Sea Grant New York

Brown Tide Research Initiative

Report #2 August 1998

1998 BTRI INFORMATIONAL SYMPOSIUM

New York Sea Grant (NYSG) hosted the second annual Brown Tide Research Initiative Informational Symposium on Saturday, April 25, 1998, at Suffolk County Community College in Riverhead, New York. Cornelia Schlenk welcomed 79 attendees, guests and investigators to the Symposium and Jack Mattice provided background history and introductory remarks about BTRI. Moderator Patrick Dooley outlined the day's events and introduced the twelve presenters including Robert Nuzzi who began the program with a review of Long Island's history of brown tide and description of Suffolk County's brown tide monitoring and research efforts. Eight BTRI (see BTRI *Report #1*) and three other brown tide investigators presented their results to an audience composed of concerned citizens, agency representatives and other investigators. Schlenk moderated a synthesis discussion by a panel of the eight BTRI investigators in which the day's results and ideas were highlighted (see side bar). The Symposium ended with Susan Banahan, a project manager from the National Oceanic and Atmospheric Administration's Coastal Ocean Program Office, reviewing the extent of harmful algal blooms (HABs) affecting the waters of the United States.

The afternoon before the Symposium, investigators and other research team members participated in a BTRI workshop. This workshop provided a networking forum the investigators used to present their results to one another and discuss new ideas and research directions. It also provided an opportunity to plan the 1998 field season so that important details could be ironed out allowing

(Continued on page 2)

PRELIMINARY RESULTS

1. Eleven strains of the brown tide organism, *Aureococcus anophagefferens* have been isolated and are available for experimental studies.
2. Gene sequence comparisons have revealed no genetic differences among the strains of *A. anophagefferens* examined thus far.
3. Dissolved organic nitrogen is the preferred nitrogen source of *A. anophagefferens.*
4. There seems to be a link between the sediment (with or without clams) and *A. anophagefferens* growth.
5. Dissolved organic nitrogen is not required for brown tide bloom onset, but rather may contribute to sustaining a bloom.
6. *A. anophagefferens* requires only very low levels of iron.
7. Although scallops spawned during brown tide, their recruitment, weight, and shell growth were reduced.
8. Various cultures of *A. anophagefferens* differ in their toxicity level to blue mussels.

Barbara Branca

The panel of BTRI researchers at the Brown Tide Informational Symposium included from left to right, Gregory Boyer, Theodore Smayda, Terry Cucci, Patricia Glibert, Robert Andersen, David Caron, Gary Wikfors and Christopher Gobler.

Brown Tide Research Initiative
Report #2

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1998 BTRI Symposium (cont'd)

coordination between the various projects. During this BTRI workshop, in addition to all of Saturday's speakers, Joseph Stabile, working with Isaac Wirgin, presented results from their **ECOHAB** project examining "population genetics of brown tide blooms." Using the latest techniques, their results suggest variability in various bloom samples. However, genetic differences in *Aureococcus anophagefferens*, the brown tide organism, are still under investigation (see Boyer, page 9).

According to Patricia Glibert, "The strength of BTRI is the multi-faceted approach," combining a focused research effort of biological, chemical and physical oceanographical expertise together with a unique networking opportunity for information and idea exchange among the investigators. This format avoids unnecessary experimental and data collection repetition while maximizing results and resources across this suite of investigators and their associated universities. Theodore Smayda enthusiastically said, "The exchange of laboratory and field biological and physiological information is great!"

This report reflects the progress made part-way through the BTRI projects, now in their second field season, as well as results from other related brown tide efforts. During this year's Symposium, not only were important questions answered, but exciting and unexpected results were described that have prompted new directions for current and future investigations. *Report #2* follows the same format as BTRI *Report #1,* March 1998, allowing for easy project progress tracking. For ease of reading, boldfaced terms are defined under *Key Terms* building upon those in *Report #1.*

Barbara Branca

BTRI Outreach Specialist Patrick Dooley moderated the Symposium.

Research Project Briefs: Culturing

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

Andersen's primary objectives are to establish xenic and axenic clonal cultures of *Aureococcus anophagefferens*, and to characterize the genetic diversity among *A. anophagefferens* strains. Eleven strains of *A. anophagefferens* are presently maintained at the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) and are available for use by brown tide researchers. Because experiments focusing on the physiology (e.g., nutrient requirements) of *A. anophagefferens* may be influenced by the presence of bacteria in cultures, one important goal is to establish bacteria-free (axenic) *A. anophagefferens* strains. Three cultures of *A. anophagefferens* which are believed to be axenic are now being rigorously tested to be sure they contain no bacteria.

Growth rate differences have been observed among strains of *A. anophagefferens* at the CCMP. It is possible that growth differences could be due to genetic differences among separate strains, the presence of different assemblages of bacteria in each culture, or to a combination of both factors. The cause for the growth differences is still unknown.

The second major objective of this research is to determine levels of genetic variability within *A. anophagefferens*. Are all *A. anophagefferens* cells genetically identical or nearly identical, or are there genetically distinct **subpopulations** that bloom at different locations or at different times? A measure of genetic diversity would enhance our understanding of *A. anophagefferens* bloom dynamics and, perhaps, provide a basis for control measures.

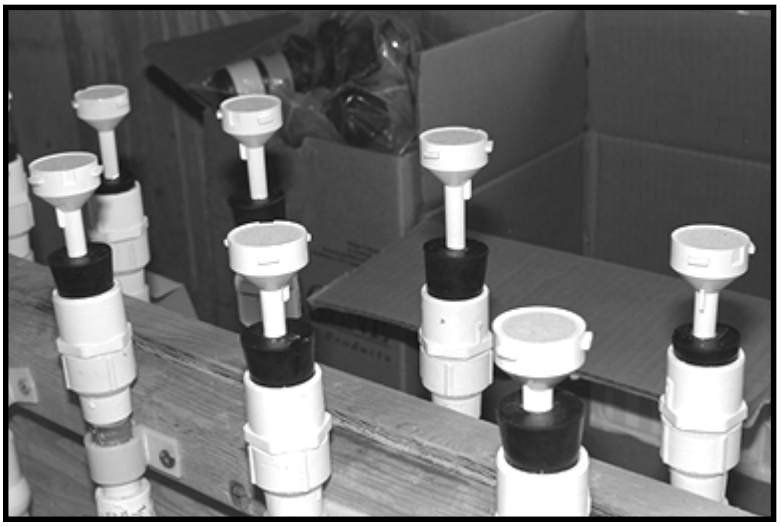
Initial studies of genetic diversity have focused on comparisons of **gene sequences**. For each strain, the DNA sequences for two different genes were determined. One gene (18S rRNA) is located within the nucleus of the cell and the second (*rbc*L) is located in the **chloroplast**. Also, a third non-coding region of DNA (the RuBisCO spacer between the *rbc*L and *rbc*S genes), also found on the chloroplast **genome**, was examined for each isolate. These three DNAs almost certainly evolve at different rates. The rate of change, or mutation, also varies within different portions of the two genes. The 18S rRNA gene has an identical nucleotide sequence in all isolates examined; similarly, the *rbc*L gene is identical in these isolates, and even the RuBiSCO spacer sequences are identical across all isolates examined thus far.

These data confirm that all isolates examined belong to a single species that apparently has little genetic variation. The apparent lack of genetic variation within *A. anophagefferens* is consistent with the proposal that the species may have recently evolved. However, it is also possible that *A. anophagefferens* possesses a highly-coadapted genome that is maintained by intense stabilizing selection.

A new molecular approach, DNA fingerprinting, is now being explored for use as a tool for assessing fine-scale genetic variability within *A. anophagefferens.* Unlike methods previously employed which focus on a particular gene or locus, DNA fingerprints are based on a survey of variation across the entire genome and can be used to examine genetic differences among populations of individuals.

Key Terms

Look for the definitions of key terms in **boldface** on page 15.

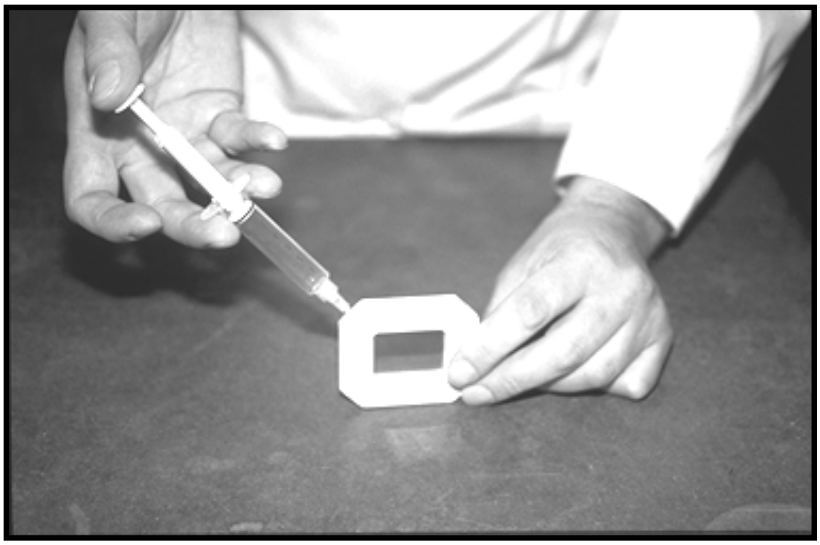


Barbara Branca

Samples of seawater being checked for chlorophyll content on the Caron and Lonsdale research project (page 7).

Research Project Briefs: Culturing

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



Barry Smith

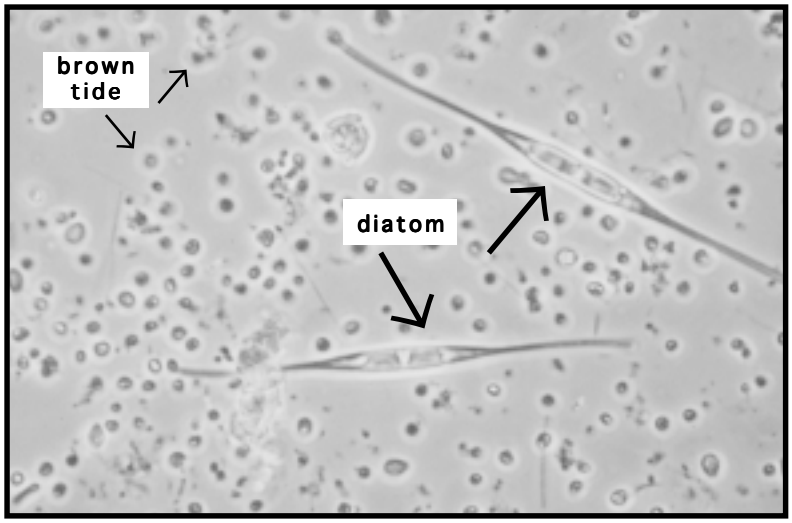
Wikfors injects purified culture of *Aureococcus anophagefferens* into a dialysis cassette. The "caged culture" will be immersed in a bath of natural seawater containing bacteria that can supply *A. anophagefferens* with organic nutrients through the dialysis membrane.

This team of investigators is perfecting a technique to isolate *Aureococcus anophagefferens* from water samples while at the same time providing a means to culture the **alga**. The method is called dialysis culture. A membrane is used to physically separate a selected organism, such as *A. anophagefferens*, from other species in a water sample, such as plankton although, brown tide culture strains can still contain bacteria. The membrane allows chemical nutrients in the water to freely pass across providing a suitable growing environment for *A. anophagefferens*, even if chemicals produced by bacteria are needed for *A. anophagefferens* growth. To date, these investigators have successfully grown and maintained several small algal species in addition to *A. anophagefferens* cultures from Barnegat Bay, New Jersey, the Peconic Bays, New York, and from CCMP cultures (see Andersen).

Producing bacteria-free cultures using the dialysis technique is problematic. A number of procedural methods have been refined and current plans are to conduct antibiotic **treatments** with the Barnegat Bay and Peconic Bay strains to ultimately provide bacteria-free cultures for subsequent studies and for other investigators.

How big are plankton?

The photomicrograph shows a phytoplankton assemblage under magnification which includes *Aureococcus anophagefferens* and diatoms.



Robert Nuzzi

Research Project Briefs: Ecology

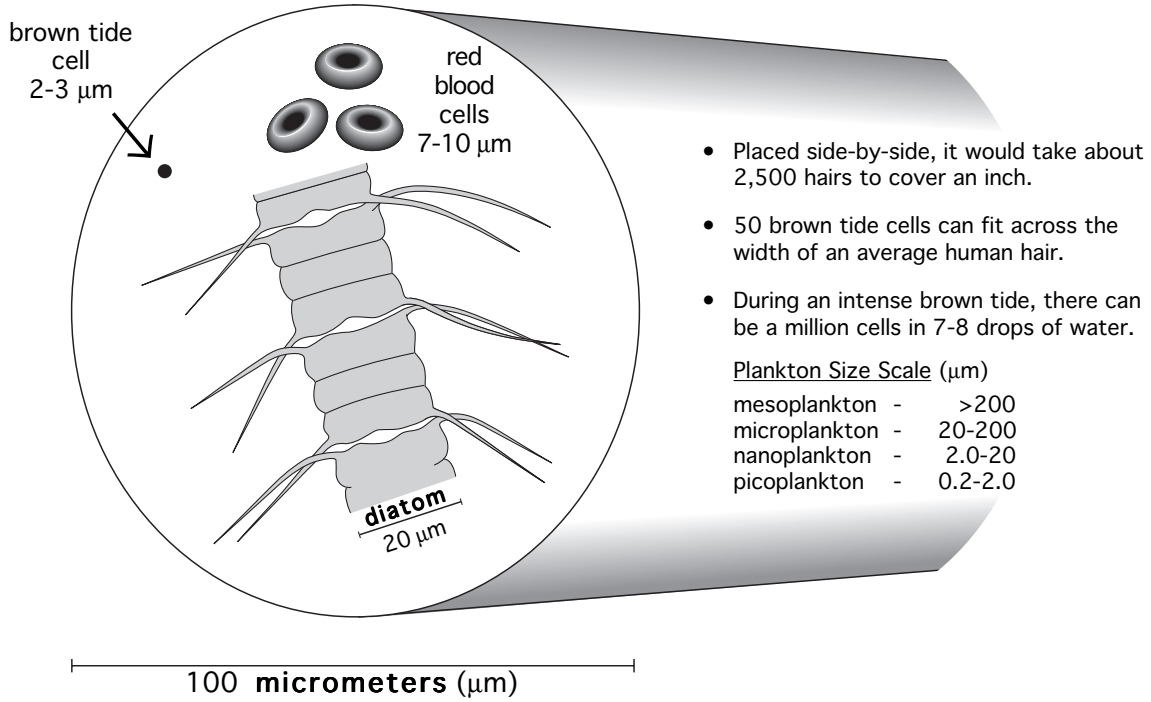
Keller & Sieracki: Physiological Ecology of the Brown Tide Organism, *Aureococcus anophagefferens*.

The first year of this project had three goals: to establish cultures of *Aureococcus anophagefferens* and other algae species which can bloom at similar times as *A. anophagefferens;* to establish cultures of micrograzers that feed on **picoplankton** (see relative size diagram below); and to characterize and count *A. anophagefferens,* co-occurring phytoplankton, and the grazing community throughout the pre-bloom period. Multi-year characterization of this type will help assess differences during bloom and non-bloom years in areas where blooms do or do not occur.

From Long Island bays, numerous cultures of different picoplankton and **ciliates** have been established. However, due to the alga's small size, isolation and identification have been difficult. An existing antibody was used in field samples to distinguish *A. anophagefferens* from other picoplankton. After modifications to the **antibody test**, previously uncharacterized strains of picoplankton from the Peconic Bays were identified as *A. anophagefferens*; other strains represent a number of algal types. Additionally, enumeration of *A. anophagefferens,* co-occurring algae, and grazers was completed in field samples, thus meeting all Year One goals.

Analysis of the 1997 samples demonstrated the seasonal **succession** of plankton under non-brown tide conditions. In April, larger **nanoplankton** dominated. In June, the dominance shifted to the smaller picoplankton, a size class which includes *A. anophagefferens*. During this time period in Shinnecock Bay, picoplankton other than *A. anophagefferens* were abundant. Laboratory work will focus on the dynamics between *A. anophagefferens* and co-occurring algal species to understand why one form dominates over another. Preliminary observations from Shinnecock Bay have shown high bacteria numbers in 1997, a limited bloom year, possibly indicating that bacteria may also compete with *A. anophagefferens* for organic nutrients. This idea will require further sample analysis and experimentation to confirm.

Compare plankton size to this cross section of an average human hair.



- Placed side-by-side, it would take about 2,500 hairs to cover an inch.
- 50 brown tide cells can fit across the width of an average human hair.
- During an intense brown tide, there can be a million cells in 7-8 drops of water.

Plankton Size Scale (μm)

mesoplankton -	>200
microplankton -	20-200
nanoplankton -	2.0-20
picoplankton -	0.2-2.0

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, respiratory and nitrogen uptake capabilities of laboratory cultures of *Aureococcus anophagefferens* (cultures supplied by Andersen). It has been suggested that in low light environments such as the Peconic Bays, photosynthesis alone may not supply enough energy for cell growth. If *A. anophagefferens* can supplement photosynthesis with uptake of organic compounds, such as urea, then in low light environments *A. anophagefferens* may possess a competitive edge over co-occurring phytoplankton potentially leading to a bloom. Although this project is still in the early stages, 1995 field data have shown *A. anophagefferens* preferred urea as its nitrogen source.

Barbara Branca

BTRI researcher Patricia Glibert of Horn Point Laboratory.

To determine photosynthetic performance, future experiments will assess the photosynthetic response to light intensity with *A. anophagefferens* grown in the absence or addition of organic compounds. To determine how the **autotrophic** behavior of *A. anophagefferens* may be different from other co-occurring species, these experiments will be conducted with representatives from other major groups of marine phytoplankton.

In the photo below, graduate students Becky Shaffner of SUNY Stony Brook (left) and Katie Rose Boissoneault-Cellineri of the MIT/WHOI Joint program (right) check experimental clam feeding during the Caron & Lonsdale research project in Coecles Harbor, Shelter Island, New York. The photo to the right shows these graduate students checking mesocosms at the same site.

Barbara Branca

Research Project Briefs: Ecology

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

Previous research in Long Island waters has shown that, under non-bloom conditions, grazers can consume up to 75% of phytoplankton production per day. However, the role grazers play in brown tides is still a question. The goal of this project is to determine the importance of planktonic grazing as a factor leading to the initiation of brown tide. During the earliest stages of bloom conditions, these investigators examined how different sized **microplankton** and nanozooplankton grazers affected the growth and grazing relationships involving *Aureococcus anophagefferens*.

Last summer, **mesocosm** experiments with six different treatments were conducted in the Shelter Island area to examine the factors leading to the initiation of brown tide. Interactions regarding the **pelagic** plankton community structure, the nutrient environment (see Sañudo-Wilhelmy, Hutchins & Donat) and the role of benthic filter feeders (or **benthos**) were examined. The table below summarizes the mesocosm treatments.

The results of this study were surprising. *A. anophagefferens* sustained positive growth only in the benthos and the benthos **control** treatments (Table 1 shaded treatments). *A. anophagefferens* did not grow in either the inorganic or organic nitrogen treatments. Although the data for the pelagic nanoplankton community aspect of this experiment are still being analyzed, initial evaluation of the microzooplankton and mesozooplankton did not show any differences in the composition or amount of zooplankton among control, benthos control, or benthos treatments.

During the summer of 1998, these investigators are continuing the mesocosm experiments and augmenting the study to address the role of filter-feeding clams in the initiation of brown tide. The combination of the 1997 and 1998 mesocosm results will add vital information into understanding the dynamics of brown tide bloom initiation.

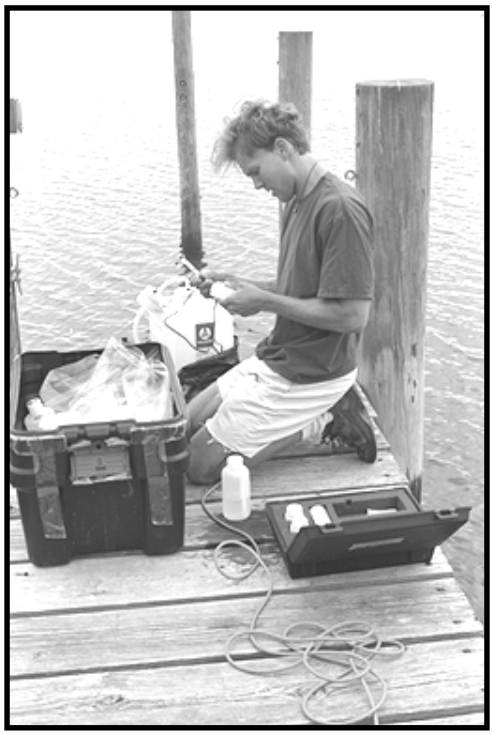
Table 1: Environmental mesocosm treatments used to investigate factors which may lead to brown tide. (√) indicates factor was added to treatment. Brown tide growth occurred only in benthos control and benthos treatments (shaded).

TREATMENT DESCRIPTIONS

EXPERIMENTAL MESOCOSM TREATMENTS	Seawater with *Aureococcus anophagefferens*	Nutrients	Sediments	Water Circulation Pump	Clams: *Mercenaria mercenaria*
1. CONTROL	√				
2. NANOPLANKTON (seawater filtered through a 20 μm sieve)	√				
3. INORGANIC NITROGEN (nitrate and phosphate)	√	√			
4. ORGANIC NITROGEN (urea and phosphate)	√	√			
5. BENTHOS CONTROL	√		√	√	
6. BENTHOS	√		√	√	√

Research Project Briefs:

Sañudo-Wilhelmy, Hutchins & Donat: Biogeochemical and Anthropogenic Factors that Control Brown Tide Blooms: The Effects of Metals and Organic Nutrients in Long Island Embayments.



Barbara Branca

Sea Grant Scholar Christopher Gobler checks water samples at West Neck Bay, Shelter Island, New York.

This team is completing the second year of field work to assess the role of metals and organic nutrients in controlling brown tide blooms in Long Island's embayments. Long-term sampling sites now exist in West Neck Bay on Shelter Island, in Flanders Bay in the Peconics and Great Cove in Great South Bay. Typical seasonal distributions of water constituents (such as trace metals and nutrients) possibly involved in brown tide stimulation or inhibition throughout the Peconic Estuary under non-bloom conditions have been established for comparison to brown tide bloom events. This group of investigators has now begun collaborating closely with the Caron and Lonsdale team by completing the chemical analyses for their mesocosm experiments (see Caron and Lonsdale).

Trace metals such as iron and organic nutrients such as dissolved organic nitrogen (DON), dissolved organic carbon (DOC) and urea have been theorized as components responsible for brown tide. To gain insight into the role these components play in brown tide, Christopher Gobler (a graduate student and Sea Grant Scholar at the Marine Sciences Research Center-MSRC under the guidance of Sañudo-Wilhelmy) conducted a field study in West Neck Bay, June, 1997, during a short but strong brown tide bloom. The month before the bloom, levels of DOC, DON, urea and inorganic nitrogen were relatively low and did not increase. During the bloom, however, levels of inorganic nitrogen fell while levels of organic nutrients did not change. When the bloom subsided, levels of both organic and inorganic nutrients increased. During the intense 1995 West Neck Bay bloom, DON levels started at comparable levels to the 1997 bloom, however, DON levels doubled before the bloom, dropped during the bloom and then began to

(Continued on next page)

Barbara Branca

MIT student Mausmi Mehta measures salinity in West Neck Bay.

Bloom Triggers

rise again upon bloom subsidence. These results suggest that DON is not required for bloom initiation, but rather may contribute to sustaining a bloom.

Other investigations have implicated the relative flow of groundwater as another potentially important factor in brown tide bloom onset. This team began analysis of all the major constituents of groundwater surrounding the Peconic Bays last autumn. The importance of groundwater inputs to brown tide bloom events will be assessed by correlating the concentrations of constituents in groundwater from various bays with how fast the groundwater flows.

This summer's field data collection and experiments schedule is very intensive and includes: continued chemical analysis of the established sites; groundwater sampling at eight wells throughout the Peconic Bay area; culture work examining hypothesized bloom initiation factors, and increased collaboration with the Caron and Lonsdale team's mesocosm work. If brown tide occurs this year, the results from this study will provide a solid framework for comparison helping to formulate trends and cycles.

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

The objective of this project is to evaluate the role of iron in brown tide growth. A **metabolic marker** for iron stress is currently under development that will be used to test the hypothesis that low iron availability can limit *Aureococcus anophagefferens* growth.

To develop the metabolic **iron stress** marker, purified ferredoxin (an iron-containing protein) and flavodoxin (a protein without iron produced in this organism when it is iron limited) from laboratory cultures are needed for use as standards and for antibody development. Successful culture growth under iron-sufficient conditions for two different *A. anophagefferens* strains (from the CCMP, see Andersen) have produced sufficient amounts of cell material for the isolation of ferrodoxin. Iron-limited cultures have proven to be more problematic. Ferredoxin is produced only under conditions of low iron availability, therefore, trace-metal clean conditions including specially treated iron-free growth media and trace-metal clean polycarbonate flasks must be used.

Despite these efforts, *A. anophagefferens'* growth rate cannot be decreased under iron limited conditions. Using natural seawater from the iron-poor Sargasso Sea and artificial seawater, both Boyer and La Roche working independently have grown *A. anophagefferens* under low iron concentrations (10-20 nM). Although it has been difficult to limit *A. anophagefferens'* growth rate with low iron concentrations, the organism may still be iron stressed and induce flavodoxin production. Work currently underway indicates that these iron-limited cultures of *A. anophagefferens* can be used successfully to isolate flavodoxin.

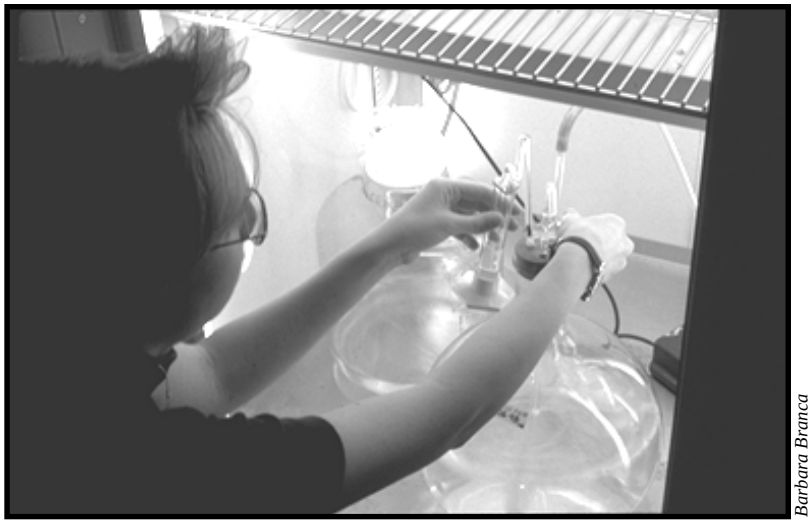
(Continued on page 10)

Gregory Boyer

Research assistant Jeff Alexander checks growth of *Aureococcus.*

(Boyer & La Roche continued)

Sea Grant scholar Darlene Szmyr has been looking at the levels of a key **enzyme** for the **assimilation** of nitrate, called **nitrate reductase**. She has found that *A. anophagefferens* from the Peconic Bays shows very traditional nitrate reductase activity and growth on nitrate. Her work will continue examining nitrate reductase activity, the iron status of the cell and the role of nitrate reductase in iron uptake.



Barbara Branca

Sea Grant scholar Darlene Szmyr monitors a flask of brown tide culture.

While examining a new research area, the **sterol** composition of *A. anophagefferens*, this team, working in collaboration with other investigators at the SUNY College of Environmental Science and Forestry, has shown that *A. anophagefferens* is the first alga to contain a novel sterol that has previously been isolated from marine **invertebrate**s. Presence of this sterol supports changing the taxonomic class in which *A. anophagefferens* belongs from **Chrysophyceae** to **Pelagophyceae**. Sterols, such as these, may provide a simple method for identification of *A. anophagefferens* in water samples.

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

To understand the dynamics of the 1985 brown tide bloom in Narragansett Bay, Smayda is analyzing a comprehensive 1985-1987 data set for brown tide with special focus on flushing, wind intensity, groundwater and many other environmental variables.

Reduced flushing rates of estuaries and coastal embayments may influence the onset of brown tide. Generally during a bloom event, *Aureococcus anophagefferens* abundance tends to increase as the flushing rate decreases. Since flushing rate can change over the course of a week, *A. anophagefferens* abundance can also fluctuate from week to week depending upon the flushing rate during the bloom event. However, evidence from the 1985 Narragansett Bay brown tide bloom does not support reduced flushing rate as the primary cause for the Narragansett Bay bloom event.

Through the evaluation of wind speed and direction, Smayda also determined that higher than average wind speeds occurred in 1985, favoring bay water mixing. The effect of a mixed bay would be to keep *A. anophagefferens* mixed in the water with continual exposure to available nutrients and light.

This investigator also looked at the influence of groundwater on *A. anophagefferens.* La Roche (in a project independent of Boyer and La Roche's BTRI project) hypothesized that the supply of inorganic and organic nitrogen supplied from groundwater can regulate the start of a brown tide bloom. The 1985 data from Narragansett Bay is consistent with this hypothesis.

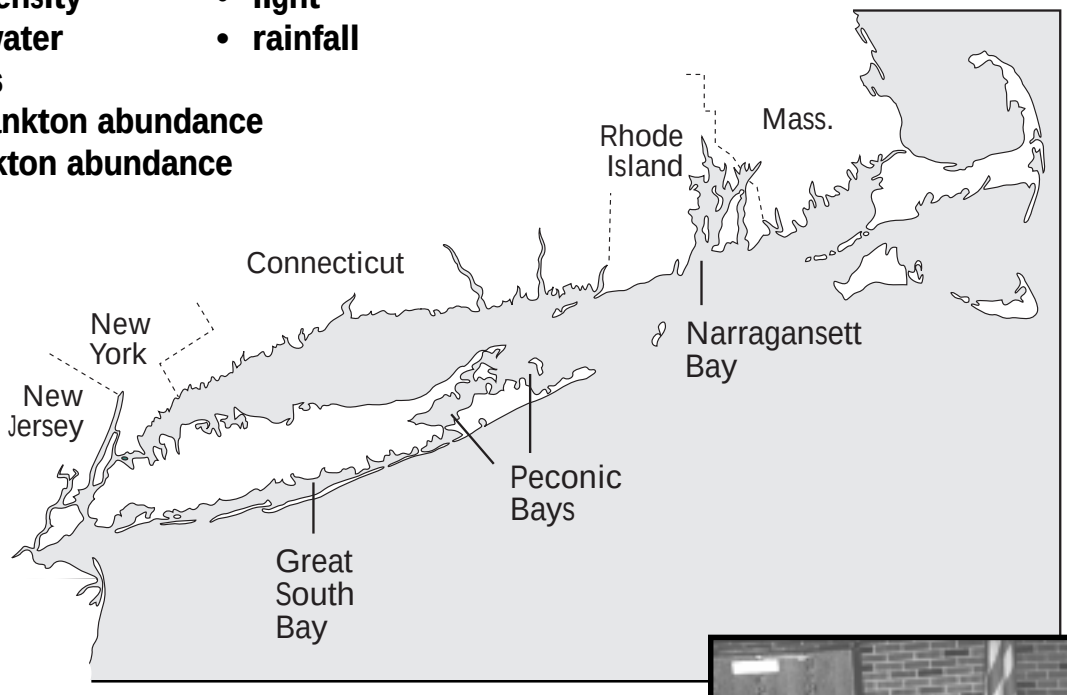
Bloom Triggers

However, groundwater cannot solely explain the simultaneous occurrence of brown tide in Long Island Bays and Narragansett Bay. Growth factors which influence *A. anophagefferens* locally may be important within some coexisting regional factor. It should be noted that 1985 also stands out from other years with higher than average **salinity**.

Currently, Smayda is in the process of examining 30 other environmental variables for the 1985 *A. anophagefferens* Narragansett Bay bloom year and two additional post-bloom years. These variables will be compared with their long-term averages established between 1959-1990. Some of these variables include temperature, light, rainfall, nutrients and phytoplankton and zooplankton abundance. Preliminary results suggest that 1985, along with 1965 and 1966, are characterized by the lowest river flow and highest irradiance of the years examined. Additional analysis has begun on bay-wide differences in physical and chemical features and their influence on the relationship between *A. anophagefferens* and other plankton species.

Comparing variables such as:

- **flushing rates**
- **wind intensity**
- **groundwater**
- **nutrients**
- **phytoplankton abundance**
- **zooplankton abundance**
- **temperature**
- **light**
- **rainfall**



Barbara Branca

Theodore Smayda presenting at the Brown Tide Symposium.

OTHER BROWN

This year's Informational Symposium also presented the results of other brown tide research funded through New York Sea Grant, the Suffolk County Department of Health Services, and other sources.

Carpenter & Lin: Cell Cycle Proteins in the Brown Tide Alga: A Potential Tool for Growth Rate Estimation.

Barbara Branca

Senjie Lin

Senjie Lin, working with Edward Carpenter at MSRC, presented results from their work to develop a technique to measure *Aureococcus anophagefferens'* growth rate in the field Currently, there is no way of assessing the ***in situ*** growth rate of *A. anophagefferens*. Knowing *A. anophagefferens'* growth rate will aid in bloom monitoring and in determining the conditions and locations under which *A. anophagefferens* blooms. This team of investigators is modifying an existing *in situ* phytoplankton growth rate technique by using antibodies specific to a protein produced as algal cells divide and grow. These investigators have identified specific *A. anophagefferens'* growth proteins from laboratory cultures and are currently perfecting staining and antibody modifications needed due to *A. anophagefferens'* small size. Once completed, methodology streamlining and field testing will begin.

Tettelbach & Smith: Effects of the 1995 Brown Tide on Bay Scallop Survival, Growth and Reproduction.

Stephen Tettelbach, working with Christopher Smith, presented results regarding the effects of brown tide on bay scallop reproduction. Tettelbach employed three different scallop culture nets and free planting of scallops on the bottom at different water depths to examine scallop spawning, growth and **recruitment** in the Peconic Bays. The 1995 Peconic Bays' brown tide bloom peaked between mid-June and late July and a second time between late August and mid-September. Very high water temperatures were recorded in late July and may be associated with the temporary brown tide subsidence. Tettelbach found that scallops spawned twice during his study, both times during the peaks of the brown tide blooms, and that the scallops spawned at all measured water depths. His results, along with anecdotal information from local baymen, showed very low scallop recruitment. Tettelbach also found a dramatic increase in tissue weight when the bloom subsided in late July. However, tissue weight fell with the second brown tide peak. It was also noted that overall shell growth was reduced compared to non-bloom years.

Stephen Tettelbach

Examining the effects of brown tide on scallop reproduction in Peconic Bay.

TIDE RESEARCH

Bricelj: An Update on the Effects of Brown Tide on Bivalve Molluscs

V. Monica Bricelj started her brown tide work while at MSRC and has since relocated to the National Research Council of Canada in Halifax, Nova Scotia. Her work focuses on how a diet of brown tide (alone or in combination with nutritious algae) affects feeding, growth and survival of commercially important **bivalves**. Previous laboratory work regarding the effects of *Aureococcus anophagefferens* on bivalves used the original 1986 brown tide cultured strain now 12 years old. Since the toxicity of various brown tide cultures, including newly isolated cultures, has not been characterized, Bricelj has focused her work on the development of a short-term **bioassay** using mussels, that would allow comparison of the toxicity of various *A. anophagefferens* cultures.

When mussels were fed only a diet of *A. anophagefferens* at bloom concentrations, the two 1995, but *not* the old 1986, cultures caused a severe inhibition in the mussels' feeding rate.

V. Monica Bricelj

Scott MacQuarrie, lab technician working on Bricelj's bivalve toxicity project.

When the mussels were fed *A. anophagefferens* 1995 isolate cells mixed with a non-toxic algal species diet, their feeding rate were also reduced significantly. Two conclusions can be drawn from these findings. Since the mussels showed considerable differences in their feeding response to the three *A. anophagefferens* cultures, this suggests that toxicity also differs markedly among isolates. Secondly, the presence of a non-toxic, nutritious alga in the water does not alleviate the effects of brown tide on mussel feeding during bloom conditions.

Barbara Branca

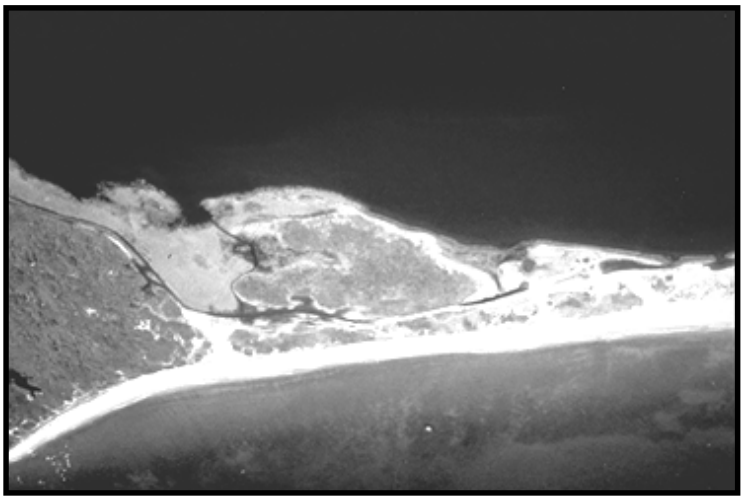
V. Monica Bricelj

In previous studies, bivalves were exposed to *A. anophagefferens* cells for only brief periods of time (up to a few hours), due to limitations in the availability of mass cultures of *A. anophagefferens*. These are now routinely produced in Bricelj's laboratory. In longer-term experiments with juvenile hard clams (*Mercenaria mercenaria*) designed to test density and the toxicity affects to growth and survival of *A. anophagefferens* over a three-week period, Bricelj's team concluded that the 1985 brown tide culture is not highly toxic to juvenile hard clams. This agrees with results of the mussel bioassay. However, growth of clams was inhibited following prolonged exposure to bloom levels of this isolate. These results suggest that the 1985 *A. anophagefferens* culture may have lost its toxicity over time.

Additional work in 1998 will investigate the effects on growth and survival of juvenile bivalves of the 1995 isolates, identified with some degree of toxicity by the mussel bioassay.

SYNOPSIS

This year's Symposium introduced many results and new ideas. Taking into consideration all these findings, new questions must be asked. Why, during years when conditions would seem to be favorable for brown tide, have there been only sporadic bloom events or none at all? Approaching this question from an ecosystem perspective, Smayda feels that the *Aureococcus anophagefferens* bloom in Narragansett Bay was a stochastic or "chance" event. There is likely some regional commonality among all the brown tide blooms, in addition to local controlling mechanisms all of which are still under investigation. Thus far, it seems that there are three general controlling mechanisms involved, to varying degrees, with brown tide bloom events: physical parameters, such as bay flushing and wind conditions, the interaction between nutrients and benthic dynamics and chemical factors such as iron. Although combined BTRI, Suffolk County and other research efforts results have greatly increased the brown tide information base, collecting and arranging all the pieces of this complex puzzle still needs work.



Brown tide in Long Island's Peconic Bay (top) contrasts with the lighter Atlantic Ocean (below).

The 1998 BTRI field season promises to be very active for continuing the ongoing studies, testing new ideas, and confirming prior results leading to a better understanding of brown tide and its effects on the environment. BTRI *Report #3,* scheduled for the Fall/Winter of 1998, will highlight the results of these activities.

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KEY TERMS

alga(e) primitive, often aquatic, plants that carry on photosynthesis but lack the flowers, roots, stems, and leaves of higher plants.

antibody test an indication test that uses antibodies to determine the presence of an organism or substance.

assimilation conversion of nutritive materials into a living organism.

autotrophic an organism capable of synthesizing organic nutrients directly from simple inorganic substances such as carbon dioxide and inorganic nitrogen.

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

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

bivalve animals having a soft body enclosed in a calcareous two-part shell, e.g., clams, scallops and oysters.

A scallop is a bivalve.

chlorophyll light-harvesting pigments that make it possible for plants to photosynthesize.

chloroplast the structure in a plant or algal cell that contains **chlorophyll**.

Chrysophyceae unicellular golden-brown algae that inhabit fresh and salt water environments.

ciliates single-celled protozoa often found in plankton that move by beating hair-like structures called cilia.

control observations made in an experiment which have not undergone treatment, to use in comparison with observations made on subjects which have undergone treatment.

diatoms single-celled algae, mostly photosynthetic, that form silica cell walls, can grow singly, in chains or in simple colonies.

ECOHAB ECology and Oceanography of Harmful Algal Blooms.

enzyme protein produced by living cells that catalyze the biochemical processes necessary to sustain life.

gene sequence the specific order in which the structural components of DNA are arranged for a particular gene.

genome the genetic endowment of a species.

in situ in the original location (e.g., water column or within the organism).

invertebrate organism lacking a backbone and internal skeleton.

iron stress the condition of an organism lacking the necessary level of the trace-metal iron for growth.

Mesocosms on Shelter Island.

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

metabolic marker a change in proteins in the cell that reflect a nutrient deficiency.

micrometer (μm) one millionth of a meter (1 inch = 25,400 μm).

microplankton small, single-celled planktonic organisms in a size range 20 - 200 μm.

nanoplankton small, single-celled planktonic organisms in a size range 2.0 - 20 μm.

nitrate reductase an enzyme necessary for growth on nitrate.

pelagic open water that is above the bottom and below the surface.

Pelagophyceae the name of a group of very small free-floating golden-brown algae.

picoplankton small, single-celled planktonic organisms in a size range 0.2 - 2.0 μm.

recruitment to increase or maintain by supplying anew (e.g., either by reproducing or migration).

salinity the total quantity of dissolved salts in sea water measured by weight.

sterol any of the natural products derived from the steroid nucleus; all are waxy, colorless solids soluble in most organic solvents but not in water.

subpopulation a group of individuals that can be set apart from a larger group of individuals to which they also belong.

succession changes in the composition of an ecosystem as the available competing organisms and plants respond to and modify the environment.

treatment controlled technique or action applied in a specified process or experimentation.

INSIDE

The Brown Tide Research Initiative 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 peer-reviewed research projects that were selected from a national call 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 (see side bar, page 2). The research projects selected for BTRI funding 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 and background information about brown tide and focus on introducing and updating the BTRI projects. 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 would like to be added to our mailing list, please contact Patrick Dooley at New York Sea Grant (patrick.dooley@sunysb.edu).

Sea Grant New York

Brown Tide Research Initiative

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