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Viruses as Potential Regulators of Regional Brown Tide Blooms Caused by the Alga, *Aureococcus anophagefferens*

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ABSTRACT: Blooms of the brown tide organism *Aureococcus anophagefferens* have recurred in the coastal bays in New Jersey since 1995 and in the coastal bays of Long Island since 1985. Intracellular viral-like particles (VLPs) were documented during 1999–2000 brown tide blooms in Little Egg Harbor, New Jersey, but it was not determined whether cells were infected during the termination of the bloom. The objective of this study was to determine if VLPs infected and lysed natural populations of *A. anophagefferens* in coastal bays of New Jersey and New York in 2002 with the same frequency as in 1999–2000 and especially at the termination of the bloom. Our results confirmed that the highest percentage (37.5%) of VLP-infected cells occurred at the termination of the brown tide bloom in New Jersey in 2002. Intracellular VLPs were present throughout the bloom event. The percentage of visibly infected cells was higher at the beginning of the bloom than during the peak of the bloom. The intracellular VLPs in natural populations of *A. anophagefferens* were consistent in size and shape (approximately 140 nm in diameter) and comparable to those in previous studies. Concentrated viral isolates, prepared from waters during brown tide blooms in New York and New Jersey in 2002, infected healthy laboratory *A. anophagefferens* cultures in vitro. The viral isolates associated with the highest laboratory viral activity (lysis positive) were concentrated from water samples having the highest viral and bacteria concentrations. The intracellular viruses in these virally infected laboratory cultures of *A. anophagefferens* were similar in size and shape to those found in natural populations. The successful isolation of a virus specific to *A. anophagefferens* from a brown tide bloom in the field, the similarity of ultrastructure of VLPs infecting both natural populations and laboratory infected cultures, and the pattern of VLP infection during bloom activity in combination with the observed high percentage of VLP-infected cells during bloom termination, supports the hypothesis that viruses may be a major source of mortality for brown tide blooms in regional coastal bays of New Jersey and New York.

Introduction

Brown tide blooms, caused by *Aureococcus anophagefferens,* have recurred in the coastal bays in New Jersey since 1995 and in the Long Island coastal bays since 1985. *A. anophagefferens* bloom abundances, mainly Category 2 ($\geq 3.5 \times 10^4$ and $< 2.0 \times 10^5$ cells ml^{-1}) and Category 3 blooms ($\geq 2.0 \times 10^5$ cells ml^{-1}; Gastrich and Wazniak 2002), were reported in Little Egg Harbor, New Jersey, in 2000–2002 (New Jersey Department of Environmental Protection [NJDEP] 2001; Gastrich et al. in press). Recent studies have indicated that viruses may play important roles as recycling agents in marine systems and may also be an important biological control mechanism in phytoplankton bloom events (see reviews by Bratbak et al. 1993; Fuhrman 1999; Wilhelm and Suttle 1999; Wommack and Colwell 2000). Viral infection in natural populations of phytoplankton has been observed in numerous species including *Micromonas pusilla* (Cottrell and Suttle 1995; Zingone et al. 1999), *Emiliani huxleyi* (Bratbak et al. 1993) and the red-tide organism, *Heterosigma akashiwo* (Raphidophyceae) in Hiroshima Bay, Japan (Nagasaki et al. 1994). Intracellular viral particles were first observed in the initial descriptions of natural populations of *A. anophagefferens* isolated from Narragansett Bay, Rhode Island (Sieburth et al. 1988), but the abundance of infected cells and the presence of viruses in brown tide blooms from other geographic areas were not examined. Subsequently, intracellular viral-like particles (VLPs) were documented in natural populations of *A. anophagefferens* during brown

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tide blooms in 1999–2000 in Little Egg Harbor, New Jersey (Gastrich et al. 2002). The presence of a lytic virus, isolated from West Neck Bay, New York, which lysed healthy laboratory cultures of *A. anophagefferens,* was also previously demonstrated (Milligan and Cosper 1994; Benmayor 1996; Garry et al. 1998; Gastrich et al. 1998). Lysis of a single infected host cell produces hundreds of virus progeny, and lytic cycles are typically on the order of hours to days (Gobler et al. 1997). Since recent research has demonstrated that microzooplankton avoid *A. anophagefferens* cells and consume other phytoplankton during brown tide blooms (Gobler et al. 2002) and that *A. anophagefferens* cells inhibit filter feeding of juvenile hard clams (*Mercenaria mercenaria*) at low cell densities of $\geq 3.5 \times 10^4$ cells ml^{-1} (Bricelj et al. 2001), the potential for viruses to act as the major mechanism of mortality is significant. The main objective of this study was to determine the potential role that viruses may play in the biological control of brown tides. The specific objectives of the current study were to assess viral and bacterial concentrations during brown tide blooms; determine if viral-like particles (VLPs) infect and lyse natural populations of *A. anophagefferens* in coastal bays of New York and New Jersey in 2002 with the same frequency as that in 1999–2000 (especially at the termination of the bloom); and investigate if isolated viruses from brown tide waters in 2002 are specific to *A. anophagefferens* and if viral activity could influence the bloom proliferation and termination in vitro.

Materials and Methods

Collection of Water Samples

In 2002, the New Jersey Department of Environmental Protection (NJDEP) collected water samples at eleven stations in Barnegat Bay and Little Egg Harbor, New Jersey. Water samples were collected from April through September to enumerate *A. anophagefferens* and environmental data. A subset of water samples were collected from 1–2 stations in Tuckerton and Long Beach township (Beach Haven Terrace) in New Jersey and one station in New York (Bay Shore Cove). Samples were prepared for transmission electron microscopy (TEM), enumeration of brown tide, bacteria, and viruses. Samples enumerated for *A. anophagefferens* were pre-fixed in a 1% research grade cacodylate-buffered glutaraldehyde solution. New Jersey samples were enumerated for *A. anophagefferens* using a monoclonal antibody (Enzyme-linked immunosorbent assay [ELIZA]) method (Caron et al. 2003). The duplicate samples taken at one station in Bay Shore Cove, New York, on three dates were enumerated using a polyclonal method (Anderson et al. 1989).

Enumeration of Bacteria and Viruses in Whole Water Samples

Because it has been hypothesized that cyanobacteria may compete for a niche with *A. anophagefferens* in Long Island bays (Sieracki et al. 1999), we measured the abundance of viruses, bacteria, and *A. anophagefferens* in our samples. Water samples containing viruses and bacteria were preserved in glutaraldehyde (2.5% final concentration, stored at 4°C) and enumerated by epifluorescence microscopy. For viruses, 800 μl aliquots of diluted (8 to 16 fold) sample were collected onto 25-mm diameter, 0.02-μm nominal pore-size Anodisc (Whatman) filters. The filters were stained with SYBR Green 1 (Noble and Fuhrman 1998). Viruses in these samples were enumerated manually using a Leica DMRXA epifluorescent microscope equipped with the appropriate optical filter set (Ex_λ = 450–490 nm; Em_λ = 510 nm) and a calibrated 10 × 10 ocular grid. Bacterial particles were likewise enumerated from 2 ml subsamples collected onto 25-mm diameter, 0.20-μm nominal pore-size black polycarbonate filters (Hobbie et al. 1977) and enumerated as above. For all samples, 20 full grids or minimally 200 particles were enumerated in order to ensure statistical accuracy.

Determination of Lytic Activity in Natural Viral Populations

The activity of viruses capable of infecting *A. anophagefferens* was determined by standard infection assays (Suttle 1993). Triplicate screenings of two samples collected on June 17 and June 26 in Little Egg Harbor, New Jersey, were used. Briefly, 6-ml cultures were maintained in ESAW medium (Berges et al. 2001) at 21°C under a 12/12 h light/dark cycle of growth (light intensity of c. 200 μmols photons m^{-2} s^{-1}). Freshly transferred cultures (24–48 h after inoculation) were used for the screening assays as follows. Water samples (1 l) collected from the NJDEP monitoring sites were maintained in the dark and transported to the lab for processing within 24–48 h. After prefiltration through 0.2 μm polycarbonate filters, viruses in 38 ml aliquots were collected by ultracentrifugation (105,000 × G, 3 h, 4°C) (Wilhelm et al. 1998). Viruses from up to 6 aliquots (c. 240 ml in total) were resuspended in sterile ESAW. Samples were pooled and amended to the screening cultures to determine whether lytic viruses may be present. Growth of the screening cultures (included unamended controls) was monitored using a Turner Designs TD-700 fluorometer equipped with the in vivo chlorophyll monitoring set. Replicate samples were screened for each sampling date as well as for the controls. Viral activity (lysis positive) was consid-

TABLE 1. *A. anophagefferens,* viral and bacterial abundances, and TEM results of percent VLP-infected *A. anophagefferens* in New Jersey and New York coastal waters (2002).

State/Site	Collection Date	N = Total[1] A.a. Cells Counted (TEM)	Percent VLP-Infected A.a. (TEM)	[2][A.a.] (cells ml^{-1})	[3]Projection No. of VLP-Infected A.a. Cells ml^{-1} in Field	Virus/ml ± SD ($\times 10^8$ ml^{-1})	Bacteria/ml ± SD ($\times 10^6$ ml^{-1})
[4]NJ/1818D	April 23, 2002	18	ND[5]	5,000	0	1.03 ± 0.30	6.62 ± 1.92
[6]NY/BSC	April 24, 2002	217	1.4	272	3.8	0.66 ± 0.12	4.34 ± 0.14
NJ/1818D	May 20, 2002	217	7.0	784,000	54,880	1.81 ± 0.53	8.14 ± 2.74
NY/BSC	May 21, 2002	25	8.0	8,559	684.7	1.31 ± 0.46	3.07 ± 0.08
[7]NJ/1719E	June 3, 2002	223	5.0	419,000	20,950	3.65 ± 1.07	8.80 ± 1.65
NJ/1818D	June 3, 2002	227	1.8	447,000	8,046	1.90 ± 0.29	6.17 ± 0.35
NY/BSC	June 6, 2002	26	0.0	1,951	0	1.61 ± 0.77	4.02 ± 0.34
NJ/1719E	June 10, 2002	223	2.7	546,000	14,742	3.91 ± 0.37	10.9 ± 0.96
[8]NJ/1719E	June 17, 2002	265	5.3	842,000	44,626	4.14 ± 0.47	11.4 ± 1.13
NJ/1818D	June 17, 2002	ND[5]	ND[5]	455,000	ND[5]	ND[5]	ND[5]
[9]NJ/1719E	June 26, 2002	549	0.60	1,228,000	7,368	3.56 ± 0.20	4.56 ± 1.09
NJ/1818D	June 26, 2002	ND	ND	272,000	ND	ND	ND
NJ/1719E	July 8, 2002	36	36.1	56,000	20,216	3.19 (± 0.75)	6.96 ± 0.53
NJ/1719E	July 24, 2002	8	37.5	98,000	36,750	3.45 (± 0.12)	8.01 ± 1.79
NJ/1719E	August 15, 2002	10	20	72,000	14,400	4.16 (± 0.59)	5.04 ± 1.07
NJ/1719E	September 12, 2002	28	21.4	36,000	7,704	3.93 (± 0.28)	7.08 ± 0.52
TOTAL		2,072					

[1] A.a. = *Aureococcus anophagefferens.*
[2] *A. anophagefferens* abundances were enumerated using monoclonal antibody methods (New Jersey) and polyclonal antibody methods (New York).
[3] Projected number of VLP-infected A.a. in natural populations = Percent VLP-infected TEM sample × ambient [A.a.].
[4] Station 1818D located in Tuckerton Bay, New Jersey.
[5] ND = not determined (or no fixed samples or very low A.a. abundance).
[6] Station BSC located in Bay Shore Cove, New York.
[7] Station 1719E located in Long Beach township, New Jersey (near Beach Haven Terrace).
[8] Samples were lysed (1719E collected June 17, 2002).
[9] Samples were not lysed (1719E collected June 26, 2002).

ered present when the amended cultures showed a sudden decrease in growth. Unamended samples continued to show growth. Confirmation that the agent was potentially a virus was made by filtering the lysis positive cultures and amending the filtrate to demonstrate that the lytic agent was a 0.2-μm filterable entity (ruling out predatory bacteria).

Transmission Electron Microscopy (TEM)

Duplicate water samples were taken at 1–2 sites in Little Egg Harbor, New Jersey (Tuckerton Bay, Station 1818D and Long Beach TWP, Station 1719E), and one site in New York (Bay Shore Cove). Samples were prepared for TEM as previously published (Gastrich et al. 2002). Samples were pre-fixed in a 2% research grade buffered (0.2 M cacodylate, pH = 7.8) glutaraldehyde solution, and post-fixed in 2% osmium tetroxide (0.2 M cacodylate, pH = 7.8), dehydrated and embedded in epon. Ultrathin sections were collected on copper grids every 50–100 sections to prevent duplication of observations. Over 2,000 individual cells were individually examined in natural populations of *A. anophagefferens* for the presence of VLPs using methods previously described by Gastrich et al. (2002). For samples containing more than 10^5 *A. anophagefferens* cells ml^{-1}, at least 200 individual cells were counted per sample (from 2 replicate embedded blocks) using several grids. For samples with lower abundances ($< 1.0 \times 10^5$ cells ml^{-1}) of *A. anophagefferens,* which were more difficult to enumerate, 5 to 6 grids were scanned for viral-containing *A. anophagefferens* cells and the presence or absence of viruses was noted. A viral isolate specific to brown tide was isolated in 2002 from Quantuck Bay and inoculated into a healthy *A. anophagefferens* laboratory culture and fixed for TEM, using previously described methods (Gastrich et al. 1998).

Results

Water Quality Parameters and Abundances of *A. anophagefferens*

Field salinity ranged from 27.1 to 33.1 ppt at New Jersey sites (Table 1) and was similar to those reported in areas having brown tide blooms in previous years (Gastrich et al. 2002). Temperatures ranged from 13°C on April 23 to over 27.3°C on June 26 (at the peak of the bloom) as abundances peaked in June (Category 3 bloom) and decreased to 21°C at the end of the bloom in September. *A. anophagefferens* abundances were similar to previous bloom years in New Jersey (Gastrich et al. 2002).

TABLE 2. Summary of the Brown Tide Bloom Index that relates *Aureococcus anophagefferens* abundances to potential impacts on natural resources (after Gastrich and Wazniak 2002).

Brown Tide Bloom Category	*A. anophagefferens* Abundance and Potential Impact
CATEGORY 1	<35,000 *A. anophagefferens* cells ml^{-1} (no observed impacts)
CATEGORY 2	≥35,000 to <200,000 cells ml^{-1} • Reduction in growth of juvenile hard clams, *Mercenaria mercenaria* • Reduced feeding rates in adult hard clams • Growth reduction in mussels (*Mytilus edulis*) and bay scallops (*Argopecten irradians*)
CATEGORY 3	≥200,000 cells ml^{-1} • Water becomes discolored yellow-brown • Feeding rates of mussels severely reduced • Recruitment failures of bay scallops and high mortalities • No significant growth of juvenile hard clams • Negative impacts to eelgrass due to algal shading • Copepod production reduced and negative impacts to protozoa

The lowest abundance of *A. anophagefferens* in New Jersey was 5.0×10^3 cells ml^{-1} (April 23) which increased to 7.8×10^5 cells ml^{-1} (May 20). Elevated abundances, including Category 2 and Category 3 blooms (Table 2; Gastrich and Wazniak 2002) ranged from 4.2×10^5 to 8.4×10^5 cells ml^{-1} through mid-June peaking at 1.2×10^6 cells ml^{-1} on June 26 at New Jersey stations. After the June 26 peak, *A. anophagefferens* cell levels remained $< 10^5$ ml^{-1} until July when the abundances decreased to 3.6×10^4 cells ml^{-1} at the end of the bloom in September. Unlike previous years (Gastrich et al. 2002), this study documented the decline of the brown tide bloom from peak abundances on June 26 and declines through September 12.

Percent VLP-infected *A. anophagefferens*, Viruses and Bacteria

Table 1 shows the percent VLP-infected *A. anophagefferens* cells as well as viral and bacterial concentrations, found at the sampling sites during the year 2002. Bacterial densities increased from 6×10^6 ml^{-1} in April and May 11.4×10^6 ml^{-1} in June. Bacterial levels decreased during the *A. anophagefferens* bloom peak on June 26 and then increased slightly following this peak. Bacteria abundances ranged from 3.07×10^6 ml^{-1} to 11.4×10^6 ml^{-1} (Table 1). Viral abundances remained fairly static during our study and ranged from 0.66×10^8 ml^{-1} to 4.16×10^8 ml^{-1}. Figure 1 shows the pattern of the percentage of VLP-infected *A. anophagefferens* in natural populations during 1999–2000 and 2002 in sites in New Jersey and New York. In 2002, we

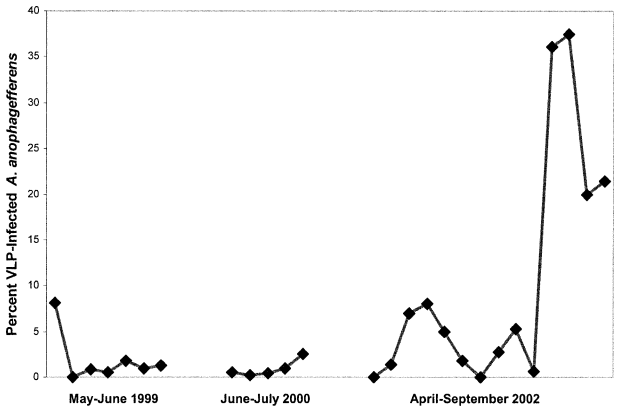

Fig. 1. TEM results: Percent VLP-infected *A. anophagefferens* in New Jersey 1999–2000 (Gastrich et al. 2002) and in New Jersey and New York 2002 (total 3 yr N = 10,037 cells counted).

observed a higher percentage of VLP-infected cells at the end of the bloom than in previous years possibly because the termination of the bloom was fully documented in 2002. The percent of VLP-infected cells, based on TEM counts, was less than 2% in April and increased to 8% in May followed by decreases from about 5% to less than 1% in June. As the bloom started to wane, with lower abundances (c. 9.8×10^4 cells ml^{-1}) in July, up to 37.5% of cells observed were VLP-infected.

TEM

Our 2002 samples displayed several stages of VLP infection ranging from a few viral capsids per cell, with intact organelles such as the nucleus or chloroplasts, to later stages of infection with viral capsids occupying most of the cross-sectional cell area. The intracellular viral capsids are approximately 140 nm in diameter, hexagonal in cross section (suggesting icosahedral geometry), and in some cases there are over 50 capsids in a cross section (Fig. 2). The VLPs were similar in size and shape to those found in a previous study (Gastrich et al. 2002, plate 1). All visibly VLP-infected *A. anophagefferens* cells had an electron dense plasma membrane but usually lacked a substantial exopolymer secretion (EPS), a common characteristic of healthy *A. anophagefferens* cells (Sieburth et al. 1988). The thickness of any fragmented remnants of the EPS in infected cells was similar to that in healthy cells (c. 0.1 μm). Free-living viruses were not observed in field samples probably due to loss during fixation or laboratory sedimentation under gravity that was used to initially concentrate the fixed sample. No viruses were observed attached to the *A. anophagefferens* plasmalemma probably due to the low probability of fixing the cells at the immediate point in time of VLP attachment and

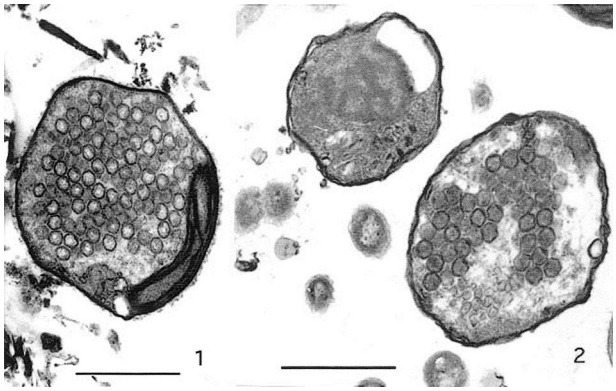

Fig. 2. (1). TEM of VLP-infected *A. anophagefferens* cell in natural populations during 2002 brown tide bloom in Little Egg Harbor, New Jersey. *A. anophagefferens* has electron-dense plasmalemma lacking an EPS, typical of infected cells, and a tightly packed geometric array of more than 50 VLPs in cross section. Each VLP is approximately 140 nm in diameter. The remaining chloroplast is usually the last organelle visible in a highly infected cell. Scale bar = 1 μm. (2). TEM of two laboratory *A. anophagefferens* cells infected with a viral isolate taken from Quantuck Bay in 2002. Both cells have an electron dense plasmalemma. The smaller cell on the left has an intact nucleus with no visible viral capsids while the larger cell to the right has no visible organelles except viral capsids, approximately 140 nm in diameter, that are similar to those seen in natural populations in (1). Scale bar = 1 μm.

the further low probability of an ultrathin section passing through a plane where the VLP was attached to a cell. Some VLP-infected cells had degraded organelles, especially chloroplasts, while other sections of cells that were tightly packed in a geometric array of capsids had no remaining organelles.

Viral Activity

Viruses isolated from New Jersey waters during the 2002 *A. anophagefferens* bloom were shown to infect and lyse healthy *A. anophagefferens* cultures. Viral activity (lysis positive) was highest on June 17. This preparation was made using viral isolates from two New Jersey sites, with one the highest reported viral concentrations ($4.14 \pm 0.47 \times 10^8$ ml^{-1}) and the highest bacteria concentration ($11.4 \pm 1.13 \times 10^6$ ml^{-1}) but at a lower abundance of brown tide (8.4×10^5 ml^{-1}) than the peak abundance cells. On June 26, there seemed to be lower viral activity as infected cultures did not lyse but there were lower viral ($3.56 \pm 0.20 \times 10^8$ ml^{-1}) and bacterial ($4.6 \pm 1.09 \times 10^6$ ml^{-1}) abundances at this time with the highest abundance of *A. anophagefferens* ($> 1.2 \times 10^6$ cells ml^{-1} (Table 1). Our experiments show that healthy cultures of *A. anophagefferens* contain intracellular VLPs after a 24 h incubation with a viral isolate freshly prepared from waters in New York and New Jersey in 2002 (Fig. 2). The VLPs (in cross section) in laboratory-infected cultures are virtually identical in size (c. 140 nm) and hexagonal shape as VLPs found in natural populations during the same sampling period. The EPS is not prominent in infected cells and we observed a range of infection after a 24 h incubation period including some cells without capsids, some with a few capsids and many cells with numerous VLPs that appeared to be in a late stage of infection.

Discussion

Our results confirm for the first time that the percentage of VLP-infected *A. anophagefferens* is highest, up to 37.5%, at the end of the bloom period. This, combined with the results of lytic activity, indicate that viruses may play a major role in bloom termination. The finding that natural populations of *A. anophagefferens* during brown tide blooms in New York and New Jersey continue to be infected with intracellular VLPs that are of the same size and shape as those found in 1999–2000 blooms in Little Egg Harbor, New Jersey (Gastrich et al. 2002), provides additional evidence of the potential role of viral control of brown tide blooms.

During the initiation of the bloom in New Jersey in 2002, the percent of VLP-infected cells reached 7% by May 20, which is consistent to previous findings (Gastrich et al. 2002). From June 3–26, as the *A. anophagefferens* reached peak abundances, the percentage of VLP-infected cells decreased to less than 1%, which was also similar to previous studies (Gastrich et al. 2002). The difference between 2002 and previous years can be explained by the fact that we were able to capture the end of the bloom in 2002. We found that the percentage of VLP-infected cells increased sharply to 37.5% when *A. anophagefferens* abundances decreased from peak levels. The percentage of VLP-infected cells was still as high as 21% even as the *A. anophagefferens* abundances dropped to 3.6×10^4 cells ml^{-1} at the end of the bloom.

Our results demonstrated that viral isolates, prepared from waters during brown tide blooms in New York and New Jersey in 2002, infected healthy laboratory *A. anophagefferens* cultures in vitro. The results indicated that the viral isolates demonstrating the highest laboratory viral activity (lysis positive) were concentrated from water samples having the highest viral concentrations ($4.14 \pm 0.47 \times 10^8$ virus ml^{-1}) and the highest bacteria concentration ($11.4 \pm 1.13 \times 10^6$ ml^{-1}), albeit at a lower abundance of brown tide (8.4×10^5 ml^{-1}) cells. Viral isolates associated with low viral activity (lysis negative) were concentrated from water samples having lower viral and bacteria concentrations but

peak *A. anophagefferens* abundances. These results appear consistent with the hypothesis that bacteria may be the primary source for viruses in marine environments (Cochlan et al. 1993; Wommack and Colwell 2000). Cyanobacteria may also play an important role in brown tide occurrence (Sieracki et al. 1999). Since bacteria, like the brown tide, can obtain most of their cellular N from organic forms (Wheeler and Kirchman 1986; Kirchman et al. 1994; Gobler and Sañudo-Wilhelmy 2001; Mullholland et al. 2002), it is possible that *A. anophagefferens* competes with bacteria for N-sources or organic C during bloom events. Results of viral lysis of *A. anophagefferens* laboratory cultures from a virus isolated in 1992 from West Neck Bay, Long Island, New York, suggested that viral lysis of field populations of *A. anophagefferens* could release dissolved organic nitrogen (DON) and other elements to bacteria which could promote changes in water column chemistry and species composition and succession (Gobler et al. 1997). Moreover, the limited number of protozoa known to consume *A. anophagefferens* (Caron et al. 1989; Mehran 1996), also efficiently graze heterotrophic bacteria (Sherr et al. 1986; Caron et al. 1991). While both top-down and bottom-up controls on bacterial and *A. anophagefferens* populations may be similar, their dynamics during brown tide events are not well known.

Our results are consistent with previous studies that reported the occurrence of intracellular VLPs in natural populations of *A. anophagefferens* in 1999–2000 in New Jersey (Gastrich et al. 2002) and studies demonstrating lytic activity in healthy *A. anophagefferens* cultures infected with viral isolates from waters during brown tide blooms in West Neck Bay, New York, in 1992 (Milligan and Cosper 1994; Garry et al. 1998; Gastrich et al. 1998). Our results also provide new information on brown tide blooms and also compare favorably with studies showing that viral infection has been observed in the final stages of blooms of the red-tide organism *H. akashiwo* (Raphidophyceae) in Hiroshima Bay, Japan (Nagasaki et al. 1994). The observation of the maximal number of infected cells during collapse is consistent with observations of a virally infected red tide bloom of *H. akashiwo* in Japan (Nagasaki et al. 1994).

These results support previous studies but also lend new insights into the important role viruses may play in the dynamics of brown tide blooms. Peaks in VLP-infection of *A. anophagefferens* occurred at two points in time: when abundances began to increase before the peak of the bloom and then again when densities decreased after the peak of the bloom (Table 1). These results suggest that for an *A. anophagefferens* bloom to occur, cellular growth rates must outpace rates of viral lysis. Low numbers of VLP-infected cells observed during the bloom peak may indicate that growth rates of *A. anophagefferens* might be outpacing the viral activity. As more cells become visibly infected, after the bloom peak, the decline in *A. anophagefferens* abundances may be affected by reduced cellular growth rates relative to viral infection rates. The cause of the decrease in cellular growth rates has not been determined, but it could be due to environmental stress such as increases in temperature and microzooplankton grazing (Gobler et al. 2002) that cooccur during this time period.

This study confirms several important observations because, for the first time, virus and bacteria abundances were determined during brown tide blooms. Natural populations of *A. anophagefferens* continue to be infected with VLPs over several years of brown tide blooms in coastal bays in the regional area of New Jersey and in New York and the intracellular VLPs are consistently similar in size and shape across the regional samples. Intracellular VLPs observed in healthy *A. anophagefferens* cultures infected with viral isolates, prepared from waters from New York and New Jersey, are also similar in size and shape to those found in the natural populations; viral activity has been positively confirmed by laboratory lytic studies. This study also demonstrates that the percentage of VLP-infected cells is highest at the end of the bloom period. The isolation of an *A. anophagefferens* specific virus from a brown tide bloom in the field in combination with the observed high percentage of VLP-infected cells during bloom termination suggests viruses may be a major source of mortality for brown tide blooms in regional coastal bays of New Jersey and New York.

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Literature Cited

Anderson, D. M., D. M. Kulis, and E. M. Cosper. 1989. Immunofluorescent detection of the brown tide organism, *A. anophagefferens,* p. 213–228. *In* E. M. Cosper, E. J. Carpenter,

and V. M. Bricelj (eds.), Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms. Springer-Verlag, Berlin, Germany.

Benmayor, S. S. 1996. Environmental conditions influencing viral-algal interactions in the picoplankter, *Aureococcus anophagefferens*. M.S. Thesis, State University of New York, Stony Brook, New York.

Berges, J. A., D. J. Franklin, and P. J. Harrison. 2001. Evolution of an artificial seawater medium: Improvement in enriched seawater, artificial water over the last two decades. *Journal of Phycology* 37:1138–1145.

Bratbak, G., J. K. Egge, and M. Heldal. 1993. Viral mortality of the marine alga *Emiliania huxleyi* (Haptophyceae) and termination of algal blooms. *Marine Ecology Progress Series* 93:39–48.

Bricelj, V. M., S. P. MacQuarrie, and R. A. Schaffner. 2001. Differential effects of *Aureococcus anophagefferens* isolates (''brown tide'') in unialgal and mixed suspensions on bivalve feeding. *Marine Biology* 39:605–615.

Caron, D. A., E. L. Lim, H. Kunze, E. M. Cosper, and D. M. Anderson. 1989. Trophic interactions between nano- and microzooplankton and the brown tide, p. 263–294. *In* E. M. Cosper, V. M. Bricelj, and E. J. Carpenter (eds.), Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms. Springer-Verlag, Berlin, Germany.

Caron, D. A., E. L. Lim, G. Miceli, J. B. Waterbury, and F. W. Valois. 1991. Grazing and utlization of chroococcoid cyanobacteria and heterotrophic bacteria by protozoa in laboratory cultures and a coastal plankton community. *Marine Ecology Progress Series* 76:205–217.

Caron, D. A., M. R. Dennett, D. M. Moran, R. A. Schaffner, D. J. Lonsdale, C. J. Gobler, R. Nuzzi, and T. I. McLean. 2003. Development and application of a monoclonal antibody technique counting *Aureococcus anophagefferens*, an algal causing recurrent brown tides in the Mid-Atlantic United States. *Applied and Environmental Microbiology* 69:5492–5502.

Cochlan, W. P., J. Wikner, G. F. Steward, D. C. Smith, and F. Azam. 1993. Spatial-distributions of viruses, bacteria, and chlorophyll *a* in neritic, oceanic, and estuarine environments. *Marine Ecology Progress Series* 92:77–87.

Cottrell, M. T. and C. A. Suttle. 1995. Dynamics of a lytic virus infecting the marine picoflagellate *Micromonas pusilla*. *Limnology and Oceanography* 40:730–739.

Fuhrman, J. A. 1999. Marine viruses and their biogeochemical and ecological effects. *Nature* 399:541–548.

Garry, R. T., P. Hearing, and E. M. Cosper. 1998. Characterization of a lytic virus infectious to the bloom-forming microalga *Aureococcus anophagefferens* (Pelagophyceae). *Phycologia* 37: 300–306.

Gastrich, M. D., R. Lathrop, S. Haag, M. P. Weinstein, M. Danko, D. A. Caron, and R. Schaffner. In press. Assessment of brown tide blooms, caused by *Aureococcus anophagefferens* and contributing factors in New Jersey coastal bays: 2000–2002. *Harmful Algae*.

Gastrich, M. D., O. R. Anderson, S. S. Benmayor, and E. M. Cosper. 1998. Ultrastructural analysis of viral infection in the brown-tide alga, *Aureococcus anophagefferens* (Pelagophyceae). *Phycologia* 37:300–306.

Gastrich, M. D., O. R. Anderson, and E. Cosper. 2002. Viral-like particles (VLPs) in the alga, *Aureococcus anophagefferens*, (pelagophyceae) during 1999–2000 brown tide blooms in Little Egg Harbor, New Jersey. *Estuaries* 25:938–943.

Gastrich, M. D. and C. E. Wazniak. 2002. A brown tide bloom index based on the potential harmful effects of the brown tide alga, *Aureococcus anophagefferens*. *Aquatic Ecosystem Health and Management* 5:1–7.

Gobler, C. J., D. A. Hutchins, N. S. Fisher, E. M. Cosper, and S. A. Sañudo-Wilhelmy. 1997. Release and bioavailability of C, N, P, Se, and Fe following viral lysis of a marine chrysophyte. *Limnology and Oceanography* 42:1492–1504.

Gobler, C. J., M. J. Renaghan, and N. J. Buck. 2002. Impacts of nutrients and grazing mortality on the abundance of *Aureococcus anophagefferens* during a New York brown tide bloom. *Limnology and Oceanography* 47:129–141.

Gobler, C. J. and S. A. Sañudo-Wilhelmy. 2001. Effects of organic carbon, organic nitrogen, inorganic nutrients, and iron additions on the growth of phytoplankton and bacteria during a brown tide bloom. *Marine Ecology Progress Series* 209:19–34.

Hobbie, J. E., R. J. Daley, and S. Jasper. 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Applied and Environmental Microbiology* 33:1225–1228.

Kirchman, D. L., H. W. Ducklow, J. J. McCarthy, and C. Garside. 1994. Biomass and nitrogen uptake by heterotrophic bacteria during the spring phytoplankton bloom in the North Atlantic Ocean. *Deep-Sea Research* 41:879–895.

Mehran, R. 1996. Effects of *A. anophagefferens* on microzooplankton grazing and growth rates in the Peconic Bays system, Long Island, New York. Master's Thesis, SUNY, Stony Brook, New York.

Milligan, K. L. D. and E. M. Cosper. 1994. Isolation of virus capable of lysing the ''brown tide'' microalga, *Aureococcus anophagefferens*. *Science* 266:805–807.

Mulholland, M. R., C. J. Gobler, and C. Lee. 2002. Peptide hydrolysis, amino acid oxidation and N uptake in communities seasonally dominated by *Aureococcus anophagefferens*. *Limnology and Oceanography* 47:1094–1108.

Nagasaki, K., M. Ando, S. Itakur, I. Imai, and Y. Ishida. 1994. Viral mortality in the final stage of *Heterosigma akashiwo* (Raphidophyceae) red tide. *Journal of Plankton Research* 16:1595–1599.

New Jersey Department of Environmental Protection (NJDEP). 2001. Annual report of phytoplankton blooms and related conditions in the New Jersey Coastal Waters: Summer of 2001. Bureau of Marine Water Classification, Leeds Point, New Jersey.

Noble, R. T. and J. A. Fuhrman. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquatic Microbial Ecology* 14:113–118.

Sherr, B. F., E. B. Sherr, T. L. Andrew, R. D. Fallon, and S. Y. Newell. 1986. Trophic interactions between heterotrophic protozoa and bacterioplankton in estuarine waters analyzed with selective metabolic inhibitors. *Marine Ecology Progress Series* 32:169–179.

Sieburth, J. M., P. W. Johnson, and P. E. Hargraves. 1988. Ultrastructure and ecology of *Aureococcus anophagefferens* genera et species nova (Chrysophyceae): The dominant picoplankter during a bloom in Narragansett Bay, Rhode Island, summer 1985. *Journal of Phycology* 24:416–425.

Sieracki, M. E., M. D. Keller, T. L. Cucci, and E. Their. 1999. Plankton community ecology during the bloom initiation period of the brown tide organism *Aureococcus anophagefferens* in coastal embayments of Long island, N.Y. *EOS* 80:285.

Suttle, C. A. 1993. Enumeration and isolation of viruses, p. 121–134. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Coli (eds.), Handbook of Methods in Aquatic Microbial Ecology. Lewis, Boca Raton, Florida.

Wheeler, P. W. and D. L. Kirchman. 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. *Limnology and Oceanography* 31:998–1009.

Wilhelm, S. W. and C. A. Suttle. 1999. Viruses and nutrient cycles in the sea. *BioScience* 49:781–788.

Wilhelm, S. W., M. G. Weinbauer, C. A. Suttle, R. J. Pledger, and D. L. Mitchell. 1998. Measurements of DNA damage and photoreactivation imply that most viruses in marine surface waters are infective. *Aquatic Microbial Ecology* 14:215–222.

WOMMACK, K. E. AND R. R. COLWELL. 2000. Virioplankton: Viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews* 64:69–114.

ZINGONE, A., D. SARNO, AND G. FORLANI. 1999. Seasonal dynamics in the abundance of *Micromonas pusilla* (Prasinophyceae) and its viruses in the Gulf of Naples (Mediterranean Sea). *Journal of Plankton Research* 121:2143–2159.

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