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Coupling of autotrophic and heterotrophic plankton food web components in the tidal-freshwater James River, USA

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COUPLING OF AUTOTROPHIC AND HETEROTROPHIC PLANKTON FOOD WEB COMPONENTS IN THE TIDAL-FRESHWATER JAMES RIVER, USA

A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science (Biology) at Virginia Commonwealth University.

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Abstract

COUPLING OF AUTOTROPHIC AND HETEROTROPHIC PLANKTON FOOD WEB COMPONENTS IN THE TIDAL-FRESHWATER JAMES RIVER, USA

By Matthew J. Beckwith, B.S.

A Thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science (Biology) at Virginia Commonwealth University.

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Empirical studies have shown that algal- and detrital-based food web components are coupled in many pelagic systems as algal carbon enhances bacterioplankton production and growth efficiencies. Such phyto-bacterioplankton coupling impacts carbon flow through plankton food webs, yet the extent of coupling is poorly understood in systems receiving large amounts of allochthonous carbon. To investigate this issue, bacterioplankton abundance (BA) and community composition were compared to

chlorophyll *a* concentrations and phytoplankton production in the tidal-freshwater James River (VA). BA averaged 10^7 cells mL^{-1} and was significantly related to chlorophyll *a*, phytoplankton production, and DOC concentrations. Analysis of DOC quality using fluorescence spectroscopy revealed that the fulvic DOC fraction was dominated by allochthonous compounds. However, estimates of DOC C:N and DOC turnover rates indicated that DOC was more labile in the lower part of the study reach where BA was highest. T-RFLP analysis of 16s rDNA showed that bacterioplankton community composition significantly varied between the upper and lower portions of the sampling reach. These findings suggest that coupling of food web components is an important pathway affecting carbon cycling within the tidal-fresh water James River.

Chapter 1: Coupling of autotrophic and heterotrophic plankton food web components in the tidal-freshwater James River, USA

INTRODUCTION

Conceptually, plankton food webs can be separated into two components: the algal- (autotroph) and detrital- (heterotroph) based pathways. The algal-grazer pathway is the classical plant-herbivore food web in which phytoplankton are grazed by zooplankton. In the detrital pathway, dissolved and particulate organic carbon (DOC and POC, respectively) are utilized by heterotrophic bacteria (Kirchman et al. 1989; Bloem et al. 1989). When bacteriovores such as protozoa and rotifers are preyed upon by larger zooplankton, organic carbon is transferred to higher trophic levels allowing the detrital pathway to form a "microbial loop" (Pomeroy 1974; Hoppe 1976; Azam et al. 1983; Scavia et al. 1986; Kankaala 1988). The extent to which the microbial loop operates as a carbon "link or sink" remains uncertain. The microbial loop may function as a link by making organic carbon that was not initially captured via the herbivore pathway available to higher trophic levels. Alternatively, organic carbon entering the detrital pathway may be lost from the system through respiration. High respiratory losses within the detrital pathway are attributed to the greater number of trophic transfers and low production efficiencies among bacteria and protists (Sherr and Sherr 1988; Legendre and Rassooulzadegan 1995). Understanding the factors governing bacterial growth efficiency

(BGE) is therefore central to assessing the function of the microbial loop and the extent to which it supports secondary production at higher trophic levels.

In diverse aquatic systems bacteria have been shown to be limited by the quality of organic carbon (Vallino et al. 1996). Autochthonous (i.e. internal) sources of carbon (particularly phytoplankton exudates) are more labile than allochthonous carbon due to their higher content of amino acids, polysaccharides, nucleic acids and lipids (Nyguyen 2005; Amon et al. 2001; Hellebust 1974; Fogg 1983). Thus, BGEs associated with algal exudates are higher in comparison to values associated with vascular plant detritus (>50% and <30% respectively; Del Giorgio and Cole 1998). As a result, bacterial production (BP), BGE and NPP co-vary in many pelagic systems (White et al. 1991). For instance, BP in Wisconsin lakes was highly correlated with algal abundance and BGE increased as the predominance of autochthonous carbon increased (Kritzberg et al. 2005). Meersche et al. (2004) demonstrated that 60% of the DOC pool following a bloom event consisted of algal cell exudates and after one day, this carbon was incorporated into bacteria-specific fatty acids. These findings suggest a strong coupling between the phytoplankton and bacterioplankton components of pelagic food webs with regards to productivity and abundance. Likewise, the composition of bacterioplankton and phytoplankton communities may be linked but the underlying mechanisms are unknown (Peng et al. 2007).

Ecologists have long questioned whether autochthonous carbon fuels secondary production in rivers where loading of allochthonous carbon is high. The River Continuum Concept (RCC) suggests that organic carbon derived from terrestrial sources supports ecosystem metabolism in small order streams while the contributions of

autochthonous production become more significant as stream order increases (Vannote et al. 1980). The importance of autochthonous carbon, and hence phyto-bacterio- plankton coupling, may be diminished in river and estuarine environments where terrestrial inputs of allochthonous carbon are large (Shia 1993; Shia and Ducklow 1994). In the tidal-freshwater portion of the Hudson River, a heterotophic system in which BP is four times greater than NPP, BP was not significantly related to algal standing stocks or NPP (Findlay 1991). In an attempt to reconcile the heterotrophic nature of rivers with findings that autochthonous carbon supports the bulk of production at higher trophic levels, Thorp and DeLong (2002) suggested that autochthonous and allochthonous carbon support different pathways within riverine food webs. Their Riverine Productivity Model states that higher trophic levels in large order rivers are sustained by labile autochthonous carbon while refractory allochthonous carbon drives high respiration rates within the detrital pathway.

In addition to constraints imposed by the source and lability of organic matter, BP and BGE may be limited by the availability of nutrients (N,P). Lake mesocosm experiments showed that BP was limited by nutrients rather than autochthonous production (Toolan et al. 1991; Morris and Lewis 1992; Le et al. 1994). Limitation by nutrients vs. organic matter may in turn affect the coupling between phyto- and bacterioplankton communities (Thingstad et al. 1997; Thingstad et al. 2008). Le et al. (1994) concluded that a gradient of coupling occurs as a function of N:P ratios. Namely, high N:P ratios (P limitation) promoted identical responses of bacteria and phytoplankton leading to co-variance between bacterial and phytoplankton biomass. Conversely, low N:P ratios (N limitation) enabled bacteria to outcompete phytoplankton for available P

causing asymmetrical patterns in abundance. Similar results were observed during a simulated algal bloom: during stationary growth when nitrogen concentrations diminished, nitrogen was immobilized by bacteria rather than remineralized, allowing bacteria to outcompete phytoplankton (Meersche et al. 2004).

Grazers (i.e. bacteriovorous zooplankton) might undermine the role of autochthonous production and nutrient availability as coupling devices. In eutrophic systems, high algal abundance can indirectly suppress bacterial biomass through maintenance of high protozoan grazing rates (Sanders et al. 1992). Rotifers, a key component of zooplankton biomass (Pourriot 1977), may mediate coupling by recycling degraded algal carbon through the microbial loop (Arndt 1993). Additionally, coupling could be regulated by temperature. Coveney and Wetzel (1995) found that seasonal patterns in BP were primarily explained by temperature, and a relationship between bacterial abundance (BA) and algal abundance was only visible with temperature effects removed.

When BP and BGE are enhanced by primary production, the algal-grazer and detrital pathways are tightly linked. Such coupling supports efficient operation of the microbial loop perhaps increasing total secondary production. However, allochthonous inputs, nutrient availability, grazing and temperature have been shown to diminish the importance of autochthonous carbon thereby de-coupling phyto- and bacterioplankton activities. A complicating factor is that relationships between bacterial and phytoplankton production may not reflect coupling, but rather a simultaneous response to environmental variables (e.g. temperature, water residence time, etc.). Clearly, there is a need to better resolve phyto-bacterioplankton interactions particularly in systems

characterized by both high autotrophic production and large allochthonous inputs. Furthermore, while many studies have characterized the effects of allochthonous and autochthonous organic carbon on BA, BP, and BGE, few have related bacterioplankton community composition to organic matter quality. The current study aims to elucidate the degree of phyto-bacterioplankton coupling within the tidal-freshwater James River (VA) by comparing phytoplankton abundance (as Chlorophyll *a;* CHL*a*) and production with BA and community composition. Based on previous work (Lederer 2008; DEQ 2007), gradients of CHL*a* and NPP existed within the sampling reach and were used to investigate the responses of BA and bacterioplankton community composition to variation of autochthonous carbon availability. This study focused primarily on conditions within the river's main channel, however a near shore and tributary site were also included. Relationships between bacterioplankton and phytoplankton were examined in the context of seasonal and spatial variability in temperature, nutrients, and DOC to explain potential mechanisms affecting the degree of coupling.

METHODS

Study reach and sampling:

This study was carried out within the tidal-freshwater James River (VA). The James River is a 7th order tributary of the Chesapeake Bay (Smock et al. 2005). Its basin (26,164 km^2) is 71% forested, 7% agricultural, 5% urban, 4% open water and 3% wetland (JRA 2009). Mean annual discharge (recorded at Cartersville, VA) was 202 m^3 s^{-1} between 1996 and 2006 (USGS 2009). Highest monthly discharge typically occurs during February and March (ca. 300 m^3 s^{-1}) and the minimum monthly discharge is in August (70 m^3 s^{-1}). The beginning of the tidal-freshwater segment is below the Fall Line

at Richmond, VA (ca. 110 river miles from the confluence with Chesapeake Bay). The upper portion of the tidal-freshwater segment is characterized by a deep (>5 m) and narrow (<1 km) channel. At approximately river mile 80 (near Hopewell, VA) the channel assumes a more estuarine morphology with broad shallow areas (<1 m depth) lateral to the main channel. The daily tidal amplitude is 1.1 m (Smock et al. 2005).

The study reach, with a total length of 50 km, begins at river mile (i.e. navigation miles) 99 and ends at river mile 69. Six main channel sites were sampled at 2- to 3- week intervals from May 25th through November 16th (**Fig. 1**). Three of the six sites were long-term monitoring locations for the VA DEQ Chesapeake Bay Program (at river miles 99, 75 and 69). In addition to the six main channel sites, occasional samples were also collected from a near-shore site (Rice Center Pier) and a tributary stream (Herring Creek). During each sampling cruise, *in situ* water quality parameters including dissolved oxygen (DO), pH, and temperature were measured at each site using a YSI multiparameter sonde. In addition, near-surface (1-2 m) water samples were collected at each site, stored on ice and processed to quantify chemical and biological variables upon return to the lab.

Sample Processing:

Within 24-48 hrs of collection, samples were prepared for measurement of turbidity, nutrient concentrations and CHL*a*. Turbidity was measured optically with a Hach Turbidimeter. Nutrient concentrations (PO_4^{-3}, NO_3^- and NH_3) were determined colorimetrically using a Skalar Segmented Flow Analyzer (APHA 1992). To quantify CHL*a* concentrations, three replicates from each site were filtered through Whatman GF/A filters. Filters were placed in 15 mL centrifuge tubes to which 10 mL of buffered

($MgCO_3$) 90% aqueous acetone were added to extract CHL*a*. After 18 hrs, CHL*a* concentrations were measured using a Turner Designs fluorometer.

Bacterial abundance (BA) was estimated using acridine orange direct counts (AODC; Hobbie et al. 1977). AODC is an epifluorescence microscopy technique utilizing acridine orange: a fluorescent stain that binds to nucleic acids allowing for visualization of bacterioplankton cells. Three whole water replicate subsamples from each site were preserved with pre-filtered formaldehyde. Following sonication, samples were vigorously vortexed and stained with acridine orange. Some samples required serial dilution to reduce cell densities to a countable range (20-200 cells/counting grid); generally, no more than a tenfold dilution factor was required. Upon staining, samples were filtered through 0.2 μm black polycarbonate membrane filters (Millipore), which were fixed to slides using non-fluorescing immersion oil and viewed under a uv-equipped compound microscope. For each replicate, the total numbers of cells within the counting grid were enumerated in 10 random fields at 1000x. The mean number of cells per field was then extrapolated to the area of the filter through which sample passed to estimate the total number of cells per filter. This value was divided by the volume of filtered sample and adjusted according to dilution factors to calculate cell densities (cells mL^{-1}).

Rates of water column net primary production (NPP) and community respiration (CR) were estimated by tracking changes in DO within light and dark bottles using the Winkler method. Whole water samples from each site were transferred to 60 mL BOD bottles, of which three were immediately fixed for determination of initial DO concentrations. Three replicates from each site were wrapped in foil ('dark' bottles), incubated at ambient water temperatures and fixed after 24 hrs to determine CR. The

remaining three replicates were left unwrapped ('light' bottles) and incubated for 8 hrs at ambient temperature and irradiance levels to determine NPP. *In situ* irradiance levels were determined using light attenuation coefficients measured at each site using a Li-Cor photometer (Lederer 2008).

To determine the quantity of dissolved organic carbon (DOC), samples were processed as follows. Whole water samples from each site were filtered using Whatman GF/A filters and the subsequent filtrate was acidified to a pH of 2 with concentrated HCl. Acidified filtrate from each sample was then processed using a Shimadzu TOC analyzer to measure DOC concentrations. DOC concentrations were divided by dissolved organic nitrogen (DON) concentrations (DEQ 2007) to estimate DOC C:N ratios as an indicator of DOC quality.

Fluorescence Spectroscopy:

To asses the quality of DOC, fluorescence spectroscopy was employed to elucidate whether the fulvic acid fraction of the DOC pool was dominated by autochthonous- or allochthonous-derived compounds. Examination of DOC using fluorescence spectroscopy was based on McKnight et al.'s (2001) end-member analysis where the fluorescent properties of purified natural fulvic acids were determined and an index (hereafter, DOC fluorescence ratio) was developed to indicate the prevalence of autochthonous and allocthonous sources. This protocol was strictly followed except for correction of the inner-filter effect: absorbance of either excitation or emission light by sample constituents causing DOC concentrations to affect metrics of fluorescence. The inner-filter effect was not corrected for because James River DOC concentrations were below the threshold (10 mg L^{-1}) at which these effects occur (Kalbitz and Geyer 2001).

In addition, the significant positive relationship between fluorescence intensity and DOC concentrations further indicates that the inner filter effect was negligible (see results).

Three-dimensional scans often referred to as Excitation-Emission Matrices (EEMs) were generated using a J. Y. Horiba Tau 3 Luminescence Spectrometer with 5.0 nm increments, excitation wavelength ranging from 260 to 460 nm and emission wavelengths between 350 and 460 nm (see above for sample preparation prior to spectral analysis). The DOC fluorescence ratio was calculated by dividing the intensity of fluoresced light observed at 450 nm emission by the intensity corresponding to 500 nm emission at a fixed excitation wavelength of 370 nm. Prior to this calculation, intensities were corrected by subtracting blank intensities from sample intensities. Ratios between 1.4 and 1.5 indicate that DOC is dominated by allochthonous sources whereas values near 1.9 suggest that DOC is autochthonous and microbial derived (McKnight et al. 2001).

Bacterioplankton Community Composition:

To investigate temporal and spatial shifts in bacterioplankton community composition, a Polymerase Chain Reaction (PCR) based technique was used to generate total-community genetic fingerprints. Specifically, Terminal Restriction Fragment Length Polymorphism (T-RFLP) PCR was employed to amplify 16s (small subunit) rDNA from total-community DNA (Liu et al. 1997; Marsh 1999). The amplification of 16s rDNA oligonucleotides is directed by universal primers, one of which is fluorescently labeled at the terminal 5' end. Following successful amplification, the PCR product is fragmented via digestion with endonuclease restriction enzymes producing fragments of varying length (i.e. size polymorphism). The size polymorphism of the fluorescently

labeled terminal restriction fragments (T-RFs), which is a function of 16s rDNA sequence variation, is assessed using an automated DNA sequencer. The final result is a figure referred to as an electropherogram that plots the length (number of base pairs) of T-RFs against corresponding fluorescence intensity serving as T-RFLP community profile that can be compared among samples to infer differences in composition. DNA extractions, PCR conditions, restriction digestion, and generation of electropherograms are detailed below.

On select dates, two 750 mL whole water replicate subsamples from river miles 99 and 75 were filtered to collect bacterioplankton for DNA extraction. To remove seston and eukaryotes, each 750 mL subsample was first pre-filtered through Whatman GF/A filters. Filtrate from each subsample was subsequently filtered through 0.2 μm Nucleopore nitrocellulose membrane filters to collect bacterioplankton cells. During both filtering steps, the 750 mL subsamples were divided into three 250 mL aliquots and filtered separately to expedite the process. All three 0.2 μm filters from each replicate were recombined and stored in sterile 50 mL centrifuge tubes at -80^{o}C. Filtering was completed within 24 hrs of sample collection using sterile techniques. To extract DNA from bacterioplankton cells, the three 0.2 μm filters constituting a single replicate were processed using UltraClean Water DNA Isolation Kits (MoBio Laboratories, Inc.). When using these kits, DNA is extracted from bacteria by repeatedly exposing cells to a series of proprietary reagents followed by centrifugation. Extracted DNA was further concentrated by centrifugation in the presence of 100% ethanol and 5M NaCl then reconstituted. DNA concentrations were measured using a NanoDrop UV/Vis

spectrophotometer in order to standardize the DNA template concentration prior to the PCR reaction.

16s rDNA was amplified under the following reaction conditions: 10 X PCR buffer, deoxynucleoside triphosphates (dNTPs) at a concentration of 1.0 mM, 1.5 mM $MgCl_2$, 0.4 μg μL^{-1} of bovine serum albumin (BSA), 1.5 U of *Taq* DNA polymerase (Applied Biosystems), and 240.0 μg μL^{-1} bacterioplankton DNA all in a final volume of 50 μL. The selected primer pair was 1492r and fluorescently labeled 27f (0.15 and 0.30 μM, respectively; Integrated DNA Technologies). The PCR reaction was amplified using a Peltier Thermal Cycler-200 (Bio-Rad) with a 5 min hot start at 95°C followed by 35 cycles of denaturation at 94°C (1 min), annealing at 49°C (1 min), and extension at 72°C for 2 min. PCR effectiveness was verified by electrophoresing 15 μL of PCR product through a 1.5% agarose gel in 1 X TBE buffer, which revealed non-specific amplification had occurred. To remove non-16s rDNA from the PCR product, 20 μL of PCR product for each sample was again electrophoresed through a 1.5% agarose gel containing ethidium bromide (0.5 μg mL^{-1} final concentration) in 1 X TBE buffer followed by excision of desired bands under blue light. DNA was recovered by centrifuging the excised gel pieces containing the desired PCR product in the presence of 1 X TBE within Ultrafree-MC (Millipore) spin columns followed by filtration and reconstitution using sterile water. Cleaned target PCR product was next digested using *Msp*I and *Rsa*I restriction enzymes following the manufacturer's instructions (2 U μL^{-1} and 1 U μL^{-1} final concentration respectively; New England BioLabs). After the restriction digest, the PCR product was further cleaned using MiniElute PCR Purification Kits (Qiagen) followed by capillary electrophoresis with a MegaBACE 1000/4000 Series Sequencer

(Amersham Biosciences). Samples were injected at 3 kV for 100 sec and ran at 10 kV with a run time of 100 min.

Statistical Analysis:

Linear regression ($\alpha = 0.05$) was used to determine if a statistically significant relationships occurred between BA and explanatory variables including CHL*a*, NPP, DOC concentrations, temperature, and nutrients. Linear regression was also used to examine the relationship between fluorescence intensity and DOC concentrations. T-RFLP electropherograms were processed and interpreted using Fragment Profiler (Amersham Biosciences). Software parameters were set so that peaks above 25 fluorescence units (FU) were marked as individual T-RFs and included in the statistical analysis of electropherograms. Anomalous peaks were manually removed from the data set if they were within one base pair of neighboring peaks as inspection of electropherograms revealed that these peaks were generally shoulders on another peak rather than representing unique T-RFs. Using the presence and absence of peaks, a binary matrix was constructed and analyzed using non-metric multidimensional scaling (NMDS) with Jacquard's distance coefficient to produce a plot in which samples are grouped by similarity. Significant differences between groups were tested for using a Mantel test (by site).

RESULTS

Longitudinal Patterns

Longitudinal gradients were characterized using average values (May-November) for the six main channel sampling locations (**Fig. 2**). Specific conductivity was generally similar among sites but was slightly higher and more variable at the most seaward sites

(river miles 75 and 69). Lowest conductivity was measured at the tributary site (Herring Creek = 0.30 mS cm^{-1}). Turbidity increased from 10 to 20 NTUs between river miles 99 and 69. Highest turbidity values occurred at the near-shore site (Rice Pier). All sites exhibited under-saturation for DO with average values between 83% and 95%.

Nutrient concentrations showed consistent longitudinal trends within the study reach, with PO_4^{-3} and NO_3^{-1} decreased in the downstream direction within the study reach (**Fig. 3**). Mean PO_4^{-3} concentrations within the main channel declined from 0.036 to 0.017 mg L^{-1} while mean NO_3^{-} concentrations declined from 0.717 to 0.151 mg L^{-1}. Mean PO_4^{-3} concentrations at the near-shore and tributary sites were similar to the values observed at adjacent main channel sites whereas mean NO_3^{-} concentrations were lowest at these sampling locations. Mean NH_3 concentrations were consistently low in comparison to nitrate concentrations and did not exhibit a longitudinal trend.

Increasing trends in CHL*a*, BA, net primary production (NPP), and community respiration (CR) occurred between river miles 99 and 75 (**Fig. 4**). The CHL*a* maximum was observed at river mile 75 (mean = 58 µg L^{-1}) while the lowest mean concentration (12 µg L^{-1}) occurred at river mile 99. The mean CHL*a* concentration at the Rice Center Pier (60 µg L^{-1}) was similar to nearby main channel sites whereas values in Herring Creek were lower. Mean BA concentrations among main channel sites ranged from 5-8 x 10^7 cells mL^{-1} with the highest cell densities at river mile 69. Mean BA concentrations were higher at the Rice Center Pier and within Herring Creek (1.0 x 10^8 cells mL^{-1}) in comparison to main channel sites. NPP generally increased between river miles 99 and 75 where mean values were 2,423 and 10,873 µg O_2 $L^{-1}d^{-1}$ respectively. Highest mean

CR among main channel sites was 1,557 µg O_2 $L^{-1}d^{-1}$ and occurred at river mile 75. Mean CR increased in the downstream direction between river miles 99 and 75.

Excitation-emission matrices (EEMs) of DOC fluorescence were similar among all sites. A single, broad peak positioned at 330-360 nm excitation and 400-460 nm emission was observed in all samples (**Fig. 5**). Maximum fluorescence intensity was consistently observed at 340 nm excitation and 430 nm emission. Peak fluorescence intensity varied among samples and was significantly related to DOC concentrations ($p<0.001$; **Fig. 6**). DOC concentrations were similar among main channel and near-shore sites with mean values between 3 and 4 mg L^{-1} (**Fig. 7**). Highest average DOC concentrations (5 mg L^{-1}) were observed in Herring Creek. Mean DOC fluorescence ratios calculated from intensities measured at 370 nm excitation ranged between 1.4 and 1.5 and were similar among sites (**Fig. 7**).

Seasonal Patterns

The discharge of the James River was highest on the first two sampling dates (May 25th and June 8th; discharge > 100 m^3 s^{-1}) and was less than 50 m^3 s^{-1} throughout the rest of the sampling period (ending November 16th). Data from stations located at the top (river mile 99) and near the bottom (river mile 75) of the study reach were used to characterize seasonal trends (**Fig. 8**). Identical seasonal patterns in temperature occurred at river miles 99 and 75 with values ranging from 22^{o}C to 31^{o}C. Temperature did not vary greatly between these sampling sites on a given date with the exception of October 18th. Turbidity and DO saturation did not exhibit pronounced seasonal patterns (**Fig. 8**). Nutrient concentrations generally increased during the period of study and this pattern was most pronounced at river mile 99 (**Fig. 9**). At river mile 99, NO_3^- concentrations

increased from 0.294 to 1.713 mg L^{-1} between May 25th and September 28th while phosphate concentrations increased from 0.018 to 0.063 mg L^{-1}. NH_3 concentrations were variable at both sites but did not exhibit consistent seasonal trends.

CHL*a* was relatively low (< 30 µg L^{-1}) throughout the summer at river mile 99. Conversely, river mile 75 exhibited an increase in CHL*a* concentration from 40 to >100 µg L^{-1} (on July 6th) after which values remained ca. 50 µg L^{-1} throughout the summer (**Fig. 10**). BA increased between May 25th and September 7th and then declined during the late summer and early fall months at both river miles 99 and 75. Peak values on August 17th were 2-fold higher relative to values at the start and end of the sampling period. NPP followed a similar temporal trend as was observed with CHL*a* at river mile 99: daily rates were greatest on October 18th at 6,226 µg O_2 $L^{-1}d^{-1}$. River mile 75 experienced an increase in NPP as the summer progressed with a maximum rate occurring on August 3rd (14,513 µg O_2 $L^{-1}d^{-1}$). At both sites, NPP values were positive on all dates except on November 16th during which river mile 99 had NPP rates of -612 µg O_2 $L^{-1}d^{-1}$. CR rates were lowest at the beginning and end of the sampling period and highest during August at both sites. Maximum CR was observed on August 3rd (2,248 µg O_2 $L^{-1}d^{-1}$) and August 17th (1684 µg O_2 $L^{-1}d^{-1}$) at river miles 75 and 99 respectively while minimum values occurred on May 25th (**Fig. 10**).

DOC concentrations were lowest at the start of the sampling period and increased by 2-3 mg L^{-1} during the summer. DOC fluorescence ratios determined using 370 nm excitation varied between 1.4 and 1.5 at both sites and did not vary seasonally. The C:N ratio of DOC was determined on four sampling dates (**Fig. 11**). At both sites, lowest values (< 10) were observed in June with higher values (> 12) occurring in August,

September and October. C:N ratios at the upper site (river mile 99) were consistently higher than those measured at the lower site (river mile 75).

BA Related to Environmental Factors and Bacterioplankton Community Composition

Linear regression ($\alpha = 0.05$), which revealed that BA was significantly related to CHL*a*, NPP, and DOC concentrations ($p = 0.01$, <0.001 and <0.0001, respectively; **Fig 12**). BA was not significantly related to temperature or nutrient concentrations. T-RFLP PCR of 16s rDNA indicated that bacterioplankton community composition varied longitudinally more so than seasonally. Specifically, Multivariate analysis (NMDS) of electropherogram peak patterns grouped bacterioplankton DNA samples by river mile regardless of sampling date (**Fig. 13**). T-RFLP peak patterns and hence bacterioplankton community composition significantly differed between river miles 99 and 75 (Mantel test, $p = 0.002$; $\alpha = 0.05$). When river mile is ignored, there is no clear discernable grouping of samples by date with the exception of samples collected during August. Seasonally, bacterioplankton community composition was more variable at river mile 99 as indicated by the larger spread of data points within this group relative to samples from river mile 75.

DISCUSSION

Traditionally, rivers have been viewed as closed, neutral pipes through which carbon is simply transported to coastal shelves. However, carbon storage via sedimentation and transformation by heterotrophic respiration are significant fluxes impacting global carbon cycling (Cole et al. 2007). Of particular importance is the metabolism of autotrophic and heterotrophic plankton, which largely regulates the flow

of carbon through pelagic food webs (Cole 1982). In many systems, autotrophic and heterotrophic food web components may be coupled as autochthonous carbon has been shown to sustain BP while enhancing BGE, presumably due to its labile nature. It has been estimated that 30%-60% of NPP is processed by bacterioplankton in marine and freshwater systems (Williams 1981; Cole et al. 1988; Ducklow and Carlson 1992). This study therefore aimed to determine whether autotrophic-heterotrophic coupling is an important pathway of carbon cycling within the tidal-freshwater James River.

Bacterioplankton cell densities in the tidal-freshwater James River (ca. 10^{10} cells L^{-1}) were on average an order of magnitude higher than the values observed within the Chesapeake Bay (Shia and Ducklow 1994), Hudson River (Findlay 1991), and the Mississippi River plume (Chin-Leo and Benner 1992). Findlay (1991) concluded that bacterial-algal coupling in the Hudson River was weak; the Hudson is a net-heterotrophic system in which terrestrial subsidies largely support bacterioplankton metabolism. Conversely, the water column of the tidal-freshwater James River exhibited net autotrophy as measurements of plankton NPP were positive and exceeded community respiration. Univariate linear regression analysis showed BA was significantly related to CHL*a*, NPP, and DOC concentrations while BA was not related to nutrient concentrations or temperature. Collectively, these results suggest that autochthonous organic matter supply was an important determinant of bacterioplankton abundance in the tidal-freshwater James River.

Given the low coefficients of determination associated with relationships among BA, CHL*a*, NPP, and DOC concentrations (r^2 = 0.1-0.2) the effects of other environmental variables on BA should not be excluded. Further exploration of the

unexplained variation surrounding the relationship between BA and CHL*a* can provide insight into the factors regulating autotrophic-heterotrophic coupling. CHL*a*, NPP and BA exhibited asymmetrical seasonal trends at river miles 99 and 75 as the seasonal pattern of BA mirrored that of temperature. Moreover, maximum temperatures (~30^{o}C) occurred between July 6th and September 7th during which BA was seasonally highest. These observations combined suggest that autotrophic-heterotrophic coupling operates within the context of temperature effects, but during summer months temperature does not chiefly determine BA. This has also been observed within the Chesapeake Bay where BA did not vary as a function of temperature during summer months when temperatures were >20^{o}C (Shiah and Ducklow 1995).

The abundance of bacterioplankton may also be influenced by nutrient availability. In Lawrence Lake (MI) phyto-bacterioplankton coupling was explained by a simultaneous response of phytoplankton and bacterioplankton to P limitation rather than the direct exchange of autochthonous carbon (Coveney and Wetzel 1992; Coveney and Wetzel 1995). Lawrence Lake is a nutrient-limited system whereas phytoplankton in the tidal-freshwater James River have been shown to be light-limited (Lederer 2008). PO_4^{-3} and NO_3^{-} concentrations among main channel sites declined in the downstream direction while CHL*a* and BA increased causing these parameters to be inversely related (though, not significantly). This pattern may be explained by nutrient uptake due to higher abundance in the lower stretch of the sampling reach or, to dilution of nutrients by tidal exchange. Moreover PO_4^{-3}, NO_3^{-1}, and NH_3 concentrations were lower in Herring Creek relative to nearby main channel sites while BA was an order of magnitude higher. Thus,

the observed relationship between phytoplankton and bacterioplankton abundance is unlikely attributed to a concurrent response to nutrient concentrations.

The significant relationship between CHL*a* and BA may also be explained by increased water residence time in the lower stretch of the sampling reach. Shen and Lin (2006) reported that freshwater advection is the dominant force regulating transport of dissolved constituents within the tidal-freshwater James River while transport within the estuarine zone is greatly influenced by tidal mixing. That is, the transit time of a water parcel slows as it nears the tidal intrusion front which, in combination with changes in channel morphometry, enhances residence time in the lower section of our study reach (river miles 75-69; Shen et al. 1999). Greater tidal mixing within this portion of the sampling reach coupled with increased river width could account for higher CHL*a* and BA via increased residence time.

The downstream portion of the sampling reach consists of a main channel flanked by large, shallow areas in which the Rice Center Pier is located. BA at the Rice Center Pier was an order of magnitude higher in comparison to cell densities at nearby main channel sites (river miles 75 and 69). Water age (i.e. time passed since a constituent is discharged into estuary head waters) is estimated to be 5 days longer within shallow areas adjacent to the main channel in the tidal James River (Shen and Lin 2006). Therefore, near-shore areas may function as bacterioplankton source areas due to their effective retention of water and dissolved constituents. This evidence collectively suggests that relationships between phytoplankton and bacterioplankton may be mediated by hydrology. Based on observational data collected in this study, we cannot determine whether these organisms are coupled through the direct exchange of carbon or exhibiting

a shared response to water residence time. Further statistical exploration of this data set would enhance elucidation of longitudinal and seasonal covariance between BA and explanatory variables.

To assess the potential for bacterial dependency on autochthonous carbon, we derived estimates of algal contributions to the DOC pool for river miles 99 and 75 during spring and summer. Baines and Pace (1991) reported that 13% of NPP is converted to DOC; this value was used to estimate the contributions to the DOC pool via NPP. NPP was on average 3- and 11- times greater at river mile 75 (summer and spring, respectively) resulting in potentially greater contributions to the DOC pool via algal exudates (**Fig. 14**). The average DOC concentration was relatively constant in comparison (varying by 1-2 mg L^{-1}) and therefore projected turnover times generally followed differences in NPP. At river mile 75, NPP was sufficient to turnover the bulk DOC pool once every 3 days (spring and summer). At river mile 99, NPP-derived DOC would require 10 and 24 days to replace the bulk DOC pool during the summer and spring, respectively. Faster turnover rates suggest that contributions of autochthonous carbon to heterotrophic bacteria are larger at river mile 75 and may in part account for higher BA observed at this site. Lower DOC C:N ratios at river mile 75 during May, July, August, and September also support this conclusion. C:N ratios are an indicator of DOC quality with algal-derived DOC typically having lower C:N ratios (Wetzel 2001).

High rates of NPP at river mile 75 suggest that autochthonous sources of DOC may be important whereas analysis of DOC fluorescence revealed that the fulvic DOC fraction was dominated by terrestrial sources. EEMs of DOC typically contain four peaks: peaks “A” and “C” corresponding to humic-like substances where peak “A”

occurs in the UV spectrum and the protein-like peaks “B” and “T” representing tyrosine and tryptophan, respectively (Coble 1996). The range of excitation and emission wavelengths used in the current study did not allow for detection of peaks “B” and “T” as these peaks are observed at emission wavelengths between 310 and 340 nm. All EEMs produced by scanning James River DOC contained peak “C”, and the position of this peak varied by only 10-20 nm across samples. Peak A observed among James River DOC EEMs is broad and featureless indicating DOC fluorescence is produced by a heterogeneous mixture of fluorescing compounds (i.e. fluorophores), most likely conjugated and aromatic molecules (Zeichman 1988; Schulten and Schnitzer 1993). The position of peak “A” was consistent with peaks observed for DOC samples from the Amazon, Maine, Mississippi, Duwamish, Columbia, and Hawaiian Rivers (Coble et al. 1996). Riverine DOC is generally regarded as being dominated by humic and fulvic substances (Hedges et al. 1994; Thurman 1985; McKnight et al. 2001).

James River DOC fluorescence ratios, as well as for other rivers, consistently ranged between 1.4 and 1.5 suggesting that the fulvic DOC fraction was dominated by allochthonous-derived compounds that are highly aromatic (McKnight et al. 2001). The ability of the DOC fluorescence ratio to resolve whether DOC is allochthonous or autochthonous may be a function of quinone moieties (i.e. group of biomolecular compounds) with redox states that vary depending on whether they are of algal, bacterial, fungal or plant cell origin. Degradation of lignin produces plant-like quinones thereby enhancing the fluorescent signature of allochthonous materials (“lignin boost”). Rather than accumulating as lignin does, materials of microbial origin are likely transformed into biomass so the fluorescence of autochthonous DOC does not experience a similar boost,

thereby effectively biasing the DOC fluorescence ratio towards the terrestrial end member (Cory and McKnight 2005; Scott et al. 1998). This might explain why James River DOC fluorescence ratios never approached the autochthonous signature (1.9) despite high rates of NPP observed at river mile 75. Further investigation of DOC fluorescence using Parallel Factor Analysis (PARAFAC) and synchronous excitation spectroscopy in combination with the metrics explored here may increase our understanding of algal contributions to the bulk DOC pool (Stedmon 2003; Chen et al. 2003).

In addition to observed coupling of bacterio-phytoplankton abundance and production, several studies have shown that bacterioplankton community composition can also vary as a function of algal attributes. Stepanauskas et al. (2003) found that bacterioplankton community composition in the Sacramento River varied seasonally due to enhancement of DOC quality by autochthonous production. Lake mesocosm experiments demonstrated that bacterioplankton community composition (Denaturing Gradient Gel Electrophoresis analysis of 16s rRNA) shifted in association with trophic status, specifically nitrogen availability and related changes in phyto-zooplankton biomass and composition (Haukka et al. 2006). In the current study, T-RFLP PCR demonstrated that bacterioplankton communities at river miles 99 and 75 differed perhaps owing to the differences in production and DOC quality that existed between these sites. Furthermore, communities may have differed between these sites due to a greater marine influence at river mile 75 causing shifts towards phylogenies adapted to estuarine conditions (Bouvier and Del Giorgio 2002), but this is unlikely because specific conductivity exhibited little variation throughout the study reach. The application of

molecular techniques for this study represents a first-level approach to understanding the factors regulating bacterioplankton community composition and highlights the potential role of coupling. Nonetheless, much work remains to fully understand the variables responsible for structuring bacterioplankton composition and consequent affects on carbon cycling.

This study provides evidence for coupling between phytoplankton and bacterioplankton within the tidal-freshwater James River during the growing season. Specifically, increased autochthonous contributions to the bulk DOC pool at river mile 75 - as indicated by higher DOC concentrations, faster turnover rates of the bulk DOC pool, and lower DOC C:N ratios - appeared to enhance BA in this portion of the sampling reach. Indirect evidence from hydrodynamic models suggests that residence time also influences phytoplankton and bacterioplankton abundance. A simultaneous response to longitudinal variation in water residence time could account for the observed correlations of BA with CHL*a* and NPP. Nutrient and temperature effects were not observed, though temperature may play a role by affecting when bacterioplankton can utilize autochthonous carbon. Food web coupling suggests that the microbial loop could play an important role in supporting overall food web production within the tidal-freshwater James River by functioning as a carbon link rather a carbon sink. Further work is needed to quantify transfer efficiencies among bacterioplankton, bacteriovores, and zooplankton to elucidate the consequences of observed coupling.

FIGURES

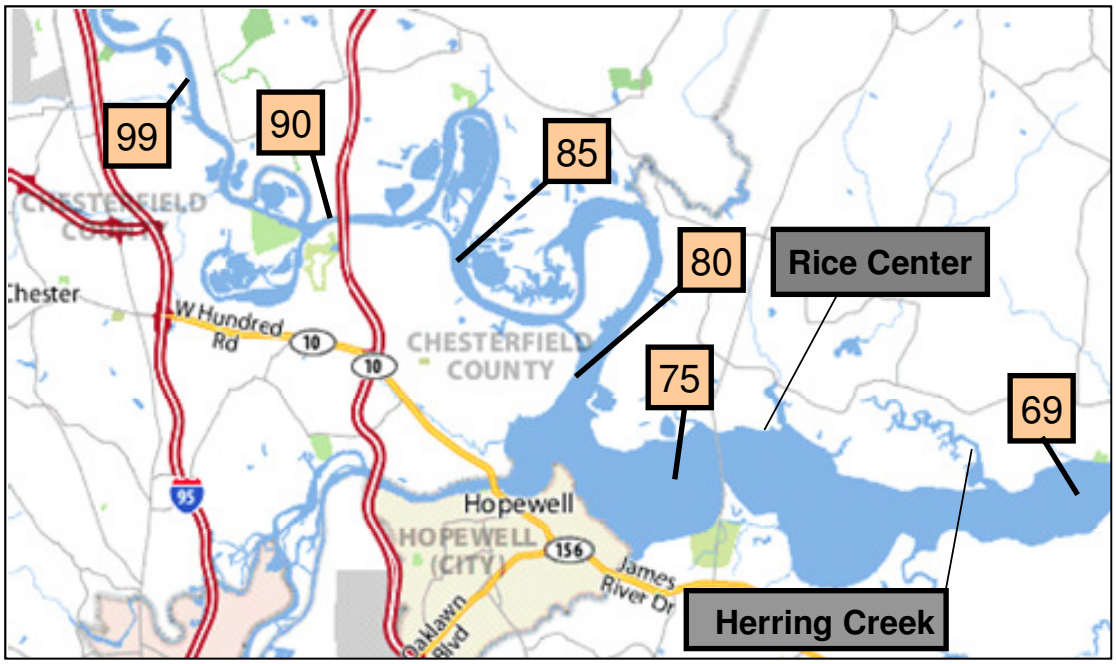

Figure 1 – Map of the tidal-freshwater James River showing sampling locations. Numbers indicate sampling locations and represent navigational river miles (i.e. miles upstream from the river mouth). Near shore samples were collected at the Rice Center pier and tributary samples were collected within Herring Creek. Map obtained using http://maps.yahoo.com.

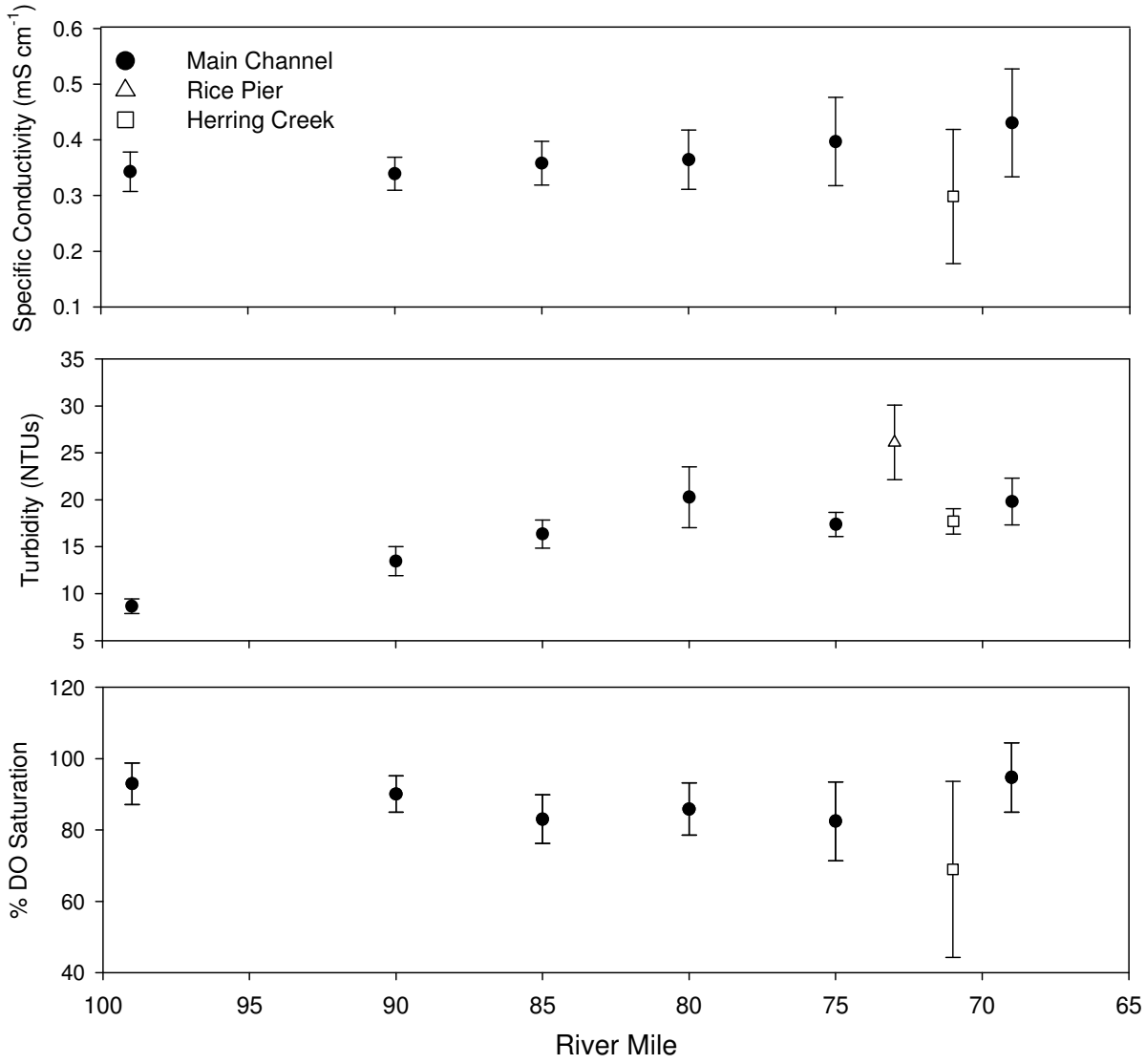

Figure 2 – Longitudinal variation in water quality parameters in the tidal freshwater James River. Values are averages for data collected at 2-3 week intervals during May-November 2007 (error bars indicate standard error).

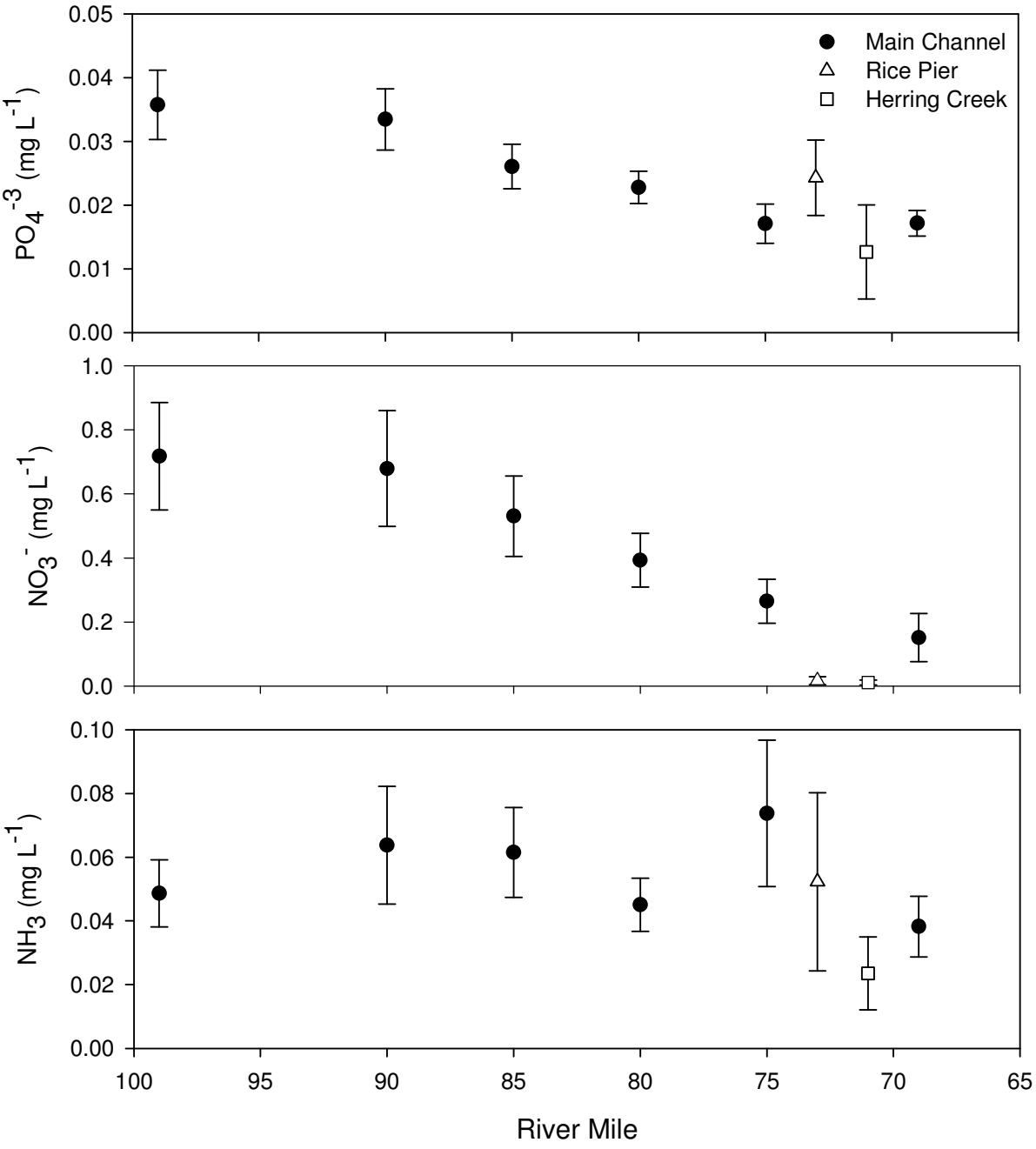

Figure 3 –Longitudinal variation in nutrient concentrations in the tidal freshwater James River during April-November 2007.

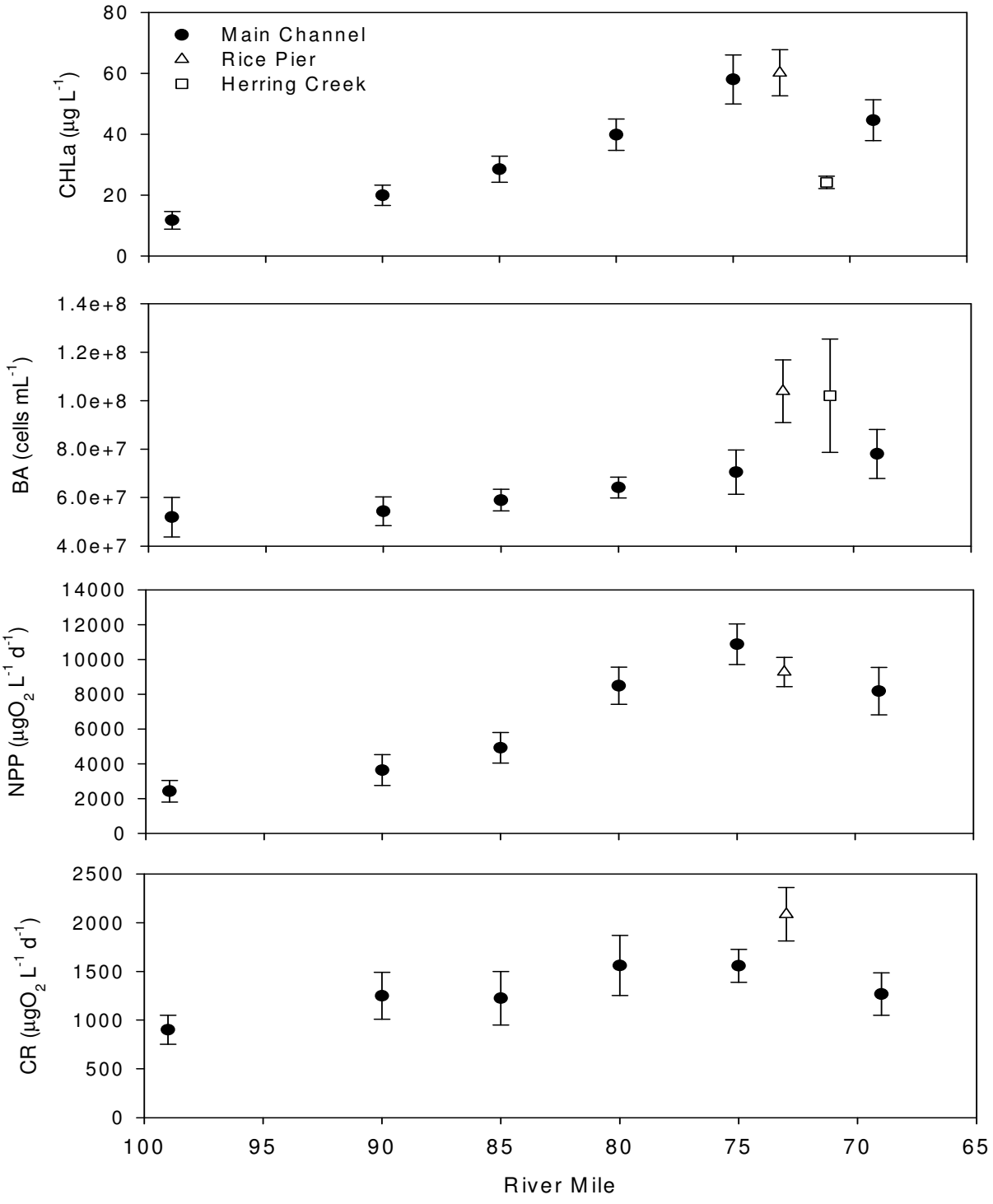

Figure 4 – Longitudinal variation in chlorophyll *a* (CHL*a*), bacterioplankton abundance (BA), net primary production (NPP) and community respiration (CR) in the tidal freshwater James River.

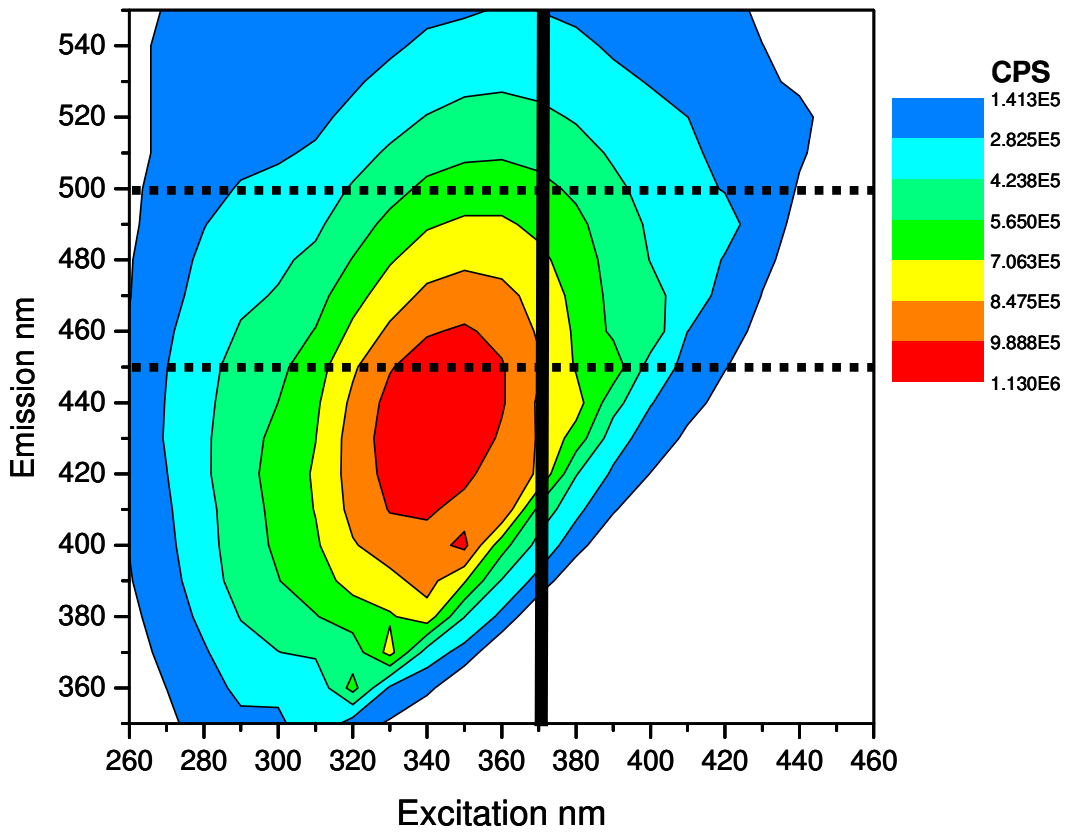

Figure 5 – Sample Excitation-Emission Matrix contour plot showing derivation of the DOC fluorescence ratio. Data shown are for the July 6th 2007 sample collected at river mile 75. Fluorescence intensity (Counts Per Second; CPS) is indicated by colored scale (z-axis). Fluorescence ratio was calculated by taking the ratio of intensities observed at 450 nm and 500 nm emission (dashed lines) at an excitation wavelength of 370 nm (solid line).

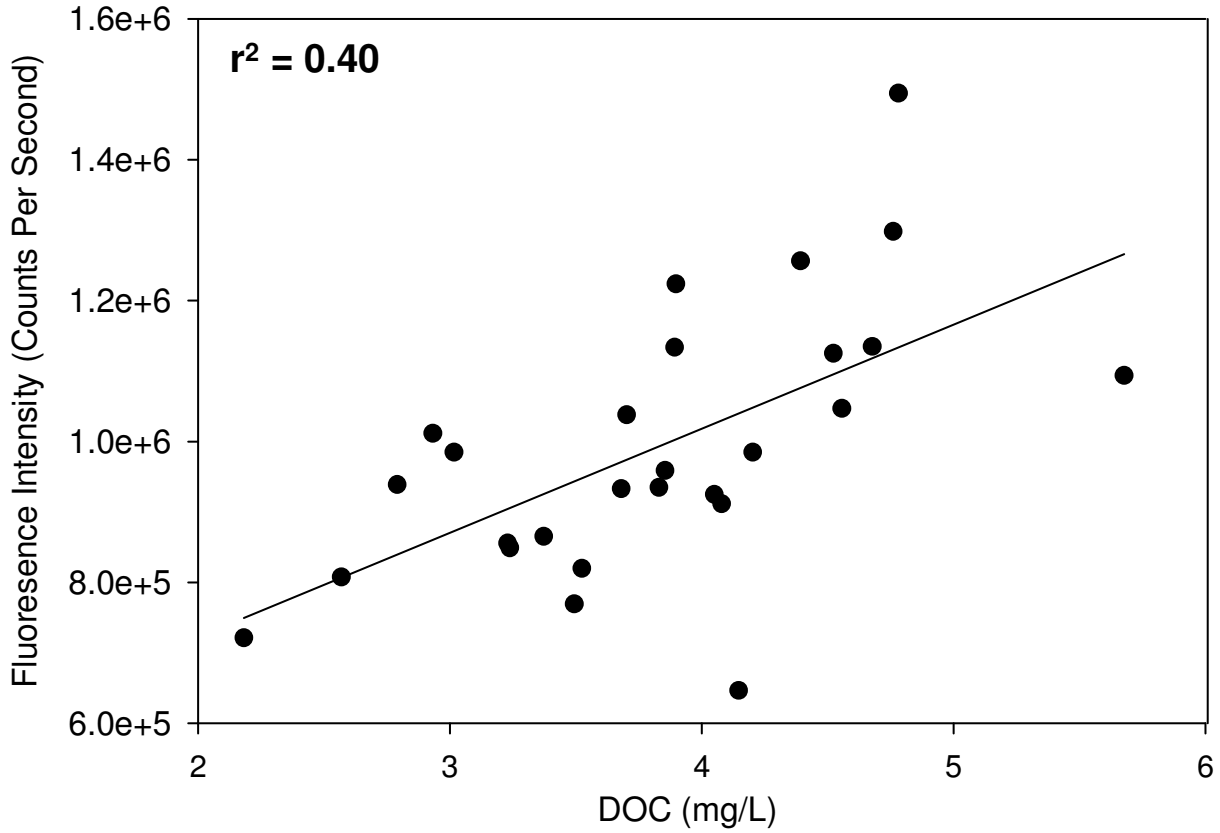

Figure 6 – Relationship between fluorescence intensity and DOC concentrations. Fluorescence intensity was measured at 450 nm emission using samples from two main channel sites (river miles 99 and 75), the near shore site and Herring Creek (May-November, 2007).

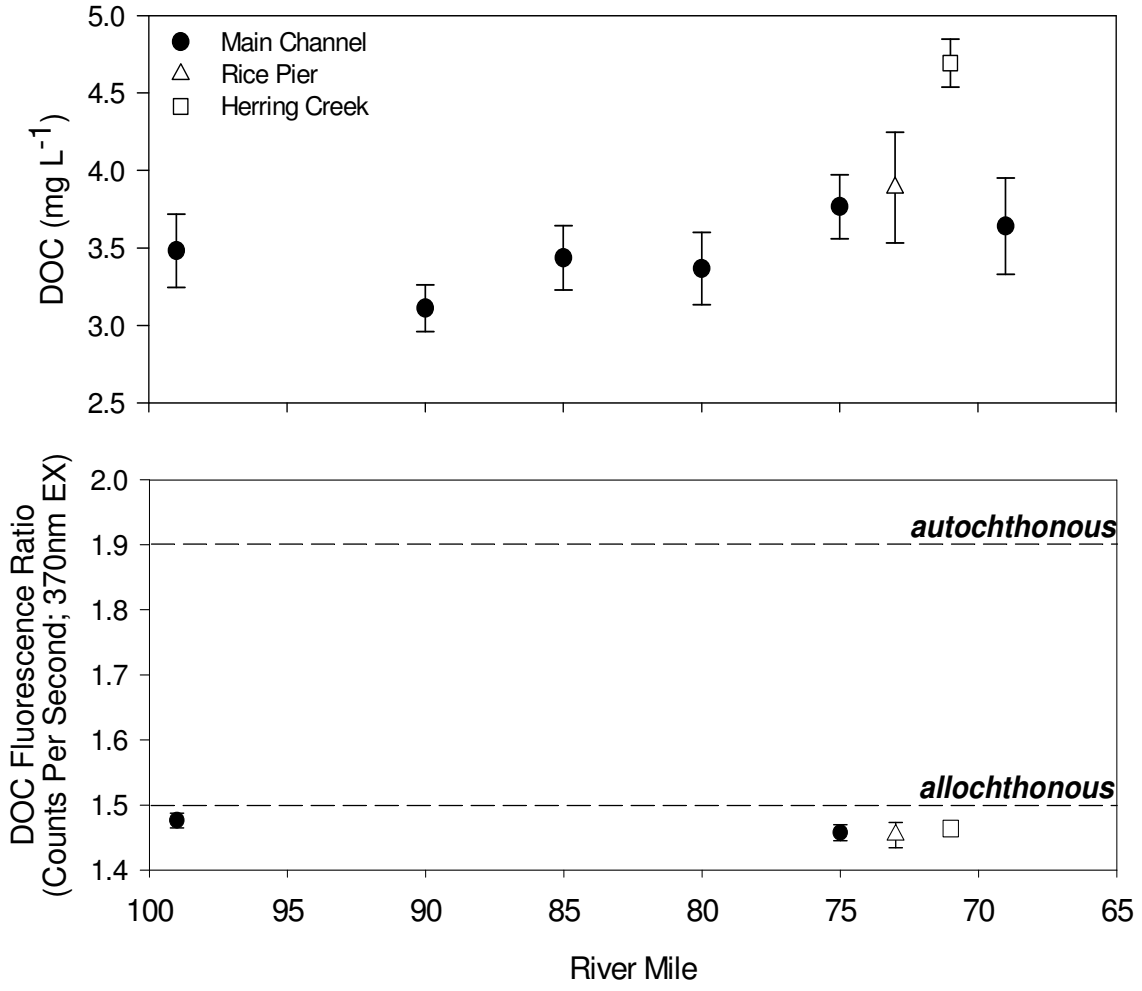

Figure 7 – Longitudinal variation in DOC concentrations and fluorescent properties in the tidal freshwater James River. DOC fluorescence ratios determined according to the procedure detailed by McKnight et al. 2001 (see methods). Horizontal dashed lines signify expected fluorescence ratios of autochthonous and allochthonous end members.

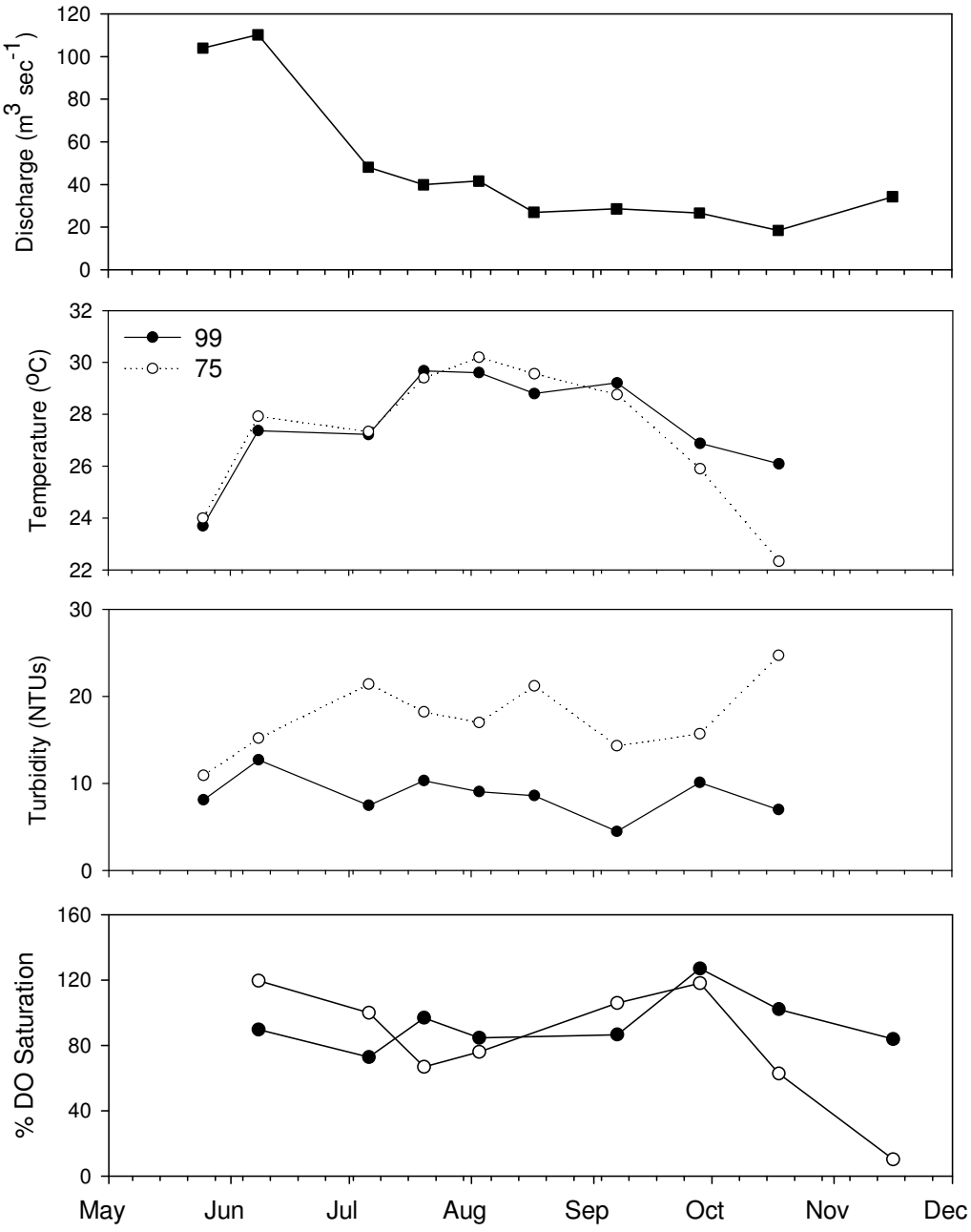

Figure 8 –Seasonal variation in discharge and water quality parameters of the tidal freshwater James River during 2007. Discharge measured at Cartersville, VA (Virginia Department of Environmental Quality) on each sampling date. Temperature, turbidity and % DO saturation are for stations at river miles 99 and 75.

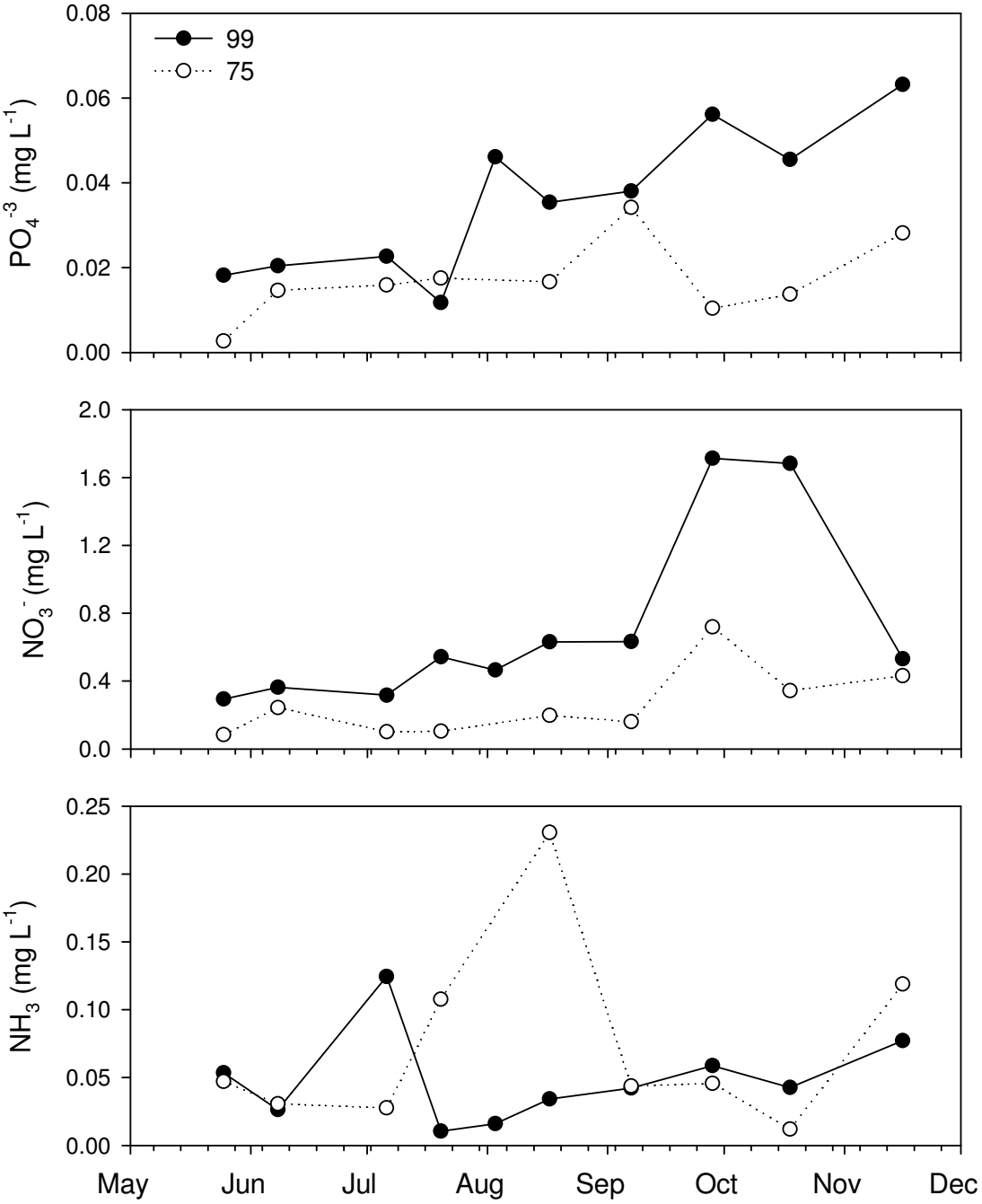

Figure 9 – Seasonal variation of ambient nutrient concentrations in the tidal freshwater James River during 2007.

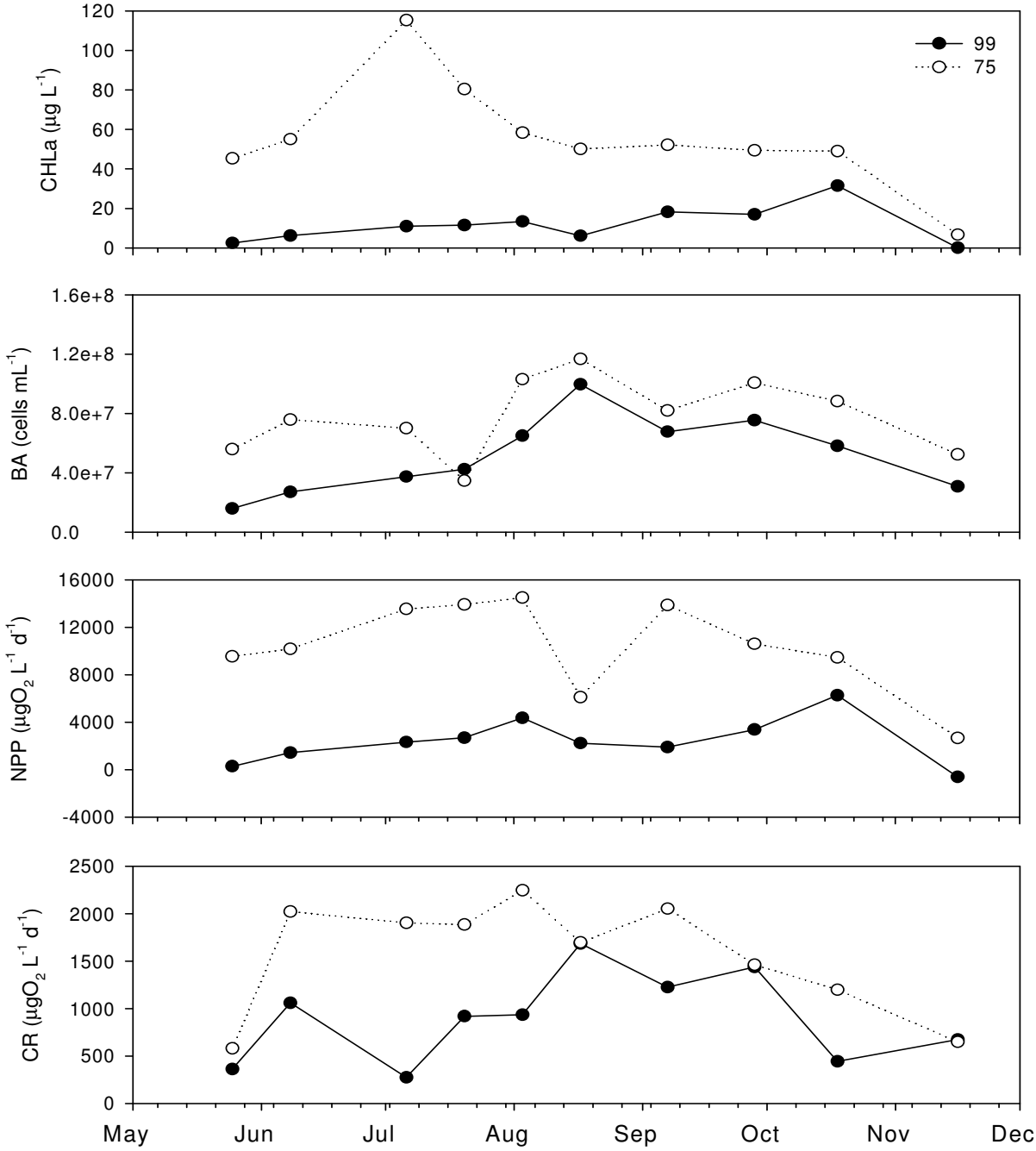

Figure 10 - Seasonal variation in chlorophyll a (CHL*a*), bacterioplankton abundance (BA), net primary production (NPP) and community respiration (CR) in the tidal freshwater James River.

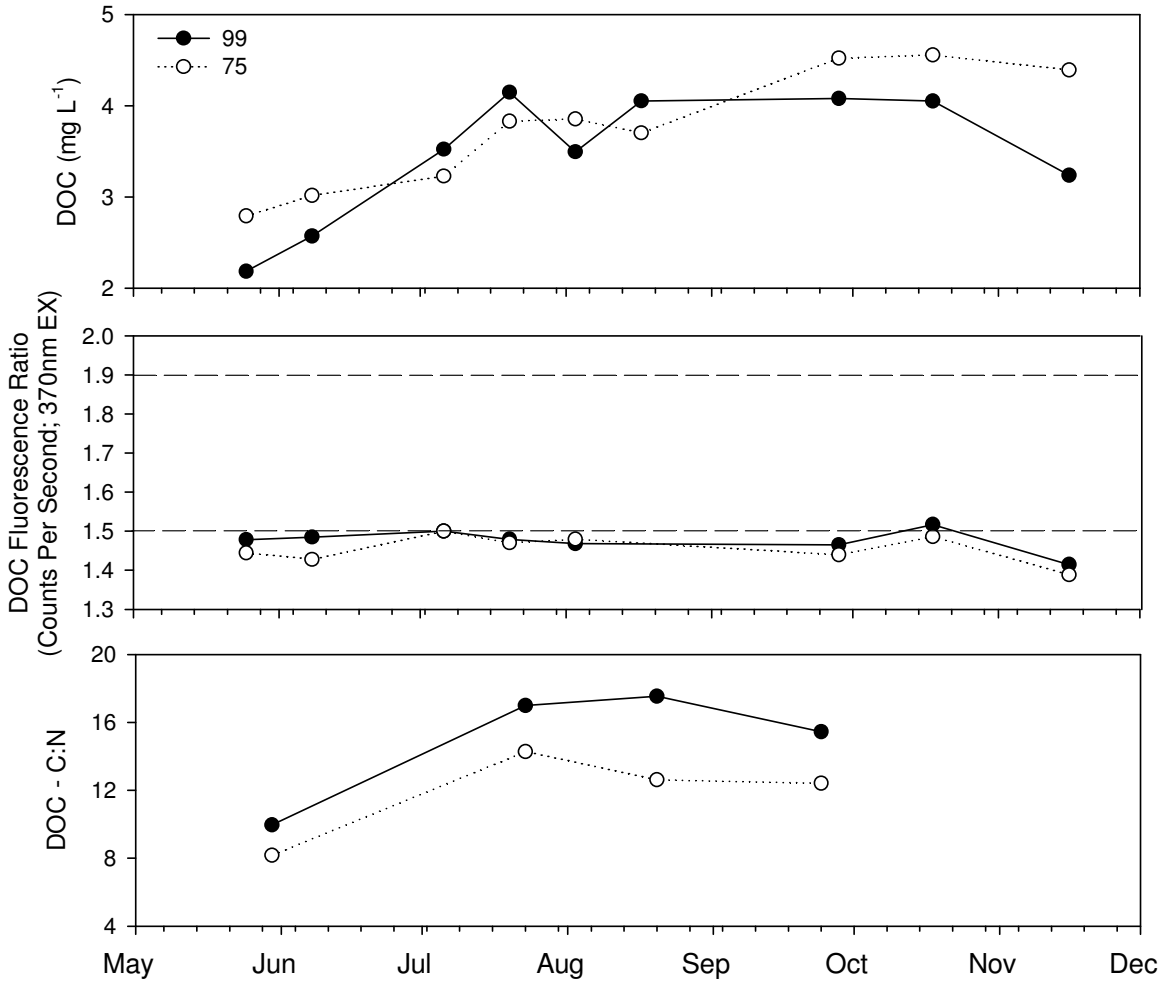

Figure 11 – Seasonal variation of DOC concentrations, fluorescent properties. and C:N ratios of DOC.

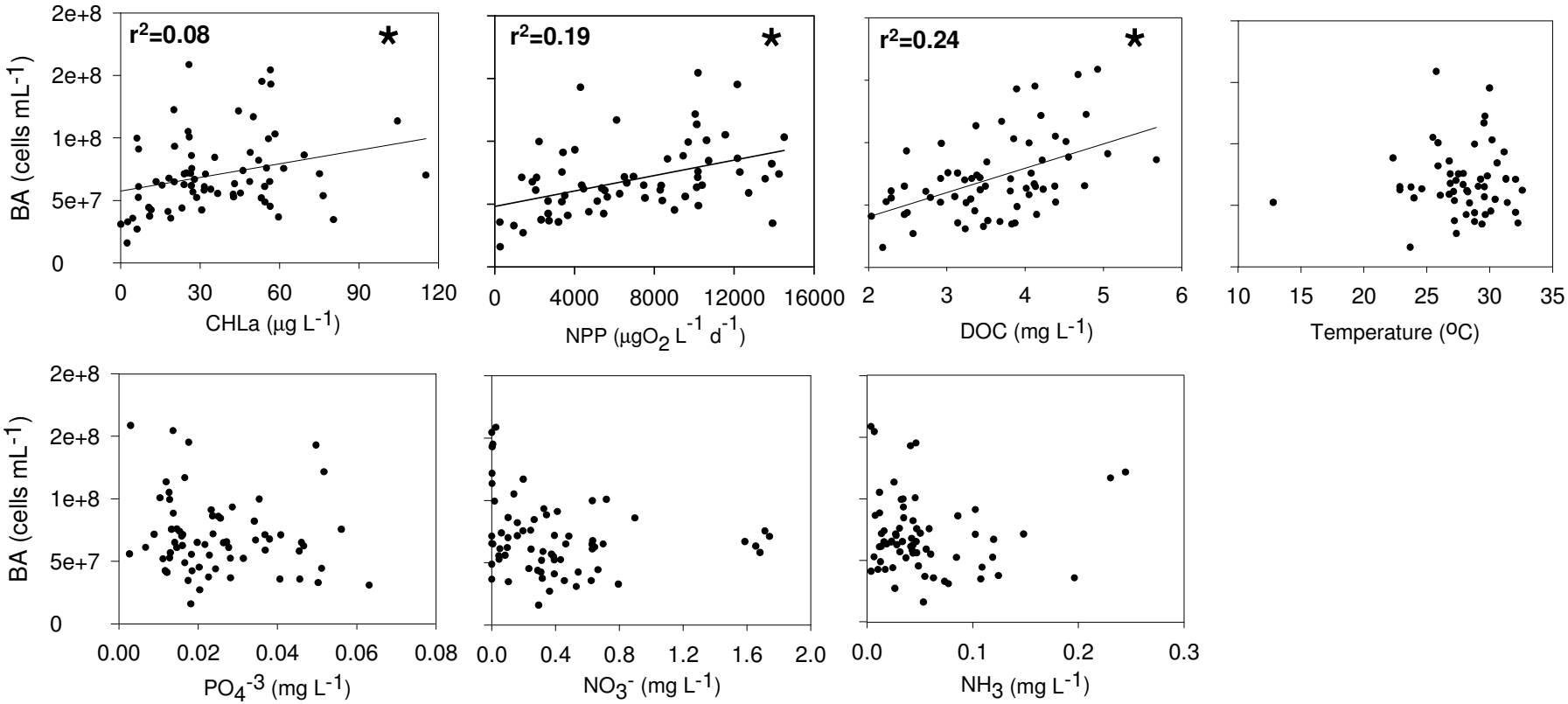

Figure 12 – Relationships between bacterioplankton abundance and environmental variables. Asterisks indicate a significant relationship for the linear regression (α=0.05). BA=bacterioplankton abundance; NPP=plankton net primary production.

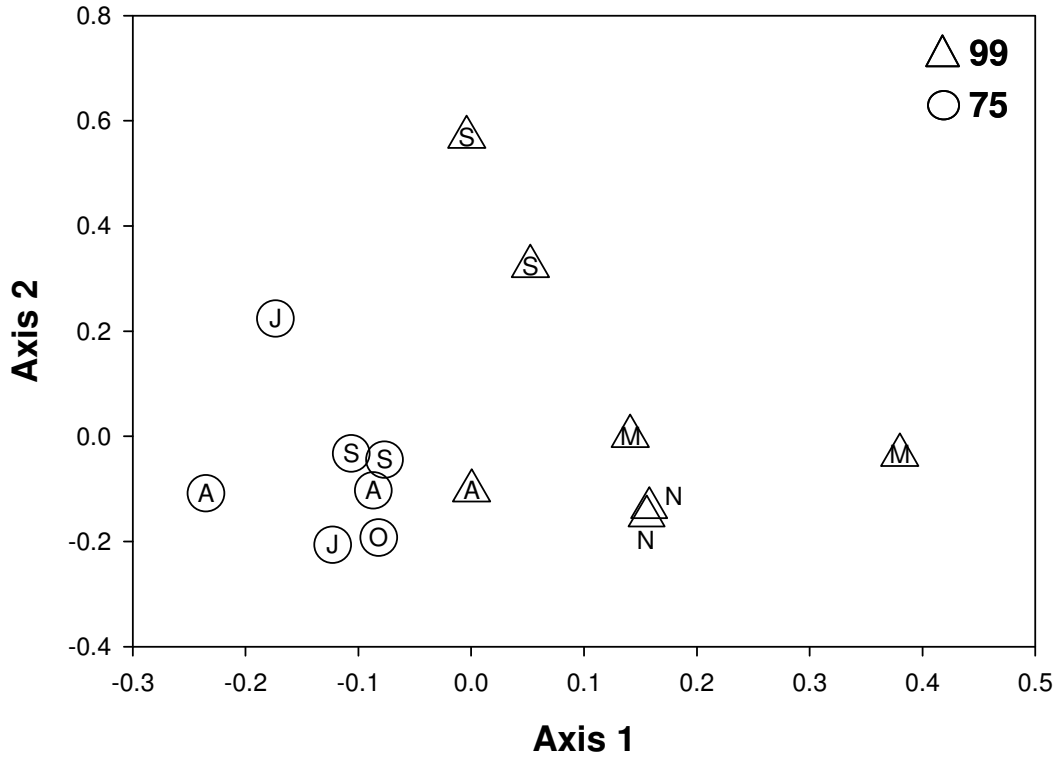

Figure 13 – Non metric multidimensional scaling plot showing longitudinal and seasonal variation in bacterioplankton community composition. Proximity of symbols denotes community similarity based on T-RFLP analyses. Letters indicate the date on which samples were collected: May 25th (M), June 22nd (J), August 3rd (A), September 7th (S), October 18th (O), and November 16th (N). Multiple months within a given site equates to a pair of replicates collected on a given date. The distance between points indicates the relatedness of bacterioplankton communities, or similarities between T-RFLP electropherograms; points closest together are more related.

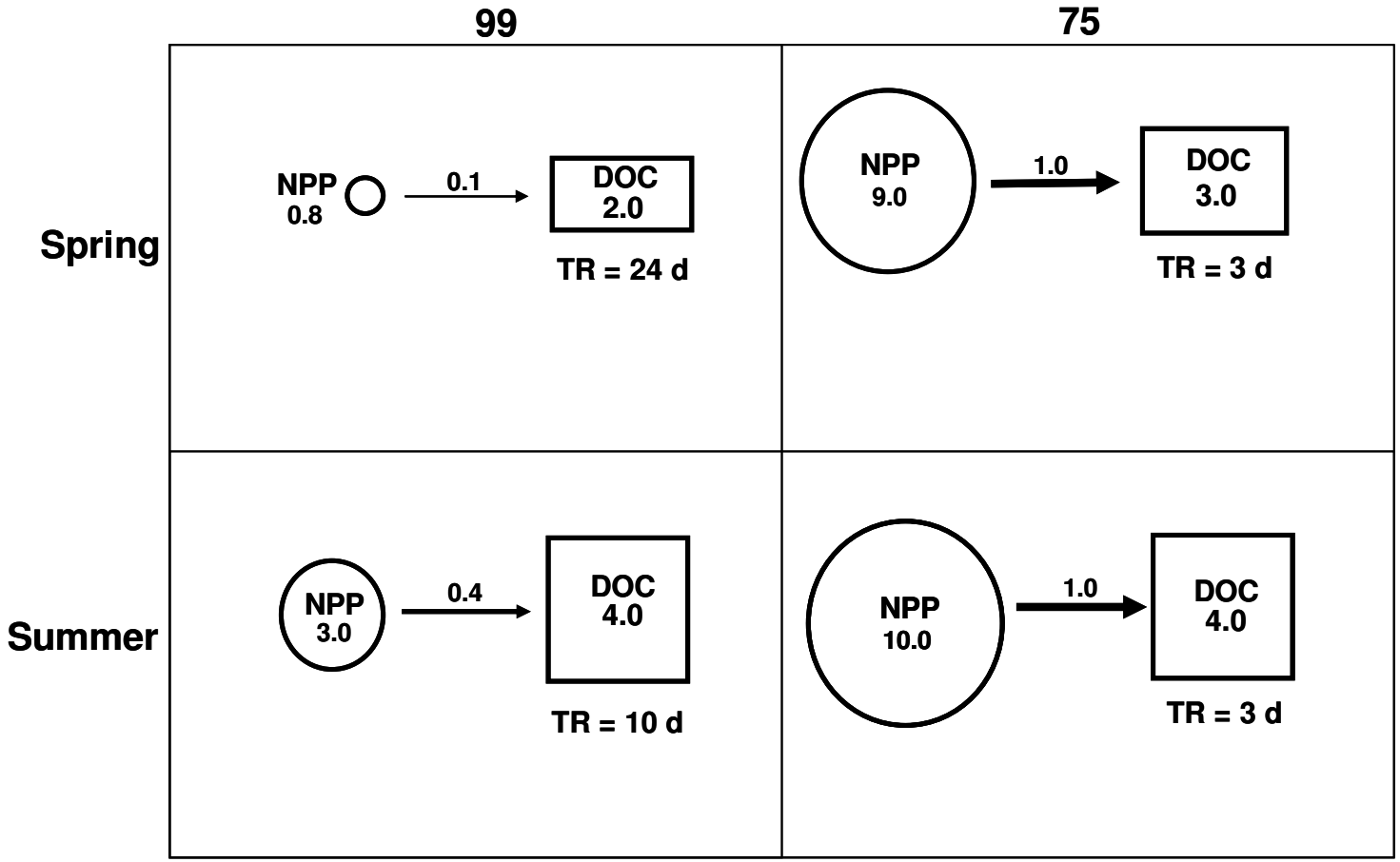

Figure 14 – Estimated phytoplankton contributions to the DOC pool of the James River at stations 99 and 75 during the spring and summer. Spring values are for data collected on May 25th and June 8th while summer averages are for July 6th, July 20th, and August 3rd. Circles = NPP (mgC L^{-1} d^{-1}); Arrows indicate the flux of NPP into the bulk DOC pool (mgC L^{-1} d^{-1}); boxes = DOC concentrations (mg L^{-1}). Turnover rates (TR) indicate the time required for NPP to replenish the DOC pool and were calculated by dividing the size of the DOC pool by the NPP conversion flux.

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Matthew Beckwith was born in Dresden, NY and graduated from Penn Yan Academy in 2002, after which he attended Keuka College. In 2006, he graduated with honors from Keuka College with a B.S. in Environmental Science. While at Keuka College, Matthew completed a senior research project through which he experimentally assessed nutrient limitations of plankton in Keuka Lake (NY). In addition, he volunteered as a laboratory technician at the Penn Yan Waste Water Treatment Plant and gained further research experience at the Kellogg Biological Station LTER (MI). Upon receiving a B.S., he began his graduate career at Virgin Commonwealth University and anticipates graduating with a M.S. (Biology) in May 2009. He was funded as a Graduate Teaching Assistant and taught introductory lab courses for majors. Teaching experience at Virgin Commonwealth University also included work with multiple environmental outreach programs. In the summer of 2009 he will be drafting manuscripts for publication.