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Controls on the Formation of Algal Blooms in the Lower Chesapeake Bay and Its Tributaries

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CONTROLS ON THE FORMATION OF ALGAL BLOOMS IN THE LOWER CHESAPEAKE BAY AND ITS TRIBUTARIES

by

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ABSTRACT

CONTROLS ON THE FORMATION OF ALGAL BLOOMS IN THE LOWER CHESAPEAKE BAY AND ITS TRIBUTARIES

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Algal blooms occur seasonally in the Chesapeake Bay and its tributaries, and while the consequences of algal blooms have been qualitatively and quantitatively assessed, the causes of algal blooms and mechanisms of bloom initiation are still not well understood despite decades of research. In order to understand nutrient dynamics and other factors that promote the initiation of algal blooms, the Lafayette River, a tidal sub-estuary of Chesapeake Bay that experiences seasonal algal blooms, was sampled daily in the fall of 2005. Three phytoplankton blooms (Chlorophyll *a* concentrations exceeding twice the average of monthly measurements from 2000-2009) occurred during this period, a mixed bloom of *Akashiwo sanguinea* and *Gymnodinium* sp., a *Skeletonema costatum* bloom, and a monospecific *Gymnodinium* sp. bloom. Over the sampling period, nutrient concentrations increased following precipitation events and were elevated between bloom periods but low during blooms. All measured forms of nitrogen were positively lag-correlated with dinoflagellate abundance between 3 and 5 days in reverse time. Concentrations of NO_2^- reached 10 μM between September and October, indicative of incomplete nitrification. Over a 24-h period, nutrient concentrations and chlorophyll *a* biomass varied by an order of magnitude and were strongly linked to the tidal phase.

Massive blooms of the harmful alga *Cochlodinium polykrikoides* Margalef occurred in the lower Chesapeake Bay and its tributaries during the summers of 2007, 2008, and 2009. The Lafayette River appears to act as initiation grounds for these blooms. However in 2008 there were also localized sites of initiation and growth of *C. polykrikoides* populations within the mesohaline portion of the James River. In 2008, bloom initiation appeared to be correlated with intense, highly localized rainfall events during neap tides. During 2009, bloom formation occurred when water temperatures had stabilized at 26°C during a period of calm winds, neap tides, high positive tidal residuals, higher salinity, low nutrient concentrations and a low dissolved inorganic nitrogen (DIN) to dissolved inorganic phosphorous (DIP) ratio (DIN:DIP). Tidal flushing transported the *C. polykrikoides* bloom from the Lafayette River into the lower James River where it was transported upriver by local estuarine circulation. A combination of physical factors including, seasonal rainfall patterns, increased stratification, nutrient loading, spring-neap tidal modulation, and complex estuarine mixing and circulation allowed *C. polykrikoides* to spread and form massive blooms over large portions of the tidal James River and lower Chesapeake Bay. The primary control on the formation of algal blooms in the Lafayette River was water column stability, and bloom formation occurred during neap tides, when there was low wind-driven mixing, and increased buoyancy from rainfall and runoff.

This dissertation is dedicated to my family

Cheryl and James, and to my parents Eric and Julie Morse

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First and foremost I'd like to thank my major advisor Margie Mulholland for taking me on as her student and encouraging the work that lead to this dissertation, as well as for looking out for me all these years. I am grateful for her patience, willingness to help (even at 11:30 pm), and guidance along the way. I am most thankful for her tireless work ethic, and I deeply appreciate the countless hours spent editing and revising this document. Many thanks also go to the Mulholland Lab members, both past and present, and especially to our lab manager and expert angler, Peter Bernhardt. Many samples, both planktonic and piscatorial, were collected and analyzed over the course of this research thanks to Pete and the *R/V Bernhardt*. Thanks go to George Boneillo, KC Filippino, Ivy Ozmon, Leo Procise, Chris Schweitzer, and Brittany Widner for help, encouragement, and distractions along the way. Many thanks are due to Jose Blanco, who was always willing to listen and offer MatLab advice. I'd like to express a great deal of gratitude to my committee: Alexander Bochdansky, Harold Marshall, and Hans Paerl, for their guidance and suggestions along the way, and I look forward to collaborating with you in the future. I also wish to express my sincere gratitude to the folks at HRSD, Will Hunley, Scott Fentress, and Mike Wiggins for all the work you have done, without which this dissertation would not have been possible. I am grateful for your continued collaboration and look forward to exploring of new avenues of research and technologies in the near future. Lastly, I'd like to express my sincere and utter gratitude to my wife, Cheryl, for putting up with me all these years! You have shown a tremendous amount of patience and love, and for that I am truly grateful. I could not have done it without you.

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CHAPTER 1

INTRODUCTION

Worldwide, algal blooms appear to be increasing in frequency due to cultural eutrophication (Paerl 1988; Pinckney et al. 2001; Smayda 1990). Since the early 1800's there has been a decrease in water quality in the Chesapeake Bay and its tributaries characterized by decreased overall diversity of diatom species, increased occurrences of anoxic events, increased rates of sedimentation (Cooper and Brush 1991; Cooper and Brush 1993; Kemp et al. 2005), and a shift from benthic to pelagic primary production. The latter has been associated with an increase in the ratio of centric to pennate diatoms and a decrease in water clarity (Cooper and Brush 1993). Over the last 20 years, sections of the lower Chesapeake Bay and its tributaries have experienced a decrease in phytoplankton diversity and an increase in the abundance of potentially harmful algal taxa (Dauer et al. 2005, Marshall et al. 2005). Algal blooms occur seasonally in the Chesapeake Bay and its tributaries, and many of the bloom forming taxa are potentially harmful either through the disruption of the normal functioning of an ecosystem (e.g. Sunda et al. 2006) or through the production of toxins (Marshall et al. 2009; Marshall et al. 2005). Since 2007, major blooms of the harmful dinoflagellate *Cochlodinium polykrikoides* have occurred annually during summer in the lower Chesapeake Bay and its tributaries (Mulholland et al. 2009, Morse et al. 2011, Morse et al. in prep.).

In coastal and estuarine environments, physical forcing due to tides and estuarine circulation play a major role in the distribution and patchiness of phytoplankton populations (Cloern et al. 1985; Cloern et al. 1989). In addition, the behavior of many

blooms organisms (e.g., vertical migration) can affect their distribution in the water column, particularly when the water column is stratified and when turbulence is low. Tidal circulation and advection tends to smear phytoplankton patches horizontally both up and down estuaries along density gradients (Lucas et al. 1999a). Further, physical boundaries within an estuary can interrupt and deflect density and wind-driven flows, often resulting in the formation of complex eddy circulation (Geyer and Signell 1992, Shen et al. 1999). The importance of tidal transport processes on estuarine phytoplankton populations is highlighted in continuous Chlorophyll *a* (Chl *a*) monitoring programs and timeseries records where Chl *a* concentrations vary with tidal periodicity, and the Chl *a* maximum often occurs at a particular stage of the tidal cycle (Mallin et al. 1999, Li and Smayda 2001). The transient and ephemeral nature of processes that occur on tidal and subtidal timescales are rarely captured in fixed-station monitoring programs in which samples are collected weekly to monthly (Dustan and Pinckney 1989; Trigueros and Orive 2000). Consequently, most monitoring programs, while able to detect long-term trends, for which they were designed to do, are temporally and spatially insufficient to capture ephemeral blooms and their progression from initiation to senescence on timescales of days to weeks. Small-scale, high frequency targeted studies of bloom initiation are required in order to gain a better understanding of the processes involved in the formation of algal blooms.

Phytoplankton blooms, and harmful algal blooms in particular, have widespread and highly variable impacts on the environment ranging from loss of aesthetic and recreational value of waterways (Anderson et al. 2002; Paerl 1988), to disruption of the normal function of ecosystems (Sunda et al. 2006), and direct toxicity (Hallegraeff 1993;

Sellner et al. 2003). Mortality of aquatic organisms can result from low dissolved oxygen concentrations, due to the degradation of algal biomass (Smayda 1997a; Tango et al. 2005), mechanical damage to organisms feeding on algae (Landsberg 2002), indirect toxicity through food chain effects (Flewelling et al. 2005), or direct toxicity (Tang and Gobler 2009). Vast economic losses (Anderson et al. 2002; Smayda 1997a) can result from trophic and community level disruption as well as direct finfish and shellfish mortality (Cloern 2001; Heil et al. 2001; Heil et al. 2005; Sunda et al. 2006).

While the consequences of algal blooms have been qualitatively and quantitatively assessed, the causes of algal blooms and mechanisms of bloom initiation are still not well understood despite decades of research, even though environmental conditions prior to bloom formation may be the key to understanding bloom initiation. This is largely because *ad hoc* sampling of blooms generally commences only after a bloom has become dense enough to discolor the water and become visible (Smayda 1998). However, environmental conditions are likely to be very different when cell densities are high in dense blooms than when they first initiate and algal biomass is still low. For example, nutrients are consumed by cells to generate biomass and may become depleted in the water column. In addition, sites of bloom initiation may be far removed from where biomass accumulates in the environment due to transport and complex circulation patterns (Lucas et al. 1999a). Finally, most routine water quality monitoring (e.g., monthly or bimonthly sampling) lacks the temporal and spatial resolution to capture bloom inception and early development. These issues represent major gaps in our knowledge regarding the causes of algal blooms and bias our view about environmental conditions promoting bloom formation.

Margalef (1978) theorized that the phytoplankton community is organized by recurrent patterns of physical forcing that select for certain life-forms based on their functional morphology (Margalef 1978). He suggested that the amount of external energy input, in particular the amount of turbulence, to a system was the dominant control on phytoplankton community structure. While the "Margalef mandala" is well respected in the scientific community, it has remained largely an untested theory due to the difficulties of quantifying the variables involved (Estrada and Berdalet 1997).

Nutrient over-enrichment is often invoked as an underlying cause for eutrophication and the increase in the frequency and/or magnitude and duration of blooms observed worldwide (Anderson et al. 2002; Cloern 2001; Kemp et al. 2005; Nixon 1995; Paerl 1997; Pinckney et al. 2001). Blooms have also been linked to perturbations in the ratios at which nutrient elements are supplied or the ratio of their concentration in the environment. In particular, DIN: DIP ratios below canonical Redfield of 16:1 are thought to select for dinoflagellates and cyanobacteria, which have highly flexible metabolisms and can use organic compounds (Hodgekiss and Ho 1997; Burkholder et al. 2008). However, elevated N:Si and P:Si ratios are thought to select for dinoflagellates over diatoms since diatoms require Si to form their characteristic frustules (Smayda 1990; Smayda 1997b). Elevated ratios of DOC:DON (Anderson et al. 2002; Heil et al. 2001; Lomas et al. 2001) have been suggested to influence bloom formation by mixotrophic flagellates that may consume organic compounds. The development of ecosystem disruptive algal blooms (EDABs) has also been linked to prolonged periods of lower than normal inorganic nutrient concentrations (Sunda et al. 2006; Gobler et al. 2005). While it is certain that nutrients play a major role in the formation of algal

blooms, there is no single nutrient or combination of nutrients that always leads to the formation blooms, and in general, the nutrient controls on blooms are still poorly understood (Anderson et al. 2002), likely because most of the nutrient to bloom organism relationships were established only after blooms were already dense and dissolved nutrients were already converted into algal biomass.

Although differing from the Chesapeake Bay system in profound ways, over 20 years of research in San Francisco Bay have led to an understanding that physical forcing controls bloom formation there (Cloern 1987; Cloern et al. 1989). Phytoplankton abundance in San Francisco Bay is strongly controlled by rates of vertical mixing, stratification, and light availability. The balance between phytoplankton production and loss is heavily controlled by benthic-pelagic coupling (Cloern 1982; Cloern 1991). Grazing losses to the benthos become important controls on phytoplankton in San Francisco Bay in the when stratification breaks down, during times of increased mixing, and during neap tides (Cloern 1982). San Francisco Bay is often considered a high nutrient, low chlorophyll system because of the relatively high dissolved nutrient concentrations and low phytoplankton standing stocks (Wilkerson et al. 2006). Nutrient availability is rarely considered a controlling factor in this system. Recent evidence suggests that uptake of NO_3^- by phytoplankton is low due to inhibition by high ambient concentrations of NH_4^+ (Wilkerson et al. 2006). Further, the ratio of NH_4^+ to NO_3^- may control when and where the spring phytoplankton bloom may occur (Dugdale et al. 2007), although these findings have been challenged and an invasive clam species is now thought to be a primary control on bloom formation in San Francisco Bay (Lucas et al.

2006a). So while San Francisco Bay may be controlled by physical processes for most of the year, biogeochemical controls can be important seasonally.

In order to better understand phytoplankton bloom dynamics at the ecosystem level, it is necessary to sample at timescales relevant to the growth of phytoplankton (hours to weeks) and on spatial scales relevant to the distribution of phytoplankton ranging from the micro-scale to the mesoscale levels (Smayda 1998). The key is in understanding both in situ bloom development – the balance of growth and loss terms on a local scale (Lucas et al. 1999b), and the transport related mechanisms that act to concentrate, diminish, or just redistribute phytoplankton biomass (Lucas et al. 1999a). The balance between phytoplankton growth and loss at a local scale will determine if it is possible for phytoplankton to form a bloom (Lucas et al. 1999b), and the transport mechanisms ultimately control when, where, and how a bloom will occur at the mesoscale level (Lucas et al. 1999a).

A general problem with sampling in an estuarine environment is the short-term variability and the heterogeneous nature of estuarine environments and the phytoplankton distribution within them. The patchiness of phytoplankton distributions in estuaries confounds sampling efforts by introducing extreme variability in time and space. Routine water quality monitoring programs that sample weekly to monthly from fixed stations do not capture the relative variability inherent in phytoplankton populations and thus often cannot explain the excursions in population or miss the bloom event altogether. The Chesapeake Bay Monitoring Program began in 1984 and over 25 years of data have been collected throughout the Bay. Geographically fixed stations are sampled twice per month in June, July, and August and once per month at all other times. While this

sampling frequency may capture the seasonal or inter-annual variability (Dauer et al. 2005) (for which it was intended), it is not sufficient for capturing bloom events that develop over days.

The Lafayette River is a shallow sub-estuary and tributary to Chesapeake Bay that is tidally dominated and has a long water residence time of 1–4 months, depending on the amount of rainfall in a given year (White 1972). As a result of the long residence time and high nutrient loads, the Lafayette River is an ideal location to study blooms. In order to better understand the temporal relationship between the supply of nutrients and physical forcing from tides and the weather and how they interact to control dinoflagellate bloom formation and transport in eutrophic estuarine environments, the Lafayette River was sampled on a daily basis for a period of 54 days during the summer 2005, when blooms were likely to form. Samples were collected to enumerate phytoplankton abundance and measure nutrient concentrations. Ancillary physical and meteorological data were also obtained to identify the primary controls on bloom formation in this eutrophic system. Results from this effort are presented in Chapter 2.

As part of its Chlorophyll Monitoring and Assessment Program, the Hampton Roads Sanitation District (HRSD) collects underway data (Chl *a*, salinity, temperature, pH, dissolved oxygen, and turbidity) in the James River using a Yellow Springs Instruments (YSI) 6600 series datasonde. Through a partnership with HRSD, Chl *a* mapping in the James River was expanded to include the Lafayette and Elizabeth Rivers in 2008 and 2009, and the data obtained during these cruises were used to assess the timing and location of bloom formation, and subsequent transport of blooms of the dinoflagellate *Cochlodinium polykrikoides*. In order understand how physical transport

processes affected the distribution of this bloom organism within the lower Chesapeake Bay region, the Virginia Institute of Marine Science three-dimensional Hydrodynamic Eutrophication Model (HEM-3D) was used to model the James River hydrodynamics and the transport of a passive tracer released in the Lafayette River under dynamic flow and wind forcing conditions. The Lafayette River was identified as the initiation grounds for the 2008 *C. polykrikoides* bloom and results from this portion of my dissertation are presented in Chapter 3.

Subsequently, during 2009, I combined the approaches used in Chapters 2 and 3 and supplemented DATAFLOW data with daily fixed station sampling of nutrients and phytoplankton in the Lafayette River. A YSI 6600 sonde was installed to capture in situ Chl *a* variability at timescales less than 1 day (measurements were recorded every 6 minutes). These data allowed for a deterministic approach to understanding the controls on bloom formation, and combine and compare data from samples collected at timescales ranging from sub-tidal to daily to weekly. Through this combined approach, I was able to capture the relevant conditions prior to *C. polykrikoides* bloom initiation during 2009, and the results from this work are presented in Chapter 4.

The goal of this project is ultimately to understand phytoplankton community structure and dynamics and to understand how and why monospecific algal blooms initiate in the environment. Bloom organisms are present in phytoplankton communities as part of the natural population but it is unclear why they are able to out-compete other phytoplankters and "bloom" at certain times. I examined bloom initiation with respect to ambient nutrient concentrations, and relevant physical controls on phytoplankton populations including stratification, water column stability, and mixing, over timescales

relevant to bloom formation. Sampling at a high frequency allows for high-resolution determination of phytoplankton biomass accumulation and accompanying nutrient drawdown. The spatio-temporal analysis afforded by underway sampling (DATAFLOW) data collected in the James, Elizabeth, and Lafayette Rivers combined with relevant physical parameters from NOAA and USGS such as wind speed and direction, current velocity and trajectories, atmospheric pressure, rain fall and river flow will show how transport related mechanisms act to concentrate, dilute, or relocate biomass over time and space, over both short term and seasonal cycles.

CHAPTER 2

DAILY VARIABILITY IN PHYTOPLANKTON ABUNDANCE AND NUTRIENT CONCENTRATIONS IN A TIDALLY DOMINATED EUTROPHIC ESTUARY

INTRODUCTION

Since the early 1800's Chesapeake Bay and its tributaries have experienced a decrease in water quality characterized by decreased overall diversity of diatom species, increased occurrences of anoxic events, increased rates of sedimentation (Cooper and Brush 1991; Cooper and Brush 1993; Kemp et al. 2005), and a shift from benthic to pelagic production. The latter has been associated with an increase in the ratio of centric to pennate diatoms and a decrease in water clarity (Cooper and Brush 1993). Over the last 20 years, sections of the lower Chesapeake Bay and its tributaries have experienced a decrease in phytoplankton diversity and an increase in the abundance of potentially harmful algal taxa (Dauer et al. 2005, Marshall et al. 2005). Algal blooms occur seasonally in the Chesapeake Bay and its tributaries, and many of the bloom forming taxa are potentially harmful or toxin-producing species (Marshall et al. 2009; Marshall et al. 2005). Since 2007, major blooms of the harmful dinoflagellate *Cochlodinium polykrikoides* have occurred annually during summer in the lower Chesapeake Bay and its tributaries (Mulholland et al. 2009, Morse et al. 2011, Morse et al. in prep.). Worldwide, algal blooms appear to be increasing in frequency due to cultural eutrophication (Paerl 1988; Pinckney et al. 2001; Smayda 1990).

Eutrophication due to nutrient over enrichment, usually attributed to nitrogen (N) and/or phosphorous (P), is often implicated as causative factor in the formation of both harmful and ecosystem disruptive algal blooms (EDABs) (Heisler et al. 2008; Anderson et al. 2008; Anderson et al. 2002; Sunda et al. 2006). Blooms have also been linked to perturbations in the ratios at which inorganic nutrients are input relative to the Redfield ratio (N:P of 16:1) (Hodgkiss and Ho 1997). Elevated ratios of dissolved organic carbon (DOC) to dissolved organic nitrogen (DON) (DOC:DON) (Anderson et al. 2002; Heil et al. 2001; Lomas et al. 2001) have also been implicated in bloom formation while elevated N:Silica (Si) or P:Si ratios are thought to select for dinoflagellates over diatoms (Smayda 1990; Smayda 1997b). In contrast, the development of many EDABs have also been linked to prolonged periods of lower than normal nutrient concentrations (Sunda et al. 2006, Gobler et al. 2005). This may be due to a positive feedback scenario where a noxious or otherwise unpalatable EDAB species experiences decreased grazing pressure, and thus nutrient recycling and availability is reduced to competing taxa, thereby prolonging bloom duration (Sunda et al. 2006). While it is certain that nutrients play a major role in the formation of algal blooms, no single nutrient or combination of nutrients has emerged as a causative factor for the formation of blooms, and the environmental conditions promoting bloom development are still poorly understood (Anderson et al. 2002).

Because algal blooms are seldom visible until cell numbers exceed 10^6 cells l^{-1}, blooms in the natural environment are usually sampled only after the bloom is already well established, nutrients have been drawn down by the bloom organism, and competing taxa are absent. Rarely are the conditions leading up to or promoting bloom formation

captured in sampling programs because the temporal resolution of sampling is insufficient. Consequently, most reports characterize fully mature or even senescent blooms; thus factors promoting blooms remain largely unknown.

In coastal and estuarine environments, physical forcing due to tides and estuarine circulation play a major role in the distribution and patchiness of phytoplankton populations (Cloern et al. 1985; Cloern et al. 1989). Tidal forcing, estuarine circulation, and behavior of many blooms organisms (e.g., vertical migration), all contribute to temporal and spatial patchiness of blooms. Tidal transport and advection tends to smear phytoplankton patches horizontally along estuarine gradients (Lucas et al. 1999a). Further, physical boundaries within an estuary can interrupt and deflect density and wind-driven flows, often resulting in the formation of complex eddy circulation (Geyer and Signell 1992, Shen et al. 1999). The importance of tidal transport processes on estuarine phytoplankton populations is highlighted in continuous Chlorophyll *a* (Chl *a*) monitoring programs and timeseries records where Chl *a* concentrations vary in conjunction with the tidal stage, and the Chl *a* maximum often occurs at a particular stage of the tidal cycle (Mallin et al. 1999, Li and Smayda 2001). The transient and ephemeral nature of these processes, which occur on tidal and subtidal timescales, are rarely captured in fixed-station monitoring programs in which samples are collected weekly to monthly (Dustan and Pinckney 1989; Trigueros and Orive 2000). Consequently, most monitoring programs are temporally and spatially insufficient to capture blooms and their progression from initiation to senescence, and small-scale, high frequency targeted studies on bloom initiation are required in order to gain a better understanding of the processes involved in the formation of algal blooms.

To better understand the timescales of variability in phytoplankton populations and conditions promoting algal blooms, I sampled the Lafayette River, a shallow, eutrophic, sub-tributary of the lower Chesapeake Bay where algal blooms regularly occur, at a fixed station on a daily basis at the same phase of the tidal cycle for a period of 54 d in Fall of 2005, a period when blooms routinely occur (Fig. 1). The ambient dissolved inorganic nitrogen (DIN) concentrations in the Lafayette River are often greater than 10 μM, and the concentration of dissolved inorganic phosphorous (DIP) is typically above 1 μM. Between 2000 and 2009, the Chesapeake Bay Monitoring Program station LFB01 in the Lafayette River had an average DIN concentration of 5.8 μM (standard deviation = 8.8 μM), and the average DIP concentration at this station was 0.74 μM (standard deviation = 0.84 μM) (Chesapeake Bay Monitoring Program: http://www.chesapeakebay.net/data_waterquality.aspx). The Lafayette River has a water residence time of 1–4 months, depending on the amount of rain in a given year (White 1972) or event-scale processes such as Nor'easters and tropical storms, which may modulate the residence time (Paerl et al. 2006). The combination of a long residence time and high nutrient loads favor the growth of dinoflagellates (Margalef 1978; Sellner et al. 2001) making this an ideal location to observe algal bloom dynamics.

The goal of this study was to identify factors promoting the initiation of algal blooms and to relate changes in phytoplankton community structure with nutrient concentrations on short timescales characteristic of developing blooms. Sampling on a daily basis allowed for higher temporal resolution of phytoplankton populations, nutrient dynamics, and physical forcing than most monitoring programs afford.

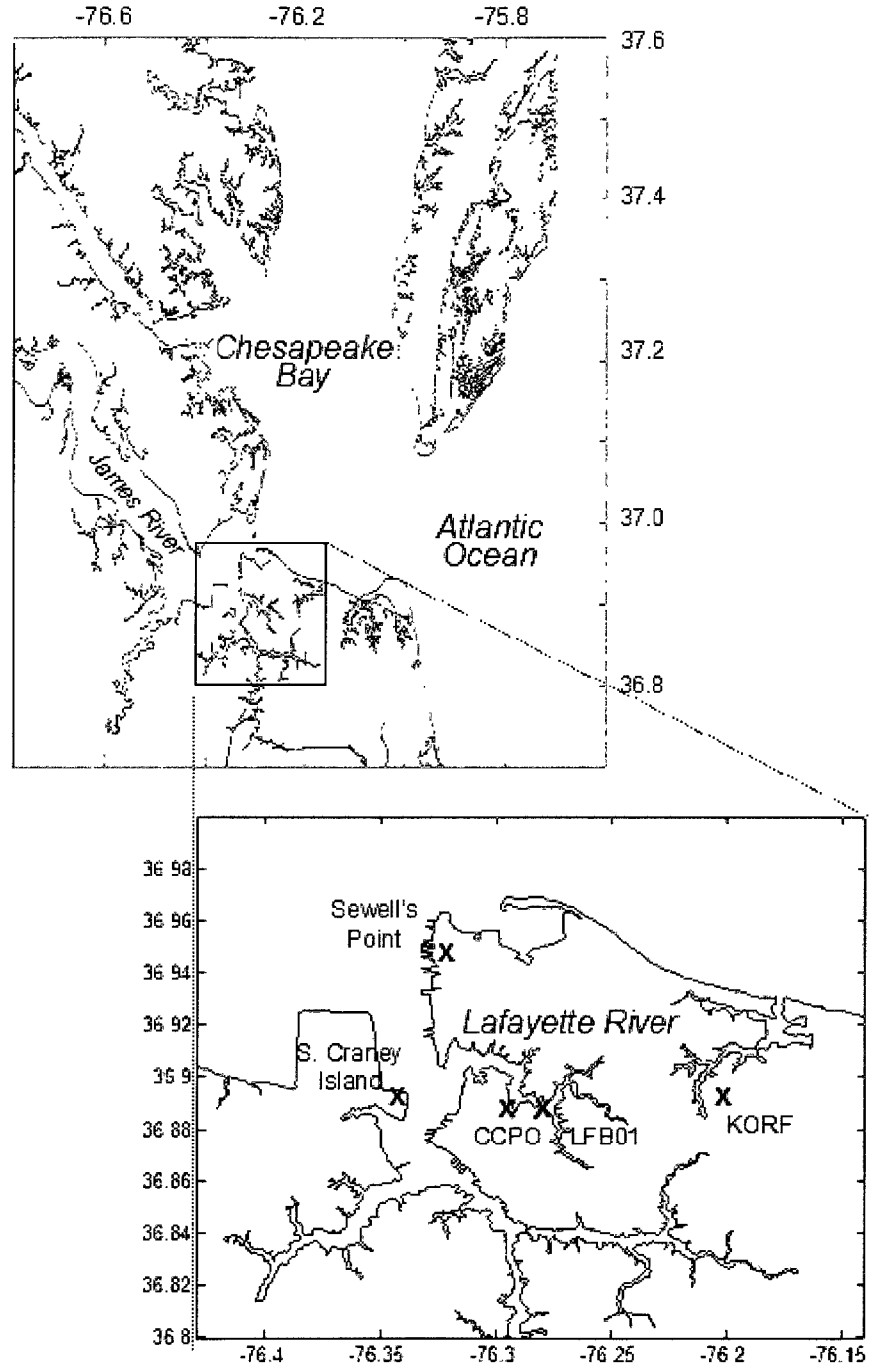


Fig. 1 Map of the study area showing the sampling site on the Lafayette River at Old Dominion University's Center for Coastal Physical Oceanography (inset, *CCPO*), Norfolk International Airport (inset, *KORF*), NOAA PORTS station at *Sewell's Point* (inset), NOAA PORTS station at *S. Craney Island* (inset), and the Virginia Department of Environmental Quality water quality station *LFB01* (inset)

METHODS

24-hour tidal phase sampling. In order to understand how algal abundance and nutrient dynamics are controlled by tidal forcing, I sampled a tidal subestuary of the lower Chesapeake Bay, the Lafayette River (Fig. 1, *CCPO*) on an hourly basis for a period of 24 hours. A Hydrolab DataSonde 4*a* Water Quality Multiprobe was used to measure conductivity and water temperature at each sampling time. Water was collected from the Lafayette River on an hourly basis beginning on July 18, 2005, at 06:00 local time in an acid-cleaned carboy. In the lab, water was withdrawn and filtered onto Whatman GF/F glass fiber filters for Chl *a* analysis. Nutrient samples were collected after filtration through 0.2 μm Supor filters. Nutrient and Chl *a* samples were immediately frozen and stored in a freezer until analysis. Tidal height data was obtained from the National Oceanic and Atmospheric Associations Physical Oceanography Real Time System (NOAA PORTS) station at Sewell's Point in the Elizabeth River (Fig. 1). The distance between the sampling site in the Lafayette River and the NOAA Sewell's Point tide gauge is less than 12 km, and on average, tidal height predictions for the Lafayette River lag those for Sewell's Point by approximately 20 minutes. Since data were collected on an hourly basis, the time of measured low water at Sewell's Point and low salinity in the Lafayette River are offset by approximately one hour.

Daily tidal phase sampling. Based on the results from the hourly sampling, daily sampling of surface water from the Lafayette River was timed to coincide with the highest observed algal biomass, which was at the incoming tide approximately two hours after the low tide in the Lafayette. Samples were only collected during daylight hours; when the flood tide occurred at night, the sampling interval was extended approximately

12 hours to coincide with the subsequent flood tide during daylight; this happened on August 22, September 2, and 7. Prior to water sampling, dissolved oxygen, salinity, and water temperature were measured *in situ* using a Hydrolab DataSonde 4*a* Water Quality Multiprobe. Due to the arrangement of the sensors on the sonde, all parameters were measured at the bottom of the water column. The average depth of the water during the sampling period was 1 m. Water samples were collected from the surface using an acid-cleaned bucket, placed into a 20 L acid-cleaned polycarbonate carboy, and transported to the laboratory less than 3 km away.

Sample handling and analyses. Once at the laboratory, water samples were kept well mixed by adding a magnetic stir bar to carboys and gently stirring their contents. Samples for nutrient analyses were immediately filtered through a 0.2 μm Pall sterile microculture capsule using a peristaltic pump. The filtrate was placed into acid-cleaned bottles and stored frozen until analysis. Nitrate + nitrite (NO_3^- + NO_2^-), nitrite (NO_2^-), urea, phosphate (PO_4^{-3}), and silicate (SiO_4^{-4}) were measured using an Astoria Pacific nutrient autoanalyzer according to manufacturer specifications and consistent with the colorimetric techniques outlined by Parsons et al. (1984). Ammonium (NH_4^+) concentrations were measured by the phenolhypochlorite method of Solorzano (1969). Total dissolved nitrogen (TDN) and total dissolved phosphate (TDP) were analyzed at Old Dominion University's Water Quality Lab, following the standard procedures and protocol outlined for the Chesapeake Bay Program Water Quality Monitoring Program (http://archive.chesapeakebay.net/pubs/quality_assurance/doc-EPA903-R-96-006.pdf). Dissolved organic nitrogen (DON) was calculated as the difference between TDN and the dissolved inorganic nitrogen (DIN). Dissolved organic phosphorous (DOP) was

calculated as the difference between TDP and dissolved inorganic phosphate (DIP). Nutrient concentrations that were below the detection limit were assigned values of the detection limit for statistical purposes.

Whole water samples (500 ml) were preserved with Utermohl's modified Lugol's solution for enumeration of microplankton and nanoplankton, and with 1% glutaraldehyde (final concentration) for enumeration of picoplankton. Phytoplankton were quantified microscopically as described by Marshall and Nesius (1996), and autotrophic picoplankton were enumerated via epifluorescent microscopy (Affronti and Marshall 1994). Chl *a* samples were collected onto glass fiber filters (Whatman GF/F) and stored frozen until analysis using the non-acidification fluorometric technique of Welschmeyer (1994), within 3 weeks of collection. Phytoplankton blooms are hereafter defined as when the cell abundance of a single taxon exceeded 0.5×10^6 cells l^{-1} for a period of three days or longer and/or daily Chl *a* concentrations exceeded 44 $\mu g\ l^{-1}$, twice the average Chl *a* concentration for the nearby Chesapeake Bay Monitoring Program station LFB01 (Fig. 1) from 2000–2009 (Chapter 3).

Correlation and statistical analyses. Phytoplankton taxonomic abundance (as phyla) was compared to nutrient concentrations by calculating the cross correlation function using The Math Works' MATLAB software, which follows the cross correlation function equation given by Box et al. (2008). Because phytoplankton growth rates are on the order of days, a time lag in the response of phytoplankton abundance to nutrient loading events was expected. Therefore, I compared nutrient concentrations with phytoplankton abundance at one-day intervals ranging from the five days previous through five days forward in time. The 95% confidence interval (CI) was calculated by

the program using 2 standard deviations of the cross correlation function. Because of the low abundance of euglenoids, cryptophytes, and cyanobacteria and chlorophytes over much of the sampling period, correlations to nutrients were only made for dinoflagellates and diatoms.

Meteorological and supplementary data. Atmospheric pressure and wind speed data were obtained from the NOAA PORTS Craney Island station near the mouth of the Lafayette River (Fig. 1, *S. Craney Island*). The wind speeds throughout the text are presented as the cube of the wind speed, which is proportional to its mixing potential (Lund-Hansen et al. 1996). Tidal height data was obtained from NOAA PORTS Sewell's Point station in the Elizabeth River (Fig. 1, *Sewell's Point*). Precipitation data were obtained from Norfolk International Airport (Fig. 1, *KORF*). Surface nutrient and Chl *a* data for Lafayette River station LFB01 (approximately 1 km upriver from the CCPO sampling site) from 2000–2009 were obtained from the Chesapeake Bay Program's data hub (http://chesapeakebay.net/data_waterquality.aspx).

RESULTS

Hourly nutrient and chlorophyll a variability. Nutrient and Chl *a* concentrations were measured hourly over a 24 h period from July 18–19, 2005, in the Lafayette River to determine the effect of tides on these water quality parameters. There was no precipitation during the sampling period and the Lafayette River has no freshwater tributaries or inputs other than runoff from precipitation. The Lafayette River experiences semidiurnal tides, and the concentrations of both Chl *a* and nutrients appear to have semidiurnal maxima and minima linked to the tidal phase (Fig. 2).

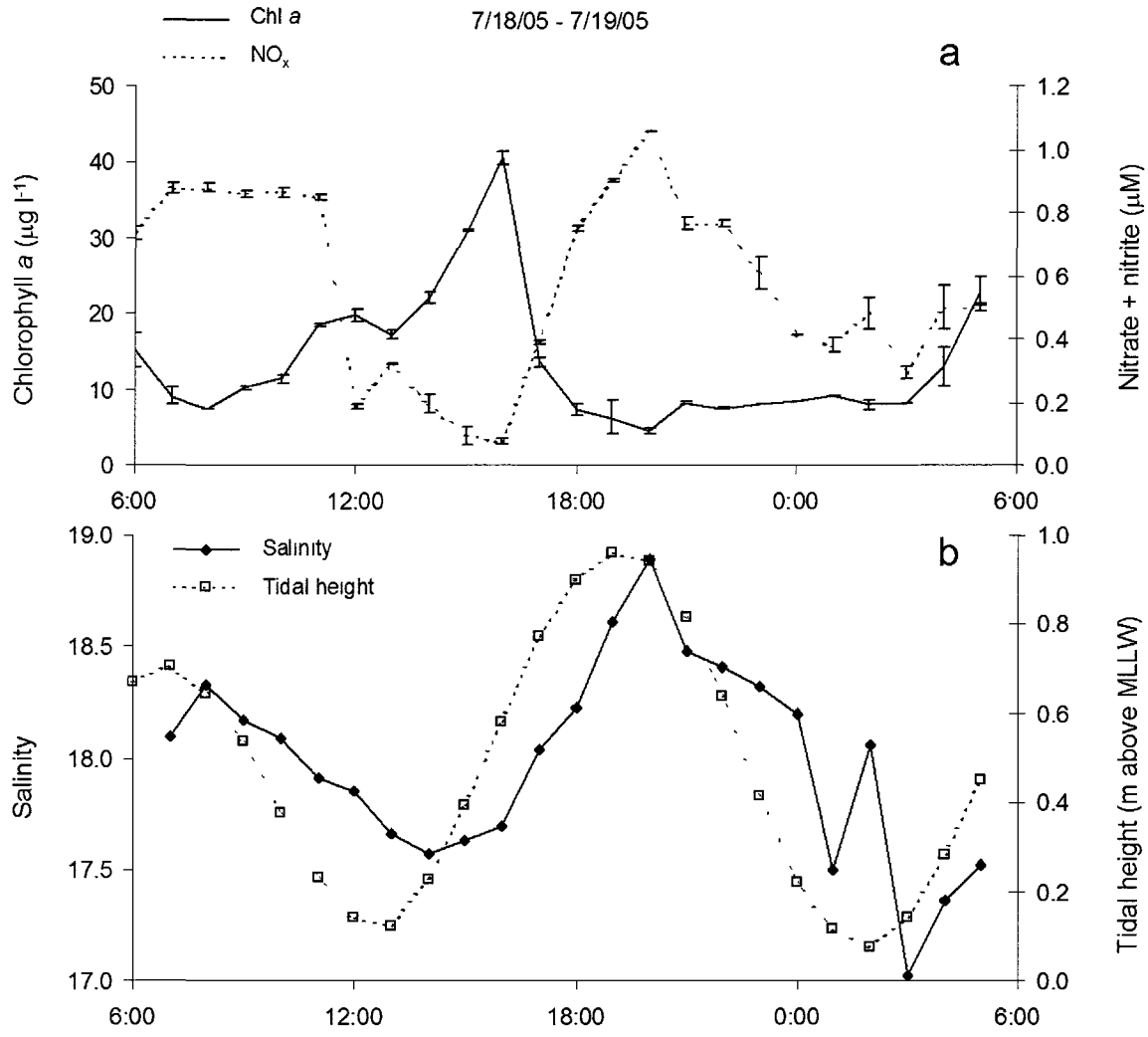


Fig. 2 Hourly chlorophyll *a*, (Chl *a*, µg l^{-1}), and nitrate plus nitrite, (NO_x, µM), measured in the Lafayette River on July 18–19, 2005 **a**; Nitrate and nitrite displayed similar concentrations and trends between time points and thus is reported as NO_x for clarity; and **b** tidal height measured at Sewell's Point in the Elizabeth River and salinity measured hourly in the Lafayette River on July 18–19, 2005

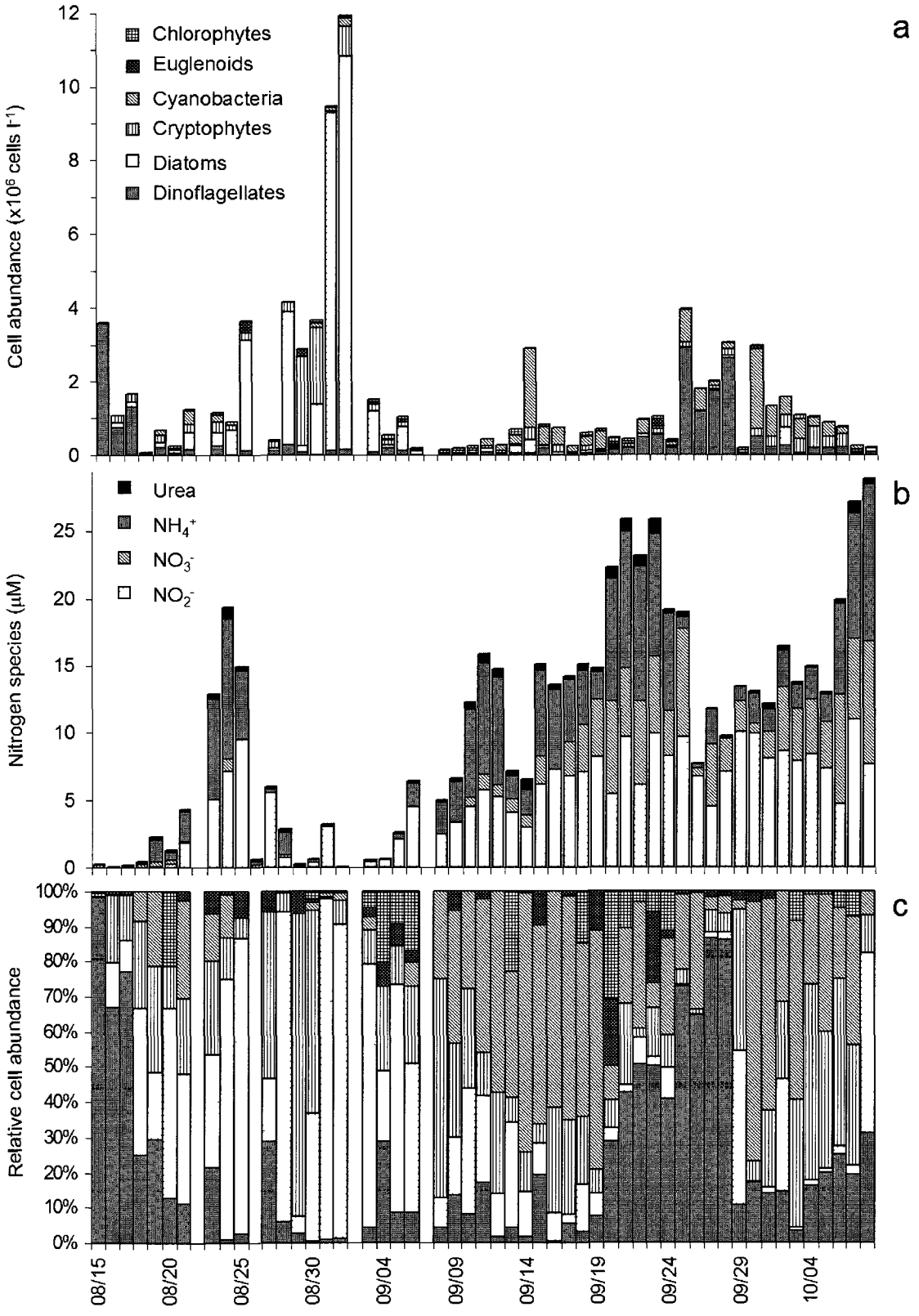
Over the 24-hour sampling period, nitrite plus nitrate concentrations (hereafter NO_x) in the Lafayette River varied by an order of magnitude, Chl *a* varied by a factor of 8, and this variability appeared to be tidally controlled (Fig. 2a, b). Chl *a* concentrations were highest approximately two to three hours after low tide (Fig. 2a, b). Nutrient concentrations were highest at maximum flood tide when Chl *a* concentrations were low. The salinity measured in the Lafayette River lagged behind tidal height observations for Sewell's Point by approximately one hour (Fig. 2b). Based on the Chl *a* variability observed over the tidal cycle, I elected to collect samples for our 54-day daily study (Aug 15–Oct 8, 2005) approximately two hours after the predicted low tide in the Lafayette River, when Chl *a*, and thus phytoplankton biomass, was highest.

Phytoplankton abundance. Between August 15 and October 8, 2005, three major blooms occurred in the Lafayette River (Fig. 3a). The first bloom, a mixed-species dinoflagellate bloom dominated by *Akashiwo sanguineua* (3.2×10^6 cells l^{-1}, >88.4% total abundance), was already in progress at the start of the daily sampling period on August 15, 2005. However, on August 16, an unidentified *Gymnodinium* sp. was the dominant species (0.5×10^6 cells l^{-1}) comprising 48% and 42% of the total phytoplankton abundance on August 16 and 17, respectively (Fig. 3a). At this time, concentrations of dissolved urea, NH_4^+, NO_3^-, and NO_2^- were at or near their limits of analytical detection (Fig. 3b). Subsequently, dinoflagellate abundance decreased until populations were <14,000 cells l^{-1} by August 18, 2005. At this time, dissolved N concentrations increased, and NO_2^- and NH_4^+ concentrations reached 7.2 μM and 10.4 μM, respectively, by August 24 (Fig. 3a, b). Diatoms and cryptophytes comprised 86% of the phytoplankton at this time but total phytoplankton abundance was still $< 1.0 \times 10^6$ cells l^{-1}.

The second bloom occurred between August 28 and September 3, 2005. Beginning about August 25 and between August 27 and September 3, the relative abundance of diatoms and cryptophytes increased, and the greatest total phytoplankton abundance observed during the study period occurred on September 1, at 11.9 x 10^6 cells l^{-1} (Fig. 3a). Diatoms were the dominant taxa on August 28, 31, and September 1, while cryptophytes were dominant on August 29–30 (Fig. 3c). Between August 31 and September 3, *Skeletonema costatum* was the dominant phytoplankter enumerated in our samples (Fig. 3a, c). Diatoms comprised 96.9% of the total phytoplankton abundance on August 31, with 9.2 x 10^6 diatom cells l^{-1}, and increased to 10.7 x 10^6 diatom cells l^{-1} on September 1, when they comprised 89.7% of the phytoplankton population (Fig. 3a, c). Diatoms remained abundant through September 5. As diatoms and cryptophytes increased in abundance, dissolved N concentrations became depleted and NO_2^- or NH_4^+ were the dominant forms of dissolved N in the system (Fig. 3b).

After September 5, the relative abundance of cyanobacteria increased (Fig. 3c) although the total cell number was much lower than that observed during the diatom bloom (Fig. 3a). At the same time, on September 6, dissolved N concentrations increased and remained > 5.0 μmol l^{-1} for the duration of the study (Fig. 3b).

The third bloom occurred between September 25 and 28, 2005. Beginning September 20, dinoflagellates relative abundance increased and dinoflagellates comprised 72.9% of the phytoplankton community by September 25 with an unidentified *Gymnodinium* sp. reaching an abundance of 1.4 x 10^6 cells l^{-1} and dominating the assemblage (Fig. 3a, c). On September 28 the abundance of *Gymnodinium* sp. reached


a
Chlorophytes
Euglenoids
Cyanobacteria
Cryptophytes
Diatoms
Dinoflagellates
Cell abundance (x10^6 cells l^-1)
0
2
4
6
8
10
12
b
Urea
NH4+
NO3-
NO2-
Nitrogen species (µM)
0
5
10
15
20
25
c
Relative cell abundance
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
08/15
08/20
08/25
08/30
09/04
09/09
09/14
09/19
09/24
09/29
10/04

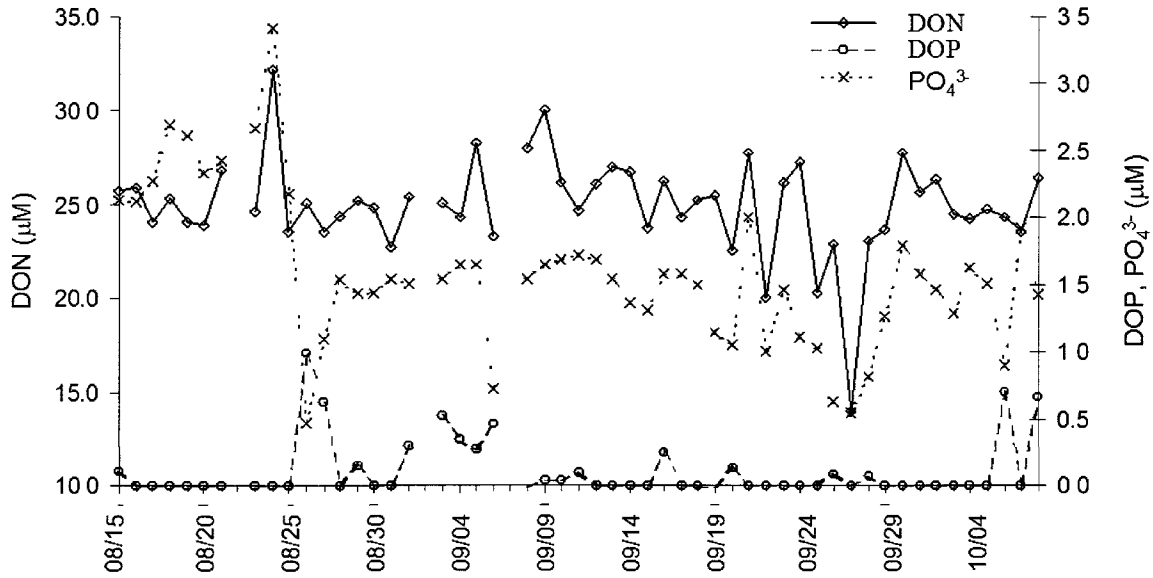


Fig. 4 Dissolved organic nitrogen (DON, μM N), dissolved organic phosphorous (DOP, μM P), and dissolved inorganic phosphorous (PO_4^{3-}, μM P) measured in the Lafayette River from August 15–October 8, 2005. DOP concentrations below detection limit (0.027 μM) were assigned values of the detection limit for statistical purposes

2.0×10^6 cells l^{-1} while cryptophyte abundance was at or near its lowest during the 54-day study.

Nutrient concentrations. DIN (NO_2^-, NO_3^-, and NH_4^+) and urea concentrations were at or near the limits of detection at the start of the study, between August 15 and 18 (Fig. 3b) when dinoflagellate abundance was high (Fig. 3a). NO_2^- concentrations increased after August 20 reaching nearly 7 μmol l^{-1} on August 25. NH_4^+ concentrations also increased, but then both NO_2^- and NH_4^+ were drawn down as phytoplankton biomass increased between Aug 24 and Sept 3 (Fig. 3a, b). Beginning Sept 5, NO_2^- concentrations increased from near the detection limit (0.02 μM) to 10 μmol l^{-1} by the end of the study period (Fig. 3b).

NO_3^- concentrations were generally low relative to other forms of N, typically less than 2 μmol l^{-1} and less than 2% of TDN until September 14 (Figs. 3b and 4). In mid-September, NO_3^- concentrations increased, reaching a maximum of 9 μmol l^{-1} by Oct 8, and NO_3^- represented a substantial fraction of the DIN pool (up to 30%) during the latter third of the study period (Fig. 3b). NO_3^-concentrations were lower during the September dinoflagellate bloom, when cyanobacterial abundance was also high (Fig. 3a, c).

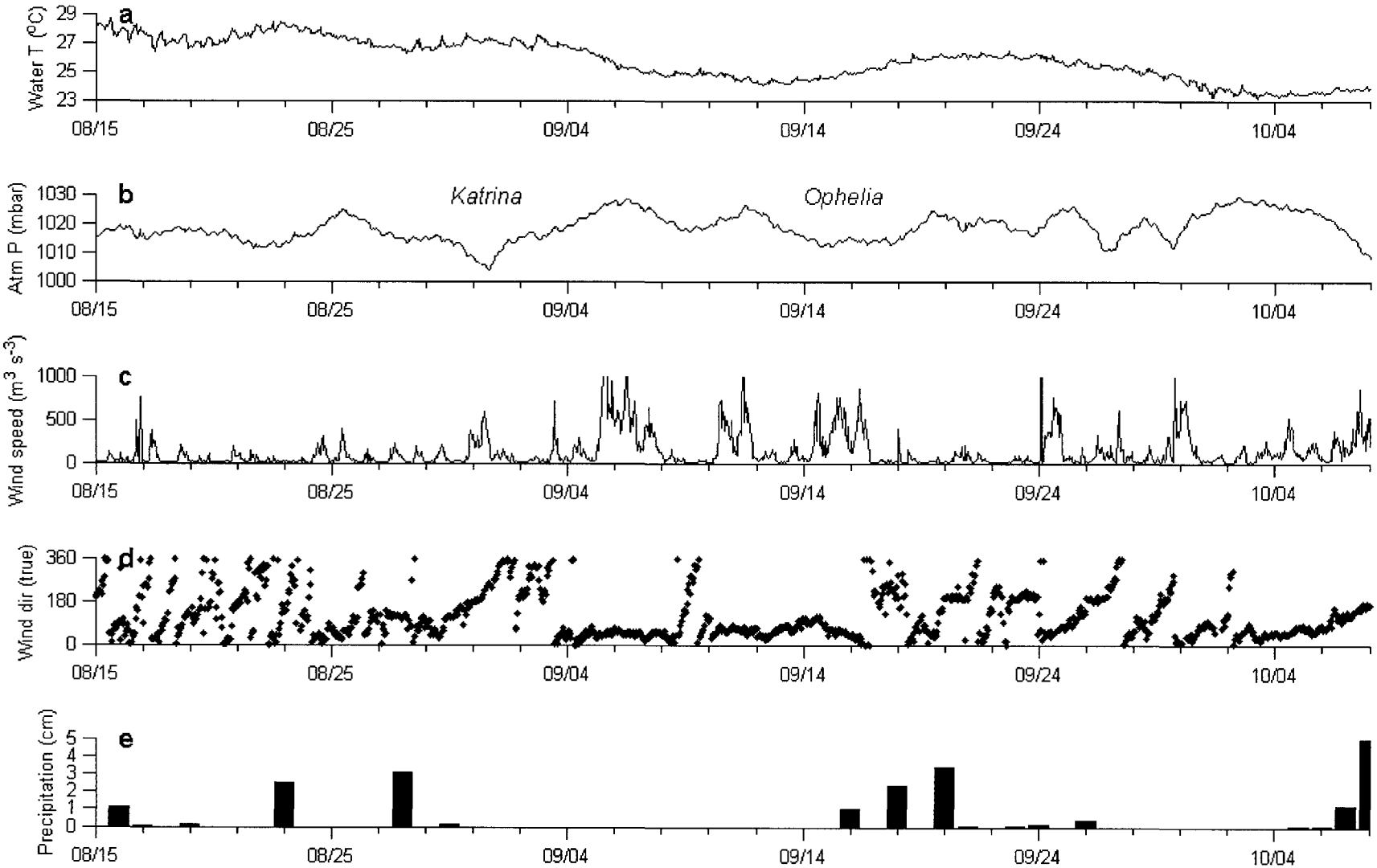
Concentrations of NH_4^+ ranged from below the detection limit (<0.02 μmol l^{-1}) to more than 10 μmol l^{-1} and were highly variable over the course of the 54-day study. The highest NH_4^+ concentrations were observed between bloom periods while large decreases in the NH_4^+ concentrations occurred during periods when phytoplankton cell abundance increased (Fig. 3a, b). NH_4^+ concentrations were highest prior to the diatom bloom at the end of August (10.4 μmol l^{-1} on August 24), and prior to and after the September dinoflagellate bloom (10.1 μmol l^{-1} on September 21–22, and 11.6 μmol l^{-1} on October

8). NH_4^+ concentrations were near or below the detection limit on August 31 during the diatom bloom and during the dinoflagellate blooms on August 15–16 and September 28.

Urea concentrations were low throughout the sampling period with a maximum concentration of 1 μmol l^{-1} on September 23 (Fig. 3b). Urea concentrations comprised only a small portion of the total dissolved nitrogen pool at any given time, (generally <1% of TDN, but always <2.5% of TDN). Silicate concentrations were high, ranging from 30–70 μmol l^{-1} throughout the study period (data not shown), and the ratio of dissolved silicate (DSi) to DIN was generally greater than 16 until September 6, indicating that silicate concentrations were not limiting to diatom growth during the study period (Conley and Malone 1992). Silicate concentrations decreased from 80 μmol l^{-1} to 60 μmol l^{-1} as a diatom bloom formed in late August, but were never depleted (data not shown).

DIP concentrations were also relatively high throughout the study period, ranging from 0.5 to 3.5 μmol l^{-1}, well above the limit of analytical detection (Fig. 4). At the onset the study in mid-August DIP concentrations were higher (maximum of 3.4 μmol l^{-1}), but decreased by nearly a factor of 2 following the diatom bloom in late August and remained lower for the remainder of the study period (average 1.6 ± 0.6 μmol l^{-1}) (Figs. 3a and 4).

DON concentrations did not change much over the 54-day study period (average 24.9 ± 2.6 μmol l^{-1}) with one exception; DON concentrations were lower during the dinoflagellate bloom from September 25–28, and the lowest concentration was observed on September 27 (13.9 μmol l^{-1}) (Figs. 4 and 3a). DOP concentrations were lower


a
Water T (°C)
29
27
25
23
08/15
08/25
09/04
09/14
09/24
10/04
b
Katrina
Ophelia
Atm P (mbar)
1030
1020
1010
1000
c
Wind speed (m3 s-3)
1000
500
0
d
Wind dir (true)
360
180
0
e
Precipitation (cm)
5
0

(maximum of 1.0 μmol l^{-1}) than DIP concentrations, and often below the limit of analytical detection (Fig. 4). Because the DOP concentrations were so low and the variance was so great, patterns in DOP concentrations relative to phytoplankton abundance could not be elucidated.

Meteorological and physical controls on estuarine variability. Between August 6–12, prior to the start of the daily sampling, 11.5 cm of precipitation was measured at Norfolk International Airport (KORF) (data not shown). Precipitation occurred on August 15 and 16 (1.1 cm) after a dinoflagellate bloom had formed in the Lafayette River (Figs. 5e and 3a), on August 23 (2.5 cm), August 28 (3.1 cm), between September 16 and 20 (6.8 cm), and between October 6 and 8 (8.0 cm) (Fig. 5e). Nutrient concentrations increased following rainfall events, except on August 15 (Figs. 3b and 5e).

There were three occasions during the study period when the cube of the wind speed was greater than 500 $m^3 s^{-3}$ for more than 12 hours (Fig. 5c). These events occurred from September 5–8, 10–12, and 14–16 (Fig. 5c). Additional high wind events where the wind velocity was >500 $m^3 s^{-3}$ for a period of less than 12 hours occurred on August 16, and 30–31, September 3, September 24, September 30, and October 7.

As the remnants of Hurricane Katrina (downgraded to a tropical storm) passed to the west of the region beginning August 30, the cube of the wind speed increased and reached 500 $m^3 s^{-3}$ as the atmospheric pressure decreased to < 1005 mbar on August 31 (Fig. 5b). This period of high wind coincided with a decrease in the abundance of cryptophytes and an increase the abundance of diatoms; a bloom of *Skeletonema costatum* followed the August 30–31 wind event (Figs. 5c and 3a, c). There was a

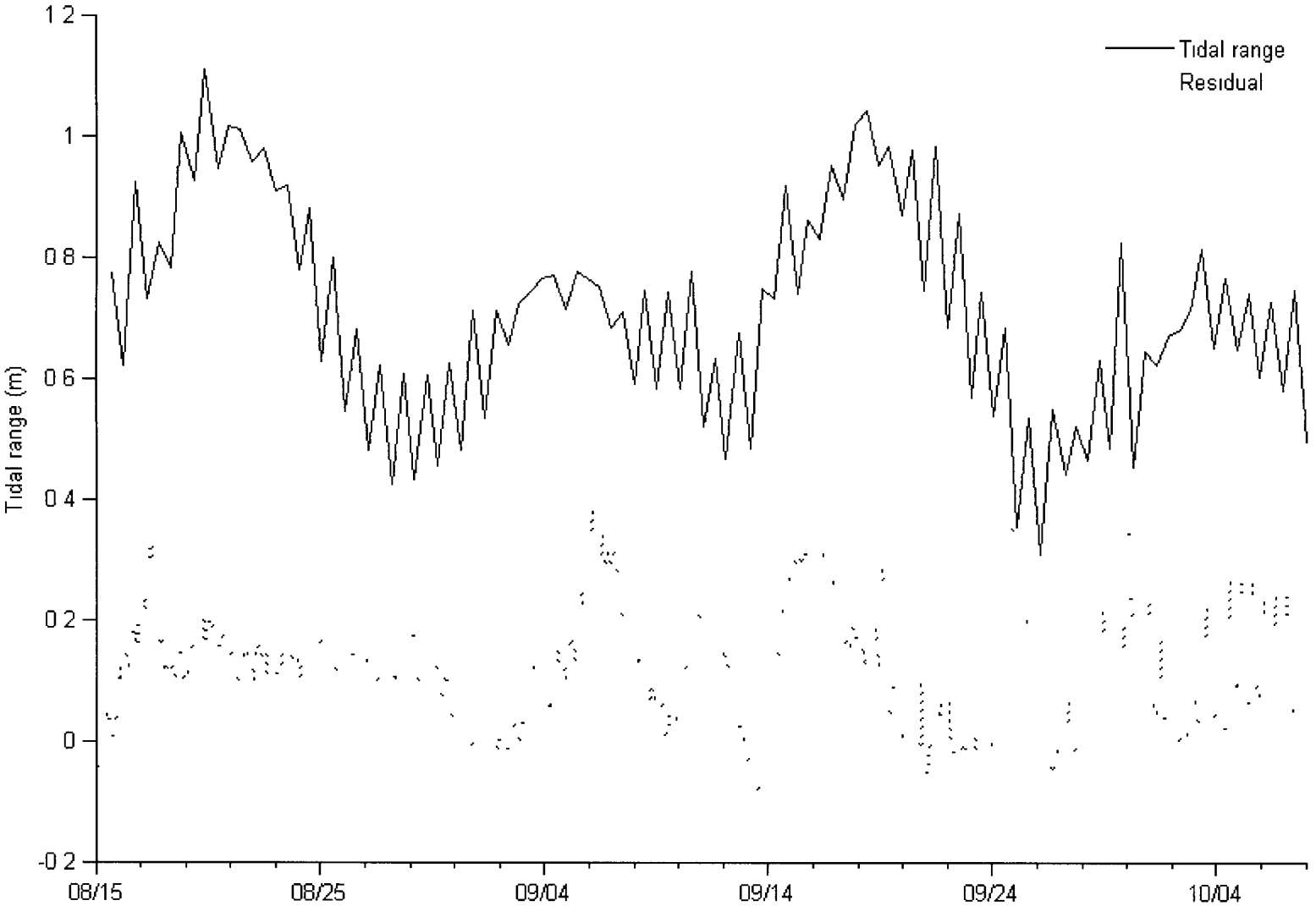


Fig. 6 Tidal range (m) and tidal residual measured at Sewell's Point in the Elizabeth River

prolonged period of high winds beginning September 3 as a high-pressure system moved through the region following the remnants of hurricane Katrina (Fig. 5b, c) and this corresponded to the demise of the diatom bloom. The high winds blew predominantly from the northeast during this period (Fig. 5d) and resulted in a positive tidal residual at Sewell's Point in the Elizabeth River (Fig. 6). This positive tidal residual also coincided with an increase in salinity in the Lafayette River after September 5 (Fig. 7). In addition, the water temperature in the Lafayette River cooled by 4°C during this event (Fig. 7).

The winds increased again from September 10–12, as another high-pressure system passed through the region, and the winds were again predominantly from the northeast (Fig. 5c and d). A third high wind event occurred as the effects from hurricane Ophelia passed over the Outer Banks of North Carolina and moved off the coast of Virginia from September 14–16 (Fig. 5b). Although below hurricane strength, this storm system was associated with substantial precipitation between September 16 and 20 (Fig. 5b, e). The predominantly northeast winds associated with this system again resulted in a positive tidal residual in the Elizabeth River at Sewell's Point (Figs. 5d and 6) as well as increased salinity and water temperature in the Lafayette River (Fig. 7). Water temperature and salinity in the Lafayette River decreased abruptly on September 20 (Fig. 7) as the remnants of hurricane Ophelia passed by the region, resulting in >3cm of precipitation (Fig. 5). Two more high wind events occurred in late September, and one in October, but the duration of the high winds was short and the direction from which it came was not constant for more than 24 hours (Fig. 5c, d).

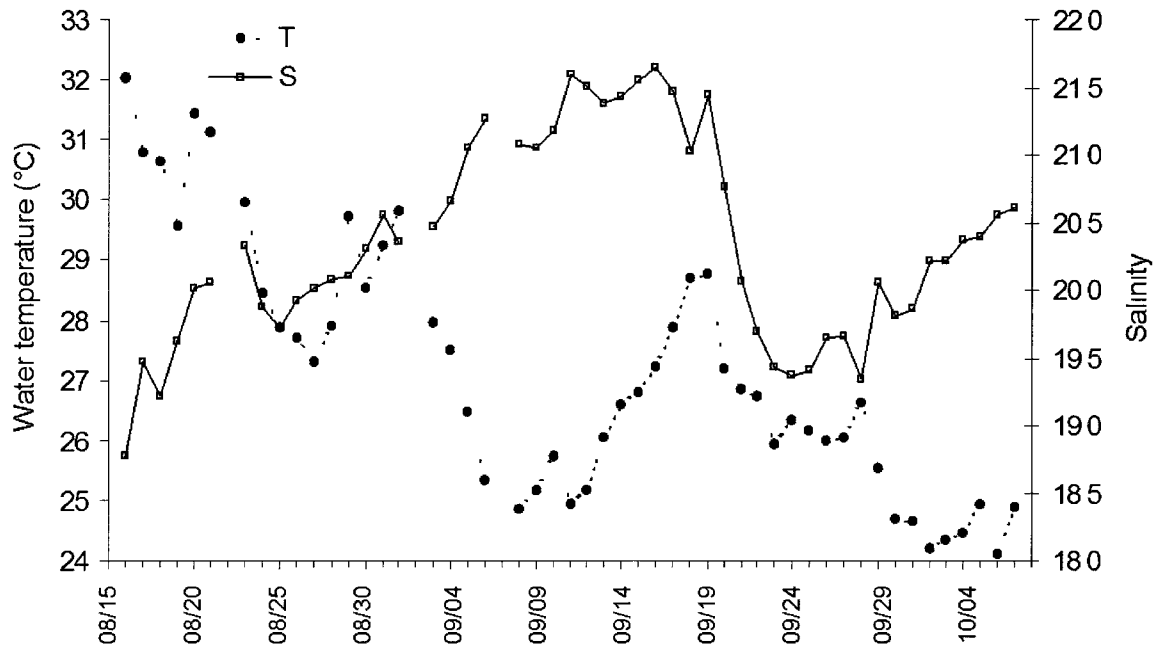


Fig. 7 Water temperature (°C) and salinity measured in the Lafayette River from August 15–October 8, 2005

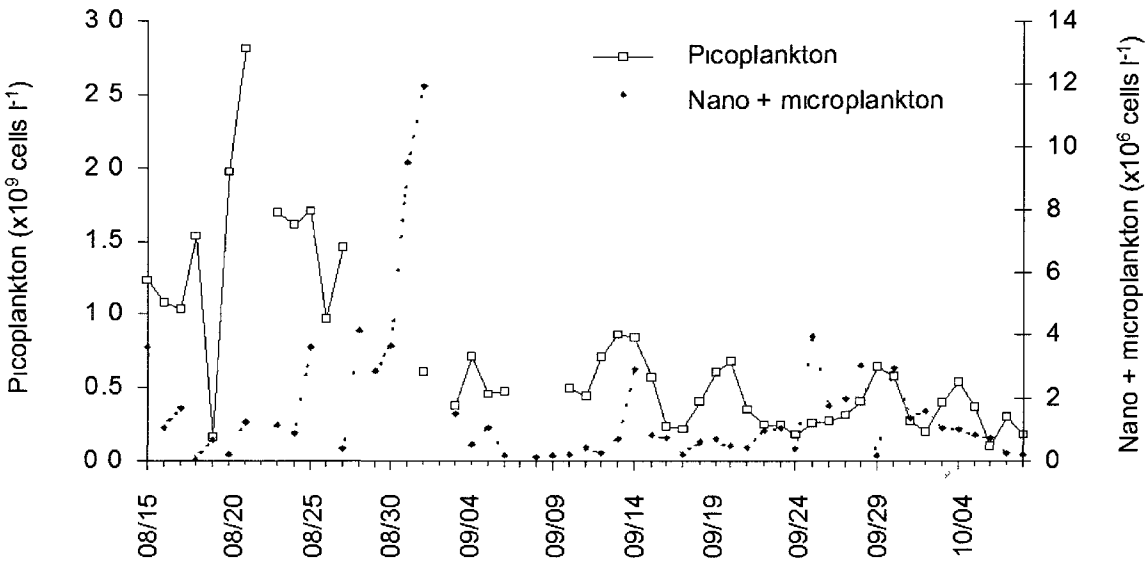


Fig. 8 Picoplankton abundance (x 10^9 cells l^{-1}) on the *left* y-axis and the total microplankton and nanoplankton abundance (x 10^6 cells l^{-1}) on the *right* y-axis. *Grayed bars* represent periods during spring tides, *non-grayed areas* occurred during neap tides

Spring-neap tidal modulation appeared to affect nanoplankton and microplankton abundance more than picoplankton abundance (Figs. 6 and 8). Total phytoplankton (nanoplankton plus microplankton) abundance was higher during neap tides and lowest during spring tides (*gray* boxes along the x-axis in Fig. 8). The dinoflagellate blooms in August and September, the diatom bloom in August, and high cyanobacterial abundance in September all occurred during neap tides (Figs. 3a, 6, and 8). Maximum and minimum picoplankton abundance occurred during a spring tide in August, with 2.8×10^9 cells l^{-1} and 0.16×10^9 cells l^{-1}, respectively. In general, picoplankton abundance was higher in August, when the winds were not as strong, than during September, when wind speeds were higher. Picoplankton abundance was not as strongly controlled by the tidal cycle and their abundance appeared to cycle on a weekly basis regardless of the tidal phase (Fig. 8).

DISCUSSION

Near-monospecific algal blooms are now common occurrences in the Chesapeake Bay and its tidal tributaries, as well as other highly eutrophic estuarine systems worldwide. However, despite decades of research, our understanding of the controls on bloom formation are poorly understood because the conditions antecedent to bloom formation are seldom characterized with the necessary temporal resolution; most nutrient monitoring programs sample too infrequently (weekly to monthly), and *ad hoc* bloom sampling is largely focused on blooms only after they have formed. In addition, Chl *a* and nutrient concentrations can vary by an order of magnitude over diurnal time scales and phytoplankton abundance is often strongly linked to the tidal phase (Fig. 2). In order to capture changing environmental conditions as blooms initiate, develop, and dissipate, I

sampled the Lafayette River on a daily basis during late summer, when blooms are common, at the same portion of the tidal cycle for a period of 54 days in 2005. During this time there were two dinoflagellate blooms and a diatom bloom. Sampling on a daily basis allowed for detailed observations regarding the sequence of events leading up to blooms as well as comparisons of phytoplankton abundance, ambient nutrient concentrations, and physical forcing (wind, precipitation, and spring-neap modulation of the tidal cycle) on timescales relevant to phytoplankton growth and bloom formation.

Nutrient dynamics and climatological controls on the formation of blooms.

Nutrient loading due to precipitation and associated runoff and subsequent water column stratification plays a key role in stimulating the formation of *Cochlodinium polykrikoides* blooms in the Lafayette River (Chapter 3). Similarly, during the present study precipitation and associated increases in ambient nutrient concentrations preceded the diatom and the dinoflagellate blooms in late August and September, respectively (Figs. 3a, b and 5e). Despite periods of intense rainfall prior to the start of this study, ambient nutrient concentrations were depleted at the start of this study, likely because the nutrient demand of a mixed bloom of *Akashiwo sanguinea* and *Gymnodinium* sp. already in progress was removing nutrients as quickly as they were supplied. *A. sanguinea* and *Gymnodinium* sp. are bloom-forming dinoflagellates typical during the summer months in the Chesapeake Bay and its tributaries (Marshall 1995, Marshall et al. 2005). Subsequent to this bloom, large increases in DIN concentrations were observed after rainfall events and increases in phytoplankton biomass were generally associated with decreases in DIN. Following precipitation on August 22–23, nutrient concentrations increased by a factor of 5 and a diatom bloom dominated by *Skeletonema costatum*

formed during a neap tide period (Figs. 3a and 5e), rapidly drawing down dissolved N concentrations to the limit of detection (Fig. 3b). The relatively high wind speed at this time (Fig. 5c) likely contributed to the formation of a diatom rather than a dinoflagellate bloom since dinoflagellates typically thrive when wind driven mixing and turbulence are low (Sellner et al. 2001, Smayda and Reynolds 2001, Margalef 1978). Rain events associated with high nutrient inputs accompanied a frontal system associated with Hurricane Ophelia in mid-September. After this system passed, nutrient concentrations were high, the wind velocity decreased and a dinoflagellate bloom ensued, likely due to high nutrient concentrations and decreased turbulence (Cloern and Dufford 2005; Margalef 1978; Sellner et al. 2001). While nutrients were not depleted during this dinoflagellate bloom, the concentrations of both DIN and DON were reduced during the bloom, consistent with previous observations that many dinoflagellates are able to use organic nutrients and grow mixotrophically (Burkholder et al. 2008, Graneli et al. 1999).

Subsequent to the diatom bloom at the end of August and the dinoflagellate bloom in September, there were numerous high wind (but low precipitation) events and this resulted in low phytoplankton abundance, higher cyanobacterial abundance (Figs. 5c and 3a), and the accumulation of NH_4^+ and NO_2^- (Fig. 3b), likely due to N regeneration as bloom organisms settled and decayed, as well as incomplete nitrification, a process common during this time of the year (McCarthy et al. 1977; McCarthy et al. 1984; Horrigan et al 1990). It is likely that regenerated nutrients were also contributed from benthic fluxes as high winds allow mixing of surface and bottom water and sediment resuspension in these shallow-water systems (Horrigan et al 1990; Rizzo 1990). In September and early October, prior to and following the September dinoflagellate bloom,

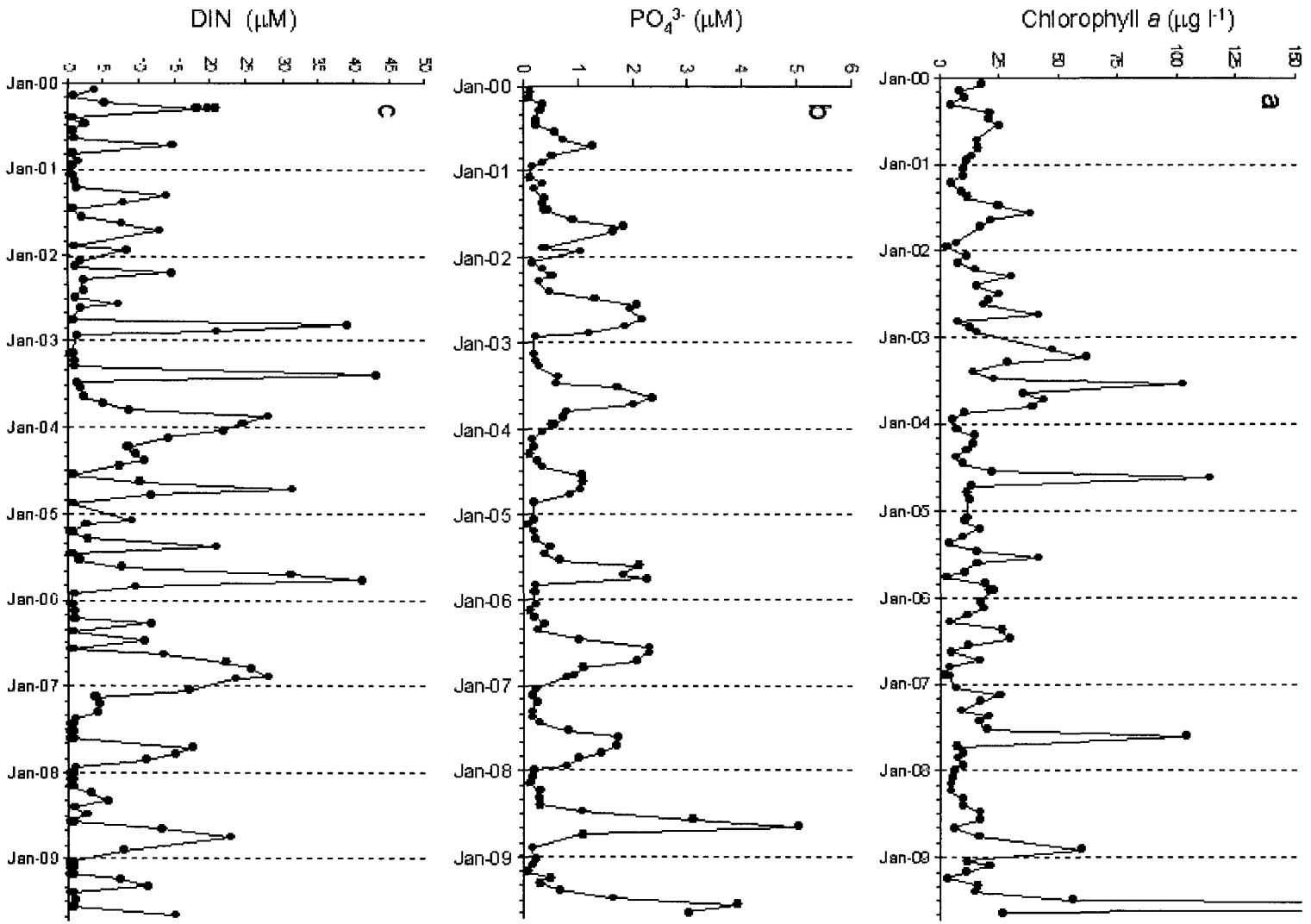
NO_3^- also accumulated in the water column, likely due to nitrification. At these times cyanobacterial abundances were high relative to other phytoplankton taxa and picoplankton abundance was also higher at these times (Figs. 5c and 8). Cyanobacteria are important components of most phytoplankton communities and thrive under stratified conditions common in the summer where they can take advantage of regenerated nutrient compounds (Paerl et al. 2006). Regenerated nitrogen is thought to fuel the bulk of primary production during summer months when new inputs of N are limited to stochastic events. Many dinoflagellate mixotrophs can also graze on picocyanobacteria including *Synechococcus* (Jeong et al. 2005a, Burkholder et al. 2008), a common component of the cyanobacterial community in the Chesapeake Bay (Marshall and Nesius 1996, Chen et al 2006), and picoplankton abundance was lowest during the September dinoflagellate bloom.

The Redfield ratio of C, N, and P nutrient elements in the environment has long been used to infer which nutrient is in shortest supply. Recently, short term changes in the ratio of dissolved N:P, and specifically, low N:P ratios, have been suggested as a causative factor for dinoflagellate blooms in Hong Kong (Hodgkiss and Ho 1997). Selection for or against diatoms has been associated with the supply of silicate relative to other nutrient elements (e.g., Si:N, and/or Si:P ratios) (Conley and Malone 1992; Smayda 1997b). During the present study period, the DIN:DIP ratio was usually less than 16, indicative of N limitation, but DIN and DIP concentrations were only depleted during the first 2 blooms, and therefore it is unlikely that phytoplankton were limited by N or P during the remainder of the study. Similarly, throughout the duration of the study, the Si:DIN ratio was always greater than 1 and Si:DIP ratio was always greater than 16,

suggesting that silicate concentrations were not limiting to diatom growth (Conley and Malone 1992).

Estuarine environments are often N limited systems (Howarth 2008), however, in contrast to our observations that Si and P were unlikely to limit productivity during our sampling period, monthly data from the Virginia Department of Environmental Quality's monitoring station in the Lafayette River (LFB01) (Fig. 1) suggest that P might limit productivity, at least seasonally. Between the years 2000 and 2009, Chl *a* and DIN concentrations at LFB01 showed some seasonality, with higher concentrations during spring and fall (Fig. 9a, c). In contrast, PO_4^{3-} concentrations were highest between August and October in all years (Fig. 9b). While DIN and DIP concentrations were positively correlated (Pearson Moment Correlation, T-test, $P < 0.05$) at this station, Chl *a* concentrations were positively correlated only with DIP (Pearson Moment correlation, T-test, $P < 0.001$) and not DIN concentrations.

Because phytoplankton growth and bloom formation often lags nutrient inputs by several days, plots of the time-lagged correlations between nutrient species and dinoflagellate abundance were constructed (Fig. 10) in order to better relate nutrient prehistory with dinoflagellate abundance. There was a strong positive correlation (correlations exceeding the 95% CI) between dinoflagellate abundance and all nitrogen compounds from two to five days in reverse time. This suggests that when nitrogen concentrations increase, dinoflagellate abundance increases two to five days later, and likewise when nitrogen concentrations decrease, dinoflagellate abundance decreases accordingly. It is important to point out that correlation does not imply cause; however,


DIN (µM)
PO_4^{3-} (µM)
Chlorophyll *a* (µg l^{-1})
Jan-00
Jan-01
Jan-02
Jan-03
Jan-04
Jan-05
Jan-06
Jan-07
Jan-08
Jan-09
c
b
a

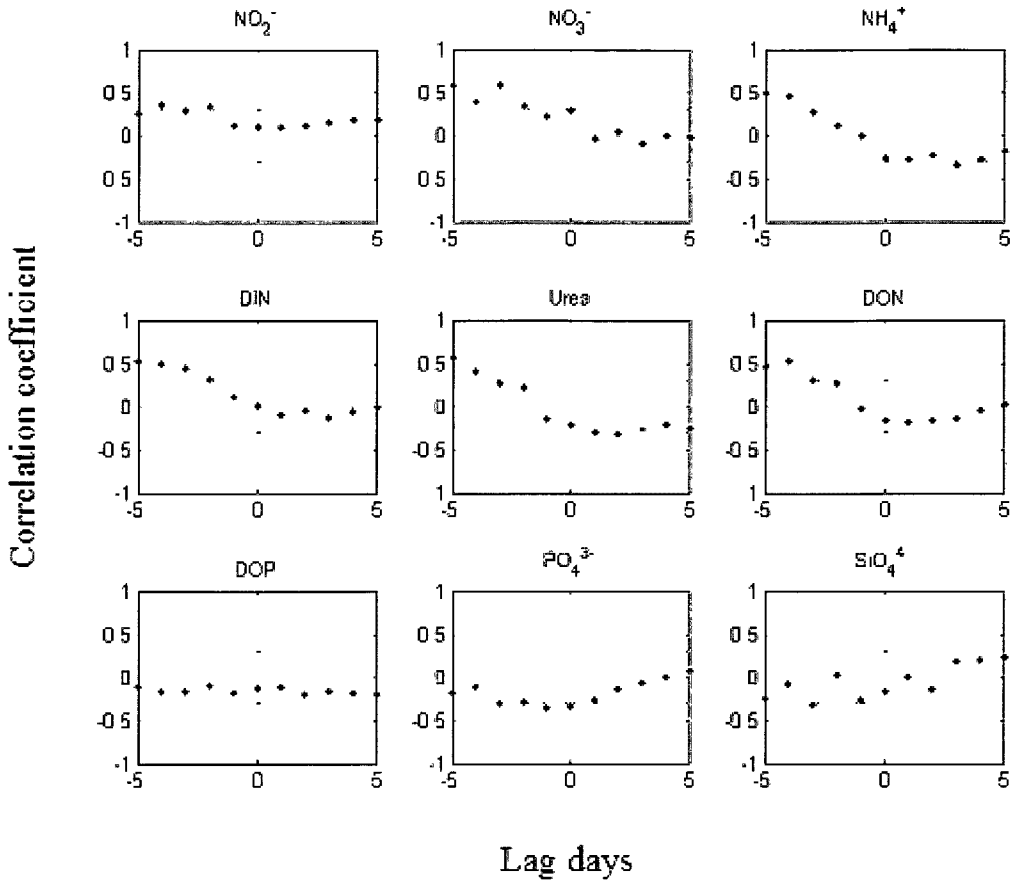


Fig. 10 Time-lagged correlation coefficient plots of nutrient compounds versus dinoflagellate abundance. The *x-axis* is a five-day forward and reverse time lag with day 0 being the present. The correlation coefficient for dinoflagellate abundance versus each nutrient compound at each time lag is shown on the *y-axis* with the 95% confidence intervals (CI) shown as *dashed lines* at 0.305 and –0.305 on the *y-axis*

because phytoplankton growth is dependent upon nutrients and an increase in biomass requires N inputs, the increase in nutrient concentration likely caused the increase in dinoflagellate abundance. The positive correlations between all forms of N measured and dinoflagellate abundance suggests that no particular nutrient species was required for bloom development, but rather that the N concentration in general (NO_3^-, NO_2^-, NH_4^+, urea and DON), regardless of N species, was important. Dinoflagellates have been shown to be nutritionally flexible (Anderson et al. 2002, Burkholder et al. 2008) and they appear to thrive in eutrophic estuarine systems where there is variability in the form of N supplied.

While the positive lagged correlation between N concentrations and dinoflagellate abundance may be indicative of growth stimulation by N, the negative correlation between PO_4^{3-} and dinoflagellate abundance with little to no lag may suggest that P is drawn down during blooms to support cellular P demand and growth, but is not growth limiting. Consistent with this observation, as dinoflagellate abundance increased during the September *Gymnodinium* sp. bloom, PO_4^+ concentrations decreased by the largest amount observed during the study period, but PO_4^+ was never depleted (Figs. 3a and 4). There was a strong positive correlation between diatom abundance and PO_4^+ concentrations from 3–5 days in reverse time, and a strong positive correlation with silicate concentrations from 2–3 days in reverse time (data not shown). Additionally, there was a strong negative correlation between diatom abundance and NO_2^- and DIN concentrations 2 days in forward time (data not shown). This suggests that diatom abundance increases in response to increased in PO_4^+ and silicate concentrations, but

when diatom abundance decreases, the concentrations of DIN, and NO_2^- increase 2 days later, which may be due to nutrient recycling following the collapse of the diatom bloom in early September.

The high concentrations of nitrite observed during the present study and the importance of nitrite to bloom formation suggests nitrite may play a larger role in estuarine environments than previously believed. The uptake of nitrite is well documented in oceanic environments where it can be an important source of N (Collos 1998; Lomas and Lipschultz 2006). While McCarthy et al. (1977, 1984) reported high NO_2^- concentrations (up to 10 μM) in Chesapeake Bay and speculated that it was derived from incomplete nitrification associated with destratification and mixing of surface and bottom waters, the abundance and utilization of this N source has not been widely examined in most estuarine systems. Concentrations of NO_2^- were observed that were consistent with those reported for the Chesapeake Bay (McCarthy et al 1977) and in the York River (Killberg and Bronk, unpublished data). Nitrite can be formed during incomplete nitrification, released by phytoplankton during NO_3^- uptake, and less commonly during incomplete denitrification (Lomas and Lipschultz 2006; Zehr and Ward 2002). Because the process of nitrification is carried out by two separate groups of organisms, ammonium-oxidizing bacteria (AOB) and/or ammonium-oxidizing archaea (AOA) and nitrite-oxidizing bacteria (NOB) (Ward et al. 2007; Zehr and Ward. 2002), the process of nitrification can become uncoupled and NO_2^- may accumulate in the water column (McCarthy et al. 1984). Ammonia-oxidizing bacteria are abundant throughout the Chesapeake Bay with the highest diversity in the oligohaline upper Bay region (Ward et al. 2007). In the polyhaline portion of the Bay, ammonium-oxidizing archaea may be

the dominant nitrifiers (Wuchter et al. 2006, Ward et al, 2007). Based on the tight coupling of NO_2^- and NH_4^+ concentrations, the low NO_3^- concentrations prior to mid-September (Fig. 3b), and the presence of sufficient oxygen in the water column (data not shown), the accumulation of high NO_2^- concentrations in the present study was likely a result of incomplete nitrification.

Physical controls on phytoplankton community dynamics.

Wind-driven mixing in shallow estuaries can both inject nutrients from the benthos (Rizzo 1990) and result in the demise (Chapter 3) or dissipation of algal biomass (Figs. 5c and 3a, b). The cube of the wind speed is proportional to its turbulent mixing potential, and as such can be used to estimate the amount of wind driven mixing and whether that mixing impinges on the bottom (Lund-Hansen et al. 1996).

Although the Lafayette River is generally sheltered from the wind, wind speed and direction may be an important factor controlling taxonomic dominance and bloom development. For example, during a period of low winds (August 28–30), phytoplankton biomass was high, and cryptophytes and diatoms were both abundant (Fig. 3a, c). However, following a period of high winds from the southwest (Fig. 5c, d), diatom abundance increased while cryptophyte abundance decreased drastically (Fig. 3a, c). The increase in wind velocity likely mixed the entire water column in the shallow Lafayette River, causing particle resuspension including diatoms, sediments and other passive particles and creating unfavorable conditions for flagellates.

In the Lower Chesapeake Bay system, wind direction influences how wind speed interacts with the estuarine circulation and mixing, and algal biomass appears to have a

threshold response dependant upon on the strength and duration of the wind, where the biomass is markedly reduced at higher wind speeds for periods >24hours (500 m^3s^{-3}, which corresponds to 15.4 knots; Figs. 3a and 5a). Following Hurricane Katrina, a high-pressure system in the region resulted in high winds from the northeast. This type of atmospheric system forces oceanic water landward, resulting in decreased riverine flushing, accumulation of oceanic water in the Chesapeake Bay, positive tidal residuals at Sewell's Point, and saltwater intrusion into the Lafayette River. Chesapeake Bay and its tributaries are more vulnerable to northeasterly winds because of the fetch over which they develop and the N–S orientation of the Bay mouth. The combined wind-driven and tidal mixing caused by this high-pressure system likely contributed to the decreased algal biomass observed during this period (Figs. 3a and 5c, d).

In contrast, winds from the southwest typically result in enhanced riverine flushing and offshore transport of water through the Bay mouth. The Lafayette River is sheltered from the southwesterly winds by the landmass, thus the effects of high wind from this direction are reduced. Therefore, although the winds were strong between August 30 and September 2, the winds were from the southwest and so did not result in the same degree of mixing and turbulence in the system, while allowing nutrient inputs from mixing to stimulate diatom growth. Diatoms characteristically thrive in higher energy environments than dinoflagellates (Smayda and Reynolds 2001). In contrast, the high-pressure system that dominated from September 4 through 9 resulted in Northeasterly winds that resulted in a large oceanic influence on the lower Chesapeake Bay and its sub-tributaries including the Lafayette River. Salinity in the Lafayette increased, there was a high positive tidal residual during this period, the phytoplankton

abundance decreased, and DIP concentrations decreased despite the lower algal biomass, suggesting increased turbidity and particle-associated nutrient removal (Froelich 1988).

Timescales of variability important to phytoplankton.

One of the problems associated with sampling blooms is coping with estuarine variability on timescales ranging from minutes to months, and biological variability associated with the lifecycles and behavior of phytoplankton cells and populations (Lucas et al 2006, Hubertz and Cahoon 1999, Glibert et al 2008). Within a 24-h period of fixed-station sampling, nutrient concentrations and phytoplankton abundance varied by an order of magnitude and nutrient and Chl *a* concentrations were strongly linked to the tidal cycle (Fig. 2) as had been observed in this system previously (Mulholland et al. in prep). Shallow estuaries and coastal systems are highly dynamic areas where a multitude of physical, chemical and biological factors synergistically control the distribution, growth, and transport of the phytoplankton community, which in turn modify the nutrient regimes of the surrounding waters. This variability makes it difficult to understand controls on blooms using data collected during most long-term monitoring programs that may sample systems only at weekly to monthly intervals; a frequency insufficient to capture ephemeral blooms. The tidal control of biomass and nutrient concentrations in estuarine environments has direct implications for interpreting monitoring data that is not tidally resolved (Cloern 1991, Lucas et al 1999a). In addition, it is now known that stochastic events are important for controlling nutrient inputs during large parts of the year and these affect nutrient loading from the water and airsheds as well as nutrient inputs from the benthos (Paerl 1997).

When daily measurements of Chl *a* concentrations from the present study are compared to Chl *a* concentrations measured monthly at LFB01 (Fig. 1), it is apparent that short-term variability is missed in the monthly sampling record. In addition, when Chl *a* concentrations are compared at the two sites (less than 1 km apart) on the same date in August, a factor of 2 difference is observed between the sites, highlighting the patchy distribution of Chl *a* in these tidally dominated systems. While the DEQ Chl *a* monitoring record between 1999 and 2009 includes periods of high Chl *a* concentrations in the Lafayette River, the magnitude of these peaks is far less than those measured during targeted studies of blooms (Mulholland et al. 2009, Morse et al. 2011). For example, in 2005, the maximum observed Chl *a* concentration was 41 $\mu g\ l^{-1}$ in July and Chl *a* was only 15.7 $\mu g\ l^{-1}$ and 10 $\mu g\ l^{-1}$ in Aug and Sept, respectively, whereas our data indicate Chl *a* concentrations above 50 $\mu g\ l^{-1}$ in August during 2 blooms and concentrations near 70 $\mu g\ l^{-1}$ in September during the dinoflagellate bloom (Figs. 10 and 11). Likewise, in August 2007 and 2008, Chl *a* concentrations in the Lafayette River during a bloom of *Cochlodinium polykrikoides* were >300 $\mu g\ l^{-1}$ (Mulholland et al. 2009, Morse et al. 2011), however, DEQ Chl *a* concentrations from the Lafayette River monitoring station during this period were 105 $\mu g\ l^{-1}$ in 2007 and just 20 $\mu g\ l^{-1}$ in 2008.

It is important to remember that the Chesapeake Bay Monitoring Program and associated sampling by the Virginia DEQ was not and is not designed to capture the dynamics of ephemeral blooms, but rather was designed as a statewide effort to understand long term changes in Chesapeake Bay phytoplankton communities. A wide suite of methods, including in-situ monitoring devices, remote sensing, and targeted sampling at a high temporal frequency can be used to supplement long term monitoring

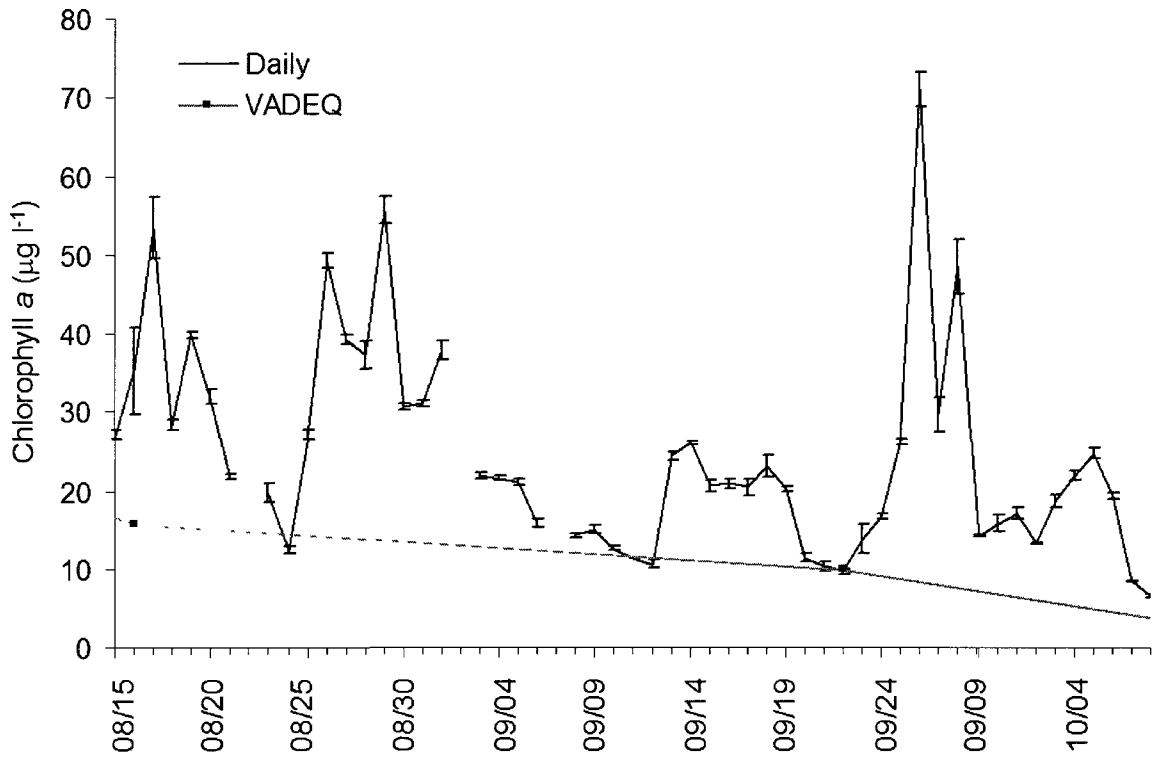


Fig. 11 Daily Chl *a* (µg l^{-1}) measured in the Lafayette River from August 15–October 8, 2005, and VADEQ monthly water quality Chl *a* (µg l-1) data from the Lafayette River station LFB01 for August through October 2005. These data were obtained through the Chesapeake Bay Program data hub:

(http://www.chesapeakebay.net/data_waterquality.aspx accessed 15 May, 2010)

systems, such as that in place in Chesapeake Bay, in order to fully capture the dynamics associated with algal populations in stochastic estuarine ecosystems. To this end, continuous monitoring of nutrients and Chl *a* provides a much more exhaustive and complete view of estuarine dynamics but these data sets are still limited (Glibert et al. 2008). In addition, most long-term monitoring programs do not collect tidally resolved data. Timing sampling to a specific portion of the tidal cycle may help to resolve processes occurring at least at tidal time scales. With the advent of technologies such as in-situ monitoring devices (e.g. Lucas et al. 2006b) and in-situ nutrient analyzers (e.g. Glibert et al. 2008), targeted sampling aimed at understanding conditions promoting the initiation of blooms will become easier. However, integrating the complex coupled climatological, physical, and biological forcings associated with blooms is likely to remain a challenge into the future.

CHAPTER 3

ENVIRONMENTAL AND PHYSICAL CONTROLS ON THE FORMATION AND TRANSPORT OF BLOOMS OF THE DINOFLAGELLATE *COCHLODINIUM POLYKRIKOIDES* MARGALEF IN LOWER CHESAPEAKE BAY AND ITS TRIBUTARIES

PREFACE

This Chapter has been accepted for publication in Estuaries and Coasts and is currently in press and available online. See Appendix for copyright information. The full citation is given below:

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DOI: 10.1007/s12237-011-9398-2

INTRODUCTION

Phytoplankton blooms have widespread and highly variable effects ranging from loss of aesthetic and recreational value of waterways (Anderson et al. 2002; Paerl 1988), to ecological deterioration (Sunda et al. 2006), and direct toxicity (Hallegraeff 1993; Sellner et al. 2003). Mortality of aquatic organisms can result from low dissolved oxygen concentrations during the degradation of excess algal blooms (Smayda 1997a; Tango et al. 2005), mechanical damage to organisms due to high algal concentrations or as a result of feeding on algae (Landsberg 2002), indirect toxicity (Flewelling et al. 2005), or direct toxicity (Tang and Gobler 2009). Vast economic losses (Anderson et al. 2002; Smayda

1997b) can result from trophic and community level disruption as well as direct finfish and shellfish mortality (Cloern 2001; Heil et al. 2001; Heil et al. 2005; Sunda et al. 2006).

While the consequences of algal blooms have been qualitatively and quantitatively assessed, the causes of algal blooms and mechanisms of bloom initiation are still not well understood despite decades of research. Additionally, even though environmental conditions prior to bloom formation may be keys to understanding bloom initiation, *ad hoc* sampling generally commences only after a bloom has become visible (Smayda 1998). Most routine water quality monitoring lacks the temporal and spatial resolution to capture bloom inception and early development. However, environmental conditions are likely to be very different when blooms first initiate and algal biomass is still low, compared to when blooms are well established and algal biomass is already very high. In addition, sites of bloom initiation may be far removed from where biomass accumulates in environments with complex circulation patterns (Lucas et al. 1999). These issues represent major gaps in our knowledge regarding the causes of algal blooms and bias our view about environmental conditions promoting bloom formation.

Cochlodinium polykrikoides Margalef is an unarmored gymnodinoid dinoflagellate that produces cysts and was first reported in the lower Chesapeake Bay region in the late 1960's in the York River (Mackiernan 1968), where it regularly forms blooms. As a result of a large bloom that initiated in the York River during the summer of 1992, *C. polykrikoides* was transported through the lower Chesapeake Bay into the lower James River (Marshall 1995) where it appears to have established a "seed" population and continues to form blooms (see Fig. 12 for location). Sediment samples taken in 1996 from the lower James River contained *C. polykrikoides* cysts at a mean

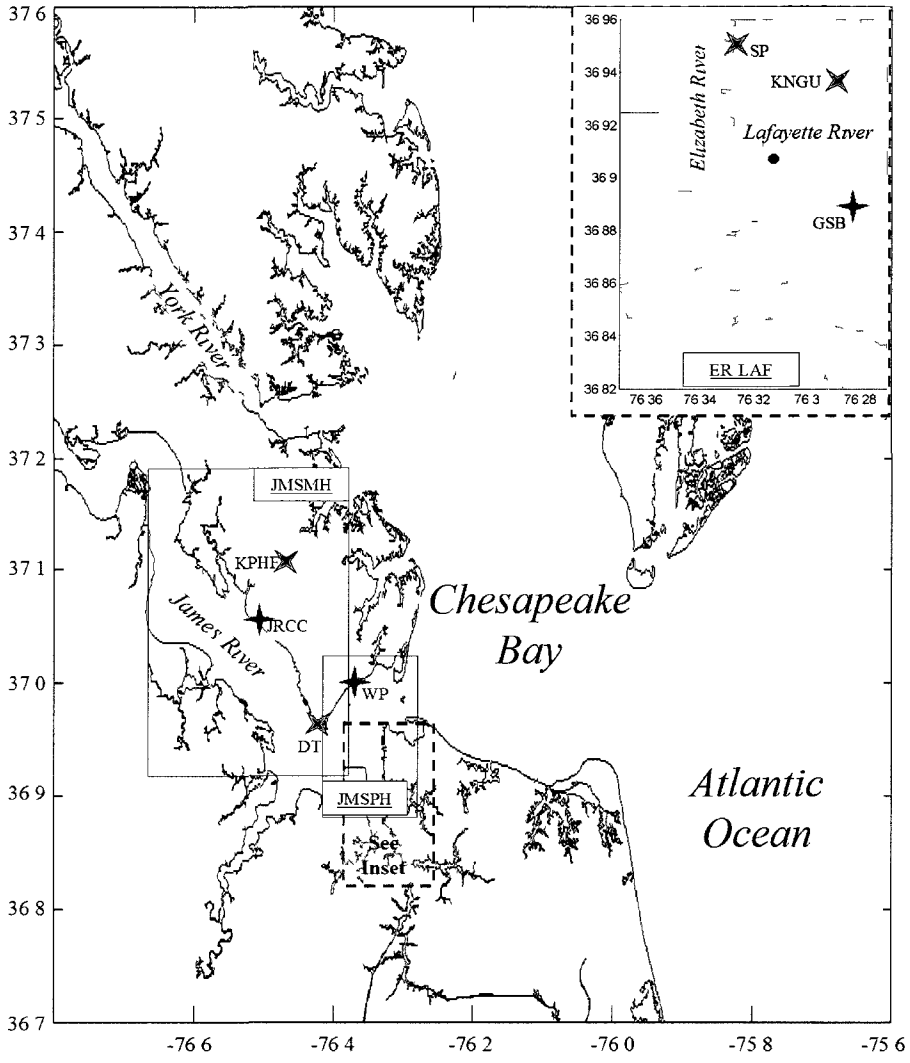


Fig. 12 Map of the lower Chesapeake Bay and its tributaries. *Boxes* indicate partitioning of cruises in the mesohaline (*JMSMH*) and polyhaline (*JMSPH*) portions of James River estuary, and in the Elizabeth and Lafayette Rivers (*ER-LAF, inset*). *Gray cross marks* indicate the location of weather and tidal stations at Newport News/Williamsburg International Airport (*KPHF*), Dominion Terminal (*DT*), Sewells Point (*SP*), and Naval Station Norfolk Chambers Field (*KNGU*). The *black cross marks* indicate the Granby Street Bridge sampling station (*GSB*) in the Lafayette River, and the VECOS YSI site locations at the James River Country Club (*JRCC*), and Wythe Point (*WP*) in the James River. The *black dot* in the Lafayette River denotes the location of the dye release for the 2007 and 2008 simulations

concentration of 96 cysts gram^{-1} of wet sediment and *C. polykrikoides* cysts were the second most abundant species identified in the sediment samples (Seaborn and Marshall 2008). Since the 1992 bloom that originated in the York River, *C. polykrikoides* has been a regular component of the phytoplankton community in the lower James River and Chesapeake Bay region (Marshall 1995).

Since 1992, *Cochlodinium polykrikoides* abundance appears to be increasing in the James and Elizabeth Rivers (Marshall et al. 2005). During 1995, the greatest abundance of *C. polykrikoides* in samples collected from the Elizabeth River was 15 cells ml^{-1} (Marshall, unpublished data). Ten years later, *C. polykrikoides* abundance in the Elizabeth River had increased to 810, 3500, and 28120 cells ml^{-1} during 2005, 2006, and 2007 blooms, respectively (Marshall, unpublished data; Mulholland et al. 2009). Chlorophyll *a*, (Chl *a*), concentrations in the James River exceeded 300 μg l^{-1} during the 2007 bloom, which persisted for more than a month in the James River and even longer in the Elizabeth River (Mulholland et al. 2009). This bloom caused multiple beach closures, and penetrated into the Atlantic Ocean where it was transported south along the Virginia coastline. Maps of surface Chl *a* concentrations were constructed for the lower James River using an underway surface water sampling system (DATAFLOW) and these suggested that bloom organisms might have entered that system from the Elizabeth River, a tributary of the lower James River (Mulholland et al. 2009).

As in 2007 (Mulholland et al. 2009), during 2008, a massive bloom of *C. polykrikoides* occurred in the lower Chesapeake Bay and its tributaries following periods of intense rainfall in late July and early August. These blooms extended for more than 30 nautical miles from the Elizabeth and Lafayette River basins into the lower James River

and the Chesapeake Bay. Based on the 2007 observation that *C. polykrikoides* blooms appeared to initiate in the Elizabeth River, the Chl *a* mapping of surface waters was expanded to include the Lafayette and Elizabeth Rivers, sub-tributaries of the Lower James River. Surface Chl *a*, salinity, temperature, and dissolved oxygen (DO) were mapped on a weekly basis from July through September 2008, when blooms of *C. polykrikoides* typically initiate, develop, and persist. In addition to the increase in our mapping coverage, surface mapping was augmented with vertical hydrographic measurements and modeling in order to better understand the initiation, development, and persistence of blooms and the transport of bloom organisms through the lower Chesapeake Bay and its tributaries.

METHODS

Surface water mapping. As part of its Chlorophyll Monitoring and Assessment Program (CMAP), the Hampton Roads Sanitation District (HRSD) collects underway-sampling data (DATAFLOW; http://www3.vims.edu/vecos/Default.aspx) during weekly cruises in the lower James River from March through September. The James River cruise segments are partitioned into two different cruise dates, and are separated into the mesohaline (JMSMH) and polyhaline (JMSPH) portions of the James River. During 2008, HRSD expanded DATAFLOW mapping of the James River into the Elizabeth and Lafayette Rivers (ER-LAF) (Fig. 12). During cruises, water was pumped continuously from 0.5 m depth into a flow through cell equipped with an YSI 6600 multiparameter datasonde. Temperature, salinity, pH, dissolved oxygen, turbidity and fluorescence were measured continuously and recorded at 0.25 Hz along the cruise track, and spatial and

temporal data were related to other data geospatially using the global positioning system (GPS). In order to calibrate the fluorescence signal to Chl *a*, discrete Chl *a* samples were taken at 5 stations on each cruise date. The data were regressed and a relationship between fluorescence and Chl *a* was determined separately for the Elizabeth River basin and for the James River. Because the Chl *a* concentrations varied greatly during the bloom, both temporally and spatially, the Chl *a* data were pooled from July 3 through September 4, 2008 for the ER-LAF calibration, and from March through September 2008 for the JMSMH and JMSPH calibration. After undergoing a quality assurance and control check, the finalized and corrected Chl *a* data were plotted along the cruise track using MathWorks MATLAB software, and the results were mapped to give a spatial representation of Chl *a* in surface waters. Maximum Chl *a* values in the figures were capped at 90 $\mu g\ l^{-1}$ in order to maintain detail in areas not affected by the bloom, however actual concentrations were often much higher within bloom patches, often exceeding 300 $\mu g\ l^{-1}$ in the ER-LAF. When viewed as a timeseries, these maps allow a visualization of the initiation and transport of blooms throughout the lower James and Elizabeth River systems.

Continuous Monitoring Stations. As part of the Virginia Estuarine and Coastal Observing System (VECOS), the Virginia Institute of Marine Science maintained two fixed continuous monitoring stations in the James River, one at Wythe Point (WP) in the JMSPH, and one at the James River Country Club (JRCC) in the JMSMH (Fig. 12). A YSI 6600 series multiparameter datasonde was located at each station at a depth of 0.5m and recorded fluorescence at 15-minute intervals. These data were used to determine the timing of bloom detection in the James River (K. Moore, personal communication).

More information can be found at the VECOS website: http://www3.vims.edu/vecos/Default.aspx.

Meteorological and tidal data. Precipitation data for the Lafayette and Elizabeth River watersheds and a 30-year average of precipitation for the city of Norfolk, VA, were obtained from Naval Station Norfolk Chambers Field (KNGU) (Fig. 12). Precipitation data for the James River continuous monitoring stations at WP and JRCC were obtained from the Newport News/Williamsburg International Airport (KPHF). Wind speed and direction for the region were obtained from the National Oceanic and Atmospheric Administration's Physical Oceanography Real Time System (NOAA PORTS) station at Dominion Terminal (DT) at Newport News Point. Tidal predictions and tidal height data were obtained from the NOAA PORTS station at Sewell's Point (SP), in Norfolk, VA located on the Elizabeth River.

Hydrographic measurements. In addition to the DATAFLOW system, a CTD (Sea-Bird Electronics SBE19plus) equipped with sensors to measure pressure, temperature, fluorescence, and conductivity was deployed at designated stations to provide vertical profiles of these physical variables during cruises and estimate stratification in the Lafayette River. A stratification index (SI) was calculated based on density profiles as the difference in density (ρ) over the water column normalized to the depth of the water column (Z):

$$SI = (\rho_{bottom} - \rho_{initial}) / (Z_{bottom} - Z_{initial}) \quad (1)$$

Additionally, a timeseries plot was constructed for the hydrographic station at the Granby Street Bridge (GSB) near the site of bloom initiation in the Lafayette River using the hydrographic data, weekly cruise data from the DATAFLOW cruises, and

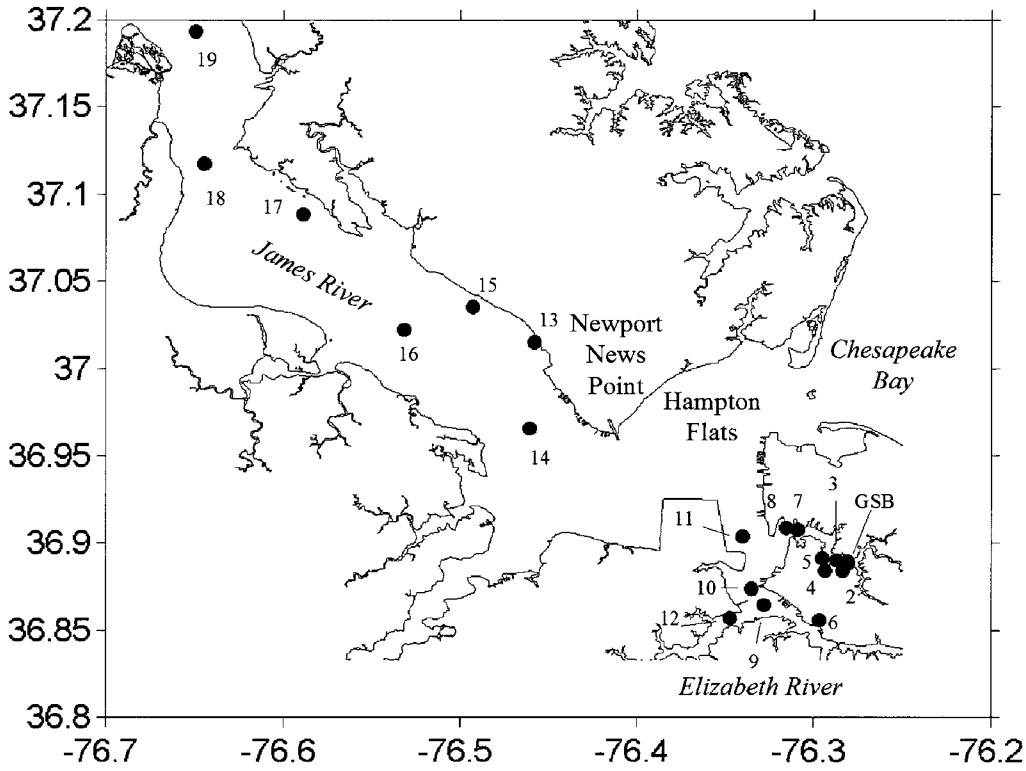


Fig. 13 Map of the study area showing stations where samples were collected for *C. polykrikoides* cell counts; the station numbers correspond to data shown in Table 1

precipitation data measured at KNGU. The DATAFLOW cruise data were sorted based on latitude and longitude, and the top five highest Chl *a* concentrations and the bottom five lowest salinity values from a specified window of GPS coordinates were averaged for each cruise. The highest Chl *a* and the lowest salinity values were selected because they were representative of the water column before mixing occurred as a result of the vessel occupying the station and the nature of the DATAFLOW sampling.

Phytoplankton. Phytoplankton samples were collected from bloom sites (Fig. 13, Table 1), and preserved to identify and confirm via microscopy that the dominant organism in samples was *C. polykrikoides*. Whole water samples were collected from the flow-through system during DATAFLOW cruises after it had passed through the YSI datasonde chamber. Duplicate samples were collected in sterile 50-ml centrifuge tubes; one sample was preserved with non-acidified Lugol's iodine solution and one sample was kept for live identification immediately upon returning from the cruises.

Phytoplankton counts for DATAFLOW cruise samples were performed by settling 300 µl of preserved sample into NUNC 8-well labtek chambered coverglass slides. Counts were made on a Zeiss Axiovert 40 CFL inverted microscope at 100x magnification. All cells were enumerated and cell abundance is expressed as cells ml^{-1}. *C. polykrikoides* abundance and total phytoplankton abundance were recorded in order to determine the percent composition of the bloom species (Table 1). Phytoplankton counts were also performed by Dr. Harold Marshall's lab at Old Dominion University on *C. polykrikoides* bloom samples received from various state agencies during the bloom period. Samples verified that the bloom organism was $\geq$ 90% of the phytoplankton, but only *C. polykrikoides* was enumerated (Marshall and Egerton, personal communication).

Table 1 *Cochlodinium polykrikoides* cell counts (cells ml^{-1}), and abundance expressed as the percent of the total phytoplankton community from stations in the James, Elizabeth, and Lafayette Rivers from July to September 2008. Samples with chlorophyll *a*, salinity and temperature data were collected during DATAFLOW cruises; all other samples were collected by the Chesapeake Bay Foundation and the VA Department of Environmental Quality. Stations are labeled as increasing numbers from east to west and the locations are shown in Fig. 13

Station	Date	*C. polykrikoides* abundance (cells ml^{-1})	*C. polykrikoides* % composition	Chl *a* ($\mu g\ l^{-1}$)	Temperature (°C)	Salinity
GSB	16 Jul	3	<1	31	29.67	20.53
	30 Jul	11149	99	376	28.4	22.14
	5 Aug	10532	93	160	27.11	21.15
	26 Aug	1828	97	104	27.51	23.41
2	6 Aug	20	>90			
3	6 Aug	110	>90			
4	6 Aug	60	>90			
	22 Aug	4290	>90			
	29 Aug	115000	>90			
5	6 Aug	870	>90			
6	20 Aug	3800	>90			
7	6 Aug	8400	>90			
8	5 Aug	2438	95	83	28.77	23.03
9	5 Aug	4932	94	169	28.67	23.59
10	12 Aug	18730	>90			
11	5 Aug	4202	94	79	28.38	23.62
12	26 Aug	3633	81	111	26.68	23.75
13	12 Aug	5990	>90			
	20 Aug	2450	>90			
	20 Aug	14030	>90			
	20 Aug	4150	>90			
	20 Aug	3920	>90			
	20 Aug	2620	>90			
	20 Aug	350	>90			
14	20 Aug	1160	>90			
15	12 Aug	17150	>90			
16	20 Aug	1080	>90			
17	2 Sep	1033	79	49	26.69	18.34
18	20 Aug	180	>90			
19	28 Jul	0	<1	85	29.31	9.7

Modeling. The Virginia Institute of Marine Science three-dimensional Hydrodynamic Eutrophication Model (VIMS HEM-3D) was used to simulate James River hydrodynamics. The estuarine hydrodynamics (including density and topographically-induced transport, and wind and tidally-driven transport) were simulated for the James River under 2008 summer-time dynamic conditions. The model was forced by observed daily freshwater discharges at upstream James River and Appomattox River stations, and by estimated freshwater runoff in the Elizabeth River watershed based on daily precipitation observations. Hourly wind data from Gloucester Point, tide data from Sewell's Point (NOAA station), and hourly salinity data generated by the large domain Chesapeake Bay 3D model at the mouth of the James River were used for the model open boundary conditions. The model simulates tide, current, salinity, and the concentrations of a conservative tracer over the duration of the simulation period in the James River. More specific details on the VIMS HEM-3D model description are given in Shen et al. (1999) and Shen and Lin (2006), and more thoroughly in Hamrick (1992), Hamrick and Wu (1997), and Park et al. (2005).

Model results were also computed under 2007 bloom conditions in order to make comparisons between bloom years and to further compare model results to surface mapping results during 2007 (Mulholland et al. 2009). In order to simulate actual tidal conditions, the tidal phase was not held constant between the 2007 and 2008 dye release simulations. However, the effect of tidal phase on the dye release should be minimal since the dye was released over a period of 12 hours, just short of one full tidal cycle. Additionally, to test the effects of the dye release location for the 2007 simulation, dye was released on August 6, 2007 in the Lafayette River at the same location used in the

2008 simulation, and, separately, at Sewell's Point in the Elizabeth River (Fig. 12) based on observed Chl *a* concentrations where the bloom was last observed on August 7, 2007 (Mulholland et al. 2009). Compared to the results when the dye was released in the Lafayette River, there was no noticeable difference in the extent of dye transport or concentration of dye observed at any given location for the Sewell's Point release (data not shown). For this reason, and to make direct comparisons of dye transport between bloom years, the Lafayette River dye release point was chosen for both 2007 and 2008 simulations.

RESULTS

Bloom Initiation and Development.

Surface Water Mapping. The 2008 *Cochlodinium polykrikoides* bloom initiated in the upper reaches of the Lafayette River between July 24 and July 30 (Table 1, Figs. 14 and 15). Phytoplankton samples from station GSB confirmed the presence of *C. polykrikoides* in the Lafayette River on July 16, with a concentration of 3 cells ml^{-1}, however the dominant species at this time was a *Gymnodinium* sp. (data not shown). The Chl *a* concentration was 36 $\mu g\ l^{-1}$, higher than the normal background concentration of 21 $\mu g\ l^{-1}$ (Table 1, Figs. 14 and 15; Chesapeake Bay Program data 2000–2009 monthly average in the Lafayette River). By July 24, Chl *a* had increased to 53 $\mu g\ l^{-1}$, more than twice the background concentration (Fig. 15). While no samples were preserved for cell counts on this date, live phytoplankton samples observed on a portable field microscope during the cruise indicated that *C. polykrikoides* was the dominant species (data not shown). Concomitant high Chl *a* concentrations in the Western Branch of the Elizabeth

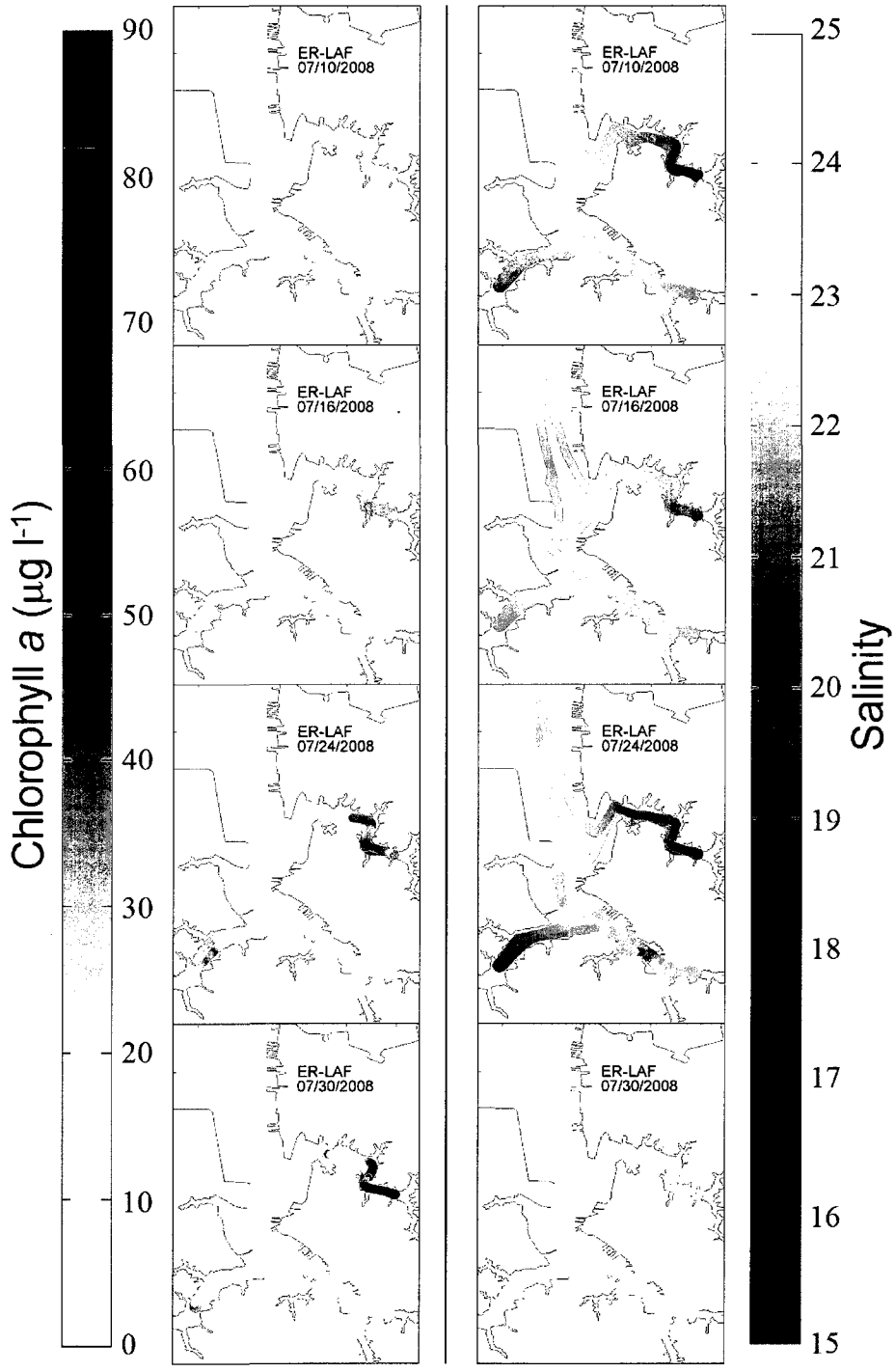


Fig. 14 Surface Chl *a* concentrations (µg l^{-1}) (*left panels*) and salinity (*right panels*) in the Lafayette and Elizabeth Rivers (*ER-LAF*) measured during cruises on July 10, 16, 24, and 30, 2008

River on July 16 and 24 were due to a mixed diatom assemblage and *C. polykrikoides* was not present in samples collected and examined microscopically from this area (data not shown).

Salinity in the upper reaches of the Lafayette River was considerably lower than salinity in the lower Lafayette on all cruise dates in July, with the lowest salinities, 19.5 and 15.1, occurring on July 10 and 24, respectively, at station GSB in the Lafayette (Figs. 14 and 15). The Lafayette River has no freshwater input other than that delivered by rainfall and runoff, and so freshening in the upper reaches was due to stormwater runoff and rainfall. Chl *a* concentrations were highest in the low salinity surface waters of the Lafayette River during July (Fig. 14).

At station GSB in the Lafayette River, Chl *a* concentrations and water column stratification increased following precipitation events in July (Fig. 15). Heavy rainfall on July 23 caused the salinity to decrease in the entire Lafayette River, and the stratification index increased to the highest values observed in the Lafayette River during the entire study period (Figs. 14 and 15). By July 30, *C. polykrikoides* was the dominant species at station GSB with 11,129 cells ml^{-1} comprising 99% of the total phytoplankton abundance and Chl *a* concentrations exceeded 300 $\mu g\ l^{-1}$ (Table 1, Fig. 15).

By August 5, *C. polykrikoides* and Chl *a* concentrations had increased in the Western and Southern Branches of the Elizabeth River suggesting that bloom organisms had been transported from sites of initiation in the Lafayette River into the Elizabeth River (Fig. 16). At the same time, the spatial extent of the bloom increased in the ER-LAF reaching the mouth of the Elizabeth River, at its confluence with the James River.

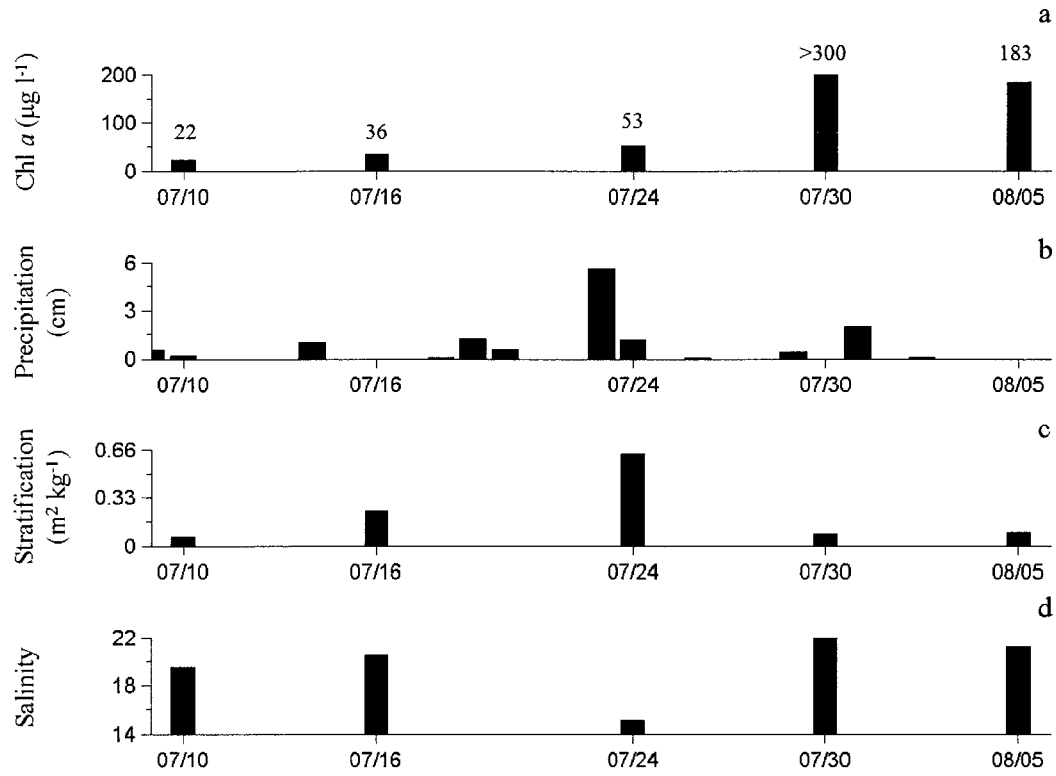


Fig. 15 Timeseries showing **a** Chl *a* (µg l^{-1}), **b** precipitation (cm), **c** water column stratification index (m^2 kg^{-1}), and **d** salinity at station GSB in the Lafayette River from July 10 to August 5, 2008. Chl *a* and salinity were taken from underway ER-LAF cruise data, precipitation was obtained from KNGU, and the stratification index was calculated based on measured density profiles during cruises

Chlorophyll a (µg l-1)
90
80
70
60
50
40
30
20
10
0
JMSMH
08/04/2008
JMSMH
08/13/2008
JMSMH
08/18/2008
JMSMH
08/25/2008
JMSMH
09/02/2008
JMSPH
08/06/2008
JMSPH
08/12/2008
JMSPH'
08/19/2008
JMSPH
08/27/2008
JMSPH
09/03/2008
ER-LAF
08/05/2008
ER-LAF
08/11/2008
ER-LAF
08/21/2008
ER-LAF
08/26/2008
ER-LAF
09/04/2008

The bloom was transported from the Elizabeth River into the James River between August 6 and 12 (Fig. 16).

Chl *a* concentrations in the JMSPH increased slightly from August 6 to August 12, with higher concentrations located upriver from the mouth of the Elizabeth River (Fig. 16). By August 13, the bloom was observed over much of the northern shoreline in the JMSMH (Figs. 16 and 17). Chl *a* in the JMSPH remained lower with a relatively patchy distribution until August 19 (Figs. 16 and 17). Uncorrected Chl *a* data recorded from the JMSPH WP continuous monitoring station shows an increase in Chl *a* beginning on August 15 (Fig. 17a), while the initial increase in Chl *a* at the JRCC station in the JMSMH occurred earlier, on August 13 (Fig. 17b). The high concentration of bloom organisms in the JMSMH combined with the patchy distribution and lower concentrations of Chl *a* in the JMSPH suggests that there may have also been sites of bloom initiation in the JMSMH (Figs. 16 and 17).

Rainfall. Yearly cumulative precipitation measured at KNGU was 18.1, 15.9, 44.7, and 22.1 cm below average during 2005, 2006, 2007, and 2008, respectively, when compared to the 30-year average (1961–1990) for rainfall in Norfolk, 116.2 cm (Fig. 18a). Between June 22 and August 31, during all four years, the pattern of precipitation was characterized by sporadic and intense rainfall with more than 5 cm of precipitation falling within 24 hours on several occasions (Fig. 18b). The timing of *C. polykrikoides* bloom initiation in the Elizabeth and Lafayette Rivers in both 2007 and 2008 coincided with intense, episodic, and highly localized rainfall events between late July and early August (Figs. 14 and 15; see also Mulholland et al. 2009). During 2008, highly localized, intense precipitation events were measured at KNGU in early July and from

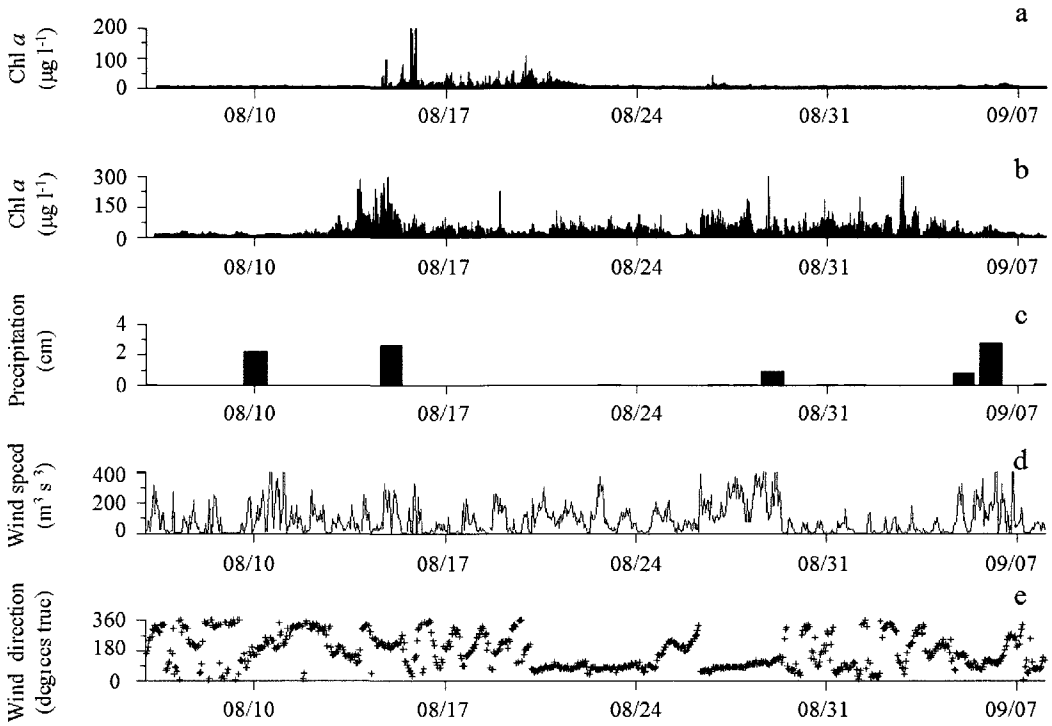


Fig. 17 Timeseries showing bloom formation at two stations in the James River. Data shown are uncorrected Chl *a* (µg l^{-1}) from VECOS YSI stations at **a** Wythe Point, WP, and **b** the James River Country Club, JRCC; **c** precipitation (cm) measured at KPHF; **d** wind speed (m^3 s^{-3}) and **e** wind direction measured at Dominion Terminal (DT)

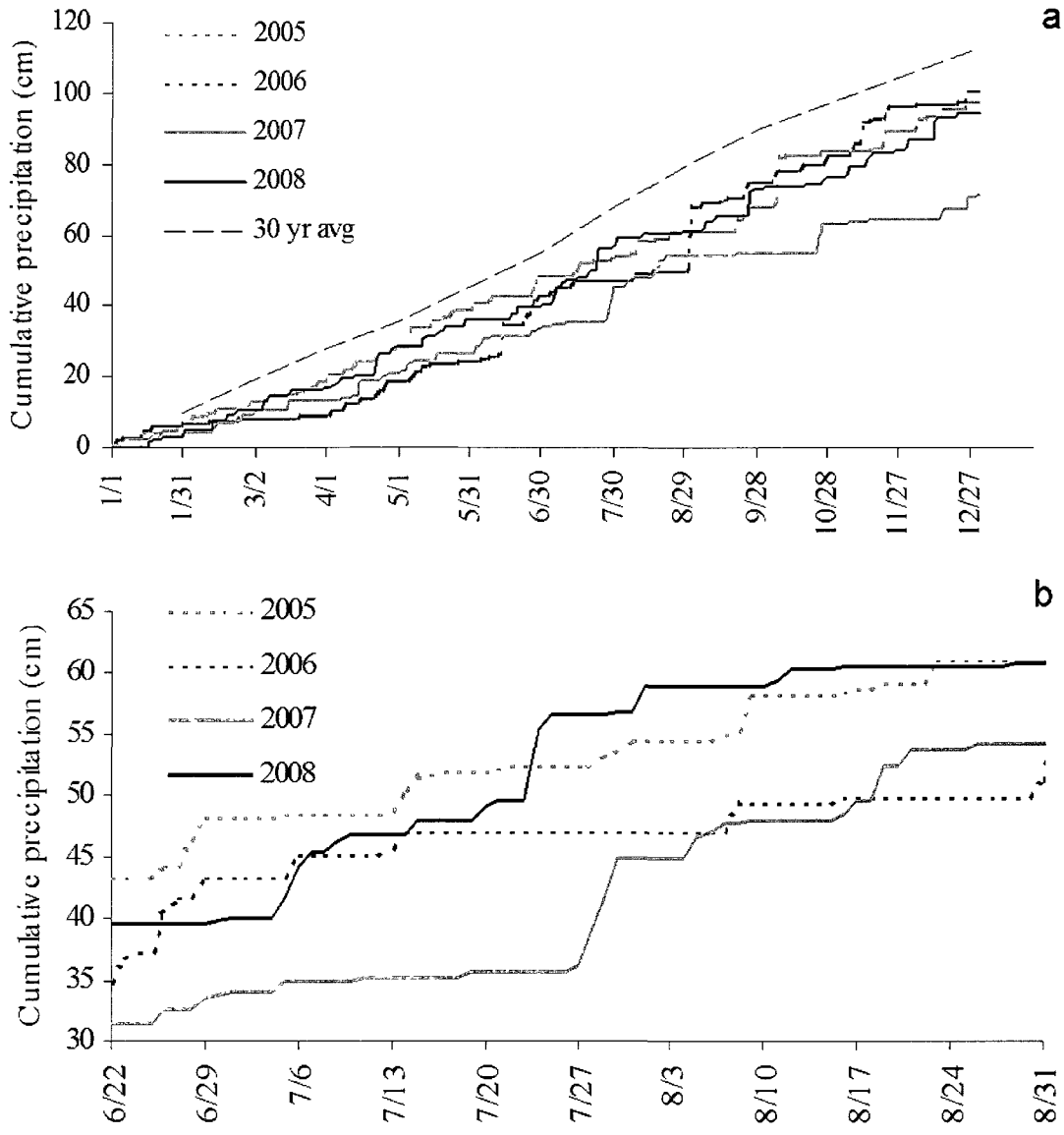


Fig. 18 **a** Yearly cumulative precipitation measured at Naval Station Norfolk Chambers Field (*KNGU*) from 2005 through 2008, and a 30-year average of precipitation for Norfolk, Virginia; **b** cumulative precipitation between June 22 and August 31 for 2005–2008 measured at KNGU

July 23–24 as the bloom formed in the Lafayette River (Figs. 14 and 18b). Precipitation events with rainfall totals of less than 5 cm occurred on July 8–10, 14, 19–20, and 31, 2008 (Figs. 15 and 18b). Decreased salinity in the upper Lafayette River was observed subsequent to these events, likely a result of runoff following these precipitation events. Daily rainfall totals measured at KPHF show rainfall events with >2 cm of precipitation occurred on August 10 and August 15, just prior to the increase in Chl *a* at the continuous monitoring stations JRCC and WP in the James River (Fig. 17a, b, and c). During 2005 and 2006, *C. polykrikoides* was present at background concentrations in the Lafayette River but never "bloomed". Rainfall in July of those years was lower than that observed during Summer, 2007 and 2008 and there were no high frequency, intense, or large rainfall events during late July or early August, the time period when blooms initiated during 2007 and 2008 (Fig. 18b).

Tidal forcing. Neap tides occurred at Sewell's Point between July 8–15, July 23–29, August 6–13, and August 22–28 (Fig. 19). Spring tides occurred from July 16–22, July 30–August 5, and August 14–21 (Fig. 19). Lunar apogee occurred during neap tides on July 13 and August 10, and lunar perigee occurred during neap tides on July 29 and August 25. The lowest tidal range during the bloom period was observed during the apogean neap tides around August 10 (Fig. 19). The residual of hourly observed tidal height minus the predicted height shows close agreement between predicted and observed tidal height, and the tidal residual was generally less than 0.2 m in magnitude between July and August (Fig. 19). The low negative residual values in early July are due to a period of high pressure that persisted from July 2–8, and the high positive residual values in late August are due to the passing of a high pressure system and the return to normal

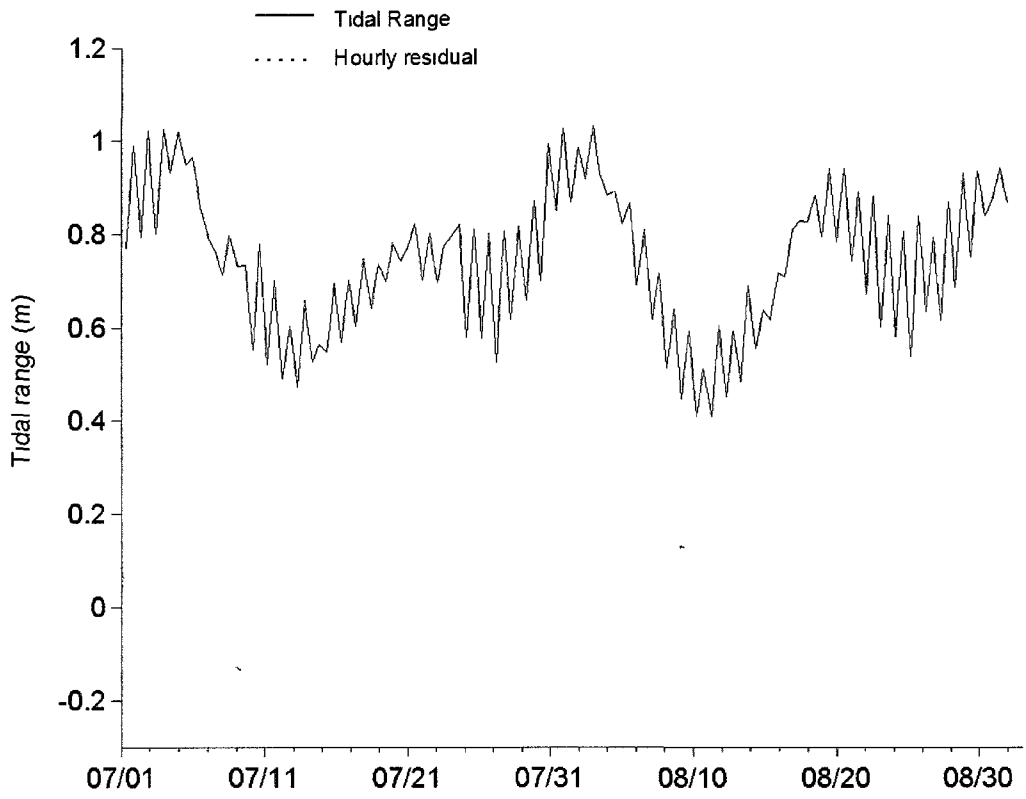


Fig. 19 The tidal range at Sewell's Point (*SP*) at the mouth of the Elizabeth River from July 1 to August 31, 2008. The *solid line* is the tidal range calculated as the difference in tidal height between consecutive high and low tides (m), and the *dashed line* is the residual of the measured hourly tidal height minus the predicted hourly height (m)

pressure, resulting in steady and strong Easterly winds for a period of several days (Figs. 17 and 19), which caused water to pile up on the western side of the Chesapeake Bay and move up into the James River and its tributaries resulting in higher than predicted tidal heights.

The initial increase in Chl *a* concentrations in the Lafayette River on July 16 coincided with a neap tide (Figs. 14 and 19). Although *C. polykrikoides* was detected, this increase in Chl *a* was due to a small bloom of an unidentified *Gymnodinium* sp. (Table 1). Decreased tidal flushing in the highly eutrophic Lafayette River during neap tides increases the residence time allowing for biomass to accumulate during this time. Heavy rainfall on July 23 during another neap tide allowed stratification to develop in this typically well-mixed system and Chl *a* in surface waters increased dramatically at station GSB (Figs. 15 and 19). The subsequent spring tide then increased tidal flushing and decreased the residence time in the upper Lafayette River facilitating transport of the bloom out of the Lafayette River and into the Elizabeth River between July 30 and August 5 (Figs. 14, 16, and 19). Transport of the bloom from the Elizabeth River into the JMSPH appeared to occur during the following neap tidal cycle from August 6 to 13 (Figs. 16 and 19).

Bloom Demise.

Climatology. Wind speed measured at KNGU was low during most of the bloom period from July 16–August 24 (data not shown). Low wind-driven mixing during summer coupled with large pulses of freshwater inputs from precipitation contributed to increased estuarine stratification and water column stability during this time. A high-pressure system passed through the region between August 20–24 with easterly winds

that strengthened and lasted from August 26 through August 29 (Fig. 17d, e). Water quality mapping showed that the bloom persisted through August 22 in the JMSPH (Fig. 19b), and August 26 in the ER-LAF (Fig. 18). The JMSPH cruise on August 27 was cut short due to high winds and heavy seas. The bloom persisted only along the Northern shore of the JMSMH in the lee of the wind through September 5 (Fig. 19a, Table 1). Between August 26 and September 6, 2008, the *C. polykrikoides* bloom in the lower Chesapeake Bay and its tributaries dissipated and returned to background Chl *a* concentrations (Figs. 16 and 17b).

Dissolved oxygen. As the bloom collapsed in the ER-LAF, dissolved oxygen concentrations, which had been elevated during the bloom due to high photosynthetic O_2 production by the *C. polykrikoides* during the initial growth and expansion of the bloom, began to drop to hypoxic and near anoxic levels in the surface waters of the Elizabeth and Lafayette Rivers (Fig. 20). *C. polykrikoides* cells were observed clumping together and forming dense aggregates at the end of the bloom, and these cell aggregates sank to the bottom where they formed a visible algal "blanket" of dead and dying cells several millimeters thick that covered large areas of the bottom near the shore. In the Elizabeth River dissolved oxygen concentrations at the surface were in the hypoxic range of 2 to 5 mg l^{-1} over much of the cruise track on August 26 (Fig. 20). Higher dissolved oxygen and Chl *a* concentrations were observed in the Lafayette River (Figs. 16 and 20). By September 4, dissolved oxygen concentrations in surface waters approached 1 mg l^{-1} in some areas of the Lafayette River and the Southern Branch of the Elizabeth River (Fig. 20). Dissolved oxygen concentrations remained low in the Lafayette and Elizabeth Rivers through September 11, 2008. Fish kills (mostly American gizzard shad,

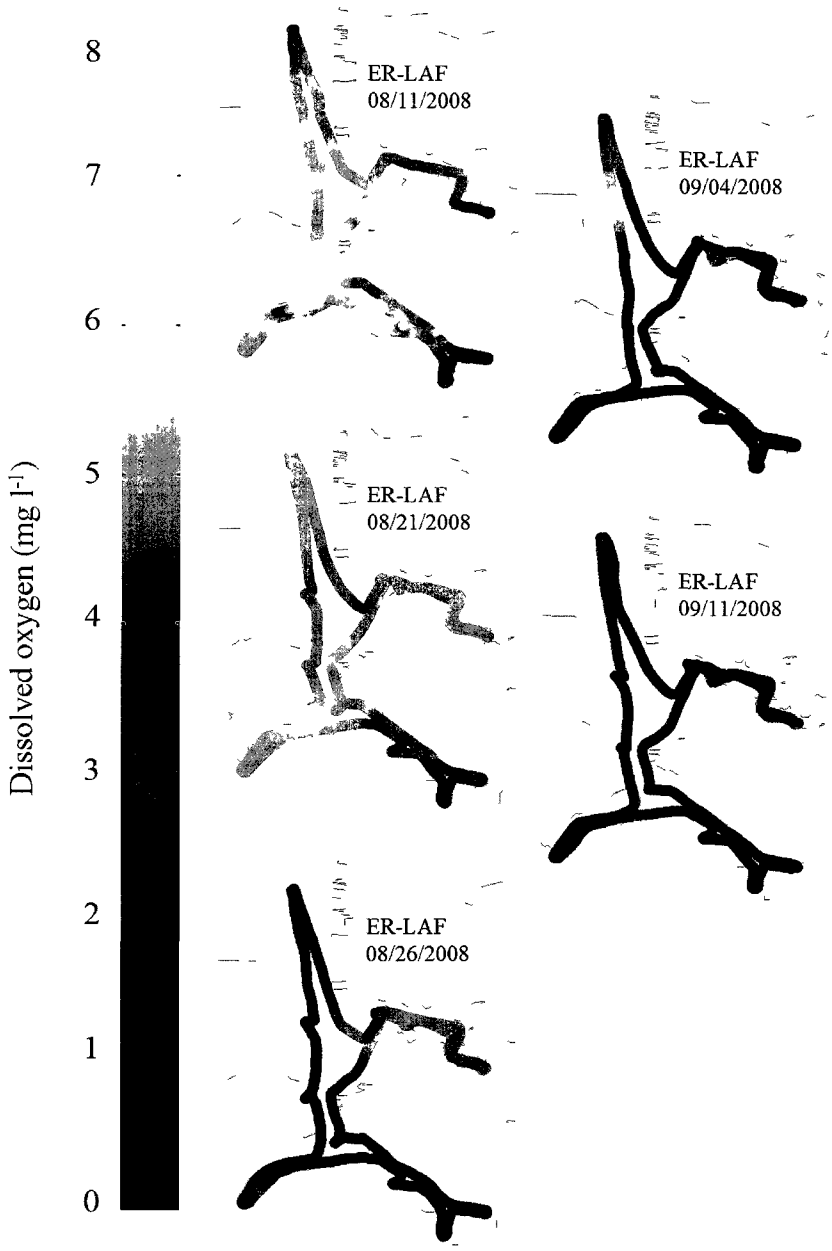


Fig. 20 Surface dissolved oxygen (*DO*) concentrations in the ER-LAF on August 11, 21, 26, and September 4, and 11 during the 2008 bloom. Dissolved oxygen concentrations on August 11 and 21 were typical of those observed during most of the bloom duration, with high DO concentrations associated with high algal biomass. DO concentrations decreased rapidly following the collapse of the bloom after August 26 and remained low through September 11

Dorosoma cepidianum) were reported in the Lafayette and Elizabeth Rivers from August 28–September 11 (Virginia Department of Environmental Quality, unpublished data).

Model results. The VIMS HEM-3D model simulated the release of a conservative tracer in the Lafayette River near its confluence with the Elizabeth River on August 3, 2008 from 00:00–12:00 (Fig 12, location denoted by the filled black circle). Throughout the simulation period, much of the dye remained concentrated in the Lafayette and Elizabeth Rivers (Fig. 21). Within 1 day of its release, the dye was transported into the Elizabeth River, and within 2 days the dye was present in the lower James River. Within 4 days, dye had been transported into the Hampton Flats area of the lower James River. By August 11, the dye was moving upriver along the Northern shore of the James River around Newport News Point, with higher dye concentrations observed on the bottom than at the surface (Fig. 21). Surface and bottom concentrations were similar on August 18 in the JMSPH and the dye was concentrated in the lower polyhaline portion although some dye was present along the Northern shore of the JMSMH (Fig. 21). By August 27 dye concentrations in the JMSPH had dissipated, while the spatial extent of the dye increased in the JMSMH (Fig. 21). The maximum spatial coverage of dye in the James River was observed 24 days after its initial release in the Lafayette River.

Model results computed under 2007 bloom conditions allow comparisons to be made between bloom years (Figs. 21 and 22) and to further compare model results to surface mapping results during 2007 (Fig. 12; Mulholland et al. 2009). Simulation of dye release in the Lafayette River (Fig. 12, *filled black circle*) on August 6, 2007 showed rapid movement of the dye into the James River, with exchange between the Elizabeth

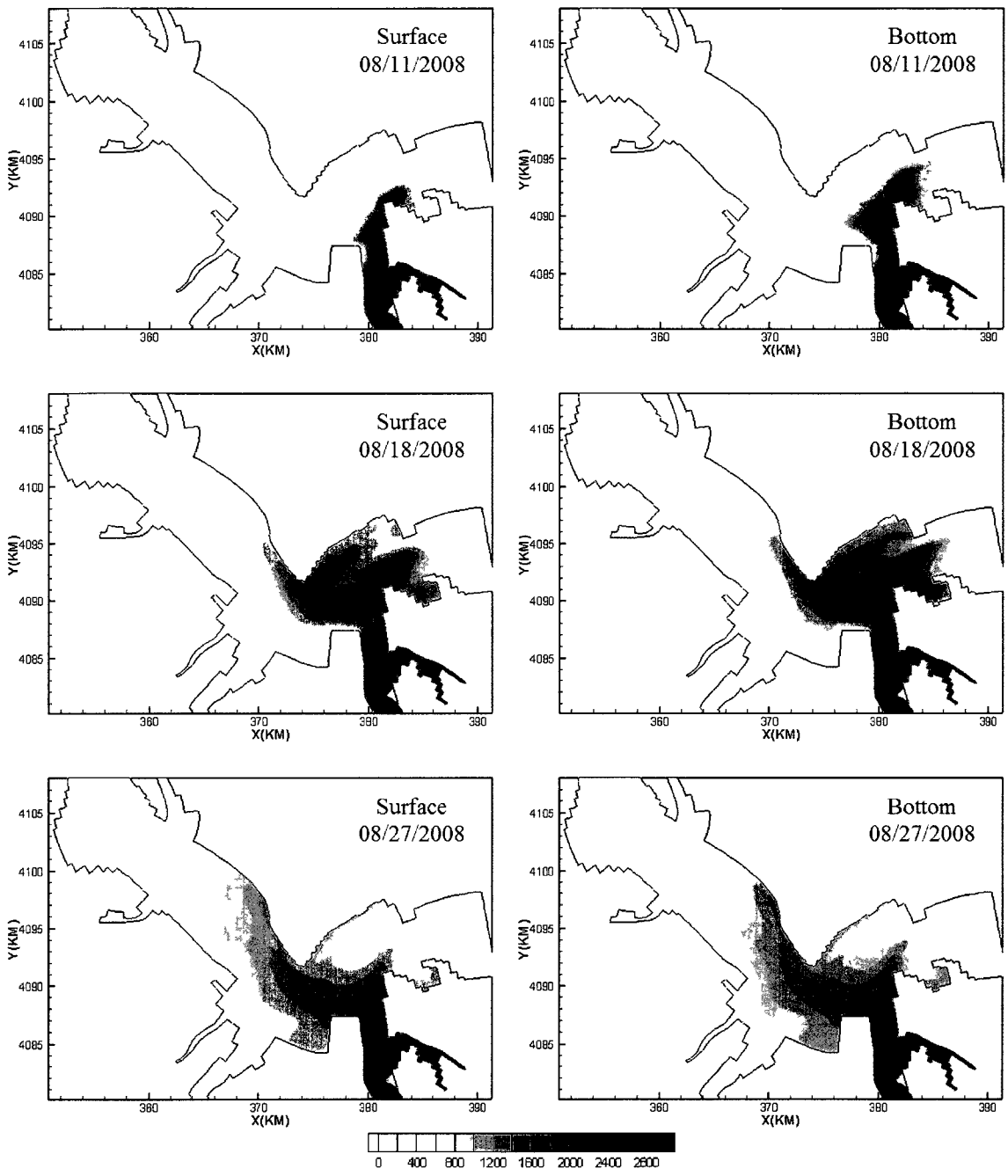


Fig. 21 VIMS HEM-3D model solutions for a dye tracer experiment run under dynamic 2008 temperature, salinity, and flow conditions observed in the James River with dye released in the Lafayette River on August 3 from 0:00 to 12:00 hours. Surface and bottom dye concentrations are shown for August 11, 18, and 27

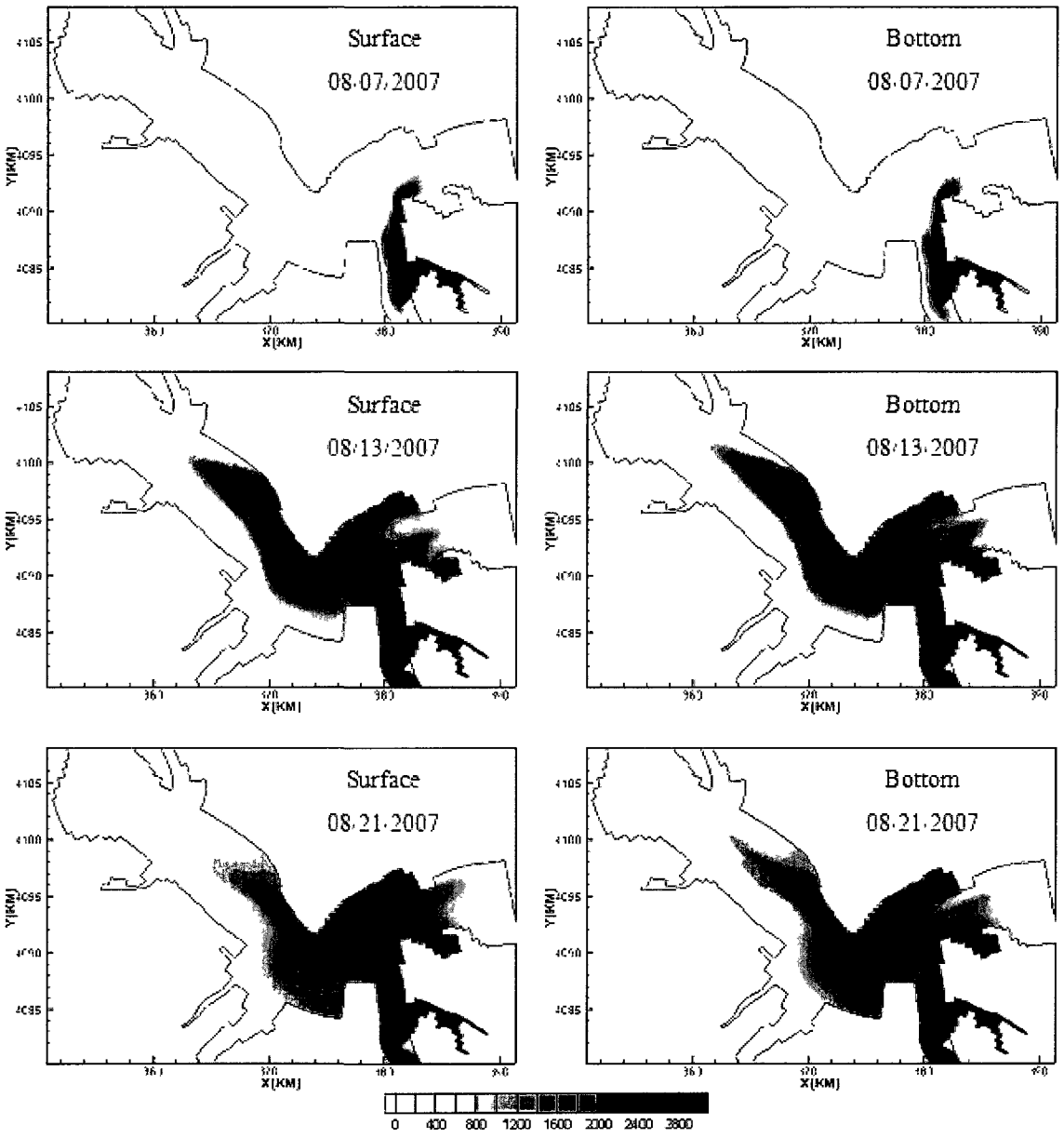


Fig. 22 VIMS HEM-3D model solutions for a dye tracer experiment run under dynamic 2007 temperature, salinity, and flow conditions observed in the James River with dye released in the Lafayette River on August 6 from 0:00 to 12:00 hours. Surface and bottom dye concentrations are shown for August 7, 13, and 21, corresponding to observed Chl *a* concentrations during the 2007 JMSPH cruises

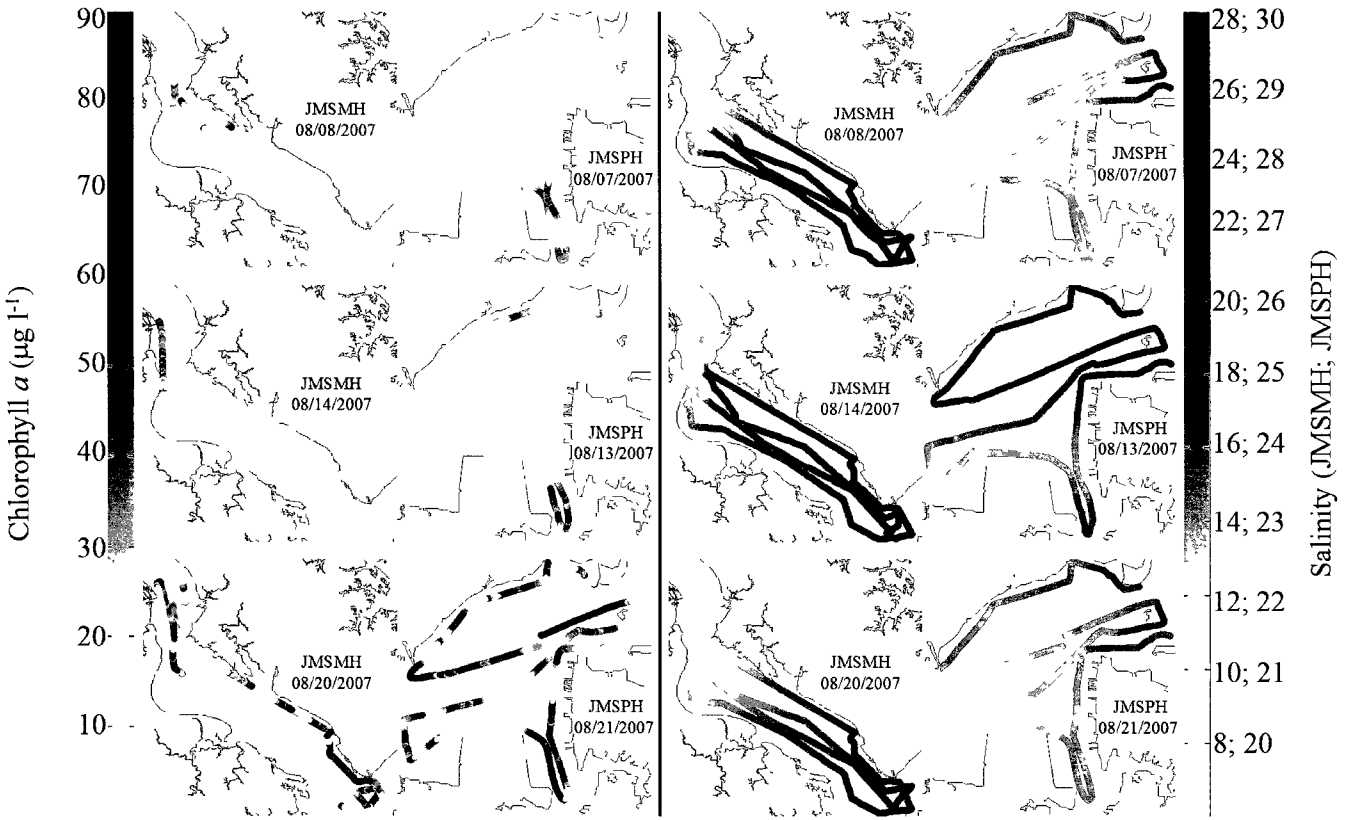


Fig. 23 Transport of the 2007 *Cochlodinium polykrikoides* bloom from the Elizabeth River into the lower James River. *Left-most panel* (*top to bottom*) shows JMSMH measured Chl *a* concentrations (µg l^{-1}) on August 8, 14, and 20, 2007. Second from the *left panel* (*top to bottom*) are JMSPH Chl *a* concentrations (µg l^{-1}) measured on August 7, 13, and 21, 2007. The *third panel* from the *left* (top to bottom) are JMSMH measured salinity for August 8, 14, and 20, 2007. The *far right panel* (*top to bottom*) are JMSPH measured salinity on August 7, 13, and 21, 2007. The salinity scale is different for JMSMH and JMSPH, as noted on the scale bar. Maps of Chl *a* distributions in the James River are redrawn from Mulholland et al. (2009)

and James River occurring over a single tidal cycle (Fig. 22). Dye was transported into the Hampton Flats area of the JMSPH 2 days after its release, and within 3 days, was present along the Northern shore of the JMSMH, upriver from Newport News Point. By August 13, the dye reached its maximum upriver intrusion in the JMSMH, near the point of the northernmost extent of *C. polykrikoides* in the JMSMH during blooms in 2007 and 2008 (Mulholland et al. 2009, Fig. 16).

Dissipation of the dye during the 2007 simulation was slow at first due to recirculation during neap tides – after five days there was very little dye lost from the system. The estimated residence time for the dye in the James River was on the order of 20–27 days, which is consistent with the freshwater travel time in this region under normal summertime flow conditions (Shen and Lin 2006). This was also the approximate duration of the *Cochlodinium* bloom in the JMSPH during 2007 (Mulholland et al. 2009), and the JMSMH in 2008 (Fig. 16).

During both 2007 and 2008, there was counterclockwise eddy circulation in the James River at Hampton Flats. In the 2008 simulation, the result of this circulation is evident as higher dye concentrations along the Northern shoreline at Hampton Flats on August 11 and 18, relative to the channel (Fig. 21). In the 2007 simulation, the model results do not readily show this pattern on the dates shown in Figure 22. However, instantaneous current velocities output at 30-minute intervals for the James River on August 7, 2007 clearly show the formation of the counterclockwise-flowing eddy around Hampton Flats (Fig. 24). The tidal control over the formation of this eddy is also apparent as the eddy develops between the waning ebb tide (Fig. 24, *13:00*) and the early flood tide (Fig. 24, *14:00*). The residual surface currents for August 7, 2007, averaged

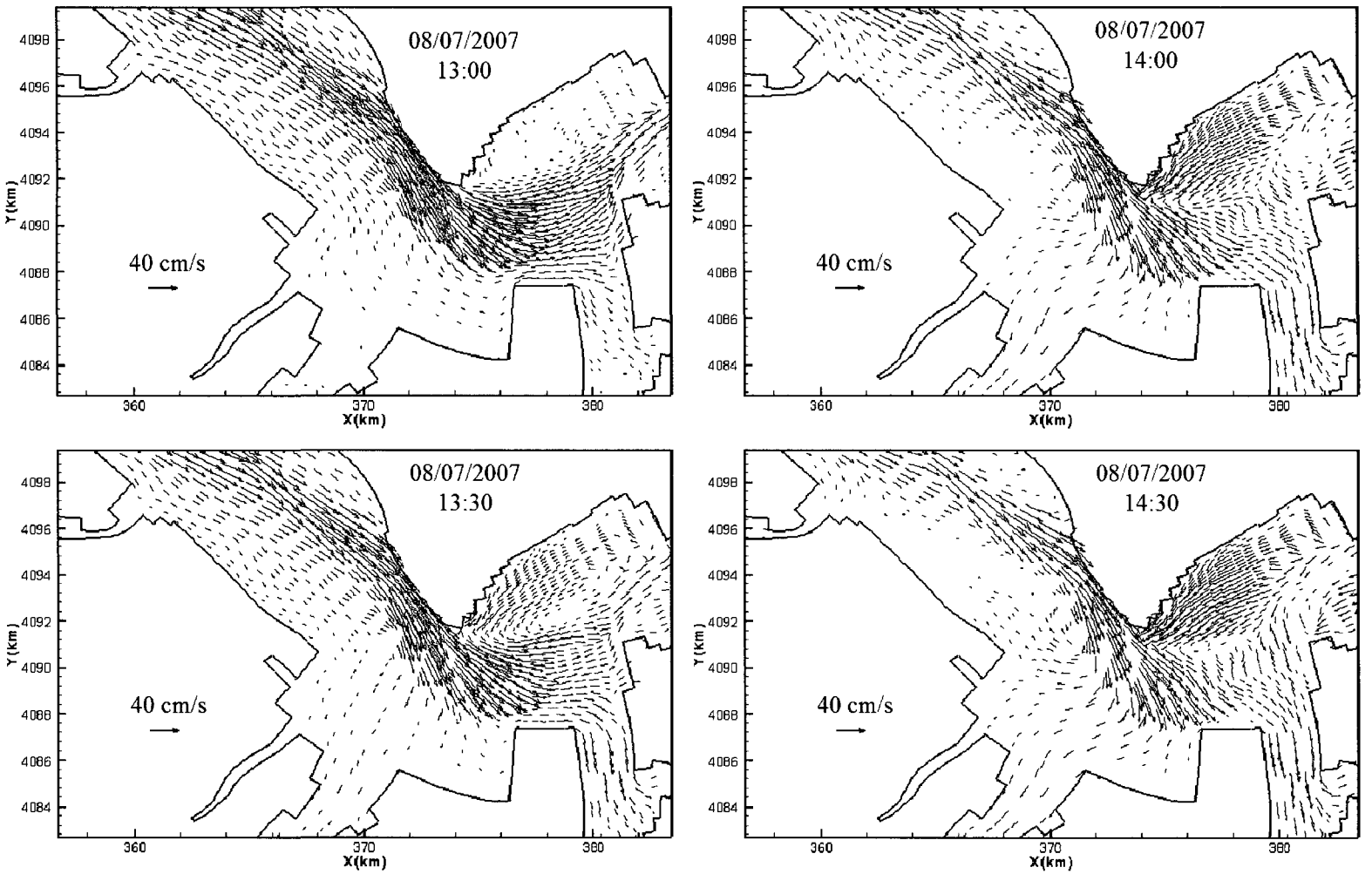


Fig. 24 Model solutions of instantaneous surface currents for the 2007 dye release experiment showing the development of eddy circulation around Hampton Flats on August 7. Eddy circulation develops between the waning ebb and early flood tides (13:00–14:00)

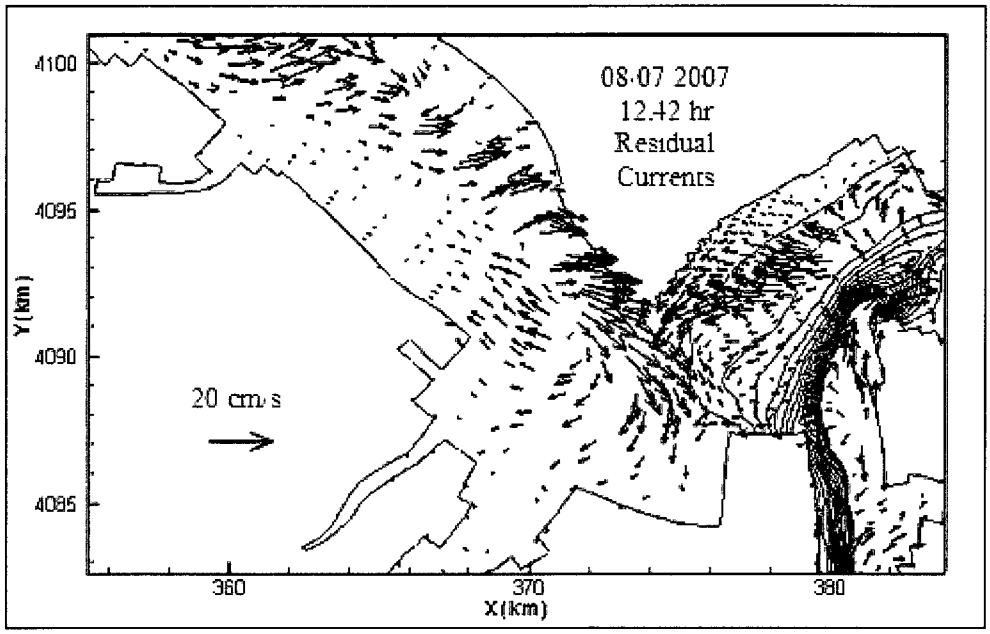


Fig. 25 Surface residual currents in the James River calculated from model results averaged over 12.42 hours on August 7, 2007 showing upriver transport at Hampton Flats toward Newport News Point. Dye concentrations are shown as *contour lines*

over 12.42 hours to remove the tidal influences, also show the influence of the eddy circulation, as the direction of net velocity in the James River at Hampton Flats is upriver (Fig. 25), and the direction of net velocity for the rest of the James River is downstream. However, the residual surface currents are highly susceptible to wind velocity and duration, and consequently other dates do not clearly show the eddy circulation, rather they mirror wind direction (data not shown).

DISCUSSION

During July and August 2007 and 2008, massive blooms of the harmful alga *Cochlodinium polykrikoides* were observed in the lower Chesapeake Bay and its tributaries. These blooms appeared to initiate in the Lafayette and Elizabeth Rivers and were transported by tidal flushing into the James River. In addition to typical downstream transport, a tidally controlled circulation feature resulted in transport of the bloom upriver in 2007, increasing its spatial distribution in the James River. While transport from the Elizabeth River was also important for the distribution of the bloom in the JMSPH in 2008, in-situ growth of *C. polykrikoides* in the JMSMH likely contributed to the initial spatial distribution of the bloom.

C. polykrikoides is a regular component of the phytoplankton community in the lower Chesapeake Bay region. The first documented report of a bloom was at the mouth of the York River in 1968 (Mackiernan 1968). *C. polykrikoides* forms resting cysts (Kim et al. 2007), and consequently regions into which blooms of *C. polykrikoides* are transported can potentially become seedbeds for future blooms. During a 1996 study, *Cochlodinium* cysts were the second most predominant dinocyst present in JMSPH and

JMSMH sediments, and cyst abundance increased near the mouth of the Elizabeth River (Seaborn and Marshall 2008), demonstrating that this region harbors seed populations of this organism. Because of the paucity of data regarding the distribution of *C. polykrikoides* cysts, and the lack of information regarding conditions promoting cyst germination, it is difficult to determine how the abundance and distribution of cysts affects blooms of this organism. Further, the current distribution of *C. polykrikoides* cysts in the James River and its tributaries is unknown because data on cyst distribution are limited to the 1996 survey, and sediments in the Lafayette and Elizabeth Rivers were not sampled. Since 1996, *C. polykrikoides* has bloomed almost annually in the Lafayette and Elizabeth Rivers (Mulholland et al. 2009; Marshall, unpublished data; Mulholland et al., unpublished data), and so it is likely that cysts have accumulated in sediments in these systems as well as the James River.

The timing of *C. polykrikoides* bloom formation in the Lafayette River appears to be controlled by a combination of spring-neap modulation, and the timing of precipitation events. The 2008 *C. polykrikoides* bloom initiated in the Lafayette River during a neap tide, when tidal straining and vertical mixing are lowest, and following a period of intense precipitation that resulted in heightened stratification. Similarly, in 2007, the bloom formed during a neap tide that followed an intense precipitation event (Mulholland et al. 2009). During and after rainfall events, stratification increases and runoff-associated nutrient inputs are likely high. These conditions favor the rapid growth of dinoflagellates (Margalef 1978; Sellner et al. 2001). The Lafayette River watershed is highly urbanized, draining much of Norfolk, Virginia. Its watershed is comprised of 46% impervious surfaces (McKee 2009), which can result in high overland runoff and nutrient

loading following intense rain events (Morse et al. in preparation, Egerton et al., in preparation, Glibert et al. 2008).

VIMS HEM-3D model predictions for the James River (Fig. 24; Shen et al. 1999) and field observations (Kuo et al. 1990) provide evidence for the tidally driven development of a counter-clockwise flowing eddy around Hampton Flats, which regularly develops between late ebb and early flood tide (Fig. 24; Shen et al. 1999). A strong tidal front also develops off Newport News Point on flood tides. The formation of the tidal front and the intensity of the counterclockwise flowing eddy around Hampton Flats are both enhanced during neap tides as a result of reduced tidal straining and increased stratification (Shen et al. 1999). Eddy flow from Hampton Flats can inject particles, including plankton, into the area of the strong tidal front centered around Newport News Point, to the west of Hampton Flats (Kuo et al., 1990, Shen et al. 1999). Saltier, denser water from Hampton Flats submerges below the fresher, less dense water at the frontal boundary and the particles suspended in the water from Hampton Flats become entrained in bottom water, where they are transported up-estuary due to gravitational circulation. This mechanism may enhance the upriver transport of *Cochlodinium* cells and allow for a greater spatial expansion of blooms than what would be possible due to typical upstream transport during flood tide.

Based on model results and observed Chl *a* distributions, it is proposed that bloom initiation occurred during a neap tide in the upper branches of the Lafayette River during July, 2008, when water residence time was longer allowing algal biomass to increase prior to flushing from the system. Bloom organisms were then transported from the Lafayette into the Elizabeth River during the subsequent spring tide as a result of

enhanced tidal flushing. The transport of bloom organisms from the Elizabeth River into the Hampton Flats area of the James River most likely happened quickly, perhaps over just one or two tidal cycles because of the proximity of the mouth of the Elizabeth River to this area of the James River (Fig. 16). Transport of the bloom from Hampton Flats upriver into the Newport News Point region could have been completed over one tidal cycle, as the distance between the two features is less than one typical tidal excursion in this area. Further upriver transport associated with deep-water injection of the bloom organism into the frontal zone off Newport News Point could have occurred on subsequent tidal cycles, and the rate of transport by this process would likely be enhanced during neap tides (Shen et al. 1999), and upriver transport could continue through the neap tidal cycle.

The duration of the *C. polykrikoides* blooms in the lower James River estuary was similar in both 2007 and 2008, lasting from early August through the first week of September during both years. However, in 2008, evidence is shown of a bloom initiating in the Lafayette River in late July, a full two weeks before the bloom entered the James River. Based on our observations and model results, it is likely that the 2007 bloom also initiated in the Lafayette River prior to its appearance at the mouth of the Elizabeth River and in the lower James River estuary (Fig. 23; Mulholland et al. 2009). High Chl *a* concentrations near the mouth of the Lafayette River in 2007 also suggest that this sub-tributary plays a role in the initiation of blooms.

During the 2007 bloom, high Chl *a* concentrations were present during August in the upper JMSMH along the westernmost section of the southern shore (Fig. 23). It is not likely that *C. polykrikoides* was responsible for these elevated Chl *a* concentrations, as

the salinity in this region of the James River was low, between 8 and 12 during this period (Fig. 23). The salinity in the area of high Chl *a* along the northern shoreline in the upper JMSMH on August 8 was 15 (Fig. 23). *C. polykrikoides* does not grow well at salinities below 18 (Kim et al. 2004). The salinities reported in the literature for water containing *C. polykrikoides* ranged from 22–30 during blooms in Long Island, New York between 2002 and 2006 (Gobler et al. 2008), 18.9–27.9 in the James River and Chesapeake Bay during 2007 (Mulholland et al. 2009), and 19–30 in Pettaquamscutt Cove in Rhode Island during blooms between 1980 and 1981, with the highest *C. polykrikoides* abundance occurring in the salinity range of 25–30 (Tomas and Smayda 2008). During the 2008 bloom in the James River (this study), *C. polykrikoides* was observed in the upper JMSMH on September 2 at a salinity of 18.3 (Fig. 13, Table 1), and at station 18 on August 20 (Table 1). Salinity was not recorded for the sample from station 18, however the salinity at that location ranged from 18 during the JMSMH cruise on August 18, to 15 during the cruise on August 25 (data not shown). During the 2008 JMSMH cruises, the salinity at the northernmost patch of high Chl *a* measured during the bloom (Fig. 16) was 18 on August 13, 19 on August 18, and 16 on August 25 (data not shown).

The model results for dye release in the Elizabeth River in 2007 show a rapid spread of the conservative tracer from the Elizabeth River into the area of Hampton Flats followed by both upriver and downriver transport (Fig. 22). High salinity in the area of Hampton Flats coincided with the high Chl *a* (Fig. 23) suggesting that the regularly occurring counterclockwise flowing eddy was present and eddy injection into the tidal front could have contributed to the upriver transport of *C. polykrikoides*. The distribution

of the dye in the model simulation closely matched the observed *C. polykrikoides* bloom distribution (Figs. 22 and 23; Mulholland et al. 2009). Additionally, model results of instantaneous current velocity show the development of the eddy circulation at Hampton Flats during 2007 (Fig. 24), and the residual currents show that the direction of net transport was upstream at Hampton Flats on August 7 (Fig. 25). The residual currents for the 2008 simulation more closely approximated wind driven flows at the surface, suggesting that eddy circulation and tidal front injection at Newport News Point may not have played as large a role in the transport of algae during the 2008 bloom.

Although the blooms were similar in extent and duration during 2007 and 2008, model results for 2008 showed less upriver transport of the dye as compared to the 2007 simulation. While the 2008 simulation still approximated the observed distribution of Chl *a* in the James and Elizabeth Rivers, there was a decreased range of dye transport in the 2008 simulation relative to the observed Chl *a* distribution in the JMSMH (Figs. 16 and 21). One reason for the observed difference in upriver transport of the dye between bloom years may be due to differences in James River flow. The mean recorded flow between August 1 and 27 for the James River at USGS station 02037500 near Richmond, VA, was 1389 cfs during 2007, but only 629 cfs during 2008 (http://waterdata.usgs.gov/usa/nwis/uv?02037500). The higher flow during August 2007 would increase stratification in the lower James River, increase the intensity of the tidal front at Newport News Point, and increase eddy circulation around Hampton Flats, resulting in increased upriver transport (Shen et al. 1999). Model results of 2007 dynamic conditions using 2008 James River flow conditions and flow-equilibrated initial salinity conditions indeed show that the upriver transport of dye under low flow

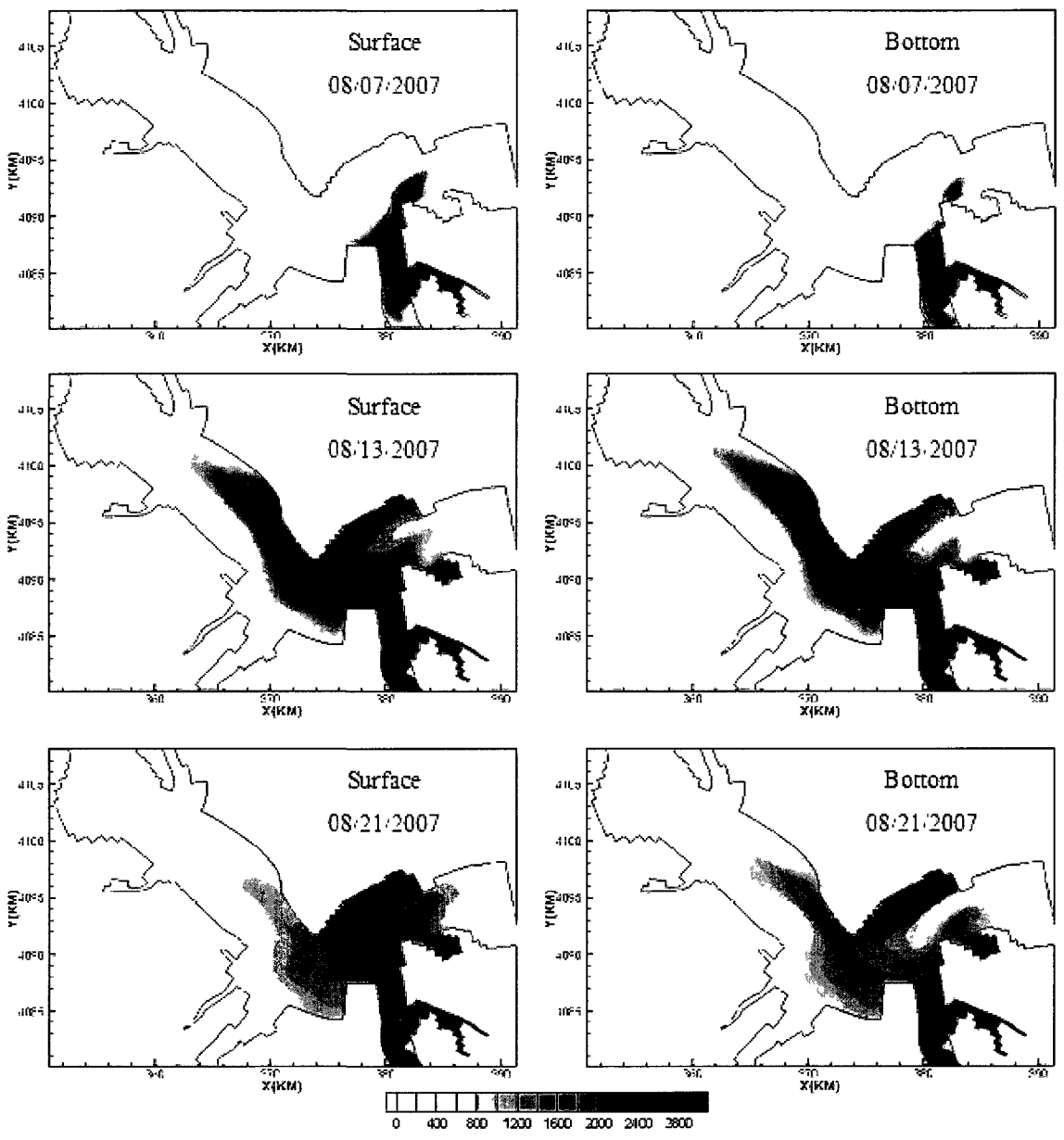


Fig. 26 Model solutions for a dye tracer experiment run under dynamic 2007 temperature and atmospheric conditions, but using 2008 James River flow (629 cfs) and low-flow equilibrated initial salinity conditions in the James River. Dye was released in the Lafayette River on August 6 from 0:00 to 12:00 hours and surface and bottom dye concentrations are shown for August 7, 13, and 21

conditions is decreased (Fig. 26). However, only the concentration of dye at any given location was reduced under low flow conditions, and the extent of dye transport remained the same between simulations (Figs. 26 and 22). This suggests that other factors such as wind velocity and duration, and dynamic conditions in Chesapeake Bay outside of the model domain control the extent of upriver transport in the James River, whereas eddy circulation around Hampton Flats and tidal front injection can influence the magnitude of the scalar transport.

Observations showed that during 2008, the high Chl *a* concentrations and *C. polykrikoides* abundance penetrated further upriver than the dye. Data from the continuous monitoring stations at JRCC and WP in the JMSMH and JMSPH, respectively, showed that the bloom was first observed in the JMSMH in 2008 (Fig. 17b), suggesting that there may have been another site of bloom initiation that contributed to the observed bloom distribution in the mesohaline portion of the estuary. Since *C. polykrikoides* was present along the northern shoreline of the JMSMH during much of the 2007 bloom (Mulholland et al. 2009), and since *C. polykrikoides* produces cysts, it is likely that cyst deposits in the JMSMH provided the seed populations in this segment of the estuary during 2008. As the bloom dissipated during 2008, it was observed that *C. polykrikoides* cells aggregated and blanketed the bottom. While the triggers for encystment and excystment for this species are still poorly understood, hyaline cysts of *C. polykrikoides* were observed to form from motile unarmored planktonic cells, and these hyaline cysts subsequently regenerated into motile cells after up to 6 months of storage in the dark at 4°C (Kim et al. 2002), suggesting that *C. polykrikoides* form hyaline cysts as an over wintering strategy. Resting cysts produced from armored motile

cells were observed in culture under laboratory conditions, and were also found in Korean coastal sediments near areas where blooms occur (Kim et al. 2007). Like the Lafayette River, the Northern shoreline of the JMSMH upriver from Newport News Point is a shallow, sheltered environment, with multiple riverine sources of nutrients. And similar to the Lafayette River bloom initiation, the timing of *C. polykrikoides* bloom formation in the JMSMH occurred during a period of neap tides (Figs. 17b and 19) following a precipitation event (Fig. 17c).

Based on the dye study, the flushing time of a conservative tracer in the lower James River under typical summertime flow condition is about 20–27 days, a figure consistent with other published values (Shen and Lin 2006). This flushing time corresponds well with the timescale of bloom dispersal observed during the *C. polykrikoides* bloom in the lower James River during 2008 (Fig. 16). The primary controls on dye removal are spring-neap modulation and tidal flushing. During spring tides, upriver transport of particles and recirculation via eddy injecting ceases, flushing is enhanced, and the tidal front at Newport News Point is drastically reduced (Shen et al. 1999, Shen and Lin 2006). This may contribute to the demise and flushing of blooms from the lower James River, particularly when combined with wind-driven mixing and destratification that would make conditions unfavorable for dinoflagellate growth (Sellner et al. 2001).

Following the collapse of the bloom, dissolved oxygen in the surface waters reached hypoxic and near-anoxic conditions in much of the Lafayette and Elizabeth Rivers (Fig. 20) resulting in extensive fish kills. It is likely there were other negative impacts from this bloom as well. *C. polykrikoides* blooms are known to negatively affect

grazer populations. *C. polykrikoides* exerts negative effects on the survivorship, feeding rates, egg production and egg hatching success of the copepod *Acartia tonsa* (Jiang et al. 2009), and under bloom concentrations can cause the mortality of juvenile fish, American oysters, *Crassostrea virginica*, (Gobler et al. 2008; Mulholland et al. 2009) and bay scallops, *Argopecten irradians*, (Gobler et al. 2008). Additionally, cell contact with *C. polykrikoides* resulted in decreased growth rates and elicited the formation of abnormal cell morphology in the dinoflagellate, *Akashiwo sanguinea*, (Yamasaki et al. 2007). Alleviating cellular competition for resources via allelopathic interactions with other phytoplankton (Tang and Gobler 2010; Kubanek et al. 2005) and relieving top down control by grazers (Gobler et al. 2008) may give *C. polykrikoides* a competitive advantage over co-occurring taxa and explain why *C. polykrikoides* is able to form near-monospecific blooms in the Chesapeake Bay region that can persist for up to six weeks when physical conditions are favorable for bloom initiation. *C. polykrikoides* has a very nutritionally flexible metabolism and can take up all inorganic N forms, as well as organic forms of N (Mulholland et al. 2009). Additionally, *C. polykrikoides* may supplement its C and N nutrition via mixotrophy. When fed cryptophyte prey, *C. polykrikoides* cultures exhibited maximum growth rates that were nearly double those of cultures grown strictly phototrophically (Jeong et al. 2004).

While similar in both timing and broad spatial extent, the model results for the spread of a conservative tracer in 2007 and 2008 do not replicate the observed patchy distribution of Chl *a* in the lower James River, particularly as the *C. polykrikoides* bloom increased to its maximum spatial extent (Figs. 16, 21, 22 and 23). The primary reason for the discrepancies between the observed Chl *a* and the predicted dye concentrations is that

the model does not account for biology, the growth and death of cells as well as their behavior. Factors such as light and nutrient availability are also not accounted for in the model. Unlike the dye, the distribution of *C. polykrikoides* is inherently patchy, partly due to small-scale circulation, but also due to vertical migration patterns that serve to concentrate or dilute the population at the surface. *C. polykrikoides* is capable of relatively rapid vertical migration with swimming velocities ranging from 1.3 to 4 m h^{-1} (Park et al. 2001). The timing of the vertical migration appears to be tied to the light cycle, however, during a bloom in Korea in 1996, *C. polykrikoides* cells began to migrate toward the surface before sunrise, and returned to depth before sunset (Park et al. 2001). Park et al. (2001) also observed populations of *C. polykrikoides* descending in the water column at 14:00 hours, concomitant with maximal irradiance. In the present study, the DATAFLOW cruises commenced in the morning and returned by early afternoon, and it is likely that abundance of *C. polykrikoides* was variable over that time within the water column, particularly in the lower James River, where the depth range is greatest.

The Lafayette River appears to act as a seedbed for *Cochlodinium* blooms in the lower Chesapeake Bay region. Once conditions are favorable for bloom initiation there, physical forcing controls the spread and transport of the bloom into surrounding waters. Controlling blooms in the Lafayette River or other sites of initiation in Bay tributaries and sub-tributaries may minimize impacts to the James River and Chesapeake Bay. While *C. polykrikoides* blooms now appear to be a regular occurrence in the lower Chesapeake Bay region, the absolute mode of bloom initiation and transport may differ between years. No single environmental variable can account for the growth and transport of bloom organisms, rather a synergistic combination of physical, chemical,

biological, and meteorological factors including periods of intense localized rainfall and runoff, spring-neap tidal forcing, calm winds, and complex estuarine circulation patterns, appear to produce conditions favorable for the growth and transport of *Cochlodinium polykrikoides* in the lower Chesapeake Bay and its tributaries during the summer.

CHAPTER 4

CONTROLS ON THE FORMATION OF BLOOMS OF THE DINOFLAGELLATE *COCHLODINIUM POLYKRIKOIDES* MARGALEF IN LOWER CHESAPEAKE BAY AND ITS TRIBUTARIES

INTRODUCTION

Massive blooms of the harmful alga *Cochlodinium polykrikoides* Margalef occurred in the lower Chesapeake Bay and its tributaries during the summers of 2007 (Mulholland et a. 2009), 2008 (Chapter 3), and 2009 (This Chapter). Maps of chlorophyll *a* (Chl *a*) constructed from underway sampling (DATAFLOW) during cruises in 2007 suggested that the Lafayette River was the initiation ground for that bloom (Mulholland et al. 2009). Subsequently in 2008, the cruise tracks were expanded to include portions of the Elizabeth and Lafayette Rivers in order to identity areas where *C. polykrikoides* bloom initiation was likely occurring (Fig. 27). The underway sampling was supplemented with a 3-dimensional hydrodynamic model in order to understand the transport of the bloom (Chapter 3). In addition to the Lafayette River, in 2008 there were also localized sites of initiation and growth of populations within the mesohaline portion of the James River and bloom initiation appeared to be correlated with intense, highly localized rainfall events during neap tides (Chapter 3). Spring tides increased the tidal flushing and transport of *C. polykrikoides* from the Lafayette and Elizabeth Rivers into the lower James River where it was transported upriver by local estuarine circulation (Chapter 3). Approximately 30 days after the bloom formed, the bloom

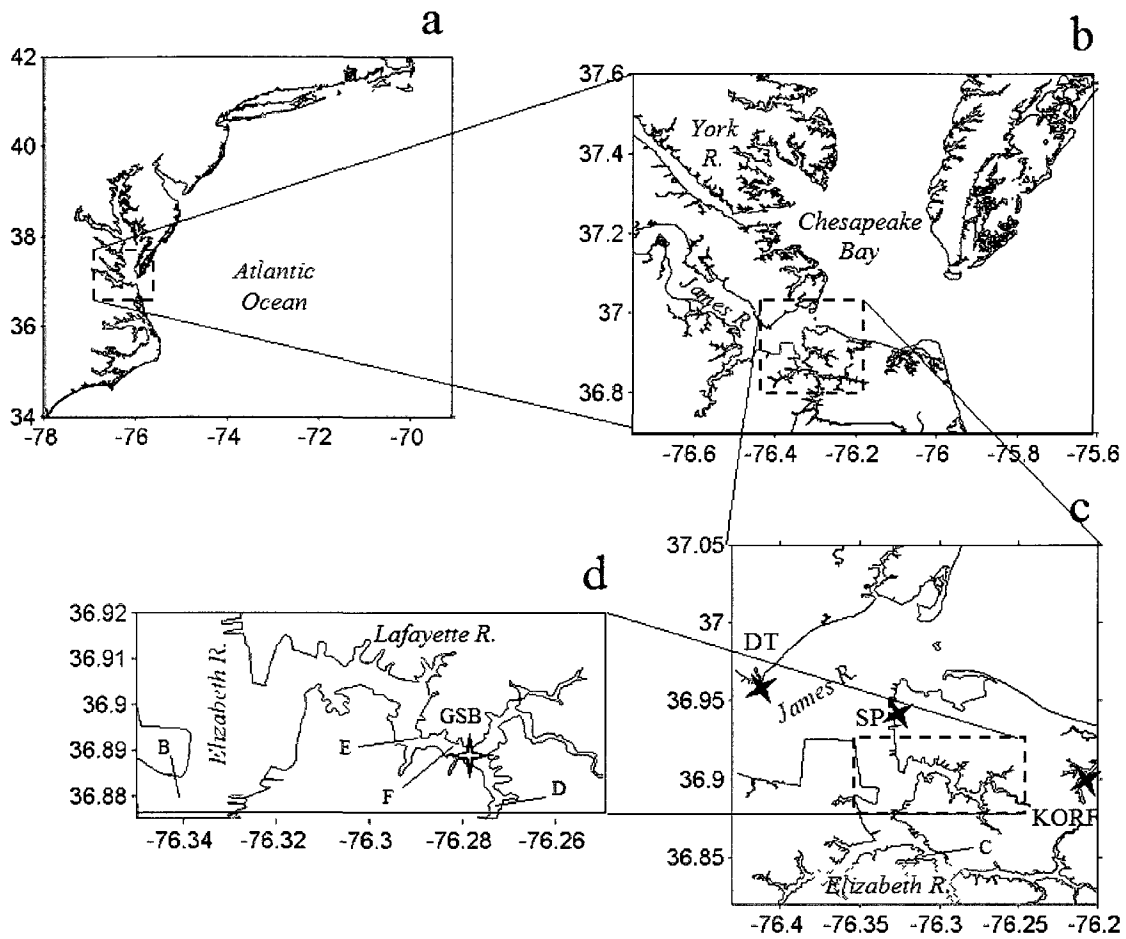


Fig. 27 **a** The Mid-Atlantic region of the United States; **b** the lower Chesapeake Bay area showing the York and James Rivers; **c** the lower James River at the confluence of the Chesapeake Bay, the Elizabeth River basin showing the location of Norfolk International Airport (*KORF*), NOAA PORTS stations at Dominion Terminal (*DT*) and Sewell's Point (*SP*) as *black crosses*, and the location of underway phytoplankton sampling station *C*, and **d** the Lafayette River and its confluence with the Elizabeth River showing the location of the fixed sampling station at the Granby Street Bridge (*GSB*) as a *white plus sign*, and the underway phytoplankton sampling stations *B, D, E,* and *F*

dissipated in the James River in response to increased wind-driven mixing associated with frontal systems moving through the region. A combination of physical factors including, seasonal rainfall patterns, increased stratification, nutrient loading, spring-neap tidal modulation, and complex estuarine mixing and circulation allowed *C. polykrikoides* to spread and form massive blooms over large portions of the tidal James River and lower Chesapeake Bay.

In order to better understand phytoplankton bloom dynamics at the ecosystem level, it is necessary to sample at timescales relevant to the growth of phytoplankton populations and on spatial scales relevant to the distribution of phytoplankton ranging from the micro-scale to the mesoscale levels (Smayda 1998). It is important to understand both in situ bloom development, the balance of growth and loss terms on a local scale (Lucas et al. 1999b), and the transport related mechanisms that act to concentrate, diminish, or just redistribute phytoplankton biomass (Lucas et al. 1999a). The balance between phytoplankton growth and loss at a local scale determines the likelihood of a phytoplankton population forming a bloom (Lucas et al. 1999b), and the transport mechanisms ultimately control when, where, and how a bloom will manifest at the mesoscale level (Lucas et al. 1999a). Using a three-dimensional hydrodynamic model, it was determined that a combination of eddy circulation and injection of the bloom into a tidal front in the James River was likely responsible for producing the observed distribution of a *Cochlodinium* bloom in the lower Chesapeake Bay during 2007, and to a lesser extent, during 2008 (Chapter 3). While the processes involved in transporting the bloom have been identified, the factors controlling the timing and location of *C. polykrikoides* bloom initiation in the Lafayette River remain elusive.

Nutrient inputs have been considered to be major drivers of eutrophication and bloom formation (Heisler et al. 2008), and *C. polykrikoides* was able to take up all inorganic and organic N substrates tested during 2007 (Mulholland et al. 2009), however the ambient dissolved nutrient concentrations measured during these uptake experiments were often low compared to the high algal biomass (0.8–2.4 μmol l^{-1} dissolved inorganic N; 680–11,137 *C. polykrikoides* cells ml^{-1}) and so appear to be insufficient for supporting further growth of the *C. polykrikoides* population. However, because N uptake was assessed only after the bloom was already well established, the nutrient controls on bloom formation could not be ascertained.

In 2009, the cruise track in the Lafayette River was expanded farther upriver in order to identify areas prone to bloom formation and the augmented weekly spatial mapping observations were enhanced with daily sampling to assess nutrient concentrations and phytoplankton abundance at a fixed site. Daily sampling began in June, prior to the time when blooms were likely to initiate in order to capture bloom initiation. In order to assess variability at the sub-tidal level, a YSI sonde was installed on a pier near the area where bloom initiation appeared to occur during 2008, and Chl *a*, salinity, turbidity and temperature were recorded at 6-minute intervals.

METHODS

Surface water mapping. The Hampton Roads Sanitation District (HRSD) collects underway-sampling data (DATAFLOW; http://www2.vims.edu/vecos) during weekly cruises in the lower James and Elizabeth Rivers from March through September. The James River cruise segments are partitioned into mesohaline (Fig. 28, *JMSMH*) and

polyhaline (Fig. 28, *JMSPH*) portions of the James River that are sampled on two consecutive days. The Elizabeth River cruise segment (Fig. 28, *ER-LAF*) is completed in one day. Because the 2008 *C. polykrikoides* bloom initiated in the upper Lafayette River (Chapter 3), cruises were expanded during 2009 to include as much of the upper river as possible using small boats. During cruises, water was pumped continuously from 0.5 m depth into a flow through cell equipped with an YSI 6600 multiparameter datasonde. Temperature, salinity, pH, dissolved oxygen, turbidity and fluorescence were measured continuously and recorded at 0.25 Hz along the cruise track, and spatial and temporal data were related geospatially via the global positioning system (GPS). Underway fluorescence was calibrated to Chl *a* with discrete samples taken at 5 stations during each cruise, and calibration data were pooled separately for the James and Elizabeth Rivers. After undergoing a quality assurance and control check, the finalized datasets were mapped in Matlab to give a spatial representation of Chl *a* in surface waters (Morse et al. 2011).

Meteorological and tidal data. Atmospheric pressure, water temperature, tidal height, and tidal predictions for the Elizabeth River were obtained from the National Oceanic and Atmospheric Administration's Physical Oceanography Real Time System (NOAA PORTS) station at Sewell's Point (SP), located on the Elizabeth River in Norfolk, VA (Fig. 27c, *SP*). Wind speed and direction data were obtained from NOAA PORTS Dominion Terminal (DT) station (Fig. 27c, *DT*) on the James River, and daily precipitation data were obtained from Norfolk International Airport (KORF) (Fig. 27c, *KORF*).

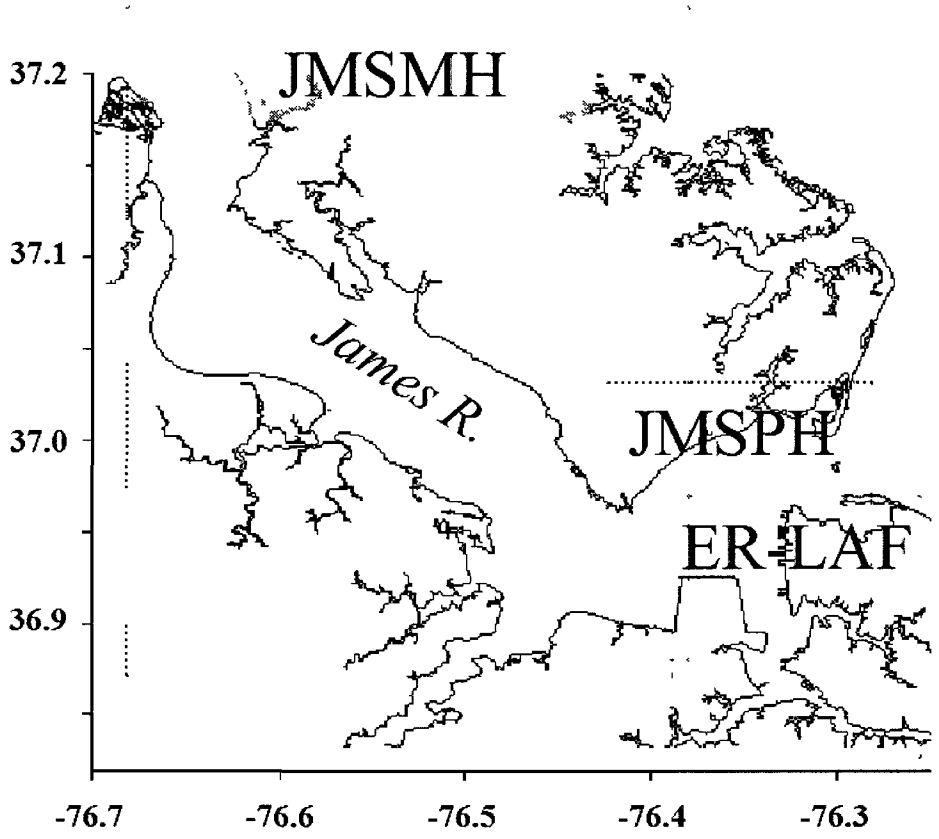


Fig. 28 Map projections showing the partitioning of the DATAFLOW cruises in the mesohaline (*JMSMH*) and polyhaline (*JMSPH*) portions of the James River, and in the Elizabeth and Lafayette Rivers (*ER-LAF*)

Phytoplankton. Phytoplankton samples were collected during DATAFLOW cruises in the ER-LAF and preserved in order to identify the dominant taxa via microscopy. The collection of phytoplankton samples was based on underway Chl *a* concentrations. During cruises between May and June 23, when Chl *a* concentrations exceeded 25 $\mu g\ l^{-1}$ whole water samples were collected from the flow-through system after it had passed through the YSI datasonde chamber. Additionally, during the cruise on July 9, phytoplankton samples were collected at lower Chl *a* concentrations in the Elizabeth River near its confluence with the JMSPH in order to determine presence of *C. polykrikoides* and the potential timing of transport of the bloom organism from the Elizabeth River into the James River. Samples were collected in sterile 50-ml centrifuge tubes and preserved with non-acidified Lugol's iodine solution. Aliquots of preserved sample (200 µl) were settled in NUNC labtek eight-well chambered coverglass slides and then enumerated on a Zeiss Axiovert 40 CFL inverted microscope at 200x magnification. The entire field was counted for each sample, and the major taxa and the dominant dinoflagellate species were enumerated.

Fixed station daily sampling. In order to determine how nutrient concentrations and phytoplankton abundance changed in relation to *C. polykrikoides* abundance during bloom formation at a fixed station, I sampled the Lafayette River from the Granby Street Bridge (GSB) (Fig. 27d, *GSB*) on a daily basis from June 6–July 18, 2009. Using a peristaltic pump and acid-cleaned teflon tubing, whole water samples were collected and preserved in sterile 50-ml centrifuge tubes using non-acidified Lugol's solution for microscopic enumeration of phytoplankton. Nutrient samples were collected by filtering whole water through a Pall cartridge filter (0.2 µm pore-size) into acid-cleaned high-

density polyethylene bottles. After returning to the laboratory less than 2 miles away, nutrient samples were frozen until analysis. Phytoplankton samples were stored in a cool dark place until counted.

Ammonium (NH_4^+) concentrations were determined using the manual phenol-hypochlorite method of Solarzano (1969). Nitrate (NO_3^-) plus nitrite (NO_2^-) (NO_x), and phosphate (PO_4^-) were determined using an Astoria Pacific nutrient autoanalyzer according to manufacturer specifications and consistent with the colorimetric methods detailed in Parsons et al. (1984). Phytoplankton were enumerated as detailed above. Salinity was measured using a portable refractometer.

Continuous monitoring. A YSI 6600 series datasonde was sited in the Lafayette River less than 50m from the Granby Street Bridge (Fig. 27, *GSB*) at a depth of approximately 0.5m relative to mean lower low water. The sonde was installed in PVC tube with adequate vent holes to allow free passage of water around the sensors, and the tube was attached to a dock piling. The sonde was installed on June 18 and was programmed to record conductivity, temperature, fluorescence, and turbidity every 6 minutes. The sonde was calibrated for conductivity, temperature, and turbidity according to manufacturer specifications, and fluorescence was zeroed against deionized water, therefore the resulting fluorescence data is uncorrected. Because of the highly eutrophic nature of the Lafayette River, the sonde was serviced weekly to prevent the instrument from fouling. During the initial deployment, random noise appeared in the conductivity channel approximately every 3–5 readings. This pattern was confirmed during recalibration using artificial seawater in the sealed calibration cup. In order to remove this noise from the data, a 24-minute moving average filter was applied to the entire

Table 2 Chlorophyll *a* (Chl *a*, μg l^{-1}), salinity (S), temperature (T, °C), and dinoflagellate abundance (cells ml^{-1}) from samples collected during ER-LAF DATAFLOW cruises during 2009. Station (Stn.) letters correspond to sample locations shown in Fig. 27c & d, while station numbers correspond to sample locations shown in Fig. 29

Date	Stn.	Chl *a* (μg l^{-1})	S	T (°C)	*Akashiwo sanguinea* (cells ml^{-1})	*Scrippsiella trochoidea* (cells ml^{-1})	*Gymnodinium uncatenum* (cells ml^{-1})	*Cochlodinium polykrikoides* (cells ml^{-1})
5/27/09	GSB	80.4	16.2	24.3	4		1794	
	B	52.4	18.0	21.8	4		26	
	C	29.3	18.2	22.1	4		50	4
6/4/09	D	29.4	15.0	26.4	12		194	7
	E	43.5	17.6	26.2	5		31	2
	F	61.9	17.2	27.2	21		147	8
6/8/09	1	63.3	13.4	24.6	320	290	90	
	2	26.7	17.7	24.1	5		15	
	3	33.2	17.7	24.4	15		85	
6/17/09	4	38.0	15.9	25.0	345	235	60	70
	5	52.5	18.7	24.3	175		10	5
	6	52.6	16.9	24.7	580		40	
6/23/09	7	50.7	16.1	26.3	215	1655		125
	8	88.8	14.5	26.0	165	2995		890
	9	49.3	15.0	26.4	175	820		280
	10	26.7	18.7	25.0	75	25		45
	11	28.7	17.4	25.3	135	115		15
7/09/09	12	25.3	21.7	25.6	50	5		10
	13	21.3	21.9	25.2	5	15		
	14	31.2	21.4	25.8	45	105		5
	15	41.0	21.3	26.0	180	125		30
	16	42.2	21.2	26.1	105	70		25

timeseries. This filter had minimal effect on the magnitude of the data and effectively removed random short-term (<12 minute) artificial noise in the salinity data. Separately, a 25-hour low pass 4th order Butterworth filter with a cutoff frequency of 1/250 was applied to the entire timeseries in order to remove semidiurnal and diurnal tidal influences on the data.

RESULTS

Meteorological and tidal forcing. The surface water temperature at SP increased rapidly from 18.4°C on May 20 to 26.4°C by June 13 (Fig. 29a, not all data shown). After this time, the water temperature decreased slightly reaching a low of 23.7 °C on June 18. Between June 19 and June 30, the water temperature again increased, reaching 26 °C by June 26. The water temperature remained >25.2°C through September 1 (Fig. 29a).

Wind velocity remained relatively low (<500 $m^3 s^{-3}$) from May 21 through June 17 (Fig. 29c), until a low-pressure system moved through the region (Fig. 29b) bringing rain (Fig. 29e) and high winds reaching 705 $m^3 s^{-3}$ and 970 $m^3 s^{-3}$ on June 18 and 22, respectively. The wind direction was from the East on June 18, shifting to the South and West, and finally to the North by June 22 (Fig. 29d), when the wind speed decreased again. A period of stable atmospheric pressure from July 24 through August 7 (Fig. 29b) coincided with high winds (Fig. 29c), which were predominantly from the SSW (Fig. 29d). The highest observed wind speeds were measured during this period on July 27 and 31, with wind speeds reaching >2300 $m^3 s^{-3}$ (>13 m s^{-1}).

Water T (°C)
20
23
26
29
a
Atm P (mbar)
1000
1010
1020
1030
b
Wind speed (m3 s-3)
0
500
1000
c
Wind dir (true)
0
180
360
d
Precipitation (cm)
0
3
6
9
e
05/21
05/31
06/10
06/20
06/30
07/10
07/20
07/30
08/09
08/19
08/29

Rainfall. Between May and September, 2009, daily precipitation at KORF totaled more than 3 cm on May 17 (3.1 cm), June 5 (6.6 cm) and 18 (3.9 cm), and August 4 (4.3 cm), 5 (6.9 cm), 12 (6.9 cm), and 22 (8.6 cm) (Fig. 29e, not all data shown). Rainfall occurred daily from June 3–5 (0.9, 1.5, and 6.6 cm, respectively), when *C. polykrikoides* was present at background concentrations in the Lafayette and Elizabeth Rivers (Fig. 29e; Table 2). The 6.6 cm of precipitation recorded on June 5 and the 3.9 cm recorded on June 18 were the highest and second-highest amounts of year-to-date rainfall measured at KORF, respectively. Daily rainfall totals of 1–3 cm were measured on June 13 (1.4 cm), July 12 (2.8 cm), 17 (1 cm), and 29 (1.2 cm) while *C. polykrikoides* was increasing in abundance in the Lafayette River (Fig. 29e, Table 2).

Spring tides occurred from June 5–12, 20–27, July 5–12, and July 19–26, with lunar apogee occurring on June 10 and July 7, and perigee occurring on June 23 and July 21 (Fig. 30). Neap tides occurred from May 27–June 4, June 13–19, and June 28–July 4. A strong positive tidal residual was measured in the Elizabeth River at SP between late May and mid-July (Fig. 30). The lowpass filtered residual shows the duration and magnitude of the high positive residual without tidal and subtidal influences (Fig. 30). The raw tidal residual shows distinct peaks coinciding with low-pressure events (e.g. May 28, June 6, June 21), and troughs coinciding with periods of high atmospheric pressure (e.g. June 3, 8, and July 12) due to the inverse barometer effect (Figs. 29b and 30).

Following a period of rainfall from June 3–5, the salinity was low in the upper branches of the Lafayette River on June 8 (Fig. 31). Between June 8 and July 9, 2009 the salinity was lower in the upper branches of the Lafayette River compared to the

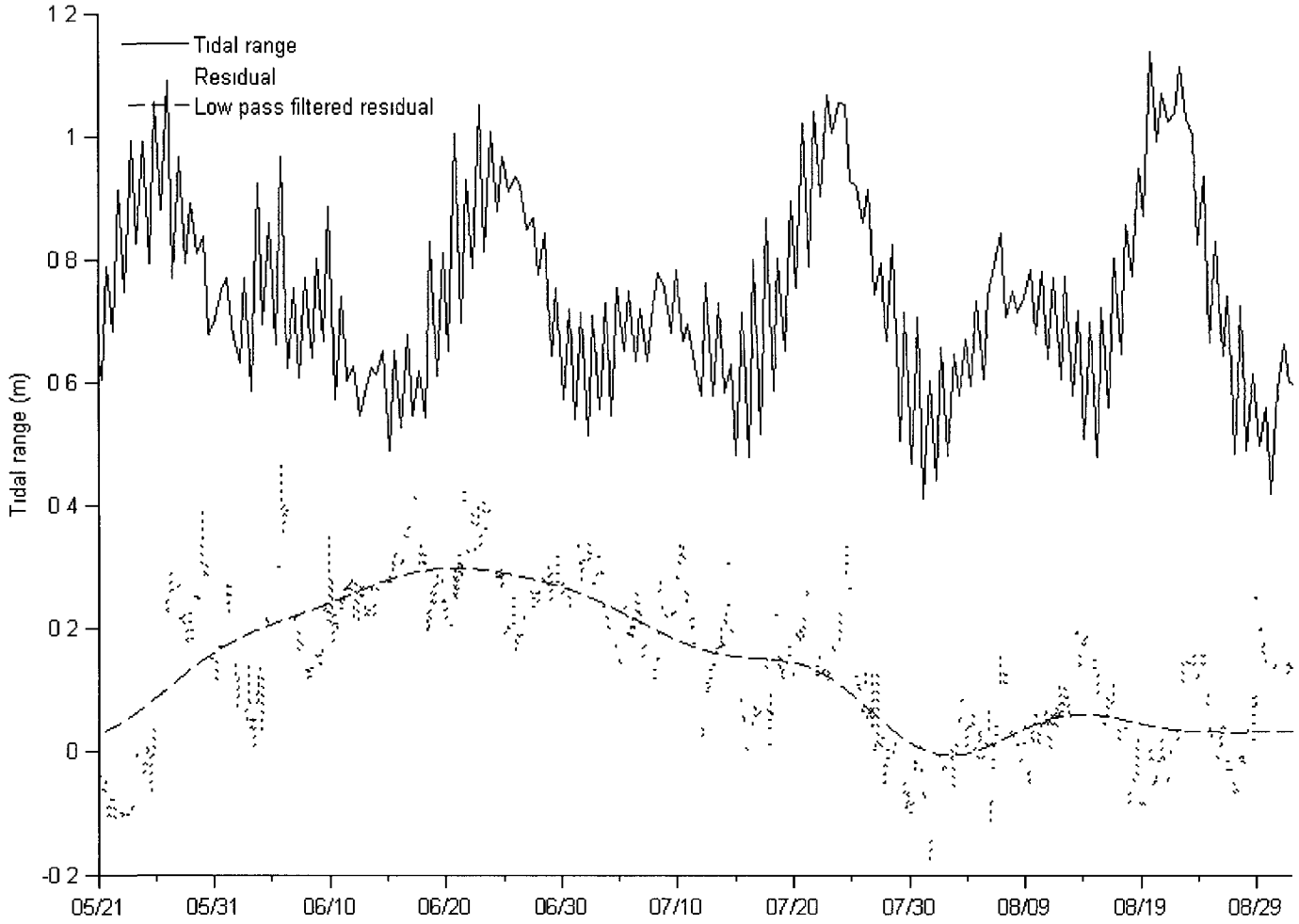
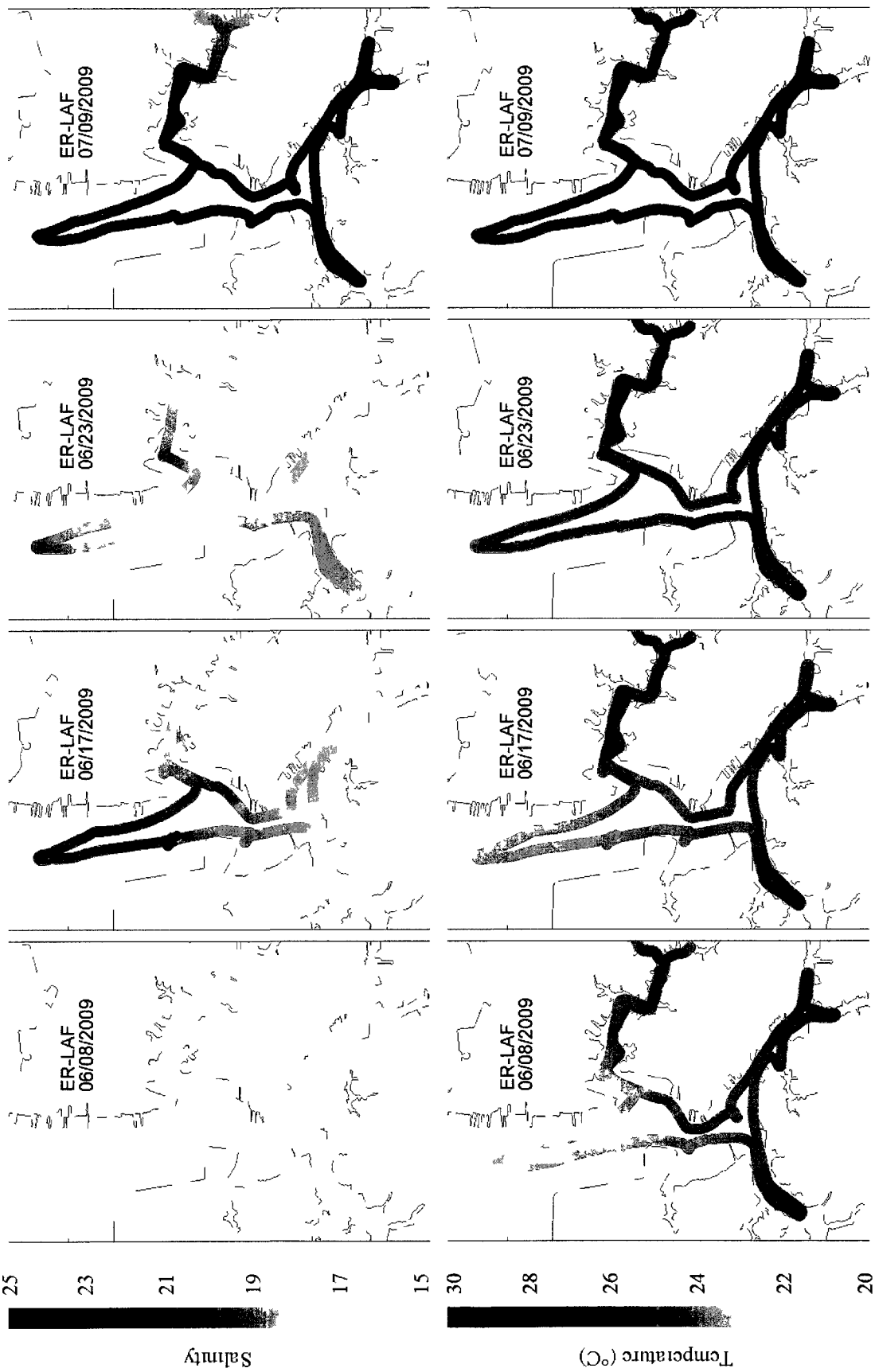


Fig. 30 Timeseries showing tidal range (m), tidal residual (m), and the low-pass filtered residual signal (m) measured at Sewell's Point from May 1–September 1, 2009

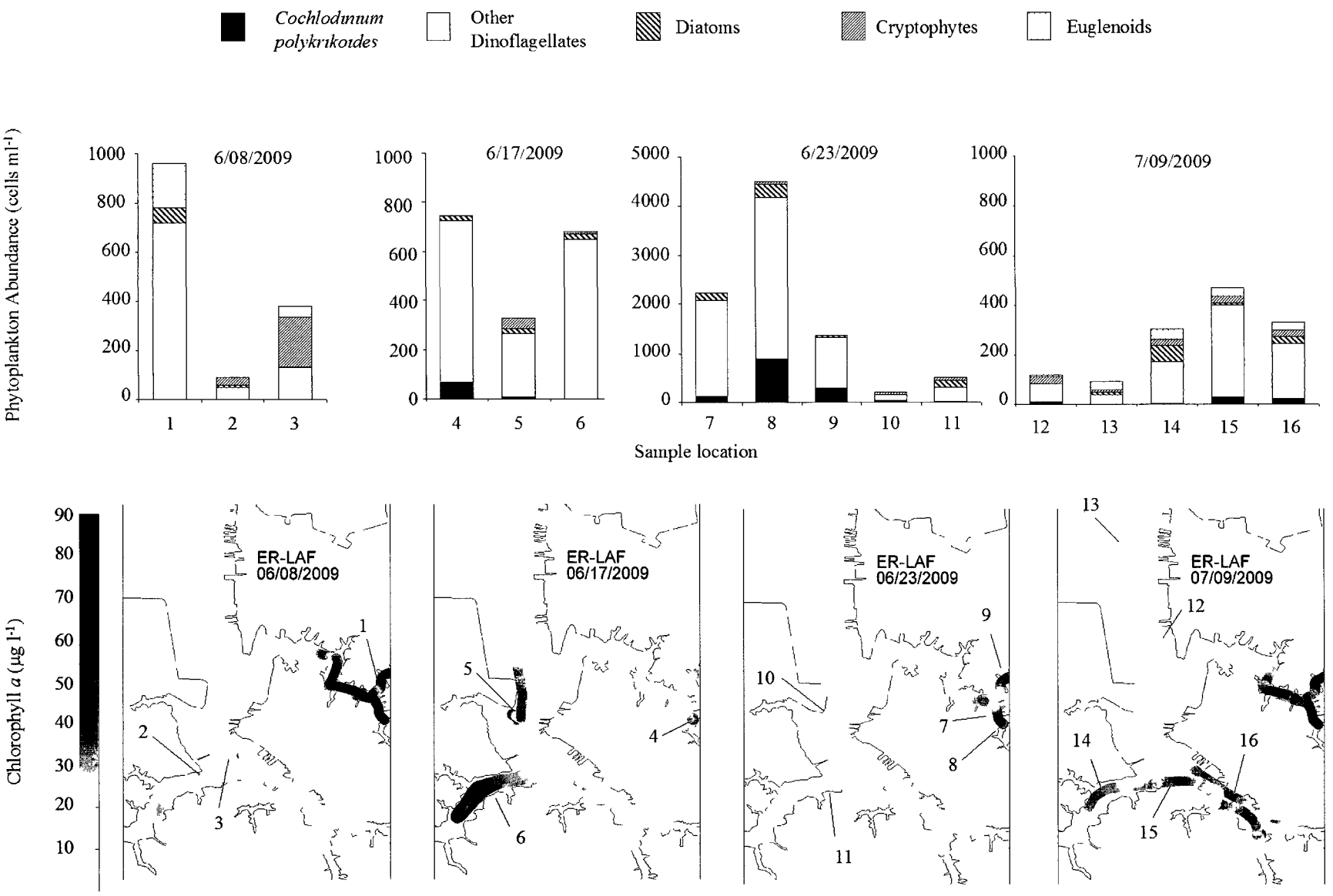

ER-LAF
06/08/2009
ER-LAF
06/17/2009
ER-LAF
06/23/2009
ER-LAF
07/09/2009
Salinity
25
23
21
19
17
15
Temperature (°C)
30
28
26
24
22
20

Elizabeth River (Fig. 31). Water temperatures were higher in the Lafayette River than in the Elizabeth River and increased between June 8 and July 9 in the upper branches of the Lafayette River, (Fig. 31).

Bloom initiation

Underway sampling. *C. polykrikoides* was present at a concentration of 4 cells ml^{-1} in a sample collected from the Elizabeth River on May 27 during a *Gyrodinium uncatenum* bloom (Fig. 27c *C*, Table 2), and on June 4 in the Lafayette River at concentrations of 2–8 cells ml^{-1} (Fig. 27d *D, E, F,* Table 2), also during the *G. uncatenum* bloom (Table 2). However, *C. polykrikoides* was not detected in samples on June 8, when there was a mixed assemblage of dinoflagellates dominated by *Scrippsiella trochoidea* and *Akashiwo sanguinea*, and *G. uncatenum* was the subdominant species (Fig. 32; Table 2). While still a subdominant species, *C. polykrikoides* abundance in the upper Lafayette River had increased by June 17 (70 cells ml^{-1}, station 1; 5 cells ml^{-1}, station 2) (Fig. 32). By June 23, *C. polykrikoides* had nearly reached bloom ($\geq$1000 cells ml^{-1}) concentrations in the Lafayette River (890 cells ml^{-1}, station 5; 280 cells ml^{-1}, station 6), however *C. polykrikoides* was still the subdominant species to *S. trochoidea* (Fig. 32; Table 2). Consistent with cell abundance data, between June 17 and July 9, surface Chl *a* concentrations increased and the spatial extent of the high Chl *a* in surface waters increased. High Chl *a* concentrations were first observed in the upper branches of the Lafayette River and then spread to the middle of the river, concomitant with an increase in abundance of *C. polykrikoides* (Fig. 32).

Samples from the Elizabeth River on July 9 confirmed the presence of *C. polykrikoides* in the channel near the confluence of the James and Elizabeth Rivers (Fig.


Cochlodinium polykrikoides
Other Dinoflagellates
Diatoms
Cryptophytes
Euglenoids
Phytoplankton Abundance (cells ml-1)
6/08/2009
6/17/2009
6/23/2009
7/09/2009
Sample location
Chlorophyll a (µg l-1)
ER-LAF 06/08/2009
ER-LAF 06/17/2009
ER-LAF 06/23/2009
ER-LAF 07/09/2009

32 station *12*), and in the Western and Southern branches of the Elizabeth River (Fig. 32 stations *14–16*; Table 2). However, *C. polykrikoides* was not detected in the James River on July 9 (Fig. 32 station *13*).

Fixed station sampling. At station GSB, dinoflagellates were the dominant taxa from June 6–July 18 (Fig. 33b), with a distinct succession of dominant species leading up to the *C. polykrikoides* bloom (Fig. 33a). *Akashiwo sanguinea* was the dominant species when sampling commenced on June 6 and remained dominant until June 11, when *S. trochoidea* took over as the dominant species from June 12–28 (Fig. 33a). *C. polykrikoides* increased in abundance beginning June 17, reaching 437 cells ml^{-1} by June 27 and 1515 cells ml^{-1} by June 30, when it was the dominant species. *C. polykrikoides* remained the dominant species at GSB through July 30 and the maximum cell abundance was observed on July 17 (13,340 *C. polykrikoides* cells ml^{-1}; Fig. 33a).

NH_4^+ concentrations in the Lafayette River ranged from 7.8 μmol l^{-1} on June 6 and 18 to below the detection limit (0.02 μmol l^{-1}) after July 3 (Fig. 34). NH_4^+ concentrations were high when sampling commenced on June 6 following a period of rainfall from June 3–5 when > 9 cm of total precipitation was measured at KORF (Figs. 29e and 34). By June 9, NH_4^+ concentrations were drawn down to 0.14 μmol l^{-1} (Fig. 34) and *A. sanguinea* had more than doubled (Fig. 33a). Rainfall on June 18 (3.9 cm) and associated winds (Fig. 29c) coincided with another large increase in NH_4^+ concentrations in the Lafayette River, and NH_4^+ again reached 7.8 μmol l^{-1}, but was quickly drawn down to 0.2 μmol l^{-1} by June 20 (Fig. 34). Additional pulses of NH_4^+ occurred on June 24 and 28 (1.7 μmol l^{-1} and 3.5 μmol l^{-1}, respectively), although no precipitation was recorded on these dates (Figs. 34 and 29e). Although there was a

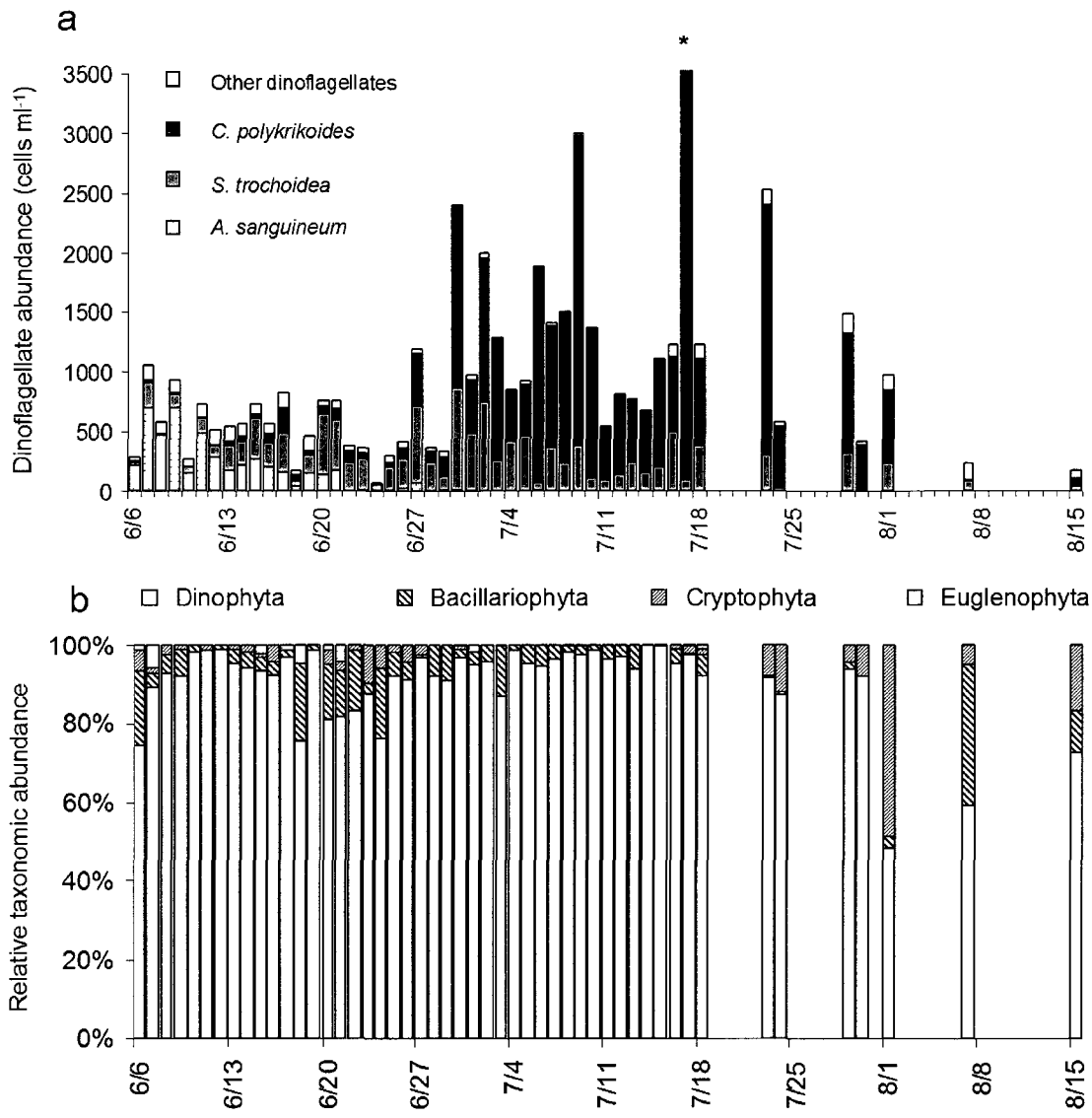


Fig. 33 **a** Dinoflagellate abundance (cells ml^{-1}) showing the dominant species measured daily at station GSB in the Lafayette River from June 6–July 18, and approximately weekly thereafter; **b** the relative taxonomic abundance of the dominant phytoplankton groups during the same period. The *asterisk* on July 17 represents an off-scale abundance of *C. polykrikoides* with 13,340 cells ml^{-1}.

large precipitation event on July 5 (0.6 cm measured at KORF), there were no detectable changes in NH_4^+ concentrations in the Lafayette River at this time. It is likely the N was rapidly consumed by the high concentration of phytoplankton already present in the system. Indeed, storm water collected from a drain at the base of the Granby Street Bridge during the rainfall event had a high NH_4^+ concentration (>23 μmol l^{-1}) (data not shown).

Nitrate plus nitrite (NO_x) concentrations were high on June 6 (2.3 μmol l^{-1}) following the rainfall on June 5, and continued to increase to 3.3 μmol l^{-1} on June 8. At the same time NH_4^+ concentrations and salinity decreased (Figs. 34 and 35). NO_x concentrations decreased to 0.4 μmol l^{-1} on June 9 and remained <1 μmol l^{-1} until a rainfall event beginning June 18. By June 19, NO_x concentrations reached 7.3 μmol l^{-1} (Fig. 35). As for NH_4^+, NO_x concentrations were below the analytical detection limit (0.02 μmol l^{-1}) between July 2 and 18 when *C. polykrikoides* abundance was high (Fig. 35).

As for dissolved inorganic nitrogen (DIN), phosphate (PO_4^+), concentrations at GSB also increased between June 6 and 8 reaching 0.8 μmol l^{-1} on June 7, and concentrations decreased to 0.3 μmol l-1 on June 9 (Fig. 35). However, unlike NO_x and NH_4^+ concentrations, PO_4^+ concentrations increased between rain events reaching 1.2 μmol l^{-1} on June 13, and PO_4^+ concentrations did not increase following the rain event on June 18 (Fig. 35). Additionally, PO_4^+ concentrations increased as the *C. polykrikoides* bloom was forming in late June, and concentrations remained high (>1 μmol l^{-1}) during the bloom from July 2–18 (Figs. 33a and 35).

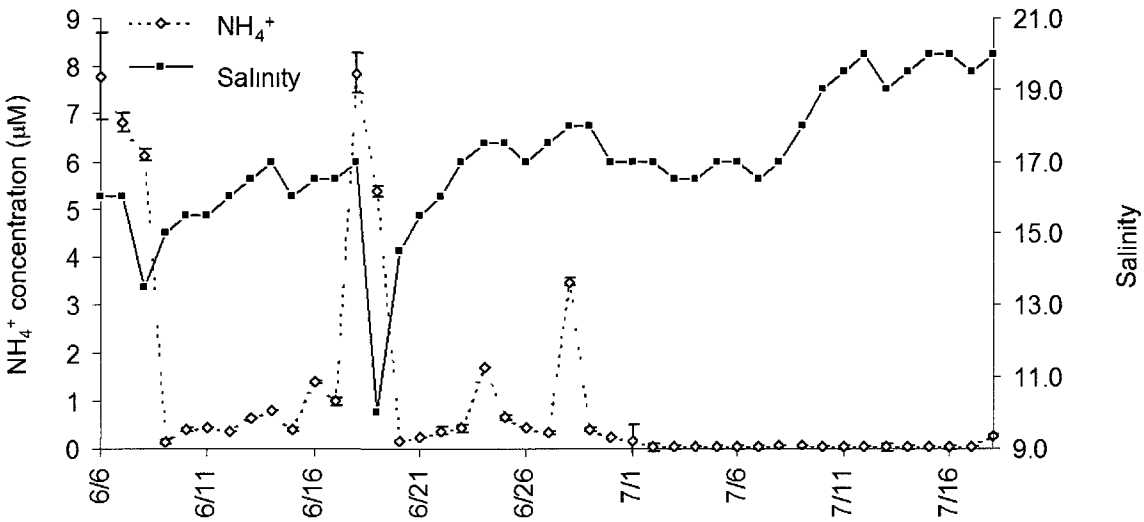


Fig. 34 Salinity and NH_4^+ concentrations (µM) measured daily at station GSB in the Lafayette River from June 6–July 18, 2009

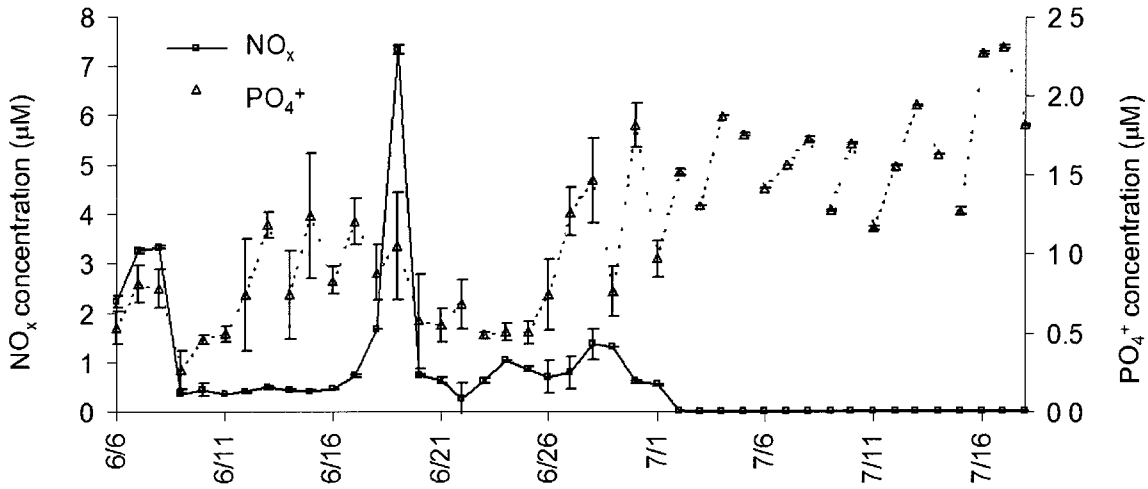
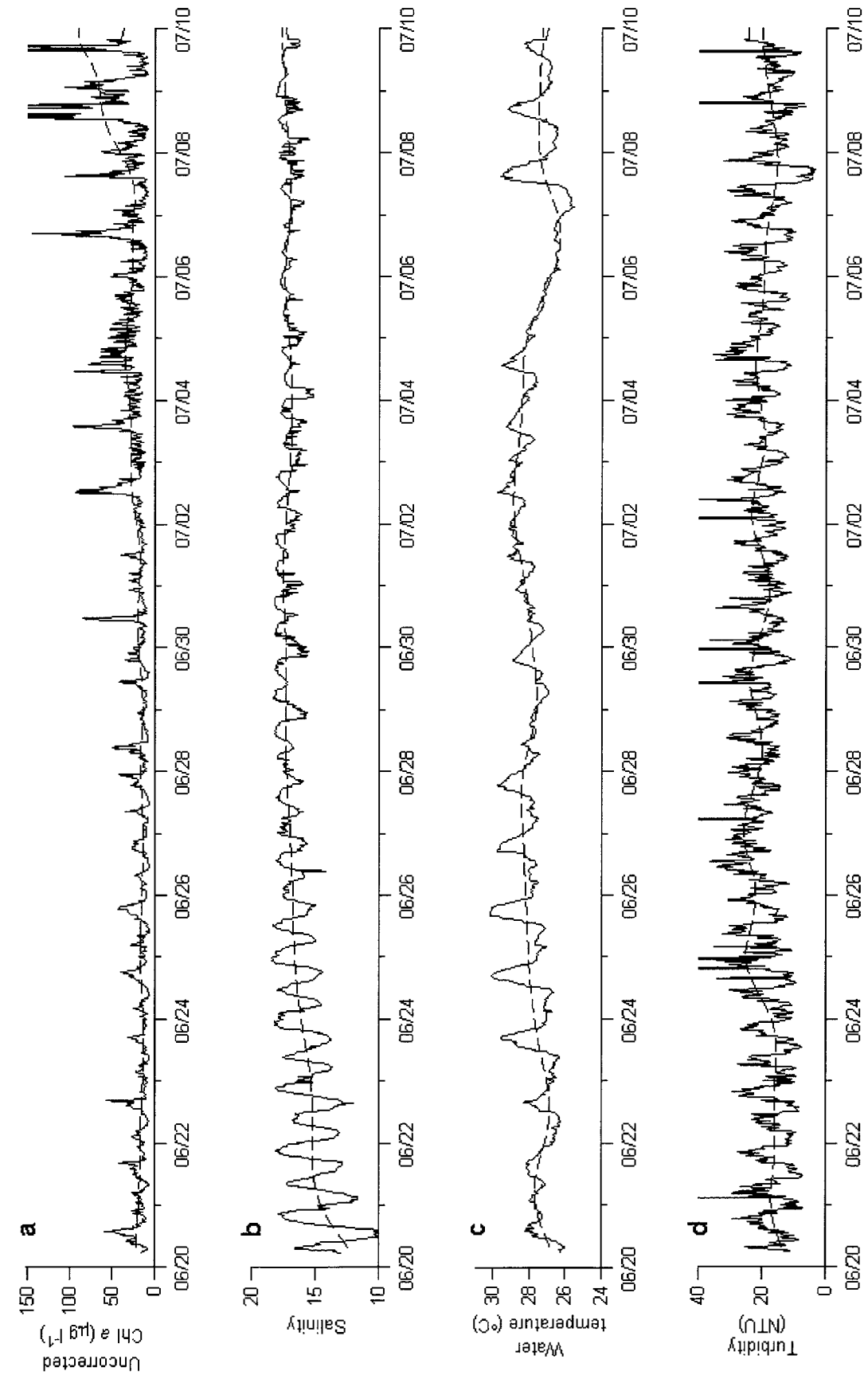


Fig. 35 Nitrate plus nitrite (NO_x) concentrations (µM) and phosphate (PO_4^+) concentrations (µM) measured at station GSB in the Lafayette River from June 6–July 18, 2009. Error bars represent standard deviation

Decreases in salinity generally lagged increases in NH_4^+ concentrations by at least a day, and the salinity at GSB was 16 when sampling commenced on June 6. By June 8, salinity had decreased to 13.5, although no precipitation was measured at KORF between June 5 and 8 (Figs. 29e and 34). This may be due to highly localized amounts of precipitation as well as the influence of groundwater intrusion and runoff following periods of heavy precipitation. Between June 18 and 19, salinity decreased from 17 to 10 following 3.9 cm of rainfall on June 18; another increase in NH_4^+ concentration was observed 1 day after this event (Fig. 34).

Continuous monitoring. The Lafayette River is influenced by semidiurnal tides that affect salinity and temperature (Fig. 36b). Following rainfall on June 18 (Fig. 29e), there was a high range of diurnal salinity variations and these were greatest on June 20 when sampling commenced, and decreased through June 26. Chl *a* concentrations were highest during periods of low salinity (low tide) and also varied on a semidiurnal pattern (Fig. 36a). Chl *a* concentrations were generally highest during low tide in the afternoon, and the magnitude of the daily high Chl *a* increased as *C. polykrikoides* abundance increased at GSB (Figs. 36a and 33a). The water temperature was 26.35 °C when sampling commenced on June 20, and the temperature increased to a high of 30.2 °C on June 25 (Fig. 36c). Following a period of warming and a return to a normal salinity range by June 27, *C. polykrikoides* abundance increased to near bloom concentration (435 cells ml^{-1}) at GSB on June 27 (Figs. 33a and 36b & c). The *C. polykrikoides* bloom was first recorded by the YSI sonde as large increase in Chl *a* on June 30 during a low tide when turbidity was low (Fig. 36 a, b, & d). Peaks in the Chl *a* timeseries at Tanners Landing match up well with *C. polykrikoides* daily abundance at


a
Uncorrected Chl a (µg l-1)
150
100
50
0
b
Salinity
20
15
10
c
Water temperature (°C)
30
28
26
24
d
Turbidity (NTU)
40
20
0
06/20
06/22
06/24
06/26
06/28
06/30
07/02
07/04
07/06
07/08
07/10

GSB after June 30 (Figs. 33a and 36a). Turbidity levels also appeared to be controlled by the tides, with high turbidity occurring during high tide (Fig. 36b, c). Daily turbidity levels generally reached 30 NTU during high tide, when Chl *a* was lowest, and the turbidity generally ranged between 10 and 15 during low tide, when Chl *a* was highest (Fig. 36a, d).

The low-pass filtered Chl *a* timeseries, which has the influence of the tides removed, shows an increase in Chl *a* beginning on July 2 and continuing through July 10 (Fig. 36a). The effect of the filter on the removal of the tidal signal is evident in the low pass filtered salinity timeseries (Fig. 36b), which shows very little change in salinity from June 26 through July 10, after the flushing of low salinity water due to rainfall on June 18 (Fig. 29d). Additionally, the low pass filtered temperature timeseries shows the removal of the tidal influence on water temperature, but a 4–7 day periodicity in the timeseries data remains (Fig. 36c). This periodicity is likely due to regional weather systems and their effect on the water temperature. Because the low pass filter removes the effect of tides on the data, peaks in the moving-average Chl *a* timeseries before July 2, when the slope of the low-pass filtered data is flat, may be attributed to tidal transport of bloom patches, rather than system-wide blooms.

DISCUSSION

During the summers of 2007–2009, massive blooms of the harmful alga *C. polykrikoides* formed in the Elizabeth and Lafayette Rivers, and these blooms were subsequently transported into the James River, where they continued to grow and spread (Chapter 3, Mulholland et al. 2009). In 2007, the bloom was first detected in the

Elizabeth River in late July, although the bloom likely initiated at an earlier date in areas not regularly monitored and so was unreported until reaching densities where it resulted in visibly discolored waters. In 2008, targeted sampling was conducted in areas where blooms were thought to initiate and better spatial and temporal resolution of data, coupled with a modeling study, and hydrographic measurements provided a more detailed picture of bloom initiation (Chapter 3). During this study, higher temporal resolution sampling at a fixed station was coupled with enhanced DATAFLOW coverage in the Lafayette River, and it was determined that *C. polykrikoides* vegetative cells were present at low concentrations in the Elizabeth River (4 cells ml^{-1}) as early as May 27, during a bloom of the dinoflagellate *Gyrodinium uncatenum*. Subsequent samples collected from the Lafayette River documented the increase in *C. polykrikoides* abundance in the upper branches of the Lafayette River from mid-June to early July when discolored waters were first observed. The 2009 *C. polykrikoides* bloom began in the Lafayette River on June 30 when water temperatures had stabilized to about 26°C and during a period of calm winds, neap tides, high positive tidal residuals, higher salinity, low nutrient concentrations and a low DIN to dissolved inorganic phosphorous (DIP) ratio (DIN:DIP). The upper Lafayette River appears to be an important area for initiation and growth of algal blooms.

Summer storm activity has previously been related to the formation of dinoflagellate blooms (Chapter 2; Chapter 3). Because the Lafayette River drains much of urban Norfolk, VA, which consists of 46% impervious surfaces (McKee 2009), storm activity and precipitation can lead to high rates of nutrient loading resulting in enhanced stratification and high nutrient concentrations in surface waters. The pulsing of nutrients associated with intense but highly localized storm activity during the summer months

when water temperatures are above 25°C may play a role in the initiation of *C. polykrikoides* blooms. During the present study more than 9 cm of rainfall accumulated between June 3 and 5, with 6.6 cm falling on June 5 alone. Between June 6 and June 8, DIN concentrations were also high (9.6–10.1 μmol l^{-1}) but by June 9, DIN had been drawn down to 0.5 μmol l^{-1} (Figs. 34 and 35) and dinoflagellate abundance had increased 3-fold from June 6 (Fig. 33). While *C. polykrikoides* was present at background concentrations (<10 cells ml^{-1}) in the Elizabeth and Lafayette Rivers at the beginning of June (Table 2), water temperatures were still below 25°C (Fig. 29a) and so this precipitation appeared to stimulate the growth of *A. sanguinea* and *S. trochoidea*, common bloom forming species during the early summer months (Marshall et al. 1995) (Table 2, Fig. 33a).

Water temperature is an important control on the pattern of dinoflagellate succession in the Lower Chesapeake Bay (Marshall 1995, Marshall and Lacouture 1986). During this study, the abundance of *A. sanguinea* decreased in the Lafayette River as water temperatures reached 24°C (Figs. 29a and 33a). As water temperatures hovered around 24°C, *S. trochoidea* became the dominant species, and then as the water temperatures reached their summertime levels, about 26°C (Fig. 29a), the population transitioned to one dominated by *C. polykrikoides*, which subsequently reached very high abundance at our fixed sampling station (Fig. 33a). In a survey of 22 years of weekly phytoplankton abundance data from Narragansett Bay, RI, water temperature was the dominant variable controlling species succession (Karentz and Smayda 1984).

Both *A. sanguinea* and *S. trochoidea* are classified as a eurythermal species, which thrive at temperatures between 10 and 30°C (Matsubara et al. 2007; Karentz and

Smayda 1984 and references therein). In contrast, *C. polykrikoides* appears to have a narrower range of temperature tolerance and grows best in cultures at temperatures between 21 and 26°C (Kim et al. 2004). However during this study, *C. polykrikoides* blooms did not initiate until water temperatures reached the upper end of this range during June in the Lafayette River during 2009 (Fig. 29a). The narrower temperature tolerance may limit the contribution of *C. polykrikoides* to the total phytoplankton composition before water temperatures have warmed in the summer. However, temperature is likely acting synergistically with other environmental variables to control species succession. Field data from Narragansett Bay show that the maximum abundance of particular species in nature often occur at temperatures much lower than their optimal growth temperatures determined in culture experiments, and the temperature coinciding with the first observance for an individual species varied widely between years (Karentz and Smayda 1984). Further, because of the relatively small change in water temperature observed (~6°C) over the sampling period relative to the large observed temperature ranges reported for these species, it is likely that water temperature was only partly responsible for the observed shift in dominance from *A. sanguinea* to *S. trochoidea* and *C. polykrikoides*, and that other variables such as nutrient availability and competition for nutrients may be important factors determining the dominant species within the community at any one time.

Following the greatest daily precipitation (on June 5) recorded at KORF for the year to date in 2009 (Fig. 29e), DIN concentrations increased to > 10 μM in the Lafayette River (Figs. 34 and 35), and the relative and absolute abundance of dinoflagellates (predominantly *A. sanguinea*) also increased (Fig. 33a). As water temperatures continued

to increase (Fig. 29a), and following an additional pulse of rainfall (Fig. 29e), and another bolus of DIN to the system (> 15 μmol l^{-1}) (Figs. 34 and 35), a shift in the phytoplankton community occurred and *S. trochoidea* became the dominant species (Fig. 33a). In contrast to observations that rainfall stimulated *C. polykrikoides* bloom initiation during 2008 in the Lafayette River (Chapter 3), *C. polykrikoides* abundance at GSB did not appear to change until 9 days after the large rainfall event after the associated DIN inputs had been drawn down. Indeed, low N concentrations (but high P concentrations) were associated with *C. polykrikoides* bloom formation during 2009, and the low (< 16, the Redfield ratio) ratio of DIN to DIP (mean DIN:DIP = 4.3) was suggestive of severe N limitation (Howarth 1988; Malone et al 1996) from June 7 through June 29. After June 30, the DIN:DIP ratio was even lower (<0.75, mean DIN:DIP = 0.1), during the time when *C. polykrikoides* was dominant. The low DIN concentrations and elevated DIP concentrations during the *C. polykrikoides* bloom may have limited the growth of phytoplankton unable to fix N_2, use dissolved organic N (DON) or other available N pools in the environment. Many bloom-forming dinoflagellates are able to thrive when DIN: DIP ratios are below 16. The optimum range of DIN:DIP reported for the growth of *S. trochoidea* is 6–13 (Hodgekiss and Ho 1997), which corresponds to the DIN:DIP range observed in the Lafayette River during the time when this organism was the dominant phytoplankter. Because of their versatile metabolisms and the ability of many dinoflagellates, including *C. polykrikoides*, to use organic N or grow mixotrophically (Burkholder et al. 2008, Mulholland et al. in prep.), these organisms may thrive when strictly photoautotrophic species cannot.

C. polykrikoides is thought to be capable of supplementing its N and C requirements via mixotrophic ingestion of prey. In studies of phagotrophy by *C. polykrikoides*, ingestion of prey was limited to prey with an equivalent spherical diameter of <12 μm (Jeong et al. 2004). This size limitation would prevent *C. polykrikoides* from grazing on the co-occurring dinoflagellates, *A. sanguineua* and *S. trochoidea*, and so while grazing could have contributed to the nutrition of *C. polykrikoides*, it was unlikely that grazing influenced the observed succession of dinoflagellate bloom species. Cryptophytes, a preferred prey for *C. polykrikoides* (Jeong et al. 2004), were generally abundant between June 6 and 27 (5–40 cells ml^{-1}) in the Lafayette River, however their abundance decreased and they were only detected on 2 dates and at low abundances (10 cells ml^{-1}) between June 30 and July 16, during the *C. polykrikoides* bloom. This could have been because they were being removed through grazing by *C. polykrikoides*. Mixotrophic grazing has been observed in Asian isolates of *C. polykrikoides* but these are genetically distinct from *C. polykrikoides* populations in the lower Chesapeake Bay (Mulholland et al. 2009), and so it remains to be determined whether mixotrophy is a significant nutrient acquisition pathway during blooms in the lower Chesapeake Bay estuary.

Ecosystem disruptive algal blooms (EDABs) often thrive when ambient nutrient concentrations are very low and nutrient recycling dominates the available nutrient pools (Sunda et al. 2006). Because EDAB species are generally unpalatable or toxic to grazers, their abundance increases relative to other co-occurring phytoplankton because biomass is not lost to grazing. Indeed, *C. polykrikoides* abundance has been shown to be inversely proportional to grazing pressure on *C. polykrikoides* (Jiang et al. 2009). Lower rates of

grazing-mediated nutrient recycling can reduce nutrient availability further, favoring growth of low-nutrient adapted EDAB populations (Sunda et al. 2006, Gobler et al. 2005). Similar to many EDAB species, *C. polykrikoides* exerts negative effects on benthic grazers including American oyster (*Crassostrea virginica*) (Mulholland et al. 2009) and bay scallops (*Argopectans irradians*) (Gobler et al. 2008). The survivorship and fecundity of the copepod *Acartia tonsa* has also been shown to decrease with increasing abundance of *C. polykrikoides* (Jiang et al. 2009). Nutritional flexibility, low losses of algal biomass to grazers through production of grazing deterrents and production of allelopathic compounds to reduce competition from co-occurring phytoplankton may all contribute to the formation of massive, enduring blooms (Sunda et al. 2006), and appears to be a strategy employed by *C. polykrikoides*.

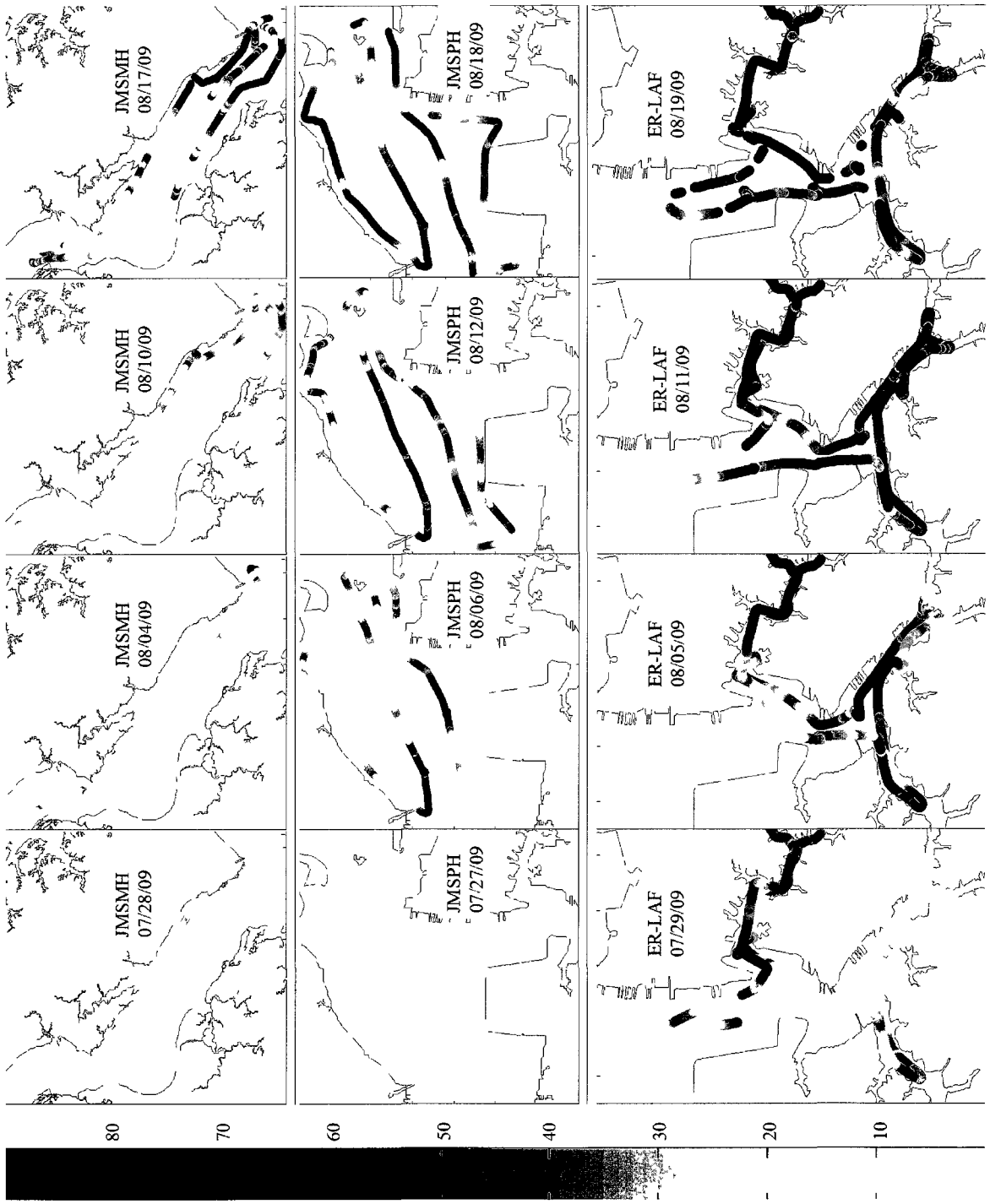
Higher than normal tides and resulting coastal flooding may have also contributed to nutrient loading in the Lafayette River toward the end of June during a period when little rain was recorded at KORF and when the *C. polykrikoides* bloom was initiating. DIN concentrations increased in the Lafayette River from June 21–24, as tidewaters flooded the streets and low-lying land in the urban Lafayette River watershed. A strong (>0.2 m) positive tidal residual existed at SP between June and July during 2009 (Fig. 30). This was not a localized event, and was experienced in varying degrees along the east coast of the United States from Florida to Maine, with higher sea levels measured between North Carolina and New Jersey (Sweet et al. 2009). This anomalous period of higher than predicted tides occurred with little associated storm activity and was attributed to predominantly NE winds over the Atlantic Ocean driving Ekman flow landward, combined with a decrease in the transport of the Florida Current (FC), which is

measured between Florida and the Bahamas (Sweet et al. 2009). A change in the rate of transport of the FC (which supplies the Gulf Stream) alters sea level along the east coast due to changes in the geostrophic balance of the FC (Sweet et al. 2009; Ezer 2001) such that decreasing FC transport results in increased sea levels along the east coast and likewise increasing FC transport causes lower sea levels. At the peak of the anomaly at SP on June 22–23, the low pass filtered tidal residual was 0.3 m, and the hourly residual peaked at 0.42 m. This period coincided with a perigean spring tide concurrent with a low-pressure system, resulting in the anomalously high tides on June 22 and 23 that flooded parts of the Lafayette River watershed. Thus, climatological forcing and far field circulation patterns can also contribute to nutrient loading at a localized watershed level.

One reason bloom initiation remains poorly understood is that areas where blooms form are rarely sampled on spatio-temporal scales relevant to bloom formation, and blooms may be first observed in areas to which they have been transported rather than the area in which they initiated. In a small system such as the Lafayette River, even daily sampling from a fixed station may not be frequent enough to resolve all of the contributing factors influencing when and where a bloom initiates (e.g. tidally dominated processes, diel variability). *C. polykrikoides* abundance reached bloom concentrations on June 30, 2009 in the Lafayette River at the Granby Street Bridge. However, *C. polykrikoides* bloom initiation likely occurred in the upper branches of the Lafayette River earlier, between June 17 and 23, following a major precipitation event when *C. polykrikoides* was a subdominant species and dissolved inorganic N concentrations increased 7-fold. The combined sampling approach employed here included high frequency in-situ monitoring, daily sampling of nutrients and the phytoplankton

community at a site where bloom initiation was likely to occur, as well as weekly surveys of temperature, salinity, and Chl *a* over a large spatial scale. This approach allowed for a more focused and detailed view of the complex factors regulating bloom initiation and formation than had been previously attempted. However, even the present study failed to determine a "smoking gun" with regard to nutrient controls on bloom formation. It may be that the sampling regime employed by some previous studies which identified such triggers on bloom formation was insufficient over spatial and temporal timescales relevant to bloom formation and thus the conclusions drawn about blooms may have been based on aliased data. Additionally, sampling at an even higher frequency in order to avoid aliasing data may be required to facilitate the use of a more quantitative approach to understanding bloom formation in tidally dominated estuarine environments.

Blooms that initiate in the Lafayette River may be transported into the Elizabeth River through tidal advection, and subsequently into the lower James River, where local circulation may result in up and downriver transport of the bloom organisms due to eddy circulation and tidal front injection (Chapter 3; Shen et al 1999; Kuo et al. 1990). This transport pathway appears to be a factor controlling not only the distribution of bloom organisms but may also affect the duration of *C. polykrikoides* blooms in the lower Chesapeake Bay area. As in previous bloom years, during 2009 the pattern of transport from the Lafayette River into the Elizabeth River followed by transport into the JMSPH and subsequently into the JMSMH was again repeated (Fig. 37). Transport of the bloom from the Elizabeth River into the JMSPH occurred between July 29 and August 6 (Fig. 37), during a neap tide (Fig. 30), and the bloom persisted in the JMSPH through August 26 (data not shown), a duration of approximately 25 days, which coincides with


JMSMH
07/28/09
JMSMH
08/04/09
JMSMH
08/10/09
JMSMH
08/17/09
JMSPH
07/27/09
JMSPH
08/06/09
JMSPH
08/12/09
JMSPH
08/18/09
ER-LAF
07/29/09
ER-LAF
08/05/09
ER-LAF
08/11/09
ER-LAF
08/19/09
80
70
60
50
40
30
20
10
Chlorophyll a (µg l-1)

the fresh water travel time in this region under normal summertime flow conditions (Shen and Lin 2006). The simulated transport of a conservative tracer under 2007 and 2008 James River dynamic conditions also showed enhanced transport from the Elizabeth River into the JMSPH during neap tides (Chapter 3). Transport of the bloom from the JMSPH into the JMSMH occurred between August 4 and 10, during a transition from neap to spring tides (Figs. 37 and 30), a time when the eddy circulation in the lower James River is weakened and upriver transport is mainly due to tidal advection and wind-driven transport (Shen et al. 1999, Chapter 3). Eddy circulation in the JMSPH may have enhanced retention of the bloom in this region despite the predominant southwesterly winds during this period (Shen et al. 1999). During blooms in 2007 and 2008, high Chl *a* concentrations were first observed in the JMSPH and later observed in the JMSMH, consistent with the modeled transport of a passive tracer released in the Lafayette River (Chapter 3), although local growth was also important in the JMSMH during 2008. Because the bloom persisted in the JMSMH from August 10 through August 24 during 2009 (Fig. 37, not all data shown), over two consecutive spring tides (Fig. 30), it is likely that tidal advection, rather than tidal front intrusion, resulted in the transport of the bloom into the JMSMH, and thus tidal flushing and wind-driven transport was responsible for the end of the bloom in the JMSMH. The winds had a predominantly southwesterly component during this period (Fig. 29d), which are likely to increase flushing in the lower James River due to its orientation.

The Lafayette River is an important zone of initiation for algal blooms that can then spread to the James River and affect the entire lower Chesapeake Bay region (Chapter 3, Mulholland et al. 2009; This Chapter). *C. polykrikoides* bloom formation

during 2009 occurred at a time when ambient DIN concentrations were low but DIP concentrations were relatively high. Consequently, the DIN:DIP ratio was low, indicative of severe N limitation. *C. polykrikoides* has the propensity to further alter the DIN:DIP ratios through allelopathic effects on co-occurring taxa (Tang et al. 2010; Yamasaki et al. 2007) and grazing deterrence (Jiang et al. 2009), and mortality of grazers (Gobler et al. 2008; Mulholland et al. 2009). In addition to forming massive blooms that likely alter ecosystem function, *C. polykrikoides* blooms can result in large areas of anoxia and hypoxia (Chapter 3). The major implications of this study are that nutrient concentrations, while essential for the growth of algae, can not be identified as a causative factor in determining when a bloom will form, and that there is no "smoking gun" with regard to nutrient controls on bloom formation in a eutrophic estuarine environment. Additionally, measurements of ambient nutrients may be less important than determining overall nutrient loads to a system, as the ambient nutrient concentrations are what is left after fueling algal growth. Further, interactions between the plankton at short timescales (e.g. successional patterns) may go unnoticed if sampling is not sufficiently frequent, and these interactions may have implications for bloom development. While the present study provides new insights regarding bloom initiation and transport of bloom organisms in the lower Chesapeake Bay watershed, additional work is required to understand the physical and chemical triggers for excystment of *C. polykrikoides* resting stages in natural environments and how cyst distribution contributes to where blooms initiate and are observed.

CHAPTER 5

CONCLUSIONS

In estuarine environments, timescales relevant to phytoplankton ecology are vast, ranging from microseconds (the timescale on which photons are captured and transmitted within a chloroplast; Falkowski and Raven 1998), to days (the scale for meteorological forcing), to many months (the dormancy period of a dinocyst buried in the sediment; Anderson et al. 1987) to years (the scale of climatological forcing). Within this wide range of timescales of variability, diel and multi-day variability is important at the population and community level, where co-occurring taxa compete for nutrients and light, and must avoid becoming prey in order to increase their net abundance within the community. Algal biomass is also strongly linked to the tidal cycle in the Lafayette River, and over a tidal cycle, nutrient concentrations were lowest on the incoming tide when phytoplankton biomass was high, highlighting the tidal influence on nutrients and phytoplankton in this system and the relationship between algal biomass and nutrient drawdown (Chapter 2). Tidally coordinated sampling can alleviate some of the variability resulting from tidal advection.

Diel cycles are also important in estuarine environments, and many dinoflagellates migrate vertically through the water column on a diel cycle, often, but not always, rising in the water column during the day and returning to depth in the evening (Park et al. 2001; Kamykowski et al. 1998). *Cochlodinium polykrikoides* is able to migrate through the water column at a speed of 1.3 to 4 m h^{-1} (Park et al. 2001), a rate much higher than other co-occurring dinoflagellates. In comparison, *Ceratium furca*, a

large mixotrophic dinoflagellate typical in Chesapeake Bay, can migrate at speeds up to 0.9 m h^{-1} (Baek et al., 2009), and *A. sanguinea* has been shown to migrate at speeds up to 1.5 m h-1 (Smayda 2002). The relatively fast migrating speed of *C. polykrikoides* may allow it to utilize nutrients at depth that are otherwise unavailable to other dinoflagellates. These migrations are often timed so that the algae are near the surface by early afternoon, and this pattern may be reflected in continuous monitoring record of Chl *a*. Vertical migration by plankton can contribute to the patchy distributions of algal biomass observed over time and space, and surface Chl *a* concentrations were usually higher in the Lafayette River later in the day rather than in the morning, when the DATAFLOW surveys began.

In addition to the semidiurnal tidal cycle, the spring-neap tidal cycles also influence algal populations through changes in the rates of vertical mixing, the breakdown of stratification, and increased turbulence during spring tides. Phytoplankton abundance was strongly linked to the spring-neap tidal cycle, and all blooms identified during the 2005 field study occurred during neap tides. Picoplankton abundance was not linked to the spring-neap cycle as increases in picoplankton abundance occurred during both spring and neap tides (Chapter 2). The picoplankton abundance maxima occurred during August, consistent with findings of a seasonal maximum occurring in late summer in the lower Chesapeake Bay and its tributaries (Affronti and Marshall 1994; Marshall and Nesius 1996). *Cochlodinium polykrikoides* bloom formation was also linked to the spring-neap cycle, and blooms initiated in the Lafayette River during neap tides in both 2008 and 2009 (Chapters 3 and 4).

Additionally, meteorological forcing influences physical stratification and the delivery of nutrients to system on timescales of days to weeks, and the duration of high wind events is as important as their magnitude on the phytoplankton community composition, with diatoms favored under high nutrient, turbulent conditions (Margalef 1978, Chapter 2). The timing and intensity of precipitation is important to the formation of algal blooms, and the timescale of variability for this parameter can range from days to weeks as frontal systems move through the region. The intensity and duration of precipitation and wind events affect salinity (and buoyancy driven stratification) and nutrient concentrations and these can contribute to species succession and bloom initiation (Chapter 4).

At the opposite end of the spectrum of meteorological forcing, infrequent large-scale events such as hurricanes can also impart drastic changes in estuaries on a much larger scale, both spatially and temporally, and the effects from a single event can have lasting effects for years. Pearl et al. (2006) documented the affects of large-scale perturbations to Chesapeake Bay, the Pamlico Sound, and the Neuse River estuary as a result of increased river discharge and decreased residence time due to the influence of hurricanes. Episodic increases in river discharge associated with large amounts of precipitation from passing hurricanes caused distinct changes in the phytoplankton community in both systems, however the Pamlico Sound sustained the greatest change in ecological function as system residence times decreased in response to pulsed fresh water discharge, likely due to a much longer residence time than for Chesapeake Bay (Paerl et al. 2006). These dramatic climatic events cause shifts in patterns of nutrient loading and

productivity, and a change in the residence time of the system appears to be the primary driver of long-term (months to years) change (Paerl et al. 2006).

In the shallow, eutrophic Lafayette River, precipitation resulted in nutrient loading from overland runoff. During summer, intense highly localized storms can inundate areas within the watershed with upwards of 6 cm of rain over periods of less than 1 hour, while some areas within the watershed do not get any rain. Without a network of rain gauges, there is no way to identify where and when these highly localized storms result in precipitation. Because the Lafayette River drains much of urban Norfolk, which consists of more than 46% impervious surface coverage (McKee 2009), much of the precipitation falling over the region reaches the watershed immediately and introduces buoyancy that results in intense stratification and nutrients that can fuel phytoplankton growth (Chapter 2). Depending on the amount of wind, and the direction it comes from, the nutrient pulsing in the Lafayette River may select for certain groups of phytoplankton over others. During summer 2005, when nutrient concentrations were high and wind-driven mixing was low, dinoflagellates were favored and blooms of *A. sanguinea* and *Gymnodinium* spp. developed. These blooms were lag correlated with all forms of inorganic and organic N from 2 to 5 days in reverse time, and so it is likely that the form of N nutrient present in the system does not have a selective effect on dinoflagellate bloom formation, but rather the amount of N in the system may be important. Many dinoflagellates have nutritionally flexible metabolisms, including the ability to grow mixotrophically and to supplement their N nutrition through the uptake of organic nutrients sources, grazing (Jeong et al. 2005b), and ability to hydrolyze and take

up large organic compounds such as peptides (Mulholland et al. 2009; Mulholland et al 2002).

Following storm events, diatoms may be favored when the wind speed increases and remains stable for an extended period of time. In 2005, diatom abundance was positively lag correlated with PO_4^+ and silicate concentrations from 2 to 4 days in reverse time, but were not significantly correlated with N concentrations (Chapter 2). Although diatom abundance in the Lafayette River was very low from June to July during 2009, when PO_4^+ concentrations were high. This may be due to the Low N:P ratios during this time, as many dinoflagellates are favored at low N:P ratios, and diatoms are more adapted to N:P near Redfield (Hodgkiss and Ho 1997). Increased wind-driven mixing, which is proportional to the cube of the wind speed (Lund-Hansen et al. 1996), causes the amount of turbulence within a system to increase and generally selects for diatom growth over the growth of dinoflagellates (Chapter 2, Margalef 1978; Sellner et al. 2001). When the wind speeds were elevated, but remained below 15 knots (cube of the wind speed $< 500\ m^3\ s^{-3}$), for a period of several days, a diatom bloom formed following nutrients loading from precipitation (Chapter 2). However, when the wind speed increased to over 15 knots (cube of the wind speed $> 500\ m^3\ s^{-3}$) for more than a few days, the total phytoplankton abundance decreased in the Lafayette, despite high nutrient concentrations. This may be a result of increased turbidity and light limitation owing to the shallow system.

Light attenuation in shallow estuarine waters is primarily influenced by high concentrations of suspended particulate material, (turbidity) (Cloern 1987). Additionally, during dense phytoplankton blooms, light is attenuated in the water columns by the increased phytoplankton biomass. During these dense blooms, it appears that light is

often scattered by the high abundance of cells rather than absorbed by Chl *a* contained within the cells (e.g. the brown tides caused by *Aureococcus anophagefferens* (Cosper et al. 1987)). Increased turbidity levels may be caused by wind-driven mixing in shallow environments, transport of sediment from riverine sources and runoff (Cloern 1987), and interactions of the tidal current with rough bottom at the water-benthic boundary resulting in turbulent mixing of sediments up into the water column (Lucas et al. 1998). In the Lafayette River during 2009, turbidity showed a semi-diurnal periodicity and turbidity levels were lowest during low tide and highest during the early stage of the flood tide (Chapter 4). Over a 24-hour period, Chl *a* concentrations were highest during low turbidity (at low tide) and lowest when turbidity was high. This may be due to increased turbulent mixing during flood tide, a feature described by the Strain Induced Periodic Stratification (SIPS) model of Simpson et al. (1990). Under the SIPS scenario in a shallow system, saltier, denser water flows over less dense river water during flood tide. This unstable condition results in turbulent mixing of the water column and the breakdown of stratification; on the ebb tide, less dense water flows over the more dense water and this shear flow results in increased stratification, which may persist until the next flood tide when stratification breaks down again (Lucas et al. 1998; Simpson et al. 1990). This recurrent pattern of tidally driven turbulent mixing, and the breakdown and buildup of stratification over tidal timescales may influence phytoplankton dynamics over short timescales, particularly when the increase in stratification occurs during solar noon (Lucas et al. 1998). However, in a modeling study of the effects of mixing on bloom formation in an estuary, periodic SIPS stratification did not increase the likelihood of

bloom formation compared to conditions of persistently non-stratified water (Lucas et al. 1998).

Although Chl *a* concentrations were highest during times of low turbidity during 2009 (Chapter 4), dinoflagellates, and particularly *C. polykrikoides*, *S. trochoidea*, and *A. sanguinea*, which were the dominant species during the 2009 bloom, are not likely to be limited by light availability in a shallow system like the Lafayette River. These species are capable of strong vertical migration, and typically form blooms in coastal frontal zones, where the rates of mixing and the water depth are much greater than in the Lafayette River (Park et al. 2001; Smayda 2002). The light attenuation caused by increased turbidity is likely to affect motile dinoflagellates to a lesser degree than for non-motile phyla such as diatoms because dinoflagellates can migrate to the surface in order to utilize the available light. Vertical migration of dinoflagellates (positive phototaxis) during daylight may alleviate light limitation caused by the periodic increased turbidity associated with SIPS turbulent mixing. Additionally, when dinoflagellate abundance is high, the accumulation of cells at the surface may result in increased light attenuation and cause further light limitation to non-motile phytoplankton (e.g. Cosper et al. 1987), perhaps perpetuating the dinoflagellate bloom through reduced competition.

During 2008, tidal advection resulted in the transport of *C. polykrikoides* from the site of initiation in the Lafayette River into the Elizabeth River, where it continued to increase in abundance (Chapter 3). This transport likely occurred over just a few days as the length of transport was approximately equal to one tidal excursion during spring tides (Sisson 1976). Subsequent transport of the bloom from the Elizabeth River into the JMSPH also occurred within 3 days, owing to the short distance relative to tidal

excursions and the strong currents typical in the Elizabeth River channel. These travel times are consistent with the model results from the hydrodynamic model (Chapter 3). An important consideration of the modeling study is that it does not apply just to algal blooms. Pollution, chemicals and nutrients would follow similar transport pathways from the Lafayette River into the JMSPH and upriver into the JMSMH, where they would slowly be flushed out of the James over a period of ~25 days. Wastewater treatment plants typically release their effluent at depth, where gravitational circulation is likely to transport the wastewater upriver as well as downriver, which may be one reason the Lafayette and Elizabeth Rivers are able to sustain large algal blooms for many weeks.

C. polykrikoides bloom formation in the Lafayette River during 2009 was linked to a period of low wind speeds, high water temperatures (>25 °C), increased salinity and decreased DIN concentrations following the return to normal salinity conditions 9 days after a large rainfall event, high DIP concentrations, and a low DIN:DIP ratio suggestive of extreme N limitation which persisted through the end of the study period. Dinoflagellate abundance was high in the Lafayette River prior to *C. polykrikoides* becoming the dominant species, and water temperature appears to influence the succession of dinoflagellate species. In late May, a bloom of *Gyrodinium uncatenum* was ongoing when *C. polykrikoides* was first detected in the Elizabeth River, and water temperatures were 22 °C during this time. As the water temperatures increased reaching 24 °C, *A. sanguinea* became the dominant species and its abundance exceeded 500 cells ml^{-1} following a period of rain from June 3–5 and increased DIN concentrations from June 6–8. *S. trochoidea* became the dominant species as water temperatures continued to increase and DIN concentrations decreased until a large precipitation event on June 18

resulted in high DIN concentrations (>15 μmol l^{-1} DIN). *C. polykrikoides* reached bloom concentration (>1000 cells ml^{-1}) and became the dominant species 9 days after this rain event, when water temperature exceeded 25 °C.

While nutrients and light are fundamental requirements for the growth of algae, the overarching control on bloom formation in the Lafayette River was water column stability, and this factor influenced the development and formation of every bloom observed during 2005, 2008, and 2009. In the shallow Lafayette River, the stability of the water column is influenced primarily by spring-neap tidal modulation, buoyancy inputs during and after rainfall events, and wind-induced turbulent mixing. Because the Lafayette River experiences heavy nutrient loads and nutrient concentrations are typically high year-round, the availability of nutrients is of secondary importance to water column stability. There are many environmental controls that may influence the timing of bloom formation in the Lafayette River, but one common variable that was similar for all blooms was the tidal range during which the blooms developed: all blooms observed during 2005, 2008, and 2009 initiated during neap tides. The lower tidal energy during neap tides may allow for increased stratification and reduced turbulent mixing (particularly when neap tides occur during and after rainfall), and the decreased flushing during neap tides may allow for accumulation of biomass. Rainfall preceded all blooms observed during 2005 and the *C. polykrikoides* bloom during 2008, but there was a 9-day period between rainfall and *C. polykrikoides* bloom initiation in the Lafayette River during 2009. Rainfall delivers nutrient loads to the Lafayette, and the pattern of summertime drought followed by intense, highly localized precipitation leads to overland runoff and nutrient loading in the Lafayette River. A third prominent and ever-present

control on bloom formation is the wind. The speed, duration, and direction of the wind can influence blooms, and may determine whether diatoms or dinoflagellates are favored. Periods of low winds favored the development of dinoflagellate blooms in the Lafayette River, and when the wind speed was elevated, diatoms became more abundant. A fourth environmental variable that must be considered is the water temperature. Water temperature may regulate species succession, as blooms regularly occur seasonally and appear to have ecological niches, although the realized and ideal ecological niche for bloom species often appears to be different (Chapter 4).

Finally, some environmental variables that have been described in the literature as having pivotal roles in the control of blooms, but which appear to have uncertain or variable roles in bloom formation in the Lafayette River are nutrient concentrations (both N, and P), and turbidity. Phosphate concentrations are generally high in the Lafayette River (often > 1 μmol l^{-1}), and during both 2005 and 2009 N: P ratios were generally less than 16, indicative of N limitation. In 2005, all measured forms of dissolved inorganic N as well as urea and bulk dissolved organic N were positively lag correlated with dinoflagellate abundance. This suggests that no particular N species is required for dinoflagellate blooms to form, thus, there is no "smoking gun" with regard to nutrient controls on bloom development, and that dinoflagellates are capable of using a multitude N forms to supplement their growth and metabolism. In 2009, dissolved inorganic nutrient concentrations fell below the detections limit (0.02 μmol l^{-1}) while the *C. polykrikoides* bloom was forming, and N concentrations remained at or near detection limits for the duration of the study, while P concentrations remained relatively high (>2 μmol l^{-1}). *C. polykrikoides* abundance was not correlated with N concentrations during

2009, although this was likely due to N concentrations below the detection limit. Mixotrophy may play a role in the N acquisition of *C. polykrikoides*, but this remains to be tested.

Turbidity showed a semi-diurnal pattern during 2009 in the Lafayette River, and Chl *a* concentrations were greatest when turbidity was lowest, which occurred during low tide. Chl *a* concentrations were lowest during high turbidity on the flood tide. This pattern of turbidity varying with tidal stage did not appear to affect the formation and development of the *C. polykrikoides* bloom, which is capable of strong vertical migrations at speeds up to 4 m h^{-1} (Park et al. 2001) and is also capable of mixotrophy (Jeong et al. 2004), and so may be able to alleviate C limitation by grazing on other cells or by migrating to the surface in order to photosynthesize. Further, many of the bloom forming taxa in the Lafayette River such as *A. sanguinea*, *S. trochoidea*, and *Gymnodinium spp.* are capable of vertically migrating (Smayda 2002), and are also mixotrophic or are capable of ingesting other cells (Bockstahler and Coats 1993; Jeong et al. 2005b). This is likely a beneficial adaptation to living in a shallow, turbid, eutrophic estuarine environment.

One of the reasons the conditions antecedent to bloom formation often remain elusive may be due to the large spatial and small temporal scales over which sampling must be carried out in order to capture all of the relevant controls on blooms. Additionally, it is important to characterize areas where bloom growth occurs versus areas into which blooms may accumulate due to wind and tidal advection (Lucas et al. 1999b, 1999a). During 2008, the Lafayette River was identified as the initiation grounds for the *C. polykrikoides* bloom. However, because the data covered a large spatial area,

but was limited to weekly cruises, the exact location and the relevant physical and chemical variables were not captured in the data. DATAFLOW underway sampling is a tool that allows a visualization of Chl *a* over vast areas, but in order to be most effective, this type of sampling needs to be augmented with fixed station sampling at a high temporal frequency in areas that are prone to blooms, as well as to include a network of sensors recording physical data at high frequency and over a large area in order to determine physical controls on the growth and transport of the bloom over short timescales. The National Oceanic and Atmospheric Administration's Physical Oceanography Real Time System (NOAA PORTS) is one such network of sensors. Data in near-real time is made available on the PORTS website, and can be downloaded for use in studies such as this. In addition to meteorological data, water temperature, tidal height, salinity, and current velocity are some of the relevant physical aspects that must be included in any attempt at identifying controls on bloom formation. Water temperature can affect seasonal successional patterns (Marshall 1995; Marshall et al. 2005, Karentz and Smayda 1984), and the spring-neap variability affects water column stability and the propensity for blooms to form.

In the Lafayette River, nutrient concentrations increased following precipitation events, which are often brief but intense during summer months, and highly sporadic over the watershed. Daily precipitation totals are comparable, but often do not match between weather stations at Norfolk International Airport (KORF) and Naval Station Norfolk at Chambers field (KNGU), even though the distance between these two stations is <10 km. This highlights the sporadic precipitation patterns typical of the summer months in this region. The pulsing of nutrients in the Lafayette River due to brief but intense summer

storms often results in a shift in the phytoplankton community structure and a bloom ensues (Chapter 2). Summertime drought conditions can exacerbate the effects of nutrient runoff, as hardened soils do not absorb as much of the precipitation and overland runoff is intensified after long periods of time between rainfall events. This sheet flow leads to enhanced nutrient loading, as well as increased sedimentation and transport of other pollutants into local waterways. Norfolk is a low-lying city, near sea level, and as such is subject to flooding during these intense summer storms. Aging infrastructure and sub-surface plumbing that is nearing the end of its useful life often results in sanitary sewer overflows during these flooding events, which is another source of nutrients and contamination to the local waterways. Additionally, the presence of local populations of non-migratory Canada geese have lead to enhanced nutrient loading and poor water quality. The Lafayette River has been closed to shellfish harvesting for decades due to high counts of fecal coliform bacteria routinely found during state monitoring (VADEQ), and is presently on the US EPA list of impaired waters due to high levels of E*nterococci.*

Unfortunately, this scenario is likely to worsen in the future, as climate models show an increased likelihood of intensified storm activity, with longer periods of drought between events, increased coastal flooding due to rising sea levels, an increase in the abundance of harmful algal species including *C. polykrikoides*, and increased occurrence of anoxia and hypoxia (Najjar et al. 2010). Norfolk is presently experiencing one of the greatest rates of sea level rise on the US east coast, with a positive trend of 0.44 mm per year between 1927 and 2006 (Barbosa and Silva 2009), which would result in sea levels approximately 0.5 m higher than normal by the year 2099. However, model predictions for sea level rise in Chesapeake Bay suggest that by the year 2099, sea level could be

even higher, with levels reaching 0.7 to 1.6 m above current heights (Najjar et al. 2010), and water temperatures in the Bay could be 2–5 °C higher than normal (Najjar et al. 2010). This scenario would have profound effects on algal populations and patterns of phytoplankton succession, eutrophication and nutrient loading, and coastal inundation. Additionally, because the base of the food web, phytoplankton, could be drastically different as a result of climate change, the ecosystem function of the Chesapeake Bay is likely to be different in profound ways. Results presented in this dissertation are consistent with the idea that blooms of organisms such as *C. polykrikoides* may increase under future climate scenarios due to changing estuarine conditions.

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