LINKING LAND USE, NUTRIENT CONDITIONS AND PHYTOPLANKTON ABUNDANCE AND DIVERSITY IN THE NON-TIDAL CREEKS OF THE NEUSE-PAMLICO ESTUARINE SYSTEM

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ABSTRACT

This project was undertaken to determine how coastal land use and land cover affect dissolved nutrient concentration and form, phytoplankton abundance and community composition, and nitrogen buffering capacity in the non-tidal oligo- to meso-haline creeks of the Neuse Pamlico Estuarine System. The research included routine sampling of four estuarine creeks and seasonal *in-situ* nutrient addition bioassays designed to mimic nutrient loading events observed in the monitoring data. The results show the land use causing the greatest degree of disturbance (agriculture) resulted in the highest in-stream nutrient concentrations, the highest inorganic to organic nitrogen ratios, the lowest phytoplankton diversity, and decreased capacity to buffer the mainstem estuary from episodic nutrient loading. The findings underscore the need for watershed management designed to mitigate increased fresh water and nutrient loading from watershed modification upstream of estuarine creeks.

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LIST OF ABBREVIATIONS AND SYMBOLS

^{14}C	carbon isotope 14
BDL	below detection limit
BMP	best management practice
°C	Celsius degrees
ChemTax	chemical taxonomy software
ΔS	change in salinity
DIN	dissolved inorganic nitrogen
DO	dissolved oxygen
DON	dissolved organic nitrogen
FAWNA	forested and agricultural watershed nitrogen attenuation
GIS	geographic information systems
h	hour
H’	Shannon-Weiner diversity index unit
HPLC	high performance liquid chromatography
l	liter
LULC	land use and land cover
M	molar
N	nitrogen
NC	North Carolina
DENR	Department of Environment and Natural Resources
LIDAR	light detection and ranging
NH_4^+	ammonium

NO_3^-	nitrate
NO_x^-	nitrate plus nitrite
NPES	Neuse Pamlico Estuarine System
NRE	Neuse River Estuary
OGF	Open Grounds Farm
OM	organic matter
P	phosphorous
π	pi
PNA	primary nursery area
PO_4^{-3}	phosphate
ppt	parts per thousand
STAR	Science to Achieve Results
T0	bioassay initial time point
T1	bioassay time point one
T2	bioassay time point two
T4	bioassay time point four
TDN	total dissolved nitrogen
TIN	triangulated irregular network
μ	micro
UNC-IMS	University of North Carolina at Chapel Hill Institute of Marine Science
USEPA	United States Environmental Protection Agency
USGS	United States Geologic Survey
YSI	Yellow Systems Instruments

CHAPTER 1

RESEARCH RATIONALE

Anthropogenic activity and development in coastal areas and upland watersheds has altered estuarine inputs including freshwater, nutrients, sediments and organic matter (Dynesius and Nilsson 1994; Hopkinson and Vallino 1995). In some cases the impacts of human population increase and changing land use on estuaries have outpaced research on estuarine function. In the 1980's, surveys of North Carolina estuarine ecosystems determined there was a deficiency in comprehensive studies of primary productivity, trophic dynamics, nutrients budgets, or any other non-commercially motivated issues for the shallow primary nursery areas of the Neuse Pamlico Estuarine System (NPES) (Epperly and Ross 1986; Stearns et al. 1989). This knowledge gap has been narrowed with understanding gleaned from studies existing at the time, and sequentially dependent research initiatives in the years since those cautionary statements (Kirby-Smith and Barber 1979; Paerl 1983; Rudek et al. 1991; Mallin et al. 1993; Boyer et al. 1994; Mallin and Paerl 1994; Pinckney et al. 1997; Paerl et al. 1998; Christian et al. 2000; Luettich 2000; Whitall and Paerl 2001; Piehler et al. 2002; Fear et al. 2004). Comparable bodies of knowledge have also been built for estuaries throughout the United States and the World (Monbet 1992; Rabalais et al. 1996; Bricker et al. 1999; Orive et al. 2002).

The current body of knowledge in estuarine science shows that anthropogenic drivers of estuarine alteration may vary across regions, but the resulting declines in water quality are

comparable; for example, nutrient loading and poor land use in North Carolina has rivaled high population densities in Florida as a threat to estuaries (Dame et al. 2000; Clement et al. 2001). One ubiquitous threat to estuaries has been eutrophication and nutrient-induced shifts in phytoplankton production and community composition (Nixon 1995; Paerl 1998; Cloern 2001). The deleterious effects of these shifts include, altered food webs, harmful algal blooms, hypoxia, and fish kills (Sanders et al. 1987; Paerl 1988; Boesch 1996; Bricker et al. 1999; Gray et al. 2002; Landsberg 2002). Such water quality declines are well documented for the open waters of the nitrogen (N) limited Neuse River Estuary (NRE) (Paerl 1983; Mallin et al. 1993; Pinckney et al. 1997; Borsuk et al. 2004).

Various studies of the NRE have examined the drivers of eutrophication that originate far from the estuary itself; these include: upper watershed driven flow, point and non-point sourced riverine nitrogen loads, and atmospheric nitrogen deposition (Mallin et al. 1993; Peierls and Paerl 1997; Pinckney et al. 1997; Whitall et al. 2003). These remote drivers are clearly influential in controlling primary production over long temporal scales; however they do not explain all observations when applied in modeling and statistical analysis (Borsuk et al. 2004). The disparity may be explained by the short temporal scale effects of proximate drivers, including meteorological conditions, internal nutrient recycling, and surface runoff loading from proximate areas (Stanley and Hobbie 1981; Luettich 2000; Luettich et al. 2002; Borsuk et al. 2004; Fear et al. 2004). Nutrient enrichment associated with proximate runoff is the subject of continuing research on watershed nutrient transport and transformation (USEPA STAR Grant #R83-0652). The critical connection between these estuarine watersheds bordering the NPES and the open waters of the NPES are the oligo- to mesohaline creeks targeted by this study.

The oligo- to mesohaline creeks bordering the NPES estuary both harbor diverse and abundant microbial communities that mediate terrestrial-to-marine material transfer and serve as critical nursery area for diverse fisheries (Bradshaw et al. 1985; Posey et al. 2002; Ross 2003). In other systems, creeks of similar reach have provided a buffer for receiving waters by assimilating 50% to 90% of the input of dissolved inorganic nitrogen from upstream watersheds (Peterson et al. 2001; Mallin et al. 2004). Impairment of these estuarine creek capacities and alterations in phytoplankton assemblages has been cited as an early indicator of impending degradation to estuarine waters (Holland et al. 1997; Paerl et al. 2003; Holland et al. 2004). Impairment can be the result of changes in nutrient and fresh water loading that have the potential to alter 1) critical nursery area function, 2) primary productivity controls, and 3) nutrient buffering capacity (Kennedy 1984; Pinckney et al. 1997; Ross 2003). In other estuarine studies, both flow rates and loading of nutrients, pathogens, and toxins have been directly tied to watershed land use and land cover (LULC) (Lerberg et al. 2000; Holland et al. 2004; Kelsey et al. 2004). In the NRE nitrogen has been identified as the primary growth limiting nutrient (Paerl 1983; Mallin et al. 1993; Pinckney et al. 1997; Borsuk et al. 2004). Consequently, I hypothesized that watershed LULC would significantly influence the abundance and forms of the biologically available dissolved nitrogen, phytoplankton abundance and diversity, and the nutrient buffering capacity in proximate estuarine creeks. This linkage between proximate land use and nutrient processing in creeks had not previously been studied directly in the NPES. This study aimed to close that gap with regular *in situ* monitoring and controlled manipulative bioassay experiments, designed to quantify how the different LULC bordering the NPES affect nutrient loading and eutrophication in these creeks. This work was supported by the US-EPA Estuarine and Great

Lakes Program STAR Grant # R82867701 that was specifically funded to develop landscape measures that serve as quantitative indicators of estuarine environmental condition and vulnerability.

CHAPTER 2

HYPOTHESIS AND OBJECTIVES

Objective and Hypothesis #1

Topic: Nutrient conditions in non-tidal NPES creeks

Objective: Quantify the concentrations and forms of biologically available nitrogen in creeks downstream of representative watersheds draining the four distinct land uses.

Hypothesis: Current land use and land cover (LULC) bordering the NPES will significantly influence allochtonous nutrient loading to the proximate estuarine creeks. Specifically, runoff originating from the agricultural areas will be enriched in total nitrogen and soluble inorganic nitrogen fractions when compared to runoff from the forested areas.

Objective and Hypothesis #2

Topic: Creek phytoplankton community

Objective: Compare and contrast the abundance, composition, and diversity of the dominant phytoplankton taxonomic groups between the creeks.

Hypothesis: Contrasting nutrient loads from different LULC will influence the abundances, composition, and diversity of the dominant phytoplankton community groups. Specifically, the reference and forested creeks will contain lower total community abundance and consist of diverse groups adapted to consistently low concentrations of DIN (e.g. cyanobacteria), whereas the agricultural creeks will contain higher total abundance and consist of groups suited to high DIN concentrations in chronic and episodic loads (e.g. chlorophytes, dinoflagellates).

Objective and Hypothesis #3

Topic: Creek nutrient buffering capacity

Objective: Determine if these non-tidal estuarine creeks serve as effective nutrient buffers for the main-stem estuary.

Hypothesis: High productivity and nutrient assimilation rates in the proximate creeks will provide an efficient nutrient buffer for the main stem of estuary during both average base flows and episodic storm-level flows. Specifically, in-stream nutrient attenuation in all creeks will be directly related to levels of phytoplankton chlorophyll *a*.

This project addressed these research questions using LULC assessments, study site selection, regular *in-situ* monitoring of creek conditions, and controlled manipulative bioassay experiments. The unifying goal was to combine the knowledge gleaned from addressing the three objectives to determine LULC influence on dissolved nutrient concentration and form, phytoplankton abundance and community composition, and nitrogen buffering capacity in the non-tidal oligo- to meso-haline creeks of the NPES.

CHAPTER 3

LITERATURE REVIEW

The literature review topics were selected and organized to address the following topics central to this research: 1) environmental controls of estuarine phytoplankton, 2) ecological significance of estuarine creeks and potential for degradation, and 3) current methods and results in studies of LULC impacts on receiving waters.

3.1 Environmental Controls of Estuarine Phytoplankton

The factors controlling phytoplankton productivity and biomass at any given point in time are numerous and complex and represent a formidable challenge to mechanistic or statistical modeling (Borsuk et al. 2004). This complexity does not suggest the dominant controls are not identified and understood; rather, that their relative influence vary enough through space and time to warrant continued examination (Turpin and Harrison 1979; Cloern 2001; Paerl et al. 2003).

Proximate creeks are connected to the main stem estuary waters; however, they do not necessarily exhibit similar nutrient limitation, nitrogen amounts and forms, and hydrologic conditions documented as main stem drivers (Noble et al. 2003). The following subsections contain reviews of existing research on three dominant drivers: *nutrient limitation, nitrogen form, and hydrology*. These controls were selected to highlight both distinct and common conditions between the estuarine open waters and estuarine creek environments.

Nutrient Limitation

The major chemical requirements for phytoplankton photosynthesis and growth are generally accepted as the Redfield (1958) molar ratios of Carbon:Nitrogen:Phosphorous (106:16:1). A deficiency in the availability of any of these three requirements has been commonly referred to as "limiting" in reference to phytoplankton growth. However, these three elements do not represent all nutrients required for growth, nor are the ratios absolute; for instance, N:P ratios have varied from 5:1 to 34:1 across a broad range of systems (Geider and La Roche 2002). In many estuarine systems, nitrogen (N) has been identified as the nutrient most often limiting phytoplankton growth, with an increasing degree of N limitation as salinity increases along the salinity gradient (Ryther and Dunstan 1971; D'Elia et al. 1986; Howarth 1988; Hinga et al. 1995). It follows that bioassay experiments have identified inorganic forms of nitrogen (NO_x^- and NH_4^+) as limiting of primary productivity in the open waters of the NPES and the tidal estuarine creeks of southern North Carolina (Rudek et al. 1991; Mallin et al. 2004; Piehler et al. 2004).

However, N enrichment does not consistently or uniformly increase the biomass of all phytoplankton; N additions have shown group specific phytoplankton response in 18 month mesocosm experiments (Sanders et al. 1987). This group specific response to varied nitrogen loadings reveals competitive differences in nutrient limited phytoplankton. Different growth rates under similar conditions are referred to as 'r' vs. 'K' selection, or affectionately "sippers" vs. "gulpers" (Morris 1980; Kilham and Hecky 1988). This concept suggests that phytoplankton may have a competitive advantage under nitrogen concentrations that are consistently high, consistently low, or fluctuating based upon uptake kinetics and cell surface area to volume ratios (Kilham and Hecky 1988; Stolte et al. 1994; Hein et al. 1995).

However, competitive advantages are not limited to varied surface area to volume ratios. Persistently low dissolved inorganic nitrogen (DIN) concentrations may also affect phytoplankton community composition. For example, the low DIN (and P sufficient) loads observed in forested creek runoff may favor cyanobacteria capable of nitrogen fixation (Piehler et al. 2002).

Nitrogen Form

Phytoplankton expend more metabolic energy assimilating N from NO_x^- versus NH_4^+. Phytoplankton can directly utilize NH_4^+, whereas NO_x^- cannot be utilized before being reduced to NH_4^+ in enzyme catalyzed reactions (Wheeler 1983; Boney 1989). The enzyme catalyzed reactions increase the energy cost of NO_x^- utilization and suggest these is a competitive advantage in NH_4^+ utilization. Selective uptake of NH_4^+ over NO_x^- has been documented in observational data (Pennock 1987). However, bioassay experiments have not consistently found selective NH_4^+ uptake causes significant differences in phytoplankton biomass or community composition (Stolte et al. 1994; Harrington 1999; Richardson et al. 2001). These findings suggest the increased energy cost of NO_x^- utilization may interact with other growth regulating factors such as light availability and vertical mixing (i.e. fluctuating light regimes), or may not be significant enough to impact primary productivity, biomass, or community composition.

Nitrogen utilization by phytoplankton is not limited to obligatory inorganic assimilation in fact, bioassay experiments have revealed stimulation of growth in response to dissolved organic nitrogen (DON) additions (Bronk and Glibert 1993; Lewitus et al. 2000). DON assimilation is a function of the chemical composition of the DON and physiological capabilities of the phytoplankton (Antia et al. 1991). The chemical composition of the DON

may determine the bioavailability of the particular DON form (Bronk and Glibert 1993; Peierls and Paerl 1997; Seitzinger et al. 2002; Twomey et al. 2005). Low molecular weight forms of DON (i.e. urea) are readily assimilated, whereas, more refractory humic substances are less available (Carpenter et al. 1972; Paerl 1988; Twomey et al. 2005). However, not all phytoplankton can utilize DON. The physiological configuration of the phytoplankton determines heterotrophic ability. Heterotrophic phytoplankton posses at least one of two different enzymes necessary for DON (urea) metabolism (Bonin and Maestrini 1981; Paerl 1988; Antia et al. 1991).

Phytoplankton DON utilization was expected in NPES creeks for three reasons. First, DON utilization has been documented in waters where inorganic N was scarce and organic N was available (Chang et al. 1995; Wafar et al. 1995). Second, Seitzinger et al. (2002) found LULC influenced the bioavailability of DON in coastal runoff. Seitzinger et al. (2002) found DON was increasingly bioavailable (i.e. less complex) in runoff from forests, agricultural land, and developed land. Third, bioavailable DON was shown to alter phytoplankton community composition in Graneli and Moreira (1990). Graneli and Moreira (1990) demonstrated that humic acid enrichment can influence a shift in species composition from diatoms to dinoflagellates in laboratory cultures.

Hydrology

Hydrology influences and often defines both main stem estuaries and nursery creeks. Hydrologic drivers include lunar tides, flow, and meteorology (Snow et al. 2000). These drivers control residence time in estuaries. Residence time can influence phytoplankton growth, biomass accumulation and community composition via many processes including nutrient cycling and bloom initiation (Paerl 1988; Monbet 1992; Koseff et al. 1993; Snow et

al. 2000; Richardson et al. 2001; Noble et al. 2003). Nutrient cycling is the nutrient utilization path in which nutrients (i.e. N) are transformed from inorganic to organic forms and *vice versa* via assimilatory, dissimilatory and degradative reactions (Stanley and Hobbie 1981). Nutrient cycling is referred to as nutrient spiraling when each successive cycle results in net downstream nutrient transport (Newbold et al. 1981; Ensign 2004). The concept of nutrient cycling helps illustrate the connection between residence time, nutrient availability, and phytoplankton. Long residence time translates into short downstream transport during each nutrient cycle. Shorter downstream transport increases the number of sedimentary regeneration cycles; thereby allowing each unit of nitrogen more "opportunity" to serve as a nutrient source in support of phytoplankton growth. Under relatively long residence conditions, a single atom of N could be assimilated in multiple bloom events before being removed from the system. This is the case in the NRE where summer residence time can be quite long (up to 90 days) due to negligible tidal influence reduced freshwater flow and restricted circulation (Robbins and Bales 1995; Pietrafesa et al. 1996; Paerl et al. 2001; Luettich et al. 2002). Conversely, macrotidal systems have shorter residence times that typically increase the system sensitivity of nitrogen inputs (Monbet 1992).

3.2 Estuarine Creeks: Ecological Significance and Vulnerability to Modification.

The soft bottom proximate creeks of the NPES function as the Primary Nursery Area (PNA) for juvenile fish species spawned both inside and outside the estuary. For many species, including North Carolina's state fish, the Red Drum, these habitats directly support key development stages that have the greatest influence on population growth (Deegan and Day 1985; Ross 1985; Ross 2003; Levin and Stunz 2005). In terms of estuarine ecology, Holland (1997; 2004) suggested that the position of estuarine creeks, as connections between

land and estuary, make them ideal early indicators of impending harm from local sources of development.

The optimum PNA for each individual species is defined by a set of parameters that are shared across a broad range of species. These parameters include adequate salinity, dissolved oxygen, temperature, sediment type, shelter from predators, rich food sources, depth, and distance from ocean inlets (Deegan and Day 1985; Ross 1985; Wolfe 1986; Hoss and Thayer 1993; Able and Kaiser 1994; Rubec et al. 1998; Kirby-Smith et al. 2003; Ross 2003). Land use in estuarine watersheds can alter these critical characteristics of PNA creeks either: directly, through abiotic processes, or, indirectly, through biologically mediated processes. Watershed runoff that lowers salinity, delivers toxins, and increases turbidity is an example of an abiotic alteration (Kirby-Smith and Barber 1979; Sanger et al. 2004). Watershed runoff that increases in-stream nitrogen concentrations is an example of an indirect biologically mediated alteration. Specifically, high nitrogen concentrations that are often found in agricultural runoff, do not directly harm juvenile fish. However, a nuisance phytoplankton bloom initiated by nitrogen laden runoff can have a measurably negative effect on juvenile fish (Paerl et al. 1998; McNatt and Rice 2004). Two examples of indirect alteration processes are *Low Dissolved Oxygen* and *Impaired Trophic Transfer*.

Low Dissolved Oxygen

In proximate estuarine creek low dissolved oxygen concentrations can be exacerbated by three factors common in estuaries. First, salinity driven density stratification can limit oxygen supply to bottom waters by isolating bottom waters from atmospheric gas exchange. Second, microbial metabolism and oxygen demand may be elevated in the shallow creeks where solar energy can readily increase water temperatures. Thirdly, watershed runoff

increases allochtonous and autochtonous OM loading (Bradshaw et al. 1985; Stanley and Nixon 1992; Able and Kaiser 1994; Pinckney et al. 1997; Ross 2003). Phytoplankton-derived OM created during a runoff stimulated bloom is an example of autochtonous organic matter loading. Phytoplankton-derived OM can settle to the bottom; either as waste products from zooplankton grazing, or as ungrazed, senescing cells. Microbially mediated decomposition of settling OM consumes dissolved oxygen driving hypoxia (DO < 2mg l^{-1}) and anoxia (no measurable DO) in stratified bottom waters. (White and Roman 1992; Mallin and Paerl 1994; Cloern 2001). Hypoxia and anoxia can have negative effects on fish and shellfish populations (Baker and Mann 1992; Stanley and Nixon 1992; Bell and Eggleston 2005). The negative impact of low oxygen on fisheries resources range across non-lethal negative costs in growth and fitness, alterations in predator-prey behavior, and death of chronically exposed communities (Bejda et al. 1992; Breitburg et al. 1997; Eby and Crowder 2002; Bell and Eggleston 2005). In addition to the aforementioned low dissolved oxygen impacts, certain creeks host highly concentrated fish populations for brief periods, suggesting that even limited spatial and temporal periods of low oxygen in PNA creeks could have a significant impact on the year class of a particular species (Able and Kaiser 1994; Ross 2003). Chronic impairments are more alarming in their ability to disrupt multiple year classes via persistent juvenile mortality (Collins et al. 2000; Campbell and Goodman 2004; Levin and Stunz 2005) The point at which such irreversible damage occurs has not been clearly defined. However, cases of limited recovery in formerly impacted systems have been clearly documented (Diaz and Rosenberg 1995; Gray et al. 2002).

Impaired Trophic Transfer

Trophic transfer studies have found high secondary and tertiary productivity in estuaries is largely supported by phytoplankton-based primary production (Ryther 1969; Deegan and Day 1985; Day 1989; Mallin and Paerl 1994; Hughes et al. 2000; Capriulo et al. 2002). Multiple trophic transfer studies have used isotopic tracers to document the enormously complex estuarine food web (Nixon 1981; Hughes et al. 2000). The complex and variable food web can be generalized as follows: Energy from primary production passes successively through zooplankton, secondary consumers (planktivores), and tertiary consumers (piscivores); eventually, being exported to the ocean as living biota or biotic detritus. The current eutrophication paradigm suggests excess production stimulated by anthropogenic eutrophication may not be transferred to higher trophic levels (Cloern 2001). Conclusive documentation of such trophic impairment in a single system has yet to be completed due to sporadic research efforts, complex food webs, and lack of baseline data. However, existing research has concluded that estuarine systems are vulnerable to this type of trophic disruption.

The vulnerabilities of estuarine trophic transfer can be drawn from evidence across series of eutrophied estuaries. In the NPES, zooplankton have been shown to graze as much as 45% of daily phytoplankton productivity (Mallin and Paerl 1994). Annual peaks in zooplankton grazing coincide with the arrival of juvenile fishes in the systems PNAs (Deegan and Day 1985; Epperly and Ross 1986; Mallin and Paerl 1994). Phytoplankton size, nutritional value, and toxicity can increase or decrease grazing by zooplankton and other primary consumers (Mallin and Paerl 1994; Haywood and Burns 2003; Leonard 2003). Moving up the next rung in the trophic ladder, Allen et al. (1995) observed selective

consumption of zooplankton by zooplanktivorous fishes species found in the NPES. Furthermore, stable isotope tracer studies have shown phytoplankton derived organic matter in juvenile fish that are present in the NEPS nursery areas (Winslow 1988; Menhinick 1991; Weinstein et al. 2000). These studies suggest alterations in phytoplankton composition in estuarine creeks may inhibit transfer of energy from primary producers to higher trophic levels. This potential loss of productivity can extend well beyond the creeks themselves, due to the migratory nature of many estuarine dependent species (Deegan and Day 1985; Diaz and Rosenberg 1995; Peterson et al. 2000).

3.3 Current Methods and Results in studies of LULC Influence on Receiving Waters

In estuarine systems, existing studies of LULC influence have focused on rates at which runoff delivers sediments, nutrients, pathogens and contaminants to receiving waters (Lerberg et al. 2000; Holland et al. 2004; Sanger et al. 2004; Van Sickle et al. 2004). Many of the methods and results from these existing studies were applicable to this study of estuarine creeks. The following section of this literature review has been divided into two parts to address how existing research benefited this research project: (1) study site selection in LULC impact assessments, and (2) results from analogous studies of LULC influence on estuarine systems.

Site Selection in LULC Impact Assessments

Ideally a monitoring study should be designed as closely to an experimental study as possible. Specifically, monitoring study should be set up to eliminate alternative explanations for observations by isolating a hypothetical causal variable. Difficulties with alternative explanations can be found in several other studies of land use and land cover influence on estuarine waters. In a tidal estuarine creek study, comparable to work presented here,

Holland et al. (2004) selected heterogeneous watersheds that required numerous caveats in LULC classification. For instance, "suburban" LULC was defined as, "*>30% but < 70% urban/suburban land cover with a human population density >5 but < 20 individuals/ha or >10% but < 50% of watershed as impervious cover*.". This mixed LULC type was related to in stream water quality observations using first order and multivariate regression techniques. Regression methods are robust and have been used in other LULC studies (Lerberg et al. 2000; Van Sickle 2003; Holland et al. 2004; Kelsey et al. 2004). However, multivariate regressions have identified relationships between parameters that were not necessary causal. For example, Kelsey et al. (2004) found a significant relationship between residential septic tank density and fecal coliform bacteria abundance in downstream estuarine waters. However, Kelsey et al. (2004) recognized domestic pets living near the septic tanks were the most likely sources of in-stream fecal coliforms because properly functioning septic tanks are not considered significant fecal coliform sources. Mixed watershed LULC also complicated an assessment of residential dock construction in Sanger et al. (2004). Specifically, in-stream toxins originating in residential dock construction could not be distinguished from originating from other anthropogenic watershed activities. Van Sickle et al. (2004) selected homogenous watersheds to model various land use impacts on streams and avoided the aforementioned problems common in heterogeneous LULC regressions. Confidence in LULC impact assessments can also be increased by selecting watersheds in close proximity to each other. For example, in the Newport River Estuary, NC Sanders and Kuenzler (1979) found greater phytoplankton biomass downstream of a sewage outfall than was found downstream of a reference watershed. However, the increased phytoplankton biomass downstream of the sewage outfall could not be conclusively linked to the sewage outfall

because of stark differences in irradiance and temperature between the sewage outfall site and the reference site.

These examples highlight site selection issues considered in planning this study of estuarine creeks. Where possible, the selection of spatially analogous and internally homogenous watersheds will reduce the number of factors influencing the study area. Reducing the possible number of influencing factors should, in turn, decrease the complexity of techniques required to interpret results.

Results of Studies in LULC Impact on Estuarine Systems

Studies of LULC impacts on creeks in other ecosystems have focused on variations in loading and in stream responses. The loading parameters have included flow, nutrients, organic matter, salinity, toxins, and turbidity (Corbett et al. 1997; Wahl et al. 1997; Sanger et al. 1999; Mallin et al. 2001; Sanger et al. 2004). The response parameters have included bacteria, phytoplankton, benthic invertebrate fauna, shellfish, and fish (Lerberg et al. 2000; Cressman et al. 2003; Arnold et al. 2004; Holland et al. 2004; Sanger et al. 2004). Studies covering parameters that influence the phytoplankton are briefly detailed below.

Mallin et al. (2004) identified nitrogen limitation and vulnerability to anthropogenic development in the macrotidal estuarine creeks located inside North Carolina barrier islands. The Mallin et al. (2004) study also found NO_3^- was the primary DIN form in creeks draining developed watersheds, while regenerated NH_4^+ dominated the waters below undisturbed lands. Similarly, Wahl et al. (1997) found higher NO_3^- concentrations in urban estuarine streams and higher NH_4^+ in forested estuarine streams of the Murrells Inlet, SC estuarine system. Wahl et al. (1997) also observed a greater than 100% per unit area load of DIN from the urbanized watershed when compared to a forested watershed. Barnes (2004) identified a

relationship between high in-creek dissolved NO_3 and increasing degrees of watershed agriculture. In the same creeks selected for this study, Kirby-Smith and Barber (1979) found in-stream turbidity increased 10 to 20 times after the conversion of forest land to agriculture. Increased DIN load has been shown to promote monotypic phytoplankton blooms and decreases in community diversity in estuarine creek phytoplankton population (Sanders and Kuenzler 1979).

3.4 Literature Review Summary

This literature review highlighted links between land use and land cover, primary production, and estuarine creek condition. Specifically, the literature review underscores four general findings relevant to the study presented in the following chapters. First, watershed LULC can quantifiably influence nutrient and allochtonous material loading. Second, phytoplankton abundance and diversity can be varied via experimental manipulation of nutrient loading. Third, altered phytoplankton communities can have negative impacts on estuarine ecosystem conditions. Finally, study site selection is a key component in linking LULC, nutrient conditions and phytoplankton response in downstream estuarine waters

CHAPTER 4

STUDY SITE

The project objectives and hypotheses were evaluated in four estuarine creeks draining to the NPES. The project creeks were selected for study based on three criteria. First, the creeks needed to be located in close proximity to each other and have similar morphometrics (e.g., length, width, and depth). Second, the upstream watersheds needed to be representative of the primary LULC types present around the NRE (agriculture, unmanaged forest, and silviculture) (Table 4.1). Third, the upstream watersheds needed to have a homogenous LULC type. These criteria were selected to eliminate alternative explanations for observed differences in nutrient conditions and phytoplankton response. The intention was to isolate the hypothetical causal variable (upstream watershed LULC).

Candidate creeks were evaluated via aerial photographs and field inspection. Creek systems that satisfied each of the three criteria listed above were selected near and within the South River sub-estuary (Figure 4.1, Figure 4.2). A sub-estuary is a system that receives direct runoff from a local coastal watershed and exchanges water at its mouth with a main-stem estuary (Gallegos et al. 1992). Sampling sites in each creek were established in the South River sub-estuary and at the headwater, mid-point, and mouth of each creek. The locations of the sampling sites and identification numbers are detailed Figure 4.2.

4.1 Creek and Watershed Descriptions

Southwest Creek receives runoff from 18.58 km^2 (4591 acres) of farm fields and riparian buffers. The fields are owned and managed by Open Grounds Farm Inc (OGF).

These fields were created in 1974 when OGF purchased 45,000 acres of pocosin and subsequently ditched, drained, and converted the land to agricultural use (Kirby-Smith and Barber 1979). Best management practices (BMP) in the Southwest Creek watershed included the use of flashboard risers. Flow was monitored at the headwaters of the creek at 30-minute intervals during the study period with a current meter and depth transducer maintained by the Forested and Agricultural Watershed Nitrogen Attenuation (FAWNA) research project currently managed through the University of North Carolina Institute of Marine Science (UNC-IMS) (Figure 4.2). This flow meter received runoff from 8.90 km^2 of the total Southwest Creek watershed.

Westfork Creek receives runoff from 13.04 km^2 (3224 acres) of OGF fields and riparian areas. In addition to the use of flashboard riser BMP's, runoff from the 6.58 km^2 of the Westfork Creek watershed passed through a constructed treatment wetland prior to reaching the creek. The FAWNA project also maintained a flow meter at the treatment wetland outfall (Figure 4.2).

Big Creek receives runoff from 4.71 km^2 (1164 acres) of actively-managed Silviculture forest. There were also five residential homes and properties within the watershed. The home lots and roads totaled only 0.03km^2 or less than 1% of the Big Creek watershed area. Two flow meters identical to those used in Westfork Creek and Southwest Creek were maintained in the headwaters of the creek. These flow meters received runoff from 2.64 km^2 of the Big Creek watershed.

Browns Creek receives runoff from 9.44 km^2 (2332 acres) of unmanaged forest. The vegetation included loblolly pine (*Pinus taeda*) and natural pond-pine pocosin species detailed in Frankenberg (1997). During the study period, the entire watershed was owned by

a hunting club with public access restricted to members. Carteret County, NC tax records show the hunt club ownership group is identified as a logging company. This suggests the land has been previously logged; however, a mature forest covered the watershed during this study.

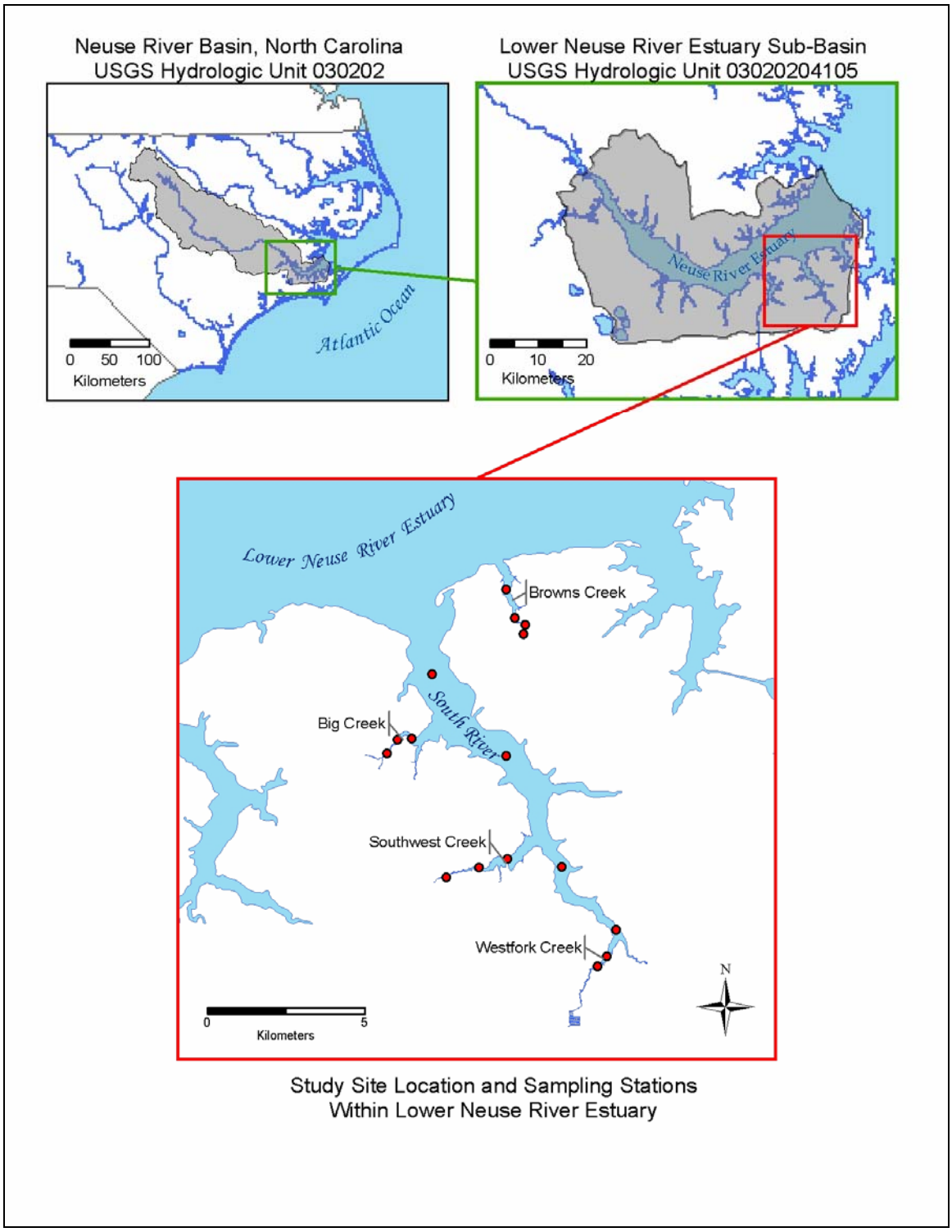

Figure 4.1 Study area location

LULC Type	Percent Coverage
Urban	5.1
Agricultural	12.6
Forested	27.0
Grassland	0.0
Open Water	26.2
Wetlands	28.9
Barren	0.3
Total	100.0

Table 4.1 LULC in the lower Neuse River Estuary watershed USGS hydrologic unit 03020204105.
Source: US-EPA SPOT and Landsat 7 ETM+ satellite sensor data and GIS software analysis (Material and Methods chapter).

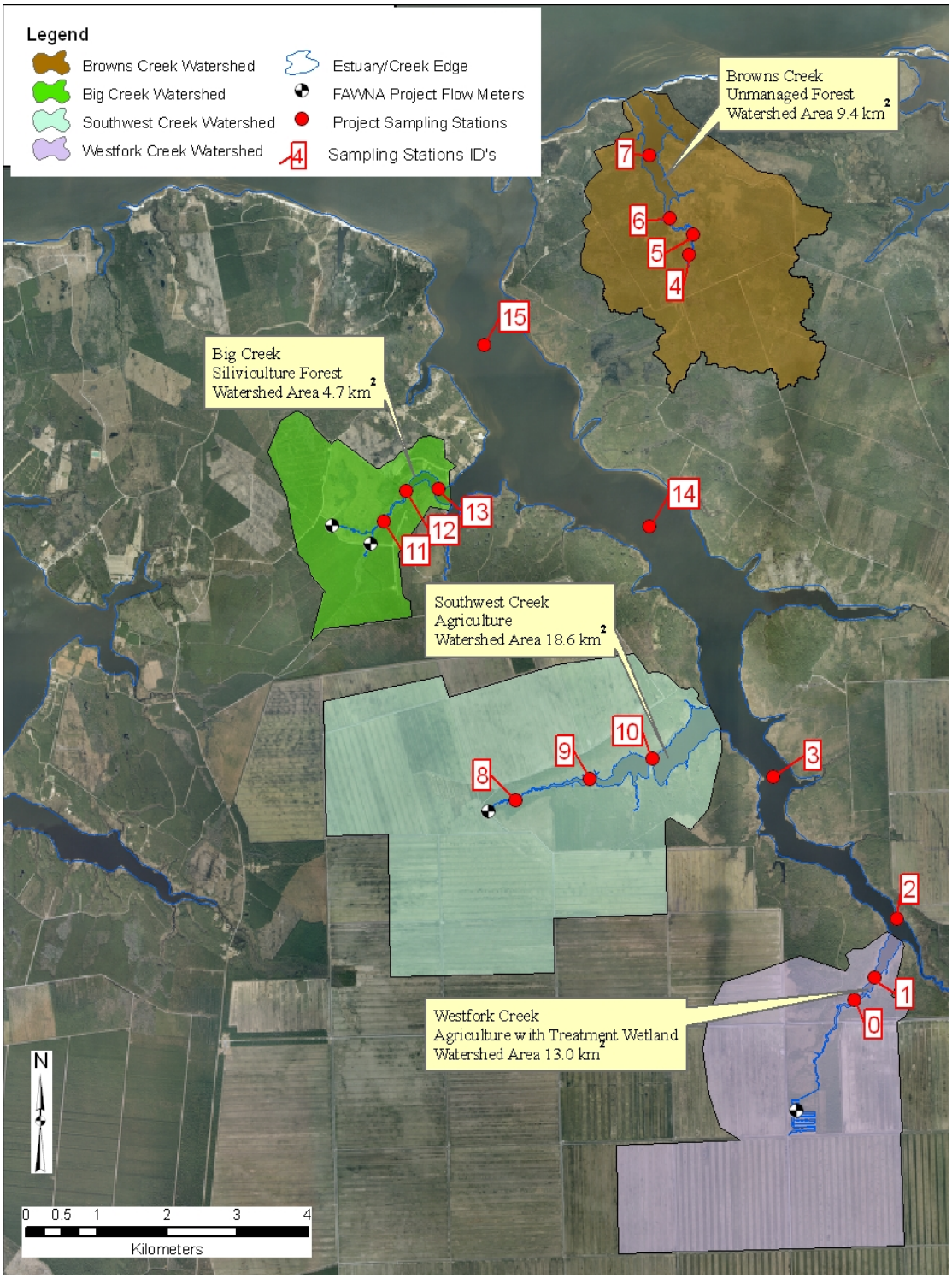

Figure 4.2 Detail of study area creeks and watersheds

CHAPTER 5

MATERIALS AND METHODS

5.1 Regular Sampling

Creek stations shown on Figure 4.2 were sampled every 14 to 21 days from February 2003 to August 2004 via shallow draft outboard skiff. Vertical profiles of in-situ salinity, turbidity, pH, dissolved oxygen, and chlorophyll *a* fluorescence were made with a Yellow Springs Instruments (YSI) Sonde model 6600. The YSI 6600 probes were calibrated the day before the each trip using methods outlined in the YSI 6600 maintenance manual. The dissolved oxygen probe and depth sensor were re-calibrated in the field on the day of sampling to compensate changes in ambient atmospheric pressure. Photic depth and light attenuation coefficient were calculated using data collected with a 4π light sensor and Sechhi disk.

At each station discrete samples of surface and bottom water were collected in translucent one liter Nalgene bottles, placed in a darkened cooler, and transported back to UNC-IMS for immediate filtration and storage until analysis. The list of parameters analyzed and methods can be found in Table 5.1.

5.2 Bioassay Water Collection, Design, and Sampling

Bioassay Design

The design of the bioassay experiments was adapted from Paerl and Bowles (1987). The water for the experimental treatments was collected from the agricultural watershed

creek (Southwest Creek), the reference forest watershed creek (Browns Creek), the silviculture watershed creek (Big Creek), and a site in the main stem of the lower NRE. The NRE site was included to provide a link to ongoing main stem monitoring and research. In treatments that received nutrient additions, the addition concentrations were selected to mimic conditions observed in the creek headwaters. Headwater runoff loading regularly increased ambient DIN concentrations to greater than 20uM (Sampling Results chapter).

Bioassays were limited to 96 hours to limit experimental artifacts (Downing et al. 1999). A DON (urea) treatment was included in the bioassay experiments to mimic the higher DON:DIN fraction associated with forested watershed runoff (Wahl et al. 1997). Urea was chosen as the DON amendment for two primary reasons: 1) previously documented urea uptake characteristics of phytoplankton and 2) urea was known to be excreted by zooplankton, bacterial processing of more complex organic compounds, and direct terrestrial inputs (Carpenter et al. 1972; Antia et al. 1991; Peierls and Paerl 1997; Harrington 1999; Twomey et al. 2005).

Bioassay Collection, Treatment, and Sampling

Bioassay waters were collected in clean and acid rinsed (0.1 N HCl) 20 liter polypropylene carboys. The carboys were immediately transported back to the UNC-IMS. Here, water from each creek was homogenized in separate cleaned 400 liter tanks. The homogenized water was dispensed via spigot into 10 liter 85% PAR transparent polyethylene cubitainers. All cubitainers were filled before solar noon and rapidly amended with nutrient additions detailed in Table 5.2. Each treatment consisted of 4 replicates for robust statistical analysis.

Cubitainers were incubated in the retention pond at UNC-IMS. Twice daily, cubitainers were manually mixed and circulated in the retention corral (Figure 5.1). Each treatment replicate was sub-sampled within 90 minutes following sunrise at T1 (24 hours), T2 (48 hours), and T4 (96 hours). Sub-samples were analyzed for: chlorophyll *a*, primary productivity, phytoplankton community composition, and dissolved nutrients (NO_x^-, NH_4^+, TDN, and PO_4^{-3}).

5.3 Laboratory Protocols and Analysis

Phytoplankton Community Analysis

Phytoplankton photopigment concentration and community composition for major algal groups were determined using reverse phase high performance liquid chromatography (HPLC) of diagnostic photopigments (Tester et al. 1995; Mackey et al. 1996; Jeffrey et al. 1997; Pinckney et al. 1998). Sample aliquots from regular sample collections and experimental treatments were filtered onto 25 mm Whatman GF/F filters and frozen until extraction. The filters were placed in a 100% acetone, sonicated with a microtip sonic dismembrator (Fisher Sonic Dismembrator, model 300), and extracted at -8° C for 18-24h (Jeffrey et al. 1997). HPLC was used to quantify selected chlorophylls and carotenoids as biomarker algal pigments. The HPLC system pumped a binary gradient under high pressure (1800-4000psi) through a single monomeric column and two polymeric C18 columns (Van Heukelem et al. 1994). The mobile phase consisted of two solvents cycling from Solvent 'A' to Solvent 'B' over a 54-minute time program. Solvent 'A' was a 80:20 solution of HPLC grade methanol and 0.5M ammonium acetate and Solvent 'B' was a 80:20 solution of HPLC grade methanol and HPLC grade acetone (Millie et al. 1993). An in-line photodiode array spectrophotometer (Shimadzu SPD M10avp) provided individual photopigment

identification and concentration. Identification was based on retention time and characteristic absorption spectra (380–700 nm), and concentration was calculated by absorbance across a 4nm bandwidth centered on 440nm. (Millie et al. 1993; Jeffrey et al. 1997; Jeffrey et al. 1999). Pigment standards were acquired from DHI Water and Environment, Denmark, and used in the calibration methods outlined by (Mantoura and Repeta 1997).

The matrix factorization program ChemTax (CHEMical TAXonomy), executed in MATLAB technical computing software (Math Works Inc., Natick, Massachusetts), was used to establish the relative biomass of major algal groups in the phytoplankton community and expressed as percentage of total chlorophyll *a*. (Mackey et al. 1996; Wright et al. 1996; Mackey et al. 1997; Pinckney et al. 1998; Schluter et al. 2000; Lewitus 2005). This program iteratively modified each element of a table containing the sample pigment concentrations obtained in HPLC analysis to "best fit" the sample data to a second matrix containing reference pigment ratios for each algal group. The seminal ChemTax work Mackey et al. (1997), stressed the importance of several factors in setting up the reference pigment ratio matrix. First, the reference matrix must include all major groups likely to be present in the samples. Second, each major group should have two reference pigments in addition to chlorophyll *a*. Third, the total number of pigments used in the ratio should outnumber the total number of expected classes by at least three. Additionally, subsequent publications have stressed that the reference matrix should be derived from phytoplankton culture isolates representative of the assemblages present in the study location (Wright et al. 1996; Schluter et al. 2000; Lewitus 2005).

Following the requirements outlined above, a reference matrix was adapted from the a matrix developed for estuaries in the southeastern US by Lewitus (2005) (Table 5.6). The

algal groups selected were those documented as the dominate algal classes present in the NRE and South River Sub-Estuary by Pinckney et al (1998) and Lapennas (1980), respectively.

Phytoplankton Chlorophyll a

In-vivo chlorophyll *a* for both bioassay and monitoring samples were determined via sample water filtration and acetone extraction. 50ml of sample water was filtered onto a 25mm Whatman GFF, folded, padded dry, and frozen until extraction. Extractions began with sonicating the frozen filter in 90% acetone solution. The samples were the extracted in the 90% acetone at -4°C for 24 hours. The extracted concentration of chlorophyll *a* was quantified with a Turner Model 10AU fluorometer calibrated with a solid standard.

Phytoplankton Productivity

Phytoplankton primary productivity was measured by assimilation of ^{14}C. As part of the bioassay experiments 20ml subsamples from each replicate were dispensed into 20ml borosilicate vials. Two additional vials were also filled from each treatment; the first vial was darkened and included in all procedures to account for non-photosynthetic assimilation while the second vial was immediately analyzed on a Shimadzu TOC 5000 for ambient dissolved inorganic carbon concentration. Each incubation vial was injected with 200uL of ^{14}C sodium bicarbonate ($NaH^{14}CO_3$) with an activity of 9.3uCi ml^{-1} (specific activity 28 Mci $mmol^{-1}$, ICN INC. (Summer and Fall Bioassays) or 10.6 uCi/ml (Winter and Spring Bioassays). Vials were incubated for 3 hours spanning solar noon in the same experiment pond as the bioassay treatments. The vials were submerged just below the surface during incubations and screened with neutral density screening when light intensities exceeded 800uE$m^{-2}s^{-1}$. At the end of the incubation period, all vials were darkened and rapidly filtered

on 25mm Whatman GFF. Filters were placed on an inert rack and fumed with concentrated HCl to remove unassimilated residual ^{14}C. The following day, air dried filters were placed in 7ml plastic scintillation vials and covered with 5ml of cytoscint liquid scintillation cocktail. The mass of assimilated ^{14}C was quantified as dissolutions per minute using a Beckman model LS5000TD scintillation counter calibrated to an unquenched ^{14}C standard. Assimilation rates were determined using the incorporated mass and the time elapsed from initial ^{14}C injections to filtration. Primary Productivity, as assimilation rate, was expressed as mgC m^{-3} h^{-1}.

Nutrient Analysis

Nutrient analyses of NO_x^-, NH_4^+, PO_4^{-3}, and TDN for the bioassays and monitoring samples were conducted with a Lachat Quick-Chem 8000 auto-analyzer using standard protocols (Lachat Quikchem methods 31-107-04-1-A, 31-107-06-1-A, 31-115-01-3-G, and 31-107-04-3-A respectively). The methods are detailed in Table 5.4. The mean DON:DIN ratios were used to assess the labile inorganic and organic fractions of nitrogen available to the creek microbial communities. The Redfield molar ratio of N:P (16:1) was applied to DIN:PO_4^{-3} and used to identify potential P and N limitation of phytoplankton growth.

Below detection limit (BDL) values were encountered during sample analysis. BDL limit values for NH_4^+ only occurred during the summer maximum productivity periods in 2003 and in during relatively high chlorophyll *a* levels in Westfork Creek on 22-Jan-04. During these times, NH_4^+ was assumed to be highly scavenged. Consequently, BDL values were set to ½ detection limit for use in analysis. In NO_x^- analysis, 22% of analyzed samples were below the detection limit of 3.68 μg l^{-1}. For these data, the values reported by the

nutrient auto-analyzer were used down to ½ detection limit in analysis. Below ½ the detection limit values were reported as BDL in analysis.

DIN utilization rates in the bioassay incubations were calculated from changes in dissolved NO_x^- and NH_4^+ over the time elapsed between sampling points. Rates were normalized to chlorophyll *a* concentrations.

5.6 Data Analysis and Calculations

Species Diversity and Dominance Methods

The Shannon-Weiner index of diversity was used to evaluate how phytoplankton groups differed spatially and temporally across the four study creeks. Both long- and short-term assessment of diversity were completed. Long-term diversity was quantified by deriving Shannon-Weiner index values from all phytoplankton community data collected during the 18 months of sampling. Short-term diversity was quantified by deriving Shannon-Weiner index values for a phytoplankton community observed on a single day in a specific creek. The diversity values from each sampling date were compared to assess the relative diversity of creek phytoplankton communities on a single sampling day. The phytoplankton community groups used in the diversity analyses were quantified by HPLC and ChemTax. The Shannon index of diversity was previously used by Sanders and Kuenzler (1979) to assess phytoplankton diversity in a tidal estuarine creek near the study area.

Geographic Information System Analysis

Geographic information system (GIS) analysis and mapping was conducted using ArcView, ArcGIS, and ArcInfo software releases 3.2 through 9.1 (Environmental Research Systems Incorporated, Redlands, California). Analysis extensions included ArcHydro,

Spatial Analyst, and 3D Analyst. The products of the GIS analysis included LULC assessments, watershed delineations, and creek volume calculations.

Areas of different LULC types were calculated for the Neuse River Basin (United States Geologic Survey [USGS] HUC 030202) and Neuse River Sub-Basin 10 (USGS HUC 03020204105). The LULC area calculations used 15 meter resolution, SPOT and Landsat 7 ETM+ imagery data developed by the USEPA Landscape Characterization Branch. All spatial data were projected into a common projection (North Carolina State Plane 1983 feet) and clipped to the extent of each watershed. Areas of each LULC type (agriculture, forest, wetland, etc) were then calculated in square meters with a visual basic code script.

Browns Creek and Big Creek watershed areas were delineated using a combination of 20ft resolution LIDAR (LIght Detection And Ranging) sensor data from the North Carolina Flood Plain Mapping System; 1/3 degree digital elevation models data from the 2005 Multi-Resolution Land Characteristics Consortium; and 1:24,000 USGS topographic quads. The graded flat topology of the agricultural watersheds had vertical variation below the resolution of available remote sensing data. Consequently, Southwest Creek and Westfork Creek watershed were delineated based on the opinion of OGF manager, Gabrelli Oranato, and onsite inspections by collaborators Suzanne Thompson and Sara McMillan. The watershed areas were calculated for each total creek watershed and also for the smaller portions for that drained to the flow meter locations (Figure 4.2).

The creek volumes were calculated using depth data collected during sampling. These depth data were used to create triangulated irregular network (TIN) models of each creek's bathymetry. The ArcGIS 9.1 3D Analyst extension and the TIN models were used to calculate the volume, benthic surface area, and select cross-sectional areas for each creek.

Creek Flushing Time and DIN Load Capacity Calculations

Creek flushing times were calculated using mean daily watershed flow and creek salinity as shown in Creek Flushing Time Equation adapted from Alperin (2003).

$$\text{Creek Flushing Time Equation} = \text{Volume}\,[Q_{watershed}\,(S_d\,(S_d - Ss)^{-1})]^{-1}$$

In Equation 1 the parameters were defined as follows: Volumes were those calculated using GIS analysis described above; $Q_{watershed}$ was the mean daily watershed water flow to the creek; S_d was the mean bottom salinity observed at the creek mouth; and S_s was the mean surface salinity observed at the creek mouth. Mean daily watershed flow was estimated using drainage area ratio method. Specifically, the ratio of the total watershed area to the sub-watershed area used to scale up the sub-watershed flow observations to the total watershed (Pope 2001). The drainage area ratio method was also used to estimate flow into Browns Creek, using Big Creek flow data.

The capacity for phytoplankton assimilation was calculated using the seasonal DIN utilization rates from the bioassay experiments, the calculated creek volumes, and calculated creek flushing times. Seasonal DIN load capacities represent the maximum DIN load each creek could assimilate given phytoplankton abundance observed during seasonal bioassays. This does not include potential assimilation by benthic algae or nutrient loading from groundwater in-flow. Calculation accuracy was improved by using the actual creek bathymetry to derive volume and cross-sectional areas via GIS software. Mulholland et al. (2002) found that using experimentally derived N uptake rates would over estimate the stream length and time required to assimilate a unit of dissolved inorganic nitrogen. Thus, the experimentally derived N uptake rates used here may actually under estimate in-creek assimilation rates. Watershed flows and loads were calculated for sub-watershed areas that

were instrumented with flow meter and automated samplers under the FAWNA project (Figure 4.2). The flow and DIN load values were then scaled up the whole watershed using drainage basin area ratios as outlined above (Pope 2001).

Statistical Methods

Statistical Software Packages SPSS 11.5 (SPSS Inc., Chicago, Illinois) and Statistix 8 (Analytical Software, Tallahassee, Florida) were used to perform statistical tests on monitoring and bioassays data. For the bioassay data, significant difference from control was evaluated with via one-way ANOVA or Kruskal-Wallis one-way nonparametric ANOVA. The methods were selected based on the results of the Shapiro-Wilk Normality Test, the Levene Test for Homogeneity of Variances, and Bartlett's Test of Equal Variances. Normally distributed data were evaluated via one-way ANOVA, with an alpha level of 0.05. Data that did not pass tests of normality and homogeneity were evaluated with the Kruskal-Wallis one-way nonparametric ANOVA. Differences were determined to be significant where $p < 0.05$.

Spatial and temporal interpolations of data were made using Surfer Version 7 (Golden Software, Golden, Colorado). The kriging interpolation method was adjusted so that spatial proximity was weighted more than temporal proximity. The spatial search windows were the total length of each creek analyzed. The temporal search window was 15 to 30 days, dependant on data availability. The spatial search window was the length of each individual project creek. This allowed only immediately preceding and succeeding sampling date data to influence interpolation results.

	Parameter	Method
Laboratory Analysis	Nitrate + Nitrite	Cadmium Reduction
	Ammonium	Phenol Hypochlorite Method
	Total Dissolved Nitrogen	Persulfate Digestion And Cadmium Reduction
	Phosphate	Molybdate Method
	Chlorophyll *a*	Fluorimetry
	Phytoplankton Accessory Pigments Chlorophyll *a*	Reverse Phase High Pressure Chromatography
	Phytoplankton Community Composition	ChemTax Analysis
In-Situ Measurements	Water Column Light Attenuation	4π LICOR Light Meter
	Dissolved Oxygen	YSI 6600 Sonde
	Chlorophyll *a* (fluorescence)	YSI 6600 Sonde
	pH	YSI 6600 Sonde
	Depth	YSI 6600 Sonde
	Salinity	YSI 6600 Sonde
	Temperature	YSI 6600 Sonde
	Turbidity	YSI 6600 Sonde

Table 5.1 Regular sampling parameters and standard analysis methods

Treatment	Experimental Concentration Increase in 10L Incubation Cubitainers			
	NO_3^-	**NH_4^+**	**DON (Urea)**	**PO_4^{-3}**
Control	--	--	--	--
Nitrate (NO_3^-)	*20uM*	--	--	--
Ammonium (NH_4^+)	--	*20uM*	--	--
Organic Nitrogen DON (Urea)	--	--	*20uM*	--
Phosphate (PO_4^{-3})	--	--	--	*5uM*
Inorganic Nitrogen + Phosphate (DIN+P)	*10uM*	*10uM*	--	*5uM*
Organic Nitrogen + Phosphate (DON+P)	--	--	*20uM*	*5uM*

Table 5.2 Increase in ambient nutrient concentrations in treatment additions

Figure 5.1 Bioassay incubation corral

	c1c2	peridinin	fucoxanthin	viola	alloxanthin	lutein	zeaxanthin	chlb	chla	anthera
Diatoms	0.239	0	0.546	0	0	0	0	0	1	0
Dinoflagellates	0.568	0.787	0	0	0	0	0	0	1	0
Cyanobacteria	0	0	0	0	0	0	0.368	0	1	0
Chlorophytes	0	0	0	0.06	0	0.221	0.002	0.322	1	0.048
Cryptomonads	0.292	0	0	0	0.389	0	0	0	1	0

Table 5.6 Reference pigment ratio matrix(Lewitus 2005) used in CHEMTAX community composition estimations for this study.

CHAPTER 6

RESULTS PART I – REGULAR SAMPLING

6.1 Dissolved Nutrients

The dissolved NH_4^+, NO_x^-, PO_4^{-3} and TDN concentrations were generally highest downstream of the agricultural watersheds and lowest downstream of the forested watersheds.

In the agricultural creeks, mean surface concentrations of NO_x^- were the highest in Westfork Creek (287 µg l^{-1}), followed by Southwest Creek (65 µg l^{-1}). The NO_x^- concentrations in the silviculture and reference forest creeks were similar at and average of 7 µg l^{-1} and 10 µg l^{-1} respectively (Figure 6.1.1). Mean NO_x^- concentrations in bottom water samples were generally lower than surface water sample concentrations in each creek. Westfork Creek had the highest bottom water NO_x^- concentrations, followed by Southwest Creek, Browns Creek, and lastly Big Creek (Figure 6.1.2).

The maximum mean surface water dissolved NH_4^+ concentrations were not found in the same creeks as the maximum mean dissolved NO_x^- concentrations. The highest mean NH_4^+ concentration was found in Browns Creek (128 µg l^{-1}) downstream of the reference forest watershed. In terms of NH_4^+ concentrations, the agricultural creeks, Westfork Creek (98 µg l^{-1}) and Southwest Creek (52 µg l^{-1}), had the second and third highest surface values, and the silviculture creek, Big Creek (24 µg l^{-1}), had the lowest (Figure 6.1.2). The highest mean bottom water NH_4^+ concentration was in Westfork Creek, followed by Browns Creek, Southwest Creek, and Big Creek. The comparatively high mean NH_4^+ concentrations in

Browns Creek were heavily influenced by data collected immediately following Hurricane Isabel on September 25, 2003. The mean NH_4^+ concentration in Browns Creek following the Hurricane was 1315 µg l^{-1}, while the mean concentration in Westfork Creek was 181 µg l^{-1}. Figure 6.1.3 shows a comparison of mean dissolved inorganic nutrient concentrations calculated with and without data from September 25, 2003. The mean surface NH_4^+ concentration in Browns Creek dropped 46% from 128 µg l^{-1} to 96 µg l^{-1} when the hurricane data were excluded. The hurricane data did not drive a similar a large percentage change in the mean inorganic nutrient concentrations from the other creeks. The hurricane data were not excluded from any analysis and were highlighted here only to emphasize the hurricane's impact.

The mean dissolved PO_4^{-3} concentrations were higher in the agricultural creeks than in the forested creeks (Figures 6.1.4 and 6.1.5).

In general, the highest mean TDN concentrations were observed below agricultural watersheds, followed by the reference watershed, and lowest below the silviculture watershed. The mean TDN concentrations for surface and bottom samples in each creek are shown in Figures 6.1.6 and 6.1.7.

Dissolved Inorganic Nitrogen Forms

Below both agricultural watersheds, NO_x^- was the primary form of surface water DIN. In contrast, NH_4^+ was the primary form of surface water DIN downstream of both forested watersheds (Figure 6.1.8). The most extreme differences in DIN form were observed downstream of the reference forest watershed (Browns Creek) and downstream of the agricultural watershed with a treatment wetland (Westfork Creek). In Browns Creek, NH_4^+ represented up to 94% of DIN, while in Westfork Creek, NH_4^+ represented up to 42% of

DIN. In the bottom water sample data, NH_4^+ was the primary DIN form in all creeks, except downstream of agricultural land in Westfork Creek where NO_x^- was the primary DIN form (Figure 6.1.9). Westfork had the lowest DON:DIN (2.0) followed by Southwest (4.6), Browns (5.8), and Big (10.4) (Figure 6.1.10).

Spatial Patterns of Dissolved Nutrients

Within each creek, dissolved NO_x^- and NH_4^+ concentrations were highest at the headwater stations (Figures 6.1.1 and 6.1.4). In the forested creeks, dissolved PO_4^{-3} in surface samples was highest at the creek mouths. In contrast PO_4^{-3} concentrations in the agricultural creeks were highest at the creek headwater stations (Figures 6.1.4 and 6.1.5).

The distribution of nutrient concentrations in the bottom water samples was more uniform than in the surface water samples. Specifically, NH_4^+ was the primary bottom water DIN form for all stations outside Westfork Creek. Westfork Creek was the only creek where NO_x^- was the primary DIN species in both surface and bottom samples.

Dissolved Inorganic N:P Ratios

The Redfield molar ratio of DIN:PO_4^{-3} (16:1) was used to identify conditions where N or P limitation of phytoplankton growth might occur. Figure 6.1.8 shows the percentage of total creek samples where DIN:PO_4^{-3} > 16, suggesting P limitation and very rapid N turn over times. The incidence of potential P limitation was highest in the reference forest creek followed by silviculture forest, μthen agriculture. Across all creeks, potential P limitation occurred mostly at the headwater creek stations where conditions were least influenced by brackish bottom water intrusion and entrainment. Potential P limitation was more prevalent during the spring and potential N limitation was more prevalent during the summer months.

The highest N:P ratios were observed immediately after the passage of Hurricane Isabel in September, 2003.

6.2 Chlorophyll *a* Concentrations

On each sampling day, the Southwest Creek mean surface chlorophyll *a* concentration was on average 1.78 times higher than Browns Creek and 1.42 times higher than Big Creek (Table 6.2.1). Spatially, chlorophyll *a* concentrations were generally highest at the mid-creek stations where the creek channels broadened and flow velocities slowed (Figures 6.2.1 and 6.2.2). Chlorophyll *a* concentrations were also highest in the surface waters. Seasonally, mean chlorophyll *a* concentrations were highest between June and September, when no creek concentrations dropped below 10 µg l^{-1}. The highest chlorophyll *a* concentration of 198 µg l^{-1} was observed in Westfork Creek during a spring 2003 dinoflagellate bloom. The lowest persistent chlorophyll *a* concentrations were found in Browns Creek throughout the spring of 2003.

6.3 Phytoplankton Community Composition

Figures 6.3.1 through 6.3.4 show spatiotemporal plots of the primary phytoplankton community groups identified in the monitoring data. The groups are shown as the percentage of total chlorophyll *a*. From top to bottom in each of the figures the fames are: dinoflagellates, cyanobacteria, chlorophyte, cryptomonad, and diatom. The bottom frame in each figure shows total chlorophyll *a* concentrations in µg l^{-1}. Across all creeks, chlorophytes were the most prevalent phytoplankton group throughout the study period. Exceptions were the two periods of cyanobacterial dominance observed in Browns Creek. In the fall of 2004 and summer of 2005, the cyanobacteria dominance persisted across several sampling dates and constituted 75% to 80% of the phytoplankton community in Browns Creek.

Dinoflagellates, cyanobacteria, cryptomonads, and diatoms, for the most part occurred as periodic blooms in each of the creeks. Consequently, the peak chlorophyll *a* concentrations observed in the monitoring data were not always attributable to chlorophytes.

The remaining distribution of total chlorophyll *a* attributed to each phytoplankton group differed across creeks. Cyanobacteria were most prevalent in the forested creeks with the lowest DIN concentrations (Figures 6.3.5, 6.3.6). The phytoplankton groups were most evenly represented in Browns Creek. The means of the Browns Creek samples were 50% chlorophytes, 24% cyanobacteria, 15% diatoms, 9% cryptomonads, and 2% dinoflagellates. By contrast, in Southwest Creek below the most disturbed project watershed, 79% of all population units were comprised of chlorophytes (Figure 6.3.5). Winter dinoflagellate blooms in Westfork Creek resulted in a relatively high mean percentage of dinoflagellates.

Within each creek, the spatial distribution of phytoplankton groups and their diagnostic indicator pigments varied with distance downstream. The samples with the highest percentage of chlorophytes were found at headwater creek stations of all creeks. In Browns Creek, the dinoflagellate indicator pigment, peridinin, was commonly detected at the creek mouth (Station 7), but was rarely present above detection levels at the creek headwater station (Figure 6.3.2). The influence of both upstream watershed forcing factors and downstream estuarine forcing factors on creek phytoplankton communities is addressed in the Discussion chapter.

6.4 Phytoplankton Group Diversity and Dominance

The Shannon-Weiner index of diversity evaluations were conducted for both long (year) and short (day) time scales, as detailed in the Materials and Methods chapter. Over the entire monitoring period, the least disturbed Browns Creek showed the highest level of

phytoplankton group diversity (H') (Figure 6.4.1). The lowest H' diversity values occurred in Southwest Creek below the most disturbed project watershed. Westfork Creek also received runoff from the same farm as Southwest Creek; however, diversity values below the treatment wetland were comparable to those found downstream of the reference forest watershed.

The mean of diversity values evaluated for phytoplankton groups at short time scales showed much less difference between creeks when compared to the long term evaluation of diversity across all sampling events (Figure 6.4.1 and 6.4.2).

6.5 Salinity

All stations in all creeks exhibited periods of vertical salinity stratification during the monitoring period. The highest incidents of strong stratification (> 3 parts per thousand [ppt]) were observed at the farthest upstream station in each creek, except for Browns Creek where the second downstream station was strongly stratified during 50% of sampling dates (Table 6.4.1). The highest surface salinities occurred in the summer months, specifically September 2003 and July 2004. The lowest surface salinity values for all stations were encountered on the June 24, 2004 sampling date.

6.6 Creek Volume and Flushing Time

The calculated volumes and flushing times for each project creek are provided in Table 6.6.1. Volumes are for the portions of the creeks between the flow meters and the creek mouth stations (Figure 4.2). The frequency at which the flushing time occurred for Big Creek and Southwest Creek at shown in Figures 6.6.1 and 6.6.2. Median flushing times better represent non-storm event conditions, while mean flushing times highlight the importance of storm events in creek hydrology. Specifically, the Big Creek mean flushing time of 1.57 days

occurred only 15% of the time (15^{th} percentile of recurrence) , while the median flushing time of 4.35 days occurred 50% of the time. This contrast shows the large influence that storm events have on the mean flushing time.

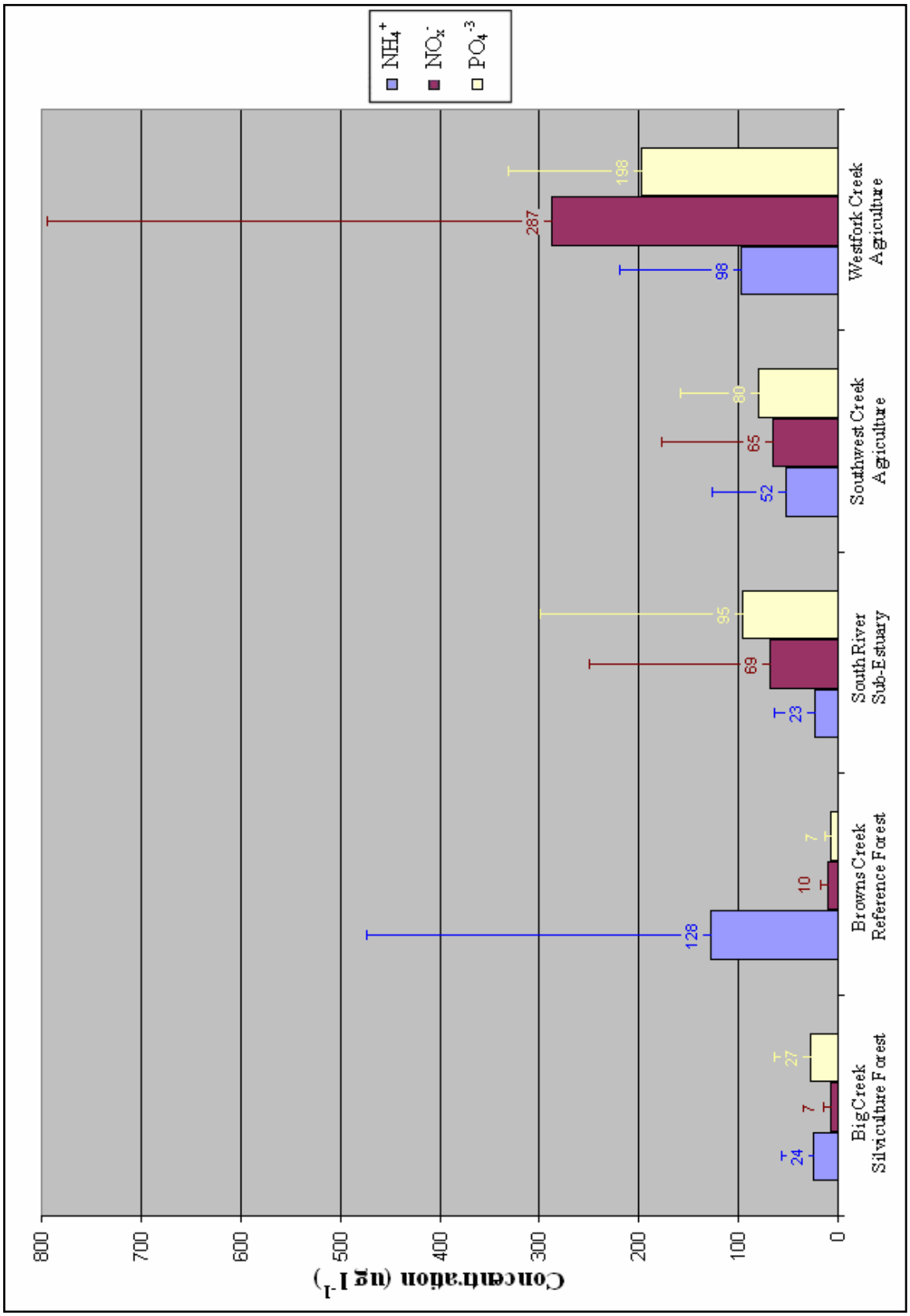

Figure 6.1.1 Mean surface water dissolved inorganic nutrient concentrations

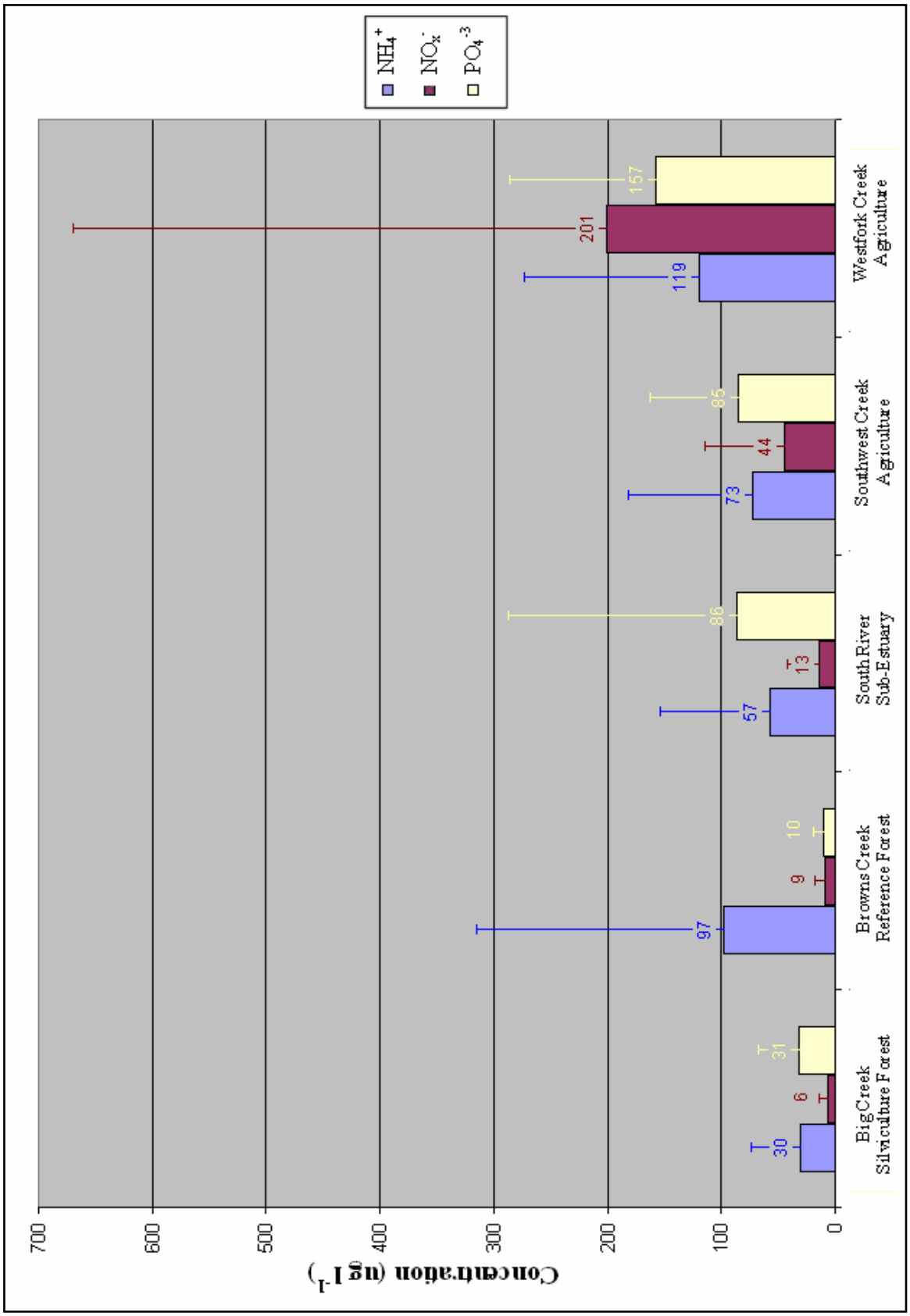

Figure 6.1.2 Mean bottom water dissolved inorganic nutrient concentrations

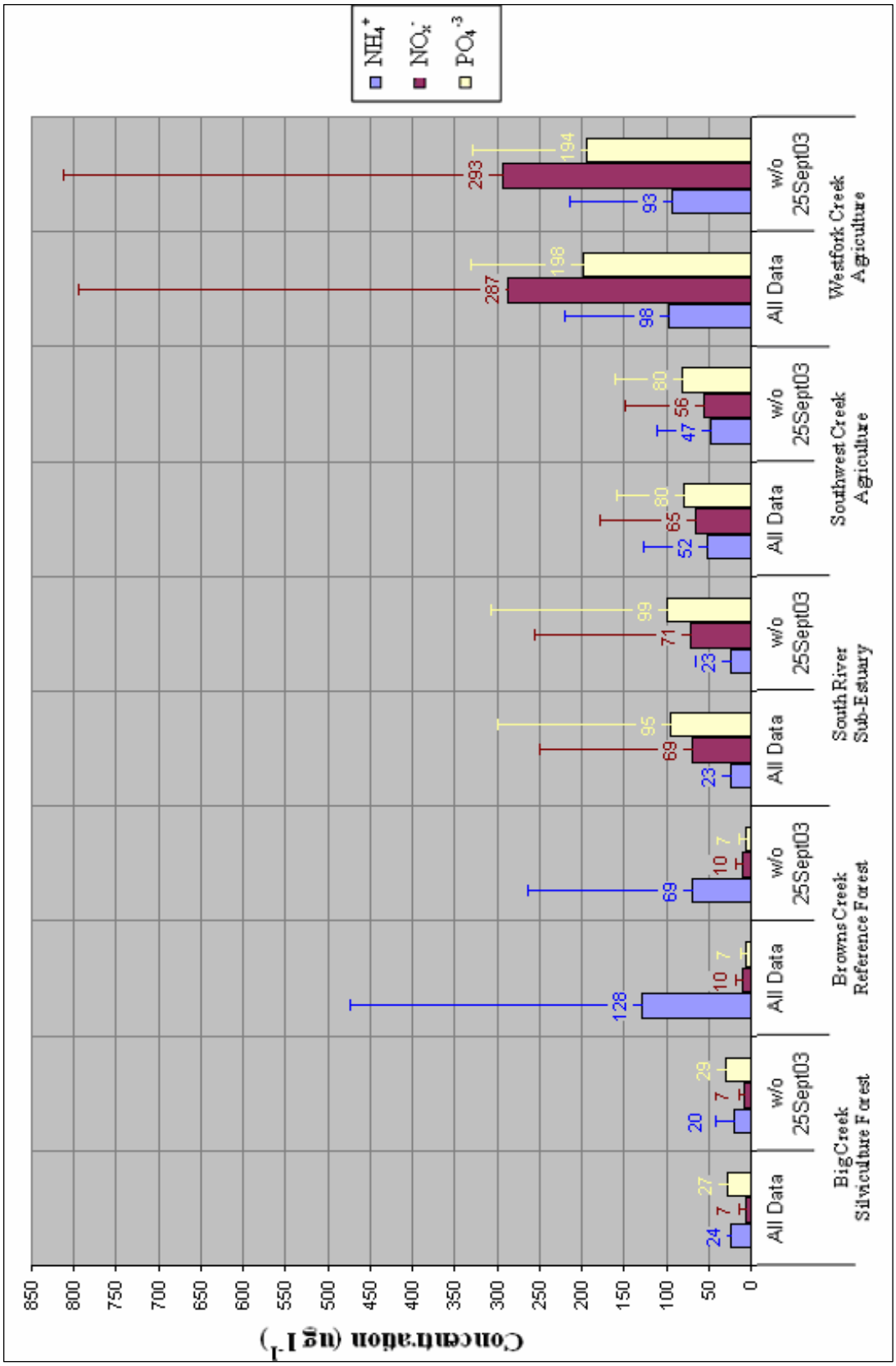

Figure 6.1.3 Effect of data collected immediately following Hurricane Isabel on mean surface nutrient concentrations

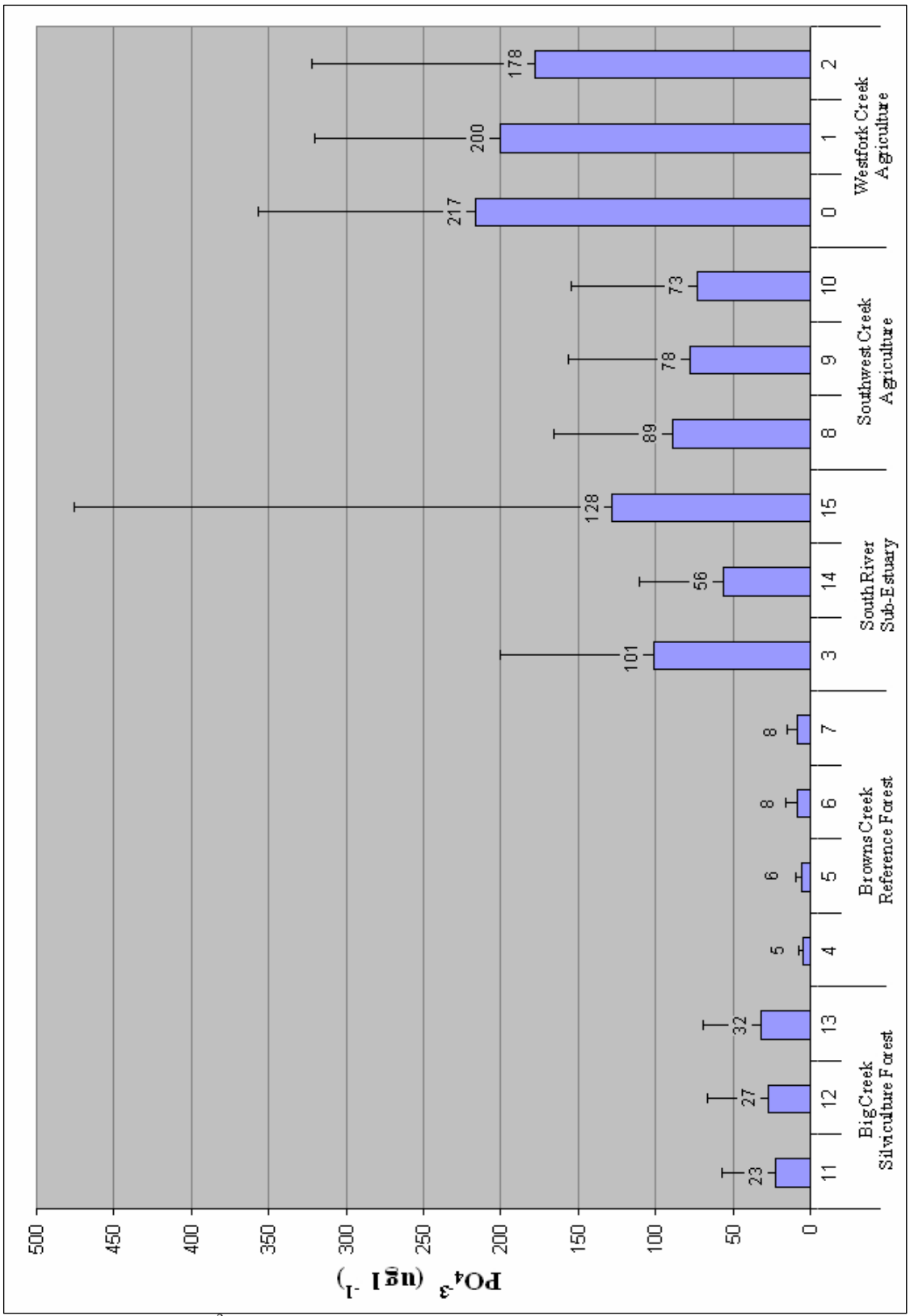

Figure 6.1.4 Mean PO_4^{-3} Surface samples. Station numbers increase from headwater to mouth within each creek

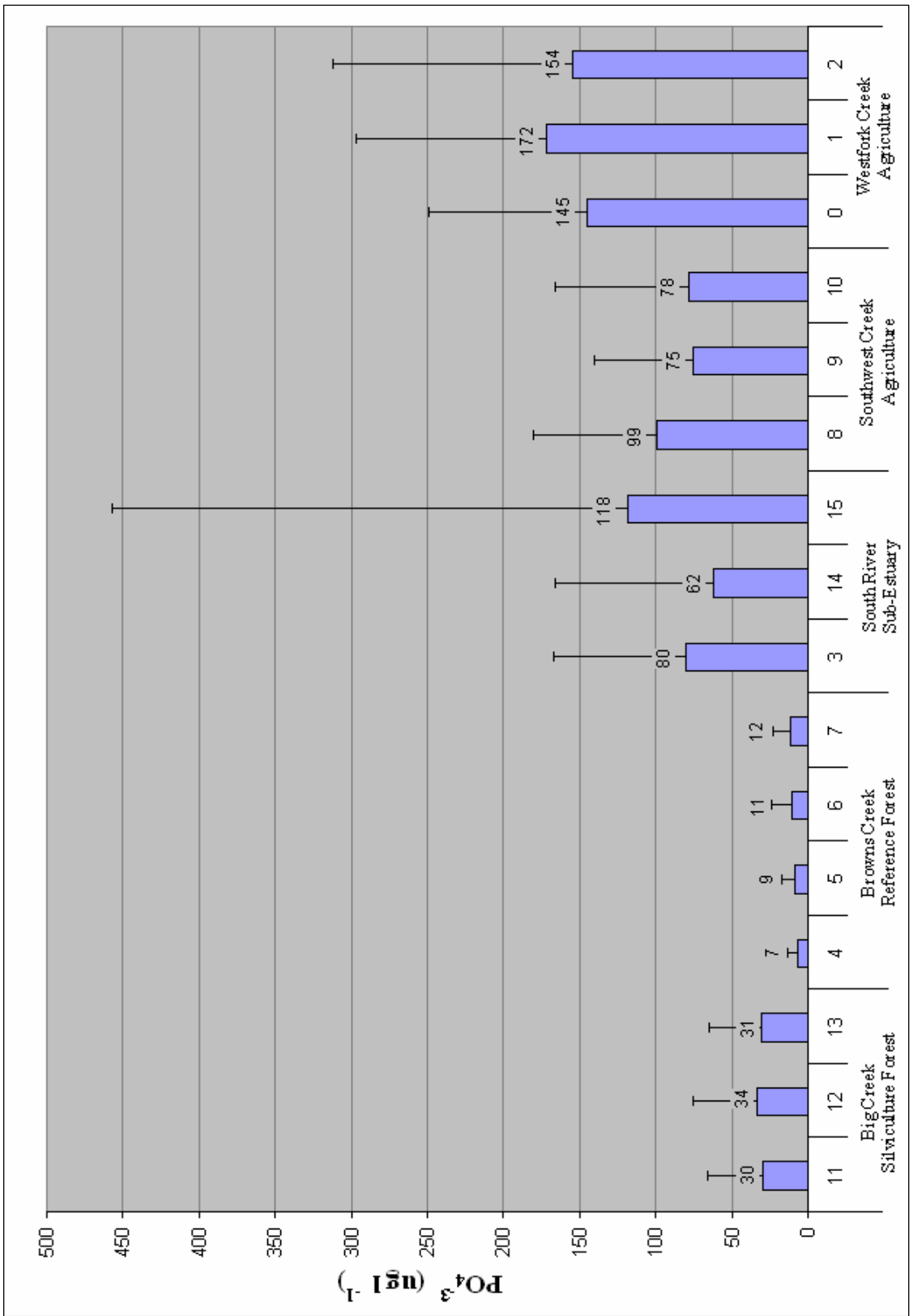

Figure 6.1.5 Mean PO_4^{-3} Bottom samples Station numbers within each creek increase from headwater to mouth

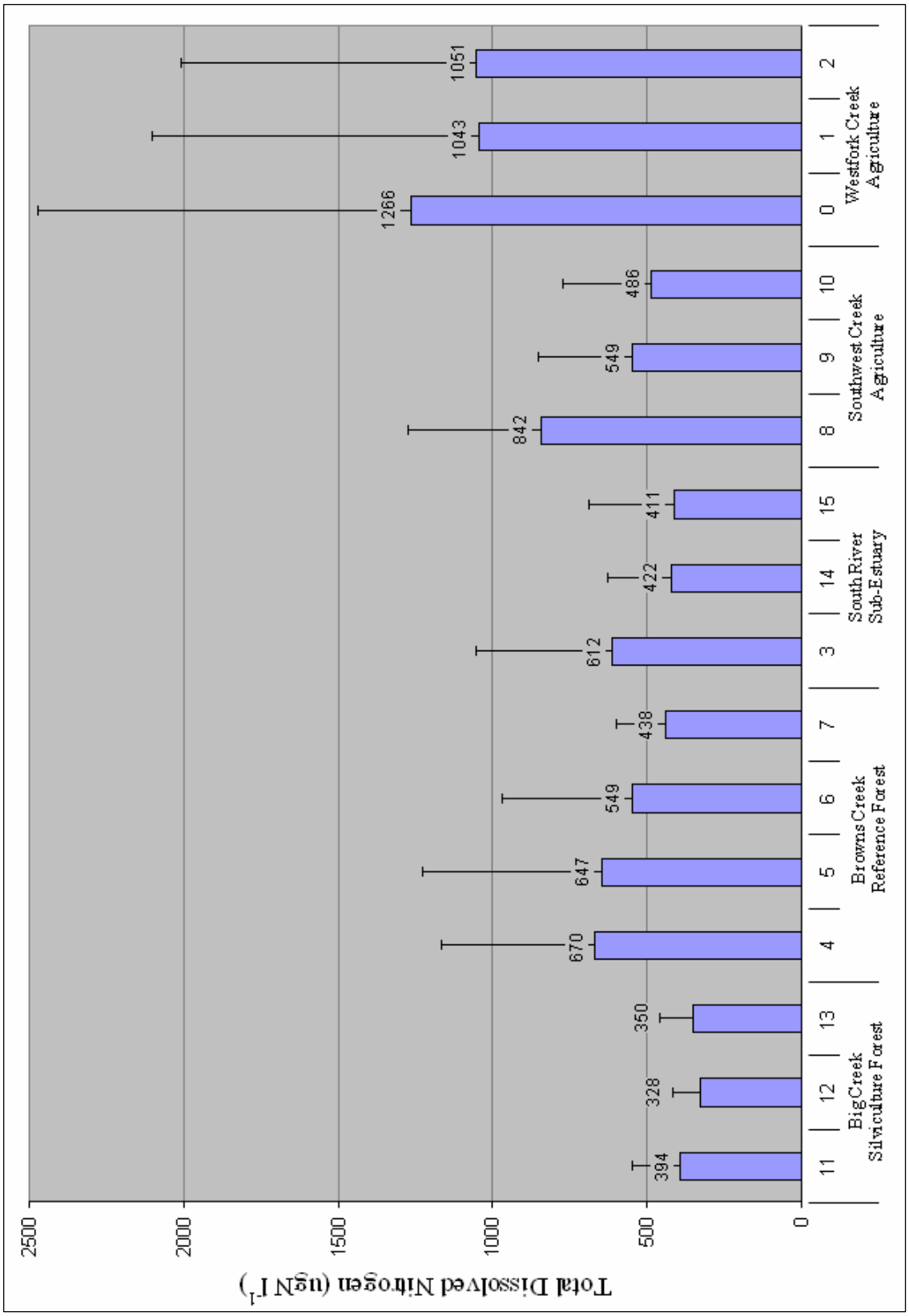

Figure 6.1.6 Mean total dissolved nitrogen (DIN + DON) in surface water samples. Station numbers increase from headwater to mouth within each creek

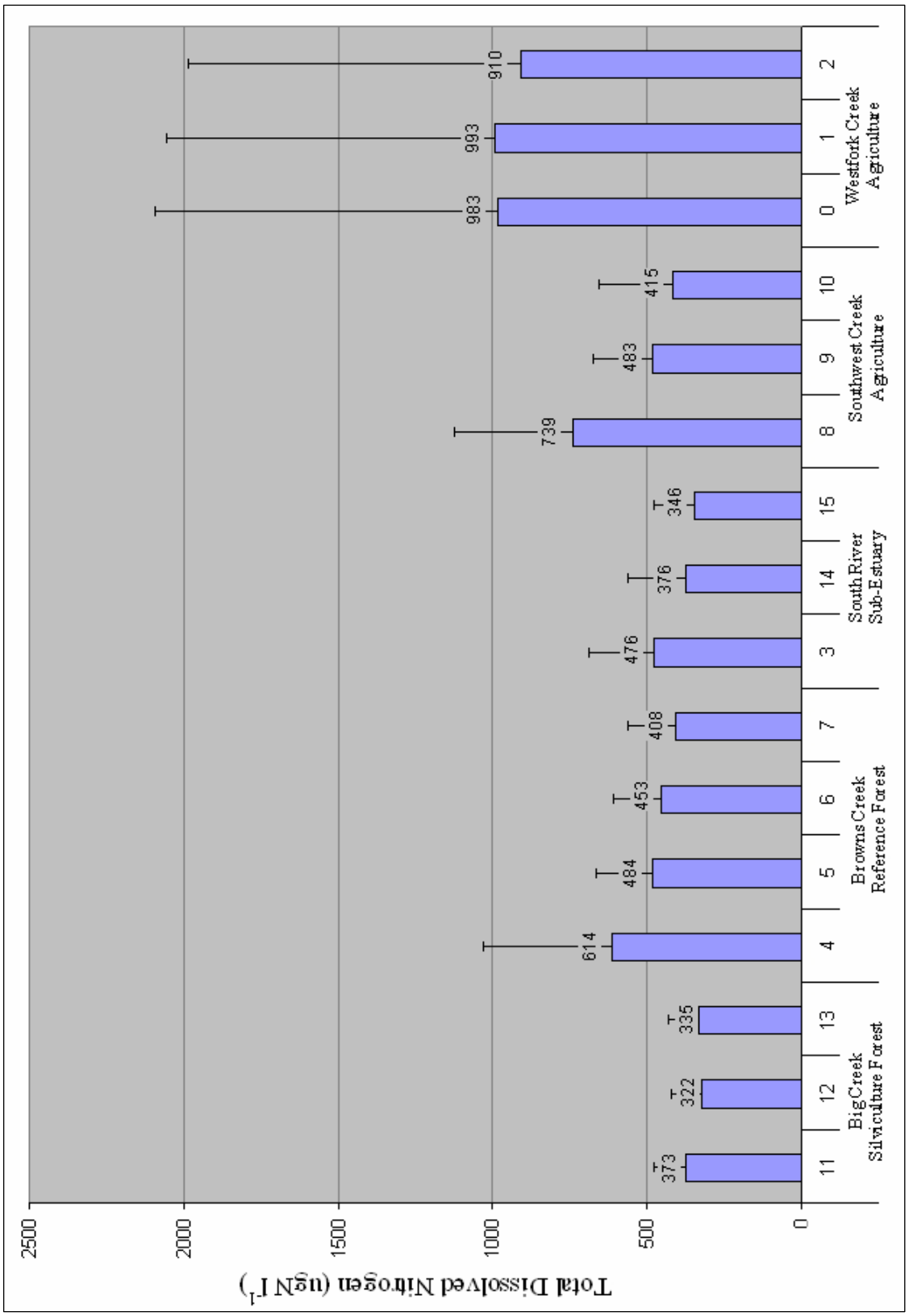

Figure 6.1.7 Mean total dissolved nitrogen in bottom water samples. Station numbers increase from headwater to mouth within each creek

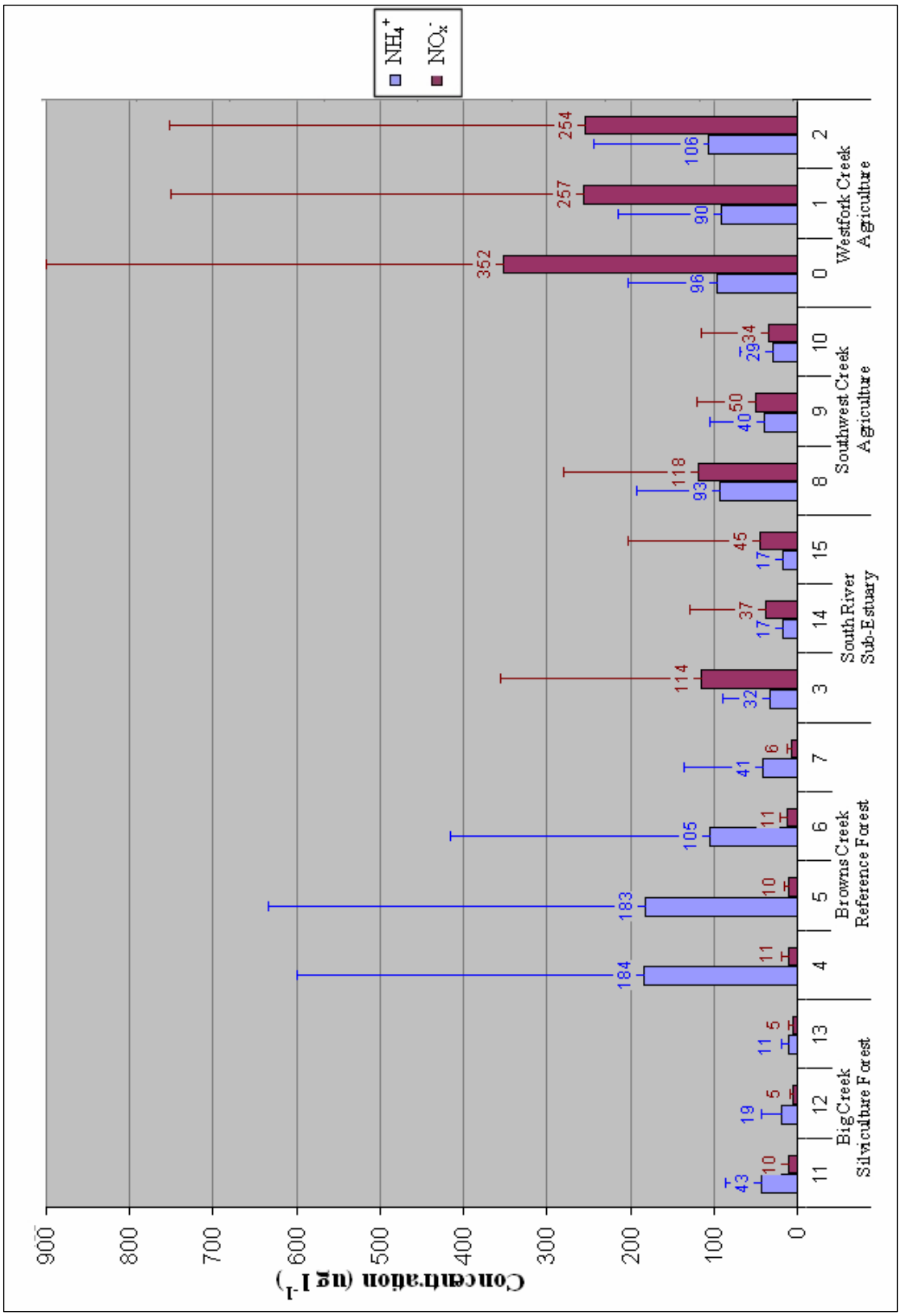

Figure 6.1.8 Mean DIN concentrations Surface Samples. Station numbers increase from headwater to mouth within each creek

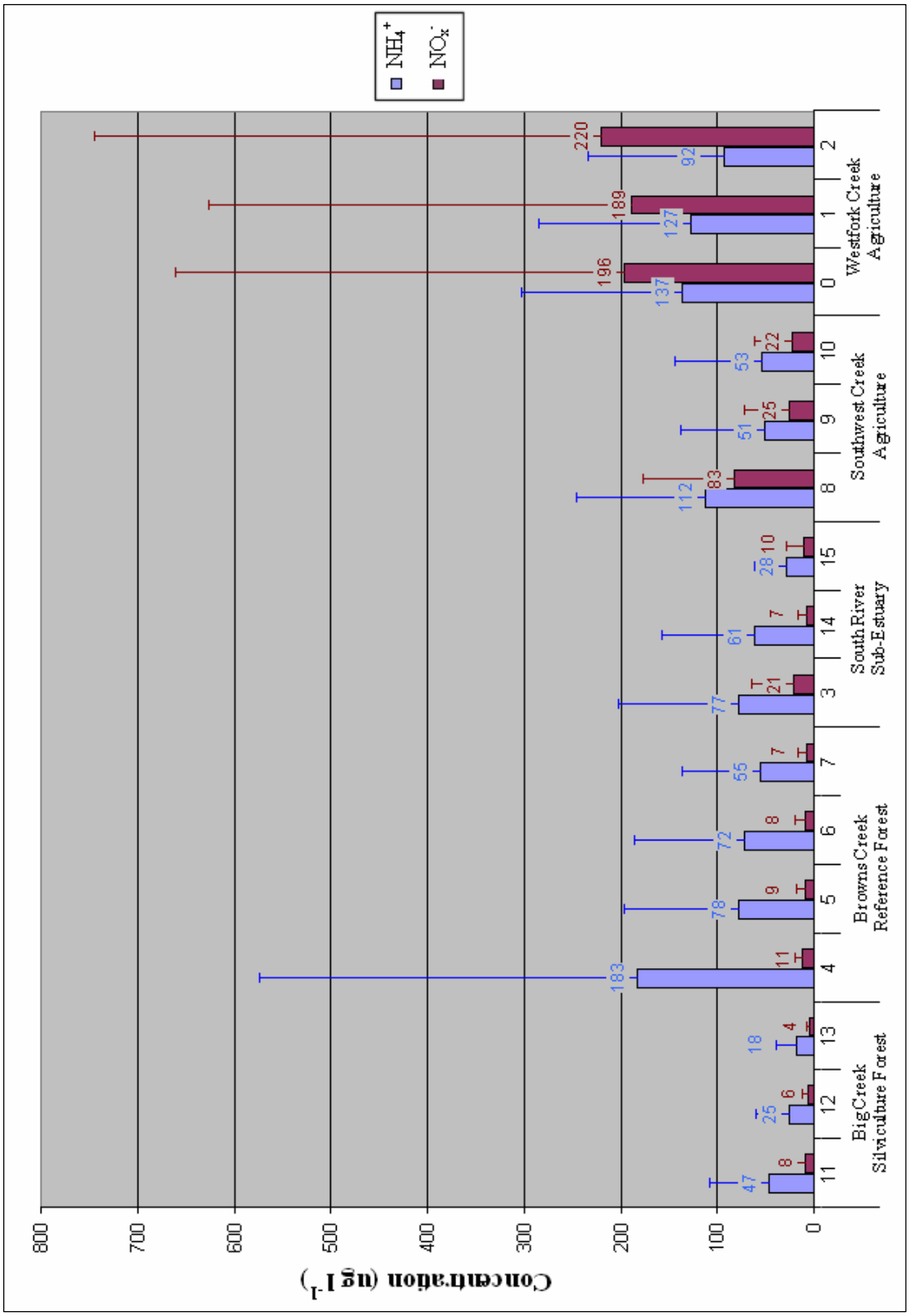

Figure 6.1.9 Mean DIN concentrations bottom samples. Station numbers increase from headwater to mouth within each creek

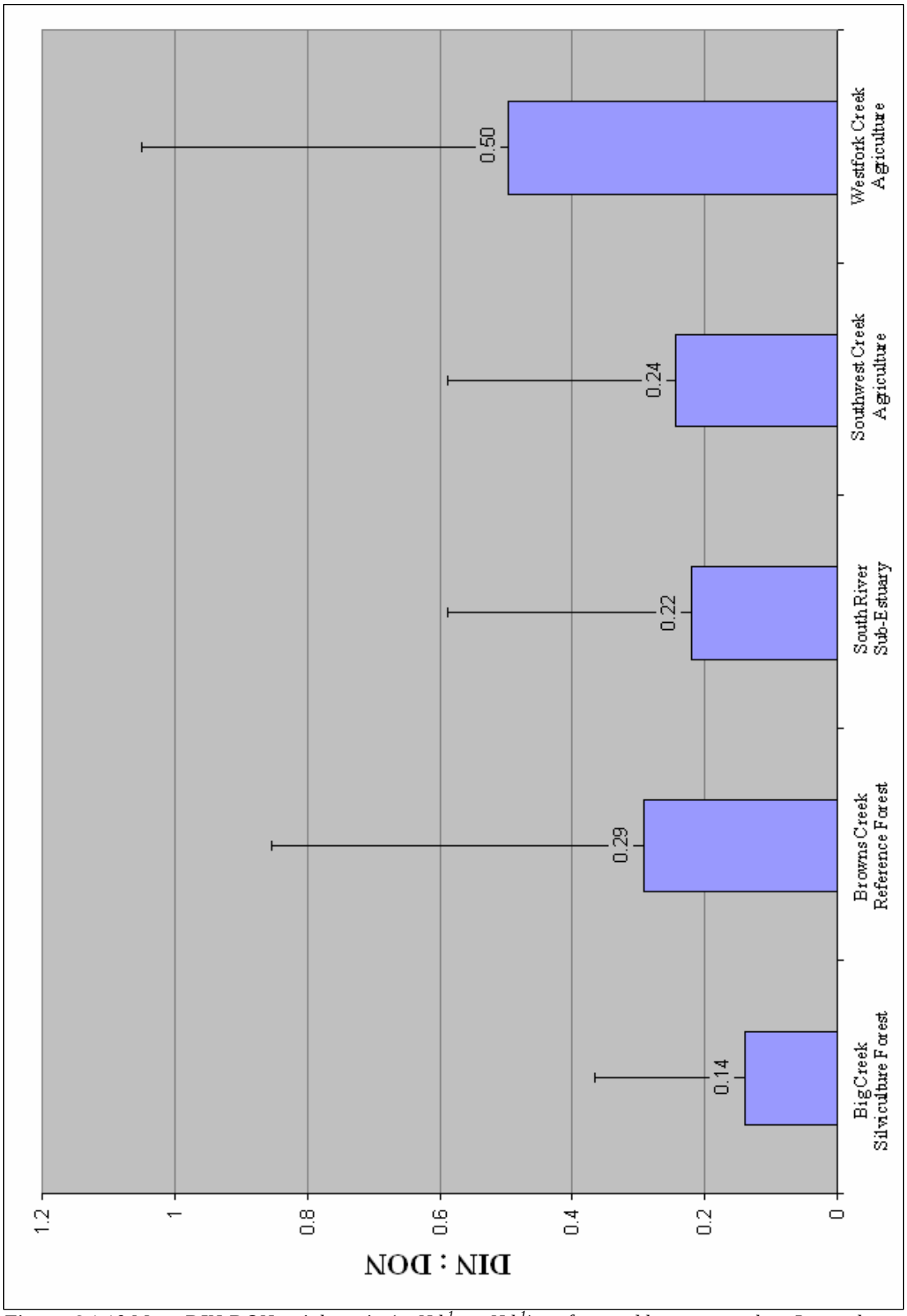

Figure 6.1.10 Mean DIN:DON weight ratio (µgN l^{-1}: µgN l^{-1}) surface and bottom samples. Low values representing the lowest fraction of DIN

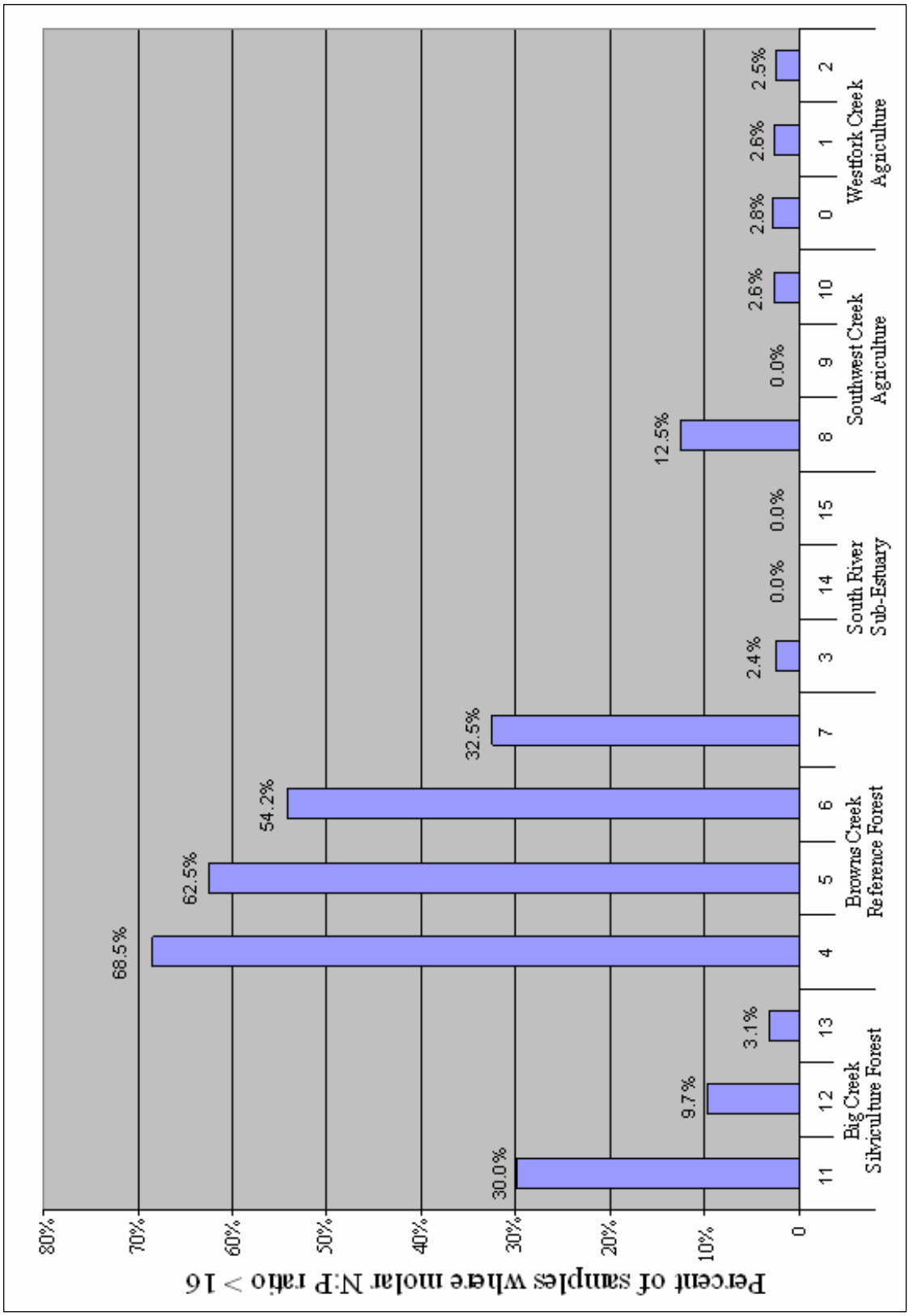

Figure 6.1.11 Percent of monitoring station samples where molar N:P ratio > 16. Surface and bottom samples combined

	Browns	**Big**	**South River**	**Southwest**	**Westfork**
LULC	Reference	Silviculture	Sub-Estuary Receiving Waters	Agriculture	Agriculture
Average *in-situ* Surface Chlorophyll *a* (μg l^{-1})	12.35	15.54	18.89	22.01	39.59

Table 6.2.1 Mean in-situ surface chlorophyll a concentrations for all stations and all sampling events

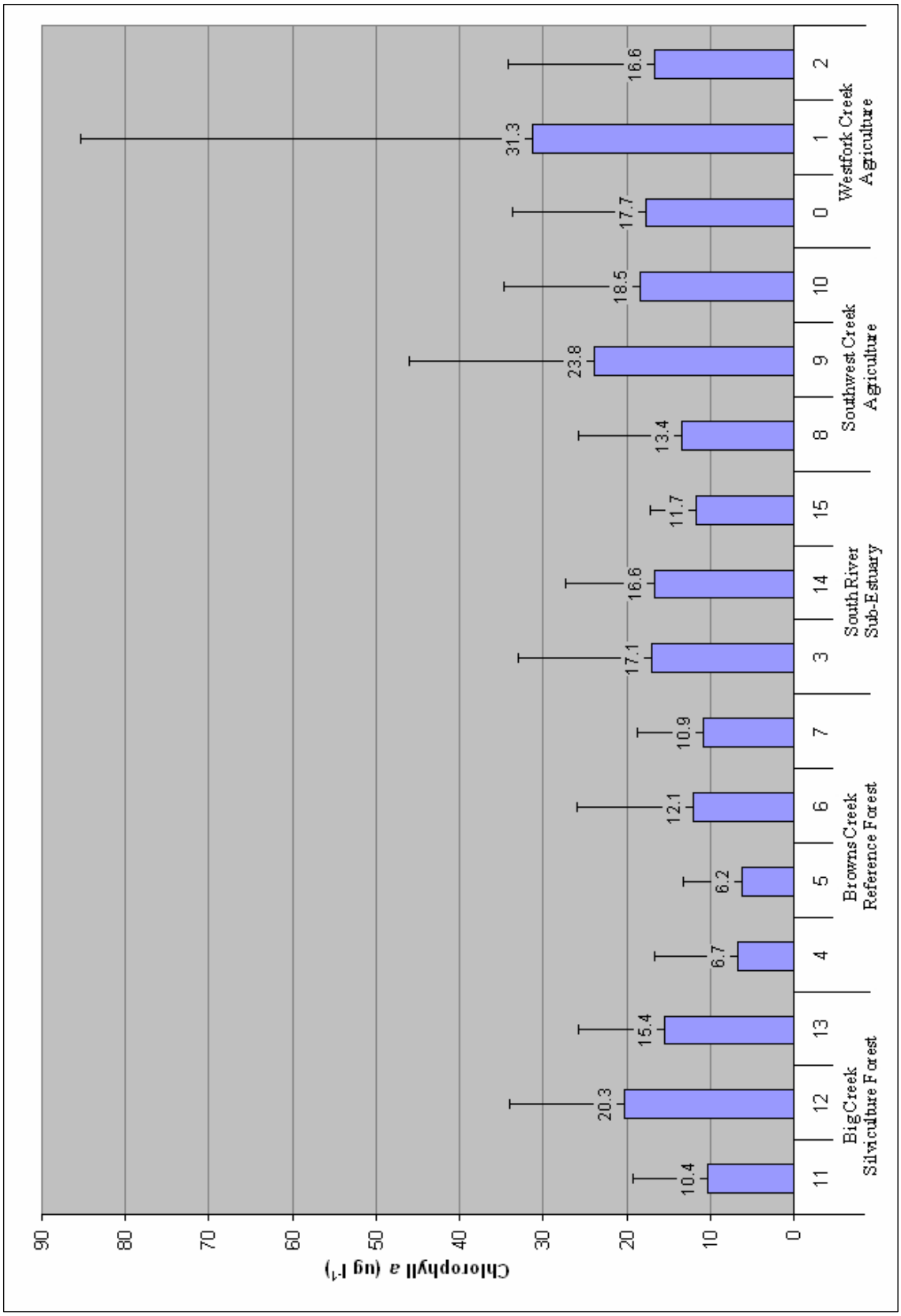

Figure 6.2.1 Mean surface chlorophyll a concentrations. Station numbers within each creek increase from headwater to mouth

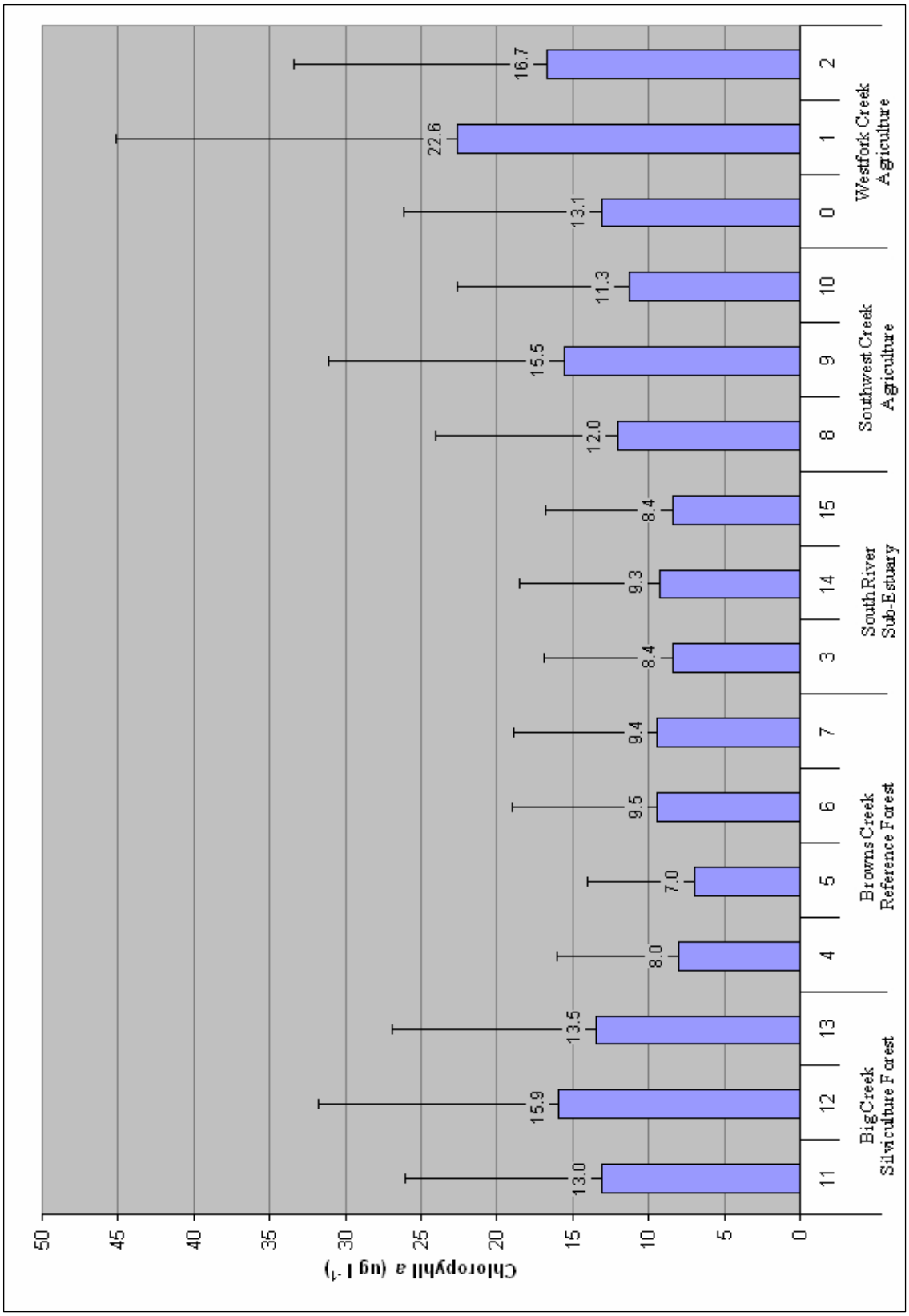

Figure 6.2.2 Mean bottom chlorophyll a concentrations. Station numbers within each creek increase from headwater to mouth

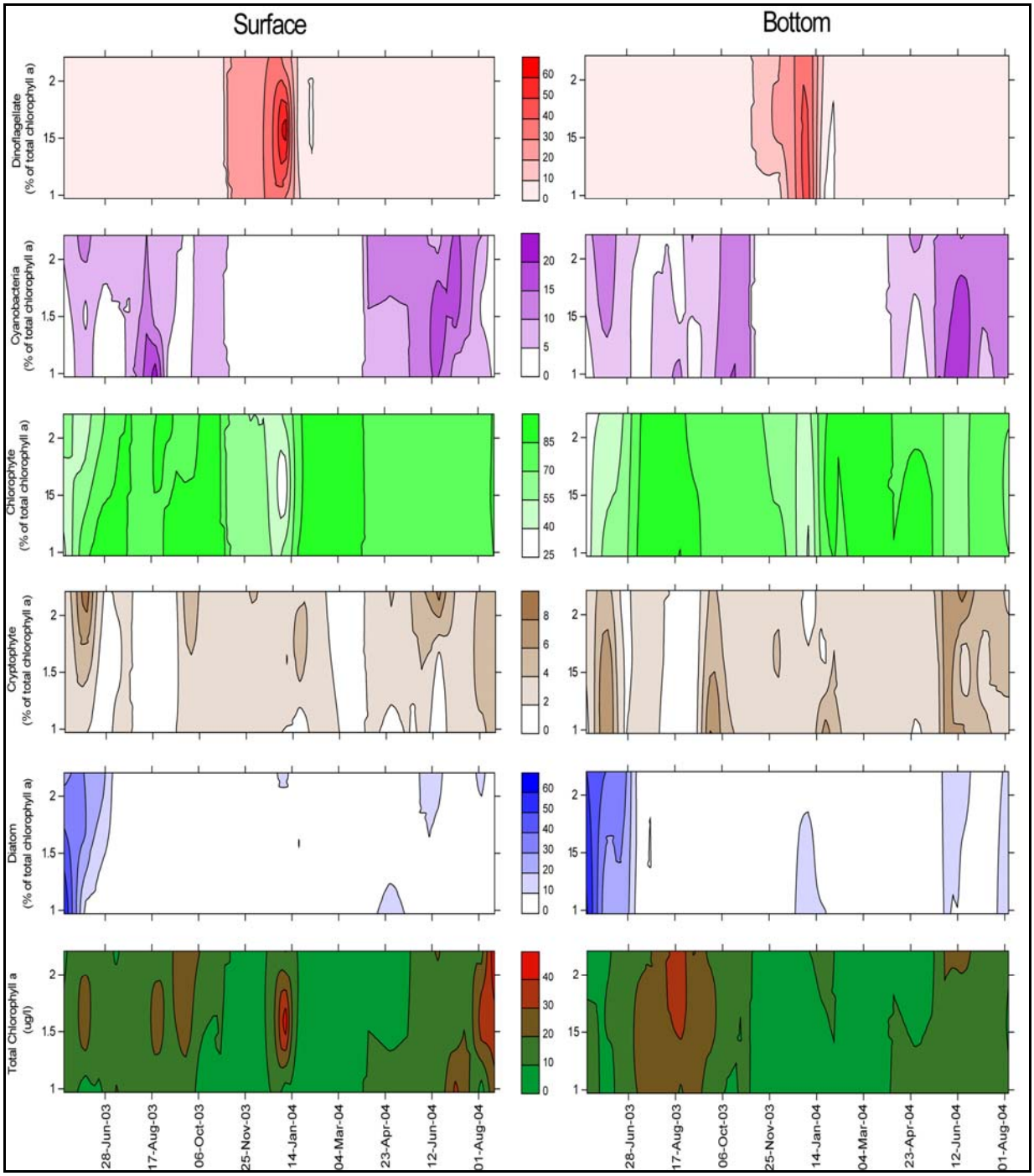

Figure 6.3.1 Big Creek phytoplankton community composition.
Spatiotemporal interpolation with 2.2 km and 30 day search windows for entire record
Groups are quantified as percentage of total chlorophyll a shown in bottom frame as µg l^{-1}.

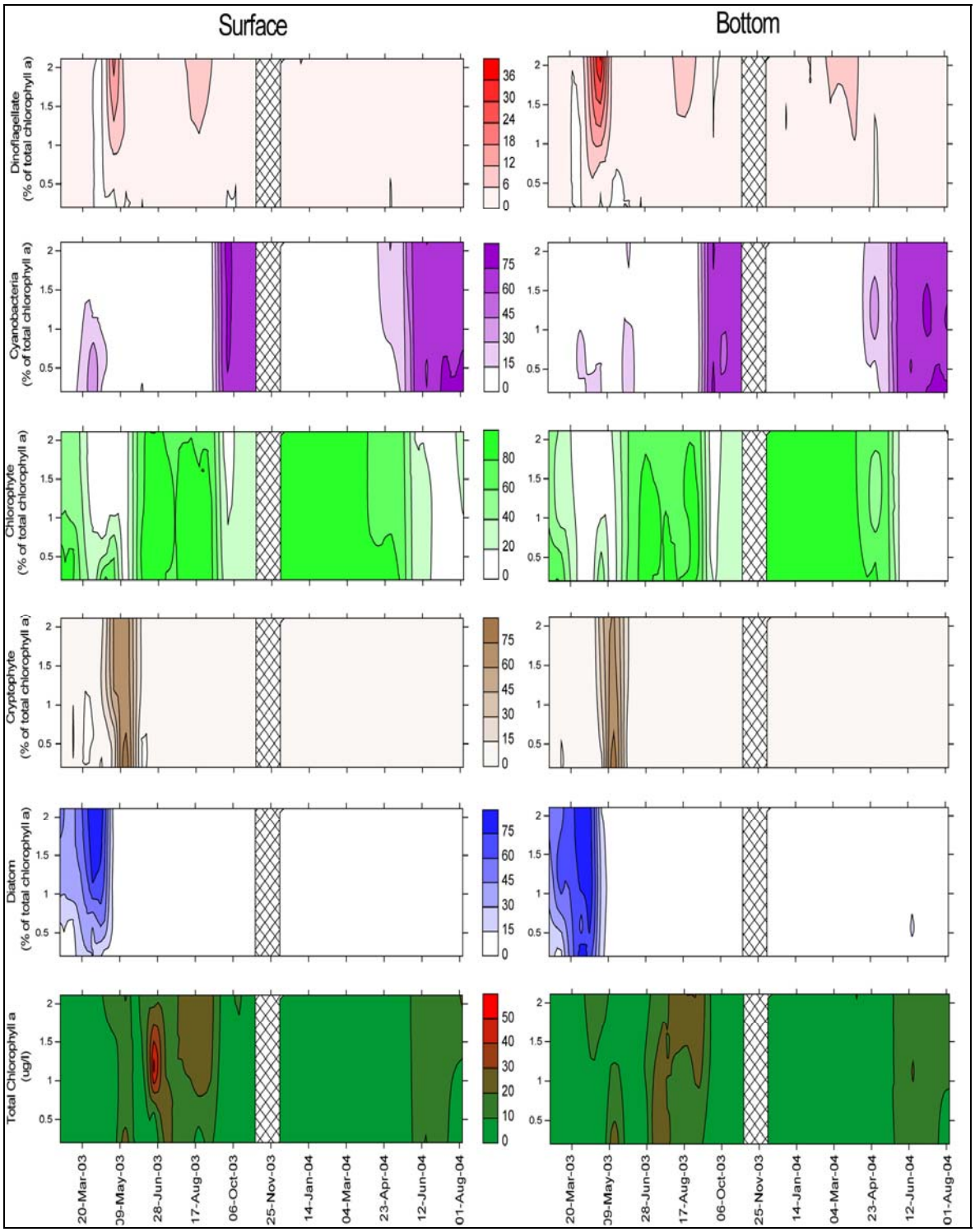

Figure 6.3.2 Browns Creek phytoplankton community composition
Spatiotemporal interpolation with 2.1 km and 30 day search windows for entire record.
X'ed regions shown period of insufficient data for interpolation.
Groups are quantified as percentage of total chlorophyll a shown in bottom frame as µg l-[1]

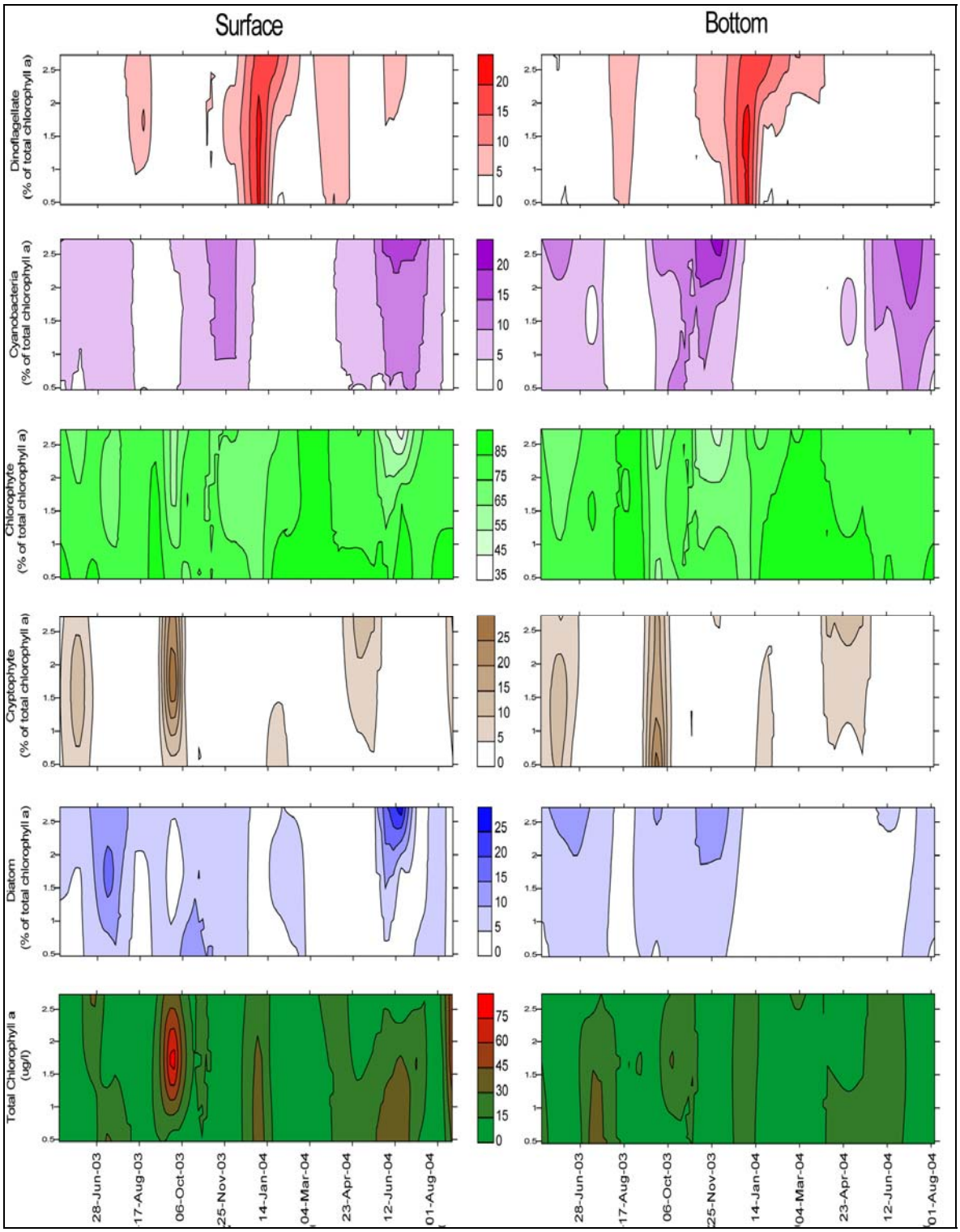

Figure 6.3.3 Southwest Creek phytoplankton community composition
Spatiotemporal interpolation with 2.7 km and 30 day search windows for entire record
Groups are quantified as percentage of total chlorophyll a shown in bottom frame as µg l^{-1}

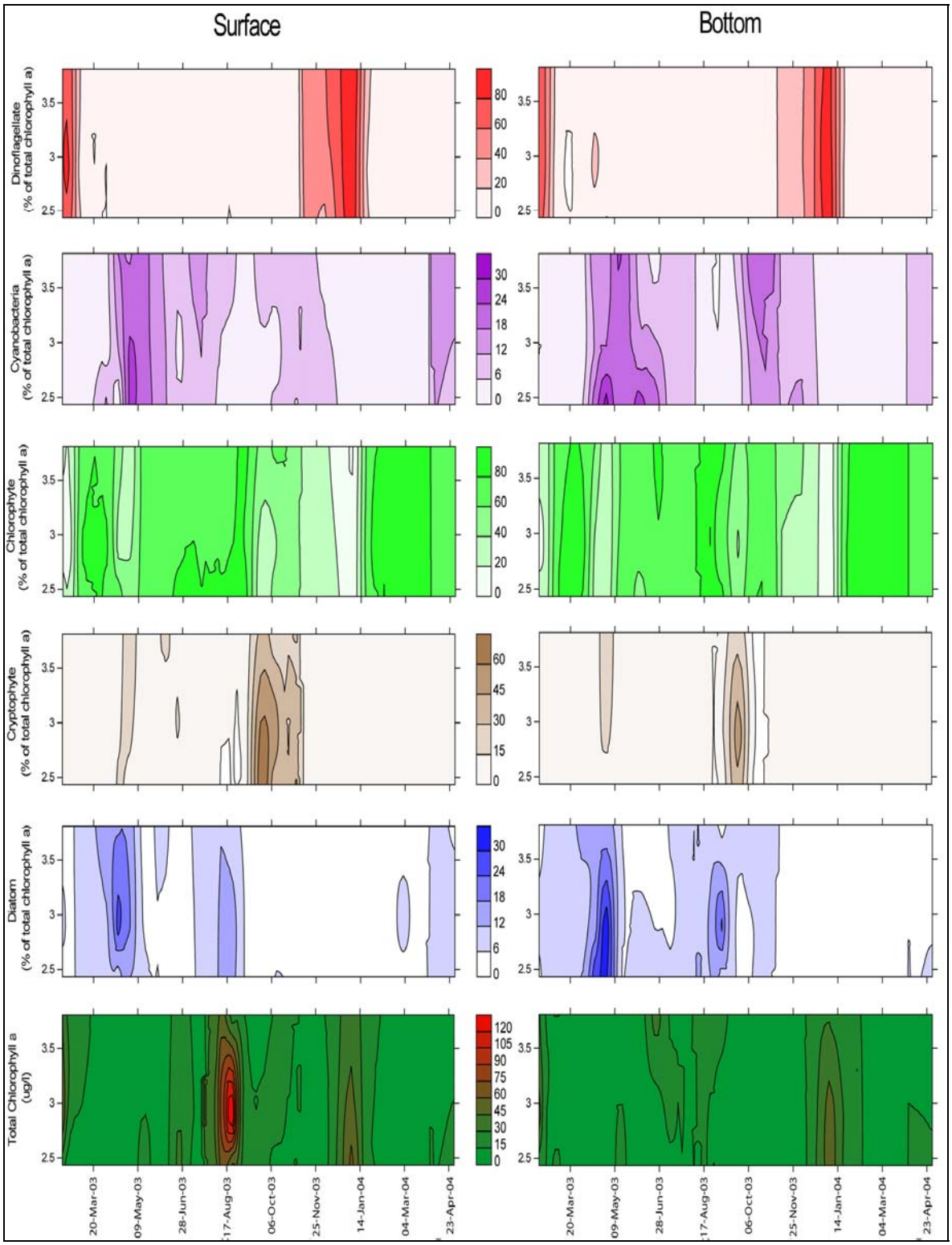

Figure 6.3.4 Westfork Creek phytoplankton community composition Spatiotemporal interpolation with 1.4 km and 30 day search windows for entire record
Groups are quantified as percentage of total chlorophyll a shown in bottom frame as µg l^{-1}

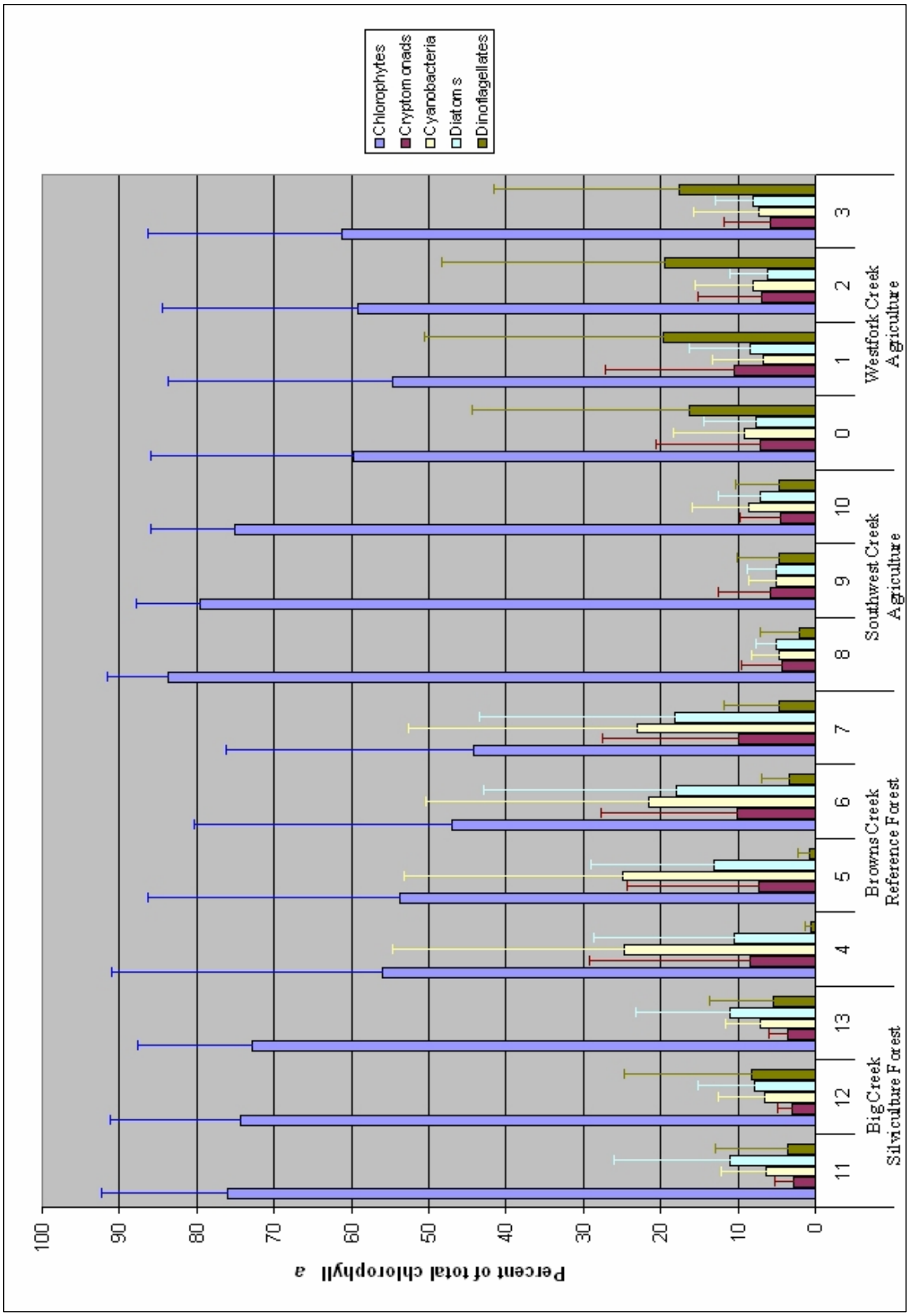

Figure 6.3.6 Mean percent contribution of each phytoplankton group to total chlorophyll a concentrations April 2003 – August 2004

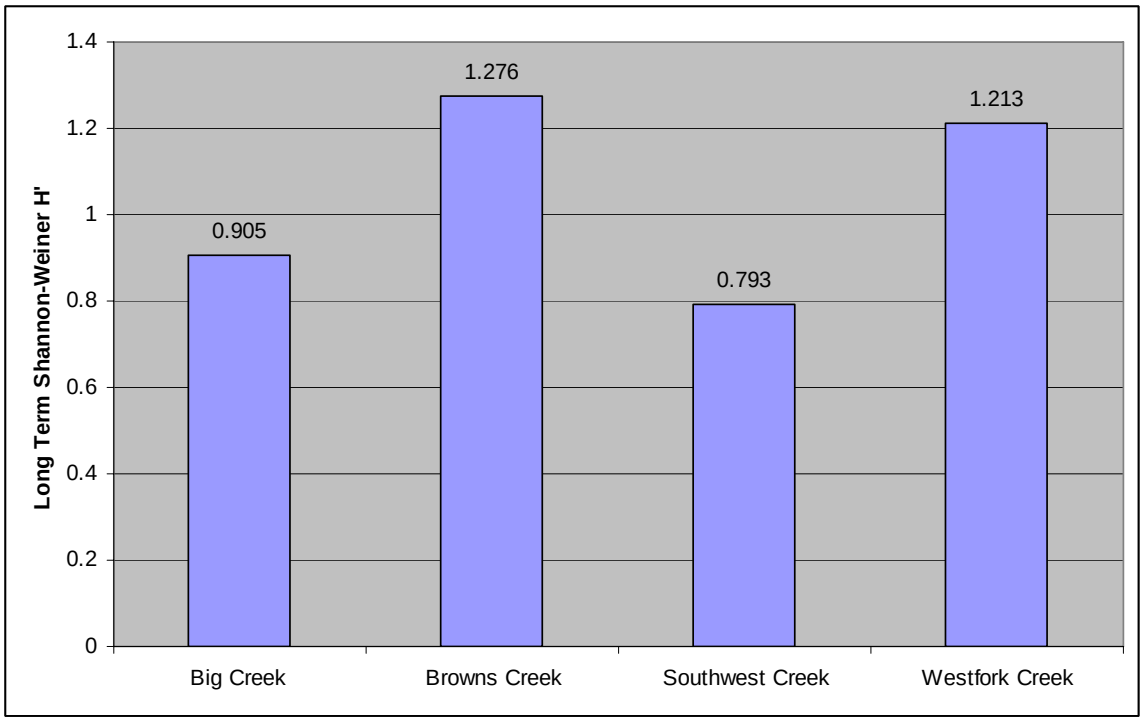

Figure 6.4.1 Long term phytoplankton group diversity Shannon-Weiner Index (H')

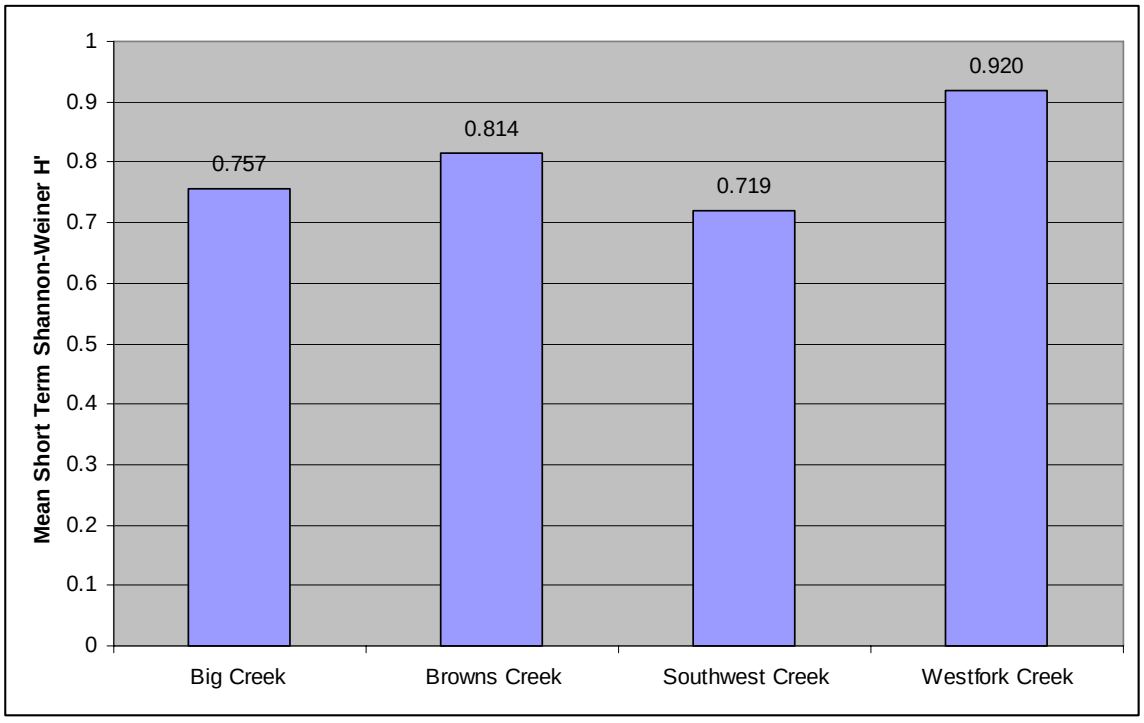

Figure 6.4.2 Short term mean phytoplankton community unit diversity Shannon-Weiner Index (H')

	Westfork Creek			Browns Creek				Southwest Creek			Big Creek			South River		
SITE	**0**	**1**	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**3**	**14**	**15**
Mean Δ S	2.9	1.0	1.9	2.0	3.9	1.8	0.7	2.6	1.6	1.6	3.4	0.4	0.6	1.7	2.5	2.6
Max Δ S	8.5	4.6	12.6	9.4	15.2	6.4	2.6	12.2	12.1	11.6	8.3	2.6	5.3	12.1	12.9	17.2
% Δ S > 2ppt	47%	21%	30%	35%	65%	33%	10%	50%	20%	11%	65%	5%	6%	24%	29%	31%
% Δ S > 3ppt	42%	5%	20%	25%	50%	17%	0%	38%	15%	11%	53%	0%	6%	19%	18%	19%

	Westfork Creek			Browns Creek				Southwest Creek			Big Creek			South River		
SITE	**0**	**1**	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**3**	**14**	**15**
Surface Mean	3.0	4.2	4.8	2.8	4.2	6.5	7.4	3.7	6.7	7.4	6.5	7.9	8.7	6.6	7.6	7.9
Surface Min	0.2	0.2	0.3	0.1	0.1	1.4	2.6	0.3	2.0	2.4	1.6	3.8	4.1	1.7	3.5	4.2
Surface Max	7.2	8.9	8.4	14.9	15.7	16.2	16.4	14.3	15.8	16.6	16.1	16.1	16.7	15.9	16.4	15.5
Bottom Mean	5.0	5.0	6.2	5.0	7.1	7.6	7.9	6.0	8.2	8.5	7.8	8.3	9.1	7.7	9.1	9.6
Bottom Min	0.2	0.2	0.3	0.1	1.3	2.0	3.3	0.9	2.5	2.9	3.4	4.0	4.1	2.3	3.8	4.4
Bottom Max	10.5	11.5	14.2	14.9	16.3	16.5	16.4	14.4	15.7	16.9	16.6	16.7	16.9	15.9	16.4	17.3

Table 6.5.1 Site salinity data (ppt). ΔS = different in ppt between surface and bottom samples

Creek	Volume (m^3)	Total Mean Flushing Time (days)	Total Median Flushing Time (days)
Big Creek (Managed Forest Watershed)	1.23×10^5	1.57	4.35
Browns Creek (Unmanaged Forest Watershed)	2.65×10^5	2.40	6.64
Southwest Creek (Agricultural Watershed)	3.52×10^5	3.79	5.63
Westfork Creek (Agricultural Watershed)	1.50×10^5	1.38	4.09

Table 6.6.1 Creek volume and flushing time values

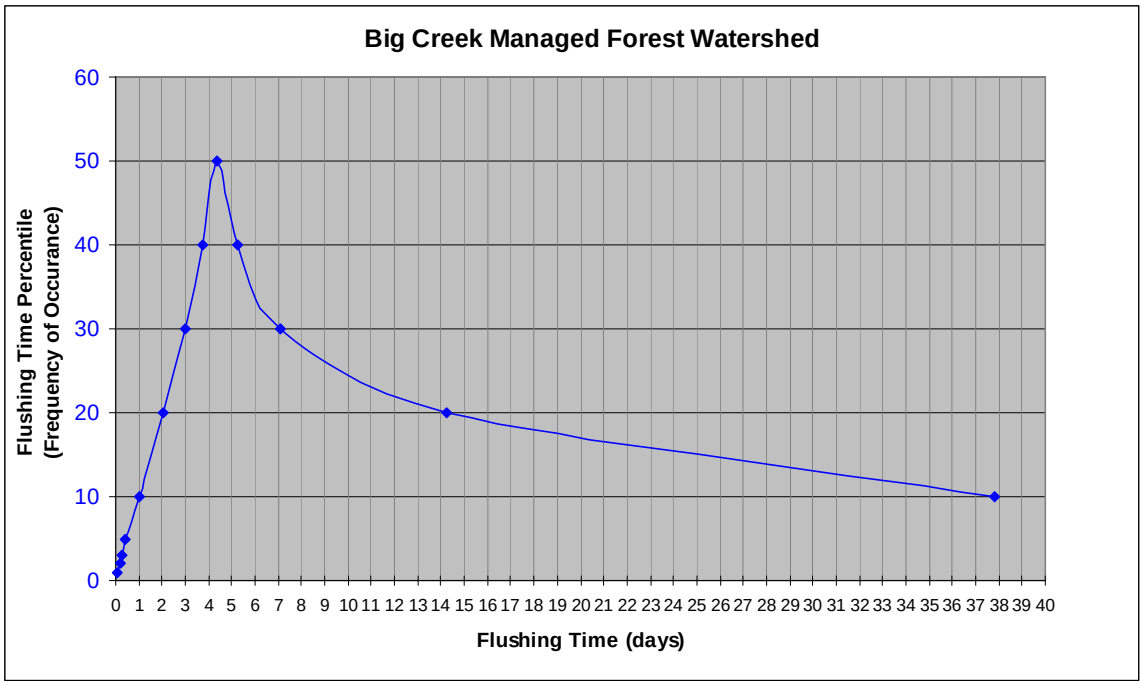

Figure 6.6.1 Big Creek flushing time recurrence distribution

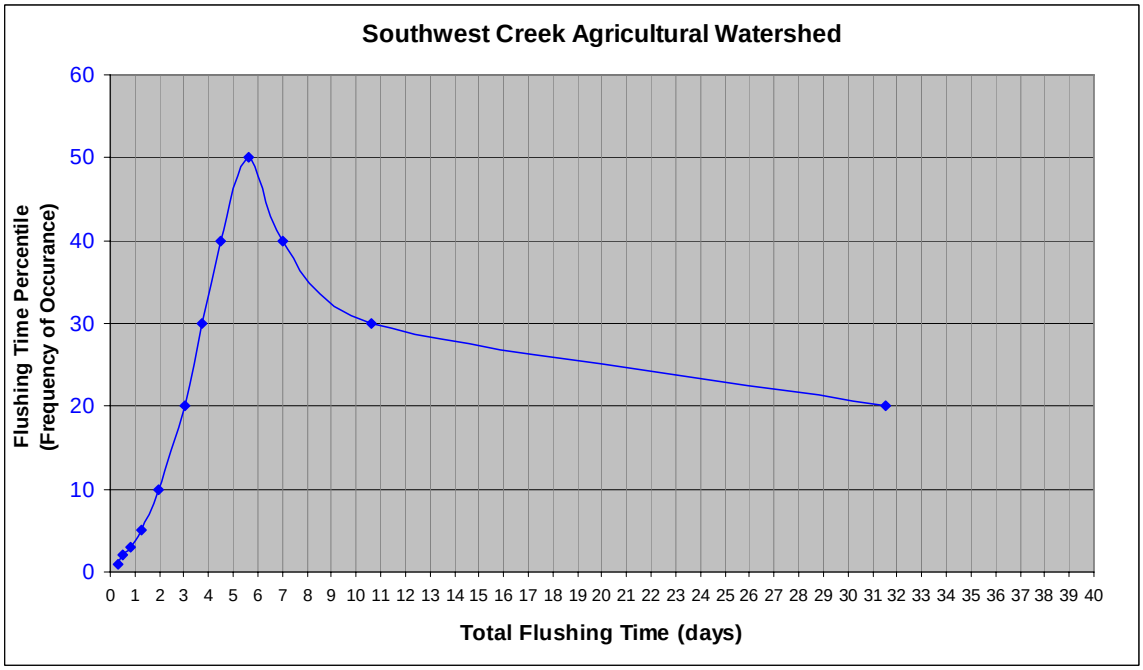

Figure 6.6.2 Southwest Creek flushing time recurrence distribution

CHAPTER 6

RESULTS PART II - BIOASSAYS

6.7 Summer Bioassay - July-August 2003

Big Creek Results

Big Creek had the second highest initial chlorophyll *a* concentration (61 µg l^{-1}) (Figure 6.7.1). Initial NO_x^-, NH_4^+ and PO_4^{-3} concentrations for each creek were similar (Table 6.7.1). The initial Big Creek phytoplankton community was dominated by cyanobacteria (42%) and chlorophytes (48%) (Figure 6.7.2). After 24 hours (T1), primary productivity was significantly greater than control in the NO_x^-, NH_4^+, and DIN+P treatments (Figure 6.7.6) (See Material and Methods chapter for statistical methods and definition of significance). There were no significantly greater than control responses in chlorophyll *a* concentration observed in any treatments until 48 hours (T2) (Figures 6.7.3, 6.7.4). At T2, primary productivity and chlorophyll *a* were significantly greater than controls in all treatments containing N additions (NO_3, NH_4^+, DON, DIN+P, and DON+P) (Figures 6.7.4, 6.7.7). At 72 hours (T4) DIN concentrations were low in all treatments, there were no primary productivity rates significantly greater than controls, and only the NO_3 and DIN+P treatments yielded chlorophyll *a* concentrations significantly greater than controls (Figures 6.7.5, 6.7.8). In the PO_4^{-3} treatments, there were no responses that were significantly greater than control at any time points. The maximum nitrogen utilization rates of both NH_4^+ and NO_x^- occurred in the first 24 hours of the experiment (Tables 6.7.2 and 6.7.3). Specifically, in the NH_4^+ additions, NH_4^+ was

utilized at 288 µgN d^{-1} (7.7 µgN µgChla$^{-1}d^{-1}$), and in the NO_3^- additions NO_x^- was utilized at 280 µgN d^{-1} (10.8 µgN µgN$^{-1}d^{-1}$). These rates were the highest observed throughout the experiment across all creeks, but they were very similar to those in Southwest Creek. The N utilization rates in the first 24 hours assimilated over 90 percent of available N, which led to much lower N utilization rates as the experiment progressed.

Southwest Creek Results

The initial Southwest Creek chlorophyll *a* concentration was (36 µg l^{-1}) (Figure 6.7.1). The Southwest Creek phytoplankton community was primarily chlorophytes (84%) (Figure 6.7.2). At T1, primary productivity and chlorophyll *a* concentrations were significantly greater than control in all N addition treatments. Primary productivity remained greater than control in all N addition treatments at T2 and T4. However, at T2 and T4 chlorophyll *a* concentrations were only significantly higher than controls in the T4 DON+P treatment. In the PO_4^{-3} treatments, there were no responses that were significantly greater than control at any time points. The PO_4^{-3} treatment chlorophyll *a* concentrations levels were less than control at T2 and T4. The maximum nitrogen utilization rates occurred in the first 24 hours of the experiment (Tables 6.7.2 and 6.7.3). As previously stated, these rates were very similar to those observed in Big Creek.

Browns Creek Results

The initial Browns Creek phytoplankton community was primarily cyanobacteria (48%), and dinoflagellates (44%). Browns Creek also had the highest initial chlorophyll *a* concentration (79 µg l^{-1}) (Figures 6.7.1 and 6.7.2). At T1, primary productivity was significantly greater than control only in treatments with additions of both N and P (DON+P, DIN+P) (Figure 6.7.6). No significantly greater than control chlorophyll *a*

concentration increases were observed until T2, and then only in the DIN+P treatment. At both T2 and T4, primary productivity rates were greater than control in all treatments containing N additions (Figures 6.7.3, 6.7.4). At T4, chlorophyll *a* concentrations were significantly higher than control in the NO3 and DON+P treatments. In the PO_4^{-3} treatments, there were no responses that were significantly greater than control at any time points. A decrease of the cyanobacteria percentage of the phytoplankton community was observed at T2 in NH_4^+ and DIN+P treatments (Figure 6.7.9). No other changes in the phytoplankton community were observed. The maximum nitrogen utilization rates occurred in the first 24 hours of the experiment. In the NH_4^+ treatments, 95% of dissolved NH_4^+ was utilized in the first 24 hours, at a rate of 288 µgN d^{-1} (7.7 µgN $µgN^{-1}$ d^{-1}). In the NO_3^- treatments, 77% of dissolved NO_x^- was utilized at a rate of 218 µgN d^{-1} (6.4 µgN $µgChla^{-1}$ d^{-1}) (Tables 6.7.2 and 6.7.3).

Neuse Estuary Results

The initial Neuse River chlorophyll *a* concentration was (28 µg l^{-1}) (Figure 6.7.1). The initial Neuse Estuary phytoplankton community was primarily cyanobacteria (80%) (Figure 6.7.2). At T1, primary productivity was significantly greater than control in the DIN+P treatments, and no significantly greater than control chlorophyll *a* concentration increases were observed (Figures 6.7.3, 6.7.4, 6.7.6). At T2, chlorophyll *a* concentrations in all treatments containing N additions were significantly higher than controls, but only the NO_3^- treatment had primary productivity rates significantly greater than controls (Figures 6.7.4 and 6.7.7). At T4, no primary productivity rates or chlorophyll *a* concentrations were significantly greater than controls, but DON+P chlorophyll *a* concentrations were significantly less than control (Figures 6.7.5 and 6.7.8). In the PO_4^{-3}

treatments, there were no responses that were significantly greater than control at any time points. The peak DIN nitrogen utilization rates (see Materials and Methods chapter) occurred between T1 and T2, which was 24 hours after the peak utilization rates were observed in all the creeks (Tables 6.7.2 and 6.7.3).

Summer Bioassay Summary

The chlorophyll *a* concentrations in the collection waters of the summer bioassay were the highest of all the experiments. Chlorophyll *a* and primary productivity values were not significantly higher than control in treatments without N additions. The chlorophyte-dominated Southwest and Big Creek N additions treatments responded 24 hours before the cyanobacteria and dinoflagellate communities in the Browns Creek and Neuse River treatments. The peak percentage of control responses in chlorophyll *a* and primary productivity were similar in all creeks and the Neuse Estuary. Additions of different forms of N did not lead to significantly different responses in chlorophyll *a* or primary productivity rates. The only community composition shift observed in the experiment was an increase in dinoflagellates in the NH_4^+, PO_4^{-3}, and DIN+P treatments.

6.8 Fall Bioassay – November 2003

Big Creek Results

The initial Big Creek chlorophyll *a* concentration was (6.7 µg l^{-1}) (Figure 6.8.1) The initial Big Creek phytoplankton community was primarily cyanobacteria (60%) and cryptomonads (32%) (Figure 6.8.2). This community was similar to that found in the Neuse Estuary collection water. At T1, increases in chlorophyll *a* concentrations in the DON treatment were the only significantly greater than control responses observed. Primary productivity rates in any treatments were not significantly higher than controls

(Figures 6.8.3, 6.8.4, 6.8.6). At T2 and T4 chlorophyll *a* in all treatments containing N additions were significantly greater than controls (Figures 6.8.4, 6.8.7). However, on T2 and T4 primary productivity rates were significantly greater than control only in the DIN+P and DON+P treatments. In the PO_4^{-3} treatments, there were no responses that were significantly greater than control at any time points. The maximum DIN utilization rates were observed at T2. Notably, at T1 all treatments, including controls, had NH_4^+ utilization rates greater than 24 µgN 1^{-1} d^{-1} and chlorophyll *a* normalized values that ranged from 4.1 µgN $chla^{-1}$ d^{-1} to 7.0 µgN $chla^{-1}$ d^{-1}. (Tables 6.8.2 and 6.8.3).

Southwest Creek Results

The initial Southwest Creek chlorophyll *a* concentration was 5.9 µg l^{-1} (Figure 6.8.1). Both DIN and PO_4 were elevated in the Southwest Creek collection water (Table 6.8.1). The majority of the initial Southwest Creek phytoplankton community was comprised of chlorophytes (72%) (Figure 6.8.2). At T1 and T2, no significantly greater than control responses were observed in any treatments except for the primary productivity rates in the T2 DIN+P additions (Figures 6.8.4, 6.8.7). At T4, all DIN chlorophyll *a* concentrations were significantly greater than control in all DIN additions (NO_x^-, NH_4^+, DIN+P) and primary productivity rates were significantly greater than control in the NH_4^+ and DIN+P additions. In the PO_4^{-3} treatments, there were no responses that were significantly greater than control at any time points. The maximum DIN utilization rates were observed at T4 (Table 6.8.2). All T2 treatments, including controls, had NH_4^+ and NO_x^- utilization rates greater than 24 µgN 1^{-1} d^{-1}.

Browns Creek Results

The initial Browns Creek chlorophyll *a* concentration was 3.2 µg l^{-1} (Figure 7.8.1). The initial molar ratio of DIN:P was greater than 16 in Browns Creek and less than 16 in water collected at other sites (Table 6.8.1). The majority of the initial Browns Creek phytoplankton community was cyanobacteria (89%) (Figure 6.8.2). The only significantly greater than control chlorophyll *a* and primary productivity responses were observed in treatments that included a PO_4^{-3} addition. Specifically, chlorophyll *a* was significantly higher than control in the T1 DON+P treatment and T4 DIN+P and DON+P treatments. Primary productivity was significantly greater than control in the T2 PO_4^{-3}, DIN+P and DON+P and T4 DIN+P and DON+P treatments. The significantly greater than control primary productivity response to the PO_4^{-3} treatment was the only such response in the experiment. In the T4 DIN+P and DON+P treatments, the percent contribution chlorophytes to total chlorophyll *a* did increase to 26% and 12% respectively. In the T0 and T4, controls chlorophytes represented less than 3% of total chlorophyll *a* (Figure 6.8.9). The maximum DIN utilization rates were observed at T4 concurrently with the highest observed chlorophyll *a* and primary productivity observations (Tables 6.8.2 and 6.8.3).

Neuse Estuary Results

The Neuse Estuary had the highest initial chlorophyll *a* concentration (25.5 µg l^{-1}) (Figure 7.8.1). The initial Neuse Estuary phytoplankton community was primarily cyanobacteria (60%) and cryptomonads (32%) (Figure 6.8.2). This community was similar to that found in Big Creek. At T1, no responses were significantly greater than control in any treatments. At T2, chlorophyll *a* concentrations were significantly greater

than control in all treatments with an N addition. At T4, chlorophyll *a* concentrations were significantly greater than control in the DIN+P and DON+P treatments. The T2 DIN+P treatment had the only primary productivity rates that were significantly greater than control during the bioassay. In the PO_4^{-3} treatments, there were no responses that were significantly greater than control at any time points. The maximum DIN utilization rates were observed at T2 in the NO_x^-, NH_4^+ and DIN+P treatments. (Tables 6.8.2 and 6.8.3).

Fall Bioassay Summary

In Big Creek, Southwest Creek, and the Neuse River, the largest increases in chlorophyll *a* and primary productivity were observed in the N+P treatments. In the Browns Creek treatments, significantly greater than control responses were only seen in N+P treatments. No temporal differences in response time were observed across the experiment. All maximum chlorophyll *a* concentrations and primary productivity rates occurred on T4. Different forms of nitrogen did not elicit significantly different responses in chlorophyll *a* or primary productivity levels.

6.9 Winter Bioassay - February 2004

Big Creek Results

The Big Creek initial chlorophyll *a* concentration was 5.5 µg l^{-1} (Figure 6.9.1). The initial nutrient concentrations in collection water from all creeks can be found in Table 6.9.1) The majority of the initial Big Creek phytoplankton community was chlorophytes (87%) (Figure 6.8.2). This community was similar to that found in the Southwest Creek collection water. The significantly greater than control responses in chlorophyll *a* and primary productivity were limited to the DIN+P treatments throughout

the experiment (Figures 6.9.3 through 6.9.9.) The exception was a significantly greater than control chlorophyll *a* response to all N addition treatments at T4 (6.9.5). In the PO_4^{-3} treatments, there were no responses that were significantly greater than control at any time points. The highest DIN utilization rates were observed at T1 (Table 6.9.2 and 6.9.3).

Southwest Creek Results

The initial Southwest Creek chlorophyll *a* concentration was 7.5 µg l^{-1} (Figure 6.9.1). The majority of the initial Southwest Creek phytoplankton community was chlorophytes (72%) (Figure 6.9.2). DIN concentrations in the Southwest Creek collection water were 15uM, and N additions increased the concentration another 20uM. All Southwest Creek treatments, including controls, showed increasing chlorophyll *a* concentrations through the experiment. There were no significantly greater than control responses in chlorophyll *a* concentrations throughout the experiment (Figures 3.9.3, 3.9.4, 3.9.5). Primary productive rates were significantly higher than control at T4 in the PO_4^{-3}, DIN+P, and DON+P treatments (Figures 3.9.6, 3.9.7, 3.9.8). The DIN utilization rates remained high throughout the experiment. The DIN utilization rate for NH_4^+ in the NH_4^+ treatment at T2 (Table 6.9.2 and 6.9.3)

Browns Creek Results

Browns Creek phytoplankton abundance was very low (0.8 µg l^{-1}) (Figure 6.9.1). Diagnostic HPLC pigment concentrations were below detection limits. Chlorophyll *a* concentrations did not exceed 1 µg l^{-1} in any treatment during the experiment. Primary productivity rates were also very low in all treatments, but were significantly greater than control in the PO_4^{-3} additions at all time points. However, the T4 primary productivity

rates normalized to chlorophyll *a* concentrations (μg C μgChla^{-1} h^{-1}) and were comparable to those measured in the other creek and estuary treatments.

Neuse Estuary Results

Initial chlorophyll *a* concentrations were highest in the Neuse River (11.9 μg l^{-1}) (Figure 6.9.1). The majority of the initial Neuse Estuary phytoplankton community was dinoflagellates (82%) (Figure 6.9.2). Chlorophyll *a* concentrations were significantly greater than control in the DIN+P treatment at T1 and significantly greater than control in all N addition treatments at T2 and T4. Primary productivity was significantly greater than control only in the DIN+P treatment at T2 and the DON+P treatment at T4. The maximum DIN utilization rates were observed at T1 in the NO_x^- and NH_4^+additions (Tables 6.9.2 and 6.9.3).

Winter Bioassay Summary

In the winter bioassay, the dinoflagellate community in the Neuse Estuary showed the greatest responses in chlorophyll *a*, primary productivity, and DIN assimilation in treatments containing N. Across all creeks, there were more significantly greater than control responses to N+P additions that to N only additions. No temporal differences in response time between the creeks were observed across the experiment. Maximum chlorophyll *a* concentrations and primary productivity rates occurred on T4.

6.10 Spring Bioassay – May 2004

Big Creek Results

Initial chlorophyll *a* concentrations were similar at all collection sites. These concentrations ranged from 8.5 μg l^{-1} in Big Creek to 5.1 μg l^{-1} in Browns Creek (Figure 6.10.1). The initial Big Creek phytoplankton community was primarily diatoms (88.9%)

(Figure 6.10.2). At T1, chlorophyll *a* concentrations were significantly greater than control in the DIN+P and DON+P treatments, and primary productivity were significantly higher than controls in all treatments containing N additions (Figures 6.10.2 and 6.10.4). At T2 chlorophyll *a* concentrations and primary productivity rates were significantly greater than control in the DIN+P and DON+P treatments (Figures 6.10.3 and 6.10.5). At T4, all chlorophyll *a* concentrations were lower than T2, but treatments containing N additions were significantly greater than control (Figure 6.10.4). No primary productivity rates were significantly greater than controls at T4 (Figure 6.10.6). In the PO_4^{-3} treatments, there were no responses that were significantly greater than control at any time points. The highest DIN utilization rates were observed at T1 in the DIN+P treatments (Tables 6.10.2 and 6.10.3).

Southwest Creek Results

A majority of the initial Southwest Creek phytoplankton community was chlorophytes (75%) (Figure 6.8.2). This community was similar to that found in Browns Creek. At T1 and T4, chlorophyll *a* concentrations were significantly higher than controls in all treatments with an N addition. At T2, chlorophyll *a* concentrations were significantly greater than control only in the DIN+P and DON+P treatments. Primary productivity was significantly higher than control only in N+P treatment at all time points. Specifically, primary productivity was significantly greater than control in the DIN+P and DON+P treatments at T1 and in the DIN+P treatments at T2 and T4. In the PO_4^{-3} treatments, there were no responses that were significantly greater than control at any time points. The highest DIN utilization rates for NH_4^+ occurred at T1 while the

highest rates for NO_x^- occurred at T2. In the DIN+P treatment, DIN utilization rates were highest for NH_4^+ at T1 and then highest for NO_x^- at T2 (Tables 6.10.2 and 6.10.3).

Browns Creek Results

A majority of the initial Browns Creek phytoplankton community was chlorophytes (89%) (Figure 6.8.2). Significantly greater than control responses in chlorophyll *a* and primary productivity were observed in treatments that included a PO_4^{-3} addition. Specifically, chlorophyll *a* in the DIN+P and DON+P treatments was significantly greater than control and PO_4^{-3} treatments at all time points. Primary productivity was significantly greater than control in the DIN+P and DON+P treatments at T2. The highest DIN utilization rates for NH_4^+ occurred at T1 while the highest rates for NO_x^- occurred at T2. In the DIN+P treatment utilization rates were highest for NH_4^+ at T1 and then highest for NO_x^- at T2 (Tables 6.10.2 and 6.10.3).

Neuse Estuary Results

The initial Neuse Estuary phytoplankton community was primarily composed of cyanobacteria (91%) (Figure 6.10.2). At T1, chlorophyll *a* concentrations were significantly higher than controls in all treatments with N additions, and primary productivity was significantly greater than control in the DIN+P treatments. At T2, chlorophyll *a* concentrations and primary productivity rates were significantly greater than control in the DIN+P and DON+P treatments. At T4, chlorophyll *a* concentrations were significantly greater than controls in all treatments with N additions. In the PO_4^{-3} treatments, there were no responses that were significantly greater than control at any time points. The highest DIN utilization rates for NH_4^+ occurred at T1, while the highest

rates for NO_x^- occurred at T2. The DIN+P treatment utilization rates were highest for NH_4^+ at T1 and then highest for NO_x^- at T2 (Tables 6.10.2 and 6.10.3).

Spring Bioassay Summary

In the spring bioassay, primary productivity rates were only found to be significantly higher than control in DIN+P and DIN+P treatments. In N only treatments, the diatom community in Big Creek was observed to have the largest response. In treatments receiving N and P, the chlorophyte community in Browns Creek showed the largest chlorophyll *a* responses. In all DIN+P treatments, peak NH_4^+ utilization occurred in the first 24 hours, followed by peak NO_x^- utilization in the second 24 hours.

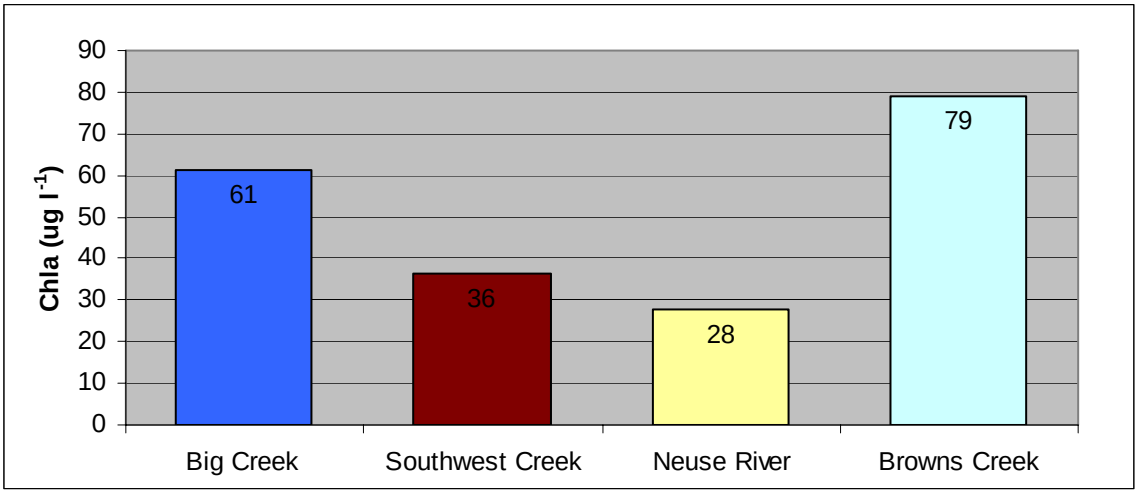

Figure 6.7.1 Summer bioassay T0 chlorophyll a concentrations

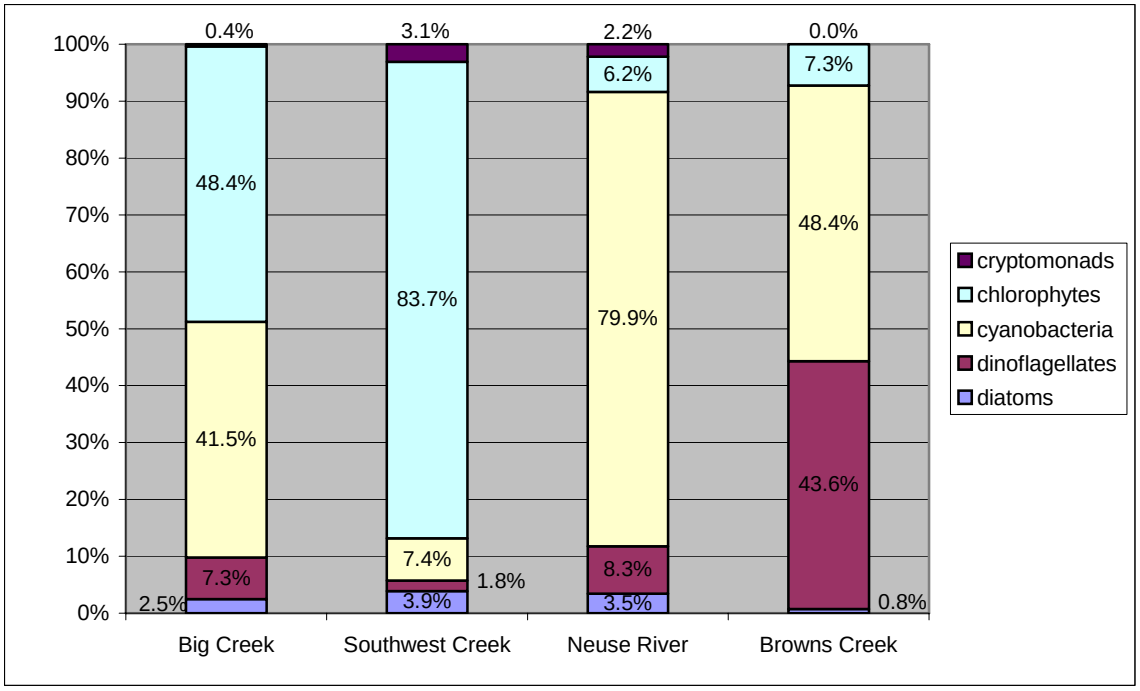

Figure 6.7.2 Summer bioassay T0 initial phytoplankton community composition

	N-NO_x µg l^{-1}	**N-NH_4 µg l^{-1}**	**P-PO_4 µg l^{-1}**	**TDN µg l^{-1}**
Browns Creek	4.3	17.6	14.2	534
Southwest Creek	2.6	18.3	160.9	511
Neuse River	3.3	12.0	58.8	346
Big Creek	2.6	16.3	51.4	408

Table 6.7.1 Summer bioassay T0 ambient nutrient concentrations

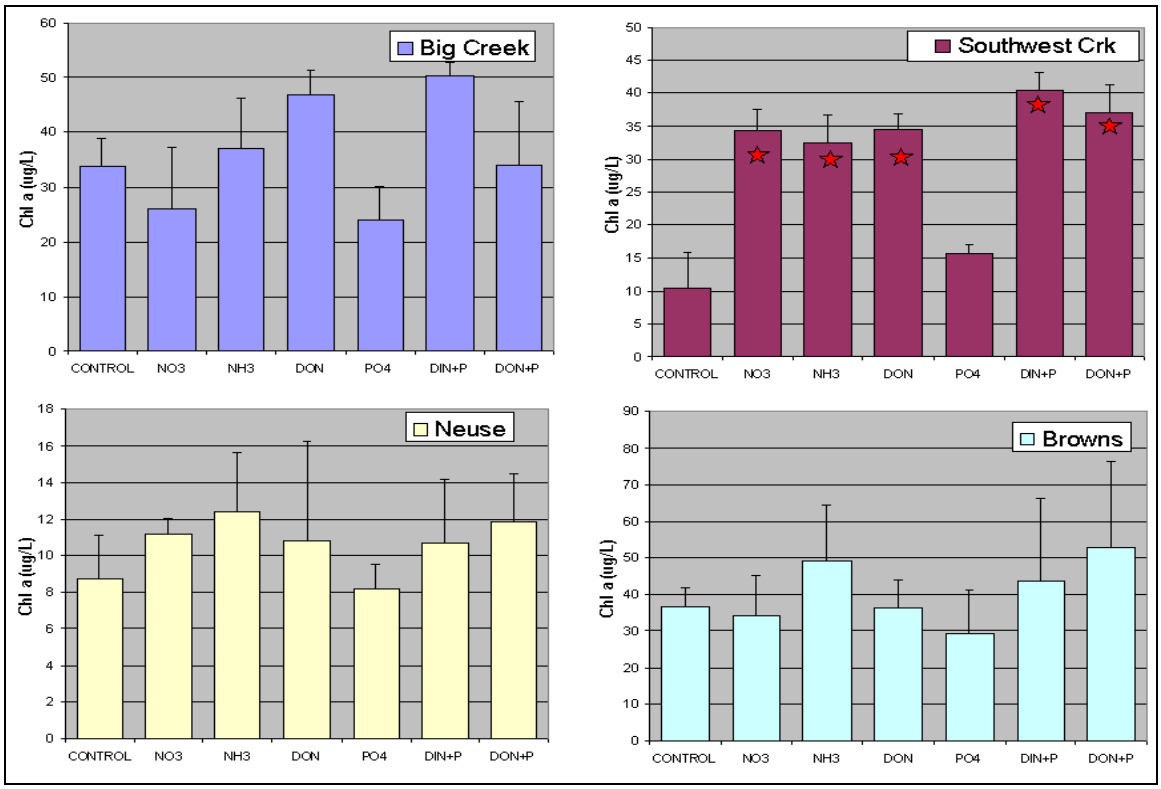

Figure 6.7.3 Summer bioassay T1 chlorophyll a results

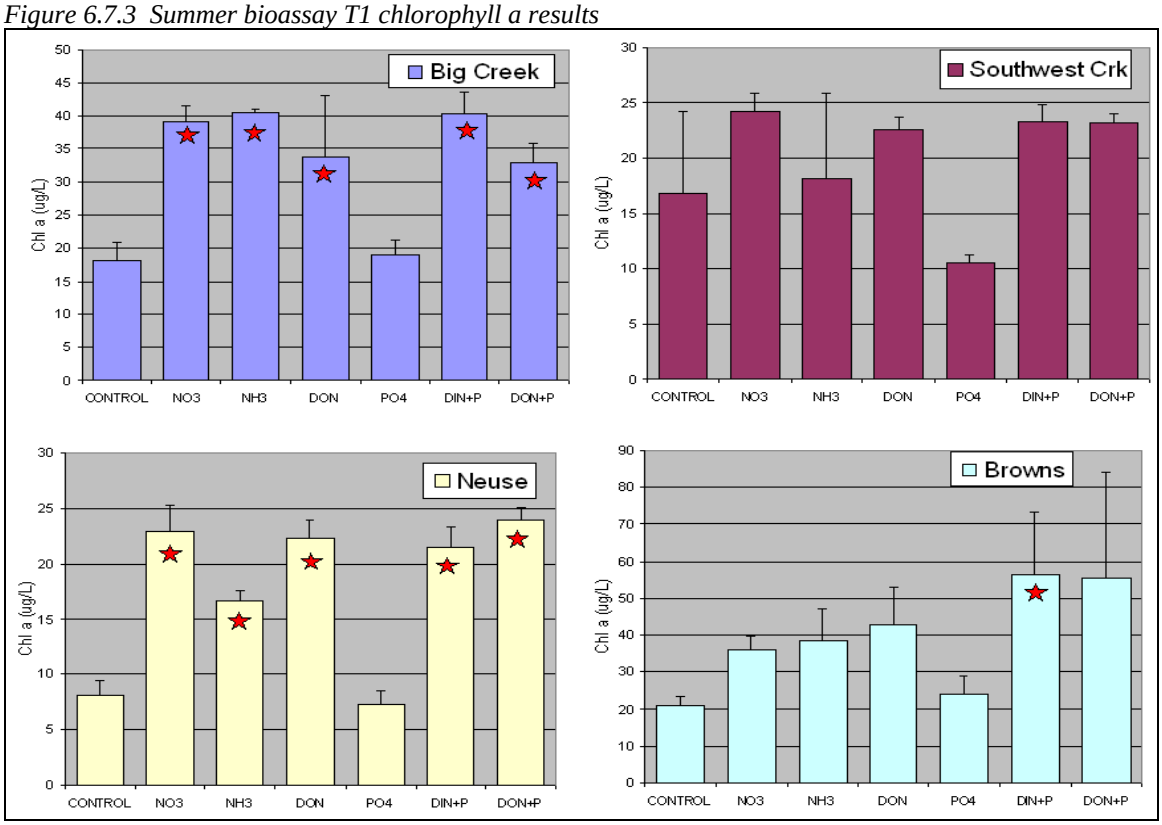

Figure 6.7.4 Summer bioassay T2 chlorophyll a results

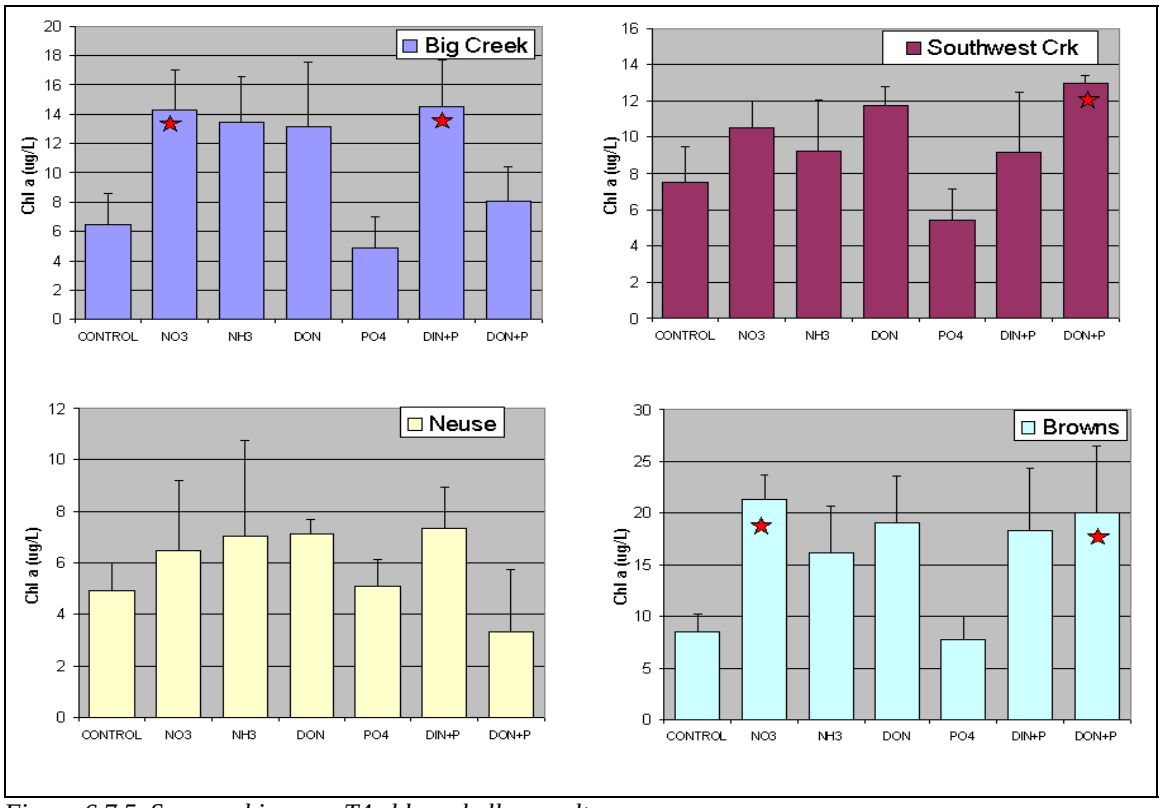

Figure 6.7.5 Summer bioassay T4 chlorophyll a results

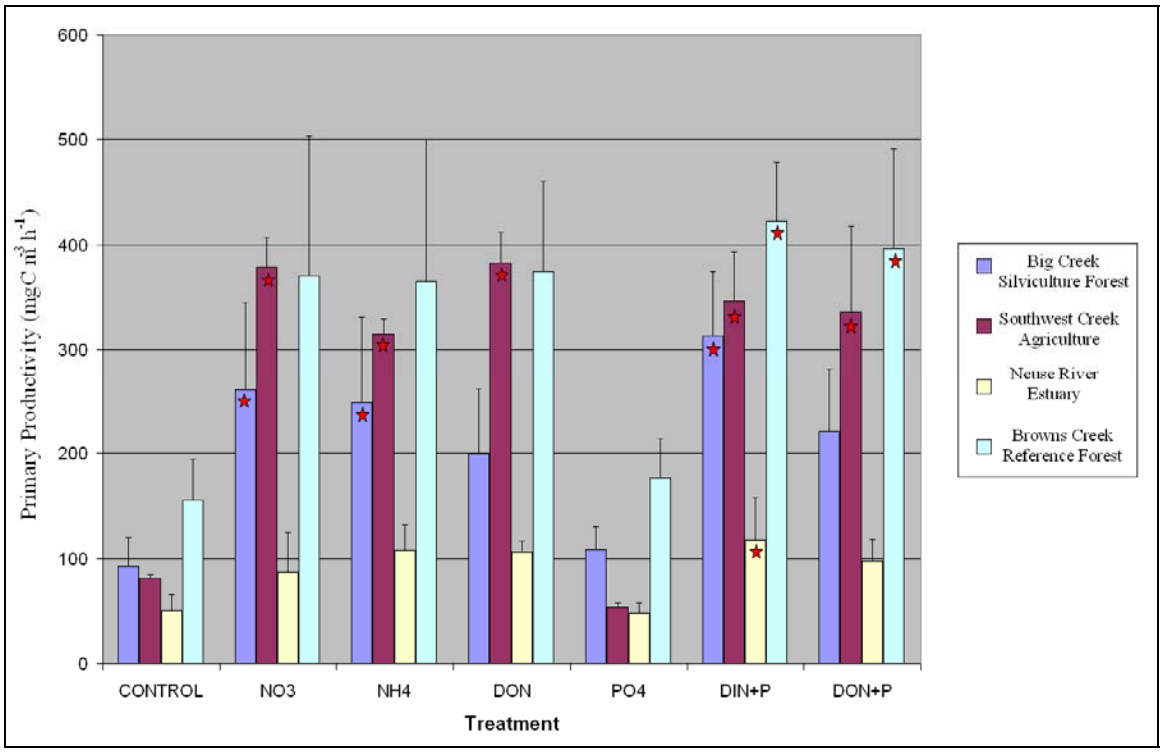

Figure 6.7.6 Summer bioassay T1 primary productivity rates
Red star indicates rate significantly greater than control ($p < 0.05$)

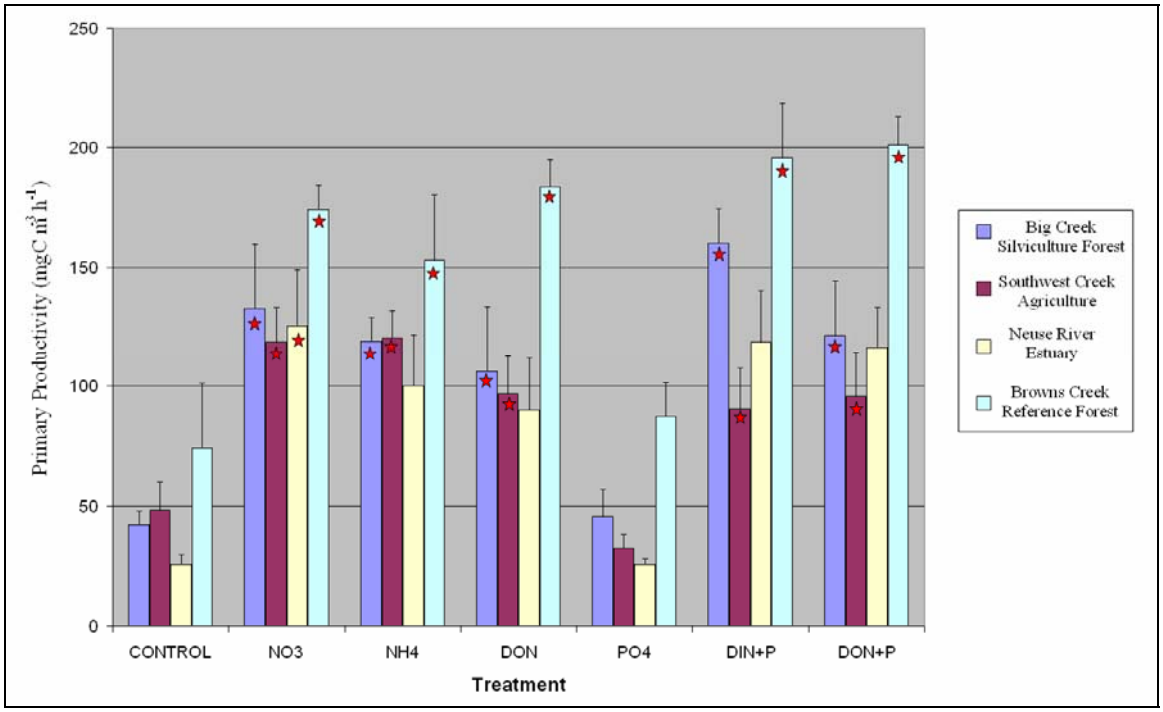

Figure 6.7.7 Summer bioassay T2 primary productivity rates
Red star indicates significantly greater than control ($p < 0.05$)

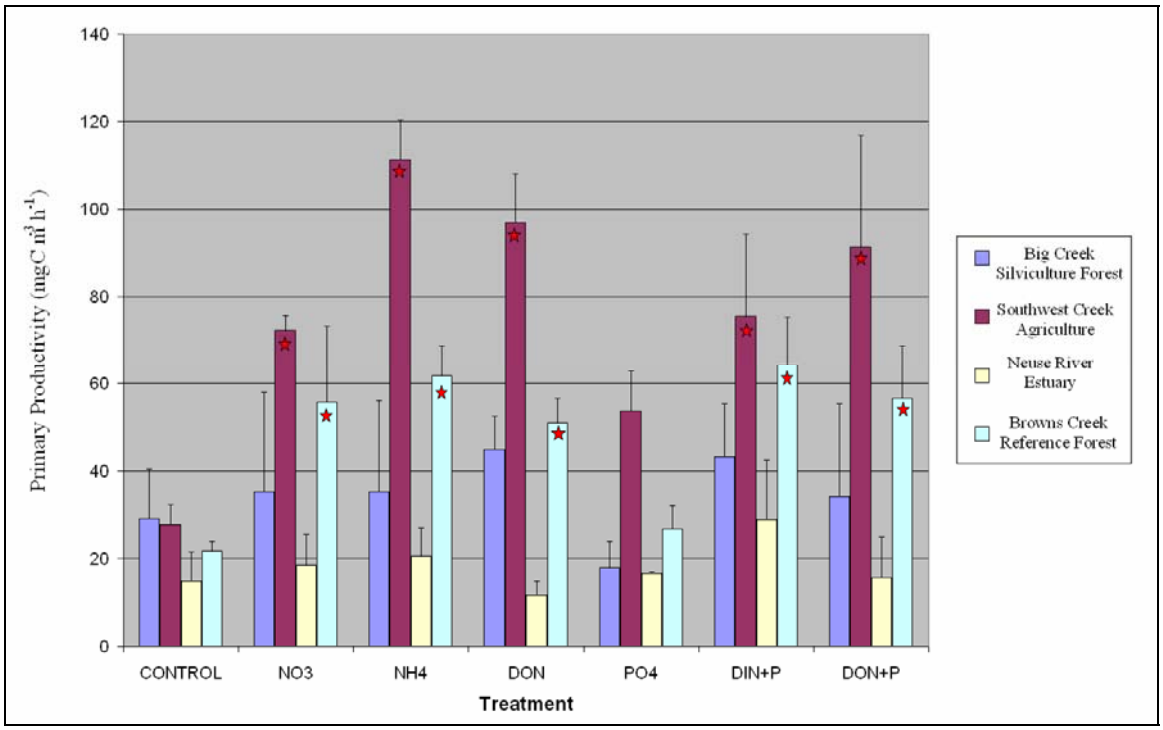

Figure 6.7.8 Summer bioassay T4 primary productivity rates
Red star indicates significantly greater than control ($p < 0.05$)

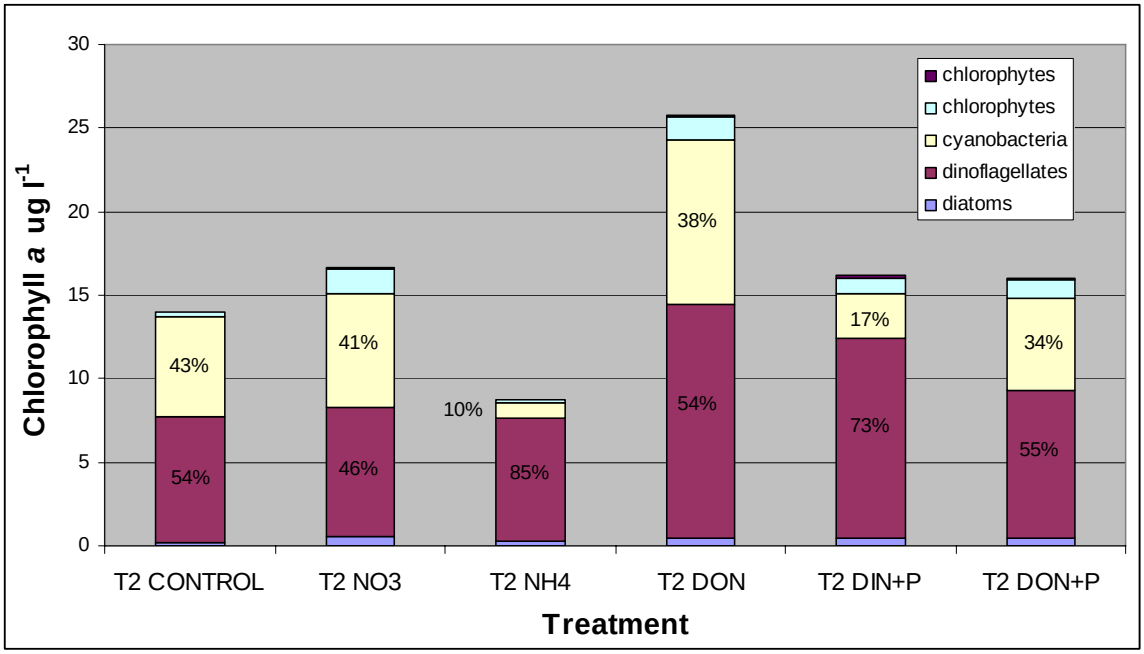

Figure 6.7.9 Summer bioassay T2 phytoplankton community

Creek	Treatment	T1 µgN l^{-1} d^{-1}	T2 µgN l^{-1} d^{-1}	T4 µgN l^{-1} d^{-1}	T1 µgN µgChla^{-1} d^{-1}	T2 µgN µgChla^{-1} d^{-1}	T4 µgN µgChla^{-1} d^{-1}
Big Creek	DIN+P	150.20	-3.77	-3.01	2.98	-0.09	-0.21
	NH_4^+	288.53	-1.25	-6.18	7.77	-0.03	-0.46
Browns Creek	DIN+P	135.23	9.99	-2.71	3.10	0.18	-0.15
	NH_4^+	280.45	5.42	-2.61	5.72	0.14	-0.16
Neuse Estuary	DIN+P	92.68	44.45	-3.89	8.70	2.07	-0.53
	NH_4^+	124.00	157.45	-8.81	9.98	9.46	-1.25
Southwest Creek	DIN+P	146.47	2.67	-3.81	3.62	0.11	-0.42
	NH_4^+	287.52	-2.56	-0.64	8.85	-0.14	-0.07

Table 6.7.2 Summer bioassay NH_4^+ utilization rates in DIN additions

Creek	Treatment	T1 µgN l^{-1} d^{-1}	T2 µgN l^{-1} d^{-1}	T4 µgN l^{-1} d^{-1}	T1 µgN µgChla^{-1} d^{-1}	T2 µgN µgChla^{-1} d^{-1}	T4 µgN µgChla^{-1} d^{-1}
Big Creek	DIN+P	141.60	-2.26	-0.25	2.81	-0.06	-0.02
	NO_3^-	281.02	-1.61	-0.58	10.76	-0.04	-0.04
Browns Creek	DIN+P	130.79	5.93	0.02	3.00	0.11	0.00
	NO_3^-	218.17	58.23	-0.02	6.38	1.61	0.00
Neuse Estuary	DIN+P	28.08	109.86	-0.26	2.64	5.12	-0.03
	NO_3^-	59.10	218.37	-0.04	5.26	9.54	-0.01
Southwest Creek	DIN+P	139.50	-1.82	-0.45	3.45	-0.08	-0.05
	NO_3^-	280.00	-2.42	-0.31	8.18	-0.10	-0.03

Table 6.7.3 Summer bioassay NO_x^- utilization rates in DIN additions

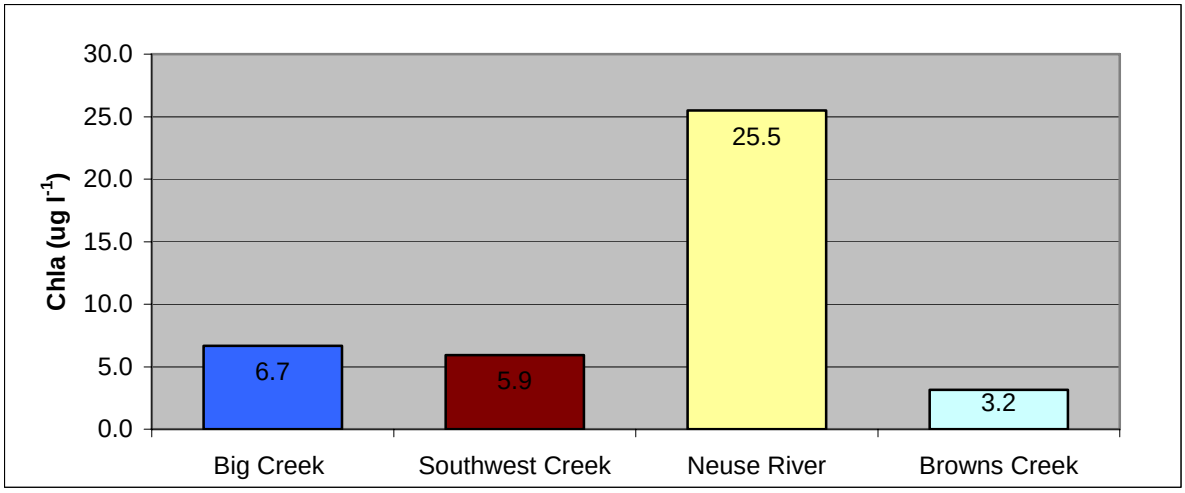

Figure 6.8.1 Fall bioassay T0 initial chlorophyll a concentrations

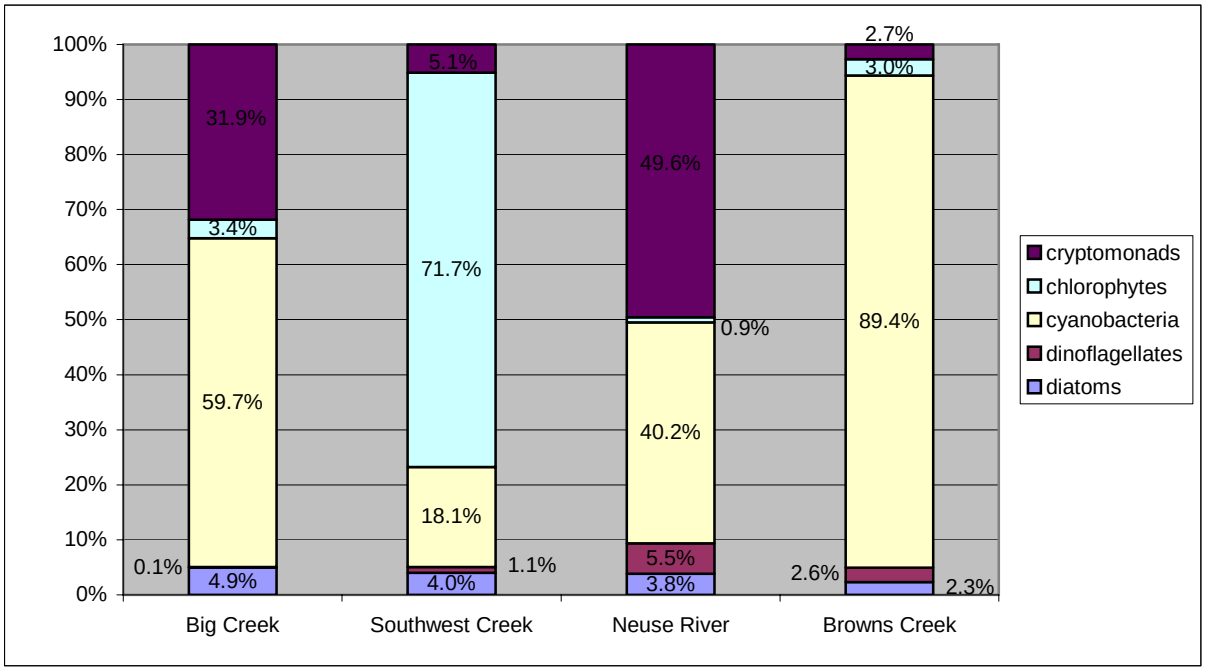

Figure 6.8.2 Fall bioassay initial phytoplankton community composition

	N-NO_x µg l^{-1}	N-NH_4 µg l^{-1}	P-PO_4 µg l^{-1}	TDN µg l^{-1}
Browns Creek	8.1	52.6	4.3	368
Southwest Creek	117.0	51.7	37.2	616
Neuse River	18.9	27.3	7.1	351
Big Creek	7.6	17.9	5.3	275

Table 6.8.1 Fall bioassay T0 ambient nutrient concentrations

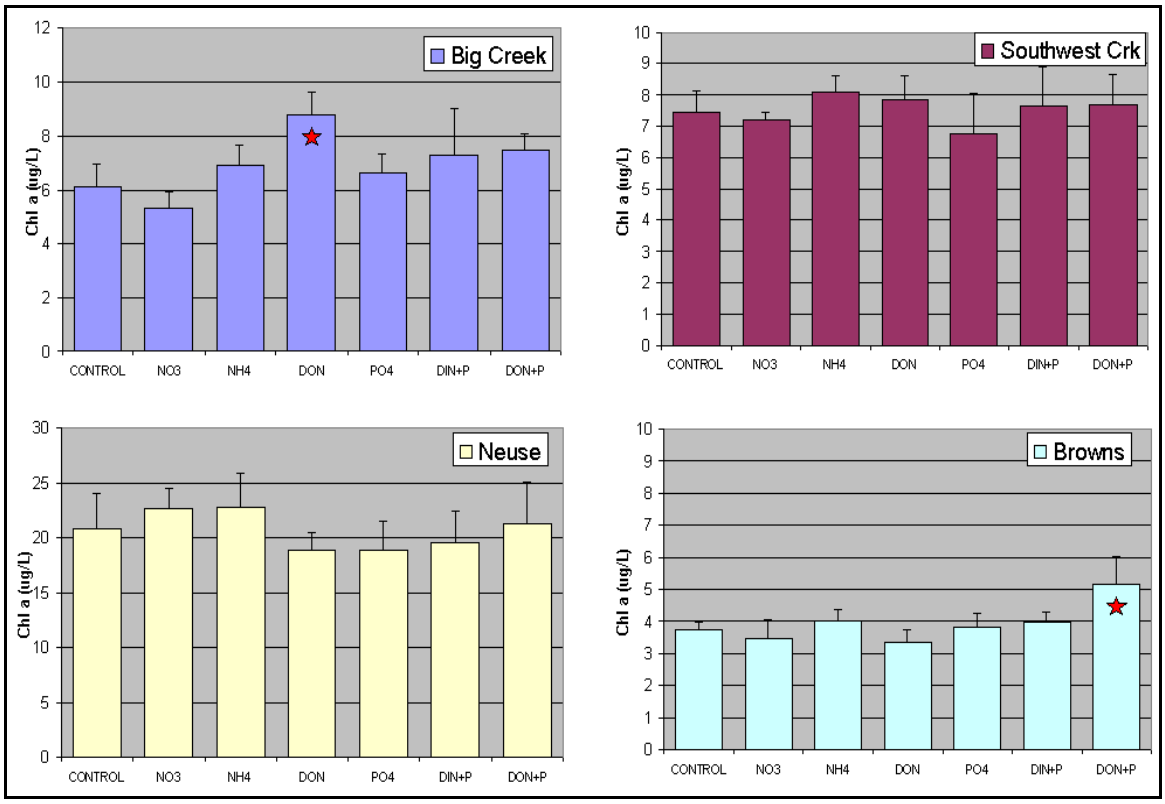

Figure 6.8.3 Fall bioassay T1 chlorophyll a results

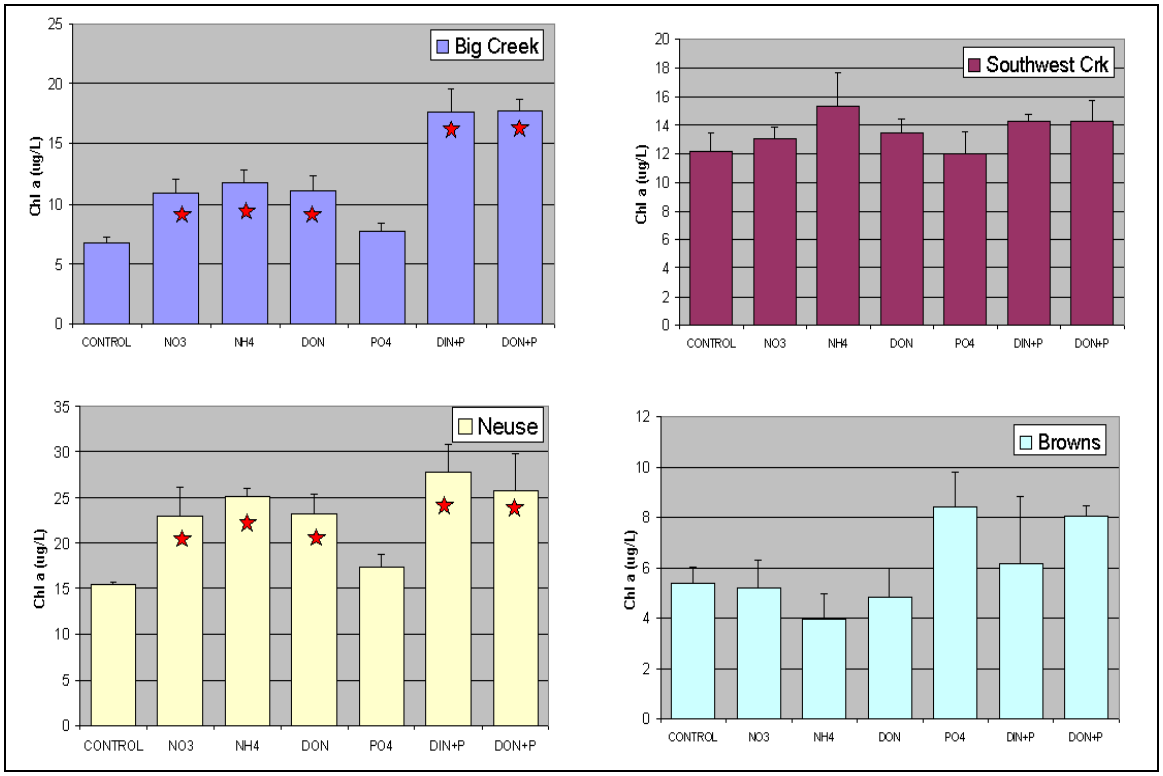

Figure 6.8.4 Fall bioassay T2 chlorophyll a results

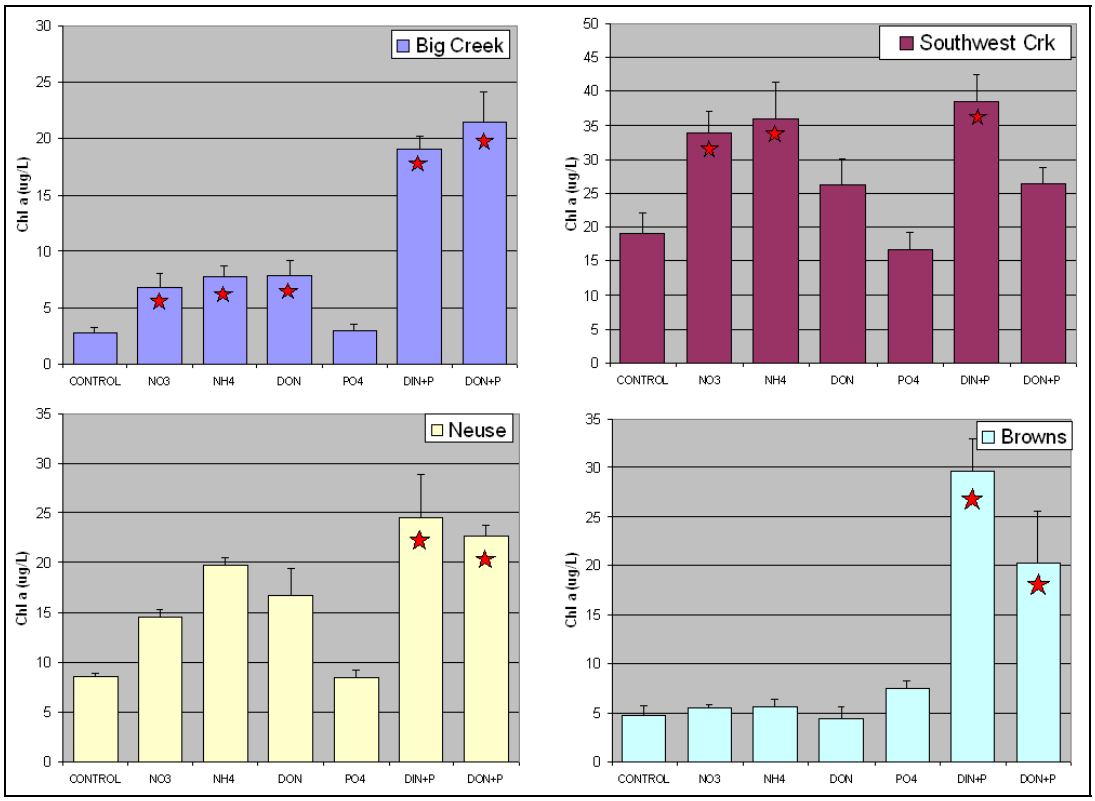

Figure 6.8.5 Fall bioassay T4 chlorophyll a results

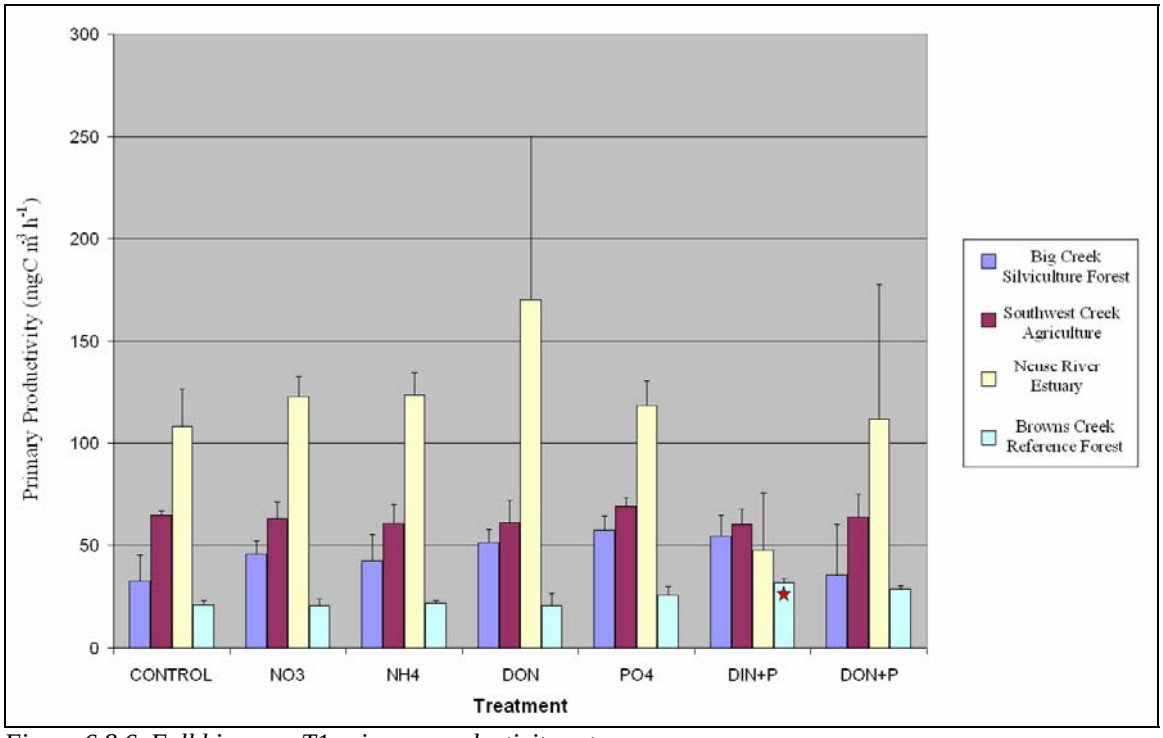

Figure 6.8.6 Fall bioassay T1 primary productivity rates
Red star indicates significantly greater than control ($p < 0.05$)

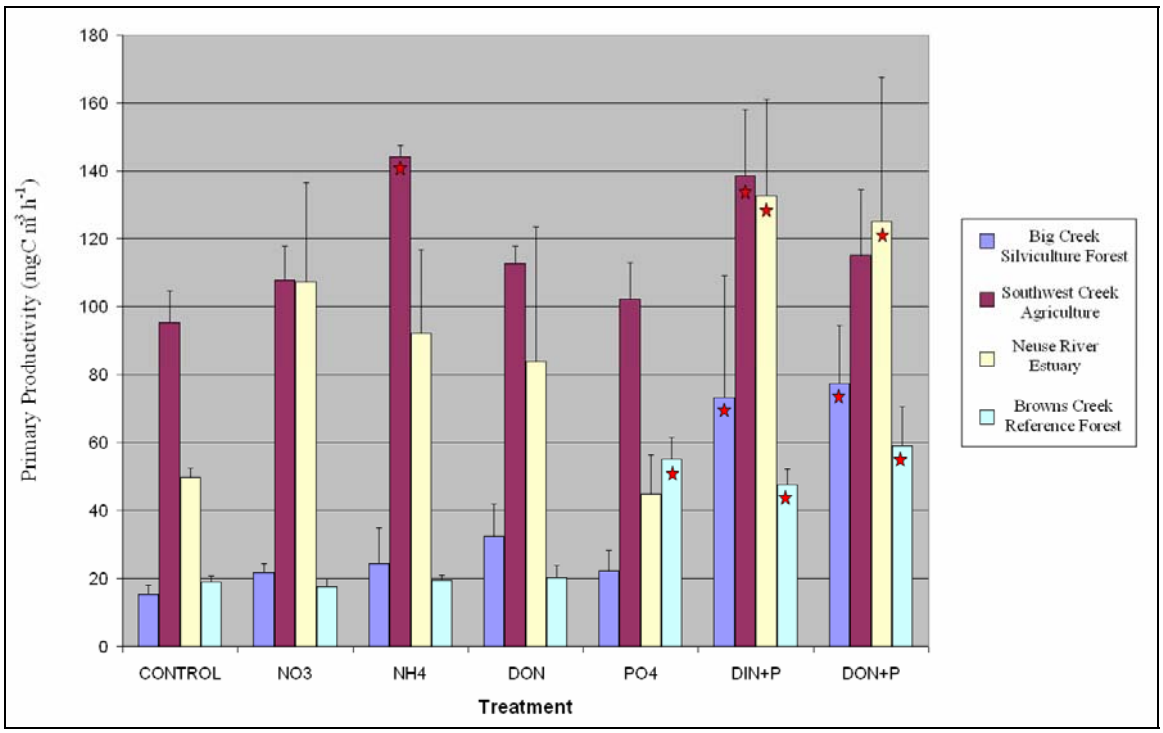

Figure 6.8.7 Fall bioassay T2 primary productivity rates
Red star indicates significantly greater than control ($p < 0.05$)

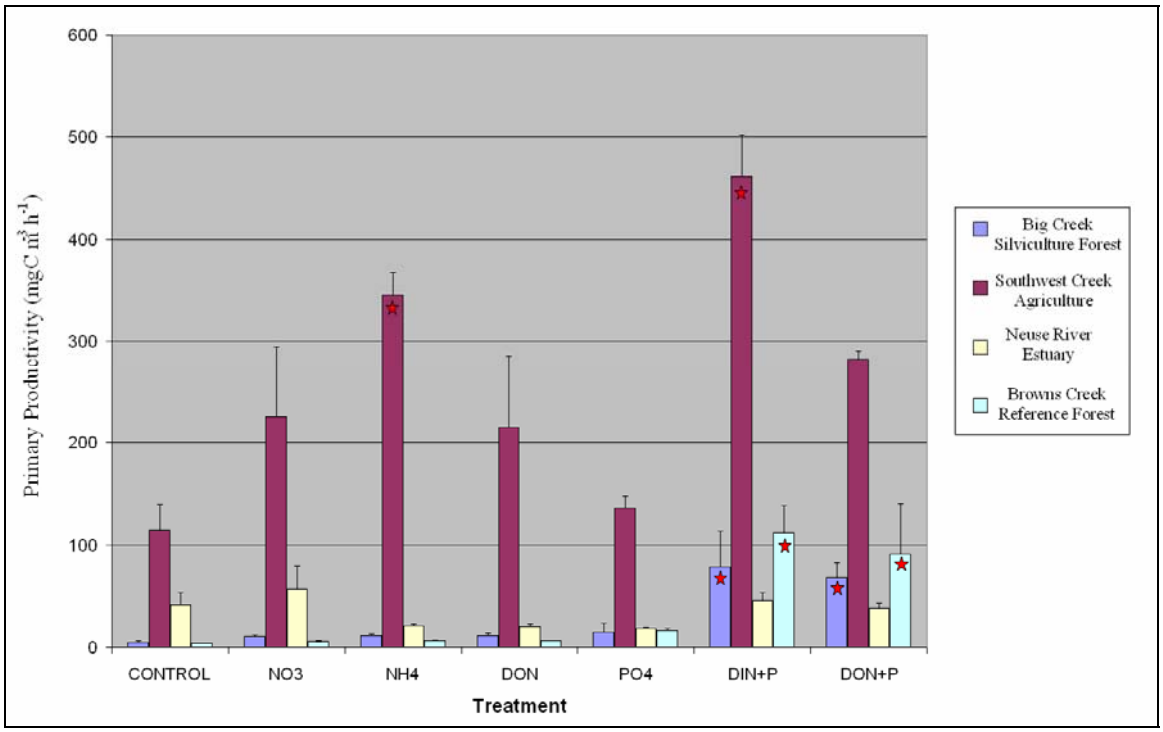

Figure 6.8.8 Fall bioassay T4 primary productivity rates
Red star indicates significantly greater than control ($p < 0.05$)

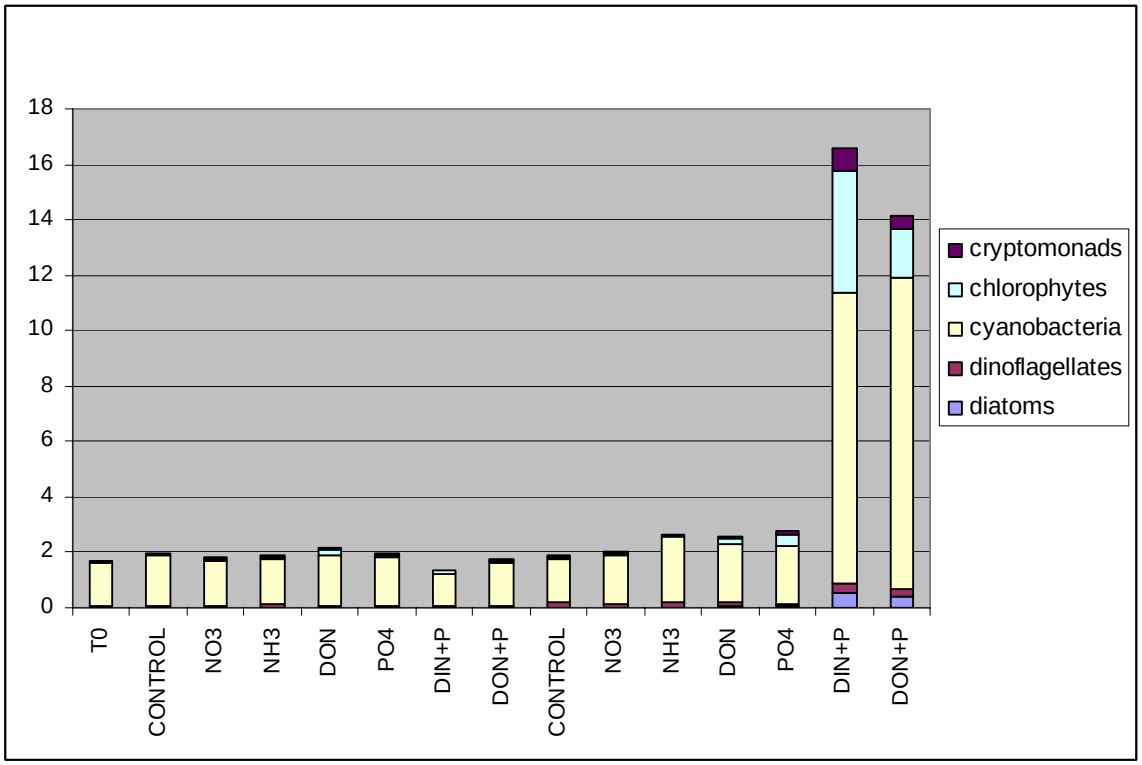

Figure 6.8.9 Fall bioassay Browns Creek phytoplankton community results

Creek	Treatment	T1 µgN l^{-1} d^{-1}	T2 µgN l^{-1} d^{-1}	T4 µgN l^{-1} d^{-1}	T1 µgN µgChla^{-1} d^{-1}	T2 µgN µgChla^{-1} d^{-1}	T4 µgN µgChla^{-1} d^{-1}
Big Creek	DIN+P	49.10	80.18	22.51	6.78	4.55	1.18
	NH_4^+	38.10	58.00	23.63	5.53	4.94	3.05
Browns Creek	DIN+P	-64.35	59.25	75.36	-16.25	9.65	2.55
	NH_4^+	-25.35	-23.75	34.38	-6.34	-6.03	6.13
Neuse Estuary	DIN+P	49.73	102.46	1.86	2.54	3.69	0.08
	NH_4^+	55.05	111.25	37.70	2.41	4.44	1.91
Southwest Creek	DIN+P	-8.15	92.70	48.06	-1.07	6.47	1.25
	NH_4^+	10.85	80.00	116.32	1.34	5.22	3.24

Table 6.8.2 Fall bioassay NH_4^+ utilization rates in DIN additions

Creek	Treatment	T1 µgN l^{-1} d^{-1}	T2 µgN l^{-1} d^{-1}	T4 µgN l^{-1} d^{-1}	T1 µgN µgChla^{-1} d^{-1}	T2 µgN µgChla^{-1} d^{-1}	T4 µgN µgChla^{-1} d^{-1}
Big Creek	DIN+P	22.40	18.03	51.81	3.09	1.02	2.72
	NO_3^-	117.85	19.45	-10.72	22.22	1.78	-1.58
Browns Creek	DIN+P	17.10	15.78	34.04	4.32	2.57	1.15
	NO_3^-	60.10	14.75	2.88	17.41	2.82	0.53
Neuse Estuary	DIN+P	21.40	74.13	29.72	1.09	2.67	1.21
	NO_3^-	53.15	113.00	18.33	2.35	4.92	1.26
Southwest Creek	DIN+P	-42.50	52.50	97.98	-5.57	3.66	2.54
	NO_3^-	120.90	-56.15	117.39	16.81	-4.29	3.47

Table 6.8.3 Fall bioassay NO_x^- utilization rates in DIN additions

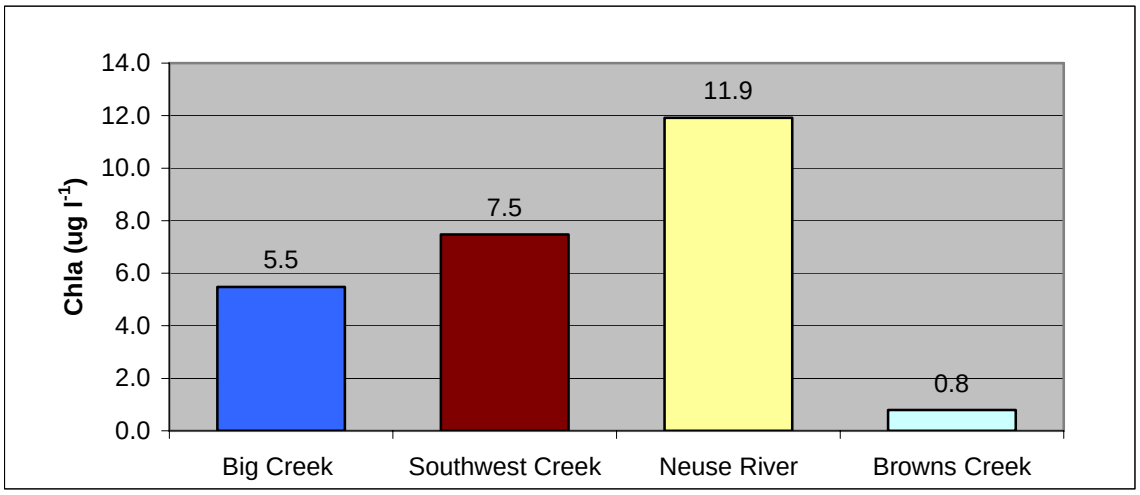

Figure 6.9.1 Winter bioassay T0 initial chlorophyll a concentrations

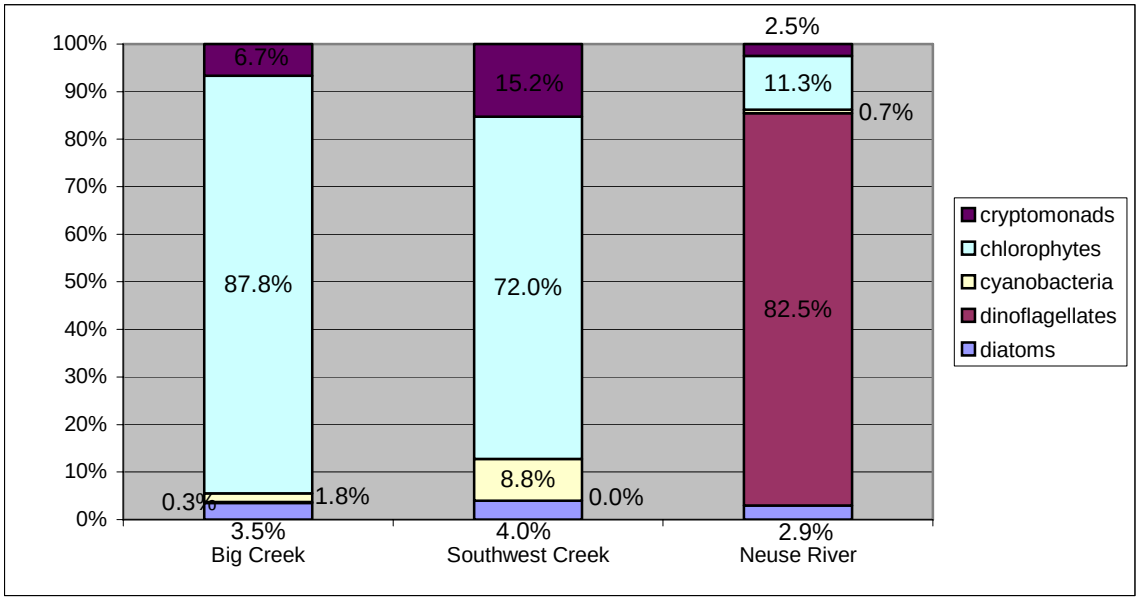

Figure 6.9.2 Winter bioassay final phytoplankton community composition

	N-NO_x µg l^{-1}	**N-NH_4 µg l^{-1}**	**P-PO_4 µg l^{-1}**	**TDN µg l^{-1}**
Browns Creek	22.8	28.8	4.0	412
Southwest Creek	147.0	70.8	46.1	640.5
Neuse River	4.3	10.7	2.5	339.5
Big Creek	6.5	5.7	2.7	367.5

Table 6.9.1 Winter bioassay T0 initial nutrient concentrations

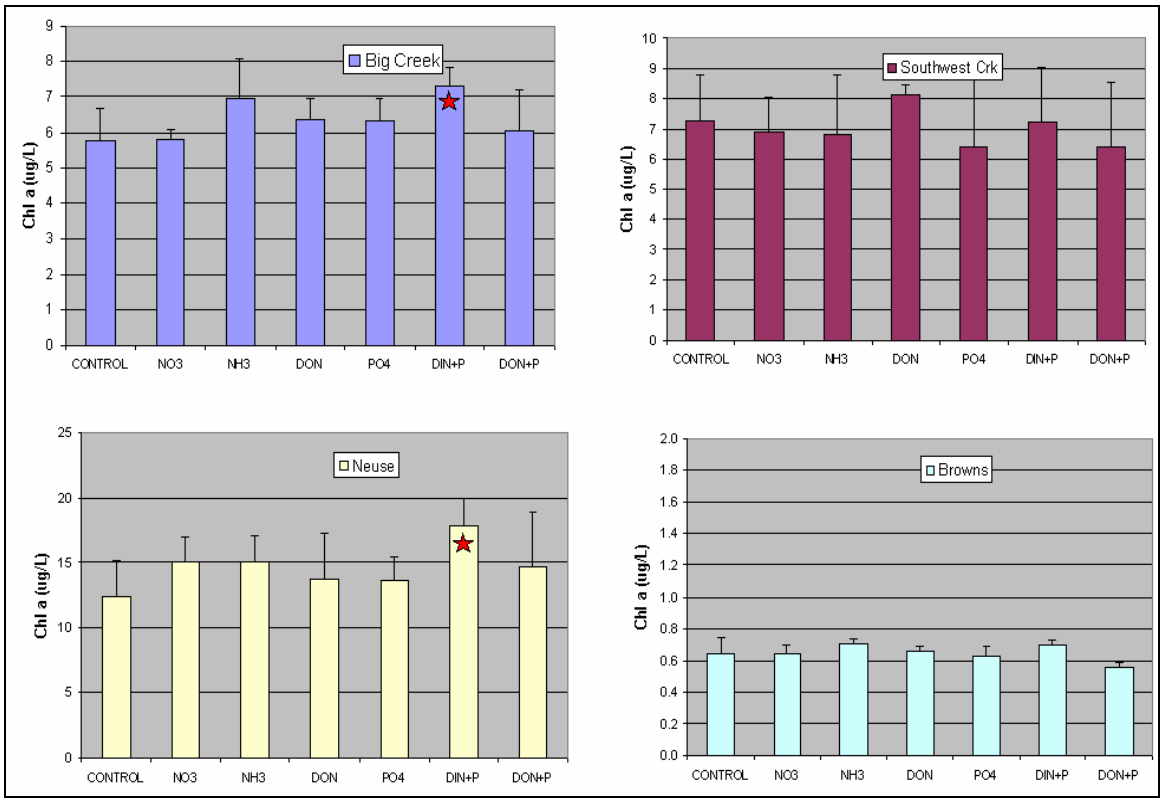

Figure 6.9.3 Winter bioassay T1 chlorophyll a results
Red star indicates significantly greater than control ($p < 0.05$)

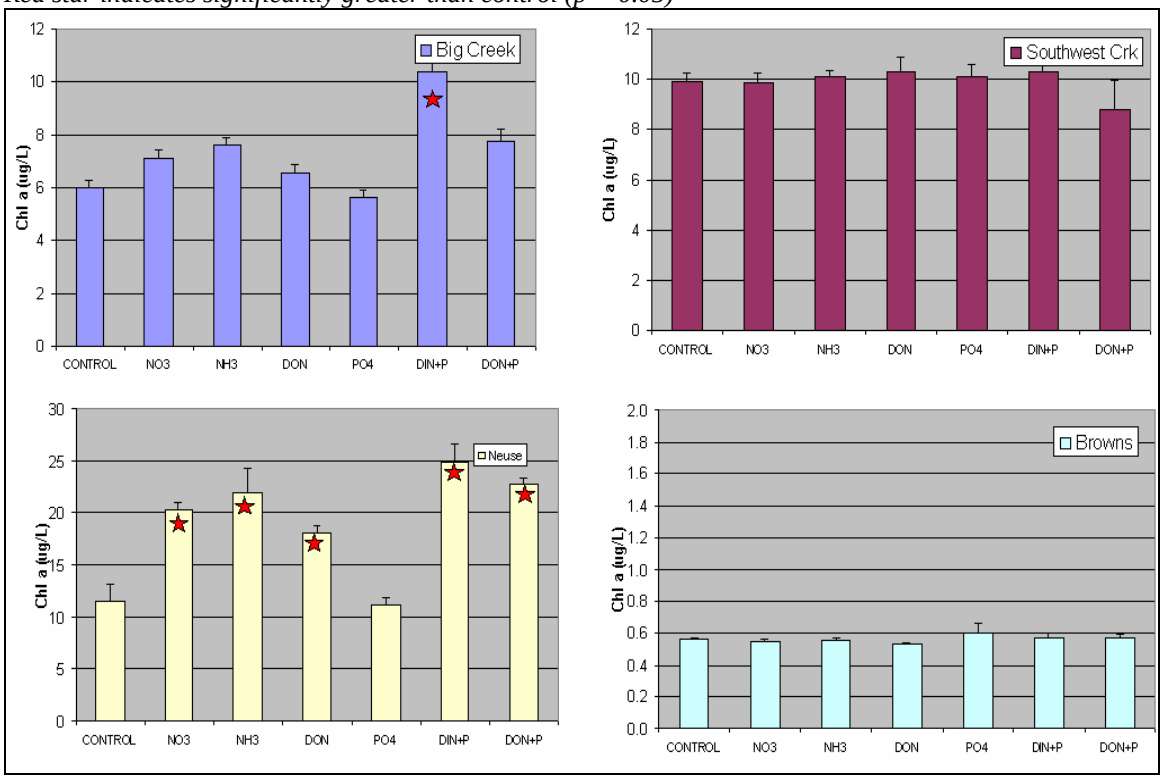

Figure 6.9.4 Winter bioassay T2 chlorophyll a results
Red star indicates significantly greater than control ($p < 0.05$)

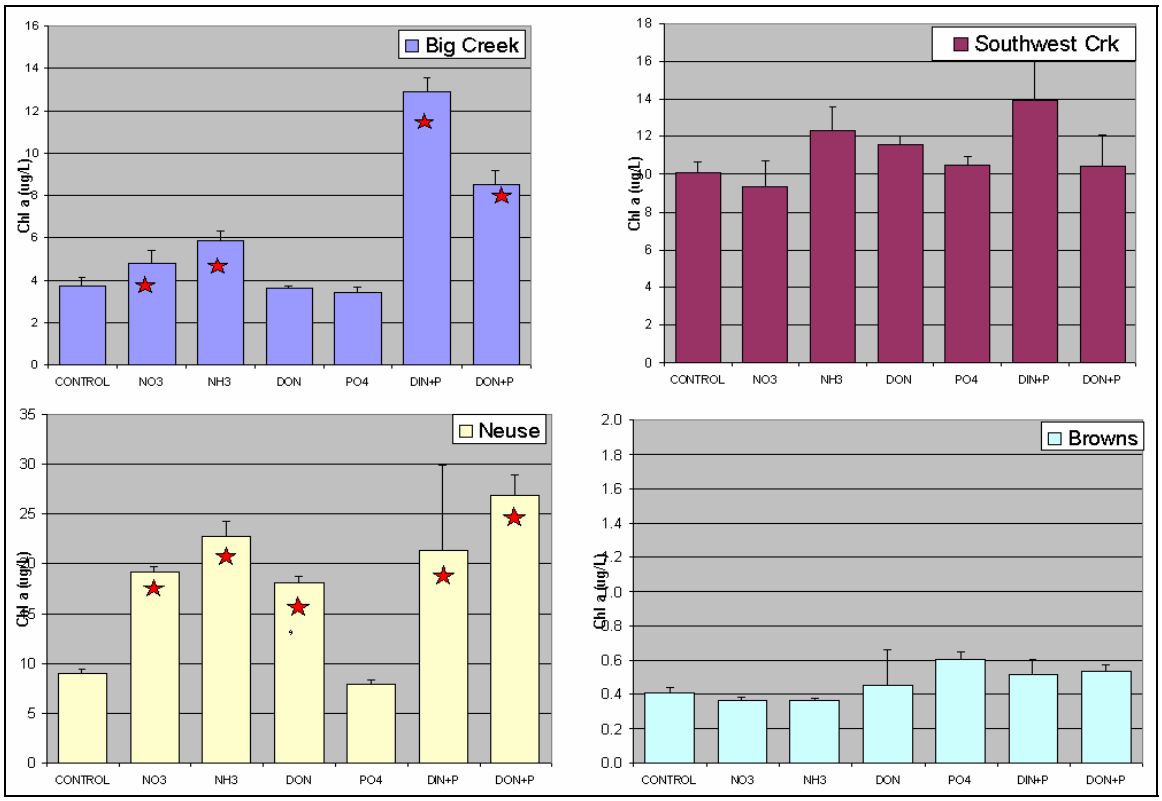

Figure 6.9.5 Winter bioassay T4 chlorophyll a results
Red star indicates significantly greater than control ($p < 0.05$)

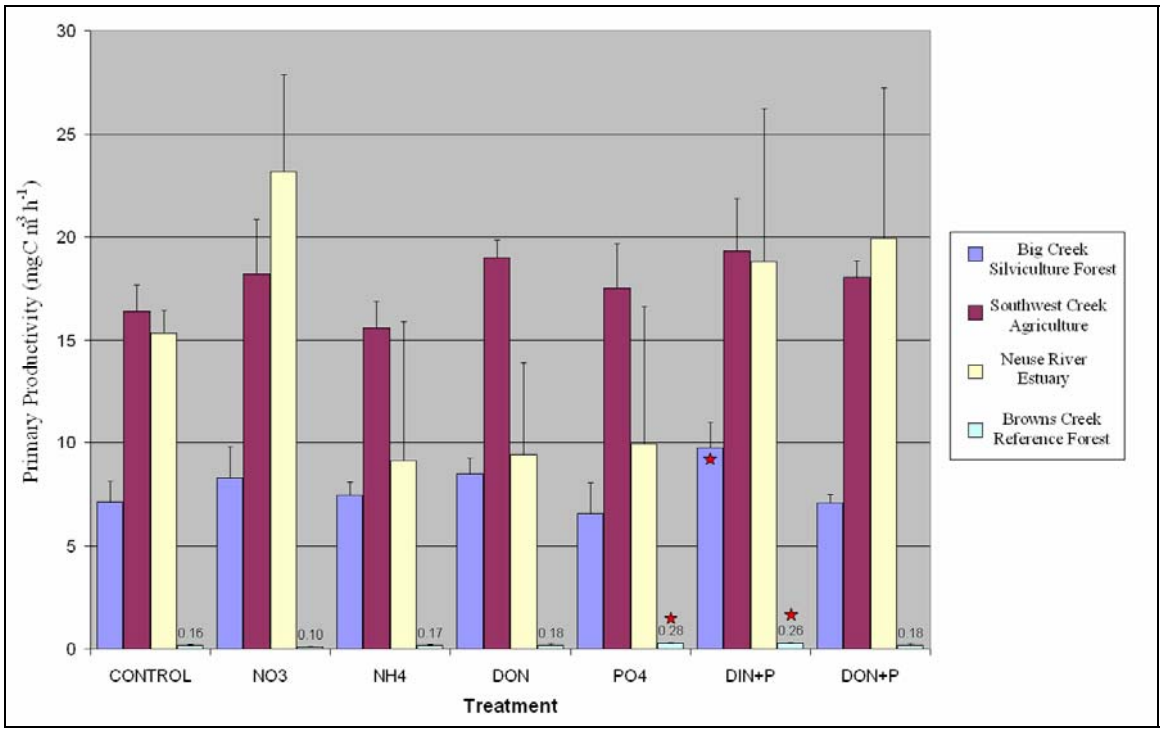

Figure 6.9.6 Winter bioassay T1 primary productivity results
Red star indicates significantly greater than control ($p < 0.05$)

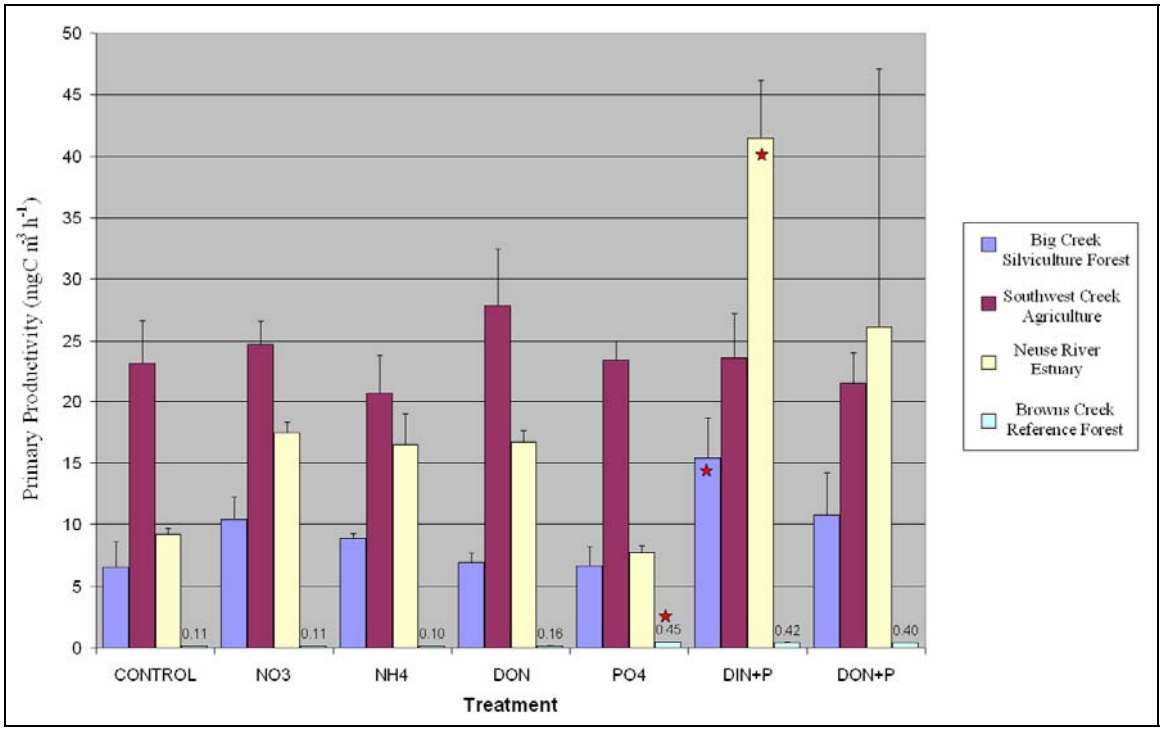

Figure 6.9.7 Winter bioassay T2 primary productivity results
Red star indicates significantly greater than control ($p < 0.05$)

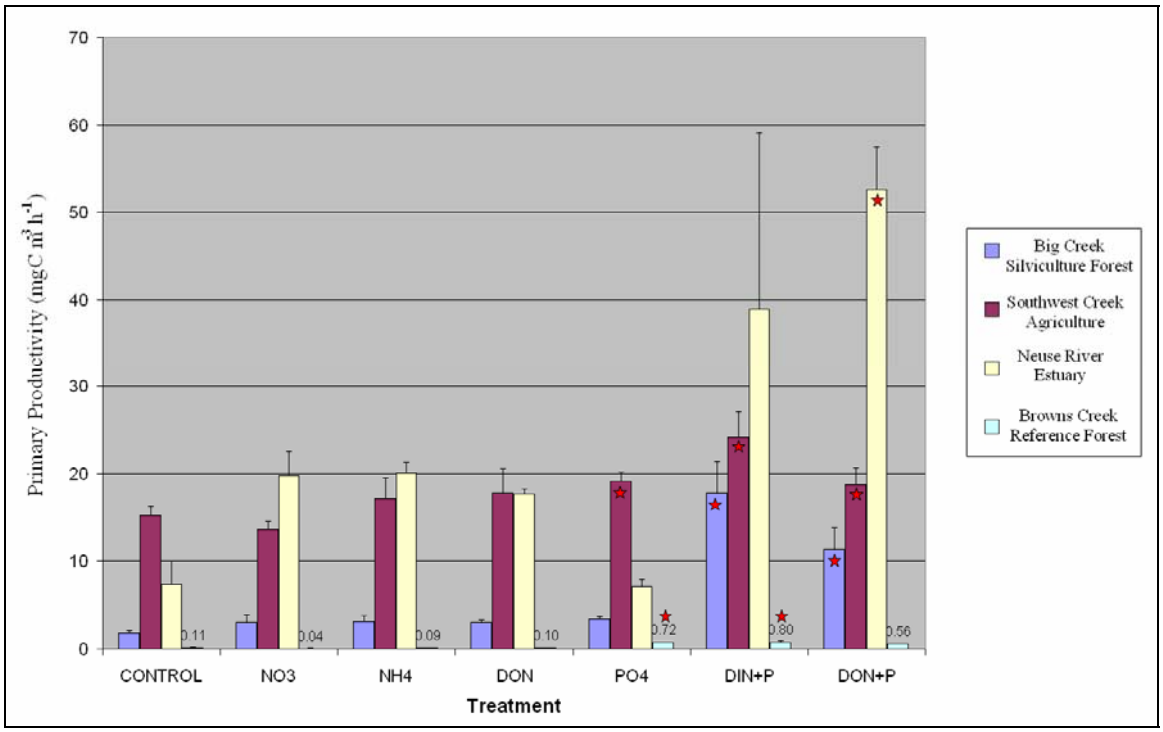

Figure 6.9.8 Winter bioassay T4 primary productivity Results
Red star indicates significantly greater than control ($p < 0.05$)

Creek	Treatment	T1 µgN l^{-1} d^{-1}	T2 µgN l^{-1} d^{-1}	T4 µgN l^{-1} d^{-1}	T1 µgN µgChla^{-1} d^{-1}	T2 µgN µgChla^{-1} d^{-1}	T4 µgN µgChla^{-1} d^{-1}
Big Creek	DIN+P	44.53	44.88	29.97	6.07	4.33	2.33
	NH_4^+	33.80	36.50	7.75	4.87	4.81	1.32
Browns Creek	DIN+P	-39.54	26.50	33.93	-56.79	46.75	65.43
	NH_4^+	17.96	-16.00	9.13	25.37	-28.85	25.28
Neuse Estuary	DIN+P	133.65	8.56	-1.27	7.50	0.34	-0.06
	NH_4^+	129.80	72.05	33.11	8.61	3.28	1.45
Southwest Creek	DIN+P	19.30	78.38	36.42	2.67	7.63	2.62
	NH_4^+	60.30	59.00	30.13	8.83	5.83	2.45

Table 6.9.2 Winter bioassay NH_4^+ utilization rates in DIN additions

Creek	Treatment	T1 µgN l^{-1} d^{-1}	T2 µgN l^{-1} d^{-1}	T4 µgN l^{-1} d^{-1}	T1 µgN µgChla^{-1} d^{-1}	T2 µgN µgChla^{-1} d^{-1}	T4 µgN µgChla^{-1} d^{-1}
Big Creek	DIN+P	44.97	-11.58	19.05	6.13	-1.12	1.48
	NO_3^-	75.14	-8.85	9.00	12.98	-1.25	1.90
Browns Creek	DIN+P	-7.69	-2.25	37.88	-11.05	-3.97	73.05
	NO_3^-	24.81	-0.50	-16.13	38.41	-0.92	-44.12
Neuse Estuary	DIN+P	38.47	76.95	14.74	2.16	3.09	0.69
	NO_3^-	122.14	19.25	40.46	8.08	0.94	2.11
Southwest Creek	DIN+P	22.48	59.50	9.90	3.11	5.79	0.71
	NO_3^-	47.23	49.75	26.25	6.83	5.04	2.82

Table 6.9.3 Winter bioassay NO_x^- utilization rates in DIN additions

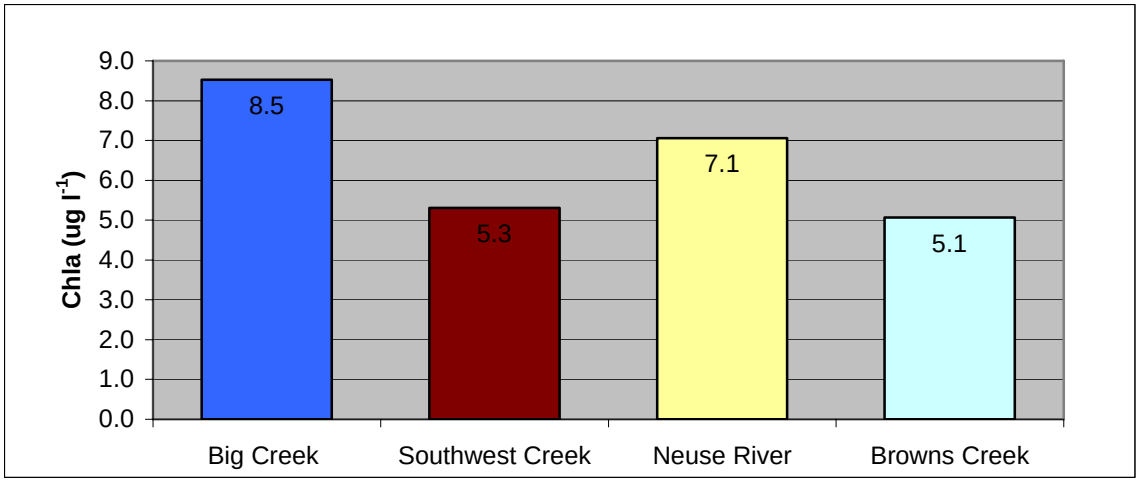

Figure 6.10.1 Spring bioassay T0 initial chlorophyll *a concentrations*

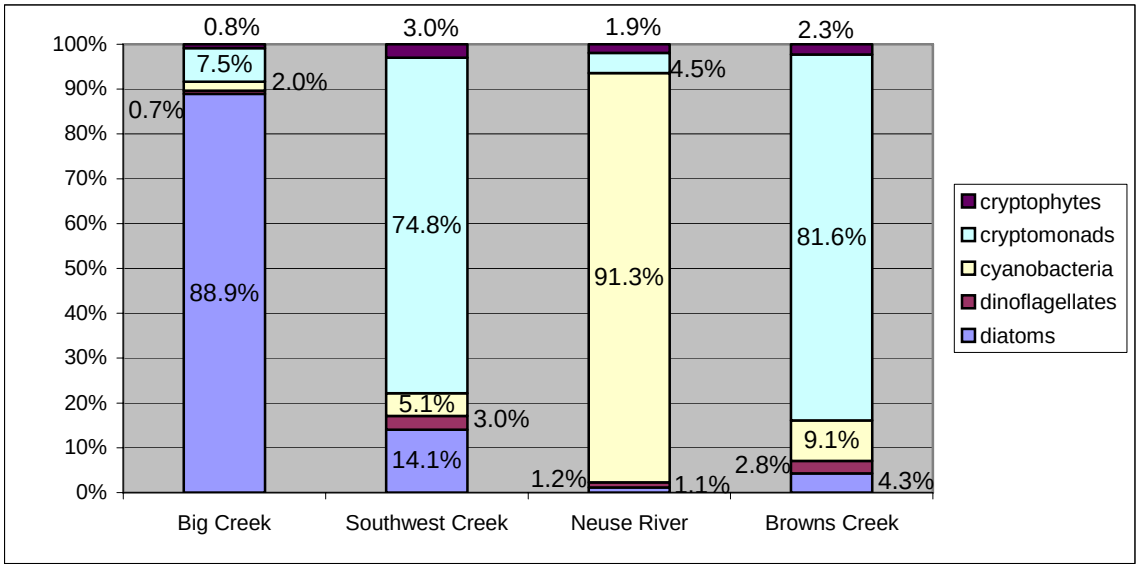

Figure 6.10.2 Spring bioassay T0 initial phytoplankton community composition

	N-NO_x µg l^{-1}	**N-NH_4 µg l^{-1}**	**P-PO_4 µg l^{-1}**	**TDN µg l^{-1}**
Browns Creek	2.9	14.0	5.6	296.5
Southwest Creek	2.4	16.9	7.4	304
Neuse River	2.0	9.5	6.2	241
Big Creek	2.2	3.6	6.5	296

Table 6.10.1 Spring bioassay T0 initial nutrient concentrations

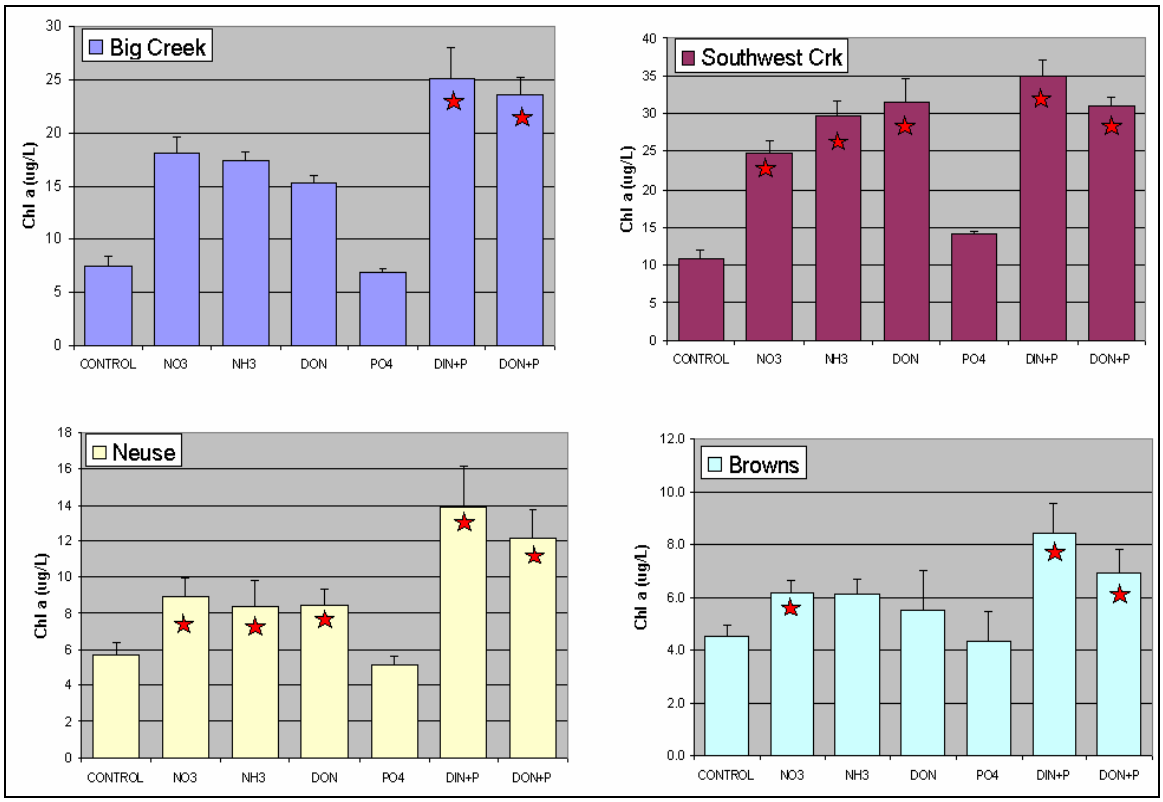

Figure 6.10.1 Spring bioassay T1 chlorophyll a results
Red star significantly greater than control ($p < 0.05$)

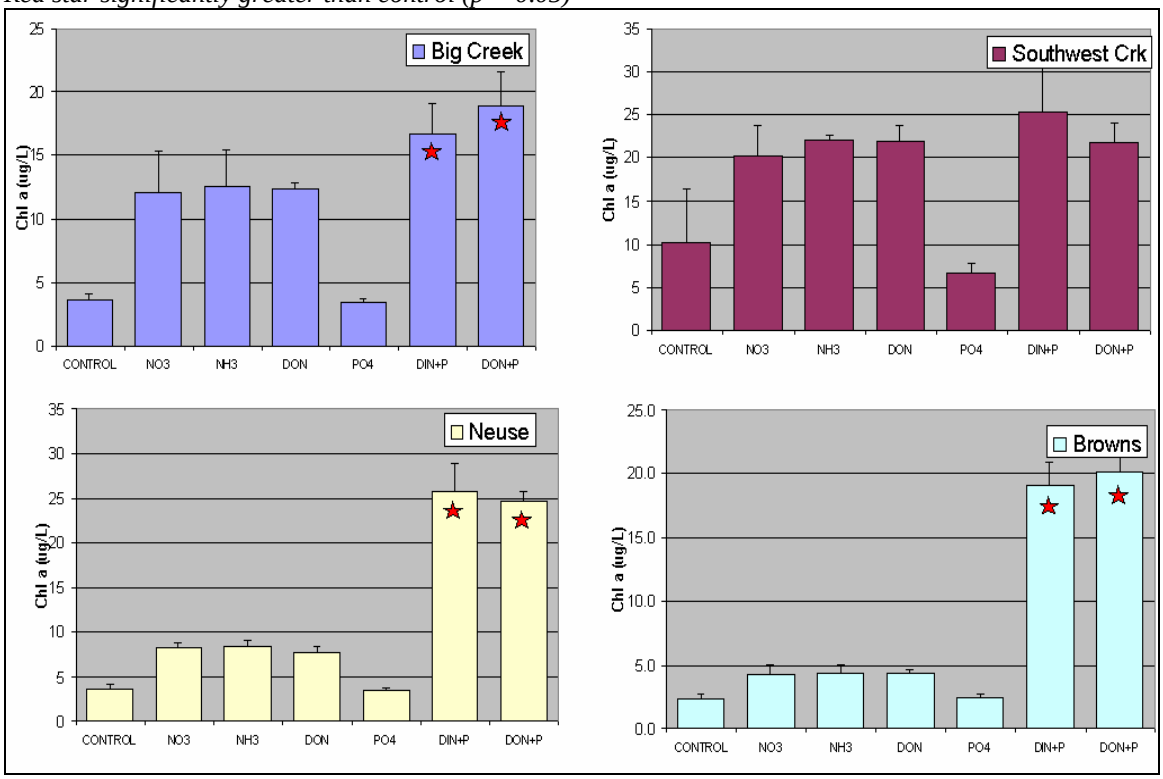

Figure 6.10.2 Spring bioassay T2 chlorophyll a results
Red star indicates significantly greater than control ($p < 0.05$)

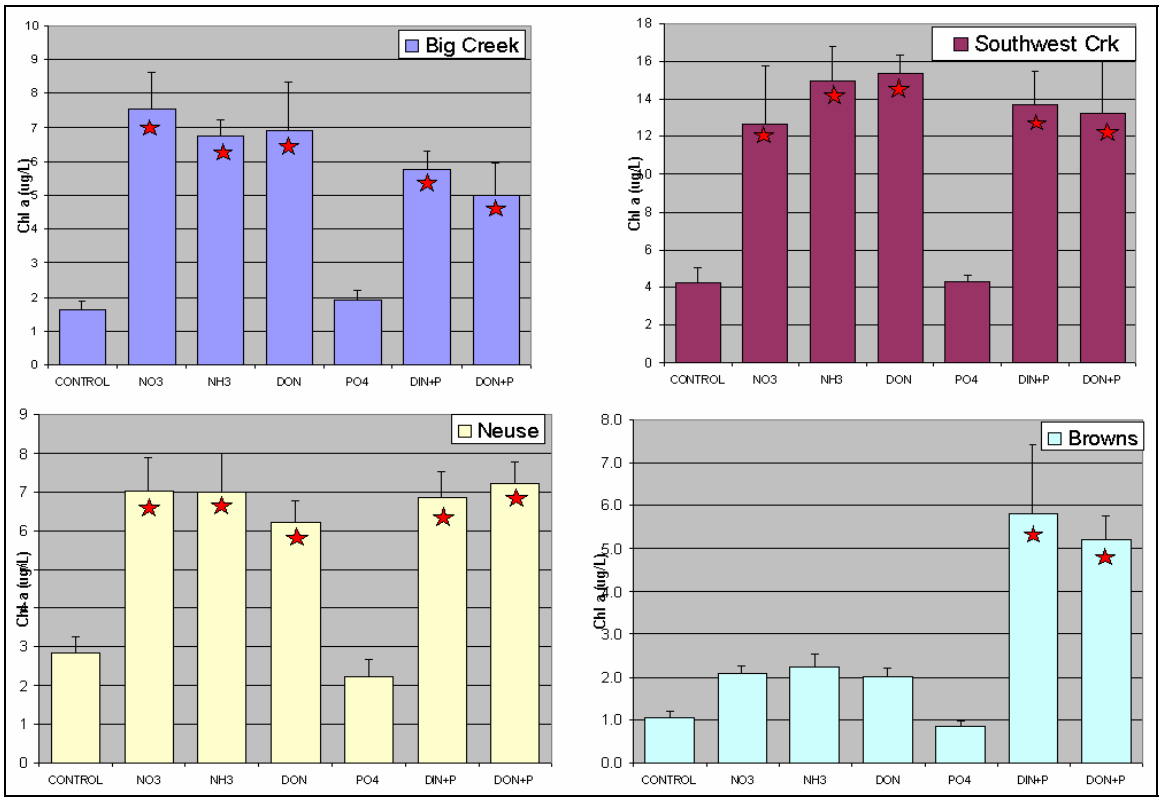

Figure 6.10.3 Spring bioassay T4 chlorophyll a results
Red star indicates significantly greater than control ($p < 0.05$)

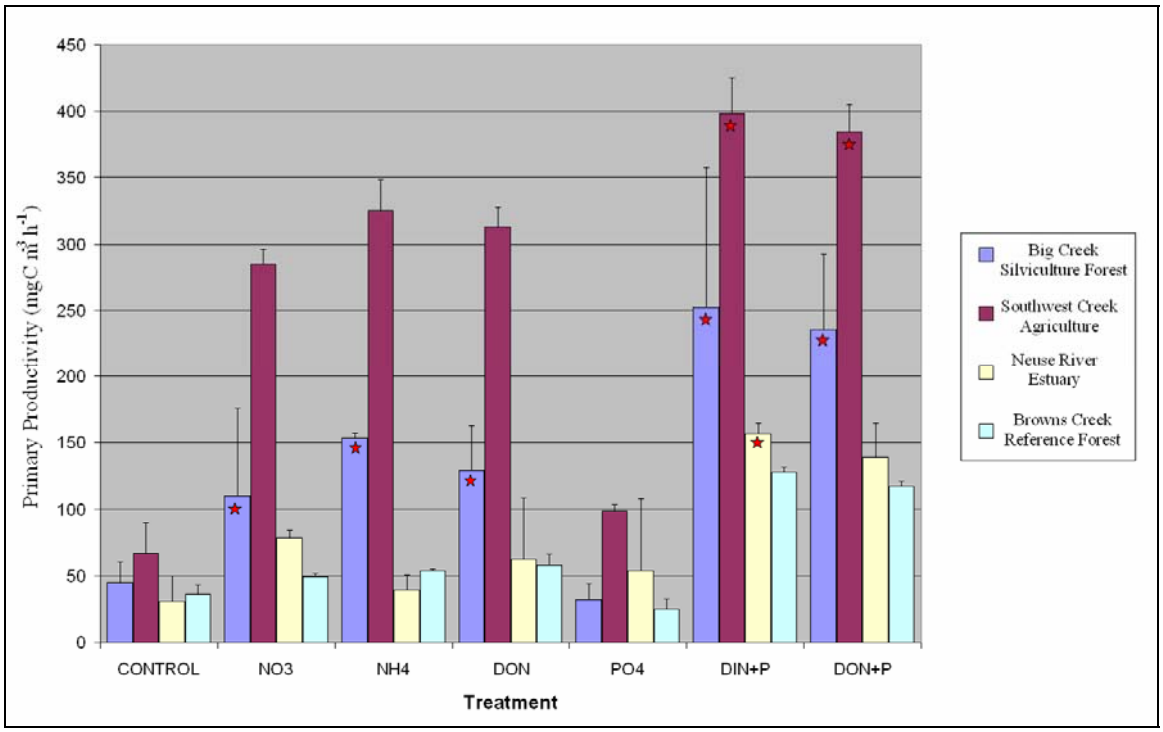

Figure 6.10.4 Spring Bioassay T1 primary productivity results
Red star indicates significantly greater than control ($p < 0.05$)

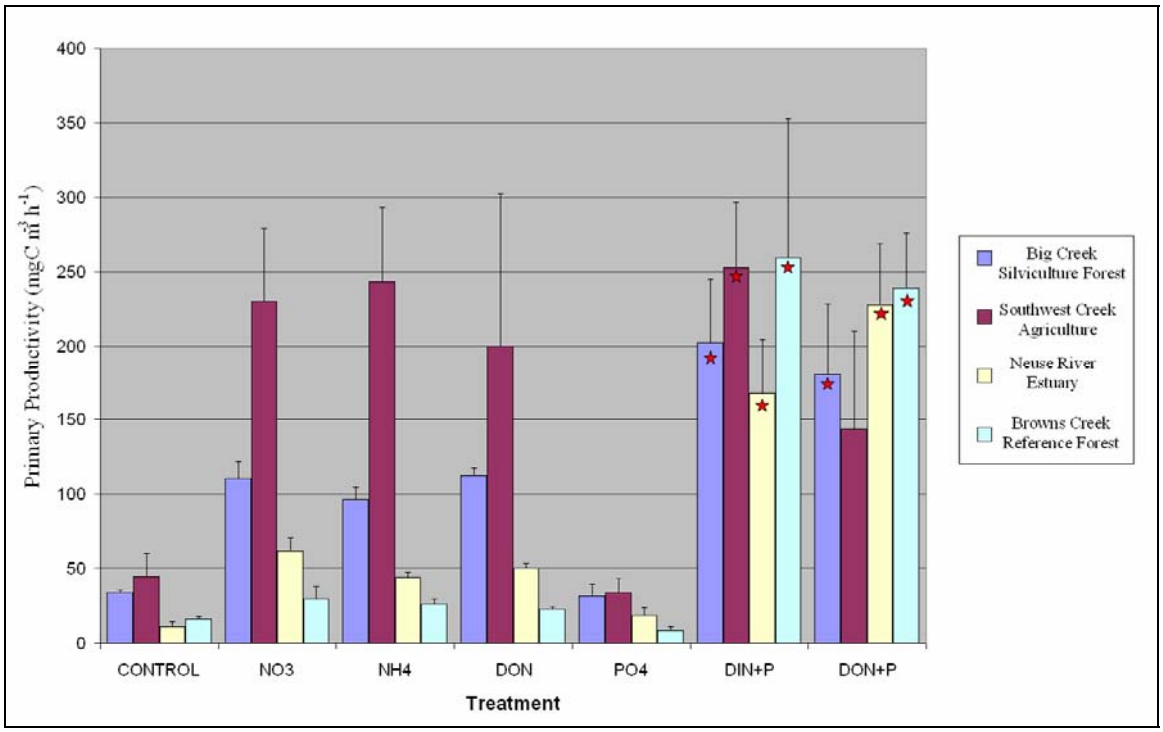

Figure 6.10.5 Spring Bioassay T2 primary productivity results
Red star indicates significantly greater than control ($p < 0.05$)

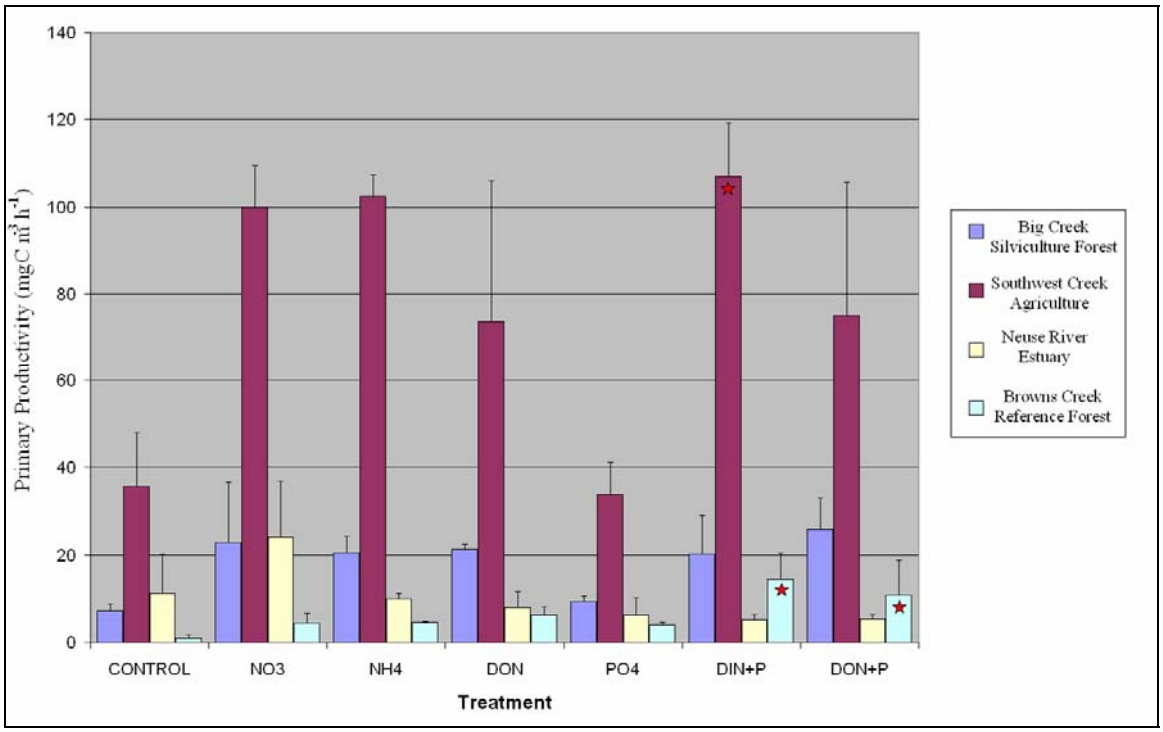

Figure 6.10.6 Spring Bioassay T4 primary productivity results
Red star indicates significantly greater than control ($p < 0.05$)

Creek	Treatment	T1 µgN l^{-1} d^{-1}	T2 µgN l^{-1} d^{-1}	T4 µgN l^{-1} d^{-1}	T1 µgN µgChla^{-1} d^{-1}	T2 µgN µgChla^{-1} d^{-1}	T4 µgN µgChla^{-1} d^{-1}
Big Creek	DIN+P	134.23	8.35	2.06	5.36	0.50	0.36
	NH_4^+	119.50	85.63	23.01	6.90	6.79	3.42
Browns Creek	DIN+P	50.95	74.70	0.93	6.06	3.90	0.16
	NH_4^+	21.82	50.50	14.88	3.58	11.67	6.66
Neuse Estuary	DIN+P	101.09	34.78	3.95	7.28	1.35	0.58
	NH_4^+	70.51	26.50	48.54	8.43	3.16	6.92
Southwest Creek	DIN+P	126.85	10.95	6.32	3.62	0.43	0.46
	NH_4^+	170.15	114.77	2.58	5.72	5.18	0.17

Table 6.10.2 Spring bioassay NH_4^+ utilization rates in DIN additions

Creek	Treatment	T1 µgN l^{-1} d^{-1}	T2 µgN l^{-1} d^{-1}	T4 µgN l^{-1} d^{-1}	T1 µgN µgChla^{-1} d^{-1}	T2 µgN µgChla^{-1} d^{-1}	T4 µgN µgChla^{-1} d^{-1}
Big Creek	DIN+P	79.02	66.60	-1.64	3.15	3.98	-0.29
	NO_3^-	115.45	43.00	31.66	6.39	3.54	4.19
Browns Creek	DIN+P	-0.07	145.06	-1.67	-0.01	7.58	-0.29
	NO_3^-	59.43	54.25	5.75	9.66	12.99	2.74
Neuse Estuary	DIN+P	19.17	125.62	-1.81	1.38	4.88	-0.27
	NO_3^-	48.67	129.50	16.96	5.44	15.61	2.41
Southwest Creek	DIN+P	44.30	100.65	-2.05	1.26	3.98	-0.15
	NO_3^-	169.70	115.96	-3.73	6.86	5.75	-0.29

Table 6.10.3 Spring bioassay NO_x^- utilization rates in DIN additions

CHAPTER 7

DISCUSSION

The following discussion sections are organized to specifically address the three project objectives. Each objective and hypothesis is first reiterated, and then evaluated, through discussion of the project results and results from similar investigations.

7.1 Land Use Influence on Nutrient Concentrations

Objective: Quantify the concentrations and forms of biologically available nitrogen in creeks downstream of representative watersheds draining the four distinct land uses.

Hypothesis: Current LULC bordering the NPES will significantly influence allochtonous nutrient loading to the proximate estuarine creeks. Specifically, runoff originating from the agricultural areas will be enriched in total nitrogen and soluble inorganic nitrogen fraction when compared to runoff from the forested areas.

Dissolved Nutrient Concentrations

Existing LULC studies have directly addressed the effects of land use on downstream dissolved nutrient concentrations in the NRE (Kirby-Smith and Barber 1979; Paerl et al. 1998; Thompson et al. 1998), and in other temperate estuaries (Hopkinson and Vallino 1995; Woodside 1995; Corbett et al. 1997; Wahl et al. 1997; Dauer et al. 2000; Cloern 2001; Gove et al. 2001; Seitzinger et al. 2002; Hagy et al. 2004). These studies found that watershed modifications, such as urban development and agricultural use, increased nutrient loading and concentrations in downstream waters. Similarly, this study of NRE estuarine creeks

compared nutrient concentrations down stream of two agricultural watersheds, a managed silviculture forest watershed, and an unmanaged reference forest watershed.

The results of this study show that dissolved inorganic nutrients (NH_4^+, NO_x^-, and PO_4^{-3}) and TDN concentrations were generally greatest downstream of the agricultural watersheds (Figure 6.1.1). An exception was high dissolved NH_4^+ concentrations observed in Browns Creek downstream of the reference forest watershed. This exception is discussed below under dissolved nitrogen form. Otherwise, these findings are consistent with the previous research on nutrient budgets and exports for agricultural and forested lands (Kirby-Smith and Barber 1979; Lowrance et al. 1985; Woodside 1995; Perry et al. 1999; Vellidis et al. 2003; Long et al. 2004). Generally, the existing research shows physical land modifications (e.g. ditching and fertilizer application) result in rapid runoff and increased dissolved nutrient loads. Woodside (1995) compared watershed land use and downstream nutrient concentrations in fresh water coastal plain creeks of the Albemarle Sound and Pamlico Sound drainage basins. Woodside (1995) found the highest median concentrations of dissolved NO_3^- were in streams draining watersheds that contained more than 45-percent cropland. The lowest median concentrations of dissolved NO_3^- were in streams draining watersheds that contained more than 90-percent forest land. The results presented here for the South River sub-estuary area also show the highest dissolved NO_x^- concentrations in creeks draining agricultural watersheds and the lowest dissolved NO_x^- concentrations in creeks draining forested watersheds. These current findings also agree with results of a previous investigation of land use and water quality in the South River sub-estuary (Kirby-Smith and Barber 1979). During the construction of OGF in the 1970's, Kirby-Smith and Barber (1979) found that concentrations of NO_x^-, NH_4^+, and PO_4^{-3} increased in the South

River sub-estuary as the watershed was converted from forest to intensive agriculture. The results from this 2003 – 2004 study show that despite advances in BMPs, such as flashboard risers and constructed wetlands, dissolved nutrient concentrations remain elevated in creeks draining the agriculture areas.

Dissolved Inorganic Nitrogen Form

The results from the monitoring data show the primary forms of DIN, NH_4^+ and NO_x^-, were different downstream of agricultural versus forested watersheds. Specifically, NH_4^+ made up the majority of the DIN in the forested watershed creeks (Browns Creek and Big Creek). In contrast, NO_x^- was the primary form of DIN in the agricultural watershed creeks (Southwest Creek and Westfork Creek) (Figure 6.1.2). Previous studies of LULC influence on downstream dissolved nutrient concentrations have also found this relationship between land use and dissolved nitrogen form (Corbett et al. 1997; Wahl et al. 1997; Mallin et al. 2004). Mallin et al. (2004) found high NO_3^- concentrations were the primary DIN form in creeks draining developed watersheds, while regenerated NH_4^+ was the primary N form in waters downstream of undisturbed lands. In the Murrells Inlet, South Carolina estuarine system, Wahl et al. (1997) also found higher NO_x^- concentrations in urban estuarine streams and higher NH_4^+ in forested estuarine streams. Wahl et al. (1997) observed a greater than 100% per unit area load of DIN from the urbanized watershed when compared to a forested watershed.

The differences in DIN form downstream of agricultural and forest watersheds observed in this study may be attributed to agricultural watershed modifications that increase NO_x^- concentrations and benthic estuarine processes that increase NH_4^+ concentrations. As mentioned above, agricultural fertilizer application and ditch networks increase rapid runoff

curves (Kirby-Smith and Barber 1979; Lowrance et al. 1985; Perry et al. 1999; Burt and Pinay 2005). The rapid runoff curves associated with the agricultural ditch networks decrease infiltration, which then also decreases the potential for NO_3^- to be converted to N_2 via denitrification (Thompson et al. 1998). Consequently, agricultural watershed modification increases in-stream nutrient loading of NO_x^-. Alternatively, NH_4^+ concentration can increase as the result of organic matter remineralization in estuarine sediments (Fear 2003; Fear et al. 2004). The persistent salinity stratification (Table 6.4.1) and chronic summer hypoxia observed in the creek monitoring data mimic the conditions that promote NH_4^+ flux from benthic sediments. However, benthic NH_4^+ flux rates were not directly measured in this study; thus, this explanation cannot be tested at this point. Other possible explanations for the elevated NH_4^+ in the Browns Creek bottom water may include intrusion of main stem estuary water in the downstream portions of the creek. Periods of downstream import of estuarine water were identified by high salinity and negative flow observations at the creek headwaters. Examples of estuary water intrusion (i.e. negative flow) during June and July of 2004 are discussed below under phytoplankton community composition.

NH_4^+ flux measurements in Southwest Creek are currently being collected as part of the related Forested and Agricultural Watershed Nitrogen Attenuation project. Upon completion, these flux data will be incorporated with the data from this project to help develop a nutrient transport model for proximate estuarine watersheds.

7.2 Creek Nutrient Buffering Capacity

Objective: Determine if these non-tidal estuarine creeks serve as effective nutrient buffers to the main-stem estuary.

Hypothesis: High productivity and nutrient assimilation rates in the proximate creeks provide an efficient nutrient buffer for the main stem of estuary during both average base flows and episodic storm-level flows. Specifically, in-stream nutrient attenuation in all creeks will be directly related to levels of phytoplankton chlorophyll *a*.

In other ecosystems, creeks of similar size have provided a buffer to receiving waters by assimilating 50% to 90% of the input of dissolved inorganic nitrogen from their watersheds (Peterson et al. 2001; Mallin 2004). This study used the sampling data and the bioassay experiments to evaluate whether these creeks are also effective buffers to the main stem Neuse River estuary from proximate nitrogen inputs.

The results show that throughout the year, the phytoplankton community in the forested watershed creeks has the capacity to assimilate DIN delivered in all but the 95th percentile of flow and loads (Figures 7.2.1 and 7.2.2). In the agricultural watershed Southwest Creek, the phytoplankton community capacity to assimilate watersheds loads was less than the forested creeks and varied throughout the year. Specifically, in spring, summer, and fall, the agricultural creek phytoplankton community has the capacity to assimilate DIN delivered in all but the 90th percentile flow and DIN loads (Figures 7.2.1 and 7.2.3). In the winter, the agricultural creek phytoplankton community has the capacity to assimilate DIN delivered in all but 80th percentile of flow and DIN loads (Figures 7.2.1 and 7.2.3).

The experimentally-derived N uptake rates used here may actually under-estimate in-creek assimilation rates. Mulholland et al. (2002) found using experimentally derived N uptake rates would over-estimate the stream length and time required to assimilate a unit of dissolved inorganic nitrogen. Also, bacterial uptakes rates were not directly measured, but bacteria were present during the bioassay experiments. However, the project creek's ability

to assimilate N does not mean the nitrogen is removed from the system. The N is assimilated into organic matter that may or may not be re-mineralized later.

The DIN assimilation capacities detailed above were validated against the sampling data for flow and load events above and below the 90th percentile. In general, moderate flows containing high inorganic loads during the warm summer months resulted in phytoplankton biomass increases and rapid nutrient attenuation within the creeks. Figure 7.2.1 is a spatiotemporal plot of flow, NO_x^- concentration increases, and subsequent chlorophyll *a* responses discussed above. Figure 7.2.1 shows the rapid attenuation of NO_x^- along the Southwest Creek axis from the headwater flow meters (0 km) to the creek mouth (2.7 km) for all but one runoff event. The exception was related to Hurricane Alex in August 2004, which brought approximately 5.5 inches of rain to the project area (Franklin 2004). The flow and DIN load observed following Hurricane Alex ranked in the 99th percentile of observations. The fact that very little of the DIN concentrations were attenuated in the stream supports the conclusion that above 90th percentile flows/loads, these creeks do not function as effective buffers to the downstream estuary. Interestingly, chlorophyll *a* peaks during winter months corresponded to nutrient attenuation and high chlorophyll *a* levels. One example of this was the spring 2003 dinoflagellate bloom discussed below under the heading *phytoplankton community composition.* This particular example suggests that the N buffering capacity of these estuarine creeks is persistent throughout the year.

The project creeks ability to assimilate N does not mean the nitrogen is removed from the system. The N is only assimilated into organic matter that may or may not be re-mineralized later. This analysis did not address the fate of the N assimilated in estuarine

creeks. These critical questions are being addressed through nitrogen fate and transport modeling under the Forested and Agricultural Watershed Nitrogen Attenuation project.

7.3 Phytoplankton Abundance and Community Composition

Objective: Compare and contrast the abundance, composition, and diversity of the dominant phytoplankton taxonomic groups between the creeks.

Hypothesis: Contrasting nutrient loads from different LULC will influence the abundance, composition, and diversity of the dominant phytoplankton community groups. Specifically, the reference and forested creeks will contain lower total community abundance and consist of groups adapted to consistently low concentrations of DIN (cyanobacteria), whereas the agricultural creeks will contain higher total abundance and consist of groups suited to high DIN concentrations in chronic and episodic loads (chlorophytes, dinoflagellates).

Phytoplankton Abundance

Chlorophyll *a* concentrations have been used as measures of phytoplankton abundance in studies of the ecology and biogeochemistry of estuarine creeks and sub-estuaries similar to those used in this study (Lapennas 1980; Gallegos et al. 1992; Mallin et al. 2004). Phytoplankton abundance investigations in estuarine creeks, sub-estuaries, and main stem estuaries have focused on nutrient, hydrologic, and other physical controls of growth and biomass (Cloern 2001). Primary productivity investigations have identified nitrogen as a phytoplankton growth limitation factor in North Carolina estuaries (Paerl and Bowles 1987; Rudek et al. 1991; Pinckney et al. 1997; Richardson et al. 2001; Mallin et al. 2004; Piehler et al. 2004). For the most part, hydrologic control investigations have focused on large scale whole estuary circulation processes (Pietrafesa et al. 1996; Luettich 2000; Luettich et al. 2002; Reynold-Fleming and Luettich 2004; Brown et al. 2005). On a smaller

scale applicable to this study, Gallegos (1992) addressed hydrologic controls in a sub-estuary of the Chesapeake Bay. Gallegos (1992) found that proximate watershed freshwater input initiated spatially-constrained, short-duration phytoplankton blooms when compared to broad, long duration blooms initiated by remote inputs. The sampling and bioassay results presented here also identified N limitation and observed short duration phytoplankton abundance increases in response to proximate watershed runoff. However, N limitation was not identified at all times or in all creeks. This result is similar to the findings in Mallin (1994), which identified alternating N and P limitation in the lower NRE.

The monitoring data showed that the Westfork Creek had the highest mean chlorophyll *a* values and the highest mean DIN concentrations. However, the lowest mean chlorophyll *a* concentration and lowest mean DIN concentration did not occur in the same creek. Specifically, the lowest mean DIN was found in Big Creek downstream of the silviculture forest, while the lowest mean chlorophyll *a* was found in Browns Creek downstream of the unmanaged forest. This finding suggests a growth-limiting factor other than nitrogen was present in Browns Creek. Field data show light availability was not a limiting factor in Browns Creek. For example, the euphotic depth reached the creek bottom in winter and was approximately ½ the water column (0.6 m) in the summer. The first project objective included the assumption, based on previous estuarine research, that phytoplankton growth in these systems would be N limited (Paerl and Bowles 1987; Mallin et al. 1991; Rudek et al. 1991; Piehler et al. 2002). However, results from the bioassays and sampling data suggest that P availability was an additional growth-limiting factor in Browns Creek. Molar nutrient concentration ratios from the sampling data showed that the mean N:P ratios at Browns Creek exceeded 16 in 32.5% of samples from the creek mouth station and 68.5%

from the creek headwater station. In comparison, N:P ratios at Big Creek exceeded 16 in 3.1% of samples from the creek mouth station and 30.0% from the creek headwater station (Figure 6.1.11). The fall and spring bioassay results show that Browns Creek responses in chlorophyll *a* and primary productivity were significantly greater than control in treatments containing additions of both N and P. Assuming Browns Creek is representative of the "natural" state of a NRE creek, the reference condition of these non-tidal estuarine creeks may include seasonal periods of N and P co-limitation.

The absence of N and P co-limitation in the agricultural creeks may have been the result of additional watershed PO_4^{-3} loading. The PO_4^{-3} concentrations observed in the sampling data (Figure 6.1.1) and the spatial distribution of PO_4^{-3} concentrations in the study creeks (Figure 6.1.5) support this theory. Specifically, Figure 6.1.5 shows that PO_4^{-3} concentrations were highest at the headwater stations in the agricultural creeks. In contrast, the mean PO_4^{-3} concentrations were highest at the creek mouth stations in the forested creeks. The combined evidence from the bioassay experiments suggests that the additional PO_4^{-3} loading to the estuarine creeks shift these systems away from co- N and P growth limitation toward N limitation. An interesting extension to this work would be to see if N_2 fixing cyanobacterial concentrations increase during periods of relatively high PO_4^{-3} concentrations.

One of the most interesting results from the monitoring data was the relationship between nutrient runoff, flow, and phytoplankton chlorophyll *a* concentrations. Figure 7.3.1 shows a spatial and temporal interpolation of surface chlorophyll *a* against an interpolation of NO_x^- concentration for Southwest Creek (data from this project, the FAWNA project, and those collected by Dr. Bill Kirby Smith, Duke University Marine Laboratory). The blue graph at the bottom of the figure shows the mean daily flow from the flow meter deployed at

the headwater monitoring station in Southwest Creek by the FAWNA project. This flow meter collects runoff from approximately 47% of the total Southwest Creek watershed. The peak flow events (July-August 2003, October 2003, December 2003, July 2004) correspond to high DIN and low chlorophyll *a* concentrations in the headwaters followed days later by low DIN and high chlorophyll *a* concentrations at mid-creek. The winter chlorophyll *a* peak in January 2004, is described below under phytoplankton community composition. In June and July of 2004 the data show the downstream transport of the summer productivity peak over the course of 15 days. The passing of Hurricane Alex on August 3, 2004 offered an example of episodic loading of water and dissolved nutrients. Approximately 5.5 inches of rain fell in the project area during the storm (Franklin 2004). More significantly, storm winds pushed estuarine water up the project creek channels and onto the watershed lands. Following the passage of the storm, watershed runoff stimulated chlorophyll *a* concentration peaks at the mid-creek stations. The location of these chlorophyll *a* peaks was similar to the locations observed under mean conditions. The relationship between headwater flow, nutrient concentrations, and phytoplankton community composition are explored further in the following sections.

Phytoplankton Community Composition

As mentioned above, Gallegos (1992) found that proximate (directly connected) watershed freshwater input initiated short duration phytoplankton blooms. Most phytoplankton have a capacity for accelerated growth under favorable chemical (nutrients), physical (light, salinity, mixing), and biological (grazers) conditions (Paerl 1988; Mallin 1994; Boesch 1996; Paerl 1998; Pinckney et al. 1998; Cloern 2001; Buchanan et al. 2005). Fresh water inputs accompanied by nutrient enrichment have been shown to stimulate

chlorophyte blooms in the NRE (Paerl 2006; Valdes 2006). Similarly, the phytoplankton community analysis results showed that chlorophytes were the dominant community constituent in all the creeks throughout the monitoring period. The phytoplankton community was also punctuated by episodic increases in cyanobacteria, dinoflagellates, cryptomonads, and diatoms. The highest concentrations of cyanobacteria were observed in the creek with the lowest DIN and second greatest DON:DIN (Browns Creek). This observation supports the project hypothesis that low DIN concentrations may results in higher cyanobacteria concentrations. The sampling results also support the project hypothesis that the agricultural creek would have high concentrations of dinoflagellates. Specifically, the highest mean concentrations of dinoflagellates were observed in the agricultural creek (Westfork Creek); however, the high dinoflagellate value was due in large part to a February 2003 *Heterocapsa* sp. bloom.

The controls of the persistent chlorophyte population and the episodic increases in cyanobacteria, dinoflagellates, cryptomonads, and diatoms were addressed by evaluating the community structure changes against headwater flow, DIN concentrations, and salinity. This evaluation shows a complex driver-response relationship. Chlorophyte percent contribution to total chlorophyll *a* was highest during periods of median headwater flow and lower salinity (Figures 7.3.2 through 7.3.5). As mentioned above, this is consistent with existing phytoplankton studies (Harris and Trimbee 1986; Paerl 2006; Valdes 2006). Other phytoplankton populations surged when controlling conditions were above or below median loading of freshwater and nutrients. The controlling conditions were either episodic events (days of high watershed runoff) or more persistent events (weeks of low or negative headwater flow and estuarine water intrusion). Dinoflagellate, cyanobacteria, cryptomonad,

and diatom population increases were mostly found following one of three types of controls: 1) blooms initiated by watershed runoff and loading of nutrient rich freshwater, 2) wind driven flow in the upstream direction (negative flow) of estuarine water containing a "seed" phytoplankton community, or 3) a rapid change from negative flow to high watershed runoff and loading. Specific examples of each of these event drivers are described in the following three paragraphs and detailed in Figures 7.3.2 through 7.3.5.

The first of the phytoplankton community controls identified in the monitoring data was the most common in estuarine phytoplankton studies: bloom initiation by upstream loading that contemporaneously delivers nutrients and dilutes biomass (Pinckney et al. 1997). An example was identified in December 2003. Three tightly clustered runoff events on December 11th, 14-15th, and 24th, 2003 were recorded at the headwaters of the project creeks instrumented with flow meters (Southwest Creek, Big Creek, and Westfork Creek) (Figure 4.2). The daily mean flows on these days were greater than the 97th percentile of recorded flows, except for the 14-15th event at the Southwest Creek location, which ranked in the 75th percentile. At the headwater stations, DIN concentrations were in excess of 100 µg l^{-1} in the agricultural creeks and between 40 µg l^{-1} and 50 µg l^{-1} in the forested creeks. The phytoplankton community was dominated by chlorophytes immediately preceding the December 2003 events. In the week immediately following these peak flows, dinoflagellate blooms were observed in Big, Westfork, and Southwest Creeks. These blooms represented the highest concentration and highest percentage of total population reached by dinoflagellates in each creek during the monitoring period. In Westfork and Big Creeks, the dinoflagellate population accounted for more than 90% of the total chlorophyll *a* concentration. These observations are similar to those common in the NRE during the winter

months. Specifically, Pinckney (1998) found that dinoflagellates reached maximum annual abundance in winter and early spring (January through March). Interestingly, the Browns Creek community composition did not change following this loading event, and chlorophyll *a* concentrations remained less than 10 µg l^{-1}. A likely cause was P limitation in Browns Creek. Specifically, the molar ratio of N:P in the other three creeks remained below 16, while the molar ratio of N:P in Browns Creek ranged from 50 at the headwater station to 32 at the creek mouth station.

The second type of observed phytoplankton community shift driver was upstream flow (negative flow) of estuarine waters and phytoplankton community groups. The salinity data presented in the Table 6.5.1 showed the creek headwater stations maintained a pycnocline with at least a three ppt salinity difference between the surface and the bottom (Δ S > 3ppt). This degree of stratification occurred least frequently at the Browns Creek headwater station (25% of sampling events) and most frequently at the Big Creek sampling station (53% of sampling events) (Table 6.5.1). Salinity at the headwater stations ranged from 0.1 ppt to 17.3ppt. (Table 6.5.1). Negative flow (flow in an upstream direction) was commonly recorded at each gauging station. Negative flow, specifically of estuarine bottom water, has also been observed in data collected in the South River sub-estuary by Dr. William Kirby-Smith of DUML (personal communication). In the data collected for this study, an example of wind driven upstream estuarine water flow and the subsequent change in phytoplankton community was observed in June and July of 2004. During this 61 day period, 16% Big Creek daily mean flow measurement and 37% of Southwest Creek daily mean flow measurements were negative, with the gage on Southwest Creek documenting 10 consecutive days of mean negative flow 2-June-2004 through 12-June-2004. Creek salinity during this

period was the highest recorded. Also during this time, chlorophyll *a* levels increased in all creeks, and the percent contribution of cyanobacteria to total chlorophyll *a* increased in all creeks. In Browns Creek, cyanobacteria contributed up to 80% of total chlorophyll *a* on the 18-June-04 and 8-July-2004 sampling dates. Cyanobacteria blooms have been documented in the NRE during periods of similar warm temperatures, low-flow/high salinity, and N limitation (Pinckney et al. 1998; Piehler et al. 2002).

The third observed phytoplankton community control was identified as a combination of the first two. Specifically, the third driver was generalized as a rapid change from upstream flow to high watershed runoff and loading. Conditions observed surrounding the passing of Hurricane Isabel in September 2003 offered a clear example of this type of driver. Sampling data collected on September 5, 2003, prior to the passing of the storm, showed high salinity concentrations in profile. At the same time, negative flow was measured at the Southwest Creek flow meter. Together, the salinity plots and flow record show a significant estuarine influence prior to the storm. As the eye of Hurricane Isabel passed to the west of the study area on September 18, 2003, northeast winds from the west side of the hurricane pushed more estuarine waters into the project creeks. During the course of the storm, approximately 5.5 inches of rain fell in the project area (Beven 2004). The subsequent runoff loading from the storm rainfall delivered the highest headwater station DIN concentrations observed throughout the entire monitoring period in Southwest, Big, and Browns Creeks. The phytoplankton community responses to this event were a cryptomonad bloom in Westfork and Southwest Creeks and a cyanobacterial bloom in Browns Creek. The cryptomonads represented over 60% of the total chlorophyll *a* concentrations in Westfork Creek, and cyanobacteria represented over 70% of total chlorophyll *a* in Browns Creek. Big

Creek did not have a distinct change in chlorophyll *a* or community composition following the hurricane; therefore, suggesting the storm flows pushed the phytoplankton community out into the South River sub-estuary. This may have happened in Big Creek and not in the other creeks due to the relatively low volume and flushing time in Big Creek.

The third type of phytoplankton community shift driver was also observed in the spring of 2003. Specifically, the salinity surface plots in the Browns Creek and Westfork Creek figures show a rapid change in salinity conditions in mid-March. The salinity change, DIN concentrations, and rainfall data collected at OGF suggest a high runoff event. Unfortunately, this event occurred prior to flow meter installation in the creek headwaters. The community response was a shift from chlorophytes to diatoms in Browns and Westfork Creeks.

Phytoplankton Community Diversity.

The Sanders and Kuenzler (1979) study of phytoplankton diversity applied the Shannon-Weiner index of diversity to North Carolina estuarine phytoplankton communities. The results of the Sander and Kuenzler (1979) study showed phytoplankton diversity was lower in a estuarine tidal creek receiving nitrogen rich sewage treatment plant effluent when compared to phytoplankton assemblages from surrounding estuaries. Similarly, the study presented here also used the Shannon-Weiner index to assess phytoplankton diversity in non-tidal estuarine creeks. The results of these analysis were similar to results from the Sanders and Kuenzler (1979) analysis. Specifically, the results showed that over the long term (18 months), the phytoplankton assemblage was most diverse in Browns Creek, downstream of the reference watershed, and least diverse in Southwest Creek, downstream of an agricultural

watershed (Figure 6.4.1). This finding supports the hypothesis that high chronic loading of DIN would reduce diversity in the creek phytoplankton community.

The results of the Shannon-Weiner index short term phytoplankton diversity assessments (described in the Materials and Methods chapter) were conflicting and less straightforward when compared to the long-term assessments. Specifically, the phytoplankton assemblages observed on a single day in Browns Creek and Big Creek (fortest watersheds) were on average less diverse than the assemblage observed in Westfork Creek, which received agricultural runoff (Figure 6.4.2). However, the phytoplankton assemblages observed on a single day in Browns Creek and Big Creek were on average more diverse than the assemblage in Southwest Creek, which also received agricultural runoff.

These diversity assessments show that over an annual cycle, the phytoplankton community was most diverse downstream of the least disturbed watershed. The diversity assessments of individual days of data do not show as clear of a conclusion. A possible explanation could be higher total chlorophyll *a* in West Fork creek. Specifically, with higher biomass, minority community constituents would be easier to detect and quantify via HPLC diagnostic pigment analysis. Also, there is the possibility that short-term diversity assessment of phytoplankton assemblages in estuarine creeks are not an appropriate application of the Shannon-Weiner index. Specifically, diversity may be better measured as longer term resilience to perturbation as opposed to a short term co-existence of many different species.

There are broad ecological impacts that can arise from phytoplankton community shifts and lower diversity. Impaired trophic transfer is one example. The current eutrophication paradigm suggests excess primary production, or a shift to a nuisance algal species, may inhibit trophic energy transfer (Cloern 2001). In the NPES, zooplankton have

been shown to graze as much as 45% of daily phytoplankton productivity with annual peaks that coincide with the arrival of juvenile fishes in estuarine creeks (Deegan and Day 1985; Epperly and Ross 1986; Mallin and Paerl 1994). Studies have also shown selective feeding of zooplankton and other primary consumers on specific phytoplankton is based on size, nutritional value, and toxicity (Mallin and Paerl 1994; Haywood and Burns 2003; Leonard 2003). This evidence supports the theory that phytoplankton community composition can influence the transfer of energy from primary producers to secondary consumers in estuarine creeks.

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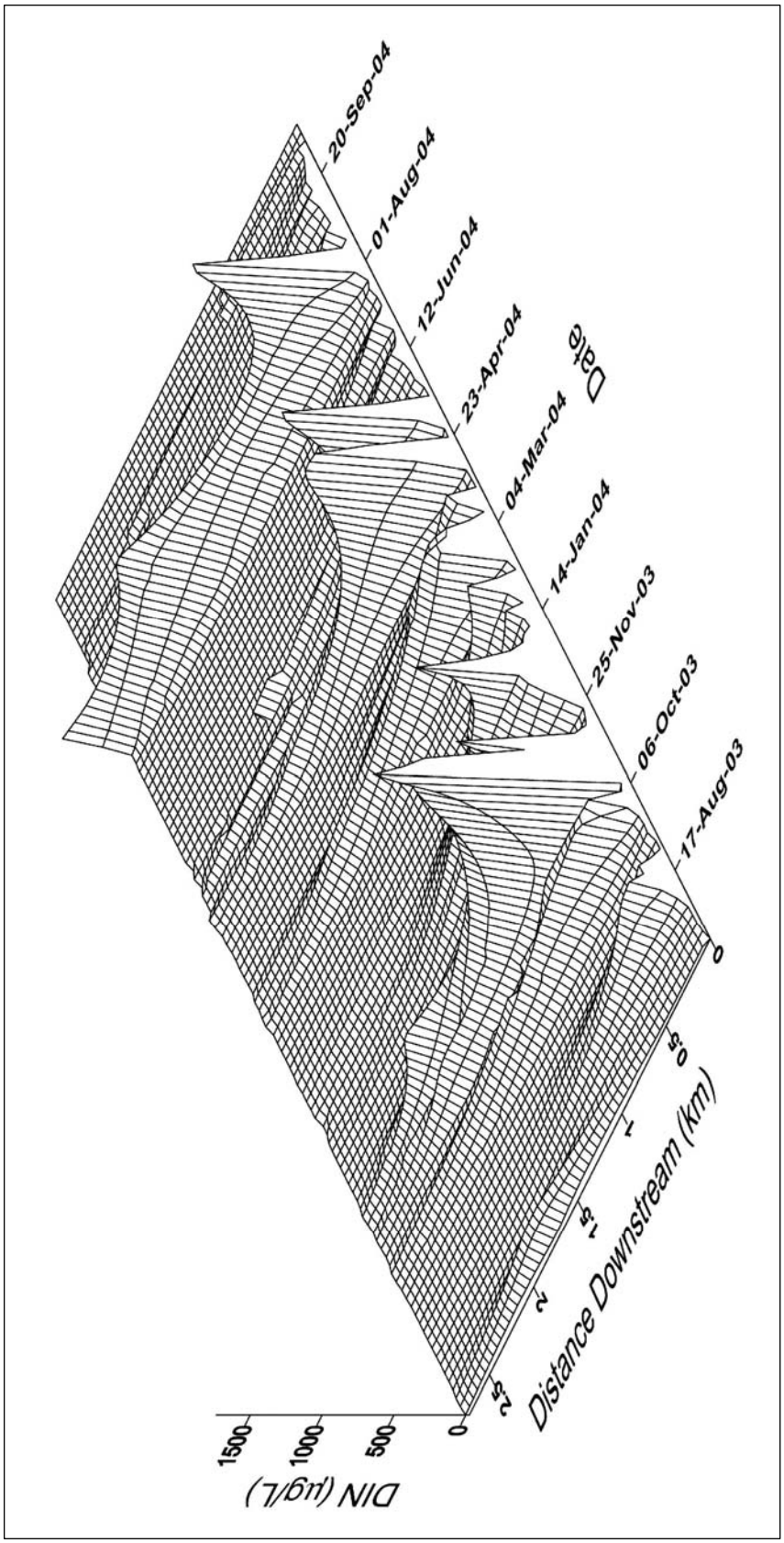

Figure 7.2.1 Nutrient attenuation down southwest creek axis July 2003 – November 2004

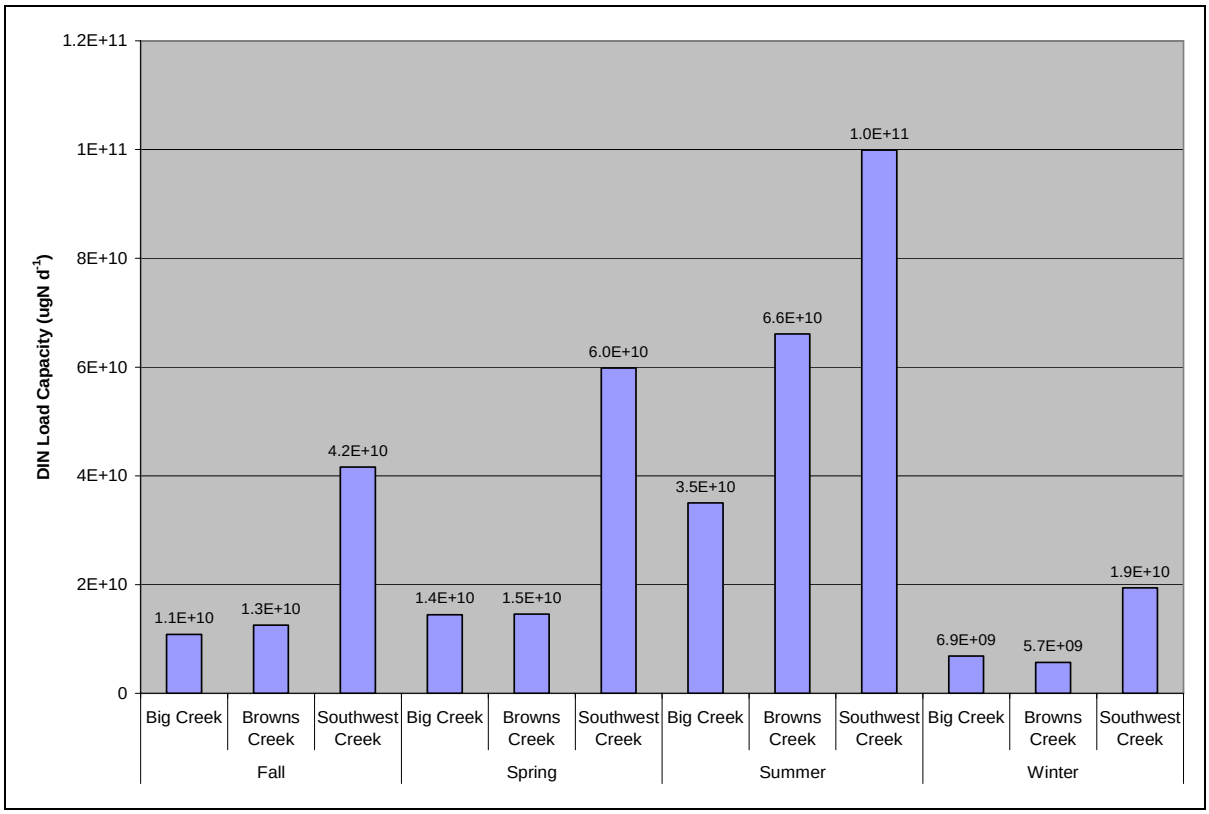

Figure 7.2.2 Estimated seasonal DIN load capacities.
Capacities represent maximum DIN load each creek could assimilate given phytoplankton abundance observed during seasonal bioassays.

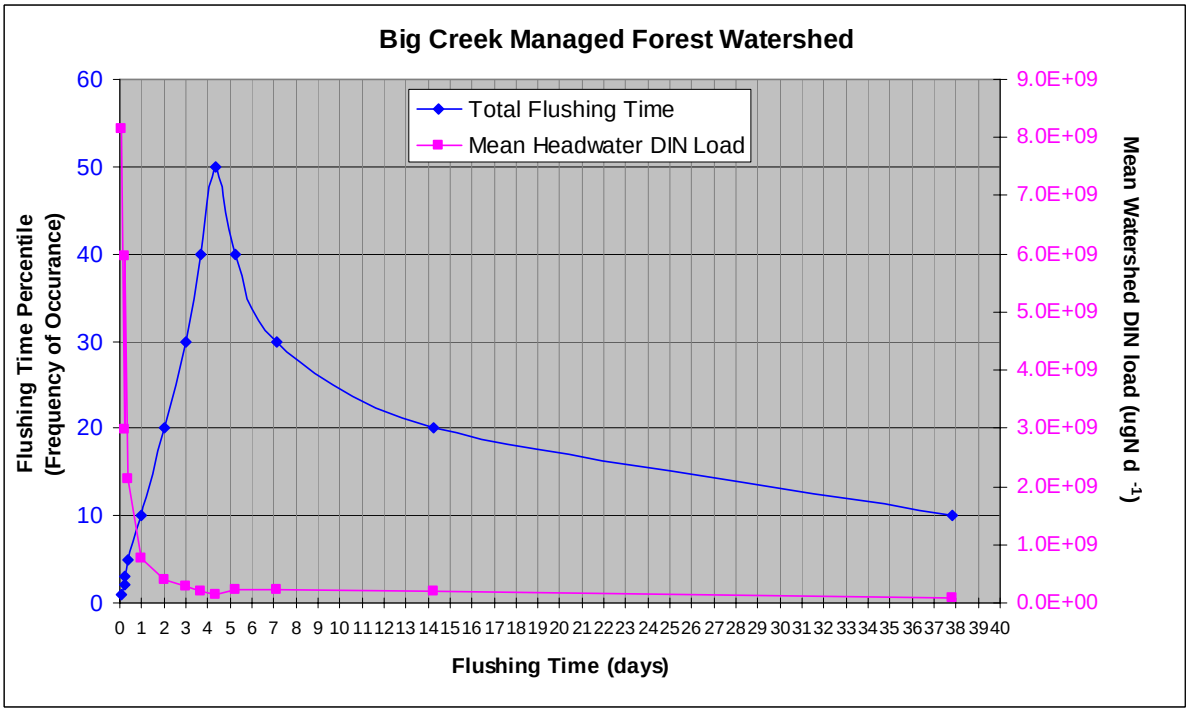

Table 7.2.3 Big Creek headwater DIN load at total creek flushing time intervals

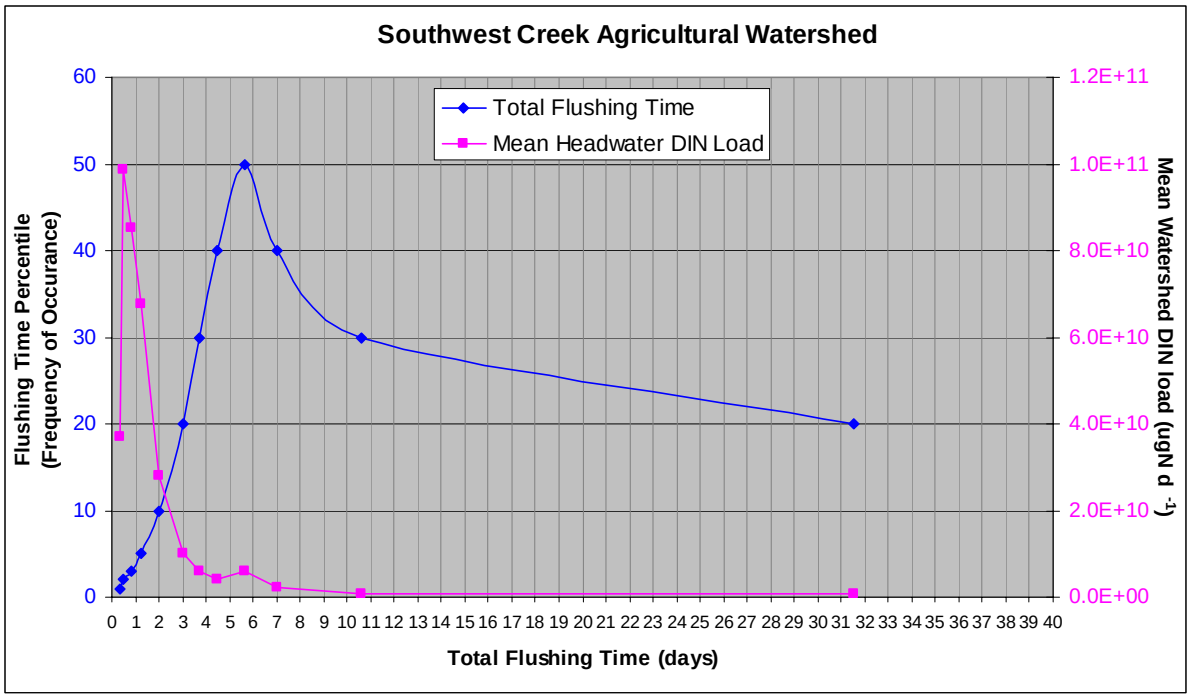

Table 7.2.4 Southwest Creek headwater DIN load at total creek flushing time intervals

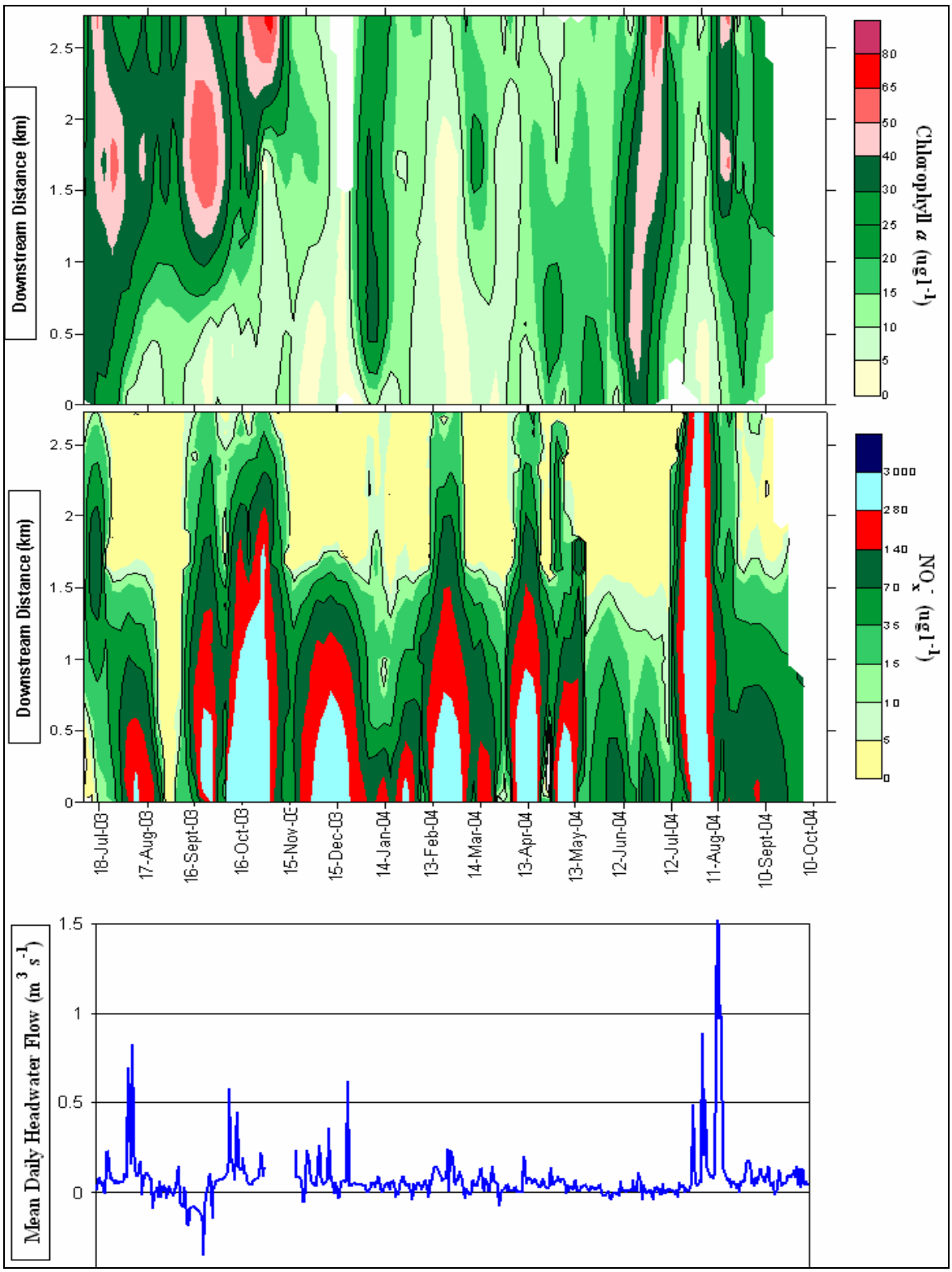

Figure 7.3.1 Spatiotemporal plot of flow, NO_x, and chlorophyll a for Southwest Creek
Search window 15 days and 2.7 km. White area indicates insufficient data. Chlorophyll a N = 251
Data from UNC-IMS and DUML Projects (This Study, FAWNA , Dr. Bill Kirby-Smith)

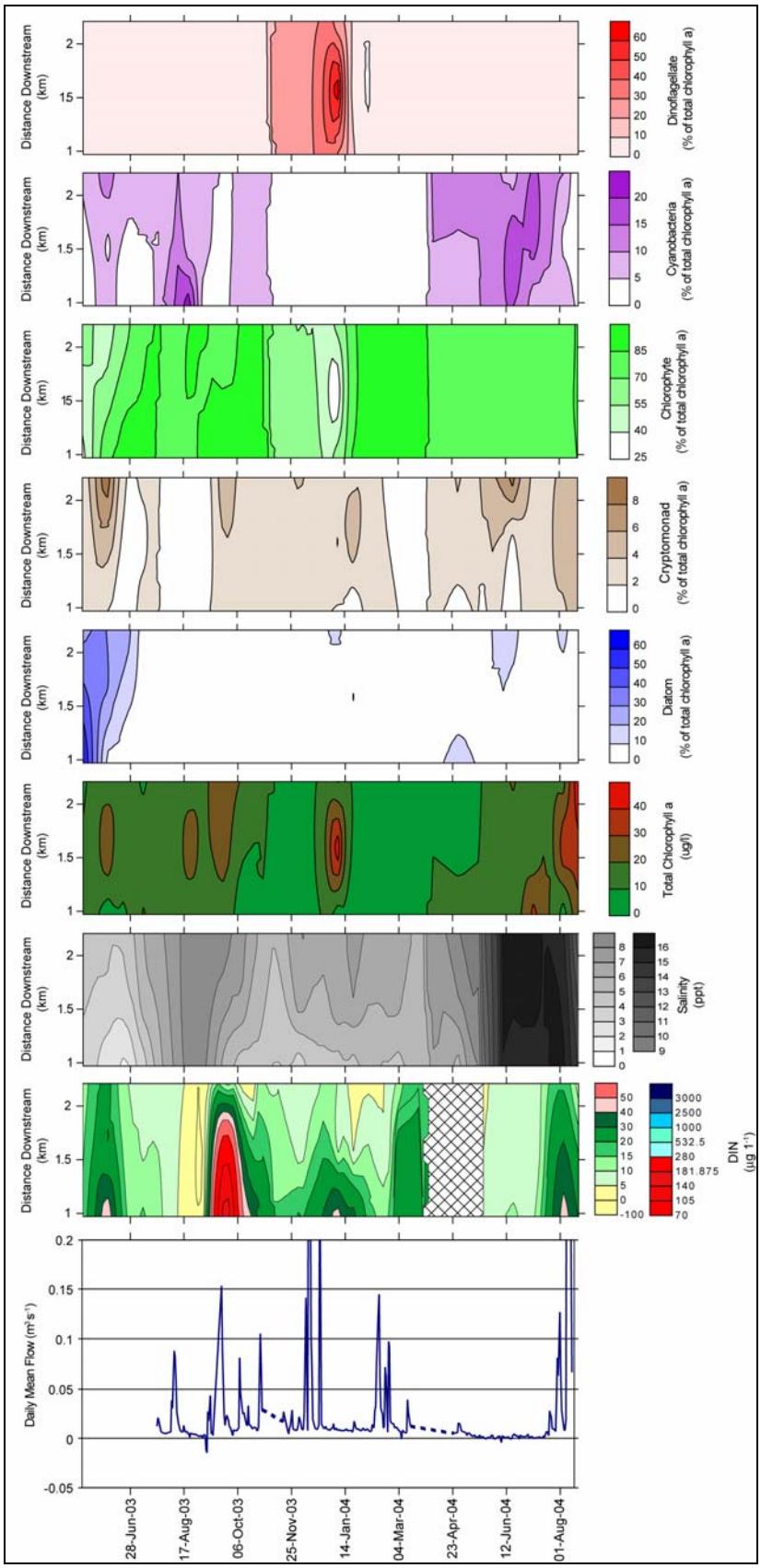

Figure 7.3.2 Big Creek surface phytoplankton community, salinity, DIN and headwater flow

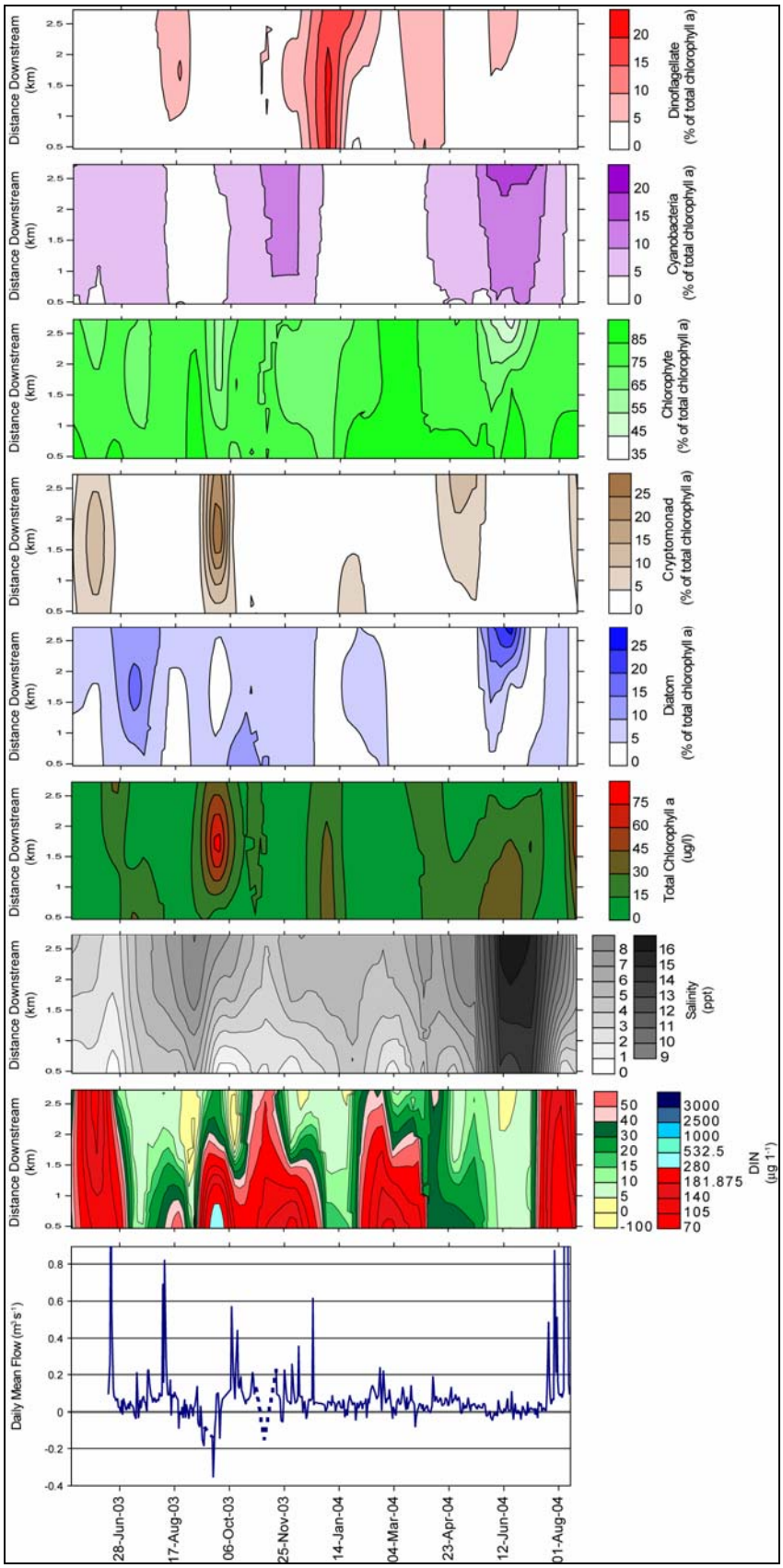

Figure 7.3.3 Southwest Creek surface phytoplankton community, salinity, DIN and headwater flow

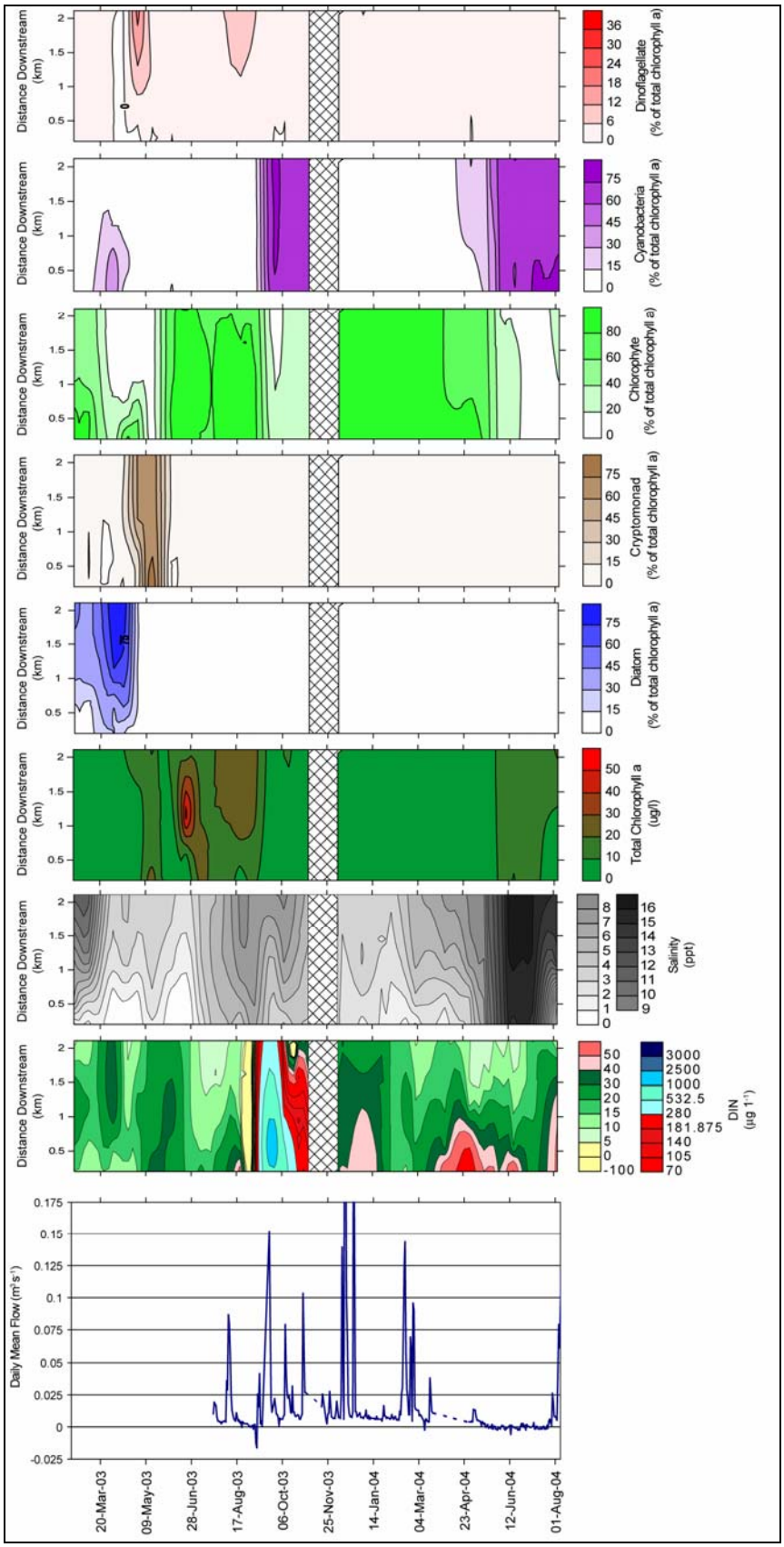

Figure 7.3.4 Browns Creek surface phytoplankton community, salinity, DIN and headwater flow

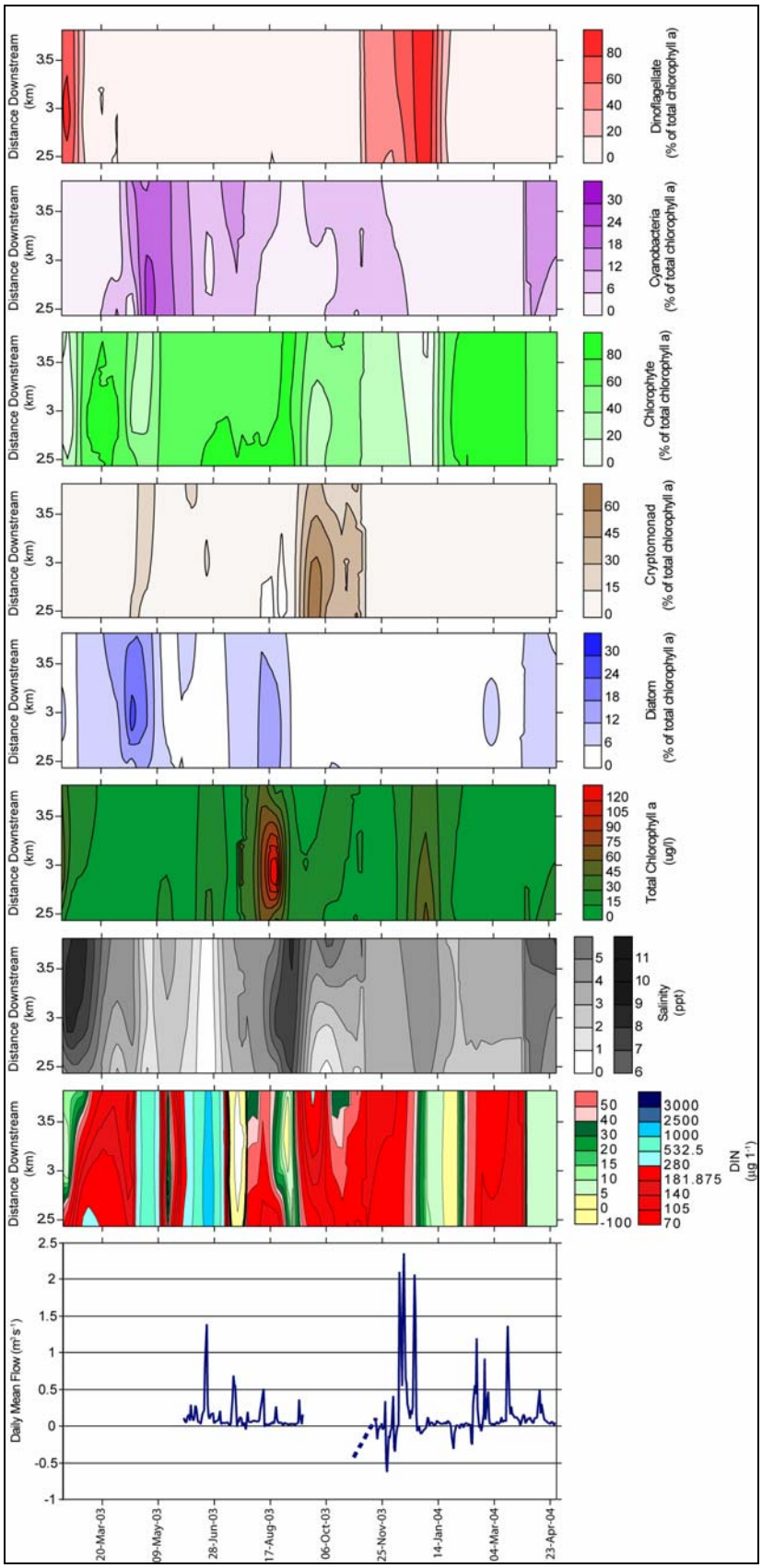

Figure 7.3.5 Westfork Creek surface phytoplankton community, salinity, DIN and headwater flow

CHAPTER 8

SYNTHESIS OF FINDINGS

Results of this study demonstrate that mechanistically, human alterations of these watersheds affect creek nutrient delivery and ecological response. Specifically, the watershed with the greatest degree of disturbance (agricultural land use) had the highest N loading, highest in-stream nutrient concentrations, lowest phytoplankton diversity, and decreased capacity to buffer the mainstem estuary from episodic nutrient loading. This work is among the few studies in NPES non-tidal estuarine creeks and confirmed the findings of studies in other systems that have shown as watershed modifications increase, downstream estuarine water quality decreases (Corbett et al. 1997; Wahl et al. 1997; Holland et al. 2004; Mallin et al. 2004; Valiela et al. 2004). This study also begins to fill a knowledge gap that has previously hindered linking watershed models to estuarine eutrophication models. Specifically, the study combined in-situ experiments on the interactions of nutrient supply and hydrodynamics with some of the first monitoring data on phytoplankton diversity and abundance in NPES non-tidal creeks. These efforts assessed the impacts of different land uses on eutrophication potential; this included not only carbon fixed and nitrogen assimilated, but also what type of organism fixed the carbon and assimilated the nitrogen (i.e. phytoplankton). This project considered a range of nitrogen forms. The data show that land use affected N load forms, but the phytoplankton responses to the different N forms were mostly the same. This information gives researchers a better chance to predict the fate of the terriginous carbon and nitrogen in estuarine waters (e.g. grazed, remineralized, transported

downstream). However, the findings must be interpreted and conveyed to decision makers in a meaningful and understandable form to make a real contribution to ecosystem management. The following sections below detail the conclusions from each project objective and close with how the findings can be applied directly applied to ecosystem research and management.

8.1 Land Use Influence on Nutrient Concentrations

Objective: Quantify the concentrations and forms of biologically available nitrogen in creeks downstream of watersheds draining four distinct land uses.

Conclusions: The results of the creek sampling data analysis show that runoff originating from the agricultural areas was higher in total nitrogen and DIN when compared to the managed and unmanaged forest creeks. NO_x^- was the primary form of DIN downstream of the agricultural watersheds. In contrast, NH_4^+ was the primary form of DIN downstream of the forested watersheds. These findings are similar to those reached in studies of watershed land use and nutrient loading to creeks and sub-estuaries in other systems, including Kirby-Smith and Barber (1979), Wahl et al. (1997), and Mallin et al (2004). In addition, the findings corroborate the paradigm that land use modification can change watershed nutrient export. Furthermore, they emphasize the need for coastal communities to focus on nutrient (and in this case, specifically N) management in land use planning.

8.2 Creek Nutrient Buffering Capacity

Objective: Determine if these non-tidal estuarine creeks serve as effective nutrient buffers to the main-stem estuary.

Conclusions: The forested creeks in the study have the capacity to assimilate DIN delivered up to the 95^{th} percentile of flow and loads year round. The agricultural creeks in the study

can assimilate DIN delivered up to the 80th percentile of flow and DIN loads in winter and up to the 90th percentile of flow and DIN loads in spring, summer, and fall.

The creek capacity to assimilate DIN does not mean that N is permanently removed from the system. The DIN assimilation capacity does mean there is limited potential for high DIN concentrations to reach the main stem estuary though the creeks. However, the possibility exists that N assimilated within the project creeks may be re-mineralized at a later time. Ensign (2004) described nutrient spiraling in the ditches and channels upstream of Southwest Creek. Specifically, phytoplankton and periphyton assimilation and senescence in shallow waters rapidly transports N rich detritus to denitrifiers in the benthos. Extending this concept downstream to the headwater estuarine creeks suggests that creek N assimilation may provide nutrient spiraling opportunities for denitrification. This potential is currently being addressed by the Forested and Agricultural Watershed Nitrogen Attenuation project via measurements of denitrification in the wetland and benthic sediments of two project creeks. At completion, the data from both studies will be used to further develop the fate of N contained in allochthonous and autochthonous organic matter found in the project creeks.

8.3 Phytoplankton Abundance and Community Composition

Objective: Compare and contrast the abundance, composition, and diversity of the dominant phytoplankton taxonomic groups between the creeks.

Conclusions: Increases in creek chlorophyll *a* concentrations were observed following watershed nutrient loading events in all creeks. However, high chlorophyll *a* observed during periods of low flow and low dissolved nutrients suggest in-stream nutrient recycling is also an important control of phytoplankton abundance. As hypothesized, the highest chlorophyll *a* concentrations were observed downstream of the agricultural watersheds. Periods of P

limitation identified in the sampling and bioassay data show that these systems may not be strictly N limited and may also explain the lower chlorophyll *a* concentrations downstream of the unmanaged forest watershed.

Within the chlorophyll *a* "green box", chlorophytes made up the vast majority of the phytoplankton community in the study creeks. This finding is not surprising, given the strong bloom potential chlorophytes have shown in the main-stem of the NRE following freshwater loading and nutrient enrichment (Paerl et al. 2006; Valdes 2006). Periods of chlorophyte dominance in the creeks were punctuated by episodic dominance of cyanobacteria, dinoflagellates, cryptomonads, and diatoms. The controls of these events were presented with examples in the Discussion chapter. The highest level of phytoplankton community diversity over the monitoring period was observed in the creek downstream of the least disturbed watershed (Browns Creek, an unmanaged forest watershed). This suggests that watershed modifications, which increase fresh water and nutrient loading, may decrease creek phytoplankton diversity and possibly community resilience (Groffman et al. 2006). The highest concentrations of cyanobacteria were observed in the creek with the lowest DIN and second greatest DON:DIN (Browns Creek, unmanaged forest watershed). An interesting extension to this work would be to determine if concentrations of N_2 fixing cyanobacteria increase during periods of low DIN concentrations and relatively high PO_4^{-3} concentrations.

8.4 Potential Application of Findings in Estuarine Research and Management

The project results show a need for watershed management designed to mitigate increased fresh water and nutrient loading from watershed modification upstream of estuarine creeks. This need is urgent, given the continued rise in urban and suburban development along U.S. coastal waters (Salvesen 2005). The acceleration of development is

expected to continue locally near the study site, where Carteret County, NC plans to develop an additional 85,000 acres by the year 2025 to accommodate increases in permanent and seasonal populations (Carteret County 2005). This increased development will be regulated in part by the Neuse River Nutrient Sensitive Waters Management Strategy (Neuse Rules), the North Carolina Coastal Area Management Act (CAMA), and local land use laws. These regulations consider freshwater and nutrient loading components; however, they are lacking in several key areas discussed below.

The Neuse Rules were established in 1997 to reduce the total annual nitrogen load to the NRE by 30% of year 1995 levels. These rules included components that addressed nitrogen loading from agriculture, stormwater dischargers, waste water dischargers, and persons applying fertilizer to over 50 acres of land (NCAC 1997). The agricultural portion of the rules applied to the project watersheds, where fertilizer management and controlled drainage measures have been installed. The Neuse Rules also mandated 50 foot riparian buffers to all perennial and intermittent streams, lakes, ponds, and estuaries in the Neuse River Basin; however, this rule does not apply to man-made ditches or stormwater channels (NCAC 1997). Also lacking in the Neuse Rules is a stormwater planning component, which does not apply to the areas surrounding the estuary downstream of New Bern, NC (Figure 4.1). The second watershed management statute in the NPES watersheds, CAMA, currently requires coastal counties and municipalities to draft land use management plans. However, these plans have limited connections to water quality and watershed runoff loading. Specifically, CAMA only requires that development projects within 575 feet of estuarine waters with greater than 30% impervious surface construct stormwater mitigation systems capable of retaining 1.5 inches of rain fall (NCDENR 2003). In creek watersheds selected for

this study, a 575 foot buffer covers only eight percent of the watershed areas. Furthermore, Carteret County does not currently operate a stormwater system or regulate stormwater dischargers beyond initial property development permit application review and approval (Carteret County 2005). This illustrates that existing regulations do not adequately address fresh water and nutrient loading which were shown as potential threats to creek buffering capacity, water quality, and ecological diversity in this study.

Threshold development is the first quantitative step that could be taken to improve the management and assessment options for the NPES nursery creeks. A threshold is a point where there is an abrupt change in an ecological condition caused by alterations in an environmental control or driver (Holling 1973; Groffman et al. 2006). The threshold concept has been applied to nutrient loading in other estuary management programs focused on habitat quality (Howes 2004). In the NPES estuarine creeks, managers must identify a threshold in freshwater and nutrient loading to avoid eutrophication and the accompanying decreased ecological quality at multiple trophic levels (Nixon 1995; Holland et al. 1997; Paerl et al. 1998; Cloern 2001; Pitois et al. 2001; Gray et al. 2002; Campbell and Goodman 2004; McNatt and Rice 2004).

Results from this study will contribute critical information for threshold development in two areas. First, the creek flushing time and recurrence intervals documented in this study provide the critical quantitative values for low (forested watershed) and high (agricultural watershed) degrees of watershed modification. Second, the observed phytoplankton abundance and diversity values and experimentally-derived primary productivity rates can be used as calibration parameters in an empirical modeling approach that relates observed creek buffering capacity, water quality, and ecological diversity to upstream watershed

characteristics. These data will most likely need to be supplemented with nutrient loading and creek flushing times data from urban and suburban watersheds on the NPES shoreline. Possessing quantitative information on land use, nutrient loading, and creek flushing times at different degrees of watershed development will allow managers and environmental engineers to identify the specific degree of development where there is an undesirable change in creek buffering capacity, water quality, or ecological diversity (Holling 1973; Howes 2004; Groffman et al. 2006). The path to threshold development outlined above will constitute a much-needed improvement to current watershed land use management in the areas surrounding the NPES. These improvements illustrate how the study findings on the deleterious effects of increased fresh water and nutrient loading can be used to effectively and realistically manage water quality in these nursery creeks with the overall goal being the preservation of acceptable water quality in the NPES mainstem.

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