

**Environmental factors promoting algal blooms in the Lower James River estuary –
Year 2**

*Subtask 1.1 and 1.3 Expand monitoring network & Determine environmental factors
favoring algal blooms*

Data Report

Submitted to

The Virginia Department of Environmental Quality

by

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Abstract

A comprehensive field study was conducted in the Lafayette River, a sub-tributary of the lower James River, in 2013 to assess the environmental factors favoring initiation and fate of high chlorophyll *a* concentrations. Through monitoring using moored sensors, surface water collection systems, and underway surface water mapping, the onset of the seasonal bloom of *Cochlodinium polykrikoides* was observed in late July, almost a month later compared to 2012, but within a time frame consistent with previous years (2008 and 2011). Localized precipitation events provided less rain in early summer in 2013 and water temperatures did not reach previously identified target temperatures of 24 to 26°C until later in the summer. However, consistent with prior years, the bloom began in the shallow headwaters of the Lafayette and moved out to the mouth through estuarine circulation. Nutrients were not statistically related to precipitation events, however increased nitrogen compounds were observed after some rain events, and decreases in ammonium concentrations were linked with increases in chlorophyll *a* concentrations. After rain events, stratification was found to be highest in the headwaters of the Lafayette compared to the mouth. During the bloom event, a sub-surface chlorophyll *a* maximum was observed that was typically not present during non-bloom periods. Diel sampling revealed two daily peaks in carbon biomass during the *Cochlodinium* bloom and a significant negative correlation was observed between chlorophyll *a* and ammonium concentrations. These findings may have implications as the current standards do not consider the vertical water column structure or diel patterns during the bloom.

Introduction

The work addressed in this report was conducted in 2013 for the Virginia Department of Environmental Quality's (DEQ) assessment of the numerical chlorophyll *a* (chl *a*) criteria in the James River. Specifically, this research focused on the Lower James River, addressing subtask 1.1 – *Expand monitoring network* and subtask 1.3 – *Determine environmental factors favoring algal blooms*. This work built on our previous research conducted in Year 1 and on past research aimed at understanding the physical and nutritional factors promoting *Cochlodinium polykrikoides* blooms in the lower James River estuary. This research responded directly to modeling needs by resolving the development and persistence of blooms on short timescales of variability (days to weeks) that aren't captured in current monitoring programs and identifying physiological, nutrient, and physical factors promoting bloom initiation. Previous research demonstrated that blooms of potentially harmful taxa are initiated at specific locations in response to stochastic events and are then transported and spread through the estuary where they can exert impacts (Mulholland et al. 2009, Morse et al. 2011). Prior to 2013, most of our efforts were spent characterizing

blooms of the dinoflagellate, *Cochlodinium polykrikoides*, despite the fact that we routinely observe a seasonal succession of potentially harmful dinoflagellate blooms in the lower James River estuary (Fig. 1) that include

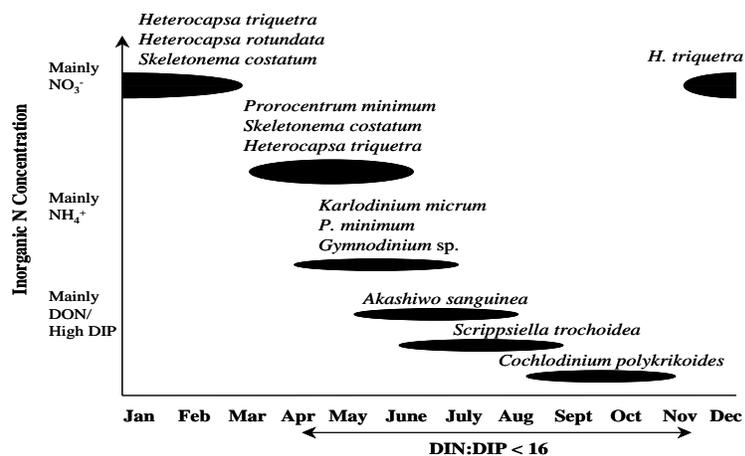


Figure 1. Species succession in the lower James River estuary and relative N concentration.

Heterocapsa triquetra in the winter and spring, *Prorocentrum minimum* later in the spring, *Gymnodinium* sp. and *Akashiwo sanguinea* in the summer followed by the dinoflagellates, *Scrippsiella trochoidea* and *Cochlodinium polykrikoides*. The purpose of this study was to identify causal factors promoting blooms of a broad spectrum of potentially harmful algae (mostly dinoflagellates) in the lower James River estuary, and identify triggers causing them to bloom. We have already found that many of these species are not dependent on one type of N compound, and that the DIN:DIP ratio may not only shift seasonally, but also limitation may vary from P to N and back to P again during an individual bloom event (see Filippino and Mulholland 2012 DEQ reports).

Intense algal blooms result in high chl *a* concentrations that result in impairments to water quality and living resources. Many algal species are also directly harmful to aquatic life or exert impacts through the food web. In addition, drawdown of dissolved oxygen (DO) commonly occurs during the decomposition of blooms and this exerts further impacts on water quality. While it is generally accepted that increased nutrient loading adversely impacts water quality through eutrophication, the direct link between nutrient loading, chl *a* impairment, and harmful algal blooms is often complicated in estuaries because of the large variability in physical forcing. Physical factors such as freshwater input, surface heating, and both wind and tidally-driven mixing can all contribute significantly to water quality impacts and nutrient dynamics in the Lower James River estuary. The inputs of nutrients into these systems are often dominated by high intensity rainfall events, which are not adequately captured by most monitoring programs. Impacts of these events (e.g., high chlorophyll) may then be transported to other parts of the estuary through estuarine circulation.

In Year 1, in conjunction with Hampton Roads Sanitation District (HRSD) and their Chlorophyll Monitoring and Assessment Program (CMAP), we conducted additional nutrient mapping in response to storms to evaluate nutrient pulses in the Lafayette River and also established two shallow water monitoring stations in the Lafayette River equipped with YSIs and automated ISCO samplers. These results showed that meteorological and physical forcing are key factors promoting the initiation of *Cochlodinium* blooms at specific sites and estuarine circulation promotes their transport and accumulation in the Lower James River (2012 data report). In Year 2, we examined the environmental triggers controlling the emergence of blooms by conducting daily sampling at the two fixed monitoring sites during 2013 and also monitored impacts on water quality (low DO and mortality of aquatic life).

In addition to the rapid shifts that occur in phytoplankton populations over the course of days, many of the bloom-forming dinoflagellates in the Lower Chesapeake Bay exhibit behavior or are influenced by tidal forcing and so their abundance in surface waters varies over the course of a day making it difficult to establish their abundance and impact. Lack of information on the diel migrations and diel variability in the distribution of phytoplankton populations currently curtails their accurate parameterization in models. In Year 2, we conducted diel sampling at one of the fixed continuous monitoring stations during blooms.

This research is needed because current regulatory efforts to restore water quality in the James River, and aquatic systems worldwide, fail to adequately account for event-driven and localized inputs that lead to water quality impairments and fail to account for the behaviors and physiology of algae that form blooms. We maintain that local stochastic events in the lower tributaries to the Chesapeake Bay, and the presence of diverse groups of bloom forming algae in the native phytoplankton community can exert large local to regional impacts on water quality

and may be drivers for algal bloom formation and chl *a* impairments. The current regulatory framework for managing the lower James River estuarine ecosystem does not adequately account for event-driven water quality impacts that result in ephemeral algal blooms that may include multiple species and the direct export of these impacts to connecting water bodies due to physical transport. The use of total maximum daily loads (TMDLs) to control algal biomass is complicated by that fact that: 1) much (perhaps most) of the nutrient loading takes place during stochastic events that are not well sampled and poorly quantified, 2) the impact of nutrients input through stochastic events may result in storage of nutrients in the system, thereby preventing the realization of short-term goals in response to management actions, 3) the inputs and impacts of nutrient loading during local stochastic events in urban regions are poorly quantified for most estuaries because few measurements are made during and after storms, weather systems are often localized near coastal systems and are not initiated in the watershed, and estuarine models do not currently feedback to watershed models. A better understanding of the impacts of storms on water quality is necessary if we are to prevent, control, or mitigate the impacts of nutrient loading and resulting high chlorophyll and harmful algal blooms.

Methods

In 2013, we expanded the monitoring and sampling that was established in the summer of Year 1, continuing to leverage existing and committed monitoring programs of HRSD. We sampled before, during, and after storms, and monitored the movement of nutrients and particle biomass and their water quality impacts (e.g., high chl *a* and low DO) through this system using underway mapping techniques, moored instruments, and grab sampling. In Year 2, we also employed the same methods to measure physical, chemical and biological processes and constituents in the Lower James River estuary in response to storms and we conducted daily

sampling at two already-established fixed, continuous monitoring sites and conducted one set of diel experiments to determine the vertical movement of algae and obtain estimates of community metabolism on daily timescales.

Fixed point monitoring

Between March and October 2013, two fixed continuous monitoring (CONMON) stations equipped with YSI 6600EDS V2 data sondes and ISCO Model 6712 sequential samplers were placed in the Lafayette River at Ashland Circle (AC) and the Norfolk Yacht and Country Club (NYCC; Fig. 2; Table 1).

These fixed stations continuously monitored water quality parameters including depth, water temperature, salinity, pH, chl *a*, turbidity, and DO. Data was collected every 6 seconds, and tidally resolved data was calculated using a low-pass Butterworth filter. The fixed stations were deployed off of existing piers within an attached 4 inch PVC housing and the sondes were secured 1-2 meters above the bottom. ISCO samplers were also placed at these sites to collect river water for nutrient analysis (described below) before, during and after rain events between July and September, 2013. The activation of the samplers was based upon forecasted storm activity which was defined as expected rainfall exceeding 0.5 inches. Upon forecast of an approaching storm the samplers were set up 24 hours in advance, sampling was continued 30 minutes and then hourly up to 24 hours after an event. If the storm did not occur as forecasted



Figure 2. Continuous monitoring stations, NYCC and AC equipped with YSI and ISCO samplers for in-river measurements, and WHRO-SW and CP-SW equipped with ISCO samplers for stormwater monitoring.

within the first 50 hours of sampling but forecasts appeared promising (definite rain) the sampler was reset for another 25 hour period. Whole water samples were collected and held on ice and filtered through a 47mm GFF (nominal pore size is 0.45 μm) filter on the same day of collection and frozen until analysis.

Storm event sampling was also conducted from land by installing two ISCO samplers, as described above, adjacent to two storm drains. Both sites were chosen for their proximity to the

Table 1. Station identification and latitude and longitude for sampling stations.

Fixed Station	Latitude (decimal degrees)	Longitude (decimal degrees)
AC	36.8804	-76.2725
NYCC	36.9065	-76.3059
CP storm drain	36.8864	-76.2905
WHRO storm drain	36.8886	-76.3008
Mouth rain gauge	36.9114	-76.316
Headwaters rain gauge	36.8774	-76.2699

Lafayette River with the WHRO site draining approximately 122 acres and Colonial Place (CP) draining a residential neighborhood,

approximately 21 acres (Fig. 2; Table 1). In 2013, flow was estimated based on hydrography and composite samples were collected for nutrient analysis. Samples were collected as described above for the in-water ISCO samplers.

Whole system monitoring and mapping

Weekly monitoring and mapping for surface temperature, salinity, DO, turbidity, pH, and chlorophyll fluorescence of the mesohaline James River (JMSMH), polyhaline James River (JMSPH), and Elizabeth and Lafayette Rivers (ERLAF) were conducted during the CMAP cruises, collecting samples for chl *a* and particulate nitrogen and carbon (PN and PC). Cruises were conducted from March 2013 through October 2013. During CMAP cruises data was collected with the dataflow system developed by VIMS as described on the VECOS website (<http://web2.vims.edu/vecos/>) using a YSI 6600 Sonde. The date, time, and position (latitude and longitude) were recorded and data collected every 4 seconds while the vessel was underway and

then consolidated and viewed on a real-time basis using LabView. During dataflow cruises, the crew followed a programmed cruise track entered in the GPS. QA/QC was conducted at five fixed sites (0.5 m depth) per cruise along cruise tracks. These stations were positioned to coincide with existing Chesapeake Bay Program (CBP) monitoring sites. At these sites samples were collected to conduct laboratory analyses of chl *a*, pheophytin, and total suspended solids (TSS). Chl *a* collected from the YSI dataflow system were corrected based upon grab samples analyzed by wet chemistry techniques taken during cruises by HRSD; Corr.Chl is the final corrected chl *a* concentration in $\mu\text{g L}^{-1}$, and YSIChl is the chl *a* concentration collected from the YSI in $\mu\text{g L}^{-1}$ (Eq. 1):

$$\text{Corr.Chl} = (\text{YSIChl} \times 1.22) - 2.50 \text{ (Eq. 1)}$$

Additional nutrient mapping was conducted by HRSD following storm events in the Lafayette River. These additional sampling events were conducted prior to storms and at intervals of one and two days after storms at

10 stations along the Lafayette River between June and August, 2013 (Fig. 3; Table 2). While underway mapping of physical parameters were collected as mentioned above, grab samples were collected from surface waters at 10 stations, filtered and analyzed for dissolved nutrients, also has described below. Vertical profiling was also conducted using a handheld YSI to assess stratification at each station.



Figure 3. Post-storm event sampling sites for surface nutrients on the Lafayette River.

Table 2. Station identification and latitude and longitude for nutrient mapping stations.

Fixed Station	Latitude (decimal degrees)	Longitude (decimal degrees)
AC	36.8804	-76.2725
1	36.8828	-76.2743
2	36.8839	-76.2747
3	36.8869	-76.277
4	36.8959	-76.2734
5	36.8894	-76.2812
6	36.8878	-76.2923
7	36.9019	-76.2938
NYCC	36.9065	-76.3059
8	36.9079	-76.315

For daily sampling, we collected samples for nutrients (nitrate + nitrite [$\text{NO}_3^- + \text{NO}_2^-$], ammonium [NH_4^+], urea, total dissolved N [TDN], and ortho-phosphate [PO_4^{3-}]) and biomass (chl *a*, particulate nitrogen [PN] and carbon [PC], and cell counts [see Egerton 2013 report]) by pumping water from the ISCO sampler into acid-cleaned bottles. Samples were then brought back to the lab and filtered (0.2 μm) and frozen until analysis. Chl *a*, PN, and PC samples were filtered on GF/F filters and immediately frozen until analysis. Twice each week we measured primary productivity rates using ^{13}C -labeled bicarbonate (see details below). We also assessed stratification during at the Norfolk Yacht and Country Club (NYCC) and the Ashland Circle (AC) sites in the Lafayette River (Fig. 2; Table 1). Both sites were equipped with continuous monitoring Sondes (YSI) and ISCO nutrient collectors. Daily samples were collected from 6/3 through 7/30 and on 8/5 at AC and 6/3 – 7/30 and 8/5, 8/12, and 8/16 at NYCC, consistently at 1000 and 1030, respectively. Vertical profiles were conducted to measure physical parameters using a CastAway CTD. In addition to the daily sampling, diel sampling was conducted during a bloom event (8/9 – 8/10) at AC, measuring primary productivity rates every 4 hours over a 24 h period. Nutrients and cell counts were collected every two hours.

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 analyses for samples filtered and frozen from ISCO samplers included inorganic nitrogen (NH_4^+ , $\text{NO}_2^- + \text{NO}_3^-$), urea N, dissolved free amino acid N (DFAA N), PO_4^{3-} , and TDN. TDN was measured after persulfate oxidation as $\text{NO}_2^- + \text{NO}_3^-$ on an Astoria Pacific Auto Analyzer (Valderrama, 1981) and the concentration of dissolved organic N (DON) was calculated by the difference between TDN and DIN. Concentrations of NH_4^+ were analyzed manually by the phenol hypochlorite method (Solorzano, 1969). Concentrations of $\text{NO}_2^- + \text{NO}_3^-$, urea, and PO_4^{3-} were measured on an Astoria Pacific autoanalyzer using standard colorimetric methods (Parsons et al., 1984; Price and Harrison, 1987).

Water was parceled into triplicate acid-cleaned 60 mL PETG light and dark bottles for primary productivity measurements using ^{13}C labeled bicarbonate (Mulholland and Capone, 2001). Primary productivity incubations were terminated after 4 hours by gentle filtration onto combusted (450 °C for 2 hours) filters (GF/F). Filters 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 ^{13}C stable isotope tracers were based on a mixing model and equations from Montoya et al. (1996) and Orcutt et al. (2001).

Results

Physical parameters, rainfall, and biomass

There were many notable differences between rainfall, temperature, and nutrients leading to differences in biomass, and bloom initiation when comparing 2012 to 2013. In the Lafayette River in 2013, overall rain totals were similar at the mouth and the headwaters between March and September (Fig. 4A) while in 2012, rainfall totals at the headwaters were greater than the mouth (Fig. 4B) and were greater overall in 2012 compared to 2013. In particular, rainfall at station AC was greater in early summer and throughout summer in 2012 compared to 2013.

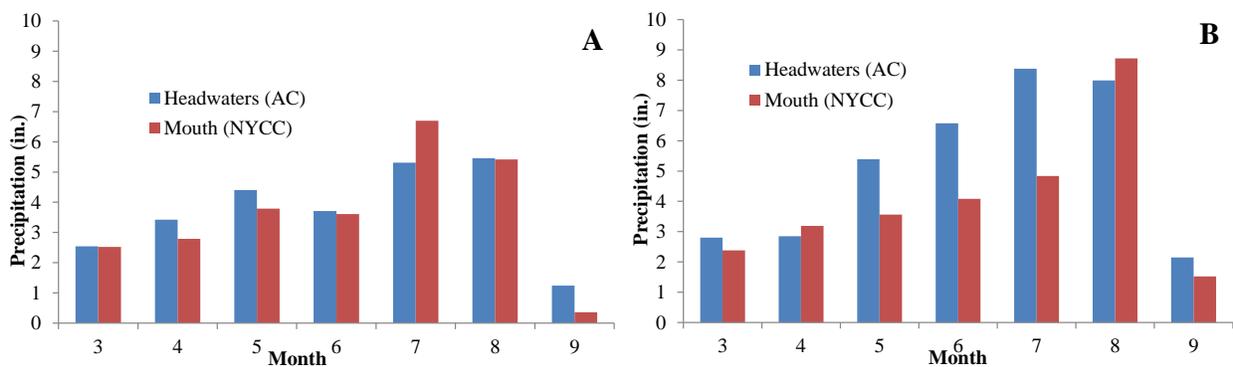


Figure 4. Monthly total precipitation (in.) in 2013 (A) and 2012 (B) for the headwaters at AC (blue bars) and the mouth at NYCC (red bars).

Temperature differences were also observed between 2012 and 2013 (Fig. 5A & 5B). Although warmer temperatures were observed over the same time frame (end of May through September) for both years, once temperatures reached 25 degrees, the average temperature in 2012 (28.51 ± 1.92) was significantly greater (t-test; $p < 0.05$) than the average temperature in 2013 (27.79 ± 1.65) at AC. Increases in chl *a* leading to bloom initiation for *Cochlodinium* were observed later, and the bloom was shorter in 2013 (7/30/13 – 9/15/13) versus 2012 (6/20/12 – 9/20/12) (red lines; Figs. 5A & 5B) at AC.

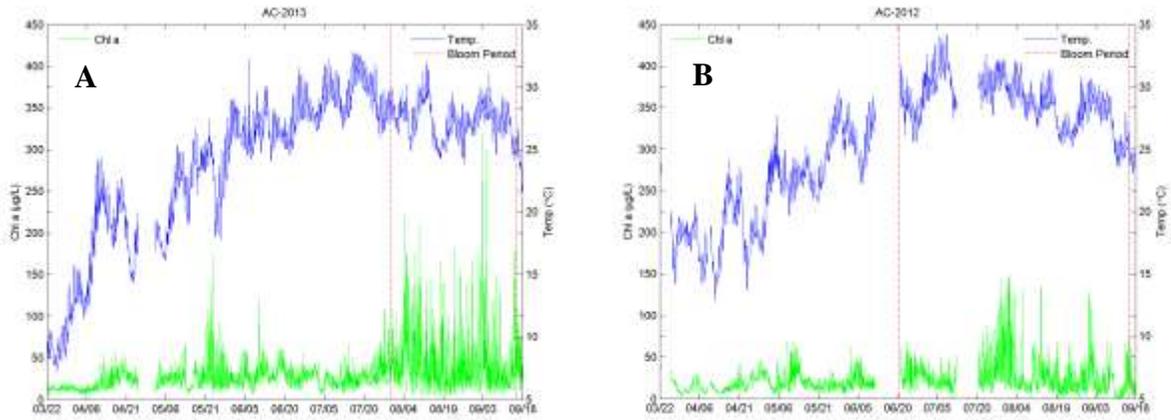


Figure 5. Chl *a* ($\mu\text{g L}^{-1}$; green line, left axis) and temperature ($^{\circ}\text{C}$; blue line, right axis) over time at station AC for 2013 (A) and 2012 (B). Red dotted lines signify *Cochlo dinium* start and end dates.

Similar observations were made at NYCC, where overall chl *a* concentrations were greater than at AC (Figs. 6A & 6B).

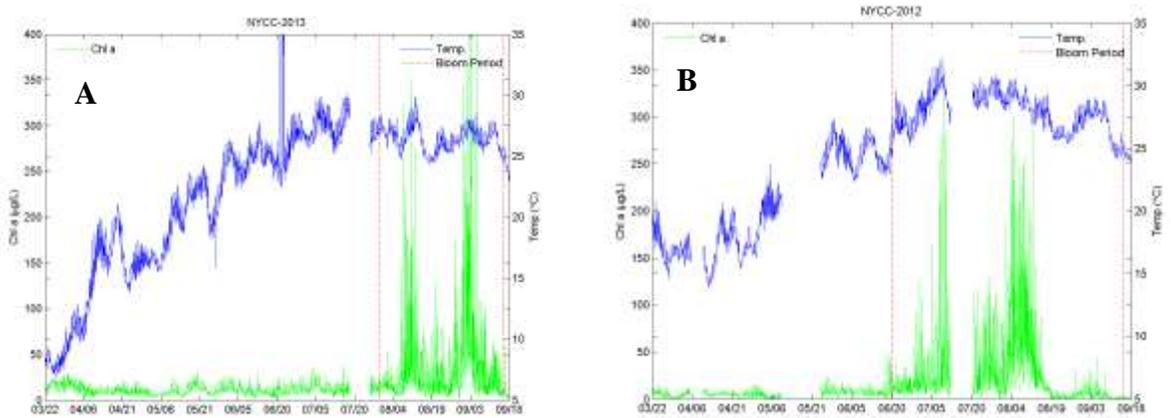


Figure 6. Chl *a* ($\mu\text{g L}^{-1}$; green line, left axis) and temperature ($^{\circ}\text{C}$; blue line, right axis) over time at station NYCC for 2013 (A) and 2012 (B). Red dotted lines signify *Cochlo dinium* start and end dates.

In 2013, a significant exponential relationship between chl *a* and temperature at both stations was consistent with what was observed in 2012 (Fig. 7) confirming our initial findings that as temperatures exceed 25 °C, chl *a* concentrations begin to rise. At AC, of the 73% of the hourly chl *a* data greater than 15 µg L⁻¹, 67% were at temperatures greater than 25°C. At NYCC, only 29% of the hourly chl *a* concentrations were greater than 15 µg L⁻¹ and 64% of those concentrations > 15µg L⁻¹ were at temperatures greater than 25°C.

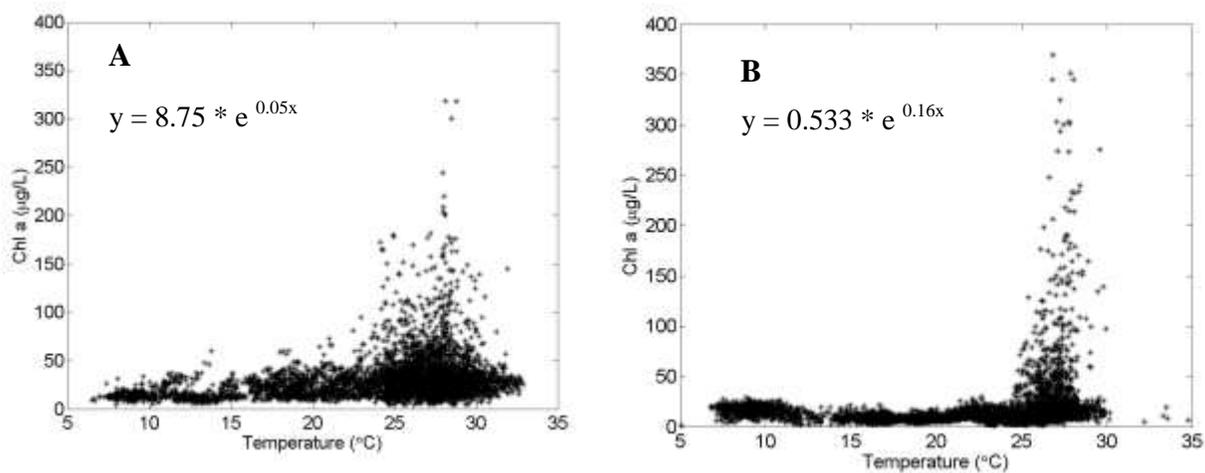


Figure 7. Chl *a* (µg L⁻¹) versus temperature (°C) station AC (A) and NYCC (B) for 2013.

Storm event sampling

A total of 8 sampling events were captured at the stormwater monitoring site WHRO, and 5 were captured at site CP (Table 3). The greatest flow event captured (~2,000,237 gal at WHRO) was not as a result of the greatest precipitation event and did not result in the greatest contribution of TSS or nutrients (Table 3). Overall, there were no correlations between flow volume and TSS or nutrients at either station.

Table 3. Water quality measurements from two stormwater drains, WHRO (drains 122 acres) and CP (drains 21 acres), after storm events. All samples are composites except at WHRO on 7/22^a and 8/11^a, first value is a composite of all grabs, the second value is a composite of only zero salinity grabs. NS = No sample.

Site/Date	TSS (mg L ⁻¹)	TP (μmol L ⁻¹)	TN (μmol L ⁻¹)	PO ₄ ³⁻ (μmol L ⁻¹)	NH ₃ (μmol L ⁻¹)	NO ₃ ⁻ +NO ₂ ⁻ (μmol L ⁻¹)	Organic N (μmol L ⁻¹)	Salinity	Precip. (in.)	Flow (gal)
WHRO 6/3/13	32.0	5.48	128.6	3.13	51.4	23.6	53.6	5.5	0.18	77,392
WHRO 6/7/13	95.0	6.45	92.9	1.23	25.0	7.90	60.0	7.0	1.02	2,000,237
WHRO 6/18/13	52.0	4.52	60.7	3.10	16.4	7.86	36.4	13.0	0.32	954,615
WHRO 7/2/13	135	7.74	61.4	1.32	18.6	3.57	39.3	17.0	0.24	951,251
WHRO 7/22/13 ^a	205 / NS	19.4 / 13.2	277.1 / 113.6	2.00 / 2.26	27.9	13.6 / 12.8	246.4 / 72.8	5.0 / 0.0	1.32	868,943
WHRO 8/1/13 ^a	211 / NS	14.8 / 11.9	136.4 / 94.3	0.84 / 1.00	17.1 / 22.1	10.0 / 10.7	106.4 / 61.4	8.5 / 0.0	1.06	637,002
WHRO 8/11/13	138	12.6	136.4	1.90	22.1 / 25.0	15.0	96.4	5.3	1.21	705,556
WHRO 8/29/13	69.5	9.03	91.4	3.77	0.71	2.14	89.3	19	0.4	NS
CP 6/7/13	62.0	6.45	88.6	3.58	25.0	7.86	55.7	0	0.72	23,954
CP 7/2/13	41.0	7.74	131	3.65	17.14	16.4	97.9	0	0.24	3,587
CP 7/11/13	36.4	6.45	101	4.55	17.14	18.6	65.7	0	1.3	NS
CP 8/1/13	47.0	7.10	116	4.03	17.14	14.3	84.3	0	0.9	28,135
CP 8/11/13	105	13.6	156	5.48	1.43	15.7	139	0	1.19	28,826

One of the largest contributions of TSS and nutrients came on 7/22/13 (measurements from WHRO only), after an extended period (11 days) with no rain over the Lafayette River (Fig. 8), similar to our findings in 2012.

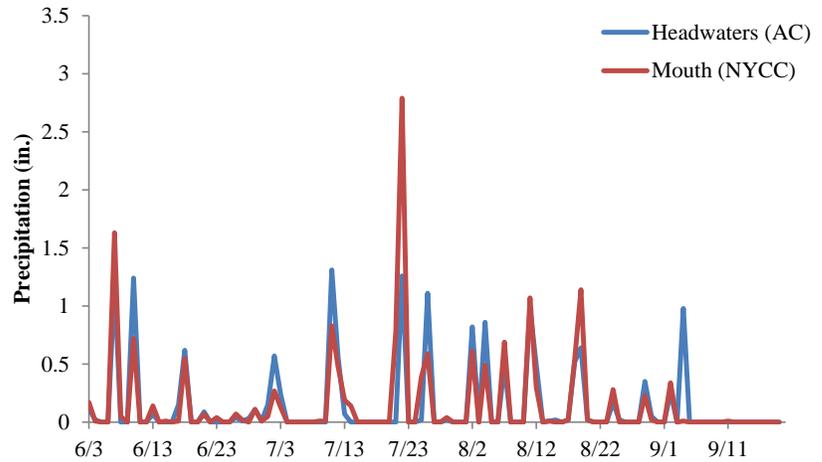


Figure 8. Daily precipitation (in.) near the headwaters and the mouth of the Lafayette River.

Prolonged periods of dry weather may

allow nutrients to build up on the

landscape, providing a large bolus of nutrients into the waterways when a rain event does occur.

In 2013, we set out to sample the surface water of the Lafayette River immediately after a rain

event and at more frequent intervals following a rain event. Six rain events were captured at AC

and NYCC, resulting in increases and decreases in nutrients within the first 30 minutes of

sampling. After the rain event on 7/22, which provided the greatest amount of nutrients in

stormwater, salinity and chl *a* concentrations decreased in the hours after the storm event, and

rain continued to fall during sample collection; ~ 0.2 in. fell in the first hour followed by ~0.5 in.

by hour 4 (Fig. 9A). As chl *a* increased in the first 2 hours, NH₄⁺ concentrations decreased (Fig.

9B). Chl *a* concentrations plateaued approximately 5 hours after the rain event, while NH₄⁺

concentrations fluctuated (Fig. 9B). NH₄⁺ may have been introduced from this rain event and

likely concentrations fluctuated as a function of regeneration and tidal cycling. Each rain event

provided different results and occurred on different tidal cycles, suggesting that a multitude of

variables (tidal state, time of day, phytoplankton community structure, wind events etc.) could be

affecting the dispersal and draw down of available nutrients from rain events.

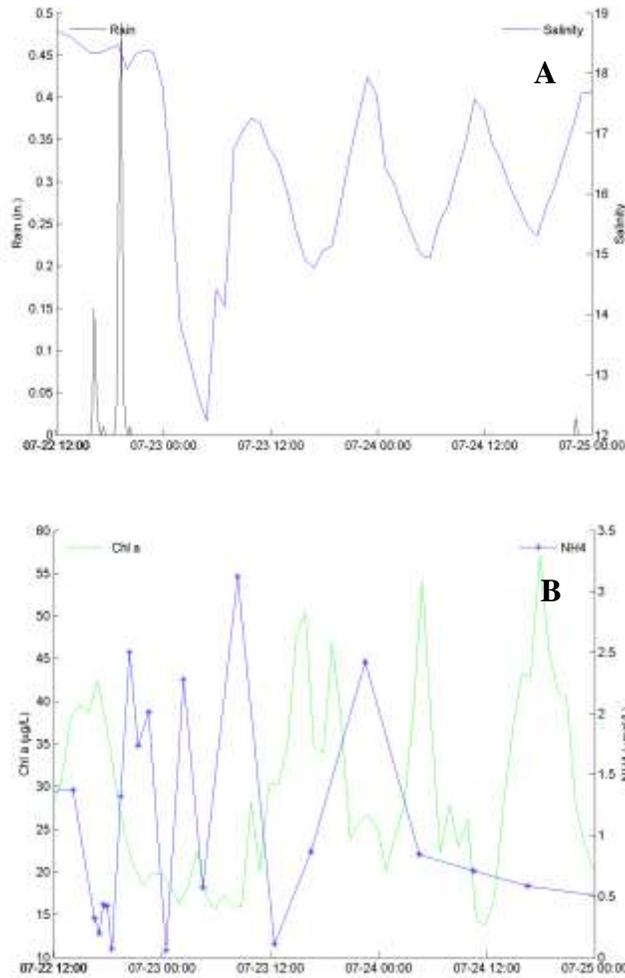


Figure 9. Precipitation (in.; left axis) and salinity (right axis) (A) and chl *a* ($\mu\text{g L}^{-1}$; left axis) and NH_4^+ ($\mu\text{mol L}^{-1}$; right axis) (B) at AC during a rain event in July, 2013.

In the days prior to bloom development, another rain event was captured (8/9 – 8/13), significant decreases in salinity were observed immediately following the rain event (Fig. 10A), chl *a* concentrations were high and fluctuated with the tide, and NH_4^+ concentrations were high after the rain event and also fluctuated according to the tide and with chl *a* (Fig. 10B). Similar findings were observed at NYCC, in which chl *a* and NH_4^+ concentrations fluctuated with the tidal cycles.

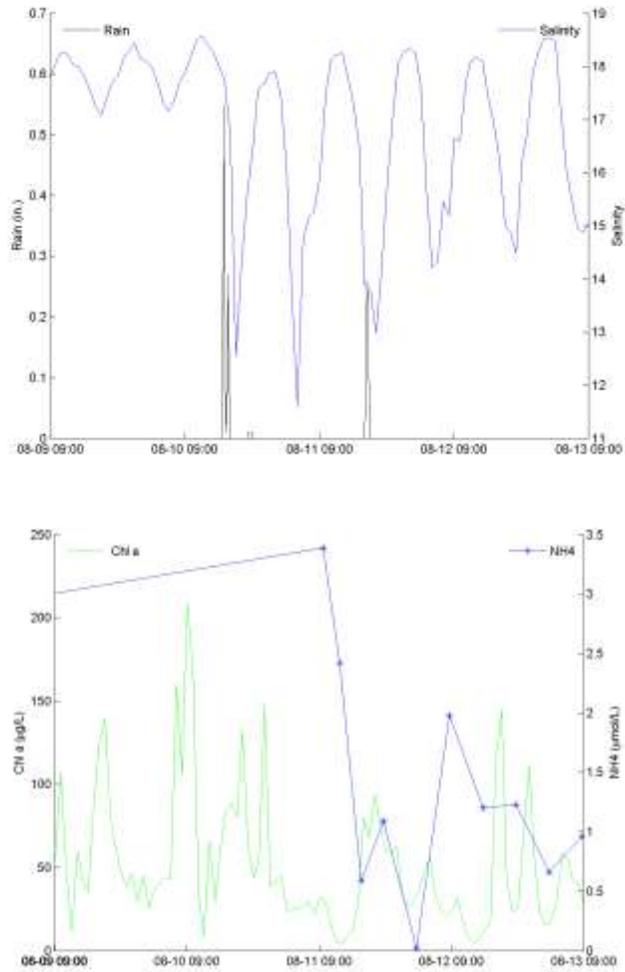


Figure 10. Precipitation (in.; left axis) and salinity (right axis) (A) and chl *a* ($\mu\text{g L}^{-1}$; left axis) and NH_4^+ ($\mu\text{mol L}^{-1}$; right axis) (B) at AC during a rain event in August, 2013.

Water quality monitoring before and after storm events was conducted along the river, and while no trends in overall TDN concentrations were observed before and after rain events, NH_4^+ concentrations were almost always greater on average one day after a rain event at most stations (Fig. 11).

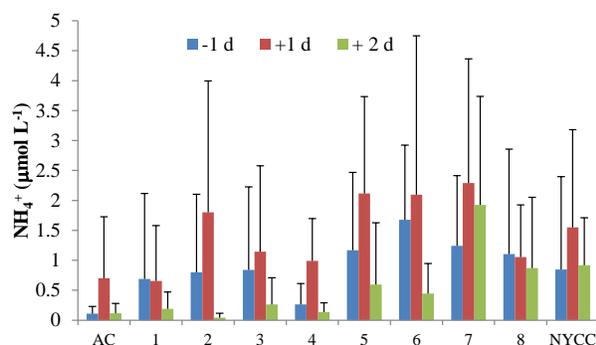


Figure 11. Average NH_4^+ concentrations ($\mu\text{mol L}^{-1}$) at each station along the Lafayette River 1 day prior (-1d), and 1 (+1d) and 2 (+2d) days after a rain event. left axis) and NH_4^+ ($\mu\text{mol L}^{-1}$; right axis) (B) at AC during a rain event in August, 2013.

Stratification was also measured at each station along the nutrient pulse cruises and salinity stratification tended to be highest at the headwater stations following a rain event compared to stations at the mouth (Table 4). This is consistent with previous observations where the shallower, headwater regions of the Lafayette are more susceptible to precipitation and stormwater runoff as they are farther removed from the tidal influence. The highest stratification index at AC also corresponds to the emergence of the *Cochlodinium* bloom in late August.

Table 4. Stratification indices at 4 stations along the Lafayette River, with associated dates and rain totals 48 hours prior to stratification measurements. AC and ER1 are representative of the headwaters while ER6 and NYCC are representative of the mouth.

Date	48 h prior rain (in.)	AC	ER1	ER6	NYCC
6/2/13	0	-0.37	4.07	-0.28	3.32
6/6/13	0.02	1.95	7.67	8.09	5.14
6/8/13	1.2	0.69	12.25	5.23	8.69
6/9/13	1.2	-40.56	-4.79	9.14	4.70
6/11/13	1.24	27.24	2.47	0.81	1.58
6/12/13	1.24	6.99	3.75	8.84	6.28
6/13/13	0	1.16	0.69	5.23	5.18
6/17/13	0	1.88	0.95	-0.71	1.05
6/19/13	0.77	2.44	0.33	3.45	2.96
6/20/13	0.62	n.d.	6.23	3.74	n.d.
7/13/13	1.86	34.98	23.52	7.54	7.72
7/14/13	0.63	26.92	0.94	14.3	15.23
7/25/13	0.02	n.d.	24.32	8.02	3.32
7/26/13	1.13	n.d.	10.2	n.d.	4.58
8/9/13	0	5.72	0.96	1.14	5.95
8/12/13	1.47	61.02	14.95	8.21	3.25

High stratification has been associated with the seasonal *Cochlodinium* bloom, chl *a* concentrations were low and no rain had come through prior to sampling on 6/17/13, and chl *a* was uniform throughout the water column at the 4 representative stations (Fig. 12A). On 8/12/13, the bloom had begun to proliferate throughout the Lafayette, and the water column was highly stratified due to prior rain events (~1.47 in.) and chl *a* concentrations were stratified at all stations (Fig. 12B). The vertical structure of chl *a* in these rivers has implications for future monitoring and regulations, as current regulations are based upon surface only concentrations. During blooms, there is a well-defined chl *a* maximum that may be missed during routine monitoring and mapping.

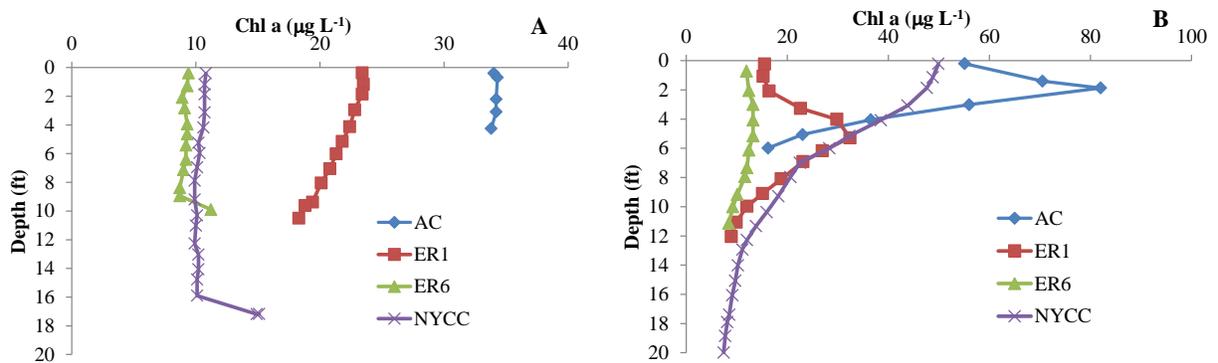


Figure 12. Chl *a* concentration ($\mu\text{g L}^{-1}$) depth profiles at representative stations along the Lafayette River during a non-rain/non-bloom event on 6/17/13 (A) and following a rain event and during the bloom on 8/12/13 (B).

Daily sampling

Daily sampling began June 3 and was conducted at the same time (1000 and 1030 at AC and NYCC, respectively) daily through June and July. Chl *a* concentrations gradually increased over the sampling period and did not appear to trend with precipitation events, but similar to what was observed from the YSI data, chl *a* had a significant relationship with temperature at AC and NYCC (Figs. 13).

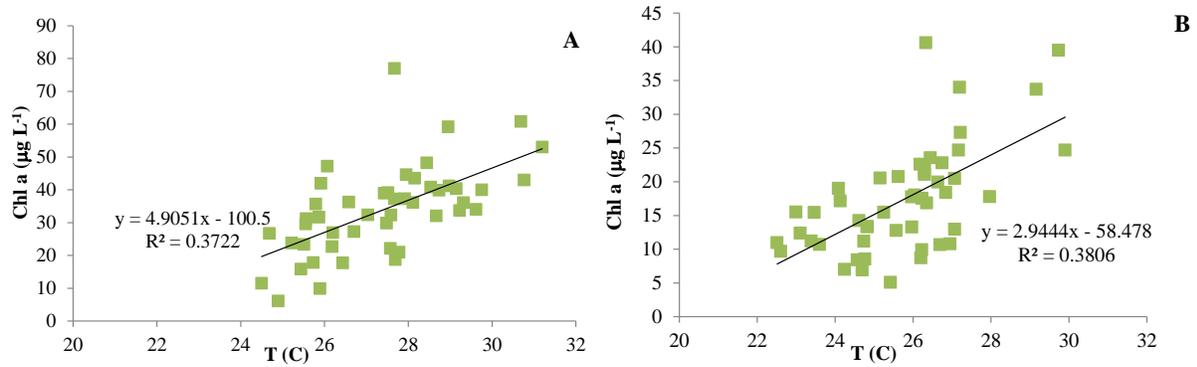


Figure 13. A positive linear relationship between Chl *a* ($\mu\text{g L}^{-1}$) and temperature ($^{\circ}\text{C}$) at AC (A) and NYCC (B) during daily sampling.

While no significant trends were observed between other conservative parameters (salinity, stratification index, or precipitation) and biomass or nutrients, there were significant relationships between N compounds and chl *a* (Fig. 14). Chl *a* increases were most significantly correlated with NH_4^+ decreases followed by $\text{NO}_2^- + \text{NO}_3^-$ decreases. The significant decrease in nutrient concentrations after 7/3 at both stations also corresponded to a 7 day period of no rain, followed by 2 days of rain (7/11 – 7/12) and again no rain for 9 more days.

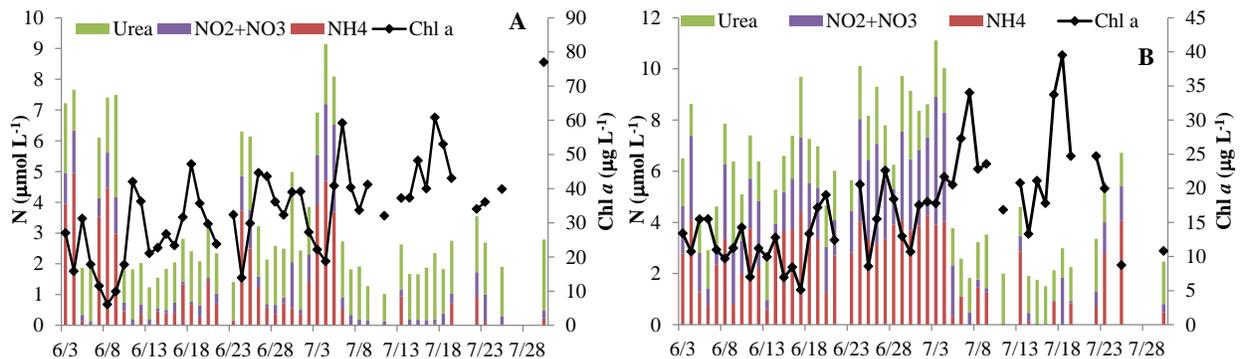


Figure 14. Urea, $\text{NO}_2^- + \text{NO}_3^-$, and NH_4^+ concentrations ($\mu\text{mol L}^{-1}$; left axis) and chl *a* concentrations ($\mu\text{g L}^{-1}$; right axis) over time at AC.

Conversely, PO_4^{3-} concentrations were positively correlated with chl *a* concentrations at both stations. At AC, $\text{DIN}:\text{PO}_4^{3-}$ was always below 16 (Redfield ratio), signifying N limitation, while at NYCC when chl *a* concentrations were between 5 and 20 $\mu\text{mol L}^{-1}$, $\text{DIN}:\text{PO}_4^{3-}$ was > 16 , suggesting P limitation at low chl *a* densities.

At AC and NYCC, diatoms were present throughout the study period, accounting for 50 - 90% of the C biomass in the beginning of June and decreasing to < 10% of the C biomass by the beginning of August (Figs. 15). Dinoflagellates, primarily *Gymnodinium instriatum*, were the dominant contributor to C biomass by the end of June at AC (Fig. 15A) and mid-July at NYCC (Fig. 15B) prior to *Cochlondinium* taking over at the end of July and beginning of August at both stations. Biomass overall was greater at AC compared to NYCC.

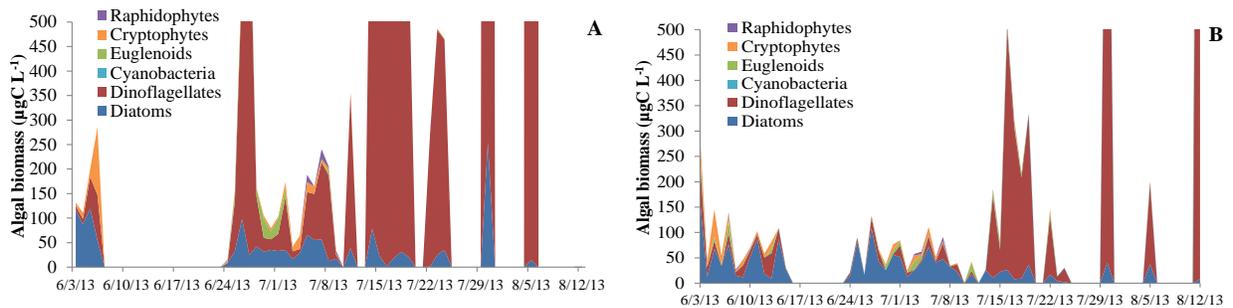


Figure 15. Algal biomass ($\mu\text{g C L}^{-1}$) at AC (A) and NYCC (B) during summer 2013.

Prior to the bloom in June, primary productivity rates did not vary averaging 8.4 ± 0.7 $\mu\text{mol C L}^{-1} \text{h}^{-1}$ at station AC and 8.5 ± 2.7 $\mu\text{mol C L}^{-1} \text{h}^{-1}$ at station NYCC. DO concentrations do not fall below 2 mg L^{-1} , even during the height of the bloom at AC, and briefly fall below 2 mg L^{-1} at NYCC when chl *a* concentrations are greatest (Fig. 16).

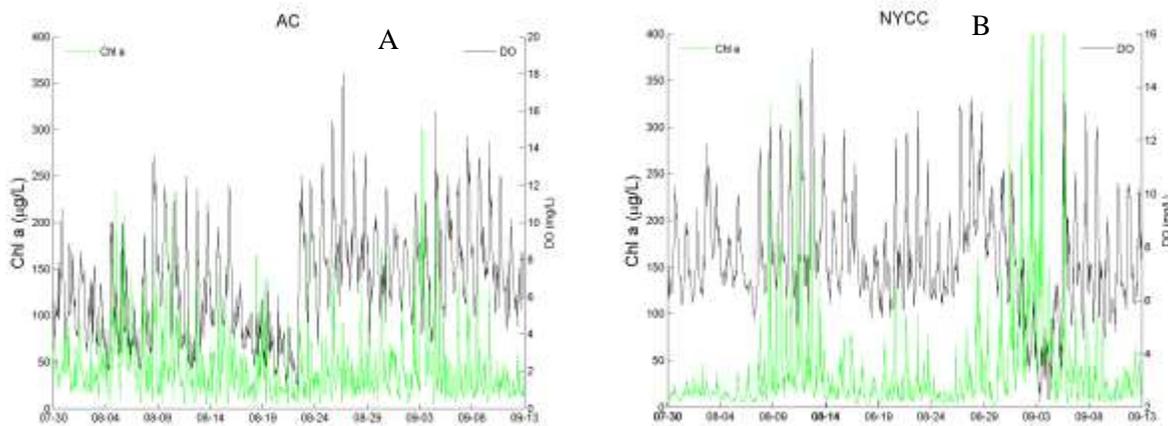


Figure 16. Chl *a* concentrations ($\mu\text{g L}^{-1}$; left axis) and DO concentrations (mg L^{-1}) over time for station AC (A) and NYCC (B).

Diel study

During the diel study at AC, sampling took place during the *Cochlodinium* bloom. High tide occurred at mid-day (1200) and midnight (0000), and C biomass concentrations peaked twice around 1000 and 1800 (Fig. 16A), both just prior to high tide and when the stratification index was greatest. Biomass was not correlated with temperature as temperatures rose throughout the day while biomass trended with the tide and daylight (Fig. 16A). TDN, DON, urea, and PO_4^{3-} were out of phase with the tides, with highest concentrations corresponding to low tides while NH_4^+ and $\text{NO}_2^- + \text{NO}_3^-$ appeared to be biomass regulated (Fig. 16B). Efforts aimed at assessing chl *a* signals must consider the nature of bloom-forming species that have diurnal patterns. Vertical migration or tidal fluctuation could impact when peak biomass is observed, and monitoring should be adjusted accordingly.

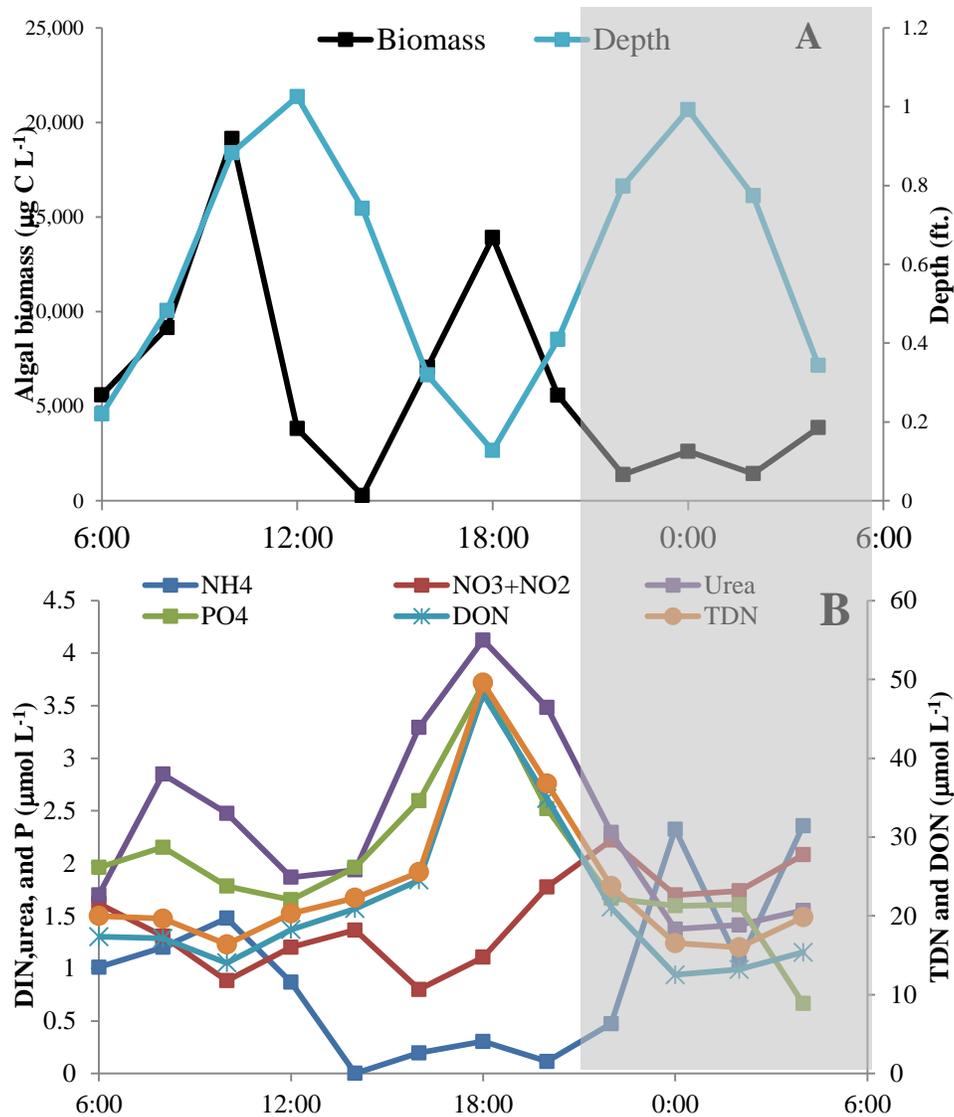


Figure 16. Algal biomass ($\mu\text{g C L}^{-1}$; left axis) and depth (ft.; right axis) (A) and DIN, urea, and PO_4^{3-} concentrations ($\mu\text{mol L}^{-1}$; left axis) and TDN and DON concentrations ($\mu\text{mol L}^{-1}$; right axis) (B) over a 24-h sampling period (8/9/13) at station AC. The shaded block denotes night time.

Key Findings

- Temperature is still considered a driving force for bloom initiation, coupled with precipitation events and increased stratification.
- Qualitatively, nutrients increase in concentration after rain events (NH_4^+ concentrations greatest one day after rain events), but there was no statistically significant relationship between precipitation and nutrients.
- NH_4^+ , and to a lesser extent, $\text{NO}_2^- + \text{NO}_3^-$ concentrations were inversely related to increases in chl *a* biomass in the headwaters of the Lafayette River.
- Nutrient concentrations in stormwater were greatest after a long dry period, resulting in a higher amount of nutrients being washed into the river when rain events were preceded by several days of dry weather.
- Vertical stratification of chl *a* may have implications as the current standards only consider surface chl *a*. A sub-surface maximum after precipitation events and during bloom events was identified.
- Diel patterns were evident with *Cochlodinium*, current standards do not account for tidal signal or time of day during sampling.

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