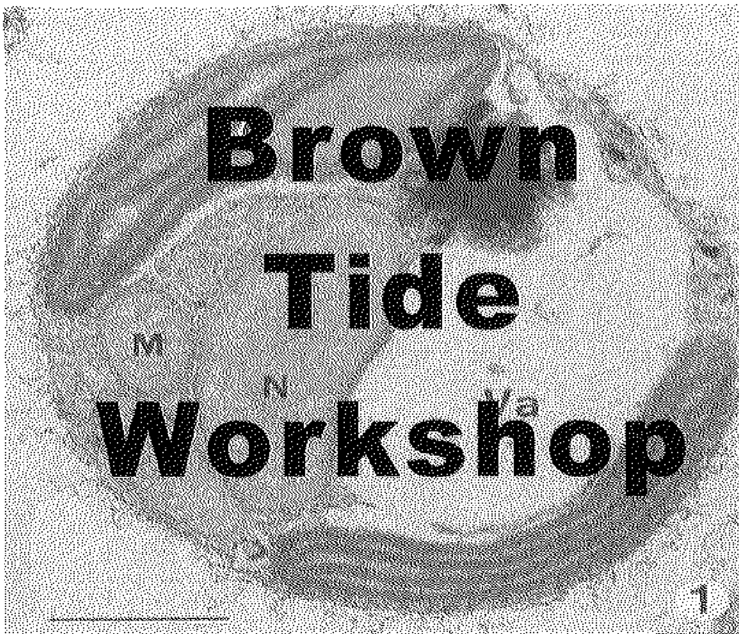
Proceedings:



Brown Tide Workshop

April 6, 2001
Monmouth University
Samuel H. Magill Commons
West Long Branch, NJ

Workshop Sponsors:
New Jersey Marine Sciences Consortium
New Jersey Sea Grant College Program
NJ DEP
Division of Science,
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Maryland Department of Natural Resources
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NEW JERSEY
MARINE SCIENCES
CONSORTIUM

Sea Grant
New Jersey

BROWN TIDE WORKSHOP
April 6, 2001

PROCEEDINGS

September 2001

Hosts
New Jersey Department of Environmental Protection
New Jersey Marine Sciences Consortium and
NJ Sea Grant College Program

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Monmouth University
Maryland Dept of Environmental Protection
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Edited by:
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Acknowledgements
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The electron micrograph © is used courtesy of Dr. Mary Downes Gastrich

We are very grateful for the time and effort of all workshop speakers, especially Dave Caron for flying in from California, including:
Mary Downes Gastrich, Ph.D., NJDEP/DSRT
Mike Weinstein, Ph.D., NJMSC/DSRT
Frank Lutz, Ph.D., Monmouth University
Patrick Dooley, NY Sea Grant
Susan Banahan, NOAA/ECOHAB
Edythe Humphries, Ph.D., DE DNREC
Robert Nuzzi, Ph.D., Suffolk County Dept. of Health Services
David Caron, Ph.D., USC (Lunch Speaker)

Follow-up Conference Calls
Conference calls were scheduled by Mary Downes Gastrich (NJDEP) and Rick Balla (USEPA) on April 30, May 9 and May 15 as a follow-up to the workshop to further discuss issues. Dr. Gastrich prepared materials reviewed in the calls. We would like to acknowledge several people involved in discussions with us on the subsequent development of a regional monitoring plan and a Brown Tide Bloom Index (Gastrich & Wazniak, submitted) including: Ted Smayda, Ph.D., URI; David Caron, Ph.D., USC; Robert Nuzzi, Ph.D., Suffolk County Department of Health Services; Patrick Dooley, NY Sea Grant; Rick Balla, U.S. EPA; Randy Braun, U.S. EPA, Helen Grebe, U.S. EPA, Ann Marie Hartzig, Ph.D., ANS, Mike Ford, NOAA/ECOHAB), and Peter Tango, MD DNR.

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I. About the Proceedings

The Brown Tide Workshop was held on April 6, 2001 at the Samuel H. Magill Commons at Monmouth University in West Long Branch, NJ from 8:00 am to 3:30 pm. A copy of the flyer and agenda are included in Appendix 1. While the workshop's facilitated discussion was recorded, these proceedings are only a summary of comments and recommendations and not a verbatim transcription of all discussions.

The purpose of these proceedings is to briefly summarize all recommendations for monitoring and scientific studies in order to provide information, which will be beneficial to the states and counties in their monitoring programs and to scientists working on the problem. These proceedings are based on facilitator notes and recorded transcripts. Following the workshop, the NJDEP scheduled several conference calls, in cooperation with US EPA Region 2, to continue dialogue on topics raised at the workshop and draft a regional monitoring plan. Included in these proceedings is a regional monitoring plan for brown tide blooms, developed in a series of three follow-up conference calls. In addition, discussions lead to the review and approval of a Brown Tide Index (Gastrich & Wazniak, submitted) by conference call participants (see Acknowledgements)

II. Purpose of the Workshop

Brown tide blooms are a regional phenomenon yet there are few efforts to determine how these blooms are being managed on a regional scale. While there is ongoing research to address the ecology of the brown tide organism, *Aureococcus anophagefferens,* and other important aspects related to brown tides, datasets that are currently being collected by various state environmental protection agencies, state health departments, and other organizations have not been coordinated on a regional basis.

This purpose of this workshop was to assess the effects of brown tides on a regional scale and to focus scientists and managers from northeastern states, where brown tide blooms occur, on management issues, data gaps, priority datasets and recommendations for monitoring of blooms on a state and regional scale. The workshop goals were to:

- *Develop a strategy to characterize brown tide blooms on a regional level based on the best available science*

- *Identify priority state data sets and data gaps*

- *Develop a regional plan for coordination, including recommendations for state monitoring of brown tide blooms and other parameters*

The workshop agenda can be found in Appendix 1 and copies of all presentations in Appendix 2.

III. Workshop Recommendations

The following recommendations were made at the workshop regarding regional monitoring plans and environmental factors that should be monitored during the year.

Part 1. Goals of a Regional Plan

Goals provide a basis for funding and support of further monitoring and scientific studies relating to brown tide blooms. Two goals relating were identified to the recurrence of brown tide blooms:

1) Characterize brown tide blooms and determine whether they are getting worse in the region (e.g., severity, geographic extent, frequency).
2) Document impacts of brown tide blooms on natural resources and/or impairments to the ecosystem.

Regarding 1) above, to document the occurrence of blooms, the only parameter that needs to be measured is *Aureococcus* concentrations. For item 2) above, negative impacts to natural resources include impacts on hard clams, shellfish, and seagrasses. Once it is initially established that brown tides in the coastal waters are getting worse and/or causing or potentially causing, a characterization of water quality in bays having brown tide blooms would provide information about the environmental factors associated with the promotion and maintenance of blooms.

Part 2. Regional Monitoring Plan

2A. General Recommendations

It was generally agreed that the monitoring that the states conduct should be consistent methodologically with each other and that these data could be put together on a regional scale to provide additional information to be used to characterize the problem on a regional scale. Another general recommendation was that monitoring for brown tide should be incorporated into the states' ongoing water quality monitoring programs in order to maximize information pertinent to these blooms.

Other long-term general considerations for states having brown tide blooms included:

- Differentiate monitoring strategies from a mechanistic understanding of causes (more difficult)
- The regional monitoring program should be designed to answer as many questions as possible
- States should have a consistent set of monitoring measurements (e.g., consistent methodology) but different monitoring strategies may be needed by different states
- Monitor areas that do not have brown tide in addition to areas that do in order to understand why it recurs in some places and not others (e.g., areas with high riverine inputs versus areas with high groundwater inputs or differing ratios of these; inlet areas; areas with changing nutrient ratios); areas where blooms do not recur (e.g., Narragansett Bay) are important to study as well as areas where bloom recur (e.g., Long Island, New Jersey)
- Long-term, high frequency data collection is needed for analysis to generate hypotheses regarding causes of brown tide blooms
- Calibrate information from different brown tide enumeration methods
- Archive water samples for future analysis

2B. Specifc Recommendations

While it would be ideal for all states to collect measurements of all the following parameters at all stations on a frequent basis (e.g., weekly, biweekly) throughout the year, at minimum, there are a combination of environmental factors that need to measured at all stations along various environmental gradients while other parameters should be considered for collection at a selected number of stations.

- First, document occurrence/recurrence of brown tide blooms and impact on natural resources (Goals above)

If blooms occur/recur:

- Consider the following issues below in B below and collect the following parameters in a tiered approach, increasing the monitoring as more resources become available.
- To the extent possible, states gear up to develop long-term monitoring programs to collect time series data at selected stations.
- States should begin to document negative impacts or potential impacts of brown tide blooms (or future blooms) on valuable ecological and economic natural resources (e.g., hard clams, eelgrass) in order to develop appropriate long-term funding commitments (e.g., Legislature, etc.)

At concentrations of 35,000 cells/mL, there are potential negative impacts of brown tide blooms. Any concentrations at or above this concentration should be considered as a bloom condition that may have potential negative ecological impact.

To document whether blooms are occurring, brown tide enumeration is necessary. Once this has been established, then other parameters need to be addressed to get a better understanding of why the blooms are recurring.

Monitoring Plan Considerations

Temporal

- Monitoring should commence in April and continue through the fall. If possible, year-round monitoring should be considered for some parameters.
- Monitor frequently during key seasons (e.g., April through September)
- Consider sampling period (e.g., water temperatures increase earlier in southern states than in northern states).
- Archive water samples for later analysis
- Determine phytoplankton composition prior to a brown tide bloom (or preserve samples for later analysis through cryopreservation and/or fixation techniques).

Spatial

- Monitoring should begin in geographic areas of known brown tide blooms and extend along an increasing radius to areas with similar characteristics that have not had Brown Tide blooms. Where possible, efforts should be made to identify where data may be present from other agencies that can be used in the Brown Tide research effort.
- Brown tide monitoring should be built upon existing water quality networks and other relevant monitoring stations
- Monitoring for brown tide organism and blooms should be conducted along various water quality gradients (e.g., salinity, temperature, riverine vs. groundwater inputs and ratios, etc.)
- Consider using USGS tide gauge stations to enumerate *Aureococcus*
- Use estuaries, and if appropriate, National Estuary Programs, and EPA's Coastal 2000 as a starting point
- Areal Extent and Density of Stations
- Monitoring should extend beyond areas of historic blooms via an expanding radius in directions beyond the bloom occurrence; increased spatial extent of monitoring should be performed in areas without brown tide

- Two identical areas with the same type of water supply (one with brown tide, the other without) should be studied intensively
- While gradients need to be monitored in areas with known blooms, appropriate use of stratified probabilistic random sampling design should be considered when conducting some causal research and/or testing the greatest number of hypotheses
- Determine time series vs. concentrated monitoring at selected stations
- Archive water samples for later analysis
- Other monitoring (i.e. *Pfiesteria*) could be coordinated so that this data could be collected and databases could be leveraged. Samples could also be stored digitally.
- The tidal flood network could be leveraged by using basic data monitors (i.e. Data Sondes with chlorophyll sensors)
- The aquaculture communities could gather data
- Citizens could also be trained to do specific monitoring.

Primary List of Environmental Parameters

- Brown tide (= *Aureococcus)* concentrations using antibody techniques, at minimum, but moving towards monoclonal analysis if possible
  This is the most important parameter in determining whether blooms are present and recurring.

Water Quality Parameters that should be assessed in the event of recurring brown tide blooms:

- Salinity (Variations of salinity methods is not a problem)
- Temperature
- Water quality data (e.g., pH, inorganic nutrients, suspended solids, Secchi disk, dissolved oxygen, irradiance/transmissivity) or use available data collected by other monitoring programs
- If not measuring nutrients and other water quality parameters, archive water samples for later analysis
- Tidal Data
- Wind Direction (National Weather Service)
- Irradiance (Are Secchi disks appropriate?)

- Dissolved Oxygen
- Concentrations and ratios of DON / DIN / Total Nitrogen / Nitrate / Nitrite / Ammonia
- Phosphorus
- Chlorophyll
- Phytoplankton
- Silicate (?)

Secondary List of Environmental Parameters

- Inputs/Water Budget
- Residence Times
- Characterization of Groundwater using historical data
- Meteorological Factors
- Ocean Impacts
  - Ratio and volumes of riverine to estuary flows including groundwater and atmospheric inputs.
- Nitrogen Characterization (nitrogen species including concentrations and ratios of DON/DIN) (Nitrates – there should be some quality control and lab inter-comparisons)
  - Urea was discussed as part of a regular State monitoring parameter. It is not in monitored in New Jersey but it is done in Suffolk County and in Maryland.
  - Water quality data (e.g., pH, inorganic nutrients, suspended solids, Secchi disk, dissolved oxygen, irradiance/transmissivity)
  - Lagoons should be mapped according to the percent of groundwater input to design studies of hypotheses
  - Water flows at tidal inlets should be measured
- Chlorophyll, both total and fractionated should be analyzed (There were no consistency issues related to chlorophyll methods)
- Total picoplankton

PART 3. Documentation of Negative Impacts

States having brown tide blooms should begin to document negative impacts to natural resources. This facilitates the characterization of the problem and builds support for future funding. Potential impacts include:

- Eelgrass Impacts (Eelgrass prevalence/absence in areas with brown tide)
- Shellfish Impacts (e.g. hard clams, scallops, mussels, etc.)
- Filter feeding biomass should be evaluated (this would include more than just clam)

PART 4. Data Management

Data Availability

Data are available through federal, state and county agencies. Data that can be used in an assessment of brown tides include:

- Land Use/Land Cover Changes (U.S. Dept. of Agriculture, state agencies, academic institutions, etc.)
- Rainfall data (US National Weather Service or state agencies)
- Meteorological data (US National Weather Service)
- Data on ocean upwellings (e.g., academic centers such as the Center for Remote Sensing at Rutgers University in NJ)
- Tidal flows (USGS)
- Land Use Changes (state agency programs, academic institutions)
- Use of benthic surveys and Mussel Watch Program (NOAA and other agencies)

Recommendations for Data Management and Funding

- EPA Grants may be available to enter nutrient data for nutrient criteria
- NOAA's data archive is a public access point that can be used for Brown Tide data. Data does not have to be generated by NOAA to be a part of the database. The input format is very open. Delaware's suite of data was the prototype. USGS data is not included in this database. The Mussel Watch Program is an easy format to use if States are interested in

including this data in NOAA's database. Excel and Access databases are preferred by NOAA's database.

- Suffolk County, Delaware, and Maryland do not have websites for sharing data; NJ publishes relevant brown tide bloom data on their website during the summer months and reports are available.
- There should be a central bibliography of data on brown tide collated by the National Sea Grant Program.

PART 5: Implementation and Communication Strategy for Brown Tides

- Common terminology should be used in the region to indicate levels of brown tide blooms in relation to ecological effects to facilitate communication among scientists and managers and between agencies and the public
- A Listserv should be developed for brown tide. The *Pfiesteria* Listserv was suggested as a possibility due to the overlap of researchers involved in each.
- Brown tide websites should be available in each state at various locations with appropriate links to other websites and provide available information about blooms in other states (e.g., NY Sea Grant, NJ Sea Grant, state environmental protection agencies, county health departments, etc.)
- Data on brown tide blooms should either be available on agency websites or available upon request
- NY Sea Grant's annual meeting for researchers could potentially be broadened to add a dual track for research concerns of governments and academics in different states; NY Sea Grant has a public meeting for citizens interested in their research
- All interested parties should be included on each other's mailing lists
- A Brown Tide Core Group, comprised of participants at the workshop and states (e.g., NY Sea Grant, NJ Sea Grant, NJDEP, Ocean Co. Health Dept., Suffolk Co. Dept. of Health Services, MD DNR, DE DNREC, academics and others) should convene to steer Brown Tide monitoring/research coordination on a regional basis; NJDEP offered to take the lead on setting up the agenda for these calls

- Maryland, NY Sea Grant, City of Baltimore, and NJ USGS offered to host discussions in the future on this topic
- Region II EPA offered to set-up telephone conferencing for the Brown Tide Core Group for up to 40 people.
- Yearly meetings should be scheduled to provide an update on brown tide monitoring in the states and research studies. Woods Hole Oceanographic Institute meets bi-yearly on harmful algal blooms and could include appropriate topics on brown tide. Other organizations also volunteered their efforts.

Part 6. Conclusions and Next Steps

Several issues were identified that need to be addressed in follow-up conference calls and meetings including:

- A federal umbrella should be identified for brown tides
- Specific state monitoring strategies should be a topic for the first teleconference.

  (e.g., What if you don't have hot spots? How would you monitor? Do we have brown tide blooms? What's the extent? Has there been any expansion? What should be the sampling design? How should states design sampling plans? How should a reconnaissance / monitoring / surveillance program be designed? How should the research be divided up on a regional basis?)
- Characterization Issues (e.g., Is Brown Tide getting worse? Is it causing economic impacts? How should this be measured if current monitoring programs don't address this?)
- Methods Issues (e.g., What concentrations indicate small and large blooms? What is a large bloom? How long does a brown tide bloom last? What is the frequency of a brown tide occurrence in the same area? Current monitoring efforts only indicate a presence/absence of a bloom)
- Development of a correction factor for relating different enumeration methods (antibody methods) will be resolved soon although standardization to one method (e.g., monoclonal analysis) would be helpful regionally. Quality Control for doing this is an issue. Water samples should be saved from different state waters.

- Development of Environmental Indicators of brown tide blooms (e.g., Can impacts to shellfish be used as an indicator of brown tide blooms? How do shellfish show stress and how can this be measured? What is the impact to fisheries? At what level does a brown tide bloom impact shellfish?).

A. Follow-up Conference Calls

Mary Gastrich (NJDEP) will take the lead to schedule follow-up conference calls with a Brown Tide Workgroup. Rick Balla (USEPA) volunteered to set up conference lines.

Topics for the First and Subsequent Conference Calls

- Listservs and websites
- Draft regional monitoring plan for brown tides and MD and NJ monitoring strategy
- Discuss and /or identify available financial resources
- Next year's Workshop
- Communication Strategies
- Regional Research Needs
- Regional bibliography of datasets and studies in the region
- Sharing information in Newsletters, etc.

B. Yearly Meetings

❖ It was recommended that this workshop should be followed by a yearly meeting to provide updates on state programs and research issues. One recommendation was that, at minimum, ECOHAB could provide a one-day seminar for updates on brown tide at the yearly Woods Hole Symposium. Several organizations volunteered to host a one-day meeting on brown tide issues including:

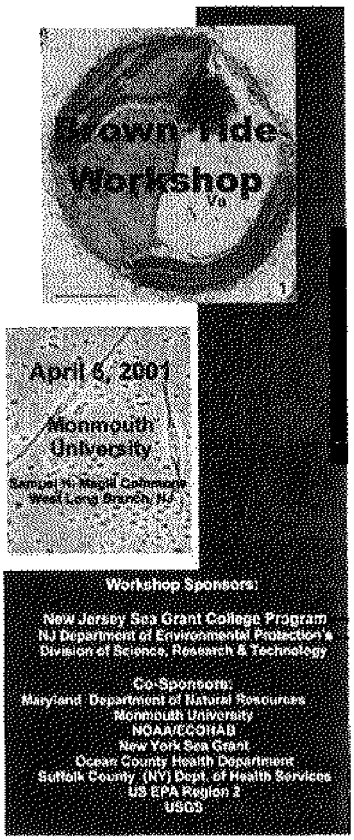
- Maryland Dept. of Natural Resources
- City of Baltimore
- NY Sea Grant (extension of yearly meetings)
- USEPA
- USGS (Maryland)

- USGS (New Jersey)
- NJ DEP

Subsequent to the Workshop on a Conference Call:
- Southampton College (Sandra Shumway)
- University of Connecticut (Senjie Lin)

APPENDIX 1

WORKSHOP FLYER AND AGENDA



AGENDA

April 6, 2001

Monmouth University
West Long Branch, NJ

8:00 am	**Registration, Continental Breakfast**
8:30 am	**Opening Remarks** Mary Downes Gastrich, NJDEP
8:45 am	**Welcome** Dr. Frank Lutz Dean of Science, Technology & Engineering Monmouth University
8:50 am	**Overview of Sea Grant Research on Harmful Algal Blooms** Michael P. Weinstein, NJ Sea Grant; Patrick Dooley, NY Sea Grant
9:10 am	**Summary of Major Scientific Findings of WHOI Workshop and Implications for a Regional Characterization** Susan Banahan, NOAA/ECOHAB, Patrick Dooley, NY Sea Grant and Dave Caron, USC
9:40 am	**Overview: Goals of State & County Programs** Robert Nuzzi, Suffolk County Department of Health Services, NY; Cathy Wazniak, MD DNR, Edythe Humphries, DE DNREC, Mary Downes Gastrich, NJDEP
10:40 am	**Break**
10:50 am	**Moderated Discussion** Mary Downes Gastrich and Becky Jones, NJ DEP Attachment: Environmental Factors Related to Brown Tide Blooms
12:30	**Lunch Speaker: Dr. David Caron, USC:** *Methods for Enumeration of Brown Tide*
1:30 pm	**Moderated Discussion (Continued)** Implementation Strategy Communications Strategy and Next Steps
3:30 pm	**Wrap Up**

A Summary of Scientific Research on Brown Tide Blooms

By Patrick Dooley[1] & Susan Banahan[2]
[1]New York Sea Grant, [2]NOAA-Coastal Ocean Program Office

Brown tide, caused by massive blooms of the pelagophyte *Aureococcus anophagefferens* is a phenomenon of interest and great concern to scientists, resource managers, government officials, anglers and users of affected embayments. In 1985, brown tide was first reported in the Peconic Bays of eastern Long Island, NY, in Narragansett Bay, RI and possibly in Barnegat Bay, NJ. Since 1985, episodic blooms have occurred with variable intensity in the eastern and southern bays of Long Island, Barnegat Bay and Little Egg Harbor, NJ. *Aureococcus* has also been positively identified in embayments along the northeast coast of the U.S., between Portsmouth, NH, and the Chesapeake Bay. Brown tides have been detrimental to the Peconic estuary bay scallop industry, with potential impacts to eelgrass beds. Although brown tides do not appear to pose a health threat to humans, its presence may have negative impacts to recreational fishing, boating and swimming.

Research investigating different aspects of brown tide has been ongoing since these unusual algal blooms first appeared in 1985. A variety of sponsors provided the funding for the early studies. New York Sea Grant, Suffolk County, New York State Department of Environmental Conservation, Stony Brook University's Marine Sciences Research Center, the Environmental Protection Agency Peconic Estuary Program, Brookhaven National Laboratory, and Southampton College have all provided sponsorship and expertise to early studies. A Brown Tide Summit was convened in 1995 to assess the state of knowledge and formulate research recommendations. In 1996 the National Oceanic and Atmospheric Administration (NOAA) Coastal Ocean Program, in cooperation with New York Sea Grant, developed the Brown Tide Research Initiative (BTRI). The BTRI is a 6-year, $3 million program of coordinated research and outreach. The overall objective is to determine the physical, chemical, and biological factors that cause, sustain, and lead to the cessation of *Aureococcus* blooms.

There are several hypotheses that have been or are currently under investigation by BTRI, New York, and other brown tide researchers. Most hypotheses are characterized as "bottom-up" or "top-down," referring to factors that stimulate growth or cause mortality of *Aureococcus*, respectively. The following is a brief description of each hypothesis and current results.

I) Hypothesis #1 – Physical Factors

Physical characteristics such as decreased rainfall prior to a bloom, reduced bay flushing and higher salinity were evaluated. The Peconic Bays and Great South Bay are shallow with a strong groundwater influence; bay flushing does not seem important in these locations for bloom formation. In Narragansett Bay, reduced bay flushing alone cannot account for the 1985-bloom event.

II) Hypothesis #2 – Bottom-Up: Iron as a Growth Factor

Initial reports suggested that *Aureococcus* had an unusually high iron requirement and that inputs of iron or a chelator stimulated *Aureococcus* growth. Current results indicate that *Aureococcus*' iron requirements are much lower than previously reported; on the order of $Fe_Q \not\subset 5$ amol/cell. *Aureococcus* also shows a very high ferric chelate reductase activity compared to the iron needs of the cell. Although iron may not be limiting to bloom initiation, it may be important in bloom maintenance.

III) Hypothesis #3 – Bottom-Up: Inorganic versus Organic Nitrogen

Aureococcus has a mixed mode of nutrition. Under low light conditions, *Aureococcus* can grow heterotrophically by supplementing photosynthesis with organic carbon and nitrogen uptake. Dissolved organic nitrogen (DON) is the preferred nitrogen source of *Aureococcus*, however it can utilize dissolved inorganic nitrogen (DIN). Urease activity has been reported in *Aureococcus* regardless of nitrogen source. It has been suggested that the ability of *Aureococcus* to utilize DON as well as DIN may allow it to out-compete "typical" phytoplankton during periods when DIN levels become limiting (e.g., during summer and fall). When DIN levels are high in early spring, phytoplankton species that use DIN can outgrow *Aureococcus*. As DIN levels drop, *Aureococcus* can continue to grow using DON, even under low light conditions such as found in bays on Long Island.

IV) Hypothesis #4 – Bottom-Up: Groundwater & Nitrogen

This hypothesis suggests that brown tide blooms are controlled by the relative amounts of DIN and DON in the system. For Long Island, groundwater is the dominant source of DIN. During low groundwater flow periods, DIN levels fall while DON levels increase, setting the stage for brown tide to bloom (La Roche *et al*, 1997, Global Change Biology, 3:397-410). This model shows some promise as a predictor of brown tides.

V) Hypothesis #5 – Top-Down: Mortality by Grazing

The role of grazing in brown tide is currently under investigation. In mesocosms containing relatively low *Aureococcus* densities (<35,000 cell/ml), filter feeding by hard clams prevented *Aureococcus* populations from dominating the mixed phytoplankton community. At densities greater than 35,000 cell/ml, *Aureococcus* can inhibit hard clam filter feeding, however no histopathological evidence of toxicity was found. The effect of brown tide is more like starvation or an anitfeedant, unlike other toxin producing harmful algae. Projects currently underway are investigating the role of pelagic food web interactions in bloom initiation and control.

VI) Hypothesis #6 – Top-Down: Mortality by Viral Infection

A virus isolated in 1992 is capable of rapidly lysing *Aureococcus* cells and may be important in the decline of brown tide blooms.

VII) Hypothesis #7 – Bottom-Up: DIN *then* DON

Brown tide blooms may be the precipitated by a succession of events favoring the ability of *Aureococcus* to utilize both inorganic and organic sources of nitrogen and carbon. Brown tide blooms appear to be preceded by a non-*Aureococcus* spring phytoplankton bloom fueled by inorganic nutrients largely supplied through groundwater seepage. As the spring bloom progresses into summer, the groundwater seepage decreases, the supply of DIN decreases, and light penetration in the water column is reduced. The spring phytoplankton bloom then starts to decompose, increasing the availability of DON. This may open a "niche" for the development of a brown tide bloom. Other

DELAWARE STATE PROGRAM

(Brown Tide Workshop, April 6, 2001, New Jersey)
Pre-Workshop Document

HAB Technical Coordinator: Edythe Humphries, Ph.D.
Pfiesteria/Fish Health Project Manager,
DNREC-DWR-ELS
ehumphries@state.de.us; 302-739-4771,FAX 302-739-3491

DNREC-DWR Agency mission as related to Brown Tides/HABs:

Routine surveillance monitoring and emergency response to events that may have a potential ecological and public health impact

Research and Monitoring Efforts: partners and primary area of responsibility

- Delaware Dept. Of Natural Resources and Environmental Control (DE DNREC), Division of Water Resources (DWR), Environmental Laboratory Section, 89 Kings Highway, Dover, DE 19901. Mission as related to Brown Tides/HABs:
    - Performs environmental water quality testing for the State
    - Participates in applied research projects (e.g. field validation of phytoplankton species specific DNA molecular probes)

- The DE DNREC does not currently have a monitoring program for HABS, including *Aureococcus*, but the agency participates in field sampling and molecular probe validation with the University of Delaware (UD), College of Marine Studies, Lewes, Delaware 19958. The DE Sea Grant funds projects on *Aureococcus*. The University of Delaware contacts are:

    David Hutchins, Ph.D.
    Assistant Professor of Oceanography
    dahutch@udel.edu
    Roles/Responsibilities: phytoplankton field studies, in-lab nutritional studies

    Craig Cary, Ph.D.
    Associate Professor of Marine Biology/Biochemistry
    caryc@udel.edu
    Roles/Responsibilities: phytoplankton species specific molecular probe development

Status of *Aureococcus* distribution and concentration in Delaware Inland Bays

- DE DNREC reports, that based on available information, the Delaware Inland Bays (Indian River/Bay, Rehoboth Bay, and Little Assawoman Bay) have NOT experienced a Brown Tide bloom

- **1998 Field Survey**
  June 24, 1998. A limited survey for *Aureococcus anophagefferens* was conducted by the

University of Delaware; species were enumerated using immunofluorescent techniques. (Map to be available at the workshop)

Rehoboth Bay - 5 stations all negative,
Indian River/Bay - 6 stations all negative
Little Assawoman Bay - 2 stations positive

December 2, 1998. Field survey for *Aureococcus anophageferens* by the University of Delaware.
Little Assawoman Bay - 2 stations positive

2001 Brown Tide Projects and Related Activities

- DE DNREC has requested grant funds from the Center for Disease Control through the Delaware Department of Public Health and Social Services to conduct a field survey in Delaware Inland Bays: *Chattonella* spp. are the target organism. Samples will also be analyzed for *Aureococcus*. Funding is pending.

- *Aureococcus* projects are to be implemented by Dave Hutchins, University of Delaware. Project details will be presented at the workshop.

- DE DNREC-DWR is currently developing a Harmful Algal Bloom Surveillance and Response Program. The draft document was completed October 27, 2000; the document currently under revision at the Division level. No state funding is currently available.
  - Currently, there are no specific management goals for any HAB species
  - Currently there are no indicators for any HAB species

Brown Tide Event Protocol

- *Aureococcus* determination will use molecular probe and immunofluorescent techniques. University of Delaware, Dr. Craig Cary and Dr. Kathy Coyne

- In-situ instantaneous field measurements:
  PH, DO at surface (min.) and bottom in mg/L and % saturation, Water Temperature at surface (min) and bottom in deg. C, Salinity at surface (min.) and bottom in ppt
- Laboratory analyses conducted on surface water sample:
  Silicate, Nitrogen (Urea, Dissolved Ammonia, Total Ammonia, Dissolved Kjeldahl, Total Kjeldahl, Total Nitrate/Nitrite, Dissolved Organic Nitrogen, Total Organic Nitrogen, Dissolved Inorganic Nitrogen, and Total Nitrogen), CBOD 5-Day, Phosphorus (Total Dissolved, Total Phosphorus, Particulate Phosphorus, and Dissolved Orthophosphate), TSS, Organic Carbon: Total

- Data stored in DNREC-DWR-ELS Microsoft Access database by activity type (e.g., bloom,)

- Communication: news releases to regional media and posted on State web page species that rely on inorganic nutrients or higher light levels for photosynthesis cannot compete in this environment.

VIII) Hypothesis #8 – Bottom-Up *and* Top-Down

Combining hypothesis #3 and #5, a DON-rich environment combined with decreased bivalve and/or microzooplankton grazing pressure can produce the necessary (or opportunistic) environment for brown tide to bloom.

IX) Culturing and Genetics

The work of several investigators funded through BTRI, Suffolk County, and the Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) program has produced the following results:

- Axenic cultures are now available.
- Seventeen strains of *Aureococcus* are also available for experimental study.
- Redefined taxonomy (Pelagophyte) based on the 18S, sterols and physiology.
- No genetic differences detected among strains of *Aureococcus* (18S rRNA, *rbc*L & RUBISCO), however there is some evidence of genetic diversity on the individual level rather than the population level.
- Laboratory growth conditions have been established.

For additional project details and a list of researchers involved, go to NY Sea Grant's BTRI website (1). For more information on the interagency program, Ecology and Oceanography of Harmful Algal Blooms (ECOHAB), check the websites for the NOAA Coastal Ocean Program and ECOHAB (2 & 3):

1. http://www.seagrant.sunysb.edu/BTRI/btri.htm
2. http://www.cop.noaa.gov/
3. http://www.redtide.whoi.edu/hab/nationplan/ECOHAB/ECOHABhtml.html

In addition, abstracts on scientific findings can be found in "Symposium on Harmful Marine Algae in the U.S., December 4-9, 2000. Symposium Agenda, Abstracts and Participants. Marine Biological Laboratory, Woods Hole, MA

Maryland Brown Tide Monitoring

Cathy Wazniak, MD Department of Natural Resources; Coastal Bays Monitoring Coordinator and contact for Brown Tide Blooms. Contact info: cwazniak@dnr.state.md.us or 410-260-8638.

The Maryland Department of Natural Resources is the lead agency responsible for routine water quality monitoring in the state. Coordination of the Brown Tide monitoring program through DNR, which conducts other state-wide aquatic and wildlife monitoring programs, will insure consistent methodologies and analyses, rigorous quality assurance, integration with state-wide monitoring data bases and other aspects of the program.

No specific environmental quality goals or management goals/obj. for addressing brown tides in MD.

Description of Program and Results:

Samples are collected at 15 stations in Maryland's coastal bays every other week from the end of April to the beginning of July (1999 - present) and in the fall of 2000. Cells counts are done using the polyclonal method.

- *Aureococcus* was first documented in Maryland on Dec. 2, 1998 in Assawoman Bay.
- Spring 1999 MD DNR began monitoring 15 sites for the distribution of *Aureococcus*
- Pigment analysis work suggests that *Aureococcus* was present in MD in 1995, 96 and 97.
- **1999**: Cell counts identified the algae at modest levels (100,000-200,000 cells/ml) in all major coastal bays and tributaries, except Sinepuxent. Significant levels (greater than 200,000 cells/ml) were observed at Ocean Pines, Tingles Island, Trappe Creek and Taylors Landing stations. WILL HAVE MAPS
- **2000**: Sampling reveals cell densities greater than 200,000 cells/ml at Green Run Bay and Tingles Island and densities greater than 500,000 cells/ml at Newport Bay (645,070 cells/ml) and Public Landing (867,003 cells/ml). All the high cell counts were recorded in mid-May through mid-June. By late June, cell densities at all stations had decreased considerably to less than 35,000 cells/ml. Fall samples were all less than 600 cells/ml.

The bloom conditions observed in May and June 2000 are of concern, but **it is currently unclear whether they were prolonged enough in duration to result in significant impacts to bivalves and seagrasses**. Assessments of possible impacts to living resources are being explored.

Major Databases:

There are several other databases that have been collected as part of the coastal bays eutrophication monitoring program that could be related to Brown Tide monitoring efforts. These data sets include atmospheric deposition, meterological data, stream gage record, groundwater flow and chemistry, monthly water quality, macroalgae abundance and sediment chemistry. Some research is being initiated on groundwater upwelling.

Coordination with other agencies/institutions:

Maryland DNR continues to work with researchers at University of Maryland to better understand the factors that control the growth of this organism in the coastal bays and the National Park Service to help conduct monitoring activities.

NEW JERSEY BROWN TIDE ASSESSMENT PROJECT

Program Manager: Mary Downes Gastrich, Ph.D.
New Jersey Department of Environmental Protection (NJDEP), Division of Science, Research and Technology, (609) 292-1895; e-mail: mdownesg@dep.state.nj.us. For Brown Tide Newsletter (summer) and related harmful algal bloom reports, e-mail: http://www.state.nj.us/dep/dsr/browntide/bt.htm

The State of New Jersey is enhancing its implementation of results-based environmental management through its continued participation in the National Environmental Performance Partnership System (NEPPS). NEPPS emphasizes management for environmental results through its use of long-term goals and indicators as measures of environmental progress. The NJDEP has also developed a strategic plan with specific goals and milestones. The following NEPPS/Strategic Plan goals and indicators may be related to brown tide blooms:

> **NJDEP Strategic Goal Area**: Clean and Plentiful Water
> **Subgoal:** Protect and **enhance aquatic life designated uses**
> Condition Indicator S9. Status and trends of phytoplankton blooms in assessed tidal waters and extent of assessment.

Description
Brown tide blooms have recurred in Barnegat Bay and Little Egg Harbor, NJ since the mid-1990s. In 1999, a significant brown tide bloom was reported in these bays (NJDEP 2000; Gastrich 2000a, 2000b). The Brown Tide Assessment Project was developed in 2000 by the New Jersey Department of Environmental Protection (NJDEP) because there are limited available data on these blooms (Gastrich, 2000b). The objectives of the Brown Tide Assessment Project are to: 1) assess the spatial and temporal distribution of *Aureococcus anophagefferens* in Barnegat Bay, Little Egg Harbor and other coastal bays and, 2) quantify the presence of viral-like particles in natural populations of *A. anophagefferens.*

2000: Water samples were collected by the Department at 44 stations from April through December 2000 from Raritan Bay south to Great Egg Harbor Inlet. Biweekly samples were also collected from May-Sept. by the USEPA helicopter. Samples were enumerated for *A. anophagefferens* by Dr. David Caron (USC) using a newly developed monoclonal antibody (ELIZA) method. Other samples were fixed for transmission electron microscopy to quantify the presence of viral-like particles at Lamont-Doherty Earth Observatory of Columbia University.

2001: NJDEP is continuing to monitor brown tide blooms in cooperation with the NJ Marine Sciences Consortium/NJ Sea Grant (NJMSC/NJSG) and Dr. David Caron (USC). Dr. Richard Lathrop (Rutgers Univ./CRSSA) will be analyzing brown tide enumeration data and other water quality data from 2000 and 2001. The NJDEP coordinates with agency scientists (e.g., USEPA, NJMSC/NJSG, Suffolk County Department of Health Services, Ocean County Health Dept in NJ, and others) and academic scientists (USC, Woods Hole Oceanographic Institute, Rutgers University, Columbia University and Southampton College at LIU).

Results: Brown Tide Assessment Project 2000
Figs. 1-3. (Attachment) show the sampling stations in Raritan Bay and northern Barnegat Bay, Little Egg Harbor (LEH) and southern Barnegat Bay, Great Bay south to Great Egg Harbor.

Objective 1. Figure 4. (Attachment) below shows the highest concentrations of *A. anophagefferens* in 2000 that were detected at most all stations sampled in 2000.

- No blooms occurred in Raritan Bay, northern Barnegat Bay, open ocean sites, or in coastal bays between Great Bay and Great Egg Harbor
- Full or significant blooms ($>10^6$ cells mL^{-1}) occurred only in LEH during June
- Highest concentrations of *A. anophagefferens* were at station 1820A (Tuckerton Bay in Little Egg Harbor) in June at $2.2 \cdot 10^6$ cells mL^{-1}. Other stations with over 2 million cells mL^{-1} occurred at stations 1818D at Tuckerton, NJ and at station 1719E near North Beach Haven on the eastern side of the Little Egg Harbor
- Smaller blooms ($>10^5$ cells mL^{-1} and $<10^6$ cells mL^{-1}) occurred in LEH and in some stations to the north in southern Barnegat Bay, to the south of LEH in Great Bay Inlet in June, July, August and at one station in Great Egg Harbor; small blooms were also recorded in three stations in LEH and in one station in southern Barnegat Bay in November with other stations in LEH in the tens of thousands cells mL^{-1}
- For the first time, smaller blooms were confirmed in areas farther south than Little Egg Harbor. The two open ocean sites had concentrations less than 10,000 cells mL^{-1} to the tens of thousands.

Objective 2: Presence of viral-like particles in natural populations of *Aureococcus*. Figure 5. below shows a healthy *A. anophagefferens* (left) and a cell infected with viral-like particles (VLPs) (right) in natural populations during the 1999-2000 brown tide blooms (Gastrich et al., submitted for publication). At least 50 VLPs, each approximately 140 nm in diameter, were observed in some cells in cross section. The number of VLPs per cell can be extrapolated to at least 500 VLPs per total cell volume. While the percentages of VLP-infected cells may have appeared low (8.1% before the blooms, less than 2% during the peak of the bloom and rising to 2.5% at the end of the bloom), the infective potential of VLPs, due to potential numbers of VLPs that could be released into the water column with subsequent reinfection, may account, in part, for the mortality of the blooms within a few days (Gastrich et al., 2000). This research is being conducted at Lamont Doherty Earth Observatory of Columbia University in collaboration with Drs. Anderson and Cosper (CES).

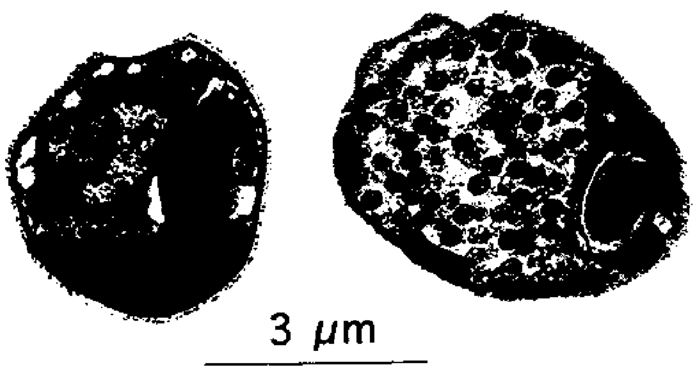
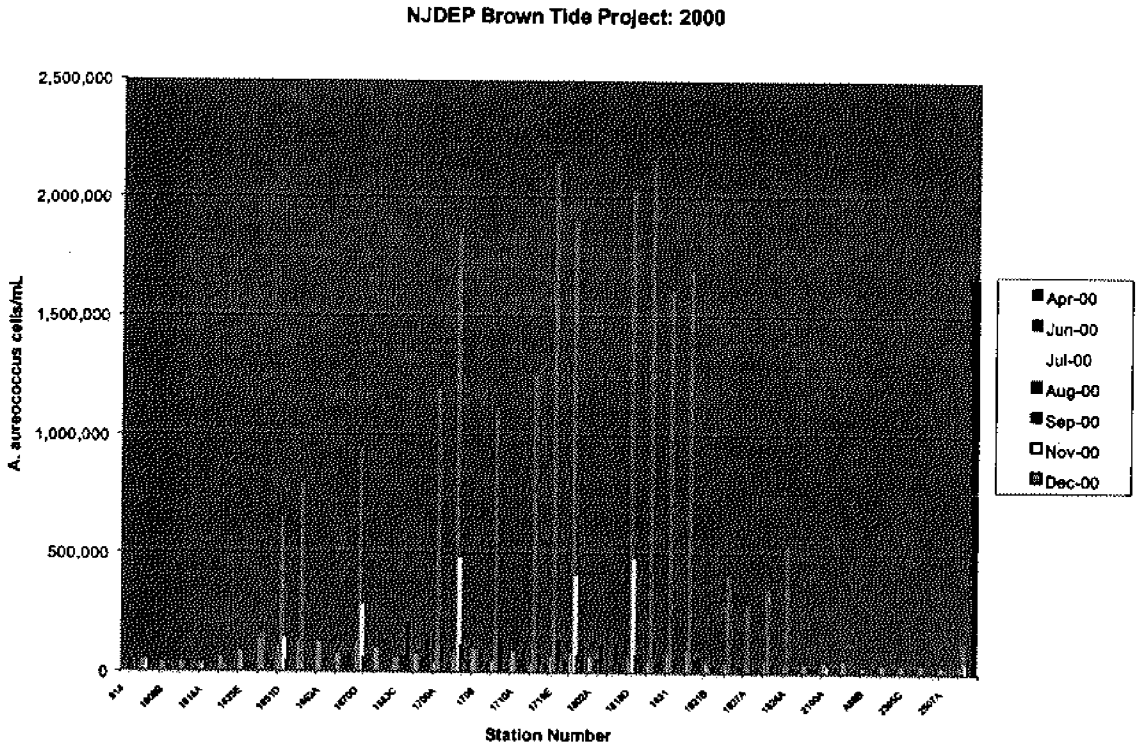


Fig. 4. Results of the NJDEP Brown Tide Assessment: 2000



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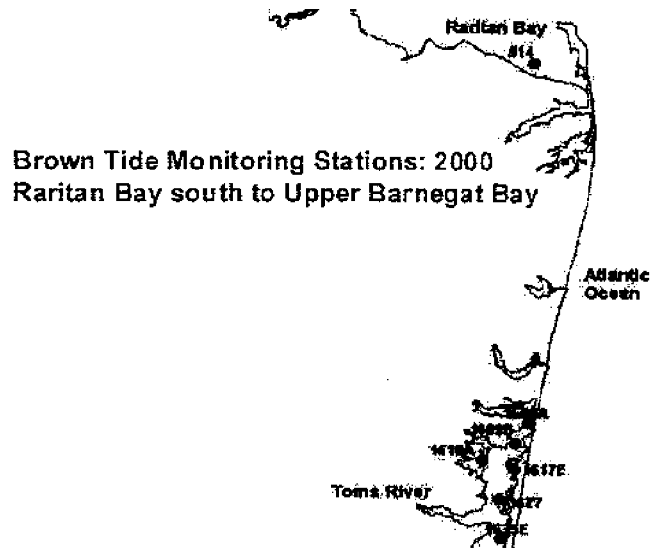
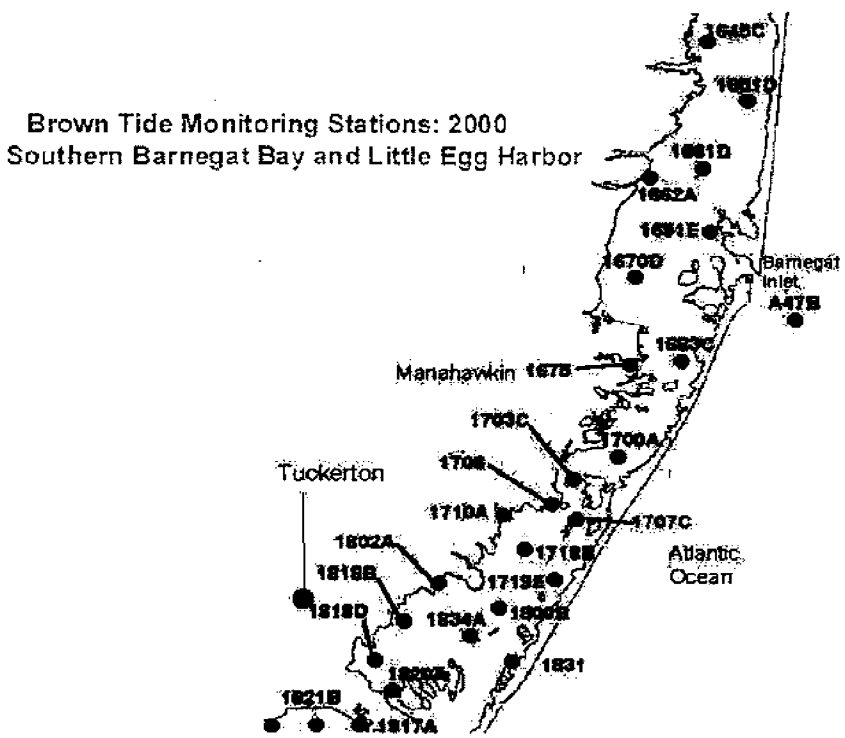
Gastrich, M.D. 2000b. Brown Tide Newsletters. June and July. NJ Dept. of Environmental Protection. Website: state.nj.us/dep/dsr

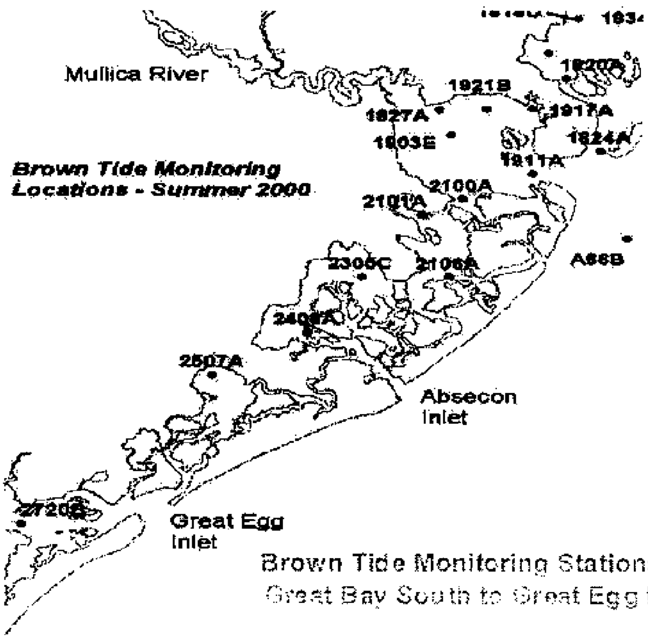
Gastrich, M.D., O.R. Anderson, and E. M. Cosper. 2000. Brown Tide Assessment Project in Barnegat Bay, NJ and the presence of viral-like particles in natural populations of *Aureococcus anophagefferens.* Abstract. Symposium on Harmful Algae in the U.S. Dec. 4-9, 2000. Marine Biological laboratory, Woods Hole, MA.

[NJDEP] New Jersey Department of Environmental Protection. 2000. Annual Report of Phytoplankton Blooms and Related Conditions in the New Jersey Coastal Waters: Summer of 1999. Bureau of Marine Water Classification, Leeds Point, NJ.

Figures 1-3. Maps of Monitoring Stations: 2000 Brown Tide Assessment Project







Brown Tide Monitoring Stations: 2000
Great Bay South to Great Egg Inlet

Brown Tide Workshop
April 6, 2001
Monmouth University

Suffolk County Department of Health Services – Summary of Brown Tide Activities

Robert Nuzzi
Chief, Bureau of Marine Resources

Data have been collected by the SCDHS since the brown tide first appeared in the Peconic and South Shore estuaries (Great South, Moriches, and Shinnecock Bays) in 1985 (Fig. 1).

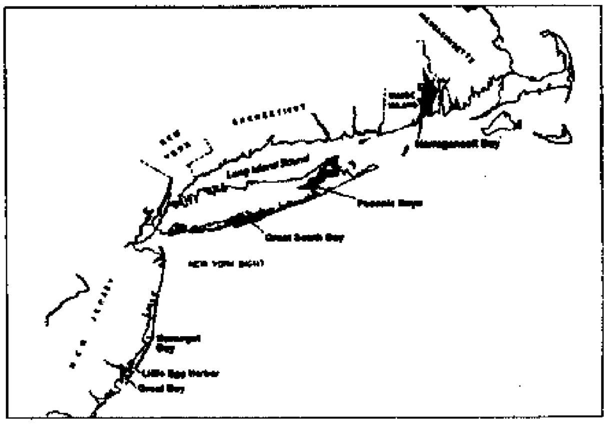


Figure 1. Sites of Brown Tide occurrence.

Because of an immediate, and drastic effect of the Brown Tide (BT) on Peconic Bay scallop (*Argopecten irradians*) landings (Fig.2), a major monitoring program, the Brown Tide Comprehensive Assessment and Management Program (BTCAMP) was initiated in the Peconic Estuary. This consisted of year-round weekly sampling of 10 stations throughout the estuary, with additional stations (tributaries and point sources) sampled less frequently

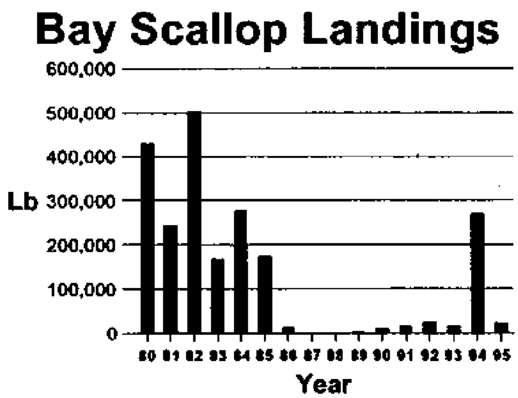


Figure 2. Bay scallop landings, 1980-1995

(Attachment A). The final report for the BTCAMP (SCDHS, 1992) served as a successful nominating document for acceptance of the Peconic Estuary into the National Estuary Program.

The availability of a decade long, high frequency data-base allowed the development of a hypothesis in 1997 (LaRoche *et al.*) suggesting that BT blooms in the Peconic Estuary were related to the relative availability of dissolved inorganic (DIN) and dissolved organic nitrogen (DON) which, in turn, was related to the interannual variability in groundwater flow. Basically, brown tides occurred when the DIN delivered to the estuary by the groundwater was not sufficient to support the "normal" flora, thus leaving an open niche to be filled by *Aureococcus*, which was found in laboratory studies to have the ability to efficiently utilize organic forms of nitrogen. The typical temporal distribution of BT in the Peconic Estuary (except for West Neck Bay, an embayment on Shelter Island) as represented by Flanders Bay (the westernmost area of the estuary), is shown in Figure 3.

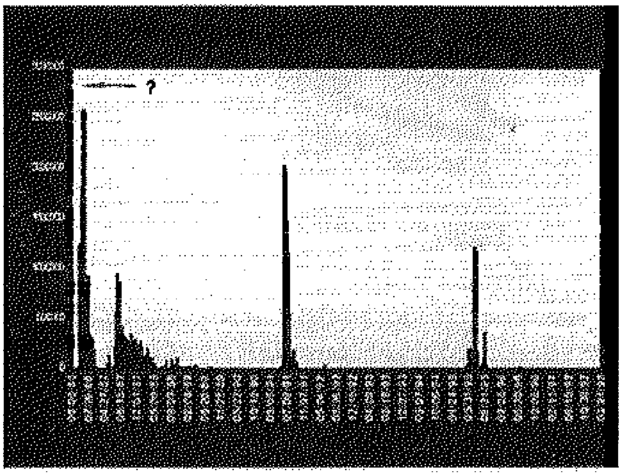


Figure 3. The occurrence of brown tide in Flanders Bay, 1985-1998

As indicated in Figure 4, the field data also indicate that *Aureococcus* cannot tolerate water temperatures above about 25° C.

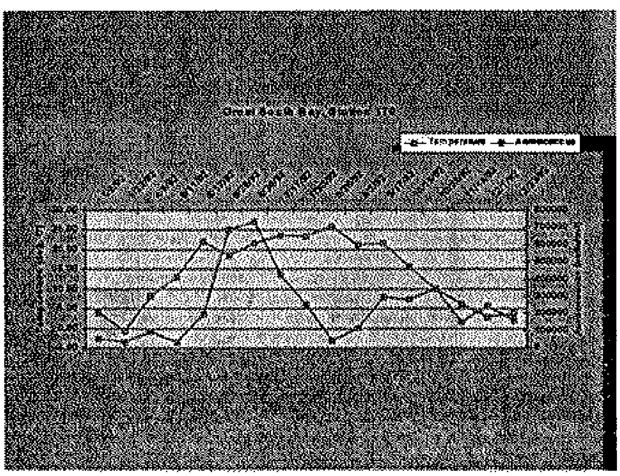


Figure 4. Apparent upper temperature tolerance of *Aureococcus*.

While biweekly monitoring of the South Shore Estuary has only recently been initiated, there has still been a considerable amount of data collected. The temporal distribution of BT at a typical station in this estuary is presented in Figure 5. Comparison of Fig. 5 with Fig. 3 reveals that blooms do not always occur concurrently in these estuaries. While the Peconic Estuary has not experienced a major bloom since 1995, the South Shore Estuary has experienced several, most recently during the fall-winter period of 1999-2000. There is little question that BT blooms have affected the growth and reproduction of shellfish, including the economically important hard clam, *Mercenaria mercenaria*, in this estuary.

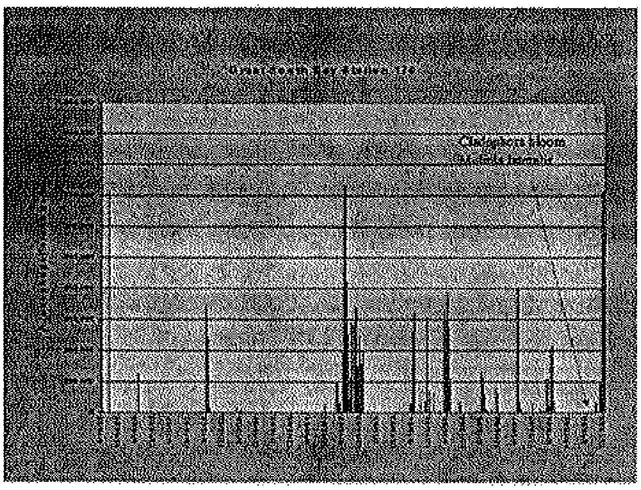


Figure 5. The occurrence of brown tide in Great South Bay, 1985-1999

Although the amount of data available may not be sufficient to provide a mechanism for BT initiation, there are indications that DIN/DON availability may also play an important role in this estuary. Those indications include:

- A decrease in the levels of inorganic nitrogen in the northwest area of Great South Bay resulting from sewering.
- The occurrence of a fall-winter bloom immediately following the die-off of a massive macroalgal (*Cladophora*) bloom in 1999.

The fall-winter bloom of 1999-2000, and previous occurrences of substantial numbers of *A. anophagefferens* in the winter months, strongly suggests that the organism can tolerate, even thrive in, low water temperatures.

In both the Peconic and South Shore estuaries, BT has not been seen within the less saline tributaries, suggesting a preference of the organism for higher salinities.

The temperature and salinity characteristics mentioned above have also been noted for non-axenic laboratory cultures of *A. anophagefferens*. An axenic culture, developed by LaRoche's laboratory with funds from Suffolk County, has recently become available and can be obtained by researchers from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP)[1]

Processed (computerized and proofed) data collected by the SCDHS are available upon request as hard copy or as Excel or Access files. Two hard copy reports (Nuzzi and Waters, 1998 and 1999) are also available, as are various reports from the BTCAMP and PEP investigations.

Suffolk County has been active in funding BT research, and in developing a "Brown Tide Workplan" (Brown Tide Steering Committee, 1998, an ad hoc advisory committee coordinated by the Office of the Suffolk County Executive). It is also represented on the steering committee of the Brown Tide Research Initiative (BTRI) administered by NY Sea Grant.

The SCDHS Bureau of Marine Resources assists researchers by providing field data and by attempting to accommodate researchers who may require special samples, or who may wish to take part in cruises to collect samples requiring special handling.

It is expected that future monitoring of both the Peconic Estuary and the South Shore Estuary will to be on an approximately biweekly basis.

References

Brown Tide Steering Committee, 1998. Brown Tide Workplan: An Overview of Ongoing and Historical Research and an Identification of Future Research Priorities, Interim Workplan, Feb. 23, 1998. SCDHS.

LaRoche, J., Nuzzi, R., Waters, R, Wyman, K., Falkowski, P.G., and D.R. Wallace, 1997. Global Change Biology, 3, 397-410.

Nuzzi, R. and R. Waters, 1998. Draft Peconic Estuary Program Surface Water Quality Monitoring Report 1976-1996, Vols.1 (Narrative) and 2 (Data). SCDHS.

____________________, 1999. Draft South Shore Estuary Reserve Surface Water Quality Monitoring Report 1977-1997, Vols. 1 (Narrative) and 2 (Data). SCDHS.

SCDHS, 1992. Brown Tide Comprehensive Assessment and Management Program, Vols. 1 and 2, and 3 (Appendices), and Summary. SCDHS.

[1] Bigelow Laboratory for Ocean Sciences, McKown Point, West Boothbay Harbor, ME 04575

BROWN TIDE WORKSHOP

LUNCH SPEAKER

Methodology for Enumerating the Causative Alga of Brown Tides in the Northeastern U.S. (*Aureococcus anophagefferens*).

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University of Southern California
Los Angeles, CA 90089-0371

Small size (3 µm) and a general lack of distinguishing morphological characters combine to make the brown tide alga *Aureococcus anophagefferens* difficult to enumerate in natural water samples. Accurate estimation of cell abundance is particularly problematic in situation where *A. anophagefferens* does not dominate the nanoplanktonic protistan assemblage (microalgae & protozoa 20 µm in size). This presentation will provide an overview of the extant methodology for enumerating this algal species, and examine the advantages and shortcomings of each approach for ecological studies and monitoring programs. Methods that are presently employed include electron microscopy, transmitted light and epifluorescence microscopy (including use of polyclonal antibodies), and a new technique employing a monoclonal antibody against *A. anophagefferens* in an Enzyme Linked Immunosorbent Assay (ELISA). These methods vary widely in their accuracy, cost and speed. In addition to these existing technologies, future developments that will improve accuracy and reduce the effort and cost of determining the abundances of *A. anophagefferens* cells will be outlined and discussed. These new advances will include the application of quantitative polymerase chain reaction assays (Q-PCR) and immuno-labelling combined with flow cytometry.

ABSTRACTS

RELATED TO BROWN TIDE

REFERENCE: "SYMPOSIUM ON HARMFUL MARINE ALGAE IN THE U.S."

DECEMBER 4-9, 2000

SYMPOSIUM AGENDA, ABSTRACTS AND PARTICIPANTS

MARINE BIOLOGICAL LABORATORY

WOODS HOLE, MA

AN OVERVIEW OF BROWN TIDE IN THE NORTHEAST U.S.

Gregory L. Boyer
College of Environmental Science and Forestry, SUNY Syracuse, NY 13210

Brown tide blooms are regional, episodic phenomena. The first major occurrences of brown tide (*Aureococcus anophagefferens*) were reported in 1985 in the eastern and southern bays of Long Island (NY), Narragansett Bay (RI), and Barnegat Bay (NJ). Since then blooms of varying severity and duration continue to occur in Long Island waters and Barnegat Bay, and as of summer 1998, brown tide cells were reported in the eastern bays of Maryland and Delaware.

Brown tide blooms can have had serious impacts on shellfish fisheries. The massive bloom of 1985 resulted in the recruitment failure of scallops in the Peconic Bay (Long Island) system. While there have been some modest harvests since that failure, bay scallop populations have not recovered to their pre-1985 levels. In recognition of a need to focus more expertise into understanding this phenomenon, a research program to understand the causes of these blooms was developed. The Brown Tide Research Initiative (BTRI) began in 1996 with two objectives: 1) to isolate, develop, and maintain axenic cultures of *Aureococcus*, and 2) identify the environmental factors that contribute to the initiation, duration, and cessation of brown tide blooms.

Multiple isolates of *Aureococcus* have been established and are maintained in culture at CCMP, however problems with maintaining axenic cultures persist. To address the broader 2nd objective, investigators have been evaluating the relative importance of factors such as DIN, DON, dissolved iron, groundwater loading, and light in *Aureococcus* growth physiology. The environmental and ecological factors examined are water column stability and residence times, changes in the species composition of the microbial plankton community, microbial and bivalve grazing, and elucidating bio-geochemical processes at the sediment-water interface.

Initial assumptions were that *Aureococcus* blooms were, in part, the result of unique growth characteristics. However, research to date suggests that *Aureococcus* shares similar growth characteristics with other picoplankton. Blooms are more likely the result of a combination of ecological and environmental factors.

THE IMPACT OF BOTTOM-UP AND TOP-DOWN PROCESSESS ON THE ABUNDANCE OF *AUREOCOCCUS ANOPHAGEFFERENS* DURING THE 1999-2000 BROWN TIDE BLOOM IN GREAT SOUTH BAY, NY, USA

Christopher J. Gobler, Natural Science Division, Southampton College of Long Island University, Southampton, NY 11968

Beginning in the fall of 1999, the most intense Brown Tide (*Aureococcus anophagefferens*) bloom in NY waters since the 1980's occurred throughout Great South Bay (GSB). The bloom persisted through the summer of 2000, with peak, monospecific cell densities exceeding 1×10^6 cells per mL. To identify factors which contributed to the initiation and persistence of this bloom, a 1-yr observational and experimental field campaign was established in October 1999 at stations in the eastern (Patchogue Bay) and western (Bay Shore Cove) portions of GSB. Nutrient bioassays were conducted in parallel with dilution-style microzooplankton grazing experiments to allow the importance of bottom-up and top-down factors to be simultaneously evaluated. During the study, dissolved organic nitrogen (DON) concentrations present in GSB were high (30 – 40 μM), while dissolved inorganic nitrogen (DIN) levels were relatively low (1 – 4 μM). Although the addition of nitrogen (nitrate or urea) during short-term (24 - 48 h) nutrient bioassays typically enhanced the growth rates of the total phytoplankton community, such additions often had no impact on or caused a decrease in growth rates of *Aureococcus* relative to unamended control treatments. These observations suggest *Aureococcus* was able to subsist on the copious DON pool in GSB, while growth of non-Brown Tide phytoplankton depended on ambient N supply rates. Dilution experiments indicated that grazing rates on *Aureococcus* were significantly lower ($P<0.05$) than those on the total phytoplankton community, suggesting that microzooplankton selectively avoided *Aureococcus* during this Brown Tide event. Significantly higher microzooplankton grazing rates ($P<0.05$) on the picoplankter, *Synechococcus sp.* compared to *Aureococcus* during this bloom event indicated that reduced grazing on the Brown Tide was likely not a function of cell size. The sum of these results demonstrates, for the first time, that both top-down (low grazing rates) and bottom-up (a high DON, low DIN nutrient regime) factors can contribute to the proliferation of Brown Tide blooms on Long Island.

CAUSES AND PREVENTION OF BROWN TIDES IN THE NORTHEASTERN UNITED STATES: THE IMPORTANCE OF TROPHIC LINKS IN THE PLANKTON AND BENTHOS.

David A. Caron[1], Darcy J. Lonsdale[2], Rebecca Schaffner[1], Robert Cerrato[2] & Julie Rose[1]
[1]Department of Biological Sciences, University of Southern California, 3616 Trousdale Parkway AHF 301, Los Angeles, CA 90089-0371 (dcaron@usc.edu)
[2]Marine Sciences Research Center, State University of New York at Stony Brook, Stony Brook, NY 11794-5000

Numerous factors have been implicated in the outbreak of harmful algal blooms of the pelagophyte *Aureococcus anophagefferens*, including specific meteorological, chemical, physical and biological conditions. Few of these factors have been examined experimentally using natural assemblages. Our group has been performing studies in 300 liter mesocosms in an effort to test specific factors that might be involved in the initiation of brown tides, and to identify means of preventing or mitigating these events. We have devised an experimental system in which we have repeatedly induced brown tides, a situation which has allowed us to investigate some of the parameters that have been proposed as factors promoting (and preventing) HABs by *A. anophagefferens*. Our work in prior years demonstrated that additions of specific inorganic (NO_3^-, NH_4^-, PO_4^{-3}) and organic (urea) nutrients, or micronutrients (Fe), were not sufficient to stimulate significant net population growth of the alga, although other phytoplankton species were definitely stimulated. In contrast, physical disturbance to the microbial food web (via submersible pumps) resulted in increases in the absolute and relative abundances of *A. anophagefferens*. Experiments carried out this past summer were aimed (in part) at determining whether or not selective grazing by microbial consumers could explain the success of *A. anophagefferens* in natural, mixed phytoplankton assemblages. The brown tide alga in these experiments reached maximal abundances of >300,000 cells ml^{-1}. Dilution experiments were performed to examine grazing on *A. anophagefferens* (via an antibody assay) and on the total phytoplankton assemblage (via chlorophyll analysis). Interestingly, the results of these studies indicated that rates of mortality for *A. anophagefferens* were generally similar to rates for the whole phytoplankton assemblage. That is, we could not demonstrate that the rejection of the brown tide cells by microbial consumers was a major factor explaining increases in its population abundance. We conclude that both growth stimulation (studies in 2000) and reduced predation (previous work) remain viable explanations for blooms of *A. anophagefferens*. Alternatively, some factor(s) unrelated to grazing that were induced by physical agitation (e.g. altered nutrient availability resulting from the action of the submersible pumps) may explain our results of previous years.

In addition to demonstrating factors involved with bloom initiation, we have repeatedly demonstrated that the presence of hard clams, *Mercenaria mercenaria*, has a dramatic effect on the absolute and relative abundance of the brown tide alga within natural phytoplankton assemblages. Population growth of *A. anophagefferens* in the presence of clams was dramatically constrained under conditions that otherwise resulted in high abundances of the alga. In addition, the presence of hard clams prevented a shift in the phytoplankton assemblage to dominance by brown tide cells. An overview of our experimental results to date will be provided.

BENTHIC-PELAGIC COUPLING AND LI BROWN TIDE

Michael W. Lomas[1], Hugh L. MacIntyre[1], Jeffrey C. Cornwell[1] and Todd M. Kana[1]
[1]UMCES, Horn Point Laboratory, Cambridge, MD 21613, USA

Blooms of the Brown Tide organism *Aureococcus anophagefferens* have been intermittent in the coastal bays of Long Island during the past 15 years. Several hypotheses have been proposed to explain these bloom events, but no single unifying hypothesis has emerged that is widely supported. There are two general working hypotheses, one relating to 'top-down' control involving grazer avoidance and one relating to 'bottom-up' control involving regulation by nutrients. Our prior work on the Brown Tide phenomenon has been focused on bottom-up regulation of *Aureococcus*' photosynthetic physiology and its ability to utilize dissolved organic nitrogen (DON).

The standing stock of inorganic nutrients in the water column is low relative to the standing stock of particulate-bound phytoplankton nitrogen during *Aureococcus* bloom events. Nutrient inputs from the shallow sediments are likely to be important although little is known about sediment fluxes in brown tide waters, particularly with regard to the organic nutrient fluxes. This research program focuses on benthic-pelagic coupling in eastern Long Island bays. Specifically, we have hypothesized that the release of DON from sediments is a significant factor in selecting for the growth and dominance of *Aureococcus* in Long Island Bays. We have developed a conceptual benthic-pelagic model in which the dominance of system level primary production can switch between benthic primary producers (microphytobenthos, macroalgae, or submerged aquatic vegetation) and pelagic primary producers depending upon the distribution of energy (i.e. light) and nutrients in the water column and sediments. These two "states" are connected by feedback mechanisms that are driven by fluctuations in the physical nature of the system.

This model is being studied in selected embayments in eastern Long Island (Quantuck and Flanders Bays). Several sites within these ecosystems were compared in May, July, and September of 2000. Both Bays served as a nutrient trap as dissolved nitrogen concentrations increased 2-fold from May to July, driven solely by increases in organic nitrogen. In accordance with this observation, the planktonic community in both bays shifted to a more heterotrophic state in July associated with increased bacterial activity.

These bays differed substantially in terms of the underwater light environment. Quantuck Bay showed substantial increases in total underwater light attenuation from May to July, whereas in Flanders Bay, total light attenuation didn't change with season although the importance of various components of light attenuation varied. This disparity in seasonal water column light attenuation between Quantuck and Flanders Bays may well have a significant impact on the balance between water column and benthic primary production.

Only Quantuck Bay in July was found to have significant populations of *Aureococcus* (>72,000 cells/ml) coincident with a substantial increase in organic nitrogen and a shift to a pelagic dominated production system. Although no conclusions can be drawn as yet, differences in ecosystem functioning between Long Island Bays are consistent with our conceptual model and the blooming of *Aureococcus*.

GROWTH OF AUREOCOCCUS ANOPHAGEFFERENS ON COMPLEX SOURCES OF DISSOLVED ORGANIC NITROGEN IN CULTURE

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Aureococcus anophagefferens repeatedly blooms in several Long Island (New York, USA) embayments, forming "brown tides" that discolor the water. Surveys of the northeast coast of the USA have shown that *A. anophagefferens* exits in several places that have no records of brown tide. Therefore, the recurrence of the brown tide in Long Island is somewhat unusual. The coastal bays in Long Island are strongly influenced by groundwater, contributing the largest input of fixed nitrogen. In years of draught and low groundwater flow, the supply of NO_3^- is sharply reduced leaving dissolved organic nitrogen (DON) as the largest source of nitrogen available to the phytoplankton. The ability of *A. anophagefferens* to grow on DON has been hypothesized to be an important factor in sustaining the brown tide during periods of dissolved inorganic nitrogen depletion. In order to test this hypothesis we prepared an axenic culture of *A. anophagefferens* and followed growth with a number of DON substrates as the sole source of nitrogen in culture. In addition to commercially available substrates we used >1 kDa ultrafiltered DON isolated from West Neck Bay (WNB) pore waters, Long Island. Efforts to characterize components of the bulk DON pool were conducted in parallel with investigations of the bioavailability of these components.

For the preparation of an axenic, artificial seawater culture of strain CCMP 1784 we modified a protocol published by Cottrell and Suttle (1995 J. Phycol. 29: 385-387). Exponentially growing cultures in F/2 media were exposed sequentially to Penicillin G, Neomycin, Streptomycin, and Penicillin G. Of the antibiotics tested, Penicillin G was the most effective in eliminating bacterial contaminants. Bacterial strains isolated from the culture medium were identified through amplification of bacterial 16S rRNA gene sequences using PCR. Two bacterial strains isolated from the culture media, belonging to the Gamma Proteobacteria and to the Cytophaga-Flavobacteria, were of marine origin.

A. anophagefferens showed good growth on > IkDa WNB DON. To date, this is the first study demonstrating that an autotrophic phytoplankton can grow on bulk DON as the sole source of nitrogen, suggesting that autrotrophs have the capability to enzymatically degrade complex DON. Future research will investigate enzyme pathways involved with DON degradation, and on interactions between *A. anophagefferens* and heterotrophic bacteria.

AMINO ACID OXIDATION AND PEPTIDE HYDROLYSIS IN POPULATIONS SEASONALLY DOMINATED BY *AUREOCOCCUS ANOPHAGEFFERENS*

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Previous studies have demonstrated that the Brown Tide species *Aureococcus anophagefferens* can use dissolved organic nitrogen (DON) to meet its N demand when growing under bloom conditions. Further, elevated levels of DON relative to DIN may create conditions favorable for bloom initiation. Recent results suggest that dissolved organic material (DOM) can be used not only as an N source but as a C source by *A. anophagefferens*; cells can thereby augment autotrophic metabolism with heterotrophy. To evaluate the relative importance of organic and inorganic nutrients to the growth of *A. anophagefferens* and associated picoplankton relative to co-occurring phytoplankton, we are conducted a seasonal study in which we measured inorganic and organic N uptake, organic C uptake, *A. anophagefferens* abundance, and rates of peptide hydrolysis and amino acid oxidation in size-fractionated samples from Quantuck Bay, Long Island. We found that rates of amino acid oxidation and peptide hydrolysis increased between April and June as Brown Tide populations developed and inorganic N sources were depleted. However rates decreased in July when Brown Tide populations collapsed. Much of the amino acid oxidase activity in June, when brown tide was present at about 350,000 cells ml^{-1}, was in the bacterial size fraction (< 1.2 μm) while the bulk of the peptide hydrolysis was in the < 5.0 μm size fraction. As seasonal Brown Tide populations developed, N uptake rates also increased; the < 5.0 μm size fraction accounted for most of the N uptake in May and June.

When dissolved inorganic N (NH_4^+ or NO_3^-) and organic compounds with different N contents (urea, glutamate and glucose) were added to incubations of natural populations, rates of extracellular enzyme activity and N and C uptake were differentially affected among size-fractions, probably as a result of relative differences in the growth stimulation among bacteria, picoplankton, and larger phytoplankton. Virtually all of the peptide hydrolysis was always accounted for in the bacterial (< 1.2 μm) and Brown Tide (< 5.0 μm) size fractions. The effect of N and C additions among size fractions shifted seasonally as did population structure and the availability of combined N sources. Our results suggest that seasonal changes in extracellular enzyme activity and N and C uptake in response to nutrient additions may reflect, 1) the degree to which C or N limits growth in various size-fractions and 2) competition among organisms for limiting nutrients.

We conclude that the relative availability of DIN, DON and DOC may be important in determining the dominant metabolism (autotrophy vs. heterotrophy) of *A. anophagefferens*. In addition, seasonal shifts in population structure affect dominant pathways through which organic material is cycled.

BROWN TIDE ASSESSMENT PROJECT IN BARNEGAT BAY, NJ AND THE PRESENCE OF VIRAL-LIKE PARTICLES IN NATURAL POPULATIONS OF *AUREOCOCCUS ANOPHAGEFFERENS*

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[3]Coastal Environmental Studies, Inc., 83 Carlough Road, Bohemia, NY 11716

Brown tide blooms, caused by *Aureococcus anophagefferens*, were documented in Barnegat Bay in 1995 and were associated with a reduction in growth of juvenile hard clams at a commercial aquaculture facility. In 1999, a significant and extensive bloom was reported in Little Egg Harbor. There are environmental factors present in Barnegat Bay which appear to be similar to other bays (e.g., south shore bays of NY) that have experienced blooms (e.g., shallow bay, elevated salinity, poor flushing time and long residence times). Because of limited data, particularly related to the 1999 brown tide bloom, the New Jersey Dept. of Environmental Protection , in cooperation from the U.S. EPA, established a Brown Tide Assessment Project in 2000 to determine the spatial and temporal extent of these blooms and ultimately to develop a predictive model leading to control strategies. Water samples were collected from up to 44 stations from Raritan Bay to areas south of Barnegat Bay and Great South Bay from April through November 2000. The brown tide organism was enumerated using a newly developed monoclonal antibody (ELIZA) technique. Selected water quality parameters were also measured (e.g., salinity, temperature, nutrients). Water samples from 1999 and 2000 were also collected and viewed, using transmission electron microscopy, to quantify the presence of viral-like particles (VLPs) in natural populations of *A. anophagefferens.*

The results of monoclonal analysis confirmed that several sites in Little Egg Harbor, NJ including Ship Bottom and Tuckerton, had a substantial brown tide bloom with the highest concentrations of *A. anophagefferens* over a million cells per mL representing full bloom conditions in early June. The highest cell counts were observed in the vicinity of Little Egg Harbor, below the Barnegat Inlet, with cell counts up to 2.2×10^6 cells per mL on June 8 which decreased to 3.0×10^4 cells per mL in early July. At Tuckerton, the counts reached two million per mL on June 15 and decreased to a low of 3.5×10^4 cells per mL on July 12. At Ship Bottom, the cell numbers reached 1.8×10^6 cells per mL on June 23 and decreased to 4.1×10^5 cells per mL on July 12. While concentrations of *A. anophagefferens* exceeded 10^5 cells/mL (representing smaller blooms) in areas near and just north of the Barnegat Inlet and south of Little Egg Harbor in Great Bay, representing an extended geographic occurrence of these blooms, full bloom concentrations were not observed in these areas. The severe brown tide bloom appeared to be concentrated in Little Egg Harbor and the southern part of Barnegat Bay between Barnegat Inlet and Little Egg Inlet.

For the first time, intracellular viral-like particles (VLPs) were quantified in the brown tide organism, *Aureococcus anophagefferens* during the 1999 brown tide bloom in Barnegat Bay and Little Egg Harbor, NJ. Up to 8% of the total individual *A. anophagefferens* cells examined (Total = 4,380) from natural populations contained VLPs (ca. 140 nm in diameter). The intracellular VLPs were similar in size and morphology to viruses reported in natural populations of *A. anophagefferens* from Narragansett Bay over a decade earlier and were also similar to observations of intracellular viruses that were inoculated previously into laboratory cultures of *A. anophagefferens.* Preliminary data also confirms the presence of VLPs in natural populations of *A. anophagefferens* sampled during the brown tide bloom in 2000 in Barnegat Bay. The presence of VLPs in natural populations of *A. anophagefferens* is significant because they have not been previously quantified in field blooms. The role of viral infection needs further study in relation to the bloom dynamics. Further sampling is needed in 2001 to continue the spatial and temporal analysis including an assessment of environmental factors that may be associated with the promotion and sustenance of brown tide blooms in Barnegat Bay.

PHYSICAL, CHEMICAL AND BIOLOGICAL CONDITIONS ASSOCIATED WITH THE NARRAGANSETT BAY BROWN TIDE

Theodore J. Smayda and David Borkman

A retrospective analysis of the 1985-1986 *Aureococcus anophagefferens* brown tide in Narragansett Bay was carried out under the auspices of the Brown Tide Research Initiative. Regional climatic events appear to have been important in triggering this event. Evidence for this includes: regional synchroneity and correlations with the North Atlantic Oscillation Index (NAO) and proxies for atmospheric/weather parameters, including wind direction, strength, rainfall, cloudiness, temperature and groundwater levels. Correlations occurred between the NAO and Groundwater Index (GW), similar to that reported for Long Island brown tide bloom sites. There is no strong evidence to suggest reduced flushing was the basic cause of the 1985 brown tide outbreak, contrary to previous views and unlike that proposed for Long Island embayments. The issue of wether Narragansett Bay was environmentally different in 1985 relative to long term patterns was addressed applying Principal Components Analysis, and revealed that 1985 was a unique year within the 32-year time series analyzed: it clusters with drought years 1956, 1966 and is among the three years of highest irradiance and lowest river flow. The role of nutrients and grazing control in this bloom event, and the commonalities and divergences in brown tide dynamics in Narragansett Bay, Long Island embayments and Laguna madre are also considered. Analysis of the 38-year time series for Narragansett Bay suggests brown tide events there will occur twice per century.

ROLE OF LONG-TERM VARIATION IN FRESHWATER INPUT AND DISSOLVED ORGANIC NITROGEN DELIVERY IN THE INITIATION AND MAINTENANCE OF THE 1985 NARRAGANSETT BAY BROWN TIDE

David Borkman and Theodore J. Smayda
Graduate School of Oceanography, University of Rhode Island

The 1985 brown tide bloom of *Aureococcus anophagefferens* in Narragansett Bay was the dominant HAB event in a nearly 40-year time series of weekly observations of Narragansett Bay phytoplankton. Mechanisms responsible for this summer-long bloom, which occurred simultaneously in several estuaries along the Northeastern US coast, are not fully known. Several features of *A. anopha*gefferens physiology and ecology indicate that freshwater input patterns with accompanying patterns in delivery of organic nutrients may play an important role in bloom initiation and maintenance. We present a time-series of estimated dissolved organic nitrogen (DON) loading and related physical data for Narragansett Bay, Rhode Island. Trends in Narragansett Bay riverine and groundwater DON input are analyzed, indicating a relative peak in riverine DON concentration accompanied by a decrease in the riverine nitrate:DON ratio in 1985. Levels of *A. anophagefferens* that may have been supported by this DON delivery are estimated and compared to observed abundance in Narragansett Bay. The spring of 1985 was marked by a departure from the usual relation between groundwater levels and salinity in Narragansett Bay, indicative of a change in Narragansett Bay estuarine circulation patterns. Changes in freshwater delivery into Narragansett Bay in 1985 and accompanying relative increases in DON delivery are implicated in the initiation and maintenance of the 1985 brown tide.

UNUSUAL STEROLS FROM HARMFUL ALGAE: MORE THAN BIOMARKERS?

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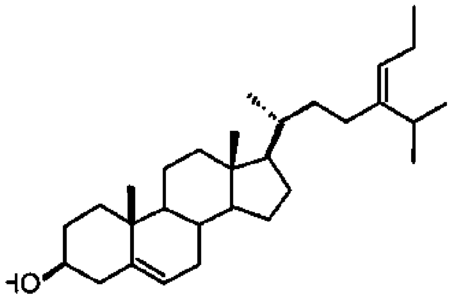
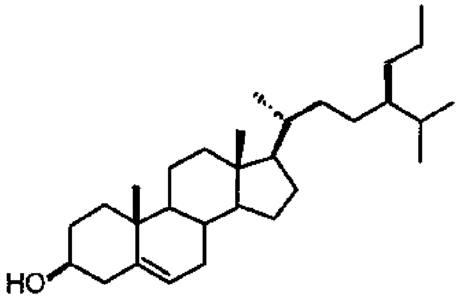
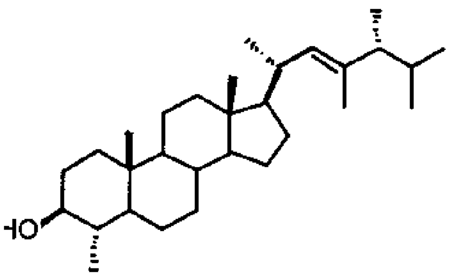
Unusual sterols found in marine algae are useful biomarkers. Dinosterol is found only in dinoflagellates and 24-propylidenecholesterol is found only in pelagophyte algae. We have found useful sterol biomarkers for *Aureococcus anophagefferens*, *Aureoumbra lagunensis*, and *Gymnodinium breve*. The biomarker for *Aureoumbra lagunensis* is an extremely rare sterol, while the sterols characteristic of *Aureococcus anophagefferens* and *Gymnodinium breve* are unique to these organisms. These biomarkers allow us to probe the sediment record for evidence of past harmful algal blooms and offer an alternative method of detection for harmful algae.



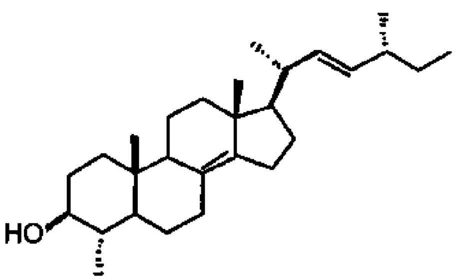
From *Aureococcus anophagefferens*



From *Aureoumbra lagunensis*



From dinoflagellates



From *Gymnodinium breve*

Why do these algae produce these unusual sterols? What benefit is it to them? Many important grazing organisms rely on dietary sterols. Arthropods and molluscs lack the capability of *de novo* sterol biosynthesis and fulfill their sterol requirement by modifying dietary sterols. It is likely that unusual algal sterols interfere with this process. The inability for grazers to meet their sterol requirements may be important to bloom formation and maintenance.

TIME SERIES OF OPTICAL PROPERTIES AND BLOOM ECOLOGY FROM A BROWN TIDE AND AN ADJACENT CONTROL SITE IN LONG ISLAND

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Since 1985 Long Island, New York embayments have been plagued with recurrent blooms of the 2.5 μm chrysophyte *Aureococcus anophagefferens*. Several hypotheses abound regarding the ecological controls on these blooms, including the ability of this species to out compete others due to its unique capacity to utilize organic nitrogen and carbon. These blooms, referred to as brown tides due to the color they impart to the water, were the focus of this study. From 17 May-8 June 2000, a time series of ocean color, particulate and dissolved absorption, dissolved fluorescence, particulate scattering, phytoplankton pigments, and particle size distributions were collected from two Long Island embayments. A brown tide developed in Quantuck Bay, whereas in West Neck Bay *A. anophagefferens* cells were in low concentrations and represented an insignificant contribution to the algal community.

During the brown tide in Quantuck Bay, spectral radiance reflectance changed in both magnitude (brightness) and shape (Fig. 1a), while phytoplankton and colored particulate organic material (CPOM, non-phytoplankton) contributed significantly to total absorption of blue photons (Fig. 1b,c). Phytoplankton size-fractionated absorption demonstrated that most of the cells were between 1-3 μm, consistent with *A. anophagefferens*. Spectral shape indicated that after the first day of the study, algal community structure remained constant. Absorption by the <0.2 μm CDOM in Quantuck Bay approximately equaled that by phytoplankton (Fig. 1d). Near the end of the time series the contribution by the 0.2-0.7 μm size fraction increased, suggesting new CDOM release or colloidal aggregation.

The control site exhibited a different suite of optical properties and size contributions. The relatively constant shape and slight magnitude fluctuations detected in radiance reflectance in West Neck Bay suggested minor community structure alterations (Fig. 1e). The <0.2 μm CDOM dominated the absorption coefficient (Fig. 1h). The concentration and spectral shape of this component remained invariant during the study. Phytoplankton and CPOM absorption were comparable (Fig. 1f,g). CPOM displayed no variation in shape; however, phytoplankton absorption, due mainly to cells between 1-3 μm and some <1 μm, changed in spectral shape indicating that the algal community varied slightly.

Further investigation of the optical properties separated into size-fractionated components provides characteristics of bloom ecophysiology. It is feasible that modeling these parameters from remotely sensed ocean color will provide a breadth of knowledge about bloom dynamics.

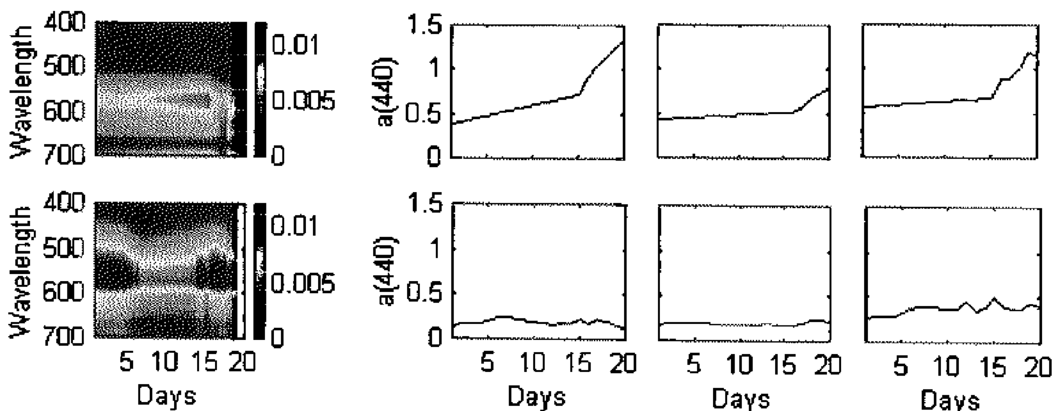

Figure 1 a) Spectral radiance reflectance, b) phytoplankton, c) CPOM, and d) CDOM absorption at 440 nm at Quantuck Bay. Plots e-h display those measurements for West Neck Bay.

MOLECULAR CLONING AND ANTISERUM DEVELOPMENT OF CYCLIN BOX IN THE BROWN TIDE ALGA *AUREOCOCCUS ANOPHAGEFFERENS*

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Cyclins can be useful cell cycle markers for growth rate studies on harmful algal blooms. In this study, a gene fragment corresponding to cyclin box was cloned for the brown tide alga, *Aureococcus anophagefferens*. This algal gene fragment, designated as *Btcyc11*, was most similar to cyclin B. Based on the deduced amino acid sequence, oligopeptides were synthesized and used to raise an antiserum which reacted on western blots with a protein of about 63 kDa, the same size as cyclin B in other organisms. The cyclin B-like protein recognized by this antiserum, and the mRNA amplified using the primers, were more abundant in exponential cultures and decreased markedly in stationary cultures. This protein also appeared to be cell cycle-dependent. Immunofluorescence labeling showed that this antiserum specifically stained a protein in *Aureococcus* cells and had no cross-reaction with bacteria that were present in the algal culture. The *Btcyc11* sequence and the antiserum will provide a useful tool for studies on regulation of in situ growth rate for this brown tide alga.

AUREOCOCCUS AND UREA METABOLISM IN LONG ISLAND BAYS

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Nutrients have been, and will continue to be, targeted for research and management in the coastal zone, due to the links with increased phytoplankton production, and the associated shifts in ecosystem functioning. Such research, until recently, has focused predominantly on dissolved inorganic nitrogen, even though dissolved organic nitrogen (DON) can be a quantitatively larger pool. The inability to characterize and quantify significant fractions of the DON pool has been a major hindrance to this research. Although poorly characterized, this DON pool can consist of low molecular weight, labile components such as urea, amino acids, and proteins. Dogma suggests that bacteria are better competitors of organic substrates than phytoplankton simply due to their smaller size, and their heterotrophic nature.

Although marine bacterial hydrolysis of urea, through the activity of the enzyme urease, has been known for nearly 70 years, more recent studies have suggested that the hydrolysis of urea by phytoplankton can be substantially more important in coastal systems. In particular, it has been suggested that an enhanced ability to hydrolyze urea and other simple organic molecules may be one physiological advantage to the formation of blooms of the Long Island brown tide organism, *Aureococcus anophagefferens*.

As part of a larger ecosystem level sampling effort to understand the bloom dynamics of *Aureococcus*, urease activity of the water column biota was measured in two Long Island bays, Quantuck and Flanders Bay, in May and July of 2000. In May, both bays were characterized by similar mean urease activities ~0.3 µmoles NH_4^+ produced /liter seawater/h. Although phytoplankton biomass (estimated as chl *a*) was twice as high in Quantuck Bay as in Flanders Bay, it was not correlated to urease activity and neither bay had significant populations of *Aureococcus*. The seasonal transition from spring, May, to summer, July, resulted in substantial changes in both the rates and patterns of urease activity, as did a significant (>4") rain event during our period of sampling. July urease activities in Flanders Bay were significantly related to chl a, and suggested that bacterial urease activity was less important as shown by the non-significant y-intercept. Additionally, *Aureococcus* was not detected in the phytoplankton assemblage. To the contrary, in Quantuck Bay, urease activities were not related to chl a and were 3-4 times greater than would have been expected based on the chl a- urease relationship observed in Flanders Bay. Although the phase of the *Aureococcus* population in Quantuck Bay in July could not be assessed, *Aureococcus* was present at substantial numbers (>72,000 cells/ml and ~14% of chl a biomass). The rainfall event in July did not appear to alter the relationship between chl a and urease as measured in Flanders Bay, but in Quantuck Bay, urease activities were reduced 4-fold, without a change in chl a, to values that concurred with the measured chl a-urease relationship in Flanders Bay.

These observations suggest that under similar nutrient and phytoplankton biomass conditions (i.e. bulk chl a), these two bays differ substantially in the metabolism of urea during the summer. The functional relationship between components of the planktonic assemblage (e.g. *Aureococcus*) and urea remain to be fully understood.

THE POTENTIAL FOR ANTHROPOGENIC TRANSPORT OF THE BROWN TIDE ORGANISM, *AUREOCOCCUS ANOPHAGEFFERENS*

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Aureococcus anophagefferens is a pelagophyte that is responsible for the harmful brown tides that have affected New Jersey, Rhode Island and New York. The known range of *A. anophagefferens* has increased since the 1990 survey (Anderson et al.), with the organisms now found as far south as Maryland and Delaware. *A. anophagefferens* has also caused blooms in Saldanha Bay in South Africa beginning in 1997 (South African Marine and Coastal Management, 1998/1999). The geographical distribution of *A. anophagefferens* appears to be increasing. Two possible ways that the brown tide could be introduced to new areas are anthropogenic transport of the organism in ballast water or water retained in recreational boats.

Experiments were conducted to determine the potential for *A. anophagefferens* to survive conditions similar to those that may be experienced in ship ballast tanks. Cultured brown tide was able to survive for at least 30 days in the dark when stored at 12°C. Temperature may play a role in survival of brown tide in the dark, as cultures recovered fastest when stored at 12 C. We are investigating the influence of environmental factors like temperature, salinity and presence of inorganic or organic nutrients on how long *A. anophagefferens* can survive in the dark.

INTRASPECIFIC VARIATION AMONG CULTURES AND BLOOM SAMPLES OF *AUREOCOCCUS ANOPHAGEFFERENS*

Joseph Stabile[1,2], Grace Montemarano[2], Frank Fazio[1] and Isaac Wirgin [2]
[1]Iona College, Department of Biology
[2]New York University School of Medicine, Department of Environmental Medicine

During the past decade blooms of the brown tide microalga, *Aureococcus anophagefferens*, have occurred sporadically in Peconic and Great South Bays of Long Island, N.Y. Blooms of the brown tide vary annually in the timing of their onset, duration and intensity. It is hypothesized that temporal and spatial variability in bloom characteristics is due to underlying genetic variation among populations of *A. anophagefferens*. This hypothesis was tested by sequence analysis of the internal transcribed spacer regions (ITS1 and ITS2) of rDNA. Brown tide homologous PCR primers were developed and used to amplify ITS sequences directly from water samples and cultured isolates. PCR products were cloned in pCR2.1 vector and 15 to 25 recombinants per sample were sequenced. Sequence data were obtained from 1995 summer bloom samples from West Neck Bay, Flanders Bay and Great South Bay and a 1999 winter bloom sample from Great South Bay. Sequence data were also obtained for cultured isolates CCMP 1784, 1785 and 1790 from L.I., N.Y. and CCMP 1794 from Barnegat Bay, N.J. *A. anophagefferens* is unique among eukaryotes in that it has extremely high levels of polymorphic ITS sequences within individuals. Cloned PCR fragments were assigned composite "types" on the basis of having unique combinations of polymorphic nucleotides. A total of 46 and 43 composite types were observed for ITS1 and ITS2, respectively. Monte Carlo based chi-square analyses were performed to determine if there were significant differences in the frequencies of ITS types between and among bloom samples and cultured isolates. Statistically significant differences in the frequency of ITS types were observed for cultured isolates CCMP 1785 (L.I.) and CCMP 1794 (N.J.) in comparison to all other cultured isolates and L.I. bloom samples. This indicates that not all cultured isolates are representative of *A. anophagefferens* blooms and that there may be some geographic differentiation between the two east coast sites. In addition, chloroplast DNA restriction fragment length polymorphism analysis has confirmed that there are genetic differences among cultured isolates. Interestingly, there were no significant differences in the frequency ITS types among the 1995 summer and 1999 winter bloom samples from L.I. This suggests variability at the peak of a bloom is low or that the resolution of the PCR-cloning technique is too low to distinguish closely related populations. Currently, Single Stranded Conformation Polymorphism analysis is being used to re-evaluate the data obtained with our cloning experiments.

APPENDIX 3

SUMMARY: BROWN TIDE CONFERENCE CALLS: FOLLOW-UP TO WORKSHOP (APRIL 30, May 9, May 15, 2001) and REGIONAL BROWN TIDE MONITORING PLAN

The purpose of the three conference calls was as follow-up to the April 6 Brown Tide Workshop held at Monmouth University on April 6, 2001. The conference call agendas included a discussion of the monitoring plans for the states of MD and NJ, the draft regional brown tide monitoring plan (sent previously by Mary Gastrich,4/10/01), development of Brown Tide LISTSERV, and development of a Brown Tide Website.

Conference Call Attendees:
Mary Gastrich (NJDEP), John Dillow and Cherie Miller (USGS-MD)
Pat Dooley (NY Sea Grant), Rick Balla (EPA), Ted Smayda, Ph.D., URI; David Caron, Ph.D., USC; Robert Nuzzi, Ph.D., Suffolk County Department of Health Services; Patrick Dooley, NY Sea Grant; Rick Balla, U.S. EPA; Randy Braun, U.S. EPA, Helen Grebe, U.S. EPA, Ann Marie Hartzig, Ph.D., ANS, Mike Ford, NOAA/ECOHAB, Peter Tango, MD DNR, and Christopher Gobler, Ph.D., Southampton College, Long Island University

REGIONAL MONITORING PLANS FOR 2001: Considerations

- John Dillow indicated that USGS-MD may monitor nutrients in freshwater streams in coordination with MD DNR; Bob Nuzzi cautioned that nutrient monitoring in relation to brown tide is not a typical "nitrogen" paradigm (organic nitrogen may be just as important as inorganic nitrogen, etc.);
- Mary Gastrich indicated that NJ revised the 2001 sampling design from 2000 to monitoring fewer stations along a gradient where blooms are not known to occur to areas where it occurs, selecting stations from 2000 that are also USGS tidal gauge stations and EPA Coastal 2000 stations. The NJDEP is conducting nutrient analysis for Coastal 2000 and may analyze nutrients, including organic nitrogen, at a selected number of brown tide stations. NJ Sea Grant asked Paul Bologna (Rutgers) to submit a proposal for funding to conduct a small field study to document negative impacts of

blooms on eelgrass. NJDEP is funding a hard clam survey this year (last one conducted in 1988) which will provide background for future experimental studies to document negative impacts of blooms on shellfish. NJDEP is also working with USGS-NJ on a proposal for 2002 to assess hydrologic time series data on ground water well altitudes, which will be used to estimate stream flows into bays that have had brown tide blooms. Rick Lathrop from Rutgers is continuing to analyze 2000-01 data. Dr. David Caron (USC) will continue to enumerate brown tide through monoclonal analysis. Bob Nuzzi suggested comparing pre-brown tide quantity, and quality data where available, to post-brown tide data.

- Cathy Wazniak, MD DNREC, reported on their monitoring plan for 2001

A draft regional plan, prepared by Mary Gastrich, was reviewed and discussed by the group. Several revisions were made and the final regional monitoring plan is provided below.

BROWN TIDE REGIONAL MONITORING, IMPLEMENTATION AND COMMUNICATION PLAN

BACKGROUND:

Brown tide blooms are caused by a minute (ca. 3 µm) pelagophye alga, *Aureococcus anophagefferens* which can be enumerated by immunofluorescent antibody techniques. For each state, the answers to management questions below will lead to the development of different objectives and different monitoring plans. A regional plan will allow each state to monitor at the level appropriate for management objectives and will provide data and information to be used in a regional characterization of brown tide blooms.

I. Management Questions Which Drive Monitoring Programs:

1) Are brown tide blooms currently occurring in the state?
2) What are the extent, severity and duration of brown tide blooms?
3) What are the ecological impacts on natural resources because of brown tide blooms?
4) What are the environmental factors/causes, which are related to the occurrence, maintenance and/or termination of brown tide blooms in specific areas?

II. Consistency of Terminology

The group agreed that using common terminology in the region would facilitate communication and understanding between scientists and managers and between agencies and the public regarding severity and concentrations of brown tide blooms. While this group initially reviewed a draft brown tide bloom index, the group eventually agreed upon a three-tiered categorical system developed by Mary Gastrich and Cathy Wazniak which related *Aureococcus* concentrations to documented negative impacts on natural resources. The Brown Tide Index includes the following:

Brown Tide Bloom Index (Gastrich & Wazniak, submitted)

Category 1 brown tide blooms: <35,000 cells per milliliter (no reported negative ecological impacts)

Category 2 brown tide blooms: >35,000 to 200,000 cells per milliliter (potential moderate to severe ecological impacts on shellfish)

Category 3 brown tide blooms: >200,000 cells per milliliter (potential severe ecological impacts to shellfish, seagrasses and plankton)

III. State Monitoring Strategies, Monitoring Objectives and Monitoring Plans

For each management question above, there are different monitoring objectives and a different monitoring plan for each state. The Monitoring Strategies below are tiered according to extent, severity and duration of brown tide blooms and reflect a corresponding level of effort.

Tier 1: Delaware Case (Focus: Measure background concentrations of brown tide organism and document any reported impacts)

Aureococcus concentrations have not yet been measured or have been measured but no brown tide blooms have been observed. No negative effects on natural resources have been observed or measured.

State Monitoring objectives:

- *Determine status/trends of ambient surface waters in the state*
    - **Recommended Monitoring Plan**: Time series data from existing state water quality monitoring networks
- *Determine background levels of brown tide organism*
    - **Recommended Monitoring Plan**: Random stratified sampling of selected stations, included in the state's water quality monitoring networks, and some focused sampling (based on known contributing factors in other states) to document background concentrations of brown tide organism in selected coastal bays
        - **Spatial**: small number of samples over a wide geographic area for enumeration of brown tide organisms
        - **Temporal**: April-May-June and perhaps in Sept.
        - **Parameters**: Brown tide enumeration

- *Document anecdotal reports of negative impacts to natural resources (e.g., clammers, Univ. of DE research results of eelgrass studies)*

Tier 2: New Jersey and Maryland Case (Focus: Document impacts and conduct monitoring along gradients)

New Jersey: Brown tide blooms are recurring in specific coastal bays (e.g., NJ). Blooms occur about the same time (June) with potential secondary blooms in fall/winter. Some negative impacts on natural resources have been documented (e.g., aquacultured clams) but other impacts not documented. Analysis to be conducted on contributory environmental factors.

Maryland: *Aureococcus* concentrations were documented but no significant blooms, perhaps small bloom concentrations at some sites.

State Monitoring Objectives:

- *Determine status/trends of ambient surface waters in the state*
    - **Recommended Monitoring Plan**: Determine concentrations of brown tide and selected water quality parameters at the existing state water quality network stations:
- *Determine Status/Trends of Brown Tide Blooms and Collection of Data*
    - **Recommended Monitoring Plan**: Determine concentrations of brown tide and selected water quality parameters at the water quality network stations representing various gradients (salinity, temperature, flushing/residence times, etc.):
        - **Spatial:**
            - **New Jersey:** Select a few stations in northern Barnegat Bay (where blooms not occurring, some stations in Little Egg Harbor (bloom occurrence), and Great Bay – coordinate with USGS on stations in terms of gradients (salinity, temp, and flushing/residence times, etc.). Extend monitoring one station southward from Great Egg Harbor Monitoring will include areas where blooms are known to occur and expand southward and beyond to geographic sites where blooms are not known to occur. This monitoring will cover a few sites in a wide geographic area.

            - **Maryland:** Monitoring at gradients will include areas where blooms are known to occur and expand southward and beyond to geographic sites where blooms are not known to occur. This monitoring will cover a few sites in a wide geographic area. Monitoring sites along gradients need to be coordinated with USGS.

- ❖ **Temporal**: Monitoring should be conducted before blooms occur (April-May), during blooms (June-July) and after blooms (Aug-Sept) and continue through the fall if fall blooms have been observed.
- ❖ **Parameters**: brown tide enumeration and other parameters (e.g., brown tide enumeration, salinity, temperature, chl a, and nutrients, others budget permitting) according to the water quality monitoring program).
  Determine environmental factors associated with or contributing to brown tide blooms

- *Determine negative impacts to natural resources in the states*
  - ❑ **Recommended Monitoring and Research Plan**

Maryland

- ❖ Develop baseline surveys of potentially affected natural resources (e.g., shellfish and eelgrass) in coastal bays
- ❖ Promote clammers to anecdotally report effects on shellfish

New Jersey

- ❖ Develop an intensive experimental monitoring or field study involving two similar (e.g., hydrologic, water quality, etc.) sites and monitor brown tide and a full suite of water quality and hydrographic parameters (time series data).

Both States: Determine negative impacts to natural resources in the states

- ❖ Develop baseline surveys of potentially affected natural resources (e.g., shellfish and eelgrass) in coastal bays
- ❖ Promote clammers in Barnegat Bay and Little Egg Harbor to anecdotally report effects on clams
- ❖ Conduct a limited study on impacts of brown tide blooms on eelgrass at two locations (one with and one without blooms).
- ❖ Analyze data collected on brown tide in previous years including water quality parameters, and data on natural resources (e.g., shellfish, eelgrass, etc.)

Tier 3: New York Case/Suffolk County (Focus: Research on causes of blooms; time series data)

Brown tide blooms have been recurring and documented over many years in several Long Island coastal bays. Monitoring programs have been in place for over 10 years. Negative impacts on natural resources are well documented.

NY State's Monitoring Strategy for Brown Tides is conducted by the Suffolk County Department of Health Services in Long Island

- Determine status/trends of ambient surface waters in the state
    - **Recommended Monitoring Plan:** Continue existing state water quality monitoring networks
- Determine time series measurements at selected stations (full suite of parameters)
    - **Recommended Monitoring plans** (e.g., Suffolk Co. Dept. of Health Services)
- Research into causes of brown tide blooms

II. Implementation and Communication Strategy

The development of a Brown Tide Listserv and general Brown Tide Website (below) will provide pertinent information to people about current brown tide blooms, major results and information on upcoming meetings and workshops.

- **Development of a Brown Tide Listserv**
  The listserv would not include legislators because legislators could go to the regional website (below) for general information and individual states send relevant materials to their legislators. All conference call participants were asked to forward lists of names and emails to Mary Gastrich (mdownesg@dep.state.nj.us) to be compiled into a listserv.

- **Development Of A Brown Tide Website**

There is currently no general website for brown tide. The group recommended the following :

❖ Establishing a generic website on brown tide which is needed to provide links to relevant websites on brown tide from many sources including a list of scientists, funded or unfunded, with a 1-2 sentence description of their research interests.

❖ Patrick Dooley, NY Sea Grant, volunteered to establish this generic website. He will send the LISTSERV group, developed by Mary Gastrich, NJDEP, a survey to solicit names and websites to be incorporated into the Brown Tide Website as per recommendations below. While it was not discussed, it is assumed that NY Sea Grant would consider maintenance and update of this website in the future.

❖ The criteria for establishing the informational "clearing-house" type Brown Tide website included the following:

1) The main purpose of the Brown Tide Website is to provide informational links. It

should be a "**brief**" website and **focus on links** – not on long descriptions of the problem or research results (for this, people will click onto other websites)

2) The focus of the website should be **as generic in geographic scope** and not be billed as "regional" in order to be of interest to people in other areas or the U.S.
3) The **names of scientists** included in the website (and their websites) should be **expanded to include all those** who have been and are currently active or interested in brown tide research – and not just currently funded by any one agency.

❖ The website would include at minimum the following information; all links will be briefly described:
  - A **generic title, "Brown Tide Website"** with perhaps a 1-2 sentence description of the organism, problem and the purpose of the website (=offers links to other websites) – this includes a standard "blurb" which can be used by all in various media and newsletters and includes the name of the brown tide website;
  - Appropriate links to federal, state and local health agencies that conduct/fund brown tide monitoring and studies including with a short description of the link. This would include the **<u>agency name, lead and/or contact, and title of any special brown tide projects.</u>**
  - Links to National and State Sea Grants and special brown tide research
  - Names and websites of scientists who have been or are active in brown tide research including a brief 1-2 sentence description of the type of research including:
    - ❖ List of scientists and e-mails from the WHOI HAB Conf.
    - ❖ List of NYS's Brown Tide Research Institute (BTRI) researchers
    - ❖ List of other scientists, who are not BTRI or WHOI, who may have been or are currently active in brown tide research
    - ❖ List of attendees from the April 6, 2001 Brown Tide Workshop
  - Click on link to the Brown Tide Listserv for brown tide mailings

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