

THE IMPACTS OF RAINFALL RUNOFF ON TIDAL CREEK ALGAL AND BACTERIAL  
PRODUCTION

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This thesis has been prepared in the style and format consistent with the journal  
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## ABSTRACT

Field and mesocosm experiments were performed to examine the effects of different nutrient concentrations on production of bacteria and phytoplankton in three New Hanover County, North Carolina, tidal creeks of differing watershed impervious cover. Radiotracer assays were performed to determine production rates using tritiated-thymidine for bacteria and  $^{14}\text{C}$  bicarbonate for phytoplankton. The field study compared production rates and nutrient concentrations monthly during dry and rain conditions and the mesocosm experiment compared production rates given different combinations of nutrients approximating concentrations from rain events (control, NP, NPSi, NPC).

Pages Creek had mean daily heterotrophic and autotrophic production rates of 36.4 and 395.4  $\text{mgC m}^{-3}\text{day}^{-1}$ , respectively. Physical parameters control production in Pages Creek, while nutrients are also a limiting factor in Howe and Bradley Creeks. Nutrient concentrations are generally similar between creeks, all of which are also nitrogen limited. Mean heterotrophic and autotrophic production rates in Howe Creek were 38.3 and 636.7  $\text{mgC m}^{-3}\text{day}^{-1}$ , respectively. Production rates in Bradley Creek were 41.1  $\text{mgC m}^{-3}\text{day}^{-1}$  heterotrophically, and 547.2  $\text{mgC m}^{-3}\text{day}^{-1}$  autotrophically. Production ratios in Bradley Creek differ from the other creeks, indicating interdependence between bacteria and algae. Both types of production correlated with dissolved carbohydrates in this creek, suggesting that phytoplankton supply dissolved carbohydrates to support bacterial growth. This research shows that though nutrient concentrations and production appear to be similar among creeks, there are differences in response to nutrient concentrations and grazing pressure, which may be anthropogenically altered.

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## DEDICATION

This thesis is dedicated to all of my nieces and nephews, in hopes that they will realize their potential, whether as scientists or artists, and endeavor to attain their dreams and goals.

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## INTRODUCTION

Algal and bacterial production are the two main paths of nutrient utilization in aquatic systems. Natural waters are populated with diatoms and flagellates, which sustain higher trophic levels (Mallin 1994), and heterotrophic bacteria, which decompose organic matter, releasing inorganic nutrients that support further production (Klug 2005). These feedback loops can maintain a healthy aquatic system, but increases in human populations and their corresponding wastes have augmented nutrient loadings and concentrations. There are two interrelated consequences of urbanization: population increase that brings with it increased nutrient, chemical, and bacterial wastes, as well as a higher demand for water, and land development, which increases the amount of impervious cover (Hurd and Civco 2004). It has been shown that urban areas with impervious cover have more runoff during rain events than rural areas (Leopold 1968; Schueler 1987; Neller 1988; CWP 1998; Loucaides 2003). Rainfall flows over impervious surfaces such as shelter, roads, and parking lots gathering contaminants, which are then directly emptied into creeks and rivers, potentially altering rates of algal and bacterial production.

Non-point source contamination comes from a variety of sources including residential and commercial land development, golf courses, tourism, and agriculture (Bailey 1996; Mallin et al. 2000a). These sources increase nutrient and bacterial loads, sedimentation, and turbidity. Higher water column turbidity has been correlated to higher counts of fecal bacteria (Mallin et al. 1999; Jeng et al. 2005). Holland et al. (2004) studied twenty three headwater tidal creeks in South Carolina within watersheds of various percent impervious cover and found that human population density and the corresponding percent impervious cover was the most important stressor on tidal creeks. They determined that with more than 10-20% of impervious cover the surrounding hydrography was altered, there was a significant salinity variance, chemical

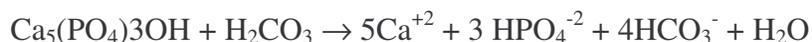
contamination increased and there was an increase in fecal coliform loadings. In areas with more than 20-30% impervious cover the number of stress-sensitive macrobenthic taxa and the abundance of commercially and recreationally important shrimp declined, altering food webs. Changes in food webs may be traced back to changes in nutrient ratios.

Nutrients enter aquatic systems through atmospheric deposition, weathering processes, stormwater runoff, groundwater, bacterial decomposition and water column transformations. Atmospheric deposition is part of the natural cycle of nutrient transfer as dry dust particles and nitrogen fixed by lightning ( $N_2 + O_2 \rightarrow 2NO$ ) dissolve in rain water, but anthropogenic sources such as fossil fuel and agricultural emissions have increased the concentrations of nutrients ( $NO_x^-$ ,  $NH_3$ ,  $PO_4^{-3}$ ) in rainwater (Paerl, 1997). Nitrogen concentrations in rainfall have been shown to directly cause primary production in a number of studies (Paerl et al. 1990; Willey and Cahoon 1991; Mallin et al. 1993), but silicon concentrations in rainfall are minimal (Cahoon 2000).

Both chemical and mechanical weathering are responsible for the bulk of the natural silicon and phosphorus inputs. Chemical weathering takes place as stormwater percolates through the soil, reacts with carbon dioxide, which is released by plant roots and maintained at a higher concentration within the soil air (0.035%) than the atmosphere, to produce carbonic acid (Schlesinger 1991):

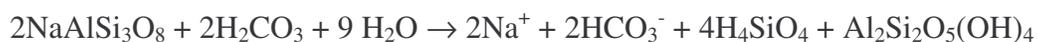


Carbonic acid weathers minerals, such as apatite ( $Ca_5(PO_4)3OH$ ), releasing a biologically available form of phosphorus ( $HPO_4^{-2}$ ), which is carried into groundwater (Schlesinger 1991):

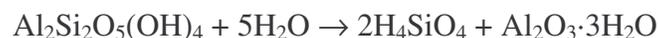


Depending on the pH of the solution, phosphorus tends to precipitate or adsorb to other soil minerals, becoming unavailable to biota. At low pH, phosphorus will precipitate with iron or aluminum oxides, and adheres to calcium carbonate or precipitates as calcium phosphate at higher pH (Schlesinger 1991). Organic acids released by organisms in the soil, such as oxalic acid released by fungi, enhance chemical weathering and inhibit precipitation of iron and aluminum oxides. These acids also combine with iron and calcium released during weathering and act as a chelating agent accelerating the weathering process further, and making more of the phosphorus biologically available (Schlesinger 1991).

Phosphorus only composes about 0.19% as P<sub>2</sub>O<sub>5</sub> of the Earth's continental crust, whereas silicon dioxide (SiO<sub>2</sub>) composes 63.2% (Schlesinger 1991). The weathering processes are the main source of silicon to the aquatic system (Weaver and Tarney 1984) therefore, groundwater discharged as stream base flow is the primary source of biologically available silicon. Carbonic acid reacts with silicate rocks such as albite (NaAlSi<sub>3</sub>O<sub>8</sub>), releasing the bioavailable form of silica, silicic acid (H<sub>4</sub>SiO<sub>4</sub>), which is carried into groundwater (Schlesinger 1991):



The secondary mineral, kaolinite (Al<sub>2</sub>Si<sub>2</sub>O<sub>5</sub>(OH)<sub>4</sub>), is left behind, which further dissolves in areas of high rainfall, such as the southeastern United States (Schlesinger 1991):



As was true of phosphorus, organic acids released by organisms in the soil also play a role in speeding up the chemical weathering processes of silica.

Mechanical weathering breaks down rocks without changing the chemical structure; these fragments can be eroded from the landscape in stormwater runoff. Stormwater runoff itself dissolves nutrients as it flows over impenetrable surfaces and areas with low infiltration rates.

Lawns and streets have been found to contribute to the majority of total and dissolved phosphorus in runoff in urban residential areas (Waschbusch et al. 2000). Although phosphates have been banned from some detergents, fertilizer use can still be a major anthropogenic source of phosphorus. A study in Wisconsin found traditionally-fertilized lawn sites had 2 times the amount of dissolved phosphorus in runoff as did unfertilized-lawn or nonphosphorus-fertilized lawn sites, but interestingly, runoff nitrate/nitrite concentrations were generally low at all sites (Garn 2002).

Eutrophication occurs when an abundance of nutrients enter the water column and stimulate algal blooms (Anderson et al. 2002). Algal blooms can lead to greater production, but they limit light to seagrasses, and ultimately result in hypoxia upon their death, since oxygen is utilized in respiration, which can lead to fish kills (Cotner et al. 2000; Mallin et al. 2000a; Anderson et al. 2002). Eutrophication has also been shown to alter nutrient ratios (Schelske and Stoermer 1971; Anderson et al. 2002). Nutrient ratios are important to understanding community structure. The Redfield ratio, 106C:16N:1P (Redfield 1963), estimates the ratio of nutrients needed for most algal growth, but diatoms also require 16Si:1P or approximately 1Si:1N (Gilpin et al. 2004) due to their hard outer silicon frustule (Busby and Lewin 1967). The enrichment of nitrogen and phosphorus can lead to silica limitation, shifting the dominant species of phytoplankton away from diatoms towards harmful algal blooms (Schelske and Stoermer 1971; Baird et al. 2004). These noxious blooms can cause finfish kills, infect shellfish, and some are toxic to humans (Burkholder and Glasgow 1997; Anderson et al. 2002; Gilpin et al. 2004).

Alterations in nutrient ratios also benefit bacterial production in two ways. Algal bloom decomposition provides energy for microbial production and bacteria may be able to out-compete phytoplankton for phosphorus (Currie 1990; Cotner et al. 2000). When silica limits

diatoms, bacteria may have the competitive advantage and a microbial bloom may result (Chrzanowski et al. 1995; Havskum et al. 2003). Some of the consequences of a predominantly microbial community include hypoxia, as bacteria utilize oxygen in decomposition of organic matter, and reduced water quality, as anthropogenic bacterial sources include enteric pathogens, which at high enough concentrations can result in shell fishery and recreational water closures (Mallin et al. 2000b).

Centric diatoms and dinoflagellates dominate healthy New Hanover County tidal creek systems; they provide sustenance for higher trophic levels and are therefore important to fisheries production (Mallin 1994). Phytoplankton communities shift dominant species given different seasons, salinities, tides, or following nutrient pulses, changing the limiting nutrient (Mallin 1994; Mallin et al. 1999). Since different species tend to tolerate different salinities, the dynamic tidal creeks have seemingly changing populations given low or high tide.

A four year study from 1993-97 of the New Hanover County tidal creeks suggested local bacterial pollution stemming from increased land development (Mallin et al. 1998), which has resulted in further research. Data from the 2003-2004 sampling period showed increased impairment, as indicated by chlorophyll *a* or bacterial increase, or dissolved oxygen decrease, following the salinity gradient upstream from the Intracoastal Waterway for all tidal creeks (Mallin et al. 2005). Increasing percent impervious cover has been shown to correlate with increasing water column fecal coliforms among Futch, Pages, Howe, Hewletts, Whiskey, and Bradley Creeks (Mallin et al. 2001). The non-toxic form of *Pfiesteria piscicida* has been identified during summer months in Hewletts, Bradley, Pages, and Futch Creeks, but they have not been linked to fish kills in this area (Mallin et al. 2004). Phytoplankton were ten times more abundant at low tide than high tide in Hewletts Creek (69% developed), flagellates dominated

the community with less than 2% diatoms at low tide only increasing to approximately 13% at high tide (Mallin et al. 1999). Futch Creek (22% developed) was also dominated by flagellates at low tide, but high tide brought in a much more diverse assemblage, and Howe Creek (39% developed) varied from dinoflagellates and pennate diatoms at low tide to centric diatoms and cryptomonads at high tide (Mallin et al. 1999). These data indicate a shift away from a diatom dominated community and an increase in fecal coliforms with increasing land development.

Phytoplankton and bacteria have been shown to compete for phosphorus, and when silicon is abundant, they support each other's growth because bacteria tend to make a greater fraction of the total phosphorous available for phytoplankton which in turn provides organic carbon to support bacterial growth (Currie 1990). Havskum et al. (2003) found that given abundant phosphorus, nitrogen, and glucose, but no silica, a community dominated by diatoms was replaced by bacteria instead of other phytoplankton species.

Due to nutrient loading, nitrogen and phosphorus are much more abundant in stormwater runoff than from natural sources (Mallin et al. 2000a; Cahoon et al. 1999) and silicon may become limiting, due to anthropogenic changes in hydrology (Loucaides 2003). Soil organic matter is thought to be labile and to contain approximately three times more carbon than land vegetation (Schlesinger 1986). With land development, it would seem that dissolved organic carbon (DOC) concentrations would increase due to erosion, but Hobbie and Likens (1973) found DOC and fine particulate carbon concentrations to be similar between a forested watershed and an experimentally clear-cut watershed. The higher amount of erosion from the clear-cut watershed was thought to provide a similar amount of carbon as the leaf litter from the forested watershed. Schlesinger (1986) proposed that since erosion could not account for carbon lost to the system after clear-cutting, that it must be given off as carbon dioxide. Bioavailable

carbohydrates have not yet been measured in runoff in this area, but Avery et al. (2003) found DOC in Wilmington, NC rainfall to range from ~20-400 $\mu$ M, of which a significant portion, 63 $\pm$ 14%, was labile. Given that stormwater would encounter terrestrial plants and leaf litter that had produced glucose through photosynthesis, runoff is another source of bioavailable DOC, but the effects of impervious cover, as it both conveys stormwater and blocks percolating ground water, are still unclear. Silicon-rich groundwater would normally flow into rivers as it is pumped in during heavy rain events. Since silicon levels from anthropogenic sources have remained constant (Gilpin et al. 2004), and runoff bypasses the natural pathway of percolating through the soil, surface water from watersheds of greater impervious cover have less of this important macronutrient (Loucaides 2003). Other species of phytoplankton that are not silicon dependant (Gilpin et al. 2004) or heterotrophic bacteria (Havskum et al. 2003) may out-compete diatoms for the other plentiful nutrients.

Therefore, it was hypothesized that with increasing impervious cover, the change in nutrient ratios could alter heterotrophic to photosynthetic growth rates, in favor of heterotrophic bacteria. This hypothesis was tested by addressing the following questions:

1. Is there a significant increase in bioavailable DOC as measured by carbohydrate analysis with rainfall/runoff?
2. Is there a significant difference in ratios of heterotrophic to autotrophic growth rates during rain events as compared to baseline levels?
3. Is there a trend in ratios of heterotrophic to autotrophic growth rates with increasing percent impervious cover in watersheds?
4. Is there a trend in ratios of heterotrophic to autotrophic growth rates with different nutrient ratios?

## METHODS

### Site Descriptions

Two sites, one upstream and one downstream, were sampled on each of three tidal creeks in New Hanover County, North Carolina, with watersheds of varying percent impervious cover (Figure 1). Pages Creek (sites PC-M and PC-BDDS) was chosen as a sampling location for its relatively low percent watershed impervious cover of 9% (NHCPD 1993), and its high midstream tidal flushing rate of 58% (Hales 2001). Howe (HW-FP and HW-GP) and Bradley (BC-76 and BC-SB) Creeks have watersheds of higher impervious cover (19% and 23% respectively; CWPD 2006) and lower midstream flushing rates (44% and 37% dry and 24% and 17% with rain, respectively), which potentially, more strongly retain nutrients and the plankton communities that utilize.

### Field Methods

Surface water samples were collected at least monthly at a high tide occurring between 9am and 12pm, from September to December of 2005 and March to August 2006. Only one creek per day could be sampled so a sampling event took place over three days. Samples were collected during dry conditions and after at least 0.1 inches of rain occurring within a 24 hour period of the first day of sampling, to compare nutrient and production trends. Triplicate surface water samples were collected in acid washed bottles for laboratory analysis of bioavailable dissolved carbohydrates, ammonium, dissolved nitrate/nitrite, dissolved orthophosphate, dissolved silica, and heterotrophic and autotrophic production, while temperature and salinity were measured *in situ* using a YSI Multiparameter Water Quality Probe. Total carbon dioxide was measured in duplicate due to time constraints. The North Carolina State Climate Office provided the precipitation data (<http://www.nc-climate.ncsu.edu/>), which was utilized as the sum

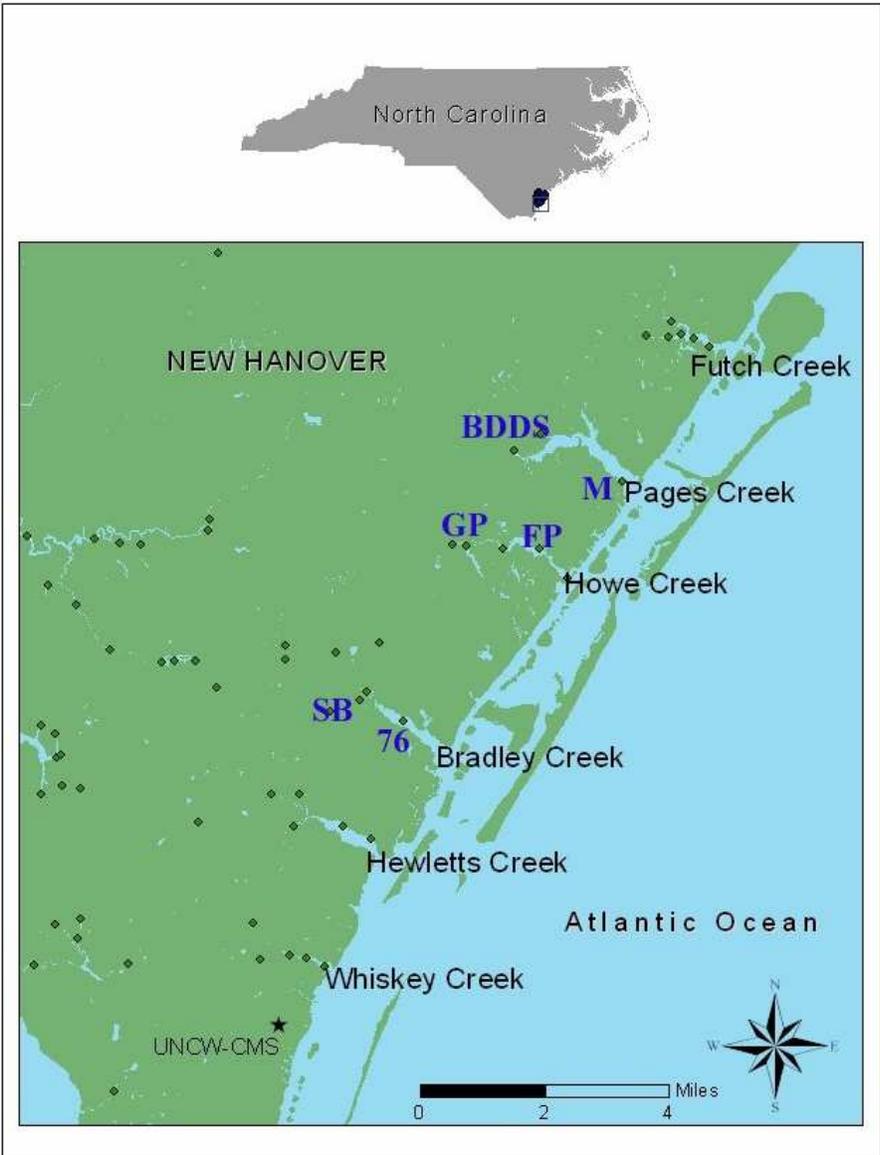


Figure 1. Map of New Hanover County, NC sampling sites.

of the precipitation in inches over a 24 hour, 48 hour, and 72 hour period previous to the sampling time.

Amber vials containing 35ml of surface water for ammonium analysis were inoculated with reagents in the field, due to its high volatility, and stored in a dark box at room temperature until analysis according to the Koroleff method (Grasshoff et al.1983). Total carbon dioxide was measured immediately upon arrival to the lab by the standard wet chemistry method of Parsons et al. (1984), unless the salinity was <5.0 or the pH was <7.3, then a Gran titration was performed (Wetzel and Likens 1991). Water samples were stored on ice, filtered through Whatman GF/F filters in the lab, and the pooled filtrates were stored frozen at -20°C (Si samples stored at 4°C) until analysis for carbohydrate (Dubois et al. 1956), phosphate and silicate by the standard wet chemistry methods of Parsons et al. (1984), and nitrate/nitrite (NO<sub>x</sub>) by a Bran-Leubbe AutoAnalyzer III.

Heterotrophic production rates were determined by the tritiated thymidine method described by Parsons et al. (1984). Triplicate samples, as well as a blank sample containing 2ml of formalin, were inoculated with 250µl of dilute tritiated [methyl-<sup>3</sup>H] thymidine (1 in 10). They were incubated in the lab for 15-30 minutes, extracted with cold 10% TCA (trichloroacetic acid), and filtered onto 0.45µ cellulose membrane filters. The filters were dissolved in 1ml of ethyl acetate, then 10ml of Scintiverse cocktail (Fischer) was added, and the amount of tritiated thymidine incorporated into DNA was measured by scintillation counting. Bacterial growth rates were determined by calculating the moles of thymidine incorporated:

$$\frac{mmol}{l\ hr} = \frac{U}{S} \times \frac{4.5 \times 10^{-13}}{t} \times \frac{1}{v}$$

Where U is the disintegrations per minute (dpm) of the sample minus the blank, S is the specific activity in Ci/mmol,  $4.5 \times 10^{-13}$  is the number of curies per dpm, t is the hours of incubation, and v

is the volume of sample incubated. This number was then converted to the number of cells produced with the conversion factor  $1.4 \times 10^{18}$  cells/mole, given by Fuhrman and Azam (1982), and then converted to the amount of carbon with the conversion factor  $2 \times 10^{-8}$   $\mu\text{g C/cell}$  in order to make a direct comparison to primary production (Lee and Fuhrman 1987; Bell 1993).

The rate of phytoplankton production was determined with the radioactive carbon method described by Parsons et al. (1984). Triplicate 255ml samples, as well as a blank sample containing 1ml of DCMU (3-(3,4-dichlorophenyl)-1,1 dimethylurea) an inhibitor of photosynthetic electron transport, were spiked with 250 $\mu\text{l}$  of  $5\mu\text{Ci NaH}^{14}\text{CO}_3$  and incubated *in situ* 2-4 hours, by floating the bottles suspended ~12 inches below the surface. Fifty milliliters was filtered through a glass fiber filter and the filter was placed into a vial containing 10 ml of Scintiverse and then the amount of  $^{14}\text{C}$  assimilated by phytoplankton was measured by scintillation counting. The algal growth rate was then calculated:

$$\frac{\text{mg C}}{\text{m}^3 \text{ hr}} = \frac{(R_S - R_B) \times W}{R \times N}$$

Where  $R_S$  is the sample count,  $R_B$  is the blank count,  $W$  is the total carbon dioxide (mg C/l),  $R$  is the specific activity of bicarbonate, and  $N$  is the number of hours incubated.

#### Autotrophic Production Correction Factor

During June of 2006, the scintillation counter needed service, which took approximately a month to complete. During this time samples were still collected in either plastic (June 28 – July 11) or glass (July 12 – 28) scintillation vials, and stored at room temperature. When the repairs were complete, the samples were run, but the “initial” vials, used to determine the specific activity of bicarbonate, were approximately 100 times lower than expected. Samples that had been run before the repair were rerun to confirm the accuracy of the instrument, and although the old samples gave approximately the same values, the “initials” were approximately 100 times

lower than previously. An experiment was performed to determine a rate of bicarbonate degassing to carbon dioxide, which was used to correct the “initial” values of stored vials, and to determine the best storage conditions for future samples. Two plastic and 2 glass vials were filled with 10ml of Scintiverse, and old “initial” vials, 2 in plastic and 2 in glass, were each adjusted to 10ml with Scintiverse. Each of these vials was run to determine the pre-addition bicarbonate level, and then 250 $\mu$ l of NaH<sup>14</sup>CO<sub>3</sub> was added to 255ml of DI water, 1ml of this solution was added to each of the vials, and they were rerun. The dpm from the first run were subtracted from the second run and the samples were stored at room temperature, except one of each of the old “initial” vials, one in plastic and one in glass, were refrigerated. All of these vials were rerun every 2-3 days for one month. When the dpm were plotted against the day since inoculation, the “initials” in plastic vials, stored at room temperature, degassed exponentially, and those in glass vials only degassed slightly linearly (Figure 2). After log transforming the values from the plastic vials, the equation of the line, one for plastic and one for glass, were used to determine the corrected dpm for each of the “initials”, since they had all been run within 30 days of sampling. This experiment showed that glass vials are superior to plastic and that storage in refrigeration is preferred to room temperature.

### Experimental Methods

A mesocosm experiment was performed to examine the effect of nutrient additions in concentrations approximating rain/runoff on algal and bacterial production, keeping physical parameters constant. Target nutrient concentrations were estimated by subtracting those measured after rain events from concentrations of dry periods. Sixteen 4L cubitainers of surface

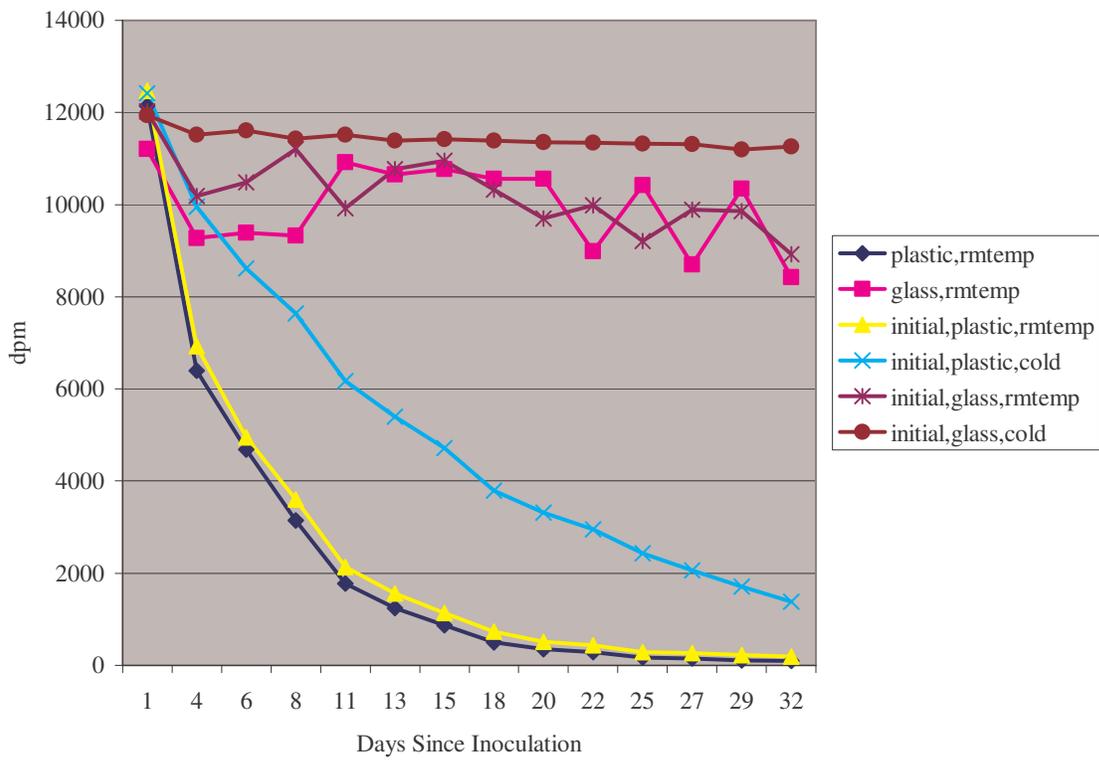


Figure 2. Storage condition affects on bicarbonate degassing to carbon dioxide. dpm = disintegrations per minute, rtemp = room temperature.

water were collected on consecutive days from September 12 – 14 of 2006, from one of the three downstream sites (PC-M, HW-FP, and BC-76, respectively), transported to the greenhouse on the UNCW campus, and spiked in quadruplicate with each of three treatments (Table 1). The remaining cubitainers were not manipulated to serve as controls. They were then placed in outdoor mesocosms and covered with two layers of neutral density screening, to prevent photo-stress, and allowed to incubate for 47 hours at ambient temperatures. Algal and bacterial production, as well as total carbon dioxide, were measured as for the field experiments, at the end of the incubation.

#### Data Analyses

SAS Institute's JMP version 4.0 was used for all statistical analyses. The Shapiro-Wilk test was used to assure all variables were normally distributed. The data were log transformed or a log was taken of (1+value) when zeros were present in the data set to satisfy the assumption of homoscedasticity. Students t-tests were used to find significant differences in all parameters after rain events from dry periods at individual sites, as well as spatial variance within each creek ( $p < 0.05$ ). An analysis of variance (ANOVA) was used to compare the combined sites of each watershed by all parameters and the Tukey- Kramer HSD was used to rank those creeks having a significant difference. A pairwise correlation was performed for the combined sites of each watershed on 12 variables to find significant correlations of both production and precipitation to nutrients using an adjusted  $\alpha = 0.0042$ , calculated by the Bonferroni method (Gotelli and Ellison 2004). Since many of the covariates were correlated a principal components analysis (PCA) was used to reduce the number of correlated variables to a few significant uncorrelated variables (Gotelli and Ellison 2004), and then regression and multiple regression analyses were performed using the significant parameters.

Table 1. Final concentration ( $\mu\text{M}$ ) of nutrients added for each treatment of the mesocosm experiment. Additions = NP (nitrate and phosphate), NPSi (nitrate, phosphate, and silicate), and NPC (nitrate, phosphate, and carbohydrate).

Treatment	$\text{NaNO}_3$	$\text{KH}_2\text{PO}_4$	$\text{Na}_2\text{SiO}_3$	Dextrose
Control				
NP	4.3	0.5		
NPSi	4.3	0.5	4.5	
NPC	4.3	0.5		19.2

## RESULTS

### Field Data

#### Physical and Chemical Parameters

There were no differences in mean temperatures among creeks (Table 2), between creek sites (Table 3), or within creeks between dry and rain events (Tables 4, 5, & 6). Seasonally, mean temperatures dropped below 20°C during the November, December, and March sampling events (Figure 3). The mean temperature of the Pages Creek sites was 21.1°C, with a range of 8.2°C in December to 28.5°C in July. The Howe Creek sites had a mean temperature of 21.6°C and a range of 8.5°C in December to 29.0°C in September. Similarly, the Bradley Creek sites had a mean temperature of 21.5°C, with a range of 9.2°C in December to 29.1°C in September.

The mean salinity of the Pages Creek sites was 29.2, which was significantly higher ( $p = 0.0011$ ) than both the Howe and Bradley Creek site means, at 24.6 and 23.0, respectively (Table 2). All of the downstream sites were euhaline, and had salinities significantly higher than the upstream mixohaline sites (Pages Creek  $p = 0.0078$ ; Howe and Bradley Creeks  $p < 0.0001$ ; Table 3). Upstream, site PC-BDDS had the widest range in salinities from 0.1 in October, during Tropical Storm Ophelia, to 34.5 in September (Figure 4). Salinities after rain events were only significantly lower in the Pages Creek sites (PC-BDDS  $p = 0.0080$ ; PC-M  $p = 0.0453$ ; Table 4). The Howe Creek sites had a mean dissolved silica concentration of 36.3 $\mu\text{M}$ , which was significantly higher than that of the Pages Creek mean of 23.5 $\mu\text{M}$  ( $p = 0.0064$ ), but not different from the Bradley Creek mean of 31.7 $\mu\text{M}$  (Table 2). All downstream site concentrations were lower and more consistent, with a range of 5.8 – 35.8 $\mu\text{M}$ , than the upstream sites, with a range of 12.8 – 99.6 $\mu\text{M}$  ( $p < 0.0001$ ; Table 3; Figure 5). There were no significant differences in dissolved silica concentration in individual sites after rain events (Tables 4, 5, & 6).

Table 2. Water quality parameters of sites pooled for each creek from September – December 2005 and March - August 2006. IC = impervious cover. Data as mean (standard deviation)/range. Nutrients given as  $\mu\text{M}$  and production as  $\text{mgC m}^{-3}\text{day}^{-1}$ . \*Indicates significantly different from other creeks. \*\*Indicates significantly different from Pages Creek only ( $p < 0.05$ ).

Parameter	Pages Creek (9% IC)	Howe Creek (19% IC)	Bradley Creek (23% IC)
Temperature ( $^{\circ}\text{C}$ )	21.1 (6.2) 8.2-28.5	21.6 (6.7) 8.5-29.0	21.5 (6.3) 9.2-29.1
Salinity	29.2 (9.0)* 0.1-36.7	24.6 (11.4) 2.1-35.5	23.0 (10.2) 0.5-34.3
Silica-Si	23.5 (16.9) 6.5-64.0	36.3 (30.8)** 5.8-99.6	31.7 (22.0) 8.1-70.8
Carbohydrate-C	147.8 (297.6) 4.0-914.3	145.9 (260.7) 5.0-777.8	136.0 (250.5) 11.1-1171.4
Ortho phosphate-P	0.6 (0.5) 0.1-1.8	0.6 (0.5) 0.1-2.0	0.6 (0.5) 0.1-2.5
Ammonium-N	2.0 (2.0) 0.1-6.6	1.9 (2.2) 0.0-7.9	1.8 (2.1) 0.0-6.9
Nitrate/Nitrite-N	1.5 (2.5) 0.0-11.8	1.1 (1.9) 0.1-9.1	1.4 (1.8) 0.0-6.3
N:P	8.7 (9.9) 0.1-28.3	7.9 (8.2) 0.9-26.5	7.9 (7.5) 1.3-26.9
Daily Heterotrophic Production	36.4 (28.1) 10.0-118.7	38.3 (31.7) 7.7-94.1	41.1 (36.4) 4.3-142.3
Daily Autotrophic Production	395.4 (462.5) 8.3-1733.8	636.7 (915.8) 14.6-3691.4	547.2 (851.5) 2.7-3469.8

Table 3. Water quality parameters of each site on Pages, Howe, and Bradley Creek from September – December 2005 and March - August 2006. Data as mean/(standard deviation)/range. Nutrients given as  $\mu\text{M}$  and production as  $\text{mgC m}^{-3}\text{day}^{-1}$ . \*Indicates significantly different from upstream site ( $p < 0.05$ ).

Parameter	PC-BDDS	PC-M	HW-GP	HW-FP	BC-SB	BC-76
Temperature (°C)	21.3 (6.5) 8.2-28.5	20.9 (6.0) 9.4-27.5	21.2 (7.1) 8.5-28.9	22.1 (6.4) 10.4-29.0	21.3 (6.4) 9.2-29.1	21.7 (6.3) 10.0-29.0
Salinity	26.4 (10.0) 0.1-34.5	32.0* (7.0) 9.8-36.7	16.2 (10.6) 2.1-31.5	33.0* (2.9) 24.5-35.5	15.7 (9.1) 0.5-31.4	30.4* (4.2) 18.6-34.3
Silica-Si	35.9 (15.4) 19.8-66.3	11.1* (4.8) 6.5-18.8	57.8 (30.5) 12.8-99.6	15.4* (8.9) 5.8-35.8	48.1 (19.7) 12.9-70.8	15.2* (6.0) 8.1-23.0
Carbohydrate- C	173.0 (309.7) 4.0-858.7	122.5 (287.1) 3.0-914.3	164.7 (229.5) 17.2-707.9	127.0 (291.0) 5.0-777.8	180.8 (311.2) 17.2- 1171.4	89.9 (158.7) 11.1- 659.6
Ortho phosphate-P	0.7 (0.5) 0.3-1.8	0.4* (0.5) 0.1-1.8	0.7 (0.6) 0.2-2.0	0.4* (0.4) 0.1-0.8	0.7 (0.7) 0.1-2.5	0.4* (0.3) 0.1-1.0
Ammonium-N	3.0 (1.9) 0.7-6.6	1.0* (1.6) 0.1-6.2	2.5 (2.2) 0.2-7.9	1.3* (2.0) 0.0-6.6	1.9 (2.2) 0.0-6.9	1.8 (2.0) 0.0-6.9
Nitrate/Nitrite- N	2.4 (3.3) 0.1-11.8	0.6* (0.6) 0.0-2.5	1.7 (2.6) 0.1-9.1	0.6* (0.4) 0.2-1.4	2.1 (2.3) 0.0-6.3	0.8* (0.7) 0.2-2.7
N:P	8.9 (5.9) 0.2-19.9	8.5 (12.7) 0.1-28.3	6.4 (6.5) 0.9-26.5	9.3 (9.4) 2.3-24.9	9.8 (8.3) 2.1-26.9	6.0* (6.1) 1.3-26.2
Daily Heterotrophic Production	43.3 (34.1) 10.0-118.7	29.5* (18.4) 10.6-59.4	43.7 (30.1) 8.9-87.1	32.8 (32.7) 7.7-94.1	51.5 (39.5) 6.9-142.3	30.8* (30.2) 4.3-103.2
Daily Autotrophic Production	469.9 (506.3) 8.3-1733.8	318.7 (405.8) 51.7-1443.1	978.5 (1167.7) 14.6-3691.4	294.9* (314.7) 36.3-1024.8	890.1 (1096.0) 2.7-3469.8	240.3* (166.2) 23.1- 518.5

Table 4. Water quality parameters of Pages Creek sites rain (n = 18) and dry (n = 18) from September – December 2005 and March - August 2006. Data as mean (standard deviation)/range. Nutrients given as  $\mu\text{M}$  and production as  $\text{mgC m}^{-3}\text{day}^{-1}$ . \*Indicates significantly different from dry ( $p < 0.05$ ).

Parameter	PC-BDDS		PC-M	
	Dry	Rain	Dry	Rain
Temperature (°C)	21.8 (5.1) 14.7-28.5	20.7 (7.7) 8.2-28.5	20.7 (4.9) 13.1-27.2	21.1 (7.2) 9.4-27.5
Salinity	30.7 (2.2) 26.7-33.4	22.1 (12.8)* 0.1-34.5	34.3 (1.5) 31.5-36.4	29.7 (9.3)* 9.8-36.7
Silica-Si	32.6 (15.6) 19.8-64.0	39.3 (15.0) 22.5-66.3	9.8 (3.5) 6.5-14.7	12.3 (5.6) 6.6-18.8
Carbohydrate-C	111.8 (138.3) 35.8-281.5	234.2 (412.9) 4.0-858.7	51.5 (55.3) 3.0-139.5	193.5 (394.9) 7.1-914.3
Ortho phosphate-P	0.4 (0.1) 0.3-0.6	0.9 (0.6)* 0.3-1.8	0.2 (0.1) 0.1-0.4	0.6 (0.6)* 0.1-1.8
Ammonium-N	2.4 (1.8) 0.7-5.3	3.5 (1.8) 1.0-6.6	0.5 (0.4) 0.1-1.2	1.5 (2.2) 0.2-6.2
Nitrate/Nitrite-N	1.1 (0.8) 0.5-3.1	3.6 (4.2)* 0.1-11.8	0.4 (0.1) 0.2-0.6	1.1 (0.9)* 0.3-2.8
N:P	9.2 (4.0) 3.9-14.7	8.5 (7.3) 0.2-19.9	10.0 (15.9) 1.6-28.3	7.0 (9.0) 0.1-24.6
Daily Heterotrophic Production	48.6 (37.7) 10.0-118.7	38.1 (30.1) 13.0-87.4	29.1 (16.9) 11.1-59.4	29.8 (20.3) 10.6-54.1
Daily Autotrophic Production	648.0 (587.0) 55.2-1733.8	301.7 (356.0)* 8.3-791.5	416.3 (522.7) 91.6-1433.1	221.1 (215.1) 51.7-626.0

Table 5. Water quality parameters of Howe Creek sites rain (n = 15) and dry (n = 18) from September – December 2005 and March - August 2006. Data as mean (standard deviation)/range. Nutrients given as  $\mu\text{M}$  and production as  $\text{mgC m}^{-3}\text{day}^{-1}$ . \*Indicates significantly different from dry ( $p < 0.05$ ).

Parameter	HW-GP		HW-FP	
	Dry	Rain	Dry	Rain
Temperature (°C)	21.6 (6.7) 12.1-28.9	20.8 (7.6) 8.5-26.8	21.9 (5.9) 13.5-29.0	22.2 (7.1) 10.4-28.0
Salinity	17.0 (9.0) 3.6-26.8	15.6 (11.9) 2.1-31.5	34.0 (0.7) 33.3-35.0	32.1 (3.7) 24.5-35.5
Silica-Si	57.0 (33.1) 12.8-96.3	58.4 (29.2) 21.2-99.6	14.7 (11.6) 5.8-35.8	16.0 (6.3) 7.6-22.6
Carbohydrate- C	109.1 (126.5) 30.3-231.1	211.0 (284.5) 17.2-707.9	64.6 (60.6) 9.9-145.7	179.0 (387.4) 5.0-777.8
Ortho phosphate-P	0.5 (0.5) 0.2-0.9	0.9 (0.6) 0.3-2.0	0.4 (0.4) 0.1-0.7	0.4 (0.3) 0.1-0.8
Ammonium-N	1.4 (1.1) 0.2-3.1	3.3 (2.5)* 0.7-7.9	0.6 (0.9) 0.0-2.2	1.8 (2.5) 0.0-6.6
Nitrate/Nitrite- N	0.5 (0.3) 0.2-0.8	2.6 (3.2)* 0.1-9.1	0.4 (0.2) 0.2-0.6	0.7 (0.4)* 0.4-1.6
N:P	5.0 (3.6) 1.5-9.5	7.6 (8.0) 0.9-26.5	11.6 (10.8) 2.4-24.9	7.4 (8.1) 2.3-22.6
Daily Heterotrophic Production	47.9 (31.9) 22.7-87.1	40.2 (29.1) 8.9-71.1	36.8 (34.7) 7.7-94.1	29.5 (31.4) 7.9-89.5
Daily Autotrophic Production	1271.9 (828.1) 94.8-2053.9	734.0 (1364.8) 14.6-3691.4	402.3 (367.4) 136.7-1024.8	205.3 (237.8) 36.3-701.8

Table 6. Water quality parameters of Bradley Creek sites rain (n = 21) and dry (n = 15) from September – December 2005 and March - August 2006. Data as mean (standard deviation)/range. Nutrients given as  $\mu\text{M}$  and production as  $\text{mgC m}^{-3}\text{day}^{-1}$ . \*Indicates significantly different from dry ( $p < 0.05$ ).

Parameter	BC-SB		BC-76	
	Dry	Rain	Dry	Rain
Temperature (°C)	20.5 (5.0) 14.4-29.1	21.9 (7.4) 9.2-27.4	20.4 (4.8) 14.3-28.6	22.6 (7.2) 10.0-29.0
Salinity	17.8 (10.0) 6.0-31.4	14.1 (8.3) 0.5-27.8	31.8 (1.6) 28.9-33.6	29.4 (5.1) 18.6-34.3
Silica-Si	43.9 (23.2) 12.9-67.3	51.2 (16.7) 23.6-70.8	14.0 (6.7) 8.1-23.0	16.1 (5.5) 8.2-23.0
Carbohydrate- C	79.9 (61.7) 17.2-161.7	252.9 (392.1) 22.2-1171.4	54.9 (47.2) 11.1-119.7	116.2 (204.3) 14.8-659.6
Ortho phosphate-P	0.3 (0.1) 0.1-0.4	1.0 (0.8)* 0.2-2.5	0.3 (0.1) 0.2-0.4	0.5 (0.3)* 0.1-1.0
Ammonium-N	0.5 (0.6) 0.0-1.3	2.8 (2.4)* 0.8-6.9	0.5 (0.6) 0.0-1.3	2.6 (2.2)* 0.4-6.9
Nitrate/Nitrite- N	0.3 (0.2) 0.0-0.5	3.2 (2.3)* 0.5-6.3	0.4 (0.2) 0.2-0.6	1.1 (0.8)* 0.3-2.7
N:P	9.6 (8.5) 2.1-21.8	10.0 (8.3) 2.2-26.9	6.8 (8.6) 1.3-26.2	5.5 (3.9) 2.1-10.1
Daily				
Heterotrophic Production	60.7 (48.4) 18.0-142.3	44.8 (31.2) 6.9-88.5	25.3 (22.8) 8.1-60.2	34.8 (34.5) 4.3-103.2
Daily				
Autotrophic Production	524.2 (299.3) 173.2-907.5	1151.5 (1366.4) 2.7-3469.8	187.8 (152.4) 30.9-452.6	216.1 (178.1) 23.1-518.5

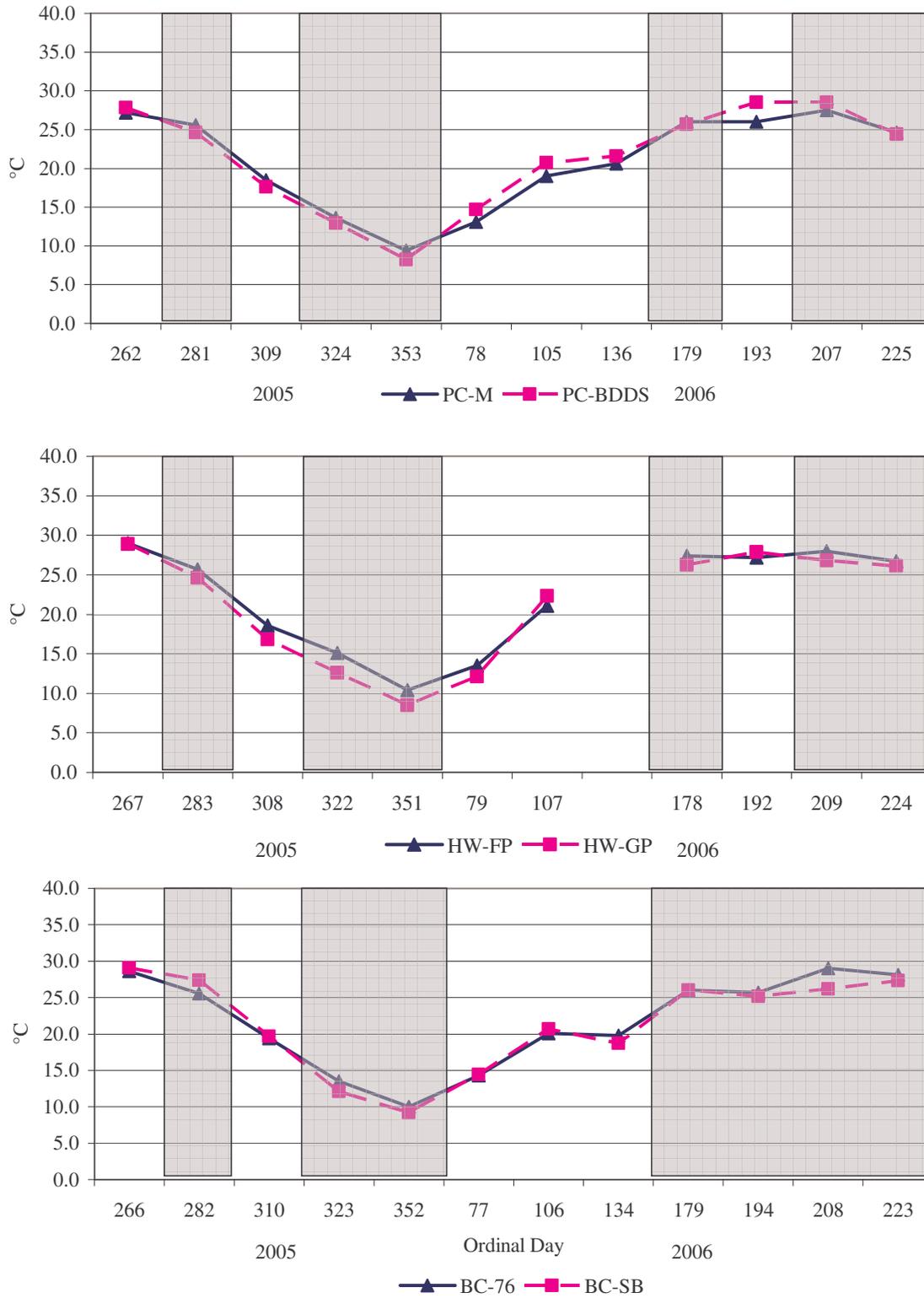


Figure 3. Mean temperature. Shaded areas indicate rain events.

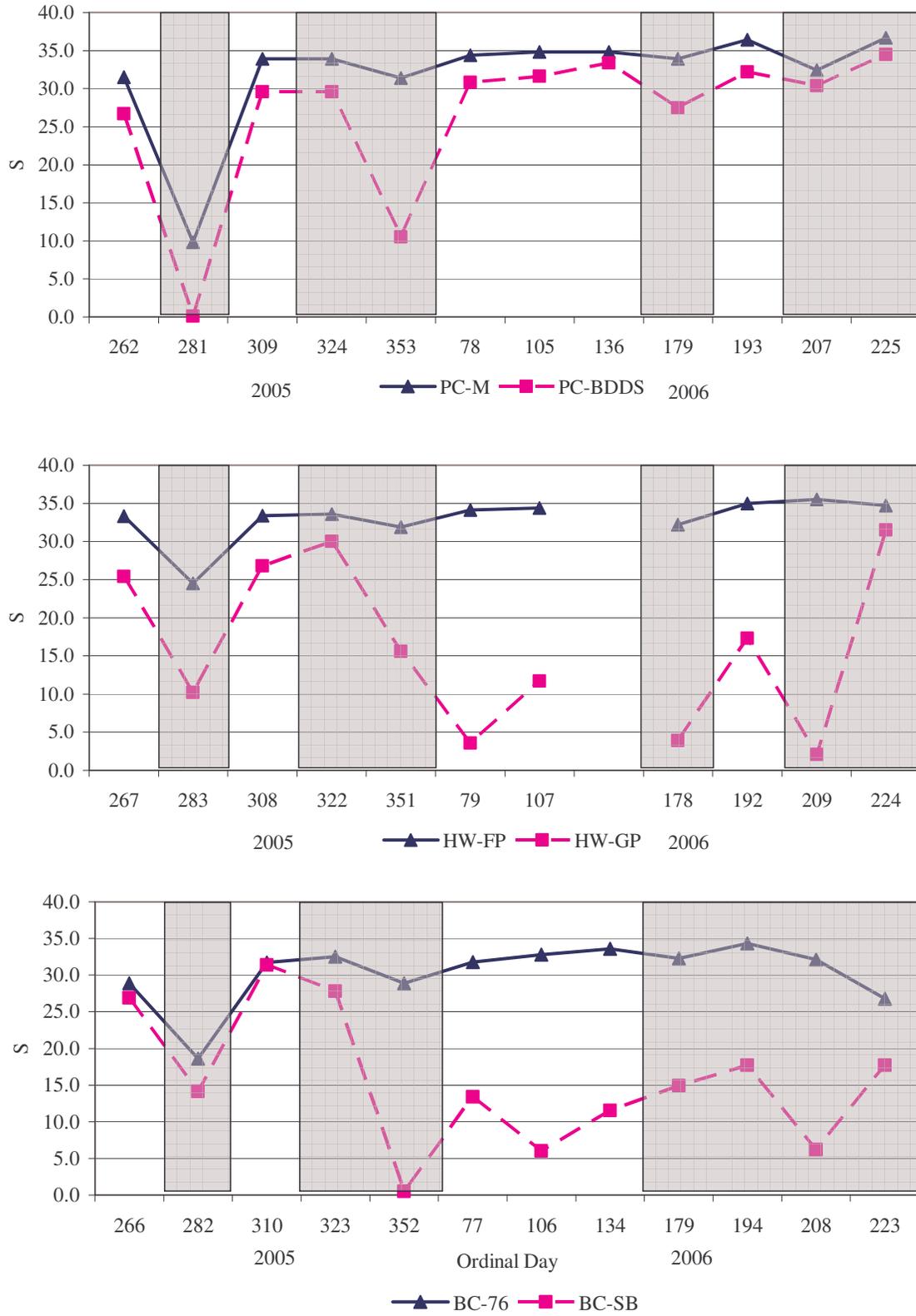


Figure 4. Mean salinity. Shaded areas indicate rain events.

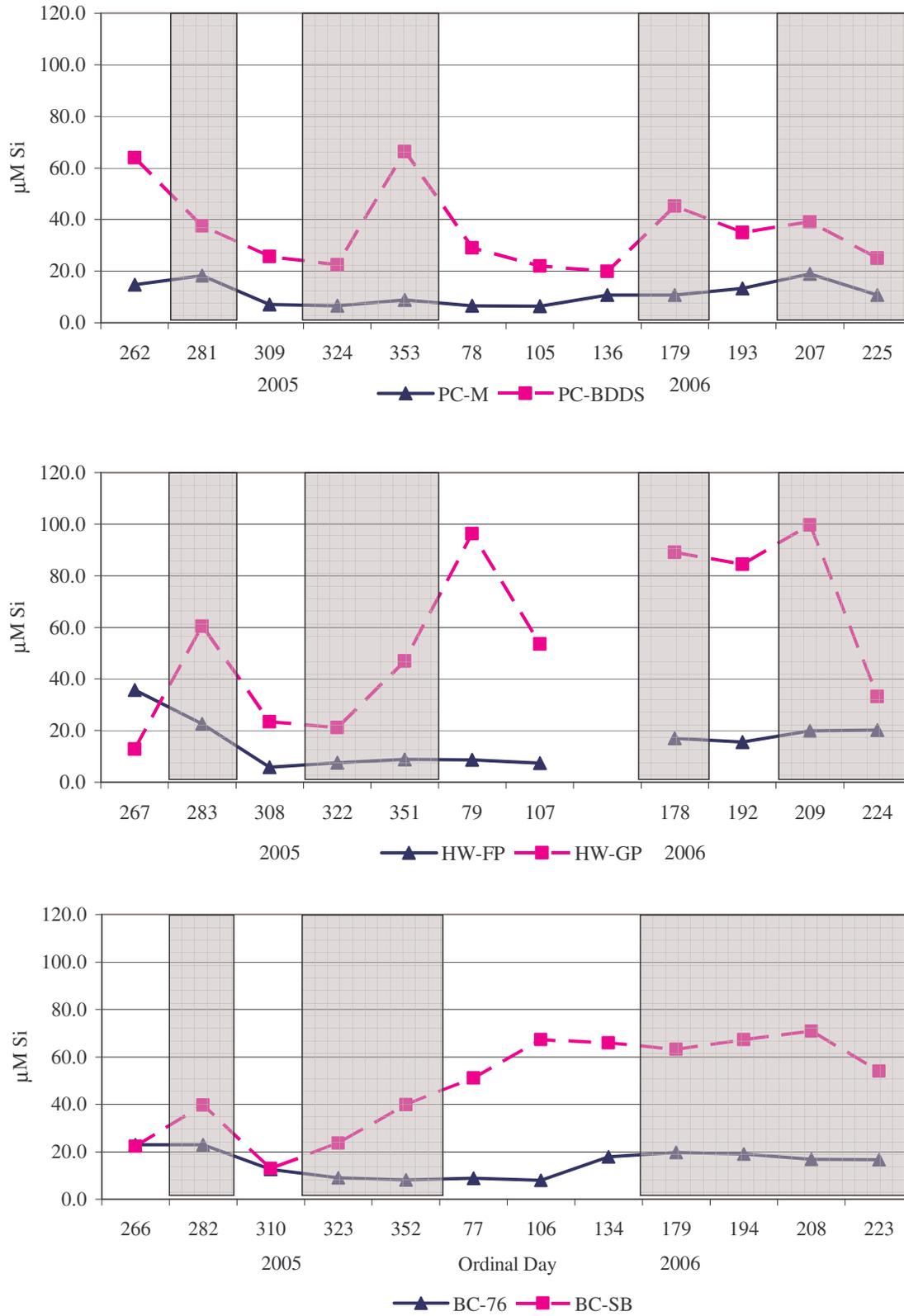


Figure 5. Mean dissolved silica concentration. Shaded areas indicate rain events.

Mean bioavailable dissolved carbohydrate concentrations were not significantly different among creeks, between creek sites, or within creeks between rain and dry periods, although mean concentrations tended to be higher upstream than downstream and they always increased after rain events (Tables 3 – 6). All sites had similar seasonal patterns with substantially higher values in June (Figure 6). Pages Creek had the highest mean concentration (147.8 $\mu$ M), with a range of 4.0 – 914.3 $\mu$ M, Howe Creek's mean was 145.9 $\mu$ M and ranged from 5.0 – 777.8 $\mu$ M, and the Bradley Creek mean was lowest at 136.0 $\mu$ M, with a range of 11.1 – 1171.4 $\mu$ M (Table 2).

Mean dissolved phosphate concentrations were remarkably similar among creeks (0.6 $\mu$ M) and all upstream (0.7 $\mu$ M) and downstream (0.4 $\mu$ M) sites (Tables 2 & 3; Figure 7). All upstream site concentrations were significantly higher than downstream (Pages Creek  $p = 0.0306$ , Howe Creek  $p = 0.0112$ , Bradley Creek  $p = 0.0213$ ). Dissolved phosphate concentrations significantly increased with rain events in PC-BDDS ( $p = 0.0006$ ), PC-M ( $p = 0.0119$ ), BC-SB ( $p = 0.0007$ ), and BC-76 ( $p = 0.0156$ ; Tables 4 & 6).

There were no significant differences in mean ammonium concentrations among creeks (Table 2), with most high values occurring during Tropical Storm Ophelia (Figure 8). The downstream sites on Pages ( $p < 0.0001$ ) and Howe ( $p = 0.0298$ ) Creeks had lower means than the upstream sites (Table 3), and rain increased ammonium concentrations within all sites, but only significantly in HW-GP ( $p = 0.0102$ ), BC-SB ( $p = 0.0030$ ), and BC-76 ( $p = 0.0022$ ; Tables 5 & 6).

Mean dissolved nitrate/nitrite concentrations were not significantly different among creeks (Table 2). All upstream sites had significantly higher concentrations than downstream (Pages Creek  $p = 0.0021$ , Howe Creek  $p = 0.0199$ , Bradley Creek  $p = 0.0032$ ; Table 3). Concentrations increased within all sites after rain events (PC-BDDS  $p = 0.0204$ , PC-M  $p = 0.0230$ , HW-GP  $p =$

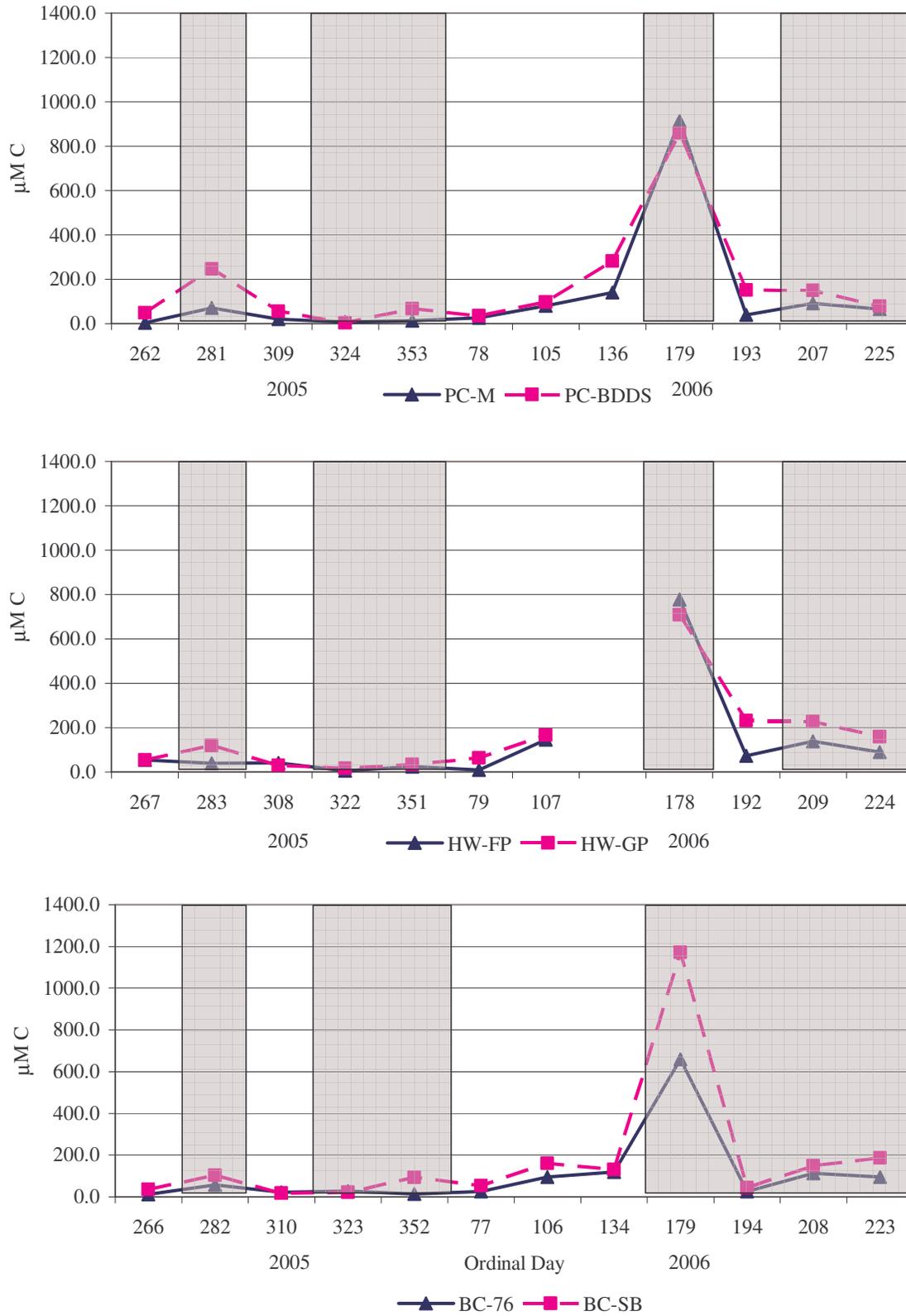


Figure 6. Mean dissolved bioavailable carbohydrate concentration. Shaded areas indicate rain events.

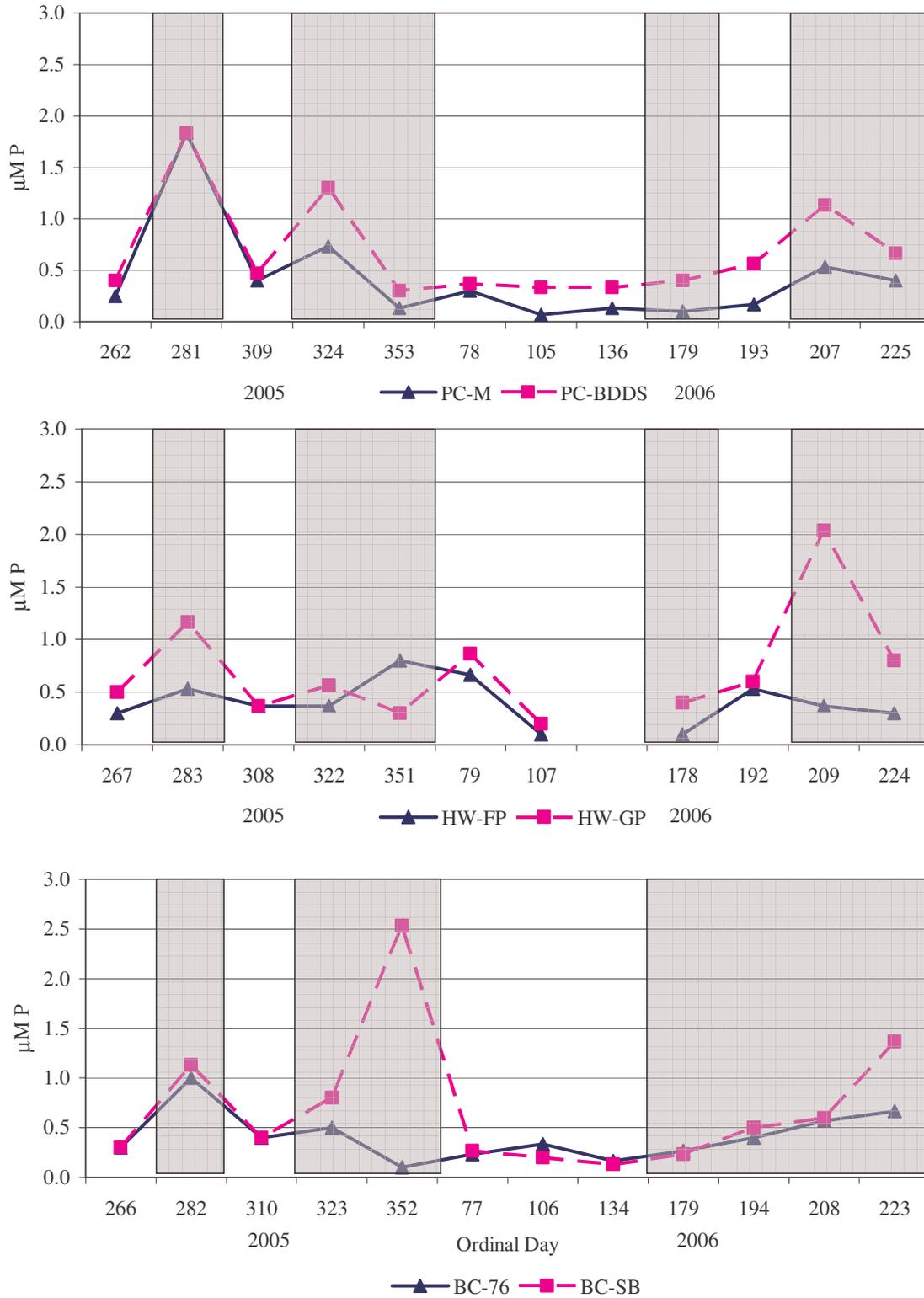


Figure 7. Mean dissolved phosphate concentration. Shaded areas indicate rain events.

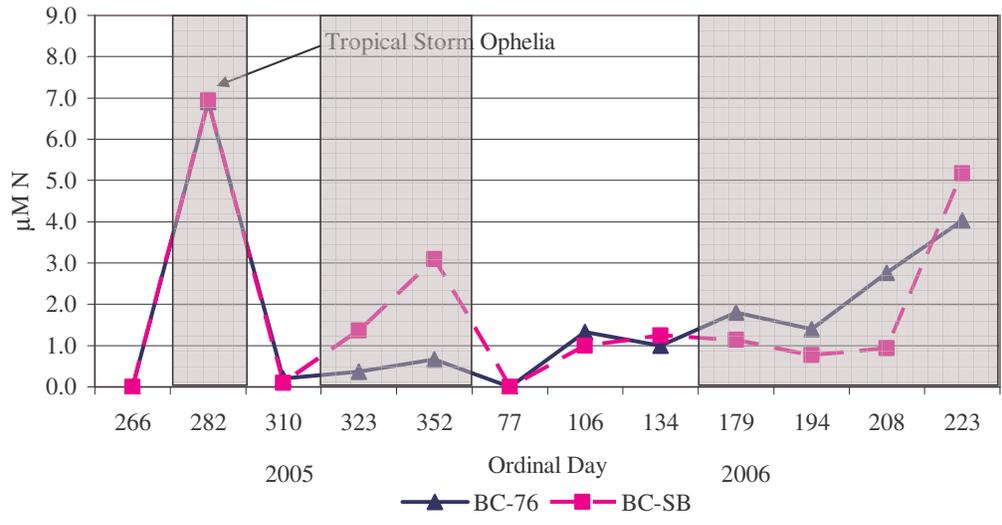
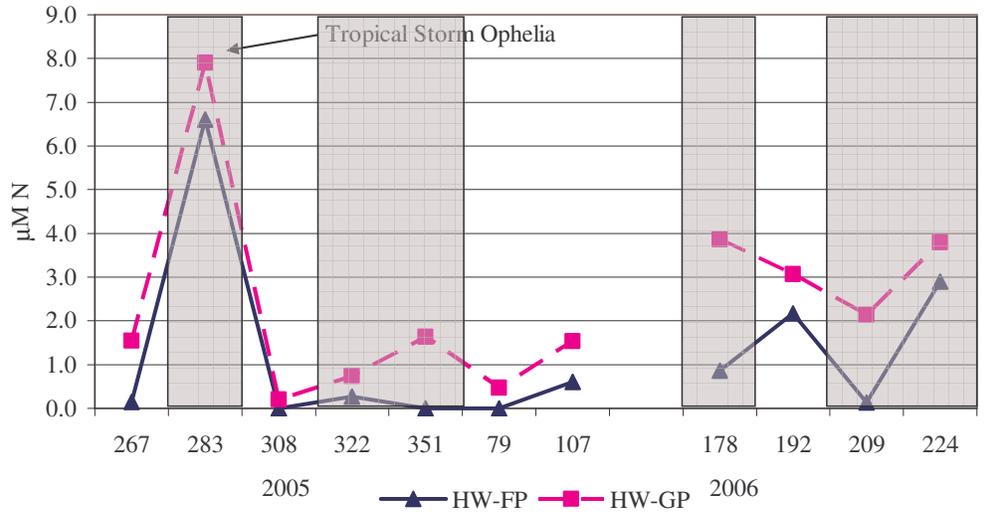
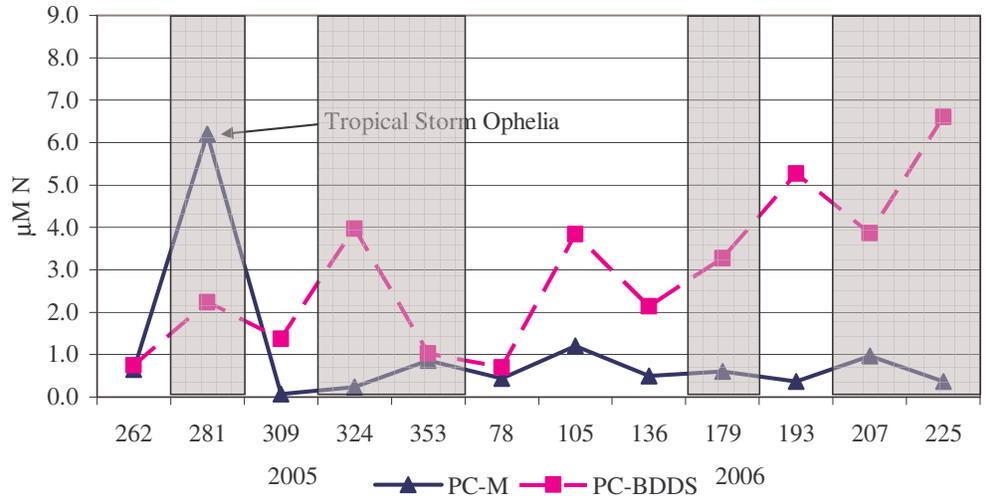


Figure 8. Mean ammonium concentration. Shaded areas indicate rain events.

0.0201, HW-FP  $p = 0.0029$ , BC-SB  $p < 0.0001$ , BC-76  $p = 0.0018$ ; Tables 4-6; Figure 9).

Mean N:P ratios were similar among creeks, but there were no consistent trends spatially or with rain events (Table 2). Site PC-BDDS had a mean N:P ratio of 8.9, PC-M was 8.5, HW-GP was only 6.4, while HW-FP was 9.3, and BC-SB was significantly higher at 9.8 than BC-76 at 6.0 ( $p = 0.0334$ ; Table 3).

#### Heterotrophic Production

Mean daily heterotrophic production was similar among creeks, between sites in Howe Creek only, and between rain and dry periods (Tables 2 – 6). Seasonally, high and low values were variable between sites, but tended to be low in the fall and higher in the spring and summer months (Figure 10). Production was significantly higher upstream in Pages ( $p = 0.0352$ ) and Bradley Creeks ( $p = 0.0151$ ).

#### Autotrophic Production

There were no significant differences in mean daily autotrophic production among creeks, although the Pages Creek mean was the lowest at  $395.4 \text{ mg C m}^{-3} \text{ day}^{-1}$ , and Howe and Bradley Creek means were  $636.7$  and  $547.2 \text{ mg C m}^{-3} \text{ day}^{-1}$ , respectively (Table 2). Spatially, production in Pages Creek was not different between sites, but was significantly higher upstream in Howe ( $p = 0.0019$ ) and Bradley Creeks ( $p = 0.0004$ ; Table 3). Seasonally, production was highest in July and lowest in December, except sites PC-BDDS and HW-FP, which had lowest production rates in October (Figure 11). Autotrophic production was not affected by rain except in site PC-BDDS, where it was significantly lower than dry periods ( $p = 0.0412$ ; Tables 4 - 6).

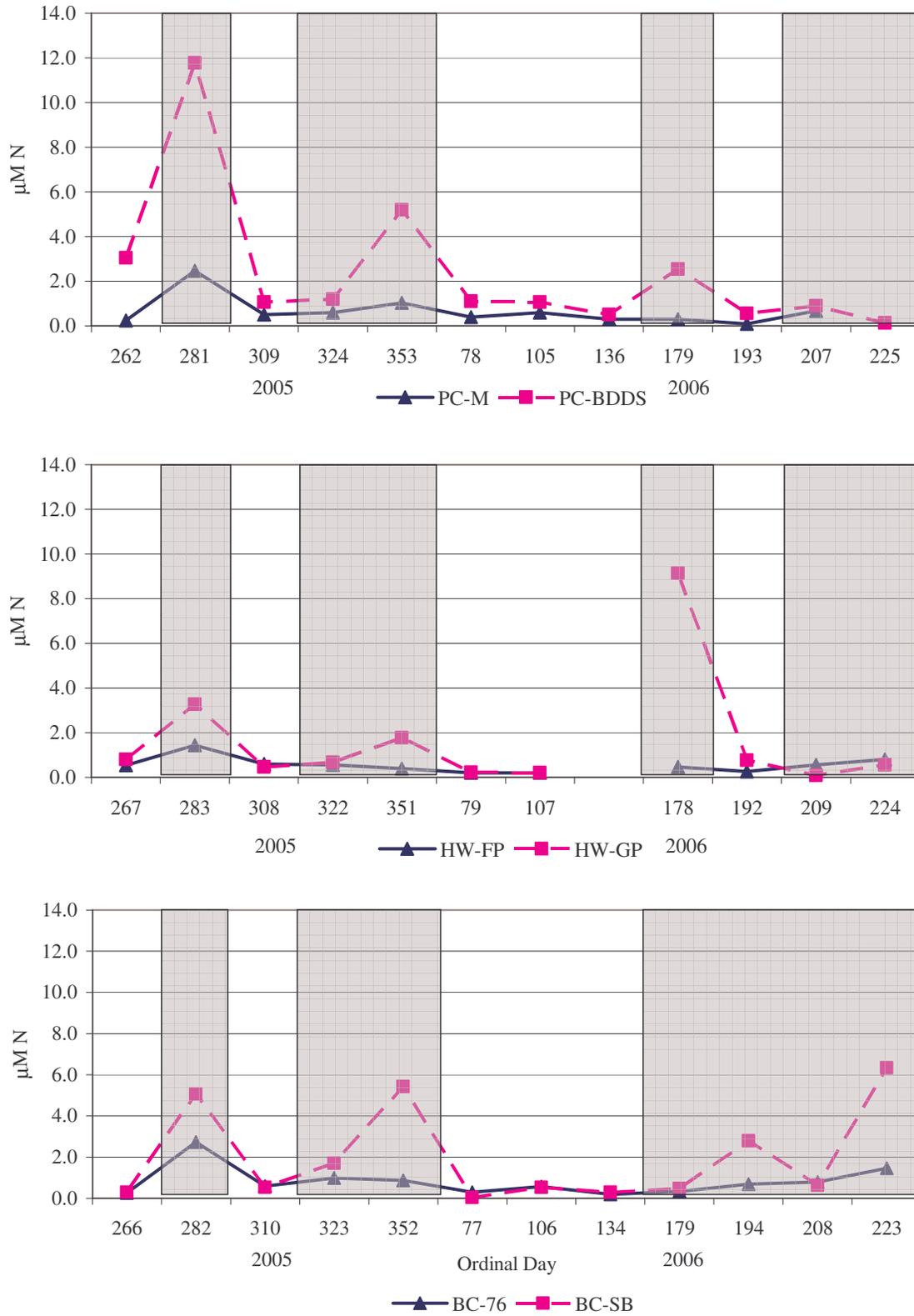


Figure 9. Mean dissolved nitrate/nitrite concentration. Shaded areas indicate rain events.

Figure 10. Mean daily heterotrophic production. Shaded areas indicate rain events.

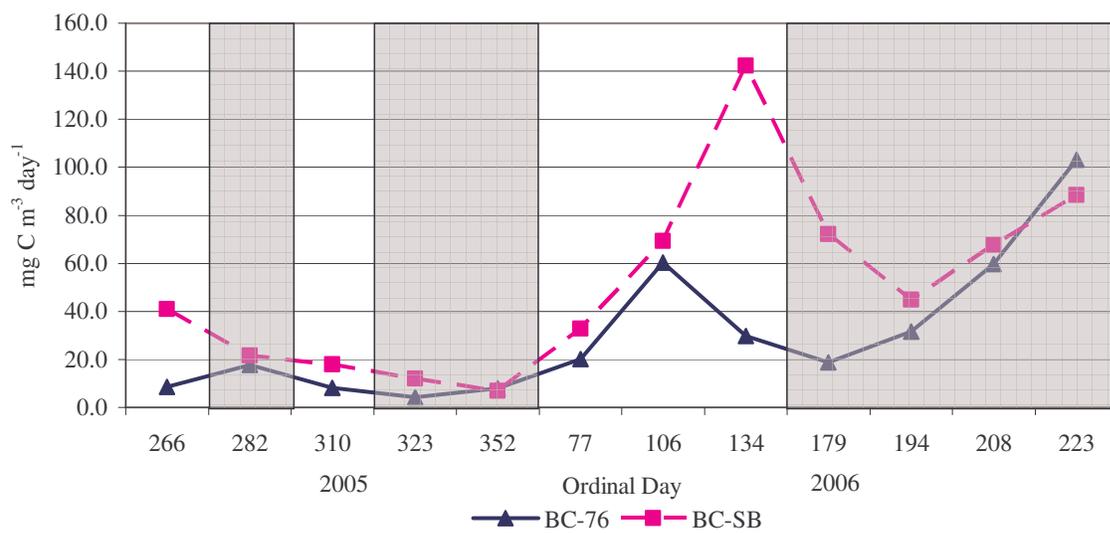
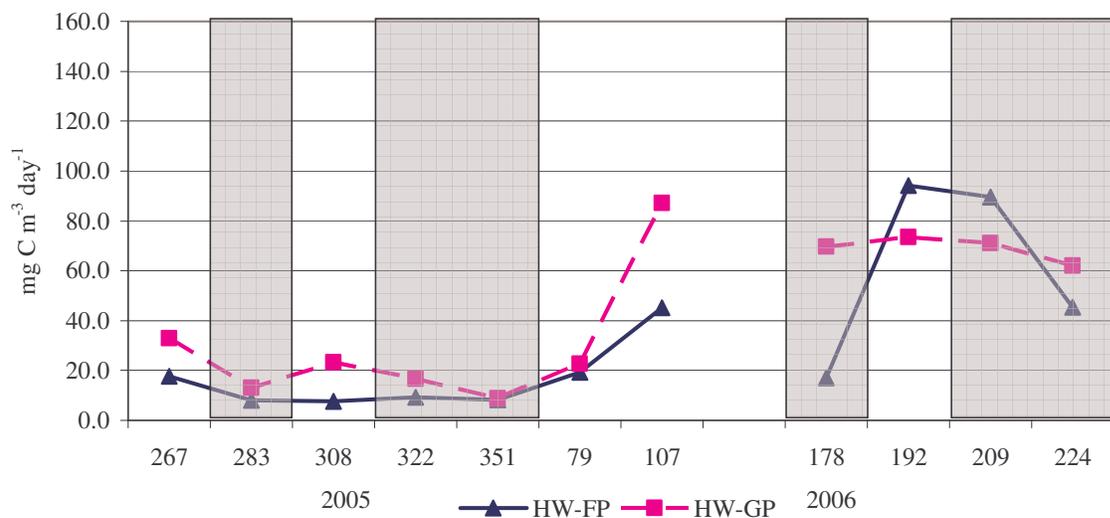
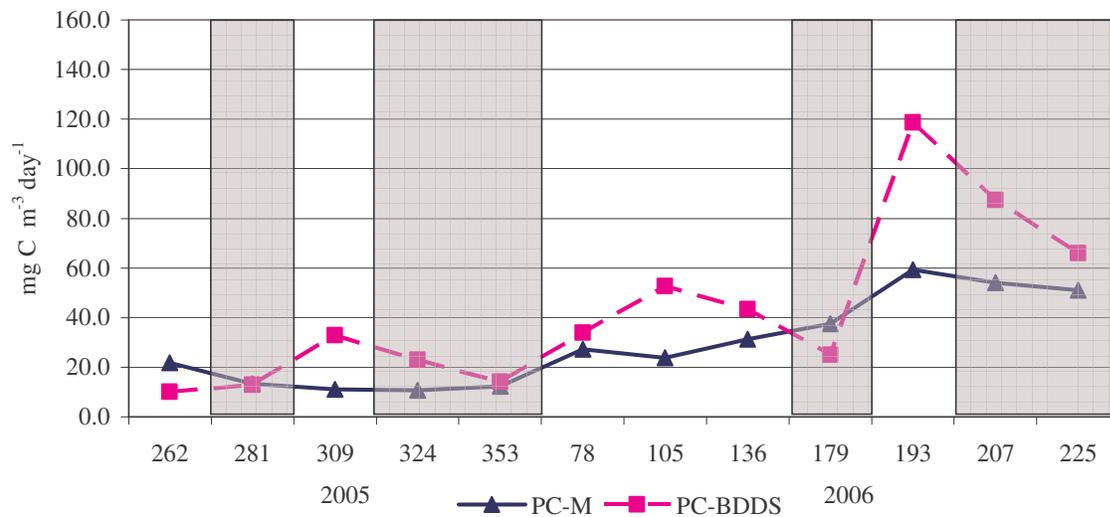
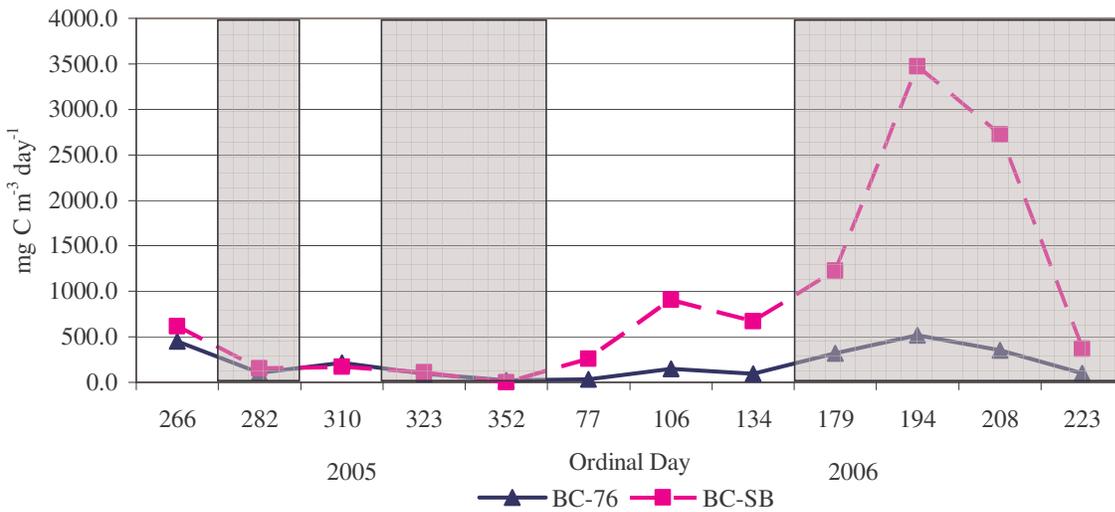
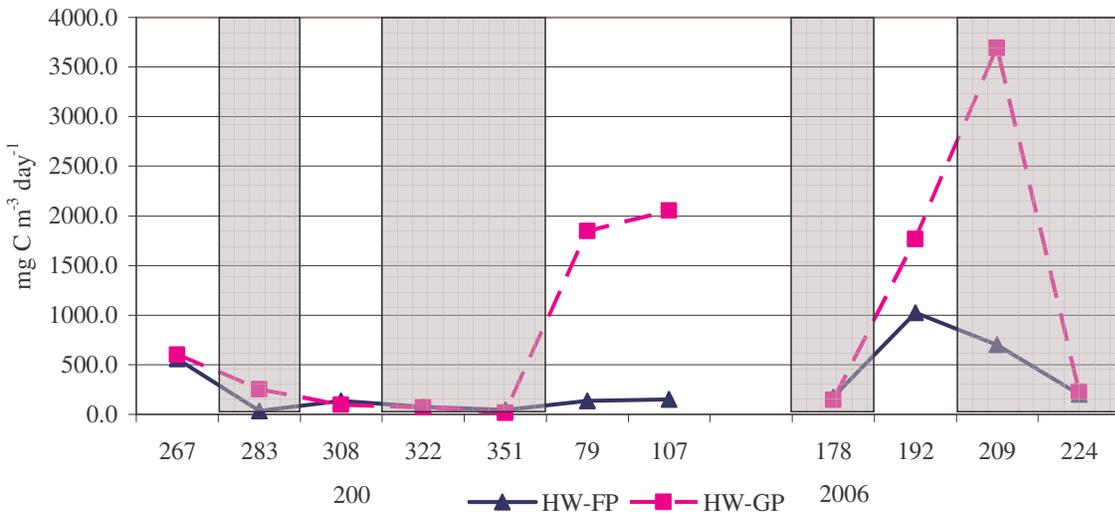
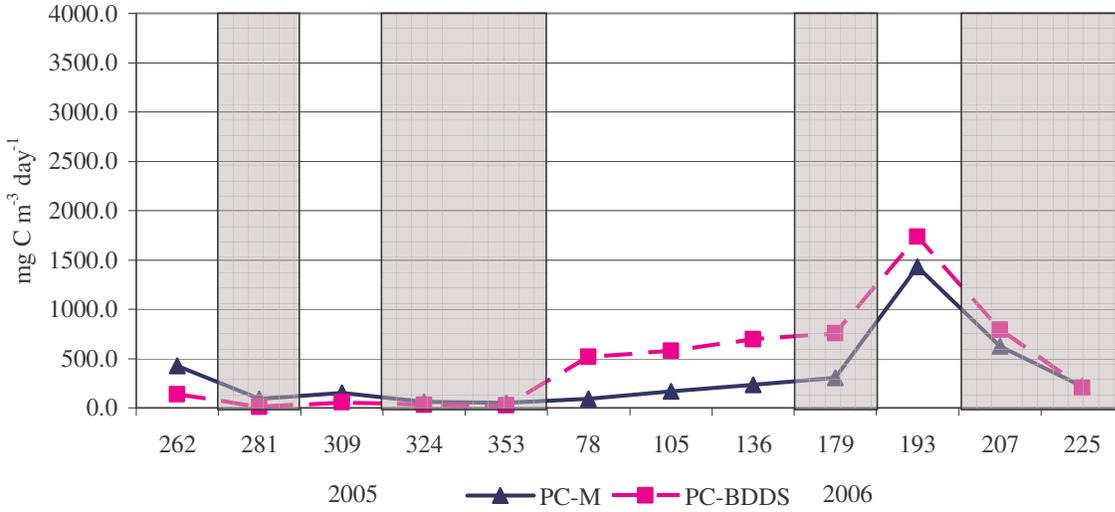


Figure 11. Mean daily autotrophic production. Shaded areas indicate rain events.



## Pairwise Correlations

A pairwise correlation found each of the nutrients to correlate with rainfall at different times in each of the creeks, except dissolved silica, which did not correlate with rainfall in any of the creeks (Tables 7, 8, & 9). Daily heterotrophic production was correlated with physical parameters in Pages Creek and a combination of physical parameters and nutrient concentrations in the other two creeks. Physical parameters also played a role in daily autotrophic production, in all creeks, but there were differences in the nutrient correlations in Howe and Bradley Creeks.

## Principal Components Analysis & Regression

Seven physical and chemical water quality parameters and the amount of rain over a 72 hour period used in a principle components analysis resulted in 4 significant components, which encompass 84% of the variability within the creeks (Table 10). The first 4 components were chosen since they group the variables into recognizable units affecting creek processes. The first 4 components were used in a factor rotation to boost the signal and significant variables to each component were considered to have a value  $\geq \pm 0.5$  (Table 11). Component 1 represents spatial variability, dominated by dissolved silica concentration and negative salinity. Component 2 represents influences of runoff, due to the sum of the rain over a 72 hour period, dissolved nitrate/nitrite and phosphate concentration, and weakly to ammonium concentration. Component 3 represents temporal variation with temperature and is weakly associated with ammonium concentration. Component 4 is dominated by dissolved carbohydrate concentration.

Each component was plotted against heterotrophic and autotrophic production of each site and the sites pooled for each creek. Heterotrophic production decreased with component 1 within the PC-BDDS site ( $p = 0.0024$ ), and increased for the combined sites of Howe and Bradley Creeks (Table 12; Figure 12). Autotrophic production decreased with component 1 in

Table 7. Pages Creek correlation analysis. Data as  $r^2/p$  - value. Shaded cells indicate significance at  $\alpha < 0.0042$ . L = log

Variable	LDaily HP	LDaily AP	Temp	Salinity	LSi	LCarb	LPO <sub>4</sub>	LNH <sub>4</sub>	LNO <sub>x</sub>
LDaily AP	0.5346 <0.0001	1.0000							
Temp	0.2985 0.0109	0.5768 <0.0001	1.0000						
Salinity	0.3593 0.0019	0.5877 <0.0001	0.0392 0.7440	1.0000					
LSi	0.0243 0.8397	0.0179 0.8837	0.2515 0.0331	-0.5125 <0.0001	1.0000				
LCarb	0.1667 0.1616	0.1680 0.1677	0.3345 0.0041	-0.2140 0.0711	0.3279 0.0049	1.0000			
LPO <sub>4</sub>	-0.0519 0.6696	-0.2755 0.0219	0.1371 0.2578	-0.5068 <0.0001	0.4096 0.0004	-0.0138 0.9096	1.0000		
LNH <sub>4</sub>	0.2895 0.0151	0.1002 0.4125	0.3137 0.0082	-0.3087 0.0093	0.5263 <0.0001	0.2569 0.0318	0.5236 <0.0001	1.0000	
LNO <sub>x</sub>	0.1741 0.1495	0.2813 0.0192	0.1737 0.1504	-0.2375 0.0478	0.4433 0.0001	0.5725 <0.0001	-0.0027 0.9826	0.3206 0.0068	1.0000
L24hr ppt	-0.1405 0.2393	-0.2654 0.0275	0.3623 0.0018	-0.7565 <0.0001	0.2219 0.0610	0.3220 0.0058	0.5454 <0.0001	0.3669 0.0018	0.2649 0.0267
L48hr ppt	-0.2069 0.0812	-0.3456 0.0036	0.2272 0.0549	-0.8104 <0.0001	0.2491 0.0349	0.3603 0.0019	0.4465 0.0001	0.3275 0.0057	0.2709 0.0233
L72hr ppt	-0.1510 0.2055	-0.3630 0.0022	0.2657 0.0241	-0.7543 <0.0001	0.2324 0.0495	0.3475 0.0028	0.4988 <0.0001	0.3752 0.0014	0.1436 0.2357

Table 8. Howe Creek correlation analysis. Data as  $r^2/p$  - value. Shaded cells indicate significance at  $\alpha < 0.0042$ .  $L = \log$

Variable	LDaily HP	LDaily AP	Temp	Salinity	LSi	LCarb	LPO <sub>4</sub>	LNH <sub>4</sub>	LNO <sub>x</sub>
LDaily AP	0.6433 <0.0001	1.0000							
Temp	0.5240 <0.0001	0.5217 <0.0001	1.0000						
Salinity	-0.1901 0.1263	-0.3586 0.0031	0.0130 0.9175	1.0000					
LSi	0.3759 0.0020	0.4677 0.0001	0.2473 0.0470	-0.8357 <0.0001	1.0000				
LCarb	0.4502 0.0001	0.3798 0.0017	0.5590 <0.0001	-0.3951 0.0010	0.4747 0.0001	1.0000			
LPO <sub>4</sub>	0.0788 0.5395	0.2316 0.0678	0.0539 0.6750	-0.3907 0.0015	0.3858 0.0018	-0.0939 0.4644	1.0000		
LNH <sub>4</sub>	0.2222 0.0752	0.0690 0.5849	0.4631 0.0001	-0.4278 0.0004	0.5387 <0.0001	0.4110 0.0007	0.3122 0.0127	1.0000	
LNO <sub>x</sub>	0.5824 <0.0001	0.2452 0.0508	0.4885 <0.0001	-0.2813 0.0243	0.4435 0.0002	0.6128 <0.0001	-0.0983 0.4436	0.4872 <0.0001	1.0000
L24hr ppt	0.2022 0.1034	0.0533 0.6706	0.2915 0.0176	-0.2003 0.1069	0.2024 0.1059	0.4866 <0.0001	-0.1012 0.4300	0.0520 0.6809	0.4880 <0.0001
L48hr ppt	0.1044 0.4043	-0.2792 0.0232	0.0495 0.6932	0.0118 0.9252	0.1027 0.4155	0.3051 0.0127	-0.0421 0.7430	0.1717 0.1715	0.4371 0.0003
L72hr ppt	-0.1452 0.2446	-0.2498 0.0431	0.2657 0.0311	-0.2234 0.0714	0.2442 0.0499	0.2549 0.0389	0.2942 0.0193	0.6531 <0.0001	0.2353 0.0612

Table 9. Bradley Creek correlation analysis. Data as  $r^2/p$  - value. Shaded cells indicate significance at  $\alpha < 0.0042$ . L = log

Variable	LDaily HP	LDaily AP	Temp	Salinity	LSi	LCarb	LPO <sub>4</sub>	LNH <sub>4</sub>	LNO <sub>x</sub>
LDaily AP	0.5725 <0.0001	1.0000							
Temp	0.5152 <0.0001	<0.0001	1.0000						
Salinity	-0.1752 0.1411	-0.0955 0.4250	0.0597 0.6186	1.0000					
LSi	0.4232 0.0002	0.4963 <0.0001	0.2702 0.0217	-0.8263 <0.0001	1.0000				
LCarb	0.4041 0.0005	0.1565 0.1924	0.2056 0.0854	-0.4249 0.0002	0.4461 0.0001	1.0000			
LPO <sub>4</sub>	-0.1510 0.2156	-0.2193 0.0703	0.1571 0.1974	-0.2911 0.0152	0.1744 0.1519	0.0441 0.7210	1.0000		
LNH <sub>4</sub>	0.1737 0.1565	-0.1303 0.2895	0.3603 0.0025	-0.2748 0.0233	0.2251 0.0649	0.3603 0.0027	0.6042 <0.0001	1.0000	
LNO <sub>x</sub>	0.5052 <0.0001	0.2701 0.0237	0.3956 0.0007	-0.2718 0.0229	0.3907 0.0008	0.3957 0.0008	0.3252 0.0064	0.5890 <0.0001	1.0000
L24hr ppt	0.0022 0.9853	-0.3009 0.0102	-0.0676 0.5728	-0.1470 0.2180	0.0403 0.7370	-0.0326 0.7874	0.3246 0.0065	0.3619 0.0024	0.4745 <0.0001
L48hr ppt	-0.0057 0.9623	-0.1104 0.3557	0.3270 0.0050	-0.2866 0.0147	0.1725 0.1472	0.2118 0.0762	0.5485 <0.0001	0.8229 <0.0001	0.3255 0.0060
L72hr ppt	0.0193 0.8724	-0.1470 0.2178	0.3267 0.0051	-0.2901 0.0134	0.1789 0.1326	0.2926 0.0133	0.5381 <0.0001	0.8489 <0.0001	0.3672 0.0018

Table 10. EigenValues and percentages of the principal components analysis.

Component	EigenValue	Percent	Cumulative Percent
1	3.2451	40.564	40.564
2	1.3862	17.328	57.892
3	1.3178	16.472	74.364
4	0.7535	9.419	83.783
5	0.4960	6.200	89.983
6	0.4271	5.338	95.321
7	0.3082	3.852	99.174
8	0.0661	0.826	100.000

Table 11. Rotated factor pattern. Shaded cells indicate variables comprising each component.

Variable	Component 1	Component 2	Component 3	Component 4
Salinity	-0.842753	-0.454525	0.075881	-0.042724
Temperature	0.030287	0.012958	0.898009	0.229996
Ammonium	0.095640	0.593483	0.617522	-0.143777
Carbohydrate	0.101421	0.003372	0.153136	0.942938
Phosphate	0.315116	0.698815	0.064879	-0.252523
Silica	0.969818	0.034945	0.133280	0.091972
Nitrate/Nitrite	0.263526	0.806189	-0.127531	0.169602
72hr ppt	-0.018956	0.873047	0.280061	0.050397

Table 12. Regression results for the effects of each component on heterotrophic and autotrophic production. The top number indicates the  $r^2$  value, the next number is the p – value, and the bottom number indicates the slope. Shaded cells indicate a significant effect given  $\alpha < 0.05$ .

Component	Production	Pages Creek	Howe Creek	Bradley Creek
1 Spatial	Heterotrophic	0.038	0.106	0.103
		0.1065	0.0093	0.0086
	Autotrophic	-0.13	0.11	0.18
		0.055	0.320	0.164
		0.0527	<0.0001	0.0007
		-0.25	0.29	0.28
2 Runoff	Heterotrophic	0.077	0.091	0.076
		0.0204	0.0162	0.0251
	Autotrophic	-0.10	-0.21	-0.13
		0.337	0.215	0.234
		<0.0001	<0.0001	<0.0001
		-0.30	-0.47	-0.31
3 Temporal	Heterotrophic	0.227	0.180	0.281
		<0.0001	0.0005	<0.0001
	Autotrophic	0.19	0.17	0.28
		0.469	0.209	0.292
		<0.0001	0.0002	<0.0001
		0.41	0.28	0.39
4 Dissolved Carbohydrate	Heterotrophic	0.002	0.004	0.079
		0.6983	0.6368	0.0223
	Autotrophic	-0.02	0.03	0.16
		0.002	0.002	0.105
		0.7462	0.7158	0.0080
		0.02	0.03	0.25

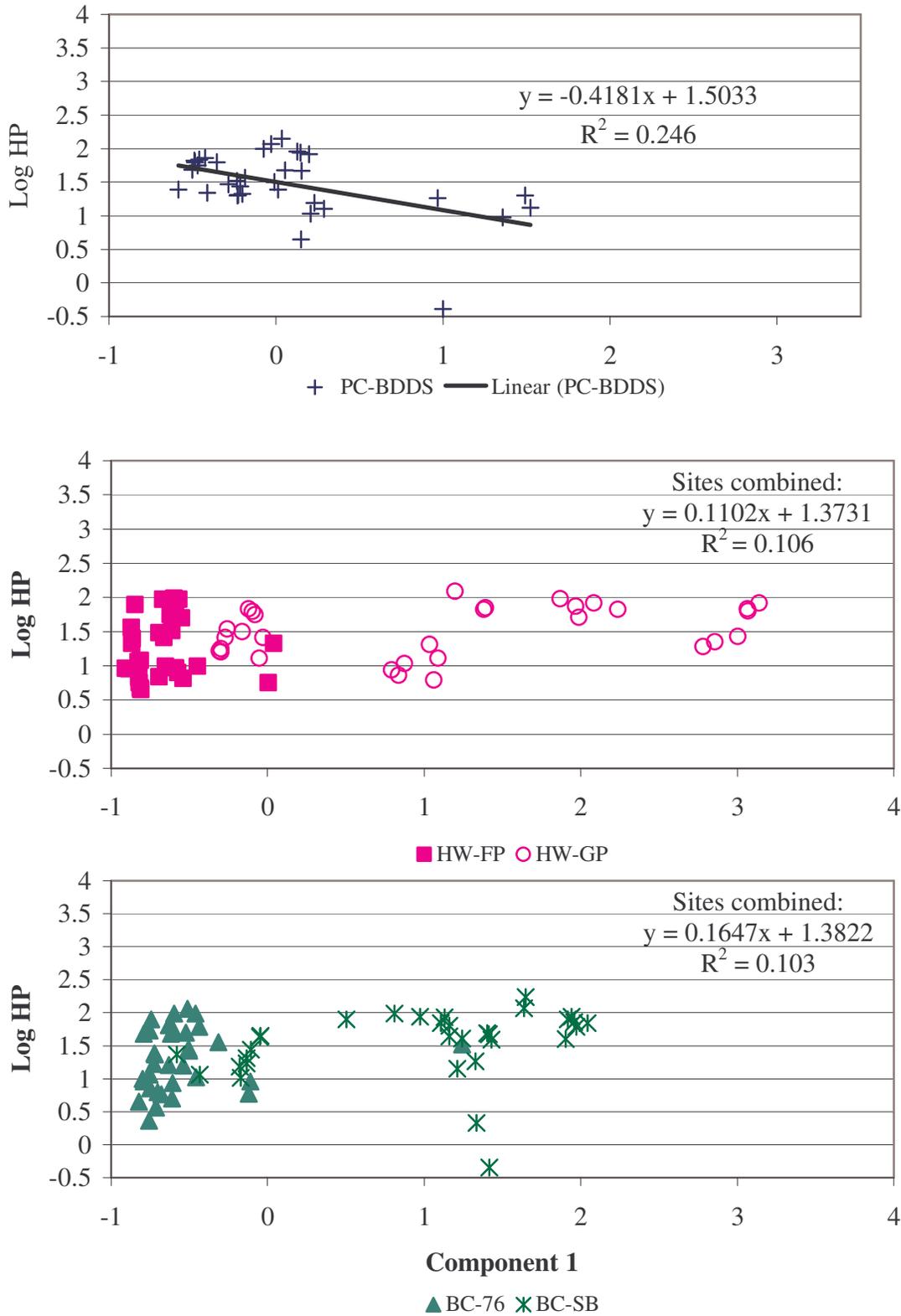


Figure 12. Regressions of log daily heterotrophic production (HP) versus component 1.

site PC-BDDS ( $p = 0.0118$ ) and increased with component 1 in Howe (HW-FP:  $r^2 = 0.126$ ,  $p = 0.0465$ ; HW-GP:  $r^2 = 0.409$ ,  $p = 0.0001$ ) and Bradley Creeks (BC-76:  $r^2 = 0.322$ ,  $p = 0.0005$ ; Table 12; Figure 13). Heterotrophic and autotrophic production decreased with component 2 in each of the creeks (Table 12; Figures 14 and 15). Heterotrophic production only significantly decreased at the individual sites PC-M ( $p = 0.0157$ ), HW-FP ( $p = 0.0335$ ), and BC-SB ( $p = 0.0027$ ). Autotrophic production decreased at all sites except BC-76; the most significant of those were the upstream sites PC-BDDS ( $r^2 = 0.436$ ,  $p < 0.0001$ ) and BC-SB ( $p < 0.0001$ ), and the downstream site HW-FP ( $r^2 = 0.501$ ,  $p < 0.0001$ ). Heterotrophic and Autotrophic production increased with component 3 by site and creek (Table 12; Figures 16 and 17). By creek, heterotrophic and autotrophic production only increased with component 4 in Bradley Creek (Table 12; Figure 18 and 19). By site, autotrophic production was not significant, but heterotrophic production showed a slight increase in sites HW-GP ( $p = 0.0541$ ) and BC-SB ( $p = 0.0328$ ).

Multiple regression was used to see if the principal components could be used to predict heterotrophic or autotrophic production. Components 1 and 3 predicted a positive response and component 2 predicted a negative response in the heterotrophic model and components 1, 3, and 4 predicted a positive response and component 2 predicted a negative response in the autotrophic model (Table 13).

## Experimental Results

Within sites, there were no significant differences in heterotrophic production among treatments for PC-M or BC-76, but the NP treatment was significantly higher than all other treatments within HW-FP ( $p = 0.0014$ ; Figure 20a). The NP treatment also led to higher autotrophic production in site PC-M compared to the control ( $p = 0.0041$ ) and treatment NPC

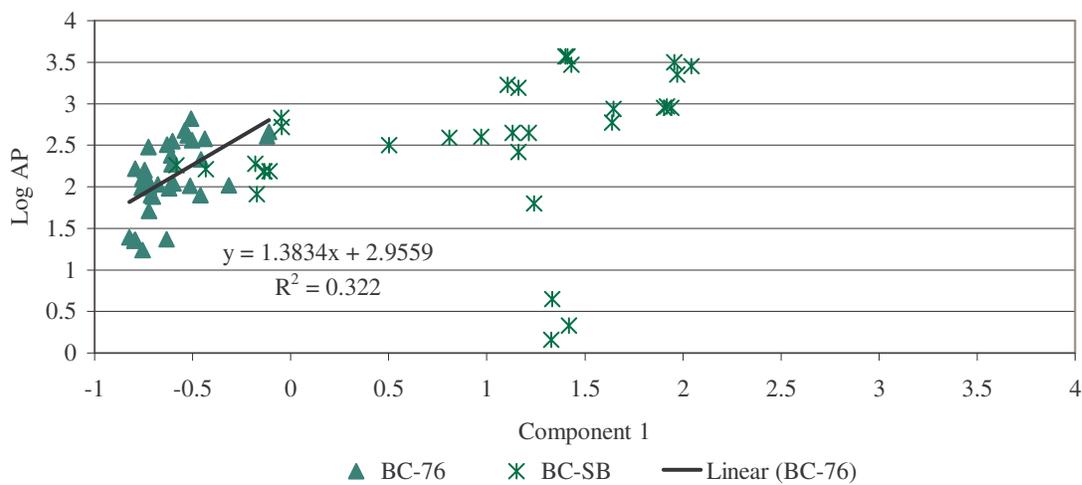
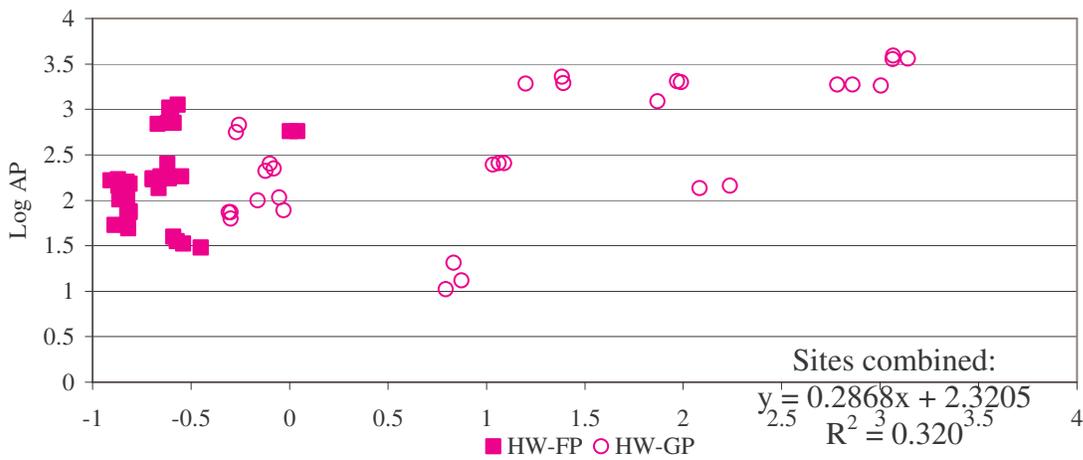
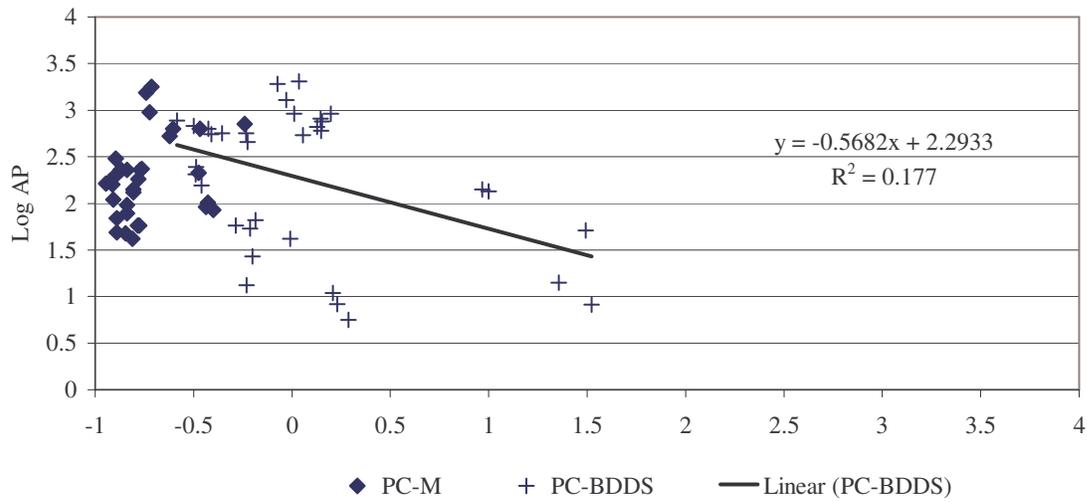


Figure 13. Regressions of log daily autotrophic production (AP) versus component 1.

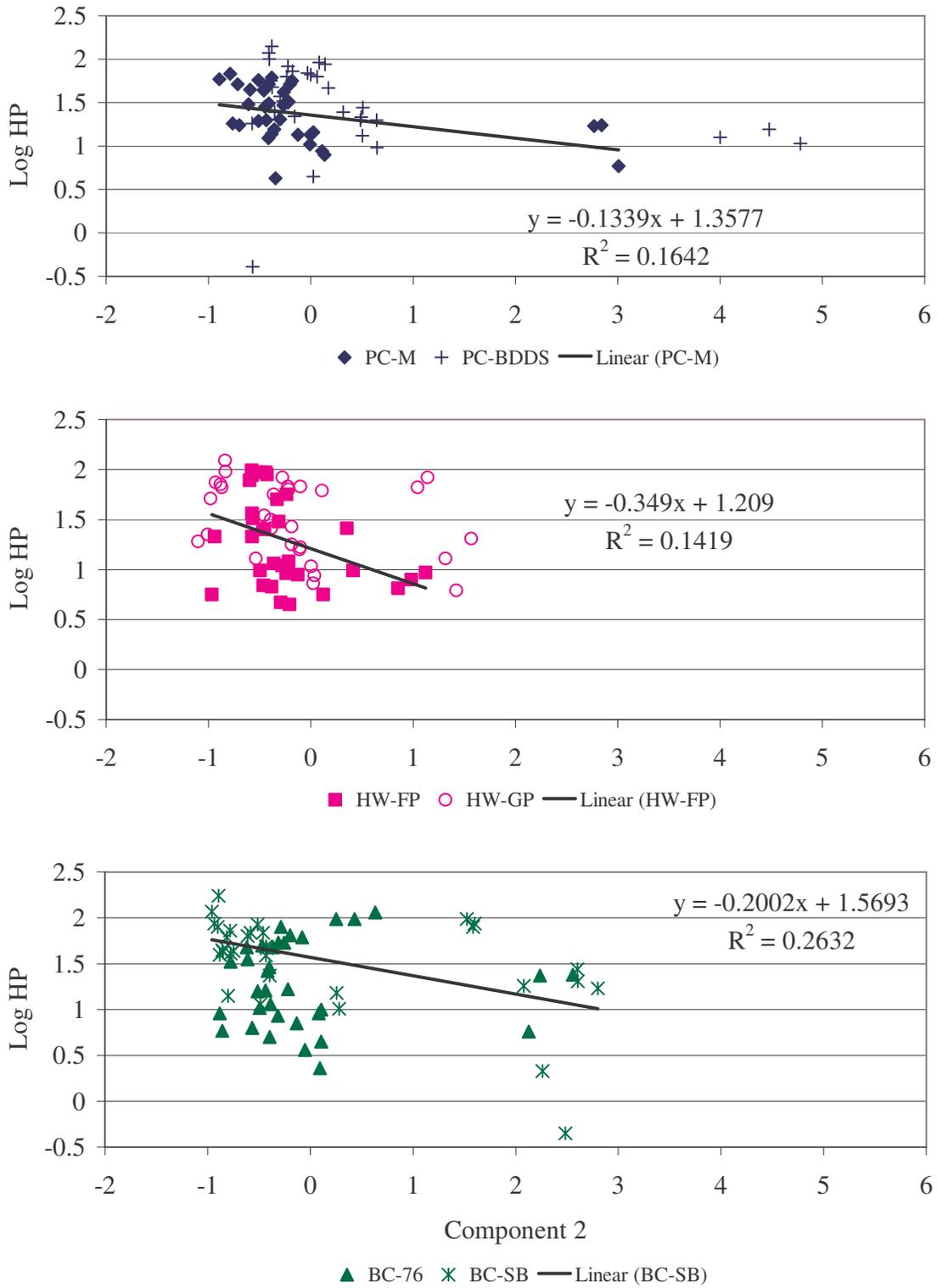


Figure 14. Regressions of log daily heterotrophic production (HP) versus component 2.

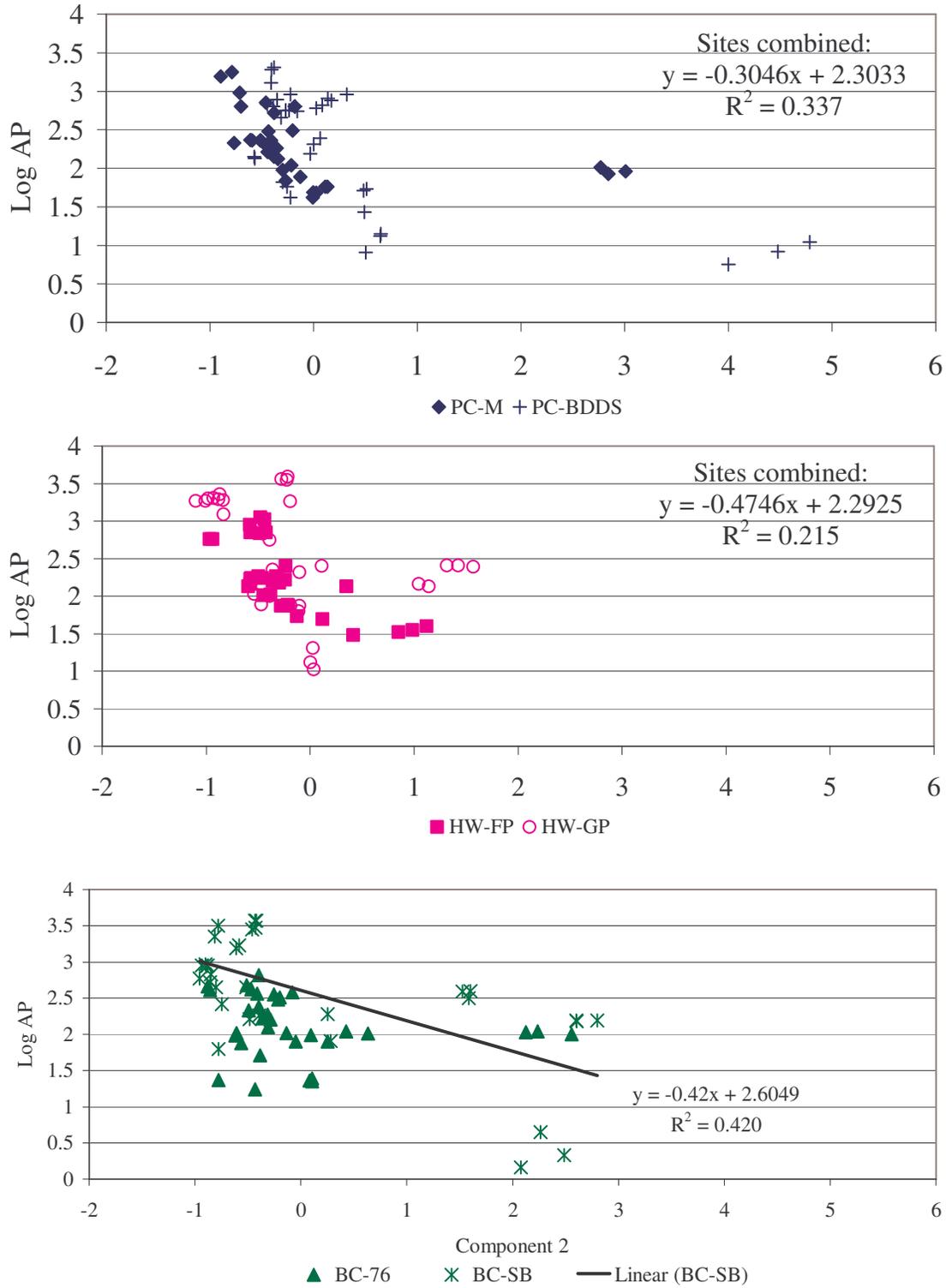


Figure 15. Regressions of log daily autotrophic production (AP) versus component 2.

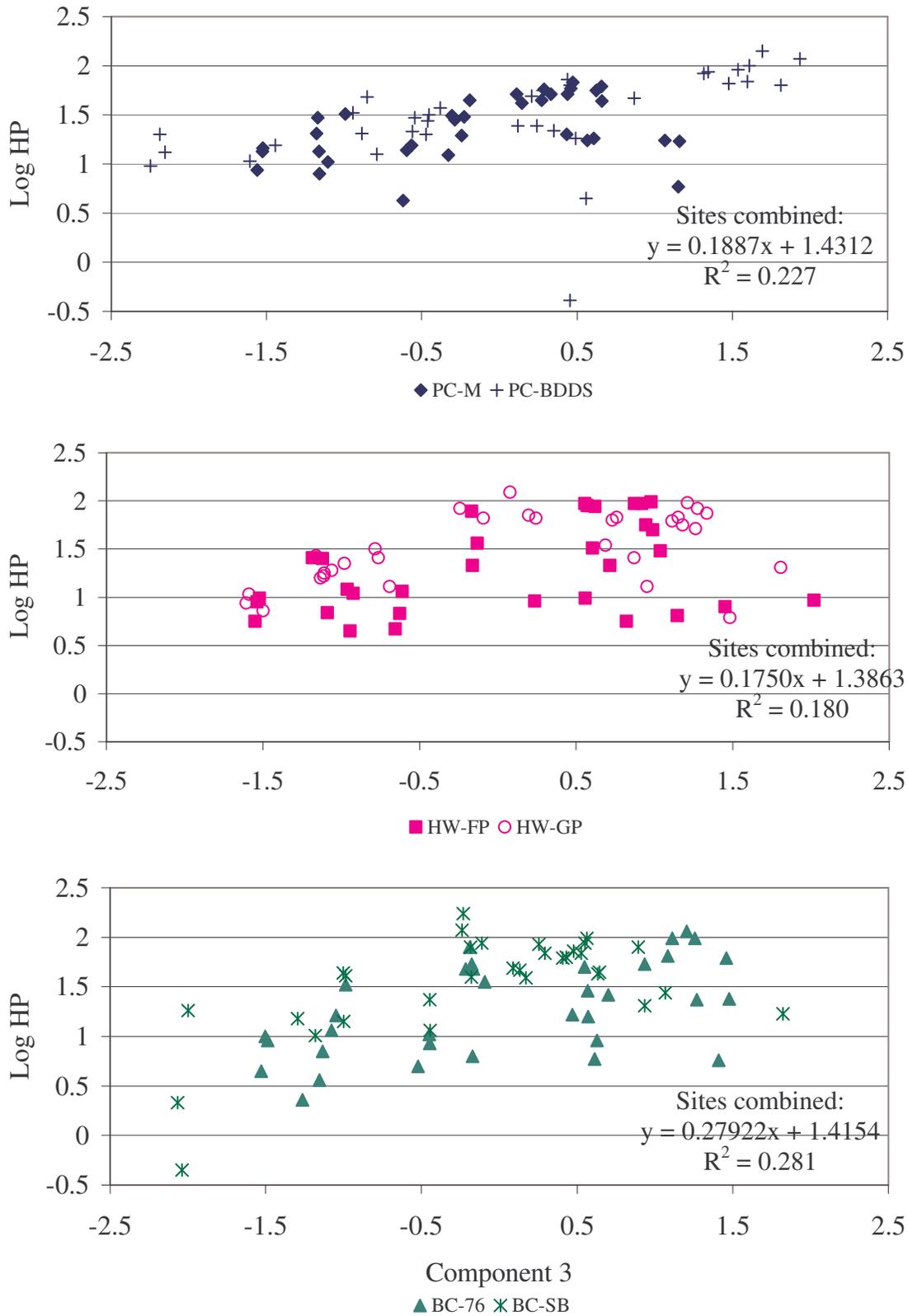


Figure 16. Regressions of log daily heterotrophic production (HP) versus component 3.

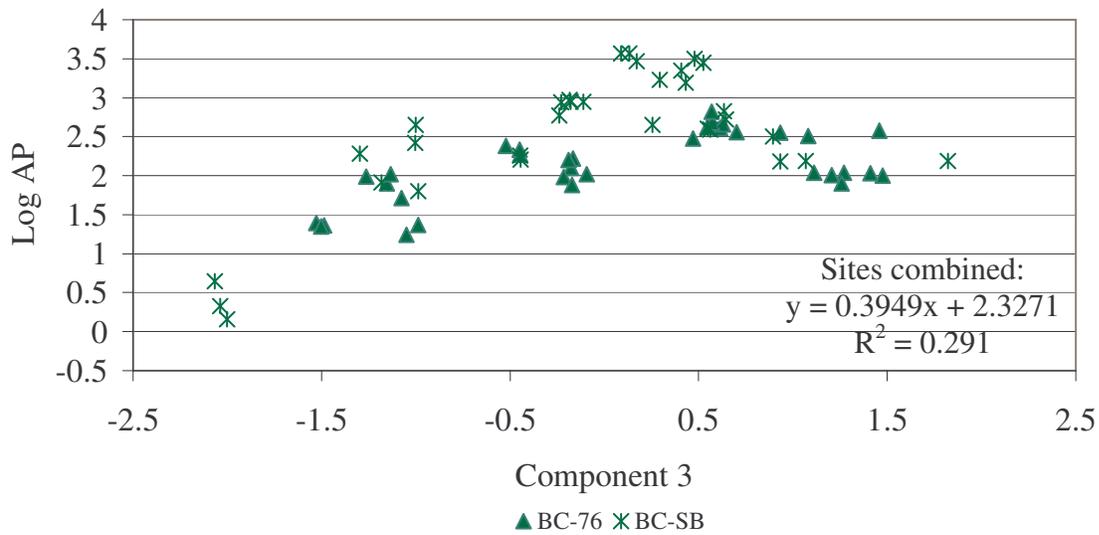
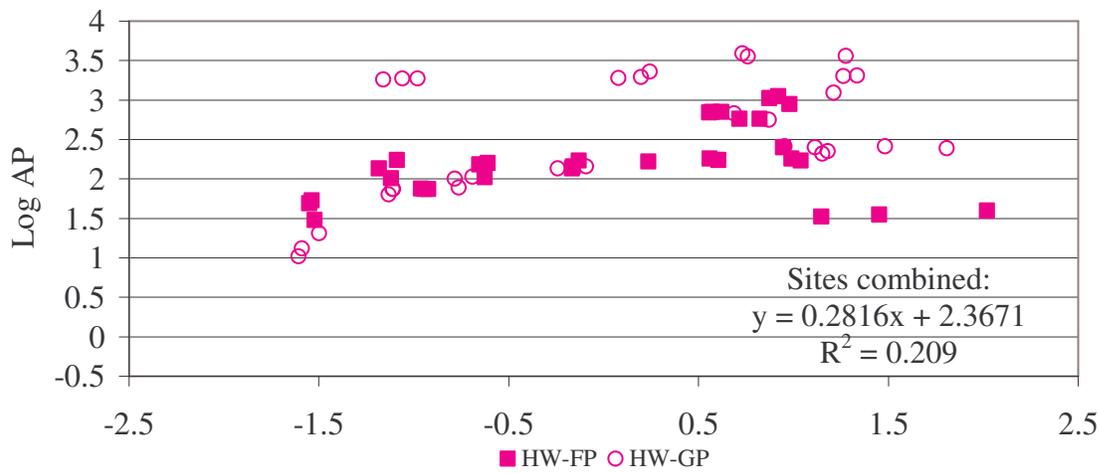
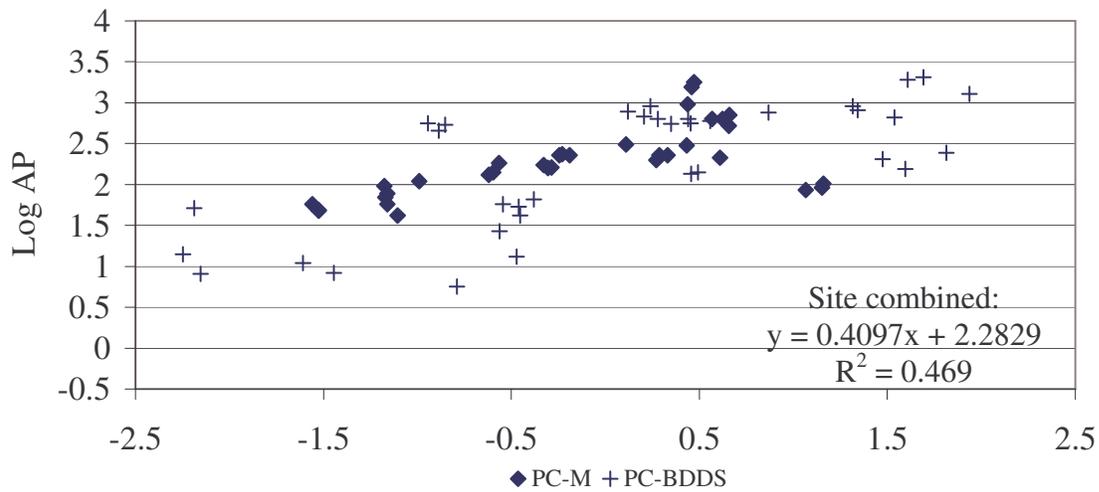


Figure 17. Regressions of log daily autotrophic production (AP) versus component 3. Experimental Results

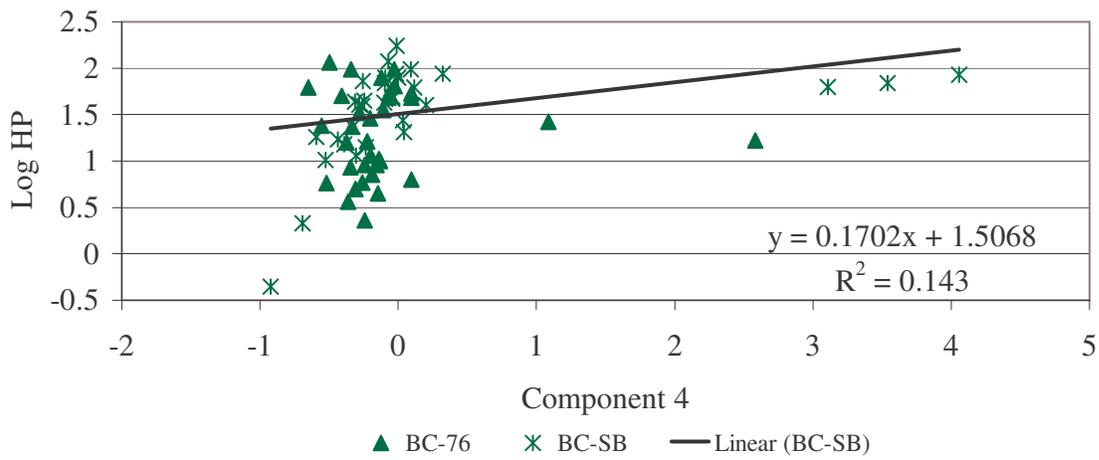
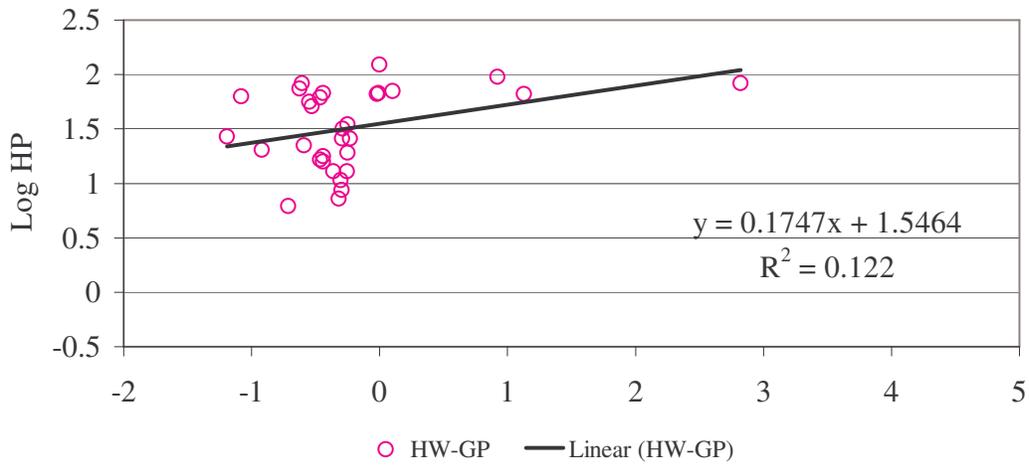


Figure 18. Regressions of log daily heterotrophic production (HP) versus component 4.

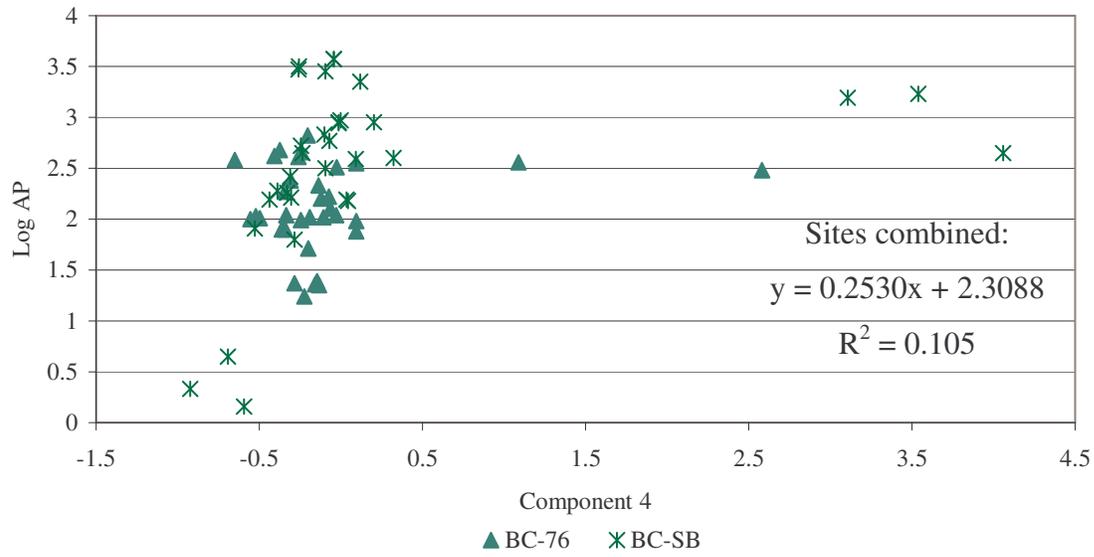


Figure 19. Regressions of log daily autotrophic production (AP) versus component 4.

Table 13. Multiple regression results for (a) heterotrophic and (b) autotrophic production ( $\alpha = 0.05$ ).

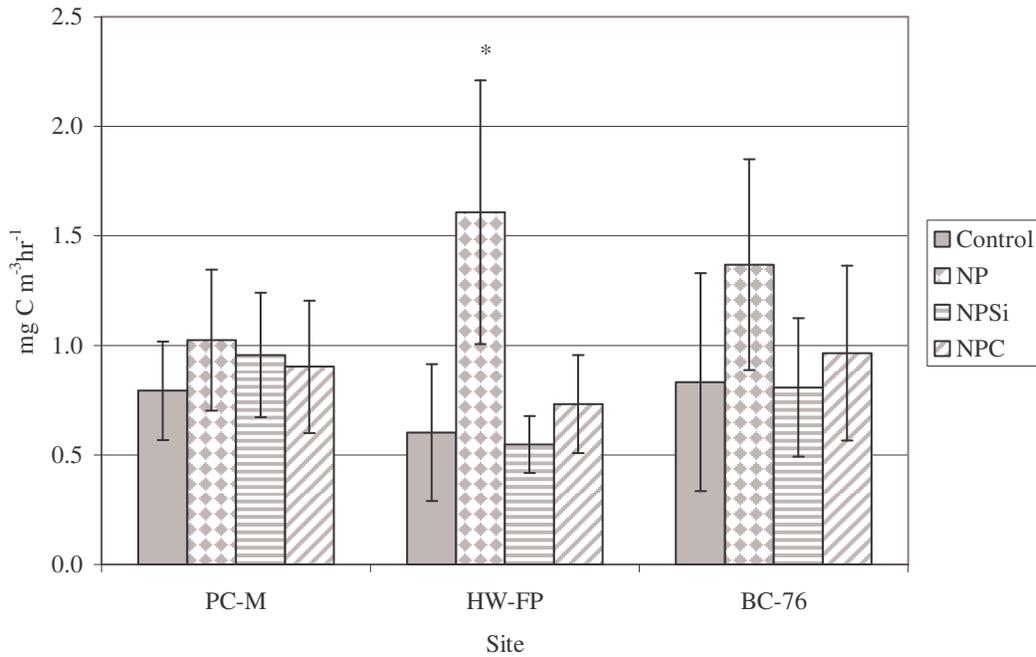
(a) Heterotrophic Production

Source	df	SS	MS	F	p - value
Model $r^2 = 0.34$	4	13.405955	3.35149	25.2549	<0.0001
Component 1 (Spatial)	1	1.5284245	1.5284245	11.5173	0.0008
Component 2 (Runoff)	1	2.7862494	2.7862494	20.9955	<0.0001
Component 3 (Temporal)	1	8.7694926	8.7694926	66.0818	<0.0001
Component 4 (Dissolved Carbohydrate)	1	0.3015577	0.3015577	2.2724	0.1333
Error	193	25.612395	13.271		
Total	197	39.018349			

(b) Autotrophic Production

Source	df	SS	MS	F	p - value
Model $r^2 = 0.69$	4	57.590728	14.3977	108.778	<0.0001
Component 1 (Spatial)	1	9.012770	9.012770	68.0941	<0.0001
Component 2 (Runoff)	1	21.411767	21.41176	161.772	<0.0001
Component 3 (Temporal)	1	26.211862	26.21186	198.038	<0.0001
Component 4 (Dissolved Carbohydrate)	1	0.850226	0.850226	6.4237	0.0121
Error	19	25.545003	0.1324		
Total	7	83.135732			

(a) Heterotrophic Production



(b) Autotrophic Production

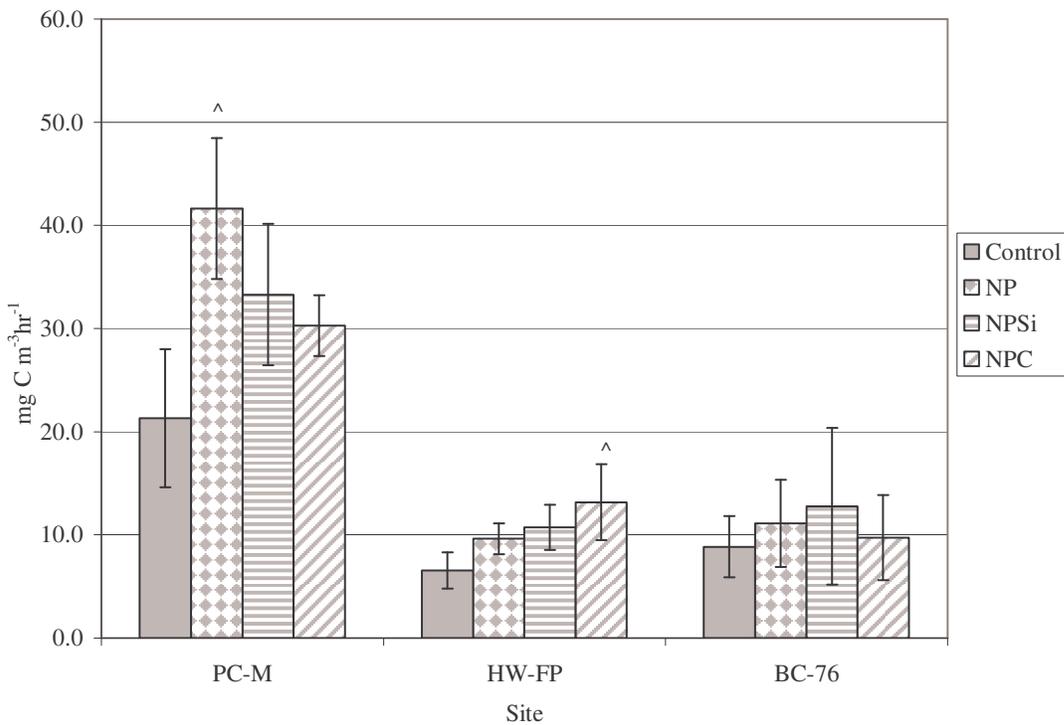


Figure 20. Mesocosm experiment response of (a) heterotrophic and (b) autotrophic production to nutrient treatments. Results of ANOVA: \*Indicates significant difference from all other treatments at that site and ^indicates significant difference from controls of that site only.

resulted in higher autotrophic production than the control in HW-FP ( $p = 0.0167$ ), but there were no differences in BC-76 (Figure 20b). Hourly heterotrophic production was lower in all of the controls than was seen in the previous rain event of the field study (August means: PC-M =  $2.1 \pm 0.3$ , HW-FP =  $1.9 \pm 0.6$ , BC-76 =  $4.3 \pm 0.4$ ). Compared to hourly autotrophic production rates of the previous sampling period (August-rain), the controls were within the same range for sites PC-M and BC-76 ( $18.3 \pm 1.6$  and  $8.2 \pm 1.3$ , respectively), but production within site HW-FP was low ( $16.7 \pm 3.6$ ). Since each site was sampled on a different day, the sites were not compared to each other, due to differences in light conditions on each of those days.

## DISCUSSION

Seasonally, phytoplankton production follows the classic pattern with low values in the winter, a spring bloom, which is grazed before another larger, summer bloom is produced (Raymont 1980). Heterotrophic production seems to follow the same pattern in Pages and Howe Creeks, but only has one peak between the two algal blooms in Bradley Creek. Thus, the heterotrophic to autotrophic production ratio in summer differs in Bradley Creek from the other creeks. High water temperatures drive the higher bacterial and algal production rates during summer months. In a cross-system review of 54 studies, heterotrophic production ranged from  $0.4 - 153 \text{ mgC m}^{-3}\text{day}^{-1}$ , averaged  $26.4 \pm 33.1 \text{ mgC m}^{-3}\text{day}^{-1}$ , and averaged 20% of phytoplankton production (Cole et al. 1988). Mean heterotrophic production in this study was slightly higher at  $38.6 \pm 32.1 \text{ mgC m}^{-3}\text{day}^{-1}$ , with an average of 14% of phytoplankton production. Differences are likely due to climate differences, since bacterial production is highly temperature dependent. Annual volumetric primary production of these creeks is comparable to other New Hanover County creeks, but tends to be greater than larger estuaries, which would be able to dilute out excess anthropogenic nutrient additions (Table 14). High plankton productivity

Table 14. Annual primary production of North Carolina estuaries and tidal creeks. Adapted from Johnson (2005).

Location	gC m <sup>-3</sup> yr <sup>-1</sup>
Beaufort Estuaries	56.0
Neuse River Estuary	75.0
Futch Creek	91.2
South River	144.0
Pages Creek	<b>144.3</b>
Pamlico River Estuary	150.0
Bradley Creek	<b>199.7</b>
Howe Creek	<b>232.4</b>
Hewletts Creek	246.6

in the summer and their resultant exudates, which contain ammonium, explain the correlation of temperature and ammonium. Large scale temporal variation does not affect individual creeks differently. The main differences within creeks can be seen in spatial variation.

As runoff enters the water column, bacterial degradation of organic matter releases the inorganic, bioavailable, forms of nutrients. Heterotrophic bacteria use organic matter as an energy source, releasing phosphate, ammonium, and dissolved silica to the water column. Phosphate is hydrolyzed to hydrogen phosphate ( $\text{HPO}_4^{-2}$ ) and phosphoric acid ( $\text{H}_2\text{PO}_4^-$ ), which tend to be taken up rapidly by organisms, but they also adsorb to colloidal particles or precipitate out of solution, so dissolved concentrations tend to be minimal (McClain et al. 1998). It is also thought that bacteria are able to mediate the release of phosphorus from particulates and some bacteria are capable of hydrolyzing polyphosphates to orthophosphate in anaerobic environments (Khoshmanesh et al. 1999). Bacterial processes also play a large role in nitrogen recycling. Nitrification takes place under aerobic conditions by bacterial oxidation of ammonium to nitrate in a two step process initially producing nitrite. This process is counteracted as nitrate/nitrite is transported to anaerobic environments, such as the sediments in which denitrification reduces it back to ammonium and nitrogen gas. Cyanobacteria are also capable of fixing nitrogen ( $\text{N}_2 \rightarrow \text{NH}_4^+$ ) when nitrate concentrations are low and phosphorus and iron are available (Schlesinger 1991).

Negatively charged clay particles in fresh, upstream water, repel each other, which suspends them in solution, keeping the water column turbid. Fresher water from upstream, with a pH from 5-7, is more productive due to higher nutrient concentrations than downstream. The chemistry of the water changes as the tide brings in saltier water from downstream, with a pH of 8. As the fresh and saltwater mix, the pH reaches 8 as the salinity approaches 5 and negatively charged

particles attract cations and silicates creating larger aggregates, which settle to the sediments keeping dissolved silica concentrations lower in all downstream sites. The reduction in particulate matter effectively reduces bacterial biomass, and the decreased turbidity increases light, which can result in a phytoplankton bloom, which is rapidly consumed, supporting estuarine fishery production (Schlesinger 1991).

Flushing rates also affect nutrient availability. Vollenweider (1976) defined  $\pi_r$  as the ratio of the average phosphorus concentration in a lake divided by that flowing into the lake, which can be derived to equal:

$$\pi_r = \frac{1}{1 + \sqrt{\tau_w}}$$

Where,  $\tau_w$  represents the filling time. The filling time can be estimated from the flushing rate and an average tidal cycle of 12.42 hours. Values of  $\pi_r < 1$  show that the incoming phosphate concentration is greater than that in the water column, suggesting that the remainder is lost to the sediments. Nutrients tend to be sequestered in sediments, because they provide a habitat for bacterial growth where they are protected from lethal UV radiation (Davies et al. 1995). Bioturbation resuspends nutrients into the water column and can enhance molecular diffusion, but the benthic community may take up a considerable amount of these nutrients before resuspension to the water column can occur (Sigmon and Cahoon 1997).

Using flushing rates given by Hales (2001), the average  $\pi_r = 0.322$  for these sites under dry conditions and  $\pi_r = 0.247$  after rain events, indicating that two-thirds of the total phosphate entering the system may support benthic communities during dry conditions and three-quarters after rain events. Given similar  $\pi_r$  ratios among creeks, it is not surprising that the N:P ratios of creeks are also similar, all of which would be considered to be nitrogen limited following the thought of Ryther and Dunstan (1971).

Urbanized tidal creeks have been shown to be nitrogen limiting to algal production, but if nitrogen were limiting production, all of the mesocosm treatments should have had substantially greater production than the controls (Mallin et al. 2004). This however, was not the case. Since light, dilution, and sedimentation were all factors controlled within the experiment, the most likely control on growth rate was grazing, which was likely altered under the conditions of the experiment, only capturing and confining a fraction of natural consumers. Bacteria are grazed upon by protozoa, heterotrophic dinoflagellates, and some bivalves. A study performed during the summers of 2005 and 2006 (Alphin and Posey 2007) of the lower portions of the tidal creeks, where oyster beds tend to have the greatest coverage, found Howe Creek to have a greater density of oysters than Pages Creek, although Pages Creek oysters were larger and had a greater percent of shell cover. Their study also examined the mortality rate from July to November of 2006. In Howe Creek more than 85% of the oysters died within the first month, which corresponds to high production rates at this time in this study and a high phytoplankton biomass of  $23.7\mu\text{g chl } a \text{ liter}^{-1}$  in July 2006 collected by the Center for Marine Science (CMS) Aquatic Ecology Lab (McIver personal communication). Pages Creek had mortality rates of 22-37% in August and September, which were probably not high enough to affect production rates, although Raymond (1980) showed that zooplankton production decreased shortly after the spring phytoplankton bloom, allowing for the summer bloom. Bivalves have not been examined in Bradley Creek since the fall of 1996 (Posey et al. 2002). Bradley Creek was found to have a high diversity of bivalves, but low density compared to Howe Creek. If grazing pressure were lower in Bradley creek, there may be more interaction between algal and bacterial production, and larger blooms would be likely to occur. In fact, the CMS Aquatic Ecology Lab reported phytoplankton blooms upstream in Bradley Creek in spring and summer of  $47$  and  $40\mu\text{g chl } a$

liter<sup>-1</sup>, respectively. Since nutrient sedimentation is high in all of the creeks, differences in grazing pressure may account for differences in the production ratios of the creeks. The proportion of heterotrophic to autotrophic production does not vary much in each of Pages and Howe Creeks, but a difference can be seen in Bradley Creek. During spring and summer months, if the rate of heterotrophic production is high, then the rate of autotrophic production tends to be lower and vice versa, suggesting dependence between the two types of production. Mutualism exists between bacteria and algae because algae exude polysaccharides utilized by bacteria, and bacteria regenerate ammonium and phosphate (Currie 1990). Currie suggested that when production was limited by phosphorus, heterotrophs and autotrophs competed for nutrients, but when bacteria were limited by DOC they had a mutualistic relationship. The same relationship has been suggested for nitrogen (Legendre and Rassoulzadegan 1995). If bacteria and phytoplankton are being grazed at a high rate, then there would not be an interaction between the two types of production, which appears to be the case in Pages and Howe Creeks (Lewitus et al. 1998). Hecky and Kilham (1988) suggested that since recycled nutrients are the main source for ocean phytoplankton, that grazing must be high in marine systems otherwise nutrients would be lost to sedimentation. Thus nutrient sedimentation and grazing seem to control downstream production, while the shallow upstream sites tend to have higher production due to the higher nutrient and light conditions, and lower grazing pressure.

Rainfall and its subsequent runoff load nutrients into the tidal creeks, which were thought to increase production. Instead, autotrophic production was negative to component 2 within all creeks and tended to decrease after rain events, although only significantly in the upstream site of Pages Creek (PC-BDDS). Cloud cover associated with rain decreases light availability. The amount of daily photosynthetically active radiation (mmol PAR m<sup>-2</sup>), only collected from

September 2005 through April 2006, was positively correlated to heterotrophic and autotrophic production and negatively correlated with rainfall in Howe and Bradley Creeks, but it was not correlated to rainfall in Pages Creek (NOAA 2006; Table 15). Runoff erodes soils, concentrating particles, which adsorb nutrients and bacteria to them, resulting in higher water column turbidity and eventually sedimentation. High turbidity of the water column decreases light availability for phytoplankton production. Future rain studies should take turbidity into consideration.

Heterotrophic production was also negatively correlated to component 2 within each of the creeks, but much less significantly than phytoplankton production, which may suggest a dependence of bacterial production to algal exudates, since bacterial production would be expected to increase with lower light and more nutrients. Table 15 also indicates a correlation of heterotrophic production with PAR in Howe and Bradley Creeks, which may also indicate some dependence on algal production.

Large rain events have produced significant chlorophyll *a* responses in Pages Creek when sampled continuously through the rain event until the salinity returned to normal (Hubertz and Cahoon 1999). Since sampling took place between 1-3 days after the rain event, as soon as the high tide occurred between 9am and 12pm, any production response may have either not occurred yet or have already taken place. Loucaides (2003) found that runoff was produced from even low amounts of rainfall in a watershed of 49.2% impervious cover compared to one of only 17.1%. Comparing the effects of low and high amounts of rain on algal and bacterial production in developed watersheds may show differences with production and different amounts of turbidity and nutrients verses flushing.

Dissolved carbohydrate concentrations were similar among creeks and between creek sites, and tend to increase after rain events, although they were not grouped in the PCA within the

Table 15. Pairwise correlations of daily heterotrophic (HP) and autotrophic (AP) production and the sum of precipitation (ppt) to daily PAR ( $\text{mmol m}^{-2}$ ) in (a) Pages Creek, (b) Howe Creek and (c) Bradley Creek. Data given as  $r^2/p$  – value; from September 2005 – April 2006 only. Shaded cells indicate significance at  $\alpha = 0.0083$ . L = log.

(a) Pages Creek

Variable	LDaily HP	LDaily AP	LDaily PAR
LDaily AP	0.5346 <0.0001	1.0000	
LDaily PAR	0.1819 0.2490	0.5675 0.0001	1.0000
L24hr ppt	-0.1405 0.2393	-0.2654 0.0275	-0.0824 0.6038
L72hr ppt	-0.1510 0.2055	-0.3630 0.0022	-0.1747 0.2686

(b) Howe Creek

Variable	LDaily HP	LDaily AP	LDaily PAR
LDaily AP	0.6433 <0.0001	1.0000	
LDaily PAR	0.5747 0.0001	0.6307 <0.0001	1.0000
L24hr ppt	0.2022 0.1034	0.0533 0.6706	0.0190 0.9050
L72hr ppt	-0.1452 0.2446	-0.2498 0.0431	-0.4685 0.0018

(c) Bradley Creek

Variable	LDaily HP	LDaily AP	LDaily PAR
LDaily AP	0.5667 <0.0001	1.0000	
LDaily PAR	0.5948 <0.0001	0.7644 <0.0001	1.0000
L24hr ppt	-0.0069 0.9522	-0.3010 0.0074	-0.9485 <0.0001
L72hr ppt	0.0025 0.9829	-0.1482 0.1955	-0.2434 0.1204

runoff component. All sites had a peak in dissolved carbohydrate concentration with the rain event in June, which could be a glucose signal from increased land primary production dissolved in runoff, but also corresponded to the decrease in heterotrophic and autotrophic production, assumed to be the crash of the spring bloom, by grazing or otherwise. The following month, production had increased again and dissolved carbohydrates were at ambient concentrations, as they had been utilized or flushed from the system. Dissolved carbohydrates were in an individual component and that component increased with heterotrophic production in Bradley Creek and the upstream site of Howe Creek, suggesting that they support bacterial growth. Autotrophic production increased with this component in Bradley Creek, which supports the idea that algal exudates are one of the sources of dissolved carbohydrates, which support bacterial growth in this creek. Any correlation between autotrophic and heterotrophic production in the other creeks may be explained indirectly by the effects of temperature on both types of production.

Pages Creek has a significantly higher salinity than Howe and Bradley Creeks, due to its wide basin and high flushing rates, driving saline water further upstream with each tide. N:P ratios are approximately 9 both upstream and downstream, which suggests nitrogen limitation of production. Algal production tends to be lower in Pages Creek, although it is not significantly different from the other creeks, and does not significantly decline downstream as in the other creeks. Bacterial production is comparable to the other creeks indicating that nutrient resources are flushed from this system. The decrease in bacterial and algal production in the upstream site with component 1, which comprises silica and negative salinity, is most likely due to the high flushing rate of this creek. The incoming freshwater from rain and runoff significantly lowers the salinity upstream and downstream, and dissolved carbohydrates, orthophosphate, and ammonium

are positively correlated with rainfall, but nitrate is not, which reduces the N:P ratio further. Bacterial production was not statistically affected by the precipitation, but production by phytoplankton is negatively correlated with rain most likely due to high turbidity visually noted at the upstream site. Autotrophic production was only higher than the controls in the NP treatment of the mesocosm experiment, which demonstrates a slight stimulation of production by concentrations found in rain and runoff, without the effects of physical variables, although grazing was likely a factor as well. Therefore, production appears to be controlled by tidal flushing and grazing in this creek.

The silica concentration is higher in Howe Creek than in Pages Creek and Howe Creek tends to have higher production. The N:P ratio is low upstream (~6), but typical of the tidal creeks at about 9 downstream, indicating the importance of regenerated nutrients downstream. Dissolved carbohydrates, ammonium, and nitrate are positively correlated with rain, but not phosphorus. Dissolved carbohydrates were not grouped in the runoff component of the PCA, so this correlation may be indicative autochthonous sources such as plankton death or exudates released. Bacterial production correlated with nitrate concentration in the field survey and increased with the NP treatment in the mesocosm experiment, which further indicates nitrogen limitation. Phytoplankton production correlated with silica and carbohydrates in the field study and increased with the NPC treatment of the mesocosm experiment, suggesting some silica limitation as well as algal heterotrophy in this system.

The N:P ratio is about 9 upstream, but significantly drops to 6 downstream within Bradley Creek. The N:P ratio does not change significantly with rain at either site since dissolved phosphate, nitrate, and ammonium are correlated with rainfall. Nutrient loadings with rainfall may have resulted in carbon limitation of bacteria during the summer resulting in algal-bacterial

mutualism in this creek. Bacterial production correlated with dissolved carbohydrate, nitrate, and silica in the field study, but did not respond to nutrient treatments in the mesocosm experiment, although grazing probably masked some responses. The correlation may be a spatial effect as nitrates, silica, and production are higher upstream, but dissolved carbohydrate and nitrate may limit production as well. Both bacterial and algal production increased with component 4 in Bradley Creek, which represented dissolved carbohydrates, indicating that autochthonous carbohydrates may be utilized more in this system.

Overall, the bioavailable dissolved carbohydrates that were analyzed were not a significant portion of the rainfall runoff. They were most likely autochthonous exudates of phytoplankton, bacteria, and other organisms not considered in this study. Even though rain and runoff are a significant source of nutrients to the water column, production decreases after rain events, likely due to light limitation as a result of cloud cover or turbidity introduced with runoff.

Sedimentation of nutrients keeps concentrations similar among creeks and limits production, which is also similar among creeks. Carbohydrate and nitrate concentrations limit bacterial production, and dissolved silica limits algal production to some extent in Howe and Bradley Creeks, suggesting that differences may still be seen with greater impervious cover. Differences in summer production ratios may signal carbon limitation due to nitrogen and phosphorus loading, but flushing and grazing may be factors as well. Heterotrophic to autotrophic production ratios may also be altered, coincidentally by the anthropogenic effects on their consumers. A mesocosm experiment that monitors the nutrient concentrations and production rates over time, including those of zooplankton, may give better results.

## CONCLUSION

Of the New Hanover County tidal creeks, only Futch and Hewletts Creeks had been measured for autotrophic production and none had been assayed for heterotrophic production. This study provides another layer to the trophic structure of the New Hanover County tidal creeks, which is remarkably similar among creeks. Although there are also similarities in nutrient concentrations among creeks, there are still distinctions, in part created by differing physical processes that contribute to the assorted limitations of production. As land development continues, it is important to monitor the effects of nutrient loading, which may still result in silicon or DOC limitation of production. An examination of tidal creek bacterial substrates and carbon cycling would benefit the study of algal and bacterial interactions with nutrient resources.

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