ABSTRACTS OF ORAL PRESENTATIONS

BROWN TIDE SESSION

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.

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

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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.

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

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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.

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

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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.

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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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 X 10^6 cells per mL on June 8 which decreased to 3.0 X 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 X 10^4 cells per mL on July 12. At Ship Bottom, the cell numbers reached 1.8 X 10^6 cells per mL on June 23 and decreased to 4.1 X 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.

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

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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 x 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.

BENTHIC-PELAGIC COUPLING AND LI BROWN TIDE

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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*.

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.

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

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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.

ABSTRACTS OF POSTERS

BROWN TIDE SESSION

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.

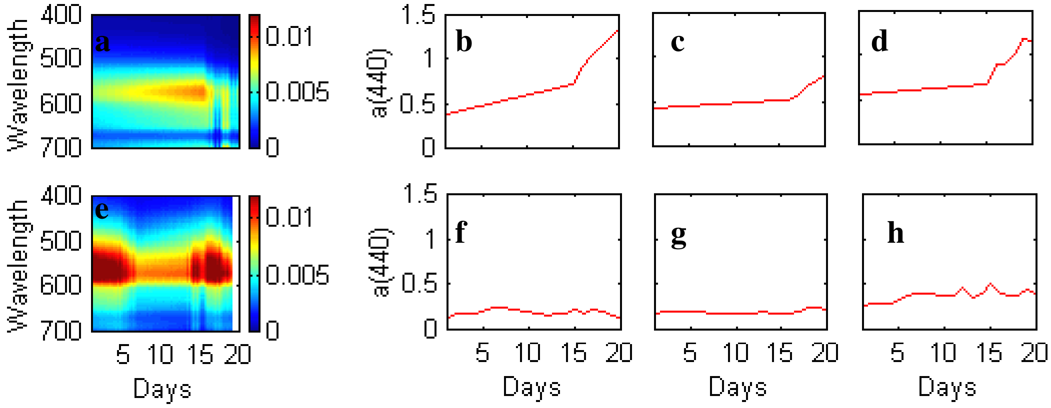

Figure 1 a) Spectral radiance reflectance, b) phytoplankton, c) CPOM, and d) CDOM absorption at 440 nm at Quantuck Bay. Plots e-h display those measurements for West Neck Bay.

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 *Btcycl1*, 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 *Btcycl1* 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*

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During the past decade blooms of the brown tide microalga*, Aureococcus anophagefferens*, have occurred sporadically in Peconic Bay and Great South Bay of Long Island (LI), NY. Blooms vary annually in the timing of their onset, duration and intensity. We hypothesize that temporal and spatial variability in bloom characteristics is due to underlying genetic variation among populations. 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 into pCR2.1 vector and 15 to 25 recombinant clones per sample were sequenced. Sequence data were obtained from 1995 summer bloom samples from two sites in Peconic Bay and one locale in 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 LI and CCMP 1794 from Barnegat Bay, NJ. *A. anophagefferens* is unique among eukaryotes in that it has extremely high numbers 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 among samples for ITS1 and ITS2, respectively. Monte Carlo based chi-square analysis was performed to determine if there were significant differences in the frequencies of ITS types within and among bloom samples and cultured isolates. Statistically significant differences in the frequency of ITS types were observed between cultured isolates CCMP 1785 (LI) and CCMP 1794 (NJ) in comparison to all other isolates and LI bloom samples. This indicates that not all cultured isolates are representative of *A. anophagefferens* blooms and that there is geographic differentiation between some coastal sites. In addition, restriction fragment length polymorphism analysis of chloroplast DNA confirmed that there are genetic differences among cultured isolates. Interestingly, there were no significant differences in the frequency of ITS types between the 1995 summer and 1999 winter bloom samples from LI. This suggests variability at the peak of a bloom is low or that the resolution of the PCR-cloning technique is insufficient to distinguish closely related populations. Currently, Single Stranded Conformation Polymorphism analysis is being used to re-evaluate the data obtained from the rRNA analyses.