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## Phytoplankton Blooms: Their Occurrence and Composition Within Virginia's Tidal Tributaries

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### ABSTRACT

Sporadic algal bloom development within a 10 year monitoring program in Virginia tidal tributaries of Chesapeake Bay is reviewed. These blooms were common events, characteristically producing a color signature to the surface water, typically short lived, occurring mainly from spring into autumn throughout different salinity regions of these rivers, and were produced primarily by dinoflagellates. The abundance threshold levels that would identify bloom status from a non-bloom presence were species specific, varied with the taxon's cell size, and ranged from ca. 10 to 10<sup>4</sup> cells mL<sup>-1</sup>. Among the most consistent sporadic bloom producers were the dinoflagellates *Akashiwo sanguinea*, *Cochlodinium polykrikoides*, *Heterocapsa rotundata*, *Heterocapsa triquetra*, *Karlodinium veneficum*, *Prorocentrum minimum*, *Scrippsiella trochoidea*, the cyanobacterium *Microcystis aeruginosa*, and two categories containing several species of often unidentified *Gymnodinium* spp. and *Gyrodinium* spp. Additional bloom producers within these tributaries are also discussed.

Keywords: Virginia, rivers, phytoplankton, blooms, Chesapeake Bay.

### INTRODUCTION

Algal blooms occur in freshwater habitats, estuaries, the world oceans, and are natural phenomena (Anderson et al., 2002). The term "algal bloom" refers to high concentrations of one or more algal species, and generally implies visual recognition of this development by color enhancement in the water column due to pigments contained in the algal cells. These colors may vary due to the different types and amount of pigments within the cells of the bloom producing species. Algal blooms have also been associated with toxic events (e.g. red tides) involving fish and shellfish mortality and human illness (Falconer, 1993; Anderson et al., 2002). Many of these species have been referred to as producing harmful algal blooms (HAB), with concern regarding their apparent increased occurrences in estuaries and oceans world-wide (Smayda, 1990; Hallegraeff, 1993; Anderson et al., 2002; Burkholder et al., 2005). In many of the toxin producing species the bloom designation becomes a secondary factor to the presence of a toxin and established toxin threshold levels of concern (Rensel and

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Whyte, 2003). Within the Chesapeake Bay estuarine system a variety of potentially harmful species and bloom producers have been identified and many of these are common constituents of the river flora in Virginia (Marshall, 1996; Marshall et al., 2005, 2008a). The presence alone of these recognized toxic species does not indicate they will cause a serious impact to the health status of these waters. Cell concentrations may not reach the abundance levels required for significant levels of toxin production that would have an environmental impact (Smayda, 1997; Marcaillou et al., 2005), or these may be non-toxin producing strains of the toxic species (Burkholder et al., 2005). However, blooms of both the toxin or non-toxin producing species can deteriorate water quality to the extent that they may impact various indigenous biota (e.g. by reducing oxygen levels, impairing gill function in fish and shellfish).

The environmental impact of an algal bloom would depend on the duration of the bloom, the taxon producing the bloom, and its cell concentrations. However, a wide range of cell concentrations have been associated with bloom status among the phytoplankton components. Paerl (1988) refers to blooms produced by different taxa ranging in abundance from  $10^4$  to  $>10^6$  cells  $\text{mL}^{-1}$ , whereas Smayda (1990) mentions bloom maxima occurring at sea of 10 cells  $\text{mL}^{-1}$  to  $>10^4$  cells  $\text{mL}^{-1}$ . Kim et al. (1993) identified variable bloom concentrations attributed to several species in the southeastern coastal waters of Korea. They noted low bloom densities of  $10^2$  to  $10^4$  cells  $\text{mL}^{-1}$  and high bloom densities for particular species ranging from  $10^2$  to  $10^5$  cells  $\text{mL}^{-1}$ . These differences are most often influenced by the cell size of the bloom producing species. Many of the smaller nanoplankters would require a greater number of cells to produce a visible bloom signature in the water compared to larger cells and filamentous taxa. Kim et al. (1993) subsequently recommended cell volume thresholds for identifying red tide blooms as  $3 \times 10^6 \mu\text{m}^3$  for nanoplankton and  $5 \times 10^6 \mu\text{m}^3$  for the larger cells of the microplankton. In another approach, Tett (1987) associated general and exceptional bloom events in reference to their chlorophyll concentrations per unit volume of water, with noticeable changes in water discoloration began when levels exceeded  $10 \text{ mg Chl m}^{-3}$ . The larger exceptional blooms had values greater than  $100 \text{ mg Chl m}^{-3}$ . Species specific criteria have also been used; for instance the Commonwealth of Virginia established a chlorophyll level of  $27.5 \mu\text{g L}^{-1}$  ( $27.5 \text{ mg Chl m}^{-3}$ ) and  $50,000 \text{ cells mL}^{-1}$  as bloom criteria for *Microcystis aeruginosa* a potential toxin producer.

A particular taxon may also have cell concentrations and biomass lower than that of other taxa within the water column, but still represent a major development in its annual productivity, yet not dominating the algal assemblage (Parker, 1987; Smayda, 1997). This is frequently noted in annual monitoring programs where background flora of usual low abundance, may seasonally achieve a modest, but often a short-lived period of high abundance, with their concentration levels and degree of color enhancement to the water lower than other more abundant or larger taxa. Reference to these abundance peaks represent an alternate method of describing bloom status that may or may not include a color signature to the water column, but relate to the seasonal population dynamics that is species specific.

Conditions associated with the inception and duration of seasonal blooms include a variety of environmental factors: e.g. concentrations of nutrients (e.g. nitrogen, phosphorus, silicon, etc.), temperature, salinity, light availability, river flow, cloud cover, grazing pressure, among other factors (Pratt, 1965; Riley, 1967; Tett, 1987;

Smayda, 1990; Keller et al., 1999, 2001; Glibert et al., 2001; Anderson et al., 2002; Iriarte and Purdie, 2004). Seasonal blooms of short or long duration are determined by various combinations of these conditions and their influence on the composition and abundance of the flora and potential bloom producers. These bloom events may, or may not be associated with foul odors, fish or shellfish mortality, reduced oxygen levels, or human illness. The degree of color enhancement to the water due to bloom development would also vary with the taxon and its abundance over time. Some blooms produce a clearly recognizable color signature in the water, whereas with other taxa the bloom presence will not be clearly visible. In general, blooms occur when one or more species respond to environmental conditions favorable to their increased development beyond their usual abundance levels. Smayda and Reynolds (2001) characterize this response as stochastic, influenced by the characters and traits innate to a species, and their ability to take advantage of prevailing conditions within the water body, and directly respond with increased concentrations.

Seasonal phytoplankton composition for Virginia tidal tributaries and the southern Chesapeake Bay have been recorded routinely by Old Dominion University (ODU) Phytoplankton Analysis Laboratory (ODUPAL) since 1985 (Marshall, 1994; Marshall et al., 2005). Phytoplankton composition and seasonal representation of taxa within the tidal rivers and Chesapeake Bay include a diverse algal representation (>1,400 taxa) and seasonal successional patterns of dominant bloom producers characteristic of temperate regions (Marshall, 1990, 1994, 1995a; Marshall and Nesius, 1996; Marshall and Burchardt, 1998, 2003, 2004a, 2004b, 2005; Marshall et al., 2005, 2009). The objectives of this paper are to provide information on sporadic bloom producing algae in Virginia tidal waters with information regarding the frequency and locations of these bloom events. In addition, cell abundance criteria are provided to formerly classify bloom status for these bloom producers.

## METHODS

The ODUPAL has closely interacted with the Virginia Department of Health Division of Shellfish Sanitation (VDHSS) and the Virginia Department of Environmental Quality (VDEQ) in providing information on the identification of algal species associated with bloom events in Virginia waters for several decades. In addition, a Virginia program initially designated in 1998 as the *Pfiesteria* Task Force (later renamed the Harmful Algal Bloom Task Force) was established to monitor potentially harmful algal blooms in Virginia waters. With the exception of 2003, routine water samples from this program were taken monthly March-October from 1998, with additional collections taken during any major algal bloom or fish-kill events. These samples were provided to the ODUPAL by VDHSS and VDEQ for determining species identification and their abundance. Data from these collections through 2008 have been incorporated in this report.

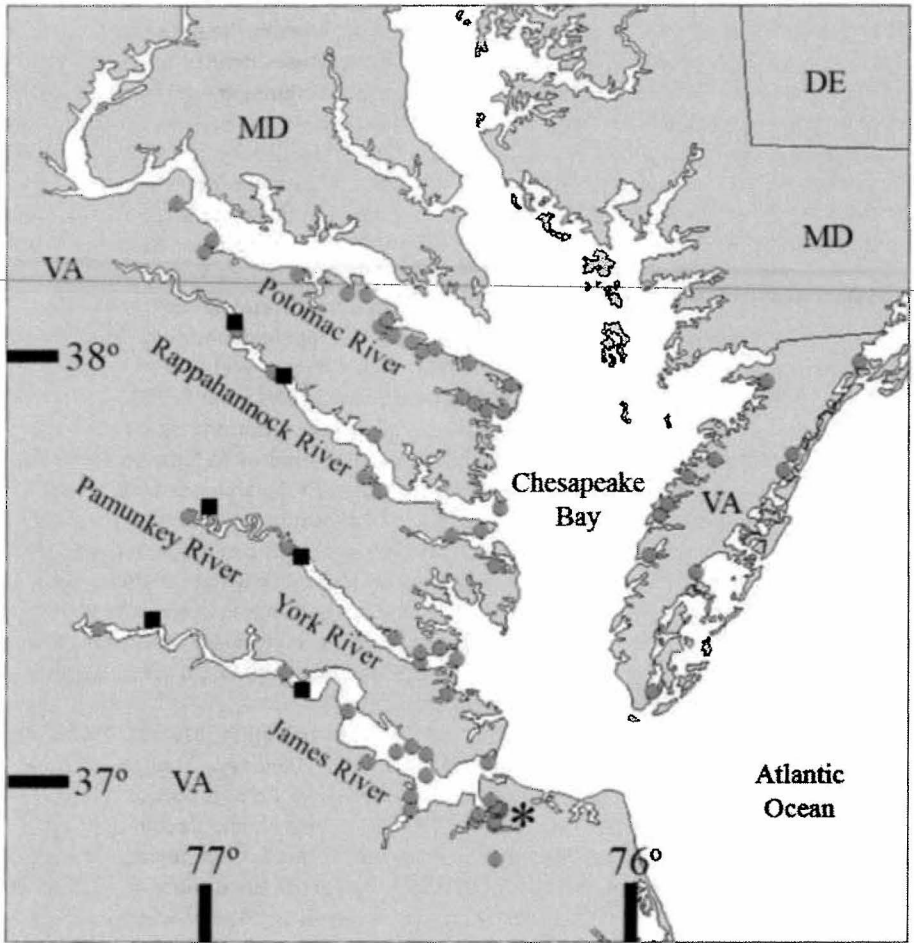


FIGURE 1. Station locations monitored 1998-2008 for algal blooms. ■ = VADEQ Stations, ● = VADH stations, VA = Virginia, MD = Maryland, DE = Delaware, \*location of Elizabeth and Lafayette rivers.

These investigations also included water quality data related to seasonal and sporadic algal blooms, and population trends within the Chesapeake Bay estuarine complex (Marshall and Burchardt, 2004a; Marshall et al., 2006, 2008a, 2009; Nesius et al., 2007). The mean number of stations monitored annually during this period was 78. A total of 4,467 preserved water samples were analyzed during these collections (1998-2008).

The water samples (0.5 or 1.0 L) were taken at the surface (< 1m) and fixed on station with Lugol's solution (2-3 ml). Standard light microscopic protocols were used with the algae examined at 300X and 600X for species identification and cell counts

(Marshall et al., 2005). This protocol was often supplemented with scanning electron microscopy, and more recently using PCR analysis to verify the presence of several potentially harmful species (Marshall et al., 2009). Water quality parameters were determined by the VDEQ and the ODU Department of Chemistry and Biochemistry.

## RESULTS

A total of 51 tributary and various sub-estuarine sites were identified where algal bloom events occurred, often repeatedly and annually at the same locations. Blooms were recorded at 26 creeks, 17 rivers, and 6 inlet bays in Virginia. Several of these blooms also progressed into lower Chesapeake Bay and to coastal waters along the Virginia Beach shoreline. Among the most common locations were the shoreline inlets, creeks, and waters of the Potomac, York, and Rappahannock rivers, plus a river complex in the lower James River that includes the James, Warwick, Lafayette and Elizabeth rivers (Fig. 1). Using the VDHDSS data base of 1998-2002, 2004-2008), and the VDEQ collections 1998-2008, the number of recorded blooms by 43 taxa ranged from 35 (2002) to 142 (2000) annually. There was a total of 685 blooms identified within the 4,467 samples examined, indicating 15.3% of the water samples contained bloom concentrations of at least one species. The highest number of blooms occurred in 2000 and 2001 which were also years of lower mean river discharge in the rivers of Chesapeake Bay (U.S. Geological Survey monthly stream flow data). During summer and early autumn, major algal development increased in the lower reaches of these rivers during periods of reduced river flow and longer phytoplankton residency time within these rivers (Marshall and Burchardt, 1998, 2003, 2004a, 2004b, 2005).

April through September was the predominant time period for blooms within these tributaries, with the lowest occurrence in December and January. These blooms were generally dominated by dinoflagellates, with the majority of blooms occurring in water temperatures between 18 and 30 °C, salinities of 8 to 18 ppt, and Secchi depths < 1.2 m. These blooms occurred over a broad range of these parameters, which was indicative of growth responses by a variety of taxa to conditions favoring their increased development. Oxygen concentrations during these blooms were consistently above dystrophic levels (> 4 mg L<sup>-1</sup>). However, no records were kept of oxygen concentrations at these sites throughout the bloom development. Using a 4-year (1998-2001) portion of the VDHDSS tributary station data, Weber and Marshall (2002) noted water quality conditions during bloom events by dinoflagellates classified as *Pfiesteria*-like organisms (PLO). This category included *Pfiesteria piscicida*, *Pfiesteria shumwayae*, and several other taxa grouped at that time as morphologically similar under light microscopy (e.g. several *Gymnodinium* spp. and *Gyrodinium* spp., plus *Cryptoperidiniopsis* sp. and *Karlodinium veneficum*). This category's bloom concentrations and color signatures in the water were associated with the following range of environmental conditions: salinity (8.0-18.4 ppt), temperature (18.0-26.1 °C), chlorophyll a (>16 µg L<sup>-1</sup>), total phosphorus (>0.01 mg L<sup>-1</sup>), TKN (>0.5 mg L<sup>-1</sup>), total dissolved nitrogen (>0.31 mg L<sup>-1</sup>), particulate carbon (>0.25 mg L<sup>-1</sup>), ammonia (>0.04 mg L<sup>-1</sup>), dissolved oxygen (6.7-13.1 mg L<sup>-1</sup>), and Secchi depth (<1.0 m). These parameters were generally similar to conditions throughout the complete data set when dinoflagellate blooms occurred in these tributaries. The concentration levels among the phytoplankton when they imparted a color pattern to the water column varied

considerably between early and later stages of the bloom, as did the color intensity, e.g. higher cell concentrations were often noted along tidal fronts or at near shore locations. There were also temporal differences in the initiation and development of blooms at stations within a river, and of similar events in adjacent rivers. The threshold abundance levels for identifying bloom status varied among the dinoflagellates and were related to their cell size and pigment content. In general, larger cells produced distinct coloration during modest bloom development in contrast to less distinct bloom color enhancement with higher cell concentrations from a smaller size bloom producer. For instance, *Akashiwo sanguinea* and *Cochlodinium polykrikoides* have larger cell sizes and pigment concentration, with lower threshold levels for bloom status than species with smaller cell sized cells (e.g. *Microcystis aeruginosa*). The threshold range for blooms between these taxa was from 10 and  $10^2$  to  $10^4$  cells  $\text{ml}^{-1}$ . Often, a major bloom of one taxon would overshadow a less conspicuous bloom of another species (*Heterocapsa rotundata*) both occurring simultaneously, and responding to favorable growth conditions for their bloom development. Several bloom producing dinoflagellates in this category were also background, or companion species to the more visual blooming taxa, resulting in multiple bloom status for several species at the same time.

Throughout the study period, sporadic bloomers were represented by a diverse assemblage of algae (43). Among these are the 28 bloom producers listed in Table 1. They include 13 dinoflagellates, 7 diatoms, 3 cyanobacteria, 2 euglenophytes, 1 chlorophyte, 1 cryptophyte, and one ciliate (Table 1), with the other species occurring less frequently during this period. Bloom events of record included only those occurring during routine sampling periods, or following special bloom notification and sampling by VDEQ and VDHDSS. Due to daily or seasonal variability in species concentrations, infrequent water analysis, or without an observed color signature, there were likely numerous algal blooms in these waters that were not recorded. Although not inclusive of all bloom occurrences, or taxa that produced blooms during this period, the long term records of these events were considered a representative indication of the bloom species and bloom events in these waters. Of these, the dinoflagellates produced 82% of the recorded blooms, followed in frequency by diatoms (6%) and cyanobacteria (5%), with the other taxa each producing ca. 1-2% of the recorded blooms. There was also the seasonal sequence of taxonomic groups that extended over monthly periods and was repeated annually. For example, the increased diatom concentrations of winter and early spring (e.g. *Skeletonema costatum*, *Skeletonema potamos*, *Cerataulina pelagica*) were subsequently followed by a diverse assemblage of dinoflagellates that produced scattered bloom events throughout these tributaries and which continued into summer and autumn (Marshall, 1994; Marshall et al., 2005). Even when these diatoms were the dominant taxa during this winter/spring period, they also exhibited short periods of sporadic increased cell concentrations at various stations. Other diatoms associated with seasonal sporadic blooms included several *Chaetoceros* spp., *Leptocylindrus minimus*, *Pleurosigma angulatum*, and *Thalassiosira nordenskiöldii*. Their blooms were more prevalent in the lower reaches of these rivers.

The dinoflagellate *Heterocapsa rotundata* was a common component of the algal flora and a sporadic bloom producer throughout the year, with a bloom threshold beginning at  $10^2$  cells  $\text{mL}^{-1}$ . Other dinoflagellates having a more dominant presence

TABLE 1. Representative bloom producers in Virginia tributaries 1998-2008. \* species more broadly distributed with seasonal bloom development; \*\*Dominant diatoms during spring diatom bloom; @ species considered harmful or toxin producers. Others composition: <sup>1</sup>Chlorophyte, <sup>2</sup>Cryptophyte, <sup>3</sup>Euglenophyte, <sup>4</sup>Ciliate.

<b>Dinoflagellates</b>
<i>Akashiwo sanguinea</i> (Hiraska) Hanse *@
<i>Alexandrium monilatum</i> (Howell) Balech @
<i>Cochlodinium polykrikoides</i> Margelef *@
<i>Gymnodinium</i> spp. *
<i>Gyrodinium</i> spp. *
<i>Heterocapsa rotundata</i> (Lohmann) Hansen *
<i>Heterocasa triquetra</i> (Ehrenberg) Stein *
<i>Karlodinium veneficum</i> (Ballantine) J. Larsen *@
<i>Pfiesteria piscicida</i> Steidinger et Burkholder @
<i>Pfiesteria shumwayae</i> Glasgow et Burkholder @
<i>Prorocentrum minimum</i> (Pavillard) Schiller *@
<i>Protoperidinium</i> spp.
<i>Scrippsiella trochoidea</i> (Stein) Loeblich III *
<b>Cyanobacteria</b>
<i>Merismopedia tenuissima</i> Lemmermann *
<i>Microcystis aeruginosa</i> Kützing *@
<i>Microcystis incerta</i> Lemmermann
<b>Diatoms</b>
<i>Cerataulina pelagica</i> (Cleve) Hendey **
<i>Chaetoceros</i> spp.
<i>Leptocylindrus minimus</i> Gran
<i>Pleurosigma angulatum</i> (Quekett) W. Smith
<i>Skeletonema costatum</i> (Greville) P.T.Cleve **
<i>Skeletonema potamos</i> (Weber) Hasle **
<i>Thalassiosira nordenskioeldii</i> P.T. Cleve
<b>Others</b>
<i>Chlamydomonas</i> spp. <sup>1</sup>
<i>Cryptomonas erosa</i> Ehrenberg <sup>2</sup>
<i>Euglena</i> spp. <sup>3</sup>
<i>Eutreptia lanowii</i> Steuer <sup>3</sup>
<i>Myrionecta rubra</i> (Lohmann) Jankowski <sup>4</sup>

from late spring into autumn included the cyst producers *Heterocapsa triquetra* and *Scrippsiella trochoidea*, plus *Akashiwo sanguinea*. Bloom threshold levels associated with *H. triquetra* and *S. trochoidea* began at  $10^3$  cells  $\text{mL}^{-1}$ , and for the larger *A. sanguinea* 10 cells  $\text{mL}^{-1}$ . The dinoflagellate blooms were also more prominent in the



lower reaches of these tributaries, whereas, the less saline regions contained increased summer/fall concentrations of cyanobacteria (*Microcystis* spp., *Merismopedia tenuissima*) and chlorophytes, e.g. *Chlamydomonas* sp. (Marshall and Burchardt, 1998, 2004a). Common components throughout these tidal regions were cryptophytes and a diverse assemblage of diatoms. The autotrophic picoplankton produced their greatest concentrations during summer, with diatoms gaining more prominence in late autumn and into winter (Marshall, 1995a; Marshall et al., 2005). Several of the dinoflagellate categories were composed of multiple species under a genus category (*Gymnodinium* spp., *Gyrodinium* spp., *Protoperidinium* spp.), with many of these taxa having sporadic seasonal occurrence with bloom thresholds of ca.  $10^2$  to  $10^3$  cells  $\text{mL}^{-1}$  depending on the particular taxon. There also existed dynamic tidal conditions between these rivers, the Chesapeake Bay, and the adjoining Atlantic coastal waters. These water movements provided access of bloom producing species from these locations to the lower reaches of these rivers and at times produced blooms. These taxa included *Eutreptia lanowii*, *Noctiluca scintillans*, *Prorocentrum micans*, and *Protoperidinium* spp. Other occasional bloomers entering from the Bay were *Ceratium furca* and *Polykrikos kofoidii*.

Among the bloom producing dinoflagellates several taxa have gained additional concern due to being potentially harmful, including *Cochlodinium polykrikoides*. This species was one of the more prolific and common bloom producer during the warm summer months in several lower Chesapeake Bay tributaries. It has been described by Mackiernan (1968), Zubkoff and Warinner (1975), and Zubkoff et al. (1979) as a re-occurring bloom producer in the lower York River, and is considered potentially toxic and associated with fish kills (Steidinger, 1993). In September 1992, *C. polykrikoides* produced a bloom that extended southward from the Rappahannock and York rivers that entered many of the tributaries and inlets along the western border of lower Chesapeake Bay. During this period the bloom spread over ca. 215  $\text{km}^2$  of the Bay's central and western regions, then continued beyond the Chesapeake Bay entrance, and progressed to the North Carolina coastal region (Marshall, 1995b). As a cyst producer, the species was able to "seed" various tributaries during this and other bloom events along the southwest shoreline of the Bay to subsequently produce reoccurring blooms in these waters (Seaborn and Marshall, 2008). Thus, *C. polykrikoides* has established itself in the Lafayette, Elizabeth, and James rivers with annual bloom concentrations appearing in mid-summer and often lasting into autumn. Early stages of the *C. polykrikoides* blooms generally began at ca.  $10^2$  cells  $\text{mL}^{-1}$  then soon escalated rapidly in abundance (e.g.  $>10^3$  cells  $\text{mL}^{-1}$ ) along with producing a reddish/brown color to the water. An especially long-lasting bloom occurred during August/September 2007 within the lower James River complex, with the bloom lasting 5 weeks at concentrations between  $10^2$  to  $>10^4$  cells  $\text{mL}^{-1}$ . Detailed discussion of this bloom entering Chesapeake Bay and related water quality relationships have been discussed by Mulholland et al. (2009). Another bloom of this species occurred August 29, 2008 in Knitting Mill Creek, a small tributary of the Lafayette River (Norfolk, VA) with the wind blown surface concentrations along the stream bank at  $11.5 \times 10^4$  cells  $\text{mL}^{-1}$  in addition to a small fish kill. For the past decade this Creek and the Lafayette River have been major bloom sites for this species. These blooms were also associated with high concentrations of cryptomonads in addition to bloom levels of other dinoflagellates (e.g. *S. trochoidea*, *H. rotundata*, and *Gymnodinium* spp.).

*Karlodinium veneficum* (*Gyrodinium galatheanum*) has produced blooms in Virginia and Maryland tidal waters from spring to early autumn (Li, et al., 2000, Goshorn, et al., 2004). The toxicity of *K. veneficum* and its association with fish kills in both agricultural ponds and Chesapeake Bay estuaries have also been reported (Li et al., 2000; Deeds et al., 2002; Goshorn et al., 2004). A major *K. veneficum* bloom developed in the Potomac River and Virginia inlets to the Potomac that lasted from June through August 2007 at concentrations of  $10\text{-}33.7 \times 10^4$  cells  $\text{ml}^{-1}$ . Bloom levels associated with this taxon would begin at ca.  $10^3$  cells  $\text{ml}^{-1}$ . To date its major blooms regionally occurred in the Potomac River and its associated tributaries. The environmental conditions during blooms of this taxon also supported increased concentrations of other dinoflagellates including *A. sanguinea* and *H. rotundata*, among others.

*Prorocentrum minimum* has been recognized as a major constituent of the flora throughout the Chesapeake Bay estuarine system, and is a common species from early spring into late autumn, with its lowest representation during winter (Tango et al., 2005; Marshall et al., 2006). This was one of the most frequent bloom producers in Virginia tributaries, with bloom thresholds at  $10^3$  cells  $\text{ml}^{-1}$ . Blooms were associated with a reddish/brown coloration to the water and these have been referred to as mahogany or red tides (Tango et al., 2005). These were more common in the higher saline regions of these rivers and less abundant at upstream tidal stations. This taxon is considered a potential toxin producer (Steidinger, 1993; Heil et al., 2005). Brownlee et al. (2005) describe its living resource impact as reducing oxygen concentrations to anoxic and hypoxic levels with Gallegos and Bergstrom (2005) emphasizing these blooms may reduce light availability to submerged plants. Mean monthly concentrations were highest during April to June at  $10^2$  cells  $\text{ml}^{-1}$ . Records these past two decades have indicated years (1998, 2000, 2003, and 2006) of higher bloom concentrations ( $10^4$  cells  $\text{ml}^{-1}$ ), with several sporadic blooms reaching  $10^5$  cells  $\text{ml}^{-1}$  in 2000. Blooms of this species have occurred most frequently in Virginia tributaries at temperatures 18-28 °C, salinities of 8-14, and Secchi depth readings < 1.0 m, but it has also been recorded over a wider range of salinities and temperatures. Threshold levels for blooms began at  $10^3$  cells  $\text{ml}^{-1}$ . Tango et al. (2005) placed this threshold at  $3 \times 10^3$  cell  $\text{ml}^{-1}$ .

Although cyanobacteria are typically associated with freshwater habitats, representative taxa are common within the tidal fresh regions of these rivers, with lower concentrations in the downstream regions of increasing salinity (Marshall and Burchardt, 1998, 2003). Several of these taxa have been associated with toxin production and extended bloom development (Tango et al., 2005; Tango and Butler, 2008). The species of most recent concern has been *Microcystis aeruginosa*. Its mean monthly concentrations in these rivers were ca.  $10^3$  cells  $\text{ml}^{-1}$ , with lowest abundance levels during winter and highest in summer and autumn. *Microcystis* has produced re-occurring annual blooms in the upper regions of the Potomac River and the adjacent Maryland and Virginia tributaries and inlets along its shoreline and on occasion was associated with high levels of microcystin and health alerts (Goshorn et al., 2004; Tango and Butler, 2008; Marshall et al., 2008a). The blooms were often during periods of rising water temperatures and increased phytoplankton residency time within rivers during summer into early autumn. Threshold status for blooms began at  $10^4$  cells  $\text{ml}^{-1}$ , with health alerts generally at concentrations greater than  $10^4$  cells  $\text{ml}^{-1}$ . Tango and

Butler (2008) reported a July 2003 toxic bloom of *M. aeruginosa* with concentrations of  $1.6 \times 10^7$  cells  $\text{ml}^{-1}$  in a Maryland estuary. To date, similar extensive and long lasting blooms have not been recorded for the Rappahannock, James, York, or Pamunkey tidal regions. Other cyanobacteria associated with blooms in the tidal fresh regions of these rivers have included *Microcystis inserta* and *Merismopedia tenuissima*. Other typical fresh water taxa associated with less frequent bloom development include *Euglena* spp. and *Chlamydomonas* spp.

Blooms also occurred in these rivers by taxa from a variety of plankton species not typically present in these waters. For instance, the diatom *Pseudo-nitzschia cuspidata* produced a bloom in the bottom downstream waters of the Potomac River that persisted for several weeks in January 1999. Also, *Dinophysis acuminata* is a common Atlantic coastal dinoflagellate and potential producer of okadaic acid, the toxin resulting in diarrhetic shellfish poisoning (Marcaillou et al., 2005). When present in the lower Chesapeake Bay *D. acuminata* concentrations are usually low, with bloom recognition beginning at 10 cells  $\text{ml}^{-1}$ . However, it had an extensive bloom in several Potomac River (Virginia) embayments from February to April 2002, reaching 236 cells  $\text{ml}^{-1}$ , with trace amounts of okadaic acid detected at Potomac River locations. Marshall et al. (2003) suggested this species was transported in sub-pycnocline waters northward in Chesapeake Bay to subsequently bloom in these tidal estuaries. Its presence was noted in sub-pycnocline waters in the lower Chesapeake Bay months prior to this bloom. Tyler and Seliger (1978) have previously identified this pathway for the re-population of *Prorocentrum minimum* into the northern regions of Chesapeake Bay. This sub-pycnocline route may likely represent a conduit for other potentially harmful species to be conveyed from the Atlantic coastal waters into Chesapeake Bay regions and its sub-estuaries. Other species that may have followed a similar path of entry would include *P. cuspidata* mentioned above and the dinoflagellate *Noctiluca scintillans*, which is common to neritic waters, and has produced blooms in the lower James River (1987, 2000) and Chesapeake Bay (2002) (Marshall, 1995b).

Blooms of the ciliate *Myrionecta rubra* (*Mesodinium rubrum*) containing the red-pigmented cryptophyte endosymbiont have occurred frequently in Chesapeake Bay and in the lower regions of the Potomac, Rappahannock, York, and James rivers. In October 1995 a major bloom of *M. rubra* developed in the lower Chesapeake Bay with concentrations of ca. 500 cells  $\text{ml}^{-1}$  (Marshall, 1996). Another more recently reported taxon in Virginia waters is the dinoflagellate *Alexandrium monilatum*. It was first identified during routine sampling in September 2007 at sites in the York River at bloom concentrations of ca. 1,200 cells  $\text{ml}^{-1}$  (Marshall et al., 2008b). This is an ichthyotoxic species and commonly produces cysts following bloom development (Walker and Steidinger, 1979). There was a September 2008 and 2009 re-occurrence of this taxon within the York River, and in September 2009 also in the lower Chesapeake Bay at concentrations 125-256 cells  $\text{ml}^{-1}$ . These sequential yearly records imply that this species has established itself in this region (possibly enhanced through cyst development) and has now become an annual bloomer with the potential of spreading its range into other tributaries of Chesapeake Bay.

## Discussion

Phytoplankton blooms were common events within Virginia's tidal tributaries. They occurred frequently and were produced by a variety of species. These results support those of Parker (1987) and Smayda (1997) in that what characterizes a bloom is species specific and is directly influenced by cell size, pigment content, and cell abundance. Each taxon will respond to those environmental conditions favorable to its continued development, which frequently results in bloom concentrations, and a visible color signature in the water. The bloom threshold concentrations given here provide standards recommended for identifying bloom status among various algae in these tidal rivers.

Depending on the taxa, the threshold range for an algal bloom in these waters varied from  $10^3$  cells  $\text{ml}^{-1}$  to  $>10^4$  cells  $\text{ml}^{-1}$ . Although many of the blooms developed annually and became common occurrences, there were others that reached bloom status infrequently or represented latent populations of earlier recorded bloom producers. *Pfiesteria piscicida* and *P. shumwayae* were associated with blooms and fish kill events in Maryland tributaries in 1997. Detailed specifics regarding their occurrence and toxicity have been reported by Glibert et al. (2001), Duncan et al. (2005), Gordon and Dyer (2005), and Moeller et al. (2007). Glibert et al. (2001) also reported the 1997 blooms of *P. piscicida* in Maryland were not repeated in 1998, but were replaced by huge *P. minimum* blooms. Our present monitoring of *Pfiesteria* spp. by molecular genetic analysis indicated only a sparse and scattered presence of these taxa (mostly *P. shumwayae*) in Virginia tributaries, with no bloom events associated with these taxa in recent years. However, these species have remained present in these tributaries and still may respond to environmental conditions favorable to bloom development. The re-occurring bloom development of other taxa remained sporadic and unpredictable (e.g., *D. acuminata*, *N. scintillans*), with other indigenous species representing a category of consistent bloom producers (including *H. triquetra*, *P. minimum*, *S. potamos*, *S. costatum*).

Marshall (1989) reviewed reports of blooms occurring 1960-1989 within the Chesapeake Bay estuarine complex and noted a greater occurrence of blooms in the creeks and rivers entering the Bay (67%), with their highest incidence (54%) taking place during summer. Bloom concentrations were generally identified with taxa having  $10^3$  to  $10^4$  cells  $\text{ml}^{-1}$ . Major bloom producers during this earlier period included *P. minimum*, *H. triquetra* and *H. rotundata*. The present results agree that these same taxa are common bloom producers with high abundance in the regional rivers and streams. Presently  $>1,400$  phytoplankton species have been identified within the Chesapeake Bay estuary system, with 38 (2.5%) recognized as potentially harmful species (Marshall et al., 2005, 2008a). This study identified 28 species associated with the more common sporadic blooms, including 8 considered potentially toxic or harmful species. These were the cyanobacterium *M. aeruginosa*, and an assemblage of dinoflagellates represented by *A. sanguinea*, *A. monilatum*, *C. polykrikoides*, *K. veneficum*, *P. piscicida*, *P. shumwayae*, and *P. minimum*. Although these species represented a fairly small component for these waters, they were a potential source of serious environmental consequences (e.g. fish kills, shellfish contamination, and human illness), with other potentially harmful taxa likely to enter and populate these waters in the future.

Blooms were seasonally produced by a resident population of indigenous taxa, plus the occasional appearance of transient species and their subsequent bloom development. In general, favorable conditions for algal growth and bloom development existed in these rivers. A variety of these blooms were associated with rising water temperatures, increased phytoplankton residency time within these rivers, and an adequate nutrient supply. These conditions provided time for expanded algal bloom development and increased opportunities for bloom taxa to enter adjacent waters and continue to reintroduce cells to the rivers and maintain bloom status.

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