

Nutrient uptake and regeneration as a means of initiating and sustaining algal blooms in the James River estuary

Abstract

Measurements of nutrient concentrations and nutrient uptake rates were collected from three stations in the James River/Elizabeth River complex. In the tidal fresh James River, monthly experiments were conducted to determine the dominant nutrients controlling cyanobacteria growth in the summer months. Weekly experiments were conducted in the mesohaline James River and in the headwaters of the Lafayette River (a tributary of the Elizabeth River) to determine nutrient controls on the annual dinoflagellate bloom, *Cochlodinium polykrikoides*. In 2012, there was no mono-specific cyanobacteria bloom of *Microcystis* as had been seen in the past, however increased chlorophyll *a* concentrations appeared to be fueled by forms of regenerated nitrogen such as ammonium, urea, and dissolved organic nitrogen. Although di-nitrogen fixation was measured, it was a very small percentage of the total nitrogen uptake for the tidal fresh station. The dinoflagellate bloom, *Cochlodinium polykrikoides*, initiated in the headwaters of the Lafayette River earlier than had been seen in years past (end of June) and lasted through August. The bloom spread to the poly- and mesohaline James River, and no particular nitrogen compound is cited as being a trigger for the bloom, however the bloom sustained itself on regenerated nitrogen compounds.

Introduction

In the Chesapeake Bay watershed total maximum daily loads (TMDL) for nitrogen (N), phosphorus (P) and sediment have been developed by the EPA (EPA 2010) to meet ambient water quality criteria for dissolved oxygen (DO), water clarity and chlorophyll *a* (chl *a*). The James River, the third largest tributary in the Chesapeake Bay watershed, does not suffer from low DO and water clarity problems are generally caused by sediment and therefore water quality criteria set for the James River have been largely aimed at chl *a* standards. In particular, increases in harmful algal blooms (HABs) suggest an unbalanced phytoplankton population and are one of the many impairments that have necessitated the development of chl *a* criteria to be protective against these blooms and other impairments. While links between nutrient loading and eutrophication have long been established, links between nutrient loading and HABs are complex. One reason is that many HAB species are dinoflagellates and these organisms have versatile metabolisms that allow them to exploit multiple nutrient sources, including organic N and carbon (C) (Stoecker 1999, Burkholder et al. 2008). These organisms often bloom when inorganic N is depleted and thus the relationship between nutrient loading and HABs is not obvious. In addition, the HAB species are diverse and distinct bloom organisms occupy different salinity denizens in the James River; for example cyanobacterial HABs affect water quality in the tidal fresh reaches of the estuary while dinoflagellate HAB organisms exert effects in the lower tidal meso- and polyhaline portions of the estuary (Marshall et al. 2006).

The James River contributes ~16% of the streamflow, 12% of the total N (TN) load, and 20% of the total P (TP) load to the Chesapeake Bay (EPA 2010). It is a partially- mixed, tidally-influenced tributary of the southern Chesapeake Bay, flowing from its headwaters in the Shenandoah Valley, through the fall line in Richmond, VA, to its confluence with the lower Chesapeake Bay in Hampton Roads, VA. The physical processes and mixing dynamics of this estuary have been studied in depth (Haas 1977, Shen et al. 1999, Shen & Lin 2006), and routine monthly monitoring by Virginia's Department of Environmental Quality (VADEQ) as part of the

Chesapeake Bay Program has provided insights on long-term trends within the estuary. Nutrient loading estimates through the watershed have long been based on streamflow at the fall line and while can predict nutrient loading for the upper estuary, it does not take into account the delivery of nutrients to the lower estuary via tidal forcing and coastal storms. In the lower James River estuary, meteorological events result in substantial nutrient inputs to the James River from overland flow and wet deposition during rainfall events and wind induced mixing of nutrients from the sediments. These nutrient inputs can trigger the initiation of blooms in the lower James River estuary (Mulholland et al. 2009, Morse et al. 2011). However, during bloom maintenance when the water column is stratified and there is little nutrient input from above or below, *in situ* regeneration of nutrients within the water column likely facilitates the persistence of these blooms. Within the James River watershed there are seasonal algae blooms that include dinoflagellates in the lower James River and cyanobacteria in the upper James River. Many species within these two groups are considered harmful either through the production of excess biomass that leads to adverse impacts to designated uses or through the production of toxins. In the upper James River, *Microcystis* commonly blooms and this organism produces microcystin, a known hepatotoxin.

Here we show results of a research focused program (2011 – 2012) designed to coordinate with modeling efforts to describe nutrient regeneration and uptake as they related to bloom development in the upper and lower James River. Specifically, we focused our research in the upper and lower James to determine which nutrient forms are important to promotion and persistence of blooms, the degree to which nutrient regeneration promotes and sustains algal blooms, and the temporal relationship between the N species utilized and bloom composition. In the upper James we also aimed to determine the importance of di-nitrogen (N₂) fixation and the role it may have on development and persistence of HABs.

Methods:

Three stations were sampled over the course of several months throughout the James River estuary (Fig. 1, Table 1). One station was sampled once a month, June through October 2011 and 2012 in the tidal fresh portion of the James River at the VCU Rice Center (JMSTF; Fig. 1). Two stations were sampled weekly beginning in June 2012 and ending in September 2012, one station was in the mesohaline section of the James River estuary (JMSMH; Fig. 1) and the second station was located in the headwaters of the Lafayette River near Ashland Circle (AC; Fig. 1). Precipitation data were obtained from Richmond and Norfolk International airports (Fig. 1) and streamflow data were obtained from the United States Geological Survey (USGS) streamflow gauge at Richmond, VA for the James River (the fall line). Monitoring and mapping of the meso- and polyhaline portions of the estuary were conducted weekly between March and October, 2012 by the Hampton Roads Sanitation District (HRSD). Technicians used an underway collection system (DATAFLOW) equipped with a YSI 6600 multiparameter DataSonde measuring fluorescence, temperature, salinity, pH, DO, and turbidity with data recorded at 0.25 Hz (Egerton & Hunley 2012). For all weekly and monthly experimental samples, surface water grab samples were collected from docks and/or a bridge using a clean bucket at all three stations. Temperature was measured immediately and samples were preserved (mercuric chloride) and sealed for dissolved inorganic carbon (DIC) measurements on-site. For each grab sample at stations JMSMH and AC, surface water was collected into an acid-cleaned 20 L carboy and kept in the dark until transport back to the laboratory for further filtration,

experiments, and analysis (described below). At station JMSTF, all incubations and experiments were conducted on site.

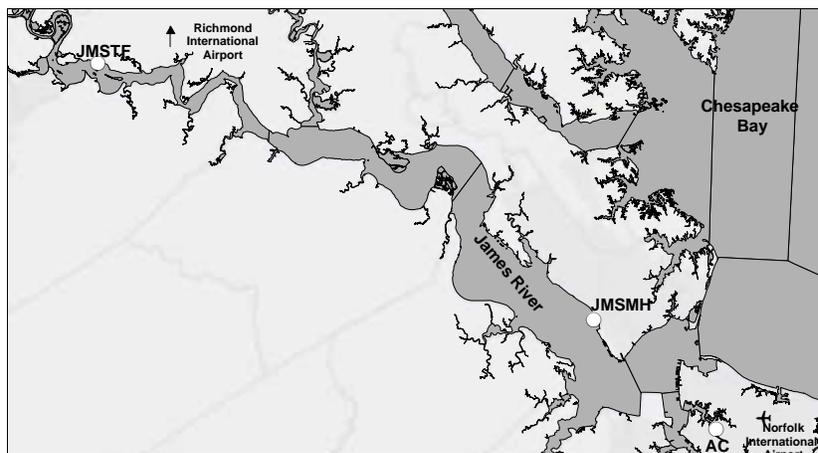


Figure 1. Map of the tidal fresh (JMSTF), mesohaline (JMSMH), and Lafayette River at Ashland Circle (AC) sampling sites for weekly and monthly experiments. Precipitation data obtained from Richmond and Norfolk International airports.

Table 1. Station identifications, locations, Chesapeake Bay Program (CBP) segment, latitude and longitude for sampling sites for weekly and monthly experiments.

Fixed Station	Location	CBP Segment	Latitude (decimal degrees)	Longitude (decimal degrees)
JMSTF	Rice Center Pier	JMSTF	37.271048	-77.079735
JMSMH	Huntington Beach Pier	JMSMH	37.005294	-76.459351
AC	Ashland Circle	LAFMH	36.880390	-76.272525

Once in the lab, nutrient samples were collected after pumping water through acid-cleaned pump tubing and filtering through a 0.2 μm cartridge filter into sterile 50 mL centrifuge tubes and immediately frozen. Two mL aliquots of the filtrate were also frozen for analysis of dissolved free amino acids (DFAA). Chl *a* samples (5 – 30 mL) were immediately vacuum filtered onto triplicate GF/F filters and placed into sterile 15 mL centrifuge tubes and frozen until analysis (within 3 days).

Water was also parceled into triplicate acid-cleaned 60 mL PETG bottles for N and C uptake experiments using highly enriched (96 – 99%) ^{15}N and ^{13}C labeled compounds (Glibert & Capone 1993, Mulholland et al. 2002): amino acid mix (aamix), NH_4^+ , nitrite (NO_2^-), nitrate (NO_3^-), and urea. Stable isotope additions were on the order of 10% of the ambient nutrient pool for each substrate. Bottles were then placed in incubators kept at temperatures similar to water column temperatures under fluorescent lighting for 30 minutes. Incubations bottles at station JMSTF were placed outdoors in a water bath, temperature-adjusted to match ambient water, and covered with black screen to match *in situ* riverine light levels. Incubations were terminated by gentle filtration onto combusted (450°C for 2 hours) filters (GF/F) and filters which were placed into sterile cryovials and frozen until analysis. Filters were dried (~2 days) at 40°C, pelletized in tin discs and analyzed using a Europa 20/20 isotope ratio mass spectrometer (IRMS) equipped with an automated N and C analyzer as a preparation model. Rate calculations for uptake of ^{15}N

and ^{13}C stable isotope tracers were based on a mixing model and equations from Montoya et al. (1996) and Orcutt et al. (2001). Primary productivity was measured using ^{13}C labeled bicarbonate in triplicate, acid-cleaned 60 mL PETG light and dark bottles (Mulholland & Capone 2001). Primary productivity incubations were terminated after 4 and 24 hours and filters were prepared and analyzed using IRMS as described above. N_2 fixation experiments were conducted in triplicate at station JMSTF only, using $^{15}\text{N}_2$ -labelled gas. Uptake experiments were initiated by adding tracer additions ($< 10\%$) of highly enriched (99%) $^{15}\text{N}_2$ to gas tight bottles filled with whole water (Montoya et al. 1996, Mulholland et al. 2004). Incubations were terminated after 4 and 24 hours by gentle filtration and filters were prepared and analyzed as described above.

Analysis of chl *a* samples was conducted within 3 days of sampling by extracting the chl *a* from the filters in 10 mL of 90% acetone for 24 hours in a freezer. After 24 hours, samples were brought to room temperature, centrifuged, and analyzed on a Turner fluorometer using a non-acidification method (Welschmeyer 1994). Nutrient samples that had been frozen until analysis were analyzed for NO_2^- , NO_3^- , urea, total dissolved N (TDN), and phosphate (PO_4^{3-}) on an Astoria Pacific autoanalyzer using standard colorimetric methods (Parsons et al. 1984). NH_4^+ was measured using the manual phenol hypochlorite method and spectrophotometric detection (Solorzano 1969). DFAAs were analyzed using high performance liquid chromatography (HPLC), modified by Cowie and Hedges (Cowie & Hedges 1992) and values were converted to N and C specific DFAA concentrations upon the average N and C content in each amino acid.

Results

James River Tidal Fresh

Although no mono-specific bloom was observed in the tidal fresh region (particularly DEQ station TF5.5) in 2011 and 2012 as HAB species did not contribute greatly to chl *a* concentrations and the community was very diverse (Marshall and Egerton DEQ report 2012), a typical seasonal increase in chl *a* was apparent. Seasonal chl *a* is likely a function of available nutrients, light, and temperature and there was a significant linear relationship between chl *a* and temperature in 2012 (Fig. 2). This is a typical relationship during the summer months, as 10 years of monitoring data from CBP station TF5.5 (closest to the Rice Center) shows a similar significant linear trend (Fig. 2).

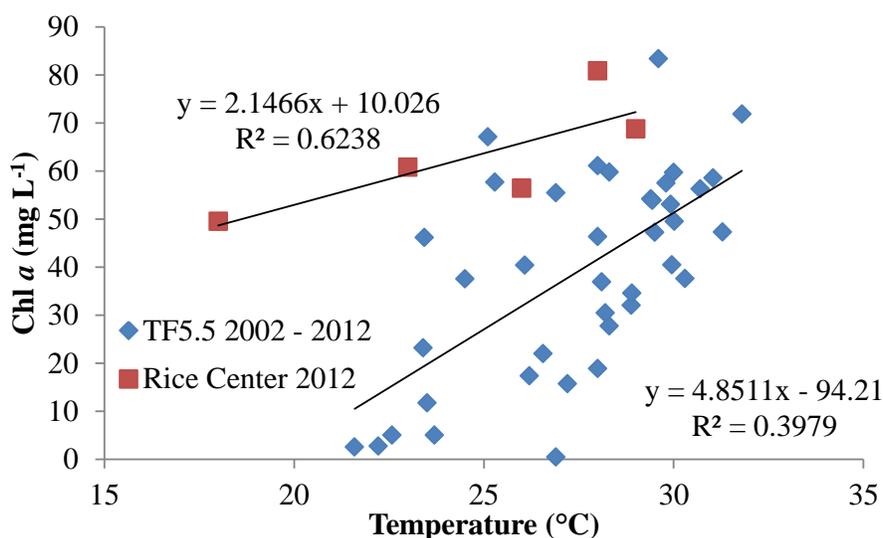


Figure 2. Significant linear relationships between chl *a* ($\mu\text{g L}^{-1}$) and temperature ($^{\circ}\text{C}$) for June, July, Aug., and Sept. between 2002 and 2012 at station CBP monitoring station TF5.5 and from the Rice Center Pier in 2012.

Concentrations of dissolved inorganic N (DIN) and dissolved phosphate (DIP) during 2011 and 2012 summer samples suggested that algal populations were N limited (DIN:DIP < 16) during times of peak chl *a* but were often P limited (DIN:DIP > 16) at other times. Dissolved N concentrations were highly variable during both bloom initiation and maintenance phases and N uptake did not always reflect the dominant form of dissolved N. In 2011, urea N and NH_4^+ were depleted as chl *a* concentrations increased (Fig. 3A). The decline of chl *a* was not captured in 2011 due to storm events (Hurricane Irene and Tropical Storm Lee) prohibiting sampling efforts. In 2012, NH_4^+ and NO_2^- became depleted after chl *a* concentrations reached a seasonal maximum, then as chl *a* concentrations decreased, NH_4^+ and NO_2^- increased significantly (Fig. 3B). NO_3^- concentrations were greatest as chl *a* was on the decline, and urea concentrations remained relatively constant throughout and were lowest when chl *a* concentrations were lowest (Fig. 3B). In both years, TDN was comprised mainly of the DON pool (68 – 95%) and TDN and DON concentrations were significantly correlated with chl *a* concentrations (Fig. 4). Presumably, as temperatures became favorable for growth, DIN compounds were assimilated and DON was produced during seasonal chl *a* growth. As DIN became depleted, chl *a* declined, and the microbial community took advantage of the available DON, subsequently regenerating DIN. However, long-term data from CBP monitoring station TF5.5 did not show any statistical trends between chl *a* and DON concentrations and only weak negative trends were observed between chl *a* and DIN and TDN (data not shown).

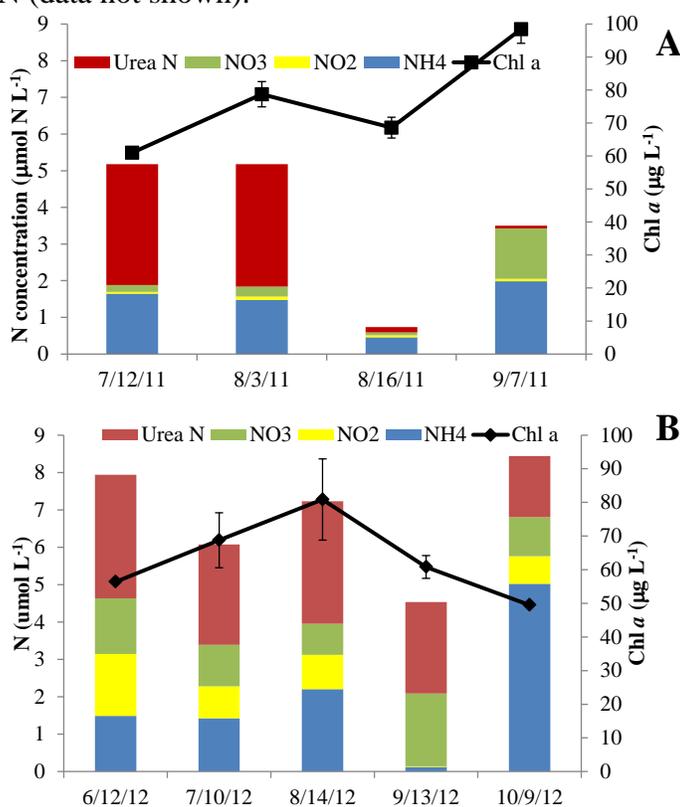


Figure 3. Monthly surface NH_4^+ , NO_2^- , NO_3^- and urea N concentrations ($\mu\text{mol N L}^{-1}$; left axis) and chl *a* concentrations ($\mu\text{g chl L}^{-1}$; right axis) in 2011 (A) and 2012 (B) at station JMSTF.

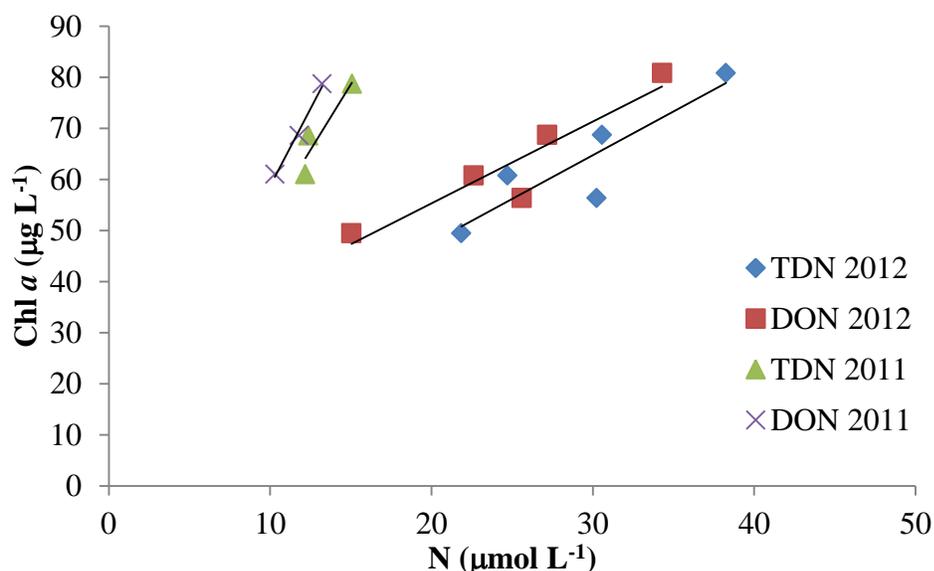


Figure 4. Chl *a* concentrations (µg chl L⁻¹) versus TDN and DON (µmol N L⁻¹) concentrations in 2011 and 2012 showing significant linear relationships at station JMSTF.

In 2011 and 2012, uptake experiments conducted at the Rice Center Pier showed that during initiation and maintenance phases (7/12/2011 and 8/3/2011 in Fig. 5A and 6/12/12, 7/10/12, and 8/14/12 in Fig. 5B) of high concentrations of chl *a*, urea N accounted 50% to 80% of the total N demand during the seasonal biomass high (Fig. 5A). Only during the chl *a* decline (8/16/2011 and 9/7/2011 in Fig. 5A, 9/13/12 and 10/9/12 in Fig. 5B), did inorganic N, particularly NH₄⁺ and NO₃⁻, account for about 50% of the total N demand of natural phytoplankton population. In 2012, similar to N uptake rates, primary productivity rates were greatest at peak chl *a* concentrations on 8/14/12 (data not shown).

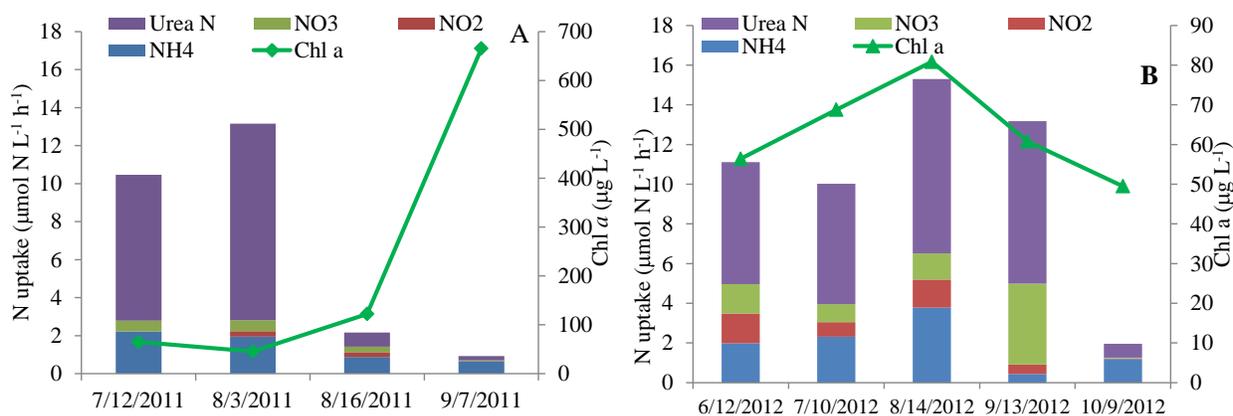


Figure 5. Weekly N uptake rates (µmol N L⁻¹ h⁻¹; left axis) and Chl *a* concentrations (µg L⁻¹; right axis) in whole water collected from the Rice Center pier in 2011 (A) and 2012 (B).

Although rates were much lower compared to total N uptake, active N₂ fixation was measured in the upper James (Table 2). Often cyanobacteria dominate the chl *a* signal during the

summer months in the tidal fresh James, and although they weren't always the dominant species, active N₂ fixation is important because N₂ fixing cyanobacteria can alleviate dissolved N limitation by fixing atmospheric N₂. These are the first measurements of active N₂ fixation in the James River and this may be a previously unquantified or emerging source of new N to phytoplankton communities, however N₂ fixation rates relative to total N uptake rates are considerably lower (Table 2).

Table 2. N₂ fixation rates (nmol L⁻¹ h⁻¹) with standard error for incubations in parentheses and % of total N uptake for sampling dates in 2011 and 2012 at station JMSTF. Reporting results from 4 hour incubations only.

Date	N ₂ fixation (nmol L ⁻¹ h ⁻¹)	% total N uptake
7/12/11	18.2 (1.56)	0.16
8/3/11	74.4 (12.9)	0.48
8/16/11	54.9 (19.4)	1.6
9/7/11	5.35 (0.85)	0.41
6/12/12	3.48 (0.91)	< 0.01
7/10/12	67.3 (5.95)	0.67
8/14/12	8.89 (1.60)	0.06
9/13/12	5.20 (0.88)	0.04
10/9/12	1.24 (0.01)	0.06

Lower James River

Weekly sampling at stations JMSMH and AC provided synoptic data between June and September 2012. Chl *a* concentrations were considerably lower at station JMSMH, compared to station AC where the chl *a* concentrations began to rise at the end of June consisting of the primary bloom former, *Cochloidium polykrikoides* (Fig. 6). Temperature was an important controller of bloom initiation at station AC in the headwaters of the Lafayette River (Fig. 6; see Filippino and Mulholland 2012 DEQ bloom report). As temperatures warmed at JMSMH and through estuarine circulation, the bloom appeared at JMSMH in mid-July (Fig. 6B).

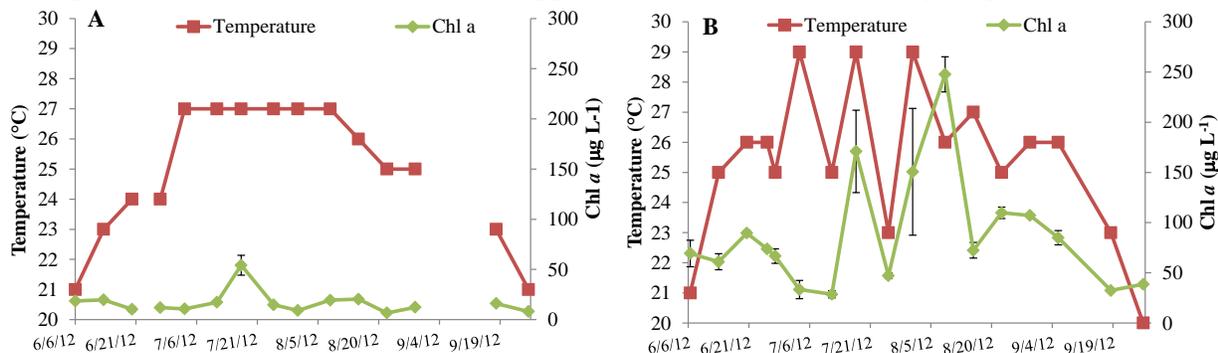


Figure 6. Weekly surface temperature (°C; left axis) and chl *a* concentrations (µg/L; right axis) at stations JMSMH (A) and AC (B).

The bloom lasted through the end of August at both stations and appeared to be sustained on recycled nutrients. In situ regeneration in the water column was likely the dominant process

contributing N for bloom sustainability. The regenerated compounds NH_4^+ and NO_2^- decreased and in the case of NO_2^- , concentrations were below the limit of detection during the bloom at both stations while urea concentrations increased during the bloom (Fig. 7). NO_3^- , NO_2^- , and NH_4^+ concentrations increased at the end of the bloom at both stations, likely due to nutrient regeneration. TDN and DON concentrations were greatest when chl *a* concentrations were greatest (Fig. 8). Precipitation events occurred consistently throughout this time period, but nutrient concentrations only increased slightly suggesting rapid utilization of any nutrient inputs.

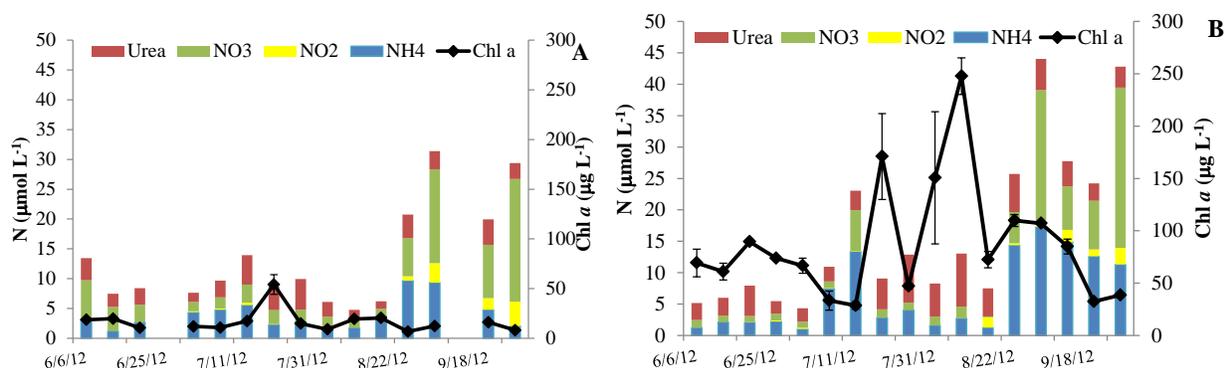


Figure 7. Weekly surface NH_4^+ , NO_2^- , NO_3^- , and urea N concentrations ($\mu\text{mol N L}^{-1}$; left axis) and chl *a* concentrations ($\mu\text{g chl L}^{-1}$; right axis) at stations JMSMH (A) and AC (B).

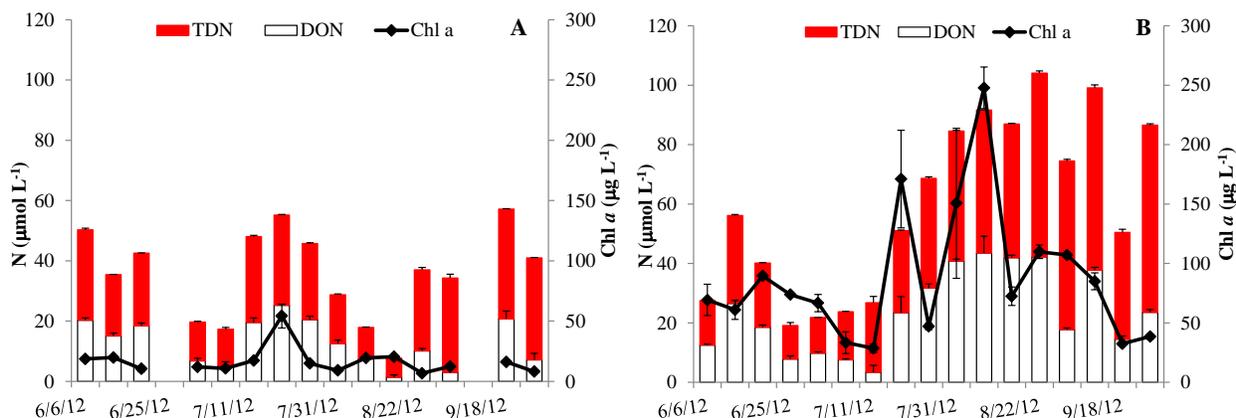


Figure 8. Weekly surface TDN and DON concentrations ($\mu\text{mol N L}^{-1}$; left axis) and chl *a* concentrations ($\mu\text{g chl L}^{-1}$; right axis) stations JMSMH (A) and AC (B).

At both stations, concentrations of PO_4^{3-} increased during the bloom, similar to TDN concentrations, and there was a shift from DIN to P limitation at various stages of the bloom (Fig. 9). At JMSMH, $\text{DIN}:\text{PO}_4^{3-}$ was on the decline during bloom initiation, then became DIN limiting briefly after bloom's demise (Fig. 9A). DIN was limiting ($\text{DIN}:\text{PO}_4^{3-} < 16$) during the height of the bloom, and P was limiting during bloom initiation and during the bloom's demise ($\text{DIN}:\text{PO}_4^{3-} > 16$) at station AC (Fig. 9B).

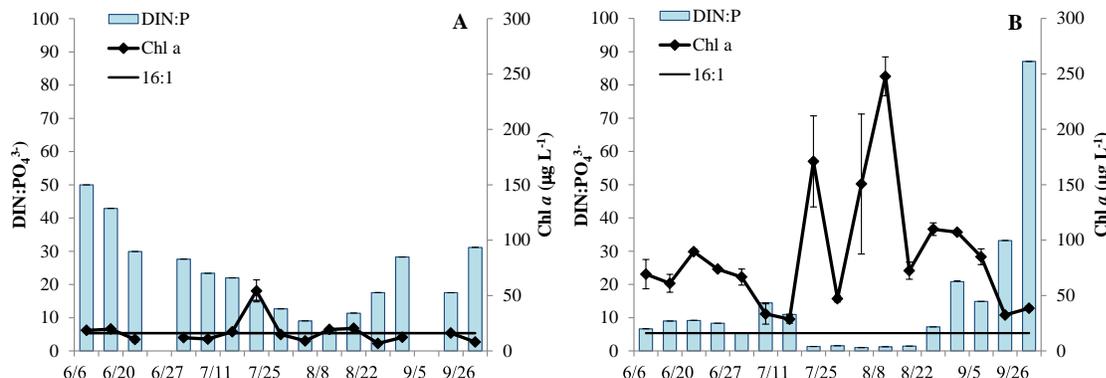


Figure 9. Weekly surface DIN:PO₄³⁻ (left axis) and chl *a* concentrations (µg chl L⁻¹; right axis) at stations JMSMH (A) and AC (B), with the solid black line denoting the 16:1 Redfield ratio.

Overall, N uptake rates were much lower at the JMSMH station (Fig. 10A) compared to the AC station (Fig. 10B). NH₄⁺ and urea contributed the most to N uptake rates before and during the bloom at both stations (Fig. 10). Primary productivity correlated positively and significantly at station AC, but here was no significant relationship at station JMSMH (data not shown).

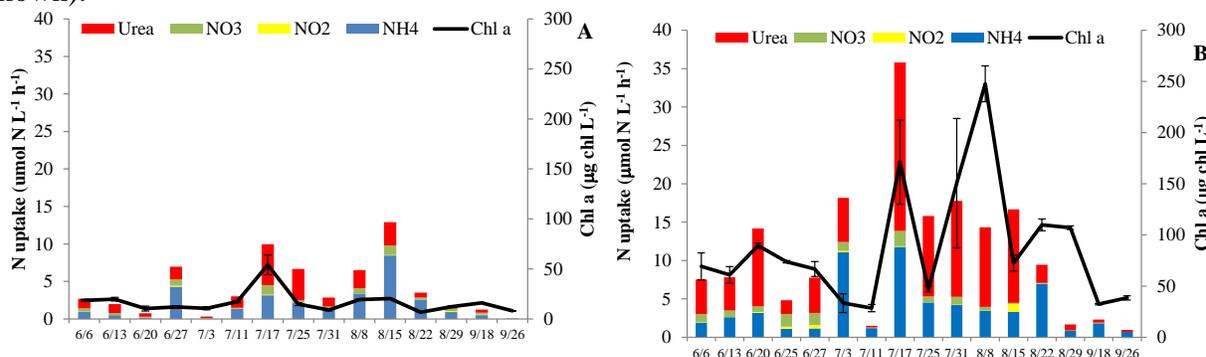


Figure 10. Weekly chl *a* normalized N uptake rates (µmol N µg chl h⁻¹; left axis) and chl *a* concentrations (µg chl L⁻¹; right axis) at stations JMSMH (A) and AC (B). Gaps in data represent samples not yet analyzed.

A pre-bloom (6/28) and bloom (8/7) event were sampled at the mouth of the Lafayette River (Norfolk Yacht and Country Club docks [NYCC]) and TDN concentrations were 1.5 times greater during the height of the bloom, and consisted mainly of urea N (Fig. 11A). The greatest uptake rates of the summer were observed during the bloom, and NH₄⁺ was the predominant form of N taken up (Fig. 11B). Primary productivity was 5.5 times greater during the bloom event, compared to the pre-bloom event at NYCC, but higher primary productivity rates were found to be higher up-river at station AC.

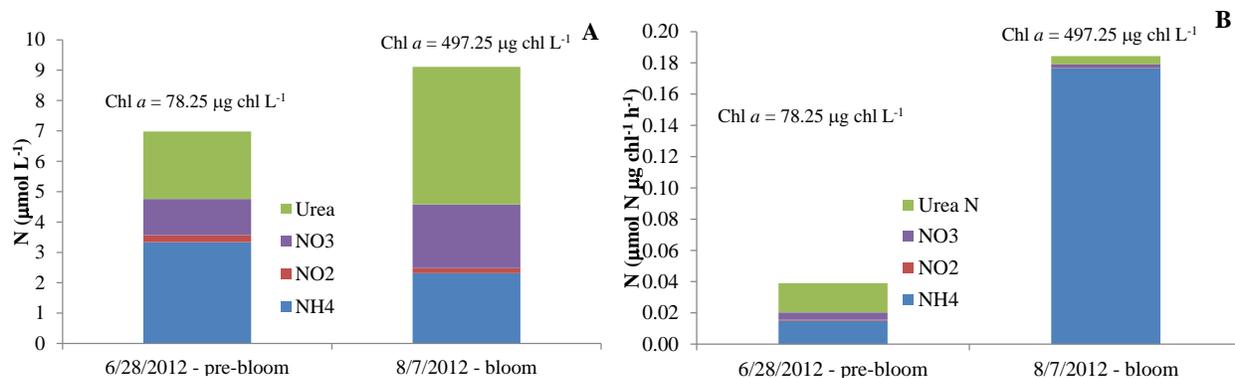


Figure 11. N concentrations ($\mu\text{mol N L}^{-1}$; A) and chl-normalized N uptake rates ($\mu\text{mol N } \mu\text{g chl}^{-1} \text{ h}^{-1}$; B) at the mouth of the Lafayette River (NYCC) during pre-bloom (6/28/12) and bloom (8/7/12) events.

Discussion and Conclusions

Nutrient concentrations in the tidal fresh James suggest that DIN was used and DON was produced during seasonal chl *a* growth. As chl *a* reached concentrations typical for the fall, NH_4^+ increased and DON, including urea, decreased, likely as a function of heterotrophic activity. Prior to the increase in chl *a* in 2011, urea N was the dominant form of N taken up, and N_2 fixation accounted for a very small percentage (< 2 %) of total N uptake. In 2012, urea N was also the dominant form of N taken up, and uptake rates remained between 10 and 16 $\mu\text{mol N L}^{-1} \text{ h}^{-1}$ throughout the summer, until chl *a* declined in October. The rates remained steady in 2012 likely due a smaller cyanobacteria population, chl *a* concentrations were an order of magnitude lower in 2012 than the seasonal high observed in 2011. This suggests nutrient preferences of the cyanobacteria and/or the rapid recycling of particular N pools. Long-term monitoring data does not show any statistically significant trends between chl *a* and N compounds in the tidal fresh James (DEQ TF5.5), and this is likely due to the artifact of monthly sampling. Nutritional requirements for chl *a* growth can change weekly, daily, and even hourly depending on the types of organisms and N sources present (Mulholland et al. 2004, Mulholland & Lomas 2008).

The weekly studies in the lower James River required a higher level of evaluation, thus principal component analysis (PCA) was conducted on all of data points at each station, AC and JMSMH). The data were weighted and transformed in order to account for the large difference in variance of the individual data points. The first and second principal component account for more than 60% of the total variance, and thus we determined the principal component coefficients and scores based on the first two principal components (Figs. 12 & 13). At station AC, each quadrant is represented by different bloom stages since data points cluster by date. During pre-bloom, and initiation phase (June 6 – July 25), DIN concentrations were lowest and NO_3^- and NO_2^- uptake rates and temperature were greatest (Fig. 12). As bloom concentrations increased, DON, urea, and PO_4^{3-} concentrations were at their highest. As the bloom declined, DIN concentrations increased, likely due to extensive regeneration of cellular material during the bloom decay. Almost daily precipitation occurred throughout the summer, but DIN concentrations did not increase in the hours following precipitation events, suggesting rapid utilization of any inorganic nutrient inputs. The dominant forms of N taken up by microbial

communities in the lower James River estuary were urea and NH_4^+ which together comprised about 90% of the observed N uptake during the *Cochlodinium* bloom. This is consistent with previous observations (Mulholland et al. 2009). In the lower James River, while bloom initiation is often tied to a meteorological event such as a storm or a period of intense wind-mixing and benthic nutrient injection, blooms thrive under physically quiescent conditions when in situ nutrient regeneration is likely to fuel the growth and proliferation of blooms.

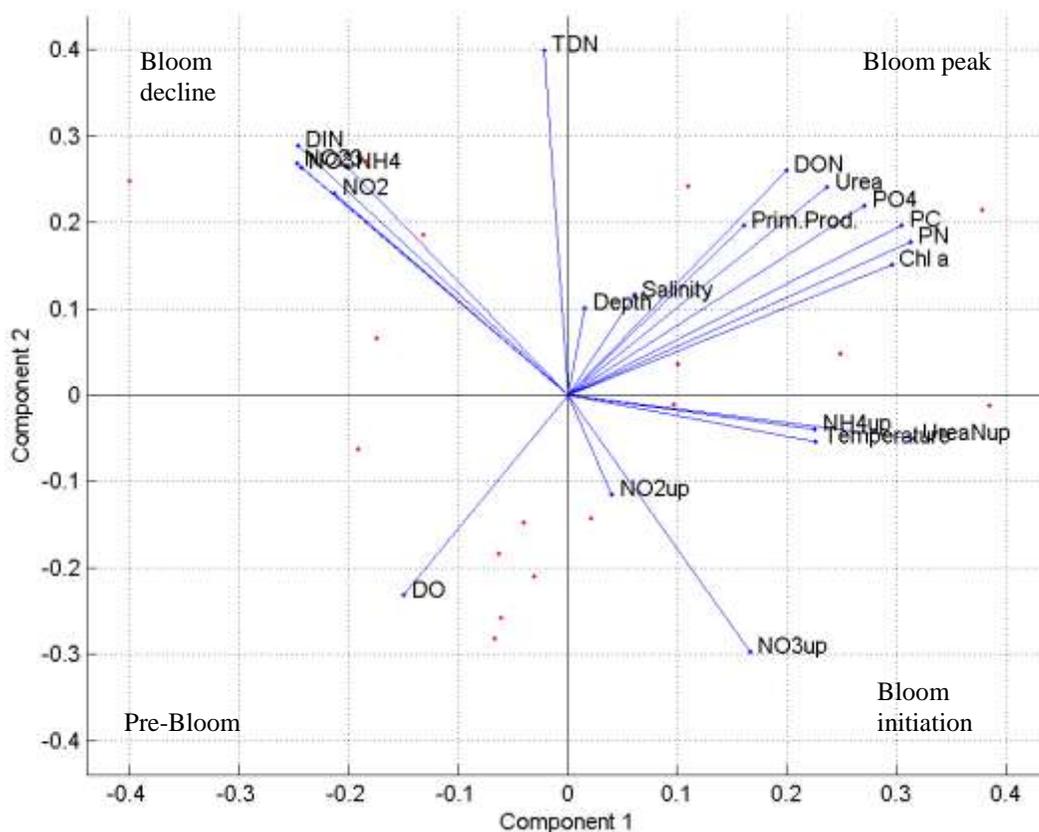
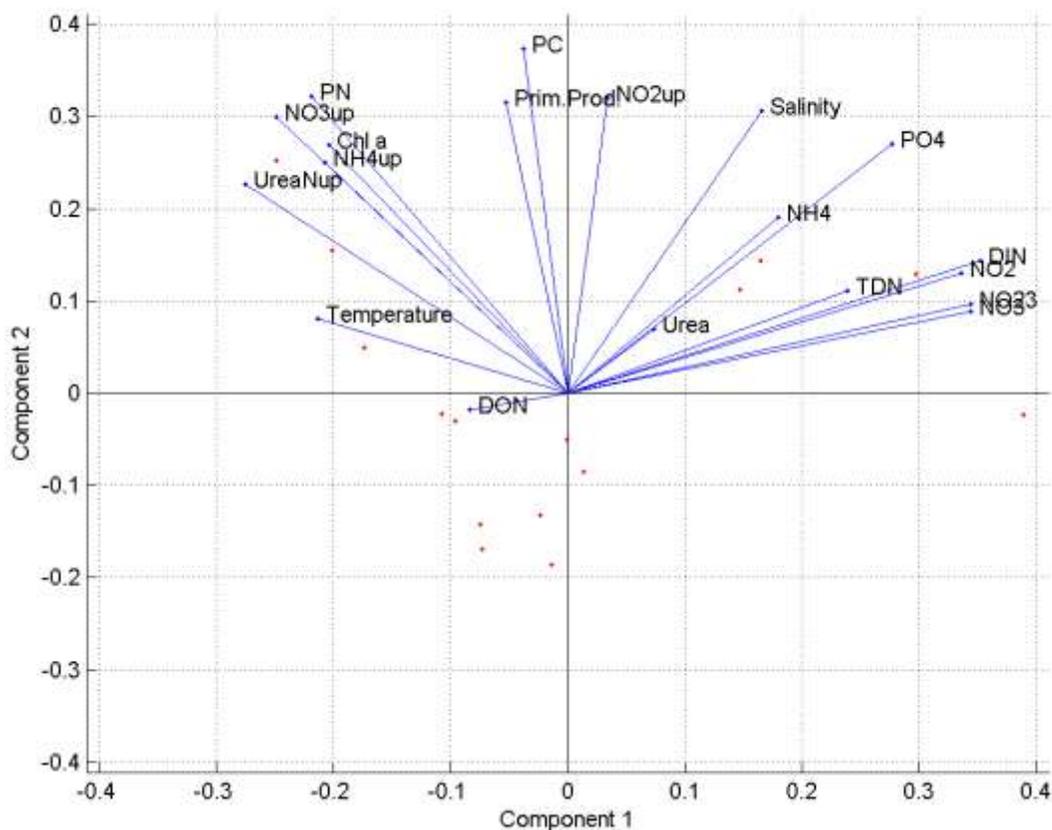


Figure 12. Biplot of principal component coefficients for each variable and the principal component scores for weekly observations at AC.

At station JMSMH, the bloom was not as intense, and weekly observations did not capture distinct pre-bloom/bloom/post-bloom periods (Fig. 13). However, the high chl *a* concentrations cluster in the upper left quadrant, where the vectors for NH_4^+ , NO_3^- , urea uptake and primary productivity rates are greatest (Fig. 13). Also similar to site AC, high DIN concentrations correlate with sample dates in the end of August and the beginning of September (Fig. 13). Bloom dynamics are not as distinct in this deeper station that is influenced by larger-scale Bay hydrography like oceanic and freshwater influences from a variety of sources.



Summary

- No significant bloom event was observed in the tidal fresh James River in 2011 and 2012. Seasonal chl *a* dynamics appear to be temperature driven with urea N fueling growth
- Although the headwaters of the Lafayette River (AC) are fundamentally different in terms of bathymetry and hydrography compared to the main stem James River MH station (JMSMH), bloom initiation of the dinoflagellate *Cochlodinium polykrikoides* was triggered by temperature at both stations.
- At both stations, bloom maintenance was controlled by N regeneration, and DIN concentrations increased as the bloom declined.
- Also at both stations, PO_4^{3-} was the limiting nutrient ($\text{DIN}:\text{PO}_4^{3-} > 16$) prior to and just after the blooms while DIN became limiting during the bloom ($\text{DIN}:\text{PO}_4^{3-} < 16$).
- PCA could be a valuable statistical tool to evaluate the importance of all variables in each stage of bloom development.

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