Sea Grant
New York

Brown Tide Research Initiative

Report #4 November 1999

1999 BROWN TIDE and SYMPOSIUM OVERVIEW

The third annual Brown Tide Research Initiative Informational Symposium, hosted by New York Sea Grant, was held on April 10, 1999 at Westhampton Beach High School in Westhampton, New York. The sixty researchers, guests and interested citizens who attended made this year's symposium a success. Presenters traveled from as far north as Maine and as far south as Virginia.

During the symposium, researchers summarized progress on the eight BTRI projects, other New York Sea Grant funded research related to brown tide, and an Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) investigation (see Andersen, page 3). Dr. Michael Reynolds, from Brookhaven National Laboratory, presented the BTRI network of investigators with a description of BNL's capabilities and the latest data in his talk entitled "Brown Tide Monitoring Buoys: Real Time Observations on the World Wide Web" (http://www.oasd.bnl.gov/peconic). As was true last year, an investigator workshop was held prior to the public symposium to provide the BTRI teams and other brown tide researchers a networking forum. They presented their results, discussed new ideas, mapped new research directions, and planned the final field season of their projects

At the symposium, a Newsday reporter (far right) discusses BTRI initiatives with (clockwise) NYSG's Assistant Director Cornelia Schlenk, Brown Tide Outreach Specialist Patrick Dooley and Susan Banahan, project manager from NOAA's Coastal Ocean Program and member of the BTRI steering committee.

BTRI investigators Drs. Patricia Glibert and David Hutchins reported scattered and relatively low brown tide numbers of *Aureococcus anophagefferens* in Barnegat Bay, NJ, and Delaware's Assawoman and Chincoteague Bays. Investigators from the BTRI network will assist in the investigation of these blooms. As of July 1999, the bays of Long Island remained relatively free from brown tide. A complete summary of 1999 brown tide bloom activity will be presented in the next BTRI report.

This report builds on BTRI Reports Numbers 1, 2 and 3, presents results from other brown tide research and sets the stage for the final field season of the BTRI Phase I research effort. Report #4 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.

Brown tide researchers (l. to r.) José L. Giner, Robert Andersen, David Caron, Maureen Keller and Darcy Lonsdale at the 1999 BTRI Informational Symposium.

Brown Tide Research Initiative
Report #4

Writer: Patrick Dooley

Editors: Barbara Branca
Cornelia Schlenk
Paul Focazio

Designers: Barbara Branca
Sharon O'Donovan

BTRI and Other Brown Tide Investigators

Bigelow Laboratory for Ocean Sciences, ME
Dr. Robert A. Andersen
Dr. Maureen Keller
Dr. Michael Sieracki

Brookhaven National Laboratory, NY
Dr. Julie La Roche (guest scientist)

College of Marine Studies, DE
University of Delaware
Dr. David A. Hutchins

Graduate School of Oceanography, RI
University of Rhode Island
Dr. Theodore J. Smayda

Horn Point Environmental Laboratories, MD
University of Maryland
Dr. Patricia M. Glibert
Dr. Todd M. Kana

Iona College, NY
Dr. Joseph Stabile

Institute for Marine Biosciences,
Halifax, Nova Scotia
Dr. V. Monica Bricelj

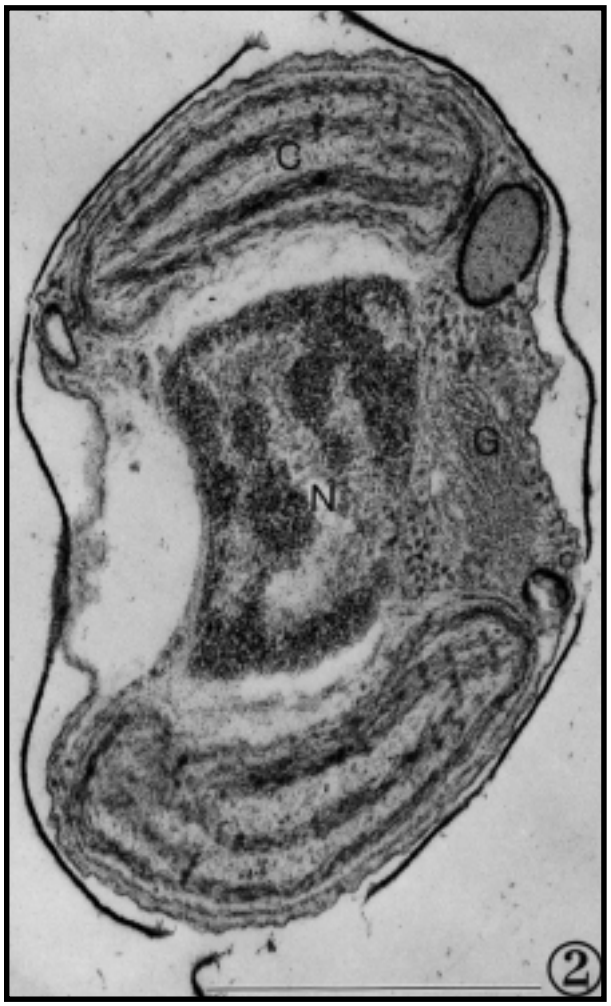
Marine Sciences Research Center, NY
SUNY at Stony Brook
Dr. Darcy J. Lonsdale
Dr. Sergio Sañudo-Wilhelmy

Northeast Fisheries Science Center, CT
NOAA/NMFS
Dr. Richard A. Robohm
Dr. Gary H. Wikfors

Old Dominion University, VA
Dr. John Donat

SUNY College of Environmental Science and Forestry, NY
Dr. Gregory L. Boyer
Dr. José L. Giner

Woods Hole Oceanographic Institution, MA
Dr. David A. Caron



Aureococcus anaphagefferens cell with a distinct cell wall.

Photo by Robert Andersen

To continue receiving the brown tide reports, see pg. 12.

Research Project Briefs: Culturing

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

While investigating the genetic variability of *Aureococcus anophagefferens*, this team of researchers made two new discoveries. In one strain of *A. anophagefferens*, they found a cell wall (see figure on p. 2); and in all strains studied, they documented genetic variability in the internal transcribed spacer (ITS) sequences of the ribosomal DNA. The Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) has 17 strains of *A. anophagefferens* from various bays across Long Island from which investigators can choose samples (or cultures) for experimental purposes. This team is also hopeful that a new suite of antibiotics will help to establish in the lab the still elusive axenic strain of brown tide.

Previously, *A. anophagefferens* was described as naked or without a cell wall. While examining a previously studied strain from Long Island, however, a distinct cell wall was discovered. The makeup and structure of this cell wall are as yet unknown. The 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, then it may act to protect the brown tide cells in the sediments during non-bloom periods. A detailed description of the cell wall's structure and molecular composition requires considerable work outside the original scope of this project.

Although it is not known if *Aureococcus* undergoes sexual reproduction at any point during its life history, ITS sequences appear to be important for sexually reproducing organisms in that organisms that differ in their ITS sequences are not able to mate successfully. To investigate differences in ITS sequences, this team employed a new technique called heteroduplex mobility assay (HMA), which amplifies gene sequences. Another technique used, called denaturing gradient gel electrophoresis (DGGE), can resolve single base differences. The results showed that several different sequences were present, but not all the strains have the same "different" sequence. Furthermore, all of the strains seemed to share one sequence hypothesized to be the functional ITS sequence. These results demonstrate that *Aureococcus* has highly unusual ITS regions compared to other **eukaryotic** organisms.

Also examining the ITS region, Drs. Joseph Stabile and Isaac Wirgin (in their ECOHAB project examining "Genetic Variation in Brown Tide Cultures and Water Samples from Long Island and New Jersey" see BTRI Report Number 2 for a summary), obtained results consistent with those of Andersen's team. By examining both ITS (ITS1 and ITS2) regions, and two genes from the ribosomal DNA, this project set out to determine if there are genetic differences among New York and New Jersey brown tide isolates and DNA obtained directly from brown tide water samples before and during a bloom. Their DNA sequencing results confirm that there is genetic variation within and among cultured isolates, and among field samples. Analysis of the Long Island isolates and field samples suggested that the 1985 strain from Long Island (CCMP strain #1784) was more similar to 1995 brown tide bloom samples than the other Long Island cultured isolates. Additional experiments are being performed to confirm these results. In addition, the Long Island and New Jersey isolates are genetically distinct. One interpretation of these results is that *A. anophagefferens* is a recently evolved species. This is consistent with the appearance of brown tides in northeastern US embayments.

Wikfors & Robohm: Isolation and Propagation of the Brown Tide Alga, *Aureococcus anophagefferens*, Using Dialysis Culture Techniques.

The dialysis culture technique was successfully used to produce bacteria-free cultures of two non-brown tide isolates. Nevertheless, by the end of this project, establishing bacteria-free cultures of *A. anophagefferens* had still proven unsuccessful. Efforts to establish new isolates of *A. anophagefferens* were hampered by relatively low densities in the Peconic Estuary during the sampling seasons (1997-98). The investigators suggested an explanation for their results. *A. anophagefferens* appeared to increase production of mucus when stressed (see Keller & Sieracki page 4), such as when exposed to antibiotics. This mucus production caused *A. anophagefferens* populations introduced into the dialysis cassettes to stick to inside surfaces, rather than remaining in suspension where they could grow normally.

Ultimately, the dialysis culture technique did work successfully with non-*A. anophagefferens* algae producing single algae, bacteria-free cultures. However, since this technique did not work with *A. anophagefferens,* no further dialysis culture work will continue with brown tide. This project has been concluded.

Research Project Briefs: Ecology

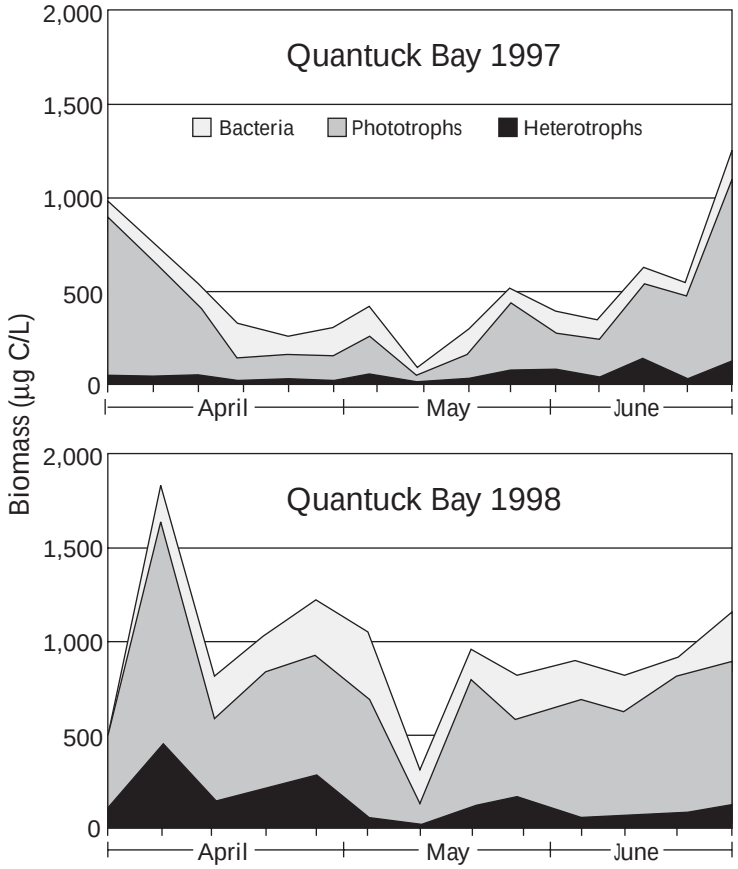
Keller & Sieracki: Physiological Ecology of the Brown Tide Organism, *Aureococcus anophagefferens* & their new initiative: Measurement of Bacterial Biomass in the Brown Tide Study Area.

This study focuses on the period of bloom initiation and the factors that result in the eventual dominance of *A. anophagefferens* in Long Island coastal embayments. The approach was two-pronged. First, this team examined the complete microbial community throughout the prebloom period in several areas where brown tides have occurred. They were searching for differences between brown and non-brown tide populations. Secondly, this team established cultures of *A. anophagefferens*, other co-occurring picoalgae, and the **microzooplankton** that may feed on these algae to examine interaction and competition between the microbial players.

This team has also established a variety of picoalgae and micrograzers cultures from Long Island Bays: Quantuck, Shinnecock and Flanders. This community work revealed that the relative diversity and seasonal succession of phytoplankton in these systems was similar between locations and between years (1997-98). There appears to be a well-developed succession from larger cells in April to smaller picoalgae cells in late May (see Quantuck Bay graphs). For both years, a niche developed in early May that was filled by a non-brown tide picoalga called *Synechococcus*, which appears to be the superior competitor in these systems. For the niche to open to other picoalgae, including *A. anophagefferens*, some factor(s) such as nutrient limitation or grazing activity may be required to eliminate or dampen *Synechococcus* growth and accumulation, therefore allowing other algae to fill the picoalgal niche.

In an accompanying non-BTRI project, funded directly by New York Sea Grant, these researchers are investigating the bacteria associated with brown tide by comparing samples from brown tide blooms and in non-bloom areas. Higher bacterial numbers were consistently found in Quantuck Bay, a non-bloom area, in both 1997 and 1998. In the *Aureococcus* bloom areas (West Neck Bay in 1997 and Great South Bay in 1998), bacterial numbers were much lower but the cells were very large and elongate. As a result, bacterial biomass was comparable for the two areas. Associated with the elongate bacteria was a matrix of extracellular mucus that could result in filtration problems and clogging of the feeding mechanisms of shellfish and other filter-feeding organisms living in these environments. It now appears that the abundance of bacteria is not correlated with brown tide. Instead, a unique bacterial assemblage may exist in a **symbiotic** relationship with *A. anophagefferens*, rather than in competition for resources (see BTRI Report Number 3, p. 8).

Utilizing these cultures, the final year of this project will examine the photosynthetic and growth characteristics of *Aureococcus* in comparison to co-occurring phytoplankton that are similar in physiology and size. This team will also investigate grazing by the microzooplankton on the brown tide organisms and the co-occurring phytoplankton to determine if *Aureococcus* is selectively avoided.



Measurements of carbon biomass in Quantuck Bay in two sampling years 1997-1998. A biomass low point was observed in early May in both years and appeared to represent a transition period from larger to small algae in the system. These patterns were also observed in Flanders and Shinnecock Bays. (Phototrophs = phytoplankton; heterotrophs = micrograzers).

Research Project Briefs: Ecology

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

The overall goal of this project is to characterize the photosynthetic and respiratory rates and nitrogen uptake capabilities of laboratory clones of *A. anophagefferens* under a range of light and nutrient growth conditions. The hypothesis has been that competitive success may be contingent on the ability of *A. anophagefferens* to supplement photosynthesis with heterotrophic uptake of organic compounds. This team has made considerable progress attaining these goals.

As previously reported, *A. anophagefferens* has proven to be exceedingly difficult to culture. Although bubbling the cultures significantly enhances *A. anophagefferens'* growth, maintaining consistent growth has been difficult. To overcome this problem, this team developed a continuous culture system based on maintaining the culture at constant **turbidity,** called a turbidostat (see photo). Each culture flask has two fiber-optic sensors linked to a computer that monitors turbidity, chlorophyll, and regulates the flow of growth media. Utilizing this new technology, it could be seen that *A. anophagefferens* showed a **negative growth** upon the onset of darkness. During the light cycle, growth resumed. When the investigators added an organic substrate (glucose + acetate) to the system, there was a two to three fold net enhancement of growth relative to the controls. Nighttime growth loss was also sharply diminished with the addition of organics. These results clearly demonstrate that organic supplements can increase *A. anophagefferens's* growth rate.

Using a range of methods to compare *A. anophagefferens'* photosynthetic physiology to similar algae this team found that *A. anophagefferens* and another dinoflagellate, *Prorocentrum minimum*, have a carbon to oxygen ratio reduced by about half compared to many other phytoplankton. This signifies that these two algal species do not maintain a balance between photosynthesis and respiration. There is either a carbon deficit or excessive oxygen production. A carbon deficit is consistent with a need to supplement photosynthesis with an additional carbon source, such as by organic uptake as described in the previous paragraph.

This team also found that *A. anophagefferens* grows well on nitrate, urea, and some amino acids, but does not grow well on ammonium. For *Aureococcus* to grow on nitrate or urea, it requires enzymes called nitrate reductases (see BTRI Report Number 2: Boyer) and ureases respectively. Typically, when cells are grown on nitrate, the enzyme to process urea (urease) is not found. However, *A. anophagefferens* expresses the urease enzyme when grown on both nitrate and urea. Since ureases can be measured under growth on a range of nitrogen sources, this suggests that ureases may serve more than one function in *A. anophagefferens*. Work will continue examining the regulation of ureases and other nitrogen assimilation enzymes.

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

In 1998, this team conducted two mesocosm experiments as described in BTRI Reports Number 2 & 3. Repeatable conditions were created in some mesocosm treatments that led to the initiation of brown tide. Contrary to 1997 results, mesocosms with hard clams did not develop brown tide. Some laboratory experiments may offer an explanation. At non-bloom concentrations of *A. anophagefferens* (e.g., less than 100,000 cells/ml), hard clam feeding rates were depressed even if an alternate algal food source was also present. When brown tide cell densities surpassed 35,000 cells/ml, filter feeding ceased (see Bricelj this report). These investigators suggest that, compared to 1997, the lower brown tide starting concentrations (5,000 to 6,000 cells/ml) and the relatively higher grazing pressure by the larger hard clams prevented bloom formation in the 1998 experiments.

This group has developed a bay-wide working hypothesis from these results. The rapid decline in bivalve population in Long Island bays during the decades prior to the first brown tide in 1985 may have led to a significant reduction of grazing pressure on phytoplankton, including the size-class of *A. anophagefferens*, allowing the onset of brown tide. This reduction in benthic grazing resulted in a shift in control from the filter-feeding benthic organisms to a pelagic microalgal consumer. According to these investigators, the interactions among the pelagic microalgal consumers may play a central role in determining the success of *Aureococcus* in planktonic communities. If this hypothesis holds true, then the overall health of the benthic community has significant consequences for the occurrence of brown tides in Long Island waters.

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

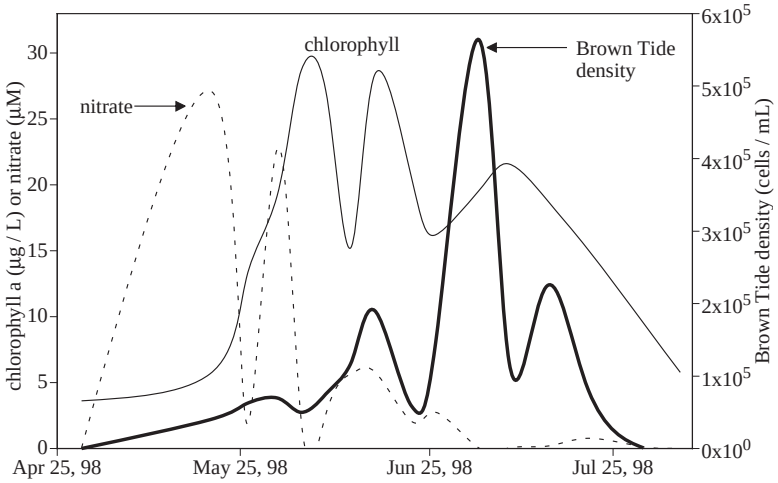
This team of investigators has performed considerable field and laboratory research on the dynamics of brown tide bloom initiation and the **biogeochemistry** of the Long Island estuaries in which blooms occur. In the final year of this study, they will continue their work in West Neck Bay, Great South Bay, Quantuck Bay and again collaborate with the Caron & Lonsdale group's mesocosm study measuring an array of inorganic and organic parameters.

During 1998, elevated nitrate was measured in West Neck Bay during the peak groundwater flow period. This nitrate input was followed by a phytoplankton bloom of various species. In July, brown tide bloomed when the supply of nitrate from groundwater decreased. Phytoplankton blooms preceding brown tide may have supplied *Aureococcus* with organic nutrients. This study concludes that rather than repressing brown tide, groundwater inputs to West Neck Bay can stimulate *Aureococcus* growth by initiating phytoplankton blooms prior to brown tide which supply to *Aureococcus* remineralized organic nitrogen (see graph below).

Results from field experiments, designed to evaluate the importance of DOC, DON, DIN, iron and phosphate on the growth of *A. anophagefferens* during a two month brown tide bloom in West Neck Bay, support other BTRI results. This team found that the nutrients, which stimulated the growth of *A. anophagefferens*, changed over the course of the bloom from organic carbon to nitrogen. In contrast, non-brown tide phytoplankton growth was consistently enhanced by nitrogen additions. Hence, nitrogen additions decreased or had no effect on the relative abundance of *A. anophagefferens* among phytoplankton. Brown tide's growth was increased by additions of dissolved organic carbon, which also augmented the growth of heterotrophic bacteria (see Glibert).

The NYSG funded (non-BTRI) new initiative project introduced in BTRI Report #3 investigating the groundwater seepage in Flanders Bay has now been completed. This study looked at how nutrients, organics, and trace metals (aluminum, copper, magnesium, cadmium, iron and silver) in groundwater can change and influence bay levels of these parameters based on groundwater seepage rates. Results indicate that groundwater can supply nitrate and copper at concentrations over three times greater than levels found in the open water of Flanders Bay. Seepage rates can also play a significant role in altering the **chemical flux** to groundwater entering the bay and can thus impact bay productivity.

Brown Tide Bloom Development in West Neck Bay



West Neck Bay's groundwater-supplied nitrate during the late spring fueled a mixed phytoplankton bloom shown on the graph as increased chlorophyll levels. In June, groundwater and light levels decreased ending this mixed phytoplankton bloom and resulted in a single-species brown tide bloom.

Graph and data by Christopher Gobler

Research Project Briefs: Bloom Triggers

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

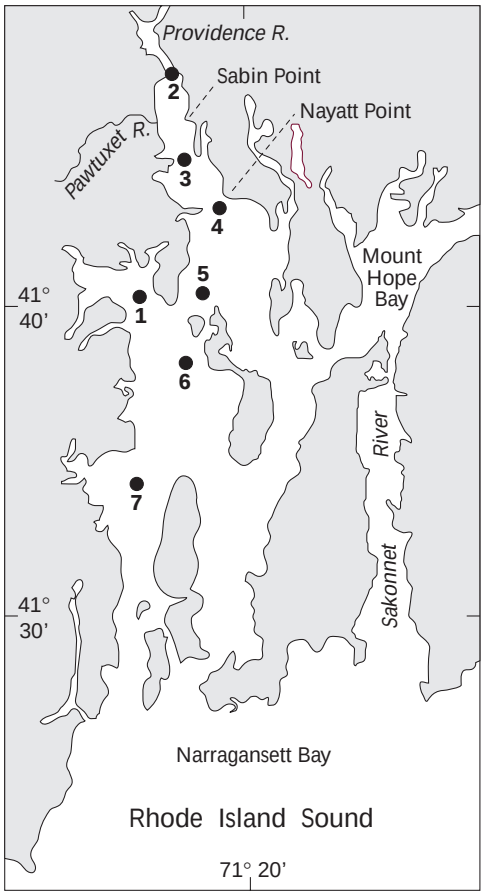
As reported in previous BTRI Reports, this team of researchers continues to explore several methods to develop a metabolic marker for iron-stress to determine if iron limits growth of *A. anophagefferens.* Currently, the best results have been obtained using a ratio that indicates a change in photosynthetic efficiency. This method has been used by members of the Sañudo-Wilhelmy team and is routinely incorporated into sampling protocols as a measure of iron limitation in Boyer's laboratory.

Preliminary studies have shown that *Aureococcus* also produces flavodoxin under iron limitation. This team's focus has been on establishing methods to measure both ferredoxin and flavodoxin using both **HPLC** and antibody techniques. For ferredoxin measurement, several HPLC ion exchange columns are currently being evaluated. In order to perfect isolation techniques for these proteins, large amounts of cellular material are needed. Since isolating the proteins has been problematic, this group is currently exploring the use of antibodies for the purification of ferredoxin and flavodoxin. Work has begun to test the use of antibodies prepared against ferredoxin from spinach and antibodies against flavodoxin isolated from *Phaeodactylum*, marine algae used in previous studies by La Roche and coworkers. These flavodoxin antibodies cross-react with flavodoxin isolated from *Aureococcus.* Once these analytical details have been worked out, the methods can be combined and used as an indicator of iron limitation.

Boyer's laboratory has also looked at the mechanisms by which *A. anophagefferens* obtains its needed iron. Other recent work has shown that over half of the marine bacteria tested produce **siderophores** to assist in iron uptake described in BTRI Report #3. However, there is no evidence that *A. anophagefferens* uses siderophore production to obtain iron. Any siderophores produced thus far would likely stem from the bacteria associated with brown tide (see Keller and Sieracki). Ferric chelate reductase (FCR) is a key enzyme for the uptake of iron. Extensive FCR characterization in *Aureococcus* has suggested that FCR activity is extremely important in the iron dynamics of *Aureococcus.* Results suggest that field populations contain about 10,000 times the minimum FCR activity needed to support maximum *Aureococcus* growth. This may be important in obtaining necessary iron and may change the iron story for *Aureococcus.* Little is know about the iron pool available to *Aureococcus*, and most organic iron complexes can be reduced by FCR. Accordingly, further field experiments will be designed considering these new results.

Smayda: Analysis of Physical, Chemical and Biological Conditions Associated with the Narragansett Bay Brown Tide.

This study examines a two -year data set, collected in Narragansett Bay from 1985-87, from a physical, chemical and biological perspective, to explain the 1985 Rhode Island brown tide (see BTRI Report Number 2). Results do not support bay flushing as the primary cause of the 1985 brown tide outbreak. Thus, this group has turned its focus to the role nutrients may have played in this bloom event. Supporting other BTRI findings, *Aureococcus* abundance in Narragansett Bay was generally greater in higher salinity bay waters with lower nutrient levels. However, nutrient and salinity levels in this system are associated with river discharge, complicating the brown tide story. An analysis of calculated growth rates suggests two possibilities. First, a possible "washout" of cells occurred in the more rapidly flushed regions of the bay preventing an accumulation of *Aureococcus* that might be expected for the nutrient-salinity conditions present. Secondly, growth losses (brown tide losses due to cell transport downstream) rather than growth repression could account for the observed spatial distribution of *Aureococcus* abundance. Preliminary results on the relationship between the dominant grazer on brown tide (the copepod *Acartia tonsa* in this system) and *Aureococcus* abundance suggest that grazing processes prior to and during the 1985 event controlled the observed *Aureococcus* abundance and distribution in the bay. Continued analyses of this data set can solidify these results and possibly present insight into the Long Island and New Jersey bloom events.



Stations in Narragansett Bay, RI, sampled during the 1985-1987 brown tide surveys.

NEW INITIATIVES

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

This team of investigators is developing another type of biomarker based on sterols to identify brown tide in field water and sediment samples. Within the short duration of this project and after developing laboratory procedures, these researchers successfully established a convenient and sensitive method for the Gas Chromatography-Mass Spectrometric (GCMS) analysis of *Aureococcus* sterols. Three *A. anophagefferens* cultures supplied from the Boyer and Andersen groups and the Texas brown tide organism, *Aureoumbra lagunensis,* have been successfully analyzed. To date, the Z-isomer of 24-propylidene-cholesterol remains a unique biomarker for *Aureococcus* (see BTRI Report Number 3). To better establish this, the sterol composition of a related species will also be analyzed.

Photo by Barbara Branca

Dr. José L. Giner

The 1999 field season will find this team very busy. All 17 strains of *Aureococcus* available from the CCMP (Andersen) will be tested with this new technique. Since much of the work so far was carried out in the winter, detection of *Aureococcus* in seawater will be tested this summer. In future projects this team plans to collaborate with geochemists to explore the historic occurrence of brown tide and see if climatic changes have influenced these blooms by examining sediments for these unique sterols.

Although sterols are essential nutrients to many marine organisms, it is unknown whether any marine organisms can metabolize the highly unusual sterols found in *Aureococcus*. The inability of shellfish to metabolize *Aureococcus* sterols could help explain the shellfish population decline and other ecological impacts of brown tide. This team hopes to collaborate with others to study the metabolism of uncommon marine algal sterols.

Bricelj: Cytotoxic Effects of Brown Tide & other brown tide work.

This team has been looking at brown tide affects on several bivalve species and is trying to gain insight into the nature of any observed toxicity by **histopathologically** evaluating bivalves exposed to brown tide. By measuring mussel feeding, this team found a marked difference in cell toxicity among the three brown tide isolates tested. Two 1995 isolates showed 100-to 250-fold inhibition of feeding relative to the controls. The third isolate, from 1985, did not affect mussel feeding. The presence of other nutritious food did not alleviate the effects of brown tide on feeding Experiments evaluating the toxicity of *Aureococcus* over its life stages showed that "older" algae (**stationary growth phase**) caused a greater inhibition of feeding rates than did the "younger" (early **logarithmic growth phase**) even in the presence of nutritious algae. Although feeding rates were affected, no histopathological evidence of toxicity was found in any of the four bivalve species tested. The digestive glands of bivalves exposed to *Aureococcus* were comparable to controls (starved animals) indicating that the effect of brown tide is more like starvation, and thus different from other harmful algae.

Short-term growth experiments on hard clams showed that *Aureococcus* densities of only 35,000 cells per milliliter were sufficient to cause harmful effects such as inhibition of clam feeding, even in the presence of nutritious algae. Long-term growth experiments on hard clams and mussels showed that the 1995 brown tide isolate mixed with nutritious algae was highly toxic to both bivalve species at high (one million cells per milliliter) and moderate (400,000 cells per milliliter) concentrations. Tissue growth rates of hard clams were completely suppressed at cell densities of 400,000 cells per milliliter. Growth by the third week did improve, suggesting some acclimation over time to relatively low *Aureococcus* concentrations (80,000 cells/ml). In summary, feeding rates of some bivalves are suppressed at moderate to high concentrations of toxic *Aureococcus* strains but were maintained at cell densities below about 20,000 cell per milliliter.

KEY TERMS

biogeochemistry a branch of geochemistry that is concerned with biologic materials and their relation to earth chemicals in an area; the science studying changes in the earth's chemical constituents as mediated by living organisms (e.g., bacteria).

chemical flux the concentration of a particular constituent multiplied by the flow or seepage rate of the water entering the bay. If the flow rate is high, then the flux of that constituent is also high. The flux of a particular constituent is a function of its concentration and flow or seepage rate.

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 cell a cell with a distinct membrane-bound nucleus.

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

HPLC High Performance Liquid Chromatography, commonly used for the separation, identification, purification and quantification of chemical compounds.

Iogarithmic growth phase the period of growth during which the population grows at an exponential rate.

microzooplankton small animals (or animal plankton) in the size class 20-200µm that are carried with the motion of the currents.

negative growth reflects a decrease in the optical or light transmission signal of the turbidostat (only observed at night). The reduction in signal may reflect a change in the cellular optical properties (such as the carbohydrate reserves) during the night. At this time, this reduction is not believed to be due to a loss in cell density (numbers).

reticulated having or resembling a network of fiber or lines.

siderophore high affinity iron chelators, produced by freshwater and marine bacteria, to aid in iron uptake under iron-limited conditions.

stationary growth phase the period following logarithmic growth phase when cell division remains relatively constant for at time.

symbiotic an interrelationship between two different organisms in which the effects of that relationship is expressed as being harmful or beneficial; an intimate association in which organisms of more than one species live together. The association may be beneficial to both (mutualism), beneficial to one with no effect on the other (commensalism) or beneficial to one with harmful effects on the other (parasitism).

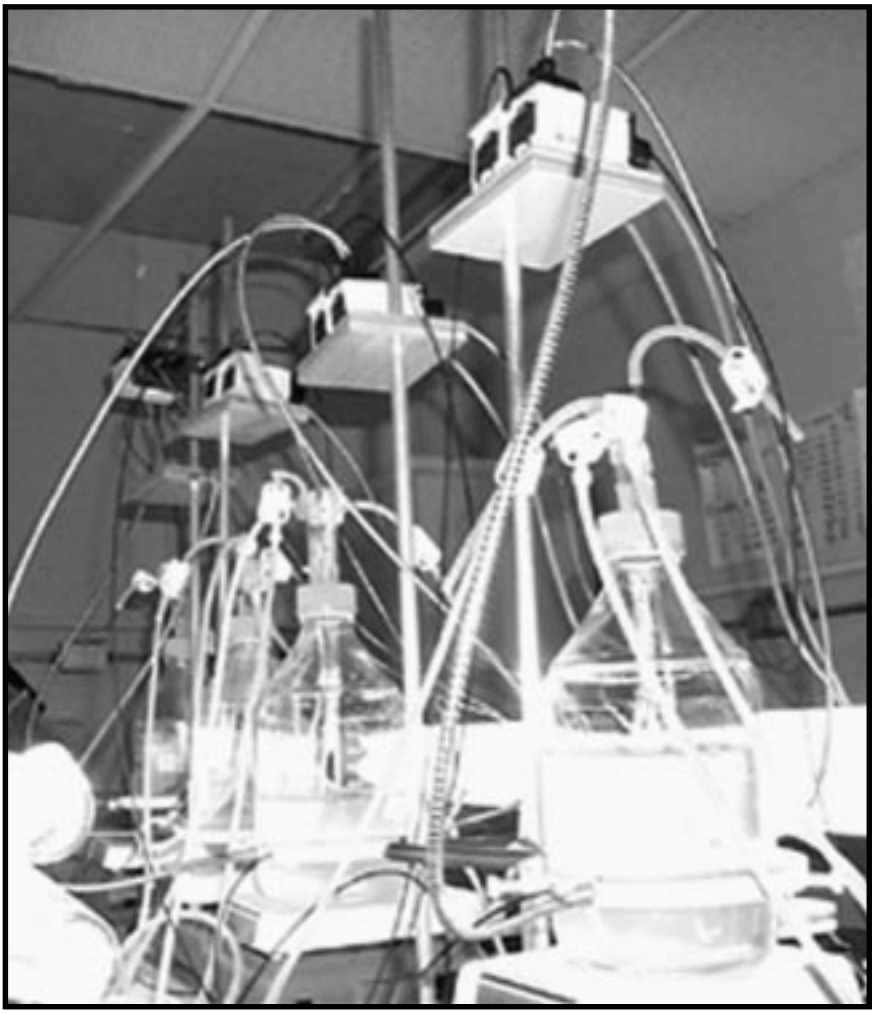
turbidity the clarity of a liquid as measured by the amount of suspended material (i.e., particulates such as sediments, phytoplankton, colloids, etc.) in a volume of water. Turbidity reduces the depth of light penetration in a water column.

SUMMARY

The summer of 1999 represents the last field season for most of these projects. Since the first BTRI report in March of 1998, the research directions of individual projects have been greatly influenced by the communication among teams in the BTRI network, other investigators, and by the data and samples provided to the researchers by the Suffolk County Department of Health Services, all of which highlight the strength of this scientific network.

Different strains of *A. anophagefferens* clearly demonstrate signs of genetic variability and a cell wall may be present at some point during *Aureococcus*' life history. In the bays of Long Island, a seasonal size-class shift in phytoplankton from larger sized cells in April to smaller sized cells in May can play a factor in setting the stage for a brown tide bloom. Although still in the preliminary research phase, investigations into brown tide-associated bacteria may provide insight into the mechanisms affecting filter feeding organisms and its persistence in Long Island bays. *Aureococcus*'s ability to exploit organic substrates, possibly as supplied by other phytoplankton, and to grow on multiple nitrogen sources seems to give this alga a competitive edge when other environmental conditions are right. While toxicity varies among brown tide culture strains, some strains clearly inhibit filter feeding and suppress tissue growth of some bivalves. The finding that some bivalve filter feeders are affected by *Aureococcus* numbers of about 35,000 cells per milliliter, an order of magnitude lower than previously suspected, suggests that *A. anophagefferens* may have been impacting Long Island bays before the first blooms appearing in 1985. The role groundwater plays in bloom development is still unclear and may be site specific. However, it is clear that groundwater flow can greatly affect the chemical constituents in Long Island bays. Grazing pressure, not bay flushing, seems to have controlled Rhode Island's Narragansett Bay brown tide event. Progress has been made in development of a brown tide biomarker, especially one based on sterols.

These results have provided a few more brown tide puzzle pieces and changed the shape of previous pieces helping to identify their proper place in the bloom puzzle. The next BTRI Report, scheduled for the spring of 2000, will present the conclusion of BTRI Phase I and introduce the new projects of BTRI Phase II.



Glibert's turbidostat, a continuous culture system that has two sensors on each culture flask monitoring turbidity and chlorophyll. See Glibert report, page 5.

The Next Phase: ECOHAB/BTRI Projects

Three new projects have been funded under ECOHAB/ BTRI II

1. Keller & Sieracki: The Effects of Microbial Food Web Dynamics on the Initiation of Brown Tide Blooms.
2. Kana, Macintyre, Cornwell & Lomas: Benthic-Pelagic Coupling and LI Brown Tide.
3. Lonsdale, Caron & Cerrato: Causes and Prevention of Long Island Brown Tides.

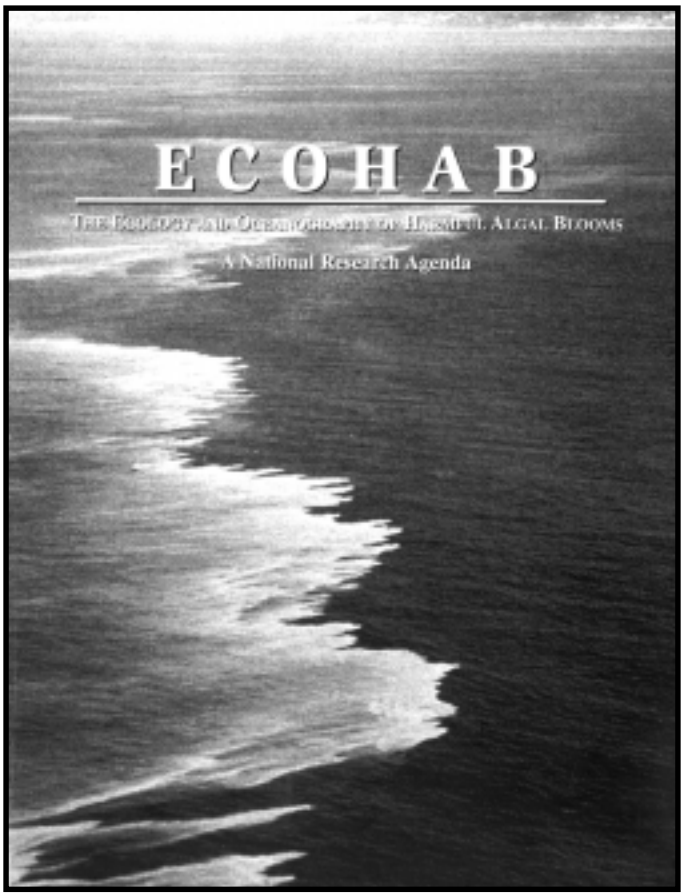
Continued funding for BTRI (1999-2002), as a $1.5 million three-year effort, comes from the National Oceanographic and Atmospheric Administration's (NOAA) Coastal Ocean Program (COP). The COP, National Sea Grant Office, National Science Foundation, Environmental Protection Agency, Office of Naval Research, and National Aeronautics and Space Administration are jointly sponsoring research on Harmful Algal Booms (HAB) ecology and oceanography in the interagency research program, *Ecology and Oceanography of Harmful Algal Blooms* (ECOHAB). Efforts include field and laboratory studies and simulation modeling to address information gaps related harmful algal species.

The primary goal of ECOHAB research is to develop the means to forecast bloom development, persistence, and toxicity. Once a clear understanding of how physical and biological processes interact to promote HABs have been developed, reliable models can be designed that can identify systems and areas potentially susceptible to outbreaks. The COP funded brown tide research in Long Island began in 1996 while other ECOHAB projects in the multi-agency study started in 1997. Including the BTRI projects as part of the larger ECOHAB effort gives BTRI national visibility, links the BTRI investigators with other HAB researchers across the country, and increases the potential for continued funding.

Regional ECOHAB studies focusing on physical and biological conditions favoring the development and expression of toxicity in several HAB species have begun for *Alexandrium* (paralytic shellfish poisoning) in the Gulf of Maine, *Gymnodinium breve* (neurotoxic shellfish poisoning) in the Gulf of Mexico, and Pfiesteria-like organisms in the mid-Atlantic states. In addition to brown tide efforts, targeted research projects have also been initiated along the U.S. coast, including California, Washington, and Alaska (*Pseudo-nitzschia* and *Alexandrium*), Connecticut (*Prorocentrum minimum, Gyrodinium aureolum*), and Guam (macroalgal overgrowth).

Web address for ECOHAB: http://www.redtide.whoi.edu/hab/nationplan/ECOHAB/ECOHABhtml.html

Web address for COP: http://www.cop.noaa.gov/



ECOHAB: The Ecology and Oceanography of Harmful Algal Blooms, was launched with this research agenda published December 1995.

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, 2, 3 or 4,* 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.

Sea Grant New York

Brown Tide Research Initiative

NOAA National Oceanic and Atmospheric Administration U.S. Department of Commerce

NONPROFIT
U.S. Postage Paid
Permit No. 1924
Ronkonkoma, NY

Bringing Science to the Shore

121 Discovery Hall
SUNY at Stony Brook
Stony Brook, NY 11794-5001

Address Correction Requested

Your FREE brown tide report subscription has run out!

To continue receiving brown tide reports, you MUST fax (631 632-6917) or email us by February 1, 2000.