ABSTRACT

Title of Document:	EFFECT OF pH ON PHYTOPLANKTON AND BACTERIA PRODUCTION
	Desmond J. Johns, M.S., 2009
Directed By:	Professor Diane Stoecker, Marine, Estuarine and Environmental Sciences

In recent decades eutrophication has fueled intense phytoplankton blooms that frequently negatively impact ecosystems. Dramatic pH elevations are commonly overlooked during such blooms, which may also be harmful. Cyanobacteria blooms in the Sassafras River, MD were sampled during Autumn 2008 and measured for primary and bacterial production (PP and BP), and differences in bacteria communities composition were examined. pH elevations above 8.8 in the field corresponded to decreased PP, but had no effect on BP. Laboratory experiments demonstrated that negative effects of pH were dependant on light intensity; PP increased with pH at moderate light intensities, but decreased at low irradiance. There was some evidence that BP is affected by high pH, although bacteria community differences as determined by DGGE were not. Negative effects of high pH are probably most important during spring and summer in low salinity environments when pH fluctuations are more common and last longer.

EFFECT OF pH ON PHYTOPLANKTON AND BACTERIA PRODUCTION

By

Desmond J. Johns

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2009

Advisory Committee:
Professor Diane K. Stoecker, Chair
Dr. Byron C. Crump
Dr. Judith M. O'Neil

Acknowledgements

Table of Contents

List of Tables

List of Figures

Introduction

Intense phytoplankton blooms, which are considered a nuisance or harmful, are increasing in scope, duration and intensity (Hallegraeff 1993, Anderson et al. 2002, Sunda et a. 2006). Cyanobacteria blooms in particular have proliferated globally in coastal low salinity areas and in fresh water systems in recent decades. This increase is largely attributed to anthropogenic nutrient input (Correll 1998, Paerl et al 2001). High biomass blooms can degrade water quality when they end abruptly when nutrient supplies are exhausted or through viral mortality, causing the bloom to sink and microbial respiration rapidly consumes oxygen and drives hypoxia and anoxia (Vitousek et al. 1997, Cloern 2001, Hewson et al. 2001, Kemp et al. 2005). Fish kills, and food web disruptions are frequent indicators of such blooms (Irigoien 2005, Sunda et al. 2006, Livingston 2007). Bacterial production (BP) is often stimulated by DOC release via phytoplankton productivity and release (Bratbak and Thingstad 1985, Mindl et al. 2005).

In tidal and fresh water systems a consequence of cyanobacteria blooms that is commonly overlooked is the dramatic increases in pH that frequently result from highly productive blooms. The pH influences several abiotic and biotic processes including phosphorus release from the benthos (Seitzinger 1991) and survival and growth of the algae themselves (Hansen 2002), as well as the food web as a whole. pH tolerance during blooms may shape microbial food web dynamics, including phytoplankton competition, grazing, and BP (Pace and Cole 1996, Hansen 2002, Hinga 2002). The pH rises as phytoplankton deplete CO_2 in the water column faster

than respiration replaces it (Paerl 1984, Rost et al. 2006). In both freshwater and marine systems greater rates of primary production (PP) generally increase the rate at which phytoplankton "leak" dissolved organic carbon (DOC) into the water column, which stimulate bacterial production and the nature of these compounds may influence community structure (Baines and Pace 1991, Horňák et al. 2008). The competing influences of DOC stimulating production, and stress-inducing pH are poorly understood in community dynamics. The aim of this study is to illuminate the effects of pH on phytoplankton and bacteria.

The pH in coastal environments is generally between 7.5-8.5, but the pH will often fluctuate > 0.5 around this range (Hinga 2002). In freshwater systems the pH may vary over a much wider scale, from less than 2 to 12 (Weisse and Stadler 2006). The pH revolves around the carbonate balance of a water body. As CO_2 dissolves from the atmosphere into water it enters on the left hand side of the following equation:

$$CO_2 + H_2O \Leftrightarrow H_2CO_3^- \Leftrightarrow HCO_3 + H^+ \Leftrightarrow CO_3 + H^+ \text{ (Libes 1992).}$$
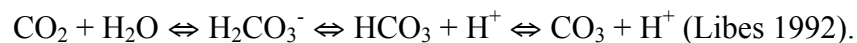
Several factors influence the pH in coastal systems. The concentration of CO_2 in the water and the alkalinity of the system are the main determinants of pH. Marked increases in pH occur because CO_2 uptake by phytoplankton exceeds the sum of water column respiration and CO_2 diffusion into the water column. At the water surface wind and turbulence aid diffusion of CO_2 from the atmosphere into the water column (Havskum and Hansen 2006). Temperature increases slightly depress pH.

Uptake of NH_4^+ decreases alkalinity and lowers pH, while NO_3^- uptake increases

alkalinity and pH (Hinga 2002).

Effects of pH on phytoplankton

A few studies have concluded that availability of inorganic carbon ($CO_2 +$

HCO_3^-) may frequently limit phytoplankton in coastal systems where nutrient inputs

are ample, and productivity is high enough to deplete CO_2 in the water column (Paerl

1984, Hinga 2002, Moisander et al. 2002, Badger et al. 2005). CO_2 is the essential

substrate for carbon fixation by autotrophs, and as pH rises the carbonate balance

shifts from CO_2 to HCO_3^- (Hinga 2002, Badger et al. 2006) (Figure 1). When pH is

below 8.5 the proportion of CO_2 in the water is generally less than 2% of the

inorganic carbon pool (C_i), and decreases as pH rises (Badger et al. 2006).

Drawdown of CO_2 by primary producers reduces the buffering capacity of the

carbonates in the water and results in an increase in pH (Hinga 2002, Martin and

Tortell 2006).

Alterations in pH may have several negative effects on phytoplankton.

Phytoplankton species that are unable to utilize HCO_3^- will probably experience

inorganic carbon (C_i) limitation as pH rises (Havskum and Hansen 2006). Increases

in pH may alter the speciation of some elements, such as Cu, which may inhibit

phytoplankton growth (Granéli and Haraldsson 1993). Enzyme activity is also

sensitive to pH and values outside an organism's range of tolerance will impair

cellular function. In order to preserve function organisms may pump ions to maintain

a constant internal pH, which would require significant energetic investment (Hinga

2002).

Figure 1. Change in Ci balance drive changes in pH. The scenario below is for a water body at 15°C

and a salinity of 30. Modified from Hinga et al. 2002
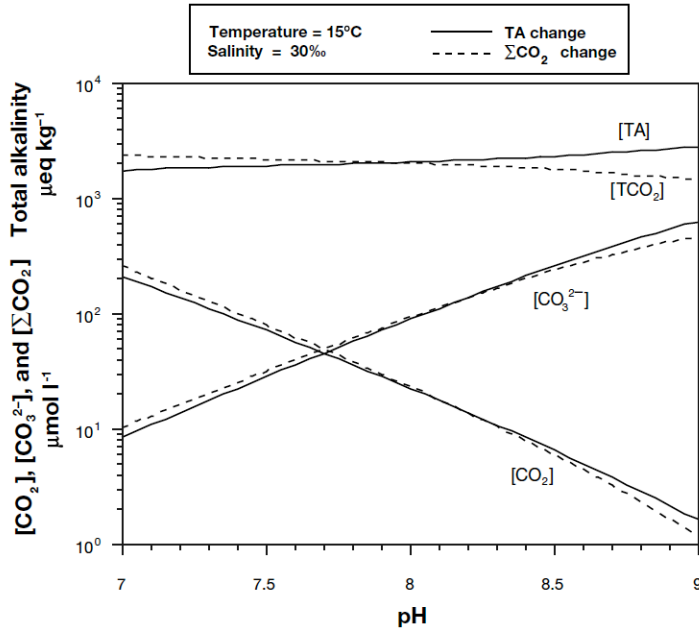
While pH may negatively affect organisms in several ways, the best

understood is the effect of C_i limitation. The proportion of inorganic carbon (C_i) that

is CO_2 is rarely favorable to phytoplankton uptake, even within a moderate pH range.

Nearly 2.5 billion years ago early photosynthesisers drew down CO_2 concentrations,

and this event is though to be primarily responsible for oxygenating the atmosphere.

The carbon fixation enzyme, Rubisco, evolved under high atmospheric CO_2

conditions and is far more inefficient in today's oxygenated atmosphere than it was

when it first evolved (Badger et al. 2006). Many phytoplankton have since evolved

various mechanisms to allow them to convert HCO_3- to CO_2, either through uptake of

and concentration of HCO_3^- or by use of extracellular carbonic anhydrase (CA)

(Aizawa and Miyachi 1986, Martin and Tortell 2008, Trimborn et al 2008). HCO_3^- is

taken into the cell and transported to the carboyxsome, which contains CA. CA

converts $HCO_3^- \rightarrow CO_2$. CO_2 is prevented from diffusing out of the carboxysome,

providing a CO_2-rich environment for Rubisco to function in, with minimum

interference from oxygen (Badger et al. 2006). Extracellular CA performs this same

function outside the cell in many phytoplankton, but cyanobacteria appear to utilize

extracellular CA minimally (Paerl 1984). Instead, cyanobacteria appear to depend on

various modes of active transport, referred to as the carbon concentrating mechanisms

(CCMs), to move HCO_3^- into the cell (Badger et al. 2005)

Phytoplankton utilization of CCMs increase as C_i becomes depleted in the

water column, but the total investment in CCMs is highly dependant on external

environmental factors that facilitate growth. In general, cells grown under low CO_2

conditions have higher affinity for HCO_3^- and are saturated for growth at a lower

concentration than cells grown at high CO_2 concentrations (Badger and Andrews

1982). Nutrient conditions favorable to growth are necessary for induction of protein

complexes necessary to CCM machinery (Burns et al. 2005). Macromolecular

reallocations also occur within the chloroplast to cope with limitations in electron flux

when CCMs are active, which also require energy and protein resources. In N-fixing

cyanobacteria, the CCMs may compete for energy with the cell's nitrogen fixation

(Paerl 1984). Light intensity is important in providing energy to the CCMs.

However, photoinhibition may be common in many surface dwelling cells, such as

cyanobacteria (Ibelings 1996, Berman-Frank et al. 2003). When light intensity is saturating in the cyanobacterium *Synechococcus* sp, internal C_i concentrations were > 30 mM, which was more than 1,000 times greater then the C_i in the external environment. However, Ibelings and Maberly (1998) point out that many cyanobacteria that dwell at the surface are likely to experience photoinhibition if they rise to the surface too quickly. If cells were not acclimated to high irradiance before exposure to such conditions the combination of C_i limitation and photoinhibition may cause the bloom to collapse. Temperature is also important in determining the efficiency of the CCMs. Optimum growth was achieved under both high and low C_i in *Synechococcus* grown at the upper limit of the strain's temperature range (35°C). At the low end of the strain's temperature range (23°C) and under high C_i, cells successfully fixed C, but were unable to divide and experienced premature senescence, probably due to an imbalance in photosystem II that produced a greater electron flux than could be utilized (Burns et al. 2005).

Several researchers have suggested that cyanobacteria have evolved various strategies to maximize access to C_i. Phytoplankton that are not sensitive to shear stress from turbulence may experience enhanced growth under turbulent conditions due to enhanced diffusion of CO_2 (Havskum and Hansen 2006). Some cyanobacteria have sturdy gas vesicles that make them buoyant and allow them to maintain a position at the surface (Oliver and Walsby 1984). Surface blooms that have elevated the pH are in prime position to intercept CO_2 diffusing from the atmosphere into the water, which proceeds at a faster rate when pH is elevated. However, interception of

CO_2 from the atmosphere probably does not allow for significant production; Ibelings and Maberly (1998) concluded that both the atmosphere and the water served as a source of C_i during intense blooms, but that HCO_3^- served as the greatest source. Cyanobacteria-bacteria associations may exist in part to supply CO_2 from bacterial respiration to the algae (Kuentzel 1969, Lange 1971). Bacteria frequently colonize the mucilaginous sheaths surrounding cyanobacteria heterocysts. N-fixing cyanobacteria may excrete N to attract or promote bacterial growth and thus bacterial respiration. Bacteria near the heterocyst may proliferate on the organic excretions and will also consume oxygen, minimizing oxygen pollution within the anaerobic N-fixing heterocyst (Paerl 1984). There is some evidence for this contention. Paerl (1982) found that the heterotrophic bacterium *Pseudomonas aeruginosa* exhibited chemotaxis towards heterocysts. During a bloom of *Microcystis aeruginosa* colonial bacteria were most abundant during the peak of the bloom, and *Microcystis* cells furthest from bacterial colonists displayed the lowest rates of CO_2 fixation (Paerl 1996).

Bacteria in aquatic ecosystems: factors controlling production and questions yet to be investigated

The complexity of bacteria dynamics and their contribution to biogeochemical cycles and food webs is well recognized (Cole et al. 1988, Cho and Azam 1988, Villa et al. 2004). Bacteria in many systems constitute a large fraction of the plankton biomass that turns over very quickly (Azam et al. 1983, Cho and Azam 1988). This

large pool remineralizes organic matter, much of which is incorporated into bacterial biomass and may be transferred to higher trophic levels (Cole 1998).

Factors controlling bacterial production (BP), abundance, and community structure are the subject of many studies. Several factors are thought to influence bacterial dynamics, although the factors influencing the bacterial community composition vary widely with location and time (Kan et al. 2006). BP and abundance frequently correlate strongly with various parameters associated with the phytoplankton community such as PP (Cole et al. 1998, Heinanen et al. 1995), Chl a (Kan et al. 2006), DOC excretion by phytoplankton (Jardillier et al. 2004), or phytoplankton community type (Rooney-Varga et al. 2005, Tuomainen et al. 2006). Other environmental factors that may shape bacterial communities involve bacterial nutrition and include nutrient concentrations (Pace and Cole 1996, Carvalho et al. 2003) and input of allochthonous substrates (Hoch and Kirchman 1993, Comerma et al. 2003). Abiotic factors such as irradiance (Pakulski 1998, Hernandez et al. 2006) and temperature (Shiah and Ducklow 1994, Kan et al. 2006) have been found to shape bacterial production and community dynamics. Biological controls may also be added to this list of factors influencing bacterial dynamics. Viral lysis influences aquatic bacteria (Suttle 1994), as do top down control by phagotrophic microbes (Jardillier et al 2004, Simek et al. 2008).

Freshwater ecologists may also add pH to the long list of factors shaping bacterial dynamics. Several freshwater studies have found significant effects of pH

on bacteria. Pace and Cole (1996) found that pH correlated best with BP in three North American lakes over a two year study period. Low pH also negatively affected BP by bacteria growing on rocks in two studies of streams in the Adirondack Mountains (Osgood and Boylen 1990, Mulholland et al. 1992). A study of 20 freshwater lagoons along the Paraná River floodplain in Brazil found that bacterioplankton abundance was best explained by DOC, nitrogen, phosphorus, and pH (Carvalho et al. 2003). In all these studies, the parameter measured (bacterial production or abundance) increased over a pH range of approximately 4-8, although the authors did not venture explanation of why pH increases over this range would positively correlate.

While the freshwater literature suggests that pH may influence bacterial dynamics, this question seems to have been largely ignored in the coastal and estuarine literature. While the pH in seawater is fairly constant, the pH in the upper reaches of estuarine systems, where the buffering capacity of the water is much less, may experience variations similar to those observed in freshwater lakes and streams (Hinga, 2002). It seems plausible that bacterial dynamics may be influenced by pH in these systems where primary production is high and buffering capacity is low.

The microbial food web and pH

Interactions between cyanobacteria and bacteria are numerous and complex. PP during cyanobacteria blooms often correlates tightly with BP rather than with physical factors (Heinanen et al. 1995, Jardillier et al. 2004). This may be due to a

symbiotic relationship between the algae and the bacteria. Blooms of toxic cyanobacteria may provide a grazing shelter to bacteria during times of toxicity (Tuomainen et al. 2006). Carbon remineralized through respiration would be immediately available to the algae and may prevent C_i limitation, as would other nutrients remineralized by heterotrophic bacteria. Paerl et. al. (1984) found evidence for this. Cyanobacteria that were spatially farthest from bacterial colonies had the lowest rates of carbon fixation. In this situation, bacteria are likely to benefit by using labile compounds that are exuded by the cyanobacteria (Heinanen et al. 1995, Tuomainen et al. 2006). Under C_i limitation, the diazotroph *Anabaena oscillariodes* will release proportionally more N-rich compounds than C-rich compounds into the water column. Exudation of N-rich compounds may have the dual benefit of shunting photochemical energy towards N-fixation (thus preventing photoinhibition), and stimulating BP and respiration that may alleviate C_i limitation (Paerl 1984). This symbiosis may extend the duration of the cyanobacteria bloom by sustaining active algal growth and toxin production. For example, when nutrient limitation occurs, the cyanobacteria *Microcystis* ceases to express the mycE gene, which is expressed when microcystins are being produced, and macrozooplankton that were previously inhibited or deterred may begin to graze down the bloom (Gobler et al. 2007)

Unique bacterial assemblages are often associated with cyanobacteria (Salomon et. al 2003, Kasputina 2006). The mucilage of microcystin-producing *Microcystis* are often colonized by bacteria that are able to degrade the toxin, but these bacteria are absent when no toxin is detected (Maruyama et al. 2003). Bacterial

communities growing on the cyanobacterium *Nodularia* spp. differed depending on if the algae were actively growing or were beginning to show signs of decay (Tuomainen et al. 2006)

While some studies have found evidence of symbioses between bacteria and cyanobacteria, other studies suggest that cyanobacteria do not provide a grazing shelter to bacteria, and that bacterivory may even increase within a bloom (Engström-Öst et al. 2002, Hornak et al. 2008). While macrozooplankton may be deterred from grazing on algal biomass during a toxic bloom, microzooplankton have been observed to actively feed at all times (Gobler et al. 2007). Some studies have also noted that DOC production by cyanobacteria or other phytoplankton assemblages is not sufficient to meet bacterial demand (Christoffersen et al. 1990, Comerma et al. 2003). Residence time and allochthonous inputs must be factored into this relationship (Comerma et al. 2003, Kan et al. 2006). Enhanced bacterivory and turnover in the microbial food web may also benefit persistence of cyanobacteria by providing nutrients though sloppy feeding, zooplankton respiration, and zooplankton remineralization of nutrients.

Cyanobacteria's high pH tolerance, coupled with high affinity for C_i uptake at low concentrations, may allow a bloom to persist for long periods of time which likely influences dynamics within the microbial food web (Badger and Andrews 1982, Møgelhøg et. al. 2006, Hansen 2002). While *Nodularia spumigena* frequently forms dense blooms in the Baltic Sea and produces the toxin nodularin, a culture

11

study found no allelopathic effects of nodularin-producing *N. spumigena* on six phytoplankton species. However, under conditions of elevated pH (up to 10.6), *N. spumigena* out-competed five of the six test species (Møgelhøj et. al. 2006). Microzooplankton may also be negatively affected by pH. Several species of ciliates and dinoflagellates show inhibition and/or death at elevated pHs (Hansen 2002, Pedersen and Hansen 2003, Weisse and Stadler 2006). Tolerance of ciliates to pH appears to correspond to temperature tolerance. Ciliate species that grow over a wide temperature range tolerate a wider range of pHs than do species that are stenothermic (Weisse and Stadler 2006). Culture experiments with dinoflagellates grown at elevated pH levels found that the species with the greatest pH tolerance always became dominant, regardless of initial species composition (Hansen 2002). Cyanobacterial elevation of pH may play a greater role in shaping microbial food web dynamics than is commonly recognized (Hansen 2002, Møgelhøg et. al. 2006). The influence of pH in shaping such dynamics may increase with eutrophication of coastal areas; moderate N and P fertilization in a marine mesocosm amplified the pH range (Hinga 2002).

It is apparent that cyanobacterial interactions with the microbial food web are diverse, but the effect of pH elevation during a bloom on the cyanobacteria and bacteria remains unclear. This study sought to investigate the effect of elevated pH on phytoplankton and bacterial production in a temperate brackish river. The hypotheses that were tested are:

1. Primary production, as measured by ^{14}C incorporation, will decrease with increasing pH. Negative effects will be more acute at saturating light intensities because C_i limitation may limit electron flow away from photosynthetic reaction centers and cause photoinhibition.

2. Elevated pH will negatively affect BP. This could occur either directly through physiological stress, or indirectly through decreased DOC availability due to impaired PP.

3. Elevated pH will shape bacterial communities, possibly selecting for bacterial populations that are pH tolerant.

Fieldwork to test these hypotheses was conducted in the Sassafras River, which is a tributary to the Chesapeake Bay on the Eastern Shore of Maryland. The watershed of this river is sparsely populated and is relatively small (approximately 97 square miles) (Sassafrassriver.org). Land use is predominately agricultural, and runoff of nitrogen and phosphorus from farm fields fuels cyanobacteria blooms during the summer and fall. High biomass blooms of cyanobacteria, accompanied by large pH variations (eyesonthebay.net), are frequent in this river. The frequency and

intensity of cyanobacteria blooms changes from year to year and the impact of elevated pH may be more important in some years than others. For example, pH was elevated throughout most of 2007. In contrast, phytoplankton blooms elevated the pH periodically in 2008, but this occurred less frequently than in the previous year. Frequent blooms and dramatic pH changes make the Sassafras an ideal location to study the effects of pH on phytoplankton and bacterial communities.

Methods

Sampling information

Samples were collected during late summer 2008 along the upper reaches of the Sassafras River in Maryland, USA (Figure 2). The Sassafras is an eastern shore tributary to the Chesapeake Bay that is approximately 20 miles long and has a watershed area of ~97 square miles. The Sassafras watershed is largely surrounded by agricultural land (68.4%) and forest (22.6%). Urban development comprises just 4.1% of the watershed. Three small municipalities lie within the watershed boundaries and are Galena, Cecilton, and Betterton. The population of these three towns is less than 1,300 residents (Sassafrassriver.org). There are approximately 2,500 households, but there are also 1,800 boat slips that are occupied almost continuously during the summer (Kascie Herron, pers. comm.). This increases the apparent population by nearly 60%, and illegal sewage dumping from boats may be a frequent problem. Sewage treatment plants are located at Galena and Betterton Beach.

The Maryland Department of Natural Resources classified the river as "nutrient saturated" in their most recent report in 2007, although the overall water quality of this tributary is increasing since the last report in 1985 (eyesonthebay.net). Agricultural fields and a few chicken farms are a major source of nutrients, especially nitrogen, to the Sassafras. However, phosphorus, which is commonly limiting in freshwater ecosystems may be released from the sediments during intense blooms in

which the pH has risen to > 9.2 (Seitzinger 1992). Phytoplankton abundance in this

river is well above the acceptable limit set by Maryland DNR, and is classified as

poor. Year-to-year variation in intensity and frequency of cyanobacteria blooms may

be great, as may the observed extremes of pH. Despite ample nutrient concentrations

and frequent and intense phytoplankton blooms, dissolved oxygen concentrations in

the Sassafras are within acceptable limits, and in fact are often saturated

(eyesontheybay.net).

Samples were collected at 4 sites upstream from the Georgetown drawbridge

at MD Route 213. Moving in the upriver direction, the sampling sites were

Drawbridge, Cove, Budd's Landing, and Powerline and spanned between N39 22.646

W75 49.712 to N39 21.981 W75 52.405 (Figure 2). The salinity is this region was

less than 4 during the entire sampling period and phytoplankton communities were

dominated by cyanobacteria. Samples were collected from surface waters in acid-

washed 1-liter glass jars during the morning. Replicate bottles were collected from

each station. Water temperature, salinity, and pH were determined during sampling

for each site using a YSI 63. After all samples had been collected, bottles were

transferred to a large cooler that was filled with water collected from off the dock to

maintain ambient temperature during transport to the Horn Point Laboratory in

Cambridge, Maryland.

Experiments to determine bacteria and phytoplankton production rates were

immediately initiated upon arrival back to the lab to obtain the most accurate

measurements possible. Additionally, samples were filtered in order to collect

samples that would be analyzed for dissolved nutrients (nitrate, ammonium, urea,

phosphate), dissolved inorganic carbon (DIC), DOC, and Chl *a* determination.

Figure 2. Map of sampling sites on the Sassafras River, a tidal tributary of the Chesapeake Bay.
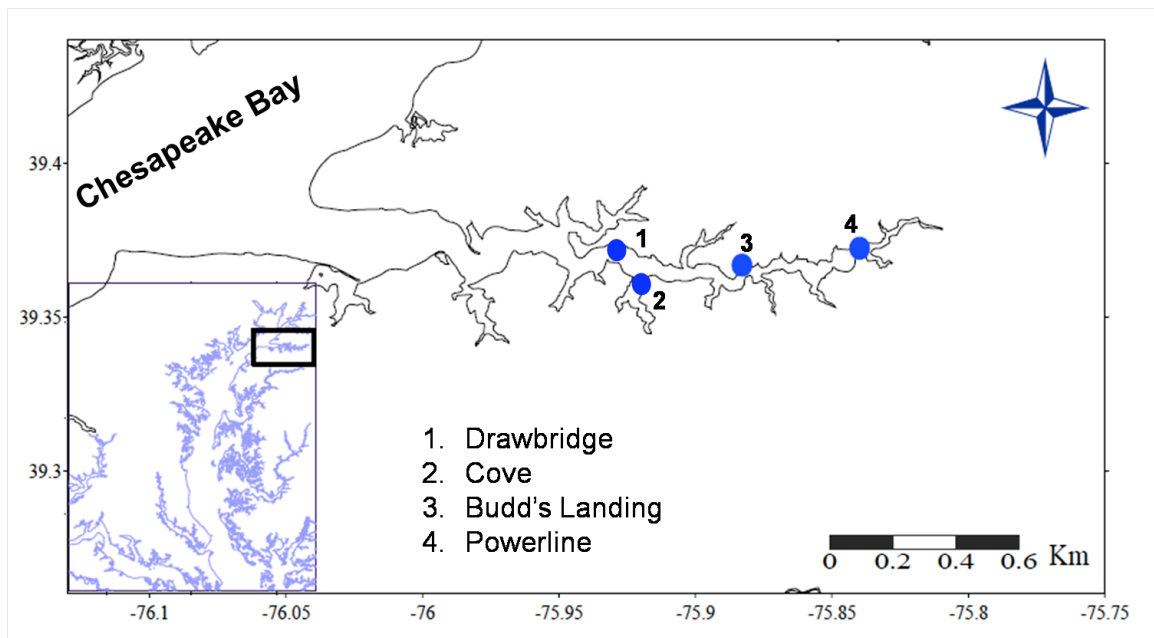
After filtering, all samples were immediately placed in the dark and frozen.

Approximately 20 ml of sample water was preserved with Lugol's acid solution and

stored in the dark for future microscopy. An additional 3 ml of water was collected

after filtration through a 0.3 μm membrane filter and fixed with 25% glutaraldehyde

to preserve bacterial communities. Finally, cells from each station were preserved for

molecular enumeration.   All cellular preservation methods for microscopy were

similar to those described in Anderson (2005). Samples for molecular work were

collected on a 0.2 µm Sterivex filter and 1ml of filter-sterilized DNA extraction was added (Crump et al. 2003). Samples for molecular work were stored at -80°C .

The Maryland Department of Natural Resources routinely collects water samples and identifies the phytoplankton via microscopy from the Sassafras River at Georgetown, which is the location of the Drawbridge site. During 2008, several blooms were observed at Budd's Landing that were also sampled and the phytoplankton communities identified by Maryland DNR (Walter Butler, pers. comm.). These data were provided upon request from Walter Butler. The sampling dates for which phytoplankton data are available at Georgetown during 2008 include September 17 and October 22. Phytoplankton community information is available from Budd's Landing for September 2, 10, 16, and 30.

Dissolved carbon, Chl a and nutrient analyses

Samples for Chl a were collected onto a 25 mm GF/F filter. Samples for NH_4^+, NO_3^-, urea, and PO_4, and DIC were collected after filtration through a GF/F filter and stored in a 50 ml acid-washed tube. DOC samples were filtered through a 0.3 µm membrane filter and stored in a 20 ml glass vial that had been pre-combusted at 450°C for 5 hrs. All these samples were stored in a dark freezer and preserved using the methods of Parsons et al. (1984).

Chl a was extracted in the dark in 90% acetone for 24 hours at 4°C. Chl a concentrations were determined fluorometrically on a Turner Designs 10AU

fluorometer. Urea concentrations were determined colorimetrically using methods

from Mulvenna and Savidge (1992) that were modified to be read in a microplate at

520 nm. Ammonia and nitrate concentrations were determined colorimetrically using

methods modified for a microplate from Parsons et al. (1984). Soluble reactive

phosphate concentrations were determined at Horn Point Laboratory Analytical

Services using their standard operating procedures (Whitledge et al. 1981).

DOC was determined at Horn Point Analytical Services using the methods of

D'Elia et al. (1997). DIC concentrations were determined using gas chromatography

using methods of Stainton (1973). Samples to determine DIC were collected in acid-

washed glass vials and stored frozen in the dark. For the analysis, 4 ml of sample was

transferred to a glass tube that had been purged with N_2. The sample was acidified

with 1 ml 1N H_2SO_4. A 1 ml gas injection was read on a Shimadzu GC-14B gas

chromatograph at 580 nm.

Bacterial production

BP rates were determined by calculating the rate of tritiated thymidine

incorporation as described by Bell (1993). Thymidine was chosen because at least

one common cyanobacteria, *Microcystis aeruginosa*, has been shown to incorporate

significant amounts of radio-labeled leucine (Kamjunke and Jähnichen 2000), but do

not incorporate thymidine. After collection in the field, sample bottles were

transported to the lab and bacterial production experiments were completed within 2

hours of collection. A saturation curve was run on the first samples collection date

19

and determined that 60 mM ^{3}H-thymidine was a saturating concentration, and was therefore the concentration used in all experiments. The procedure for determining BP is that of Kirchman (2001). Triplicate tubes from each sample plus a TCA-killed control were spiked with ^{3}H-thymidine. After a 30-minute incubation period at 26°C the rest of the vials were killed with 75 μl of ice-cold 100% TCA. After terminating production all samples were either processed through the methanol wash the same day or else were stored in a dark refrigerator overnight. To complete processing, samples were centrifuged for 10 minutes at 13,000 RPM and the radioactive supernatant was poured off. The samples were washed first with 5% cold TCA and second with 80% ethanol. Between washes samples were centrifuged for 5 minutes at high speed. After the methanol wash samples were left to air dry in a fume hood. Finally, 1 ml of ULTIMA Gold scintillation cocktail was added and the samples were radioassayed.

Thymidine incorporation was calculated using the following equation from Bell (1993).

$$\text{moles thymidine L}^{-1} \text{ hr}^{-1} = \frac{(\text{dpm}_{\text{sample}} - \text{dpm}_{\text{blank}}) * 4.5\text{x } 10^{-13}}{\text{SA} * t * v} * 10^{-3} * 1.03$$

Where SA is the specific activity in curies/mM of the ^{3}H-thymidine, t is the time incubated in hours, and v is the sample volume in liters.

Further, carbon incorporation was calculated using the equation:

$$\text{μg C L}^{-1} \text{ h}^{-1} = \text{moles thymidine L}^{-1} \text{ hr}^{-1} * \text{cells mole}^{-1} * \text{carbon cell}^{-1}$$

The number of cells mole^{-1}, also commonly referred to as the thymidine conversion factor, has been empirically derived in many marine and freshwater studies. The average value 2×10^{18} cells mol^{-1} was used in this study. The carbon cell^{-1} has been empirically derived in several studies and was approximated to be 25 fg C cell^{-1} for bacteria in eutrophic systems (Bell 1993).

Phytoplankton Production Rates

Phytoplankton incorporation of ^{14}C-bicarbonate was measured using the methods of Parsons et al. (1984). Briefly, 50 ml from each replicate were poured into triplicate clear polycarbonate bottles. Each bottle was spiked with 20 µl of ^{14}C-bicarbonate (specific activity = 10.5 µCi 100 µl^{-1}), which yielded a final addition of 2.1 µCi ^{14}C-bicarbonate. Each sample was incubated at 3 light levels: one in the dark as a control, 30 and 100 µmol photons m^{-2} sec^{-1}. Light was measured in water using a 4π Biospherical Instruments light sensor (model QSL2101) to determine light intensity. All bottles were immediately placed in the same incubator at 26°C for 4 hours. After incubation 20 ml were filtered onto a GF/F filter and placed into scintillation vial. Two drops of 5N HCl were added to each vial to drive off any excess ^{14}C-bicarbonate overnight. The next day 10 ml of Ecoscint scintillation fluid was added and the samples were radioassayed.

Incorporation of ^{14}C-bicarbonate was determined using the equations from Parsons et al. (1984).

$$\text{Photosynthesis in mg C m}^{-1}\text{ hr}^{-1} = \frac{(R_S - R_B) \times W}{R \times N}$$

Where R_S is the dpm of the sample, R_B is the dpm of the dark blank, W is the DIC concentration in mg C m^{-3} hr^{-1}, R is the dpm of the initial ^{14}C spike, and N is the time the sample was incubated in hours.

pH Experiment

While the pH in the Sassafras River may climb as high as 10, this was not observed during the sampling period. In order to better understand the effect of the most extreme naturally occurring pH levels, a series of experiments were performed to determine the effect of pH on bacterial and phytoplankton production over a range of relevant pH levels. Water for these experiments was collected on September 4th and on September 16, 2008. During field sampling water was collected in a 10 L plastic carboy from the site that appeared to have the highest cyanobacteria density, which was judged by pH and water color. The carboy was transported back to Horn Point Lab and gently poured through a 156 μm mesh to remove most large grazers. The water was also spiked with J1 media to prevent nutrient limitation. The nutrient addition was approximately 10% of the recommended protocol addition for culture media in Anderson (2005). The carboy was stored at 26°C in an incubator on a 14:10 light/dark cycle until the experiment began.

The effect of pH on phytoplankton and bacteria production at pH 8, 9, and 10 was tested. The first experiment altered the pH via acid/base titration. This method ensures that the initial bacteria and phytoplankton communities are identical between pH treatments, but makes the assumption that differences in production rates are due to prolonged pH exposure rather than acute effects due to the acid/base addition. This assumption seemed somewhat dubious, so the experiment was repeated a second time and a different method of pH manipulation was performed. The pH was altered in the second experiment by manipulating the supply of air (and thus carbon dioxide) to phytoplankton, which altered the carbonate balance and the pH of the water. To do this one carboy was capped to prevent air exchange. This resulted in the phytoplankton drawing down the carbon dioxide in the carboy and causing the pH to rise. The other carboy was bubbled to ensure sufficient CO_2 supply, thus dropping the pH. This method removes the risk of immediate damage to cells through a drastic pH change, but is more difficult to compare production rates since the phytoplankton and bacteria communities are likely to have diverged during the time the carboys were incubated to alter the pH. Any community divergence may represent what would occur naturally in the field, but bottle effects make this a difficult assumption.

In the first experiment, which was initiated on September 6th with water collected from the Powerline station, the pH was altered in the carboy by using 0.1 M HCl or NaOH to lower and raise the pH of the river water. The initial pH was 9.16. The water in the carboy was split into 3 containers and the pH of two containers was altered to achieve a pH of 8 and 10. Single drops of acid or base were added while

gently swirling the river water to minimize damage to individual cells and while

monitoring the pH with a YSI 63 probe. After titration the river water at the

beginning of the experiment was at pH 8.1, 9.16, and 10.11. For each pH level river

water was poured into triplicate 1 liter bottles and placed in a 26° C incubator at two

light levels: 30 and 300 µmol photons m^{-2} sec^{-1} for 24 hours. This design yielded 6

bottles within each pH level; three of which were growing at 30 µmol photons m^{-2}

sec^{-1} and three that were growing at 300 µmol photons m^{-2} sec^{-1} (Figure 3). When the

experiment was initiated samples were collected to assess Chl a, DOC, DIC, PO$_4^-$,

NH$_4^+$, NO$_3^-$, urea, and BP. After 24 hours incubation, BP and PP was measured, and

the same nutrient and carbon samples were again collected.

Figure 3. Summary of experimental design to test the effect of pH on PP and BP.

1.River water at pH 9 collected from the Sassafras was manipulated to yield the three treatment levels
2.Cells were allowed to grow under 2 light levels for 24 hours
3.14C primary production was measured by incubating each treatment flask in #2 under 3 light levels.
Bacterial production was also measured using 3H-Thymidine
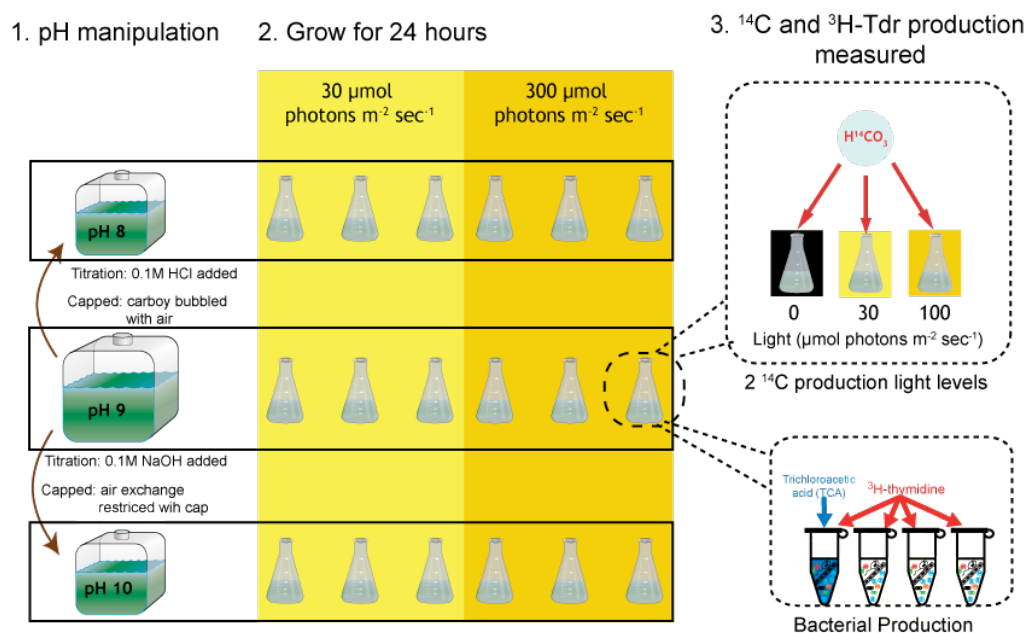
This same procedure was initiated on September 16th, 2008 in the second pH experiment in which the pH was manipulated in a different manner. Two carboys were collected from Budd's Landing on September 14th and transported back to Horn Point Laboratory where the water was gently poured through a 156 μm mesh to remove large grazers. One carboy was then capped to drive the pH up, while the other was bubbled with air to drive the pH down. Both were incubated at 26°C for approximately 16 hours until both carboys had attained the appropriate pH. A portion of the high and low pH carboys were mixed to create the pH 9 treatment level. At the beginning of the experiment the pH of each treatment level was 7.95, 9.06, and 10.01.

It should be noted that cells were grown at 30 and 300 μmol photons m^{-2} sec^{-1}, and the original intention was to measure PP in each treatment at approximately the same light level (factorial treatment design). However, it was discovered after the experiments were conducted that the maximum irradiance in the production measurements was only 100 μmol photons m^{-2} sec^{-1}, which unfortunately alters the treatment design, and must be considered when examining the results.

DOC Excretion

Anabaena crassa (AN1B) that had been isolated from the Sassafras River in 2007 was obtained to investigate the effect of pH and light intensity on DOC excretion. Triplicate bottles of 500 ml DI water were autoclaved and J1 nutrients were added to the media. Cells were inoculated and incubated at 20°C on a 14:10 light dark cycle. The cells were grown at 30, 50, 150, and 300 μmol photons m^{-2} sec^{-1}. The pH was monitored weekly and when the culture reached a high biomass a

portion of the culture was removed to test for production of particulate organic carbon (POC) and DOC using ^{14}C-bicarbonate. A portion of the sample was also filtered for Chl *a*, DOC, and DIC. PP experiments were initiated after the lights had been on for ~12 hours.

DOC excretion from phytoplankton was assessed using methods modified from Šimek et al. (2008). To assess production and exudation 20 ml of sample water was spiked with 2.1 μCi ^{14}C-bicarbonate. Each sample was paired with a dark control and incubated in full light and with two shade screens over it at 26°C for 4 hours. After incubation the entire sample was filtered through a GF/F filter and processed in the same way as the other ^{14}C production samples. To determine excretion, 10 ml of filtrate was transferred to a new scintillation vial and acidified with ~1 ml of 5N HCl. Vials were placed on a heating block at 60°C and dried overnight in a fume hood. In the morning 10 ml of Ecoscint scintillation fluid was added. All vials sat overnight before ^{14}C radioassay.

Molecular Work

DNA extraction, PCR amplification, and DGGE were performed using modified methods of Crump et al. (2003) and Muyzer et al. (1993). DNA was extracted from Sterivex filters using a chloroform extraction. Samples were frozen at -80°C and thawed at 37°C three times and then incubated for 30 minutes at 37°C after addition of 20 μl each of proteinase K (1%) and lysozyme (10%). Sodium dodecyl sulfate (SDS, 50 μl of 20% filter sterilized) was added and tubes were inverted to mix

before a further 2 hour incubation at 65°C. Samples were next washed with phenol:chloroform:isoamyl alcohol 2x (25:24:1, pH 8.0) two times. Between washes samples were centrifuged at 3,000 rpm for 5 minutes and the supernatant pipeted off and transferred to a new microcentrifuge tube. All samples were precipitated with 0.6 parts 100% isopropyl alcohol in the dark at room temperature overnight. The next day samples were centrifuged at 13,000 rpm for 30 minutes to concentrate the DNA pellet at the bottom of the tube. The isopropyl alcohol was poured off and the pellet was washed with 70% ethanol two times. Between washes the samples were centrifuged at 13,000 rpm for 10 minutes. Finally, the DNA pellet was dried in a roto-evaporator until dry (approximately 30 minutes). DNA was resuspended in 250 μl sterilized DI and stored at -80°C.

PCR amplification was performed with a GoTaq Flexi DNA Polymerase kit supplied by Promega. The cocktail consisted of $MgCl_2$ (2 mM), buffer (1x), deoxynucleoside triphosphates (dNTPs) (0.2 μM), a bacteria-specific primer 357f(g+c) (0.25 μM), a universal primer 519r (0.25 μM), Go-Taq polymerase (0.25 units per reaction), and the DNA template. Bovine serum albumin (BSA, 0.2 μM) was added to samples that did not amplify on the initial PCR attempt. PCR conditions consisted of an initial 4 min at 94°C, followed by 30 cycles of 1 min at 94°C; 1 min at 65–55°C (reducing temperature by 1° per cycle for 10 cycles plus 20 cycles at 55°C); and 1 min at 72°C, followed by 1 h at 72°C. Steps involving a temperature reduction were carried out at a rate of 0.3°C per second. PCR products were stored at 4°C.

Denaturing gradient acrylamide gels that were 1 mm thick and 16 cm long

were used for DGGE. Acrylamide gels (8%) were prepared with 0% acrylamide-

disacrylamide (37.5:1) and TAE buffer (40 mM Tris, pH 8.0), acetic acid (20 mM),

and 1 mM EDTA. The denaturing gradient consisted of urea and formamide between

35-60%. Gels were loaded on a Bio-Rad D-code system and electrophoresis was run

for 24 hours at 70 V. Gels were magnified and photographed using a ChemImager

4000 system and gel images were reconstructed with Adobe Photoshop to ensure

adequate resolution. Gels were analyzed with GelcomparII software (Bionumerics),

and each sample was scored for presence and absence of bands. A pairwise distance

matrix was then constructed after putting data into StatSoft's Statistica package. A

pairwise distance matrix was calculated and visualized with multidimensional scaling

analysis so that samples with more similar bands (highest community similarity) are

plotted closely, and less-similar communities are plotted farther away, providing a

visual representation of community similarity.

Results

Field Observations:

Physical factors had a large effect on both primary and bacterial production measurements from the field. Temperature decreased steadily from early September until the last sampling date on October 20 (Figure 4). During September the temperature varied little (~1°C) among stations, while there was greater spatial variability in temperature during October. On October 20 the Powerline site was 3.1°C cooler than the Drawbridge site (17°C).

Figure 4. Surface water temperature on sampling dates for each site.
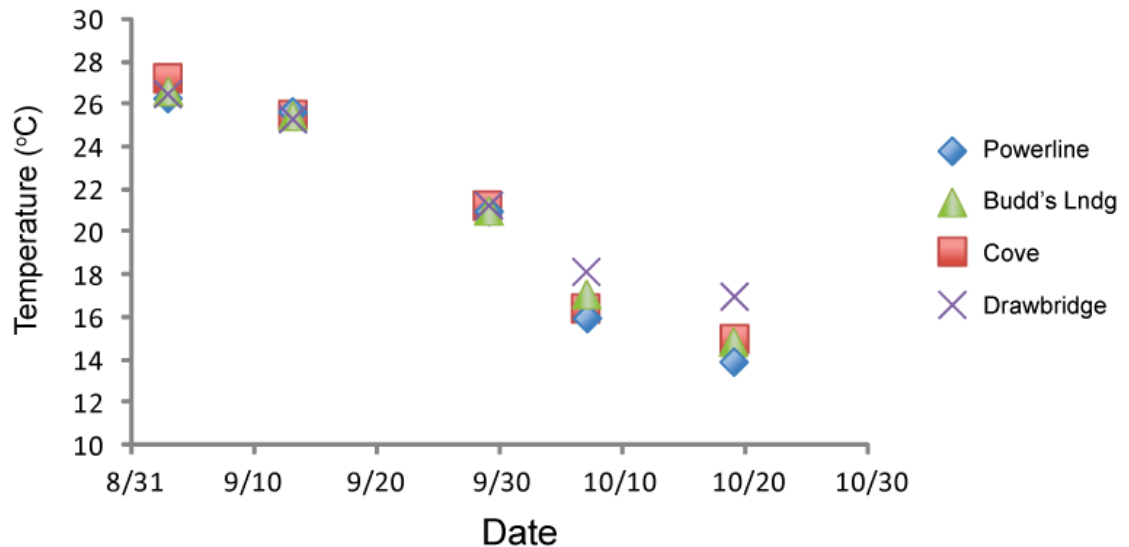
A clear increasing salinity gradient was observed in the downstream direction. The salinity range between stations increased throughout the sampling period (Figure 5). In early September the sites' salinity variability was within 0.5 among the stations,

whereas in late October the salinity ranged from 2.4 upriver at Powerline to 4
downriver at the Drawbridge. Overall, salinity also increased during the sampling
period. Salinity was lowest during early September (<1) and was as high as 4 on
October 20. Below-average rainfall in October may partially explain the enhanced
salinity gradient observed over that month.

Figure 5. Surface water salinity on each sampling date at the four sampling stations.
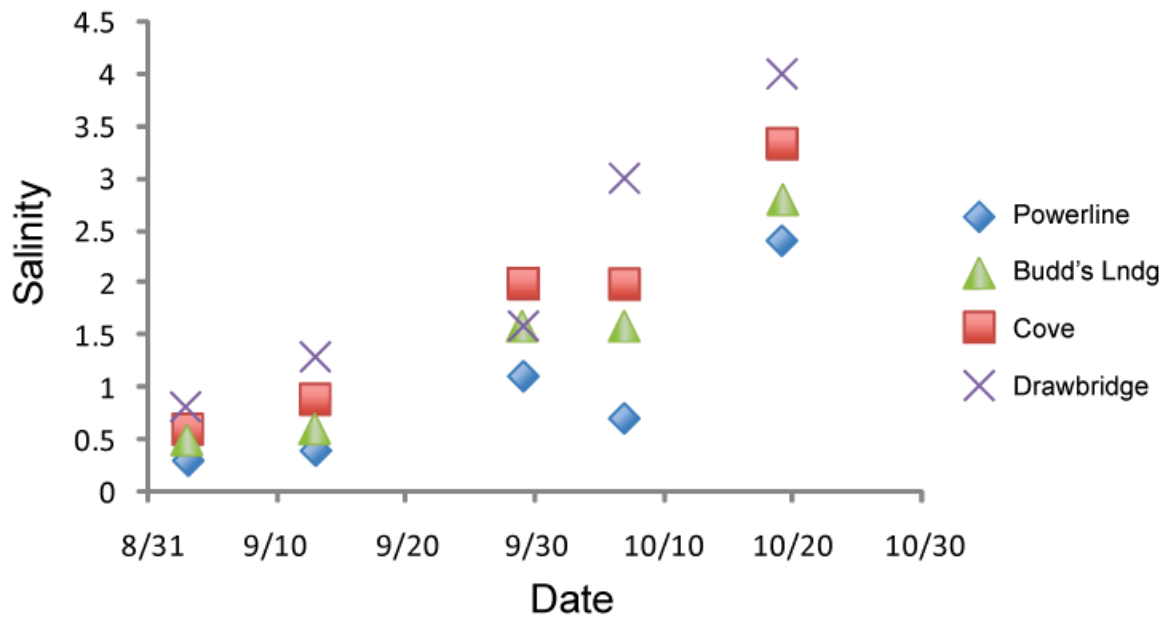
Nutrient concentrations also varied during the sampling period (Figure 6).
PO_4 concentrations generally fell during the sampling period until October 8th and
then remained low. This trend was not observed in the Cove sampling site, where
PO_4 concentrations remained relatively low and variable. Urea and NH_4^+ were
largely below the detection limit (0.5 and 1 µM, respectively) during this period.
NO_3^- was the dominant nitrogen source in this region and varied widely between 2.8-

10 μM. Anomalously high NO_3^- concentrations (42 μM) were observed on October

8th at the Powerline station. DIC concentrations ranged from < 4,000 to > 10,000 μM

and generally decreased during the sampling period. DOC also decreased during the

sampling period. Variability in DOC in early September was approximately 2-fold

among stations, but variability dramatically decreased by the end of the sampling

period in late October.

Figure 6. a.) Phosphate and b.) Nitrate concentrations (μM) over the sampling period for each station. Ammonium and urea concentrations were consistently below detection limits and are not shown.
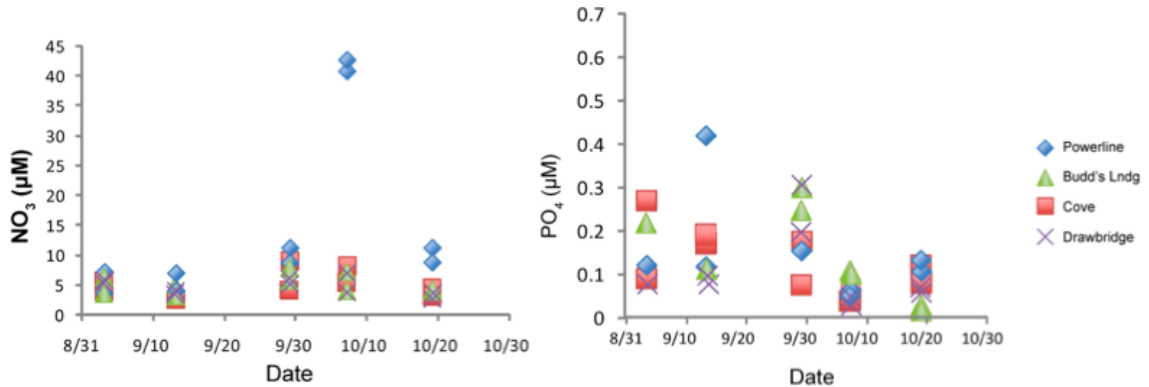

Figure 6. d.) Dissolved organic carbon concentrations (mg l^{-1}) and e.) dissolved inorganic carbon (mg m^{-3}) over the sampling period for each station.
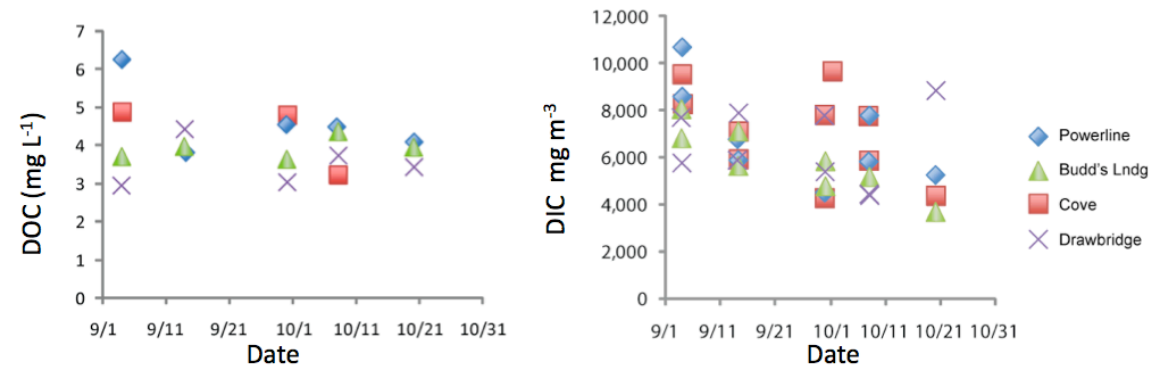
The pH values observed were as low as 7.35 and as high as 9.19. The downriver sites at Georgetown and Cove had lower pH on average (8.036 and 8.18 respectively) then the upriver sites at Budd's Landing and Powerline (8.8 and 8.68 respectively) (Figure 7). Sustained winds of 5 mph over September 14-15 may have depressed pH values at some sampling sites by increasing diffusion of CO_2 from the atmosphere into the water (weather.com). While the pH at Drawbridge and Cove were near 7.5, the pH at Budd's Landing and Powerline remained above 8.4 during this period. Winds also picked up from October 1-4 to between 5-19 mph, and may have influenced pH on this date.

Figure 7. pH at each station during the sampling period.
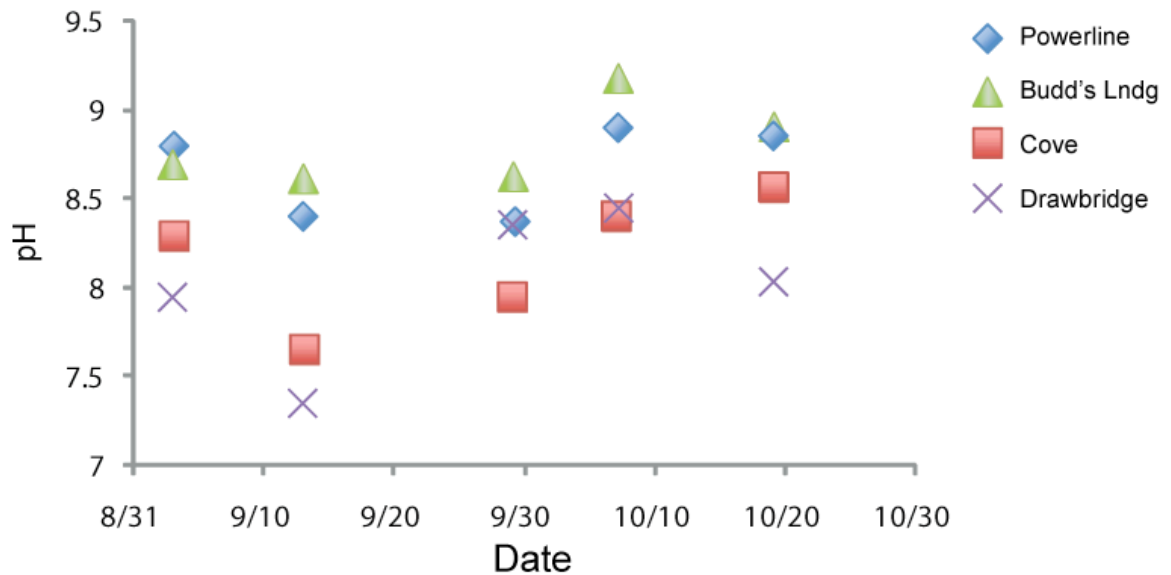
Chl a ranged widely from ~40 to 100 µg Chl a l^{-1} among the sites during the sampling period (Figure 8). Chl a correlated positively with

pH (p = 0.0175, R^2 = .1363). Temperature, salinity, NO_3^-, and PO_4 did not

significantly correlate with Chl a. The downriver site at Drawbridge consistently had

low Chl a values relative to the upriver stations, except on October 4, when Chl a at

this site equaled or exceeded the other sites. Chl a values gradually decreased at all

sites after mid-September, but still remained relatively high at the end of the sampling

period in late October. Chl a and pH values were both generally greatest at Budd's

Landing and Powerline.

Figure 8. Chlorophyll a concentrations (μg l^{-1}) on each sampling date for each station in the Sassafras.
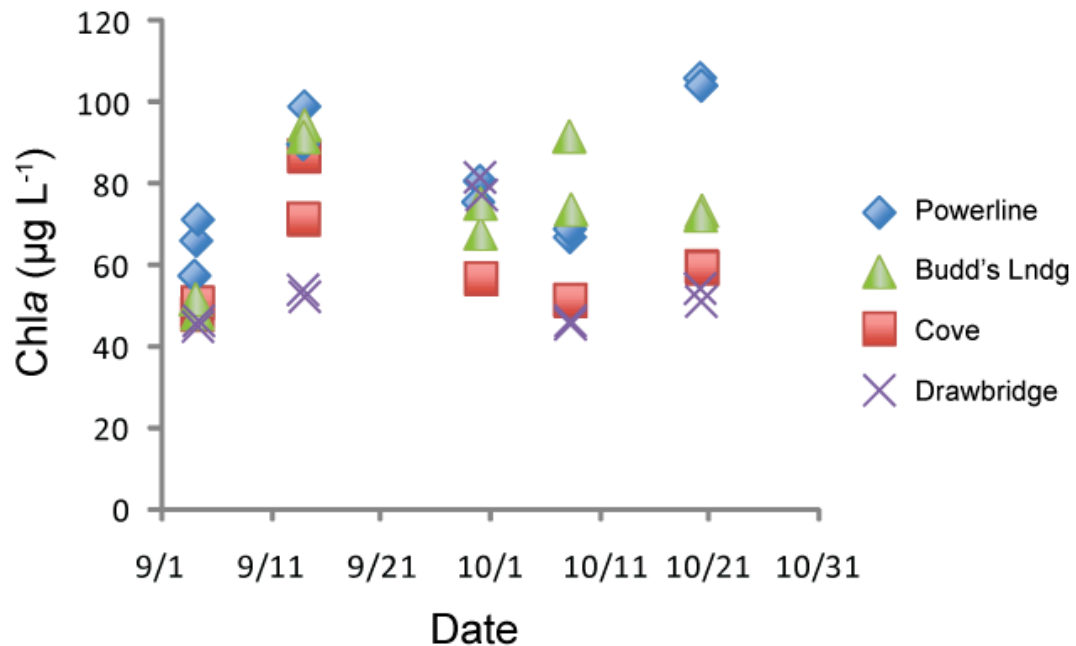
PP rates at 26°C decreased steadily as the fall season progressed (Figure 9).

Temperature, salinity, and DIC strongly correlated with primary production in both

30 and 100 μmol photons m^{-2} sec^{-1} light treatments over the sampling period (Figure

10). Unless otherwise specified, correlations are made on PP measurements taken at

100 μmol photons m^{-2} sec^{-1} at 26°C (Table 1). Water temperature at the time of

sampling was positively correlated with PP, while salinity correlated negatively. Low

DIC concentrations correlated with decreases in PP. Phosphorus also had a

significant, but weak positive correlation with PP, but nitrogen did not. A multiple

regression using stepwise selection, which required a significance level of 0.2 to enter

the model, determined that temperature, salinity and phosphate all correlated with PP

during early fall (p < .0001, R^2 = .9390).

Figure 9. Primary production (mg C m^{-3} hr^{-1}) when measured at a) at 30 μmol photons m^{-2} sec^{-1} and b)
100 μmol photons m^{-2} sec^{-1} measured as ^{14}C incorporation from each station during the sampling
period.
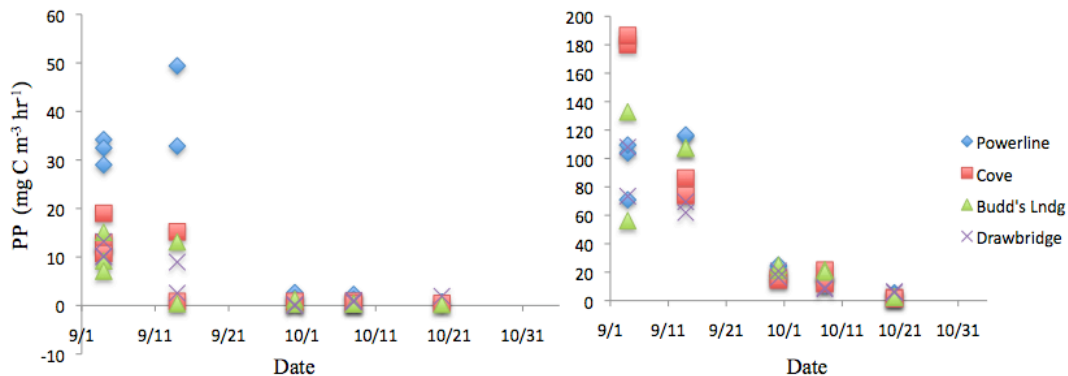
PP also did not correlate with Chl *a* or pH. However, PP rates were relatively

low at the highest observed pHs, so an ANOVA was performed in which three pH

ranges (7.6-8.2, 8.3-8.6, and 8.7-9.2) were tested. A significant effect of pH on both

low and high light measured PP was detected. When the pH ranged from 8.7-9.2

phytoplankton exhibited less PP than at lower pHs. It seems that the concurrent fall

in temperature and PO_4, combined with increasing salinity and pH stress at some sites explain PP in the Sassafras River.

Table 1: Field correlations of in situ conditions with PP at 100 μmol photons m^{-2} sec^{-1}

Parameter	slope	R^2	F	p
†temperature	0.116	0.8619	243.48	<.0001 ***
†salinity	-0.478	0.7475	115.46	<.0001 ***
PO_4	19129	0.1460	6.5	0.015 **
NO_3^-	n.s.	n.s.	1.83	0.1837
DIC	1.86	0.2563	13.44	0.0007 **
DOC	2240	0.0823	3.14	0.0851 *
Chl$a$	n.s.	n.s.	2.67	0.1949
pH	n.s.	n.s.	1.59	0.2142

†correlations performed on log transformed PP

*** significant at α= .01

**significant at α=.05

*significant at α=.1

On September 30th PP rates dropped sharply to between one half and one third of previous production measurements at all stations. This was concurrent with water temperatures dropping between 4 and 5°C from the previous sampling date approximately 2 weeks earlier. However, it should be noted that all production measurements were performed at 26°C. Salinity had also increased by September 30th. The pH measurements were variable among the stations on this date. The upriver stations had similar pH to the previous sampling date (8.4-8.6), while both the downriver stations' pH had increased.

Figure 10a. Correlations of log-transformed primary production against temperature in the field. PP at 100 μmol photons m^{-2} sec^{-1} was used.
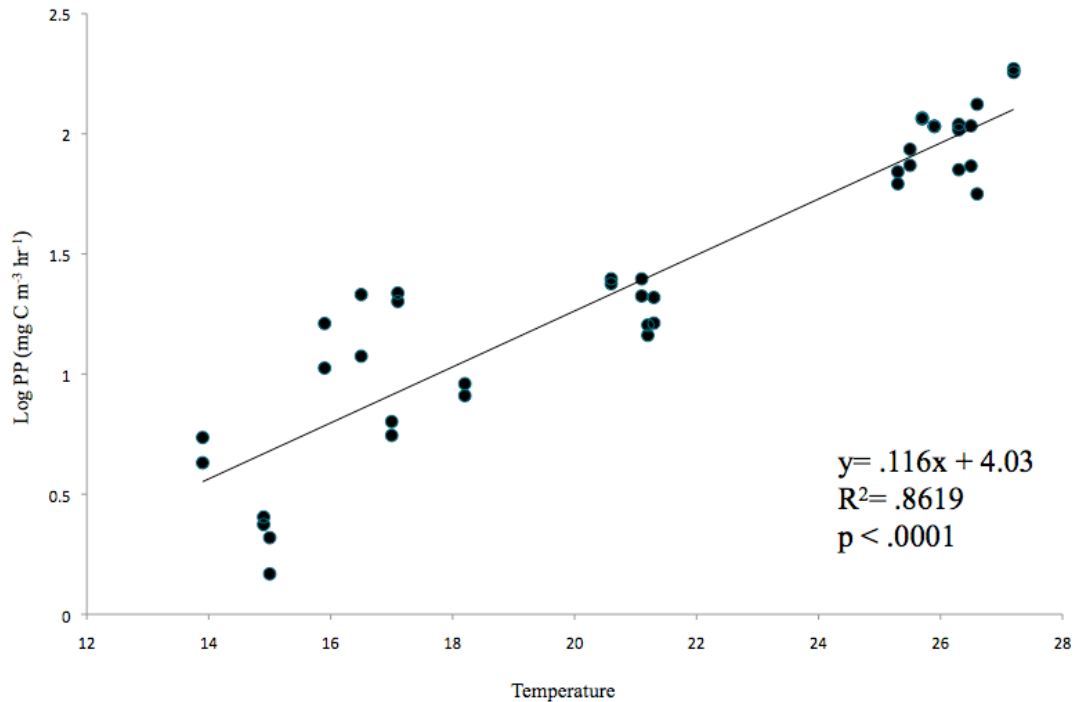
PP varied as expected between measurements taken at 30 μmol photons m^{-2} sec^{-1} and 100 μmol photons m^{-2} sec^{-1}. Greater ^{14}C production was observed in 100 μmol photons m^{-2} sec^{-1} light treatment than in the 30 μmol photons m^{-2} sec^{-1} treatment (Figure 9). However, temperature, salinity, and pH were correlated with carbon fixation in cells exposed to the lower light level more than the higher light level, suggesting non-linear responses in photosynthesis to environmental stressors. At the Powerline station phytoplankton collected from a salinity of 0.3 and temperature 26.3°C where able to incorporate nearly half as much ^{14}C-bicarbonate when incubated at 30 μmol photons m^{-2} sec^{-1} as cells measured at 100 μmol photons

m^{-2} sec^{-1}. When the temperature dropped to 20.6 and the salinity nearly quadrupled to

1.1, low light production fell to 10% of 100 light level production.

PP was not significantly correlated with pH, but when plotting PP vs. pH it is

clear that PP was lower at elevated pH, so three pH levels were chosen and tested as

an ANOVA. The pH levels tested were <8.3, 8.3-8.7, and >8.7. The ANOVA shows

that carbon fixation at 30 µmol photons m^{-2} sec^{-1} was significantly lower beginning at

pH 8.3. PP measured at 100 µmol photons m^{-2} sec^{-1} also declined with pH, but the

negative effect of pH did not occur until pH 8.7.

Figure 10b. Correlations of log-transformed primary production against salinity in the field. PP at 100 µmol photons m^{-2} sec^{-1} were used.

$y = -0.478 \ x - 7.23$
$R^2 = .7475$
$p < .0001$
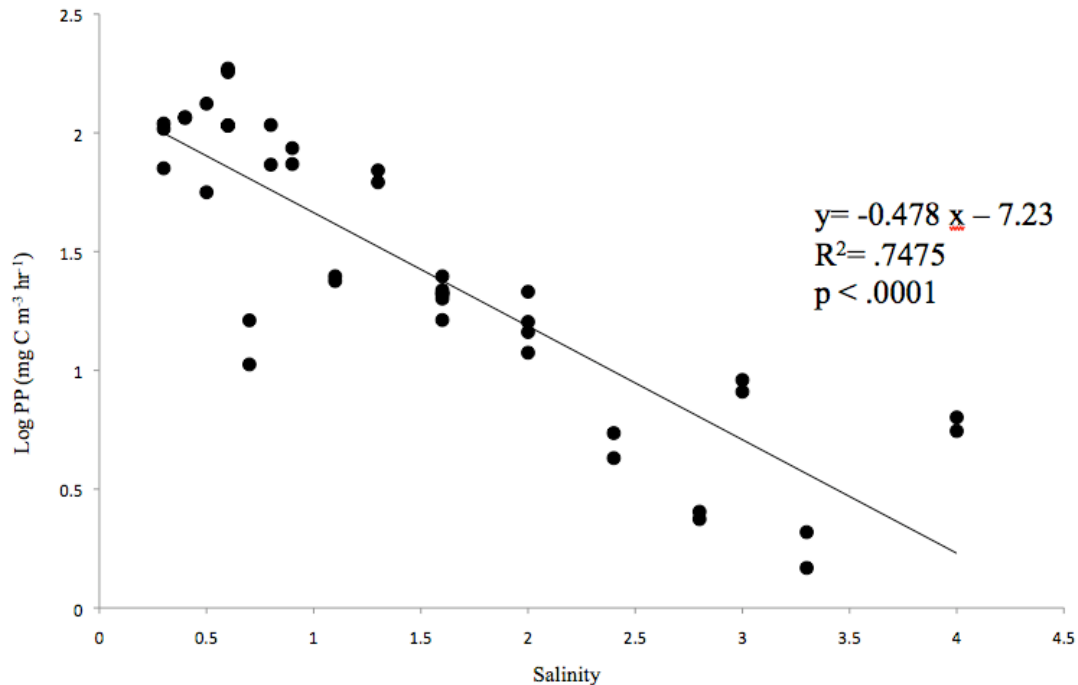
Figure 10c. Correlations of log-transformed primary production against DIC in the field. PP at 100 μmol photons m^{-2} sec^{-1} were used.

$$y = 1.86\ x - 5610$$
$$R^2 = .2563$$
$$p < .0007$$
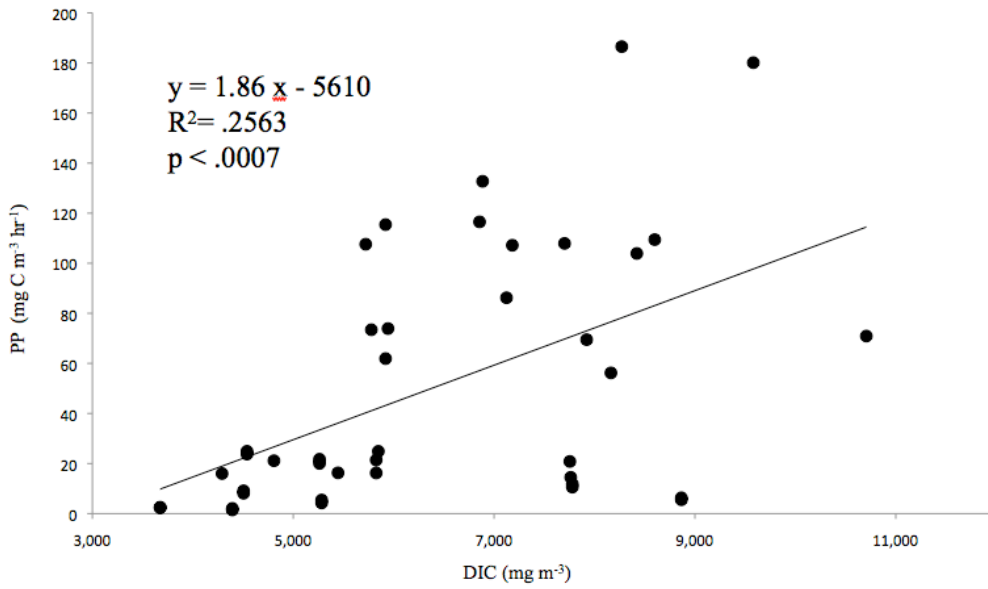
Figure 10d. Correlations of log-transformed primary production against soluble reactive phosphorus in the field. PP at 100 μmol photons m^{-2} sec^{-1} were used.

$$y = 19129x + 3275$$
$$R^2 = .1460$$
$$p < .0150$$
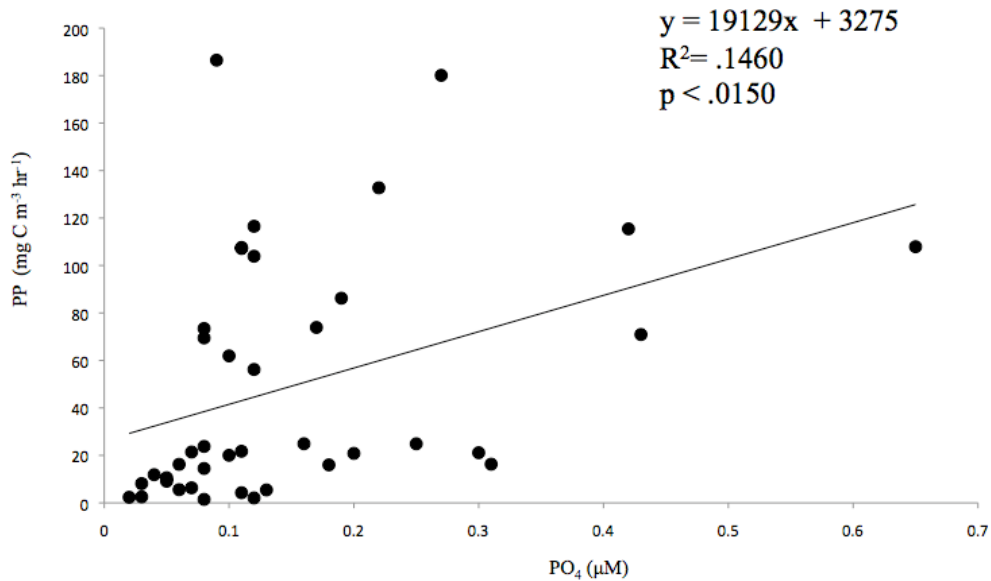
Figure 11. PP measured at a) 30 and b) 100 μmol photons m^{-2} sec^{-1} normalized to Chl a.
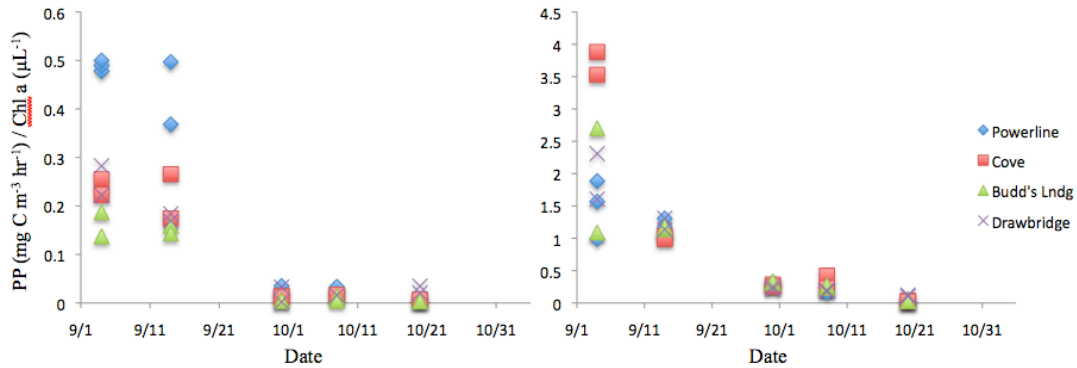
PP was normalized to Chl a in order to compare between the stations (Figure 11). Production differed between stations and depended strongly on the light intensity the measurement was performed at. For example, a phytoplankton community at one station was often far more productive then the other stations at 100 μmol photons m^{-2} sec^{-1}, but became less productive then the other communities when exposed to 30 μmol photons m^{-2} sec^{-1}. The phytoplankton communities at the upriver station were more productive then other communities at low light on all days but October 20, but became relatively less productive than the downriver stations at the higher light intensity. Since Chl a was often elevated at the upriver stations, this may indicate that the cells were experiencing self-shading and were adapted to low light intensities. The mid-river stations (Budd's Landing and Cove) were generally the most productive communities when incubated at 100 μmol photons m^{-2} sec^{-1}, but became the least productive stations when measured at the lower light level.

PP rates correlated weakly with DOC (p= .0851, R2= .0823) among all the stations, suggesting that phytoplankton were not the main contributors to DOC

availability. Groundwater, terrestrial and benthic fluxes were not measured, and their contribution to DOC availability is unknown, but is probably important.

BP correlated positively with several environmental factors. BP correlated with parameters associated with PP including the strongest correlation with ^{14}C uptake ($p < 0.0001$, $R^2 = 0.4150$), DOC ($p < .0001$, $R^2 = 0.3581$), and DIC ($p = .0004$, $R^2 = 0.2869$) (Figure 12, Table 2). Temperature ($p < 0.0001$, $R^2 = 0.3439$) positively correlated with BP, while higher salinity values corresponded to relatively low BP measurements ($p < 0.0001$, $R^2 = 0.3057$). PO_4 availability also significantly correlated with BP, although to a lesser degree ($p = .0040$, $R^2 = 0.2030$) (Figure 13). No significant relationship existed between Chl a or any of the nitrogenous nutrients. BP followed the same general decreasing trend with primary production as fall progressed. However, on the last sampling date BP increased at all stations. BP was not correlated with pH ($p = 0.1448$).

Table 2: Field correlations with BP

Parameter	slope	R^2	F	p
temperature	62239	0.3439	61.84	<.0001***
salinity	-256402	0.3057	51.95	<.0001 ***
PO$_4$	1547	0.2030	9.42	0.004 ***
NO$_3^-$	n.s.	n.s.	1.04	0.3133
DIC	0.14776	0.2869	15.29	0.0004 ***
DOC	359	0.3581	18.97	0.0001 ***
PP	0.0492	0.4150	83.71	<.0001 ***
Chl*a*	n.s.	n.s.	0.42	0.5196
pH	n.s.	n.s.	2.15	0.1448

*** significant at α= .01

**significant at α=.05

*significant at α=.1

Figure 12a. Correlation of BP vs PP from field data. PP was measured at 100 μmol photons m^{-2} sec^{-1}.

$y = 0.0403x - 602234$
$R^2 = .4150$
$p < .0001$
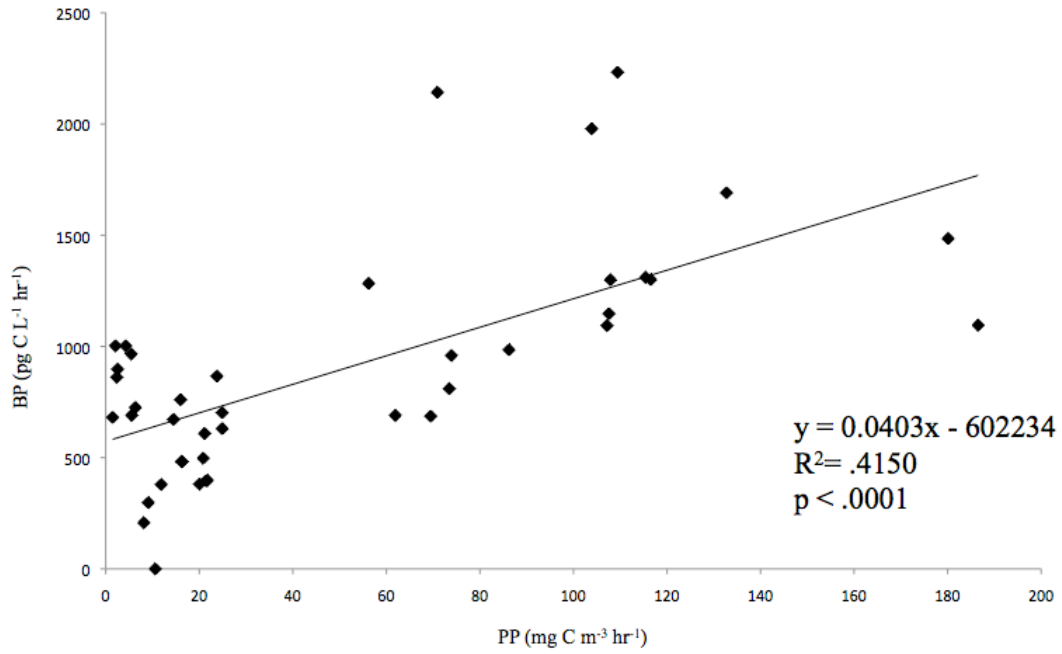
Figure 12b. Correlation between BP and DOC from all field samples collected from the Sassafras.

$y = 359x - 564$
$R^2 = .3581$
$p < .0001$
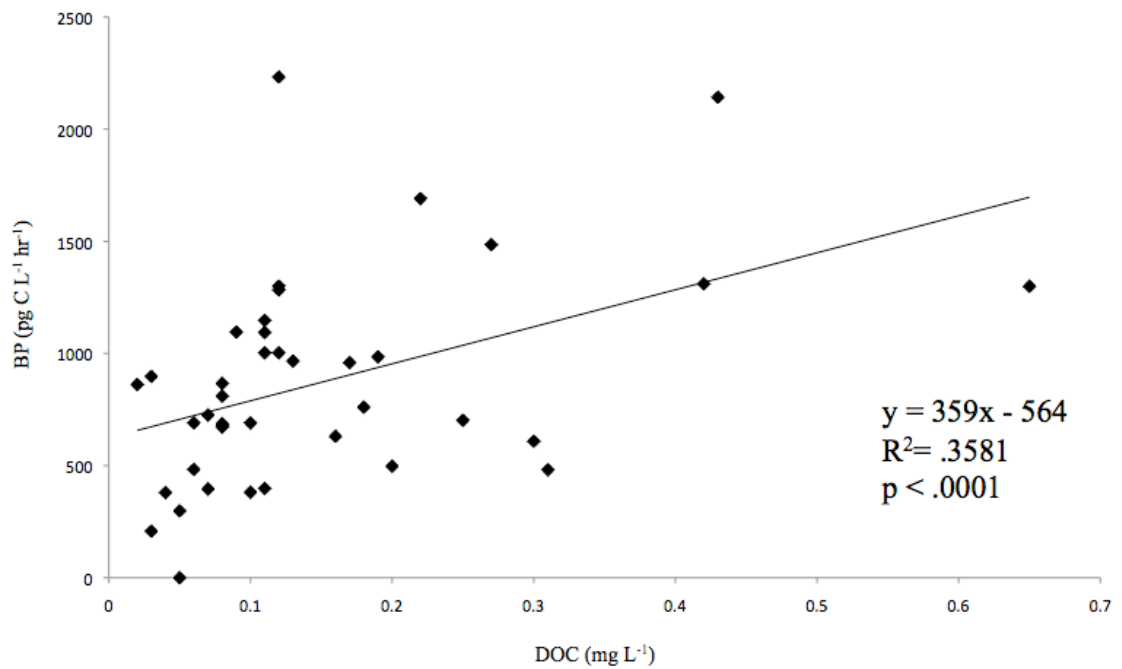
Figure 12c. Correlation between BP and DIC from all field samples collected from the Sassafras.

$$y = 0.1448x - 22.71$$
$$R^2 = .2869$$
$$p = .0004$$
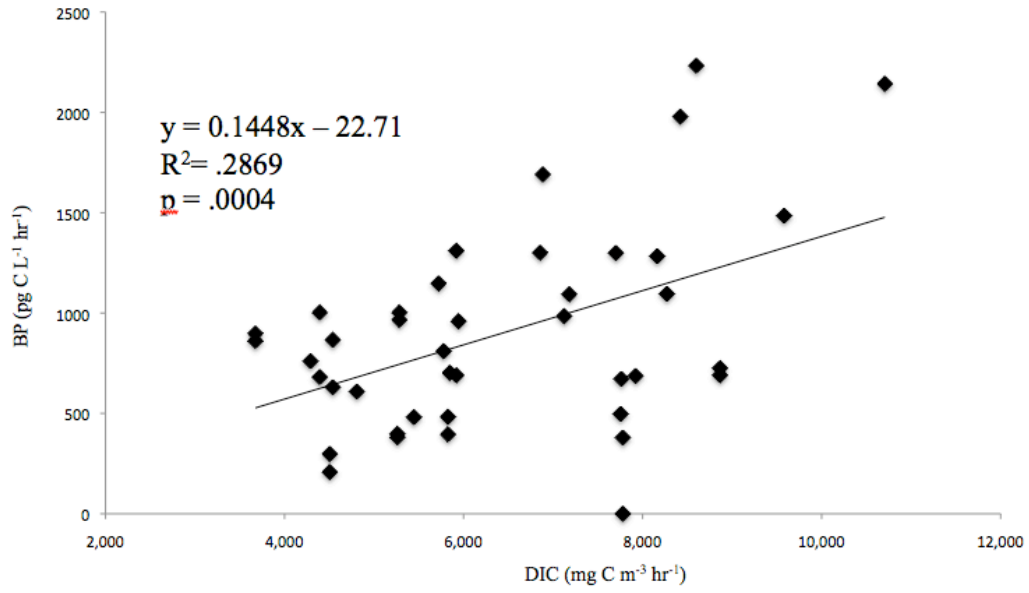
Figure 13a. Correlation between BP and temperature from all field samples collected from the Sassafras.

$$y = 62239 x - 408910$$
$$R^2 = .3439$$
$$p < .0001$$
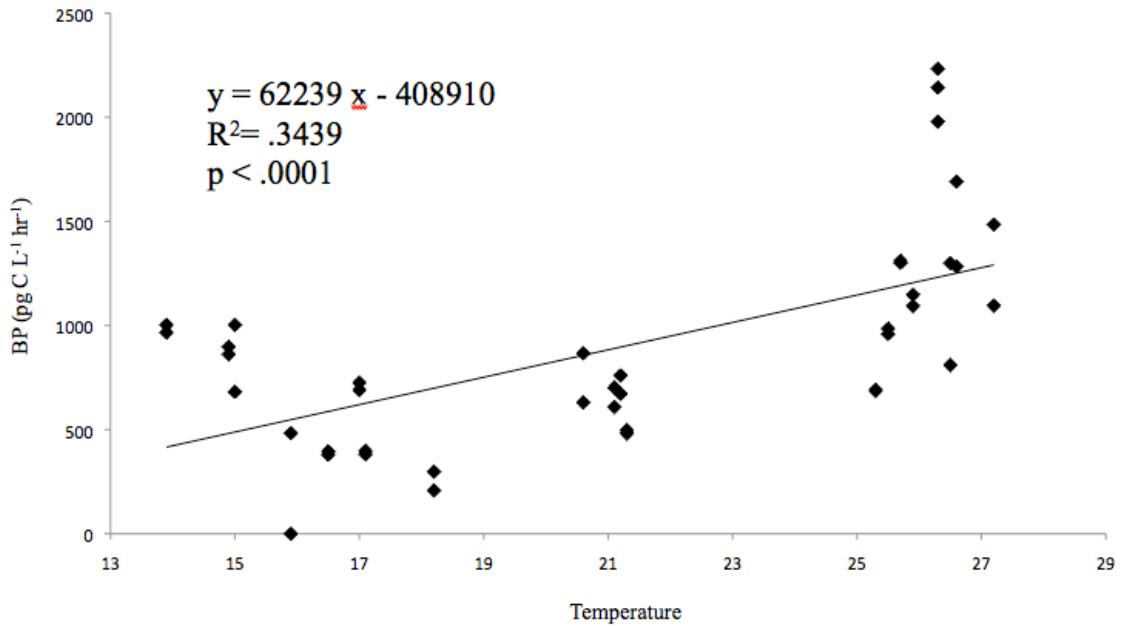
Figure 13b. Correlation between BP and salinity from all field samples collected from the Sassafras.

$$y = -256402x + 1320847$$
$$R^2 = .3057$$
$$p < .0001$$
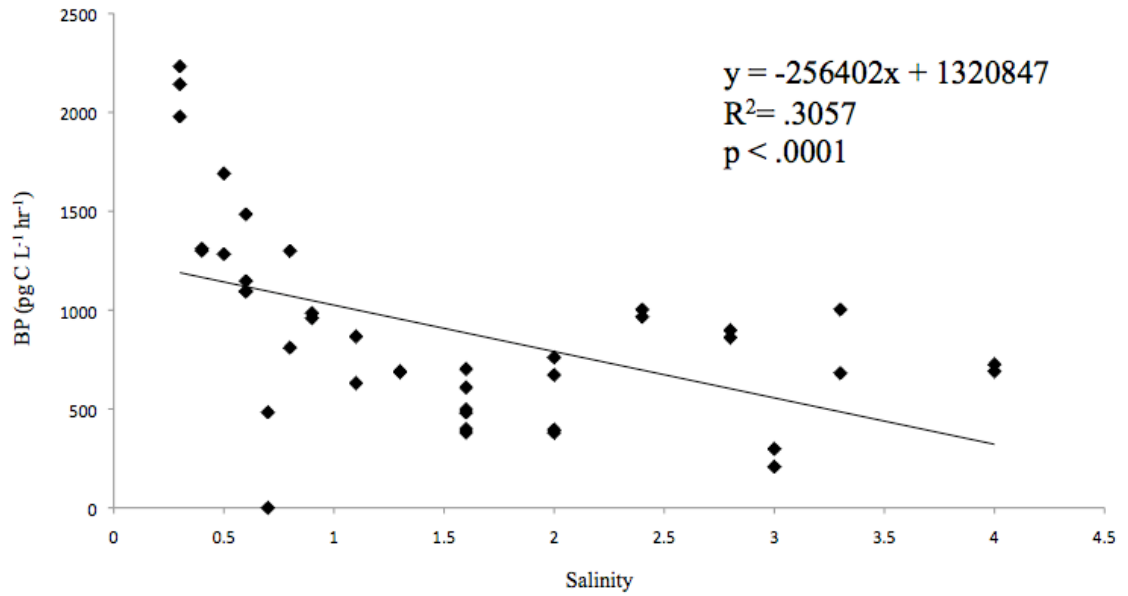
Figure 13c. Correlation between BP and PO$_4$ from all field samples collected from the Sassafras.

$$y = 1547\,x + 651$$
$$R^2 = .2030$$
$$p < .0040$$

44
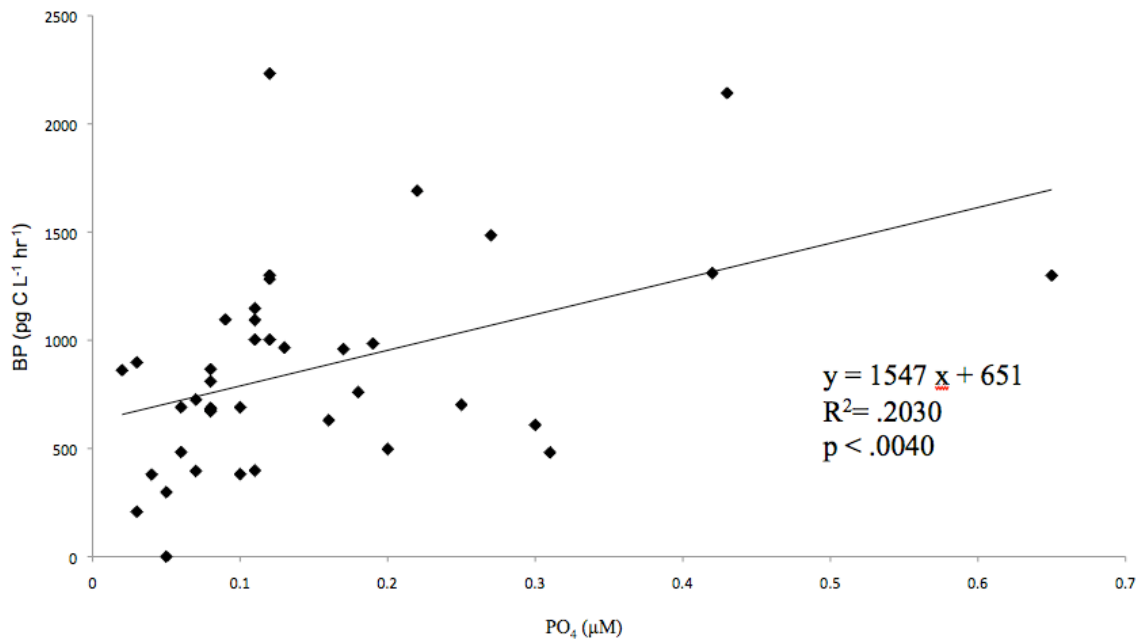
Spatial differences in BP were observed (Figure 14). Among the stations bacterial production was highest at the station located furthest upriver (Powerline) and decreased to a minimum downriver at Drawbridge. Variability in bacterial production between stations was much greater (2-3 fold) in September, and decreased in October.

Figure 14. Bacterial production at each station over the sampling period.
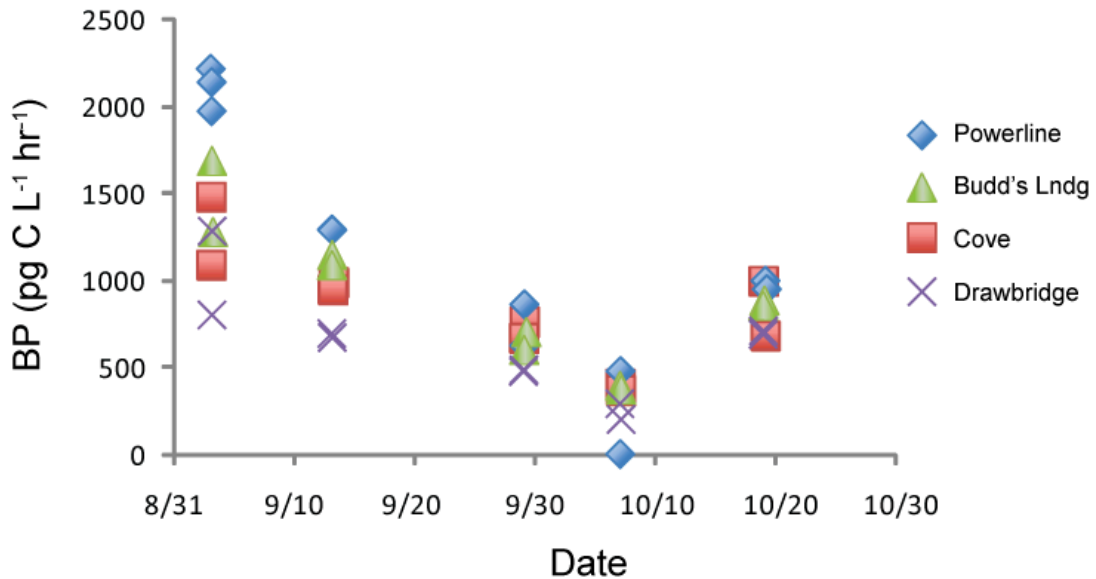
Different bacterial communities as determined by DGGE analysis exist among stations and dates (Figure 15). Bacteria samples from October clustered separately from the rest of the samples. Bacterial communities present on September 4 and 14 formed another cluster and overlapped within the cluster. Temperature differences seemed to correspond best with differences between the clusters. Nearly all parameters measured, with the exception of some nutrient data, decreased between

September and October, so it is impossible to tie a shift in community to any dominant parameter based on these data alone.

Figure 15. Plot from a pairwise distance matrix showing bacteria community similarity. Points that are plotted more closely share similar DNA, while points that are farther apart are less similar.
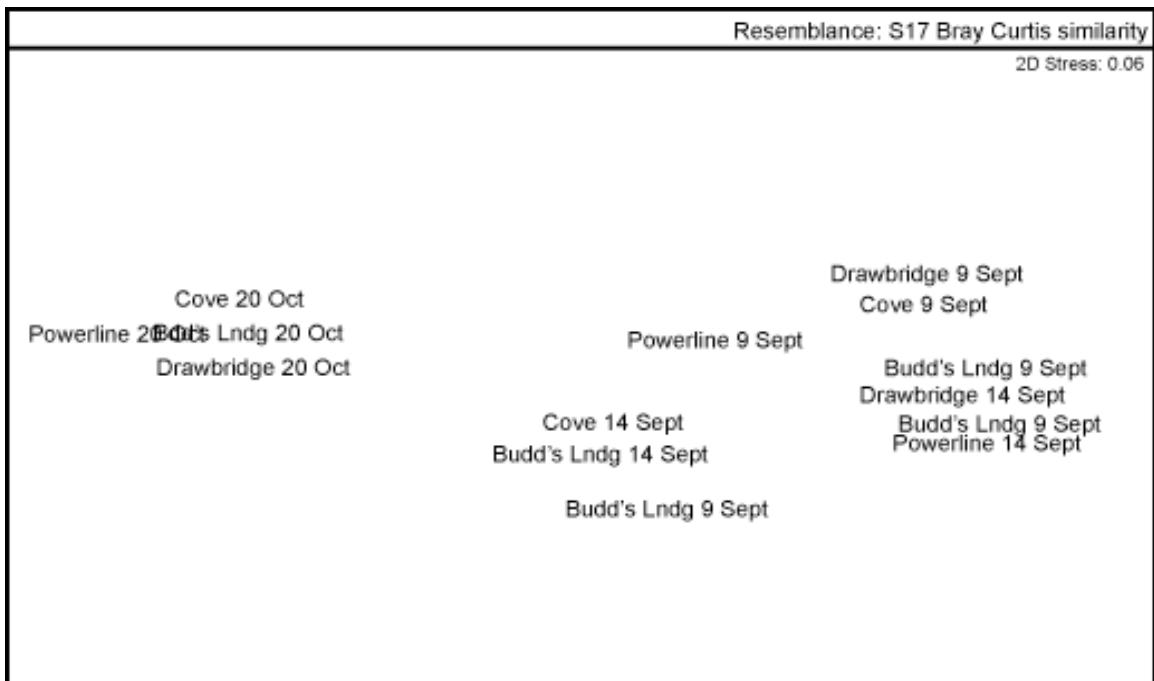
Phytoplankton communities sampled from the two endpoints of the sampling area (Drawbridge and Powerline) were dominated throughout the sampling period by cyanobacteria (Figure 16). However, at both stations the proportion of cyanobacteria decreased throughout the fall. At the Drawbridge in Georgetown cyanobacteria represented 84% of the phytoplankton, and decreased to 51% on October 22nd. During that time the proportion of small flagellates increased substantially from 6% to 32% of the phytoplankton. The same decreasing trend was observed upriver at

Powerline. Cyanobacteria dominance slipped from >90% in early September to 51% at the end of the month. During this time the proportion of chlorophytes and other flagellates increased substantially in the phytoplankton. The proportion of diatoms also increased modestly at Budd's Landing in September as well.

Figure 16. Phytoplankton community composition from samples taken by Maryland DNR that are located within the sampling area. Budd's Landing is a site shared between the data sets, and Georgetown is near the Drawbridge station.

a. Phytoplankton composition at Georgetown (near Drawbridge)

Diatom
Chlorophyceae
Cyanophyceae
Other

b. Phytoplankton composition at Budd's Landing
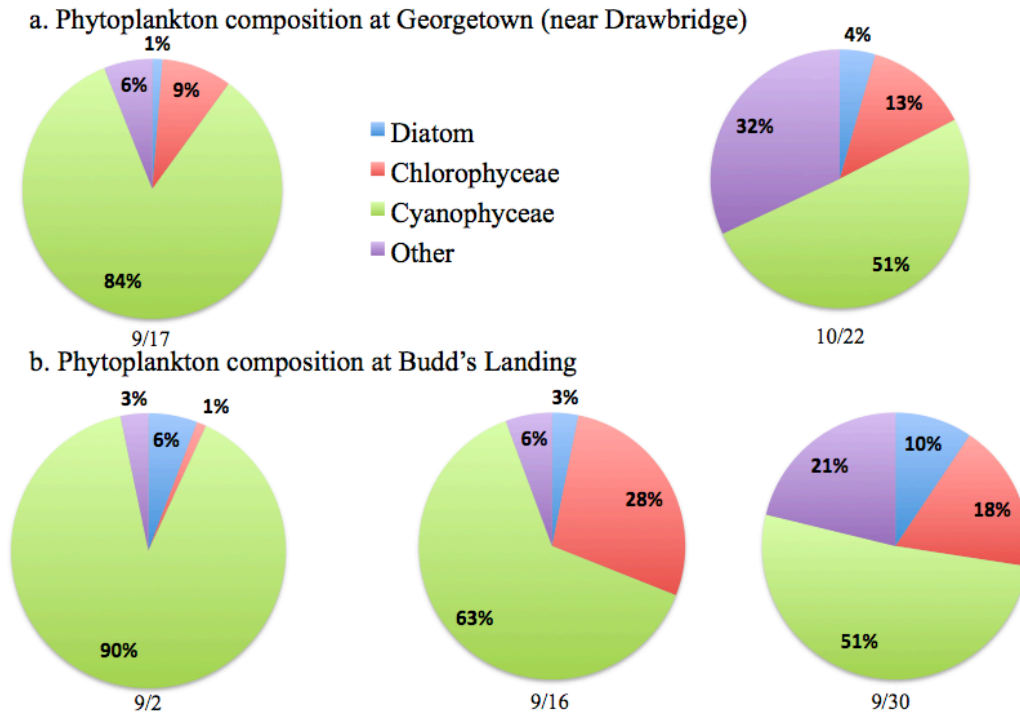
The cyanobacteria community was dynamic between stations and sampling dates. In early September at Georgetown cyanobacteria in the genera *Pseudoanabaenaceae* dominated (54%), with significant populations of *Merismopediaceae* and *Synechoccaceae* present as well (Figure 17). By the end of the month *Pseudoanabaenaceae* was still present, but *Merismopediaceae* and

Synechoccaceae had become dominant. *Merismopediaceae* was only present at Budd's Landing through September 10th, and never comprised more than 8% of the cyanobacteria. The bloom that was sampled in this study on September 4th was sampled by Maryland DNR two days earlier for taxonomic identification. Cyanobacteria represented > 90% of the species at this time. At least 6 cyanobacteria species were abundant during this time, including *Microcystis*, which occasionally produces toxins. The dominant cyanobacteria at Budd's Landing were mainly *Pseudoanabaenaceae* and *Synechoccaceae*; by September 30th *Pseudoanabaenaceae* represented 87% of the cyanobacteria community.

Figure 17. Phytoplankton community composition from samples taken by Maryland DNR that are located within the sampling area. Budd's Landing is a site shared between the data sets, and Georgetown is near the Drawbridge station.

A. Cyanobacteria composition at Georgetown (near Drawbridge)

B. Cyanobacteria composition at Budd's Landing
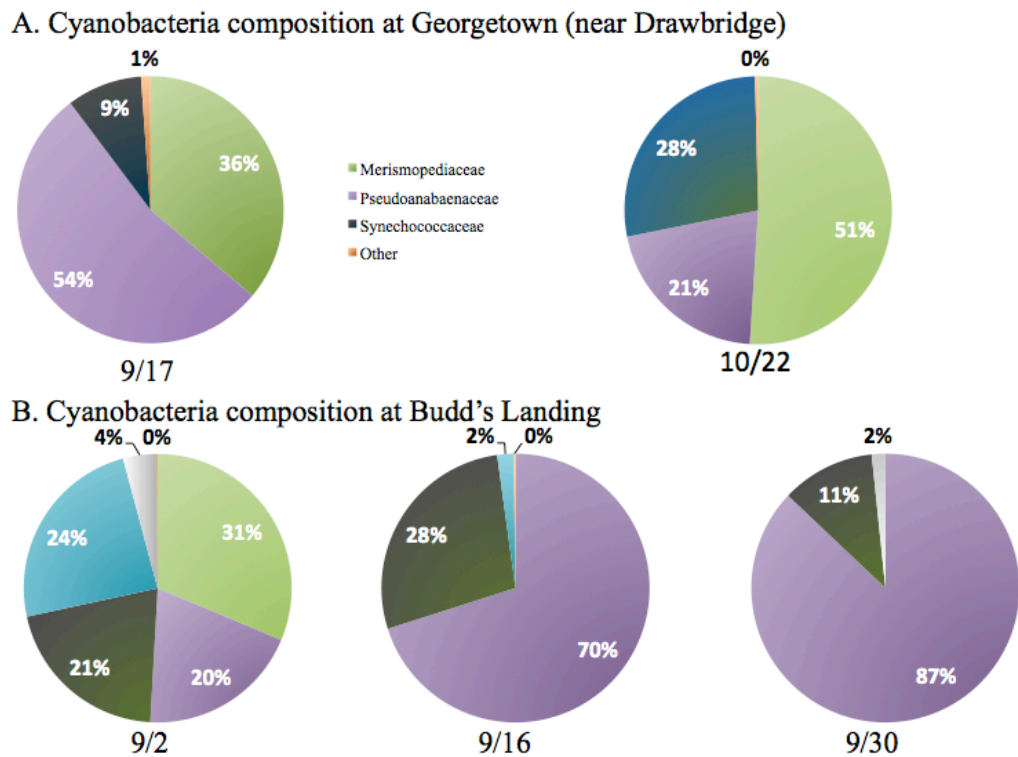
Figure 18. Ratio of PP:BP in field samples during September and October. PP rate was measured at 100 μmol photons m^{-2} sec^{-1}.
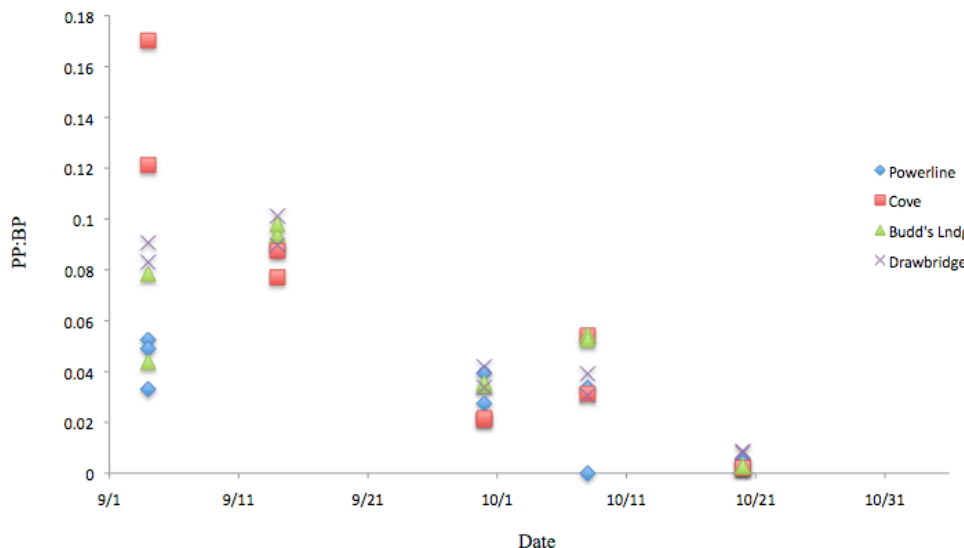
PP:BP were well below 1 at all stations throughout the sampling period (Figure 18). PP:BP ratios may be used as an indicator of the trophic status of a system, although this ratio does not account for respiration. PP:BP values > 1 may indicate autotrophy during the measurement period at the surface, while PP:BP values < 1 indicate that BP exceeds PP and may indicate that the system is heterotrophic. PP: BP fell throughout early fall, and the range of observed PP:BP values also decreased as fall progressed. There was an overall significant increase in PP:BP with temperature (p < .0001, R^2= 0.6092) (Figure 19) and a decrease with salinity (p < .0001, R^2= 0.4745). The pH also correlated significantly, but weakly (p = .0239, R^2= 0.1273). Neither nitrogenous nutrients, PO_4, DOC, nor DIC correlated with PP:BP. However, a multiple regression using stepwise selection (0.20 significance

necessary for entrance into model) isolate temperature as the sole factor driving this relationship.

Figure 19. Correlation between the ratio of PP:BP in field samples to temperature. PP rate was measured at 100 μmol photons m^{-2} sec^{-1}.
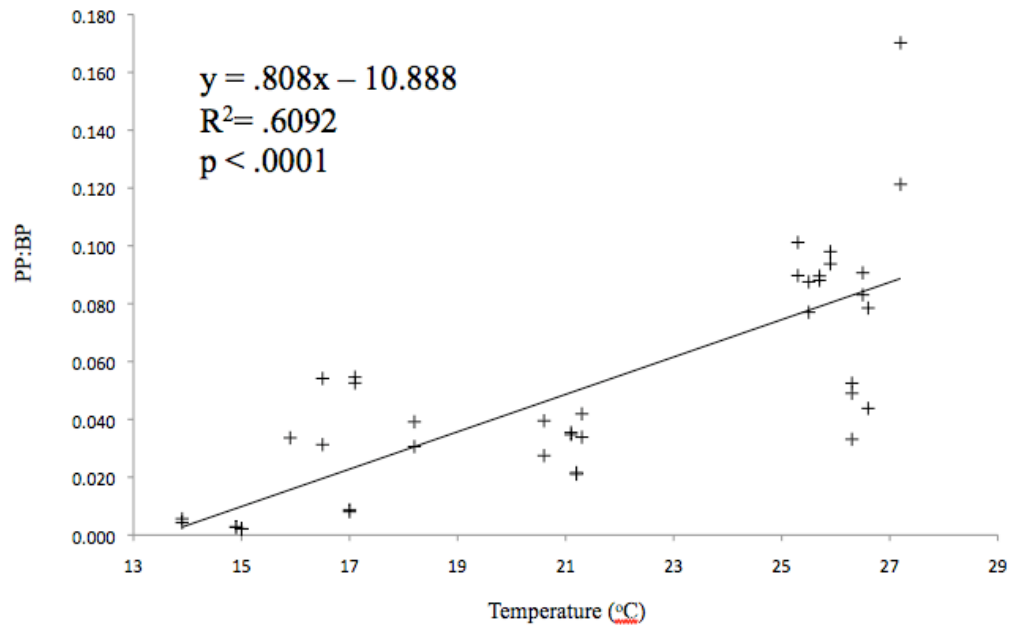
In the field PP was affected by pH, but the main factors that explained the observed production rates were salinity, temperature, and DIC. In order to better understand the effect of pH on primary and bacterial production an experiment was performed in which temperature and salinity were kept constant, but the pH was varied. All production measurements were performed at low (30 μmol photons m^{-2} sec^{-1}) and moderate (100 μmol photons m^{-2} sec^{-1}) conditions. In all experiments PP measurements were greater when measured at 30 μmol photons m^{-2} sec^{-1} than at 100 μmol photons m^{-2} sec^{-1} in both experiments.

When measured at 30 μmol photons m^{-2} sec^{-1} PP decreased as pH increased in both experiments, regardless of the light intensity the cells were grown at. However, PP was nearly 5 times greater when the cells were grown at 30 μmol photons m^{-2} sec^{-1} than cells grown at 300 μmol photons m^{-2} sec^{-1}. In the capped experiment, PP significantly decreased with each increasing pH level (Figure 20a, Table 3). Also in the capped experiment, cells grown at the higher light level fixed carbon at similar rates at pH 8 and 9, but PP was significantly lower at pH 10. In the titration experiment variability was much greater in the low light treatment. Still, PP was significantly higher at pH 8, but there was no difference between pH 9 and 10 (Figure 20b, Table 4).

When PP was measured in the same field samples at 100 μmol photons m^{-2} sec^{-1} the effect of pH was opposite of the effect seen when PP was measured at 30 μmol photons m^{-2} sec^{-1}. However, production in cells grown at 30 μmol photons m^{-2} sec^{-1} was still far greater than production in cells grown at 300 μmol photons m^{-2} sec^{-1} (Figure 21).

In both experiments cells that were grown at 30 μmol photons m^{-2} sec^{-1} at pH 8 fixed significantly less carbon than cells at pH 9. Production peaked at pH 9 and declined at pH 10, although this difference was not significant in either experiment. In the capped experiment, a significant difference was not detected between pH 8 and 10, although average PP was greater in pH 10 (Table 4). In the titration experiment production was significantly higher at pH 10 than at pH 8 in cells grown at 30 μmol

photons m^{-2} sec^{-1} (Table 6). When examining PP in the cells grown at 300 μmol

photons m^{-2} sec^{-1} a plateau in production emerged at the higher pHs in both

experiments. In the capped experiment, this increase was significant. However, high

variability in PP in the titration experiment may have obscured the trend; average PP

at pH 10 is greater than pH 8, but this difference was not significant. However, PP at

pH 9 was significantly greater than pH 8 in the titration experiment (Table 6).

Table 3: Capped lab experiment ANOVA on PP at 30 μmol photons m^{-2} sec^{-1}

F= 3.98 Pr > F 0.0615

	8H	9L	9H	10L	10H
8L	**0.0389**	0.3262	**0.0386**	**0.0102**	**0.0164**
8H	.	0.1687	0.9965	0.3301	0.5293
9L	.	.	0.1676	**0.0393**	**0.0671***
9H	.	.	.	0.3320	0.5320
10L	.	.	.	.	0.7084

Table 4: Titration lab experiment ANOVA on PP at 30 μmol photons m^{-2} sec^{-1}

F= 6.07 Pr > F 0.0025

	8H	9L	9H	10L	10H
8L	**0.0018***	**0.0388**	**0.0089***	**0.0029***	**0.0045***
8H	.	0.3853	0.8224	0.2578	0.4286
9L	.	.	0.2861	**0.0714***	0.1245
9H	.	.	.	0.349	0.5616
10L	.	.	.	.	0.7019

*** significant at α= .01

**significant at α=.05

*significant at α=.1

Figure 20. PP measured at 30 µmol photons m^{-2} sec-^{1} in a.) the capped experiment on September 4th and b.) the titration experiment on September 16th. Grey columns represent cells that were grown at 30 µmol photons m-^{2} sec-^{1} and white columns represent cells grown at 300 µmol photons m-^{2} sec-^{1}. Letters above bars indicate if there is a significant difference from other treatments. Like letters are similar and different letters indicate statistical difference. Please refer to tables 3 and 4 for level of statistical significance. Brackets indicate standard deviations.
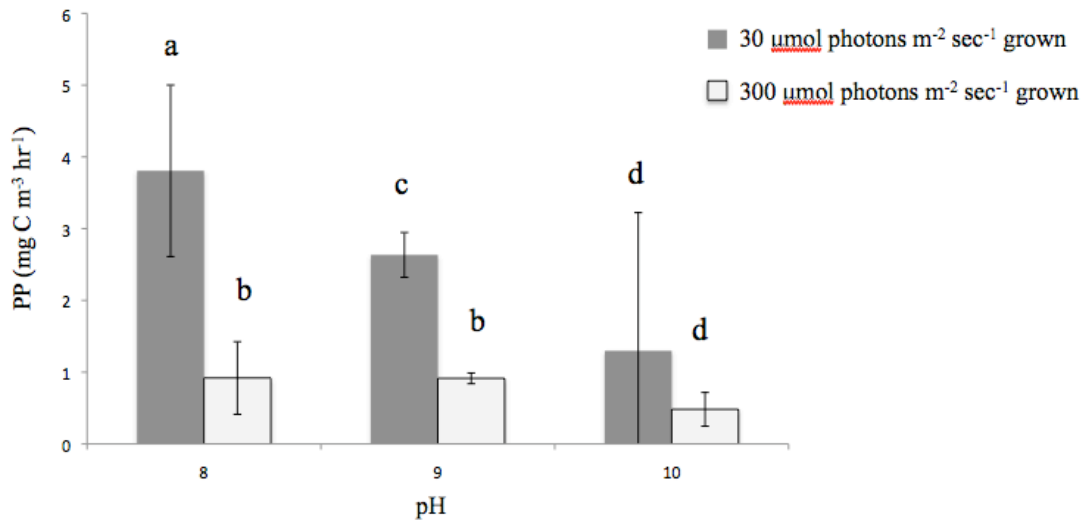
a) Capped

b) Titration

Table 5: Capped lab experiment ANOVA on PP at 100 μmol photons m^{-2} sec^{-1}

F- 16.21   Pr > F   0.002

	8H	9L	9H	10L	10H
8L	**0.0028*****	**0.0494****	**0.0467****	0.1300	**0.0389****
8H	.	**0.0003*****	**0.0543***	*0.0006****	**0.0653***
9L	.	.	**0.0026*****	0.5089	*0.0022****
9H	.	.	.	*0.0054****	0.8965
10L	.	.	.	.	*0.0046****

Table 6: Titration lab experiment ANOVA on PP at 100 μmol photons m^{-2} sec^{-1}

F= 14.80   Pr > F   0.0025

	8H	9L	9H	10L	10H
8L	*0.0036****	**0.0898***	**0.0773***	**0.035****	0.2685
8H	.	*0.0006****	**0.0475****	**0.0003*****	0.2685
9L	.	.	*0.006****	0.5154	**0.0177****
9H	.	.	.	**0.0029*****	0.3982
10L	.	.	.	.	**0.0077*****

*** significant at α= .01

**significant at α=.05

*significant at α=.1

Figure 21. PP measured at 100 µmol photons m-^{2} sec-^{1} in a.) the capped experiment and b.) the titration experiment. Grey columns represent cells that were grown at 30 µmol photons m-^{2} sec-^{1} and white columns represent cells grown at 300 µmol photons m-^{2} sec-^{1}. Letters above bars indicate if there is a significant difference from other treatments. Like letters are similar and different letters indicate statistical difference. Please refer to table 5 and 6 for level of statistical significance. Brackets indicate standard deviations.
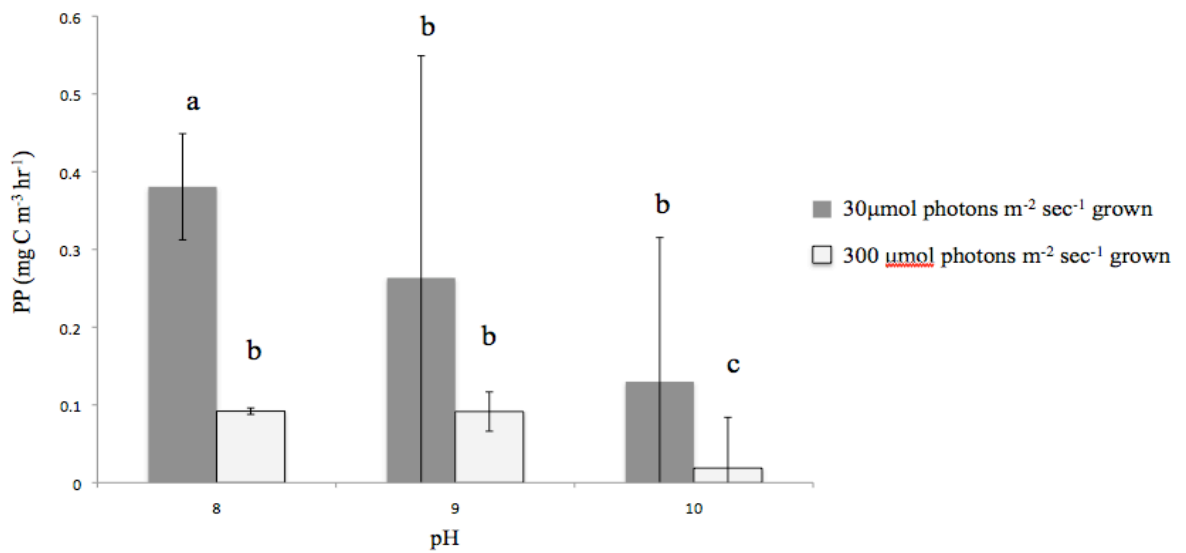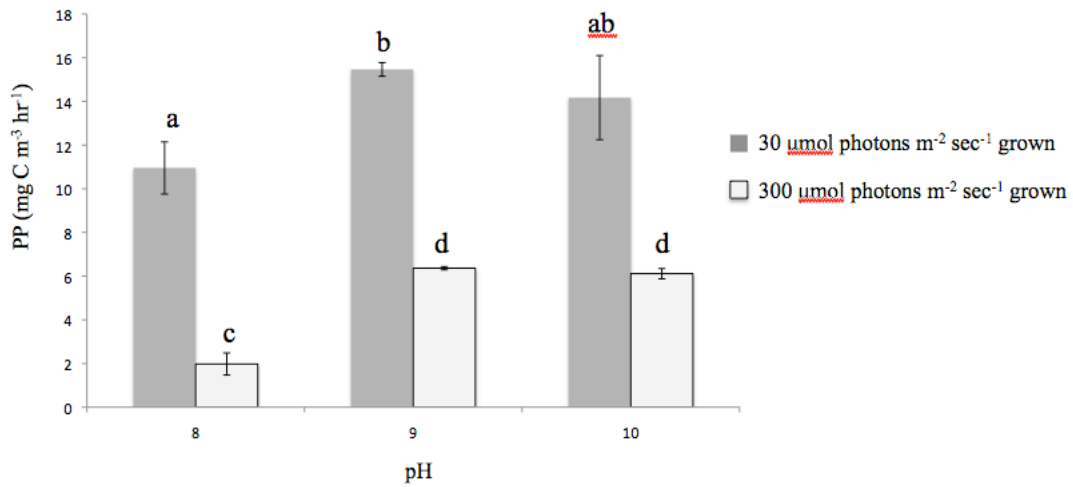
a.) Capped
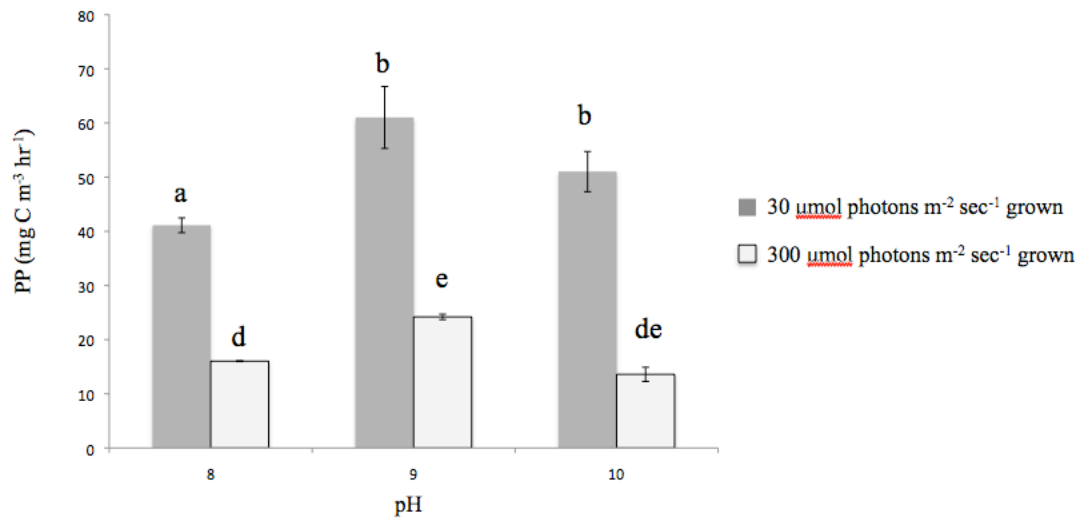
b.) Titration

Effect of pH on BP in the laboratory:

Bacterial production varied over a wider range in the titration experiment than in the capped experiment.   In the titration experiment bacterial production in bottles that were incubated at low light were 1.5-2 times greater than production in bottles incubated at high light. Light and pH exerted a significant effect on bacterial production. Significant differences existed between all light and pH levels except cells grown at high light at pH 8 and 9 (Figure 22, Table 7). At low light, bacterial production was greatest at pH 9, 10 and 8 respectively. At high light BP was greatest at pH 8, and there was a slight, non-significant increase in production with increasing pH.

Light intensity and pH had less effect on BP in the capped experiment. BP was approximately 1000 pmol C l^{-1} hr^{-1} in this experiment, and was comparable to production at the higher light level in the titration experiment, but lower than BP measured at the lower light level. There was no significant difference between light or pH treatments except for bottles that were grown at high light at pH 10 ($p < .0001$) (Figure 23, Table 8). BP was significantly reduced at pH 10 in bottles that were incubated at high light. Unlike the titration experiment, there did not appear to be any relationship between BP and PP. In both experiments the water was spiked with L1 media to prevent nutrient limitation, so this should not have been a factor in determining primary or bacterial production.

DOC values ranged from 4-5 mg l^{-1} after 24 hours in the capped experiment.
In the titration experiment DOC ranged from ~2.5-4, although a relationship between
primary production or bacterial production and DOC was not evident in this
experiment.

Table 7: Titration lab experiment ANOVA on BP
F=275.64   Pr>F <.0001

	8H	9L	9H	10L	10H
8L	<.0001***	<.0001***	<.0001***	0.0037	<.0001***
8H	.	<.0001***	0.0766*	<.0001***	<.0001***
9L	.	.	<.0001***	<.0001***	<.0001***
9H	.	.	.	<.0001***	<.0001***
10L	.	.	.	.	<.0001***

Table 8: Capped lab experiment ANOVA on BP
F=7.87   Pr > F   <.0001   F=7.88

	8H	9L	9H	10L	
8L	0.8288	0.8936	0.3495	0.7401	8L
8H	.	0.7264	0.2509	0.5841	8H
9L	.	.	0.4218	0.8430	<.0001***
9H	.	.	.	0.5440	<.0001***
10L	.	.	.	.	<.0001***

*** significant at α= .01

**significant at α=.05

*significant at α=.1

Figure 22. BP in the titration experiment. Grey columns represent cells that were grown at 30 μmol photons m-^{2} sec-^{1} and white columns represent cells grown at 300 μmol photons m-^{2} sec-^{1}. Brackets indicate standard deviations. All comparisons are significant at α= .05 except between like symbols. The model is significant, p<.0001.
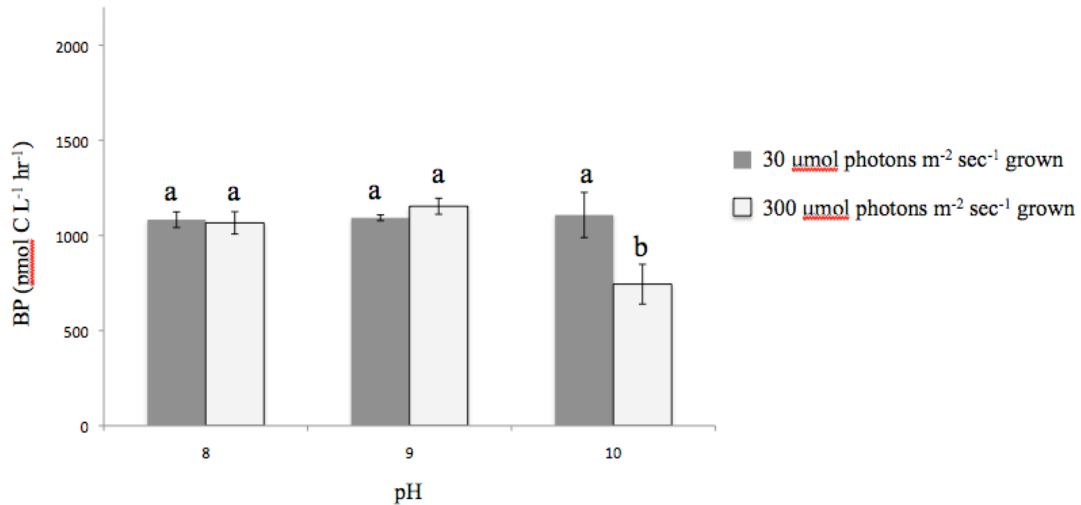
Figure 23. BP in the capped experiment. Grey columns represent cells that were grown at 30 μmol photons m-^{2} sec-^{1} and white columns represent cells grown at 300 μmol photons m-^{2} sec-^{1}. Brackets indicate standard deviations. Letters that differ indicate statistical significance at α=.05. The model is significant, p=.0001.
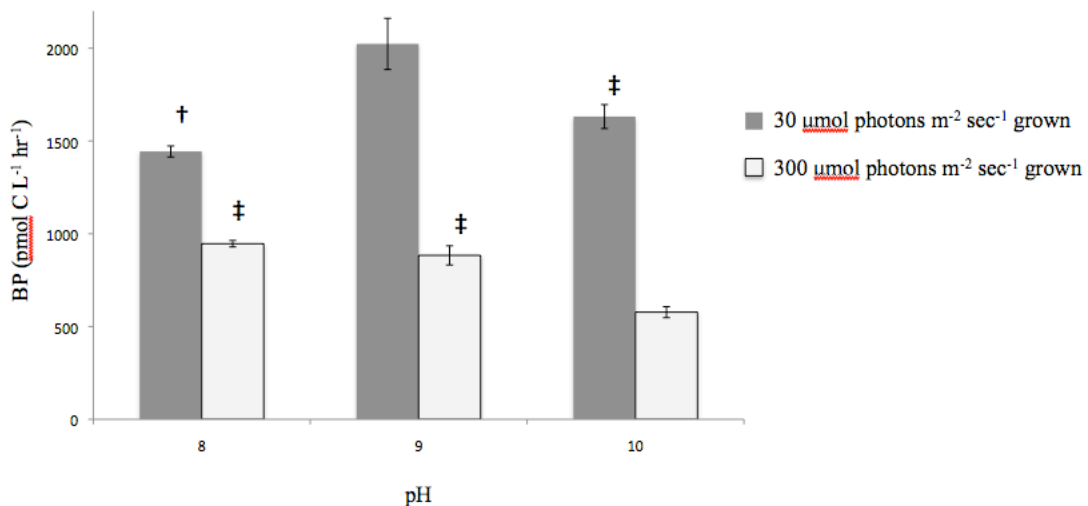
Differences in bacterial production in the field correlated positively with primary production, but this trend was not observed in the laboratory. Bacterial productivity is often affected by DOC availability, so an experiment was performed to determine if DOC exudation differed in *A. crassa* when grown at different light intensities.

Relationship between ^{14}C- uptake and excretion:

Incorporation of ^{14}C-bicarbonate by *A. crassa* grown in semi-continuous culture was predictably higher in cells grown at the higher light intensities. Data was log transformed for statistical analysis to uphold the assumption that data are normally distributed in regression. However, non-log transformed data are discussed. Cells grown at 30 μmol photons m^{-2} sec^{-1} incorporated as little as ~2 mg C m^{-3} hr^{-1}, while cells grown at 10 times greater light intensity incorporated nearly 100 mg C m^{-2} hr^{-1} (Figure 24a).

Fig 24a. Variation in ^{14}C uptake over varying light intensities by *A. crassa* in a laboratory experiment. Brackets indicate standard deviations.
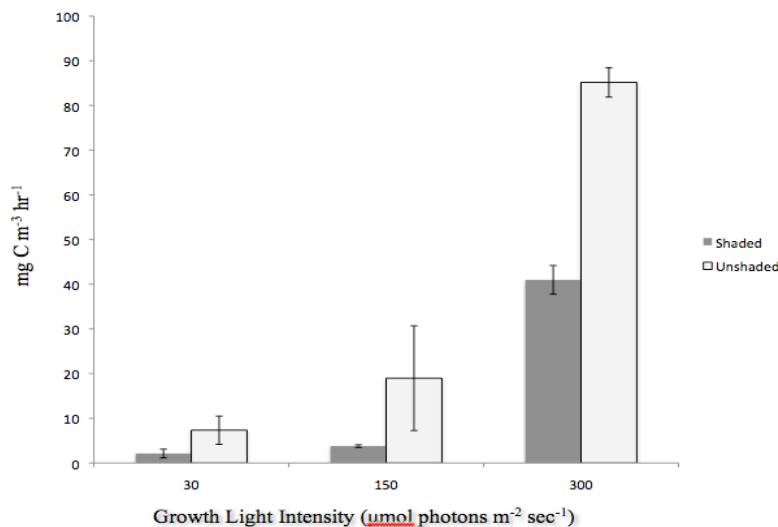
When production was measured in the shade between 0.5-2% of the carbon that was taken up was excreted (Figure 24c). In cultures grown at 30 and 150 μmol photons m^{-2} sec^{-1}, DOC excretion was higher when measured in the shade than at the growth light intensity (Figure 24b). Surprisingly, this DOC excretion pattern did not occur in cultures grown at 300 μmol photons m^{-2} sec^{-1}. In fact, cells grown at 300 μmol photons m^{-2} sec^{-1} appeared to have negative ^{14}C incorporation (Figure 24b). This is likely because the calculation involves subtracting a dark blank. The dark blanks from cells that were grown at 300 μmol photons m^{-2} sec^{-1} were much larger than the exudation measurement, causing calculated ^{14}C-DOC excretion to be negative.

Figure 24b. Variation in ^{14}C excretion over varying light intensities by *A. crassa* in a laboratory experiment. Brackets indicate standard deviations.
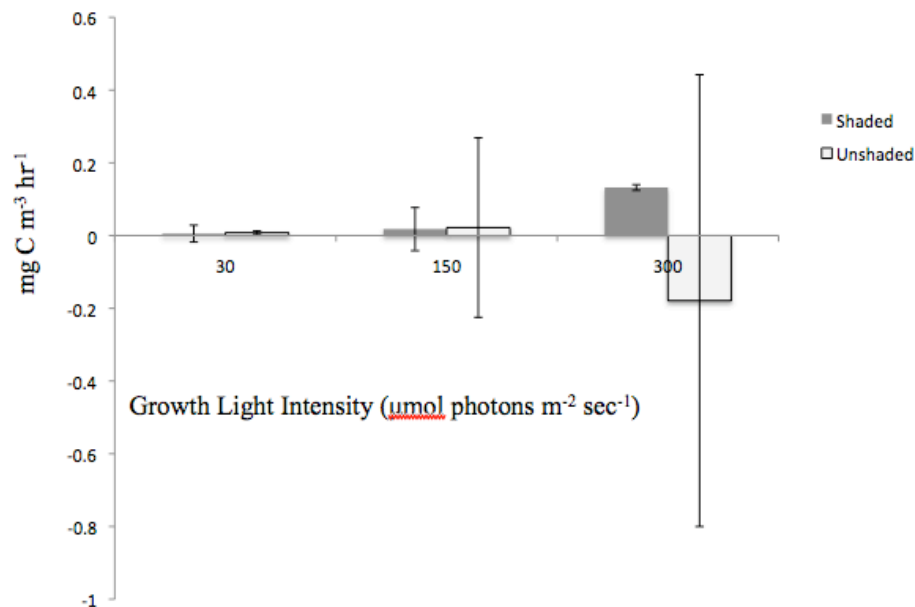
Figure 24c. Percent of ^{14}C uptake that was excreted by *A. crassa* in a laboratory experiment. Brackets indicate standard deviations.
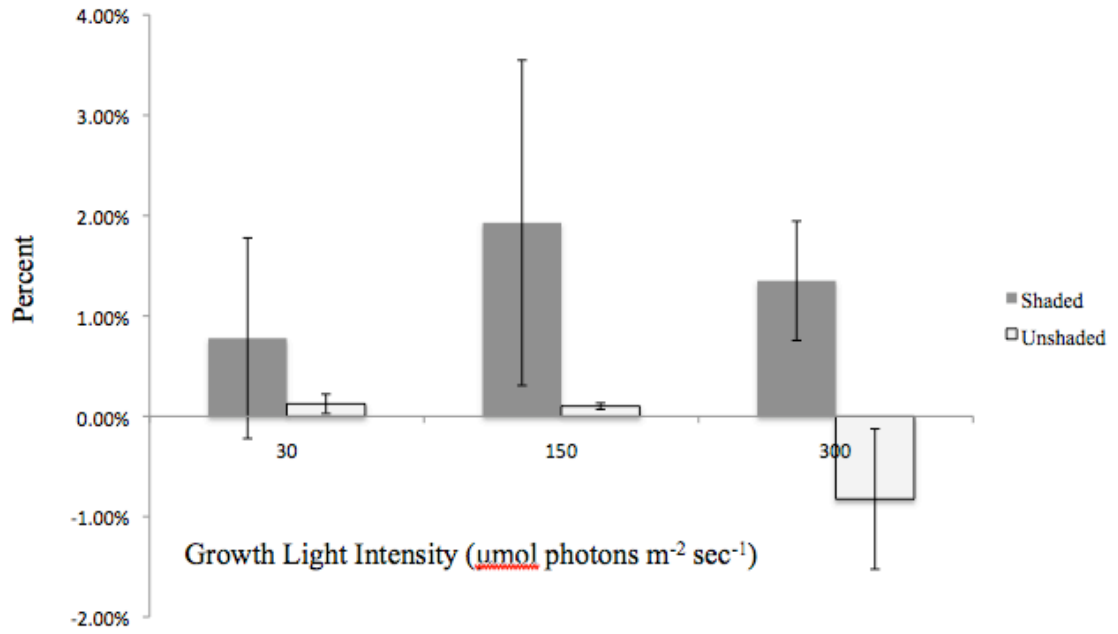
Figure 25. Variation in ^{14}C a) uptake and b) excretion from dark blanks from the laboratory experiment with *A. crassa*.
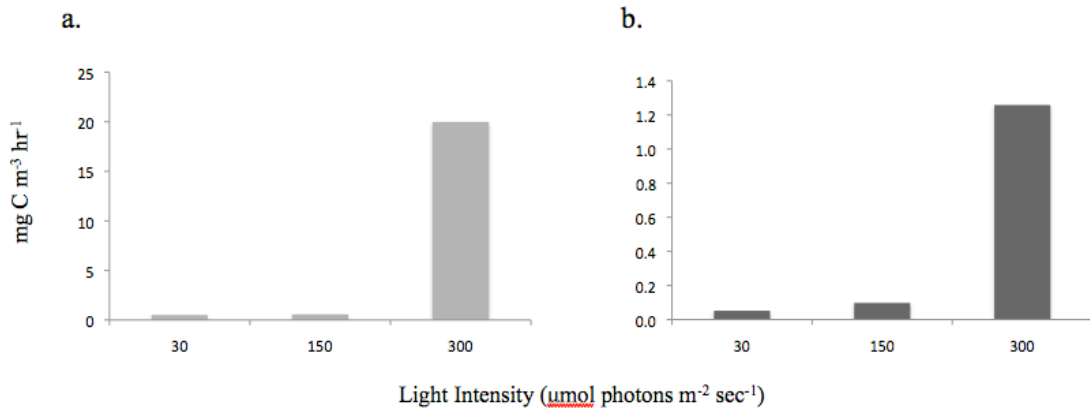
Discussion

This study examined the main factors that correlated with primary and bacterial production in the Sassafras River during autumn 2008, with particular emphasis on the effects of pH. The factors that correlated most tightly with ^{14}C-incorporation by cyanobacteria-dominated phytoplankton communities in the Sassafras River during fall were temperature and salinity. PP fell with temperature as autumn progressed. This idea has long been supported by the Q_{10} theory, which is the change in metabolic rate with every 10°C change in temperature (Rose, 1967). For photosynthesis, temperature is rarely limiting to the light reactions that generate ATP and other forms of chemical energy, although photosynthetic enzymes are affected by temperature (Raven and Geider, 2003). The dark reactions however, which include carbon fixation and other metabolic processes, are heavily dependant upon metabolic rate and are sensitive to temperature. The Calvin cycle turns over more slowly as temperature falls, limiting the rate of carbon fixation. Methodological effects of incubation temperature may have influenced PP measurements. PP measurements were conducted over 4 hours at 26°C. During August, when water temperatures were $> 20^{o}$C the influence of altered temperature was probably minimal. Several cyanobacteria are greatly affected by downward shifts in temperature. For example, Coles and Jones (2000) studied three cyanobacteria in cultures and found that Q_{10} for maximum photosynthetic rate normalized to biomass ranged from $1.79 - 2.67$. While the species studied were present in the Sassafras phytoplankton, a direct comparison cannot be made; Q_{10} was measured in this study in cultures that had been acclimated to varying temperature. Additionally, Coles and Jones (2000) note that their Q_{10} rates

were relatively high compared to other studies, probably because these cultures were also acclimated to low light intensity (40 μmol photons m^{-2} sec^{-1}). However, the effect of temperature on multiple cyanobacteria species is clear, and an effect of shifting phytoplankton from temperatures in the mid-teens to 26°C over a period of hours may have stressed the phytoplankton and affected photosynthetic rates.

Researchers have addressed the physiological effects of salinity on PP, although their conclusions conflict somewhat. In laboratory experiments *Spirulina platensis* and *Chlamydomonas* (a green alga) decreased maximum photosynthetic rate (P_{max}) and experienced photoinhibition when placed under salinity stress (Vonshak et al. 1989, Neale and Melis 1989). However, strains of *Anabaenopsis*, *Anabaena*, and two strains of *Nodularia* did not experience photoinhibition in response to salinity stress experiments, and in fact displayed an increased P_{max}. An increase in P_{max} could indicate increased carbon demand by the salinity acclimation mechanisms (Moisander et al. 2002), while a decrease in P_{max} accompanied by photoinhibition indicates the salinity stress is too great to be compensated for, or that there are no active salinity acclimation mechanisms (Zeng and Vonshak, 1997). The ecological outcome when P_{max} is decreased is clear: the cyanobacteria will be outcompeted by more euryhaline species. When P_{max} increases due to increased energetic demand the cyanobacteria may be able to persist so long as light intensity is adequate to meet the increased energy demand. However, if light intensity is too low, then the cyanobacteria may lose their competitive ability and be replaced by other more efficient phytoplankton.

The dual influence of increasing salinity and decreasing temperature on primary production should be considered during this period. Sub-optimal temperatures will retard production in all phytoplankton species when light intensity is saturating. It seems surprising that salinity correlated so tightly with PP as it has not been determined as a factor limiting photosynthesis. Cyanobacteria dominated this system during the entire sampling period, although their relative abundance in the phytoplankton decreased as fall progressed. In this study, the maximum salinity observed was 4, which is well within the range of tolerance for many cyanobacteria (Tonk et al. 2007). Cyanobacteria decreased proportionally in the phytoplankton at all sites as fall progressed, and this decrease may be due to the combination of temperature and salinity decreasing metabolic rate at a time when demand was increasing. However, total phytoplankton PP decreased, which means that all phytoplankton, including non-cyanobacteria were fixing carbon at a decreased rate. Therefore, while these conditions may have led to decreased prevalence of cyanobacteria, the rate of PP for the system still decreased overall.

PO_4 concentrations also significantly correlated with PP, although the correlation was weaker than with temperature and salinity. However, lack of correlation with Chl a suggests that PO_4 did not restrict biomass, but did limit carbon fixation. Phytoplankton subjected to P limitation in culture studies have displayed lowered maximum photosynthetic rates (P_{max}), increased sensitivity to photoinhibition, and reduced ability to take advantage of fluctuations in light intensity (Geider et al. 1998, Litchman et al. 2003). The Sassafras River is considered to be

nutrient enriched, especially in nitrogenous forms and this data supports this. If

nitrogen were limiting it should correlate with PP. However, PP may be limited at

times by phosphorus availability. In this situation phosphorus release from benthic

sediments when the pH is elevated above 9.5 may be particularly important in

sustaining productivity in high biomass blooms. During a dense *Microcystis* bloom

in the Potomac River elevated P concentrations observed within the bloom were

attributed to P release from the sediments due to elevated pH, which supported further

bloom production (Seitzinger 1991).

None of the parameters measured correlated with Chl *a* except for pH. High

biomass blooms, indicated by elevated levels of Chl *a* above 60 µg l^{-1}, effectively

drove up pH in this system. Chl *a* is often affected by nutrient concentrations in

aquatic systems (Hallegraeff 1993, Cloern 2001,Anderson et al. 2002), but neither the

nitrogenous nor phosphorus forms correlated with Chl *a* during the sampling period.

Maryland DNR classified the Sassafras as nutrient saturated during the years 1987-

2005 (Karrh et al. Tidewater ecosystem assessment 2007), and this data set appears to

support this classification, except that P may have occasionally limited PP. Top-

down controls are often important in controlling phytoplankton biomass in aquatic

systems (Frost 1991, Landry and Calbet 2004) and grazing pressure is a likely factor

that may control Chl *a* in the Sassafras, although this question has not yet been

addressed. The maintenance of high Chl a levels during autumn, despite decreasing

PP rates, may indicate weak grazing control.

The ability of the phytoplankton community to fix carbon at elevated pH was dependant on light intensity. Carbon fixation decreased significantly in cells experiencing a pH above 8.3 when ^{14}C-photosynthesis was measured at 30 μmol photons m^{-2} sec^{-1}, whereas a drop in production was not seen until pH 8.7 in phytoplankton measured at 100 μmol photons m^{-2} sec^{-1}. Experiments performed at varying levels of light and pH on phytoplankton communities confirmed that cells photosynthesizing at 100 μmol photons m^{-2} sec^{-1} were more pH tolerant than cells photosynthesizing at low light intensities. While pH had a negative effect on primary production it is impossible discern if this effect is due to C_i limitation, or due to physiological stress induced by elevated pH.

In the laboratory, cells acclimated to low light had consistently higher production then cells that were adapted to high light. Phytoplankton acclimated to low light increase the number of photosynthetic reaction centers and accessory pigments to increase efficiency of photon capture (Kana and Glibert 1987a), and this is probably the case here. Exposure to 100 μmol photons m^{-2} sec^{-1} was probably a near-saturating irradiance, but was not high enough to cause photoinhibition. Instead these cells were more efficient at harvesting light to provide energy for carbon fixation than cells that were adapted to high light. However, it is not likely that all the carbon that these cultures fixed was incorporated into biomass; some of it was probably excreted as DOC. Kana and Glibert (1987b) found that *Synechococcus* (WH7803) grown between 50-100 μmol photons m^{-2} sec^{-1} greatly increased ^{14}C-incorporation when light intensity was shifted upwards to approximately 500 μmol

photons m^{-2} sec^{-1}. Carbon fixation rates are dependant upon rate of light absorption,

electron flow, and the dark enzymatic reactions. In this situation, low light grown

cells are able to harvest more photons than moderate and high-light grown

counterparts, but the dark reactions are adapted to lower electron flow and cannot turn

over quickly enough to incorporate all the carbon which was reduced in low-light

grown cells. This response probably indicates the ability of low light-adapted cells to

take advantage of short-term increases in irradiance for growth (Kana and Glibert

1987b).

The ability of the cyanobacteria community to increase ^{14}C-incorporation at

elevated pH when exposed to a moderate light intensity may facilitate bloom

formation and persistence. First, increased rates of carbon fixation (like those found

in this study), even if it is not all incorporated into biomass, may provide a

competitive advantage over other species of phytoplankton that are less tolerant of

pH. Both low and high-light adapted cells increase carbon fixation rates as pH rises

(up to a critical threshold) which may allow these pH-tolerant species to persist in

conditions that are inhospitable to other phytoplankton.

Further, laboratory experiments on cultures of *A. crassa* suggest that this

cyanobacterium actively takes up bicarbonate in the dark. Cultures that were grown at

300 µmol photons m^{-2} sec^{-1} took up more radiolabeled H^{14}CO$_3$ than samples

incubated in the light. Dark incubations are normally done in order to obtain a

"background" value for calculation of primary production rates, and such high dpm

values were surprising. This result is either due to an artifact of the method or indicates that *A. crassa* has some mechanism to incorporate bicarbonate in the dark. The latter scenario seems to be plausible for two reasons. First, cells that were growing at a greater light intensity had taken up more radioactive label in the dark then cells growing at a lower light intensity (Figure 25), which clearly suggests that some biological process is driving the apparent dark uptake. Second, all extracellular ^{14}C-bicarbonate should be driven off upon addition of a few drops of 5N HCl. Since this procedure has been used for decades in other studies, and no evidence of methodological artifact was found previously, this seems unlikely.

A multiple regression using stepwise selection isolated temperature, PO_4, DIC and pH as the most significant factors correlating with BP. However, PP, DOC and salinity also correlated strongly in single linear regressions. Excretion of DOC by phytoplankton has been identified as a primary factor that influences BP in many aquatic systems (Cole et al. 1988, Jardillier et al. 2004, Apple 2006). BP is tightly coupled with DOC availability and with PP, but phytoplankton appear to minimally contribute to the total DOC pool. Several studies have found that bacteria are less dependent on carbon supplied by primary producers in systems where terrestrial and benthic fluxes contribute increasingly to the carbon pool, and this may be the case of DOC in the Sassafras (Jansson et al. 2000, Comerma et al. 2003). Possible allochthonous sources in this system include terrestrial runoff, benthic fluxes, and groundwater.

Temperature has long been recognized as an important factor in regulating BP, but whether temperature can be considered the main factor controlling BP is still a subject of debate (Shiah and Ducklow 1994, Apple et al. 2006). In this study BP decreased with temperature as fall progressed. Some studies show that BP is substrate dependant when the temperature is above 20°C, and becomes more limited by temperature as the temperature dips below that threshold (Hoch and Kirchman 1993, Shiah and Ducklow 1994). This study spans the predicted transition between substrate and temperature limitation. However, substrate concentrations fell concurrently with temperature, making it impossible to discern between substrate and temperature effects. It is possible that a lack of rainfall resulted in lower allochthonous inputs to the river, thus causing decreased nutrient concentrations and increased salinity. Decreased freshwater input would also result in lower flushing rates, and this may also explain the observed (and counter-intuitive decrease) in DIC. BP and DIC concentrations may have fallen during fall due to lack of rainfall, which would recharge DIC and nutrients to the water column. If this is the case, the importance of allochthonous inputs from the watershed are probably important in fueling BP in the upper Sassafras River.

Concern that methodological effects due to the incubation temperature may have affected the data must be considered for BP as it was for PP. All BP samples were incubated at 26°C, and during October the temperature difference between the river water and the incubation temperature may have been significant. The sudden increase in temperature may have inhibited the bacterial communities and contributed

69

to the decline in BP at colder temperatures. Conversely, increased temperature may have facilitated greater BP rates, and the decrease in BP may have actually been much larger, and perhaps temperature dependant, than the data suggests.

There was a weak correlation between PP and DOC, but high correlation between BP and DOC, which suggests that allochthonous inputs are important in providing substrate to support BP in this system, which is in agreement with other studies (Jansson et al. 2000, Comerma et al. 2003). Estuarine and coastal systems are frequently heterotrophic due to increased OM availability, and therefore BP exceeds PP (Hoch and Kirchman 1993, Glibert et al. 2006). The ratio of PP:BP was generally below 0.1, which may also suggest that allochthonous sources of OM are ample.   The ratio of PP:BP may be used to discern the trophic state of an ecosystem, but this ratio should be interpreted cautiously because it does not consider respiration, which is often important. The PP:BP ratio at all stations decreased during autumn. While PO_4 concentrations were decreasing during this period, it seems likely that the steady drop in temperature drove this ratio down. This may suggest that temperature exerts greater control in the autumn over PP than BP.

Associations between bacteria assemblages and the cyanobacteria community were not found in this study, and other factors shape bacteria assemblages. September bacterial communities differed slightly and samples collected in October formed an independent cluster, but no pattern could be discerned from any of the parameters measured. Numerous factors have been identified to partially shape

bacterial communities and include several parameters associated with phytoplankton and primary production (Cole et al. 1988, Apple 2006), as well as nutrient concentrations (Carvalho et al. 2003, Kan et al. 2006), physical factors (Pace and Cole 1996), and input of allochthonous bacteria and organic matter (Jansson et al. 2000). The contribution of bacterial communities and OM from runoff, groundwater, and atmospheric deposition are not yet known in the Sassafras, and it is possible that external inputs are the main determinants of bacterial communities in the upper Sassafras river. Crump et al. (2004) found that distinct bacterial communities only formed in Plum Island Sound (Massachusets) when bacterial doubling time exceeded residence time. Otherwise, advected microbial communities dominated. The influence of bactivory was not quantified in this study and is also another factor that may have influenced bacteria communities during this time. One study in a eutrophic reservoir found that the bacterial community was shaped by the presence of a *Microcystis aeruginosa* bloom, and the authors attributed this effect to the effect of enhanced grazing on bacteria within the bloom (Hornak et al. 2008).

Decreasing temperature, photoperiod, and PO_4 concentrations, coupled with increasing salinity likely decreased cyanobacterial dominance in the Sassafras during the fall season. Cyanobacterial dominance in the phytoplankton fell from >85% at either end of the sampling areas to just over half the phytoplankton community by the last sampling date (September 30th at Budd's Landing, and October 22nd at Georgetown). Flagellates and other green algae had greatly increased in the phytoplankton at both stations. Due to the limited sample size and the fact that all

significant variables were changing during that time it is not possible to choose one variable that best explains shifts in phytoplankton community dominance.

The cyanobacteria community was dynamic between stations and dates. Either *Pseudoanabaenacea* or *Merismopediaceae* consistently dominated the cyanobacteria at both end members of the sampling site, although various *Synechococcus* species were frequently observed. Factors influencing cyanobacteria community dynamics in the Sassafras cannot be discerned here due to the small sample size.

Examination of data from the Maryland DNR continuous monitoring station at Budd's Landing during 2007 and 2008 may put data from September and October into a larger context (Figure 26). These data reveal periods of elevated pH beginning in spring and extending through the summer. For example, during 2008 elevated Chl *a* levels at Budd's Landing correspond to pH levels between 9.5 and 10 for one week in April. Fluctuations in pH were more frequent and more extreme during 2007. The pH at Budd's Landing remained elevated above 9 from May through August, and pH values in excess of 10 were common. It is possible that during the spring and summer, when elevation in pH was higher and more persistent than during September and October that pH may have had more of an effect on PP and BP. It may also have been responsible in altering the grazing community, but unfortunately there is no data for this time period.

Figure 26. a) 2007 and b) 2008 pH at Budd's Landing data from Maryland DNR continuous monitor.
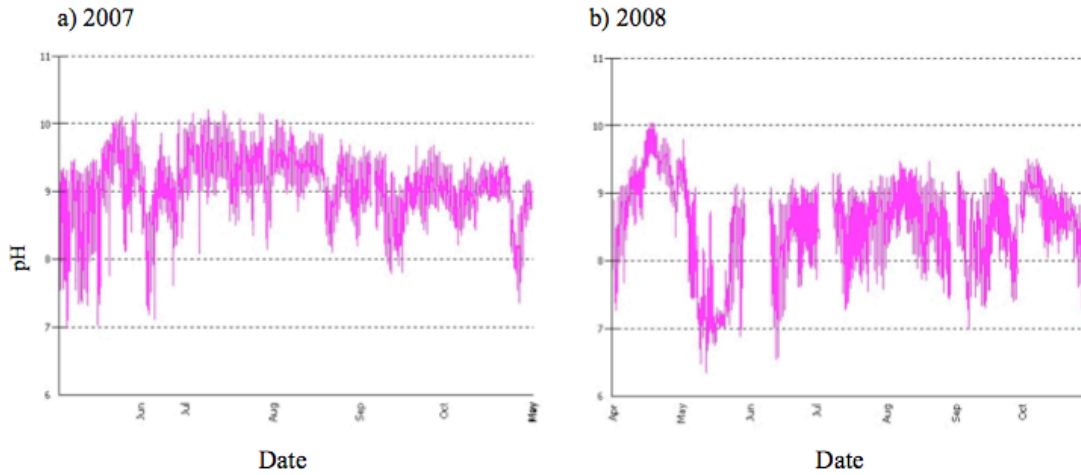
Elevated pH may contribute to the dominance of cyanobacteria in eutrophic waterways through 4 mechanisms. First, some cyanobacteria may have a higher pH tolerance than other phytoplankton. Variations in pH may induce physiological stress through enzyme and protein denaturation and by upsetting osmotic balance within the cell (Hoffman and Decho 2000, Hinga 2002). Second, increased efficiency of CCMs at higher pH may facilitate increased production and growth, also providing a competitive advantage. This thesis shows that when light intensity is above 100 μmol photons m^{-2} sec^{-1} carbon fixation increases with pH. Since pH experiments were performed with field samples containing mixed cyanobacteria communities highly efficient CCMs may be a feature common among most cyanobacteria. CCM performance under high light intensities is an area in need of investigation. A study of carbon and nitrogen fixation in several cyanobacteria species (Moisander 2002) showed that under high light intensities photochemical energy was shunted towards N-fixation, even when N was not limiting, which may have prevented

73

photoinhibition. This may select for N-fixing genera.   Third, phosphorus flux from the benthos may prolong blooms of cyanobacteria after pH is elevated above 9.5. This is likely due to solubilization of aluminum and iron-phosphate complexes (Seitzinger 1991). This may be particularly beneficial to N-fixing cyanobacteria and could effectively release cyanobacteria from bottom-up nutrient limitation. Fourth, microzooplankton may be suppressed at high pH and release cyanobacteria from top-down control. Under this scenario, C_i and other micronutrients may exert bottom-up control and limit cyanobacteria production rather than nitrogen and phosphorus. Additionally, bacteriophages and/or viruses may ultimately prove to be important in terminating such blooms.

Much research is required to address the role of pH in waterways that experience intense cyanobacteria blooms. This thesis demonstrates evidence for varying effects of pH on primary production in cyanobacteria-dominated phytoplankton communities that is dependent on light intensity. In the Sassafras River during September and October the main factors that correlated with PP were temperature, salinity, PO_4 availability, and pH. Bacteria community composition was not affected by pH, but rather by temperature, DOC, and salinity. BP was also relatively insensitive to pH, except at pH 10 in one experiment. However it is possible that during the spring and summer, when PP is greater and pH elevation is more frequent and sustained for longer, that effects on PP may be stronger and may also influence BP. This remains to be investigated.

Literature Cited

Aizawa, K. and S. Miyachi (1986). "Carbonic anhydrase and CO2 concentrating mechanisms in microalgae and cyanobacteria." FEMS Microbiology Reviews 39: 215-233.

Andersen, R. (2005). Algal Culturing Techniques. Burlington, MA, Elsevier Academic Press.

Anderson, D. M., P. M. Glibert, et al. (2002). "Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences." Estuaries 25(4b): 704-726.

Apple, J., P. del Giorgio, et al. (2006). "Temperature regulation of bacterial production, respiration, and growth effeciency in a temperatre salt-marsh estuary." Aquatic Microbial Ecology 43: 243-254.

Azam, F., T. Fenchel, et al. (1983). "The ecological role of water-column microbes in the sea." Marine Ecology Progress Series 10: 257-263.

Badger, M., G. Price, et al. (2006). "The environmental plasticity and ecological genomics of the cyanobacterial CO2 concentrating mechanism." Journal of Experimental Botany 57(2): 249-265.

Badger, M. and A. TJ (1982). "Photosynthesis and inorganic carbon usage by the marine cyanobacterium, Synechococcus sp." Plant Physiology 70: 517-523.

Baines, S. and M. Pace (1991). "The production of dissolved organic matter by phytoplankton and its importance to bacteria: Patterns across marine and freshwater systems." Limnology and Oceanography 36(6): 1078-1090.

Bell, R. (1993). Estimating production of heterotrophic bacterioplankton via incorporation of tritiated thymidine. Handbook of Methods in Aquatic microbial ecology. W. Kemp, B. Sherr, E. Sherr and J. Cole. Boca Raton, FL, Lewis Publishers.

Bratbak, G. and T. Thingstad (1985). "Phytoplankton-bacteria interactions: an apparent paradox? Analysis of a model system with both competition and commensalism." Marine Ecology Progress Series 25: 23-30.

Berman-Frank, I., P. Lundgren, P. Falkowski. (2003). "Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria." Research in Microbiology 154: 157-164.

Burns, R., C. MacDonald, et al. (2005). "Inorganic carbon repletion disrupts photosynthetic acclimation to low temperature in the cyanobacterium Synechococcus elongatus." Journal of Phycology 41: 322-334.

Carrasco, M., J. Mercado, et al. (2008). "Diversity of inorganic carbon acquisition mechanisms by intact microbial mats Microcoleus chthonoplastes (Cyanobacteriae, Oscillatoriaceae)." Physiologia Plantarum 133: 49-58.

Carvalho, P., S. Thomaz, et al. (2003). "Effects of water level, abiotic and biotic factros on bacteriolankton abundance in lagoons of a tropical floodplain (Parana River, Brazil)." Hydrobiologia 510: 67-74.

Cho, B. and F. Azam (1988). "Major role of bacteria in biogeochemical fluxes in the ocean's interior." Nature 332(31): 441-443.

Christoffersen, K., S. Lyck, et al. (2002). "Microbial activity and bacterial community

    structure during degredation of microcystins." Aquatic Microbial Ecology 27:

    125-136.

Christoffersen, K., B. Riemann, et al. (1990). "Qualitative importance of the

    microbial loop and plankton community structure in a eutrophic lake during a

    bloom of cyanobacteria." Microbial Ecology 20: 253-272.

Coles, J.F., C.C. Jones. (2000). "Effect of temperature on photosynthesis-light

    response and growth of four phytoplankton species isolated from a tidal

    freshwater river.' Journal of Phycology 36: 7-16.

Crump, B., C. Hopkinson, et al. (2004). "Microbial biogeography along an estuarine

    salinity gradient: combinged influences of bacterial growth and residence

    time." Applied and Environmental Microbiology 70: 1494-1505.

Crump, B., G. Kling, et al. (2003). "Bacterioplankton community shifts in an arctic

    lake correlate with seasonal changes in organic matter sources." Applied and

    Environmental Microbiology 69(4): 2253-2268.

D'Elia, C., E. Connor, et al. (1997). "Chesapeake Biological Laboratory Nutrient

    Analytical Servieces." Standard Operating Procedures. Technical Report

    Series No. 158-97.

Hewson, I., JM O'Neil, et al. (2001) "Effects of concentrated viral communities on

    photosynthesis and community composition of co-occurring benthic

    microalgae and phytoplankton." Aquatic Microbial Ecology 25: 1-10.

Hoffman, M. and A. Decho (2000). "Proteolytic enzymes in the marine bacterium

    Pseudoalteromonas atlantica: post-secretional activation and effects of

environmental conditions." Applied and Environmental Microbiology 23: 29-39.

Ibelings, B. (1996). "Changes in photosynthesis in response to combined irradiance and temperature stress in cyanobacterial surface waterblooms." Journal of Phycology 32: 549-557.

Ibelings, B. and S. Maberly (1998). "Photoinhibition and the availability of inorganic carbon restrict photosynthesis by surface blooms of cyanobacteria." Limnology and Oceanography 43(3): 408-419.

Irigoien, X., K. J. Flynn, et al. (2005). "Phytoplankton blooms: a 'loophole' in microzooplankton grazing impact? ." Journal of Plankton Research 27: 313-321.

Jansson, M., A. Bergstrom, et al. (2000). "Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes." Ecology 81(11): 3250-3255.

Jardillier, L., M. Basset, et al. (2004). "Bottom-up and top-down control of bacterial community composition in the euphotic zone of a reservoir." Aquatic Microbial Ecology 35: 259-273.

Jiang, Y., B. Ji, et al. (2008). "Statistical study onthe effects of environmental factors on the growth and microcystins production of bloom-forming cyanobacterium - Microcystis aeruginosa." Harmful Algae 7: 127-136.

Judd, K., B. Crump, et al. (2006). "Variation in dissolved organic matter controls bacterial production and community composition." Ecology 87(8): 2068-2079.

Kamjunke, N. and S. Jähnichen (2000). "Leucine incorporation by Microcystis aeruginosa." Limnology and Oceanography 45(3): 741-743.

Kan, J., B. Crump, et al. (2006). "Bacterioplankton community in Chesapeake Bay: predictable or random assemblages." Limnology and Oceanography 51(5): 2157-2169.

Kana, T. and P. Glibert (1987). "Effect of irradiances up to 200μE m-2 sec-1 on marine Synechococcus WH7803. II. Photosynthetic responses and mechanisms." Deep Sea Research 34(4): 497-516.

Kana, T. and P. Glibert (1987). "Effect of irradiances up to 2000μE m-2 sec-1 on marine Synechococcus WH7803. I. Growth, pigmentation, and cell composition." Deep Sea Research 34(4): 479-495.

Karrh, R., W. Romano, et al. (2007). Maryland tributary strategy upper eastern shore basin summary report for 1985-2005 data. Tidewater ecosystem assessment. Annapolis, MD 21401, Maryland Department of Natural Resources.

Kasputina, L. (2006). "Experimental study of Microcystis-associated free-living bacteria." Microbiology 75(5): 606-610.

Kemp, W., W. Boynton, et al. (2005). "Eutrophication of Chesapeake Bay: historical trends and ecological interactions." Marine Ecology Progress Series 303: 1-29.

Kirchman, D. (2001). Measuring bacterial biomass productionand growth rates from leucine incorporation in natural aquatic environments. Methods in microbiology. J. Paul. San Diego, Academic Press. 30: 227-237.

Kirchman, D., Y. Suzuki, et al. (1991). Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. Methods in microbial biology. J. Paul. San Diego, CA, Academic Press. 30: 227-237.

Kirk, J. (1994). Light and photosynthesis in aquatic ecosystems. New York, Cambridge University Press.

Kisand, V. and J. Wikner (2003). "Limited resolution of 16S rDNA DGGE caused by melting properties and closely related DNA sequences." Journal of Microbiological Methods: 183-191.

Kozolowsky-Suzuki, B., M. Karjalainen, et al. (2007). "Disruption of the microbial food web and inhibition of metazooplankton development in the presence of iron- and DOM-stimulated Baltic Sea cyanobacteria." Marine Ecology Progress Series 337: 15-26.

Kuentzel (1969). "Bacteria, carbon dioxide, and algal blooms." Journal of Water Pollution Control Federation 41(10): 1737-1747.

Lancelot, C. (1983). "Factors affefcting phytoplankton extracellular release in teh Souther Bight of the Norht Sea." Marine Ecology Progress Series 12: 115-121.

Landry, M. and A. Calbet (2004). "Microzooplankton production in the oceans." ICES Journal of Marine Science 61: 501-507.

Lane, L., S. Rhoades, et al. (2000). "Standard Operating Procedures." Horn Point Analytical Services Laboratory Technical Report. No. TS-263-00.

Lange, W. (1971). "Enhancement of algal growth in Cyanophyta-bacteria systems by caronaceous compounds." Canadian Journal of MIcrobiology 17(3): 303-313.

Libes, S. (1992). An Introduction to Marine Biogeochemistry. New York, John Wiley & Sons, Inc.

Litchman, E., D. Steiner, et al. (2003). "Photosynthetic and growth responses of three freshwater algae to phosphorus limitation and daylength." Freshwater biology 48: 2141-2148.

Livingston, R. (2007). "Phytoplankton bloom effects on a gulf estuary: water quality changes and biological response." Ecological Applications 17(5 Supplement): S110-S128.

Maruyama, T., K. Kato, et al. (2003). "Dynamics of microcystin-degrading bacteria in mucilage of Microcystis." Microbial Ecology 46: 279-288.

Michelou, V., M. Cottrell, et al. (2007). "Light-stimulated bacterial production and amino acid assimilation by cyanobacteria and other microbes in teh North Atlantic Ocean." Applied and Environmental Microbiology 73(17): 5539-5546.

Mindl, B., B. Sonntag, et al. (2005). "Effects of phosphorus loading on interactions of algae and bacteria: reinvestigation of the 'phytoplankton-bacteria paradox' in a continuous cultivation system." Aquatic Microbial Ecology 38: 203-213.

MK, M., P. Hansen, et al. (2006). "High pH and not allelopathy may be responsible for negative effects of Nodularia spumigena on other algae." Aquatic Microbial Ecology 43: 43-54.

Muyzer, G., E. De Waal, et al. (1993). "Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA." Applied and Environmental Microbiology 59(3): 695-700.

Parsons, T., M. Yoshiaki, et al. (1984). A manual on chemical and biological methods for seawater analysis, Pergamon Press.

Stainton, M. (1973). "A syringe gas-stipping procedure for gas chromatographic determination of organic and inorganic carbon in water." Journal of Fisheries Research Board Canada 30: 1441-1445.

Vonshak, A., R. Guy, et al. (1988). "The response of the filamentous cyanobacterium Spriulina platensis to salt stress." Archives of Microbiology 150(5): 417-420.

Zeng, M. and A. Vonshak (1998). "Adaptation of Spirulina platensis to salinity-stress." Comparative Biochemistry and Physiology Part A 120: 113-118.