



HPLC pigment records provide evidence of past blooms of *Aureococcus anophagefferens* in the Coastal Bays of Maryland and Virginia, USA

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Abstract

Concentrations of the accessory phytoplankton pigment 19'-butanoyloxyfucoxanthin (but-fuco), derived from archived high performance liquid chromatography (HPLC) data from the Atlantic coastal bays of Maryland and Virginia (1993–1995 and 1999–2002), were used to determine the presence of *Aureococcus anophagefferens* at 18 stations. Paired data of direct cell counts of *A. anophagefferens* and but-fuco concentration data from 2000 to 2002 were linearly regressed ($R^2 = 0.78$). This regression was used to estimate historical cell densities from 1994 to 1995 and to improve the spatial resolution from 1999 to 2002. Although the HPLC method used did not permit quantification of but-fuco before 1994, the records indicate that qualitatively *A. anophagefferens* was present in 1993. Quantitative measurements grouped into bloom index categories showed that annually, peak densities occurred in May to July. Severe Category 3 blooms ($>200,000$ cells ml^{-1}) were found in 1995, 2001, and 2002. Spatially, concentrations of but-fuco were higher in the northern extent of the study region than in the lower Chincoteague Bay, and along the western shore of Chincoteague Bay than on the eastern side. On an interannual basis, it appeared that *A. anophagefferens* became more geographically widespread and blooms more intense throughout the study period. © 2004 Elsevier B.V. All rights reserved.

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1. Introduction

One of the major questions of concern in the field of harmful algal bloom (HAB) research is whether HABs are increasing in extent and frequency. There is considerable evidence that eutrophication-related blooms

are indeed increasing in frequency in many parts of the world (Anderson et al., 2002). In some cases, however, it has been argued that our detection methods are improving and therefore we are only recognizing blooms now that may have existed for long periods of time.

The occurrence of the brown tide organism *Aureococcus anophagefferens* was first reported in Narragansett Bay, Rhode Island, in the Great South Bay and Peconic Bays, New York, and in Barnegat Bay,

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New Jersey, in 1985 (Casper et al., 1987; Sieburth et al., 1988; Bricelj and Lonsdale, 1997). During the past nearly 15 years, it has bloomed frequently in the waters of New York, with many of those blooms being of significant concentration (Category 3, $>200,000$ cells ml^{-1} ; Gastrich and Wasniak 2002). In contrast, there are no reports that *A. anophagefferens* occurred in Maryland or Virginia prior to 1998. The first formal sampling for *A. anophagefferens* in the Chesapeake and coastal bays of Maryland and Virginia was conducted during a survey of the waters of the east coast of the United States by Anderson et al. (1993). They detected no presence of the organism in any of their samples from Delaware, Maryland, or Virginia waters; all sampling in these waters was conducted during the month of August 1990. The Anderson et al. study thus concluded that the southern extent of *A. anophagefferens* was Great Bay, New Jersey.

New molecular probes were applied to the detection of *A. anophagefferens* in the Delaware coastal bays in 1998. The first positive response was recorded on 24 June 1998, in a sub-embayment of Little Assawoman Bay and later that year on 2 December 1998, in the Maryland portion of Little Assawoman Bay using immunofluorescent techniques (Gastrich et al., 2001). As a result, routine sampling programs for brown tide in portions of the coastal bays were instituted and its presence has been detected each year since 1999. Without a regular brown tide sampling program in place prior to 1999 in Maryland and Virginia, it is questionable whether the 1998 finding was the first occurrence of *A. anophagefferens* in the region, or rather the first detection. The recent surveys of *A. anophagefferens*, from 1999 to 2002, indicate that brown tides are a common annual event in the coastal bays, and that they generally occur in May and June. Although samples for direct enumeration of *A. anophagefferens*, and other HAB species did not formally begin until 1999, water quality parameters in these embayments have routinely been collected for more than a decade. These samples and analyses provided the opportunity to examine the historical record for evidence of *A. anophagefferens* prior to its first detection in 1998.

Photosynthetic phytoplankton pigments can be used as chemotaxonomical markers to identify and enumerate phytoplankton communities (e.g. Andersen et al., 1996; Suzuki et al., 1995; Ansoetegi et al., 2001) and can be especially useful as a first approx-

imation of species composition for small eukaryotic cells (Jeffrey and Vesk, 1997). The accessory xanthophyll pigment 19'-butanoyloxyfucoxanthin (hereafter but-fuco; Jeffrey and Mantoura, 1997) can be used as a marker for pelagophytes, such as *Aureococcus* (Jeffrey and Vesk, 1997; Bidigare, 1989) and *Pelagococcus* (Bjørnland and Liaaen-Jensen, 1989; Suzuki et al., 1997).

Through analysis of historical pigment data acquired by high performance liquid chromatography (HPLC), we show that *A. anophagefferens* most likely did exist prior to 1998 in Maryland and Virginia coastal bays. In this study but-fuco concentrations, derived from archived HPLC chromatograms, were used to approximate *A. anophagefferens* distribution and density in space and time in the coastal bays of Maryland and Virginia. This approach is taken with the recognition that but-fuco is not exclusive to *A. anophagefferens* and can be found in some marine chrysophytes as well as some prymnesiophytes (Barlow et al., 1993; Jeffrey and Wright, 1994; Jeffrey and Vesk, 1997). *A. anophagefferens* was formerly classified as a chrysophyte (Sieburth et al., 1988). While we have no direct counts for the earlier years of this analysis, such data are available for the more recent years, giving us confidence that *A. anophagefferens* was indeed the organism associated with the but-fuco pigment signatures.

2. Methods

2.1. Study area

The National Park Service (NPS) at Assateague Island National Seashore has conducted a routine monthly water quality sampling program at 18 stations throughout Newport and Sinepuxent Bays, Maryland; and Chincoteague Bay, Maryland and Virginia since the early 1990s (Fig. 1). These three bays, collectively known as coastal bays, are shallow coastal lagoon systems with average depths ranging between 0.67 and 1.22 m (Boynton et al., 1996). The systems are poorly flushed (Quinn et al., 1989; Boynton et al., 1996) with generally non-stratified waters. The northern and southern extents of the coastal bays are bordered by inlets to the Atlantic Ocean, and salinities range from near 0 at the headwaters of Trappe Creek that feeds

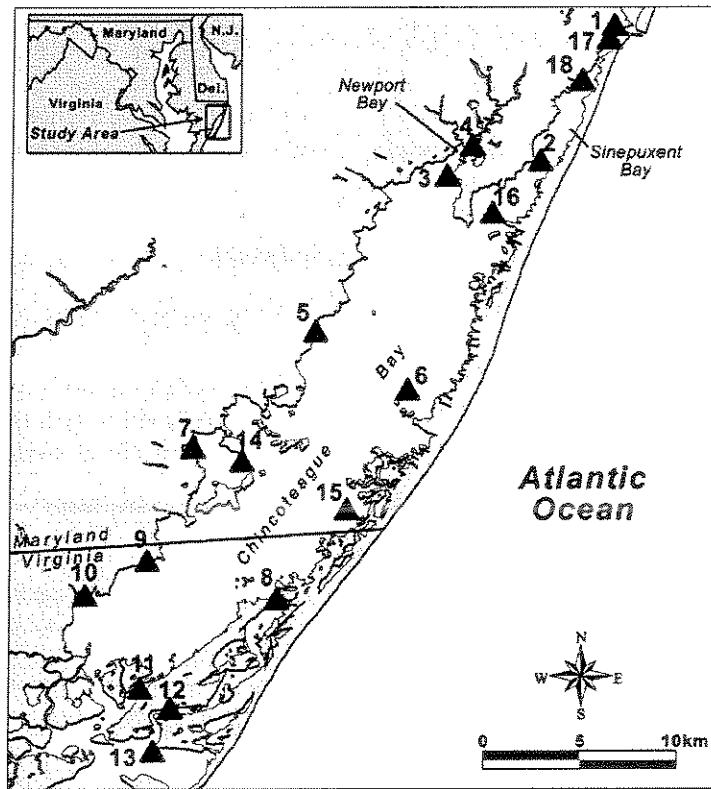


Fig. 1. Station map showing the location of the stations surveyed by the National Park Service at Assateague Island National Seashore for pigments during the study period.

Newport Bay, to greater than 32 in areas in Chincoteague Bay where entrained seawater can evaporate. Sediments generally contain finer grain sizes from east to west reflecting beach overwash processes on the east and finer silt particles from terrestrial runoff on the west (Bohlen and Boynton, 1996).

Land use in the relatively small watersheds is a mix of intensive poultry growing operations, agriculture, forests, extensively ditched wetland systems, a national park barrier island system, and growing residential development to support the seasonal tourism of nearly 8 million visitors traveling to nearby Ocean City, Maryland (Maryland Coastal Bays Program, 1997). Development pressures are generally greater in the northern reaches of the watershed. The majority of nutrient loadings to the system come from non-point sources, with only an estimated 4% of nitrogen and phosphorus coming from point sources (Jacobs et al., 1993).

2.2. Pigment analysis

During the monthly sampling for water quality, samples were collected for pigment analysis. Although the objective was analysis of chlorophylls *a*, *b* and *c*, the methods applied, as described below, during the years 1993–1995 and 1999–2002 allowed the resolution of but-fuco. Water samples collected for pigment analysis were filtered in the field through syringe cartridges containing 25 mm 934 AH glass fiber filters. Filters were removed from the filter cartridge, folded in half, wrapped in aluminum packets and placed on ice for several hours and delivered to the University of Maryland Center for Environmental Science, Horn Point Laboratory, and placed in a freezer (-80°C) until analyzed.

Pigments were extracted from the filtered material by disruption in cold acetone with a sonic probe (Branson 450). The filter homogenates were clarified

with HPLC syringe cartridges containing Teflon membrane filters. Sample extracts were then placed in the HPLC automated injector sample compartment (4 °C) for analysis.

Pigment calibration standards were either purchased (Sigma-Aldrich, St. Louis, MI; Fluka Chemical, Milwaukee, WI; DHI Water and Environment Institute, Høsholm Denmark) or isolated from natural sources (Van Heukelem and Thomas, 2001). Quality control check standards were analyzed, at minimum, after 10 sample analyses. Except for 1993 when chlorophylls, but not carotenoids were quantified, calibration practices have remained essentially the same (1994–2002) and are consistent with protocols suggested for use with HPLC remote-sensing objectives (Bidigare et al., 2002). Pigments were quantified by peak area at wavelengths near the peak absorbance maxima (but-fuco was monitored at 452 nm). Calibration factors were derived from the linear relationship between amounts injected and peak area.

The HPLC separation methods evolved during the decade of monitoring. Samples collected in 1993 were analyzed using HPLC methods modified from Van Heukelem et al. (1992), those collected in 1994–1995 were analyzed according to Van Heukelem et al. (1994), and those from 1999 to 2002 were analyzed according to Van Heukelem and Thomas (2001). As a consequence, in the 1993 sample record, it is only possible to resolve presence or absence of but-fuco, whereas during the subsequent years, quantification of but-fuco was possible. Sample analysis for 1996–1998 did not quantify the but-fuco pigment and therefore were not included in this study.

2.3. Direct enumerations of *Aureococcus anophagefferens*

From 2000 to 2002, samples were collected from just below the surface at stations 3, 4, 5, and 7 (Fig. 1), fixed immediately with 1% glutaraldehyde, and held on ice until return to the laboratory. Epifluorescence microscopy was later (within 14 days) used to determine *A. anophagefferens* cell counts using the polyclonal technique (Anderson et al., 1993). In as much as the direct cell monitoring was conducted before the archived chromatograms were investigated, and the monitoring programs had different overall objectives, cell counts and but-fuco samples were not always con-

current. To hindcast *A. anophagefferens* abundance, cell counts and but-fuco data from 2000 to 2002 were regressed. Samples that fell within a 7-day range of each other were used in the regression, with the majority of samples (79.4%) falling within a 1-day range.

3. Results

3.1. Pigment and direct count comparison

The regression of direct cell counts of *A. anophagefferens* and but-fuco concentration (Fig. 2) yielded an R^2 of 0.78 ($n = 39$) according to the equation:

$$\text{cells (ml}^{-1}\text{)} = \frac{(\text{but-fuco}) - 0.2443}{1.73 \times 10^{-5}}$$

Cell counts were then classified as zero, or into three bloom categories, as previously defined by Gastrich and Wazniak (2002). Category 1 blooms are specified as $<35,000$ cells ml^{-1} causing no reported impacts. Category 2 blooms are between 35,000 and 2×10^5 cells ml^{-1} and have potential negative feeding and growth impacts upon shellfish. Category 3 blooms are $>2 \times 10^5$ cells ml^{-1} and may cause severe impacts or mortality on shellfish and reduction in submerged aquatic vegetation. Therefore, but-fuco concentrations $<0.85 \mu\text{g l}^{-1}$ were placed within Category 1, concentrations between 0.85 and $3.71 \mu\text{g l}^{-1}$ were placed in Category 2, and those $>3.71 \mu\text{g l}^{-1}$ were placed into Category 3.

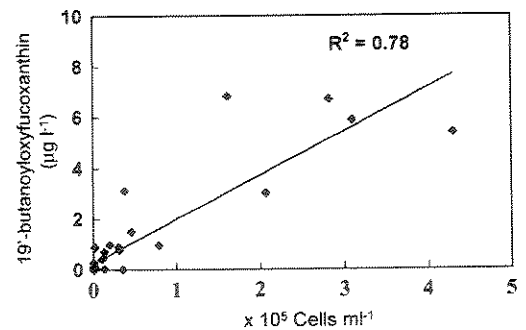


Fig. 2. Regression of 19'-butanoyloxyfucoxanthin ($\mu\text{g l}^{-1}$) concentrations vs. direct cell counts of *Aureococcus anophagefferens*. Regression is based on samples collected from 2000 to 2002 that fell within a 7-day range of each other (see text).

Table 1

Maximum observed concentrations of 19'-butanoyloxyfucoxanthin ($\mu\text{g l}^{-1}$) in the coastal bays for the station and year indicated. In 1993, the calibration used only permits an estimate of presence (+) or absence (-)

Region	Station number	1993	1994	1995	1999	2000	2001	2002
Newport Bay	3	+	0.37	6.04	1.51	1.49	5.37	5.89
	4	+	0.19	3.73	1.28	0.48	0.98	3.00
Sinepuxent Bay	1	+	0.00	0.00	0.05	0.03	0.70	0.70
	2	+	0.00	1.62	0.27	0.34	1.88	2.10
	16	+	0.36	2.43	0.23	1.09	4.75	2.72
	17	-	0.00	0.00	0.04	0.02	0.09	0.52
	18	-	0.00	0.43	0.05	0.02	4.46	1.09
Mid Chincoteague Bay	5	+	0.00	4.70	2.26	3.10	6.71	6.85
	6	+	0.00	4.06	0.39	2.59	2.10	1.33
	7	-	0.00	0.00	1.33	0.06	0.41	0.05
	14	+	0.00	0.00	2.44	0.26	0.40	0.04
Lower Chincoteague Bay	15	+	0.80	4.61	1.19	1.90	2.16	1.65
	8	-	0.00	0.00	0.17	0.00	0.02	0.03
	9	-	0.00	0.00	0.88	0.02	0.00	0.18
	10	-	0.00	0.00	0.40	0.03	0.02	0.00
	11	-	0.00	0.00	0.25	0.03	0.06	0.07
	12	-	0.00	0.00	0.19	0.06	0.04	0.06
	13	-	0.00	0.27	0.18	0.18	0.23	0.05

3.2. Temporal trends

In every year of this study, the presence of but-fuco was recorded, thus indicating that outbreaks of *A. anophagefferens* likely occurred as early as 1993 (Table 1). Maximum direct cell counts for the entire study period were 4.3×10^5 cells ml^{-1} and the maximum but-fuco concentration was $6.85 \mu\text{g l}^{-1}$. However, outbreaks from year to year varied in intensity. In 1993, the only year for which presence and absence was recorded, but cell densities could not be estimated, but-fuco was present at 50% of the stations sampled at some point during the year (Table 1). In 1994, concentrations of but-fuco were non-detectable at all but four stations (Table 1). Similarly, in 1995, there was no detectable but-fuco in half of the stations sampled, although both Category 2 and Category 3 blooms did occur (Fig. 3). In the years 1999–2002, however, over 96% of the stations had at least Category 1 blooms, and 20–44% of the stations had Category 2 or 3 blooms. Thus, based on the years sampled, the general temporal trend was that of brown tide becoming more widespread and blooms more intense (Table 1, Fig. 3).

As shown for the years having significant outbreaks (1999–2002), concentrations of but-fuco increased in May or June of each year and rapidly dissipated each

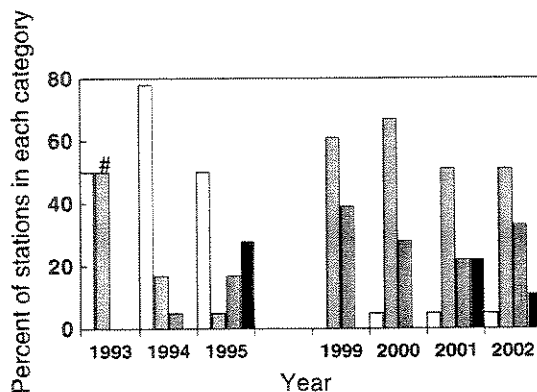


Fig. 3. Percent of stations with maximum concentrations of 19'-butanoyloxyfucoxanthin within the ranges that define different levels of brown tide blooms for the years indicated. Concentrations $<0.85 \mu\text{g l}^{-1}$ are defined herein as Category 1 blooms; concentrations $0.85\text{--}3.71 \mu\text{g l}^{-1}$ are defined as Category 2 blooms, and those $>3.71 \mu\text{g l}^{-1}$ are defined as Category 3. Open bars are frequency of measurements with no detectable 19'-butanoyloxyfucoxanthin; gray bars are frequency of Category 1 blooms; hatched bars are frequency of Category 2, and black bars are frequency of Category 3 blooms. Note that for 1993, all positive records are shown as Category 1 blooms although their actual strength may have been greater; the method used in that year did not permit further differentiation (see text). That bar is shown with a # to indicate potential underestimation.

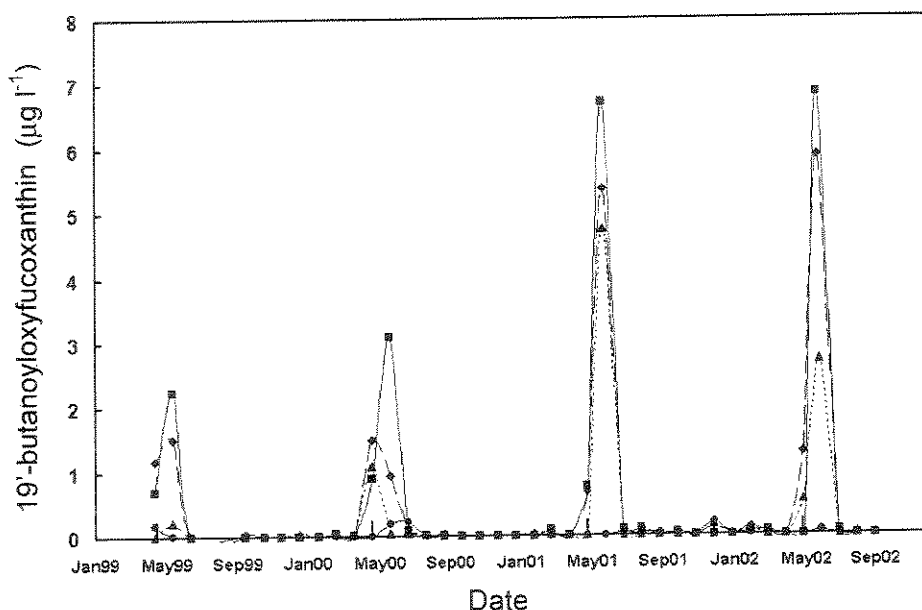


Fig. 4. Concentration of 19'-butanoyloxyfucoxanthin ($\mu\text{g l}^{-1}$) for the month and year indicated. Results shown are from Station 3 (diamonds), Station 5 (squares), Station 13 (circles), and Station 16 (triangles).

July (Fig. 4). Concentrations were generally nominal throughout the remainder of the year although low concentrations of but-fuco were detected in some early spring and late fall samples.

3.3. Spatial and temporal variability

Geographically there were differences both between the strength of the concentrations of but-fuco and also the time of first appearance annually. On a north-south basis, higher per station maxima of but-fuco were generally found in Newport Bay, and mid-Chincoteague Bay, with lower values found in the lower Chincoteague Bay (Fig. 5). However, there were year-to-year differences in these patterns, such as in 2001 when similar maximum but-fuco concentrations were found in all regions except the lower Chincoteague Bay (Fig. 5). On an east-west basis, Station 3 in Newport Bay and Station 5 near Public Landing on the western shore of Chincoteague Bay generally appeared to have higher concentrations than stations on the eastern basin (Fig. 4).

There were differences in the timing of the blooms as well, as shown by an interannual comparison of May to July for years 1994 and 1995 (Fig. 6). In 1994,

Category 1 blooms first appeared in May in Newport Bay, the lower Sinepuxent and one mid Chincoteague Bay station. In June 1994, similar low level bloom conditions existed with the addition of two more Category 1 stations. During July 1994, concentrations of but-fuco fell to non-detectable levels. In May 1995, similar Category 1 bloom conditions existed as in May 1994. In June 1995, however, large scale Category 2

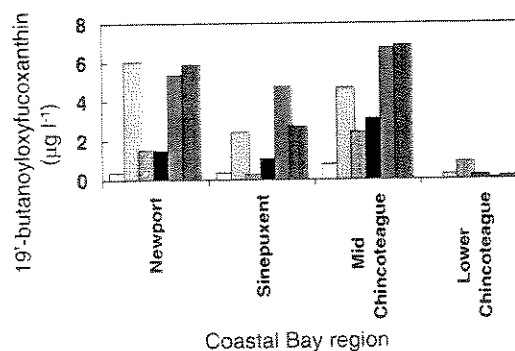


Fig. 5. Maximum observed concentration of 19'-butanoyloxyfucoxanthin ($\mu\text{g l}^{-1}$) for the regions of the coastal bays indicated. The six bars for each region represent 1994, 1995, 1999, 2000, 2001, and 2002, respectively.

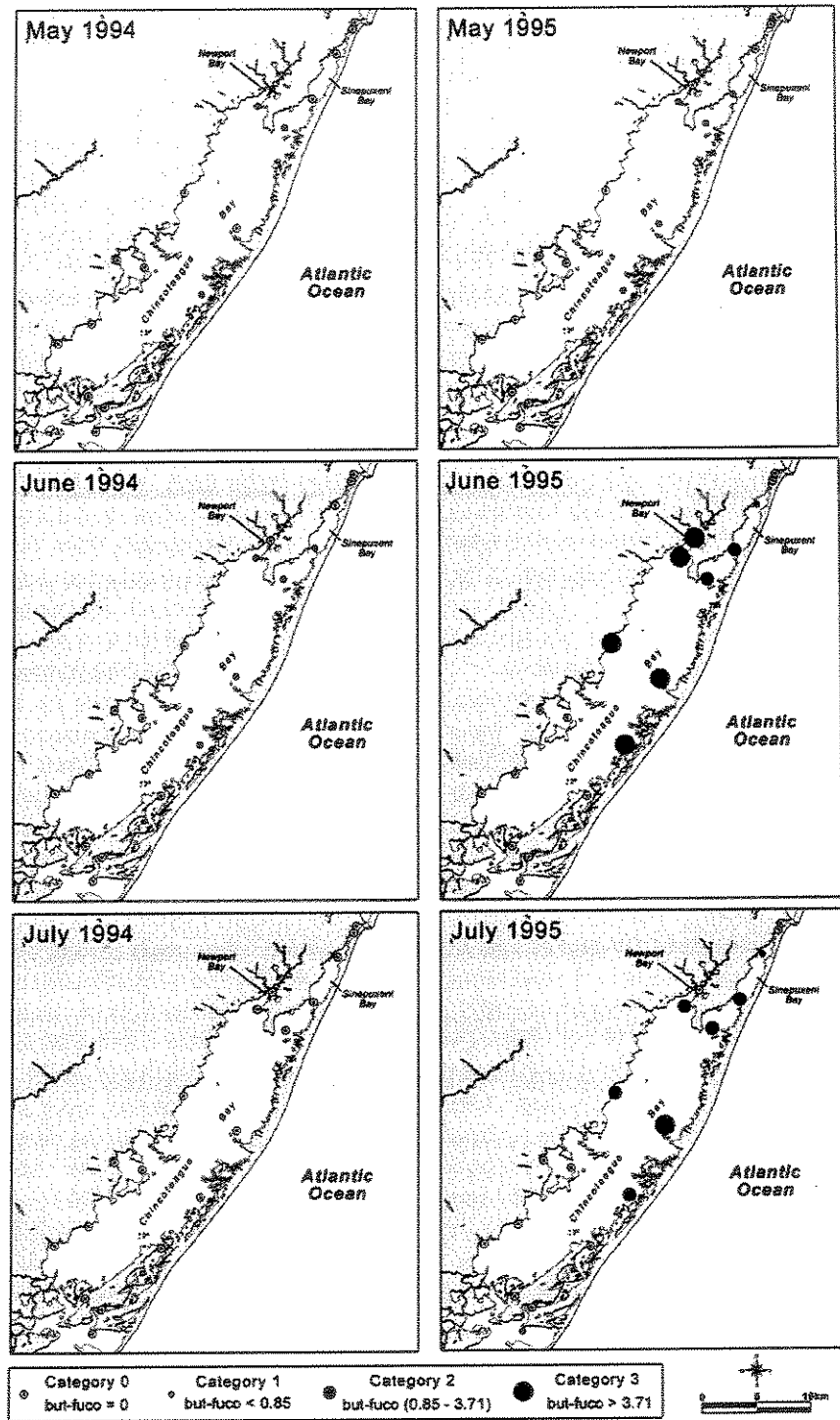


Fig. 6. Seasonal and spatial comparison of concentrations of 19'-butanoyloxyfucoxanthin ($\mu\text{g l}^{-1}$) for the years 1994 and 1995. The relative size of the symbols is indicative of the strength of the bloom. Open symbols indicate stations that were sampled, but no 19'-butanoyloxyfucoxanthin detected.

and 3 blooms were observed in the northern study areas. Category 2 bloom conditions persisted into July 1995 in the lower Sinepuxent and mid-Chincoteague Bays.

4. Discussion

In the absence of cell count data, but-fuco concentrations proved useful in reconstructing the likely presence of *A. anophagefferens* in the Maryland and Virginia coastal bays as early as 1993, the first year of available HPLC data. Although the HPLC data were collected with a different purpose, this is one example where retrospective examination, in conjunction with new data, including direct cell enumerations, has yielded insight into likely previous dynamics of this bloom-forming organism. These results raise the question that the conclusion of Anderson et al. (1993), that no brown tide existed in the coastal bays in 1990, may have been biased by their late summer sampling or choice of one sampling location.

The annual patterns in brown tide reported here, albeit inferred from but-fuco concentrations, show variable relationships with meteorological conditions found to be associated with some other brown tide blooms. It has previously been found that brown tides in Peconic Bay, New York, are correlated with years of low rainfall and groundwater flow (e.g. LaRoche et al., 1997). When groundwater levels are low, organic nitrogen inputs tend to be higher, and *A. anophagefferens* is a strong competitor for this form of nitrogen (Dzurica et al., 1989, Berg et al., 1997, Lomas et al., 2001, Gobler et al., 2002). Although this correlation has not held for all systems examined (e.g. Gobler and Sanudo-Wilhelmy, 2001), both 1995 and 2002 were particularly dry years. The highest intensity blooms, with estimated cell counts reaching Category 3 levels, were found in 1995, 2001, and 2002. A large brown tide bloom was also observed in 1995 in Long Island (Lomas et al., 1996, Berg et al., 1997) and was also associated with drought conditions (Bricelj and Lonsdale, 1997). Cell count maxima at some Long Island locations in 1995 were $>1.7 \times 10^6$ cells ml⁻¹ (Bricelj and Lonsdale, 1997) whereas maximum estimated Maryland concentrations were 3.7×10^5 cells ml⁻¹. Some of this difference may be attributable to sparser temporal and spatial sampling

in Maryland at that time. In contrast, however, large blooms (with >30% of stations at Category 2 levels) were found in 1999 and 2000, years that were not considered drought years. In another analysis of groundwater inputs and relationship to brown tides, Gobler and Sanudo-Wilhelmy (2001) found that fluctuations in groundwater on a short time scale can also be related to brown tide development mediated by other blooms that develop and subsequently lead to increases in organic nitrogen. In that regard, it is interesting that while large blooms of brown tide were documented in Maryland in 2001, only relatively small blooms were found off Long Island that year (Lomas et al., 2004). In coastal bays, there are also direct inputs of organic nitrogen from runoff, as urea and manures are used for fertilizer in the agricultural regions of the more northern region, so short term variability may be important in this system. Thus, the general trend toward increasing frequency and strength of brown tide blooms over the years of this study seems to override the effects of rainfall or flow, but continued monitoring in the future is required to confirm this pattern.

The effects of brown tide on Maryland and Virginia coastal bays' living resources are still relatively unknown. Wazniak and Glibert (2004) found, during a 2002 brown tide event, that the rate of growth of juvenile hard clams *Mercentaria mercenaria* in the coastal bays was negatively impacted at brown tide densities as low as 20,000 cells ml⁻¹, but that growth rates recovered following the collapse of the brown tide bloom. That study, along with Bricelj et al.'s (2001) findings of a 35,000 cells ml⁻¹ growth inhibition threshold for hard clams, stresses the importance of quantifying lower concentration blooms.

Results of a hard clam survey by Tarnowski and Bussell (2002) suggested some negative impacts of brown tide. In 2000, 12% of their 111 randomly selected stations throughout the coastal bays contained juvenile clams, while the 2001 survey only observed 6%. Overall 2001 had higher maxima of but-fuco and *A. anophagefferens* concentrations (direct count data) than 2000. Their study also indicates a general decline in hard clam densities in Newport, Sinepuxent, and Chincoteague Bays between 2001 and 2002, which both had 44% of stations reporting maxima in Category 2 or 3 of the bloom index.

This study has thus shown the usefulness of HPLC pigment records in estimating past blooms of *A.*

anophagefferens. It has also shown that severe Category 3 blooms of *A. anophagefferens* in the coastal bays have likely occurred in the past, and that the frequency and intensity of blooms have apparently increased over this decade. Furthermore, these blooms appear to have had some negative impacts on the growth rates of juvenile clams.

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