try to fit all the new pieces of information together.

A Call for Proposals addressing Long Island Brown Tides is being issued as part of the broader ECOHAB (Ecology and Oceanography of Harmful Algal Blooms) program, supported by NOAA, EPA, NSF, NASA and the Office of Naval Research. Local input is being maintained through the continued involvement of the BTRI Steering Committee and administration of the program by New York Sea Grant. Potential researchers may find the Call on the World Wide Web at <<http://habserv1.whoi.edu/hab/announcements/ECOHAB99.html>>. The goal of BTRI continues to be to understand and predict the onset of brown tide blooms and advance strategies for mitigating its environmental impacts. The COP expects to commit approximately $1.5 million over the next three years to support the activities of the Brown Tide Research Initiative.

Pat Glibert inspecting *Aureococcus* cultures. *Photo by Todd Kana*

Research Project Briefs: Ecology

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

Using nine culture isolates of *A. anophagefferens* from the CCMP (see Andersen page 6), this team has developed methods for monitoring and maintaining growth and survival of *A. anophagefferens*. Rapid *A. anophagefferens* growth has been difficult to maintain in culture and the focus of efforts thus far has been in establishing the basic growth and culture conditions. Bubbling air through the cultures significantly improves culture success. Employing these “in-house” culture techniques, this team compared culture growth of four isolates at different salinities and temperatures. The results showed growth differences among the various conditions tested.

To select the least stressed culture isolates for subsequent growth experiments, this team measured “**variable chlorophyll fluorescence**” in *A. anophagefferens.* Four isolates were chosen and growth rates measured at various temperatures and salinities. Growth rates for the four isolates at 16°C were approximately the same. However, they were about 50 percent less than at 20°C. At 20°C the four different culture isolates showed different growth rates. Differences in culture growth rates measured in this study are consistent with growth rate differences seen in Andersen’s project. Salinity also affected *A. anophagefferens*’ growth rate. Generally speaking, the highest growth rates measured under these experimental conditions were seen in the warmer and saltier waters.

Insert: Close-up of the culture chamber for on-line experiments with the membrane inlet mass spectrometer.

Photos by Todd Kana

Pat Glibert recording data from the membrane inlet mass spectrometer.

In May 1998, tributaries of the Chesapeake Bay were the sites of blooms of *Prorocentrum minimum,* an alga unrelated to *A. anophagefferens* . This bloom appeared to follow a period of wet weather with a large amount of runoff. Bay eelgrasses and shellfish populations were negatively impacted in a similar fashion to brown tide’s effects on the Peconic Estuary in 1985 and subsequent years. Since *P. minimum’s* effects were similar to *A. anophagefferens’*, some of the physiological growth characteristics of *P. minimum* are being analyzed for comparison to brown tide. Comparison between these two species may provide valuable insights in understanding brown tide.

Key Terms

Look for the definitions of key terms in **boldface** on page 13.

Research Project Briefs: Ecology

Caron & Lonsdale Microzooplankton-Mesozooplankton Coupling and Its Role in the Initiation of Blooms of *Aureococcus anophagefferens* (Brown Tides).

Most of the 1997 zooplankton community samples have been processed. Initial evaluation of the larger mesozooplankton (>200 mm) and smaller microzooplankton (between 20-200 mm) population data do not show substantial differences in the population numbers or species type between the two treatments that maintained brown tide growth (sediment and sediment with hard clams), and the control treatment (only seawater with *A. anophagefferens*). The small nanoplankton samples (between 2.0-20 mm), however, are still being processed. Nanoplankton grazing dynamics may play an important role in bloom development. Accordingly, assessing the relationship between brown tide and planktonic grazing will continue.

Photo by Barbara Branca

Graduate student Becky Schaffner monitoring a mesocosm experiment on Coecles Harbor, Shelter Island, during the 1998 field season.

This team completed two mesocosm experiments during the 1998 field season. Although samples are still being processed, they found that when various numbers of hard clams were present, algal population numbers and *A. anophagefferens* growth rates were significantly reduced. For the experimental conditions, it seems that the presence of high numbers of filter feeding bivalves may act to limit brown tide bloom formation.

In mesocosms with pumps, brown tide became the dominant phytoplankton. The pumps created an artificial situation by possibly eliminating grazing on *A. anophagefferens,* thus allowing brown tide to bloom. The ability to create an *A. anophagefferens* bloom in mesocosms developed through this study will greatly enhance the next round of experiments in the 1999 field season.

Hard clam (*Mercenaria mercenaria*) **feeding rates** were measured in another set of experiments designed to assess toxic effects of *A. anophagefferens*. When brown tide concentrations reached 35,000 cells per milliliter, the clams' clearance rates were significantly reduced. At concentrations greater than 400,000 cells per milliliter, the clams stopped filtering. These results are consistent with Bricelj's findings (see *BTRI Report Number 2*) that brown tide can inhibit mussel and hard clam feeding rates.

Research Project Briefs: Culturing

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

Andersen and co-workers have made steady progress in establishing different culture **strains** (or **isolates**) of *A. anophagefferens* and investigating genetic variability among these strains. Eight new *A. anophagefferens* strains have been established from samples collected in Great South Bay, Long Island. These strains are available for study and have been deposited at the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP). The identification of these strains as *A. anophagefferens* has been confirmed by DNA sequence comparisons.

Establishing bacteria-free (axenic) cultures of *A. anophagefferens* has proven difficult. After an extended period of brown tide growth, bacteria have arisen in cultures that initially appeared bacteria-free. Continued efforts to establish axenic strains are now underway. These efforts include the use of different antibiotics as well as new combinations of antibiotics. A problem is that *A. anophagefferens* is itself sensitive to antibiotics, and concentrations routinely used to establish axenic cultures of other marine phytoplankton appear to inhibit the growth of *A. anophagefferens*.

Growth differences have been noted among cultured isolates of *A. anophagefferens*. Cultures that grow best are often characterized by increased amounts of bacteria in the medium. This seems to suggest that bacteria may perhaps play a fundamental role in maintaining brown tide blooms, but this hypothesis has yet to be rigorously tested. Growth of some strains is promoted by the addition of **peptone** and **VA vitamins** to the medium. However, the amounts of these organic compounds must be kept low because they also promote bacterial growth. *A. anophagefferens* cultures initiated with a large innoculum of cells that subsequently are maintained in relatively high light conditions also generally exhibit higher rates of growth.

To examine genetic variability within *A. anophagefferens*, nucleotide sequences for three different DNA regions were determined. These include the nuclear-encoded 18S rRNA gene and the *rbc*L gene and RUBISCO spacer region that are found in the chloroplast genome. No genetic differences among strains of *A. anophagefferens* have been found in these regions. These data imply that blooms of *A. anophagefferens* are comprised of cells that are very similar. It is possible that variation within the species may be revealed using other techniques or by examining DNA regions that are more variable. Two new finer-scale methods, heteroduplex DNA analysis (HAD) and denaturing gradient **gel electrophoresis** (DGGE), are now being tested in an effort to find genetic differences among *A. anophagefferens* isolates.

These data imply that blooms of *A. anophagefferens* are comprised of cells that are very similar.

The *rbc*L and RUBISCO spacer sequences were also determined for a second brown tide alga, *Aureoumbra lagunensis*, isolated from Laguna Madre, Texas. These data were compared to that for *A. anophagefferens*. Differences between the *A. anophagefferens* and *A. lagunensis* sequences support the conclusion of morphological studies that these organisms are separate species. The nucleotide sequence data place *A. anophagefferens* and *A. lagunensis* within the class **Pelagophyceae**. This implies that they are not necessarily closely related.

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.

This team of investigators conducted a 21-station water column sampling cruise across Great South Bay in September 1998. The goal was to continue their assessment of the role of metals and organic nutrients in controlling brown tide blooms in Long Island's embayments. Flanders Bay did not experience a bloom this past year and will, therefore, be used as a control site for comparison to Great South Bay and West Neck Bay. Groundwater samples were collected from seven wells around Flanders Bay and from ten new wells surrounding West Neck Bay. (See figure below.)These samples are currently being processed.

A new survey has been started with the help of Suffolk County Department of Health Services (SCDHS) Division of Water Resources and the United States Geological Survey. Groundwater monitoring has been established around West Neck Bay for the purpose of comparing the chemical composition of local groundwater to that of bay waters. To understand how chemical composition of groundwater may alter as it seeps through sediments into coastal embayments, this project now includes sampling of **intertidal** wells and **seepage chambers** in West Neck Bay. (See Assessing Groundwater diagram, page 9).

Continuing their successful collaboration with the Caron and Lonsdale project (see page 5), members of this team sampled the mesocosms for an array of chemical factors. This collaboration will continue in the 1999 field season. New field research investigating nutrient limitation of *A. anophagefferens* at West Neck Bay shows that nitrogen (either urea or nitrate) and organic carbon additions can stimulate *A. anophagefferens* growth rates during bloom events.

Hutchins is investigating the effects of light and organic carbon on brown tide growth. Preliminary laboratory results indicate that for short periods of time, organic carbon can stimulate the growth of brown tide. These results are consistent with other field observations and, with them, suggest that *A. anophagefferens* may have a specific requirement for materials produced by other plankton.

Development of Brown Tide in West Neck Bay.

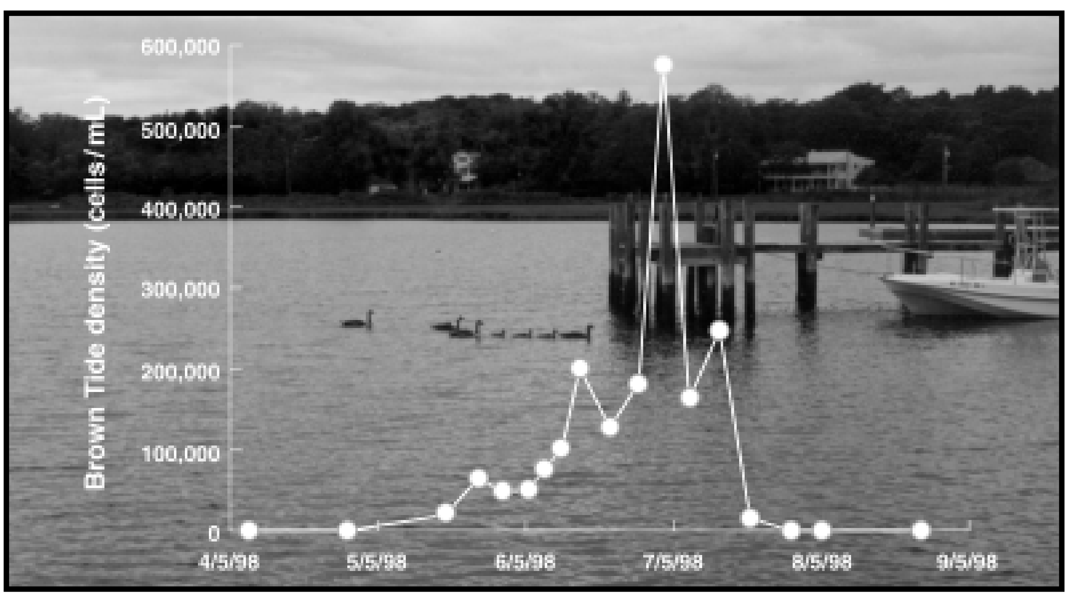


Photo by Barbara Branca

*Data from SCDHS and Gobler

Research Project Briefs: Bloom Triggers

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

The trace metal iron has been hypothesized to be a limiting factor that may influence brown tide bloom events. However, the role of iron in brown tide growth remains unclear. In order to establish iron's influence in brown tides, a method must first be developed to measure "iron stress" in *A. anophagefferens*. This team of investigators has been working on four possible markers for brown tide.

There are a number of different ways to measure chlorophyll, the photosynthetic pigment used to represent population abundance in phytoplankton. A number of these could potentially be used in the field to determine iron stress in *A. anophagefferens*. Promising results were obtained using a ratio that indicates the change in photosynthetic efficiency. Using this ratio with laboratory cultures has shown that *A. anophagefferens'* photosynthetic capacity diminishes as iron levels decrease. This occurred even though the cells' growth rates did not change. Work will continue to determine if this indicator can be applied to field populations to determine iron stress.

In higher plants and other green algae, the enzyme ferric chelate reductase (FCR) has been reported to be produced when the plant is iron-limited. Thus, the production or activity of FCR may be useful as a marker for iron stress. Laboratory results with cultured *A. anophagefferens* grown under iron-limited conditions did not show an increase in FCR activity. Field samples containing *A. anophagefferens* and other marine phytoplankton, however, showed a high level of FCR activity. These results imply that FCR activity is not very useful as an iron-stress marker in *A. anophagefferens*.

Under iron-limited conditions, freshwater and marine bac.teria produce high affinity iron chelators called siderophores that aid in iron uptake. Results from several experiments with iron-stressed *A. anophagefferens* showed no evidence of siderophore production. If siderophores are produced in *A. anophagefferens,* they are likely below the detection limits of the methods used to measure them. Accordingly, they are not useful as markers for iron stress.

As reported in *BTRI Reports Numbers 1 & 2*, a primary focus of this project has been evaluating the use of the ratio of flavodoxin to ferredoxin as an indicator of iron stress. Under iron-limited conditions, the iron containing protein ferredoxin is replaced with the non-iron containing protein flavodoxin formulating the basis for this marker. Brown tide cultures grown under iron-rich conditions have produced ferredoxin. Under iron-limited conditions, *A. anophagefferens* has produced a "flavodoxin-like" protein. Neither protein, however, has been sufficiently purified for use as standards in detection experiments. This team continues its work to purify these two proteins so they can be used as markers.

Experiments also continue with nitrate reductase, the key enzyme for phytoplankton use of dissolved inorganic nitrogen. *A. anophagefferens* shows very typical nitrate reductase activity. Culture experiments have shown very similar growth rates on DIN (nitrate) and DON (urea). Field samples, however, show low nitrate reductase activity. This suggests that *A. anophagefferens* may not use nitrate as its primary nitrogen supply in its natural habitat.

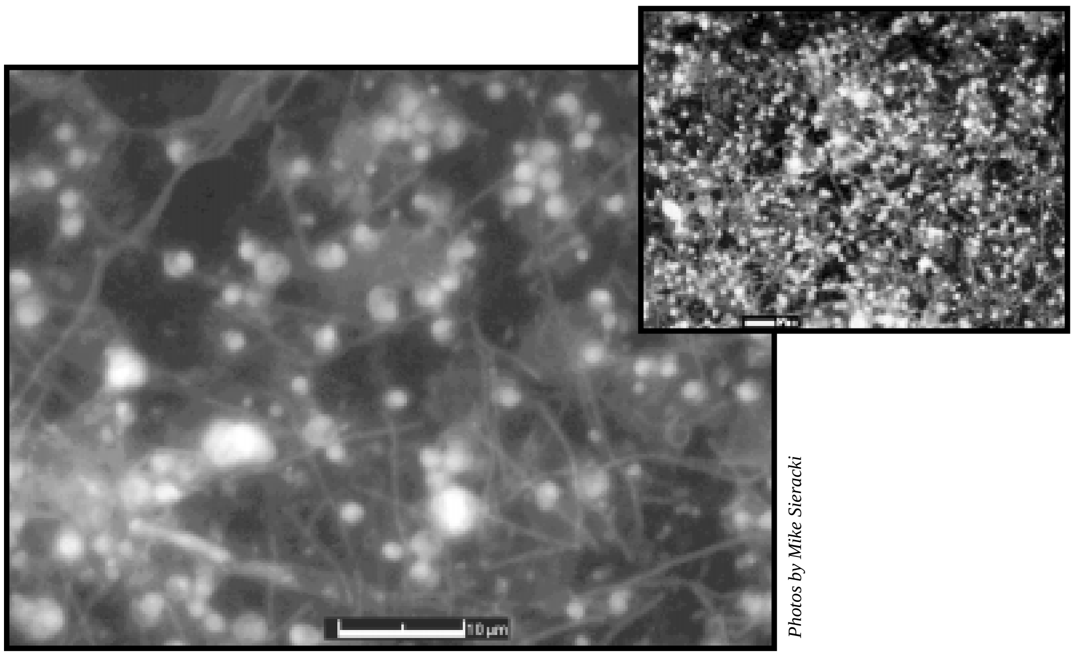
In order to determine iron's role in bloom formation, a method must first be established to assess if the growth of natural populations of *A. anophagefferens* is limited by iron. Boyer and his team will continue their efforts to develop a method to measure iron stress.

Aureococcus anophagefferens **shows very typical nitrate reductase activity.**

Culture experiments have shown very similar growth rates on DIN (nitrate) and DON (urea).

NEW INITIATIVES

Resulting from topics and questions discussed at the *Spring 1998 BTRI Informational Symposium* (see *BTRI Report Number 2*), New York Sea Grant will sponsor additional brown tide research. These efforts have been derived as offshoots or enhancements of current BTRI or other brown tide projects. The following five projects represent new initiatives whose results will help in understanding brown tide.



Photos by Mike Sieracki

These microscope images of an *Aureococcus* bloom in Great South Bay, May 1998, were prepared using fluorescent antibody methods. The *Aureococcus* are the smallest cells and the material between them is mucopolysaccharide (MPS) which is produced by *Aureococcus.* Of particular interest are the filamentous bacteria associated with the MPS which form a mesh that could affect filter-feeding organisms.

Keller & Sieracki: Measurement of Bacterial Biomass in the Brown Tide Study Area

Building on their current BTRI project (see Keller & Sieracki BTRI Report #2), this team of investigators plans to explore the relationship between *A. anophagefferens* and **heterotrophic** bacteria. *A. anophagefferens* and these heterotrophic bacteria efficiently utilize DON. Therefore, they compete for this nitrogen source. In theory, this could imply that under bloom conditions, *A. anophagefferens* must be able to out-compete these bacteria for DON. If this were true, bacterial growth and biomass would vary with bloom activity. Results from 1997 suggest that this may be true. This team will use a state-of-the-art image analysis system to measure heterotrophic bacterial abundance, biomass, size(s), and **morphology** from 1997 and 1998 samples. David Caron (see Caron and Lonsdale page 5) and Robert Nuzzi of the Suffolk County Department of Health Services, have agreed to supply additional samples for the bacterial analysis.

NEW INITIATIVES

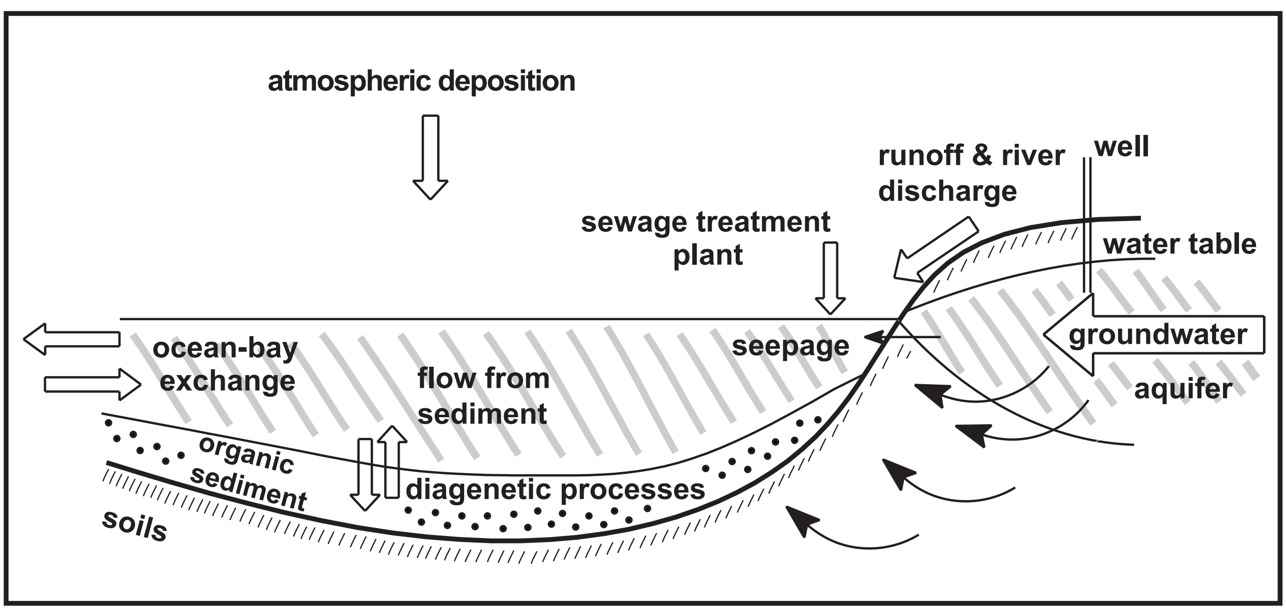
Sañudo-Wilhelmy: Sources of Nitrogen and Bioactive Trace Metals to the Great South Bay, Long Island: Effects on Brown Tide Blooms

Sañudo-Wilhelmy and his team will try to establish the relative importance of natural processes versus anthropogenic inputs on the water quality of Great South Bay. The levels and types of organic nutrients (organic nitrogen and urea), inorganic nutrients (nitrate) and trace metals (iron, nickel and zinc) have not been characterized for Great South Bay. This type of information is required to understand the major processes controlling the levels of several factors that may influence brown tide growth. These results will also help determine if results from the Peconics can be extrapolated to other systems, such as Great South Bay. Seasonal and temporal patterns and possible sources of these chemical constituents will be determined and correlated to field measurements of growth rates and cell densities. The results from this work are critical for understanding when, where and under what conditions brown tide blooms will be predicted to occur.

Sañudo-Wilhelmy: Impact of Interstitial and Groundwater on the Chemical Composition of Surface Waters of Long Island's Embayments

Sañudo-Wilhelmy will also be directing a New York Sea Grant Scholar graduate student who will supplement his current BTRI project (see Sañudo-Wilhelmy page 4 for an overview of these activities). The scope of this effort will be expanded to include more water quality sampling of groundwater. This student will investigate the chemical and physical changes occurring in sediments that influence the chemical quality of groundwater seepage in the study area.

Assessing Groundwater



This schematic shows how measuring the quality of groundwater at wells only shows part of the picture of water quality in coastal embayments. As groundwater flows through the sediment and out into embayments, it is influenced by diagenetic processes. Diagenetic processes are transformations of materials that occur within the sediments after deposition. Bacterial decomposition of organic matter or formation of an animal burrow are examples of such processes. Added to the mix are river discharge (runoff) and atmospheric deposition (either dry or in the form of precipitation).

NEW INITIATIVES

Giner: GCMS Detection of Sterol Biomarkers for *Aureococcus anophagefferens*

Dr. José-L. Giner, from the Department of Chemistry at the State University of New York College of Environmental Science and Forestry in Syracuse, NY is a new brown tide investigator. Giner is developing another type of **biomarker** using **sterols** to help identify brown tide in the field (see Boyer & La Roche page 7 for another type of a biomarker). Since *A. anophagefferens* is a very small alga with no particularly distinguishing features, a highly specialized form of microscopy involving **immunofluorescence** is used for its detection and quantification. Immunofluorescence microscopy, however, requires expensive instrumentation, antibodies and large amounts of technician time. Giner has found that *A. anophagefferens* is the first microalga to contain the rare sterols E- and Z- propylidenecholesterol. Furthermore, the relative proportions of the two sterols appears to be constant for *A. anophagefferens.* Since these sterols are rare, they can be used to identify and quantify brown tide. Sterols are identified in the laboratory using **Gas Chromatography-Mass Spectrometry (GCMS)**. This method is fast, relatively easy to do, requires little technician time and can be automated. Using GCMS, Giner will work towards developing an analytical protocol based on sterol biomarkers to specifically detect and quantify *A. anophagefferens* in seawater samples. Fostering the cooperation which is the hallmark of the BTRI network, investigator Boyer is supplying *A. anophagefferens* cell material for this study.

Bricelj: Cytotoxic Effects of Brown Tide

This new project builds on Bricelj's current New York Sea Grant funded project, "Relative Susceptibility of Bivalves to the Brown Tide Alga *Aureococcus anophagefferens*: Comparison Among Species and Life History Stages" which she presented at the Spring 1998 BTRI Symposium. She has developed a short-term **bioassay** using mussels that allows **toxicity** comparison among the various *A. anophagefferens* cultures. The bioassay has shown remarkable differences in the toxicity of three available isolates of *A. anophagefferens* from Long Island estuaries. The reason for culture toxicity differences may be due to culture age. Culture toxicity could change over time, or the initial toxicity in older cultures (e.g., from 1985) could be less. Bricelj's team will investigate the varying brown tide toxicity and explore the possible mechanism(s) of **cytotoxicity.** This work will utilize **histopathological** analysis of tissue samples from hard clams and mussels exposed to the various isolates.

Groundwater Hypothesis:
Does brown tide "go with the flow?"

The BTRI projects are investigating numerous hypotheses to explain the occurrence of brown tide. The hypothesis below demonstrates the complexity of the brown tide story and does not imply that this is the only hypothesis under investigation. An additional hypothesis, the "Groundwater Hypothesis" proposed by investigators from Brookhaven National Laboratory and the Suffolk County Department of Health Services (SCDHS), suggests a mechanism for the development of brown tide in the Peconic Estuary where brown tide is regulated by the relative availability of dissolved inorganic nitrogen which, in turn, is regulated by groundwater flow into the estuary. The relevance of this hypothesis to other estuaries affected by brown tide, such as Great South Bay, has not been determined. Some of the BTRI results mesh with this hypothesis, but others indicate that different factors are involved. The picture of what causes brown tide is still evolving.

Defining the terms

- Groundwater results from precipitation that passes through the surface of the ground to become the subsurface water supply. Groundwater within the Peconic Estuary watershed moves toward, and into the estuary, supplying it with fresh water.
- Dissolved Inorganic Nitrogen (DIN) is composed of small nitrogen molecules that do not contain carbon. Nitrate, often found in fertilizers, is an example of DIN. This is a form of nitrogen easily utilized by most phytoplankton.
- Dissolved Organic Nitrogen (DON) is a larger, carbon-containing molecule not easily used by most plants. It results from animal waste products such as urea and organic matter (animal and plant biomass) containing particulate nitrogen decomposed by bacteria.

Setting the stage

- Due to farming activities and, more recently, urbanization, the groundwater flowing into the Peconic Estuary is high in nitrate (DIN), providing one to two times more DIN to the estuary than any other source.
- Unlike most phytoplankton, *Aureococcus anophagefferens* can utilize DON as well as DIN. When DIN levels are limited, *A. anophagefferens* can outcompete the "typical" phytoplankton. Accordingly, when DIN levels are high, competing species which use DIN will outgrow *A. anophagefferens* (see Boyer & La Roche and Sañudo-Wilhelmy).
- Depending upon climatological factors, such as precipitation, the volume of high DIN-containing groundwater into the Peconic Estuary varies from year to year. Therefore, the supply of DIN to the estuary can be limited during years of low groundwater flow.
- The long term time-series (1950-1990) of annual average groundwater flow shows variability from year to year.
- Data collected by the SCDHS over an eleven-year period suggest an inverse relationship between groundwater flow and *A. anophagefferens* abundance. In other words, brown tide occurred during years of low groundwater flow and was mostly absent during years of high groundwater flow.

Understanding the hypothesis

The groundwater hypothesis suggests that brown tide blooms are controlled by the relative amounts of DIN and DON in the system, which are tied to the flow of groundwater. For example, 1985 was preceded by several years of high groundwater flow (presumably high DIN conditions), then two years of low groundwater flow. During the low groundwater flow period, measured salinities increased. The decrease in the amount of DIN, accompanied by increased DON, may have set the stage for brown tide to bloom. Additional work still needs to be completed to test this theory, likely requiring several more bloom events to collect data.

In addition to the groundwater hypothesis, there are other hypotheses that attempt to explain brown tides in different locations (e.g., Narragansett Bay and Great South Bay), the mechanisms controlling bloom formation (e.g., bay flushing and grazing pressure), and how and why *A. anophagefferens* first appeared in the bays of Long Island. In all likelihood, a combination of factors such as DON availability, poor bay flushing, changes in the grazing community and other chemical factors may all play a role with brown tide.

Brown Tide's "Family Tree"

Kingdom: ***Protista***

Phylum: ***Chrysophyta***

Class: ***Pelagophyceae***

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

Order: ***Pelagomonadales***

One of two taxonomic orders classified within the pelagophyceae. This order includes *A. anophagefferens* and is the name of a group of very small free-floating golden-brown algae.

Order: ***Sarcinochrysidales***

A second taxonomic order classified within the pelagophyceae. This order includes *Aureoumbra lagunensis*, the organism which causes brown tide in bays along the Texas Coast.

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KEY TERMS

bioassay a method for quantitatively determining the concentration of a substance by its effect on the survival, development, growth, behavior, or measurable physiological response of a suitable animal, plant, or microorganism under controlled conditions.

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

cytotoxicity the characteristic of being **toxic** to living cells.

DIN Dissolved Inorganic Nitrogen, (e.g., chemical fertilizer).

DON Dissolved Organic Nitrogen, (e.g., urea).

feeding rates rate at which a predator consumes its prey.

Gas Chromatography-Mass Spectrometry (GCMS) a technique used to separate, identify and quantify chemicals.

gel electrophoresis an electrochemical process in which charged molecules migrate in a gel under the influence of an electric current: typically used as studies of genetics.

heterotrophic characteristic of an organism that obtains nourishment from the ingestion and breakdown of organic matter such as plants and animals. This is unlike **autotrophic** organisms such as plants and some bacteria which produce their own food.

histopathology a branch of pathology that deals with tissue changes associated with disease or toxic effects.

hypothesis an idea or statement that must be tested before it can be stated as fact.

immunofluorescence microscopy a technique for identifying or counting organisms under a microscope using antibodies that glow under blue or other wavelengths of light.

intertidal the zone between high and low tide.

isolates single species of algae picked from a natural population and established in culture.

morphology the study of a form appearance and structure of an organism such as shape, size and color. The way the structure or form of an organism looks.

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

peptone an organic carbon source used to grow bacteria or other heterotrophic organisms.

seepage chambers a devise used to collect groundwater seeping through intertidal sediments.

sterols a type of lipid, such as cholesterol, present in the cell membranes of plants and animals.

strain a group of organisms of the same species of presumed common ancestry with clear-cut physiological but usually not morphological distinctions (i.e., a stock or line).

toxic the kind and amount of a poison or toxin produced by a microorganism or a chemical substance not of biological origin.

toxicity the state or effect of being toxic.

VA Vitamins a mixture of vitamins added to nutrient media for the growth of algae.

variable chlorophyll fluorescence a measurement of the energy from sunlight that is absorbed by the cells, and then released again as light.

SUMMARY

Since brown tide first appeared in Long Island waters in 1985, combined efforts of many agencies, organizations and investigators have made steady progress in understanding *Aureococcus anophagefferens* and what causes it to bloom. Although the specific mechanisms surrounding the bloom cycle remain hypothetical, some general factors which seem conducive for brown tide development in shallow estuaries have been identified.

Continuing work by Keller, Sieracki and other investigators indicates that *A. anophagefferens* seems to grow best at higher salinities and in warmer water but that it also can survive low over-winter temperatures. Progress made by Sañudo-Wilhelmy's group, Glibert's team and other researchers also demonstrate that, unlike other coastal marine algae, *A. anophagefferens* prefers organic nutrients over inorganic nutrients. It has also been shown that *A. anophagefferens* can maintain growth in low nutrient environments.

While specific bloom triggers are still unknown, factors have been identified which seem to influence blooms. Smayda and other researchers agree that reduced embayment flushing rates seem to help set the stage for bloom development. Boyer's team has shown a low iron requirement for *A. anophagefferens* and continue to investigate iron's role in bloom formation. Other work by researchers, including Lonsdale, investigating the dynamics between *A. anophagefferens* and other plankton has shown that some plankton avoid consuming *A. anophagefferens*. Bricelj's work suggests some degree of toxicity for *A. anophagefferens* in mussels and hard clams. However, it is possible that culture toxicity may vary. Yet, other work by members of Lonsdale's team as shown that hard clams can graze on brown tide in low densities. This grazing pressure can potentially control *A. anophagefferens* blooms. Clearly, additional research and time are still needed to fit all these brown tide pieces into the bloom puzzle.

Innovative BTRI research may explain a local bloom

The following hypothesis, specific for West Neck Bay, is yet another example of the growing number of hypotheses put forth to explain brown tide.

Sañudo-Wilhelmy has a possible explanation for 1998's bloom activity in that area and why it has been a brown tide "hot spot" in previous years. The wet spring of 1998 likely resulted in a large nitrate input into West Neck Bay. Groundwater analyses in that area showed enriched nitrate levels. The high nitrate fueled an early June bloom of phytoplankton other than *Aureococcus*, which, in turn, potentially supplied a large amount of organic nutrients (DOC) into the bay through decomposition. *A. anophagefferens* may have exploited the available DOC to obtain maximum densities in July (see figure on page 9). The narrow channel connecting West Neck Bay to the Peconics results in a relatively long water residence time of 15 days (relatively poor flushing). This allows phytoplankton to maximize uptake of nutrients and dissolved organic matter. This could account for brown tide, even during years of high rainfall.

Brown Tide Research Initiative

BTRI Informational Symposium

Saturday, April 10th 1999

From 8:30 am Registration to 3:00 pm

Westhampton Beach High School Cafetorium,

Westhampton, New York

Brown Tide Research Initiative

INSIDE

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 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. The program will help us better understand brown tide and advance strategies for minimizing its impact.

The BTRI is composed of eight research projects that were selected from a national call for proposals in 1996. 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 (see side bar page 2). The research projects chosen for BTRI funding were selected following peer review and evaluation by a technical review panel and the BTRI Steering Committee. Projects were submitted by investigators from along the east coast including: Maine, Massachusetts, Rhode Island, Connecticut, New York, Delaware, Maryland and Virginia.

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 any questions about brown tide, would like a copy of *Report #1 or #2,* or would like to be added to our mailing list, please contact Patrick Dooley at New York Sea Grant (patrick.dooley@sunysb.edu or 516 632-9123) . 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.

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